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Sewage sludge hydrolysates and organic waste as alternative phosphate fertilizers in a circular economy strategy

Presentata da: Silvia Mancarella

Coordinatore Dottorato

Prof. Giovanni Dinelli

Supervisore

Prof. Giorgio Prosdocimi Gianquinto

Co-supervisore

Dott. Luciano Cavani

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Thesis abstract

Phosphorus (P) is one of the main plant nutrients. In soil, though in high concentration, P has very low solubility, due to strong bonds with Al and Fe in acidic soils and with Ca and Mg in alkaline soils. Soil P can be mineralized or immobilized by microbial biomass, which participates actively to the soil-plant P system. The plant roots can also increase the P solubility with organic acid extrusion, which solubilizes P acidifying the rhizosphere or protecting P from metal precipitation. The higher food demand of the growing world population has led to an increase of the use of fertilizers, among them the phosphate one. Until now, the raw source of P fertilizers has been mainly the phosphate rocks, which are non-renewable resources and are predicted to be depleted in the next decades. Thus, there is the improving need to close the P cycle in order to reduce the P input from mineral source, recycling P rich organic products such as animal manure and human excreta. However, P is usually not highly available in organic waste for plant uptake. In calcareous soil, where struvite, sewage ash or phosphate rocks are not effective, different treatments on sewage sludge are needed in order to recover P. Therefore, the aim of this work was to study the P availability in soil and plants of different bio-waste and to develop a new fertilizer from sewage sludge, which could not only recover a high amount of P from the waste, but could also be easily assimilated from plants.

In this study, different bio-waste was tested for their P soil availability, their microbial P immobilization and the plant P uptake. We found different characteristics that can help predict the P availability of an organic product. Firstly, the metal concentration and the Al:P, Fe:P and Ca:P ratios are easy and economic tools that give a good prevision. The highest Al, Fe and Ca concentration, the lowest will be the short-term P availability. The organic C can improve the microbial biomass and ameliorate the rhizosphere conditions, also protecting P from precipitation with Ca. Furthermore, microbial biomass can release P from metals, increasing metal and P phytoavailability, without competing for P with plants, storing P for long-term nutrition and preventing P fixation. Therefore, products with low Al, Ca and Fe concentration, such as swine slurries and their derived products, can be more suitable for short-term fertilization, while bio-waste with high metal concentration, for example compost, and products with high organic matter content, like manure, can be more convenient for long-term fertilization.

Afterwards, many hydrolysis of sewage sludge with chemical and enzymatic extractants had been tested in term of P recovery. Two sewage sludge samples were used: an anaerobic and Fe-rich sludge and an aerobic and Al-rich sludge. The results were very promising, with the highest P recovery provided by the sulphuric acid in combination with acid phosphatase and citric acid in combination with acid

phosphatase. Due to its lower heavy metal concentration, the treatment with citric acid and acid phosphatase was chosen. In the trial, the extraction with only citric acid was inserted as a comparison. The hydrolysates were then tried in a soilless system, on floating lettuce. Nutrients in the solution as well as the leaf area of the plants were measured periodically during the lettuce growth. At the end of the experiment, plants biomass and plants metal concentration were assessed. It resulted that the alternative fertilizer did not performed as the chemical reference, but provided a good plant growth, even when the meso and micronutrient were not added to the nutrient solution, showing that they contained a good amount of Mg, Fe, S and B. Nevertheless, the problems encountered in the soilless cultivation were a pH increase of the nutrient solution, which caused a nutrient precipitation and an Al toxicity that depleted the roots development. The problems of pH and Al toxicity were overcome with the addition of a calcareous soil to the system. A soil incubation test was carried out to understand the P availability kinetics, the soil pH, the phosphatase activity and the microbial growth. After that, lettuce, var. *Romana*, was grown in the calcareous soil and harvested every 40 days in order to measure plant growth and metal concentration. Additionally, the soil of the rhizosphere was tested for the same traits previously described. The hydrolysates in calcareous soil provided better results in comparison not only to raw sludge but also to the chemical reference. The higher plant growth and P uptake were provided by the more constant P availability, decreased soil pH, higher phosphatase activity and microbial growth in the soil. In order to understand the increase of P solubility in the products going from the raw sludge to the hydrolysates, both were tested at the same amount of sludge, rather than at a fixed P dose. Exhausted sludge samples, after the hydrolysis, were tested at the same dose. In this way, it was possible to calculate the P use efficiency of the fertilizers. The soil incubation with these different amounts of fertilizers was repeated as previously described. For this trial it was used a baby leaf lettuce in order to have the same plants for the entire duration of the test and to do not interfere with the P balance. From the results, it was shown a very high P use efficiency of the aerobic sludge hydrolysates and a very good plant growth for every hydrolysates treatments. On the contrary, the exhausted sludge provided a plant P uptake lower than the not amended plants, acting as a sink of P from the soil solutions. However, they were tested as soil conditioner (at N fixed dose) and resulted comparable to the raw sludge in terms of plant growth with the benefit of a less heavy metal uptake.

In conclusion, the hydrolysates resulted suitable for lettuce nutrition as an alternative to chemical fertilizers, although further studies on the use of solubilizing bacteria for the P extraction and even more on the environmental impact of the chemical and enzymatic hydrolysis with life cycle assessment method (LCA) are needed.

Riassunto

Il fosforo (P) è uno dei principali nutrienti per le piante. Nel suolo, sebbene spesso in alte concentrazioni, è scarsamente disponibile, poiché si lega ad Al e Fe (in caso di suoli acidi) e Ca e Mg (in caso di suoli alcalini). Il fosforo può essere mineralizzato o immobilizzato dalla biomassa microbica che partecipa attivamente al ciclo del fosforo nel sistema suolo-pianta. Anche la pianta è in grado di aumentare la solubilità del fosforo nel suolo, attraverso l'estrusione di acidi organici e fosfatasi dalle radici, che acidificano la rizosfera e proteggono il P dalla precipitazione con metalli e carbonati. La crescita esponenziale della popolazione mondiale ha causato una crescita nella necessità di cibo. È perciò aumentato a dismisura l'utilizzo di fertilizzanti, tra cui i fertilizzanti fosfatici, per ottenere una maggiore resa produttiva dei campi coltivati. Finora la principale materia prima per la produzione di fertilizzanti fosfatici sono state le rocce fosfatiche, le quali però sono una risorsa non rinnovabile. Le previsioni sostengono che le riserve di rocce fosfatiche si esauriranno nei prossimi decenni. Il ciclo del fosforo, che al momento inizia dai fertilizzanti per finire nei residui vegetali, nei liquami o nei fanghi di depurazione civili con scarso o nullo riciclo, dovrebbe essere chiuso utilizzando i rifiuti come primo input. Il fosforo all'interno dei rifiuti organici è però scarsamente disponibile per l'assimilazione da parte delle piante. Inoltre, in suoli calcarei, dove struvite, ceneri e rocce fosfatiche hanno scarso effetto fertilizzante, sono necessarie alternative per il recupero del fosforo dai fanghi civili. L'obiettivo dello studio è, perciò di studiare la disponibilità del fosforo nel suolo e per le piante di differenti rifiuti organici e di sviluppare un nuovo fertilizzante dai fanghi di depurazione, che abbia sia un'alta percentuale di recupero di P, ma che possa anche essere facilmente assimilato dalle piante.

In questo studio, differenti prodotti organici sono stati testati per la loro solubilità di P, per l'immobilizzazione microbica di P e per l' assimilazione da parte delle piante. Si è scoperto che diverse caratteristiche sono importanti per predire la solubilità del fosforo di un prodotto organico. La prima caratteristica utile è il contenuto di metalli e il rapporto Al:P, Fe:P e Ca:P, i quali possono dare già una buona stima della solubilità in modo veloce ed economico. Maggiore è il contenuto di Al, Fe e Ca minore sarà la solubilità del fosforo nel prodotto nel breve periodo. Il carbonio organico stimola la crescita microbica e forma dei complessi con il P proteggendolo dalla precipitazione. Inoltre, la biomassa microbica può rilasciare gradualmente P e metalli nella soluzione del suolo, senza competere per l'assimilazione del P con la pianta, agendo così da accumulo di P per la nutrizione a lungo termine. Pertanto i prodotti con basso contenuto di Al, Fe e Ca, come liquami suini e prodotti derivanti da questi sono adatti alla fertilizzazione a breve termine, mentre quelli con alto contenuto di metalli, come i compost, o ancora meglio ad alto contenuto di sostanza organica, come i letami

bovini, sono migliori per un rilascio graduale del fosforo e una fertilizzazione di colture a ciclo lungo.

Successivamente, diverse soluzioni per l'idrolisi di fanghi di depurazione sono state testate, con estraenti chimici ed enzimatici, per il recupero di P. Sono stati utilizzati due tipi di fanghi: un fango anaerobico ricco in Fe a un fango aerobico ricco in Al. I risultati sono stati promettenti, con le combinazioni di acido citrico e fosfatasi acida e acido solforico e fosfatasi acida che hanno mostrato le percentuali di recupero maggiori. Tuttavia, per il minor contenuto di metalli pesanti in soluzione, è stato scelto il trattamento con acido citrico e fosfatasi acida. Negli esperimenti con le piante è stato inserito anche il trattamento con il solo acido citrico. Gli idrolizzati sono stati perciò provati come fertilizzanti in un sistema fuori suolo, con la lattuga in floating system. Gli elementi nella soluzione nutritiva e l'area fogliare delle piante sono stati monitorati periodicamente durante tutta la durata dell'esperimento. Alla fine del test sono stati misurati anche biomassa vegetale e contenuto di metalli nelle piante. Dai risultati si denota che gli idrolizzati non hanno una performance ancora comparabile con i concimi chimi, anche se riescono comunque a promuovere una buona crescita delle piante pur nei trattamenti con la sola aggiunta di azoto e potassio invece che la soluzione standard con meso e microelementi. Questo ha rimarcato il buon contenuto di elementi nutritivi negli idrolizzati, oltre al P, tra cui soprattutto Mg, Fe, S e B. I problemi incontrati, invece, sono stati un aumento del pH della soluzione nutritiva, che ha causato la precipitazione di molti nutrienti, e un eccesso di Al a livelli tossici per lo sviluppo radicale delle piante. Queste criticità sono state, però, superate con l'inserimento nel sistema del suolo calcareo. Il suolo addizionato degli idrolizzati è stato incubato e testato per la cinetica della disponibilità di P, per il pH, l'attività fosfatasica e la crescita microbica nel tempo. Contemporaneamente una prova su piante, lattuga var. *Romana*, è stata portata avanti per misurare crescita e concentrazione di metalli nella biomassa vegetale. Inoltre anche il suolo rizosferico (campionato in prossimità delle radici) è stato testato per le stesse caratteristiche precedentemente menzionate per la prova di incubazione del suolo. Dopodiché piante di lattuga, var. *Romana*, sono state cresciute nel suolo calcareo fertilizzato con idrolizzati e con i fanghi grezzi e raccolte ogni 40 giorni per misurare la crescita e la concentrazione di metalli. Gli idrolizzati nel suolo calcareo hanno avuto risultati migliori rispetto non solo ai fanghi prima delle idrolisi ma anche rispetto al testimone chimico. La crescita maggiore delle piante è stata favorita dalla disponibilità più constante di P, dal leggero abbassamento del pH, da una attività fosfatasica e una crescita microbica maggiore. Per delineare meglio l'avvenuto aumento della solubilità del fosforo passando dai fanghi grezzi agli idrolizzati, sono stati testati entrambi ad una dose fissa di fango, invece che ad una dose fissa di P, come in precedenza. Anche i fanghi esausti, dopo l'idrolisi, sono stati testati alla stessa dose. In questo modo è

stato possibile calcolare l'efficienza d'uso del fosforo dei diversi fertilizzanti. La prova di incubazione del suolo con queste dosi fisse di fertilizzante è stata ripetuta come precedentemente descritto. Per la prova con le piante si è scelta una lattuga da taglio, invece della lattuga romana usata nelle prove precedenti, per avere le stesse piante durante tutta la durata del test e non andare così ad interferire con il bilancio di massa del P. I risultati hanno mostrato un'alta efficienza di uso del fosforo negli idrolizzati provenienti dal fango aerobico ricco in Al e una buona crescita delle piante trattate con ognuno dei 4 diversi idrolizzati. Al contrario, i fanghi esausti hanno evidenziato un'assimilazione di P minore rispetto al testimone non trattato, agendo come un sink di P dalla soluzione del suolo. Quando però questi sono stati testati come ammendanti (ad una dose fissa di N) hanno avuto risultati comparabili ai fanghi grezzi rispetto alla crescita vegetale, ma con il vantaggio di un minore assorbimento di metalli pesanti.

In conclusione, gli idrolizzati sono risultati una valida alternativa ai fertilizzanti chimici per la nutrizione della lattuga, benché siano necessari ulteriori studi sull'utilizzo di batteri solubilizzatori per l'estrazione di P dai fanghi e ancor più sull'impatto ambientale delle idrolisi chimiche ed enzimatiche con il metodo dell'analisi del ciclo di vita (LCA).

Index

Chapter 1

Introduction

Abstract

Phosphorus (P) is one of the main plant nutrients. In soil, P, although in high concentration, has very low solubility, due to strong bonds with Al and Fe in acidic soils and with Ca and Mg in alkaline soils. Soil P can be mineralized or immobilized by microbial biomass, which participates actively to the soil-plant P system. The plant roots can also increase the P solubility with organic acid extrusion, which solubilizes P acidifying the rhizosphere or protecting P from metal precipitation. Phosphorus has a fundamental role in the physiology of the plants being contained in nucleic acids (DNA and RNA), in all the membranes of the cells as phospholipids and in the P-rich energy bonds of ATP. Plants react to P deficiency with lower leaf expansion and increased root hair proliferation in order to increase the exploration volume and the organic acid and phosphatase extrusion and consequently P uptake.

The higher food demands of the growing world population has led to an increase of the use of fertilizers, among which phosphate fertilizers. The raw source of P fertilizers until now was mainly the phosphate rocks, which are non-renewable resources and which are predicted to be depleted in the next decades. Thus, there is the improving needs to close the P cycle in order to reduce the P input for mineral source, recycling P rich organic products such as animal manure and human excreta.

Plant P use efficiency can be increased studying the symbiotic relation with mycorrhizae and different strategies developed by the P deficiency tolerant plants, such as cluster roots, which are able to extrude phosphatase and organic acid to increase P solubilisation. Furthermore, also the efficiency of fertilizers can be increased, using organic waste for plant nutrition or also developing new fertilizers from bio-waste. Thus, the aim of this work was to study the P availability in soil and plants of different bio-waste and to develop a new fertilizer from sewage sludge, which can recover a high amount of P from the waste, but also can be easily assimilated from plants.

Phosphorus in soil

Phosphorus (P) is present in soil in amount ranging between 100 and 3000 mg P kg^{-1} (*Frossard et al.*, 2000), mostly orthophosphate compounds, in inorganic and organic forms. The inorganic P pool usually account for 35% to 70% of the total P (*Shen et al.*, 2011), while the organic P ranges from 30 to 65% (*Frossard et al.*, 2000). The primary source of inorganic P are apatite, strengite and variscite, which are very stable; while secondary P minerals includes Ca, Fe and Al phosphates (*Shen et al.*, 2011). The stabilized forms of organic soil P are inositol phosphates and phosphonates, and the active forms are orthophosphate diesters, labile orthophosphate monoesters, and organic polyphosphates (*Shen et al.*, 2011).

Soil P is poorly mobile in soil (*Hinsinger*, 2001). The high reactivity of phosphate ions with the soil constituent caused the very low mobility of these ions (*Hinsinger*, 2001), which are generally found in a low amount in the soil solution (between 0.01 and 3 mg P L^{-1}) (*Frossard et al.*, 2000). Thus, the available P generally do not meet the crop demand, causing severe yield loss in crops (*Shen et al.*, 2011), particularly in tropical and subtropical region where the soils are highly weathered and in the Mediterranean areas where the soils are generally calcareous (*Hinsinger*, 2001).

The P transformation can be driven by abiotic or biological process (Fig. 1.1). Within the abiotic P transformation there are precipitation-dissolution and adsorption-desorption, while the biological immobilization-mineralization transfer P from inorganic form to organic and *vice versa* (*Frossard et al.*, 2000).

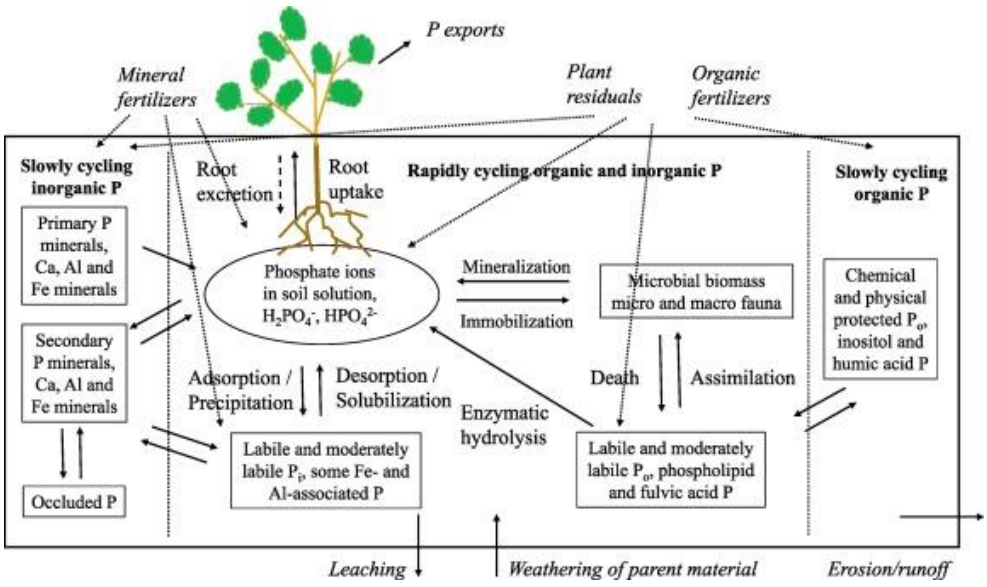


Figure 1.1: Phosphorus soil cycle (*Zhu et al.*, 2017).

The P availability and the P speciation is strongly correlated to the soil pH, with a higher amount of $H_2PO_4^-$ in acidic soil and HPO_4^{2-} in soil with a pH above 7.2 (*Hinsinger*, 2001). In acidic soils, the high reactive P ions form bonds with Fe and Al oxides, which are more soluble at low pH. On the other hand, in neutral and alkaline soil, Ca and Mg are the more soluble cations, which are likely to ligate with P (*Hinsinger*, 2001).

The P bonds depend also from the organic ligands presents, such as citrate and oxalate, which are able to form stable complexes with P and avoid precipitation and adsorption (*Guppy et al.*, 2005; *Hinsinger*, 2001). In calcareous alkaline soils, P tend to precipitate as Ca phosphates (dicalcium phosphates, octacalcium phosphates, apatites and hydroxyapatites), with a decreasing solubility from pH 7 to 8 and an increasing solubility for pH above 8. In acidic soil, however, P precipitates in Fe and Al phosphatases (strengite, vivianite, variscite) with an increasing solubility as the pH decrease (*Hinsinger*, 2001).

In addition to precipitation, P can also be adsorbed by the positively charged soil constituents, such as hydroxyl (Fe and Al oxides), carboxyl (organic compounds) and clays. The P release from these constituents should be easier than in the case of precipitated P, via the substitution of P with competitive anions as ligands. However, the P affinity with positively charged constituents is generally higher than other anions, making the P release difficult nonetheless (*Hinsinger*, 2001).

The transformation of organic soil P is usually mediated by the soil microorganisms (Fig. 1.2) or plant roots secretions (*Shen et al.*, 2011). Microorganisms can increase the P solubilisation with the extrusion of phosphatase and cellulolytic enzymes, able to solubilise organic P, or the mineralization of organic matter (Fig. 1.2) (*Richardson and Simpson*, 2011). They can also release protons and organic anions which can solubilise the precipitated forms of P, chelating the metal ions that are ligated to P or substituting the P in the bonds (*Richardson and Simpson*, 2011).

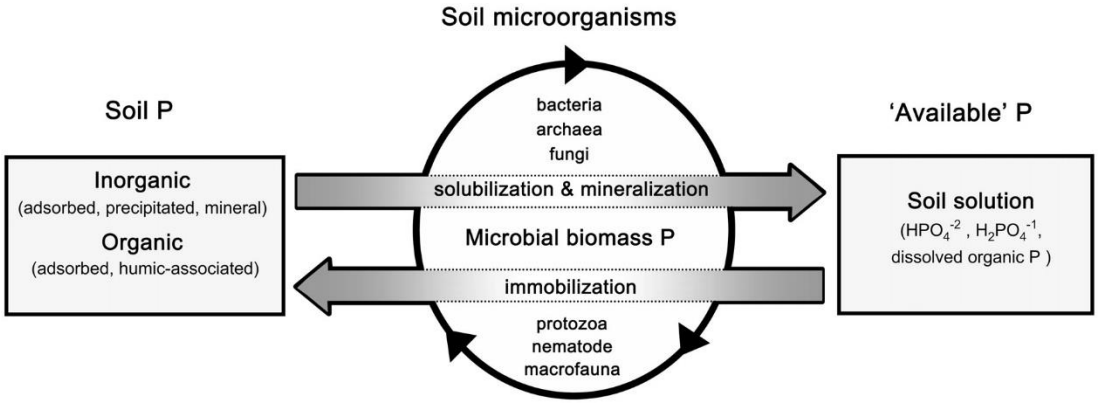


Figure 1.2: Microorganisms activity in mobilizing soil P (*Richardson and Simpson*, 2011).

Plant roots had an active role in solubilizing P in the rhizosphere, extruding organic acids, such as citrate, malate and oxalate when under P deficiency (*Jones*, 1998), but also enzymes, such as acid and alkaline phosphatase, which can actively contribute to the mineralization from organic P to inorganic P (*Nannipieri et al.*, 2011). Thus, the P cycle in the soil is not only driven by chemical and biochemical change within the soil, but the soil-microorganism-plant system should be considered as a whole (Fig. 1.3).

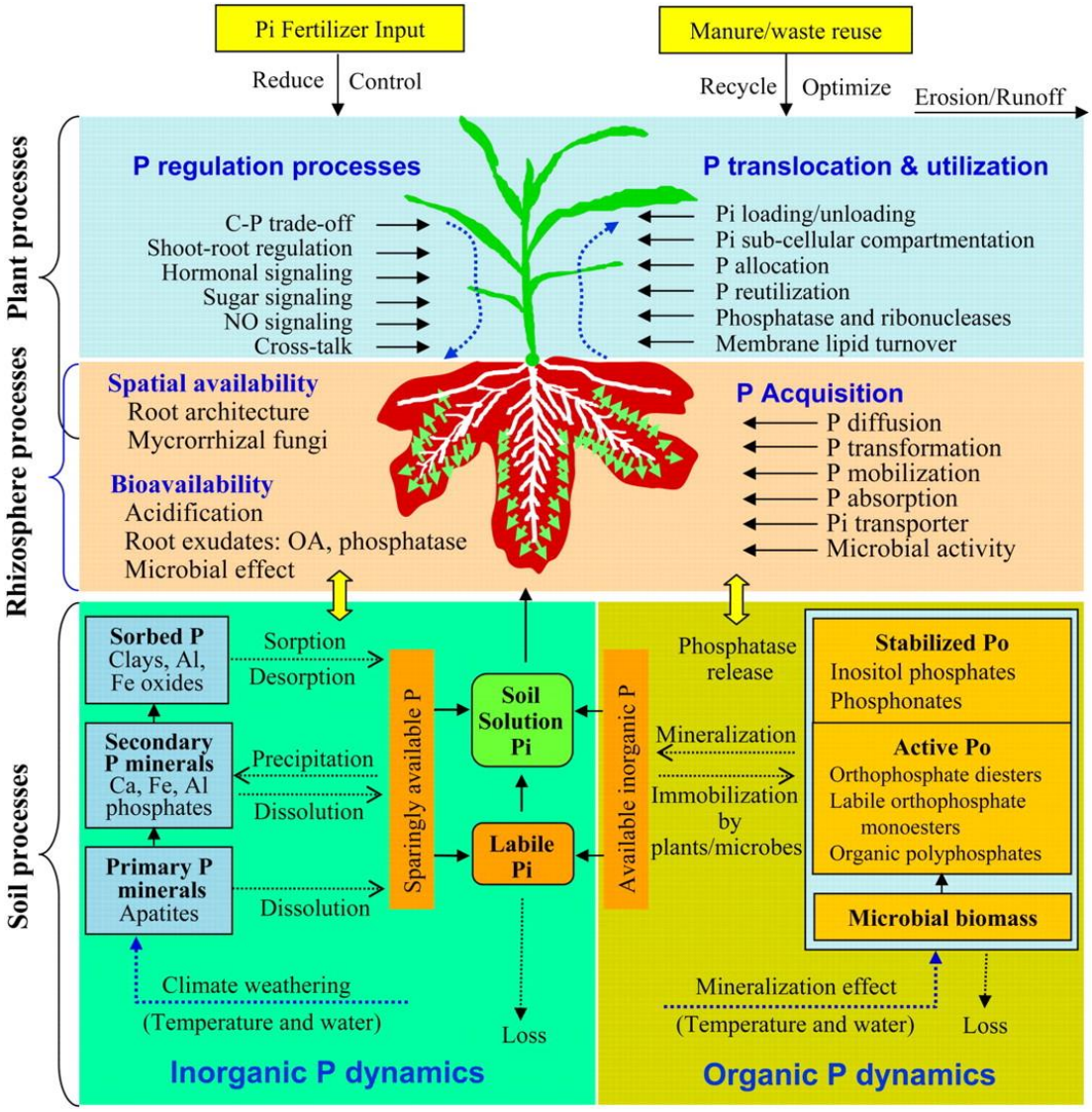


Figure 1.3: Soil, rhizosphere and plant processes in P cycle (*Shen et al.*, 2011).

Phosphorus and plants

Plants do not reduce phosphate, unlike nitrate and sulphate, but utilize the highest oxidised form. The uptake is mainly as $H_2PO_4^-$ when plants grow at physiological pH (*Marschner and Marschner*, 2012). In plants, P can change its form from inorganic to organic, with an esterification or with the bond to another phosphate in the energy-rich pyrophosphate (such as ATP). From organic it can also return to inorganic very quickly when the plant needs to transport P through xylem (*Marschner and Marschner*, 2012). P in plants has numerous and fundamental functions (Fig. 1.4). The function of P is mainly in nucleic acids (DNA and RNA), where it forms a bridge between ribonucleotide units. The concentration of P in the nucleic acid differs highly between leaves at various maturity stage, with a high concentration in young leaves where the RNA is in a large amount for the protein synthesis, and lower in mature and in senescing leaves (*Marschner and Marschner*, 2012). P diesters are also highly present in phospholipids of biomembranes. Under P deficiency, plants can replace phospholipids with galactolipids or sulpholipids (*Marschner and Marschner*, 2012). Furthermore, although the concentration of phosphate esters (C-P) and energy-rich phosphates is very low, these forms are of extreme importance for the plant physiology, because they represent the only metabolic energy of cells (*Marschner and Marschner*, 2012).

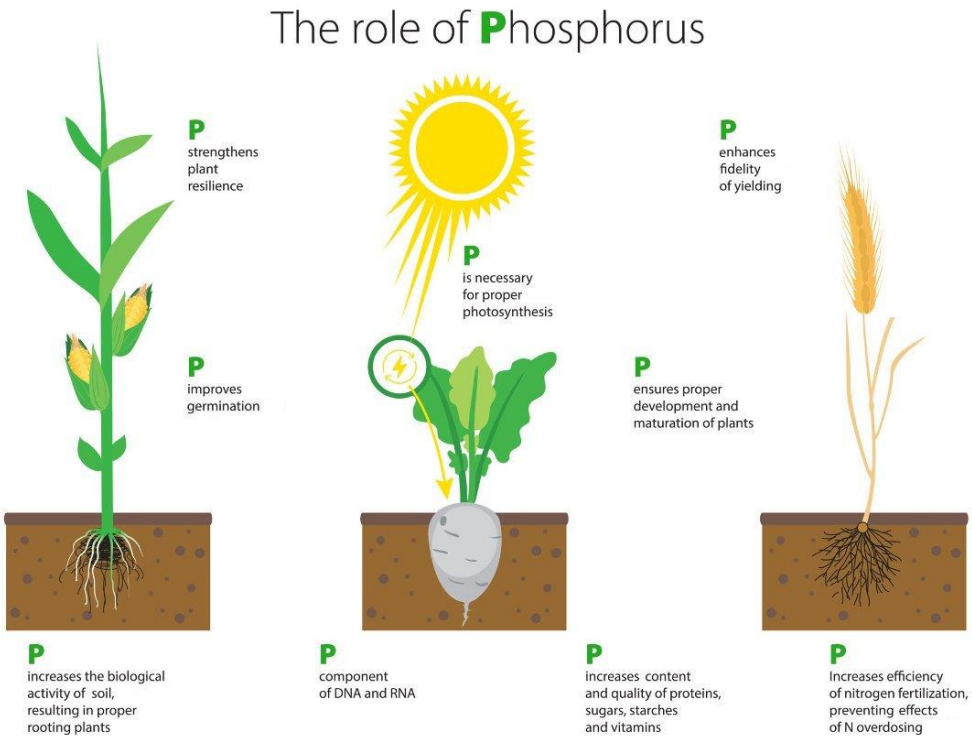


Figure 1.4: Phosphorus functions in plants (*AEEP*, 2017).

In adequate P supply, about 85-95% of inorganic P (Pi) is located within the vacuoles (as shown in the ^{31}P NMR spectrum in Fig. 1.5), while under P deficiency this is contained in cytosol and chloroplast, where its metabolic function is active (*Raghothama*, 1999).

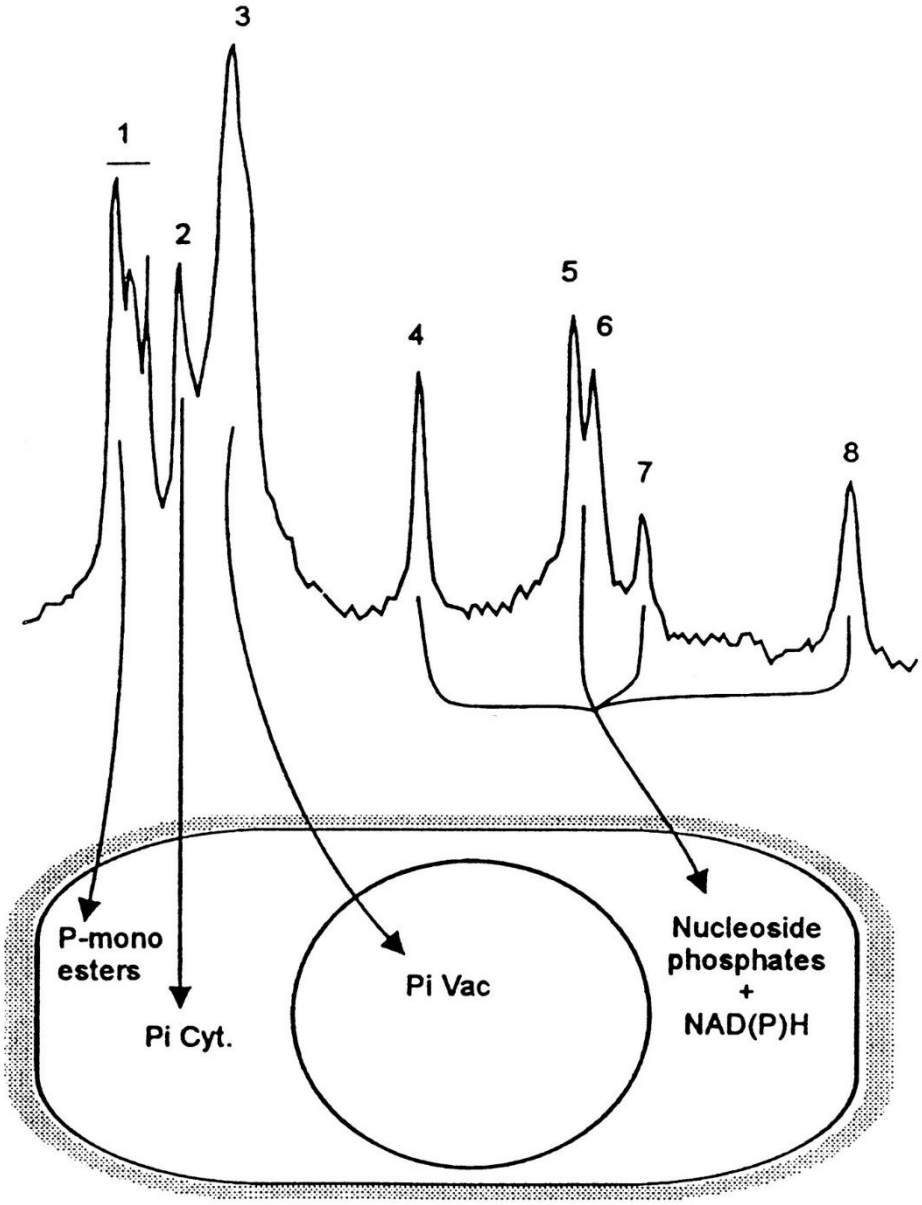


Figure 1.5: 31P-NMR of carrot cells, in which the higher amount of P is assigned to the resonance of vacuolar Pi (peak 3). The peak assignments are: 1, P-monoesters (including Glc-6-P and phosphocholine); 2, cytoplasmic Pi; 3, vacuolar Pi; 4, nucleoside triphosphates (principally ATP); 5, α-P of NTPs; 6, NDP-hexose and NAD(P)H; 7, NDP-hexose; and 8, β-P of NTPs (*Delhaize et al.*, 1995).

The optimal concentration in leaves is between 3 and 5 mg g^{-1} (*Lambers et al.*, 2011). The photosynthesis is strongly depleted when the P concentration is below 1.4-1.0 mM, rather than the usual 2.5 mM (*Marschner and Marschner*, 2012). In case of P excess, the concentration in leaves can increase without major consequences, due to

the storage in vacuoles or in two alternative forms, phytate (mostly in grains, seeds and roots) and inorganic polyphosphates (*Marschner and Marschner*, 2012). In P deficient plants, reduction of leaf expansion (*Fredeen et al.*, 1989) and leaves number (*Lynch et al.*, 1991) are the primary effects, while the chlorophyll per unit of leaf area and also the root growth are less affected, causing a decrease in shoot:root ratio (*Marschner and Marschner*, 2012). On the contrary, formation and elongation of root hairs is generally promoted under P starvation, increase the P influx (*Bates and Lynch*, 1996; *Raghothama*, 1999). Roots increase also the extrusion of organic acid, such as malate and citrate in order to acidify the rhizosphere and increase the P solubility (*Raghothama*, 1999). In the first phase of P deficiency, sensible plants, such as tomato, start to increase the P influx rate and concurrently to decrease the P concentration in leaves and the stomatal conductance, in the second phase the first symptoms on leaves expansion are visible. The less sensible plants show symptoms only in a third phase (*Clarkson and Scattergood*, 1982). On the other hand, in a non-limiting P environment the P uptake is controlled by a higher P efflux, rather than a reduced P influx (*Cogliatti and Santa Marìa*, 1990).

In conclusion, many factors influence the plants P uptake (Fig. 1.6). Among these, the already mentioned soil factors, such as pH and mineral concentration, the rhizosphere community with enzymes and organic acid extrusion and the ability of P mineralization or the root architecture (*Hunter et al.*, 2014). However, factors above the soil are also important, with agronomic factors, such as soil management and input of P fertilizer, which can affect P plant uptake, changing the P soil concentration and modifying slightly the soil pH.

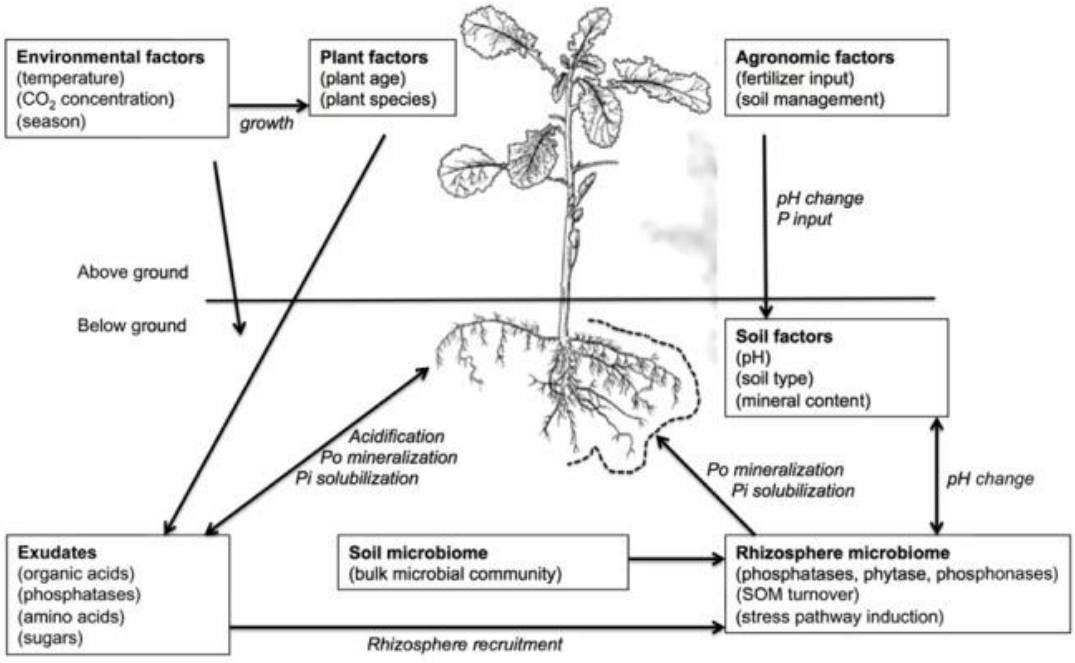


Figure 1.6: Factors influencing plant P uptake (*Hunter et al.*, 2014).

Phosphorus crisis

The world population has grown exponentially in the last century and the estimations reveal that it will continue growing at this rate in the next 100 years (Fig. 1.7) (*FAO*, 2016). With the increase of the global population the food demands has continuously growing in the last century, with an increase of primary crop production, substantial in the ’80s (Fig. 1.8), mostly due to the rise of animal products consumption (Fig. 1.9b) which caused a huge fodder demands (Fig. 1.9c). The cereal production (Fig. 1.9a), however, increased with a steady rate (*FAO*, 2016). This higher demand in food has increased the need for higher yield in crop production, which was obtained with genetic plant enhancement but also with better agronomic managements. Within the agronomic tools, the fertilization had a fundamental role. Thus, the use of fertilizers in the arable lands has increased. Among the fertilizers, phosphate use had an exponential escalation (Fig. 1.10).

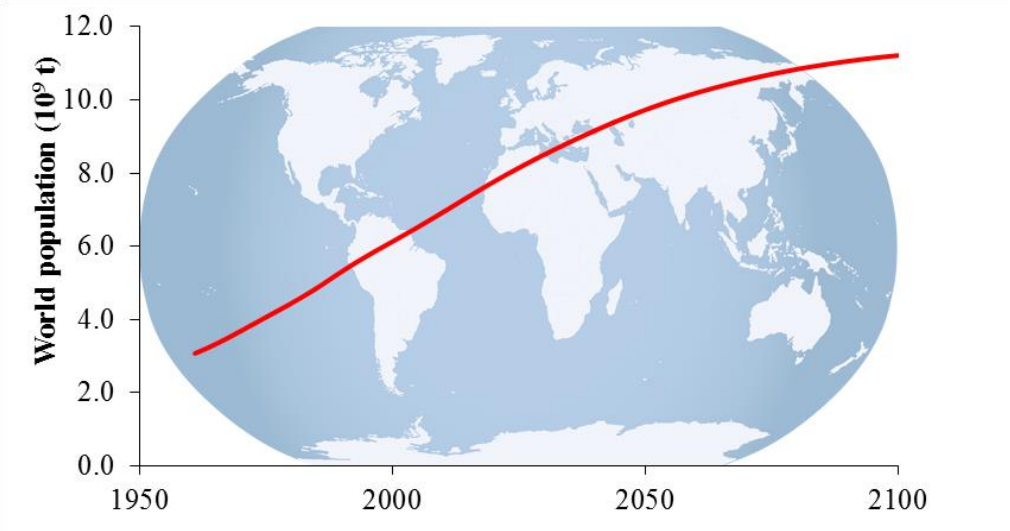


Figure 1.7: World population prediction from 1950 to 2100. Data from *FAO* (2016).

Figure 1.8: Primary crop production. Data from *FAO* (2016).

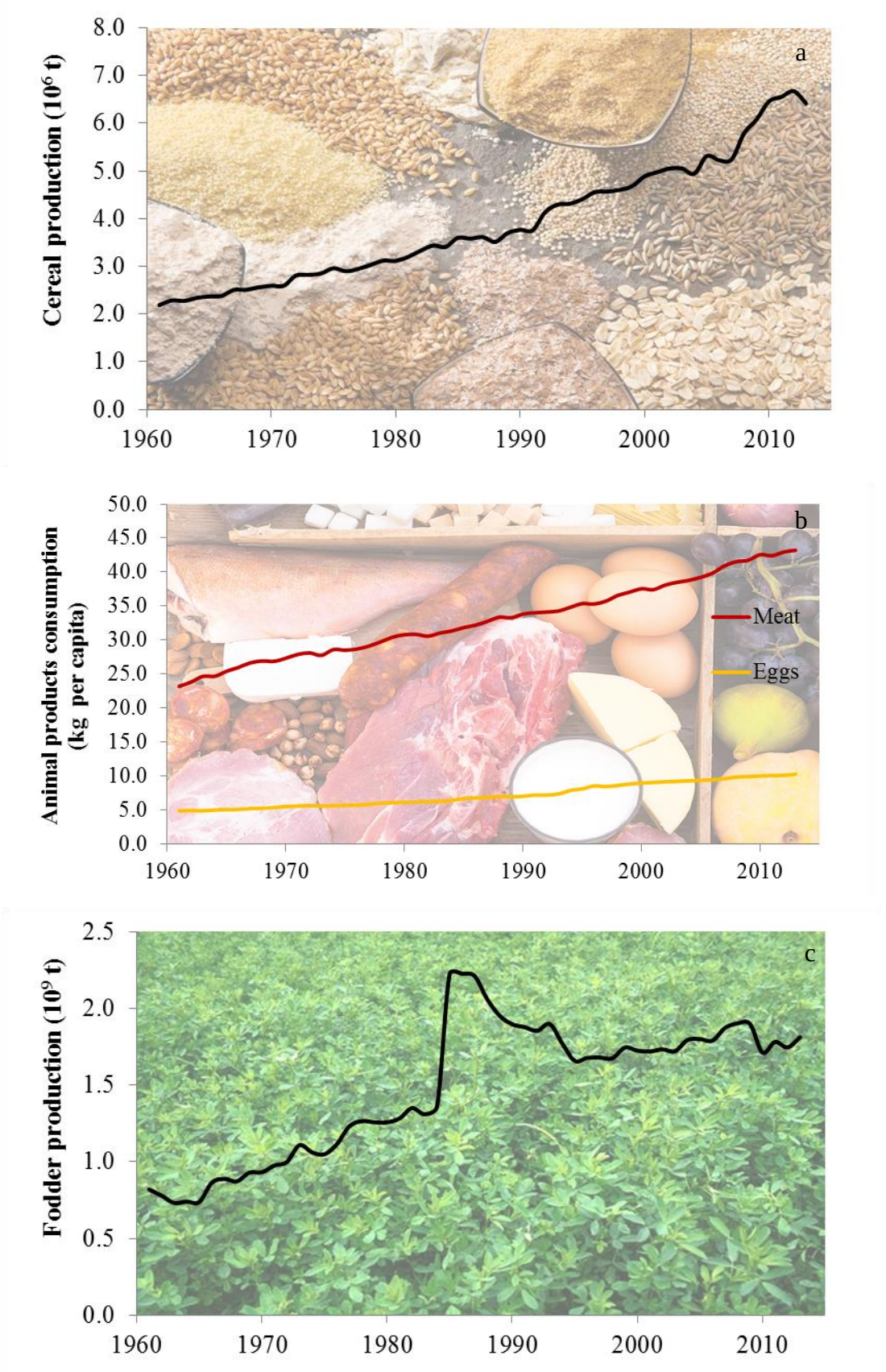


Figure 1.9: Global food production from 1960 to 2014. Data from *FAO*, (2016). a) Cereal production. b) Animal derived products consumption. C) Fodder production.

The P fertilizers, which use had increased exponentially in the last 50 years, had been mostly derived from phosphate rocks mining. While, the use of manure increased only slightly as a P fertilizers source. (Fig. 1.11) (*Cordell et al.*, 2009).

The phosphate rocks are a non-renewable resource, and thus, at this mining rate they will deplete in the next future. The estimation of the peak of phosphorus (Fig. 1.12), the moment when the high quality and highly accessible resource will finish, were pointed as 2033 (*Cordell et al.*, 2009). This dramatic estimation has led to a rocketing of the fertilizers prices after 2008 (Fig. 1.13) which had caused a reduction of P fertilizers use (Fig. 1.14) and a consequent reduction of P balance in soil (input minus outputs) (Fig. 1.15). In some countries, such as Italy, the P balance became negative after the decrease of P use (Fig. 1.15). The P concentration in soil, as already mentioned, is always above crop demands, but the P solubility is often so scarce that without a fertilizers input the crop yield can easily decrease (*Vance*, 2001). In addition, the Italian soils are generally calcareous, which decrease the P solubility due to the high amount of Ca. Thus, the negative P balance should be taken more into account for the future crop production.

The rock phosphates mines are located in very few countries, such as USA, China and Morocco (*Cooper et al.*, 2011). China has already stop the exports, privileging the internal use of the P fertilizers (*Childers et al.*, 2011). Recently, new source of phosphate rocks were found in Morocco (*Walan et al.*, 2014). These new mines had postponed the peak of phosphorus of some decades. However, the quality of these new phosphate rocks is very poor, with a high Cd contamination. Furthermore, the political instability of the region, make these reserves even less accessible for world lands fertilization (*Walan et al.*, 2014).

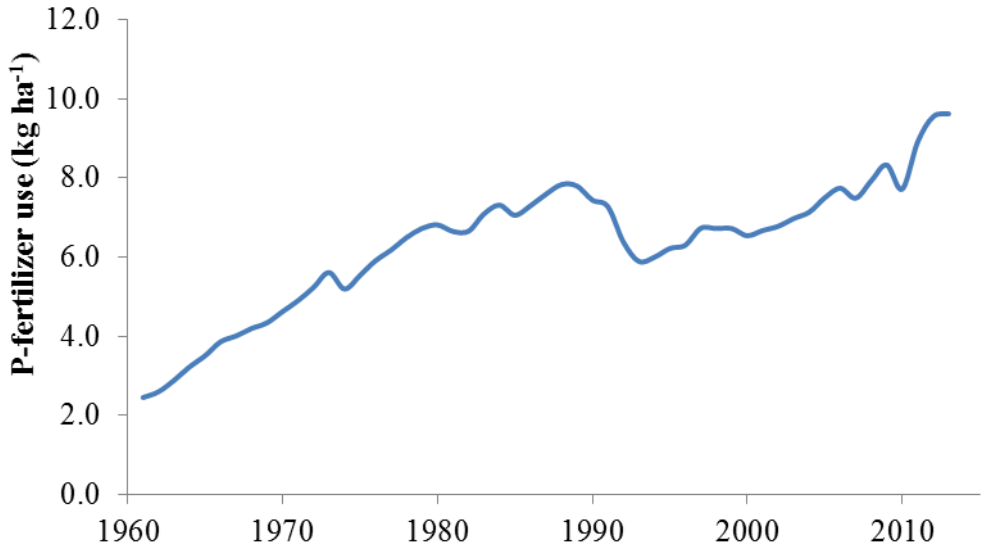


Figure 1.10: P-fertilizer use from 1960 to 2014. Data from *FAO* (2016).

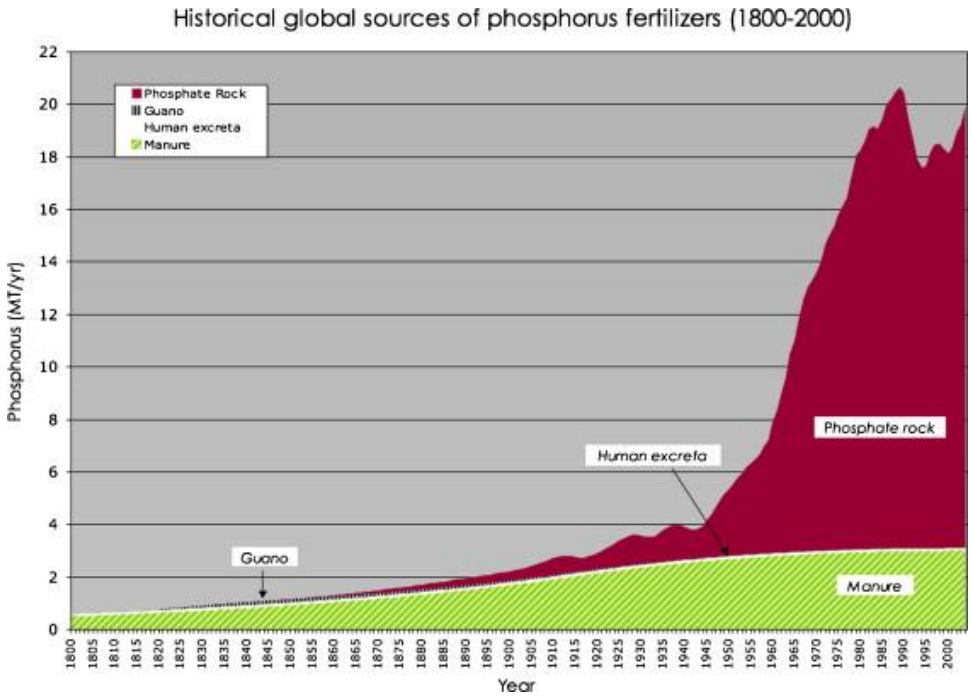


Figure 1.11: Source of phosphorus fertilizers (*Cordell et al.*, 2009).

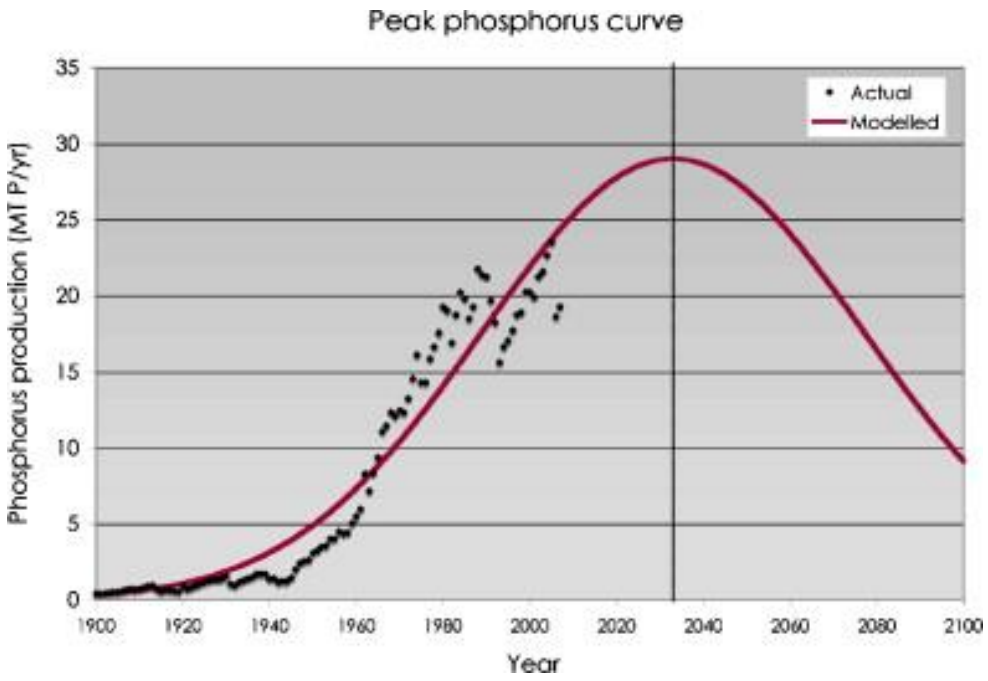


Figure 1.12: the peak of phosphorus (*Cordell et al.*, 2009).

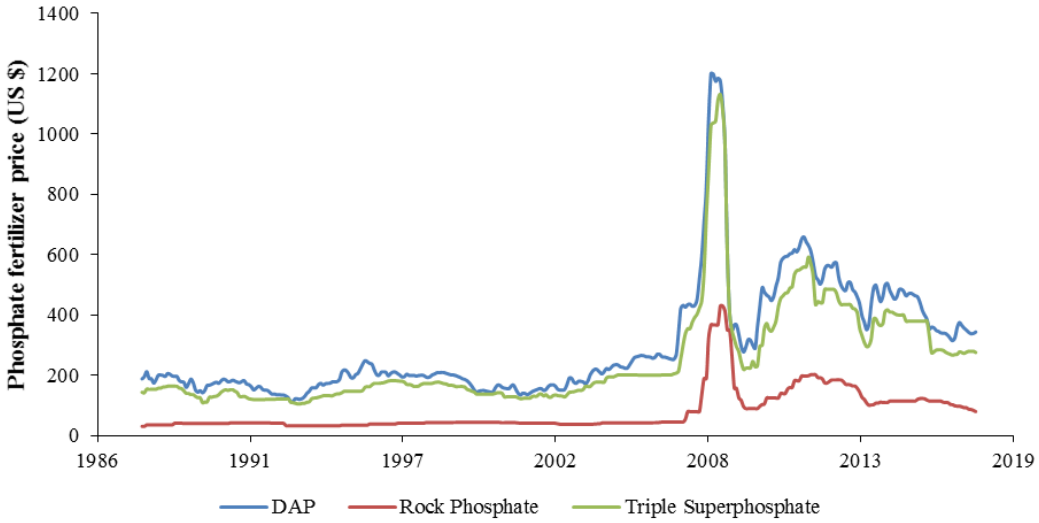


Figure 1.13: Phosphate fertilizers price (*Indexmundi*, 2016).

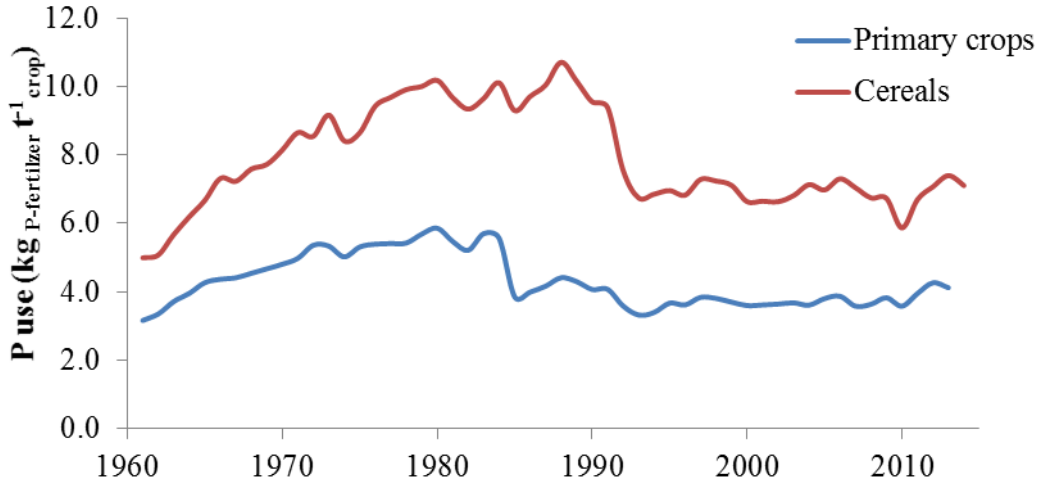


Figure 1.14: P use (kg P fertilizers t^{-1} of crop). Data from *FAO* (2016).

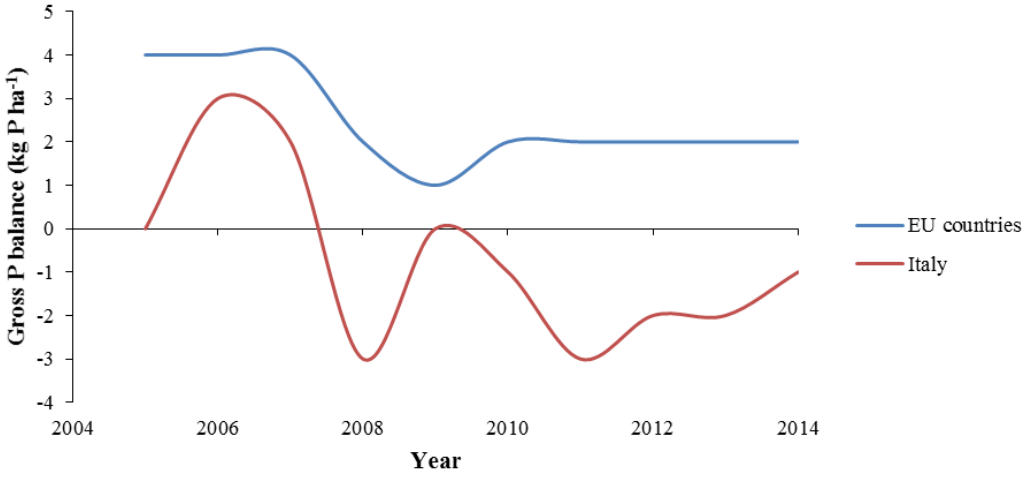


Figure 1.15: Gross P balance. Data from *Eurostat* (2016).

Circular economy – Closing the P cycle

The concept of circular economy was introduced in the 1990s. The fundamental ideas of this concept is the reuse of the waste as primary input of a cycle. In this view, the waste become a resource and the input should be lowered as much as possible, with the use of renewable sources of energy. The recycle, which obviously cannot endure without continuity solution, is the base of the circular economy (*Andersen*, 2007).

In this frame, the P cycle, which nowadays is open, can be closed using the circular economy concept. Nowadays, the P cycle starts from the input of mineral fertilizers and finish with P leaching from soil to the ground water, from food to human excreta and in the water basins after wastewater treatments (Fig. 1.16) (*Cordell et al.*, 2009). The P losses in the water basins is the major cause of eutrophication (*Childers et al.*, 2011). Thus, closing the P cycle could not only reduce the problem of the scarcity of phosphate rocks, but also reduce the environmental problem of P losses in water bodies.

In the near future, the agricultural management will be the easiest way of reducing the P losses. The first way is the reduction of fertilizer input, due to better fertilization management or to genetically enhanced plants, which can have lower P demand or higher capacity of P uptake from soil. The agricultural improvements can also reduce the erosion of P-rich soil, wasting P and contaminating ground water. Furthermore the last agricultural process to reduce the P losses can be the return on soil of crop and food residue and animal and human excreta (*Childers et al.*, 2011; *Cordell et al.*, 2011).

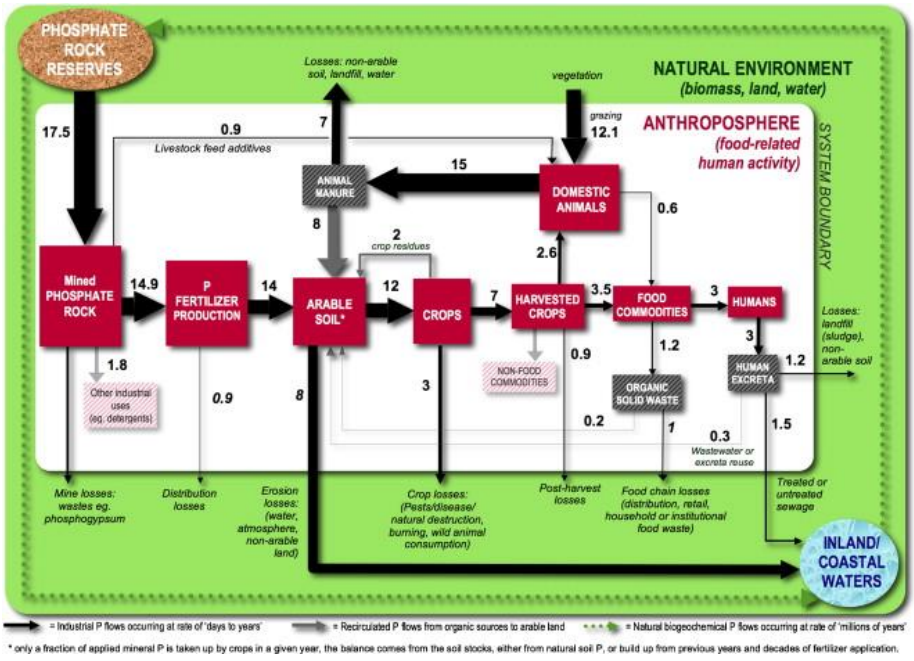


Figure 1.16: The open cycle of phosphorus (*Cordell et al.*, 2009).

From Fig. 1.17 it is clear that the P recycle can be enhanced at every step of the P cycle starting from the disposal of crop residue and food waste on soil and finishing with the recycle of animal manure and human excreta (*Cordell et al.*, 2011). However, the reuse of recycled resource will account only of 1/3 of the needed P in the future (*Cordell et al.*, 2011). Major changing are required to decrease the P use, such as: changing diet, with less animal products, which need high amount of P to be produced; improving food chains in order to avoid food waste; agricultural managements such as conservative agriculture, for erosion reduction, and precision agriculture for increased P efficiency of fertilizers.

Nowadays, the P recovery is still not very widespread, because its costs are generally higher than the phosphate rocks. However, for the total value of P recovery, it should take into account also the value of the recovery of energy, nitrogen, metals, minerals and waters that are associated with the reuse of waste as a source of P (*Mayer et al.*, 2016). In addition, the environmental cost should be considered, because the recycle of P decrease the eutrophication, enhancing the wastewater treatment efficiency. Also the social costs should be accounted, because the P recycle can improve food security and social equity (*Mayer et al.*, 2016), due to less use of phosphate rocks which will become a limiting factors and probably a challenging resource (due to the concentration of sources and political instability of the mining regions).

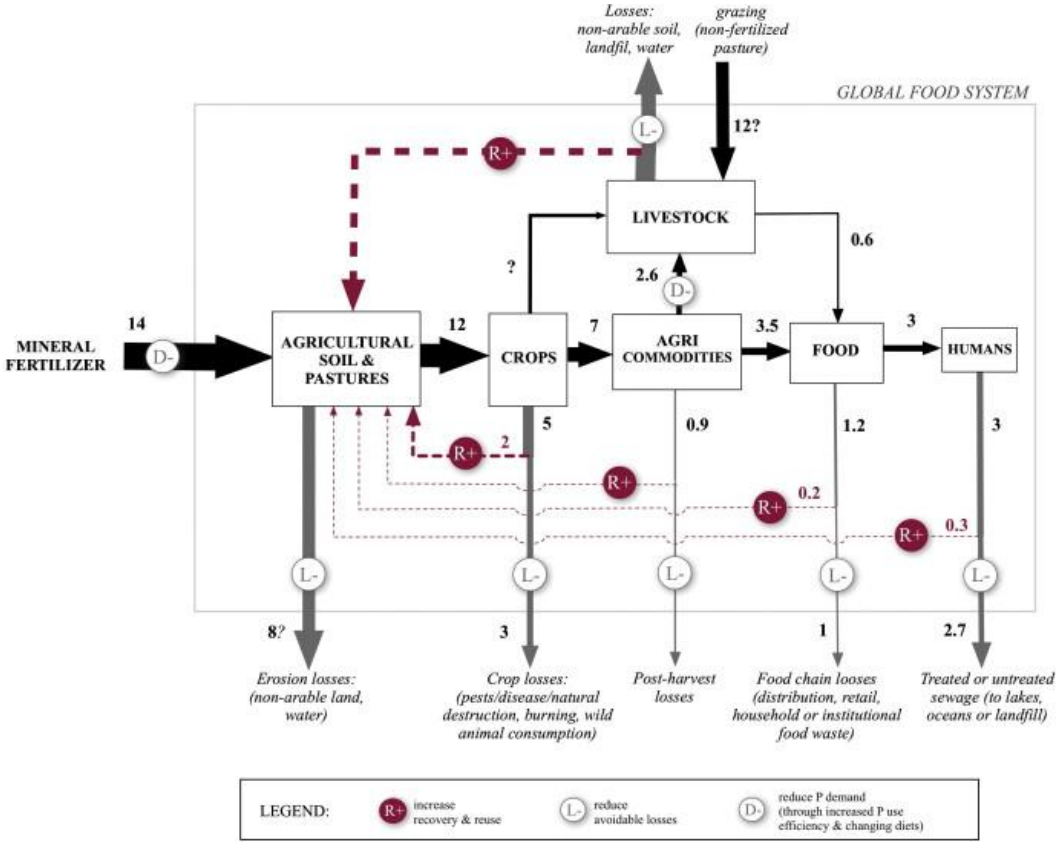


Figure 1.17: P cycle with possible recovery (R+), reduced losses (L-) and reduced P demand (D-). (*Cordell et al.*, 2011).

A well-developed method to assess the environmental impact is the Life Cycle Assessment (LCA). The LCA is a technique to quantify the impacts of a product or a service, with the "cradle-to-grave" vision (*Corominas et al.*, 2013). The first studies on wastewater management with LCA are reported in the 1990's (*Corominas et al.*, 2013). The categories of impacts generally taken into account in the LCA method are eutrophication potential, global warming potential, toxicity-related impacts, energy balance, water and land use (*Zang et al.*, 2015). Even though more improvements in the study of wastewater treatments with LCA are required (*Zang et al.*, 2015), many studies were focused on revealing the best solution for wastewater treatments in term of environmental impact. *Suh and Rousseaux* (2002) compared three different main processes (incineration, agricultural land application and landfill disposal) in combination with three stabilization processes (lime stabilization, composting and anaerobic digestion) and the final transport of the residue of the wastewater treatment plants. The combination of anaerobic digestion and agricultural use resulted the best scenario in term of environmental impact and the highest human toxicity was found in the incineration processes or the direct sludge application on field. Another study (*Hospido et al.*, 2008) confirmed that the addition of a secondary process such as anaerobic digestion to the wastewater treatment sensibly reduced the impact on the environment decreasing the leaching of ammonium and phosphate, reducing the volume of the product to dispose. The increase of energy consumption for this secondary process did not produce impacts comparable to the reduction generated with the digestion. Furthermore, if the biogas produced by the anaerobic digestion are used to produce electricity or heat, replacing the fossil fuels, the impact reduction would increase even more (*Pasqualino et al.*, 2009).

Beside the environmental impact, LCA can assess also the human health risks. The pathogen risks of using the sewage sludge has only recently been added to the possible risks. A study calculated that the pathogen risks account for 20% of the total human health risks of the wastewater management (*Heimersson et al.*, 2014).

Therefore, LCA analysis has already highlighted the problems of wastewater managements, among which the greenhouse gases production derived from the energy used for the treatments and after for the transport of the waste. However, as already evidenced, the wastewater treatments can drastically reduce the impact on the leaching of ammonium and phosphate, with a general impact reduction that is well above the environmental cost. To reduce the problems generated with pathogen risks and waste disposal secondary processes such as anaerobic digestion or composting or even new practices are needed.

Enhancing P use efficiency

One of the abovementioned strategies to reduce P input is the increase P use efficiency of plants, which can be achieved with plants able to improve the P uptake from soil, with particular roots (such as cluster roots) or with symbiosis with mycorrhizae. The increase of plants P use efficiency was not the focus of this research, thus the strategies will be addressed briefly.

Changes in plants physiology can occur with the reduction of superfluous ribosomal RNA and the ability of replace phospholipids with sulfolipids and galactolipids, recognized in plants highly tolerant to P starvation. Furthermore, plants can mobilized P from senescing leaves to new leaves or grains. Genetic enhancements in moving traits from high tolerant plant to cultivated crops are highly needed in order to reduce the P crop demands (*Veneklaas et al.*, 2012).

Mycorrhizae

Symbiotic association of fungi and plants roots are called mycorrhizae. These are widespread in all type of soils and occurred in almost all plant species (*Bolan*, 1991).

The benefit provided by the symbiosis with mycorrhizae are the larger exploration of soil volume, faster P movement into mycorrhizal hyphae and P solubilisation (*Bolan*, 1991). The soil exploration provided by the mycorrhizal hyphae expansion decrease the distance between the P ions and the plants roots. The hyphae also increase the surface area of P absorption. Furthermore, the mycorrhizae needs lower concentration of P for uptake, due to higher P affinity. The P solubilisation is achieved with the extrusion of P from mycorrhizal hyphae (*Bolan*, 1991).

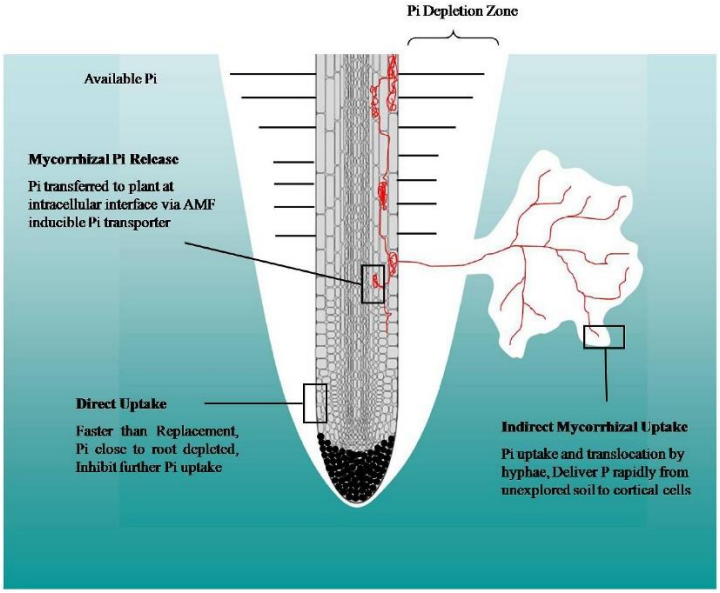


Figure 1.18: Direct P uptake and P uptake mediated by mycorrhizae (*Johri et al.*, 2015).

Cluster roots

Among the plant mechanisms to increase the P uptake the mycorrhizal symbiosis is common in almost the 80% of the plants (*Burleigh et al.*, 2002). Within the plants that do not create symbiosis with mycorrhizae there are some, mostly belonging to the family of Proteaceae, which are able to create cluster roots (*Neumann*, 2010). These are a dense clusters of short lateral roots covered by hairs (*Marschner and Marschner*, 2012). These radical structures are greatly specialized and are able to decrease the pH of the rhizosphere, extruding organic acids and phosphatases. In the rhizosphere of these plants, elements such as P, Fe, Zn and Mn are more mobile and available for plant uptake (*Neumann and Martinoia*, 2002).

Figure 1.19: White lupin grown under P deficiency developed cluster roots.

White lupin (*Lupinus albus*, L.) is a model plant to study the cluster root formation. Few data were found on the regulation factors involved in the induction and development of cluster root (*Neumann*, 2010). In *Wang et al.* (2014) some genes were shown to be involved in the regulation of citrate exudation, such as genes that regulate the production of MATE and ALMT proteins. The transcriptome sequencing identified MATE proteins were found also in the regulation of phenolic exudations (*Wang et al.*, 2014). However, stable genetic transformation with *Agrobacterium tumefaciens* of white lupin has not been successful yet, due to its recalcitrant characteristics to transformation and regeneration in vitro (*Uhde-Stone et al.*, 2005). Green fluorescence protein (GFP) constructs has been used in order to localize the expression of *LaMATE* proteins. The results showed that these proteins were produced under nutrient deficiency of phosphorus, but also in nitrogen, iron and manganese deficiency and aluminum toxicity. (*Uhde-Stone et al.*, 2005).

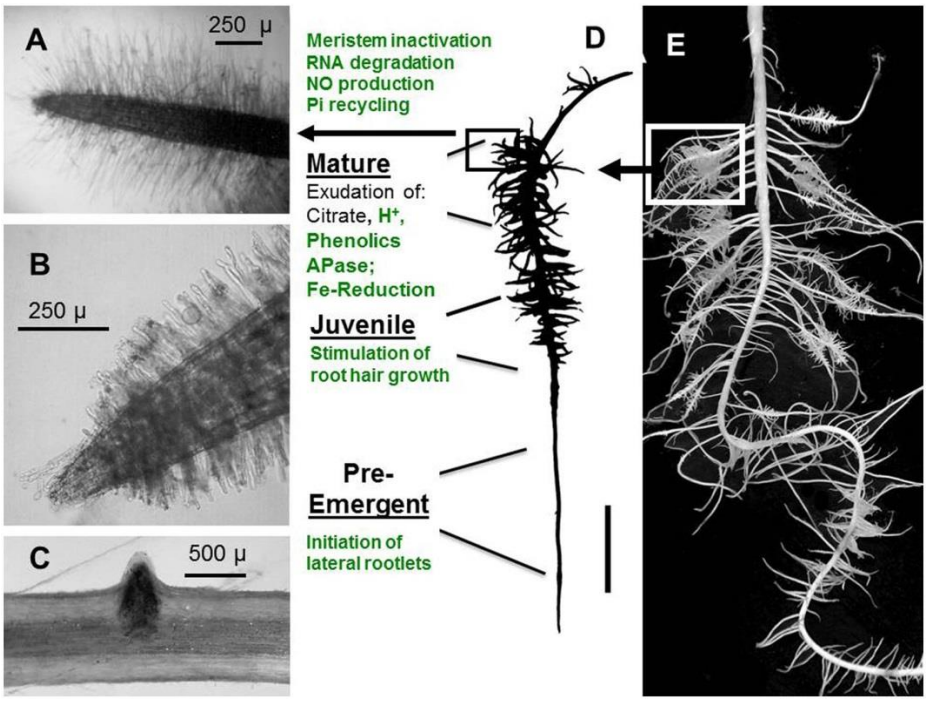


Figure 1.20: Cluster roots functions dependent to their age (*Neumann*, 2016).

Genetic transformation

The low solubility of P in soil, the scarcity of raw material for fertilizers and the need to avoid P losses in water had highly increased the attention on improving genetically the nutrient use efficiency of plants (*López-Arredondo et al.*, 2013). Genes encoding sensors, transcription factors, transporters and metabolic enzymes had been suggested as potential tools for genetic transformation focused on P use efficiency. In addition, also the study of bacteria and fungi nutrient assimilation can be interesting for a genetic transformation (*López-Arredondo et al.*, 2013). An example of candidate genes for increasing P use efficiency was found in a study on tomato, where a phosphate starvation induced gene, *LePS2*, representing an acid phosphatase, was isolated and characterized (*Baldwin et al.*, 2001).

An example of a genetic transformation and regeneration of white lupin is described in box 1.

Box 1.1: **GENETIC TRANSFORMATION AND *IN VITRO* REGENERATION OF WHITE LUPIN**

In our research, a genetic transformation was tried on white lupin. The *LaMate* gene, previously described, responsible for the citric acid extrusion channel of cluster roots has been knock out. The white lupin *in vitro* regeneration was found difficult and the effectiveness of the transformation was proved using GFP protein in the *CRISP Cas-9* construct. The regeneration was tried with calli induction and consequent *Agrobacterium tumefaciens* infection, using a revised protocol of *Ewa Sroga*, (1987) and *Mamani et al.*, (2014) (experiment #1); infected embryo slices using a revised protocol developed by *Polowick et al.* (2014) (experiment #2); and infected "truncated seedlings" and successive calli induction as described in *Pniewski et al.* (2006) (experiment 3#). The results showed that i*n vitro* regeneration was not successful in experiment 1# and 3#. In experiment 1# calli had grown but no shoots were formed (Fig. 1.21). In experiment 3#, instead, after the co-cultivation with truncated seedlings and bacteria it was not possible the induction of calli.

Figure 1.21: Calli from experiment#1 on genetic transformation of white lupin.

Box 1 continue: **GENETIC TRANSFORMATION AND *IN VITRO* REGENERATION OF WHITE LUPIN**

Experiment 2# had different results and whole plants were grown and acclimated in soil. Plants were selected in the selected media with antibiotics at the end of the regeneration period. The shoot regeneration efficiency for the experiment 2# with embryo slices reached 40% in the first two tests and it were even higher in the third test were the seeds were cut in just two slices, reaching 56% of the shoot regeneration efficiency. In the three months experience plants regenerated during the experiment 2# developed roots and were acclimated in a growth chamber (Fig. 1.22) with an efficiency of 11% of the total explants, which can be considered a good results due to the very low capacity of rooting of white lupin (*Uhde-Stone et al.*, 2005).

Figure 1.22: Inducted shoots, elongated shoots, rooted shoots and acclimated plants from experiment 2# on genetic transformation of white lupin.

Box 1 continue: **GENETIC TRANSFORMATION AND *IN VITRO* REGENERATION OF WHITE LUPIN**

In order to assess the effective genetic transformation GFP constructs, which is green fluorescent, was inserted in the *Agrobacterium tumefaciens*. Confocal images of leaves of plants of the experiment 2# (Fig. 1.23) showed signs of transformation. GFP construct in the empty vector could be localized in every part of the plants and in this experiment seemed to be localized in the leaf hairs.

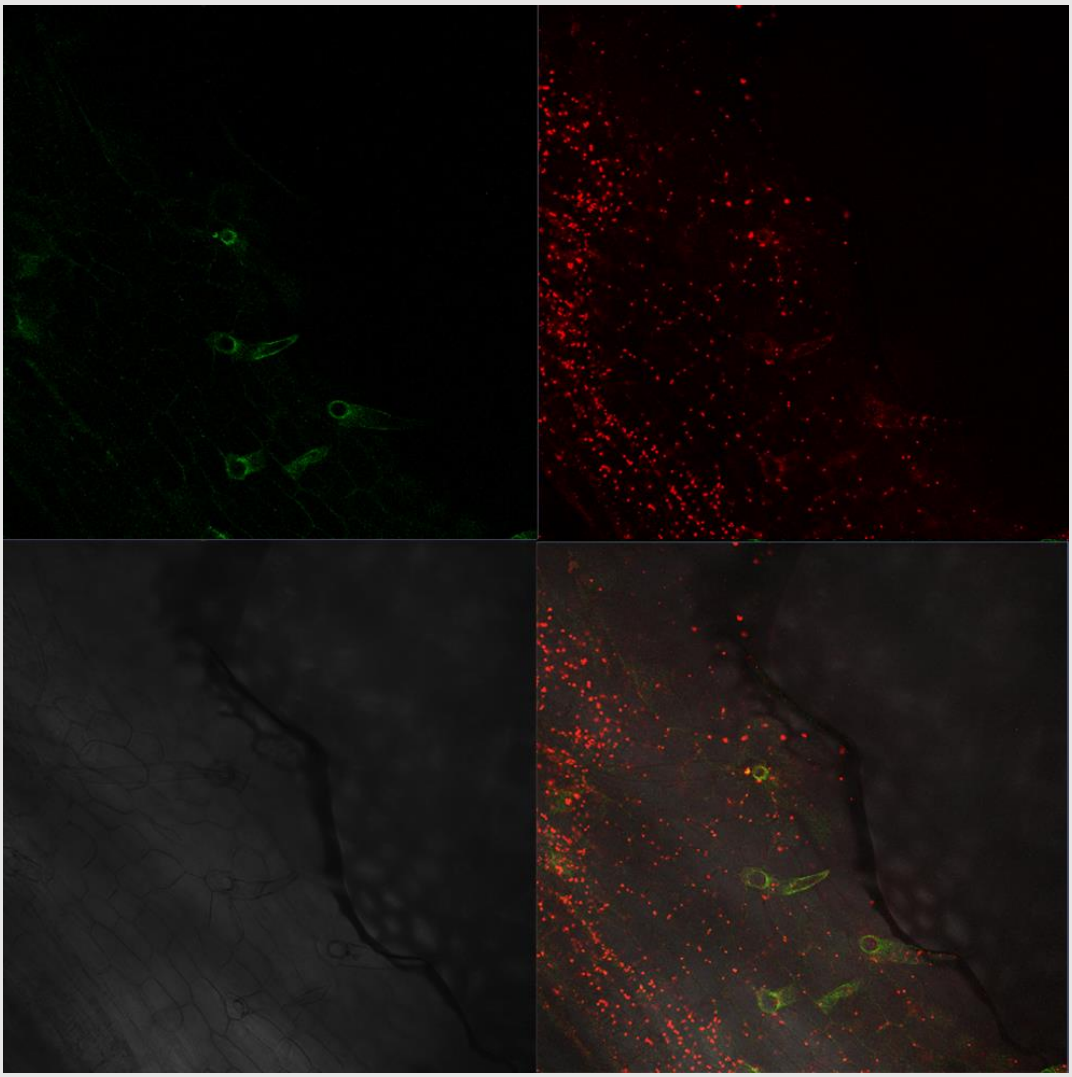


Figure 1.23: Confocal images of GFP of genetic transformed plants of white lupin. Fluorescence was localized in the leaf hair, resulted green in the first and fourth pictures.

P use efficiency for fertilizers

The efficiency of phosphate fertilizers is generally very low, attesting between 10 and 25% (*Khan et al.*, 2009). A possible improvement for reducing the phosphate rocks mining can be, thus, to reach a higher efficiency of the fertilizers. In order to assess the fertilizers P use efficiency the P uptake from the plants should be compared to the P added from fertilizers in a input-output balance method (*Syers and Johnston*, 2008). Obviously, the efficiency of fertilizers depend on the characteristics of the soil: so a NPK application to an already acid soil can further decrease the pH causing a lower P solubility (*Tang et al.*, 2008). In similar cases, a pH correction of the soil is suggested to increase the P efficiency, with lime addition to increase pH above 5.5 (*Syers and Johnston*, 2008). The soil pH changes also the efficiency of fertilizers types: the phosphate rocks are very effective in acid soil but almost useless in neutral and calcareous soil (*Syers and Johnston*, 2008). In some cases, the addition to the soil of silicon (e.g. in the form of zeolite), which act as a competitor to P for adsorption sites, can increase the P solubility (*Syers and Johnston*, 2008). Furthermore, also the use of particular agricultural managements such as placed or fractionated fertilization can have positive effect on the nutrient use efficiency of the fertilizers (*Nkebiwe et al.*, 2016). While fractionated fertilization, using fertigation, is not much common for phosphate nutrition, the localized P fertilization, where fertilizers are placed closely to the seeds or the plant roots, is generally highly widespread. Localized fertilization increased the yield of 3.7% as compared to broadcast fertilisation with respect of a broad meta-analysis on 772 dataset (*Nkebiwe et al.*, 2016). Focusing on phosphate fertilisation the placed mix of urea and phosphorus showed 27% yield increase, while ammonium and phosphate enhanced yield of 14% as compared to broadcast fertilization. Furthermore, the localisation of the fertilization has increased also the nutrient (N, P or K) content of the plants (*Nkebiwe et al.*, 2016). Nutrient concentration and yield increase was not found for placed fertilization of lettuce, while placed fertilization was more effective in wheat and maize (*Nkebiwe et al.*, 2016). A possibility to reduce the P run-off, increasing the P efficiency and reducing the risk of eutrophication, is the use of a minimum or zero tillage (*Syers and Johnston*, 2008). Another way to improve the fertilizer use efficiency is to inoculate them with plant promoting bacteria or mycorrhizal fungi (*Adesemoye and Kloepper*, 2009), with very good results in P deficient soils, but lower efficacy when the soil P concentration is sufficient (*Syers and Johnston*, 2008).

In conclusion, the use of better agricultural managements can increase easily the P use efficiency of the fertilizers. Among these, also the use of precision farming can assess the actual need of P of the crops and balancing better the use of fertilizers in the right phenological phase and in a localised way.

Organic products as an alternative to phosphate rocks

There are many possibilities of reuse of P-rich waste (Fig. 1.24). Human excreta and animal manure, animal meal, food waste and crop residues can be disposed directly on soil or after composting (composting processes described below). Civil, livestock and industrial grey water can be used diluted as irrigation water and the sludge after treatments can be directly disposed on soil, used as primary feed for biogas plants or composted. In addition, the wastewater can be treated for struvite crystallisation (below the description) or incinerated and the ashes used as a source of P (*Cordell et al.*, 2011).

Phosphorus sources		Phosphorus recovery and reuse process					
		i. Source separation and reuse	ii. Wastewater mixing and reuse	iii. Recovery and reuse of byproducts/residuals	iv. Struvite generation and reuse	v. Virgin extraction and processing	vi. Incineration/ burning and reuse
Type A: used sources	A1. Human excreta	e.g. Urine (storage and direct reuse), composted/dry faeces	e.g. Direct use of diluted wastewater; use of treated effluent as irrigation water	e.g. Activated sewage sludge from wastewater treatment plant; sludge from biogas/ biofuel digester; composted filter cake from sugar factories	e.g. From mixed wastewater at the treatment plant		e.g. Incinerating toilet[a]
	A2. Greywater	e.g. Treatment and non-potable reuse					
	A3. Animal manure	e.g. Direct application of manure			e.g. From dairy waste		e.g. Ashes from burning manure
	A4. Other industrial waste						
	A5. Animal meal	e.g. Ground bonemeal, meatmeal, bloodmeal		e.g. Ground bonemeal, meatmeal, bloodmeal			
	A6. Food waste	e.g. Composted food waste	e.g. In-sink garbage grinder	e.g. Composted residues from food processing			
	A7. Crop residues	e.g. Crop residues ploughed back into field		e.g. Oil press cakes[c]			e.g. Ashes from burning crop residues
Type B: new sources	B1. Crops	e.g. Green manure[b]		e.g. Sludge from anaerobic digestion of virgin crops			e.g. Slash and burn
	B2. Phosphate rock			e.g. Extracting P from phosphogypsum stockpiles		e.g. Mining existing and potential reserves; seabed phosphate	
	B3. Aquatic vegetation (e.g. algae, seaweed), sediments and seawater					e.g. Compostable source of nutrients in coastal areas where algae accumulate	

Figure 1.24: (*Cordell et al.*, 2011)

Manure

Eutrophication problems are usually correlated to areas with high density of livestock (*Sharpley and Moyer*, 2000). Swine slurry has generally the highest P concentration, followed by poultry manure, whereas, dairy manure has very low P concentration. The higher total P concentration, but mostly the water extractable P, in swine slurry causes a higher P runoff (*Kleinman et al.*, 2002). The livestock rich areas, thus, have the problems of disposing manure, to avoid P losses, while areas without animal farms have generally soils highly P deficient. Therefore, there is the need of reduce the volume of animal manure in order to make sustainable the transport of manure. Some possibilities are pelleting or composting (*Sharpley and Moyer*, 2000).

Compost

The composting is the biological decomposition and stabilization of organic substrates. The conditions of this process, aeration and moisture, should allow the increase in temperature operated by thermophilic microorganisms that will create a stable product, pathogens and vital seeds free (*Haug*, 1993). The application of compost on crops at the N needs of the plants can cause P accumulation in soil, because the N/P ratio of the compost is generally lower than the N/P ratio of the plant needs (*Eghball and Power*, 1999). On the other hand, compost has shown very low P solubility in previous studies, depending on the waste used for compost production, with sewage sludge compost having higher soluble P and green waste compost and municipal solid waste compost showing the lowest P solubility (*Grigatti et al.*, 2017, 2015).

Biodigestate

Biodigestate is the final waste of the biogas production. The EU politic to incentivise renewable resource for energy production has led to an increase of biogas plants and consequently biodigestate waste (*Grigatti et al.*, 2015). Biodigestate can be disposed directly to soil or composted (*Wellinger et al.*, 2013). As compost, the P concentration and its solubility is highly dependent on the feed of the biogas plants.

Sewage sludge

Sewage sludge are the solid waste of the wastewater treatment plants. They are generally rich in P, due to the necessity of municipality to precipitate the P contained in wastewater to avoid P accumulation in water basin and the consequent eutrophication. Sewage sludge production is increasing every year and their disposal is a concern of the governments. Sewage sludge can be disposed directly on soil, composted, incinerated or disposed in landfill (*Smith*, 2009). Tring to avoid the landfill, the soil dispose needs some attention, due the high risk of heavy metal contamination (*Kidd et al.*, 2007). Although P concentration is very high in the sewage sludge, its solubility remains generally very low, also because of the metals used in the wastewater treatment plants to precipitate P (*Tarayre et al.*, 2016).

Struvite

Struvite is a crystalline substance composed of magnesium ammonium phosphate (in equal molar concentration), which can be found in sewage sludge after the wastewater treatments (*Doyle and Parsons*, 2002). After the EU directive on wastewater treatment (91/271/EC), the demands of P removal from wastewater had increasing, causing the beginning of the use of mineral salts to precipitate P. The struvite formation, caused by the salts addition, has always been a problem for the wastewater treatment plants,

due to the accumulation of the mineral in the basins and tubes (*Doyle and Parsons*, 2002). Recently, an increasing attention was focused on the use of struvite as a slow release fertilizer (*Talboys et al.*, 2016). Struvite formation depend highly on pH of the process which should be slightly alkaline and on the addition of $MgCl_2$ in order to reach the right Mg:NH_4:P molar ratio (*Rahman et al.*, 2014).

Although the difficulties in struvite crystallisation, the high content of P coupled with the nitrogen concentration make it a possible source for P fertilization (*Talboys et al.*, 2016). For its concentration of P (13%) struvite is considered a P fertilizer (*Rahman et al.*, 2014). In a pot trial on lettuce struvite increased the yield as compared to triple superphosphate, probably because of its concurrent Mg supply (*Gonzalez-Ponce et al.*, 2009). On the other hand, Mg accumulation in soil can occur when highly fertilized with struvite, due to the lower demand in Mg as compared to N and P(*Kataki et al.*, 2016).

Objective

In the frame of the circular economy, of the necessity to close the P cycle and to reuse the waste as a new input, the aims of the work were, thus:

(I) to study the phosphorus soil availability and plant assimilation of different organic bio-waste. To reach this aim, compost, biodigestate and livestock manure were analysed in a soil test for P availability and microbial biomass P kinetics and in a pot trial with ryegrass for plant P uptake (chap. 2);

(II) to create a new alternative P fertilizers deriving from sewage sludge and to test it on lettuce in a soil-plant system. To do so, two different sewage sludge were chemically characterized, P speciation was performed with sequential extraction. Afterwards, different chemical and enzymatic extractants was used to solubilise P from the sewage sludge (chap. 3). The best hydrolysing solution in respect of P recovery and heavy metal concentration was tried in a soilless system lettuce (chap. 4), later in soil, and in a soil-plant system (chap. 5). Finally, the fate of P from the sludge to plants was described with a mass balance view (chap. 6).

Reference

Achat, D.L., Bakker, M.R., Zeller, B., Pellerin, S., Bienaimé, S., Morel, C. (2010): Long-term organic phosphorus mineralization in Spodosols under forests and its relation to carbon and nitrogen mineralization. *Soil Biol. Biochem.* 42, 1479–1490.

Adam, C., Peplinski, B., Michaelis, M., Kley, G., Simon, F.-G. (2008): Thermochemical treatment of sewage sludge ashes for phosphorus recovery. *Waste Manag.* 29, 1122–1128.

Adesemoye, A.O., Kloepper, J.W. (2009): Plant–microbes interactions in enhanced fertilizer-use efficiency. *Appl. Microbiol. Biotechnol.* 85, 1–12.

AEEP (2017): Phosphorus: Key to Plant Life [WWW Document]. URL http://aeep.eu/phosphorus/ (accessed 11.29.17).

Ajiboye, B., Akinremi, O.O., Hu, Y., Flaten, D.N. (2007): Phosphorus Speciation of Sequential Extracts of Organic Amendments Using Nuclear Magnetic Resonance and X-ray Absorption Near-Edge Structure Spectroscopies. *J. Environ. Qual.* 36, 1563.

Alborés, A.F., Cid, B.P., Gómez, E.F., López, E.F. (2000): Comparison between sequential extraction procedures and single extractions for metal partitioning in sewage sludge samples. *Analyst* 125, 1353–1357.

Alvarenga, P., Mourinha, C., Farto, M., Santos, T., Palma, P., Sengo, J., Morais, M.-C., Cunha-Queda, C. (2015): Sewage sludge, compost and other representative organic wastes as agricultural soil amendments: Benefits versus limiting factors.

Alvarez, A.E., Mochon, C.M., Sanchez, J.C., Rodriguez, T.M. (2002): Heavy metal extractable forms in sludge from wastewater treatment plants. *Chemosphere* 47, 765–775.

Andersen, M.S. (2007): An introductory note on the environmental economics of the circular economy. *Sustain. Sci.* 2, 133–140.

Ayaga, G., Todd, A., Brookes, P.C. (2006): Enhanced biological cycling of phosphorus increases its availability to crops in low-input sub-Saharan farming systems. *Soil Biol. Biochem.* 38, 81–90.

Baek, D., Kim, M.C., Chun, H.J., Kang, S., Park, H.C., Shin, G., Park, J., Shen, M., Hong, H., Kim, W.-Y., Kim, D.H., Lee, S.Y., Bressan, R.A., Bohnert, H.J., Yun, D.-J. (2013): Regulation of miR399f Transcription by AtMYB2 Affects Phosphate Starvation Responses in Arabidopsis. *Plant Physiol.* 161, 362–373.

Baldwin, J.C., Karthikeyan, A.S., Raghothama, K.G. (2001): LEPS2, a phosphorus starvation-induced novel acid phosphatase from tomato. *Plant Physiol.* 125, 728–37.

Bates, T.R., Lynch, J.P. (1996): Stimulation of root hair elongation in Arabidopsis thaliana by low phosphorus availability. *Plant, Cell Environ.* 19, 529–538.

Becana, M., Moran, J.F., Iturbe-Ormaetxe, I. (1998): Iron-dependent oxygen free radical generation in plants subjected to environmental stress: Toxicity and antioxidant protection. *Plant Soil* 201, 137–147.

Becker, M., Asch, F. (2005): Iron toxicity in rice-conditions and management concepts.

J. Plant Nutr. Soil Sci. 168, 558–573.

Bernal, M.P., Alburquerque, J.A., Moral, R. (2009): Composting of animal manures and chemical criteria for compost maturity assessment. A review. *Bioresour. Technol.* 100, 5444–5453.

Bhadoria, P.S., Steingrobe, B., Claassen, N., Liebersbach, H. (2002): Phosphorus efficiency of wheat and sugar beet seedlings grown in soils with mainly calcium, or iron and aluminium phosphate. *Plant Soil* 246, 41–52.

Biswas, B.K., Inoue, K., Harada, H., Ohto, K., Kawakita, H. (2009): Leaching of phosphorus from incinerated sewage sludge ash by means of acid extraction followed by adsorption on orange waste gel. *J. Environ. Sci.* 21, 1753–1760.

Blöcher, C., Niewersch, C., Melin, T. (2012): Phosphorus recovery from sewage sludge with a hybrid process of low pressure wet oxidation and nanofiltration. *Water Res.* 46, 2009–2019.

Bolan, N.S. (1991): A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. *Plant Soil*.

Breeuwsma, A., Reijerink, J.G.A., Schoumans, O.F. (1995): Impact of manure on accumulation and leaching of phosphate in areas of intensive livestock farming, in: Animal Waste and the Land-Water Interface. pp. 239–249.

Brod, E., Øgaard, A.F., Hansen, E., Wragg, D., Haraldsen, T.K., Krogstad, T. (2015): Waste products as alternative phosphorus fertilisers part I: inorganic P species affect fertilisation effects depending on soil pH. *Nutr. Cycl. Agroecosystems* 103, 167–185.

Brookes, P., Beyaert, R., Voroney, R. (2007): Soil Microbial Biomass C, N, P, and S, in: Soil Sampling and Methods of Analysis, Second Edition. CRC Press.

Brookes, P.C., Powlson, D.S., Jenkinson, D.S. (1982): Measurement of microbial biomass phosphorus in soil. *Soil Biol. Biochem.* 14, 319–329.

Brown, T.A., Shrift, A. (1982): SELENIUM: TOXICITY AND TOLERANCE IN HIGHER PLANTS. *Biol. Rev.* 57, 59–84.

Bünemann, E.K. (2015): Assessment of gross and net mineralization rates of soil organic phosphorus – A review. *Soil Biol. Biochem.* 89, 82–98.

Burleigh, S.H., Cavagnaro, T., Jakobsen, I. (2002): Functional diversity of arbuscular mycorrhizas extends to the expression of plant genes involved in P nutrition. *J. Exp. Bot.* 53, 1593–1601.

Cakmak, I., Hengeler, C., Marschner, H. (1994): Partitioning of shoot and root dry matter and carbohydrates in bean plants suffering from phosphorus, potassium and magnesium deficiency. *J. Exp. Bot.* 45, 1245–1250.

CCIA (2016): Prezzi - News — Camera di Commercio di Modena [WWW Document]. URL http://www.mo.camcom.it/tutela-del-mercato/ufficio-prezzi (accessed 11.9.16).

Chen, C.R., Condron, L.M., Davis, M.R., Sherlock, R.R. (2002): Phosphorus dynamics in the rhizosphere of perennial ryegrass (Lolium perenne L.) and radiata pine (Pinus Radiata D. Don.). *Soil Biol. Biochem.* 34, 487–499.

Childers, D.L., Corman, J., Edwards, M., Elser, J.J. (2011): Sustainability Challenges

of Phosphorus and Food: Solutions from Closing the Human Phosphorus Cycle. *Bioscience* 61, 117–124.

Christodoulou, A., Stamatelatou, K. (2016): Overview of legislation on sewage sludge management in developed countries worldwide. *Water Sci. Technol.* 73.

Cieslik, B., Konieczka, P. (2017): A review of phosphorus recovery methods at various steps of wastewater treatment and sewage sludge management. The concept of " no solid waste generation " and analytical methods. *J. Clean. Prod.* 142, 1728–1740.

Clarkson, D.T., Scattergood, C.B. (1982): Growth and Phosphate Transport in Barley and Tomato Plants During the Development of, and Recovery from, Phosphate-stress. *J. Exp. Bot.* 33, 865–875.

Cogliatti, D.H., Santa Marìa, G.E. (1990): Influx and Efflux of Phosphorus in Roots of Wheat Plants in Non-Growth-Limiting Concentrations of Phosphorus. *J. Exp. Bot.* 41, 601–607.

Cooper, J., Lombardi, R., Boardman, D., Carliell-Marquet, C. (2011): The future distribution and production of global phosphate rock reserves. *Resour. Conserv. Recycl.* 57, 78–86.

Cordell, D., Drangert, J., White, S. (2009): The story of phosphorus: global food security and food for thought. *Glob. Environ. Chang.*

Cordell, D., Rosemarin, A., Schroder, J.J., Smit, A.L. (2011): Towards global phosphorus security: A systems framework for phosphorus recovery and reuse options. *Chemosphere* 84, 747–758.

Corominas, L., Foley, J., Guest, J.S., Hospido, A., Larsen, H.F., Morera, S., Shaw, A. (2013): Life cycle assessment applied to wastewater treatment: State of the art. *Water Res.* 47, 5480–5492.

Da Ros, C., Cavinato, C., Pavan, P., Bolzonella, D. (2016): Mesophilic and thermophilic anaerobic co-digestion of winery wastewater sludge and wine lees: An integrated approach for sustainable wine production. *J. Environ. Manage.*

Dakora, F.D., Phillips, D.A. (2002): Root exudates as mediators of mineral acquisition in low-nutrient environments. *Plant Soil* 245, 35–47.

Delhaize, E., Randall, P.J., Laurie, S., Phillips, R., Ratcliffe, R., Stewart, G. (1995): Characterization of a Phosphate-Accumulator Mutant of Arabidopsis thaliana. *Plant Physiol.* 107, 207–213.

Direzione Generale Ambiente, D. del suolo e della costa-D.A.R.E.-R. (2004): Linee di Intervento in materia di utilizzo dei fanghi di depurazione in agricoltura. Bologna.

Domenech, C.E., Lisa, T.A., Salvano, M.A., Garrido, M.N. (2015): Selective release of phosphorus and nitrogen from waste activated sludge with combined thermal and alkali treatment. *Bioresour. Technol.* 190, 522–528.

Donatello, S., Tong, D., Cheeseman, C.R. (2010): Production of technical grade phosphoric acid from incinerator sewage sludge ash (ISSA). *Waste Manag.* 30, 1634–1642.

Doyle, J.D., Parsons, S.A. (2002): Struvite formation, control and recovery. *Water Res.* 36, 3925–3940.

Duboc, O., Santner, J., Fard, A.G., Zehetner, F., Tacconi, J., Wenzel, W.W. (2017): Predicting phosphorus availability from chemically diverse conventional and recycling fertilizers. *Sci. Total Environ.* 599–600, 1160–1170.

Easlon, H.M., Bloom, A.J. (2014): Easy Leaf Area: Automated Digital Image Analysis for Rapid and Accurate Measurement of Leaf Area. *Appl. Plant Sci.* 2, 1400033.

Eghball, B. (2002): Soil Properties as Influenced by Phosphorus-and Nitrogen-Based Manure and Compost Applications WASTE MANAGEMENT Soil Properties as Influenced by Phosphorus-and Nitrogen-Based Manure and Compost Applications. *Waste Manag.* 94, 128–135.

Eghball, B., Power, J.F. (1999): Phosphorus- and Nitrogen-Based Manure and Compost Applications: Corn Production and Soil Phosphorus. *Soil Sci. Soc. Am. J.* 63, 895–901.

Ellis, D.R., Salt, D.E. (2003): Plants, selenium and human health. *Curr. Opin. Plant Biol.*

Eneji, A.E., Honna, T., Yamamoto, S., Masuda, T., Endo, T., Irshad, M. (2003): Changes in Humic Substances and Phosphorus Fractions During Composting. *Commun. Soil Sci. Plant Anal.* 34, 2303–2314.

European Biogas Association (2015): EBA Biomethane & Biogas Report 2015 [WWW Document]. URL http://european-biogas.eu/2015/12/16/biogasreport2015/ (accessed 11.9.16).

Eurostat (2016): Eurostat [WWW Document]. URL http://ec.europa.eu/eurostat/web/main/home (accessed 11.9.16).

Ewa Sroga, G. (1987): Plant regeneration of two Lupinus spp. from callus cultures via organogenesis. *Plant Sci.* 51, 245–249.

FAO (2016): FAOSTAT [WWW Document]. URL http://www.fao.org/faostat/en/#home (accessed 12.2.17).

Feng, R., Wei, C., Tu, S. (2013): The roles of selenium in protecting plants against abiotic stresses. *Environ. Exp. Bot.*

Fertilisers Working Group Meeting (2014): Essential safety and quality requirements for fertilising materials.

Foehse, D., Jungk, A. (1983): Influence of phosphate and nitrate supply on root hair formation of rape, spinach and tomato plants. *Plant Soil* 74, 359–368.

Fredeen, A., Rao, I., Terry, N. (1989): Influence of phosphorus nutrition on growth and carbon partitioning in Glycine max. *Plant Physiol.*

Frossard, E., Condron, L.M., Oberson, A., Sinaj, S., Fardeau, J.C. (2000): Processes Governing Phosphorus Availability in Temperate Soils. *J. Environ. Qual.* 29, 15.

Frossard, E., Sinaj, S., Zhang, L.-M., Morel, J.L. (1996): The Fate of Sludge Phosphorus in Soil-Plant Systems. *Soil Sci. Soc. Am. J.* 60, 1248.

Fuentes, B., Bolan, N., Naidu, R., de la Luz Mora, M., Australia, A. (2006): PHOSPHORUS IN ORGANIC WASTE-SOIL SYSTEMS Fósforo en los sistemas suelo – residuos orgánicos. *J.Soil Sc. Plant. Nutr* 66, 64–8364.

García-Albacete, M., Martín, A., Cartagena, M.C. (2012): Fractionation of phosphorus biowastes: Characterisation and environmental risk. *Waste Manag.*

32, 1061–1068.

Gianquinto, G., Orsini, F., Sambo, P., D'Urzo, M.P. (2011): The Use of Diagnostic Optical Tools to Assess Nitrogen Status and to Guide Fertilization of Vegetables. *Horttechnology* 21, 287–292.

Gonzalez-Ponce, R., López-de-Sá, E.G., Plaza, C. (2009): Lettuce Response to Phosphorus Fertilization with Struvite Recovered from Municipal Wastewater. *HortScience* 44, 426–430.

Grigatti, M., Boanini, E., Cavani, L., Ciavatta, C., Marzadori, C. (2015): Phosphorus in Digestate-Based Compost: Chemical Speciation and Plant-Availability. *Waste and Biomass Valorization* 6, 481–493.

Grigatti, M., Boanini, E., Mancarella, S., Simoni, A., Centemero, M., Veeken, A.H.M. (2017): Phosphorous extractability and ryegrass availability from bio-waste composts in a calcareous soil. *Chemosphere* 174, 722–731.

Grigatti, M., Di Girolamo, G., Chincarini, R., Ciavatta, C., Barbanti, L. (2011): Potential nitrogen mineralization, plant utilization efficiency and soil CO2 emissions following the addition of anaerobic digested slurries. *Biomass and Bioenergy* 35, 4619–4629.

Grusak, M. a., DellaPenna, D. (1999): Improving the Nutrient Composition of Plants To Enhance Human Nutrition and Health1. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 50, 133–161.

Guppy, C., Menzies, N., Moody, P., Blamey, F. (2005): Competitive sorption reactions between phosphorus and organic matter in soil: a review. *Soil Res.*

Haug, R.T. (1993): The practical handbook of compost engineering. Lewis Publishers.

Heimersson, S., Harder, R., Peters, G.M., Svanström, M. (2014): Including pathogen risk in life cycle assessment of wastewater management. 2. Quantitative comparison of pathogen risk to other impacts on human health. *Environ. Sci. Technol.* 48, 9446–9453.

Herzel, H., Kruger, O., Hermann, L., Adam, C. (2016): Sewage sludge ash - A promising secondary phosphorus source for fertilizer production. *Sci. Total Environ.* 542, 1136–1143.

Hinsinger, P. (2001): Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. *Plant Soil* 237, 173–195.

Hooda, P.S., Truesdale, V.W., Edwards, A.C., Withers, P.J.A., Aitken, M.N., Miller, A., Rendell, A.R. (2001): Manuring and fertilization effects on phosphorus accumulation in soils and potential environmental implications. *Adv. Environ. Res.* 5, 13–21.

Hospido, A., Moreira, M.T., Feijoo, G. (2008): A comparison of municipal wastewater treatment plants for big centres of population in Galicia (Spain). *Int. J. Life Cycle Assess.* 13, 57–64.

Hukari, S., Hermann, L., Nättorp, A. (2016): From wastewater to fertilisers — Technical overview and critical review of European legislation governing phosphorus recycling. *Sci. Total Environ.* 542, 1127–1135.

Hunter, P.J., Teakle, G.R., Bending, G.D. (2014): Root traits and microbial community interactions in relation to phosphorus availability and acquisition, with particular

reference to Brassica. *Front. Plant Sci.* 5, 27.

Indexmundi (2016): DAP fertilizer - Monthly Price - Commodity Prices - Price Charts, Data, and News - IndexMundi [WWW Document]. URL http://www.indexmundi.com/commodities/?commodity=dap-fertilizer&months=180 (accessed 11.9.16).

Jaffer, Y., Clark, T.A., Pearce, P., Parsons, S.A. (2002): Potential phosphorus recovery by struvite formation. *Water Res.* 36, 1834–1842.

Jeng, A.S., Haraldsen, T.K., Grønlund, A., Pedersen, P.A. (2006): Meat and bone meal as nitrogen and phosphorus fertilizer to cereals and rye grass. *Nutr. Cycl. Agroecosystems* 76, 183–191.

Johri, A.K., Oelmüller, R., Dua, M., Yadav, V., Kumar, M., Tuteja, N., Varma, A., Bonfante, P., Persson, B.L., Stroud, R.M. (2015): Fungal association and utilization of phosphate by plants: success, limitations, and future prospects. *Front. Microbiol.* 6, 984.

Jones, D.L. (1998): Organic acids in the rhizosphere – a critical review. *Plant Soil* 205, 25–44.

Kabata-Pendias, A. (2011): Trace elements in soils and plants. Fourth Edition, CRC Press.

Kataki, S., West, H., Clarke, M., Baruah, D.C. (2016): Phosphorus recovery as struvite: Recent concerns for use of seed, alternative Mg source, nitrogen conservation and fertilizer potential. *Resour. Conserv. Recycl.* 107, 142–156.

Khan, A., Jilani, G., Akhtar, M.S., Naqvi, S.M.S., Rasheed, M., Khan, A.A., Jilani, G., Akhtar, M.S., Muhammad, S., Naqvi, S., Rasheed, M. (2009): Phosphorus Solubilizing Bacteria: Occurrence, Mechanisms and their Role in Crop Production. *Publ. J. agric. biol. sci* 1, 48–58.

Khan, K.S., Joergensen, R.G. (2012a): Relationships between P fractions and the microbial biomass in soils under different land use management. *Geoderma* 173, 274–281.

Khan, K.S., Joergensen, R.G. (2012b): Compost and phosphorus amendments for stimulating microorganisms and growth of ryegrass in a Ferralsol and a Luvisol. *J. Plant Nutr. Soil Sci.* 175, 108–114.

Kidd, P.S., Domínguez-Rodríguez, M.J., Díez, J., Monterroso, C. (2007): Bioavailability and plant accumulation of heavy metals and phosphorus in agricultural soils amended by long-term application of sewage sludge. *Chemosphere* 66, 1458–1467.

Kim, M., Han, D.W., Kim, D.J. (2015): Selective release of phosphorus and nitrogen from waste activated sludge with combined thermal and alkali treatment. *Bioresour. Technol.*

Kleinman, P.J.A., Sharpley, A.N., Wolf, A.M., Beegle, D.B., Moore, P.A. (2002): Measuring Water-Extractable Phosphorus in Manure as an Indicator of Phosphorus in Runoff. *Soil Sci. Soc. Am. J.* 66, 2009.

Kochian, L. V (1995): Cellular Mechanisms of Aluminum Toxicity and Resistance in Plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 46, 237–260.

Koppelaar, R.H.E.M., Weikard, H.P. (2013): Assessing phosphate rock depletion and

phosphorus recycling options. *Glob. Environ. Chang.* 23, 1454–1466.

Krüger, O., Adam, C. (2015): Recovery potential of German sewage sludge ash. *Waste Manag.* 45, 400–406.

Kumar, V., Gilkes, R.J., Bolland, M.D.A. (1992): A comparison of seven soil P tests for plant species with different external P requirements grown on soils containing rock phosphate and superphosphate residues. *Fertil. Res.* 33, 35–45.

Lambers, H., Brundrett, M.C., Raven, J.A., Hopper, S.D. (2011): Plant mineral nutrition in ancient landscapes: high plant species diversity on infertile soils is linked to functional diversity for nutritional strategies. *Plant Soil* 348, 7–27.

Laturnus, F., von Arnold, K., Grøn, C. (2007): Organic Contaminants from Sewage Sludge Applied to Agricultural Soils. False Alarm Regarding Possible Problems for Food Safety? (8 pp). *Environ. Sci. Pollut. Res. Int.* 14 Suppl 1, 53–60.

Leite, W.R.M., Gottardo, M., Pavan, P., Belli Filho, P., Bolzonella, D. (2016): Performance and energy aspects of single and two phase thermophilic anaerobic digestion of waste activated sludge. *Renew. Energy* 86, 1324–1331.

Leytem, A.B., Westermann, D.T. (2003): PHOSPHATE SORPTION BY PACIFIC NORTHWEST CALCAREOUS SOILS. *Soil Sci.* 168.

Leytem, A.B., Westermann, D.T. (2005): PHOSPHORUS AVAILABILITY TO BARLEY FROM MANURES AND FERTILIZERS ON A CALCAREOUS SOIL. *Soil* 170, 401–412.

Liang, Y., Sun, W., Zhu, Y.G., Christie, P. (2007): Mechanisms of silicon-mediated alleviation of abiotic stresses in higher plants: A review. *Environ. Pollut.* 147, 422–428.

López-Arredondo, D.L., Leyva-González, M.A., Alatorre-Cobos, F., Herrera-Estrella, L. (2013): Biotechnology of nutrient uptake and assimilation in plants. *Int. J. Dev. Biol.* 57, 595–610.

Lynch, J., Läuchli, A., Epstein, E. (1991): Vegetative Growth of the Common Bean in Response to Phosphorus Nutrition. *Crop Sci.* 31, 380.

Ma, J.F. (2004): Role of silicon in enhancing the resistance of plants to biotic and abiotic stresses. *Soil Sci. Plant Nutr.* 50, 11–18.

Ma, Z., Bielenberg, D.G., Brown, K.M., Lynch, J.P. (2001): Regulation of root hair density by phosphorus availability in Arabidopsis thaliana. *Plant, Cell Environ.* 24, 459–467.

Malik, M., Khan, K., Marschner, P. (2013): Microbial biomass, nutrient availability and nutrient uptake by wheat in two soils with organic amendments. *J. Soil Sci. Plant Nutr.* 13, 955–966.

Mamani, I., Averanga, Kattia, Boris, E., Terrazas, E. (2014): Establecimiento del sistema de generación in vitro de callos de Lupinus mutabilis sweet (tarwi) a partir de hipocótilos. *Rev. CON-CIENCIA* 2.

Marchetti, R., Castelli, F., Orsi, A., Sghedoni, L., Bochicchio, D. (2012): Biochar from swine manure solids: influence on carbon sequestration and Olsen phosphorus and mineral nitrogen dynamics in soil with and without digestate incorporation. *Ital. J. Agron.* 7, 26.

Marschner, H., Kirkby, E.A., Cakmak, I. (1996): Effect of mineral nutritional status on shoot-root partitioning of photoassimilates and cycling of mineral nutrients. *J. Exp. Bot.* 47, 1255–1263.

Marschner, H., Marschner, P. (2012): Marschner's mineral nutrition of higher plants. Academic Press.

Mattenberger, H., Fraissler, G., Brunner, T., Herk, P., Hermann, L., Obernberger, I. (2008): Sewage sludge ash to phosphorus fertiliser: Variables influencing heavy metal removal during thermochemical treatment. *Waste Manag.* 28, 2709–2722.

Mayer, B.K., Baker, L.A., Boyer, T.H., Drechsel, P., Gifford, M., Hanjra, M.A., Parameswaran, P., Stoltzfus, J., Westerhoff, P., Rittmann, B.E. (2016): Total Value of Phosphorus Recovery. *Environ. Sci. Technol.* 50, 6606–6620.

McBride, M., Sauve, S., Hendershot, W. (1997): Solubility control of Cu, Zn, Cd and Pb in contaminated soils. *Eur. J. Soil Sci.* 48, 337–346.

McCoy, J.L., Sikora, L.J., Weil, R.R. (1986): Plant Availability of Phosphorus in Sewage Sludge Compost1. *J. Environ. Qual.* 15, 403.

Møller, J., Boldrin, A., Christensen, T.H. (2009): Anaerobic digestion and digestate use: accounting of greenhouse gases and global warming contribution. *Waste Manag. Res.* 27, 813–824.

Möller, K., Müller, T. (2012): Effects of anaerobic digestion on digestate nutrient availability and crop growth: A review. *Eng. Life Sci.* 12, 242–257.

Mosier, N.S., Ladisch, C.M., Ladisch, M.R. (2002): Characterization of acid catalytic domains for cellulose hydrolysis and glucose degradation. *Biotechnol. Bioeng.* 79, 610–618.

Muhammad, S., Müller, T., Joergensen, R.G. (2006): Decomposition of pea and maize straw in Pakistani soils along a gradient in salinity. *Biol. Fertil. Soils* 43, 93–101.

Murphy, J., Riley, J.P. (1962): A modified single solution method for the determination of phosphate in natural waters. *Anal. Chim. Acta* 27, 31–36.

Nagul, E.A., McKelvie, I.D., Worsfold, P., Kolev, S.D. (2015): The molybdenum blue reaction for the determination of orthophosphate revisited: Opening the black box. *Anal. Chim. Acta*.

Nannipieri, P., Giagnoni, L., Landi, L., Renella, G. (2011): Role of Phosphatase Enzymes in Soil, in: Phosphorus in Action. Springer, Berlin, Heidelberg, pp. 215–243.

Neumann, G. (2010): Mining for nutrients - regulatory aspects of cluster root function and development. *New Phytol.* 187, 879–882.

Neumann, G. (2016): The Role of Ethylene in Plant Adaptations for Phosphate Acquisition in Soils – A Review. *Front. Plant Sci.* 6, 1224.

Neumann, G., Martinoia, E. (2002): Cluster roots – an underground adaptation for survival in extreme environments. *Trends Plant Sci.* 7, 162–167.

Nkebiwe, P.M., Weinmann, M., Bar-Tal, A., Müller, T. (2016): Fertilizer placement to improve crop nutrient acquisition and yield: A review and meta-analysis. *F. Crop. Res.* 196, 389–401.

Øgaard, A.F. (1996): Effect of Phosphorus Fertilization on the Concentration of Total

and Algal-available Phosphorus in Different Particle-size Fractions in Norwegian Agricultural Soils. *Acta Agric. Scand. Sect. B - Soil Plant Sci.* 46, 24–29.

Olsen, S.R. (1954): Estimation Of Available Phosphorus In Soils By Extraction With Sodium Bicarbonate.

Ottosen, L.M., Kirkelund, G.M., Jensen, P.E. (2013): Extracting phosphorous from incinerated sewage sludge ash rich in iron or aluminum. *Chemosphere* 91, 963–969.

Pant, H.K., Warman, P.R. (2000): Enzymatic hydrolysis of soil organic phosphorus by immobilized phosphatases. *Biol. Fertil. Soils* 30, 306–311.

Pasqualino, J.C., Meneses, M., Abella, M., Castells, F. (2009): LCA as a Decision Support Tool for the Environmental Improvement of the Operation of a Municipal Wastewater Treatment Plant. *Environ. Sci. Technol.* 43, 3300–3307.

Pastor, L., Marti, N., Bouzas, A., Seco, A. (2008): Sewage sludge management for phosphorus recovery as struvite in EBPR wastewater treatment plants. *Bioresour. Technol.* 99, 4817–4824.

Petronici, C. (1989): Gli elementi nutritivi, in: Paolo Sequi (ed.): Chimica Del Suolo. Patron Editore, Bologna, pp. 357–380.

Pniewski, T., Kapusta, J., Płucienniczak, A. (2006): Agrobacterium-mediated transformation of yellow lupin to generate callus tissue producing HBV surface antigen in a long-term culture. *J. Appl. Genet.* 47, 309–318.

Polowick, P.L., Loukanina, N.N., Doshi, K.M. (2014): Agrobacterium-mediated transformation of tarwi (Lupinus mutabilis Sweet), a potential platform for the production of plant-made proteins. *Vitr. Cell. Dev. Biol. - Plant* 50, 401–411.

Pote, D.H., Daniel, T.C., Sharpley, A.N., Moore, P.A., Edwards, D.R., Nichols, D.J. (1996): Relating Extractable Soil Phosphorus to Phosphorus Losses in Runoff t'. *Soil Sci. Soc. Am. J.* 60, 855–859.

Raghothama, K.G. (1999): PHOSPHATE ACQUISITION. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 50, 665–693.

Rahman, M.M., Salleh, M.A.M., Rashid, U., Ahsan, A., Hossain, M.M., Ra, C.S. (2014): Production of slow release crystal fertilizer from wastewaters through struvite crystallization – A review. *Arab. J. Chem.* 7, 139–155.

Reijnders, L. (2014): Phosphorus resources, their depletion and conservation, a review. *Resour. Conserv. Recycl.*

Richardson, A.E., Simpson, R.J. (2011): Soil microorganisms mediating phosphorus availability update on microbial phosphorus. *Plant Physiol.* 156, 989–96.

Russo, A. (2012): ANALISI E MIGLIORAMENTO DELL'IMPIANTO DI DEPURAZIONE DI LEGNAGO. Università degli Studi di Padova.

Shannon, M.C., Grieve, C.M. (1999): Tolerance of vegetable crops to salinity. *Sci. Hortic. (Amsterdam).* 78, 5–38.

Sharpley, A., Moyer, B. (2000): Phosphorus Forms in Manure and Compost and Their Release during Simulated Rainfall. *J. Environ. Qual.* 29, 1462.

Sharpley, A.N., Chapra, S.C., Wedepohl, R., Sims, J.T., Daniel, T.C., Reddy, K.R. (1994): Managing Agricultural Phosphorus for Protection of Surface Waters:

Issues and Options. *J. Environ. Qual.* 23, 437.

Shen, J., Yuan, L., Zhang, J., Li, H., Bai, Z., Chen, X., Zhang, W., Zhang, F. (2011): Phosphorus dynamics: from soil to plant. *Plant Physiol.* 156, 997–1005.

Shenoy, V. V., Kalagudi, G.M. (2005): Enhancing plant phosphorus use efficiency for sustainable cropping. *Biotechnol. Adv.* 23, 501–513.

Siddique, M.T., Robinson, J.S. (2003): Phosphorus Sorption and Availability in Soils Amended with Animal Manures and Sewage Sludge. *J. Environ. Qual.* 32, 1114.

Simpson, M., McLenaghen, R.D., Chirino-Valle, I., Condron, L.M. (2012): Effects of long-term grassland management on the chemical nature and bioavailability of soil phosphorus. *Biol. Fertil. Soils* 48, 607–611.

Sims, J.T., Simard, R.R., Joern, B.C. (1998): Phosphorus Loss in Agricultural Drainage: Historical Perspective and Current Research. *J. Environ. Qual.* 27, 277.

Smith, K.A., Van Dijk, T.A. (1987): Utilisation of phosphorus and potassium from animal manures on grassland and forage crops, in: Animal Manure on Grassland and Fodder Crops. Fertilizer or Waste? Springer Netherlands, Dordrecht, pp. 87–102.

Smith, S.R. (2009): A critical review of the bioavailability and impacts of heavy metals in municipal solid waste composts compared to sewage sludge. *Environ. Int.* 35, 142–156.

Sonneveld, C., Straver, N. (1994): Nutrient solutions for vegetables and flowers grown in water or substrates, Tenth. ed.

Stewart, J.W.B., Tiessen, H. (1987): Dynamics of soil organic phosphorus. *Biogeochemistry* 4, 41–60.

Suh, Y.J., Rousseaux, P. (2002): An LCA of alternative wastewater sludge treatment scenarios. *Resour. Conserv. Recycl.* 35, 191–200.

Syers, D.J., Johnston, A. (2008): Efficiency of soil and fertilizer phosphorus use Reconciling changing concepts of soil phosphorus behaviour with agronomic information. *FAO Fertil. Plant Nutr. Bull.*

Tabatabai, M.A., Bremner, J.M. (1969): Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biol. Biochem.* 1, 301–307.

Takahashi, M., Kato, S., Shima, H., Sarai, E., Ichioka, T., Hatyakawa, S., Miyajiri, H. (2001): Technology for recovering phosphorus from incinerated wastewater treatment sludge, in: Chemosphere. pp. 23–29.

Takahashi, S. (2013): Phosphorus characterization of manure composts and combined organic fertilizers by a sequential-fractionation method. *J. Plant Nutr. Soil Sci.* 176, 494–496.

Talboys, P.J., Heppell, J., Roose, T., Healey, J.R., Jones, D.L., Withers, P.J.A. (2016): Struvite: a slow-release fertiliser for sustainable phosphorus management? *Plant Soil* 401, 109–123.

Tang, X., Li, J., Ma, Y., Hao, X., Li, X. (2008): Phosphorus efficiency in long-term (15 years) wheat–maize cropping systems with various soil and climate conditions. *F. Crop. Res.* 108, 231–237.

Tarayre, C., De Clercq, L., Charlier, R., Michels, E., Meers, E., Camargo-Valero, M.,

Delvigne, F. (2016): New perspectives for the design of sustainable bioprocesses for phosphorus recovery from waste. *Bioresour. Technol.*

Tejada, M., García-Martínez, A.M., Rodríguez-Morgado, B., Carballo, M., García-Antras, D., Aragón, C., Parrado, J. (2013): Obtaining biostimulant products for land application from the sewage sludge of small populations. *Ecol. Eng.* 50, 31–36.

Toor, G.S., Peak, J.D., Sims, J.T. (2005): Phosphorus Speciation in Broiler Litter and Turkey Manure Produced from Modified Diets. *J. Environ. Qual.* 34, 687.

Torri, S.I., Correa, R.S., Renella, G. (2017): Biosolid Application to Agricultural Land—a Contribution to Global Phosphorus Recycle: A Review. *Pedosphere* 27, 1–16.

Turner, B.L., Cade-Menun, B.J., Condron, L.M., Newman, S. (2005): Extraction of soil organic phosphorus. *Talanta* 66, 294–306.

Turner, B.L., McKelvie, I.D., Haygarth, P.M. (2002): Characterisation of water-extractable soil organic phosphorus by phosphatase hydrolysis. *Soil Biol. Biochem.* 34, 27–35.

Uhde-Stone, C., Liu, J., Zinn, K.E., Allan, D.L., Vance, C.P. (2005): Transgenic proteoid roots of white lupin: a vehicle for characterizing and silencing root genes involved in adaptation to P stress. *Plant J.* 44, 840–853.

Uysal, A., Dilsad Yilmazel, Y., Demirer, G.N. (2010): The determination of fertilizer quality of the formed struvite from effluent of a sewage sludge anaerobic digester. *J. Hazard. Mater.* 181, 248–254.

Vance, C.P. (2001): Symbiotic Nitrogen Fixation and Phosphorus Acquisition. Plant Nutrition in a World of Declining Renewable Resources. *Plant Physiol.* 127.

Vance, C.P., Uhde-Stone, C., Allan, D.L. (2003): Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. *New Phytol.* 157, 423–447.

Veneklaas, E.J., Lambers, H., Bragg, J., Finnegan, P.M., Lovelock, C.E., Plaxton, W.C., Price, C.A., Scheible, W.-R., Shane, M.W., White, P.J., Raven, J.A. (2012): Opportunities for improving phosphorus-use efficiency in crop plants. *New Phytol.* 195, 306–320.

Walan, P., Davidsson, S., Johansson, S., Höök, M. (2014): Phosphate rock production and depletion: Regional disaggregated modeling and global implications. *Resour. Conserv. Recycl.* 93, 178–187.

Wang, Z., Straub, D., Yang, H., Kania, A., Shen, J., Ludewig, U., Neumann, G. (2014): The regulatory network of cluster-root function and development in phosphate-deficient white lupin (*Lupinus albus*) identified by transcriptome sequencing. *Physiol. Plant.* 151, 323–338.

Wellinger, A., Murphy, J., Baxter, D. (2013): The biogas handbook : science, production and applications.

Wheeler, B.D., Al-Farraj, M.M., Cook, R.E.D. (1985): Iron toxicity to plants in base-rich wetlands: comparative effects on the distribution and growth of Epilobium hirsutum l. and Juncus subnodulosus Schrank. *New Phytol.* 100, 653–669.

White, P.J., Brown, P.H. (2010): Plant nutrition for sustainable development and

global health. *Ann. Bot.* 105, 1073–1080.

Wollmann, I., Gauro, A., Müller, T., Möller, K. (2017): Phosphorus bioavailability of sewage sludge-based recycled fertilizers. *J. Plant Nutr. Soil Sci.*

Xu, H., He, P., Gu, W., Wang, G., Shao, L. (2012): Recovery of phosphorus as struvite from sewage sludge ash. *J. Environ. Sci. (China)* 24, 1533–1538.

Ye, Y., Ngo, H.H., Guo, W., Liu, Y., Li, J., Liu, Y., Zhang, X., Jia, H., Ngo, H.H. (2016): Insight into chemical phosphate recovery from municipal wastewater. *Sci. Total Environ.* 576, 159–171.

Zang, Y., Li, Y., Wang, C., Zhang, W., Xiong, W. (2015): Towards more accurate life cycle assessment of biological wastewater treatment plants: A review. *J. Clean. Prod.*

Zhu, J., Li, M., Whelan, M., Gan, J. (2017): Phosphorus activators contribute to legacy phosphorus availability in agricultural soils: A review. *Sci. Total Environ.* 612, 522–537.

Chapter 2

Phosphorus availability and uptake in a plant-soil system after treatments with organic waste products

Abstract

In order to close the P cycle, organic waste products are a useful alternative to the mineral fertilizer. Generally, though, P in the organic amendants is not highly available for plant uptake. In this study, we have found that there are different characteristics that can help predict the P availability of an organic product. Firstly, the metal concentration and the Al:P, Fe:P and Ca:P ratios are easy and economic tools that give an already good prevision. The highest Al, Fe and Ca concentration, the lowest will be the short-term availability. The organic products with high metal concentration can be more suitable for long-term fertilization. In our study, the organic C can improve the microbial biomass and ameliorate the rhizosphere conditions, also protecting P from precipitation with Ca. Furthermore, microbial biomass can release P from metals, increasing metal and P phytoavailability, without competing for P with plants, storing P for long-term nutrition and preventing P fixation.

Introduction

It is widely recognized that phosphorus (P) is an essential element for plant growth (*Baek et al.*, 2013; *Fredeen et al.*, 1989). In this frame, P fertilization has been fundamental to reach the ever-increasing food demand in the last 100 years. Phosphate fertilizers are mainly derived from the non-renewable phosphate rocks that are claimed to become very scarce in the next 50-100 years (*Cordell et al.*, 2009). Hence, partially replacing phosphate with alternative sources can be part of the solution to P scarcity problem. In this scenario, the European Union (EU) planned a strategic action to deal with the facing phosphorus deficiency by improving the soil application of organic products, such as animal slurries, composts and also sewage sludge (*Eurostat*, 2016). Organic wastes, such as animal slurries, urban and agro-industrial sewage sludge can be directly utilized on agricultural fields. Nowadays the anaerobic digestion (AD) is rapidly increasing as a strategy for the management of the abovementioned kind of waste (*Møller et al.*, 2009; *Möller and Müller*, 2012). AD can reduce the easily degradable organic matter (OM) concentration being this feature important for their further utilization in agricultural soils. On the other hand AD can promote the precipitation of P-based compounds (Fe-P, Ca-P, Mg-P besides to struvite), thus affecting P solubility (*Möller and Müller*, 2012). Bio-waste cannot be directly disposed by soil spreading, to this aim those are often composted. Composting promote the OM mineralization giving stable and safe final products (*Bernal et al.*, 2009), however the composting process can affect their P solubility (*Eneji et al.*, 2003).

The nutrient efficiency of P from chemical fertilizer is reported in literature at range varying between 10 and 25% (*Khan et al.*, 2009). Cultivated plants mainly utilize orthophosphate, which is generally at low level in agricultural soils. Furthermore, P is poorly available, especially in calcareous soils where it is rapidly fixed forming sparingly soluble compounds with Ca and Mg (*Bolan*, 1991; *Hinsinger*, 2001). Thus, only a small fraction of soil P is easily available for plants (*Hinsinger*, 2001). In this frame, the addition of OM to the soils through recycled organic waste can increase P phytoavailability by a competition mechanism for available P-sorption sites (*Guppy et al.*, 2005; *Hinsinger*, 2001). Useful information on P solubility in the products can be also obtained by the study of Al:P, Fe:P, Ca:P ratios.

Beside to the physical-chemical interaction after the OM addition, the increased soil microbial growth can be registered. Microorganisms are involved in P cycling and in the varied P plant availability. C:P ratio may give some additional information on potential microbial P mineralization/immobilization. Effectively, a high C:P ratio organic product can increase the P immobilization following microbial growth (*Leytem and Westermann*, 2005). Microbial biomass P, which is the active portion of the organic P pool in soil (*Stewart and Tiessen*, 1987), increases the P turnover. The

presence of actively growing plants decreasing the soil solution P concentration induces further organic P mineralization inhibiting the P fixation to the soil (*Stewart and Tiessen*, 1987).

The assessment of these processes can be done via the study of inorganic soil P course in time, beside to the investigation of microbial P following the addition of organic wastes. In this frame, the study of plant P uptake can be helpful to a better description of the P release pattern in soil. To describe this point ryegrass has been widely used in literature (*Chen et al.*, 2002; *Foehse and Jungk*, 1983; *Jeng et al.*, 2006), being this fast growing species very adapted to multiple harvests arrangement. To our knowledge few are the studies focussing on the coupled soil microbial P and plant P uptake after the addition on recycled OM (*Khan and Joergensen*, 2012a; *Simpson et al.*, 2012).

The aim of the present study is to investigate the Olsen-P and the microbial P course after addition of different organic product to a calcareous soil. The ryegrass P apparent uptake was utilized to support this approach.

To do this we have selected six organic wastes with different origin: three anaerobic digestates, two animal slurries and one compost. We have, thus, assessed the soil microbial-P course and the potential available P (Olsen-P) in a soil incubation over 112 days. The plant apparent P utilization efficiency was determined in a pot test on ryegrass, in comparison with a chemical grade P source (KH_2PO_4) and struvite ($MgNH_4PO_4 \cdot 6H_2O$).

Material and methods

Tested products

The three anaerobic digestates compared in this work were: D1, from a two-phase thermophilic AD process of urban wastewater sludge (*Leite et al.*, 2016); D2 from a mesophilic AD process of winery sludge and wine lees (*Da Ros et al.*, 2016); BD from the mesophilic AD of bovine slurry and agricultural waste. The two slurries were BS, a bovine slurry and SS, a swine slurry. A municipal solid waste compost (MSWC), obtained from bio-waste, green waste and urban sewage sludge, was used as reference. A chemical fertilizer (KH_2PO_4) (Chem) and struvite ($MgNH_4PO_4 \cdot 6H_2O$) (Stru) derived from an industrial plant (CNP, Berlin, Germany) were used as chemical reference beside to not amended soil (CTRL).

Soil

The soil used for the incubation and the pot trial was collected from the top layer in a field of the University of Bologna Experimental Farm (Cadriano, Bologna, Italy). This was air-dried and seized at 2 mm. The soil characteristics were analysed in *Grigatti et al.* (2015). Briefly the soil presented pH (H_2O, 1:2.5) = 8.16 and silty clay loam texture. Total $CaCO_3$ was 85 g kg^{-1}, total organic carbon (TOC) was 10.2 g kg^{-1}, total Kjeldahl nitrogen (TKN) 1.60 g kg^{-1}, C:N 8.3 and exchangeable K 330 mg kg^{-1} as K_2O. The total Al was 36 g kg^{-1}, total Fe 22 g kg^{-1}, and total P 0.8 g kg^{-1}.
The soil was chosen for the low amount of available P (P-Olsen) which was 3.81 mg kg^{-1}.

Soil incubation experiments

A quantity of soil corresponding to 200 g of dry soil was pre-incubated for 2 weeks at 25°C at 60% water hold capacity (WHC) in 500 mL plastic vessels. The products were added to the soil, by thorough mixing, in order to have a final concentration of 30 mg P kg^{-1}. Each treatment was repeated in triplicate in a completed randomized design. The soils were then incubated at 25°C for 4 months (112 days); moisture was checked twice a week by weighing and adjusted adding deionized water. Available P (P_{olsen}) and microbial biomass P (P_{mic}) was assessed 7 times during incubation (0, 14, 28, 42, 56, 84, and 112).

Olsen-P and microbial biomass P

Microbial biomass P was determined through the fumigation/extraction method, according to *Brookes et al.* (2007, 1982), as follows. An amount of soil corresponding to 4.00 g (TS) was placed in a desiccator into tinfoil crucibles, together with two

beckers with 25 mL of chloroform and 50 g of soda lime. Then samples were fumigated under vacuum (10 min.) and placed in the dark for 24 hours. Samples were then transferred into 50 mL centrifuge tubes, added with 40 mL of 0.5 M $NaHCO_3$ pH 8.5, end to end shacked for 30 min. and filtered through Whatman #42 filter paper. The amount of P in this solution was determined via molybdenum blue method (*Murphy and Riley*, 1962) and called fumigate-P (P_f). Another aliquot of 4.00 g (TS) of soil was directly weighed into centrifuge tubes and placed in a desiccator for 24 h, and then it was extracted in the same way of P_f. This solution was analysed as abovementioned for P_f in order to determine the unfumigated P (P_{uf}). The values of unfumigated soils also represented the concentration of available P, as described in *Olsen* (1954).

To assess the potentially fixed P (P_s) by soil during the procedure three extra samples of control soils were spiked just before the extraction with 0.5 $NaHCO_3$ (pH 8.5) solution with 1 mL of 100 mg P L^{-1} as KH_2PO_4 (to obtain 25 μg P g^{-1} TS).

The microbial biomass phosphorous (P_{mic}) was calculated through the following expression:

$$P_{mic} = \frac{P_f - P_{uf}}{k} * \frac{100}{R}$$

where k= 0.40= efficiency of extraction of microbial biomass P;

R= percent recovery of P_i spiked, which was calculated as:

$$\mathrm{R} = \frac{P_i - P_{uf}}{25} * 100$$

2.7. Plant test

Organic products (D1, D2, BD, BS, SS), reference compost (MSWC) and chemical references (Chem and Stru) were added to 1 kg of soil at the rate of 30 mg P kg^{-1}. Then, the amended soil was placed in 2 L pots (ø 16 cm), previously filled for ½ volume (1 L) with expanded clay. An unamended soil was used as control (CTRL). Three replicates per treatment in a completed randomized design were used. Seeds of ryegrass (*Lolium multiflorum*, subsp. *Italicum*), cv. Sprint were sown at the amount of 1.5 g pot^{-1} and then covered with a thin layer of sand to prevent dehydration. Pots were placed in a growth chamber with 14 h of photoperiod and a temperature of 23°C/13°C day/night. The light was ensured by Master Tld 58 W-840 tubes (Philips, Amsterdam, The Netherlands). In order to limit the differences in nitrogen applied by the products, plants were supplied by an overestimated amount of nitrogen with one application of 50 mg kg^{-1} NH_4NO_3 every four weeks. Pots were regularly watered with tap water. Every 4 weeks, 28, 56, 84, 112 days after sowing (DAS), the plants were cut at 2 cm above the soil and the fresh material was dried at 60°C for 72 h. Dry matter were

weighted, finely grinded and digested with 6 ml of HNO_3 and 2 ml of H_2O_2 in a microwave. The digested samples were analysed for metal concentration analysis with ARCOS ICP-OES (Spectro Analytical Instruments, Kleve, Germany).

The plant P availability was calculated with the apparent recovery fraction (ARF) method as:

$$ARF\ (\%) = \sum_{t=1}^{4} \frac{P\ uptake\ treatment - Puptake\ control}{P\ added}\ x\ 100$$

where P uptake treatment is the total phosphorus uptake (mg pot^{-1}) of the plants treated with organic and mineral products (BD, D1, D2, BS, LS, MSWC, Stru, Chem) at time t (n = harvest 1- 4); P uptake control is the total phosphorus uptake (mg pot^{-1}) of the unfertilized control at time t (n= harvest=1 – 4); P added is the total phosphorus added to the pot (mg pot^{-1}).

Statistical analysis

Plant data, dry weight, P uptake and plant metal concentration were statistically analysed with R (https://cran.r-project.org) with two-way ANOVA and Tukey test with $P \leq 0.05$. Normality of population was assessed with Kolmogorov-Smirnov test and equality of variance with Levene test.

Pearson's correlation with two tails $P \leq 0.05$ was performed between all the analyses performed with IBM SPSS (IBM, Armonk, North Castle, New York, USA).

Results

Main characteristics of the tested products

The main characteristics of the fresh digestates D1 and D2 are reported in *Da Ros et al.* (2016) and *Leite et al.* (2016), BD is described in *Grigatti et al.* (2011) along with MSWC. BS and SS were analysed for total organic carbon (TOC), Total Kjeldahl Nitrogen (TKN) and metal concentration for this study.

The chemical characteristics of the freeze-dried products tested in this work are exposed in Table 2.1. Amongst nutrients P was at the highest level in SS (33 mg g^{-1}) then followed D2 (18.4 mg g^{-1})> BS (8.0 mg g^{-1})> D1 (7.1 mg g^{-1})> BD (6.2 mg g^{-1})> MSWC (4.3 mg g^{-1}). The products showed varying C:N ratios (>12 in BD and BS; <10 in D1, D2, SS and MSWC), Struvite attained to C:N <1. The C:P ratio of all products were between 12 and 86 (SS and BD, respectively), well below 200, which is considered the threshold for the P immobilization. The different elements concentration reflected on the higher Ca:P ratio for BS and D2 (>2), attaining to lower values in BD, D1 and SS (Ca:P, 1-2). Different products on the contrary showed similar Fe:P (≈0.20), except D2 (0.46) and SS showing the lowest value (0.07). The Al:P ratio was in D1 and D2 at ≈0.45, being at the lower level in BD, BS and SS (≈0.10). MSWC showed the highest Ca:P ratio (>10), Fe:P (>4) and Al:P ratio (4.38 and 3.60) compared to the other organic products.

Table 2.1: Main physic-chemical characteristics of the tested products

Product	**P** mg g^{-1}	**C:N**	**C:P**	**Ca:P**	**Fe:P**	**Al:P**
BD	6.2	12.0	83	1.7	0.16	0.06
D1	7.1	7.2	48	1.4	0.26	0.47
D2	18.4	8.9	22	2.0	0.46	0.48
BS	8.0	15.0	61	2.6	0.16	0.09
SS	33.0	8.5	12	1.1	0.07	0.11
MSWC	4.3	9.8	52	10.7	4.38	3.6
Struvite	100	0.5	0.29	0	0	0

3.5. Available P and microbial biomass P

Available P (Fig. 2.1a) was the lowest and fairly constant in control (<5 mg kg^{-1}). In control soil, also P_{mic} (Fig. 2.1b) was at the lowest level (≈8 mg kg^{-1}) with little variation in time. Compared to the control soil, MSWC showed similar trend of both available P (≈6 mg kg^{-1}), and microbial P (≈8 mg kg^{-1}). Aside unamended and MSWC-amended soils, all the treated soils showed a decreasing P_{Olsen} pattern during the incubation, reaching after two months the background level (-57%, as mean of the treated soils). The soils amended with SS, Stru, BD and BS showed higher initial values (mean 25.5 mg P kg^{-1}), while digestates, D1 and D2, showed similar pattern to Chem (from 15.8 mg kg^{-1} at 0 DAT down to 7.0 mg kg^{-1}at 114 DAT as a mean of D1 and D2). Beside to the rapid P_{olsen} reduction BD and BS, showed increasing P_{mic} during the first month of incubation thus suggesting the utilization of available P by microbial biomass. D1 showed increasing P_{mic} in the first two weeks of incubation then followed by a decrease down to control level after two months.

Regarding the two slurries, both BS and SS had a decreasing trend of available P (-65% and -70% in BS and SS from 0 to 114 DAT, respectively). The major part of the consumption of available P was found in the first two weeks of incubation. Conversely, BS accumulated P_{mic} from the initial 3 mg $kg^{-1}$to the final 10 mg kg^{-1}. SS had a different behaviour regarding P_{mic}, as it decreased from 9.5 to 3.5 mg kg^{-1} in the first two weeks of incubation and then increased stabilizing around 8 mg kg^{-1}. It seems that the depleted available P was not accumulated into microbial biomass. Considering the chemical amendments, the soils treated with potassium phosphate and struvite showed a decreasing trend for P_{olsen}. On the other hand, P_{mic} remained, except some fluctuations, almost constant in *Chem* and even decreased during the experiment course for Stru.

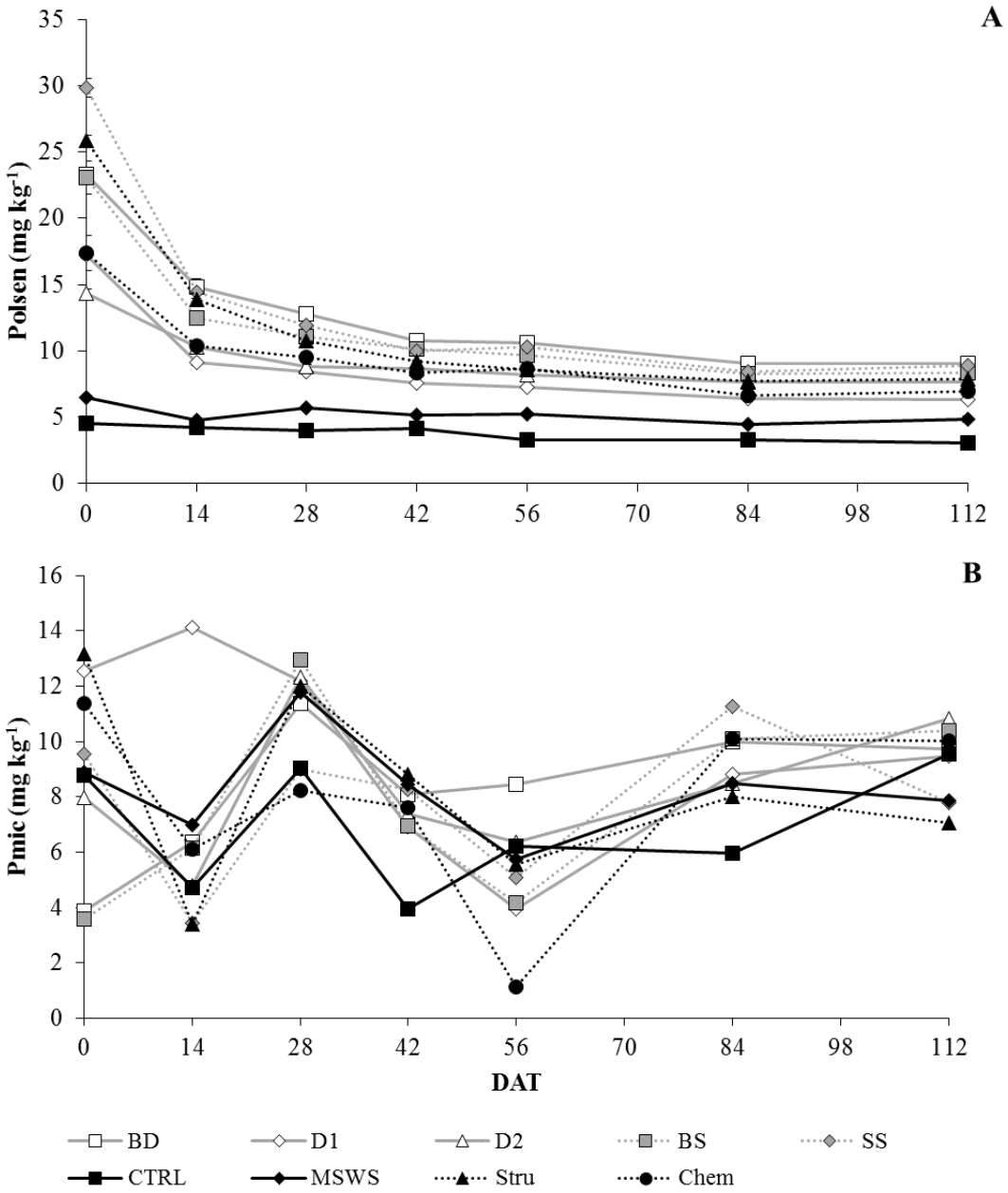


Figure 2.1: A) Available phosphorus (mg kg^{-1}) and B) phosphorus in microbial biomass (mg kg^{-1}) in soil amended with organic and chemical products.

Plant growth and P uptake

Plant shoots dry weight showed significant differences between treatments ($P<0.001$), harvest ($P<0.001$) and their interaction ($P<0.05$), resulting significantly higher in plants treated with BD (1.87 g pot^{-1} as a mean of the four harvests) (Table 2.2). The addition of all the organic products caused an increase of plant biomass compared to the control plants. Considering the different harvests the higher yield was reached in the second harvest at 56 DAS (1.87 g pot^{-1} as a mean of different treatments). The cumulated value of the shoot biomass resulted similar in plants treated with BD, D1 and Stru (7.47, 6.85 and 6.67 g pot^{-1}, respectively), whereas the control plant showed the lower growth (5.55 mg pot^{-1}) (Table 2.2). The P concentration showed a significant decrease in time ($P<0.001$) with the first harvest reached higher concentration (2.35 mg g^{-1} as a mean of the different treatments) and the following reduced by 20% (56 DAS) and 24% (mean of 84 and 112 DAS). Significant differences in P concentration were found in treatments and interactions ($P<0.001$) (Table 2.2). Plants treated with SS, Stru and Chem showed the highest P concentration (1.99, 1.86 and 2.11 mg g^{-1} as a mean of different harvest, respectively). Lowest values, instead, were assessed in untreated plants and MSWC treated plants. P uptake was also significantly different between treatments, harvest and interactions ($P<0.001$) (Table 2.2). The uptake decrease in time by 41% comparing the mean of the first two harvests and the mean of the third and fourth harvest. The plants treated with Chem, BD, SS, Stru, D1 and D2 showed higher uptake considering both the mean of the different cuts and the cumulated values. In roots dry weight, P concentration and P uptake did not differ between treatments (Table 2.2). Cumulated apparent recovery fraction (ARF) (Fig. 2.2a) showed different behaviour in the treatments, it increased rapidly in Chem, BD and SS, whereas showed very slow increment in MSWC and D2. The slope of cumulated ARF of D1, BS and Stru resulted similar to the curve of Chem, BD and D1 but the plants were able to recover less P during the time showing a lower total ARF (Fig. 2.2b).

Table 2.2: Dry biomass (DW) (g pot^{-1}), total P concentration (mg g^{-1}) and uptake (mg pot^{-1}) in ryegrass shoots at four successive harvests, and roots at final harvest.

Treatment	**Days after sowing**								
	Shoots								**Roots**
	28	**56**	**84**	**112**	**Mean**		**0-112**		**112**
DW (g pot^{-1})									
Control	1.14	1.72	1.30	1.39	1.39	d	5.55	d	2.93
BD	1.77	2.30	1.75	1.65	1.87	a	7.47	a	3.25
D1	1.60	2.00	1.62	1.63	1.71	ab	6.85	ab	3.72
D2	1.41	1.82	1.37	1.56	1.54	bd	6.16	bd	3.85
BS	1.54	1.96	1.38	1.56	1.61	bc	6.44	bd	3.95
SS	1.49	1.95	1.40	1.69	1.63	b	6.52	bc	3.15
Struvite	1.47	1.77	1.65	1.77	1.67	ab	6.67	ab	2.97
MSWC	1.51	1.53	1.14	1.51	1.42	cd	5.69	cd	3.27
Chem	1.47	1.72	1.61	1.73	1.63	b	6.53	bc	3.23
Mean	1.49 c	1.87 a	1.47c	1.61 b					
P Concentration (mg g^{-1})									
Control	1.53	1.54	1.07	1.22	1.34	c	-		1.24
BD	2.27	1.92	1.46	1.29	1.73	b	-		1.34
D1	2.21	1.87	1.56	1.27	1.73	b	-		1.22
D2	2.30	1.68	1.27	1.37	1.66	b	-		1.29
BS	2.46	1.93	1.35	1.20	1.74	b	-		1.15
SS	2.96	2.16	1.52	1.32	1.99	a	-		1.43
Struvite	2.66	1.87	1.51	1.41	1.86	ab	-		1.46
MSWC	1.71	1.49	1.17	1.26	1.41	c	-		1.33
Chem	3.12	2.27	1.65	1.39	2.11	a	-		1.48
Mean	2.35 a	1.86 B	1.40 c	1.31 c					

Two-way ANOVA way applied on treatment*harvest on shoots data resulting always significant per treatment ($P<0.001$), harvest ($P<0.001$) and interaction ($P<0.05$). One-way ANOVA on cumulated (0-112) harvests (significant differences with $P<0.001$) and roots data (non-significant differences). Different letters mean significant differences with $P<0.05$ Tukey test, which was carried out on population with significant difference with ANOVA.

Table 2.2 continue: Dry biomass (DW) (g pot^{-1}), total P concentration (mg g^{-1}) and uptake (mg pot^{-1}) in ryegrass shoots at four successive harvests, and roots at final harvest.

Treatment	Days after sowing							
	Shoots							Roots
	28	56	84	112	Mean		0-112	112
P Uptake (mg pot^{-1})								
Control	1.74	2.67	1.39	1.68	1.87	d	7.48 d	3.58
BD	4.02	4.45	2.59	2.13	3.20	a	13.19 a	4.35
D1	3.54	3.71	2.54	2.06	2.96	ab	11.85 ab	4.40
D2	3.25	3.05	1.74	2.13	2.54	bc	10.17 bc	4.88
BS	3.80	3.79	1.86	1.87	2.83	ab	11.32 ab	4.51
SS	4.41	4.22	2.12	2.24	3.25	a	12.99 a	4.46
Struvite	3.92	3.31	2.49	2.50	3.06	ab	12.22 ab	4.33
MSWC	2.58	2.26	1.34	1.90	2.02	cd	8.09 cd	4.35
Chem	4.57	3.92	2.64	2.43	3.39	a	13.55 a	4.77
Mean	3.53	3.48	2.61	2.07				
	a	a	b	b				

Two-way ANOVA way applied on treatment*harvest on shoots data resulting always significant per treatment ($P<0.001$), harvest ($P<0.001$) and interaction ($P<0.05$). One-way ANOVA on cumulated (0-112) harvests (significant differences with $P<0.001$) and roots data (non-significant differences). Different letters mean significant differences with $P<0.05$ Tukey test, which was carried out on population with significant difference with ANOVA.

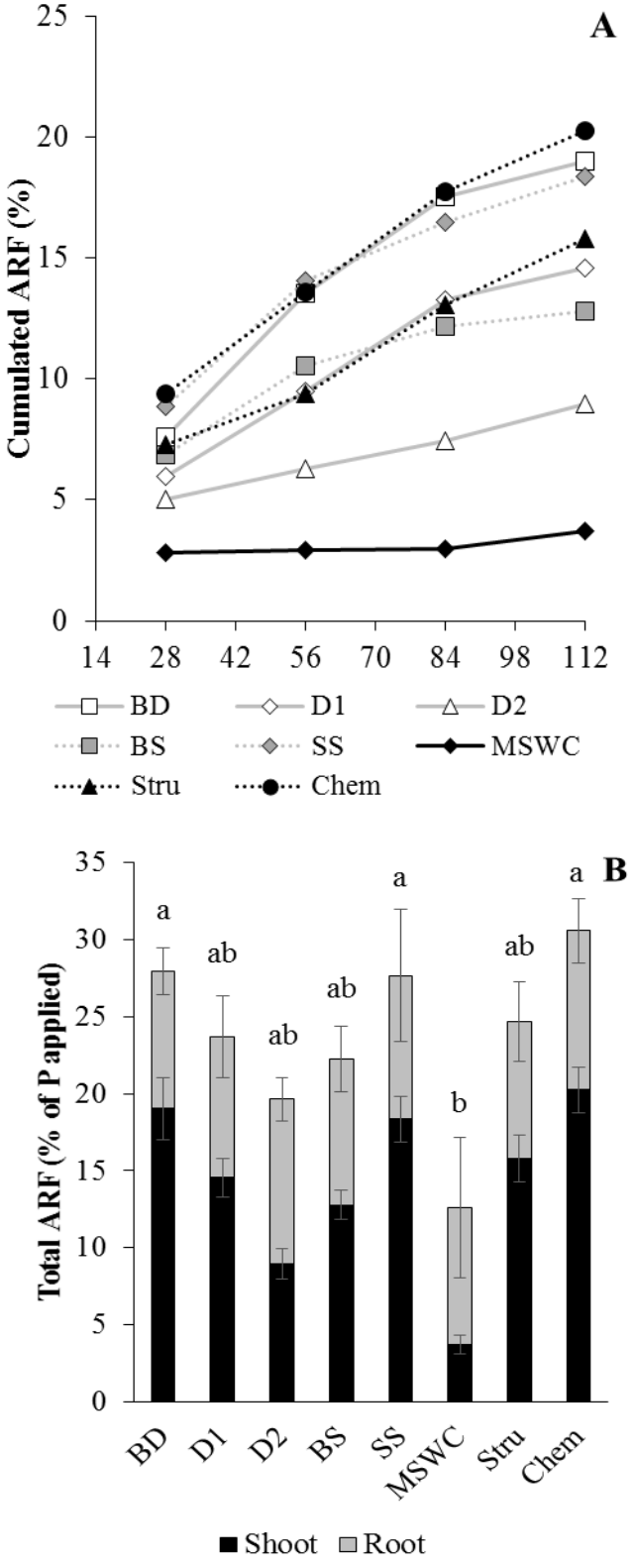


Figure 2.2: A) Apparent recovery fraction of phosphorus (ARF) (% of the P applied) cumulated in the 4 harvests (28, 56, 84, 122 DAT) in shoots. Significant differences with two-way ANOVA in treatments $P<0.001$, harvest $P<0.001$, no significant differences for interactions. B) Total ARF in shoots and roots. Error bars, SE (n=3), different letters mean significant differences with Tukey test at $P<0.05$.

Plant metal concentration and uptake

Plant metal concentration at 112 DAS (Table 2.3) did not show any significant differences in roots concentration. The results, also, did not show significant differences in Al and Fe concentration in shoots.

Ca shoots concentration resulted higher in control plants, D1, D2, SS plants (42.2 mg g^{-1} as a mean), while resulted lower in BD plants (-11.3% as compared to control).

Cu concentration in shoots was higher in D1 and BS plants (+21.7% and +17.0% vs control, respectively), while MSWC and control showed the lowest concentration (0.06 mg g^{-1} as a mean).

Mg concentration in SS and Stru shoots reached the highest values (+12.1% in SS and +10.5% in Stru, vs control). On the other hand, BD plants resulted in the lowest Mg concentration (-9.4% as compared to control).

Mn shoots concentration was significantly higher in control, as compared to all the treated plants, while SS, Stru and Chem plants showed the lowest values (-25.3% in SS, -23.4% in Stru and -24.5% in Chem, as compared to control).

Plants metal uptake did not show significant differences in roots, while significant differences was found in Ca, Cu, Mg, Mn total shoots uptake (Table 2.3). Al and Fe did not show significant differences in shoots.

Plants treated with BD, D1. SS and Stru showed the highest Ca uptake (+20% as a mean compared to control), while plants treated with D2 and BD showed Ca uptake similar to Chem (-16.3% as an average of D2 and BD as compared to control). While plants treated with MSWC showed Ca uptake similar to control, and lower than the other treatments.

Cu uptake resulted higher in BD treated plants (+59.1% as compared to control), while control and MSWC showed the lowest concentration. D2 resulted similar to Chem (+30.7% in D2 vs CTRL).

Mg uptake was higher in BD, D1, SS treated plants (+24.2% as the average of BD, D1 and SS vs control) and similar to Stru and Chem. Control and MSWC, again resulted the lowest in Mg uptake.

Table 2.3: Shoot and root total metal concentration (mg g^{-1}) and uptake (mg pot^{-1}) at 112 DAS.

Treatment	Shoots						Roots					
	Plant metal concentration (mg g^{-1})											
	Al	**Ca**	**Cu**	**Fe**	**Mg**	**Mn**	**Al**	**Ca**	**Cu**	**Fe**	**Mg**	**Mn**
CTRL	0.12	41.81 a	0.058 c	0.37	15.81 bc	0.20 a	4.90	13.60	0.245	4.17	2.60	0.15
BD	0.13	37.08 b	0.068 ab	0.38	14.32 d	0.19 ab	5.92	16.17	0.089	5.03	2.87	0.19
D1	0.09	41.40 a	0.071 a	0.33	15.66 cd	0.17 bc	7.72	18.68	0.075	6.81	3.23	0.25
D2	0.10	42.45 a	0.068 ab	0.33	16.75 ac	0.16 bc	9.05	18.82	0.064	7.50	3.58	0.27
BS	0.11	39.62 ab	0.070 a	0.35	15.83 bc	0.17 bc	8.00	17.27	0.056	6.79	3.25	0.25
SS	0.14	43.30 a	0.069 ab	0.37	17.72 a	0.15 c	6.23	15.54	0.059	5.15	3.05	0.18
MSWC	0.09	40.63 ab	0.059 c	0.33	15.62 cd	0.19 ab	6.84	15.86	0.060	5.95	3.29	0.22
Stru	0.16	40.84 ab	0.066 ab	0.40	17.47 a	0.16 c	5.30	15.58	0.060	4.55	2.74	0.16
Chem	0.10	40.03 ab	0.063 bc	0.32	17.08 ab	0.15 c	5.42	14.69	0.056	4.95	2.85	0.16
ANOVA Sign.	*0.435*	*0.001*	*0.000*	*0.530*	*0.000*	*0.000*	*0.204*	*0.255*	*0.273*	*0.296*	*0.486*	*0.176*
	Plant uptake (mg pot^{-1})											
CTRL	0.17	57.90 b	0.08 d	0.52	22.0 b	0.29 b	15.63	41.43	0.55	13.34	8.07	0.48
BD	0.27	69.42 a	0.13 a	0.75	26.4 a	0.35 a	19.61	52.97	0.28	16.65	9.43	0.62
D1	0.16	70.57 a	0.12 ab	0.56	26.7 a	0.28 b	31.25	73.32	0.27	27.79	12.67	1.05
D2	0.16	65.28 ab	0.10 c	0.50	25.7 ab	0.25 b	36.24	73.88	0.24	29.98	14.10	1.09
BS	0.17	63.90 ab	0.11 ac	0.55	25.2 ab	0.27 b	31.89	68.15	0.22	27.13	12.92	1.00
SS	0.22	70.37 a	0.11 ac	0.59	28.8 a	0.25 b	20.55	50.36	0.18	17.14	9.93	0.60
MSWC	0.13	57.68 b	0.08 d	0.47	21.9 b	0.26 b	23.49	53.03	0.20	20.55	11.15	0.75
Stru	0.27	67.59 a	0.11 bc	0.66	29.1 a	0.26 b	15.92	46.53	0.18	13.70	8.17	0.47
Chem	0.16	64.99 ab	0.10 c	0.51	28.0 a	0.25 b	17.30	47.31	0.18	15.95	9.21	0.51
ANOVA Sign.	*0.106*	*0.000*	*0.000*	*0.052*	*0.000*	*0.001*	*0.315*	*0.481*	*0.143*	*0.384*	*0.635*	*0.285*

One-way ANOVA performed on each metal, with significance reported in the last row. On metals with ANOVA significance $P \leq 0.05$, different letters represents different groups with Tukey test at $P \leq 0.05$.

Pearson's correlations

Amount of metals in the products, microbial P, Olsen P and Plant dry weight were correlated with Pearson's correlation (Fig. 2.3). Then the same characteristic were correlated to the plant metal concentration (Fig. 2.4 and 2.5). The correlation with a $P<0.05$ were marked as green (if the correlation was positive) and red (for negative correlation).

The amount of Fe, Al and Ca in the organic products affects negatively the P_{Olsen} (correlation between organic product metals and P ratio and the mean P_{Olsen}, Al:P- P_{Olsen} R^2= 0.880, P=0.009; Fe:P- P_{Olsen} R^2= 0.859, P=0.13; Ca:P- P_{Olsen} R^2= 0.817, P=0.025) (Fig. 2.3).

Consequently, P_{Olsen} affected the P uptake (correlation between the mean P_{Olsen} and the mean P leaves concentration, R^2=0.766; P=0.016) and plant growth (correlation between the mean P_{Olsen} and the plant total dry weight, R^2=0.724, P= 0.027) (Fig. 2.5). P_{Olsen} showed a positive correlation with Cu concentration (correlation between the mean of P_{Olsen} and the mean of Cu concentration R^2= 0.79, P=0.011) (Fig. 2.4). The plant P uptake affected positively the plant growth at 112 DAS (correlation between mean P concentration and plant growth at 112 DAS R^2=0.89 P=0.001).

Furthermore, the organic carbon concentration in the products affects the microbial P biomass, negatively at 0 DAT (R^2=0.788, P=0.036) and positively at 112 DAT (R^2=0.764, P=0.046) (data not shown), while P_{mic} did not affect the plant growth (Fig. 2.3).

Simultaneously to the increase of microbial P at 28 DAT, we have observed an increase of metal uptake from plants especially in roots (Al, Fe, Ca and Mn), with a high correlation (Al R^2=0.674, P=0.046; Ca R^2=0.750, P=0.020; Fe R^2=0.668, P=0.049 and Mn R^2=0.730, P=0.026) (Fig. 2.4 and 2.5).

Mn concentration showed negative correlation with Mg concentration and P concentration in plants. In addition, Mn concentration showed also a negative correlation with plant growth at 112 DAS (correlation between mean Mn concentration and plant growth at 112 DAS R^2=0.79 P=0.011).

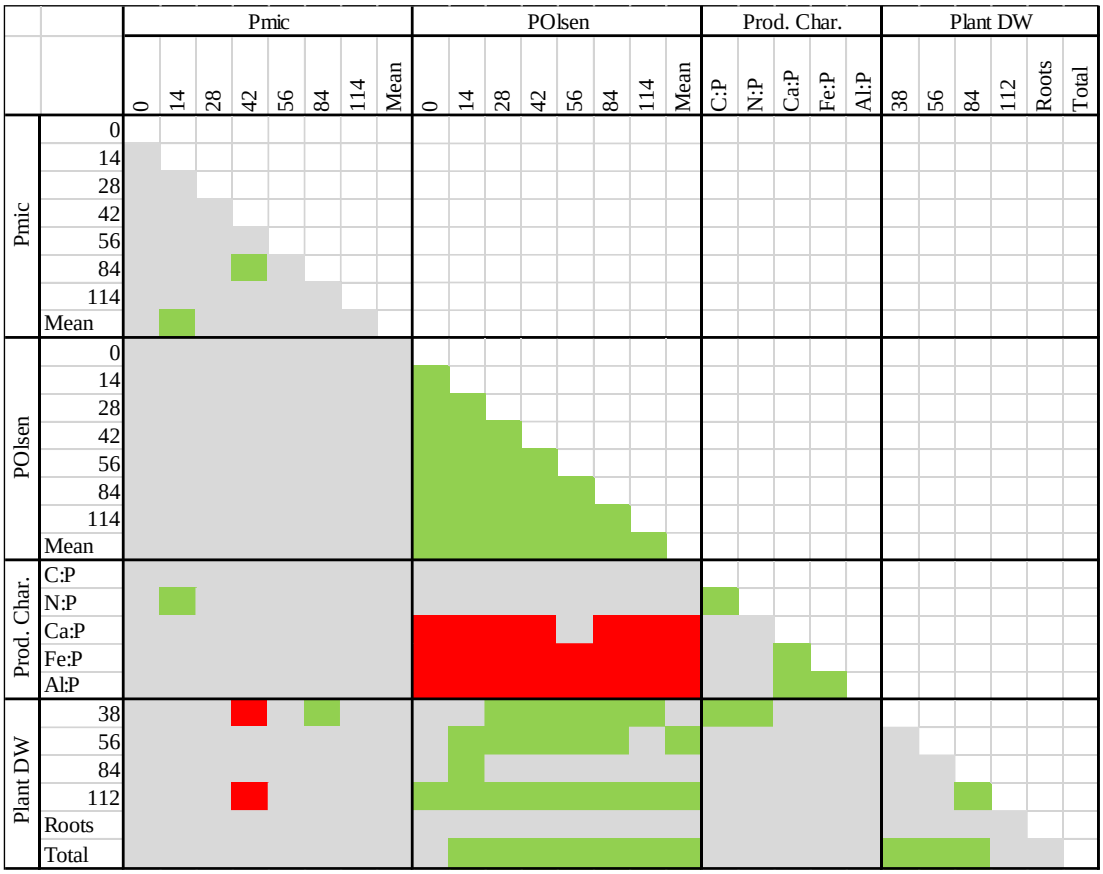


Figure 2.3: Pearson's correlation heat map with all the analysis performed. In green the positive correlation and in red the negative correlation resulted significant (two tails) with $P \leq 0.05$.

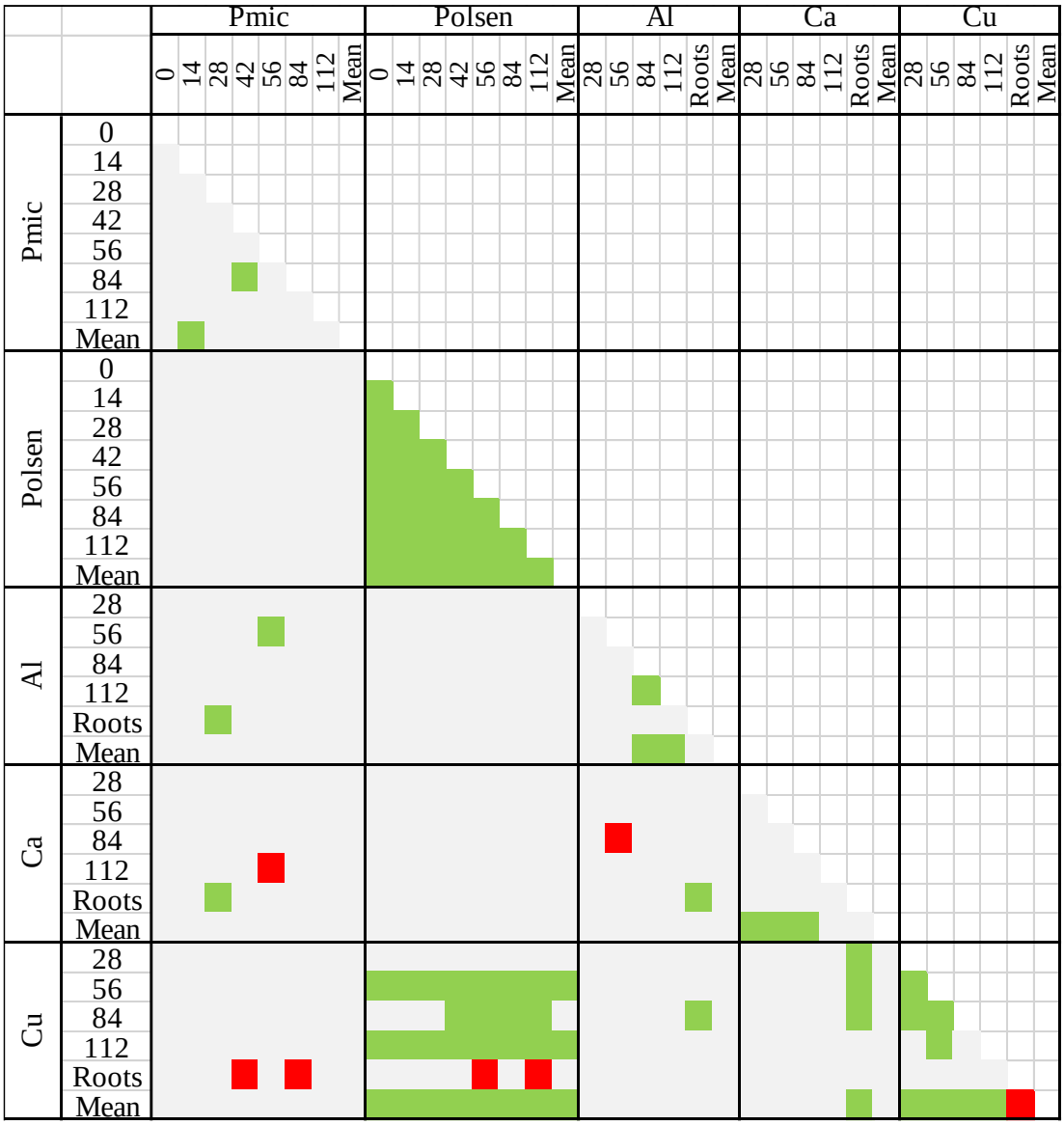


Figure 2.4: Pearson's correlation heat map with all the analysis performed. In green the positive correlation and in red the negative correlation resulted significant (two tails) with $P \leq 0.05$.

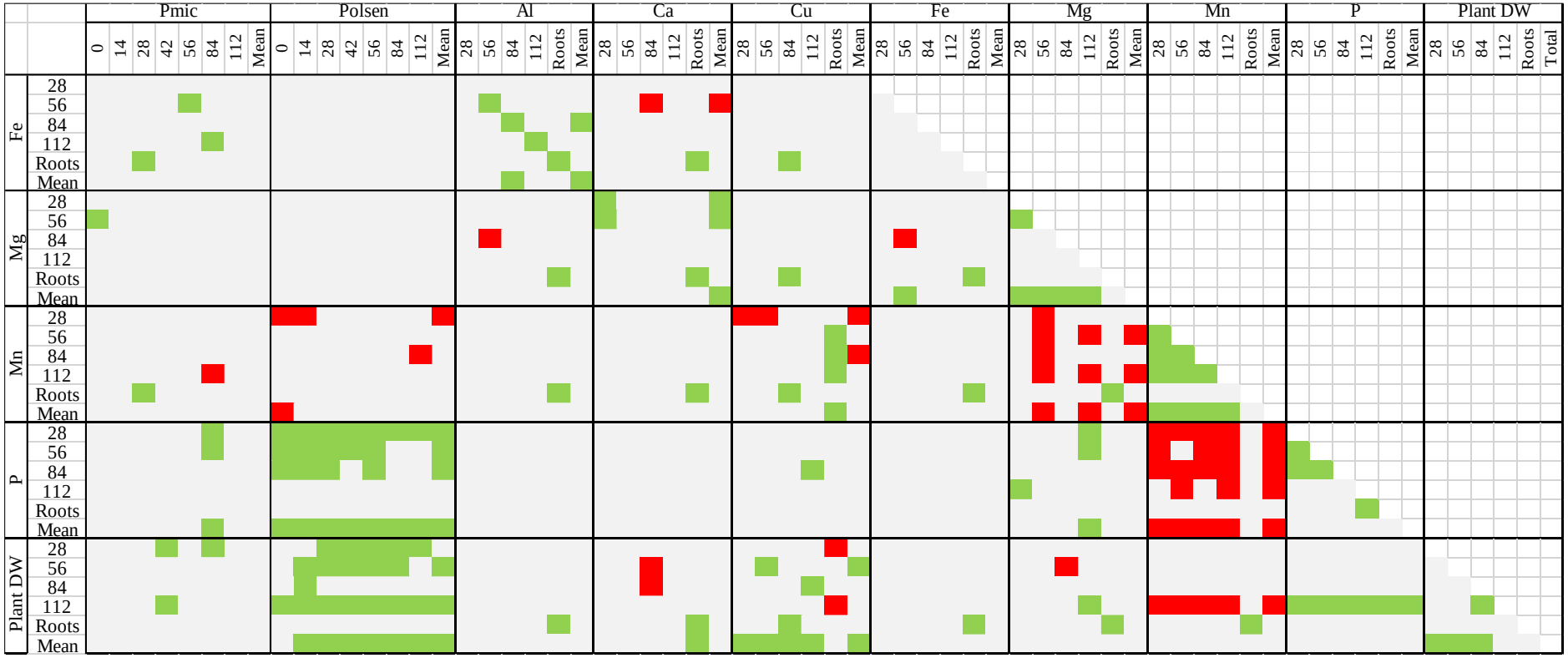


Figure 2.5: Pearson's correlation heat map with all the analysis performed. In green the positive correlation and in red the negative correlation resulted significant (two tails) with P≤0.05.

Discussion

In the latest years, the organic waste management via anaerobic digestion and/or composting strongly increased in the EU. Anaerobic digestion plants in EU is growing exponentially reaching in 2015 the amount of 17240 plants (+18% compared to the previous year) (*European Biogas Association*, 2015) and in 2014 in EU 38% of the municipal waste was composted (+170% compared to 1995) (*Eurostat*, 2016). Digestates and composts spread to agricultural field may represent a partial solution to the scarcity problems of phosphate rocks, adding P in soil and increasing its solubility with the addition of organic matter. Furthermore, they showed a lower environmental impact and human toxicity (calculated with life cycle assessment method) when disposed in agricultural field as compared to the direct disposal of sewage sludge or even more to sewage ash (*Hospido et al.*, 2008; *Suh and Rousseaux*, 2002). The use of organic products as phosphate fertilizer is even more important in countries, such as Italy, where the peak of the mineral phosphate fertilizer prices in 2008 (*CCIA*, 2016; *Indexmundi*, 2016) has led to a rapid decrease of the use of these fertilizer causing a deficit in the gross P balance in the soil (*Eurostat*, 2016). The present work had assessed the characteristics of anaerobic digestates and slurries as P organic fertilizers in comparison with compost, struvite derived from an industrial wastewater treatment plant and with a chemical reference. The products characteristics were then correlated with the plant P availability and the plant growth.

Metal concentration of the organic products affects their P availability

Bio-waste effects on phosphorus availability are well studied on acidic soil (*Brod et al.*, 2015; *Eghball*, 2002; *Eghball and Power*, 1999; *Pote et al.*, 1996; *Siddique and Robinson*, 2003) but very few are the studies on calcareous soil (*Malik et al.*, 2013; *Marchetti et al.*, 2012). In acidic soils trials (*Brod et al.*, 2015) wood ashes with high pH (pH= 13) and high metal concentration (Al = 19 g kg^{-1} DM; Fe = 7.6 g kg^{-1} DM.), resulted the best P fertilizer regarding plant growth. In calcareous and alkaline soil, instead, the P added as fertilizer, despite the soil has an already high total P concentration, meet a fate of precipitation and formation of Ca-phosphates, resulting in low availability (*Muhammad et al.*, 2006). The P availability and P use efficiency (PUE), resulted, however, better in fish sludge and dairy manure where the metal concentration were lower and the P extractable with H_20 and $NaCOH_3$ were higher (*Brod et al.*, 2015). Accordingly, in our study the concentration of Ca, Fe and Al and the ratio between these elements and P affected negatively the P phytoavailability. Indeed, the plants treated with BD and SS, which showed lower Ca:P, Fe:P and Al:P resulted in the highest P uptake. Bovine manure (BS), although generally recognized with good P fertilization effects (*Smith and Van Dijk*, 1987), due to the concentration

of low molecular weight organic acid able to reduce P retention in soil (*Guppy et al.*, 2005; *Øgaard*, 1996), did not show the best growth in our calcareous soil. This result is probably caused by its high Ca:P ratio, which decreased the P availability faster compared to the other organic products. The amount of Fe, Al and Ca in the organic products affects negatively the P_{Olsen}. Consequently, this affects the P uptake and plant growth. Thus, we can assume that Fe, Al and Ca concentrations in the organic waste are a good indicator for P availability as fertilizer, as already found in a previous study (*García-Albacete et al.*, 2012). Although the differences in the Al and Fe concentration in the products, there were not significant differences in the Al and Fe accumulation in plant tissues, probably due to a fast absorption of these metal from the soil. On the other hand, BD plants showed very low Ca and Mg uptake, which were probably less soluble in the products or complexed with organic matter, causing a lower P sorption and a higher protection against P precipitation (*Guppy et al.*, 2005). The products which provided the highest available P, such as SS, Stru and Chem, showed also a very low Mn uptake from the plants, probably due to a low concentration in the raw products. However, the Mn plant uptake was lower in all the organic products treatments than in control plants, showing that also Mn is easily complexed with organic matter and decrease its solubility in case of organic products addition. Furthermore, Mn plant availability is strongly affected by its oxidation state, which is generally affected by the pH and the aerobic condition of the soil, thus probably in treatments with low Mn uptake, the soil pH or the oxygen had increased (*Feng et al.*, 2013; *Petronici*, 1989). Instead, MSWC, which added very high amount of Al, Ca and Fe in soil, did not show very high metal uptake in plants, enlightening the strong bonds between Al, Ca, Fe and P in the organic product, which did not become available for plants.

Microbial biomass can release P from metals, increasing metal and P phytoavailability

The estimation of net release of nutrient from the organic matter, which is calculated as the difference between gross mineralization and gross immobilization for phosphorus is very difficult because in most soils the phosphate released by the mineralization is quickly adsorbed on colloid surface or precipitated (*Bünemann*, 2015). Furthermore, measurement of microbial P had to be calibrated for each soil because the P released from the lysis of the cells during the chloroform treatment can be adsorbed to the soil colloidal surfaces (*Stewart and Tiessen*, 1987).

Although microbial biomass P has been considered as an easily available P fraction, it cannot be directly taken up by plants (*Khan and Joergensen*, 2012b). Considering together the data of all treatments, we can observe that the addition of the products led

to an increase of microbial biomass P for all amendment with respect to the control, which was larger for D1, D2, BS and SS and slighter for BD and MSWC. The increase of P_{mic} in response to amendment addition is in accordance with the findings by Khan and Joergensen (2012) and Malik et al. (2013), who described P_{mic} values for treated soils similar to the ones of the present work. Simultaneously to the increase of microbial P at 28 DAT, we have observed an increase of metal uptake from plants especially in roots (Al, Fe, Ca and Mn). This simultaneous increase of P_{mic} and metal roots uptake can exhibit the capability of microbial biomass of immobilize P cutting the bond with metals and consequently the plant uptake the released metals. This effect was also visible at 56 DAT where microbial P showed the same behaviour of Al and Fe concentration in leaves at the same time and at 84 DAT where the microbial P highly correlated with Fe and Mn concentration at 112 DAT. The higher capability of microorganisms to uptake P from soil with high Fe and Al concentration compared to plants were already pointed out in previous studies (*Bhadoria et al.*, 2002; *Khan and Joergensen*, 2012a).

The fact that the increase of P_{mic} did not cause decrease in plant P uptake, indicating that microbial biomass did not compete with P uptake with plants, due to the high amount of nutrient applied with the organic fertilization (*Malik et al.*, 2013). The P stocked in the microbial biomass, could be useful, hence, for the long term nutrition and could prevent the fixation of P in soil (*Ayaga et al.*, 2006) and even be able to uptake P from the non-labile pool and transform it in an available source of P (*Achat et al.*, 2010).

Carbon and nitrogen concentration in organic products provide the best plant growth and affects the microbial P biomass

Biodigestate had shown the best crop growth, even if the P uptake was not the highest. This result can be explained by the highest N:P and C:P ratio. Besides the obvious effects on plant growth of N, the highest C:P ratio can imply a higher immobilization of P, but also a lower P soil sorption, due to the completion of the organic carbon decomposition products and P for the sorption sites (*Guppy et al.*, 2005).

Although the amount of metals and organic carbon in the products influenced the P solubility and the P plant availability, increasing the P soil sorption in the short term, it can protect P from precipitation with Ca (*Leytem and Westermann*, 2003). Thus, the products with high Fe:P and Al:P ratio, such as MSWC, may not have agronomic value for crops with short life cycle, but their fertilization value would increase considering long-term effects and plant with longer cycle, such as fruit tree. This attitude is also detectable by the very slight slope of the cumulated curve of ARF, which showed a very slow but continuous P uptake by plants.

Furthermore, the organic carbon concentration in the products affects the microbial P biomass, negatively at 0 DAT and positively at 112 DAT. This suggests that the organic carbon availability affects the soil microorganisms and mostly the microbial immobilization of P in the long-term, rather than shortly after the organic products addition. In BD, the high organic carbon made the P available also for microbial biomass, which remained always high during the 112 days period. This was true also at 56 DAT when all the treatment showed a decrease, which was even more visible in the soil treated with the chemical fertilizer, where no organic matter was added.

Consistently, BD resulted the organic product with the most available P for plants and microorganism. However, SS showed a behaviour similar to Chem with very high available P in the first period and microbial P always low and with a very consistent drop at 56 DAT, revealing few microbial growth and P immobilization, probably due to their low concentration in organic carbon. D1 instead had a performance analogous to Stru, with a medium P_{Olsen} and P_{mic}, but a great P solubilisation after 28 days, which can have caused the great increase of ARF of the plants between the first and the third month. Furthermore, D2 had a comparable behaviour to MSWC, with always very low P_{Olsen} and constant P_{mic}, thus the P remain not available for both plants and microorganisms.

The best fertilizer for short-term crops were, thus, biodigestate of pig slurry and agricultural wastes (BD) providing high amount of C and P available, and swine slurry (SS), both having low amount of Fe, Al and Ca and resulting in the highest shoot:root ratio in plants. A high shoot:root ratio shows that plants did not experience N and P deficiency and did not have the need to grow root in order to explore soil searching for nutrient (*Cakmak et al.*, 1994; *Marschner et al.*, 1996). The bovine slurry (BS), though proving high P available, was rich in Ca, thus resulting in low P recovery. Plant treated with digestate from civil wastewater sludge (D1) achieved good growth but low ARF due to the considerable amount of Fe, Al and Ca added to the soil. This effect is even more visible in the digestate from winery sludge and wine less (D2) where the plant uptake of Al, Fe, Ca and even Mn were higher, if compared to the other organic products.

References

Achat, D.L., Bakker, M.R., Zeller, B., Pellerin, S., Bienaimé, S., Morel, C. (2010): Long-term organic phosphorus mineralization in Spodosols under forests and its relation to carbon and nitrogen mineralization. *Soil Biol. Biochem.* 42, 1479–1490.

Adam, C., Peplinski, B., Michaelis, M., Kley, G., Simon, F.-G. (2008): Thermochemical treatment of sewage sludge ashes for phosphorus recovery. *Waste Manag.* 29, 1122–1128.

Adesemoye, A.O., Kloepper, J.W. (2009): Plant–microbes interactions in enhanced fertilizer-use efficiency. *Appl. Microbiol. Biotechnol.* 85, 1–12.

AEEP (2017): Phosphorus: Key to Plant Life [WWW Document]. URL http://aeep.eu/phosphorus/ (accessed 11.29.17).

Ajiboye, B., Akinremi, O.O., Hu, Y., Flaten, D.N. (2007): Phosphorus Speciation of Sequential Extracts of Organic Amendments Using Nuclear Magnetic Resonance and X-ray Absorption Near-Edge Structure Spectroscopies. *J. Environ. Qual.* 36, 1563.

Alborés, A.F., Cid, B.P., Gómez, E.F., López, E.F. (2000): Comparison between sequential extraction procedures and single extractions for metal partitioning in sewage sludge samples. *Analyst* 125, 1353–1357.

Alvarenga, P., Mourinha, C., Farto, M., Santos, T., Palma, P., Sengo, J., Morais, M.-C., Cunha-Queda, C. (2015): Sewage sludge, compost and other representative organic wastes as agricultural soil amendments: Benefits versus limiting factors.

Alvarez, A.E., Mochon, C.M., Sanchez, J.C., Rodriguez, T.M. (2002): Heavy metal extractable forms in sludge from wastewater treatment plants. *Chemosphere* 47, 765–775.

Andersen, M.S. (2007): An introductory note on the environmental economics of the circular economy. *Sustain. Sci.* 2, 133–140.

Ayaga, G., Todd, A., Brookes, P.C. (2006): Enhanced biological cycling of phosphorus increases its availability to crops in low-input sub-Saharan farming systems. *Soil Biol. Biochem.* 38, 81–90.

Baek, D., Kim, M.C., Chun, H.J., Kang, S., Park, H.C., Shin, G., Park, J., Shen, M., Hong, H., Kim, W.-Y., Kim, D.H., Lee, S.Y., Bressan, R.A., Bohnert, H.J., Yun, D.-J. (2013): Regulation of miR399f Transcription by AtMYB2 Affects Phosphate Starvation Responses in Arabidopsis. *Plant Physiol.* 161, 362–373.

Baldwin, J.C., Karthikeyan, A.S., Raghothama, K.G. (2001): LEPS2, a phosphorus starvation-induced novel acid phosphatase from tomato. *Plant Physiol.* 125, 728–37.

Bates, T.R., Lynch, J.P. (1996): Stimulation of root hair elongation in Arabidopsis thaliana by low phosphorus availability. *Plant, Cell Environ.* 19, 529–538.

Becana, M., Moran, J.F., Iturbe-Ormaetxe, I. (1998): Iron-dependent oxygen free radical generation in plants subjected to environmental stress: Toxicity and antioxidant protection. *Plant Soil* 201, 137–147.

Becker, M., Asch, F. (2005): Iron toxicity in rice-conditions and management concepts.

J. Plant Nutr. Soil Sci. 168, 558–573.

Bernal, M.P., Alburquerque, J.A., Moral, R. (2009): Composting of animal manures and chemical criteria for compost maturity assessment. A review. *Bioresour. Technol.* 100, 5444–5453.

Bhadoria, P.S., Steingrobe, B., Claassen, N., Liebersbach, H. (2002): Phosphorus efficiency of wheat and sugar beet seedlings grown in soils with mainly calcium, or iron and aluminium phosphate. *Plant Soil* 246, 41–52.

Biswas, B.K., Inoue, K., Harada, H., Ohto, K., Kawakita, H. (2009): Leaching of phosphorus from incinerated sewage sludge ash by means of acid extraction followed by adsorption on orange waste gel. *J. Environ. Sci.* 21, 1753–1760.

Blöcher, C., Niewersch, C., Melin, T. (2012): Phosphorus recovery from sewage sludge with a hybrid process of low pressure wet oxidation and nanofiltration. *Water Res.* 46, 2009–2019.

Bolan, N.S. (1991): A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. *Plant Soil*.

Breeuwsma, A., Reijerink, J.G.A., Schoumans, O.F. (1995): Impact of manure on accumulation and leaching of phosphate in areas of intensive livestock farming, in: Animal Waste and the Land-Water Interface. pp. 239–249.

Brod, E., Øgaard, A.F., Hansen, E., Wragg, D., Haraldsen, T.K., Krogstad, T. (2015): Waste products as alternative phosphorus fertilisers part I: inorganic P species affect fertilisation effects depending on soil pH. *Nutr. Cycl. Agroecosystems* 103, 167–185.

Brookes, P., Beyaert, R., Voroney, R. (2007): Soil Microbial Biomass C, N, P, and S, in: Soil Sampling and Methods of Analysis, Second Edition. CRC Press.

Brookes, P.C., Powlson, D.S., Jenkinson, D.S. (1982): Measurement of microbial biomass phosphorus in soil. *Soil Biol. Biochem.* 14, 319–329.

Brown, T.A., Shrift, A. (1982): SELENIUM: TOXICITY AND TOLERANCE IN HIGHER PLANTS. *Biol. Rev.* 57, 59–84.

Bünemann, E.K. (2015): Assessment of gross and net mineralization rates of soil organic phosphorus – A review. *Soil Biol. Biochem.* 89, 82–98.

Burleigh, S.H., Cavagnaro, T., Jakobsen, I. (2002): Functional diversity of arbuscular mycorrhizas extends to the expression of plant genes involved in P nutrition. *J. Exp. Bot.* 53, 1593–1601.

Cakmak, I., Hengeler, C., Marschner, H. (1994): Partitioning of shoot and root dry matter and carbohydrates in bean plants suffering from phosphorus, potassium and magnesium deficiency. *J. Exp. Bot.* 45, 1245–1250.

CCIA (2016): Prezzi - News — Camera di Commercio di Modena [WWW Document]. URL http://www.mo.camcom.it/tutela-del-mercato/ufficio-prezzi (accessed 11.9.16).

Chen, C.R., Condron, L.M., Davis, M.R., Sherlock, R.R. (2002): Phosphorus dynamics in the rhizosphere of perennial ryegrass (Lolium perenne L.) and radiata pine (Pinus Radiata D. Don.). *Soil Biol. Biochem.* 34, 487–499.

Childers, D.L., Corman, J., Edwards, M., Elser, J.J. (2011): Sustainability Challenges

of Phosphorus and Food: Solutions from Closing the Human Phosphorus Cycle. *Bioscience* 61, 117–124.

Christodoulou, A., Stamatelatou, K. (2016): Overview of legislation on sewage sludge management in developed countries worldwide. *Water Sci. Technol.* 73.

Cieslik, B., Konieczka, P. (2017): A review of phosphorus recovery methods at various steps of wastewater treatment and sewage sludge management. The concept of " no solid waste generation " and analytical methods. *J. Clean. Prod.* 142, 1728–1740.

Clarkson, D.T., Scattergood, C.B. (1982): Growth and Phosphate Transport in Barley and Tomato Plants During the Development of, and Recovery from, Phosphate-stress. *J. Exp. Bot.* 33, 865–875.

Cogliatti, D.H., Santa Marìa, G.E. (1990): Influx and Efflux of Phosphorus in Roots of Wheat Plants in Non-Growth-Limiting Concentrations of Phosphorus. *J. Exp. Bot.* 41, 601–607.

Cooper, J., Lombardi, R., Boardman, D., Carliell-Marquet, C. (2011): The future distribution and production of global phosphate rock reserves. *Resour. Conserv. Recycl.* 57, 78–86.

Cordell, D., Drangert, J., White, S. (2009): The story of phosphorus: global food security and food for thought. *Glob. Environ. Chang.*

Cordell, D., Rosemarin, A., Schroder, J.J., Smit, A.L. (2011): Towards global phosphorus security: A systems framework for phosphorus recovery and reuse options. *Chemosphere* 84, 747–758.

Corominas, L., Foley, J., Guest, J.S., Hospido, A., Larsen, H.F., Morera, S., Shaw, A. (2013): Life cycle assessment applied to wastewater treatment: State of the art. *Water Res.* 47, 5480–5492.

Da Ros, C., Cavinato, C., Pavan, P., Bolzonella, D. (2016): Mesophilic and thermophilic anaerobic co-digestion of winery wastewater sludge and wine lees: An integrated approach for sustainable wine production. *J. Environ. Manage.*

Dakora, F.D., Phillips, D.A. (2002): Root exudates as mediators of mineral acquisition in low-nutrient environments. *Plant Soil* 245, 35–47.

Delhaize, E., Randall, P.J., Laurie, S., Phillips, R., Ratcliffe, R., Stewart, G. (1995): Characterization of a Phosphate-Accumulator Mutant of Arabidopsis thaliana. *Plant Physiol.* 107, 207–213.

Direzione Generale Ambiente, D. del suolo e della costa-D.A.R.E.-R. (2004): Linee di Intervento in materia di utilizzo dei fanghi di depurazione in agricoltura. Bologna.

Domenech, C.E., Lisa, T.A., Salvano, M.A., Garrido, M.N. (2015): Selective release of phosphorus and nitrogen from waste activated sludge with combined thermal and alkali treatment. *Bioresour. Technol.* 190, 522–528.

Donatello, S., Tong, D., Cheeseman, C.R. (2010): Production of technical grade phosphoric acid from incinerator sewage sludge ash (ISSA). *Waste Manag.* 30, 1634–1642.

Doyle, J.D., Parsons, S.A. (2002): Struvite formation, control and recovery. *Water Res.* 36, 3925–3940.

Duboc, O., Santner, J., Fard, A.G., Zehetner, F., Tacconi, J., Wenzel, W.W. (2017): Predicting phosphorus availability from chemically diverse conventional and recycling fertilizers. *Sci. Total Environ.* 599–600, 1160–1170.

Easlon, H.M., Bloom, A.J. (2014): Easy Leaf Area: Automated Digital Image Analysis for Rapid and Accurate Measurement of Leaf Area. *Appl. Plant Sci.* 2, 1400033.

Eghball, B. (2002): Soil Properties as Influenced by Phosphorus-and Nitrogen-Based Manure and Compost Applications WASTE MANAGEMENT Soil Properties as Influenced by Phosphorus-and Nitrogen-Based Manure and Compost Applications. *Waste Manag.* 94, 128–135.

Eghball, B., Power, J.F. (1999): Phosphorus- and Nitrogen-Based Manure and Compost Applications: Corn Production and Soil Phosphorus. *Soil Sci. Soc. Am. J.* 63, 895–901.

Ellis, D.R., Salt, D.E. (2003): Plants, selenium and human health. *Curr. Opin. Plant Biol.*

Eneji, A.E., Honna, T., Yamamoto, S., Masuda, T., Endo, T., Irshad, M. (2003): Changes in Humic Substances and Phosphorus Fractions During Composting. *Commun. Soil Sci. Plant Anal.* 34, 2303–2314.

European Biogas Association (2015): EBA Biomethane & Biogas Report 2015 [WWW Document]. URL http://european-biogas.eu/2015/12/16/biogasreport2015/ (accessed 11.9.16).

Eurostat (2016): Eurostat [WWW Document]. URL http://ec.europa.eu/eurostat/web/main/home (accessed 11.9.16).

Ewa Sroga, G. (1987): Plant regeneration of two Lupinus spp. from callus cultures via organogenesis. *Plant Sci.* 51, 245–249.

FAO (2016): FAOSTAT [WWW Document]. URL http://www.fao.org/faostat/en/#home (accessed 12.2.17).

Feng, R., Wei, C., Tu, S. (2013): The roles of selenium in protecting plants against abiotic stresses. *Environ. Exp. Bot.*

Fertilisers Working Group Meeting (2014): Essential safety and quality requirements for fertilising materials.

Foehse, D., Jungk, A. (1983): Influence of phosphate and nitrate supply on root hair formation of rape, spinach and tomato plants. *Plant Soil* 74, 359–368.

Fredeen, A., Rao, I., Terry, N. (1989): Influence of phosphorus nutrition on growth and carbon partitioning in Glycine max. *Plant Physiol.*

Frossard, E., Condron, L.M., Oberson, A., Sinaj, S., Fardeau, J.C. (2000): Processes Governing Phosphorus Availability in Temperate Soils. *J. Environ. Qual.* 29, 15.

Frossard, E., Sinaj, S., Zhang, L.-M., Morel, J.L. (1996): The Fate of Sludge Phosphorus in Soil-Plant Systems. *Soil Sci. Soc. Am. J.* 60, 1248.

Fuentes, B., Bolan, N., Naidu, R., de la Luz Mora, M., Australia, A. (2006): PHOSPHORUS IN ORGANIC WASTE-SOIL SYSTEMS Fósforo en los sistemas suelo – residuos orgánicos. *J.Soil Sc. Plant. Nutr* 66, 64–8364.

García-Albacete, M., Martín, A., Cartagena, M.C. (2012): Fractionation of phosphorus biowastes: Characterisation and environmental risk. *Waste Manag.*

32, 1061–1068.

Gianquinto, G., Orsini, F., Sambo, P., D'Urzo, M.P. (2011): The Use of Diagnostic Optical Tools to Assess Nitrogen Status and to Guide Fertilization of Vegetables. *Horttechnology* 21, 287–292.

Gonzalez-Ponce, R., López-de-Sá, E.G., Plaza, C. (2009): Lettuce Response to Phosphorus Fertilization with Struvite Recovered from Municipal Wastewater. *HortScience* 44, 426–430.

Grigatti, M., Boanini, E., Cavani, L., Ciavatta, C., Marzadori, C. (2015): Phosphorus in Digestate-Based Compost: Chemical Speciation and Plant-Availability. *Waste and Biomass Valorization* 6, 481–493.

Grigatti, M., Boanini, E., Mancarella, S., Simoni, A., Centemero, M., Veeken, A.H.M. (2017): Phosphorous extractability and ryegrass availability from bio-waste composts in a calcareous soil. *Chemosphere* 174, 722–731.

Grigatti, M., Di Girolamo, G., Chincarini, R., Ciavatta, C., Barbanti, L. (2011): Potential nitrogen mineralization, plant utilization efficiency and soil CO2 emissions following the addition of anaerobic digested slurries. *Biomass and Bioenergy* 35, 4619–4629.

Grusak, M. a., DellaPenna, D. (1999): Improving the Nutrient Composition of Plants To Enhance Human Nutrition and Health1. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 50, 133–161.

Guppy, C., Menzies, N., Moody, P., Blamey, F. (2005): Competitive sorption reactions between phosphorus and organic matter in soil: a review. *Soil Res.*

Haug, R.T. (1993): The practical handbook of compost engineering. Lewis Publishers.

Heimersson, S., Harder, R., Peters, G.M., Svanström, M. (2014): Including pathogen risk in life cycle assessment of wastewater management. 2. Quantitative comparison of pathogen risk to other impacts on human health. *Environ. Sci. Technol.* 48, 9446–9453.

Herzel, H., Kruger, O., Hermann, L., Adam, C. (2016): Sewage sludge ash - A promising secondary phosphorus source for fertilizer production. *Sci. Total Environ.* 542, 1136–1143.

Hinsinger, P. (2001): Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. *Plant Soil* 237, 173–195.

Hooda, P.S., Truesdale, V.W., Edwards, A.C., Withers, P.J.A., Aitken, M.N., Miller, A., Rendell, A.R. (2001): Manuring and fertilization effects on phosphorus accumulation in soils and potential environmental implications. *Adv. Environ. Res.* 5, 13–21.

Hospido, A., Moreira, M.T., Feijoo, G. (2008): A comparison of municipal wastewater treatment plants for big centres of population in Galicia (Spain). *Int. J. Life Cycle Assess.* 13, 57–64.

Hukari, S., Hermann, L., Nättorp, A. (2016): From wastewater to fertilisers — Technical overview and critical review of European legislation governing phosphorus recycling. *Sci. Total Environ.* 542, 1127–1135.

Hunter, P.J., Teakle, G.R., Bending, G.D. (2014): Root traits and microbial community interactions in relation to phosphorus availability and acquisition, with particular

reference to Brassica. *Front. Plant Sci.* 5, 27.

Indexmundi (2016): DAP fertilizer - Monthly Price - Commodity Prices - Price Charts, Data, and News - IndexMundi [WWW Document]. URL http://www.indexmundi.com/commodities/?commodity=dap-fertilizer&months=180 (accessed 11.9.16).

Jaffer, Y., Clark, T.A., Pearce, P., Parsons, S.A. (2002): Potential phosphorus recovery by struvite formation. *Water Res.* 36, 1834–1842.

Jeng, A.S., Haraldsen, T.K., Grønlund, A., Pedersen, P.A. (2006): Meat and bone meal as nitrogen and phosphorus fertilizer to cereals and rye grass. *Nutr. Cycl. Agroecosystems* 76, 183–191.

Johri, A.K., Oelmüller, R., Dua, M., Yadav, V., Kumar, M., Tuteja, N., Varma, A., Bonfante, P., Persson, B.L., Stroud, R.M. (2015): Fungal association and utilization of phosphate by plants: success, limitations, and future prospects. *Front. Microbiol.* 6, 984.

Jones, D.L. (1998): Organic acids in the rhizosphere – a critical review. *Plant Soil* 205, 25–44.

Kabata-Pendias, A. (2011): Trace elements in soils and plants. Fourth Edition, CRC Press.

Kataki, S., West, H., Clarke, M., Baruah, D.C. (2016): Phosphorus recovery as struvite: Recent concerns for use of seed, alternative Mg source, nitrogen conservation and fertilizer potential. *Resour. Conserv. Recycl.* 107, 142–156.

Khan, A., Jilani, G., Akhtar, M.S., Naqvi, S.M.S., Rasheed, M., Khan, A.A., Jilani, G., Akhtar, M.S., Muhammad, S., Naqvi, S., Rasheed, M. (2009): Phosphorus Solubilizing Bacteria: Occurrence, Mechanisms and their Role in Crop Production. *Publ. J. agric. biol. sci* 1, 48–58.

Khan, K.S., Joergensen, R.G. (2012a): Relationships between P fractions and the microbial biomass in soils under different land use management. *Geoderma* 173, 274–281.

Khan, K.S., Joergensen, R.G. (2012b): Compost and phosphorus amendments for stimulating microorganisms and growth of ryegrass in a Ferralsol and a Luvisol. *J. Plant Nutr. Soil Sci.* 175, 108–114.

Kidd, P.S., Domínguez-Rodríguez, M.J., Díez, J., Monterroso, C. (2007): Bioavailability and plant accumulation of heavy metals and phosphorus in agricultural soils amended by long-term application of sewage sludge. *Chemosphere* 66, 1458–1467.

Kim, M., Han, D.W., Kim, D.J. (2015): Selective release of phosphorus and nitrogen from waste activated sludge with combined thermal and alkali treatment. *Bioresour. Technol.*

Kleinman, P.J.A., Sharpley, A.N., Wolf, A.M., Beegle, D.B., Moore, P.A. (2002): Measuring Water-Extractable Phosphorus in Manure as an Indicator of Phosphorus in Runoff. *Soil Sci. Soc. Am. J.* 66, 2009.

Kochian, L. V (1995): Cellular Mechanisms of Aluminum Toxicity and Resistance in Plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 46, 237–260.

Koppelaar, R.H.E.M., Weikard, H.P. (2013): Assessing phosphate rock depletion and

phosphorus recycling options. *Glob. Environ. Chang.* 23, 1454–1466.

Krüger, O., Adam, C. (2015): Recovery potential of German sewage sludge ash. *Waste Manag.* 45, 400–406.

Kumar, V., Gilkes, R.J., Bolland, M.D.A. (1992): A comparison of seven soil P tests for plant species with different external P requirements grown on soils containing rock phosphate and superphosphate residues. *Fertil. Res.* 33, 35–45.

Lambers, H., Brundrett, M.C., Raven, J.A., Hopper, S.D. (2011): Plant mineral nutrition in ancient landscapes: high plant species diversity on infertile soils is linked to functional diversity for nutritional strategies. *Plant Soil* 348, 7–27.

Laturnus, F., von Arnold, K., Grøn, C. (2007): Organic Contaminants from Sewage Sludge Applied to Agricultural Soils. False Alarm Regarding Possible Problems for Food Safety? (8 pp). *Environ. Sci. Pollut. Res. Int.* 14 Suppl 1, 53–60.

Leite, W.R.M., Gottardo, M., Pavan, P., Belli Filho, P., Bolzonella, D. (2016): Performance and energy aspects of single and two phase thermophilic anaerobic digestion of waste activated sludge. *Renew. Energy* 86, 1324–1331.

Leytem, A.B., Westermann, D.T. (2003): PHOSPHATE SORPTION BY PACIFIC NORTHWEST CALCAREOUS SOILS. *Soil Sci.* 168.

Leytem, A.B., Westermann, D.T. (2005): PHOSPHORUS AVAILABILITY TO BARLEY FROM MANURES AND FERTILIZERS ON A CALCAREOUS SOIL. *Soil* 170, 401–412.

Liang, Y., Sun, W., Zhu, Y.G., Christie, P. (2007): Mechanisms of silicon-mediated alleviation of abiotic stresses in higher plants: A review. *Environ. Pollut.* 147, 422–428.

López-Arredondo, D.L., Leyva-González, M.A., Alatorre-Cobos, F., Herrera-Estrella, L. (2013): Biotechnology of nutrient uptake and assimilation in plants. *Int. J. Dev. Biol.* 57, 595–610.

Lynch, J., Läuchli, A., Epstein, E. (1991): Vegetative Growth of the Common Bean in Response to Phosphorus Nutrition. *Crop Sci.* 31, 380.

Ma, J.F. (2004): Role of silicon in enhancing the resistance of plants to biotic and abiotic stresses. *Soil Sci. Plant Nutr.* 50, 11–18.

Ma, Z., Bielenberg, D.G., Brown, K.M., Lynch, J.P. (2001): Regulation of root hair density by phosphorus availability in Arabidopsis thaliana. *Plant, Cell Environ.* 24, 459–467.

Malik, M., Khan, K., Marschner, P. (2013): Microbial biomass, nutrient availability and nutrient uptake by wheat in two soils with organic amendments. *J. Soil Sci. Plant Nutr.* 13, 955–966.

Mamani, I., Averanga, Kattia, Boris, E., Terrazas, E. (2014): Establecimiento del sistema de generación in vitro de callos de Lupinus mutabilis sweet (tarwi) a partir de hipocótilos. *Rev. CON-CIENCIA* 2.

Marchetti, R., Castelli, F., Orsi, A., Sghedoni, L., Bochicchio, D. (2012): Biochar from swine manure solids: influence on carbon sequestration and Olsen phosphorus and mineral nitrogen dynamics in soil with and without digestate incorporation. *Ital. J. Agron.* 7, 26.

Marschner, H., Kirkby, E.A., Cakmak, I. (1996): Effect of mineral nutritional status on shoot-root partitioning of photoassimilates and cycling of mineral nutrients. *J. Exp. Bot.* 47, 1255–1263.

Marschner, H., Marschner, P. (2012): Marschner's mineral nutrition of higher plants. Academic Press.

Mattenberger, H., Fraissler, G., Brunner, T., Herk, P., Hermann, L., Obernberger, I. (2008): Sewage sludge ash to phosphorus fertiliser: Variables influencing heavy metal removal during thermochemical treatment. *Waste Manag.* 28, 2709–2722.

Mayer, B.K., Baker, L.A., Boyer, T.H., Drechsel, P., Gifford, M., Hanjra, M.A., Parameswaran, P., Stoltzfus, J., Westerhoff, P., Rittmann, B.E. (2016): Total Value of Phosphorus Recovery. *Environ. Sci. Technol.* 50, 6606–6620.

McBride, M., Sauve, S., Hendershot, W. (1997): Solubility control of Cu, Zn, Cd and Pb in contaminated soils. *Eur. J. Soil Sci.* 48, 337–346.

McCoy, J.L., Sikora, L.J., Weil, R.R. (1986): Plant Availability of Phosphorus in Sewage Sludge Compost1. *J. Environ. Qual.* 15, 403.

Møller, J., Boldrin, A., Christensen, T.H. (2009): Anaerobic digestion and digestate use: accounting of greenhouse gases and global warming contribution. *Waste Manag. Res.* 27, 813–824.

Möller, K., Müller, T. (2012): Effects of anaerobic digestion on digestate nutrient availability and crop growth: A review. *Eng. Life Sci.* 12, 242–257.

Mosier, N.S., Ladisch, C.M., Ladisch, M.R. (2002): Characterization of acid catalytic domains for cellulose hydrolysis and glucose degradation. *Biotechnol. Bioeng.* 79, 610–618.

Muhammad, S., Müller, T., Joergensen, R.G. (2006): Decomposition of pea and maize straw in Pakistani soils along a gradient in salinity. *Biol. Fertil. Soils* 43, 93–101.

Murphy, J., Riley, J.P. (1962): A modified single solution method for the determination of phosphate in natural waters. *Anal. Chim. Acta* 27, 31–36.

Nagul, E.A., McKelvie, I.D., Worsfold, P., Kolev, S.D. (2015): The molybdenum blue reaction for the determination of orthophosphate revisited: Opening the black box. *Anal. Chim. Acta*.

Nannipieri, P., Giagnoni, L., Landi, L., Renella, G. (2011): Role of Phosphatase Enzymes in Soil, in: Phosphorus in Action. Springer, Berlin, Heidelberg, pp. 215–243.

Neumann, G. (2010): Mining for nutrients - regulatory aspects of cluster root function and development. *New Phytol.* 187, 879–882.

Neumann, G. (2016): The Role of Ethylene in Plant Adaptations for Phosphate Acquisition in Soils – A Review. *Front. Plant Sci.* 6, 1224.

Neumann, G., Martinoia, E. (2002): Cluster roots – an underground adaptation for survival in extreme environments. *Trends Plant Sci.* 7, 162–167.

Nkebiwe, P.M., Weinmann, M., Bar-Tal, A., Müller, T. (2016): Fertilizer placement to improve crop nutrient acquisition and yield: A review and meta-analysis. *F. Crop. Res.* 196, 389–401.

Øgaard, A.F. (1996): Effect of Phosphorus Fertilization on the Concentration of Total

and Algal-available Phosphorus in Different Particle-size Fractions in Norwegian Agricultural Soils. *Acta Agric. Scand. Sect. B - Soil Plant Sci.* 46, 24–29.

Olsen, S.R. (1954): Estimation Of Available Phosphorus In Soils By Extraction With Sodium Bicarbonate.

Ottosen, L.M., Kirkelund, G.M., Jensen, P.E. (2013): Extracting phosphorous from incinerated sewage sludge ash rich in iron or aluminum. *Chemosphere* 91, 963–969.

Pant, H.K., Warman, P.R. (2000): Enzymatic hydrolysis of soil organic phosphorus by immobilized phosphatases. *Biol. Fertil. Soils* 30, 306–311.

Pasqualino, J.C., Meneses, M., Abella, M., Castells, F. (2009): LCA as a Decision Support Tool for the Environmental Improvement of the Operation of a Municipal Wastewater Treatment Plant. *Environ. Sci. Technol.* 43, 3300–3307.

Pastor, L., Marti, N., Bouzas, A., Seco, A. (2008): Sewage sludge management for phosphorus recovery as struvite in EBPR wastewater treatment plants. *Bioresour. Technol.* 99, 4817–4824.

Petronici, C. (1989): Gli elementi nutritivi, in: Paolo Sequi (ed.): Chimica Del Suolo. Patron Editore, Bologna, pp. 357–380.

Pniewski, T., Kapusta, J., Płucienniczak, A. (2006): Agrobacterium-mediated transformation of yellow lupin to generate callus tissue producing HBV surface antigen in a long-term culture. *J. Appl. Genet.* 47, 309–318.

Polowick, P.L., Loukanina, N.N., Doshi, K.M. (2014): Agrobacterium-mediated transformation of tarwi (Lupinus mutabilis Sweet), a potential platform for the production of plant-made proteins. *Vitr. Cell. Dev. Biol. - Plant* 50, 401–411.

Pote, D.H., Daniel, T.C., Sharpley, A.N., Moore, P.A., Edwards, D.R., Nichols, D.J. (1996): Relating Extractable Soil Phosphorus to Phosphorus Losses in Runoff t'. *Soil Sci. Soc. Am. J.* 60, 855–859.

Raghothama, K.G. (1999): PHOSPHATE ACQUISITION. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 50, 665–693.

Rahman, M.M., Salleh, M.A.M., Rashid, U., Ahsan, A., Hossain, M.M., Ra, C.S. (2014): Production of slow release crystal fertilizer from wastewaters through struvite crystallization – A review. *Arab. J. Chem.* 7, 139–155.

Reijnders, L. (2014): Phosphorus resources, their depletion and conservation, a review. *Resour. Conserv. Recycl.*

Richardson, A.E., Simpson, R.J. (2011): Soil microorganisms mediating phosphorus availability update on microbial phosphorus. *Plant Physiol.* 156, 989–96.

Russo, A. (2012): ANALISI E MIGLIORAMENTO DELL'IMPIANTO DI DEPURAZIONE DI LEGNAGO. Università degli Studi di Padova.

Shannon, M.C., Grieve, C.M. (1999): Tolerance of vegetable crops to salinity. *Sci. Hortic. (Amsterdam).* 78, 5–38.

Sharpley, A., Moyer, B. (2000): Phosphorus Forms in Manure and Compost and Their Release during Simulated Rainfall. *J. Environ. Qual.* 29, 1462.

Sharpley, A.N., Chapra, S.C., Wedepohl, R., Sims, J.T., Daniel, T.C., Reddy, K.R. (1994): Managing Agricultural Phosphorus for Protection of Surface Waters:

Issues and Options. *J. Environ. Qual.* 23, 437.

Shen, J., Yuan, L., Zhang, J., Li, H., Bai, Z., Chen, X., Zhang, W., Zhang, F. (2011): Phosphorus dynamics: from soil to plant. *Plant Physiol.* 156, 997–1005.

Shenoy, V. V., Kalagudi, G.M. (2005): Enhancing plant phosphorus use efficiency for sustainable cropping. *Biotechnol. Adv.* 23, 501–513.

Siddique, M.T., Robinson, J.S. (2003): Phosphorus Sorption and Availability in Soils Amended with Animal Manures and Sewage Sludge. *J. Environ. Qual.* 32, 1114.

Simpson, M., McLenaghen, R.D., Chirino-Valle, I., Condron, L.M. (2012): Effects of long-term grassland management on the chemical nature and bioavailability of soil phosphorus. *Biol. Fertil. Soils* 48, 607–611.

Sims, J.T., Simard, R.R., Joern, B.C. (1998): Phosphorus Loss in Agricultural Drainage: Historical Perspective and Current Research. *J. Environ. Qual.* 27, 277.

Smith, K.A., Van Dijk, T.A. (1987): Utilisation of phosphorus and potassium from animal manures on grassland and forage crops, in: Animal Manure on Grassland and Fodder Crops. Fertilizer or Waste? Springer Netherlands, Dordrecht, pp. 87–102.

Smith, S.R. (2009): A critical review of the bioavailability and impacts of heavy metals in municipal solid waste composts compared to sewage sludge. *Environ. Int.* 35, 142–156.

Sonneveld, C., Straver, N. (1994): Nutrient solutions for vegetables and flowers grown in water or substrates, Tenth. ed.

Stewart, J.W.B., Tiessen, H. (1987): Dynamics of soil organic phosphorus. *Biogeochemistry* 4, 41–60.

Suh, Y.J., Rousseaux, P. (2002): An LCA of alternative wastewater sludge treatment scenarios. *Resour. Conserv. Recycl.* 35, 191–200.

Syers, D.J., Johnston, A. (2008): Efficiency of soil and fertilizer phosphorus use Reconciling changing concepts of soil phosphorus behaviour with agronomic information. *FAO Fertil. Plant Nutr. Bull.*

Tabatabai, M.A., Bremner, J.M. (1969): Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biol. Biochem.* 1, 301–307.

Takahashi, M., Kato, S., Shima, H., Sarai, E., Ichioka, T., Hatyakawa, S., Miyajiri, H. (2001): Technology for recovering phosphorus from incinerated wastewater treatment sludge, in: Chemosphere. pp. 23–29.

Takahashi, S. (2013): Phosphorus characterization of manure composts and combined organic fertilizers by a sequential-fractionation method. *J. Plant Nutr. Soil Sci.* 176, 494–496.

Talboys, P.J., Heppell, J., Roose, T., Healey, J.R., Jones, D.L., Withers, P.J.A. (2016): Struvite: a slow-release fertiliser for sustainable phosphorus management? *Plant Soil* 401, 109–123.

Tang, X., Li, J., Ma, Y., Hao, X., Li, X. (2008): Phosphorus efficiency in long-term (15 years) wheat–maize cropping systems with various soil and climate conditions. *F. Crop. Res.* 108, 231–237.

Tarayre, C., De Clercq, L., Charlier, R., Michels, E., Meers, E., Camargo-Valero, M.,

Delvigne, F. (2016): New perspectives for the design of sustainable bioprocesses for phosphorus recovery from waste. *Bioresour. Technol.*

Tejada, M., García-Martínez, A.M., Rodríguez-Morgado, B., Carballo, M., García-Antras, D., Aragón, C., Parrado, J. (2013): Obtaining biostimulant products for land application from the sewage sludge of small populations. *Ecol. Eng.* 50, 31–36.

Toor, G.S., Peak, J.D., Sims, J.T. (2005): Phosphorus Speciation in Broiler Litter and Turkey Manure Produced from Modified Diets. *J. Environ. Qual.* 34, 687.

Torri, S.I., Correa, R.S., Renella, G. (2017): Biosolid Application to Agricultural Land—a Contribution to Global Phosphorus Recycle: A Review. *Pedosphere* 27, 1–16.

Turner, B.L., Cade-Menun, B.J., Condron, L.M., Newman, S. (2005): Extraction of soil organic phosphorus. *Talanta* 66, 294–306.

Turner, B.L., McKelvie, I.D., Haygarth, P.M. (2002): Characterisation of water-extractable soil organic phosphorus by phosphatase hydrolysis. *Soil Biol. Biochem.* 34, 27–35.

Uhde-Stone, C., Liu, J., Zinn, K.E., Allan, D.L., Vance, C.P. (2005): Transgenic proteoid roots of white lupin: a vehicle for characterizing and silencing root genes involved in adaptation to P stress. *Plant J.* 44, 840–853.

Uysal, A., Dilsad Yilmazel, Y., Demirer, G.N. (2010): The determination of fertilizer quality of the formed struvite from effluent of a sewage sludge anaerobic digester. *J. Hazard. Mater.* 181, 248–254.

Vance, C.P. (2001): Symbiotic Nitrogen Fixation and Phosphorus Acquisition. Plant Nutrition in a World of Declining Renewable Resources. *Plant Physiol.* 127.

Vance, C.P., Uhde-Stone, C., Allan, D.L. (2003): Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. *New Phytol.* 157, 423–447.

Veneklaas, E.J., Lambers, H., Bragg, J., Finnegan, P.M., Lovelock, C.E., Plaxton, W.C., Price, C.A., Scheible, W.-R., Shane, M.W., White, P.J., Raven, J.A. (2012): Opportunities for improving phosphorus-use efficiency in crop plants. *New Phytol.* 195, 306–320.

Walan, P., Davidsson, S., Johansson, S., Höök, M. (2014): Phosphate rock production and depletion: Regional disaggregated modeling and global implications. *Resour. Conserv. Recycl.* 93, 178–187.

Wang, Z., Straub, D., Yang, H., Kania, A., Shen, J., Ludewig, U., Neumann, G. (2014): The regulatory network of cluster-root function and development in phosphate-deficient white lupin (*Lupinus albus*) identified by transcriptome sequencing. *Physiol. Plant.* 151, 323–338.

Wellinger, A., Murphy, J., Baxter, D. (2013): The biogas handbook : science, production and applications.

Wheeler, B.D., Al-Farraj, M.M., Cook, R.E.D. (1985): Iron toxicity to plants in base-rich wetlands: comparative effects on the distribution and growth of Epilobium hirsutum l. and Juncus subnodulosus Schrank. *New Phytol.* 100, 653–669.

White, P.J., Brown, P.H. (2010): Plant nutrition for sustainable development and

global health. *Ann. Bot.* 105, 1073–1080.

Wollmann, I., Gauro, A., Müller, T., Möller, K. (2017): Phosphorus bioavailability of sewage sludge-based recycled fertilizers. *J. Plant Nutr. Soil Sci.*

Xu, H., He, P., Gu, W., Wang, G., Shao, L. (2012): Recovery of phosphorus as struvite from sewage sludge ash. *J. Environ. Sci. (China)* 24, 1533–1538.

Ye, Y., Ngo, H.H., Guo, W., Liu, Y., Li, J., Liu, Y., Zhang, X., Jia, H., Ngo, H.H. (2016): Insight into chemical phosphate recovery from municipal wastewater. *Sci. Total Environ.* 576, 159–171.

Zang, Y., Li, Y., Wang, C., Zhang, W., Xiong, W. (2015): Towards more accurate life cycle assessment of biological wastewater treatment plants: A review. *J. Clean. Prod.*

Zhu, J., Li, M., Whelan, M., Gan, J. (2017): Phosphorus activators contribute to legacy phosphorus availability in agricultural soils: A review. *Sci. Total Environ.* 612, 522–537.

Chapter 3

Phosphorus solubilisation from urban sewage sludge through enzymatic hydrolysis

Abstract

In the circular economy point of view, recycling of a waste in the best way for solving a raw material scarcity. Within the frame of decreasing availability of phosphate rocks as a source of phosphorus for fertilizers, the use of sewage sludge as an alternative for plant nutrition is thus an important achievements. Sewage sludge is an available and renewable resource, which is generally very difficult to dispose. On the other hand, the P solubility and phytoavailability in sludge is generally very low. In this study, we have evaluated enzymatic, chemical and combined treatments in order to increase the P extraction from two different sewage sludge. The sludge were an anaerobic and Fe-rich sludge and an aerobic and Al-rich sludge. Acid and alkaline phosphatase, in combination with phytase, cellulase and pronase were used as enzymatic extractants. Maleic, citric and sulfuric acid and potassium hydroxide were also tested alone and in combination with phosphatase. Metal release in the hydrolysates was also evaluated. The results were very different between the two sludge, with the anaerobic one having higher recovery with enzymatic extraction and organic acid extraction, while the aerobic sludge P recovery resulted very high only with sulfuric acid treatments.

Introduction

Phosphorous (P) is the second main element for plant nutrition after nitrogen (N), playing a key role in plant growth. P fertilizers are usually obtained from phosphate rocks or phosphorites (fluorapatites, apatites and hydroxyapatites) which are not renewable resources (*Grigatti et al.*, 2015): it is estimated that these P-rocks will be exhausted in about 100 years at the current consumption rate (*Cooper et al.*, 2011). Moreover, phosphate rocks could be contaminated by heavy metals such as cadmium (Cd) and nickel (Ni), causing a serious issue on its safety in the perspective on further massive agricultural utilization of P fertilizers (i.e. simple and triple superphosphates) (*Grigatti et al.*, 2015). Therefore, the extraction of P from organic wastes, such as wastewaters, sewage sludge or manures, could represent an interesting and alternative way to recover P for agricultural use, closing the cycle of nutrients in soils from renewable resources (*Koppelaar and Weikard*, 2013) within the circular economy strategy. Among organic wastes, sewage sludge, resulting from activated sludge wastewater treatment plants, deserve particular attention as they are produced in large amounts in the European Union (about 10 million tons in 2012) (*Eurostat*, 2016). It is a matrix that should not be employed directly to the soil in many European nations (*Laturnus et al.*, 2007) and, at the same time, has an interesting P concentration (about 3% of dry matter). However, the amount of P occurring in sewage sludge is only in part soluble in water, the main part of P available for plants. The insoluble part of P, present in organic (i.e. phytates) and inorganic forms (i.e. insoluble phosphates of Ca, Fe and Al), could be solubilized through chemical or biochemical treatment, such as hydrolysis, incineration, co-precipitation. Although chemical hydrolysis with acid or alkaline solutions and thermal treatment had already been investigated (*Ye et al.*, 2016), biochemical hydrolysis through specific enzymes has been less studied till now (*Kim et al.*, 2015). Many studies are focused on recovery of P from sewage sludge ashes (*Adam et al.*, 2008; *Herzel et al.*, 2016; *Krüger and Adam*, 2015; *Mattenberger et al.*, 2008; *Xu et al.*, 2012), or on precipitation of P as struvite (*Jaffer et al.*, 2002; *Pastor et al.*, 2008; *Uysal et al.*, 2010). However, wastewater treatment plants often did not have the technology to precipitate P as struvite, or country legislation did not permit the incineration of sewage sludge (*Christodoulou and Stamatelatou*, 2016; *Hukari et al.*, 2016; *Ye et al.*, 2016). Furthermore, struvite precipitation need specific ratio between ammonium, magnesium and phosphorus in order to have the complete precipitation (*Cieslik and Konieczka*, 2017) and the right alkaline pH. The high amount of ammonium, the use of $MgCl_2$ and the unbalanced ratio between N, P and Mg are not appropriate for plant growing, increasing Mg concentration in soil and alkalinising the soil (*Kataki et al.*, 2016). In addition, struvite did not result as the most suitable fertilizer for P phytoavailability as previously shown in chap. 2, due to the addition to calcareous soil in which the struvite fertilizing efficiency drastically decrease (*Kataki et al.*, 2016).

The enzymatic hydrolysis of sewage sludge can be directed to the organic P forms (*Nannipieri et al.*, 2011; *Pant and Warman*, 2000; *Turner et al.*, 2002), while the acid or alkaline can solubilise P ligated to metals and carbonates (*Grigatti et al.*, 2015). In order to understand the mechanisms on which P release from sewage sludge is based, metal concentration and phosphorus sequential extraction can speciate the different forms of P (*Grigatti et al.*, 2017, 2015) and help to speculate the P bonds in the raw material. Moreover, the use of different type of extractants, time and temperature of extraction can be linked with the sludge characteristics, creating different solutions for different raw materials.

Therefore, the aim of this work was to evaluate the efficiency of enzymatic hydrolysis, alone and in combination with chemical extractants, in the solubilisation of P from two urban sewage sludge, which differed for metal concentration and digestion treatment.

Material and methods

In order to analyse the efficiency of the hydrolysis on different types of sludge, two sewage sludge from civil wastewater plants were chosen: the first deriving from an anaerobic wastewater treatment plants (ANA), the second from an aerobic one (AER). They were analysed for total P concentration and total metal concentration with ARCOS ICP-OES (Spectro Analytical Instruments, Kleve, Germany). In addition, pH (H_2O 1:2.5) was analysed and ash concentration was measured after drying the samples in a muffle furnace at 660°C. Organic P was calculated as the difference between the total P and the inorganic P measured after the muffle furnace drying with ascorbic acid method (*Murphy and Riley*, 1962). For the water soluble concentration an amount of sludge corresponding to 1 g in dry weight was put in a falcon tube with 20 mL of deionized water and shaken for 1 hour. After filtration with Whatman no. 42, the supernatant was analysed with the ascorbic acid method (*Murphy and Riley*, 1962). The sludge were, then, subjected to different treatments summarized in Table 3.1.

Sequential extraction on raw sludge

The raw products were sequentially extracted as previously described in *Grigatti et al.* (2015), in order to better understand the nature of the P bonds in the raw material. Briefly, a sample (corresponding to 0.3 g of dry weight) of product was extracted at a ratio of (1:100 w/v) for 24 h with deionized water then centrifuged, the supernatant was filtered with Whatman no. 42 paper filter and the pellets was extracted with 0.5 M $NaHCO_3$ (pH 8.5) for 24 h. The same procedure was repeated using 0.1 M NaOH and then 1 M HCl. The residual pellets was extracted with 96% H_2SO_4 + 30% H_2O_2 hot digestion. The inorganic P was colorimetrically determined with Murphy and Riley (1962) method. As references, a municipal solid waste compost (MSWC), previously described in chap. 2, and a mineral P fertilizer, $Ca(H_2PO_4)_2{*}H_2O$, (Chem), were used.

Enzymatic extraction

Firstly, sludge was subjected to enzymatic hydrolysis. The primary enzymes used were phosphatase: an acid phosphatase from potato (Ac PHO) (CAS Number 9001-77-8; EC Number 232-630-9; Enzyme Commission (EC) Number 3.1.3.2 EC 3.1.3.2; 3.0 unit/mg solid) and an alkaline phosphatase from bovine intestinal mucosa (Alk PHO) (CAS Number 9001-78-9; EC Number 232-631-4; Enzyme Commission (EC) Number 3.1.3.1; 10 DEA units/mg solid), both from Sigma Aldrich (Saint Louis, Missouri, USA). The enzymes are very sensitive to pH and temperature, thus the enzymatic hydrolysis needed to be strictly correlated with the enzymes requirements. Hence, the hydrolysis used the following procedure. An aliquot of 10 g of fresh sludge (corresponding to about 2.5 g of dry matter) was weighed and added with 50 mL (ratio 1:20 w/v) of enzyme solution in a concentration of 1 unit/50 mL, which is one unit per sample. The suspension was shaken

for 1 h at 37 °C in a water bath, then it was centrifuged at 10,000 rpm for 20 min at 4 °C and filtered through Whatman no. 42 paper filters. The liquid fraction was analysed for the determination of orthophosphate through the ascorbic acid method (*Murphy and Riley*, 1962) in order to evaluate P extraction efficiency, which was calculated as the ratio between P extracted and total P concentration of the sludge, in percentage.

In the first test, the enzymes were added directly to deionized water, whereas in all the following experiment they were put in a buffer solution, in order to maintain the right pH. The buffers were constituted by 90 mM citrate buffer at pH 4.8 and 1.0 M diethanolamine buffer at pH 9.8, for Ac PHO and Alk PHO, respectively. The enzymatic treatments are summarized in Table 3.1.

Later a sequence of two different enzymatic extraction was tested, a biochemical pre-treatment with different enzymes followed by the phosphatase extraction. For the biochemical pre-treatment, sludge was hydrolysed with phytase (Phy) in acetate buffer at pH 5.15, cellulase (Cel) in acetate buffer at pH 5.0 and pronase (Pro) in buffer at pH 7.5. The pre-treatments enzymes precisely were: phytase from wheat (CAS Number 9001-89-2; Enzyme Commission (EC) Number 3.1.3.26; ≥0.01 unit/mg solid); cellulase from *Aspergillus niger* (CAS Number 9012-54-8; EC Number 232-734-4; Enzyme Commission (EC) Number 3.2.1.4; ≥0.3 units/mg solid) and pronase from *Streptomyces griseus* (CAS Number 9036-06-0; EC Number 232-909-5; Enzyme Commission (EC) Number 3.4.24.4; 4,000,000 PU/g). The three enzymes were form Sigma Aldrich (Saint Louis, Missouri, USA), and they hydrolysed the sludge with the ratio 1:20 w/v for 1 h at 37°C in a concentration of 1 unit/50 mL. After this treatment, the suspensions were centrifuged, then the supernatant was withdrawn and analysed for P concentration, while the pellet was subjected to hydrolysis with acid or alkaline phosphatase as already described. In this way, the efficiency of the different enzymes, singularly or sequentially employed, was assessed. The sequenced enzymatic treatments are summarized in Table 3.1.

For the enzymatic mixed treatments, Ac PHO was used simultaneously with Phy and Cel whereas Alk PHO was used in mix with Pro, in a hydrolysis with the ratio sludge/solution of 1:20 w/v for 1 h at 37°C in a concentration of 1 unit/50 mL for each enzymes. The enzymes mixes was chosen for similarity of pH and buffers. The pH of the buffers was adjusted to pH 5 and pH 8 in order have a good environment for all the enzymes in the two different mixes. The enzymatic mixed treatments are summarized in Table 3.1.

The efficacy of the treatment was evaluated in terms of inorganic P recovered in the aqueous solution and compared to the ones obtained with extraction in deionized water at the same temperature, extraction time and ratio. The calculated P concentration in the solutions after the hydrolysis and filtration was referred to the total P concentration of the sludge (%) and called P recovery.

Chemical extraction

Anaerobic and aerobic sludge were also treated with chemical extractants: 0.5 M sulfuric acid (H_2SO_4), 1 M potassium hydroxide (KOH), 0.1 M citric (Cit) and 0.1 M maleic acid (Mal) were used with the same procedure of the enzymatic extraction. Briefly, 10 g of sludge were put in solutions with 50 ml of chemical extractants and hydrolysates for 1 h in a water bath at 37°C, then centrifuged and filtered. The filtrated solutions were analysed spectrophotometrically for orthophosphate concentration (*Murphy and Riley*, 1962).

The chemical extractions, which were not tied to the enzymatic working temperature (37°C), were repeated with different temperature of extraction (90°C for H_2SO_4 and 121°C for citric and maleic acids). Chemical treatments are summarized in Table 3.1.

Chemical and enzymatic combined extraction

Afterwards, chemical and enzymatic extractants were combined in order to reach the highest efficiency in term of P extraction from sludge. Firstly, H_2SO_4, KOH, Mal and Cit were used singularly with the same extraction procedure as previously described and then the supernatant was filtered and analysed. Subsequently, the pellet was hydrolysed with Ac PHO and Alk PHO. For the first extraction with chemical extractants the different temperature of extraction previously described were also tried. Chemical and enzymatic sequence treatments are summarized in Table 3.1.

Further, a mixing solution was tested: 0.1 M citric acid and 0.5 M sulfuric acid was used for 1h at 37°C with the normal procedure. After the centrifuge the solution of citric and sulfuric acid were not filtered but adjusted for pH 4.8, in order to add the acid phosphatase. Then, the samples were treated as usual. The control treatments in this case were citric and sulfuric acid for 2 h of extraction. The chemical and enzymatic mixed treatments are summarized in Table 3.1.

Metal, total and organic P concentration in hydrolysates

All the extracted solutions were analysed with an inductively coupled plasma mass spectrometer ARCOS ICP-OES (Spectro Analytical Instruments, Kleve, Germany) for metal and total P determination. The PO_4 (%) was calculated as the ratio between the P concentration measured with ascorbic acid method and the total P from ICP.

Table 3.1: Enzymatic and chemical treatments for sludge hydrolysis.

Treatment code	Solution	Enzyme	Temperature	Time
H_2O	H_2O		20°C	1 h
Enzymatic treatments				
Ac PHO	Buffer pH 4.80	Acid phosphatase	37°C	1 h
Alk PHO	Buffer pH 9.80	Alkaline phosphatase	37°C	1 h
Ac PHO 121°C	Buffer pH 4.80	Acid phosphatase	37°C	1 h
Alk PHO 121°C	Buffer pH 9.80	Alkaline phosphatase	37°C	1 h
Phy	Buffer pH 5.15	Phytase	37°C	1 h
Cel	Buffer pH 5.00	Cellulase	37°C	1 h
Pro	Buffer pH 7.50	Pronase	37°C	1 h
Sequenced enzymatic treatments				
Phy + Ac PHO	Buffer pH 5.15	Phytase	37°C +	1 h
	+ Buffer pH 4.80	+ Acid Phosphatase	37°C	+1 h
Phy+Alk PHO	Buffer pH 5.15	Phytase	37°C +	1 h
	+ Buffer pH 9.80	+ Alkaline Phosphatase	37°C	+1 h
Cel+Ac PHO	Buffer pH 5.00	Cellulase	37°C +	1 h
	+ Buffer pH 4.80	+ Acid Phosphatase	37°C	+1 h
Cel+Alk PHO	Buffer pH 5.00	Cellulase	37°C +	1 h
	+ Buffer pH 9.80	+ Alkaline Phosphatase	37°C	+1 h
Mixed enzymatic treatments				
Phy +Cel +AcPHO	Buffer pH 5	Phytase + Cellulase + Acid phosphatase	37°C	1 h
Pro + Alk PHO	Buffer pH 8	Pronase + alkaline phosphatase	37°C	1 h
Chemical treatments				
Mal	Maleic acid	---	37°C	1 h
Cit	Citric acid	---	37°C	1 h
H_2SO_4	H_2SO_4	---	37°C	1 h
KOH	KOH	---	37°C	1 h
Mal 121°C	Maleic acid	---	121°C	1 h
Cit 121°C	Citric acid	---	121°C	1 h
H_2SO_4 90°C	H_2SO_4	---	90°C	1 h
Cit 2 h	Citric acid	---	37°C	2 h
H_2SO_4 2 h	H_2SO_4	---	37°C	2 h
Chemical and enzymatic sequenced treatment				
Mal + Ac PHO	Maleic acid	---	37°C	1 h
	+ buffer pH 4.80	acid phosphatase		+ 1 h
Cit + Ac PHO	Citric acid	---	37°C	1 h
	+ buffer pH 4.80	acid phosphatase		+ 1 h
H_2SO_4 +Ac PHO	H_2SO_4	---	37°C	1 h
	+ buffer pH 4.80	acid phosphatase		+ 1 h

Treatment code	Solution	Enzyme	Temperature	Time
KOH + Alk PHO	KOH	---	37°C	1 h
	+ buffer pH 9.80	alkaline phosphatase		+ 1 h
Mal 121°C + Ac	Maleic acid	---	121°C	1 h
PHO	+ buffer pH 4.80	acid phosphatase		+ 1 h
Cit 121°C + Ac	Citric acid	---	121°C	1 h
PHO	+ buffer pH 4.80	acid phosphatase		+ 1 h
H_2SO_4 90°C + Ac	H_2SO_4	---	90°C	1 h
PHO	+ buffer pH 4.80	acid phosphatase		+ 1 h
Chemical and enzymatic mixed treatments				
Cit mix Ac PHO	Citric acid	---	37°C	2 h
	(citric acid pH 4.8)	acid phosphatase		
H_2SO_4 mix Ac	H_2SO_4	---	37°C	2 h
PHO	(H_2SO_4 pH 4.8)	acid phosphatase		

Results

Raw sludge characteristics

From the preliminary results (Table 3.2), we observed that ANA and AER samples presented similar moisture, ash content and pH, when analysed as raw materials. Both products had a concentration of total P of about 20000 mg kg^{-1}, but only 133 mg kg^{-1} and 4 mg kg^{-1} (corresponding to 6.65 mg L^{-1} and 0.20 mg L^{-1} considering the extraction ratio of 20 g ml^{-1}) were available in water, in ANA and AER respectively, which is less than 1% of total P (Table 3.2).

The sequential extraction (Fig. 3.1) revealed different type of P fractions in the two sludge. Both of them had very low amount of water extractable P (2.5% in ANA and 0% in AER), the water extractable P was very low also in MSWC (0%) and Chem (3.7%). The phytoavailable P and P labile bonded with Ca (Ca2-P) were extracted with sodium bicarbonate, can be representative of the available P in the sludge. The results showed that ANA had a higher available fraction compared to AER (12.6% and 8.9%, respectively); however, MSWC and Chem showed higher labile bonded P (16.7% and 16.4% in MSWC and Chem) (Fig. 3.1). The NaOH extractable P characterise the phosphorus bonded in complexes Fe-P and complexes soluble in alkaline solutions. In this fraction, it can be found also organic P. Both sludge showed high portion of P in this fraction, with AER showing 41.1% of NaOH extractable P and ANA 30.9%, whereas very low amount of P was extractable with sodium hydroxide in MSWC (9.5%) and Chem (21.4%). The scarcely labile P portion was lower in sludge (29.8% and 22.6%, in ANA and AER, respectively) compared to MSWC (69%) and Chem (55.8%). On the other hand, the residual P fraction resulted very higher in sludge (24.3% and 27.2%, in ANA and AER) compared to MSWC (5%) and Chem (2.3%), showing a big portion of non-extractable P in sludge.

In Fig. 3.2, the metal concentration of the different extractant solutions is shown as recovery of the total Al, Ca, Fe, Mg and Mn concentration of the sludge. The differences between the sludge is evident in Al extraction, with ANA resulting with a high percentage of Al in the residual portion, as compared to AER (+286%). When comparing the net release the residual Al (Fig. 3.3) in ANA resulted still higher (+80%), even without taking into account the higher Al concentration of AER sludge (Table 3.2), whereas less Al was found in ANA more easily extractable fraction (-71% in $NaHCO_3$, -61% in NaOH and -24% in HCl). A higher amount, both as net release (Fig. 3.3) and relative recovery (Fig. 3.2), was found in Ca, with also big difference between sludge. The Ca concentration was slightly lower in ANA, and also the net release were consistently lower in all the fraction (-44% in H_2O, -50% in $NaHCO_3$, -35% in NaOH, -11% in HCl and -37% in the residual fraction). The Ca recovery were, also, lower in ANA, except for HCl portion, where Ca where 12% higher (Fig. 3.2). Fe concentration in extracted solution were lower in the

labile P pools in ANA, although a higher Fe concentration. In $NaHCO_3$ extraction, the Fe extracted were 35% lower in ANA. On the other hand, the Fe were released in high amount in NaOH portion in ANA (+161% as compared to AER). High amount of Mg was also released in all the portions of the sequential extraction, with higher amount in ANA in the non-labile or difficulty extractable fractions as compared to AER (+18% in NaOH, +14% in HCl and +17% in the residual), whereas smaller amount in the labile fraction (-57% in water and -20% in sodium bicarbonate). Mn, which is generally acid extractable, was mostly released in the HCl fraction in both sludge samples (15% in ANA and 17% in AER).

Table 3.2: Principal characteristics of the raw products

	Anaerobic sludge (ANA)	**Aerobic sludge (AER)**
Moisture (% fresh matter)	72.4%	77.3%
Ash (% dry matter)	45.7%	46.3%
pH-H_2O	7.3	7.8
P_{tot} (mg kg^{-1})	20,040	20,323
P_{in} (mg kg^{-1})	18,154	18,468
P_{org} (mg kg^{-1})	1,311	1,429
P_{H2O} (mg kg^{-1})	50	<0,1
Al (mg kg^{-1})	19347	41366
Ca (mg kg^{-1})	23611	29711
Cd (mg kg^{-1})	2.15	0.61
Co (mg kg^{-1})	5.62	6.49
Cr (mg kg^{-1})	101	50
Cu (mg kg^{-1})	390	296
Fe (mg kg^{-1})	32885	15175
Mg (mg kg^{-1})	6854	6825
Mn (mg kg^{-1})	233	429
Ni (mg kg^{-1})	50	31
Pb (mg kg^{-1})	61	37
Zn (mg kg^{-1})	826	528

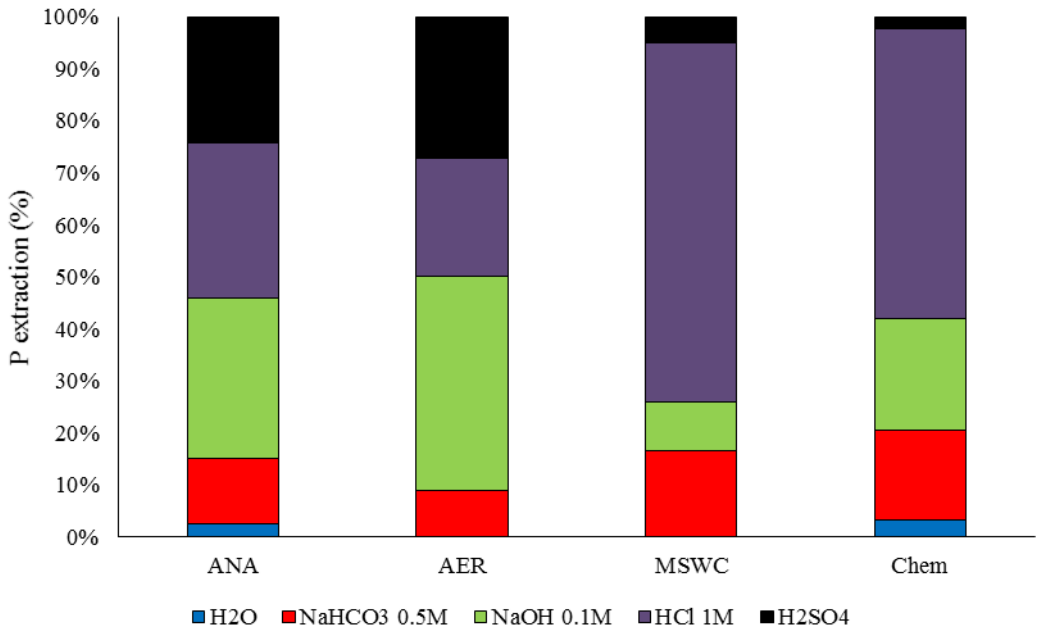


Figure 3.1: Phosphorus sequential extraction with extractant at different strength: water (H_2O) in blue, sodium bicarbonate ($NaHCO_3$ 0.5 M) in red, sodium hydroxide (NaOH 0.1 M) in green, hydrochloric acid (HCl 1M) in violet and hot digestion with sulfuric acid ($H2SO_4$ 96%) in black.

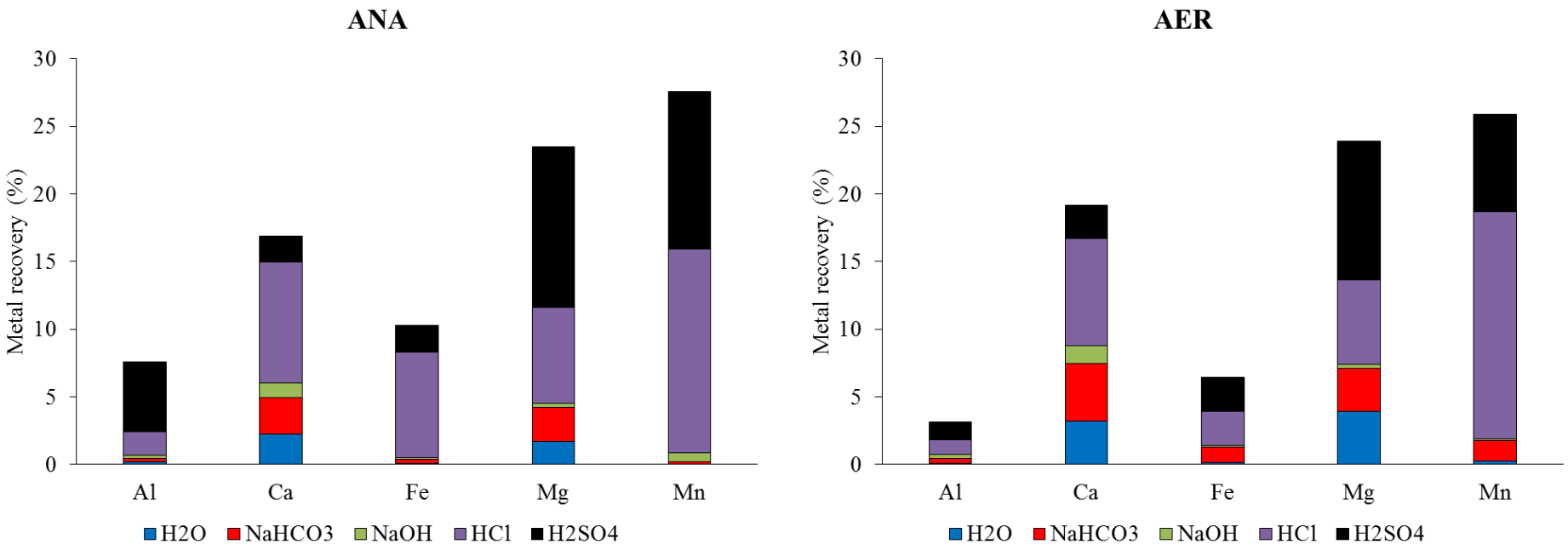


Figure 3.2: ANA and AER sequential extraction metal recovery, with extractant at different strength: water (H_2O) in blue, sodium bicarbonate ($NaHCO_3$ 0.5 M) in red, sodium hydroxide (NaOH 0.1 M) in green, Hydrochloric acid (HCl 1M) in violet and hot digestion with sulfuric acid ($H2SO_4$ 96%) in black.

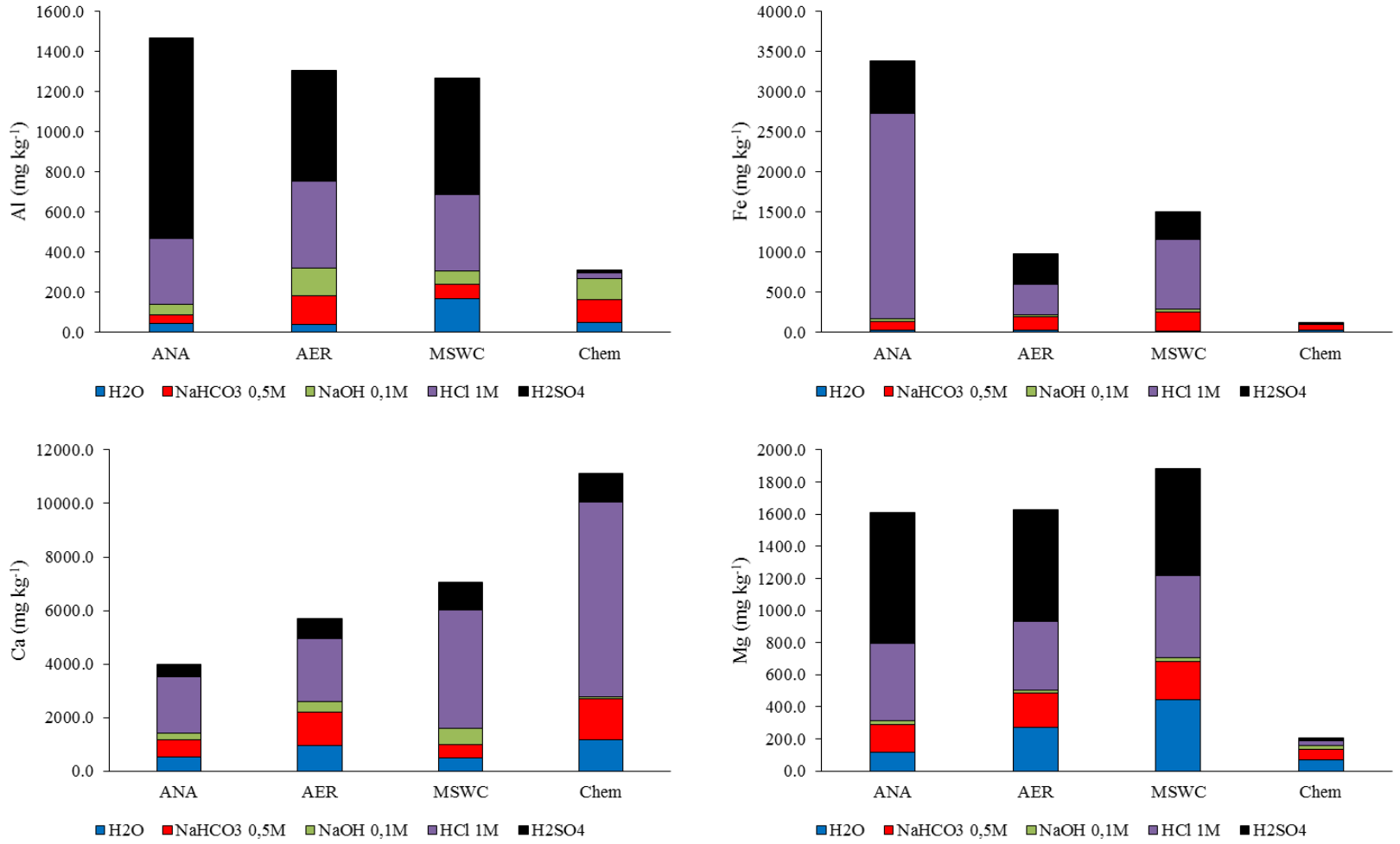


Figure 3.3: Metal extracted with P sequential extraction, with extractant at different strength: water (H_2O) in blue, sodium bicarbonate ($NaHCO_3$ 0.5 M) in red, sodium hydroxide (NaOH 0.1 M) in green, Hydrochloric acid (HCl 1M) in violet and hot digestion with sulfuric acid ($H2SO_4$ 96%) in black.

Enzymatic extraction

The results of enzymatic extraction with acid and alkaline phosphatase in water solution showed very low efficiency, similar to the extraction with only water (data not shown), showing that the enzymes did not increase the extractability if the pH is not managed. Controlling the pH of the solution with the buffer the P recovery efficiency sensibly increased. The results of enzymatic extraction (Fig. 3.4) with phosphatase in buffer solution increased comparing to the extraction with only water (8.4 times in ANA with Ac PHO and almost 4000 times in AER with Ac PHO). However, the P recovery still resulted lower than 2% of the total P, with no sensible increase with the thermal pre-treatments. Ac PHO, generally resulted better compared to Alk PHO. Thermal pre-treatment in autoclave did not allow any improvement of extraction yield as the values of extracted P after autoclaving and enzymatic hydrolysis were the same of the single hydrolysis. However, the results were more interesting when considering only the organic P in sludge, indeed the P recovery with acid phosphatase reached 32% and 28% in ANA and AER, while alkaline phosphatase reached 25% and 11% in ANA and AER.

In addition to phosphatase it was tested also the P recovery efficiency of phytase (Phy), cellulase (Cel) and pronase (Pro) (Fig. 3.5). Phy recovered 1.5% in ANA and 0.3% in AER, Cel recovered 1.2% and 0.1% in ANA and AER, Pro recovered 0.3% and 0.1% in ANA and AER. These results were lower of the phosphatase, mostly in AER sludge and compared to Ac PHO (Ac PHO 2.1% and 1.9% in ANA and AER; Alk PHO 1.6% and 0.8% in ANA and AER) showing that singularly the best solution for enzymatic extraction of phosphorus is acid phosphatase.

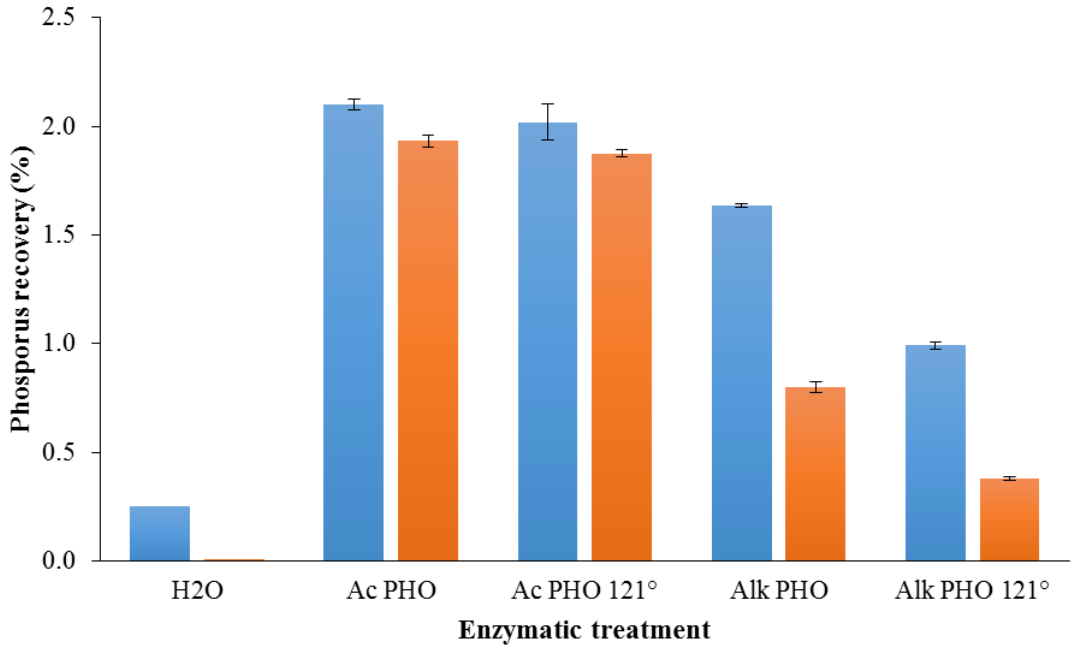


Figure 3.5: Enzymatic extraction in buffered solution with water (H_2O), acid phosphatase (Ac PHO), alkaline phosphatase (Alk PHO), with only the extraction at 37°C or with the thermal pre-treatment at 121°C. Blue bars represents ANA and orange bars AER. Error bars represent standard errors.

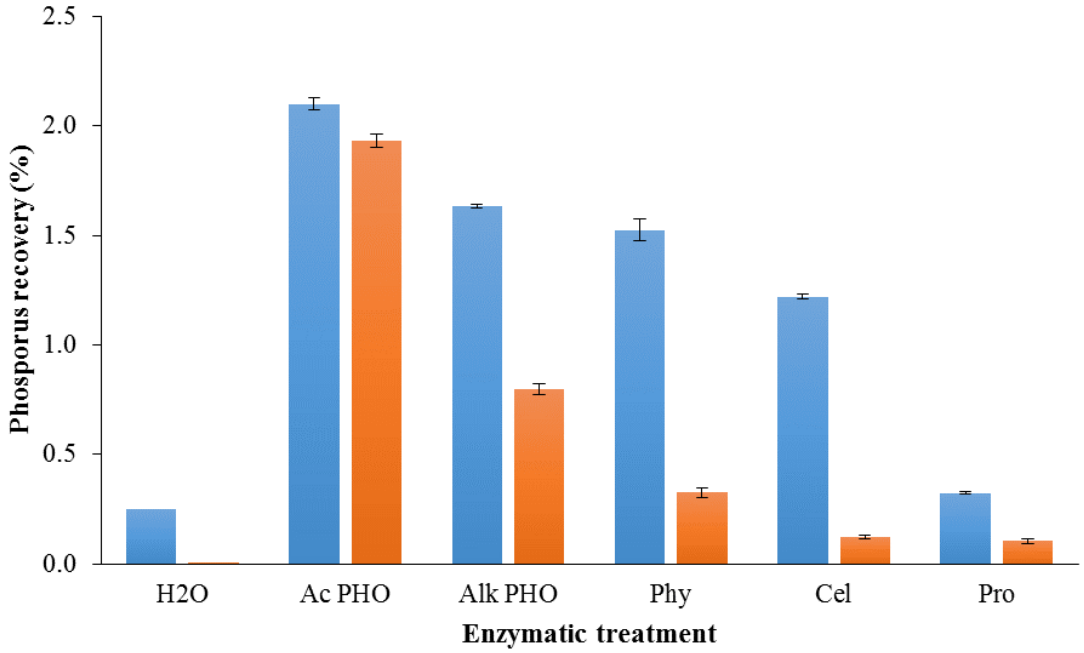


Figure 3.6: Enzymatic extraction in buffered solutions, with water (H_2O), acid phosphatase (Ac PHO), alkaline phosphatase (Alk PHO), phytase (Phy), cellulase (Cel) and pronase (Pro). Blue bars represents ANA and orange bars AER. Error bars represent standard errors.

On the contrary, interesting results were obtained if hydrolysis with acid phosphatase was preceded by hydrolysis with phytase or cellulase (Fig. 3.6). Indeed, although phytase and cellulase themselves granted a very low P extraction, the following hydrolysis of ANA sludge with acid phosphatase allowed to reach very high P extraction, corresponding to 39.1% and 40.3% of the total P present in the sludge, when the Ac PHO was preceded by Phy and Cel, respectively. The sum of the two P extraction reached, thus, 40.6% and 41.5% for Phy+Ac Pho and Cel+Ac PHO. These values, however, were obtained only for the anaerobic sludge, while the maximum efficiency of P for AER was 1.3%. The enzyme sequence increased the efficiency also of Alk PHO, but in a lower measure, with efficiency reaching 4% and 3.2% in ANA with Phy and Cel followed by Alk PHO. It is evident that the action of enzymes as phytase or cellulase had a positive effect on the following action of phosphatase, but only for the acid one and only for the anaerobic sludge; instead, the aerobic one had a lower P release even if hydrolysed with phytase or cellulase and acid phosphatase in sequence.

Furthermore, it was considered to perform the simultaneous hydrolysis with phytase, cellulase and phosphatase at acidic pH and with pronase and phosphatase at alkaline pH for P extraction from both sludge samples (Fig. 3.7). Using enzymes at same time provided the problem of choosing the best buffer and pH, it was decided to use pH 5 for the acidic enzymes and pH 8 for the alkaline ones. In acidic pH with Phy, Cel and Ac Pho the P release was attested at 35.7% in ANA and only 3.7% in AER. In alkaline pH with Pro and Alk PHO the maximum efficiency was 1.3% for both sludge types. These results, however slightly lower than the one in sequence, were reached with only one hour of extraction compared to the two hours of the sequence.

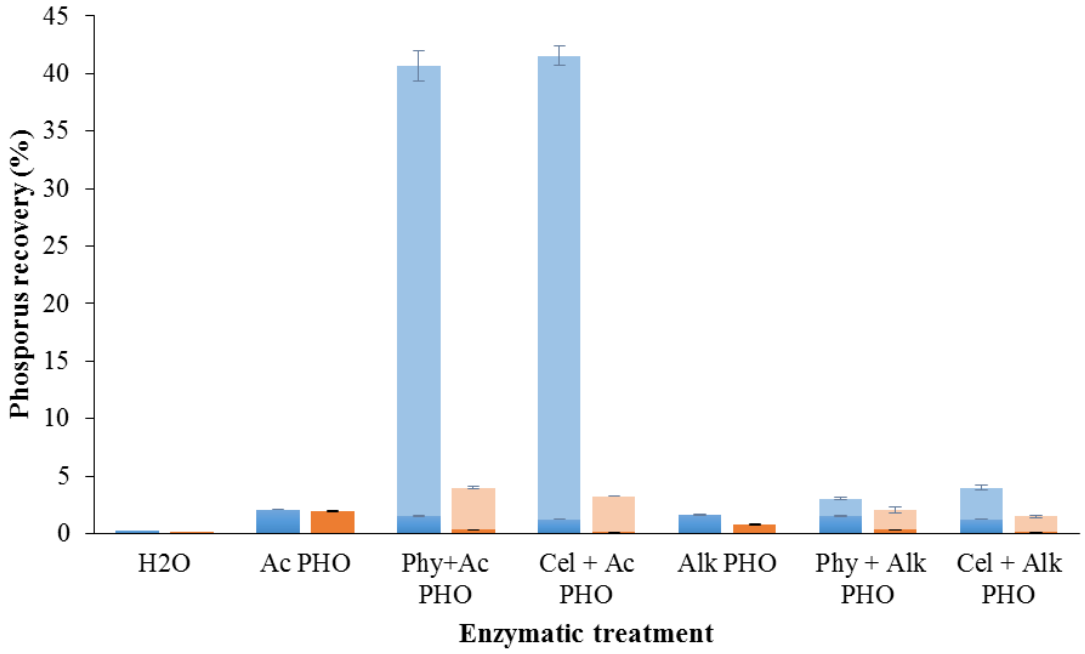


Figure 3.7: Enzymatic extraction in sequence. The dark colours represent the first extraction with water (H_2O), acid phosphatase (Ac PHO), alkaline phosphatase (Alk PHO), phytase (Phy), cellulase (Cel) and pronase (Pro), whereas the light colours represent the second extraction with acid phosphatase (Ac PHO) and alkaline phosphatase (Alk PHO). Blue bars represents ANA and orange bars AER. Error bars represent standard errors.

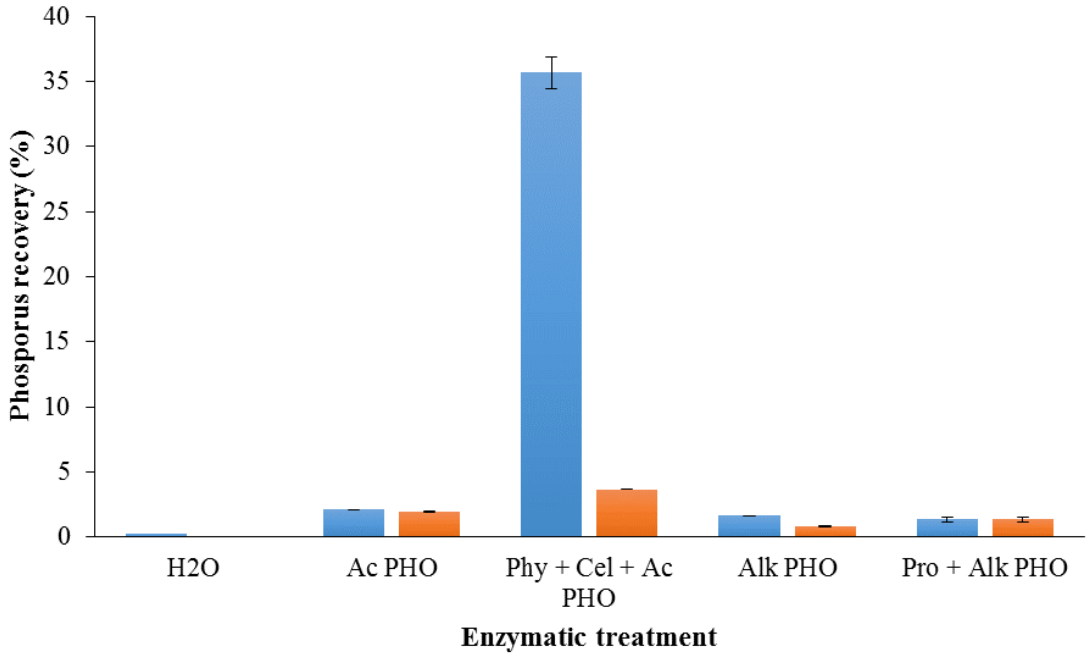


Figure 3.8: Mixed enzymatic extraction with water (H_2O), acid phosphatase (Ac PHO), alkaline phosphatase (Alk PHO), and the mixed solution of phytase (Phy), cellulase (Cel) and acid phosphatase (Ac PHO) and pronase (Pro) and alkaline phosphatase (Alk PHO). Blue bars represents ANA and orange bars AER. Error bars represent standard errors.

Chemical extraction

Chemical extraction (Fig. 3.8) resulted immediately very efficient, with maleic acid (Mal) reaching 27.1%, citric acid (Cit) 41.5%, sulfuric acid (H_2SO_4) 42.2% and potassium hydroxide (KOH) 31.7% of the total P present in anaerobic sludge. The results, however, were very different in AER, in which P was not extracted with organic acids such as maleic (0.7%) and citric (0.1%). The mineral acids instead were able to increase highly the P release of AER, reaching 53.2% and 23.4% with sulfuric acid and potassium hydroxide, respectively.

The use of chemical extraction at different temperature (Fig. 3.9) increased the efficiency in ANA: using Mal and Cit at 121°C the efficiency increased of 11%, instead H_2SO_4 at 90°C increase the efficiency of 59% in ANA. However, in AER the high temperature did not have any effect for citric and maleic acid, whereas it did increase of 54% the P release with H_2SO_4 reaching the efficiency of 82% of the total P present in the aerobic sludge.

Similarly to the high temperature, also the effect of increasing the time of extraction had sensible effect on the P release (Fig. 3.10). Citric acid in 2 hours of extraction increased only of 0.8% in ANA (from 41.5 to 44.9% of the total P released) and 8 times in AER (from 0.1% to 0.5%). Instead, the extraction with sulfuric acid increased the P recovery efficiency of 76% in both sludge samples, reaching 74.4% of P recovery in ANA and 93.8% in AER, the highest P recovery recorded in this study.

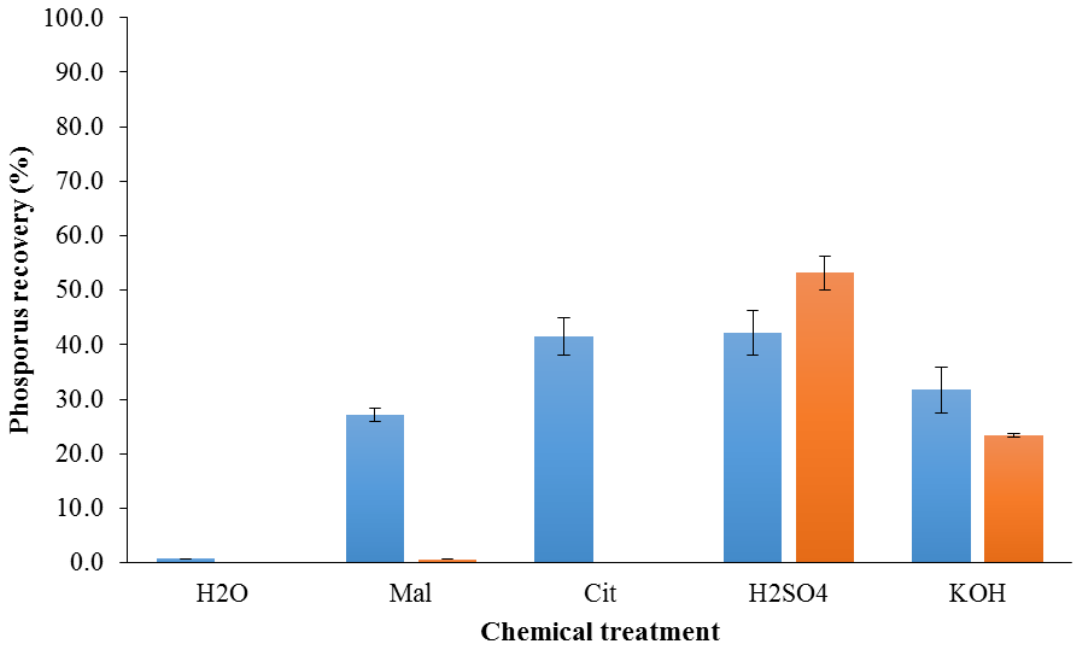


Figure 3.9: Chemical extraction with water (H_2O) as control, maleic acid (Mal), citric acid (Cit) sulfuric acid (H_2SO_4) and potassium hydroxide (KOH). Blue bars represents ANA and orange bars AER. Error bars represent standard errors.

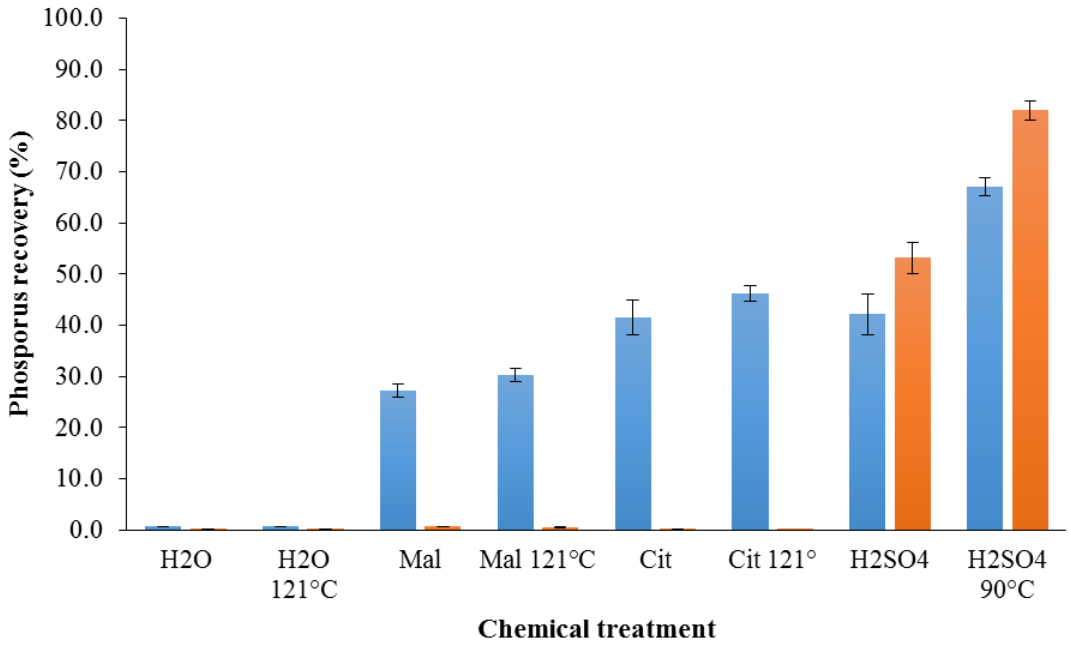


Figure 3.10: Chemical extraction at different temperature with water (H_2O) as control, maleic acid at 37°C (Mal) and at 121°C (Mal 121°C), citric acid at 37°C (Cit) and at 121°C (Cit 121°C) sulfuric acid at 37°C (H_2SO_4) and at 90°C (H_2SO_4 90°C). Blue bars represents anaerobic sludge ANA and orange bars aerobic sludge AER. Error bars represent standard errors.

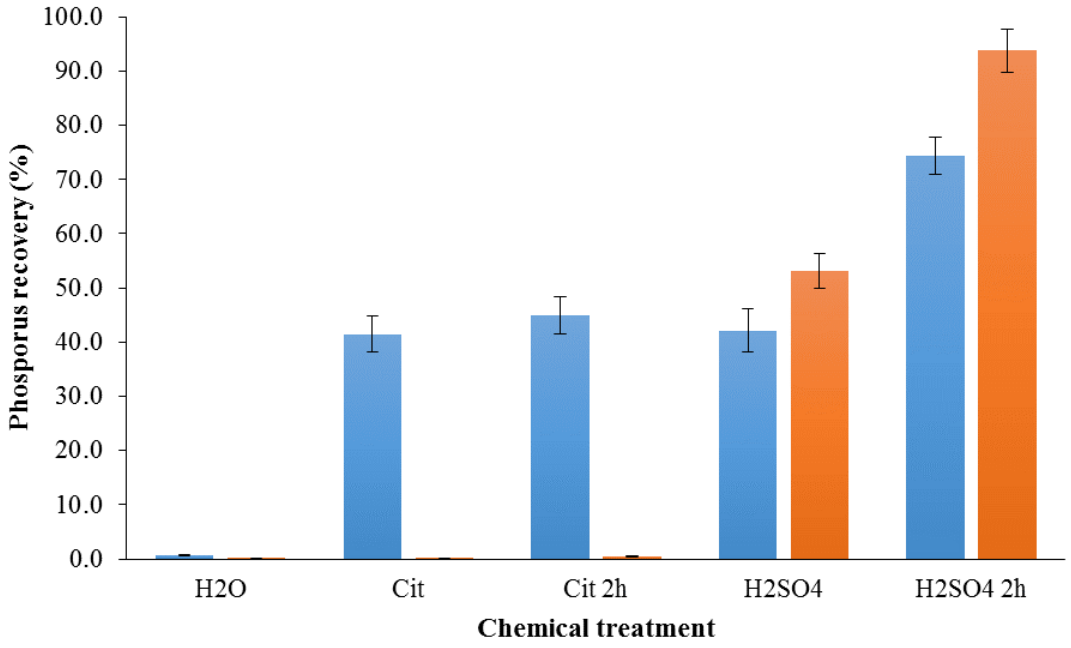


Figure 3.11: Chemical extraction at different time of treatment with water (H_2O) as control, citric acid in 1 hour (Cit) and in 2 hours (Cit 2h) sulfuric acid in 1 hour (H_2SO_4) and in 2 hours (H_2SO_4 2h). Blue bars represents anaerobic sludge ANA and orange bars aerobic sludge AER. Error bars represent standard errors.

Chemical and enzymatic combined extraction

The use of chemical and enzymatic combined treatments was tried after the results previously shown. In Fig. 3.11, the chemical treatment was followed by the enzymatic treatment with acid and alkaline phosphatase. The treatments were done in sequence, with the analysis of the solution of the chemical hydrolysis followed by a successive hydrolysis with enzymatic solution on the pellet of the previously hydrolysates sludge. The results showed that the second enzymatic treatment slightly increased the already high efficiency of the chemical treatment. In anaerobic sludge Cit, which alone hydrolysed 41.5% of the total P followed by Ac PHO reached 44.4%; Mal (alone 27.1%) followed by Ac PHO resulted 30.9%; sulfuric acid (alone 42.2%) increase with Ac PHO reaching 45.1% and KOH followed by Alk PHO reached 33.9% (instead of 31.6% of the only KOH). In aerobic sludge the addition of a second treatment with phosphatase increased slightly the efficiency of Mal (from 0.65% to 2.2% when followed by Ac PHO) and of potassium hydroxide (from 23.3% to 25.2% when followed by Alk PHO). An increase of only 0.5% was found for Ac PHO treatment after sulfuric acid in aerobic sludge.

Using different temperature for the chemical treatment there was a slight increase in the overall results, mostly due to the higher efficiency of the chemical extraction (Fig. 3.12). The highest effect of the temperature on the sequenced treatments is shown in Mal treatment. Although Mal alone did not differ a lot between 37°C and 121°C, the higher temperature increased the P recovery efficiency of the following Ac PHO extraction, reaching as a sum the efficiency of 67.7% in anaerobic sludge (+148% as compared to the Mal 37°C + Ac PHO). The same effect was not remarkable in aerobic sludge. The temperature effect on ANA sludge P release was less evident in Cit at 121°C followed by Ac PHO (11% increase compared to Cit at 37°C + Ac PHO) and in sulfuric acid followed by Ac PHO (64% increase compared to the chemical treatment at 37°C followed by the enzymatic one). In aerobic sludge the temperature increase the P release sensibly only in sulfuric acid at 90°C followed by Ac PHO the chemical treatment at 37°C (82.2% H_2SO_4 90°C+Ac PHO compared to 53.8% H_2SO_4 + Ac PHO).

The sequence of chemical and enzymatic treatments, however, increased the time of extraction to 2 hours, due to the two different hydrolysis. Thus, the increase of the enzymatic treatment that followed the chemical treatments needed to be compared to the chemical treatments at the same time of extraction. In Fig. 3.13, it is possible to see that using Cit for 2 hours it is possible to reach a similar result of the sequence of Cit + Ac PHO. The 2 hours of extraction with sulfuric acid reached even better results compared to the sequence of sulfuric acid and acid phosphatase (64% and 74% more P recovery efficiency in 2 hours of sulfuric acid in ANA and AER compared to the sequence of H_2SO_4 + Ac PHO).

In the last experiment, the Ac PHO was added directly in the citric acid solution and the sulfuric acid solution after 1 hours of hydrolysis and after the adjustment of the pH of the

solution (Fig. 3.14). Thus, the final time of extraction was also 2 hours, but the solution were not replaced after the first hydrolysis as in the sequenced treatments previously described. The results were very interesting and contrasting: in ANA sludge, the addition of Ac PHO to the Cit solution increased the P recovery efficiency of 80% as compared to the Cit 2 hours extraction (from 45% of Cit 2 h to 81% of Cit mix Ac PHO). This great increase was not found instead in AER sludge. Furthermore, in the sulfuric acid treatment in both sludge, the 2 hours treatment with H_2SO_4 resulted higher than the mixed solution of H_2SO_4 and Ac PHO (16% and 79% more in ANA and AER respectively).

Summarized data on P recovery of enzymatic, chemical and combined treatments are reported in Table 3.3 in the PO_4 column in mg P kg^{-1} of sludge and in Table 3.4 in the PO_4 recovery column as percentage of PO_4 in the hydrolysates respect to the total P in the sludge.

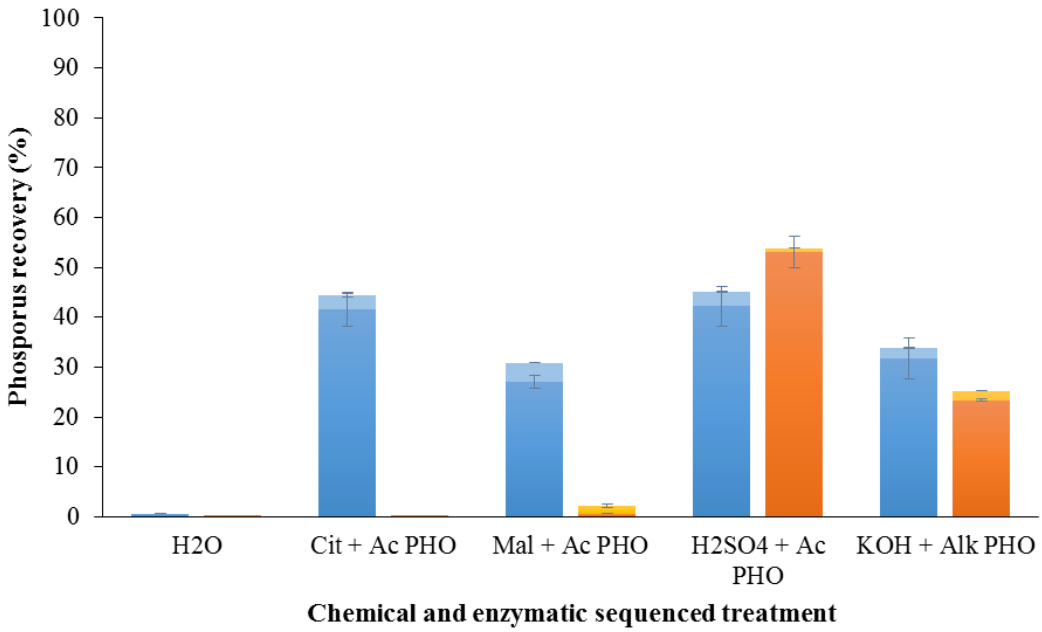


Figure 3.12: Sequence of chemical treatments (in dark colours) with citric (Cit) and maleic (Mal) acid, sulfuric acid (H_2SO_4) and potassium hydroxide (KOH) and enzymatic treatments (in light colours) with acid (Ac PHO) and alkaline (Alk PHO) phosphatase. Blue bars represents anaerobic sludge ANA and orange bars aerobic sludge AER. Error bars represent standard errors.

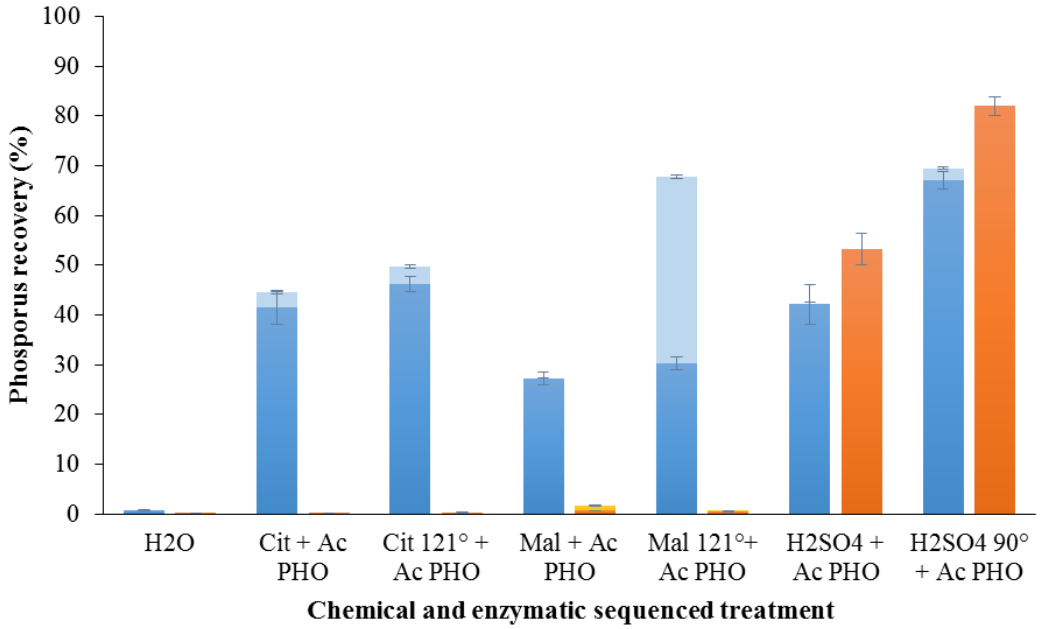


Figure 3.13: Sequence of chemical treatments (in dark colours) at different temperature with citric acid at 37°C (Cit) and at 121°C (Cit 121°C), maleic acid at 37°C (Mal) and at 121°C (Mal 121°C), sulfuric acid at 37°C (H_2SO_4) and at 90°C (H_2SO_4 90°C). The chemical treatments were followed by enzymatic treatments (in light colours) with acid (Ac PHO) and alkaline (Alk PHO) phosphatase. Blue bars represents anaerobic sludge ANA and orange bars aerobic sludge AER. Error bars represent standard errors.

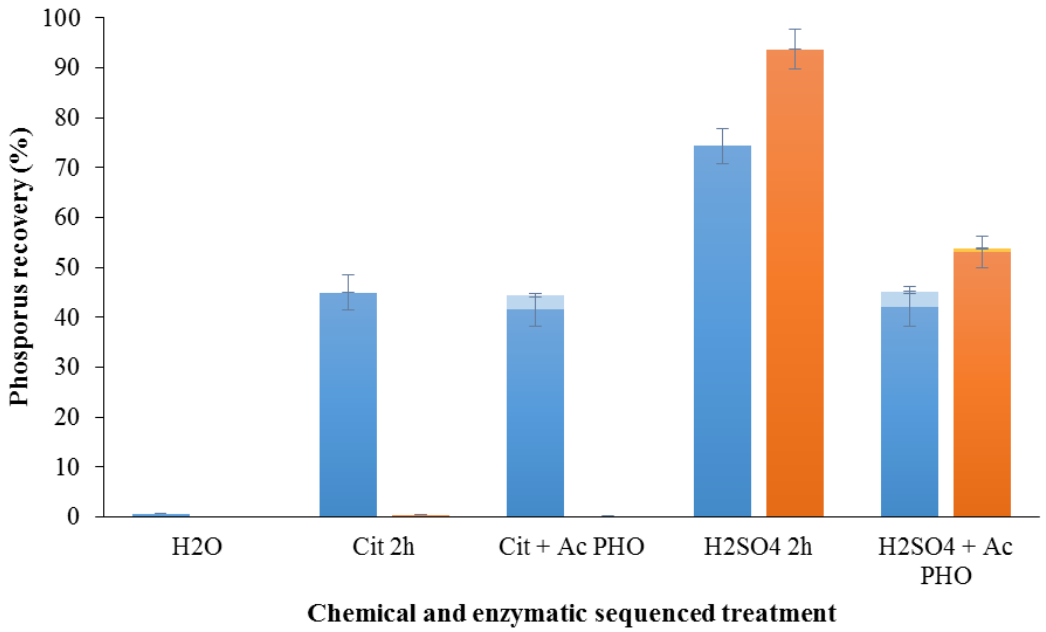


Figure 3.14: Chemical and enzymatic treatment in sequence compared to two hours of chemical treatment. The treatments were: water (H2O) as control, citric acid with 2 hours of extraction (Cit 2 h); citric acid with 1 hour of extraction followed by 1 hour of extraction with acid phosphatase (Cit + Ac PHO); sulfuric acid with 2 hours of extraction (H_2SO_4 2h); sulfuric acid with 1 hour of extraction followed by 1 hour of extraction with acid phosphatase (H_2SO_4 + Ac PHO). Blue bars represents anaerobic sludge ANA and orange bars aerobic sludge AER. Error bars represent standard errors.

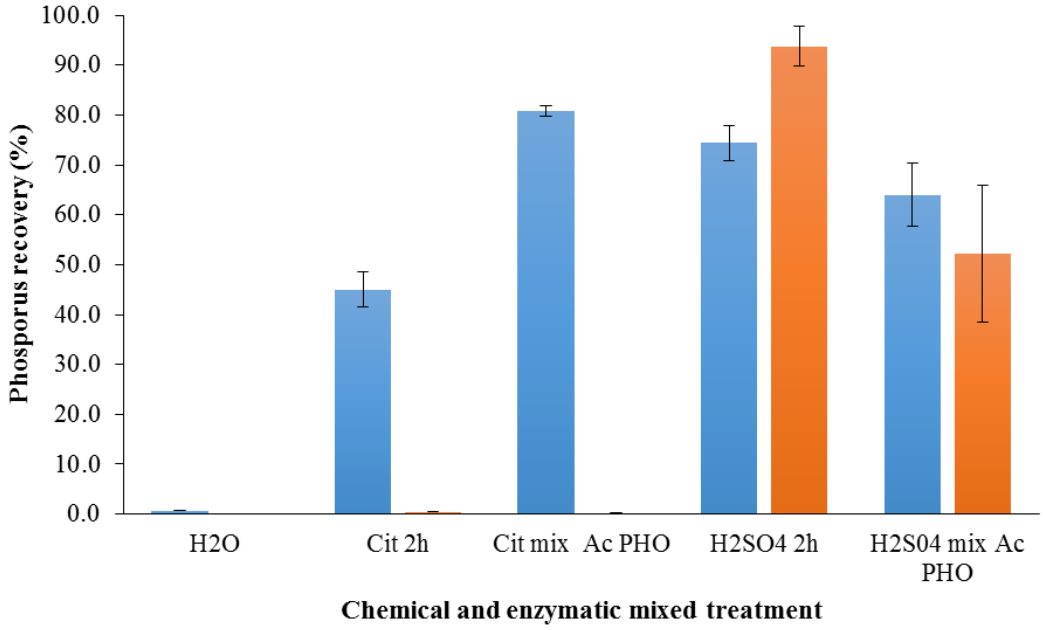


Figure 3.15: Chemical and enzymatic treatment mixed. The treatments were: water (H2O) as control, 2 hours of citric acid (Cit 2 h); 1 hour with citric acid and 1 hour with acid phosphatase mixed with citric acid (Cit + Ac PHO); 2 hours of sulfuric acid (H_2SO_4 2h); 1 hour with sulfuric acid and 1 hour of acid phosphatase mixed with sulfuric acid (H_2SO_4 + Ac PHO). Blue bars represents anaerobic sludge ANA and orange bars aerobic sludge AER. Error bars represent standard errors.

Total P and PO_4 concentration

The hydrolysates solution were analysed with ICP-OES to assess the total P concentration. The results showed a huge increase of the total P concentration in AER hydrolysates as compared to the P measured with the ascorbic acid method, which can be considered the inorganic P (Table 3.3). In the enzymatic sequenced extraction of AER sludge the Ac PHO, which followed phytase and cellulase, contained a high amount of P, which was, however, not in the form as PO_4^-, the only one considered available for plants and measurable with the spectrophotometric method. Thus, in AER hydrolysates less than 10% of the P released was in orthophosphate forms, measurable with ascorbic acid method. Even more evident is this effect in the organic acid treatments, where 3% of the P released was PO_4 in the acid phosphatase treatment after maleic acid at 121°C and less than 1% in the treatment with citric acid (Cit, Cit + Ac PHO and Cit mix AC PHO) (Table 3.3).

On the other hand, the results of the treatment with sulfuric acid showed clearly that the strong acid were able to mineralize all the P released, with results of total P which were often lower than the inorganic P measured spectrophotometrically. In this case, the effect was detectable in both sludge, with lower total P in all the sulfuric acid treatment except for the AER treated with H_2SO_4 at 90°C (0.4% of P not in the form of PO_4) (Table 3.3).

The total P concentration in hydrolysates was also used to calculate the total P recovery (Table 3.4), which resulted higher in AER as compared to the PO_4 recovery, with the same proportion described above.

Table 3.3: Phosphorus concentration in different hydrolysates measured as total (P tot) and orthophosphate (PO_4).

Sludge	**Hydrolysates treatment**	***P tot*** mg kg^{-1}	***PO_4*** mg kg^{-1}	***PO_4*** (% of P_{tot})
ANA	Ac PHO	684	421	61.5
	Alk PHO	394	328	83.2
	Buffer pH 5.1	446	288	64.5
	Buffer pH 5.1 + 4.8 (seq)	14560	7646	52.5
	Phy	568	311	54.8
	Phy+Ac PHO (seq)	15680	7836	50.0
	Phy + Alk PHO (seq)	1410	741	52.6
	Cel	397	243	61.2
	Cel + Ac PHO (seq)	15320	8082	52.8
	Phy + Cel + Ac PHO (mix)	13660	7148	52.3
	Mal 121°C	9600	6052	63.0
	Mal 121°C + Ac PHO (seq)	10420	7512	72.1
	Cit	17520	8442	48.2
	Cit + Ac PHO (seq)	3396	586	17.2
	Cit + Ac PHO (mix)	14380	16199	100.0
	H2SO4 90°C	12795	13440	100.0
	H2SO4 90°C + Ac PHO (seq)	1882	480	25.5
	H2SO4 90°C + Alk PHO (seq)	1718	631	36.7
	H2SO4 90°C + Ac PHO (mix)	9910	12818	100.0
	H2SO4 2 h	14203	14903	100.0
AER	Ac PHO	14980	393	2.6
	Alk PHO	163	163	99.7
	Buffer pH 5.1	23	21	91.7
	Buffer pH 5.1 + 4.8 (seq)	6620	1785	27.0
	Phy	97	69	71.4
	Phy+Ac PHO (seq)	7140	302	4.2
	Phy + Alk PHO (seq)	622	349	56.1
	Cel	42	25	58.2
	Cel + Ac PHO (seq)	6120	553	9.0
	Phy + Cel + Ac PHO (mix)	5540	747	13.5
	Mal 121°C	377	105	27.9
	Mal 121°C + Ac PHO (seq)	796	24	3.1
	Cit	19220	10	0.1
	Cit + Ac PHO (seq)	3480	22	0.6
	Cit + Ac PHO (mix)	16300	49	0.3
	H2SO4 90°C	16490	16426	99.6
	H2SO4 90°C + Ac PHO (seq)	2284	92	4.0
	H2SO4 90°C + Alk PHO (seq)	460	166	36.1
	H2SO4 90°C + Ac PHO (mix)	7390	10459	100.0
	H2SO4 2 h	15300	18790	100.0

Seq: the treatments are in sequences; Mix: the treatments are in the same solution. In grey the cells in which the percentage was forced to 100% due to higher inorganic P rather than total P.

Table 3.4: P recovery measured as total P and PO_4 concentration in hydrolysates on the total P in sludge.

Sludge	Treatments	Total P recovery (% of total P in sludge)	PO_4 recovery (% of total P in sludge)
ANA	Ac PHO	3.4	2.1
	Alk PHO	2.0	1.6
	Buffer pH 5.1	2.2	1.4
	Buffer pH 5.1 + 4.8 (seq)	72.7	38.2
	Phy	2.8	1.6
	Phy+Ac PHO (seq)	78.2	39.1
	Phy + Alk PHO (seq)	7.0	3.7
	Cel	2.0	1.2
	Cel + Ac PHO (seq)	76.4	40.3
	Phy + Cel + Ac PHO (mix)	68.2	35.7
	Mal 121°C	47.9	30.2
	Mal 121°C + Ac PHO (seq)	52.0	37.5
	Cit	87.4	42.1
	Cit + Ac PHO (seq)	16.9	2.9
	Cit + Ac PHO (mix)	80.8	80.8
	H2SO4 90°C	67.1	67.1
	H2SO4 90°C + Ac PHO (seq)	9.4	2.4
	H2SO4 90°C + Alk PHO (seq)	8.6	3.1
	H2SO4 90°C + Ac PHO (mix)	64.0	64.0
	H2SO4 2 h	74.4	74.4
AER	Ac PHO	73.7	1.9
	Alk PHO	0.8	0.8
	Buffer pH 5.1	0.1	0.1
	Buffer pH 5.1 + 4.8 (seq)	32.6	8.8
	Phy	0.5	0.3
	Phy+Ac PHO (seq)	35.1	1.5
	Phy + Alk PHO (seq)	3.1	1.7
	Cel	0.2	0.1
	Cel + Ac PHO (seq)	30.1	2.7
	Phy + Cel + Ac PHO (mix)	27.3	3.7
	Mal 121°C	1.9	0.5
	Mal 121°C + Ac PHO (seq)	3.9	0.1
	Cit	94.6	0.0
	Cit + Ac PHO (seq)	17.1	0.1
	Cit + Ac PHO (mix)	80.2	0.2
	H2SO4 90°C	81.1	80.8
	H2SO4 90°C + Ac PHO (seq)	11.2	0.5
	H2SO4 90°C + Alk PHO (seq)	2.3	0.8
	H2SO4 90°C + Ac PHO (mix)	51.5	51.5
	H2SO4 2 h	92.5	92.5

Seq: the treatments are in sequences; Mix: the treatments are in the same solution.

Metals release after sludge hydrolysis

The hydrolysis of sludge released also other metals in the hydrolysing solutions (Table 3.5). The total content of metals could be potentially useful in a nutrient solution for plants (Table 3.6) or potentially toxic (Table 3.7). In Table 3.6 there was also Cu and Zn which can be considered useful only in very low amount and phytotoxic in high concentration, which are dependent on the metal solubility (*McBride et al.*, 1997). Acid phosphatase in ANA sludge used alone compared to alkaline phosphatase, in addition to a slightly higher P recovery, released also more Ca and Mg (14 times more), more Mn (16 times more), whereas less Fe (-97%) less Cu (-47%) and less Zn (-89%). It had also extracted less toxic elements such as Al (-95%), and it had no released Pb in solution compared to the 1.3 mg kg^{-1} released by alkaline phosphatase. The enzymatic extraction with higher P recovery were the sequence with phytase and acid phosphatase (Phy + Ac PHO) and the sequence with cellulase and acid phosphatase (Cel + Ac PHO) in ANA sludge. The released metals were higher in Phy+Ac PHO, in term of Ca (+9%), Fe (+35), Mg (+5%), Mn (+6%) and Zn (+7%). The sequence with phytase had extracted also more Al (+4%), more Co (+1%), more Cr (6%) and more Pb (+17%), whereas less Cu (-2%) and Ni (-2%). The mix of the enzymes, phytase, cellulase and acid phosphatase had a similar P recovery of sulfuric acid in ANA sludge (35.7% vs 42.2%). The mix of enzymes extracted more Ca (+78%), more Fe (+65%), but less Mg (-22%), Mn (-42%), Zn (-71%), Al (-53%), Co (-33%), Cr (-50%), Ni (-30%); it had no extracted Cd (0 vs 1.6 mg kg^{-1}) but it had extracted more Pb (11.2 vs 3.7 mg kg^{-1}). Good results of P recovery were also detectable in maleic acid extraction at 121°C in ANA sludge. This methodology was compared to the extraction with H_2SO_4 at 90°C. The sulfuric acid released more Mg (+7%), Mn (26%), Zn (+356%), Co (+69%) and Ni (163%), but also much more Al (292 times), Cr (70 times), Cu (443 times) and Pb (17 times). In addition it had released less Ca (-65%) and less Fe (-4%).

Two hours of extraction with sulfuric acid compared to the extraction with citric acid mixed with acid phosphatase released more Mg (+42%), Mn (+34%), Zn (131%), but also more Al (+55%), more Co (+124%), Cr (+64%), Ni (+60%), and much more Cu (1116 times). On the other hand it had extracted less Ca (-51%) and Fe (-35%) and less Pb (-73%). Similar results were found when comparing the mix of sulfuric acid at 90°C and acid phosphatase and the mix of citric acid and acid phosphatase.

Furthermore, all the sulfuric acid extractions released a small amount of Cd, which is toxic even in small concentration. The cadmium extracted were in the H_2SO_4 2 hour extraction 1.6 and 0.5 mg kg^{-1}, in ANA and AER, respectively; in the H_2SO_4 at 90°C 1.5 and 0.6 mg kg^{-1} in ANA and AER; and in the mix with H2SO4 and acid phosphatase 1.3 and 0.4 mg kg^{-1} in ANA and AER.

Table 3.5: Elements concentration in the hydrolysates solutions.

Treatment	Sludge	Elements in mg L^{-1} of hydrolysis solution												
		Al	Ca	Cd	Co	Cr	Cu	Fe	Mg	Mn	Ni	P	Pb	Zn
Ac PHO	**ANA**	2.1	5040.0	0.0	0.5	0.1	1.0	22.1	1226.0	4.6	11.1	684.0	0.0	3.9
	AER	n.a.	n.a.	0.0	0.7	26.8	0.7	n.a.	1910.0	117.5	18.2	n.a.	12.8	218.3
Alk PHO	**ANA**	47.0	347.4	0.0	0.0	0.0	2.0	633.4	87.2	0.3	1.9	394.0	1.3	35.6
	AER	234.3	262.0	0.0	0.0	0.0	0.8	289.8	49.7	0.1	0.3	93.4	0.7	7.2
Phy	**ANA**	1.4	5820.0	0.0	0.4	0.2	2.0	23.3	1290.0	5.2	15.5	568.0	0.0	6.3
	AER	7.3	8520.0	0.0	0.3	0.0	0.2	13.0	2300.0	32.1	1.9	97.0	0.0	0.6
Cel	**ANA**	2.5	3804.0	0.0	0.4	0.1	2.2	24.8	976.0	2.8	16.1	397.0	0.0	4.8
	AER	6.5	6080.0	0.0	0.2	0.0	0.0	9.0	1834.0	17.8	1.5	42.2	0.0	0.1
Phy + Ac PHO	**ANA**	1513.4	22640.0	0.0	1.1	27.6	2.7	16672.8	3240.0	125.0	31.2	16248.0	13.1	233.3
	AER	9834.3	25480.0	0.0	1.2	3.9	2.3	4358.2	3898.0	318.8	8.2	7237.0	7.4	86.2
Phy + Alk PHO	**ANA**	93.0	6183.6	0.0	0.6	0.6	33.5	697.9	1334.2	5.6	24.9	1978.0	1.1	25.3
	AER	503.0	8844.8	0.0	0.4	0.0	7.4	512.6	2346.0	33.5	4.1	719.0	1.1	8.0
Cel + Ac PHO	**ANA**	1457.6	20784.0	0.0	1.1	26.2	2.7	16232.3	3080.0	118.1	31.7	15717.0	11.9	217.6
	AER	8847.8	23620.0	0.0	1.1	3.4	1.1	4139.6	3520.0	294.0	7.7	6162.2	6.3	73.9
Phy + Cel + Ac PHO	**ANA**	1238.8	16080.0	0.0	0.9	24.1	1.2	14530.7	2578.0	98.1	20.1	13660.0	11.2	186.3
	AER	7994.1	19180.0	0.0	1.1	3.2	7.4	4393.9	2748.0	284.1	7.9	5540.0	4.9	51.6
H2SO4 2h	**ANA**	2623.5	9030.0	1.6	1.4	48.0	258.1	8802.2	3320.0	168.5	28.8	14230.0	3.7	653.3
	AER	22849.7	9640.0	0.5	2.3	13.5	202.7	5555.7	4050.0	362.5	15.2	15300.0	9.4	437.1
H2SO4 90°C	**ANA**	4088.3	8490.0	1.5	3.1	52.5	273.9	8237.2	4001.5	168.7	35.3	12795.0	4.7	606.1
	AER	23622.1	10310.0	0.6	4.8	22.2	249.8	7917.0	5230.0	391.9	24.3	16490.0	9.8	506.2
H2SO4 + Ac PHO	**ANA**	411.0	1784.0	0.1	0.3	4.8	5.4	2430.1	378.0	14.2	3.2	1882.0	4.4	60.1
	AER	3086.5	7120.0	0.0	0.5	2.3	7.8	1226.0	566.0	40.1	2.4	2284.0	1.9	53.8
H2SO4 + Alk PHO	**ANA**	51.3	512.0	0.0	0.0	1.3	7.8	737.2	169.2	1.5	0.4	1718.0	1.4	16.9
	AER	482.9	1588.0	0.0	0.0	0.0	6.2	231.1	146.2	1.4	0.0	460.0	1.4	9.1
H2SO4 mix Ac PHO	**ANA**	3233.4	5620.0	1.3	2.1	37.5	206.4	8447.6	3070.0	134.7	24.1	9910.0	2.6	545.7
	AER	13135.5	5930.0	0.4	2.7	11.6	98.7	5236.3	3130.0	242.4	13.9	7390.0	4.8	348.2

Treatment	Sludge	Elements in mg L^{-1} of hydrolysis solution												
		Al	Ca	Cd	Co	Cr	Cu	Fe	Mg	Mn	Ni	P	Pb	Zn
Mal 121°C	**ANA**	14.0	24160.0	0.0	1.8	0.7	0.6	8622.5	3750.0	134.3	13.4	9600.0	0.3	132.9
	AER	126.4	29140.0	0.0	0.9	0.0	0.9	3677.2	4180.0	289.8	0.5	377.2	0.1	42.5
Mal 121°C + Ac PHO	**ANA**	1268.6	9640.0	0.0	0.7	20.3	0.0	11946.2	954.0	75.1	7.4	10420.0	6.3	388.0
	AER	1212.0	8620.0	0.0	0.5	0.4	0.0	2023.3	1046.0	99.9	1.3	796.0	0.7	65.7
Cit	**ANA**	2008.0	23880.0	0.0	0.9	32.8	0.6	15070.4	3068.0	169.7	25.8	17520.0	11.4	391.4
	AER	6.1	1076.0	1.2	9.0	1.6	n.a.	n.a.	n.a.	3.1	18.8	19220.0	2.1	252.4
Cit + Ac PHO	**ANA**	2589.3	28560.0	0.0	1.3	47.0	3.9	20473.5	3666.0	204.4	32.3	20916.0	28.5	502.4
	AER	5271.9	7176.0	1.2	9.6	3.9	3.8	1252.3	672.0	77.7	22.6	22700.0	10.5	336.0
Cit mix Ac PHO	**ANA**	1696.9	18300.0	0.0	0.6	29.3	0.2	13477.1	2332.0	125.4	18.0	14380.0	13.7	283.1
	AER	23379.1	25600.0	0.0	1.0	7.7	2.8	5896.5	3042.0	307.2	7.6	16300.0	19.2	216.3

n.a.= not available data

Table 3.6: Fertilizing metal recovery in hydrolysates (% of the total metal in sludge)

Plant nutrients RECOVERY (%)												
	Ca		Cu		Fe		Mg		Mn		Zn	
	ANA	AER	ANA	AER	ANA	AER	ANA	AER	ANA	AER	ANA	AER
Metal in sludge mg kg^{-1}	23611	29711	390	296	32884	15175	6854	6824	233	429	826	528
Enzymes - Phosphatase												
Ac PHO	21.3	53.6	0.3	0.2	0.1	105.0	17.9	28.0	2.0	27.4	0.5	41.4
Alk PHO	1.5	0.9	0.5	0.3	1.9	1.9	1.3	0.7	0.1	0.0	4.3	1.4
Enzymes Sequence												
PHY + Ac. PHO	95.9	14.0	0.7	11.4	50.7	4.6	47.3	14.9	53.6	0.7	28.3	4.5
PHY+ Alk PHO	26.2	70.0	8.6	0.9	2.1	107.0	19.5	45.1	2.4	27.5	3.1	41.2
CEL + Ac. PHO	88.0	85.3	0.7	0.3	49.4	109.8	44.9	62.3	50.6	35.5	26.3	43.2
Enzymes -Mix												
PHY + CEL + Ac Pho	68.1	64.6	0.3	2.5	44.2	29.0	37.6	40.3	42.1	66.3	22.6	9.8
Acid - H_2SO_4												
H_2SO_4 2h	38.2	32.4	66.2	68.6	26.8	36.6	48.4	59.3	72.3	84.6	79.1	82.8
H_2SO_4 90°C	36.0	33.5	70.3	84.0	25.0	51.9	58.4	75.5	72.3	89.8	73.4	94.1
Acid + enzymes - Sequence												
H_2SO_4 90°C + Ac. Pho	47.9	47.0	58.7	87.1	28.9	58.9	54.9	82.5	71.6	97.0	67.3	103.5
H_2SO_4 90°C + Alk. Pho	44.2	37.7	85.6	85.7	29.2	53.1	71.6	76.4	86.4	88.5	88.9	93.9
Acid + enzymes - Mix												
H_2SO_4 90° + Ac PHO	23.8	20.0	53.0	33.4	25.7	34.5	44.8	45.9	57.8	56.6	66.1	66.0
Organic acids												
Mal 121°C	81.3	98.1	0.2	0.3	56.8	24.2	54.9	61.2	31.3	67.6	25.2	8.1
Cit	80.4	3.6	0.2	n.a.	99.3	0.0	45.0	n.a.	39.6	0.7	74.2	47.8
Organic acids + enzymes - Sequence												
Mal 121°C + Ac Pho	32.4	29.0	0.0	0.0	78.7	13.3	14.0	15.3	17.5	23.3	73.5	12.5
Cit + Ac PHO	96.1	24.2	1.3	n.a.	134.9	8.3	53.7	n.a.	47.7	18.1	95.2	63.7
Organic acid + enzymes - Mix												
Cit+ Ac PHO	61.6	86.2	0.1	0.9	88.8	38.9	34.2	44.6	29.3	71.7	53.6	41.0

Table 3.7: Potential toxic element extraction in hydrolysates (% of the total metal in sludge)

Potential toxic element RECOVERY (%)												
	Al		Cd		Co		Cr		Ni		Pb	
	ANA	AER	ANA	AER	ANA	AER	ANA	AER	ANA	AER	ANA	AER
Metal in sludge mg kg^{-1}	19347	41366	2.1	0.6	5.6	6.5	101	50	50	31	61	37
Enzymes - Phosphatase												
Ac PHO	0.0	3.5	0.0	0.0	8.3	10.4	0.1	54.0	22.4	58.2	0.0	34.8
Alk PHO	0.2	0.6	0.0	0.0	0.0	0.0	0.0	0.0	3.8	0.9	2.2	1.8
Enzymes Sequence												
PHY + Ac. PHO	7.8	0.2	0.0	0.0	19.7	9.3	27.3	1.2	62.7	81.5	21.4	3.1
PHY+ Alk PHO	0.5	3.5	0.0	0.0	10.7	16.9	0.6	52.7	50.1	101.3	1.9	32.5
CEL + Ac. PHO	7.5	3.7	0.0	0.0	19.5	14.5	25.8	55.3	63.6	56.0	19.5	35.6
Enzymes -Mix												
PHY + CEL + Ac Pho	6.4	19.3	0.0	0.0	16.7	16.7	23.8	6.5	40.4	25.4	18.3	13.4
Acid - H_2SO_4												
H_2SO_4 2h	13.6	55.2	75.1	83.2	25.1	35.4	47.4	27.2	57.9	48.7	6.0	25.5
H_2SO_4 90°C	21.1	57.1	70.6	93.0	54.9	72.9	51.9	44.1	71.0	76.3	7.6	25.8
Acid + enzymes - sequence												
H_2SO_4 90°C + Ac. Pho	27.0	62.4	65.6	98.9	56.3	78.3	45.7	48.6	67.2	82.7	12.5	31.0
H_2SO_4 90°C + Alk. Pho	25.3	57.1	78.3	90.9	60.3	71.5	62.9	43.5	81.5	74.9	10.5	28.5
Acid + enzymes - mix												
H_2SO_4 90° + Ac PHO	16.7	31.8	60.7	61.5	38.1	42.1	37.1	23.3	48.4	44.5	4.3	12.9
Organic acids												
Mal 121°C	0.0	0.3	0.0	0.0	28.1	13.6	1.5	0.0	43.0	1.6	0.7	0.2
Cit	4.9	0.0	0.0	189.7	13.7	138.9	66.0	3.2	82.6	60.1	30.9	5.6
Organic acids + enzymes - Sequence												
Mal 121°C + Ac Pho	3.1	3.2	0.0	0.0	10.7	7.9	40.9	0.9	23.8	4.1	17.1	2.0
Cit + Ac PHO	6.3	12.7	1.4	197.6	19.6	147.3	94.6	7.9	103.1	72.3	77.5	28.6
Organic acid + enzymes - Mix												
Cit+ Ac PHO	4.1	56.5	0.0	0.0	9.7	15.0	58.9	15.5	57.7	24.4	37.3	52.2

Discussion

The crisis of phosphorus (*Cordell et al.*, 2009), with the resource depletion and the price of the raw material for phosphate fertilizer that has rocketed in the last 20 years (*Reijnders*, 2014), has increased the attention on alternative solutions to phosphate rocks. Furthermore, the market in the future will be highly dependent on the new reserves discovered recently in Morocco, which are extremely contaminated with Cd and which are insecure due to political instability of the country (*Walan et al.*, 2014). As alternative solutions, organic wastes are the most appreciated due to their need to be recycled and their evident abundance (*Cordell et al.*, 2011). Sewage sludge is one of the most abundant organic waste and one of the most difficult to dispose (*Smith*, 2009). In sewage sludge, the P is highly concentrated, because municipalities need to decrease the free P in the wastewater, to avoid the pollution of the hydric basins and eutrophication: thus, in wastewater plants, Fe and Al salts are used in order to precipitate P in the solid fraction (*Tarayre et al.*, 2016). Hence, the P contained in sewage sludge is poorly available for plant nutrition, due to its strong bonds with Fe and Al (*Ye et al.*, 2016). Many processes tried to recovery the P in the sewage sludge, mostly on ashes, with chemical treatment such as sulfuric acid (*Takahashi et al.*, 2001), with microbial systems (*Tarayre et al.*, 2016) or thermochemical processes (*Herzel et al.*, 2016). In this study, the treatment tried to make the P contained in the sewage sludge again available and create a liquid fertilizer that can be used in plant nutrition, using enzymatic and chemical treatments combined. We have used two sludge samples, one from an anaerobic treatment plant and one from an aerobic one. The total P contained, which was 20 g kg^{-1} in both sludge, was available in water for only 0.7%. The sequential extraction of both the sludge, helped to understand the different P portion in the sludge.

The anaerobic sludge had a higher portion of P that is in the available fraction compared to the aerobic sludge, but both sludge samples attested their available fraction lower than a municipal solid waste compost used as an organic reference and a chemical P fertilizer. Another important difference is the higher amount in the AER sludge of P that is ligated to metals such as Fe, Al and Mn, which is soluble in the alkaline fraction. Furthermore, in alkaline soluble fraction also organic P compounds such as phosphonates and phosphate di- and tri-esters, deriving from detergents can be present. Their occurrence could be assessed with ^{31}NMR spectroscopy (*Turner et al.*, 2005). On the other hand, the portion extractable with HCl was lower in AER and lower in both sludge comparing to the municipal solid waste compost. This fraction contain P considered non labile and include P from hydroxyapatite (*Toor et al.*, 2005), from dicalcium phosphate not $NaHCO_3$ soluble, from struvite or phytate (*Takahashi*, 2013), but also from Mn (*Alborés et al.*, 2000; *Kidd et al.*, 2007). Both of the sludge

had also a sensible amount of P, which remained in the residual P portion, in which Ca phytase, Al and Fe phytase (*Ajiboye et al.*, 2007; *Toor et al.*, 2005) and P bonded to stable humic substance (*Ajiboye et al.*, 2007) are contained. This residual fraction accounted for more than 25% of the P concentration in the sludge. Thus, the efficiency in P recovery with enzymatic and chemical extraction can be predicted not higher of the 75% of the total P, due to the residual P fraction which can be extracted only with hot digestion with concentrated sulfuric acid. The other large difference between the sludge is the Al and Fe ratio. The anaerobic sludge contain almost double amount of Fe of the aerobic sludge, which, on the other hand, contain the double amount of Al compared to the anaerobic. This Al:Fe ratio (0.59 in ANA and 2.73 in AER) described also a difference in salts used to precipitate P in the wastewater treatment plants. In the extracted solution of the sequential extraction dissimilar amount of Al, Ca, Fe and Mg were found in the different pools. The most easily extractable bonds were the ones with Ca and Mg, which were released highly in the first extractions with water and sodium bicarbonate. Phosphorus in anaerobic sludge were not easily released from the Fe-P bonds, as we did not found high amount of Fe in the less strong extractant. Otherwise, the highest effect of Fe-P bonds were increasing the strength, with HCl extraction. Al-P bonds were even less easily to cut in both sludge, with a higher portion of Al that were not released at all, and a big portion which was release only with 96% of H_2SO_4. These differences between Al and Fe in extractability in sludge was found also in (*Alvarez et al.*, 2002), in which Al and Mg resulted the highest oxidable metals with Cu and Cr, whereas Co, Mn, Ni and Zn resulted in the exchangeable and reducible fraction and Fe and Pb remained as residual. Mn resulted soluble only with hydrochloric acid, in accordance with *Alborés et al.*, (2000) and *Kidd et al. (*2007). Most probably it was in the form of oxides and not ligated with P, due the high pH and the aerobic condition of the sludge, which did not promote the formation of Mn^{2+}, which is the only form of Mn that interacts with P (*Petronici*, 1989). This difference in Al and Fe concentration can explain also the differences in the hydrolysable P in the two sludge samples. The results of enzymatic extraction with acid phosphatase, which resulted always sensibly lower in AER sludge, can be explained with the high portion of alkaline soluble P in this sludge, which in addition contained phosphonates and P esters hardly soluble, but also with the high amount of Al, which can inhibit the acid phosphatase (*Domenech et al.*, 2015). The P release in ANA had higher efficiency when preceded by phytase or cellulase or mixed with them, probably because of both the effects of the enzyme and of the acidic pH (4.8), which is favourable for P release. On the contrary, such biochemical hydrolysis was not suitable to extract P from sludge AER, in which P remained not soluble. The comparison between hydrolysis methodology with similar P extraction efficiency make possible to choose the best extractant based on plant nutrient released in solution and potential toxic elements

concentration. Between phytase and cellulase the metals extraction, also did not give a clear suggestion for the best extractant. Although the P released is very similar, the other nutrient were a bit higher in phytase and acid phosphatase sequence. However, the phytase were also able to enhance the release of heavy metals slightly more than cellulase. In aerobic sludge, on the contrary, the sequence of phytase and acid phosphatase did not released high amounts of metals (potentially good or toxic), whereas cellulase and acid phosphatase had a similar effect of the phytase and alkaline phosphatase. These two treatments had a high recovery of Fe and also of Al. Both this elements are present in the P bonds that are represented in the NaOH soluble portion of the sequential extraction, which were larger in AER. The alkaline solution results were, thus, easily explainable with this high amount of Fe and Al bond; however, the alkaline buffer increased the release of Al and Fe, but it had not released the P, which were probably strongly bonded with other Al and Fe present.

The strong chemical treatment with sulfuric acid were able to release the P also from the aerobic sludge, arriving to the maximum efficiency of 93% of the 2 hours extraction. The strong treatment, however, released a high amount of potential toxic elements, among which Cd. The same effect was not detectable in anaerobic sludge, in which the P recovery were similar to enzymatic extraction or other organic acid extraction. Among the organic acid there was the most interesting results: citric acid sequenced but mostly mixed with acid phosphatase had rocketed the P release in anaerobic sludge. The mixed solution of citric acid and acid phosphatase released more than 80% of the total P, which was higher than the prevision and 80% more than the extraction with only citric acid for 2 hours. Thus, in this treatment, there were the great effect of the enzymatic extraction. The citric acid were able to break the strong bonds and activate the phosphatase hydrolysis.

Another evidence of the success of the enzymatic extraction after an organic acid is the great effect of the acid phosphatase after the maleic acid treatment at 121°C. A study (*Mosier et al.*, 2002) revealed that maleic acid at high temperature had a biomimetic catalytic effect and it the best candidate for cellulose and organic matter hydrolysis. This were not immediately remarkable in our results, but when the maleic acid at 121°C was followed by acid phosphatase, the P release were highly increased. This could be explained by the selectivity of the phosphatase which was able to release the P from the organic matter, only when the complex structure of this matter were already strongly degraded from the maleic acid.

The differences between the two sludge types were also evident in the total P results. The ICP analysis shown a P concentration that is often quite higher than the one measured with ascorbic acid method and that can be considered a total P content. AER sludge treated with acid phosphatase (after phytase or cellulose) or organic acid (citric

and malic) released a higher percentage of P, which were not detectable with the ascorbic acid method. This can be due to a different speciation of P released, which were not in the form of PO_4 , but in organic or complexes forms (such as phosphonates and P esters) which were higher in AER sludge according to the sequential extraction, or also to an interference of metals in the spectrophotometrically methods (*Nagul et al.*, 2015). The sulfuric acid treatments, instead, were able to mineralise the P in the extracts resulting in the totality in inorganic and available form.

The citric acid treatment followed or mixed with acid phosphatase of ANA sludge and the sulfuric acid treatment of AER sludge resulted the highest in P solubilisation, but also had higher solubilisation of Zn and in case of sulfuric acid also of Pb. This can be an indication that the formation or re-precipitation as new minerals (ZnP or PbP) does not occur in these treatments.

Mineral and organic acid treatment combined with enzymatic extraction reached up to 80% of P recovery without the need of extremely high temperature. Many studies used thermochemical treatment with temperature up to 1000°C to have an almost total P recovery and in some cases also the heavy metal removal with the use of different chlorine treatment (*Adam et al.*, 2008; *Mattenberger et al.*, 2008). Acid extraction was also much studied (*Biswas et al.*, 2009; *Donatello et al.*, 2010; *Ottosen et al.*, 2013; *Takahashi et al.*, 2001; *Xu et al.*, 2012). *Ottosen et al.* (2013) used sulfuric acid on two different sludge ashes rich in Al and Fe. Consistently with our results, the Al rich ash needed more acid to reach a good P recovery, thus the recommendation was to prefer Fe for P precipitation in wastewater treatment plants (*Ottosen et al.*, 2013). Furthermore, the optimum time extraction with H_2SO_4 was found to be 2 hours and the best liquid-to-solid ratio is 25 (*Ottosen et al.*, 2013). Thus, our results remained consistent on sulfuric acid extraction, but we had found that using one hour of citric or maleic acid and an hour of acid phosphatase in the same pH adjusted solution drastically decreased the release of heavy metal.

On the other hand, using sludge instead of ashes can reduce the greenhouse gasses emission. In our study, the technology was very simple, and in case of using technical grade enzymes, it could be also not extremely expensive. *Blöcher et al.* (2012), which was able to create a clean phosphoric acid with the use of low pressure wet oxidation and a consecutive nanofiltration, claimed that their technology could be comparable in term of cost to the sludge disposal. However, their technology did not worked on iron rich sludge, but only on sludge treated with Al and they also obtained a diluted solution (*Blöcher et al.*, 2012). Another study (*Xu et al.*, 2012), after the extraction with acid (HCl in this case), tried a cation exchange resins for the removal of heavy metal and a consecutive precipitation as struvite. Hence, further developments of the solutions created could be the evaporation of part of the liquid, or the precipitation as struvite or

trying to use a lower amount of liquid for the extraction. These improvements should increase the P concentration in the fertilizer, in the point of view of a circular economy and nutrient recycling.

References

Achat, D.L., Bakker, M.R., Zeller, B., Pellerin, S., Bienaimé, S., Morel, C. (2010): Long-term organic phosphorus mineralization in Spodosols under forests and its relation to carbon and nitrogen mineralization. *Soil Biol. Biochem.* 42, 1479–1490.

Adam, C., Peplinski, B., Michaelis, M., Kley, G., Simon, F.-G. (2008): Thermochemical treatment of sewage sludge ashes for phosphorus recovery. *Waste Manag.* 29, 1122–1128.

Adesemoye, A.O., Kloepper, J.W. (2009): Plant–microbes interactions in enhanced fertilizer-use efficiency. *Appl. Microbiol. Biotechnol.* 85, 1–12.

AEEP (2017): Phosphorus: Key to Plant Life [WWW Document]. URL http://aeep.eu/phosphorus/ (accessed 11.29.17).

Ajiboye, B., Akinremi, O.O., Hu, Y., Flaten, D.N. (2007): Phosphorus Speciation of Sequential Extracts of Organic Amendments Using Nuclear Magnetic Resonance and X-ray Absorption Near-Edge Structure Spectroscopies. *J. Environ. Qual.* 36, 1563.

Alborés, A.F., Cid, B.P., Gómez, E.F., López, E.F. (2000): Comparison between sequential extraction procedures and single extractions for metal partitioning in sewage sludge samples. *Analyst* 125, 1353–1357.

Alvarenga, P., Mourinha, C., Farto, M., Santos, T., Palma, P., Sengo, J., Morais, M.-C., Cunha-Queda, C. (2015): Sewage sludge, compost and other representative organic wastes as agricultural soil amendments: Benefits versus limiting factors.

Alvarez, A.E., Mochon, C.M., Sanchez, J.C., Rodriguez, T.M. (2002): Heavy metal extractable forms in sludge from wastewater treatment plants. *Chemosphere* 47, 765–775.

Andersen, M.S. (2007): An introductory note on the environmental economics of the circular economy. *Sustain. Sci.* 2, 133–140.

Ayaga, G., Todd, A., Brookes, P.C. (2006): Enhanced biological cycling of phosphorus increases its availability to crops in low-input sub-Saharan farming systems. *Soil Biol. Biochem.* 38, 81–90.

Baek, D., Kim, M.C., Chun, H.J., Kang, S., Park, H.C., Shin, G., Park, J., Shen, M., Hong, H., Kim, W.-Y., Kim, D.H., Lee, S.Y., Bressan, R.A., Bohnert, H.J., Yun, D.-J. (2013): Regulation of miR399f Transcription by AtMYB2 Affects Phosphate Starvation Responses in Arabidopsis. *Plant Physiol.* 161, 362–373.

Baldwin, J.C., Karthikeyan, A.S., Raghothama, K.G. (2001): LEPS2, a phosphorus starvation-induced novel acid phosphatase from tomato. *Plant Physiol.* 125, 728–37.

Bates, T.R., Lynch, J.P. (1996): Stimulation of root hair elongation in Arabidopsis thaliana by low phosphorus availability. *Plant, Cell Environ.* 19, 529–538.

Becana, M., Moran, J.F., Iturbe-Ormaetxe, I. (1998): Iron-dependent oxygen free radical generation in plants subjected to environmental stress: Toxicity and antioxidant protection. *Plant Soil* 201, 137–147.

Becker, M., Asch, F. (2005): Iron toxicity in rice-conditions and management concepts. *J. Plant Nutr. Soil Sci.* 168, 558–573.

Bernal, M.P., Alburquerque, J.A., Moral, R. (2009): Composting of animal manures and chemical criteria for compost maturity assessment. A review. *Bioresour. Technol.* 100, 5444–5453.

Bhadoria, P.S., Steingrobe, B., Claassen, N., Liebersbach, H. (2002): Phosphorus efficiency of wheat and sugar beet seedlings grown in soils with mainly calcium, or iron and aluminium phosphate. *Plant Soil* 246, 41–52.

Biswas, B.K., Inoue, K., Harada, H., Ohto, K., Kawakita, H. (2009): Leaching of phosphorus from incinerated sewage sludge ash by means of acid extraction followed by adsorption on orange waste gel. *J. Environ. Sci.* 21, 1753–1760.

Blöcher, C., Niewersch, C., Melin, T. (2012): Phosphorus recovery from sewage sludge with a hybrid process of low pressure wet oxidation and nanofiltration. *Water Res.* 46, 2009–2019.

Bolan, N.S. (1991): A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. *Plant Soil*.

Breeuwsma, A., Reijerink, J.G.A., Schoumans, O.F. (1995): Impact of manure on accumulation and leaching of phosphate in areas of intensive livestock farming, in: Animal Waste and the Land-Water Interface. pp. 239–249.

Brod, E., Øgaard, A.F., Hansen, E., Wragg, D., Haraldsen, T.K., Krogstad, T. (2015): Waste products as alternative phosphorus fertilisers part I: inorganic P species affect fertilisation effects depending on soil pH. *Nutr. Cycl. Agroecosystems* 103, 167–185.

Brookes, P., Beyaert, R., Voroney, R. (2007): Soil Microbial Biomass C, N, P, and S, in: Soil Sampling and Methods of Analysis, Second Edition. CRC Press.

Brookes, P.C., Powlson, D.S., Jenkinson, D.S. (1982): Measurement of microbial biomass phosphorus in soil. *Soil Biol. Biochem.* 14, 319–329.

Brown, T.A., Shrift, A. (1982): SELENIUM: TOXICITY AND TOLERANCE IN HIGHER PLANTS. *Biol. Rev.* 57, 59–84.

Bünemann, E.K. (2015): Assessment of gross and net mineralization rates of soil organic phosphorus – A review. *Soil Biol. Biochem.* 89, 82–98.

Burleigh, S.H., Cavagnaro, T., Jakobsen, I. (2002): Functional diversity of arbuscular mycorrhizas extends to the expression of plant genes involved in P nutrition. *J. Exp. Bot.* 53, 1593–1601.

Cakmak, I., Hengeler, C., Marschner, H. (1994): Partitioning of shoot and root dry matter and carbohydrates in bean plants suffering from phosphorus, potassium and magnesium deficiency. *J. Exp. Bot.* 45, 1245–1250.

CCIA (2016): Prezzi - News — Camera di Commercio di Modena [WWW Document]. URL http://www.mo.camcom.it/tutela-del-mercato/ufficio-prezzi (accessed 11.9.16).

Chen, C.R., Condron, L.M., Davis, M.R., Sherlock, R.R. (2002): Phosphorus dynamics in the rhizosphere of perennial ryegrass (Lolium perenne L.) and radiata pine

(Pinus Radiata D. Don.). *Soil Biol. Biochem.* 34, 487–499.

Childers, D.L., Corman, J., Edwards, M., Elser, J.J. (2011): Sustainability Challenges of Phosphorus and Food: Solutions from Closing the Human Phosphorus Cycle. *Bioscience* 61, 117–124.

Christodoulou, A., Stamatelatou, K. (2016): Overview of legislation on sewage sludge management in developed countries worldwide. *Water Sci. Technol.* 73.

Cieslik, B., Konieczka, P. (2017): A review of phosphorus recovery methods at various steps of wastewater treatment and sewage sludge management. The concept of " no solid waste generation " and analytical methods. *J. Clean. Prod.* 142, 1728–1740.

Clarkson, D.T., Scattergood, C.B. (1982): Growth and Phosphate Transport in Barley and Tomato Plants During the Development of, and Recovery from, Phosphate-stress. *J. Exp. Bot.* 33, 865–875.

Cogliatti, D.H., Santa Marìa, G.E. (1990): Influx and Efflux of Phosphorus in Roots of Wheat Plants in Non-Growth-Limiting Concentrations of Phosphorus. *J. Exp. Bot.* 41, 601–607.

Cooper, J., Lombardi, R., Boardman, D., Carliell-Marquet, C. (2011): The future distribution and production of global phosphate rock reserves. *Resour. Conserv. Recycl.* 57, 78–86.

Cordell, D., Drangert, J., White, S. (2009): The story of phosphorus: global food security and food for thought. *Glob. Environ. Chang.*

Cordell, D., Rosemarin, A., Schroder, J.J., Smit, A.L. (2011): Towards global phosphorus security: A systems framework for phosphorus recovery and reuse options. *Chemosphere* 84, 747–758.

Corominas, L., Foley, J., Guest, J.S., Hospido, A., Larsen, H.F., Morera, S., Shaw, A. (2013): Life cycle assessment applied to wastewater treatment: State of the art. *Water Res.* 47, 5480–5492.

Da Ros, C., Cavinato, C., Pavan, P., Bolzonella, D. (2016): Mesophilic and thermophilic anaerobic co-digestion of winery wastewater sludge and wine lees: An integrated approach for sustainable wine production. *J. Environ. Manage.*

Dakora, F.D., Phillips, D.A. (2002): Root exudates as mediators of mineral acquisition in low-nutrient environments. *Plant Soil* 245, 35–47.

Delhaize, E., Randall, P.J., Laurie, S., Phillips, R., Ratcliffe, R., Stewart, G. (1995): Characterization of a Phosphate-Accumulator Mutant of Arabidopsis thaliana. *Plant Physiol.* 107, 207–213.

Direzione Generale Ambiente, D. del suolo e della costa-D.A.R.E.-R. (2004): Linee di Intervento in materia di utilizzo dei fanghi di depurazione in agricoltura. Bologna.

Domenech, C.E., Lisa, T.A., Salvano, M.A., Garrido, M.N. (2015): Selective release of phosphorus and nitrogen from waste activated sludge with combined thermal and alkali treatment. *Bioresour. Technol.* 190, 522–528.

Donatello, S., Tong, D., Cheeseman, C.R. (2010): Production of technical grade phosphoric acid from incinerator sewage sludge ash (ISSA). *Waste Manag.* 30,

1634–1642.

Doyle, J.D., Parsons, S.A. (2002): Struvite formation, control and recovery. *Water Res.* 36, 3925–3940.

Duboc, O., Santner, J., Fard, A.G., Zehetner, F., Tacconi, J., Wenzel, W.W. (2017): Predicting phosphorus availability from chemically diverse conventional and recycling fertilizers. *Sci. Total Environ.* 599–600, 1160–1170.

Easlon, H.M., Bloom, A.J. (2014): Easy Leaf Area: Automated Digital Image Analysis for Rapid and Accurate Measurement of Leaf Area. *Appl. Plant Sci.* 2, 1400033.

Eghball, B. (2002): Soil Properties as Influenced by Phosphorus-and Nitrogen-Based Manure and Compost Applications WASTE MANAGEMENT Soil Properties as Influenced by Phosphorus-and Nitrogen-Based Manure and Compost Applications. *Waste Manag.* 94, 128–135.

Eghball, B., Power, J.F. (1999): Phosphorus- and Nitrogen-Based Manure and Compost Applications: Corn Production and Soil Phosphorus. *Soil Sci. Soc. Am. J.* 63, 895–901.

Ellis, D.R., Salt, D.E. (2003): Plants, selenium and human health. *Curr. Opin. Plant Biol.*

Eneji, A.E., Honna, T., Yamamoto, S., Masuda, T., Endo, T., Irshad, M. (2003): Changes in Humic Substances and Phosphorus Fractions During Composting. *Commun. Soil Sci. Plant Anal.* 34, 2303–2314.

European Biogas Association (2015): EBA Biomethane & Biogas Report 2015 [WWW Document]. URL http://european-biogas.eu/2015/12/16/biogasreport2015/ (accessed 11.9.16).

Eurostat (2016): Eurostat [WWW Document]. URL http://ec.europa.eu/eurostat/web/main/home (accessed 11.9.16).

Ewa Sroga, G. (1987): Plant regeneration of two Lupinus spp. from callus cultures via organogenesis. *Plant Sci.* 51, 245–249.

FAO (2016): FAOSTAT [WWW Document]. URL http://www.fao.org/faostat/en/#home (accessed 12.2.17).

Feng, R., Wei, C., Tu, S. (2013): The roles of selenium in protecting plants against abiotic stresses. *Environ. Exp. Bot.*

Fertilisers Working Group Meeting (2014): Essential safety and quality requirements for fertilising materials.

Foehse, D., Jungk, A. (1983): Influence of phosphate and nitrate supply on root hair formation of rape, spinach and tomato plants. *Plant Soil* 74, 359–368.

Fredeen, A., Rao, I., Terry, N. (1989): Influence of phosphorus nutrition on growth and carbon partitioning in Glycine max. *Plant Physiol.*

Frossard, E., Condron, L.M., Oberson, A., Sinaj, S., Fardeau, J.C. (2000): Processes Governing Phosphorus Availability in Temperate Soils. *J. Environ. Qual.* 29, 15.

Frossard, E., Sinaj, S., Zhang, L.-M., Morel, J.L. (1996): The Fate of Sludge

Phosphorus in Soil-Plant Systems. *Soil Sci. Soc. Am. J.* 60, 1248.

Fuentes, B., Bolan, N., Naidu, R., de la Luz Mora, M., Australia, A. (2006): PHOSPHORUS IN ORGANIC WASTE-SOIL SYSTEMS Fósforo en los sistemas suelo – residuos orgánicos. *J.Soil Sc. Plant. Nutr* 66, 64–8364.

García-Albacete, M., Martín, A., Cartagena, M.C. (2012): Fractionation of phosphorus biowastes: Characterisation and environmental risk. *Waste Manag.* 32, 1061–1068.

Gianquinto, G., Orsini, F., Sambo, P., D'Urzo, M.P. (2011): The Use of Diagnostic Optical Tools to Assess Nitrogen Status and to Guide Fertilization of Vegetables. *Horttechnology* 21, 287–292.

Gonzalez-Ponce, R., López-de-Sá, E.G., Plaza, C. (2009): Lettuce Response to Phosphorus Fertilization with Struvite Recovered from Municipal Wastewater. *HortScience* 44, 426–430.

Grigatti, M., Boanini, E., Cavani, L., Ciavatta, C., Marzadori, C. (2015): Phosphorus in Digestate-Based Compost: Chemical Speciation and Plant-Availability. *Waste and Biomass Valorization* 6, 481–493.

Grigatti, M., Boanini, E., Mancarella, S., Simoni, A., Centemero, M., Veeken, A.H.M. (2017): Phosphorous extractability and ryegrass availability from bio-waste composts in a calcareous soil. *Chemosphere* 174, 722–731.

Grigatti, M., Di Girolamo, G., Chincarini, R., Ciavatta, C., Barbanti, L. (2011): Potential nitrogen mineralization, plant utilization efficiency and soil CO2 emissions following the addition of anaerobic digested slurries. *Biomass and Bioenergy* 35, 4619–4629.

Grusak, M. a., DellaPenna, D. (1999): Improving the Nutrient Composition of Plants To Enhance Human Nutrition and Health1. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 50, 133–161.

Guppy, C., Menzies, N., Moody, P., Blamey, F. (2005): Competitive sorption reactions between phosphorus and organic matter in soil: a review. *Soil Res.*

Haug, R.T. (1993): The practical handbook of compost engineering. Lewis Publishers.

Heimersson, S., Harder, R., Peters, G.M., Svanström, M. (2014): Including pathogen risk in life cycle assessment of wastewater management. 2. Quantitative comparison of pathogen risk to other impacts on human health. *Environ. Sci. Technol.* 48, 9446–9453.

Herzel, H., Kruger, O., Hermann, L., Adam, C. (2016): Sewage sludge ash - A promising secondary phosphorus source for fertilizer production. *Sci. Total Environ.* 542, 1136–1143.

Hinsinger, P. (2001): Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. *Plant Soil* 237, 173–195.

Hooda, P.S., Truesdale, V.W., Edwards, A.C., Withers, P.J.A., Aitken, M.N., Miller, A., Rendell, A.R. (2001): Manuring and fertilization effects on phosphorus accumulation in soils and potential environmental implications. *Adv. Environ. Res.* 5, 13–21.

Hospido, A., Moreira, M.T., Feijoo, G. (2008): A comparison of municipal wastewater treatment plants for big centres of population in Galicia (Spain). *Int. J. Life Cycle Assess.* 13, 57–64.

Hukari, S., Hermann, L., Nättorp, A. (2016): From wastewater to fertilisers — Technical overview and critical review of European legislation governing phosphorus recycling. *Sci. Total Environ.* 542, 1127–1135.

Hunter, P.J., Teakle, G.R., Bending, G.D. (2014): Root traits and microbial community interactions in relation to phosphorus availability and acquisition, with particular reference to Brassica. *Front. Plant Sci.* 5, 27.

Indexmundi (2016): DAP fertilizer - Monthly Price - Commodity Prices - Price Charts, Data, and News - IndexMundi [WWW Document]. URL http://www.indexmundi.com/commodities/?commodity=dap-fertilizer&months=180 (accessed 11.9.16).

Jaffer, Y., Clark, T.A., Pearce, P., Parsons, S.A. (2002): Potential phosphorus recovery by struvite formation. *Water Res.* 36, 1834–1842.

Jeng, A.S., Haraldsen, T.K., Grønlund, A., Pedersen, P.A. (2006): Meat and bone meal as nitrogen and phosphorus fertilizer to cereals and rye grass. *Nutr. Cycl. Agroecosystems* 76, 183–191.

Johri, A.K., Oelmüller, R., Dua, M., Yadav, V., Kumar, M., Tuteja, N., Varma, A., Bonfante, P., Persson, B.L., Stroud, R.M. (2015): Fungal association and utilization of phosphate by plants: success, limitations, and future prospects. *Front. Microbiol.* 6, 984.

Jones, D.L. (1998): Organic acids in the rhizosphere – a critical review. *Plant Soil* 205, 25–44.

Kabata-Pendias, A. (2011): Trace elements in soils and plants. Fourth Edition, CRC Press.

Kataki, S., West, H., Clarke, M., Baruah, D.C. (2016): Phosphorus recovery as struvite: Recent concerns for use of seed, alternative Mg source, nitrogen conservation and fertilizer potential. *Resour. Conserv. Recycl.* 107, 142–156.

Khan, A., Jilani, G., Akhtar, M.S., Naqvi, S.M.S., Rasheed, M., Khan, A.A., Jilani, G., Akhtar, M.S., Muhammad, S., Naqvi, S., Rasheed, M. (2009): Phosphorus Solubilizing Bacteria: Occurrence, Mechanisms and their Role in Crop Production. *Publ. J. agric. biol. sci* 1, 48–58.

Khan, K.S., Joergensen, R.G. (2012a): Relationships between P fractions and the microbial biomass in soils under different land use management. *Geoderma* 173, 274–281.

Khan, K.S., Joergensen, R.G. (2012b): Compost and phosphorus amendments for stimulating microorganisms and growth of ryegrass in a Ferralsol and a Luvisol. *J. Plant Nutr. Soil Sci.* 175, 108–114.

Kidd, P.S., Domínguez-Rodríguez, M.J., Díez, J., Monterroso, C. (2007): Bioavailability and plant accumulation of heavy metals and phosphorus in agricultural soils amended by long-term application of sewage sludge. *Chemosphere* 66, 1458–1467.

Kim, M., Han, D.W., Kim, D.J. (2015): Selective release of phosphorus and nitrogen from waste activated sludge with combined thermal and alkali treatment. *Bioresour. Technol.*

Kleinman, P.J.A., Sharpley, A.N., Wolf, A.M., Beegle, D.B., Moore, P.A. (2002): Measuring Water-Extractable Phosphorus in Manure as an Indicator of Phosphorus in Runoff. *Soil Sci. Soc. Am. J.* 66, 2009.

Kochian, L. V (1995): Cellular Mechanisms of Aluminum Toxicity and Resistance in Plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 46, 237–260.

Koppelaar, R.H.E.M., Weikard, H.P. (2013): Assessing phosphate rock depletion and phosphorus recycling options. *Glob. Environ. Chang.* 23, 1454–1466.

Krüger, O., Adam, C. (2015): Recovery potential of German sewage sludge ash. *Waste Manag.* 45, 400–406.

Kumar, V., Gilkes, R.J., Bolland, M.D.A. (1992): A comparison of seven soil P tests for plant species with different external P requirements grown on soils containing rock phosphate and superphosphate residues. *Fertil. Res.* 33, 35–45.

Lambers, H., Brundrett, M.C., Raven, J.A., Hopper, S.D. (2011): Plant mineral nutrition in ancient landscapes: high plant species diversity on infertile soils is linked to functional diversity for nutritional strategies. *Plant Soil* 348, 7–27.

Laturnus, F., von Arnold, K., Grøn, C. (2007): Organic Contaminants from Sewage Sludge Applied to Agricultural Soils. False Alarm Regarding Possible Problems for Food Safety? (8 pp). *Environ. Sci. Pollut. Res. Int.* 14 Suppl 1, 53–60.

Leite, W.R.M., Gottardo, M., Pavan, P., Belli Filho, P., Bolzonella, D. (2016): Performance and energy aspects of single and two phase thermophilic anaerobic digestion of waste activated sludge. *Renew. Energy* 86, 1324–1331.

Leytem, A.B., Westermann, D.T. (2003): PHOSPHATE SORPTION BY PACIFIC NORTHWEST CALCAREOUS SOILS. *Soil Sci.* 168.

Leytem, A.B., Westermann, D.T. (2005): PHOSPHORUS AVAILABILITY TO BARLEY FROM MANURES AND FERTILIZERS ON A CALCAREOUS SOIL. *Soil* 170, 401–412.

Liang, Y., Sun, W., Zhu, Y.G., Christie, P. (2007): Mechanisms of silicon-mediated alleviation of abiotic stresses in higher plants: A review. *Environ. Pollut.* 147, 422–428.

López-Arredondo, D.L., Leyva-González, M.A., Alatorre-Cobos, F., Herrera-Estrella, L. (2013): Biotechnology of nutrient uptake and assimilation in plants. *Int. J. Dev. Biol.* 57, 595–610.

Lynch, J., Läuchli, A., Epstein, E. (1991): Vegetative Growth of the Common Bean in Response to Phosphorus Nutrition. *Crop Sci.* 31, 380.

Ma, J.F. (2004): Role of silicon in enhancing the resistance of plants to biotic and abiotic stresses. *Soil Sci. Plant Nutr.* 50, 11–18.

Ma, Z., Bielenberg, D.G., Brown, K.M., Lynch, J.P. (2001): Regulation of root hair density by phosphorus availability in Arabidopsis thaliana. *Plant, Cell Environ.* 24, 459–467.

Malik, M., Khan, K., Marschner, P. (2013): Microbial biomass, nutrient availability and nutrient uptake by wheat in two soils with organic amendments. *J. Soil Sci. Plant Nutr.* 13, 955–966.

Mamani, I., Averanga, Kattia, Boris, E., Terrazas, E. (2014): Establecimiento del sistema de generación in vitro de callos de Lupinus mutabilis sweet (tarwi) a partir de hipocótilos. *Rev. CON-CIENCIA* 2.

Marchetti, R., Castelli, F., Orsi, A., Sghedoni, L., Bochicchio, D. (2012): Biochar from swine manure solids: influence on carbon sequestration and Olsen phosphorus and mineral nitrogen dynamics in soil with and without digestate incorporation. *Ital. J. Agron.* 7, 26.

Marschner, H., Kirkby, E.A., Cakmak, I. (1996): Effect of mineral nutritional status on shoot-root partitioning of photoassimilates and cycling of mineral nutrients. *J. Exp. Bot.* 47, 1255–1263.

Marschner, H., Marschner, P. (2012): Marschner's mineral nutrition of higher plants. Academic Press.

Mattenberger, H., Fraissler, G., Brunner, T., Herk, P., Hermann, L., Obernberger, I. (2008): Sewage sludge ash to phosphorus fertiliser: Variables influencing heavy metal removal during thermochemical treatment. *Waste Manag.* 28, 2709–2722.

Mayer, B.K., Baker, L.A., Boyer, T.H., Drechsel, P., Gifford, M., Hanjra, M.A., Parameswaran, P., Stoltzfus, J., Westerhoff, P., Rittmann, B.E. (2016): Total Value of Phosphorus Recovery. *Environ. Sci. Technol.* 50, 6606–6620.

McBride, M., Sauve, S., Hendershot, W. (1997): Solubility control of Cu, Zn, Cd and Pb in contaminated soils. *Eur. J. Soil Sci.* 48, 337–346.

McCoy, J.L., Sikora, L.J., Weil, R.R. (1986): Plant Availability of Phosphorus in Sewage Sludge Compost1. *J. Environ. Qual.* 15, 403.

Møller, J., Boldrin, A., Christensen, T.H. (2009): Anaerobic digestion and digestate use: accounting of greenhouse gases and global warming contribution. *Waste Manag. Res.* 27, 813–824.

Möller, K., Müller, T. (2012): Effects of anaerobic digestion on digestate nutrient availability and crop growth: A review. *Eng. Life Sci.* 12, 242–257.

Mosier, N.S., Ladisch, C.M., Ladisch, M.R. (2002): Characterization of acid catalytic domains for cellulose hydrolysis and glucose degradation. *Biotechnol. Bioeng.* 79, 610–618.

Muhammad, S., Müller, T., Joergensen, R.G. (2006): Decomposition of pea and maize straw in Pakistani soils along a gradient in salinity. *Biol. Fertil. Soils* 43, 93–101.

Murphy, J., Riley, J.P. (1962): A modified single solution method for the determination of phosphate in natural waters. *Anal. Chim. Acta* 27, 31–36.

Nagul, E.A., McKelvie, I.D., Worsfold, P., Kolev, S.D. (2015): The molybdenum blue reaction for the determination of orthophosphate revisited: Opening the black box. *Anal. Chim. Acta*.

Nannipieri, P., Giagnoni, L., Landi, L., Renella, G. (2011): Role of Phosphatase Enzymes in Soil, in: Phosphorus in Action. Springer, Berlin, Heidelberg, pp. 215–

243.

Neumann, G. (2010): Mining for nutrients - regulatory aspects of cluster root function and development. *New Phytol.* 187, 879–882.

Neumann, G. (2016): The Role of Ethylene in Plant Adaptations for Phosphate Acquisition in Soils – A Review. *Front. Plant Sci.* 6, 1224.

Neumann, G., Martinoia, E. (2002): Cluster roots – an underground adaptation for survival in extreme environments. *Trends Plant Sci.* 7, 162–167.

Nkebiwe, P.M., Weinmann, M., Bar-Tal, A., Müller, T. (2016): Fertilizer placement to improve crop nutrient acquisition and yield: A review and meta-analysis. *F. Crop. Res.* 196, 389–401.

Øgaard, A.F. (1996): Effect of Phosphorus Fertilization on the Concentration of Total and Algal-available Phosphorus in Different Particle-size Fractions in Norwegian Agricultural Soils. *Acta Agric. Scand. Sect. B - Soil Plant Sci.* 46, 24–29.

Olsen, S.R. (1954): Estimation Of Available Phosphorus In Soils By Extraction With Sodium Bicarbonate.

Ottosen, L.M., Kirkelund, G.M., Jensen, P.E. (2013): Extracting phosphorous from incinerated sewage sludge ash rich in iron or aluminum. *Chemosphere* 91, 963–969.

Pant, H.K., Warman, P.R. (2000): Enzymatic hydrolysis of soil organic phosphorus by immobilized phosphatases. *Biol. Fertil. Soils* 30, 306–311.

Pasqualino, J.C., Meneses, M., Abella, M., Castells, F. (2009): LCA as a Decision Support Tool for the Environmental Improvement of the Operation of a Municipal Wastewater Treatment Plant. *Environ. Sci. Technol.* 43, 3300–3307.

Pastor, L., Marti, N., Bouzas, A., Seco, A. (2008): Sewage sludge management for phosphorus recovery as struvite in EBPR wastewater treatment plants. *Bioresour. Technol.* 99, 4817–4824.

Petronici, C. (1989): Gli elementi nutritivi, in: Paolo Sequi (ed.): Chimica Del Suolo. Patron Editore, Bologna, pp. 357–380.

Pniewski, T., Kapusta, J., Płucienniczak, A. (2006): Agrobacterium-mediated transformation of yellow lupin to generate callus tissue producing HBV surface antigen in a long-term culture. *J. Appl. Genet.* 47, 309–318.

Polowick, P.L., Loukanina, N.N., Doshi, K.M. (2014): Agrobacterium-mediated transformation of tarwi (Lupinus mutabilis Sweet), a potential platform for the production of plant-made proteins. *Vitr. Cell. Dev. Biol. - Plant* 50, 401–411.

Pote, D.H., Daniel, T.C., Sharpley, A.N., Moore, P.A., Edwards, D.R., Nichols, D.J. (1996): Relating Extractable Soil Phosphorus to Phosphorus Losses in Runoff t'. *Soil Sci. Soc. Am. J.* 60, 855–859.

Raghothama, K.G. (1999): PHOSPHATE ACQUISITION. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 50, 665–693.

Rahman, M.M., Salleh, M.A.M., Rashid, U., Ahsan, A., Hossain, M.M., Ra, C.S. (2014): Production of slow release crystal fertilizer from wastewaters through

struvite crystallization – A review. *Arab. J. Chem.* 7, 139–155.

Reijnders, L. (2014): Phosphorus resources, their depletion and conservation, a review. *Resour. Conserv. Recycl.*

Richardson, A.E., Simpson, R.J. (2011): Soil microorganisms mediating phosphorus availability update on microbial phosphorus. *Plant Physiol.* 156, 989–96.

Russo, A. (2012): ANALISI E MIGLIORAMENTO DELL'IMPIANTO DI DEPURAZIONE DI LEGNAGO. Università degli Studi di Padova.

Shannon, M.C., Grieve, C.M. (1999): Tolerance of vegetable crops to salinity. *Sci. Hortic. (Amsterdam).* 78, 5–38.

Sharpley, A., Moyer, B. (2000): Phosphorus Forms in Manure and Compost and Their Release during Simulated Rainfall. *J. Environ. Qual.* 29, 1462.

Sharpley, A.N., Chapra, S.C., Wedepohl, R., Sims, J.T., Daniel, T.C., Reddy, K.R. (1994): Managing Agricultural Phosphorus for Protection of Surface Waters: Issues and Options. *J. Environ. Qual.* 23, 437.

Shen, J., Yuan, L., Zhang, J., Li, H., Bai, Z., Chen, X., Zhang, W., Zhang, F. (2011): Phosphorus dynamics: from soil to plant. *Plant Physiol.* 156, 997–1005.

Shenoy, V. V., Kalagudi, G.M. (2005): Enhancing plant phosphorus use efficiency for sustainable cropping. *Biotechnol. Adv.* 23, 501–513.

Siddique, M.T., Robinson, J.S. (2003): Phosphorus Sorption and Availability in Soils Amended with Animal Manures and Sewage Sludge. *J. Environ. Qual.* 32, 1114.

Simpson, M., McLenaghen, R.D., Chirino-Valle, I., Condron, L.M. (2012): Effects of long-term grassland management on the chemical nature and bioavailability of soil phosphorus. *Biol. Fertil. Soils* 48, 607–611.

Sims, J.T., Simard, R.R., Joern, B.C. (1998): Phosphorus Loss in Agricultural Drainage: Historical Perspective and Current Research. *J. Environ. Qual.* 27, 277.

Smith, K.A., Van Dijk, T.A. (1987): Utilisation of phosphorus and potassium from animal manures on grassland and forage crops, in: Animal Manure on Grassland and Fodder Crops. Fertilizer or Waste? Springer Netherlands, Dordrecht, pp. 87–102.

Smith, S.R. (2009): A critical review of the bioavailability and impacts of heavy metals in municipal solid waste composts compared to sewage sludge. *Environ. Int.* 35, 142–156.

Sonneveld, C., Straver, N. (1994): Nutrient solutions for vegetables and flowers grown in water or substrates, Tenth. ed.

Stewart, J.W.B., Tiessen, H. (1987): Dynamics of soil organic phosphorus. *Biogeochemistry* 4, 41–60.

Suh, Y.J., Rousseaux, P. (2002): An LCA of alternative wastewater sludge treatment scenarios. *Resour. Conserv. Recycl.* 35, 191–200.

Syers, D.J., Johnston, A. (2008): Efficiency of soil and fertilizer phosphorus use Reconciling changing concepts of soil phosphorus behaviour with agronomic

information. *FAO Fertil. Plant Nutr. Bull.*

Tabatabai, M.A., Bremner, J.M. (1969): Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biol. Biochem.* 1, 301–307.

Takahashi, M., Kato, S., Shima, H., Sarai, E., Ichioka, T., Hatyakawa, S., Miyajiri, H. (2001): Technology for recovering phosphorus from incinerated wastewater treatment sludge, in: Chemosphere. pp. 23–29.

Takahashi, S. (2013): Phosphorus characterization of manure composts and combined organic fertilizers by a sequential-fractionation method. *J. Plant Nutr. Soil Sci.* 176, 494–496.

Talboys, P.J., Heppell, J., Roose, T., Healey, J.R., Jones, D.L., Withers, P.J.A. (2016): Struvite: a slow-release fertiliser for sustainable phosphorus management? *Plant Soil* 401, 109–123.

Tang, X., Li, J., Ma, Y., Hao, X., Li, X. (2008): Phosphorus efficiency in long-term (15 years) wheat–maize cropping systems with various soil and climate conditions. *F. Crop. Res.* 108, 231–237.

Tarayre, C., De Clercq, L., Charlier, R., Michels, E., Meers, E., Camargo-Valero, M., Delvigne, F. (2016): New perspectives for the design of sustainable bioprocesses for phosphorus recovery from waste. *Bioresour. Technol.*

Tejada, M., García-Martínez, A.M., Rodríguez-Morgado, B., Carballo, M., García-Antras, D., Aragón, C., Parrado, J. (2013): Obtaining biostimulant products for land application from the sewage sludge of small populations. *Ecol. Eng.* 50, 31–36.

Toor, G.S., Peak, J.D., Sims, J.T. (2005): Phosphorus Speciation in Broiler Litter and Turkey Manure Produced from Modified Diets. *J. Environ. Qual.* 34, 687.

Torri, S.I., Correa, R.S., Renella, G. (2017): Biosolid Application to Agricultural Land—a Contribution to Global Phosphorus Recycle: A Review. *Pedosphere* 27, 1–16.

Turner, B.L., Cade-Menun, B.J., Condron, L.M., Newman, S. (2005): Extraction of soil organic phosphorus. *Talanta* 66, 294–306.

Turner, B.L., McKelvie, I.D., Haygarth, P.M. (2002): Characterisation of water-extractable soil organic phosphorus by phosphatase hydrolysis. *Soil Biol. Biochem.* 34, 27–35.

Uhde-Stone, C., Liu, J., Zinn, K.E., Allan, D.L., Vance, C.P. (2005): Transgenic proteoid roots of white lupin: a vehicle for characterizing and silencing root genes involved in adaptation to P stress. *Plant J.* 44, 840–853.

Uysal, A., Dilsad Yilmazel, Y., Demirer, G.N. (2010): The determination of fertilizer quality of the formed struvite from effluent of a sewage sludge anaerobic digester. *J. Hazard. Mater.* 181, 248–254.

Vance, C.P. (2001): Symbiotic Nitrogen Fixation and Phosphorus Acquisition. Plant Nutrition in a World of Declining Renewable Resources. *Plant Physiol.* 127.

Vance, C.P., Uhde-Stone, C., Allan, D.L. (2003): Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. *New Phytol.*

157, 423–447.

Veneklaas, E.J., Lambers, H., Bragg, J., Finnegan, P.M., Lovelock, C.E., Plaxton, W.C., Price, C.A., Scheible, W.-R., Shane, M.W., White, P.J., Raven, J.A. (2012): Opportunities for improving phosphorus-use efficiency in crop plants. *New Phytol.* 195, 306–320.

Walan, P., Davidsson, S., Johansson, S., Höök, M. (2014): Phosphate rock production and depletion: Regional disaggregated modeling and global implications. *Resour. Conserv. Recycl.* 93, 178–187.

Wang, Z., Straub, D., Yang, H., Kania, A., Shen, J., Ludewig, U., Neumann, G. (2014): The regulatory network of cluster-root function and development in phosphate-deficient white lupin (*Lupinus albus*) identified by transcriptome sequencing. *Physiol. Plant.* 151, 323–338.

Wellinger, A., Murphy, J., Baxter, D. (2013): The biogas handbook : science, production and applications.

Wheeler, B.D., Al-Farraj, M.M., Cook, R.E.D. (1985): Iron toxicity to plants in base-rich wetlands: comparative effects on the distribution and growth of Epilobium hirsutum l. and Juncus subnodulosus Schrank. *New Phytol.* 100, 653–669.

White, P.J., Brown, P.H. (2010): Plant nutrition for sustainable development and global health. *Ann. Bot.* 105, 1073–1080.

Wollmann, I., Gauro, A., Müller, T., Möller, K. (2017): Phosphorus bioavailability of sewage sludge-based recycled fertilizers. *J. Plant Nutr. Soil Sci.*

Xu, H., He, P., Gu, W., Wang, G., Shao, L. (2012): Recovery of phosphorus as struvite from sewage sludge ash. *J. Environ. Sci. (China)* 24, 1533–1538.

Ye, Y., Ngo, H.H., Guo, W., Liu, Y., Li, J., Liu, Y., Zhang, X., Jia, H., Ngo, H.H. (2016): Insight into chemical phosphate recovery from municipal wastewater. *Sci. Total Environ.* 576, 159–171.

Zang, Y., Li, Y., Wang, C., Zhang, W., Xiong, W. (2015): Towards more accurate life cycle assessment of biological wastewater treatment plants: A review. *J. Clean. Prod.*

Zhu, J., Li, M., Whelan, M., Gan, J. (2017): Phosphorus activators contribute to legacy phosphorus availability in agricultural soils: A review. *Sci. Total Environ.* 612, 522–537.

Chapter 4

Sewage sludge hydrolysates for soilless phosphate plant nutrition

Abstract

The objective of this work was to evaluate the efficiency of different hydrolysates for P recovery from sludge as liquid fertilizer as P source for lettuce grown in floating system. Sulfuric acid, potassium hydroxide, citric and maleic acids were previously evaluated alone and in combination with different enzymes, such as acid and alkaline phosphatase. The higher efficiency was reached by H_2SO_4 (more the 90%), which however showed high extraction of toxic elements such as Al, Cd, Cu and Zn. High P recovery efficiency was found in citric acid (45%) and citric acid in combination with acid phosphatase (80%), which were thus chosen for this plant trial. Lettuce plants were grown in a floating system using a standard nutrient solution (ST) or with only N and K (NK) for 4 weeks after transplant. Citric acid (**C+ST** and **C+NK**) and citric acid with acid phosphatase (**CF+ST** and **CF+NK**) were added as source of P, whereas KH_2PO_4 was used as mineral control (**P+ST**). The solutions were replaced every 2 weeks. Trace metals, phosphate and nitrate concentration in the nutrient solution were periodically monitored, as well as metal concentration in the leaves after destructive harvest. The results of dry matter production did not show significant differences between the treatments, showing that the production was not decreased by the use of the organic products. Nutrient solution analyses showed that **C+NK** and **CP+NK** contained similar amounts of Ca, Mg and Mn and higher amount of Fe compared to **P+ST**. Higher Al and Na were also detected in the solution with the organic products which, however, remained beyond toxicity levels in the leaf tissue. Nitrate concentration showed a fast decrease in the solution enriched with organic products probably due to a high C:N ratio, promoting greater microbial growth. From this study, although the problems encountered, it appears that sewage sludge hydrolysates are promising products for effective and sustainable supply of P and nutrients to plants.

Introduction

The phosphorus is one of the three essential nutrient (with nitrogen and potassium) for plant growth (*Grigatti et al.*, 2015). In modern agriculture, in last century the use of P fertilizer has exponentially grown and consequently also the crop yield (*Cordell et al.*, 2009). This has led to a fast consumption of the reserves of phosphate rocks, which was the primary raw material for phosphate fertilizers. The need to find alternative source of phosphorus, due to the scarcity of resources (*Cordell et al.*, 2011), has encouraged the researchers and the government to focus on finding solutions to close the cycle of phosphorus, with a circular economy view (*Koppelaar and Weikard*, 2013). In chap. 3, we had tried many combinations of chemical and enzymatic extractants in order to hydrolyse P from fresh sewage sludge to a liquid fertilizer. Between the many possibilities we have choose to test on plants the treatment with citric acid and the mix of citric acid and acid phosphatase on anaerobic sludge. This decision has been made due to the high heavy metal extraction of the sulfuric acid treatment (chap. 3). Furthermore, the treatment was more effective on the anaerobic sludge and the wastewater treatment plants that used anaerobic digestion are more diffuse in the big scale systems (*Russo*, 2012). The citric acid in combination with phosphatase in anaerobic sludge reached about 80% of the P recovery, while the only citric acid in two hours of extraction reached 45% of P recovery.

Sewage sludge contains a high amount of P, which is poorly available for plants (as previously described in chap. 3). However, the sludge disposal on agricultural fields remained one of the most frequent way of disposal in countries (such as Italy), in which incineration is not approved (*Eurostat*, 2016; *Kidd et al.*, 2007). The sewage sludge fertilization, beside its evident pros compared to landfill or incineration, has some problems, such as heavy metal accumulation (*Kidd et al.*, 2007). Hence, sewage sludge disposal in agricultural field had strict regulation (*Direzione Generale Ambiente*, 2004), about the maximum amount of sludge disposable, the metal and organic pollutants content and about the crops that can be fertilized with them. Thus, horticultural crops are not inserted in the list of crops fertilizable with sewage sludge. Another solution for sewage sludge disposal is composting, highly incentivized by the Italian municipalities. The sewage sludge compost field disposal, as P fertilizer is, though, not as effective as triple superphosphate (*Frossard et al.*, 1996; *McCoy et al.*, 1986) and as previously described in chap. 2 has not the highest P plant availability compared to other organic products. In other European countries (Italy not included) another solutions for P recovery is struvite precipitation, which has many positive effect on reducing P in the wastewater, but on the other hand increases the pH of the soil, is not effective as fertilizer in calcareous soils (chap. 2) and can led to Mg accumulation in soil (*Kataki et al.*, 2016). Therefore, a new solution for sewage sludge

use in horticultural crops need to be evaluated, such as a fertilizer which can overcame the problems of pH increase and heavy metal accumulation. In order to analyse the effect of a fertilizer on plants without the buffer effect of soil, which can decrease the P availability but also the heavy metal uptake, a soilless system experiment can be useful. Frequent sampling of the nutrient solutions could be possible and could help understand the fate of the fertilizers. Furthermore, analysing physiological traits and possible plant stress and morphological characteristics of the plants information on the fertilizers effects could be achieved. The aim of this work, thus, is the evaluation of a liquid fertilizer, derived from a chemical and enzymatic hydrolysis of sewage sludge for the growth of lettuce in a soilless system, analysing plant physiological and morphological growth and matter quality in terms of heavy metal accumulation.

Material and methods

Hydrolysates fertilizers

Anaerobic sewage sludge was used as fresh raw material for chemical and enzymatic hydrolysis. The sludge characteristic is summarized in Table 4.1. The hydrolysis of the sludge is well explained in chapter 3. Briefly, the sludge was hydrolysed in an extractant solution with a liquid:solid ratio of 20, for 1 h at 37°C. Than the solution was centrifuged, filtered and analysed. In the treatment with a combination of chemical and enzymatic treatment, after the first hour of extraction the pH of the solution was adjusted to 4.8 and acid phosphatase added in a rate of 1 unit per 2.5 g of sludge and then hydrolysed again at 37°C. Between the many treatments tried in chap. 3 (summarized in Fig. 4.1), as liquid fertilizer for plant nutrition it was chosen the hydrolysates of citric acid and the mix between citric acid and acid phosphatase due to their high P recovery and low heavy metal extraction. In Table 4.2, the composition of the two hydrolysates is described.

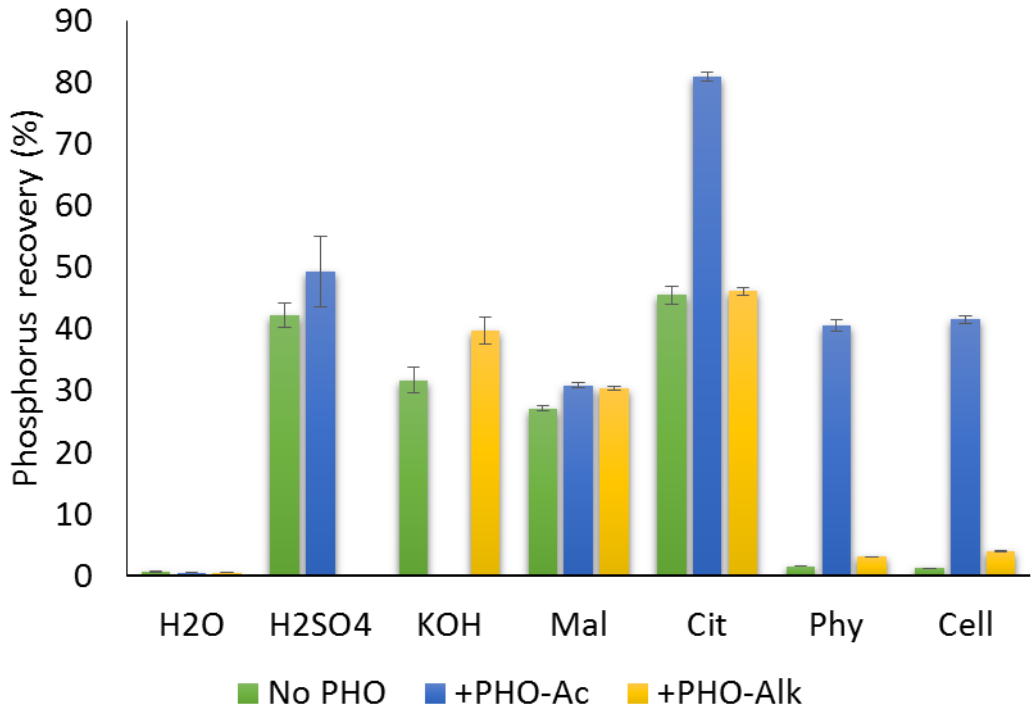


Figure 4.1: Summary of the results of different extraction on sewage sludge with water (H2O), sulfuric acid (H2SO4), potassium hydroxide (KOH), maleic acid (Mal), citric acid (Cit), phytase (Phy) and cellulose (Cel). The extraction was performed alone (green bars), in combination (or sequence) with acid phosphatase (PHO-Ac) (blue bars) or alkaline phosphatase (PHO-Alk) (yellow bars).

Table 4.1: Principal characteristics of the raw products

	Anaerobic sludge (ANA)
Moisture (% fresh matter)	72.4%
Ash (% dry matter)	45.7%
pH-H_2O	7.3
P_{tot} (mg kg^{-1})	20,040
P_{in} (mg kg^{-1})	18,154
P_{org} (mg kg^{-1})	1,311
P_{H2O} (mg kg^{-1})	50
C (%)	25.93
N (%)	4.43
Al (mg kg^{-1})	19347
Ca (mg kg^{-1})	23611
Cd (mg kg^{-1})	2.15
Co (mg kg^{-1})	5.62
Cr (mg kg^{-1})	101
Cu (mg kg^{-1})	390
Fe (mg kg^{-1})	32885
Mg (mg kg^{-1})	6854
Mn (mg kg^{-1})	233
Ni (mg kg^{-1})	50
Pb (mg kg^{-1})	61
Zn (mg kg^{-1})	826

Table 4.2: Nutrient and heavy metal concentration of hydrolysates

		Citric acid	*Citric acid mixed with acid phosphatase*
PO_4	mg kg^{-1}	8442	16199
K	mg kg^{-1}	27.2	11.6
Ca	mg kg^{-1}	23880	18300
Mg	mg kg^{-1}	3068	2332
S	mg kg^{-1}	117	81.5
Fe	mg kg^{-1}	15070.4	13477.1
Mn	mg kg^{-1}	169.7	125.4
Cu	mg kg^{-1}	0.6	0.2
Ni	mg kg^{-1}	25.8	18
Zn	mg kg^{-1}	391.4	283.1
Al	mg kg^{-1}	2008	1696.9
Cd	mg kg^{-1}	0	0
Co	mg kg^{-1}	0.9	0.6
Cr	mg kg^{-1}	32.8	29.3
Pb	mg kg^{-1}	11.4	13.7

Plant growth

Seeds of lettuce, variety *Romana* (Blumen Group S.P.A, Bologna, Italy) were sown on sterilized sponges. After 11 days after sowing (DAS) the seedling were fertilized with 8.3 mM NO_3, 1.6 mM NH_4 and 2.75 mM K. At 18 DAS they were transplanted with the sponges in thin layer of polystyrene in 4 plants randomized blocks with two replication per treatment and laid on a plastic box (4 L volume) with nutrient solution. Aeration in the boxes was provided with an aquarium oxygenator and a porous stone. The plants were grown in a growth chamber with day/night temperature of 25°/18°C and 16 h of photoperiod.

Nutrient solutions

The nutrient solution chosen for plant growth was a standard solution for floating system lettuce (*Sonneveld and Straver*, 1994). Before the addition of the nutrients, it was performed the analysis of tap water, which resulted as reported in Table 4.3.

The nutrient solution used as treatments for the hydrolysates trial were:

NK = a solution with only nitrogen and potassium as negative control;

ST = the standard solution for lettuce without phosphorus;

P+ST = normal standard solution with chemical P as a positive control

C+NK =solution with N, K from mineral source and P derived from hydrolysates with citric acid

CF+NK = solution with NK and P from citric acid and acid phosphatase;

C+ST = standard solution with P from citric acid hydrolysates

CF+ST = standard solution with P from citric acid and acid phosphatase hydrolysates.

Therefore the composition of the solutions, after the tap water analysis and considering the treatments, were as reported in Table 4.4.

The solutions were replaced once after 14 days.

Table 4.3: Tap water nutrient concentration and standard solution (*Sonneveld and Straver*, 1994).

	Tap water	**Standard solution for lettuce**	**Nutrient to add to the solution**
	mM	**mM**	**mM**
NH_4	0.03	1.25	1.22
NO_3	0.00	19.00	19.00
K	0.07	11.00	10.93
Ca	1.95	4.50	2.55
Mg	0.66	1.00	0.34
S	0.58	1.13	0.55
P	0.00	2.00	2.00
Si	0.06	0.50	0.44
	µM	**µM**	**µM**
Fe	1.37	40.00	38.63
Cu	3.43	0.75	-2.68
Zn	5.29	4.00	-1.29
B	4.72	30.00	25.28
Mn	0.00	5.00	5.00
Mo	0.00	0.50	0.50

Table 4.4: Macronutrient, micronutrients and hydrolysates concentration to add to the different nutrient solutions.

Treatments	NK	ST	P+ST	C+NK	CF+NK	C+ST	CF*ST
Macronutrients (mM)							
NH4	5.0	1.2	1.2	5.0	5.0	1.2	1.2
NO3	16.0	19.8	19.8	16.0	16.0	19.8	19.8
K	11.0	10.9	10.9	11.0	11.0	10.9	10.9
Ca	0.0	2.5	2.5			2.5	2.5
Mg	0.0	0.3	0.3			0.3	0.3
S	0.0	1.5	0.5			1.5	
P	0.0	0.0	2.0	2.0	2.0	2.0	2.0
Micronutrient (µM)							
Fe	0.0	38.6	0.0	0.0	0.0	38.6	38.6
Cu	0.0	-2.7	0.0	0.0	0.0	-2.7	-2.7
Zn	0.0	0.0	0.0	0.0	0.0	0.0	0.0
B	0.0	25.3	0.0	0.0	0.0	25.3	25.3
Mn	0.0	5.0	0.0	0.0	0.0	5.0	5.0
Mo	0.0	0.5	0.0	0.0	0.0	0.5	0.5
Hydrolysates (ml l^{-1})							
Cit	0.0	0.0	0.0	131.6	0.0	131.6	0.0
Cit mix Ac PHO	0.0	0.0	0.0	0.0	82.1	0.0	82.1

Nutrient solutions pH and metal concentration

Samples of the nutrient solution had been collected three times a week during plants growth as described in Table 4.5. The samples were collected after mixing the solutions. The samples were immediately frozen and later analyzed for PO_4 concentration with ascorbic acid method (*Murphy and Riley*, 1962), NO_3 with an AutoAnalyzer and metal concentration with ARCOS ICP-OES (Spectro Analytical Instruments, Kleve, Germany). In Table 4.5, T0.2 is the day of replacement of the nutrient solutions.

pH of the solution was adjusted to pH 5.5 with nitric acid, it was checked with a portable pH meter (Growmart-de, Hamburg, Germany) and at day 18, 25, 26, 32, 38, 39, 43, 44 and it was readjusted periodically with sulfuric acid to the right pH in all the treatments.

Table 4.5: Solutions sampling time.

	Hours from the treatment start	**Hours from the previous sampling**	**DAS**	**DAT**
T0	0	0	18	0
T1	18	18	19	1
T2	60	42	21	3
T3	144	84	24	6
T4	228	84	28	10
T5	312	84	31	13
T0.2	333	21	32	14
T6	396	63	35	17
T7	480	84	38	20
T8	564	84	42	24
T9	648	84	45	27

Plant physiological measurements

N-tester – Leaf greenness was evaluated twice with N-tester (Yara International ASA, Oslo, Norway) at 38 and 45 DAS. For data evaluation only the second measurements was used. Leaf greenness can be considered an indirect value for chlorophyll relative content (*Gianquinto et al.*, 2011).

Chlorophyll fluorescence – Chlorophyll fluorescence were analysed with a hand-held fluorometer (in development) at 45 DAS.

Plant morphological measurements

Leaf area – Leaf area of the plants was analysed through software analysis of pictures. The pictures were captured in the same days of the solution samples (Table 4.5). On the polystyrene layer it was pasted a red square of 2 cm x 2 cm, in order to have a reference for the software. The pictures taken were modified with GIMP (GNU Image Manipulation Program, www.gimp.org) to create a black background and then analysed with Easy Leaf Area (*Easlon and Bloom*, 2014) to have a leaf area measurements.

Plant fresh and dry weight – Plants were destructively sampled 45 DAS and immediately weighted for fresh weight. The plants were firstly weighted whole and then, roots and shoots were weighted separately. The samples were then dried in an oven at 70°C for 72 hours and weighted to have dry weight. The shoot-root ratio was calculated as the ratio between dry weight of the shoots and dry weight of the roots.

Leaf and root metal concentration – The dried samples were finely milled and 0.25 g of sample was digested with 6 ml of HNO_3 and 2 ml of H_2O_2 in a microwave for 40 minutes. The resulting solutions were diluted and analysed with ARCOS ICP-OES (Spectro Analytical Instruments, Kleve, Germany).

Statistical analysis

Statistical analysis was performed with R (https://cran.r-project.org) on plants data with a One-way ANOVA and Tukey test with $P \leq 0.05$. Normality of population was assessed with Kolmogorov-Smirnov test and equality of variance with Levene test.

Results

Solution pH

The pH of the solution (Fig. 4.2), which was readjusted periodically to pH 5.5, increased more in C+NK treatment (from 5.5 to 8) in the first tranche and in all the hydrolysates treatments and in NK in the second tranche (all above 7.5). It is evident the more stability of the positive control (P+ST) which remains always lower than 6.5.

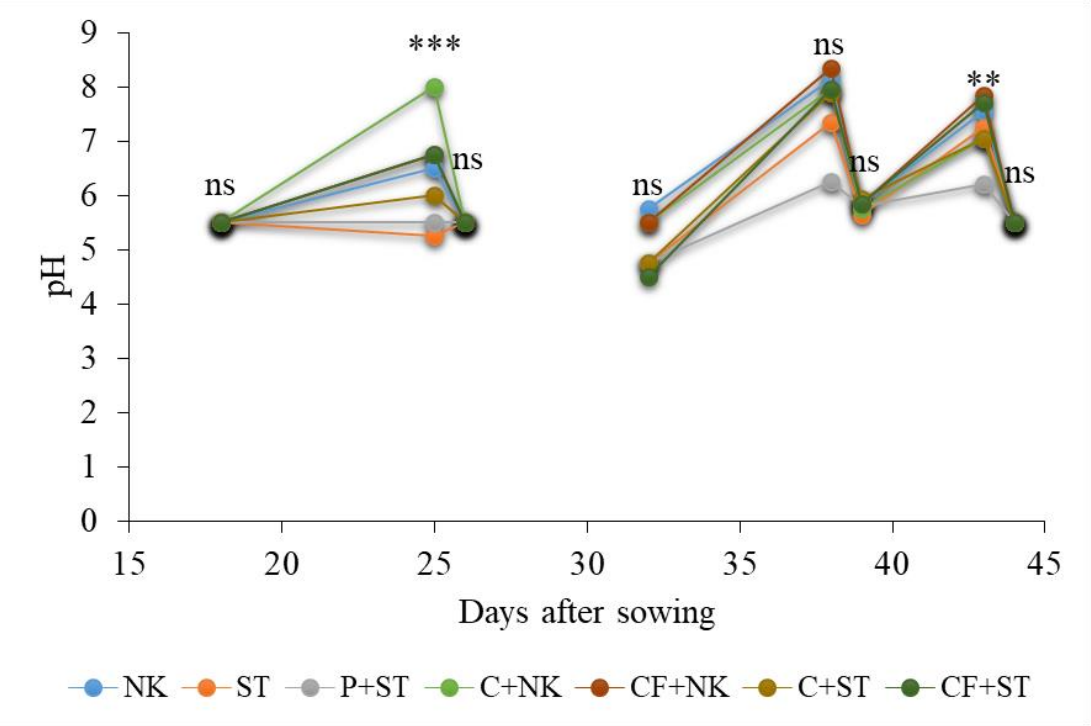


Figure 4.2: pH of different hydroponic nutrient solutions during lettuce growth. The treatment were a solution with only nitrogen and potassium as negative control (**NK**); a standard solution for lettuce without phosphorus (**ST**); a normal standard solution with chemical P as a positive control (**P+ST**); a solution with N, K from mineral source and P derived from hydrolysates with citric acid (**C+NK**); a solution with NK and P from citric acid and acid phosphatase (**CF+NK**); a standard solution with P from citric acid hydrolysates (**C+ST**) and a standard solution with P from citric acid and acid phosphatase hydrolysates (**CF+ST**). One-way ANOVA significance within time is shown as *** = P≤0.001, ** = P≤0.01, * = P≤0.05 and ns= P>0.05.

Phosphorus in solutions

The orthophosphate concentration (Fig. 4.3) in the solutions had different behaviour in the mineral controls and in hydrolysate treatments. It is evident that in the solutions without any source of P (NK and ST) the PO_4 concentration remain always close to 0. On the other hand in the citric acid hydrolysates and citric acid mixed with phosphatase hydrolysate treated solutions the PO_4 content decreased visibly from 21 DAS to 24 DAS (-55% in C+NK, - 93% in CF+NK, -63% in C+ST) and from 35 to 38 DAS (-92% in C+NK, - 78% in CF+NK, -94% in C+ST and –93% in CF+ST). In CF+NK the high decrease in the first part of the experiment was visible already from DAS 19 to 21. On the contrary, this decrease was not visible in P+ST, the positive control, in which the PO_4 remain constant during the experiment. When comparing the PO_4 concentration at T0 and T0.2 with the final sampling (T5 for the first part and T9 for the second part), the decrease was clear in all the organic treatment and higher in the second part of the experiment: in C+NK the PO_4 concentration remain similar for T0 to T5 (+3%) and decreased from T0.2 and T9 (-74%); CF+NK showed a decrease of 46% in the first solution and -78% in the second; in C+ST in the first part -19% of PO_4 decreased and -61% in the second part and in CF+ST from DAS 18 to 31 it was found a decrease of -38% and from DAS 32 to 45 of -86%.

The citric acid hydrolysates solutions showed an initial PO_4 concentration that was always higher than the other treatment (+60% at T0 and +150% at T0.2, comparing the average of C+NK and C+ST to P+ST). The content was also higher than the calculated molarity of the standard solution for lettuce (*Sonneveld and Straver*, 1994) (+34% at T0 and +65% at T0.2, comparing the average mM PO_4 of C+NK and C+ST to 2 mM of the calculated solution).

A similar pattern was recognisable in the total P (Fig. 4.3). Total P was significantly higher only in citric acid hydrolysates solution where, it was 37% and 87% higher in C+NK and C+ST respectively at T0. In the other treatments, total P did not exceed much the PO_4 concentration, showing low P, which can be considered not available for plant nutrition.

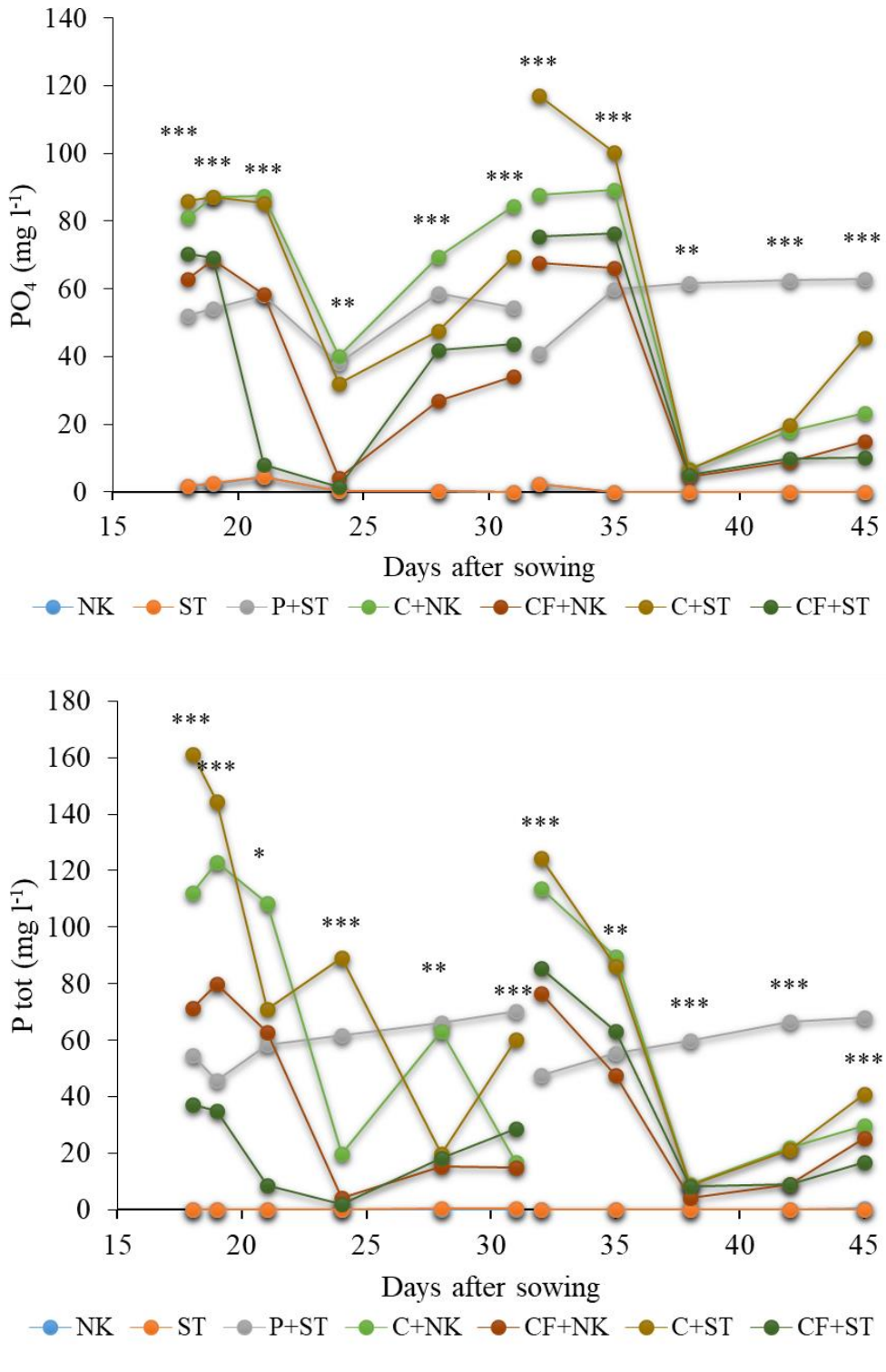


Figure 4.3: PO_4 (mg l^{-1}) and total P (mg l^{-1}) of different hydroponic nutrient solutions during lettuce growth. The treatments were: solution with N and K (**NK**); standard solution without P (**ST**); standard solution with P (**P+ST**); solution with N and K and P derived from citric acid hydrolysates (**C+NK**); solution with NK and P from citric acid and acid phosphatase (**CF+NK**); standard solution with P from citric acid hydrolysates (**C+ST**) and standard solution with P from citric acid and acid phosphatase hydrolysates (**CF+ST**). One-way ANOVA significance within time is shown as *** = P≤0.001, ** = P≤0.01, * = P≤0.05 and ns= P>0.05.

Nitrate concentration in solutions

The patterns described in PO_4 content are recognisable also in the NO_3 concentration (Fig. 4.4). The mineral solution contained an almost constant N concentration, while in the hydrolysates solution a fast decrease is shown in DAS 28 and 38. Contrarily to PO_4, the NO_3 in the first portion of the experiment decreased later (28 DAS vs 24 DAS). Furthermore, oppositely to P, the nitrate content did not re-increase after the precipitation, showing higher decrease when comparing the concentration in T0 to T5 (-99.8% in C+NK, -79.6% in CF+NK, -99.8% in C+ST, -61.5% in CF+NK) and T0.2 to T9 (-99.8% in C+NK, -93.3% in CF+NK, -100% in C+ST, -97.9% in CF+NK).

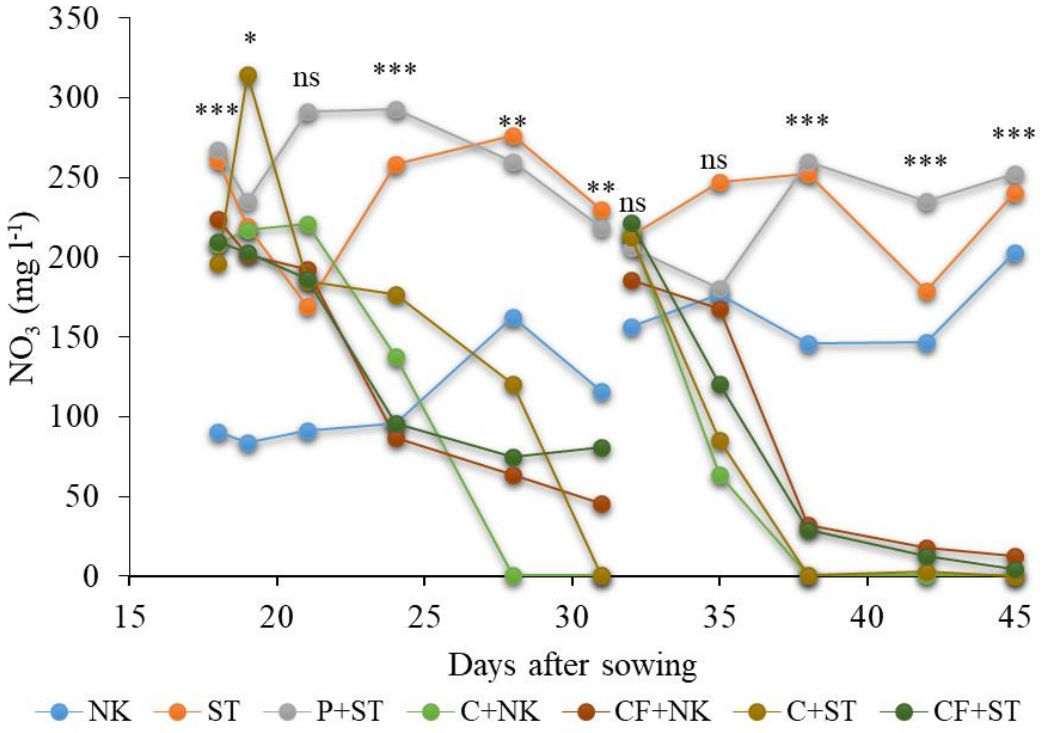


Figure 4.4: NO_3^- (mg l^{-1}) of different hydroponic nutrient solutions during lettuce growth. The treatments were: solution with N and K (**NK**); standard solution without P (**ST**); standard solution with P (**P+ST**); solution with N and K and P derived from citric acid hydrolysates (**C+NK**); solution with NK and P from citric acid and acid phosphatase (**CF+NK**); standard solution with P from citric acid hydrolysates (**C+ST**) and standard solution with P from citric acid and acid phosphatase hydrolysates (**CF+ST**). One-way ANOVA significance within time is shown as *** = P≤0.001, ** = P≤0.01, * = P≤0.05 and ns= P>0.05.

Metals concentration in nutrient solutions

Among the metals directly related to P availability (Fig. 4.5) Al and Fe showed patterns similar to P, with a rapid decrease at DAS 24 and 38. Only for C+ST treatment the decrease in the first tranche was a bit postponed to DAS 28 in both the metals. Ca concentration, although also showed a slight decrease during the time in the hydrolysates treatment, did not show such a high slope as compared to Fe and Al.

The hydrolysates treatments, however, showed a higher amount of Al and an exponentially higher amount of Fe as compared to the mineral fertilizer, whereas the Ca concentration was always comparable.

Potassium concentration was also comparable, whereas Mg concentration was slightly higher in hydrolysates treatment (+12% as average between the four hydrolysates treatment in all the sampling time and the positive control) (Fig. 4.6). On the other hand Mg curve (Fig. 4.6) showed a decrease similar to the one found in PO_4 at 24 and 38 DAS (-80% in C+NK, -96% in CF+NK and -80% in CF+ST between 21 and 24 DAS; -91% in C+NK, -97% in CF+NK, -87% in C+ST and -84% in CF+ST between 35 and 38 DAS). As measured in Al and Fe concentration, also Mg decrease in C+ST was postponed in the first part of the experiment (-80% between 24 and 28 DAS). Mn concentration (Fig. 4.7) was higher in the hydrolysate treatment (+19% as average of the hydrolysate treatment as compared to P+ST. Mo concentration (Fig. 4.7), instead, was always higher and constant in the treatment with standard treatment, thus coming from the microelements mineral fertilizer. However, when not applied the microelements fertilizer, the Mo concentration is slightly higher in hydrolysates treatment (14.36 $\mu g\ l^{-1}$ as average between C+NK and CF+NK vs 0 $\mu g\ l^{-1}$ of NK).

On the potentially toxic elements side that can be also nutrient in low dosages (Fig. 4.8), Cu concentration was only present in solutions with mineral micronutrients, with 147.7 $\mu g\ l^{-1}$ as an average of the ST treatments (ST, P+ST, C+ST and CF+ST) compared to 26.1 $\mu g\ l^{-1}$ of the NK treatments (NK, C+NK and CF+NK). Ni, however, was not added in the nutrient solution as micronutrient, thus was present only in hydrolysates solutions. Zn concentration, was instead comparable in all the treatments with the treatments with citric acid and acid phosphatase that showed a decrease at 24 DAS (-57% for CF+NK and -46% for CF+ST from 21 to 24 DAS. All the hydrolysates treatments showed a decrease of -54% (as an average of the hydrolysates treatments) from 35 to 38 DAS. Regarding heavy metal concentration (Fig. 4.9), Cd traces were found only in the treatments without hydrolysates. On the other hand, Cr was traceable only in hydrolysates solutions. Pb was also found only in solutions with the fertilizer derived from sludge, but showed a pattern similar to other metals: it decrease reaching 0 $\mu g\ l^{-1}$ in all the hydrolysates treatment at 24 and 38 DAS, except for C+ST, which reached a value under detection limits later at 28 DAS and 38 DAS.

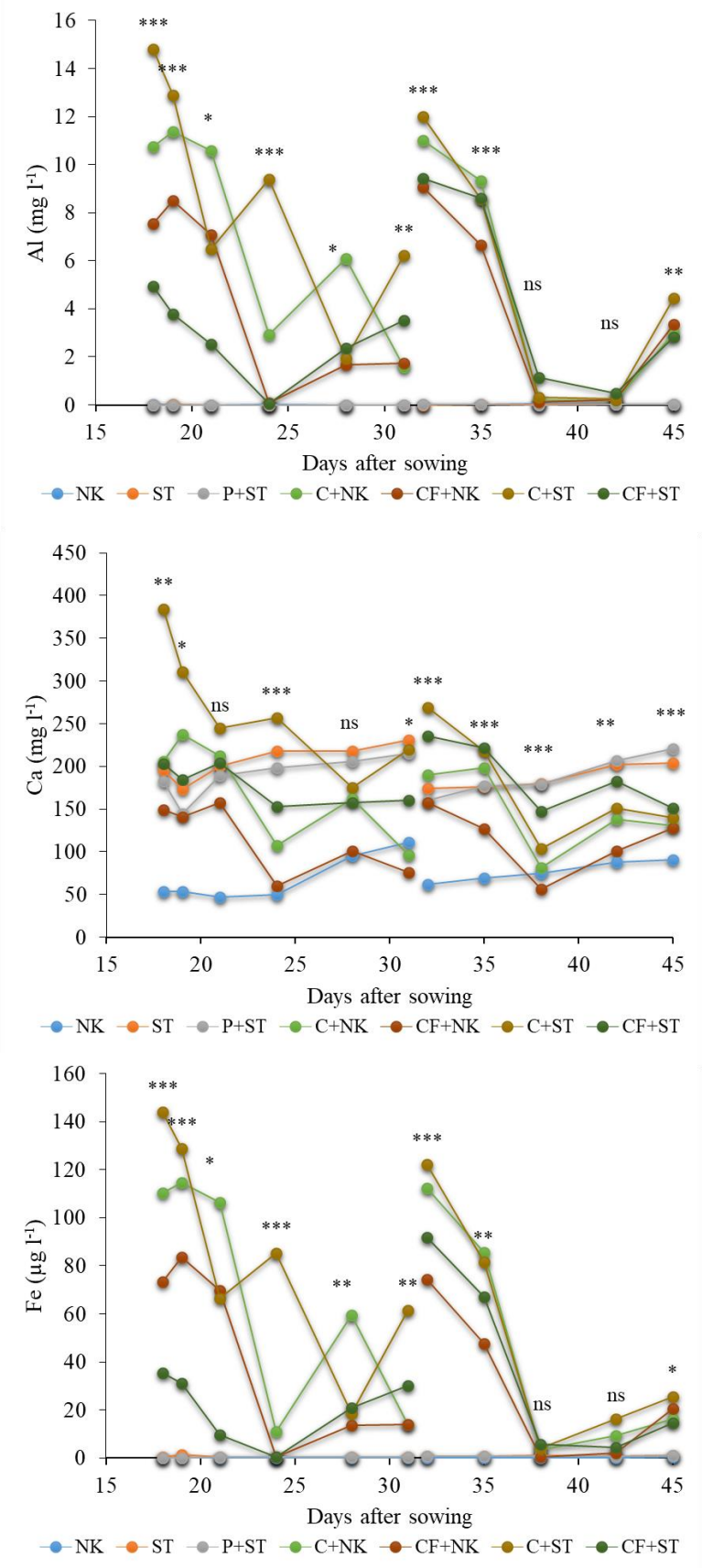


Figure 4.5: Al, Ca and Fe concentration (mg l^{-1}) of the different hydroponic nutrient solutions during the growth cycle of lettuce. One-way ANOVA significance within time is shown as *** = P≤0.001, ** = P≤0.01, * = P≤0.05 and ns= P>0.05.

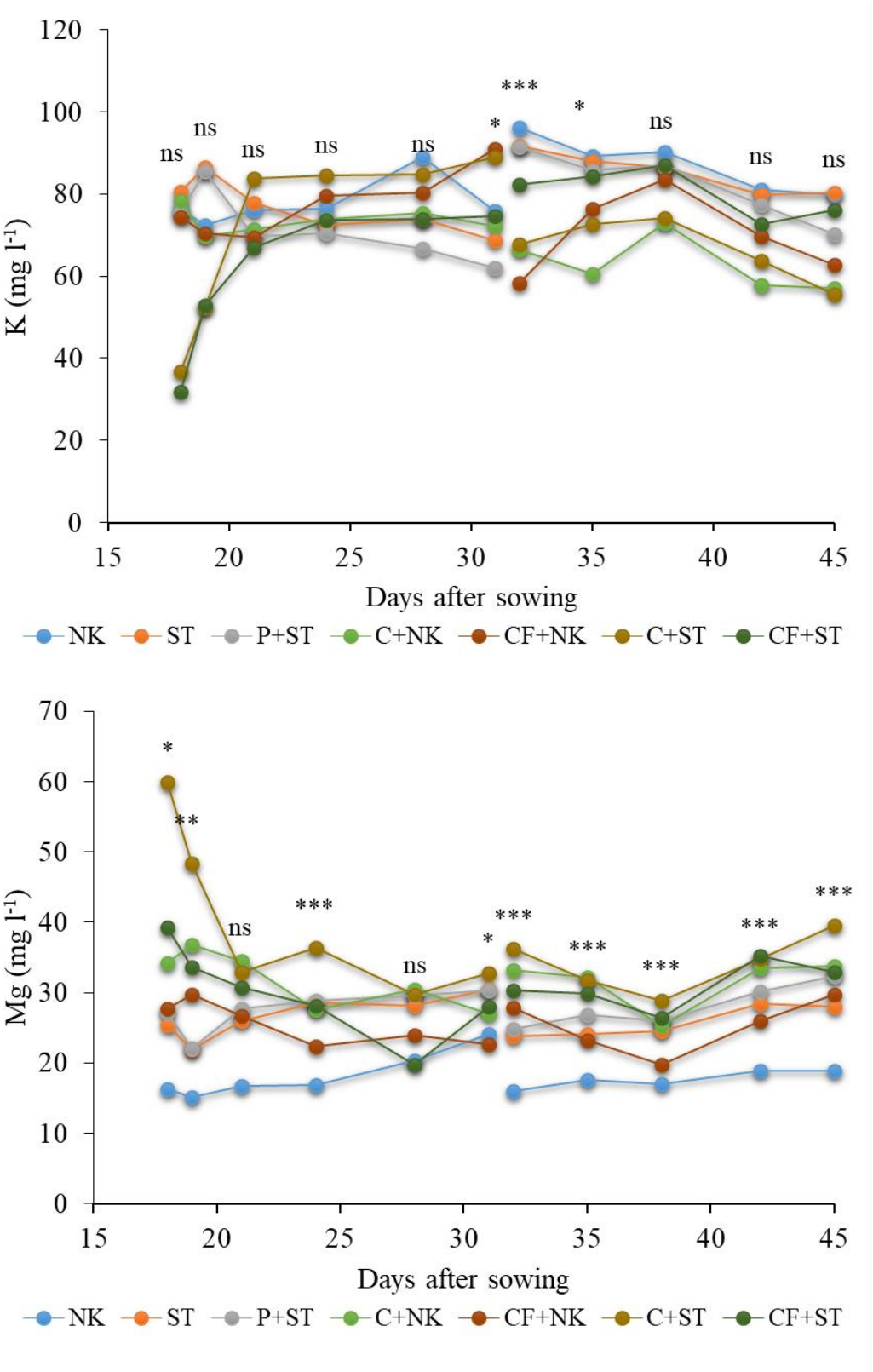


Figure 4.6: K and Mg concentration (mg l^{-1}) of the different hydroponic nutrient solutions during the growth cycle of lettuce. One-way ANOVA significance within time is shown as *** = P≤0.001, ** = P≤0.01, * = P≤0.05 and ns= P>0.05.

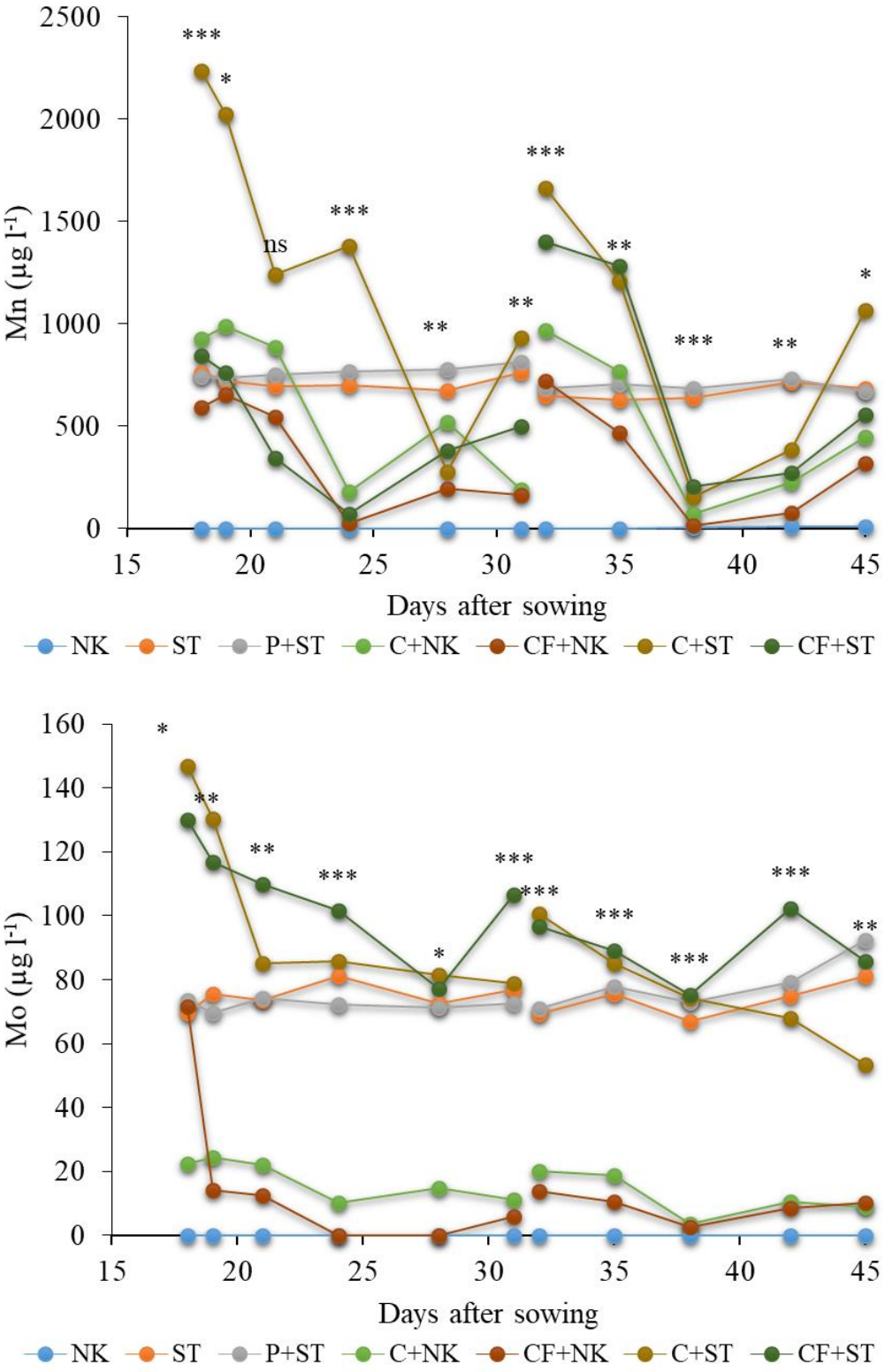


Figure 4.7: Mn and Mo concentration (µg l^{-1}) of the different hydroponic nutrient solutions during the growth cycle of lettuce. One-way ANOVA significance within time is shown as *** = P≤0.001, ** = P≤0.01, * = P≤0.05 and ns= P>0.05.

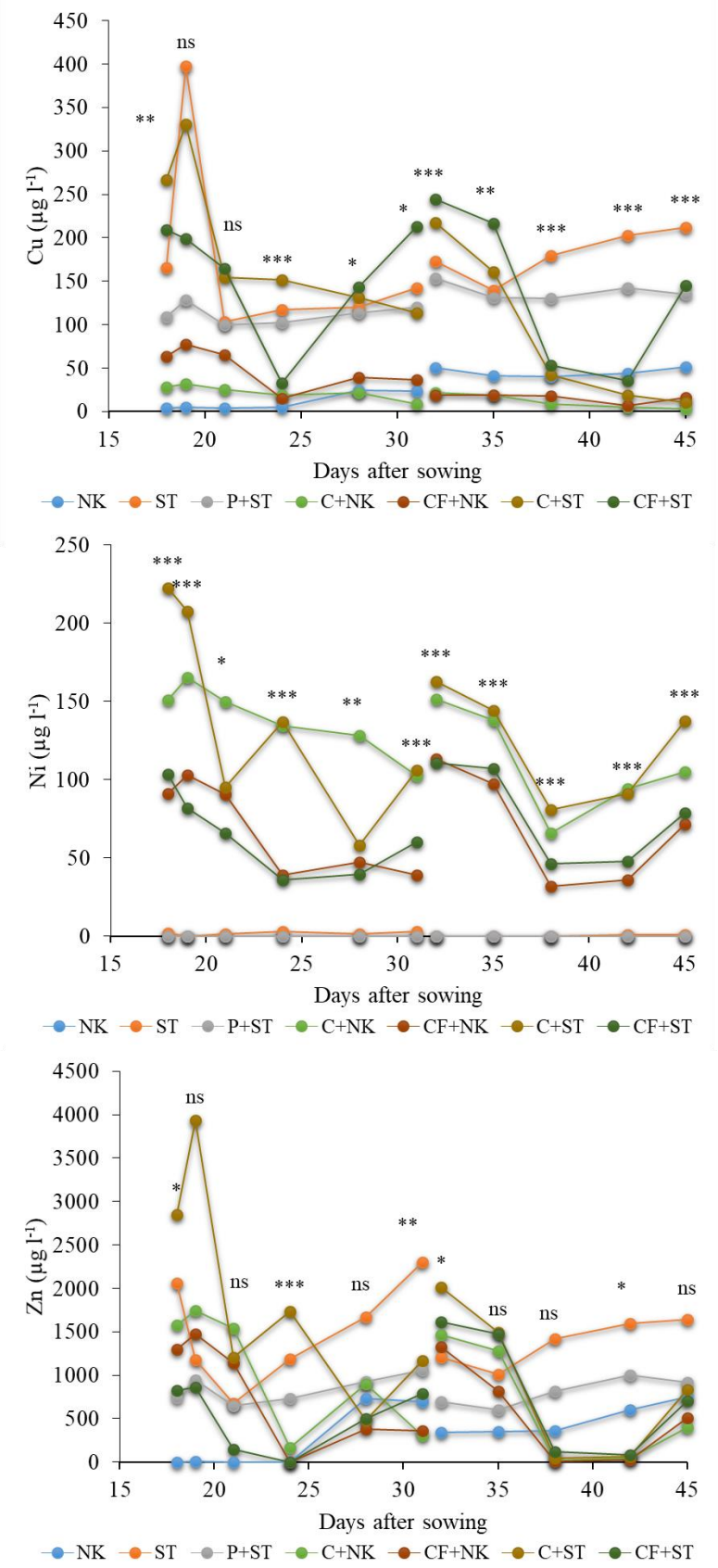


Figure 4.8: Cu, Ni and Zn concentration (µg l^{-1}) of the different hydroponic nutrient solutions during the growth cycle of lettuce. One-way ANOVA significance within time is shown as *** = P≤0.001, ** = P≤0.01, * = P≤0.05 and ns= P>0.05.

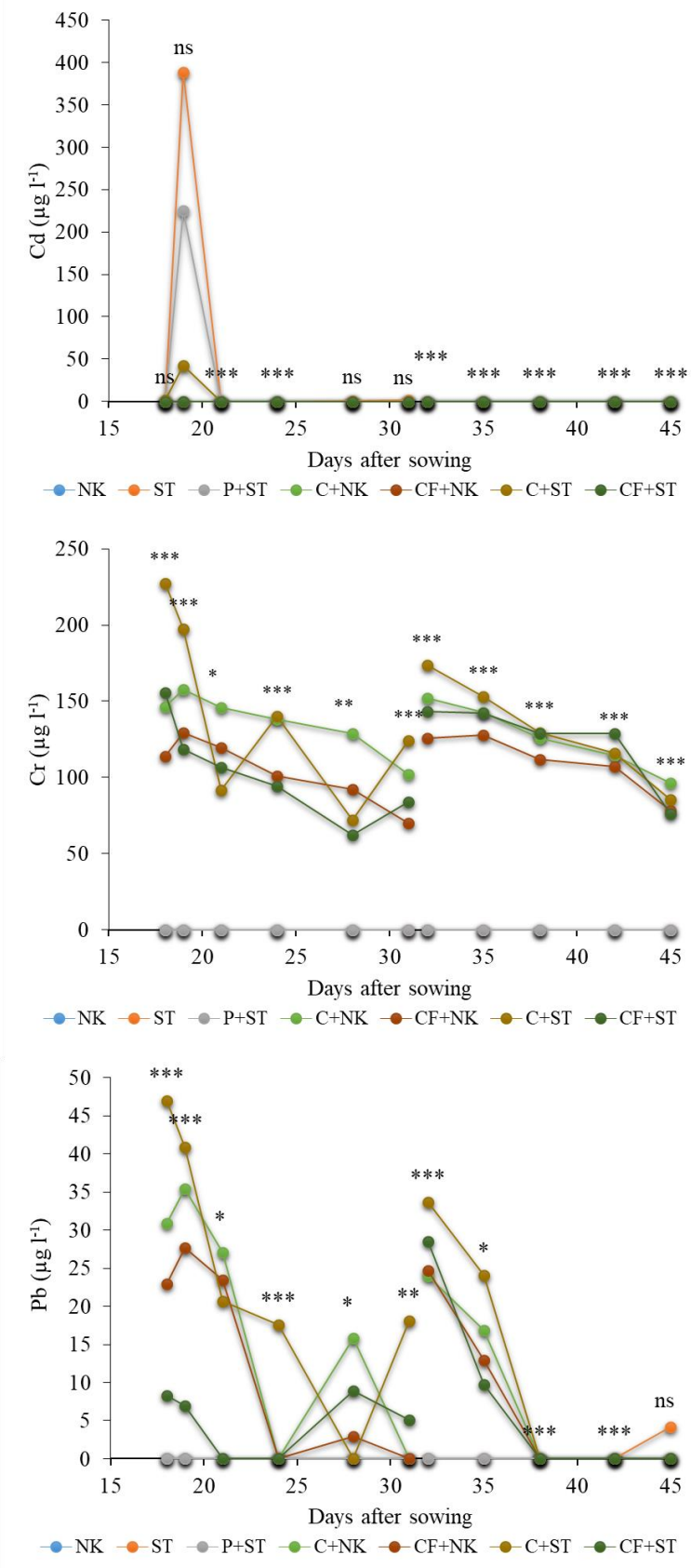


Figure 4.9: Cd, Cr and Pb concentration (µg l^{-1}) of the different hydroponic nutrient solutions during the growth cycle of lettuce. One-way ANOVA significance within time is shown as *** = P≤0.001, ** = P≤0.01, * = P≤0.05 and ns= P>0.05.

Plant physiology

Leaf greenness measurements (Fig. 4.10), an indirect measure of relative chlorophyll content, showed higher values for plants treated with C+NK (+53.8% as compared to plants treated with P+ST). The overall view showed a higher greenness in plants treated with hydrolysates solutions as compared to plants treated with the positive control solution (+35.3% as the average of the hydrolysates solution vs P+ST). No measurements were taken on NK plants, because their leaf blades were too small.

Chlorophyll fluorescence values (Fig. 4.11) showed clearly the highest values on the hydrolysates treated plants with the exception of CF+ST treated plant, with an average values of C+NK, CF+NK and C+ST that was 3.4 times the positive control P+ST and 2.9 times the value of CF+ST. No significant differences were found between plants grown with P+ST and CF+ST solutions. No measurements were taken on NK plants, because their leaf blades were too small.

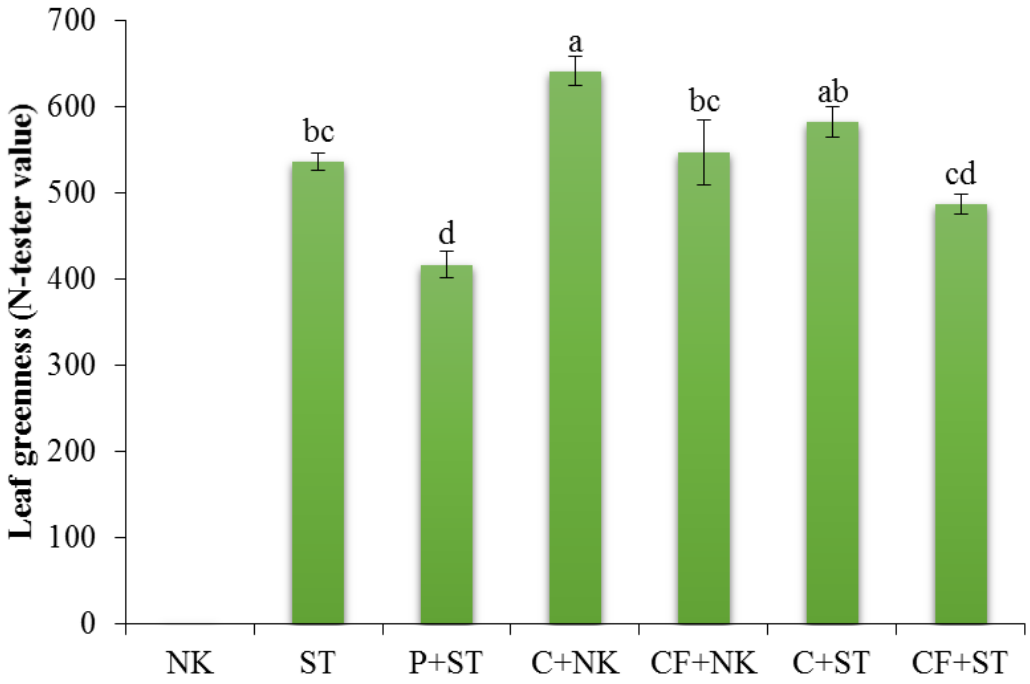


Figure 4.10: Leaf greenness (N-tester value) of lettuce grown in floating system with different nutrient solutions. The treatments were: solution with N and K (**NK**); standard solution without P (**ST**); standard solution with P (**P+ST**); solution with N and K and P derived from citric acid hydrolysates (**C+NK**); solution with NK and P from citric acid and acid phosphatase (**CF+NK**); standard solution with P from citric acid hydrolysates (**C+ST**) and standard solution with P from citric acid and acid phosphatase hydrolysates (**CF+ST**). Error bars represent standard errors (n=8) and different letters correspond to different group with Tukey test at P≤0.05.

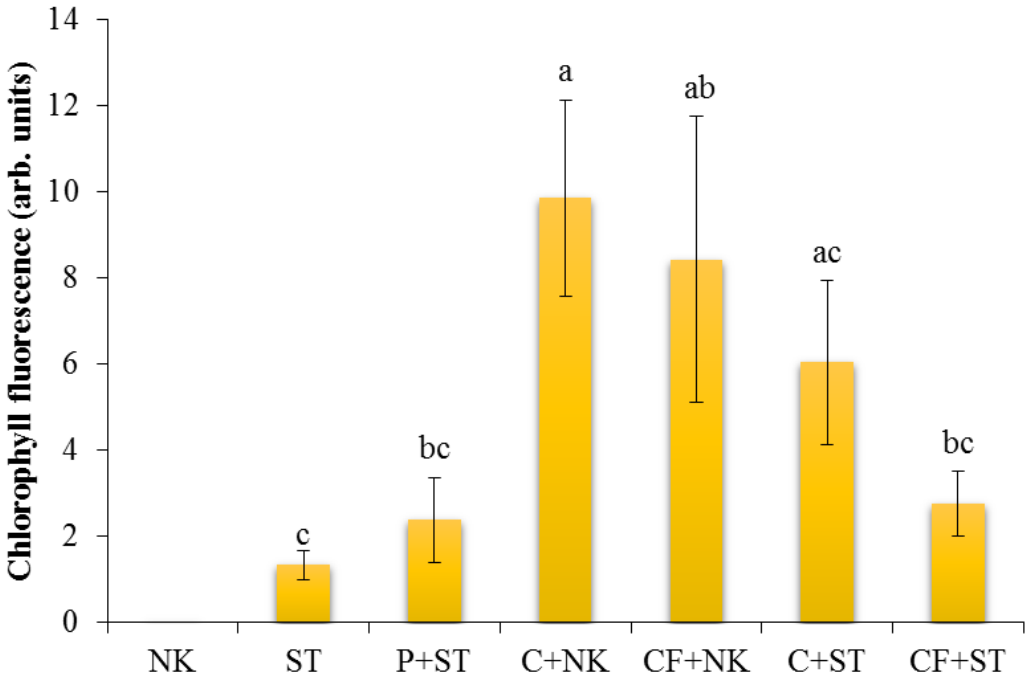


Figure 4.11: Chlorophyll fluorescence (arbitrary units) of lettuce grown in floating system with different nutrient solutions. The treatments were: solution with N and K (**NK**); standard solution without P (**ST**); standard solution with P (**P+ST**); solution with N and K and P derived from citric acid hydrolysates (**C+NK**); solution with NK and P from citric acid and acid phosphatase (**CF+NK**); standard solution with P from citric acid hydrolysates (**C+ST**) and standard solution with P from citric acid and acid phosphatase hydrolysates (**CF+ST**). Error bars represent standard errors (n=8) and different letters correspond to different group with Tukey test at $P \leq 0.05$.

Plant growth

Leaf area (Fig. 4.12), which was obviously similar at the transplanting, increased very differently between treatments: the control plants grown without P did not expanded their leaf area in the whole duration of the experiment; whereas, all the hydrolysates and the positive control increased constantly during the experiment and showed a higher slope between 24 and 32 DAS. CF+ST remained similar to the positive control until the end of the experiment, C+NK and C+ST were slightly lower, while CF+NK did not showed the exponential increase between 24 and 32 DAS, hence showed a smaller leaf area at 35 DAS, as compared to the other hydrolysates treatment and the positive control.

Plant biomass production was significantly higher in the positive control (Fig. 4.13) in both leaf and roots. In leaf fresh weight (data not shown) the decrease as compared to the positive control were -73.2% in C+NK, -84% in CF+NK, -70.6% in C+ST and -48.9% in CF+ST. When comparing the dry weight of the plants the leaf biomass production of hydrolysates solutions were also lower than the control, though with a slightly smaller decreases: -57.1% in C+NK, -72.5% in CF+NK, -49.5% in C+ST and -42.9% in CF+ST. Higher decreases, however, were found in root dry weight: -76.9% in C+NK, -82% in CF+NK, -62.9% in C+ST and -60.9% in CF+ST.

Consistently, the shoot-root ratio (Fig. 4.14) was sensibly higher in hydrolysates treated plants: +297% in C+NK, +141% in CF+NK, +220% in C+ST and +151% in CF+ST.

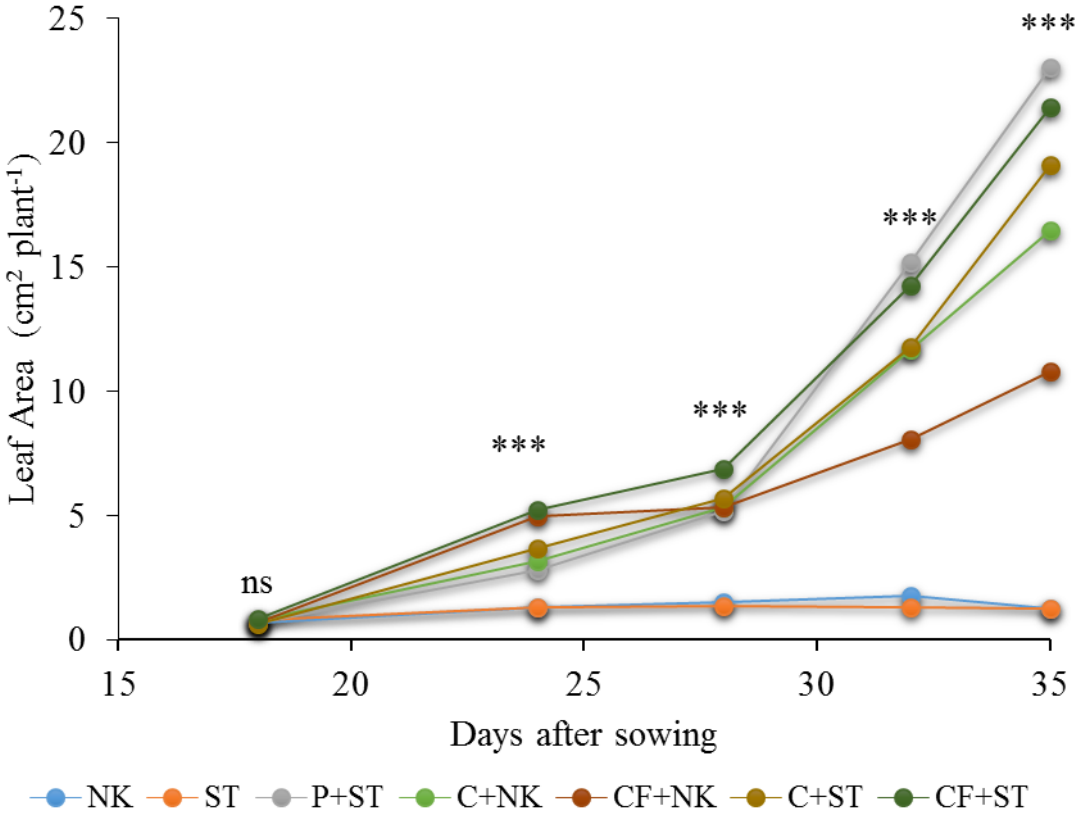


Figure 4.12: Leaf area (cm^2 $plant^{-1}$) of plants grown in different hydroponic nutrient solutions during lettuce growth. The treatments were: solution with N and K (**NK**); standard solution without P (**ST**); standard solution with P (**P+ST**); solution with N and K and P derived from citric acid hydrolysates (**C+NK**); solution with NK and P from citric acid and acid phosphatase (**CF+NK**); standard solution with P from citric acid hydrolysates (**C+ST**) and standard solution with P from citric acid and acid phosphatase hydrolysates (**CF+ST**). One-way ANOVA significance within time is shown as *** = P≤0.001, ** = P≤0.01, * = P≤0.05 and ns= P>0.05.

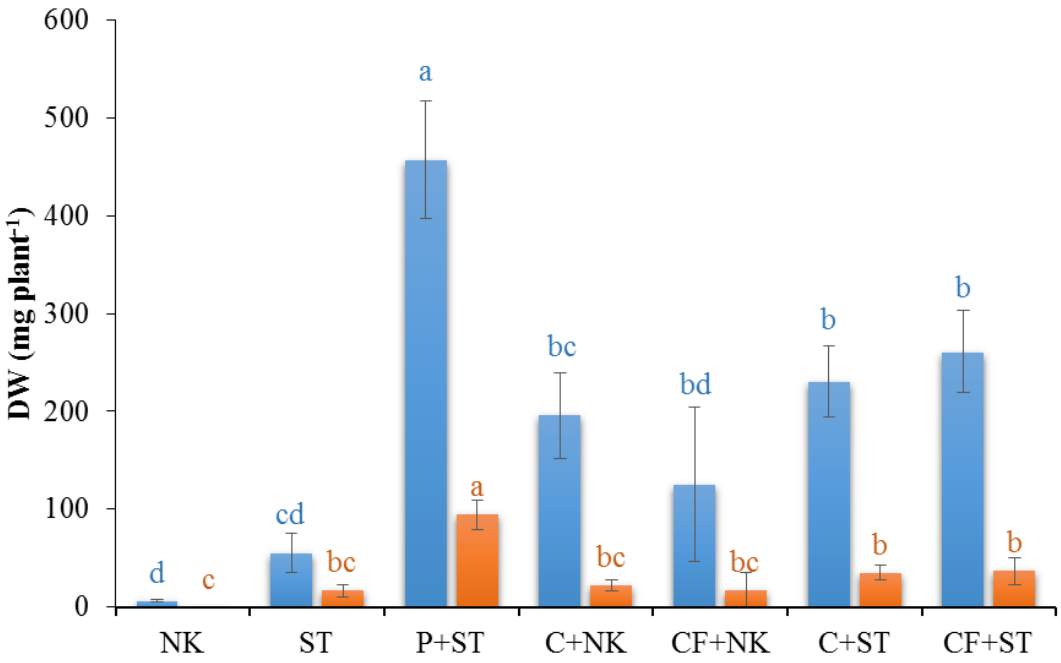


Figure 4.13: Lettuce dry weight (leaf in blue and roots in orange) grown in floating system with different nutrient solutions. The treatments were: solution with N and K (**NK**); standard solution without P (**ST**); standard solution with P (**P+ST**); solution with N and K and P derived from citric acid hydrolysates (**C+NK**); solution with NK and P from citric acid and acid phosphatase (**CF+NK**); standard solution with P from citric acid hydrolysates (**C+ST**) and standard solution with P from citric acid and acid phosphatase hydrolysates (**CF+ST**). Error bars represent standard errors (n=8) and different letters correspond to different group with Tukey test at $P \leq 0.05$.

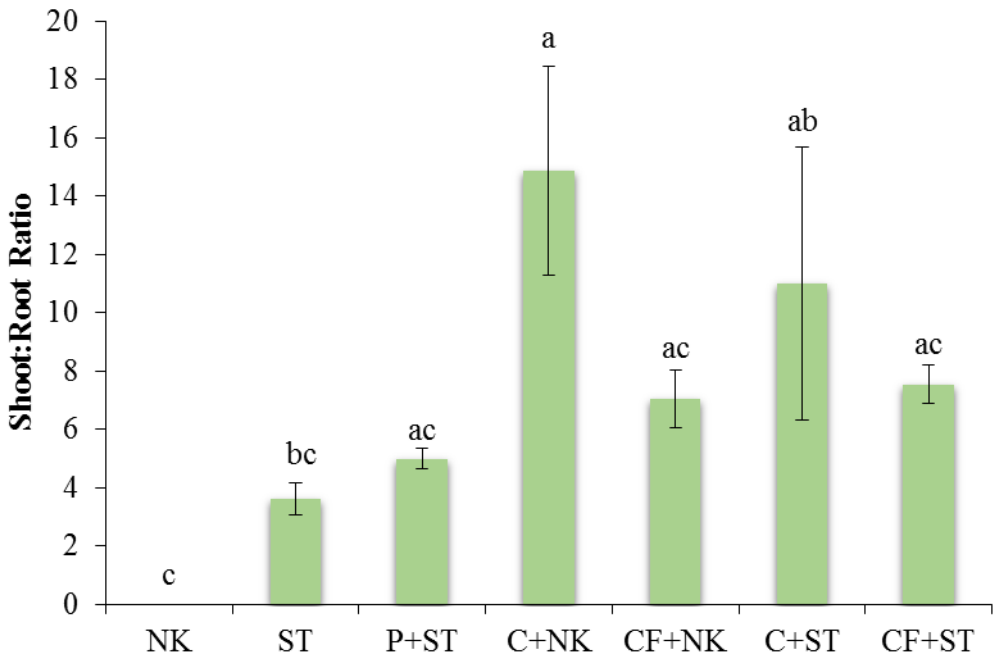


Figure 4.14: Shoot-root ratio of lettuce grown in floating system with different nutrient solutions. The treatments were: solution with N and K (**NK**); standard solution without P (**ST**); standard solution with P (**P+ST**); solution with N and K and P derived from citric acid hydrolysates (**C+NK**); solution with NK and P from citric acid and acid phosphatase (**CF+NK**); standard solution with P from citric acid hydrolysates (**C+ST**) and standard solution with P from citric acid and acid phosphatase hydrolysates (**CF+ST**). Error bars represent standard errors (n=8) and different letters correspond to different group with Tukey test at $P \leq 0.05$.

Leaf and roots metal concentration

Phosphorus concentration in plant biomass (Fig. 4.15) showed a higher P in leaves of positive control treated plants as compared to hydrolysates treated plants (+52.8%), whereas in roots P content was higher in CF+ST plants (+32.2% as compared to P+ST and +116.8% as compared to the average of C+NK, CF+NK and C+ST).

Al concentration (Fig. 4.16) resulted higher in both leaves and roots in plants treated with hydrolysates: in leaves the average of the hydrolysates treated plants were +205% higher than the positive control plants. In roots, the highest amount was found in acid phosphatase and citric acid treatments (+352% CF+ST as compared to C+ST and +101% CF+NK vs C+NK).

The highest Ca concentration (Fig. 4.17) in leaves was found in P+ST (+97.9% as compared to the average of hydrolysates treatments), whereas in roots the highest concentration was found in CF+ST (+113% compared to P+ST).

The results of Fe concentration (Fig. 4.15) in leaves and even more in roots were exponentially higher in hydrolysates treatment plants (+414% in leaves and +696% in roots of hydrolysates treated plants as compared to positive control).

On the nutrients side (Table 4.6 and 4.7), Mg concentration is comparable between the mineral control and the hydrolysates treatment, even in hydrolysates treatment without the standard solution. In roots the hydrolysates treatment without standard solutions contained more Mg than the hydrolysates treatment with standard solutions (+26% of the average of C+NK and CF+NK compared to the average of C+ST and CF+ST). Additionally, S concentration was similar between mineral and organic treatments. In roots, CF+ST had +20.2% S compared to P+ST. Another nutrient that was found similar in mineral P treatment and hydrolysates treatment was B, which was higher in citric acid treatment (+38% of the average of C+NK and C+ST compared to CF+NK and CF+ST). However, in roots, the B concentration was higher in the control (+55.3% of P+ST compared to the average of hydrolysates treatments). On the other hand, Mn concentration was higher in the mineral control (+625% in leaves and +126% in roots compared to the average of the hydrolysates treatment). In roots, however, Mn concentration in P+ST was similar to CF+ST. Mo concentration is also higher in P+ST (+81.8% in P+ST compared to the average of the hydrolysates treatment), but in roots the highest concentration was found in C+NK.

The elements which were found higher in the leaves of the mineral control plants (P+ST) as compared to the hydrolysates treatments (as average) were Cd (+31.4%), Cu (+202.2%) and Zn (+394%). On the other hand leaves of hydrolysates treated plants compared to the positive control had higher Al (+205.9%), Co (+18.9%), Na (+554%) and Pb (+138%). Many elements were also found in higher amount only in CF+ST treatment: Al concentration was higher as compared to P+ST (+250%) and to the

average of C+NK, CF+NK and C+ST (+20.4%); Cd concentration was higher in CF+ST than P+ST (+66.9%) and than the other hydrolysates (+264%); Fe (+426%), Na (+400%), Ni (+305%) and Pb (+128%) were higher in CF+ST than in P+ST, while Cu (+130%), Mg (+17.5%) and Mn (+103%) were higher in CF+ST as compared to the other hydrolysates treatments.

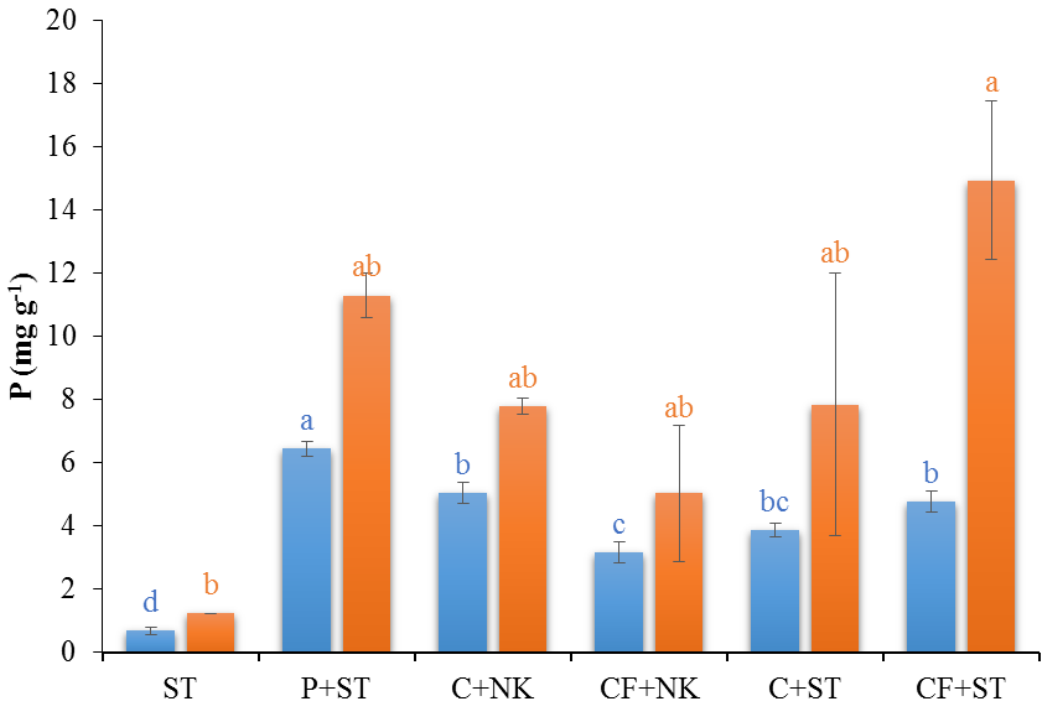


Figure 4.15: Leaf (in blue) and root (in orange) P concentration (mg g^{-1}) of lettuce grown in floating system with different nutrient solutions. The treatments were: standard solution without P (**ST**); standard solution with P (**P+ST**); solution with N and K and P derived from citric acid hydrolysates (**C+NK**); solution with NK and P from citric acid and acid phosphatase (**CF+NK**); standard solution with P from citric acid hydrolysates (**C+ST**) and standard solution with P from citric acid and acid phosphatase hydrolysates (**CF+ST**). Error bars represent standard errors (n=8) and different letters correspond to different group with Tukey test at $P \leq 0.05$.

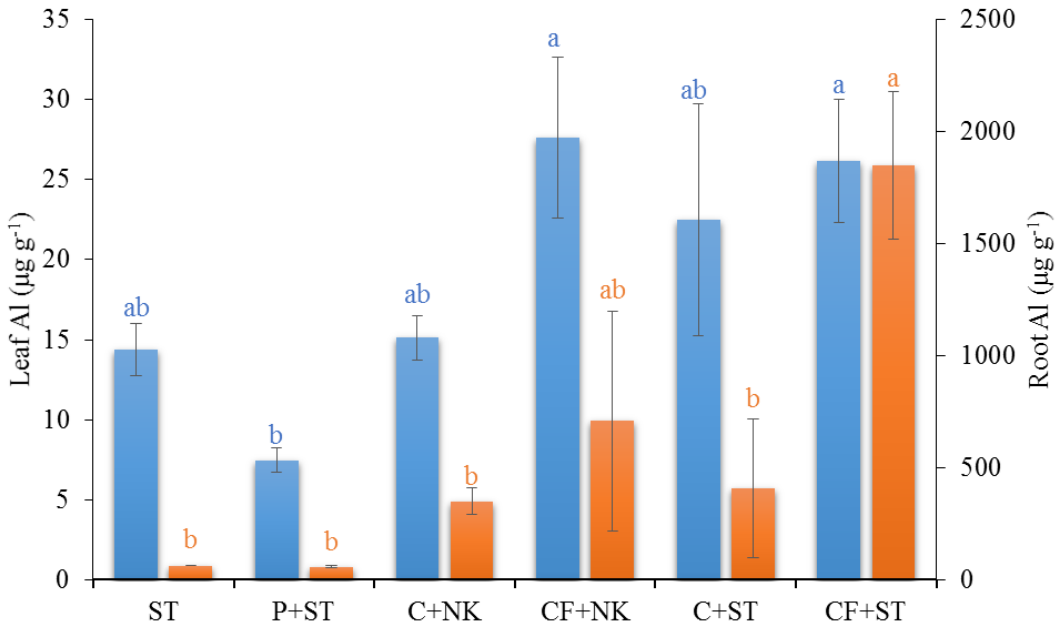


Figure 4.16: Leaf (in blue) and root (in orange) P concentration (mg g^{-1}) of lettuce grown in floating system with different nutrient solutions. The treatments were: standard solution without P (**ST**); standard solution with P (**P+ST**); solution with N and K and P derived from citric acid hydrolysates (**C+NK**); solution with NK and P from citric acid and acid phosphatase (**CF+NK**); standard solution with P from citric acid hydrolysates (**C+ST**) and standard solution with P from citric acid and acid phosphatase hydrolysates (**CF+ST**). Error bars represent standard errors (n=8) and different letters correspond to different group with Tukey test at $P \leq 0.05$.

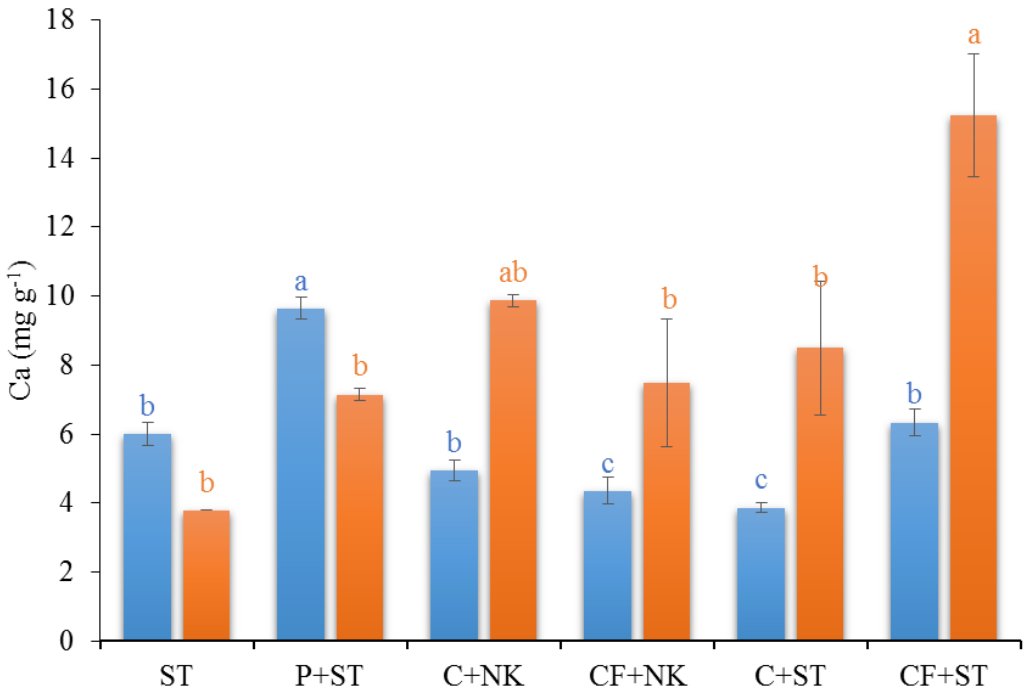


Figure 4.17: Leaf (in blue) and root (in orange) P concentration (mg g^{-1}) of lettuce grown in floating system with different nutrient solutions. The treatments were: standard solution without P (**ST**); standard solution with P (**P+ST**); solution with N and K and P derived from citric acid hydrolysates (**C+NK**); solution with NK and P from citric acid and acid phosphatase (**CF+NK**); standard solution with P from citric acid hydrolysates (**C+ST**) and standard solution with P from citric acid and acid phosphatase hydrolysates (**CF+ST**). Error bars represent standard errors (n=8) and different letters correspond to different group with Tukey test at $P \leq 0.05$.

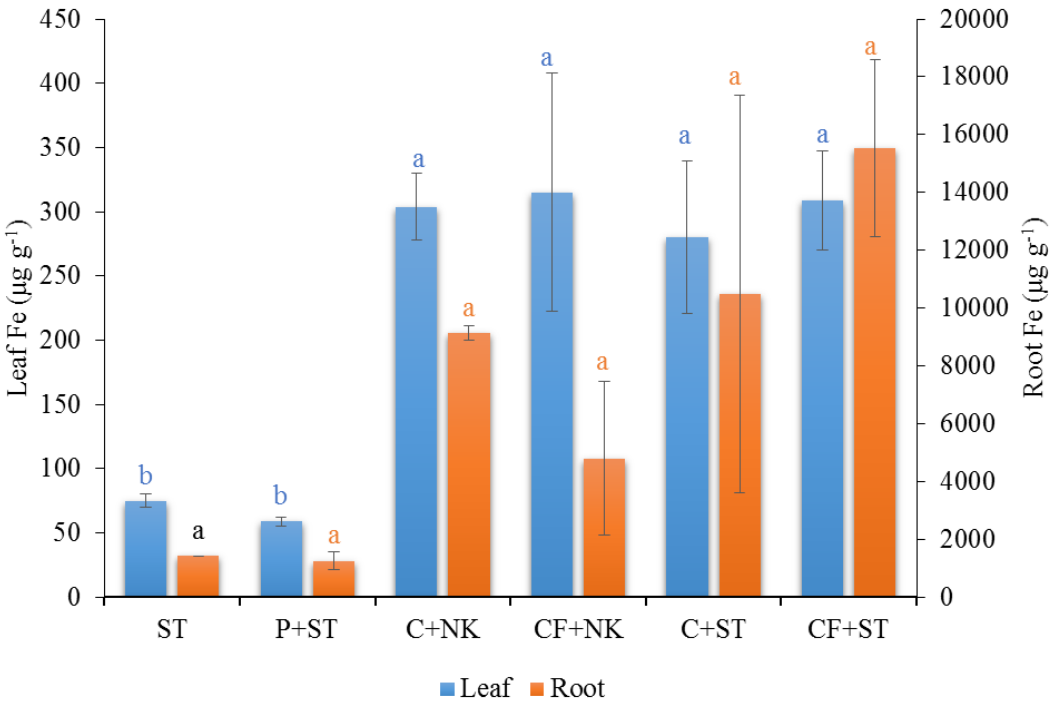


Figure 4.18: Leaf (in blue) and root (in orange) P concentration (mg g^{-1}) of lettuce grown in floating system with different nutrient solutions. The treatments were: standard solution without P (**ST**); standard solution with P (**P+ST**); solution with N and K and P derived from citric acid hydrolysates (**C+NK**); solution with NK and P from citric acid and acid phosphatase (**CF+NK**); standard solution with P from citric acid hydrolysates (**C+ST**) and standard solution with P from citric acid and acid phosphatase hydrolysates (**CF+ST**). Error bars represent standard errors (n=8) and different letters correspond to different group with Tukey test at $P \leq 0.05$.

Table 4.6: Leaf metal concentration in mg g^{-1} and µg g^{-1} in plants grown with different nutrient solutions.

	Leaf metal concentration					
	ST	**P+ST**	**C+NK**	**CF+NK**	**C+ST**	**CF+ST**
Elements in mg g^{-1}						
Ca	6.00 ab	9.65 a	4.95 bc	4.35 c	3.86 c	6.33 b
K	13.55	<DL	<DL	<DL	<DL	<DL
Na	3.39 c	1.96 c	15.66 a	9.36 b	16.58 a	9.82 b
P	0.69 d	6.45 a	5.06 b	3.17 c	3.88 cd	4.78 bc
S	1.75 b	3.45 ab	3.23 ab	3.82 a	2.46 ab	2.87 ab
Elements in µg g^{-1}						
Al	14.40 ab	7.47 b	15.12 ab	27.63 a	22.48 ab	26.17 a
B	28.45 ab	26.87 ab	34.26 a	21.48 b	29.28 ab	24.48 b
Cd	0.22 ab	0.43 ab	0.16 b	0.32 ab	0.11 b	0.72 a
Co	<DL	4.57	5.44	<DL	<DL	<DL
Cr	<DL a	<DL a	0.47 a	0.47 a	0.58 a	0.23 a
Cu	16.43 ab	20.43 a	4.59 b	5.65 b	5.09 b	11.72 ab
Fe	74.81 bc	58.70 c	303.84 a	315.23 a	280.47 ab	308.56 a
Mg	1.30 c	1.95 ab	2.12 a	1.55 bc	1.63 ac	2.08 a
Mn	85.83 bc	307.61 a	29.60 c	33.16 bc	38.57 bc	68.44 bc
Mo	1.59 ab	2.57 a	<DL b	1.37 ab	1.73 ab	1.14 ab
Ni	1.37 bc	0.59 c	3.73 a	3.87 a	1.48 bc	2.39 ab
Pb	2.29 a	0.66 b	1.65 ab	1.73 a	1.40 ab	1.50 ab
Sb	2.39 a	0.83 b	0.75 b	0.67 b	0.64 b	0.89 b
Se	<DL b	<DL b	1.26 a	1.20 a	1.24 a	1.24 a
Sn	0.56 a	0.26 b	<DL c	<DL c	<DL c	<DL c
Sr	49.16 ab	55.60 a	28.61 cd	34.37 ac	20.77 d	36.15 bc
Tl	<DL	<DL	<DL	<DL	<DL	<DL
V	<DL	<DL	<DL	<DL	<DL	<DL
Zn	140.08 b	233.44 a	46.41 cd	52.06 cd	24.15 d	66.14 c
					d	

<DL: concentration below the detection limit. Data were analysed with One-way ANOVA. Different letters represent different homogenous group with Tukey test ($P \leq 0.05$) within the metal. Metals without letters were not significant with ANOVA.

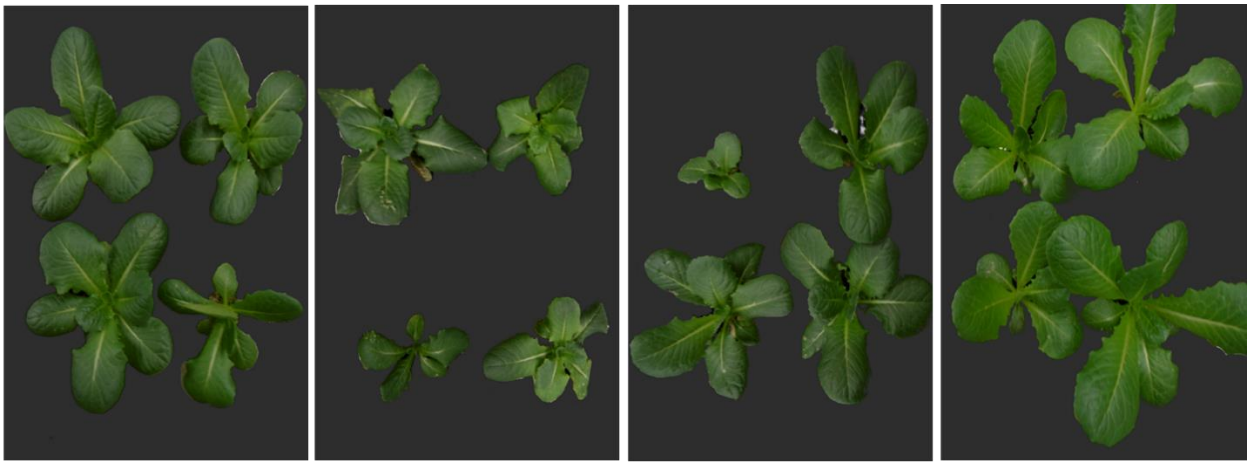
Table 4.7: Root metal concentration in mg g^{-1} and µg g^{-1} in plants grown with different nutrient solutions.

	Root metal concentration					
	ST	**P+ST**	**C+NK**	**CF+NK**	**C+ST**	**CF+ST**
Elements in mg g^{-1}						
Ca	3.79 b	7.14 b	9.86 ab	7.47 b	8.49 b	15.25 a
K	8.55	2.31	12.32	14.25	<DL	<DL
Na	1.70 b	1.99 b	15.24 a	8.36 ab	10.93 ab	11.32 ab
P	1.24 b	11.30 ab	7.80 ab	5.04 ab	7.84 ab	14.94 a
S	1.82 d	4.83 ab	4.46 ac	2.56 cd	3.69 bd	5.81 a
Elements in µg g^{-1}						
Al	64.07 b	58.71 b	350.7 b	708.0 ab	408.1 b	1847.5 a
B	15.20 b	24.28 a	16.98 b	17.42 ab	11.44 b	16.68 b
Cd	0.66	3.50	1.63	1.74	3.45	5.46
Co	<DL	1.01	<DL	<DL	0.38	1.57
Cr	4.83 b	4.19 b	4.75 b	9.35 ab	4.64 b	26.16 a
Cu	370.0 b	815.6 a	9.97 c	10.10 c	15.98 c	85.58 c
Fe	1429	1254	9133	4800	10489	15539
Mg	1.17	1.66	1.46	1.99	1.09	1.48
Mn	92.68 a	332.1 a	66.64 a	70.27 a	110.9 a	340.47 a
Mo	48.87	56.50	103.3	<DL	7.90	18.00
Ni	1.84 b	1.70 b	16.27 ab	18.50 ab	10.37 ab	28.28 a
Pb	6.96	2.73	6.13	8.15	4.05	8.03
Sb	1.58	1.26	2.67	3.36	1.69	1.88
Se	<DL	<DL	<DL	2.16	1.59	2.05
Sn	1.21	0.36	5.16	5.50	2.59	2.82
Sr	40.90 b	67.13 ab	151.0 ab	125.9 ab	154.1 ab	301.18 a
Tl	<DL	<DL	<DL	<DL	<DL	<DL
V	<DL	<DL	<DL	1.00	1.04	2.15
Zn	221.4 b	734.9 a	80.51 b	96.54 b	91.69 b	377.58 b

<DL: concentration below the detection limit.

Data were analysed with One-way ANOVA. Different letters represent different homogenous group with Tukey test ($P \leq 0.05$) within the metal. Metals without letters were not significant with ANOVA.


ST
P+ST


C+NK
CF+NK
C+ST
CF+ST

Discussion

Organic products as a source of P for plant nutrition are extensively studied nowadays, in order to find a concrete solution for replacing phosphate rocks as raw material for P fertilizers (*Torri et al.*, 2017). The organic products can be used directly on the soil, as previously described in chap 2, or can be used as raw material for a new fertilizer. In this study, we have tested a potential liquid fertilizer derived from the hydrolysis of an anaerobic sewage sludge with citric acid and a combination of citric acid and acid phosphatase. The hydrolysis was described in chap. 3. The fertilizer was used for lettuce grown in a floating system, in order to test the suitability of such hydrolysates as organic fertilizers for soilless cultivation, but mostly to assess the effects of these products on plants, excluding the influence of soil. The P and other nutrient solubility was also easily monitored with the frequent samplings of the nutrient solution. It has been shown that soil can quickly decrease the availability of P, due to precipitation of this element with Ca and Mg in alkaline soil and Fe and Al in acidic soil (*Guppy et al.*, 2005). In soilless cultivation, the pH can be controlled and the availability of the nutrients is normally the highest possible. In our study, it was shown that using the hydrolysates, which contained high concentration of organic matter, the pH could increase faster and was, thus, difficult to control. These pH fast changes had influenced all the study, with an evident precipitation of many elements and a solubilisation of others. The precipitation with the pH increase included P, which probably precipitated with Al and Fe, which were in very high amount in the hydrolysates, but also with Ca (in low amount) and Mn. On the other hand, some elements, such as Mg, did not show a decrease in availability with the pH increase, and some others, such as Se and the more dangerous As (data not shown), did increase their solubility. A slight difference was remarkable between the hydrolysates: the citric acid hydrolysate with the addition of standard solution (C+ST) in the first part of the experiment showed a metal precipitation slightly postponed. This can be due to a lower initial pH as compared to the treatment with acid phosphatase (they were adjusted to pH 4.8 for the addition of the enzymes) and to a better equilibrium of elements compared to the hydrolysates without the standard solution.

As the potential toxic elements contained in the hydrolysates solutions, they were always lower than the EU regulations (*Fertilisers Working Group Meeting*, 2014) in term of Cd, which was under detection limits in hydrolysates, but also for Cr, Pb, Ni, Zn and Cu. Furthermore they were considered safe also for Finnish and Canadian regulation for fertilizers (*Kabata-Pendias*, 2011).

In addition to the toxic metals below the limits, it was clear that the hydrolysates solutions contained also some nutrients, which can be considered meso and micronutrient for plant growth, such as Mg, Fe, S and B. That was recognizable from

the amount of these elements in the treatments without standard solution: in these treatments, the concentration of Mg, S and B was similar to the standard control and the Fe concentration was even much higher. This very high Fe concentration makes the Fe uptake from the plants very high and consequently the leaf greenness significantly increased. Besides its role in increasing the leaf chlorophyll, Fe can participate to the antioxidant activity of the plants with opposite roles: as protective species (Fe is a constituent of catalase, ascorbate peroxidase, guaiacol peroxidase, and ferro-superoxide dismutase) or as catalytic of Fenton reaction creating new toxic oxygen species (*Becana et al.*, 1998). Generally the iron toxicity is limited to the submerged lands, where the reducing conditions increase the solubility of Fe (*Wheeler et al.*, 1985), but in the conditions of this study the solubility of Fe remained higher, until the pH remained in the range 5.5-6.5. The high Fe uptake can increase, thus, the production of toxic oxygen radical (*Becker and Asch*, 2005) in the upper part of the plants. These symptoms can explain the higher chlorophyll fluorescence found in plants grown in hydrolysates solutions, caused by an oxidative stress in the leaves.

On the other hand, this high concentration in Fe and the higher concentration in Se in leaves as compared to the mineral fertilized plants can be interesting for human and plant health. Plant Fe accumulation can improve the composition of healthy food, being Fe essential for human nutrition (*Grusak and DellaPenna*, 1999). Se is known to be a useful micronutrient for humans and animals at low dosage, with anticarcinogenic proprieties (*Ellis and Salt*, 2003), but also can help the plants against abiotic stress (*Feng et al.*, 2013), up to a certain dose that is plant-specific which can be poisonous (*Brown and Shrift*, 1982).

Contrariwise, Al concentration, which is not generally included in the regulatory limits, were very high in the hydrolysates. Al concentration is known to be toxic in high concentration, mostly for roots development (*Kochian*, 1995; *Marschner and Marschner*, 2012). Therefore, this can explain the low roots dry biomass and the consequent very high shoot-root ratio of the plants grown in hydrolysates solutions. Consistently, the low roots development caused the plant growth to be compromised. However, the growth decrease was not as consistent as the Al toxicity could have caused, especially in the plants treated with the mix of citric acid and acid phosphatases (CF+ST). These plants, beside the highest growth between the hydrolysate treated plants and the lowest chlorophyll fluorescence, which showed lower plant stress, showed highest metal (among which also toxic metal) uptake and a good roots developments. The Al toxicity was more clear in the plants treated with only citric acid (C+NK and C+ST): in these plants, due to the higher amount of hydrolysates added, the Al concentration in solutions were higher and thus, the roots had great difficulties in growing, with a consequent shoot-root ratio extremely high.

The nutrient precipitation and the Al toxicity had, thus, influenced the plant growth, which were lower in hydrolysates as compared to the mineral control. However, the differences in plant biomass between hydrolysates treated plants and positive control plants became clearer only in the last week of the experiment, while the leaf area production between P+ST and CF+ST were similar until 35 days after sowing.

In conclusion, the hydrolysates were not ready yet to be used directly as P fertilizers in a soilless cultivation, due the problems of pH increase and Al toxicity. On the other hand, the content in micronutrient and Fe remains very interesting for a creation of a complex fertilizer and promising for biofortification of plants for human nutrition. A test on plants grown in soil can exacerbate the potentiality of the hydrolysates, such as the nutrient concentration for plant nutrition and human health. But it also can improve the soil quality and the enzymatic activity (*Tejada et al.*, 2013) and reduce the aluminum toxicity, reducing its plant availability with Ca precipitation (*Marschner and Marschner*, 2012).

References

Achat, D.L., Bakker, M.R., Zeller, B., Pellerin, S., Bienaimé, S., Morel, C. (2010): Long-term organic phosphorus mineralization in Spodosols under forests and its relation to carbon and nitrogen mineralization. *Soil Biol. Biochem.* 42, 1479–1490.

Adam, C., Peplinski, B., Michaelis, M., Kley, G., Simon, F.-G. (2008): Thermochemical treatment of sewage sludge ashes for phosphorus recovery. *Waste Manag.* 29, 1122–1128.

Adesemoye, A.O., Kloepper, J.W. (2009): Plant–microbes interactions in enhanced fertilizer-use efficiency. *Appl. Microbiol. Biotechnol.* 85, 1–12.

AEEP (2017): Phosphorus: Key to Plant Life [WWW Document]. URL http://aeep.eu/phosphorus/ (accessed 11.29.17).

Ajiboye, B., Akinremi, O.O., Hu, Y., Flaten, D.N. (2007): Phosphorus Speciation of Sequential Extracts of Organic Amendments Using Nuclear Magnetic Resonance and X-ray Absorption Near-Edge Structure Spectroscopies. *J. Environ. Qual.* 36, 1563.

Alborés, A.F., Cid, B.P., Gómez, E.F., López, E.F. (2000): Comparison between sequential extraction procedures and single extractions for metal partitioning in sewage sludge samples. *Analyst* 125, 1353–1357.

Alvarenga, P., Mourinha, C., Farto, M., Santos, T., Palma, P., Sengo, J., Morais, M.-C., Cunha-Queda, C. (2015): Sewage sludge, compost and other representative organic wastes as agricultural soil amendments: Benefits versus limiting factors.

Alvarez, A.E., Mochon, C.M., Sanchez, J.C., Rodriguez, T.M. (2002): Heavy metal extractable forms in sludge from wastewater treatment plants. *Chemosphere* 47, 765–775.

Andersen, M.S. (2007): An introductory note on the environmental economics of the circular economy. *Sustain. Sci.* 2, 133–140.

Ayaga, G., Todd, A., Brookes, P.C. (2006): Enhanced biological cycling of phosphorus increases its availability to crops in low-input sub-Saharan farming systems. *Soil Biol. Biochem.* 38, 81–90.

Baek, D., Kim, M.C., Chun, H.J., Kang, S., Park, H.C., Shin, G., Park, J., Shen, M., Hong, H., Kim, W.-Y., Kim, D.H., Lee, S.Y., Bressan, R.A., Bohnert, H.J., Yun, D.-J. (2013): Regulation of miR399f Transcription by AtMYB2 Affects Phosphate Starvation Responses in Arabidopsis. *Plant Physiol.* 161, 362–373.

Baldwin, J.C., Karthikeyan, A.S., Raghothama, K.G. (2001): LEPS2, a phosphorus starvation-induced novel acid phosphatase from tomato. *Plant Physiol.* 125, 728–37.

Bates, T.R., Lynch, J.P. (1996): Stimulation of root hair elongation in Arabidopsis thaliana by low phosphorus availability. *Plant, Cell Environ.* 19, 529–538.

Becana, M., Moran, J.F., Iturbe-Ormaetxe, I. (1998): Iron-dependent oxygen free radical generation in plants subjected to environmental stress: Toxicity and antioxidant protection. *Plant Soil* 201, 137–147.

Becker, M., Asch, F. (2005): Iron toxicity in rice-conditions and management concepts.

J. Plant Nutr. Soil Sci. 168, 558–573.

Bernal, M.P., Alburquerque, J.A., Moral, R. (2009): Composting of animal manures and chemical criteria for compost maturity assessment. A review. *Bioresour. Technol.* 100, 5444–5453.

Bhadoria, P.S., Steingrobe, B., Claassen, N., Liebersbach, H. (2002): Phosphorus efficiency of wheat and sugar beet seedlings grown in soils with mainly calcium, or iron and aluminium phosphate. *Plant Soil* 246, 41–52.

Biswas, B.K., Inoue, K., Harada, H., Ohto, K., Kawakita, H. (2009): Leaching of phosphorus from incinerated sewage sludge ash by means of acid extraction followed by adsorption on orange waste gel. *J. Environ. Sci.* 21, 1753–1760.

Blöcher, C., Niewersch, C., Melin, T. (2012): Phosphorus recovery from sewage sludge with a hybrid process of low pressure wet oxidation and nanofiltration. *Water Res.* 46, 2009–2019.

Bolan, N.S. (1991): A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. *Plant Soil*.

Breeuwsma, A., Reijerink, J.G.A., Schoumans, O.F. (1995): Impact of manure on accumulation and leaching of phosphate in areas of intensive livestock farming, in: Animal Waste and the Land-Water Interface. pp. 239–249.

Brod, E., Øgaard, A.F., Hansen, E., Wragg, D., Haraldsen, T.K., Krogstad, T. (2015): Waste products as alternative phosphorus fertilisers part I: inorganic P species affect fertilisation effects depending on soil pH. *Nutr. Cycl. Agroecosystems* 103, 167–185.

Brookes, P., Beyaert, R., Voroney, R. (2007): Soil Microbial Biomass C, N, P, and S, in: Soil Sampling and Methods of Analysis, Second Edition. CRC Press.

Brookes, P.C., Powlson, D.S., Jenkinson, D.S. (1982): Measurement of microbial biomass phosphorus in soil. *Soil Biol. Biochem.* 14, 319–329.

Brown, T.A., Shrift, A. (1982): SELENIUM: TOXICITY AND TOLERANCE IN HIGHER PLANTS. *Biol. Rev.* 57, 59–84.

Bünemann, E.K. (2015): Assessment of gross and net mineralization rates of soil organic phosphorus – A review. *Soil Biol. Biochem.* 89, 82–98.

Burleigh, S.H., Cavagnaro, T., Jakobsen, I. (2002): Functional diversity of arbuscular mycorrhizas extends to the expression of plant genes involved in P nutrition. *J. Exp. Bot.* 53, 1593–1601.

Cakmak, I., Hengeler, C., Marschner, H. (1994): Partitioning of shoot and root dry matter and carbohydrates in bean plants suffering from phosphorus, potassium and magnesium deficiency. *J. Exp. Bot.* 45, 1245–1250.

CCIA (2016): Prezzi - News — Camera di Commercio di Modena [WWW Document]. URL http://www.mo.camcom.it/tutela-del-mercato/ufficio-prezzi (accessed 11.9.16).

Chen, C.R., Condron, L.M., Davis, M.R., Sherlock, R.R. (2002): Phosphorus dynamics in the rhizosphere of perennial ryegrass (Lolium perenne L.) and radiata pine (Pinus Radiata D. Don.). *Soil Biol. Biochem.* 34, 487–499.

Childers, D.L., Corman, J., Edwards, M., Elser, J.J. (2011): Sustainability Challenges

of Phosphorus and Food: Solutions from Closing the Human Phosphorus Cycle. *Bioscience* 61, 117–124.

Christodoulou, A., Stamatelatou, K. (2016): Overview of legislation on sewage sludge management in developed countries worldwide. *Water Sci. Technol.* 73.

Cieslik, B., Konieczka, P. (2017): A review of phosphorus recovery methods at various steps of wastewater treatment and sewage sludge management. The concept of " no solid waste generation " and analytical methods. *J. Clean. Prod.* 142, 1728–1740.

Clarkson, D.T., Scattergood, C.B. (1982): Growth and Phosphate Transport in Barley and Tomato Plants During the Development of, and Recovery from, Phosphate-stress. *J. Exp. Bot.* 33, 865–875.

Cogliatti, D.H., Santa Marìa, G.E. (1990): Influx and Efflux of Phosphorus in Roots of Wheat Plants in Non-Growth-Limiting Concentrations of Phosphorus. *J. Exp. Bot.* 41, 601–607.

Cooper, J., Lombardi, R., Boardman, D., Carliell-Marquet, C. (2011): The future distribution and production of global phosphate rock reserves. *Resour. Conserv. Recycl.* 57, 78–86.

Cordell, D., Drangert, J., White, S. (2009): The story of phosphorus: global food security and food for thought. *Glob. Environ. Chang.*

Cordell, D., Rosemarin, A., Schroder, J.J., Smit, A.L. (2011): Towards global phosphorus security: A systems framework for phosphorus recovery and reuse options. *Chemosphere* 84, 747–758.

Corominas, L., Foley, J., Guest, J.S., Hospido, A., Larsen, H.F., Morera, S., Shaw, A. (2013): Life cycle assessment applied to wastewater treatment: State of the art. *Water Res.* 47, 5480–5492.

Da Ros, C., Cavinato, C., Pavan, P., Bolzonella, D. (2016): Mesophilic and thermophilic anaerobic co-digestion of winery wastewater sludge and wine lees: An integrated approach for sustainable wine production. *J. Environ. Manage.*

Dakora, F.D., Phillips, D.A. (2002): Root exudates as mediators of mineral acquisition in low-nutrient environments. *Plant Soil* 245, 35–47.

Delhaize, E., Randall, P.J., Laurie, S., Phillips, R., Ratcliffe, R., Stewart, G. (1995): Characterization of a Phosphate-Accumulator Mutant of Arabidopsis thaliana. *Plant Physiol.* 107, 207–213.

Direzione Generale Ambiente, D. del suolo e della costa-D.A.R.E.-R. (2004): Linee di Intervento in materia di utilizzo dei fanghi di depurazione in agricoltura. Bologna.

Domenech, C.E., Lisa, T.A., Salvano, M.A., Garrido, M.N. (2015): Selective release of phosphorus and nitrogen from waste activated sludge with combined thermal and alkali treatment. *Bioresour. Technol.* 190, 522–528.

Donatello, S., Tong, D., Cheeseman, C.R. (2010): Production of technical grade phosphoric acid from incinerator sewage sludge ash (ISSA). *Waste Manag.* 30, 1634–1642.

Doyle, J.D., Parsons, S.A. (2002): Struvite formation, control and recovery. *Water Res.* 36, 3925–3940.

Duboc, O., Santner, J., Fard, A.G., Zehetner, F., Tacconi, J., Wenzel, W.W. (2017): Predicting phosphorus availability from chemically diverse conventional and recycling fertilizers. *Sci. Total Environ.* 599–600, 1160–1170.

Easlon, H.M., Bloom, A.J. (2014): Easy Leaf Area: Automated Digital Image Analysis for Rapid and Accurate Measurement of Leaf Area. *Appl. Plant Sci.* 2, 1400033.

Eghball, B. (2002): Soil Properties as Influenced by Phosphorus-and Nitrogen-Based Manure and Compost Applications WASTE MANAGEMENT Soil Properties as Influenced by Phosphorus-and Nitrogen-Based Manure and Compost Applications. *Waste Manag.* 94, 128–135.

Eghball, B., Power, J.F. (1999): Phosphorus- and Nitrogen-Based Manure and Compost Applications: Corn Production and Soil Phosphorus. *Soil Sci. Soc. Am. J.* 63, 895–901.

Ellis, D.R., Salt, D.E. (2003): Plants, selenium and human health. *Curr. Opin. Plant Biol.*

Eneji, A.E., Honna, T., Yamamoto, S., Masuda, T., Endo, T., Irshad, M. (2003): Changes in Humic Substances and Phosphorus Fractions During Composting. *Commun. Soil Sci. Plant Anal.* 34, 2303–2314.

European Biogas Association (2015): EBA Biomethane & Biogas Report 2015 [WWW Document]. URL http://european-biogas.eu/2015/12/16/biogasreport2015/ (accessed 11.9.16).

Eurostat (2016): Eurostat [WWW Document]. URL http://ec.europa.eu/eurostat/web/main/home (accessed 11.9.16).

Ewa Sroga, G. (1987): Plant regeneration of two Lupinus spp. from callus cultures via organogenesis. *Plant Sci.* 51, 245–249.

FAO (2016): FAOSTAT [WWW Document]. URL http://www.fao.org/faostat/en/#home (accessed 12.2.17).

Feng, R., Wei, C., Tu, S. (2013): The roles of selenium in protecting plants against abiotic stresses. *Environ. Exp. Bot.*

Fertilisers Working Group Meeting (2014): Essential safety and quality requirements for fertilising materials.

Foehse, D., Jungk, A. (1983): Influence of phosphate and nitrate supply on root hair formation of rape, spinach and tomato plants. *Plant Soil* 74, 359–368.

Fredeen, A., Rao, I., Terry, N. (1989): Influence of phosphorus nutrition on growth and carbon partitioning in Glycine max. *Plant Physiol.*

Frossard, E., Condron, L.M., Oberson, A., Sinaj, S., Fardeau, J.C. (2000): Processes Governing Phosphorus Availability in Temperate Soils. *J. Environ. Qual.* 29, 15.

Frossard, E., Sinaj, S., Zhang, L.-M., Morel, J.L. (1996): The Fate of Sludge Phosphorus in Soil-Plant Systems. *Soil Sci. Soc. Am. J.* 60, 1248.

Fuentes, B., Bolan, N., Naidu, R., de la Luz Mora, M., Australia, A. (2006): PHOSPHORUS IN ORGANIC WASTE-SOIL SYSTEMS Fósforo en los sistemas suelo – residuos orgánicos. *J.Soil Sc. Plant. Nutr* 66, 64–8364.

García-Albacete, M., Martín, A., Cartagena, M.C. (2012): Fractionation of phosphorus biowastes: Characterisation and environmental risk. *Waste Manag.*

32, 1061–1068.

Gianquinto, G., Orsini, F., Sambo, P., D'Urzo, M.P. (2011): The Use of Diagnostic Optical Tools to Assess Nitrogen Status and to Guide Fertilization of Vegetables. *Horttechnology* 21, 287–292.

Gonzalez-Ponce, R., López-de-Sá, E.G., Plaza, C. (2009): Lettuce Response to Phosphorus Fertilization with Struvite Recovered from Municipal Wastewater. *HortScience* 44, 426–430.

Grigatti, M., Boanini, E., Cavani, L., Ciavatta, C., Marzadori, C. (2015): Phosphorus in Digestate-Based Compost: Chemical Speciation and Plant-Availability. *Waste and Biomass Valorization* 6, 481–493.

Grigatti, M., Boanini, E., Mancarella, S., Simoni, A., Centemero, M., Veeken, A.H.M. (2017): Phosphorous extractability and ryegrass availability from bio-waste composts in a calcareous soil. *Chemosphere* 174, 722–731.

Grigatti, M., Di Girolamo, G., Chincarini, R., Ciavatta, C., Barbanti, L. (2011): Potential nitrogen mineralization, plant utilization efficiency and soil CO2 emissions following the addition of anaerobic digested slurries. *Biomass and Bioenergy* 35, 4619–4629.

Grusak, M. a., DellaPenna, D. (1999): Improving the Nutrient Composition of Plants To Enhance Human Nutrition and Health1. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 50, 133–161.

Guppy, C., Menzies, N., Moody, P., Blamey, F. (2005): Competitive sorption reactions between phosphorus and organic matter in soil: a review. *Soil Res.*

Haug, R.T. (1993): The practical handbook of compost engineering. Lewis Publishers.

Heimersson, S., Harder, R., Peters, G.M., Svanström, M. (2014): Including pathogen risk in life cycle assessment of wastewater management. 2. Quantitative comparison of pathogen risk to other impacts on human health. *Environ. Sci. Technol.* 48, 9446–9453.

Herzel, H., Kruger, O., Hermann, L., Adam, C. (2016): Sewage sludge ash - A promising secondary phosphorus source for fertilizer production. *Sci. Total Environ.* 542, 1136–1143.

Hinsinger, P. (2001): Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. *Plant Soil* 237, 173–195.

Hooda, P.S., Truesdale, V.W., Edwards, A.C., Withers, P.J.A., Aitken, M.N., Miller, A., Rendell, A.R. (2001): Manuring and fertilization effects on phosphorus accumulation in soils and potential environmental implications. *Adv. Environ. Res.* 5, 13–21.

Hospido, A., Moreira, M.T., Feijoo, G. (2008): A comparison of municipal wastewater treatment plants for big centres of population in Galicia (Spain). *Int. J. Life Cycle Assess.* 13, 57–64.

Hukari, S., Hermann, L., Nättorp, A. (2016): From wastewater to fertilisers — Technical overview and critical review of European legislation governing phosphorus recycling. *Sci. Total Environ.* 542, 1127–1135.

Hunter, P.J., Teakle, G.R., Bending, G.D. (2014): Root traits and microbial community interactions in relation to phosphorus availability and acquisition, with particular

reference to Brassica. *Front. Plant Sci.* 5, 27.

Indexmundi (2016): DAP fertilizer - Monthly Price - Commodity Prices - Price Charts, Data, and News - IndexMundi [WWW Document]. URL http://www.indexmundi.com/commodities/?commodity=dap-fertilizer&months=180 (accessed 11.9.16).

Jaffer, Y., Clark, T.A., Pearce, P., Parsons, S.A. (2002): Potential phosphorus recovery by struvite formation. *Water Res.* 36, 1834–1842.

Jeng, A.S., Haraldsen, T.K., Grønlund, A., Pedersen, P.A. (2006): Meat and bone meal as nitrogen and phosphorus fertilizer to cereals and rye grass. *Nutr. Cycl. Agroecosystems* 76, 183–191.

Johri, A.K., Oelmüller, R., Dua, M., Yadav, V., Kumar, M., Tuteja, N., Varma, A., Bonfante, P., Persson, B.L., Stroud, R.M. (2015): Fungal association and utilization of phosphate by plants: success, limitations, and future prospects. *Front. Microbiol.* 6, 984.

Jones, D.L. (1998): Organic acids in the rhizosphere – a critical review. *Plant Soil* 205, 25–44.

Kabata-Pendias, A. (2011): Trace elements in soils and plants. Fourth Edition, CRC Press.

Kataki, S., West, H., Clarke, M., Baruah, D.C. (2016): Phosphorus recovery as struvite: Recent concerns for use of seed, alternative Mg source, nitrogen conservation and fertilizer potential. *Resour. Conserv. Recycl.* 107, 142–156.

Khan, A., Jilani, G., Akhtar, M.S., Naqvi, S.M.S., Rasheed, M., Khan, A.A., Jilani, G., Akhtar, M.S., Muhammad, S., Naqvi, S., Rasheed, M. (2009): Phosphorus Solubilizing Bacteria: Occurrence, Mechanisms and their Role in Crop Production. *Publ. J. agric. biol. sci* 1, 48–58.

Khan, K.S., Joergensen, R.G. (2012a): Relationships between P fractions and the microbial biomass in soils under different land use management. *Geoderma* 173, 274–281.

Khan, K.S., Joergensen, R.G. (2012b): Compost and phosphorus amendments for stimulating microorganisms and growth of ryegrass in a Ferralsol and a Luvisol. *J. Plant Nutr. Soil Sci.* 175, 108–114.

Kidd, P.S., Domínguez-Rodríguez, M.J., Díez, J., Monterroso, C. (2007): Bioavailability and plant accumulation of heavy metals and phosphorus in agricultural soils amended by long-term application of sewage sludge. *Chemosphere* 66, 1458–1467.

Kim, M., Han, D.W., Kim, D.J. (2015): Selective release of phosphorus and nitrogen from waste activated sludge with combined thermal and alkali treatment. *Bioresour. Technol.*

Kleinman, P.J.A., Sharpley, A.N., Wolf, A.M., Beegle, D.B., Moore, P.A. (2002): Measuring Water-Extractable Phosphorus in Manure as an Indicator of Phosphorus in Runoff. *Soil Sci. Soc. Am. J.* 66, 2009.

Kochian, L. V (1995): Cellular Mechanisms of Aluminum Toxicity and Resistance in Plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 46, 237–260.

Koppelaar, R.H.E.M., Weikard, H.P. (2013): Assessing phosphate rock depletion and

phosphorus recycling options. *Glob. Environ. Chang.* 23, 1454–1466.

Krüger, O., Adam, C. (2015): Recovery potential of German sewage sludge ash. *Waste Manag.* 45, 400–406.

Kumar, V., Gilkes, R.J., Bolland, M.D.A. (1992): A comparison of seven soil P tests for plant species with different external P requirements grown on soils containing rock phosphate and superphosphate residues. *Fertil. Res.* 33, 35–45.

Lambers, H., Brundrett, M.C., Raven, J.A., Hopper, S.D. (2011): Plant mineral nutrition in ancient landscapes: high plant species diversity on infertile soils is linked to functional diversity for nutritional strategies. *Plant Soil* 348, 7–27.

Laturnus, F., von Arnold, K., Grøn, C. (2007): Organic Contaminants from Sewage Sludge Applied to Agricultural Soils. False Alarm Regarding Possible Problems for Food Safety? (8 pp). *Environ. Sci. Pollut. Res. Int.* 14 Suppl 1, 53–60.

Leite, W.R.M., Gottardo, M., Pavan, P., Belli Filho, P., Bolzonella, D. (2016): Performance and energy aspects of single and two phase thermophilic anaerobic digestion of waste activated sludge. *Renew. Energy* 86, 1324–1331.

Leytem, A.B., Westermann, D.T. (2003): PHOSPHATE SORPTION BY PACIFIC NORTHWEST CALCAREOUS SOILS. *Soil Sci.* 168.

Leytem, A.B., Westermann, D.T. (2005): PHOSPHORUS AVAILABILITY TO BARLEY FROM MANURES AND FERTILIZERS ON A CALCAREOUS SOIL. *Soil* 170, 401–412.

Liang, Y., Sun, W., Zhu, Y.G., Christie, P. (2007): Mechanisms of silicon-mediated alleviation of abiotic stresses in higher plants: A review. *Environ. Pollut.* 147, 422–428.

López-Arredondo, D.L., Leyva-González, M.A., Alatorre-Cobos, F., Herrera-Estrella, L. (2013): Biotechnology of nutrient uptake and assimilation in plants. *Int. J. Dev. Biol.* 57, 595–610.

Lynch, J., Läuchli, A., Epstein, E. (1991): Vegetative Growth of the Common Bean in Response to Phosphorus Nutrition. *Crop Sci.* 31, 380.

Ma, J.F. (2004): Role of silicon in enhancing the resistance of plants to biotic and abiotic stresses. *Soil Sci. Plant Nutr.* 50, 11–18.

Ma, Z., Bielenberg, D.G., Brown, K.M., Lynch, J.P. (2001): Regulation of root hair density by phosphorus availability in Arabidopsis thaliana. *Plant, Cell Environ.* 24, 459–467.

Malik, M., Khan, K., Marschner, P. (2013): Microbial biomass, nutrient availability and nutrient uptake by wheat in two soils with organic amendments. *J. Soil Sci. Plant Nutr.* 13, 955–966.

Mamani, I., Averanga, Kattia, Boris, E., Terrazas, E. (2014): Establecimiento del sistema de generación in vitro de callos de Lupinus mutabilis sweet (tarwi) a partir de hipocótilos. *Rev. CON-CIENCIA* 2.

Marchetti, R., Castelli, F., Orsi, A., Sghedoni, L., Bochicchio, D. (2012): Biochar from swine manure solids: influence on carbon sequestration and Olsen phosphorus and mineral nitrogen dynamics in soil with and without digestate incorporation. *Ital. J. Agron.* 7, 26.

Marschner, H., Kirkby, E.A., Cakmak, I. (1996): Effect of mineral nutritional status on shoot-root partitioning of photoassimilates and cycling of mineral nutrients. *J. Exp. Bot.* 47, 1255–1263.

Marschner, H., Marschner, P. (2012): Marschner's mineral nutrition of higher plants. Academic Press.

Mattenberger, H., Fraissler, G., Brunner, T., Herk, P., Hermann, L., Obernberger, I. (2008): Sewage sludge ash to phosphorus fertiliser: Variables influencing heavy metal removal during thermochemical treatment. *Waste Manag.* 28, 2709–2722.

Mayer, B.K., Baker, L.A., Boyer, T.H., Drechsel, P., Gifford, M., Hanjra, M.A., Parameswaran, P., Stoltzfus, J., Westerhoff, P., Rittmann, B.E. (2016): Total Value of Phosphorus Recovery. *Environ. Sci. Technol.* 50, 6606–6620.

McBride, M., Sauve, S., Hendershot, W. (1997): Solubility control of Cu, Zn, Cd and Pb in contaminated soils. *Eur. J. Soil Sci.* 48, 337–346.

McCoy, J.L., Sikora, L.J., Weil, R.R. (1986): Plant Availability of Phosphorus in Sewage Sludge Compost1. *J. Environ. Qual.* 15, 403.

Møller, J., Boldrin, A., Christensen, T.H. (2009): Anaerobic digestion and digestate use: accounting of greenhouse gases and global warming contribution. *Waste Manag. Res.* 27, 813–824.

Möller, K., Müller, T. (2012): Effects of anaerobic digestion on digestate nutrient availability and crop growth: A review. *Eng. Life Sci.* 12, 242–257.

Mosier, N.S., Ladisch, C.M., Ladisch, M.R. (2002): Characterization of acid catalytic domains for cellulose hydrolysis and glucose degradation. *Biotechnol. Bioeng.* 79, 610–618.

Muhammad, S., Müller, T., Joergensen, R.G. (2006): Decomposition of pea and maize straw in Pakistani soils along a gradient in salinity. *Biol. Fertil. Soils* 43, 93–101.

Murphy, J., Riley, J.P. (1962): A modified single solution method for the determination of phosphate in natural waters. *Anal. Chim. Acta* 27, 31–36.

Nagul, E.A., McKelvie, I.D., Worsfold, P., Kolev, S.D. (2015): The molybdenum blue reaction for the determination of orthophosphate revisited: Opening the black box. *Anal. Chim. Acta*.

Nannipieri, P., Giagnoni, L., Landi, L., Renella, G. (2011): Role of Phosphatase Enzymes in Soil, in: Phosphorus in Action. Springer, Berlin, Heidelberg, pp. 215–243.

Neumann, G. (2010): Mining for nutrients - regulatory aspects of cluster root function and development. *New Phytol.* 187, 879–882.

Neumann, G. (2016): The Role of Ethylene in Plant Adaptations for Phosphate Acquisition in Soils – A Review. *Front. Plant Sci.* 6, 1224.

Neumann, G., Martinoia, E. (2002): Cluster roots – an underground adaptation for survival in extreme environments. *Trends Plant Sci.* 7, 162–167.

Nkebiwe, P.M., Weinmann, M., Bar-Tal, A., Müller, T. (2016): Fertilizer placement to improve crop nutrient acquisition and yield: A review and meta-analysis. *F. Crop. Res.* 196, 389–401.

Øgaard, A.F. (1996): Effect of Phosphorus Fertilization on the Concentration of Total

and Algal-available Phosphorus in Different Particle-size Fractions in Norwegian Agricultural Soils. *Acta Agric. Scand. Sect. B - Soil Plant Sci.* 46, 24–29.

Olsen, S.R. (1954): Estimation Of Available Phosphorus In Soils By Extraction With Sodium Bicarbonate.

Ottosen, L.M., Kirkelund, G.M., Jensen, P.E. (2013): Extracting phosphorous from incinerated sewage sludge ash rich in iron or aluminum. *Chemosphere* 91, 963–969.

Pant, H.K., Warman, P.R. (2000): Enzymatic hydrolysis of soil organic phosphorus by immobilized phosphatases. *Biol. Fertil. Soils* 30, 306–311.

Pasqualino, J.C., Meneses, M., Abella, M., Castells, F. (2009): LCA as a Decision Support Tool for the Environmental Improvement of the Operation of a Municipal Wastewater Treatment Plant. *Environ. Sci. Technol.* 43, 3300–3307.

Pastor, L., Marti, N., Bouzas, A., Seco, A. (2008): Sewage sludge management for phosphorus recovery as struvite in EBPR wastewater treatment plants. *Bioresour. Technol.* 99, 4817–4824.

Petronici, C. (1989): Gli elementi nutritivi, in: Paolo Sequi (ed.): Chimica Del Suolo. Patron Editore, Bologna, pp. 357–380.

Pniewski, T., Kapusta, J., Płucienniczak, A. (2006): Agrobacterium-mediated transformation of yellow lupin to generate callus tissue producing HBV surface antigen in a long-term culture. *J. Appl. Genet.* 47, 309–318.

Polowick, P.L., Loukanina, N.N., Doshi, K.M. (2014): Agrobacterium-mediated transformation of tarwi (Lupinus mutabilis Sweet), a potential platform for the production of plant-made proteins. *Vitr. Cell. Dev. Biol. - Plant* 50, 401–411.

Pote, D.H., Daniel, T.C., Sharpley, A.N., Moore, P.A., Edwards, D.R., Nichols, D.J. (1996): Relating Extractable Soil Phosphorus to Phosphorus Losses in Runoff t'. *Soil Sci. Soc. Am. J.* 60, 855–859.

Raghothama, K.G. (1999): PHOSPHATE ACQUISITION. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 50, 665–693.

Rahman, M.M., Salleh, M.A.M., Rashid, U., Ahsan, A., Hossain, M.M., Ra, C.S. (2014): Production of slow release crystal fertilizer from wastewaters through struvite crystallization – A review. *Arab. J. Chem.* 7, 139–155.

Reijnders, L. (2014): Phosphorus resources, their depletion and conservation, a review. *Resour. Conserv. Recycl.*

Richardson, A.E., Simpson, R.J. (2011): Soil microorganisms mediating phosphorus availability update on microbial phosphorus. *Plant Physiol.* 156, 989–96.

Russo, A. (2012): ANALISI E MIGLIORAMENTO DELL'IMPIANTO DI DEPURAZIONE DI LEGNAGO. Università degli Studi di Padova.

Shannon, M.C., Grieve, C.M. (1999): Tolerance of vegetable crops to salinity. *Sci. Hortic. (Amsterdam).* 78, 5–38.

Sharpley, A., Moyer, B. (2000): Phosphorus Forms in Manure and Compost and Their Release during Simulated Rainfall. *J. Environ. Qual.* 29, 1462.

Sharpley, A.N., Chapra, S.C., Wedepohl, R., Sims, J.T., Daniel, T.C., Reddy, K.R. (1994): Managing Agricultural Phosphorus for Protection of Surface Waters:

Issues and Options. *J. Environ. Qual.* 23, 437.

Shen, J., Yuan, L., Zhang, J., Li, H., Bai, Z., Chen, X., Zhang, W., Zhang, F. (2011): Phosphorus dynamics: from soil to plant. *Plant Physiol.* 156, 997–1005.

Shenoy, V. V., Kalagudi, G.M. (2005): Enhancing plant phosphorus use efficiency for sustainable cropping. *Biotechnol. Adv.* 23, 501–513.

Siddique, M.T., Robinson, J.S. (2003): Phosphorus Sorption and Availability in Soils Amended with Animal Manures and Sewage Sludge. *J. Environ. Qual.* 32, 1114.

Simpson, M., McLenaghen, R.D., Chirino-Valle, I., Condron, L.M. (2012): Effects of long-term grassland management on the chemical nature and bioavailability of soil phosphorus. *Biol. Fertil. Soils* 48, 607–611.

Sims, J.T., Simard, R.R., Joern, B.C. (1998): Phosphorus Loss in Agricultural Drainage: Historical Perspective and Current Research. *J. Environ. Qual.* 27, 277.

Smith, K.A., Van Dijk, T.A. (1987): Utilisation of phosphorus and potassium from animal manures on grassland and forage crops, in: Animal Manure on Grassland and Fodder Crops. Fertilizer or Waste? Springer Netherlands, Dordrecht, pp. 87–102.

Smith, S.R. (2009): A critical review of the bioavailability and impacts of heavy metals in municipal solid waste composts compared to sewage sludge. *Environ. Int.* 35, 142–156.

Sonneveld, C., Straver, N. (1994): Nutrient solutions for vegetables and flowers grown in water or substrates, Tenth. ed.

Stewart, J.W.B., Tiessen, H. (1987): Dynamics of soil organic phosphorus. *Biogeochemistry* 4, 41–60.

Suh, Y.J., Rousseaux, P. (2002): An LCA of alternative wastewater sludge treatment scenarios. *Resour. Conserv. Recycl.* 35, 191–200.

Syers, D.J., Johnston, A. (2008): Efficiency of soil and fertilizer phosphorus use Reconciling changing concepts of soil phosphorus behaviour with agronomic information. *FAO Fertil. Plant Nutr. Bull.*

Tabatabai, M.A., Bremner, J.M. (1969): Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biol. Biochem.* 1, 301–307.

Takahashi, M., Kato, S., Shima, H., Sarai, E., Ichioka, T., Hatyakawa, S., Miyajiri, H. (2001): Technology for recovering phosphorus from incinerated wastewater treatment sludge, in: Chemosphere. pp. 23–29.

Takahashi, S. (2013): Phosphorus characterization of manure composts and combined organic fertilizers by a sequential-fractionation method. *J. Plant Nutr. Soil Sci.* 176, 494–496.

Talboys, P.J., Heppell, J., Roose, T., Healey, J.R., Jones, D.L., Withers, P.J.A. (2016): Struvite: a slow-release fertiliser for sustainable phosphorus management? *Plant Soil* 401, 109–123.

Tang, X., Li, J., Ma, Y., Hao, X., Li, X. (2008): Phosphorus efficiency in long-term (15 years) wheat–maize cropping systems with various soil and climate conditions. *F. Crop. Res.* 108, 231–237.

Tarayre, C., De Clercq, L., Charlier, R., Michels, E., Meers, E., Camargo-Valero, M.,

Delvigne, F. (2016): New perspectives for the design of sustainable bioprocesses for phosphorus recovery from waste. *Bioresour. Technol.*

Tejada, M., García-Martínez, A.M., Rodríguez-Morgado, B., Carballo, M., García-Antras, D., Aragón, C., Parrado, J. (2013): Obtaining biostimulant products for land application from the sewage sludge of small populations. *Ecol. Eng.* 50, 31–36.

Toor, G.S., Peak, J.D., Sims, J.T. (2005): Phosphorus Speciation in Broiler Litter and Turkey Manure Produced from Modified Diets. *J. Environ. Qual.* 34, 687.

Torri, S.I., Correa, R.S., Renella, G. (2017): Biosolid Application to Agricultural Land—a Contribution to Global Phosphorus Recycle: A Review. *Pedosphere* 27, 1–16.

Turner, B.L., Cade-Menun, B.J., Condron, L.M., Newman, S. (2005): Extraction of soil organic phosphorus. *Talanta* 66, 294–306.

Turner, B.L., McKelvie, I.D., Haygarth, P.M. (2002): Characterisation of water-extractable soil organic phosphorus by phosphatase hydrolysis. *Soil Biol. Biochem.* 34, 27–35.

Uhde-Stone, C., Liu, J., Zinn, K.E., Allan, D.L., Vance, C.P. (2005): Transgenic proteoid roots of white lupin: a vehicle for characterizing and silencing root genes involved in adaptation to P stress. *Plant J.* 44, 840–853.

Uysal, A., Dilsad Yilmazel, Y., Demirer, G.N. (2010): The determination of fertilizer quality of the formed struvite from effluent of a sewage sludge anaerobic digester. *J. Hazard. Mater.* 181, 248–254.

Vance, C.P. (2001): Symbiotic Nitrogen Fixation and Phosphorus Acquisition. Plant Nutrition in a World of Declining Renewable Resources. *Plant Physiol.* 127.

Vance, C.P., Uhde-Stone, C., Allan, D.L. (2003): Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. *New Phytol.* 157, 423–447.

Veneklaas, E.J., Lambers, H., Bragg, J., Finnegan, P.M., Lovelock, C.E., Plaxton, W.C., Price, C.A., Scheible, W.-R., Shane, M.W., White, P.J., Raven, J.A. (2012): Opportunities for improving phosphorus-use efficiency in crop plants. *New Phytol.* 195, 306–320.

Walan, P., Davidsson, S., Johansson, S., Höök, M. (2014): Phosphate rock production and depletion: Regional disaggregated modeling and global implications. *Resour. Conserv. Recycl.* 93, 178–187.

Wang, Z., Straub, D., Yang, H., Kania, A., Shen, J., Ludewig, U., Neumann, G. (2014): The regulatory network of cluster-root function and development in phosphate-deficient white lupin (*Lupinus albus*) identified by transcriptome sequencing. *Physiol. Plant.* 151, 323–338.

Wellinger, A., Murphy, J., Baxter, D. (2013): The biogas handbook : science, production and applications.

Wheeler, B.D., Al-Farraj, M.M., Cook, R.E.D. (1985): Iron toxicity to plants in base-rich wetlands: comparative effects on the distribution and growth of Epilobium hirsutum l. and Juncus subnodulosus Schrank. *New Phytol.* 100, 653–669.

White, P.J., Brown, P.H. (2010): Plant nutrition for sustainable development and

global health. *Ann. Bot.* 105, 1073–1080.

Wollmann, I., Gauro, A., Müller, T., Möller, K. (2017): Phosphorus bioavailability of sewage sludge-based recycled fertilizers. *J. Plant Nutr. Soil Sci.*

Xu, H., He, P., Gu, W., Wang, G., Shao, L. (2012): Recovery of phosphorus as struvite from sewage sludge ash. *J. Environ. Sci. (China)* 24, 1533–1538.

Ye, Y., Ngo, H.H., Guo, W., Liu, Y., Li, J., Liu, Y., Zhang, X., Jia, H., Ngo, H.H. (2016): Insight into chemical phosphate recovery from municipal wastewater. *Sci. Total Environ.* 576, 159–171.

Zang, Y., Li, Y., Wang, C., Zhang, W., Xiong, W. (2015): Towards more accurate life cycle assessment of biological wastewater treatment plants: A review. *J. Clean. Prod.*

Zhu, J., Li, M., Whelan, M., Gan, J. (2017): Phosphorus activators contribute to legacy phosphorus availability in agricultural soils: A review. *Sci. Total Environ.* 612, 522–537.

Chapter 5

Sewage sludge hydrolysates for P nutrition in a soil-plant system

Abstract

The scarcity of phosphate rocks mines has increased the attention on alternative source of phosphorus (P) for plant nutrition. Previously chemical and enzymatic hydrolysis of sewage sludge was tested for P recovery from a waste product. Citric acid and acid phosphatase hydrolysates of sewage sludge was tried as a source of P for plant nutrition in a soil-plant system. After the problems occurred in soilless cultivation, the system was implemented with the addition of the soil. A soil incubation test was performed comparing an unamended soil (CTRL), a chemical P reference (Chem), the two raw sewage sludge (ANA and AER), two hydrolysates with citric acid alone (ANA Cit and AER Cit) and two hydrolysates with the combination of citric acid and acid phosphatase (ANA Cit+AcPHO and AER Cit+AcPHO). Simultaneously, a plant pot test was performed with lettuce and plants and pots soil were sampled. Soil P availability were maintained higher, microbial growth has enhanced and phosphatase activity was modified in the hydrolysates treatments concurring to increase the P uptake in plants and in some cases also the plant growth.

Introduction

Phosphate is the second main element for plant nutrition. The need to create an alternative solution to phosphate rock mining for P fertilization has been extensively described in the previous chapters. The solution proposed in this study was the use of organic acid extraction of sewage sludge in combination with the addition of acid phosphatase (chap. 3). The liquid fertilizer, derived from the hydrolysis of P from sewage sludge, has been tested in a soilless cultivation of lettuce, with contrasting results (chap. 4). The hydrolysates were not efficient as the mineral fertilizer for sustaining the growth of the plants, probably due to a fast increase of pH and Al toxicity for root growth. On the other hand, the potential of the hydrolysates as complex fertilizers was evident. The hydrolysates solutions contained, besides P, some of the meso and microelements necessary to the plants growth, such as Fe, Mg, B and S. However, the Fe uptake of plants were much higher than the normal needs.

As previously described in chap. 2 the phosphate availability in soil is dependent on many factors, among which the soil pH and cation concentration. High concentration of Ca or Mg and an alkaline pH make the P strongly ligated to the soil particles, as high concentration of Fe and Al and an acidic soil (*Guppy et al.*, 2005; *Hinsinger*, 2001). We have formerly demonstrated that in calcareous soil the addition of P from mineral or organic fertilizer undergo to a fast decrease in P availability, due to the bonds that P forms with Ca (chap. 2). This decrease in availability was not found in soilless cultivation (chap. 4), where the P remained in solutions until the uncontrolled pH increase. The buffer capacity of soil can be useful to contain the microbial growth promoted from the hydrolysate solution and also to avoid high pH changes. Furthermore, the high Ca concentration, can also ligate the Al contained in the solutions and limit the Al toxicity and thus, enhance the root growth (*Marschner and Marschner*, 2012).

Therefore, the aim of this study is to test the liquid fertilizer derived from sewage sludge in soil and soil-plant system, in order to confirm the potentiality found in the soilless cultivation and to overcome the problems of microbial growth and high metal availability noticed in hydroponic system.

Materials and Methods

Hydrolysates fertilizers

The hydrolysates fertilizers were prepared as previously described. For this experiment, anaerobic (ANA) and aerobic (AER) sludge were used. The principal characteristics of the sludge are described in Table 5.1. The sludge samples were hydrolysed with citric acid at the beginning and for the mixed treatments added of acid phosphatase after pH adjustment. Due to the high amount of hydrolysates, it was decided to use glass filter and a vacuum pump to filter the hydrolysates, in order to shorten the time of filtration. In this way, four different solutions were obtained: anaerobic sludge hydrolysed with citric acid (ANA Cit), anaerobic sludge hydrolysed with citric acid and acid phosphatase (ANA Cit+AcPHO), aerobic sludge hydrolysed with citric acid (AER Cit) and aerobic sludge hydrolysed with citric acid and acid phosphatase (AER Cit+AcPHO).

The hydrolysates were then analysed for phosphate with ascorbic acid method (*Murphy and Riley*, 1962) and for metal concentration with ARCOS-ICP OES (Spectro Analytical Instruments, Kleve, Germany). The metal concentration of the different hydrolysates are described in Table 5.2.

Table 5.1: Principal characteristics of anaerobic (ANA) and aerobic (AER) sludge.

		ANA	AER
Moisture	(% fresh matter)	72.40%	77.30%
Ash	(% dry matter)	45.70%	46.30%
pH-H_2O		7.3	7.8
P tot	mg kg^{-1}	20039.8	20322.7
N (%)	(%)	4.4	3.6
C (%)	(%)	25.9	24.2
Ag	mg kg^{-1}	2.6	4.4
Al	mg kg^{-1}	19346.9	41365.6
As	mg kg^{-1}	4.5	11.2
B	mg kg^{-1}	35.1	37.6
Ba	mg kg^{-1}	479.6	337.8
Be	mg kg^{-1}	0.6	0.7
Ca	mg kg^{-1}	23610.7	29710.6
Cd	mg kg^{-1}	2.1	0.6
Co	mg kg^{-1}	5.6	6.5
Cr	mg kg^{-1}	101.3	49.7
Cu	mg kg^{-1}	389.8	295.5
Fe	mg kg^{-1}	32884.5	15175.4
Hg	mg kg^{-1}	0.4	0.3
K	mg kg^{-1}	5241.7	4560.6
Li	mg kg^{-1}	22.9	20.7
Mg	mg kg^{-1}	6854.0	6824.9
Mn	mg kg^{-1}	233.2	428.6
Mo	mg kg^{-1}	5.5	4.8
Na	mg kg^{-1}	1169.9	5755.4
Ni	mg kg^{-1}	49.8	31.3
Pb	mg kg^{-1}	61.2	36.8
S	mg kg^{-1}	7880.0	8078.4
Sb	mg kg^{-1}	3.0	2.2
Se	mg kg^{-1}	6.7	1.9
Sn	mg kg^{-1}	25.5	19.1
Sr	mg kg^{-1}	336.3	301.4
Ti	mg kg^{-1}	246.6	218.2
V	mg kg^{-1}	33.5	27.8
Zn	mg kg^{-1}	825.7	527.7

Table 5.2: Elements concentration of the four hydrolysates derived from two different sewage sludge.

		ANA Cit	*ANA Cit + Ac PHO*	*AER Cit*	*AER Cit+AC PHO*
PO_4	mg l^{-1}	871.02	860.73	592.39	715.85
Al	mg l^{-1}	54.4	62.6	508.2	615.9
Ba	mg l^{-1}	6.5	7.1	7.6	10.6
Ca	mg l^{-1}	471.0	412.0	506.0	474.0
Fe	mg l^{-1}	356.4	359.2	188.7	204.2
K	mg l^{-1}	59.6	69.5	67.5	68.8
Mg	mg l^{-1}	90.8	76.8	97.6	88.0
Mn	mg l^{-1}	5.4	5.1	9.8	10.7
Na	mg l^{-1}	43.0	85.9	177.0	122.0
P	mg l^{-1}	492.0	479.0	376.0	444.0
S	mg l^{-1}	30.3	32.9	51.2	60.2
Si	mg l^{-1}	6.1	6.3	30.1	32.5
Sr	mg l^{-1}	11.6	12.0	9.1	9.7
Zn	mg l^{-1}	8.1	8.0	5.0	5.5
Ag	µg l^{-1}	<DL	<DL	<DL	<DL
As	µg l^{-1}	54.2	42.5	172.0	198.0
B	µg l^{-1}	445.0	316.0	717.0	585.0
Be	µg l^{-1}	1.7	1.5	5.9	7.1
Cd	µg l^{-1}	<DL	<DL	<DL	<DL
Co	µg l^{-1}	33.0	33.4	35.1	41.8
Cr	µg l^{-1}	811.0	1074.0	190.0	231.0
Cu	µg l^{-1}	<DL	<DL	24.7	61.8
Hg	µg l^{-1}	<DL	<DL	<DL	<DL
Li	µg l^{-1}	31.1	48.3	132.0	169.0
Mo	µg l^{-1}	218.0	260.0	118.0	122.0
Ni	µg l^{-1}	828.0	713.0	227.0	216.0
Pb	µg l^{-1}	194.0	291.0	317.0	484.0
Sb	µg l^{-1}	50.8	47.4	28.4	29.6
Se	µg l^{-1}	65.7	58.8	45.1	40.2
Sn	µg l^{-1}	81.8	44.7	120.0	107.0
Ti	µg l^{-1}	535.0	509.0	459.0	478.0
Tl	µg l^{-1}	<DL	<DL	<DL	<DL
V	µg l^{-1}	134.0	129.0	159.0	156.0

<DL: concentration under detection limits

Soil test

Soil incubation

Soil was collected in the top layer in a field of the experimental farm of the University of Bologna, Northern Italy, in Po valley. The soil was air-died and seized at 2 mm. The characteristics of the soil were previously described in chap 2. Briefly the soil presented pH (H_2O, 1:2.5) = 8.16 and silty clay loam texture. This soil was chosen due to its low concentration of available P (3.81 mg kg^{-1}).

The soil was incubated for two weeks at 25°C and watered periodically at 20% humidity. Then a quantity of fertilized soil corresponding to 200 g of dry weight were put in a 500 mL plastic vessel, with dry sludge or liquid fertilizers as treatments and 3 replication per treatments. The 8 treatments were:

- **CTRL**: soil without the addition of P sources;
- **ANA**: soil with the addition of ANA dried raw sludge in the proportion to add 30 mg P kg^{-1} of soil;
- **AER**: soil with the addition of AER dried raw sludge in the proportion to add 30 mg P kg^{-1} of soil;
- **ANA Cit**: soil with the addition of the hydrolysates of ANA with citric acid in the proportion to add 30 mg P kg^{-1} of soil;
- **ANA Cit+AcPHO**: soil with the addition of the hydrolysates of ANA with citric acid and acid phosphatase in the proportion to add 30 mg P kg^{-1} of soil;
- **AER Cit**: soil with the addition of the hydrolysates of AER with citric acid in the proportion to add 30 mg P kg^{-1} of soil;
- **AER Cit+AcPHO**: soil with the addition of the hydrolysates of AER with citric acid and acid phosphatase in the proportion to add 30 mg P kg^{-1} of soil;
- **CHEM**: soil with the addition of KH_2PO_4 in the proportion to add 30 mg P kg^{-1} of soil.

The dry sludge were added to the soil and hand mixed, whereas the liquid hydrolysates and the chemical solution were added to the soil after this was already in the plastic vessels. The vessels were put in an incubator at 25°C.

Soil pH

The equivalent of 10 g in dry weight of the incubated soil were sampled at 0 and 40 DAT and analysed for pH using 25 ml of milliQ water. The falcon tubes with the soil and water solution were shaken for one hour and then left vertically for two hours before performing the pH analysis. The pH was measured with a glass electrode. Sampling for analysis were done at 0 and 40 days after the start of the treatment (DAT).

Phosphorus availability

Phosphorus availability was measured with Olsen method (*Olsen*, 1954). The equivalent of 2 g (in dry weight) of incubated soil (corresponding to 2 g of dry weight) and 40 ml of 0.5 M $NaHCO_3$ at pH 8.5 were shaken for 30 mins and then filtered with Whatman 42 filters. Then the solutions were analysed spectrophotometrically with the ascorbic acid method (*Murphy and Riley*, 1962). Sampling for analysis were done at 0, 20, 40, 80 and 120 DAT.

Phosphatase activity

An amount of incubated soil corresponding to 1 g of dry weight was analysed for phosphatase activity spectrophotometrically using the p-nitrofenyl phosphate method (*Tabatabai and Bremner*, 1969). Sampling for analysis were done at 0, 40, 80 and 120 DAT.

Carbon in microbial biomass

The equivalent of 4 g in dry weight of incubated soil were weighted in centrifuge tubes and added of 16 mL of 0.5 M K_2SO_4. Then the tubes were shaken for one hour, centrifuges at 10000 rpm for 10 min and filtered. The same amount of soil was weighted in thin foil caps, then fumigated under vacuum with chloroform for 24 hours. After fumigation, the samples were put in centrifuge tubes and extracted as the unfumigated samples. The unfumigated and the fumigated samples were analysed with an elemental analyser (TOC-VCPH/CPN, Shimadzu) for C concentration. The microbial C was calculated as the difference between fumigated and unfumigated concentration, without correction factors. Sampling for analysis were done at 0, 40, 80 and 120 DAT.

Plant test

Seeds of lettuce, variety *Romana* (Blumen Group S.P.A, Bologna, Italy), were sown in pots of 10 cm Ø, filled with 500 g of soil with the same characteristics of the one used for the incubation test. The seeds were covered with a thin layer of sand. After germination, the plants were thinned out until only one plant per pot remained. The plants were grown in a growth chamber with a day/night temperature of 25/18°C and a photoperiod of 16 h. At the beginning of the experiment, 12 plants per treatment were organized in a completely randomized design. The treatments were the same as in the incubation soil test: raw sludge and hydrolysates were added at the dose to reach the concentration of 30 mg P kg^{-1}. Three destructive sampling were done at 40, 80 and 120 days after sowing (DAS), using for each sampling 4 plants per treatment. The plants were fertilized with urea at the dose of 50 mg kg^{-1} N and KNO_3 at the dose of 50 mg kg^{-1} N at the sowing. Then at 40 and 80 DAS, the remaining pots were fertilized with KNO_3 at the dose of 50 mg kg^{-1} N.

Leaf greenness

At the day of destructive sampling (40, 80 and 120 DAS), the plants were measured for leaf greenness with N-tester (Yara International ASA, Oslo, Norway).

Plant fresh and dry weight

The plants at 40, 80 and 120 DAS were extracted from pots, the roots were gently washed from soil and dried with paper. Then the fresh weight was taken. Later, the plants were put in an oven at 65°C for 72 hours and weighted for dry weight.

Plant metal concentration

At 40 and 80 DAS, the whole plants were finely grinded, whereas at 120 DAS, when enough roots had grown, roots and leaves were grinded separately. Then, 0.25 g of sample was digested with 6 ml of HNO_3 and 2 ml of H_2O_2 in a microwave for 40 minutes. The resulting solutions were diluted and analysed with ARCOS ICP-OES (Spectro Analytical Instruments, Kleve, Germany).

Apparent P recovery fraction

Apparent recovery fraction was calculated as previously described in chap. 2.

Statistical analysis

Statistical analysis was performed with IBM SPSS (IBM, Armonk, North Castle, New York, USA) on plants and soil data. Normality of population was assessed with Kolmogorov-Smirnov test and equality of variance with Levene test. Two-way ANOVA was performed in traits with different sampling time on treatments and time as fixed factors and the interaction between factors. Later, One-way ANOVA and Tukey test with $P \leq 0.05$ were performed within time and in one-factor traits.

Results

Soil pH

Soil pH (Fig. 5.1) was significantly lower at DAT 0 in hydrolysates treatments (-6% as an average of ANA Cit, AER Cit and AER Cit+AcPHO compared to the average of the controls and the sludge amended soil), except for ANA Cit+AcPHO which was similar to the controls. After 40 DAT, only AER Cit remained significantly lower than the other treatments (-2%).

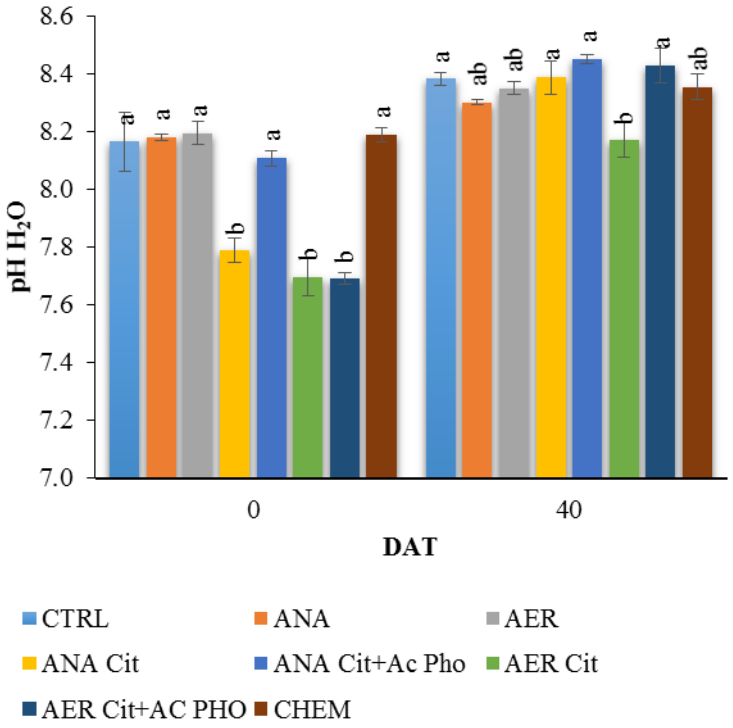


Figure 5.1: pH of different sludge and hydrolysates treatments at 0 and 40 DAT. Error bars represent standard errors and different letters represent different groups within the sampling time with One-way ANOVA and Tukey test (P≤0.05).

Soil available P

Soil available P decrease significantly during time, form 0 to 120 DAT (Fig. 5.2), with also the differences between treatments that flattened (ANOVA significance decreased from 0 to 120 DAT) (Fig. 5.3). At 0 DAT there were the highest differences between treatments with Chem and hydrolysates of aerobic sludge showing the highest P available (+181%, +131%, +139% in Chem, AER Cit and AER Cit+AcPHO, as compared to control). Below, hydrolysates of anaerobic sludge followed (+75% and +107% of ANA Cit and ANA Cit+AcPHO as compared to control). Already at 20 DAT, the differences decreased, but still aerobic hydrolysates and Chem resulted higher than other treatments (+146%, +171%, +183% in Chem, AER Cit and AER Cit+AcPHO, as compared to control). On the other hand, these treatments showed the highest decrease comparing to 0 DAT (-55%, -39%, -39% in Chem, AER Cit and AER Cit+AcPHO, at 20 DAT compared to 0 DAT). At both 0 and 20 DAT, control showed the lowest P available. At 40 DAT the hydrolysates treatment, with the exception of ANA Cit showed similar P available to Chem (+95% as an average of ANA Cit+AcPHO, AER Cit and AER Cit+AcPHO and +88% Chem as compared to control) while the raw sludge conditioners resulted with P available similar to control. At 80 DAT only Chem resulted higher of control, while all the other treatments (hydrolysates and raw sludge conditioners) did not differ from control. At 120 DAT, no significant differences were found between treatments.

Soil phosphatase activity

Soil phosphatase activity (Fig. 5.3) was significantly different between treatments at 0, 40 and 80 DAT (Fig. 5.6). At 0 DAT, unamended soil, AER and Chem showed the highest phosphatase activity. While ANA Cit showed the lowest phosphatase activity. However, at 40 DAT AER Cit+AcPHO showed the highest phosphatase activity (+85% as compared to control), while all the other treatments resulted similar. At 80 DAT ANA showed high phosphatase activity similar to the hydrolysates treated pots (except for ANA Cit), while AER results the lowest, similar to ANA Cit and Chem.

Soil microbial biomass

Soil microbial biomass (C_{mic}) (Fig. 5.4), C_{mic} decreased from 0 DAS to 40 DAT, more evident in ANA hydrolysates and chemical reference and less pronounced for raw sludge and AER hydrolysates. ANOVA was significant only at 40 DAT, where ANA Cit+AcPHO and Chem resulted the lowest (-46% and –50%, respectively, as compared to control). After 40 DAT, the treatments did not showed differences, remaining stable at 80 and 120 DAT.

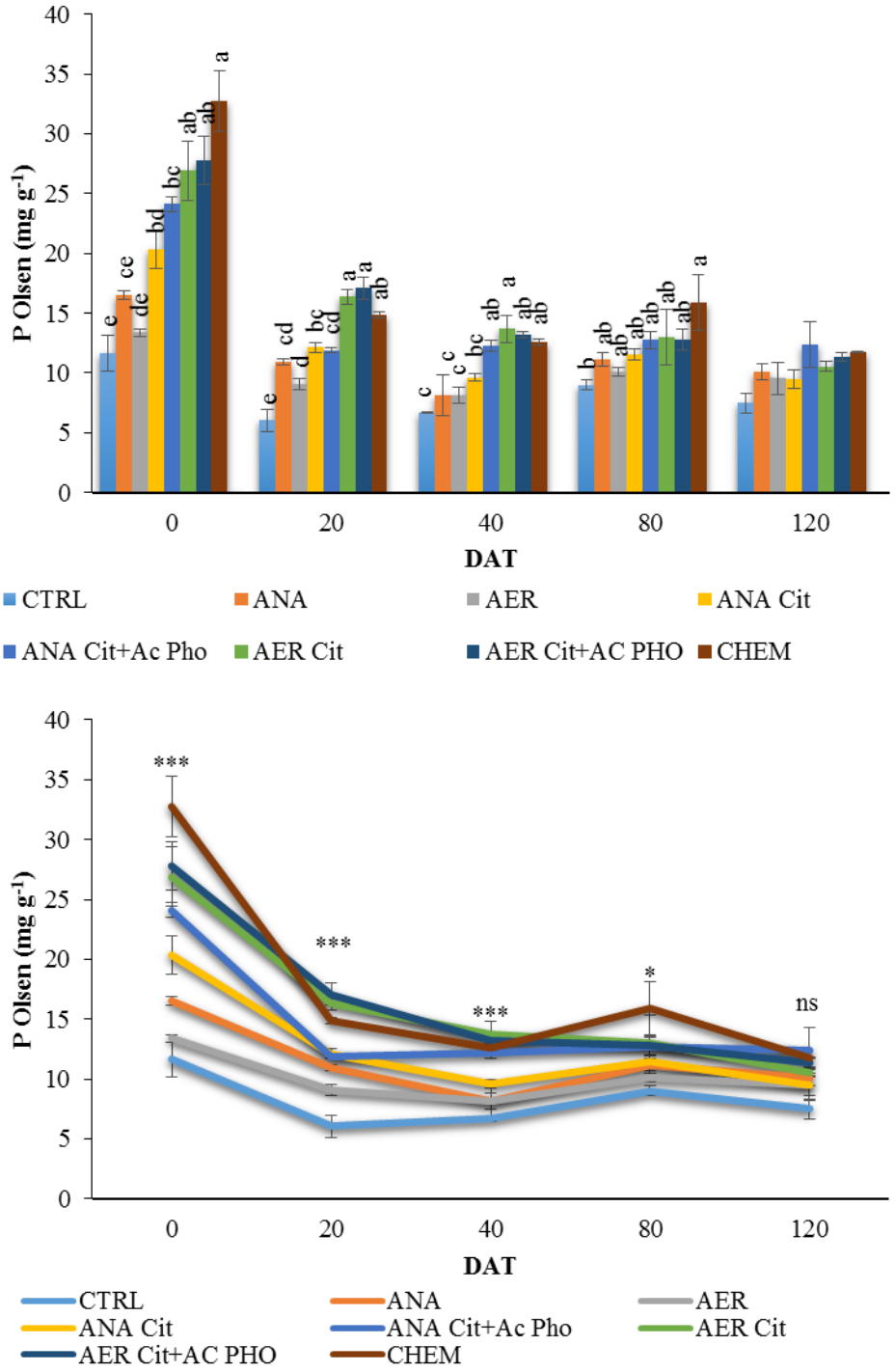


Figure 5.2: Available P (mg kg^{-1}) on incubated soil amended with different sludge and hydrolysates at different sampling time. Error bars represent standard errors. In first pictures different letters represent different groups within the sampling time with One-way ANOVA within time and Tukey test (P≤0.05), in second picture One-way ANOVA significance is shown as *** = P≤0.001, ** = P≤0.01, * = P≤0.05 and ns= P>0.05. Two-way ANOVA was significant (P≤0.001) in treatment, time and treatment*time.

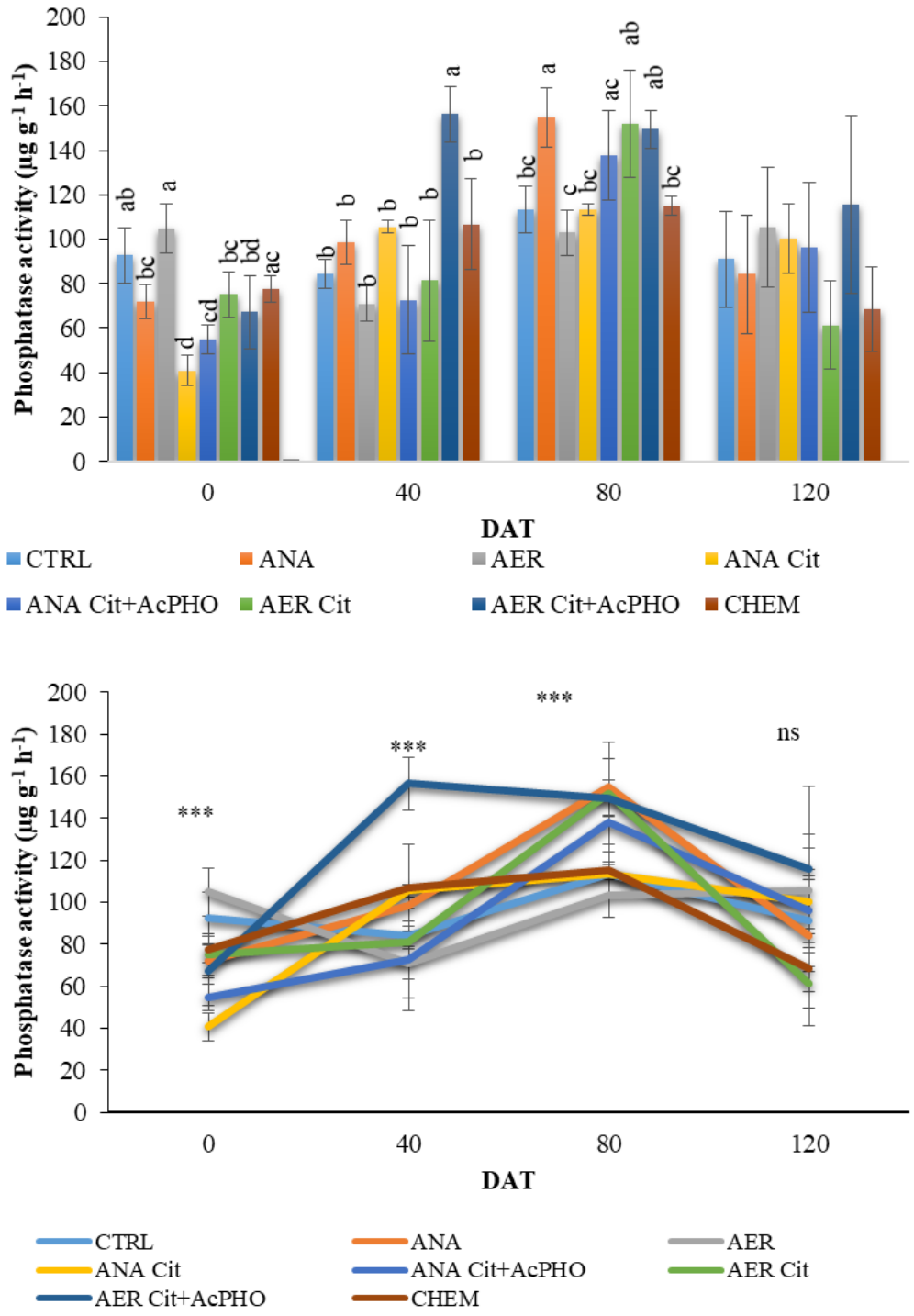


Figure 5.3: Phosphatase activity (µg g^{-1} h^{-1}) on soil amended with different sludge and hydrolysates at different sampling time. Error bars represent standard errors. In first pictures different letters represent different groups within the sampling time with One-way ANOVA within time and Tukey test (P≤0.05), in second picture One-way ANOVA significance is shown as *** = P≤0.001, ** = P≤0.01, * = P≤0.05 and ns= P>0.05. Two-way ANOVA was significant (P≤0.001) in treatment, time and treatment*time.

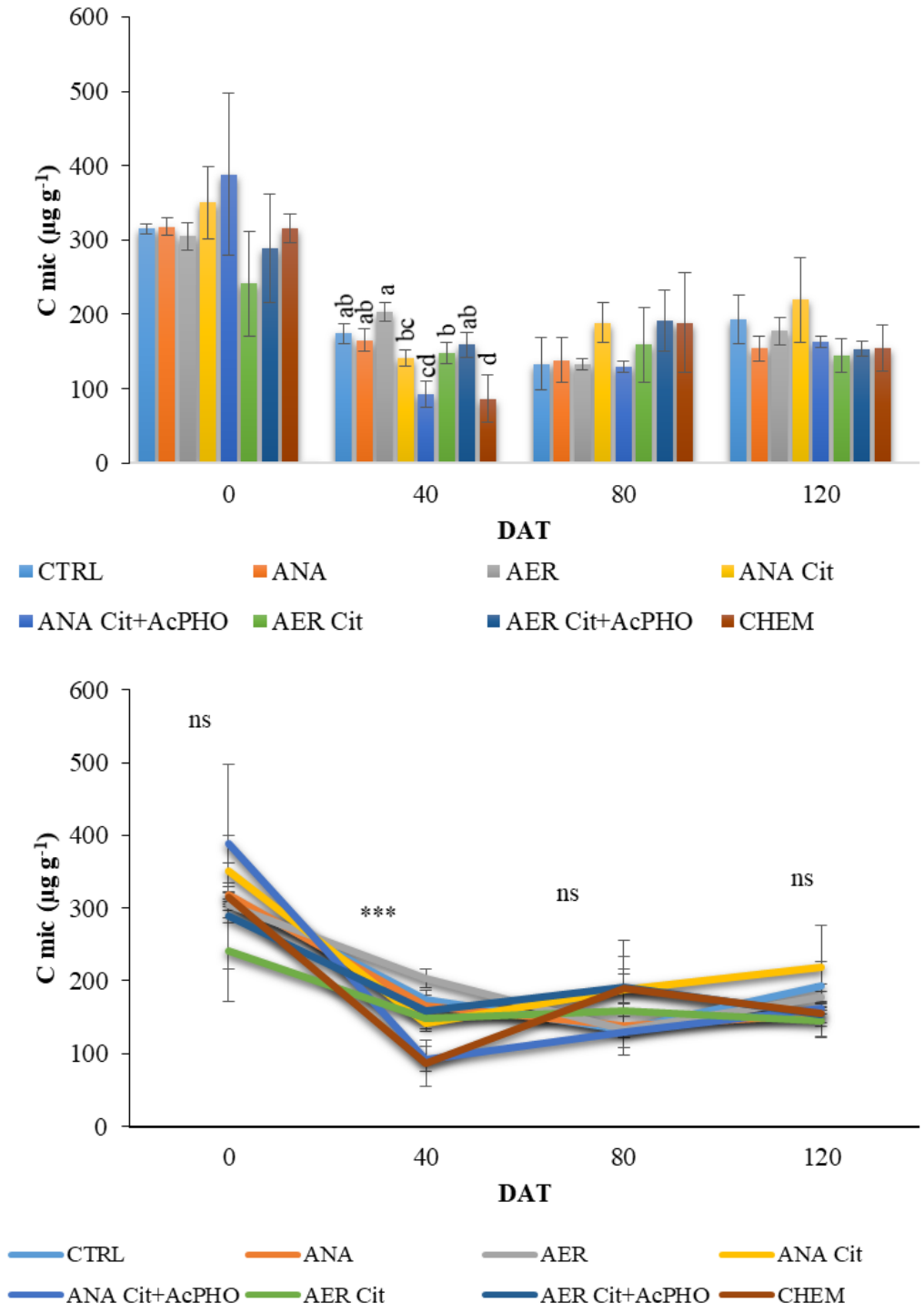


Figure 5.4: Microbial biomass C (µg g^{-1}) on soil amended with different sludge and hydrolysates at different sampling time. Error bars represent standard errors. In first pictures different letters represent different groups within the sampling time with One-way ANOVA within time and Tukey test (P≤0.05), in second picture One-way ANOVA significance is shown as *** = P≤0.001, ** = P≤0.01, * = P≤0.05 and ns= P>0.05. Two-way ANOVA was significant in treatment (P≤0.01), and time (P≤0.001).

Plant growth

Plant dry weight (Fig. 5.5) increased exponentially between 40 and 80 DAS and also between 80 and 120 DAS. At 40 DAS Chem showed the highest plant growth, while all the treatments with raw sludge conditioners and hydrolysates resulted lower (-45% as an average of hydrolysates and -56% as average of both raw sludge compared to Chem). At 80 DAS AER Cit and ANA Cit showed the highest dry weight (+52% and +77%, respectively, as compared to Chem). Similar results were found at 120 DAS, where ANA Cit and AER Cit showed the highest growth (+44% and +36% as compared to control), while control and ANA Cit+AcPHO showed the lowest plant biomass.

In Chem and ANA Cit the lowest shoot:root ratio was found (Fig. 5.6) (-39% and –36% as compared to control) whereas in AER Cit the results were the highest (+30% as compared to control).

Leaf greenness

The leaf greenness (Fig. 5.7) increased from 40 to 80 DAS and decreased from 80 to 120 DAS. At 40 DAS AER Cit showed the highest leaf greenness (+16% as compared to control). While Chem, raw sludge and ANA Cit+AcPHO treated plants resulted the lowest. At 80 DAS, instead, the highest leaf greenness was found in ANA Cit+AcPHO (+15% as compared to control). At 120 DAS Chem and hydrolysates (with the exception of ANA Cit) resulted lower and ANA Cit resulted the highest (+20% as compared to Chem).

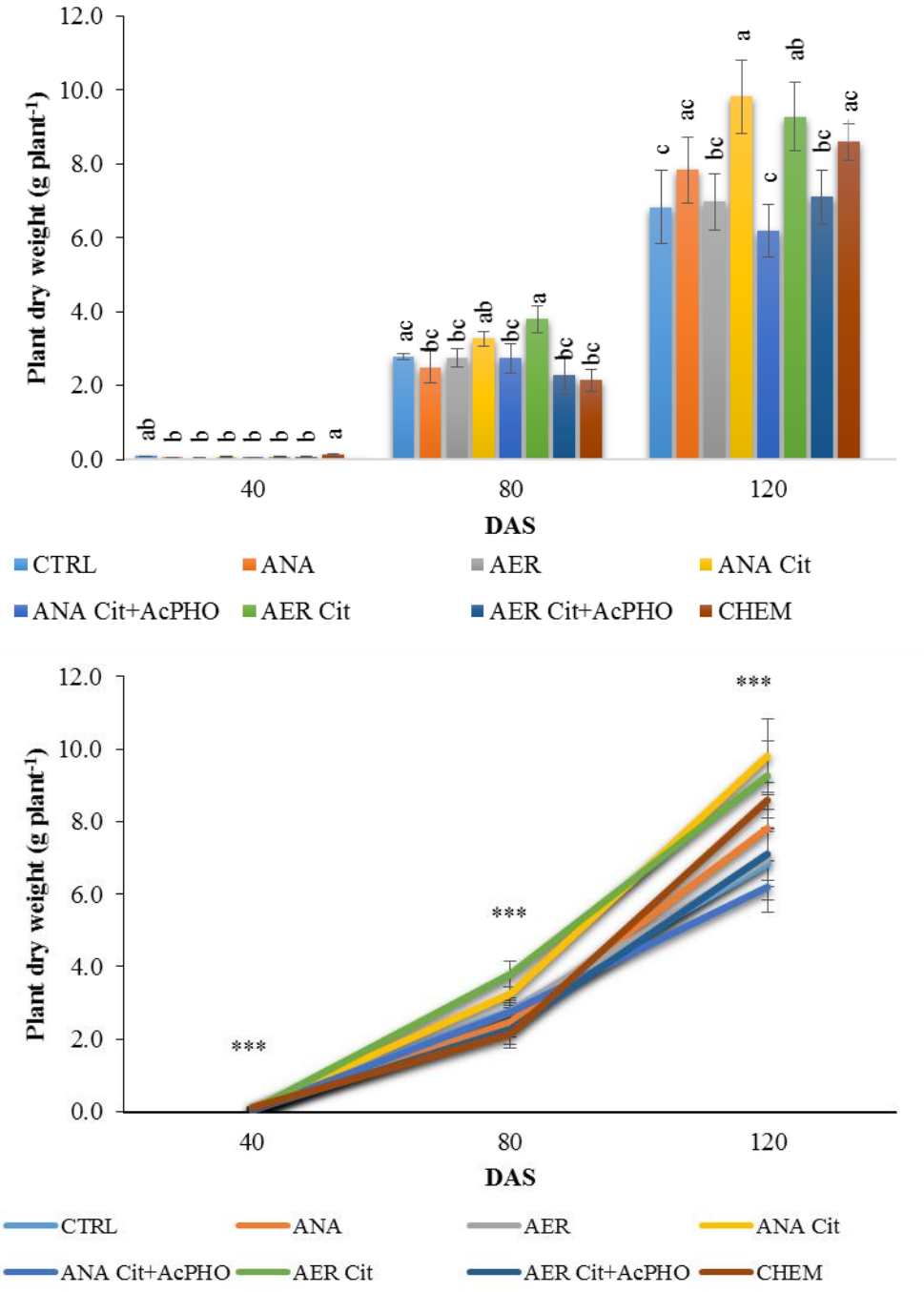


Figure 5.5: Dry weight (g plant^{-1}) of plants grown in soil amended with different sludge and hydrolysates at different sampling time. Error bars represent standard errors. In first pictures different letters represent different groups within the sampling time with One-way ANOVA and Tukey test (P≤0.05), in second picture One-way ANOVA significance is shown as *** = P≤0.001, ** = P≤0.01, * = P≤0.05 and ns= P>0.05. Two-way ANOVA was significant (P≤0.001) in treatment, time and treatment*time.

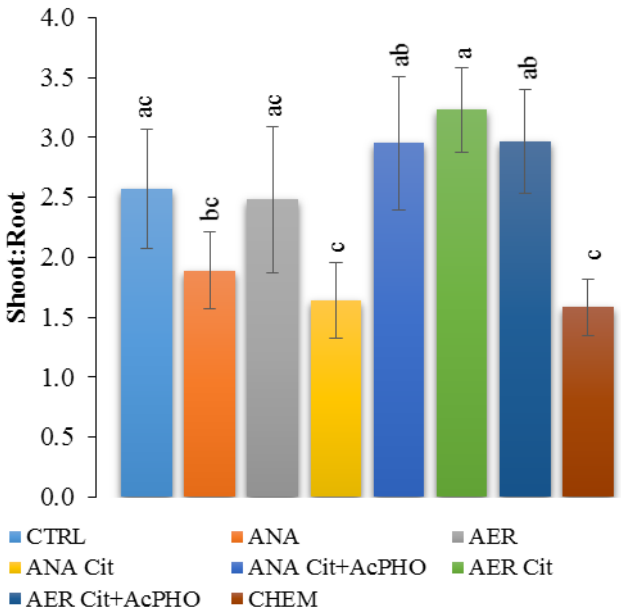


Figure 5.6: Shoot-root ratio of plants grown in soil amended with different sludge and hydrolysates. Error bars represent standard errors and different letters represent different groups within the sampling time with One-way ANOVA and Tukey test ($P \leq 0.05$).

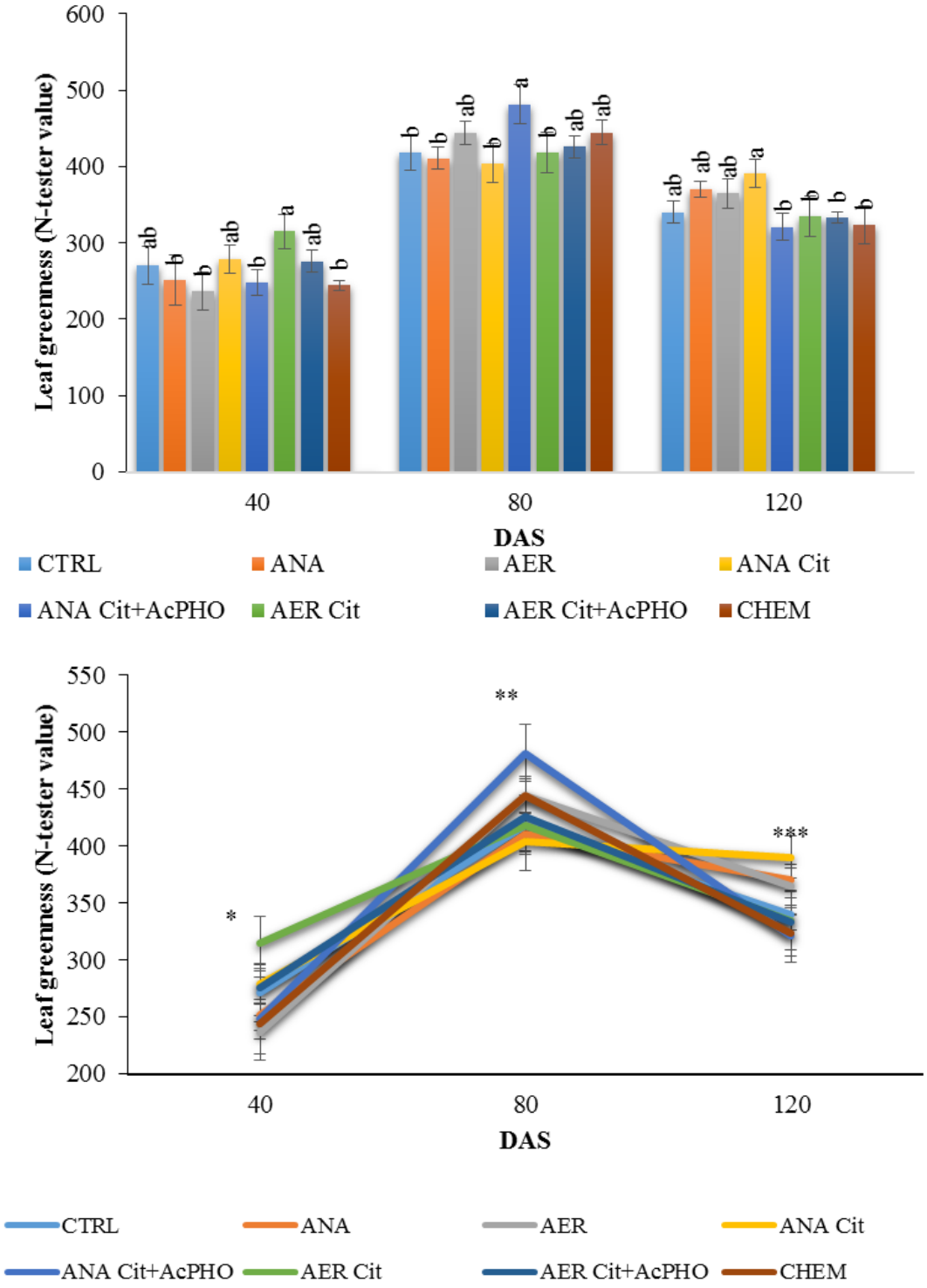


Figure 5.7: Leaf greenness (N-tester value) of plants grown in soil amended with different sludge and hydrolysates at different sampling time. Error bars represent standard errors. In first pictures different letters represent different groups within the sampling time with One-way ANOVA and Tukey test (P≤0.05), in second picture One-way ANOVA significance is shown as *** = P≤0.001, ** = P≤0.01, * = P≤0.05 and ns= P>0.05. Two-way ANOVA was significant (P≤0.001) in time and treatment*time.

Plant metal concentration

P concentration (Fig. 5.8) decreased in time from 40 to 120 DAS. At 40 DAS, all the treatments were higher than control. At 80 DAS, the differences were smaller and the highest P concentration was found in ANA Cit (+ 15% as compared to control). At 120 DAS, the differences were more evident with AER Cit+AcPHO and ANA Cit+AcPHO showing the highest leaf P concentration (+72% and +66% as compared to control) and control, ANA and ANA Cit showing the lowest P concentration. In roots at 120 DAS, there were no significant differences.

Al concentration (Table 5.3) showed differences only in leaves at 120 DAS (where leaves and roots where divided) (Fig. 5.9). At 120 DAS (Fig. 5.18) the highest Al concentration were found in ANA Cit and ANA Cit+AcPHO plants (+107% and +117% as compared to control). Whereas ANA showed the lowest content.

Also in Ca concentration (Table 5.3), the only time where there were significant differences between treatments was 120 DAS (where the roots were analysed separately) (Fig. 5.9). Hydrolysates treatments showed the highest Ca concentration (+48% as an average compared to control), similar to chemical reference.

No significant differences in Fe concentration (Table 5.3) were found during the whole experiment.

Mg concentration (Table 5.3), similarly to Al and Ca, was significantly different at 120 DAS. (Fig. 5.9). The highest Mg concentration was found in AER Cit+AcPHO (+54% as compared to control), while the lowest was found in Control and Chem.

In Table 5.3, some elements accumulated in leaves and roots are reported. Besides the already commented elements Cd was always higher in control plants; Cu showed differences oly at 120 DAS with AER Cit showing the highest amount and CHEM the lowest; K was not detected at 40 and 80 DAS, while at 120 DAS was higher in CHEM and lower in CTRL, ANA, AER and ANA Cit; Na at 40 and 120 DAS was higher in ANA Cit+AcPHO and AER Cit+AcPHO; Pb at 120 DAS in leaves and roots was higher in ANA and not different in all the other treatments. Cr, Ni; S and Zn did not show any significant differences.

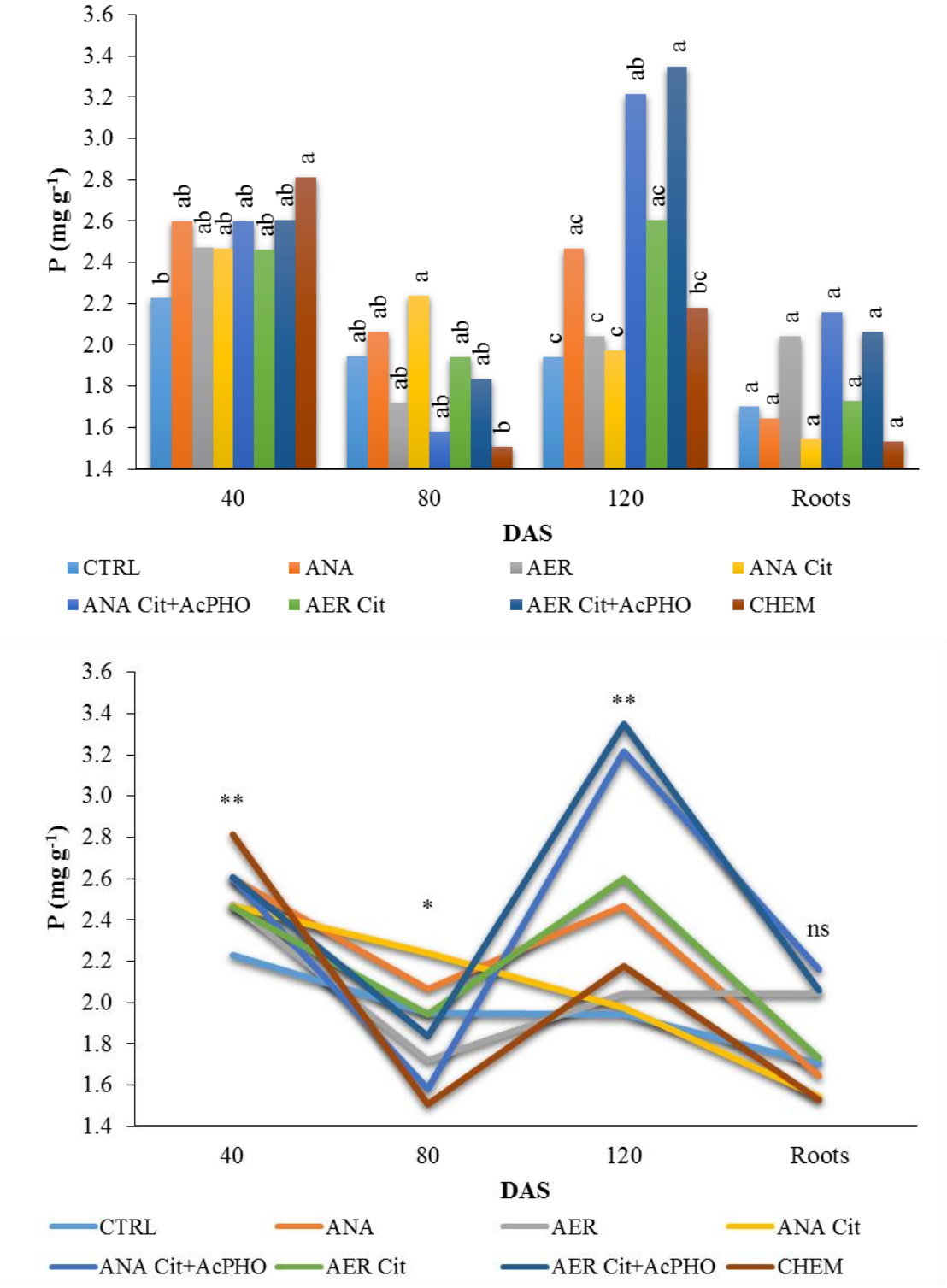


Figure 5.8: P concentration (mg g^{-1}) in plants grown in soil amended with different sludge and hydrolysates at different sampling time. Error bars represent standard errors. In first pictures different letters represent different groups within the sampling time with One-way ANOVA and Tukey test (P≤0.05), in second picture One-way ANOVA significance is shown as *** = P≤0.001, ** = P≤0.01, * = P≤0.05 and ns= P>0.05.

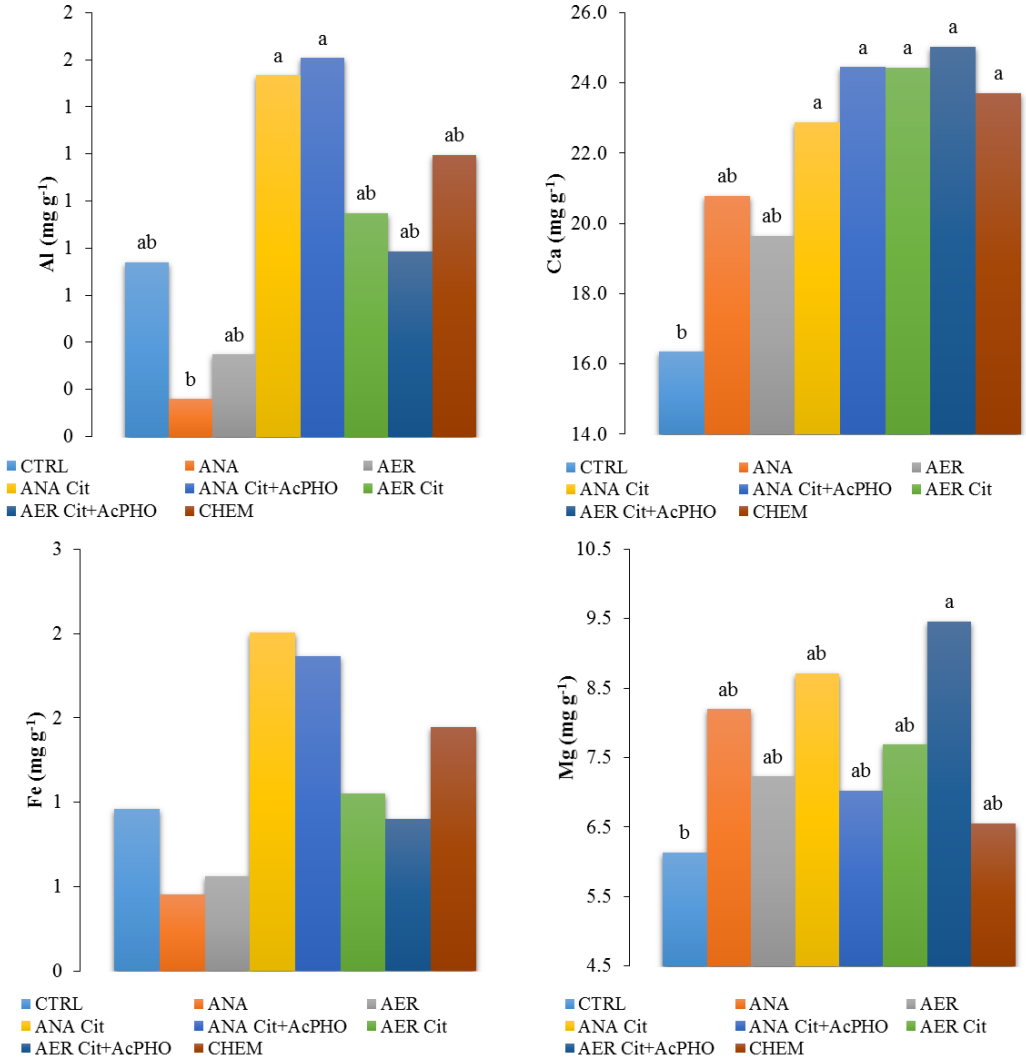


Figure 5.9: Al (µg kg^{-1}), Ca (mg kg^{-1}), Fe (µg kg^{-1}) and Mg (mg kg^{-1}) concentration in leaves of plants grown in soil amended with different sludge and hydrolysates at 120 DAS. Different letters represent different group with Tukey test (P≤0.05).

Table 5.3: Leaf and roots metal concentration.

Element	DAT	*Sig.*	CTRL	ANA	AER	ANA Cit	ANA Cit + Ac PHO	AER cit	AER cit+AcPHO	CHEM
Al µg g^{-1}	**40**	ns	1800.4	1635	2227.7	1829	1650.1	1942.2	1090.3	1772.9
	80	*ns*	1687.3	1766.5	2325.9	2811.5	2642.7	2862	1921.5	2417.5
	120	*	740.5 ab	158.9 b	348.7 ab	1533.4 a	1606.1 a	948.9 ab	786.2 ab	1194.2 ab
	roots	*ns*	6563.4	9308	8081.8	7163.2	6641.7	6524.3	9730.6	7868
Ca mg g^{-1}	**40**	ns	17.5	19.5	19.3	18.6	17.4	17.9	16.1	18.2
	80	*ns*	16.8	17.3	17.8	19.9	17.8	18.8	17.1	17.2
	120	***	16.3 b	20.8 ab	19.6 ab	22.9 a	24.4 a	24.4 a	25 a	23.7 a
	roots	*ns*	35.1	41.1	41.4	41.9	37.6	39.7	46.2	43.1
Cd µg g^{-1}	**40**	**	4.3 a	2 b	1.9 b	2.2 b	2.1 b	3.2 ab	2.2 b	1.9 b
	80	*	4.1 a	1.2 b	1.2 ab	1.7 ab	1.2 b	3.6 ab	2.4 ab	2 ab
	120	***	3.7 a	1.1 b	0.8 b	1.5 b	0.9 b	1.7 b	1.9 b	2.2 b
	roots	**	2.1 a	0.5 b	0.3 b	0.4 b	0.4 b	0.8 ab	0.9 ab	0.9 ab
Cr µg g^{-1}	**40**	ns	13.4	12.3	14.7	11.5	9.9	9.5	11.2	17.7
	80	*ns*	7.7	8.9	9.4	10.6	12	11.8	8.6	11.7
	120	*ns*	3.7	2.4	1.8	6.9	8	3.3	2.7	5
	roots	*ns*	19.7	25.9	23.5	21.3	19.8	18.2	27.9	24.5
Cu µg g^{-1}	**40**	ns	13.8	15.2	16.3	13.4	14.1	14	12.9	14.1
	80	*	24 a	24 a	20.8 a	24.2 a	15.5 a	21.7 a	18.4 a	16.4 a
	120	*	14.5 ab	16 ab	13.7 ab	14.4 ab	17.8 ab	25.4 a	19.6 ab	10.5 b
	roots	*	73.7 a	74 a	66 a	51.8 a	59.9 a	47.8 a	56.5 a	48.3 a

Element		DAT	*Sig.*	CTRL	ANA	AER	ANA Cit	ANA Cit + Ac PHO	AER cit	AER cit+AcPHO	CHEM
Fe	**µg g^{-1}**	**40**	ns	2508.6	2142.1	2874.5	2312.1	2093	2453.7	1557	2505.2
		80	*ns*	2109.5	2148.2	2887	3352.7	3356.3	3461.1	2351.9	2872.9
		120	*ns*	961.9	455.4	561.1	2007.1	1867.3	1052.6	901.5	1443.8
		roots	*ns*	7820	9327.5	8462.4	8126.4	7561.4	6844.4	10546.2	8948.2
K	**mg g^{-1}**	**40**		<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
		80		<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
		120	***	<DL c	<DL c	0.3 c	3.6 c	5.2 ab	5.4 ab	2.9 bc	6.4 a
		roots	*ns*	6.7	7.3	7.2	7.2	7.7	7.5	6.7	6.9
Mg	**mg g^{-1}**	**40**	**	5.4 ab	5.9 ab	6.2 a	5 ab	5.1 ab	5.1 ab	4.9 b	6.1 a
		80	**	6.9 ab	6.9 ab	6.3 ab	7.3 a	5.7 b	6.8 ab	5.6 b	5.6 b
		120	*	6.1 b	8.2 ab	7.2 ab	8.7 ab	7 ab	7.7 ab	9.5 a	6.5 ab
		roots	*ns*	5.1	5.9	6	5.9	5.3	5.6	7.2	5.8
Mn	**mg g^{-1}**	**40**	ns	107.6	97.5	126	91.5	98.2	105.1	77.8	113
		80	*ns*	99.4	102.1	126.3	126.8	146.6	138.9	105.1	122.9
		120	*	81.3 a	85.3 a	83.2 a	127.9 a	122.9 a	101.1 a	83.7 a	100.4 a
		roots	*ns*	310.7	370.6	357	361.9	310.8	362	379.2	393.2
Mo	**µg g^{-1}**	**40**		<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
		80		<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
		120	*ns*	<DL	<DL	<DL	<DL	0.6	<DL	<DL	<DL
		roots	*ns*	1.5	2.2	2	1.7	1.9	1.4	2.7	2
Na	**mg g^{-1}**	**40**	***	4.5 b	5.6 ab	5.7 ab	4.5 b	6.5 a	5.5 ab	6.4 a	4.8 b
		80	*ns*	4.5	5.3	5.6	4.1	4.1	3.7	4.2	6
		120	***	5.8 bc	4.5 c	5.2 bc	4.6 c	11.7 a	10.4 a	10.9 a	8.5 ab
		roots	*ns*	8.6	10.7	10	11.1	6.2	10.2	10.3	10.3

Element		DAT	*Sig.*	CTRL	ANA	AER	ANA Cit	ANA Cit + Ac PHO	AER cit	AER cit+AcPHO	CHEM
Ni	**µg g^{-1}**	**40**	ns	11.2	8.6	12.6	9.2	7.9	9.7	7.8	12.1
		80	*ns*	12.2	10.8	10.9	12.8	12.8	14.1	10.3	11.8
		120	*ns*	5	4.2	5.8	6.7	7.6	4.9	4.5	5.2
		roots	*ns*	25	28.7	26.4	25.6	26.5	22.6	28.5	28.1
P	**mg g^{-1}**	**40**	**	2.2 b	2.6 ab	2.5 ab	2.5 ab	2.6 ab	2.5 ab	2.6 ab	2.8 a
		80	*	1.9 ab	2.1 ab	1.7 ab	2.2 a	1.6 ab	1.9 ab	1.8 ab	1.5 b
		120	**	1.9 c	2.5 abc	2 c	2 c	3.2 ab	2.6 abc	3.4 a	2.2 bc
		roots	*ns*	1.7	1.6	2	1.5	2.2	1.7	2.1	1.5
Pb	**µg g^{-1}**	**40**	ns	3.7	3.9	4.4	3.9	3.9	4.2	3.2	4.2
		80	*ns*	6.1	4.7	5.3	5.5	5.6	5.6	4.6	4.9
		120	***	10.7 b	27 a	7.9 b	5 b	2.9 b	2.3 b	1.7 b	2.4 b
		roots	***	10.7 b	27 a	7.9 b	5 b	2.9 b	2.3 b	1.7 b	2.4 b
S	**mg g^{-1}**	**40**	ns	1.9	2	1.9	1.8	1.9	1.8	1.9	2.1
		80	*	2.3 a	2.2 a	1.9 a	2.1 a	1.6 a	2 a	1.9 a	1.6 a
		120	*ns*	1.9	2.9	2.6	2.7	2.9	2.9	3.7	2.6
		roots	*ns*	1.9	2.9	2.6	2.7	2.9	2.9	3.7	2.6
Zn	**µg g^{-1}**	**40**	*ns*	56.9	68.4	50.8	63	51.2	54.5	59.5	60.1
		80	*ns*	65.8	72.7	67	68.9	55.3	66.1	53.2	57.4
		120	*ns*	68.2	97.4	71.9	85.5	79.2	92.7	102.2	77
		roots	*ns*	68.2	97.4	71.9	85.5	79.2	92.7	102.2	77

<DL: metal concentration under detection limits

Plant apparent P recovery fraction

Cumulated apparent P recovery fraction (Fig. 5.10) showed that AER Cit and ANA Cit assimilated similar concentration of P until 80 DAS, after ANA Cit decrease the assimilation and AER Cit+AcPHO fastened the accumulation in the last 40 days of experiment, going from a recovery lower than control at 80 DAS to 20% at 120 DAS (the second highest value). ANA Cit, however, showed a curve that did not change its slope from 80 to 120 DAS. ANA and AER sludge, ANA Cit+AcPHO and Chem remained always lower, with AER being the lowest at the end of the experiment.

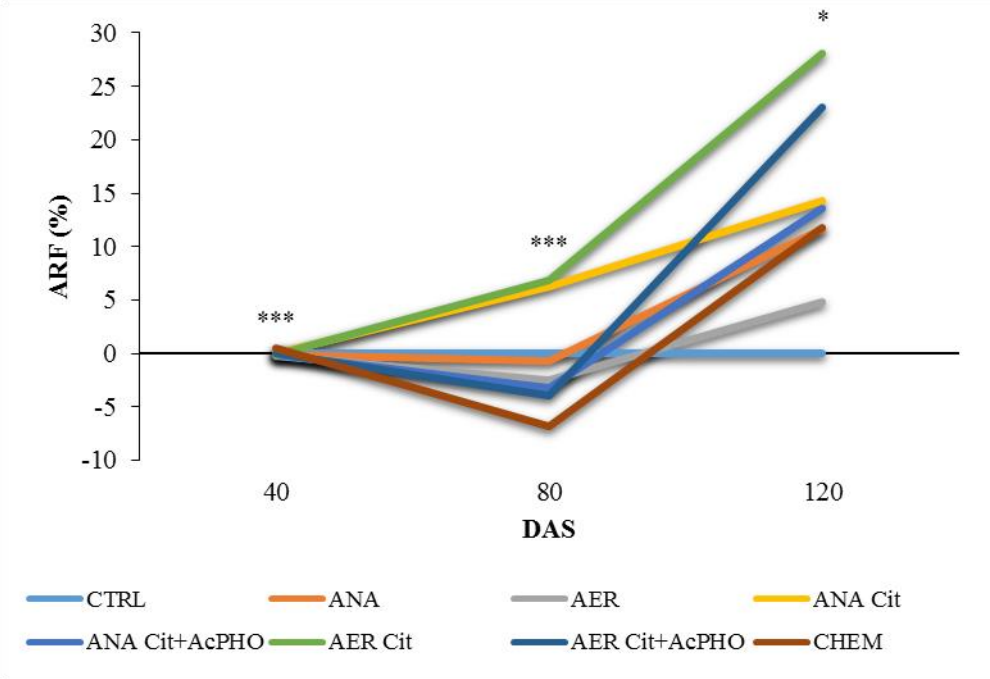


Figure 5.10: Cumulated plant apparent P recovery fraction (%) at different samplings. One-way ANOVA significance within time is shown as *** = P≤0.001, ** = P≤0.01, * = P≤0.05 and ns= P>0.05. Two-way ANOVA was significant in treatment (P≤0.001), time (P≤0.001) and treatment*time (P≤0.01).

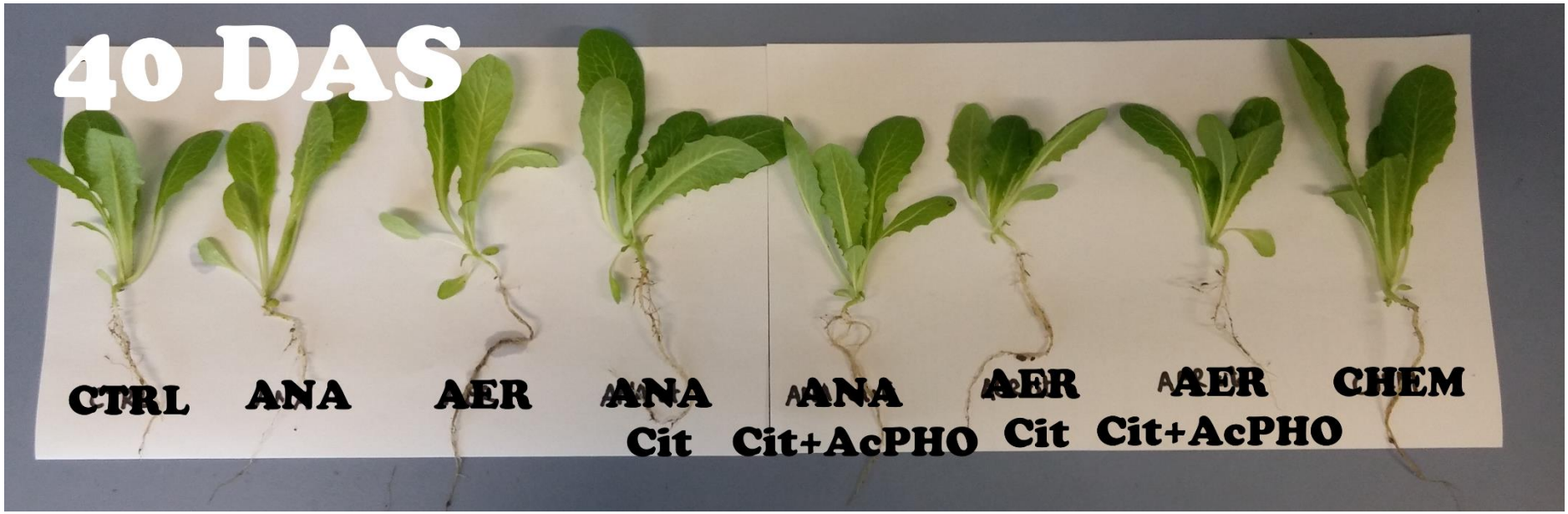


Figure 5.11: Whole plants of different treatments at 40 DAS.

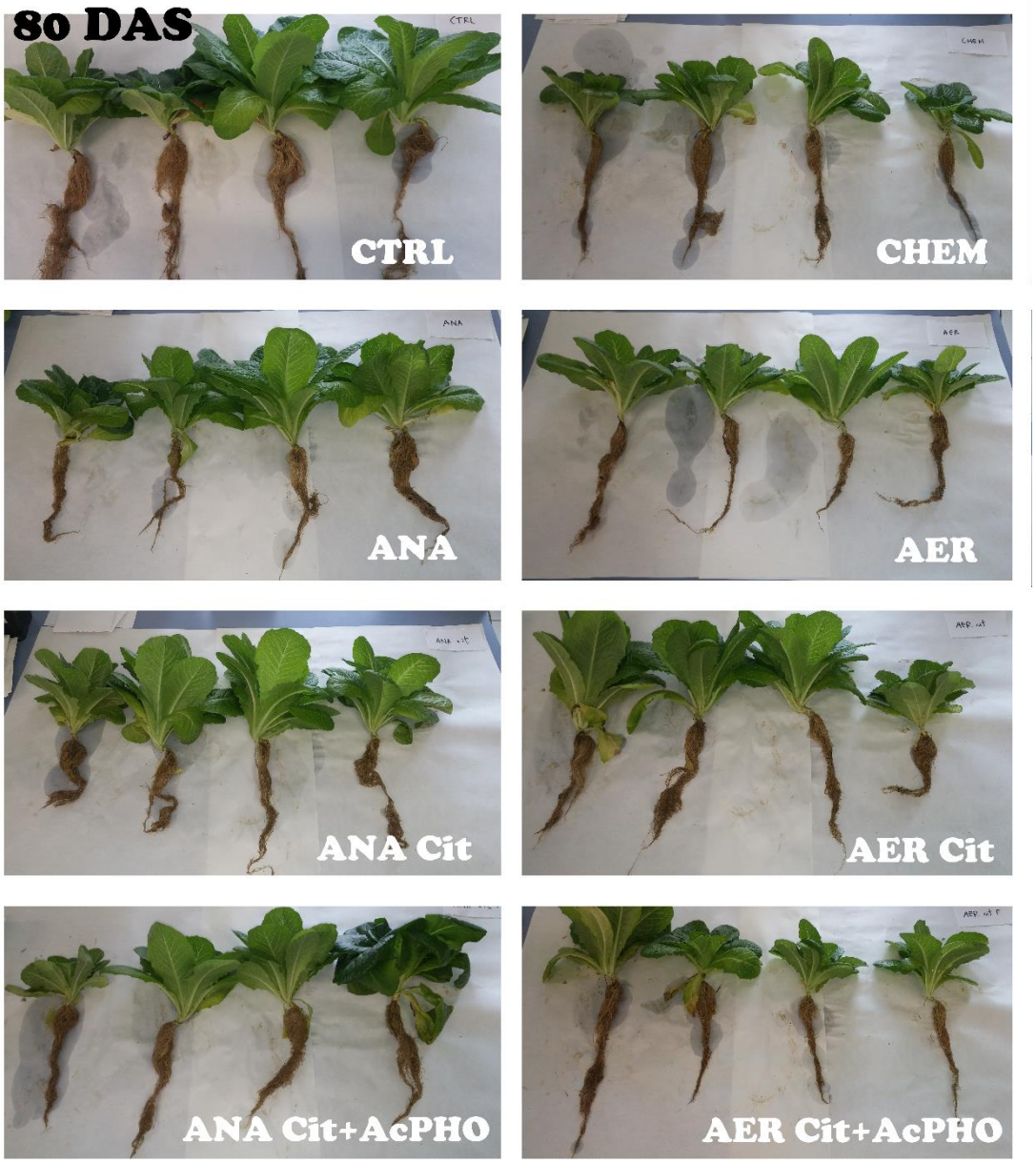


Figure 5.12: Whole plants of different treatments at 80 DAS.

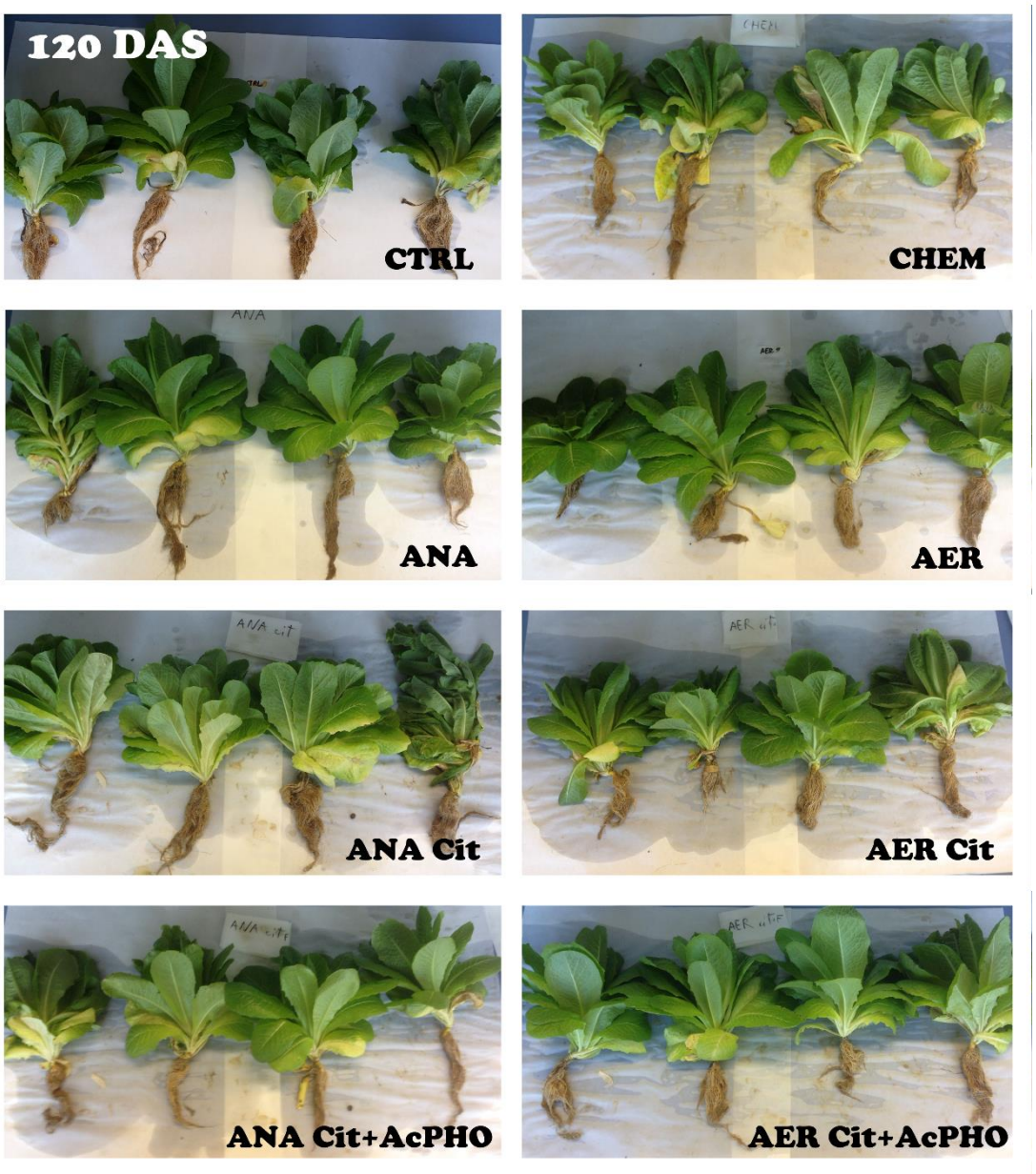


Figure 5.13: Whole plants of different treatments at 120 DAS.

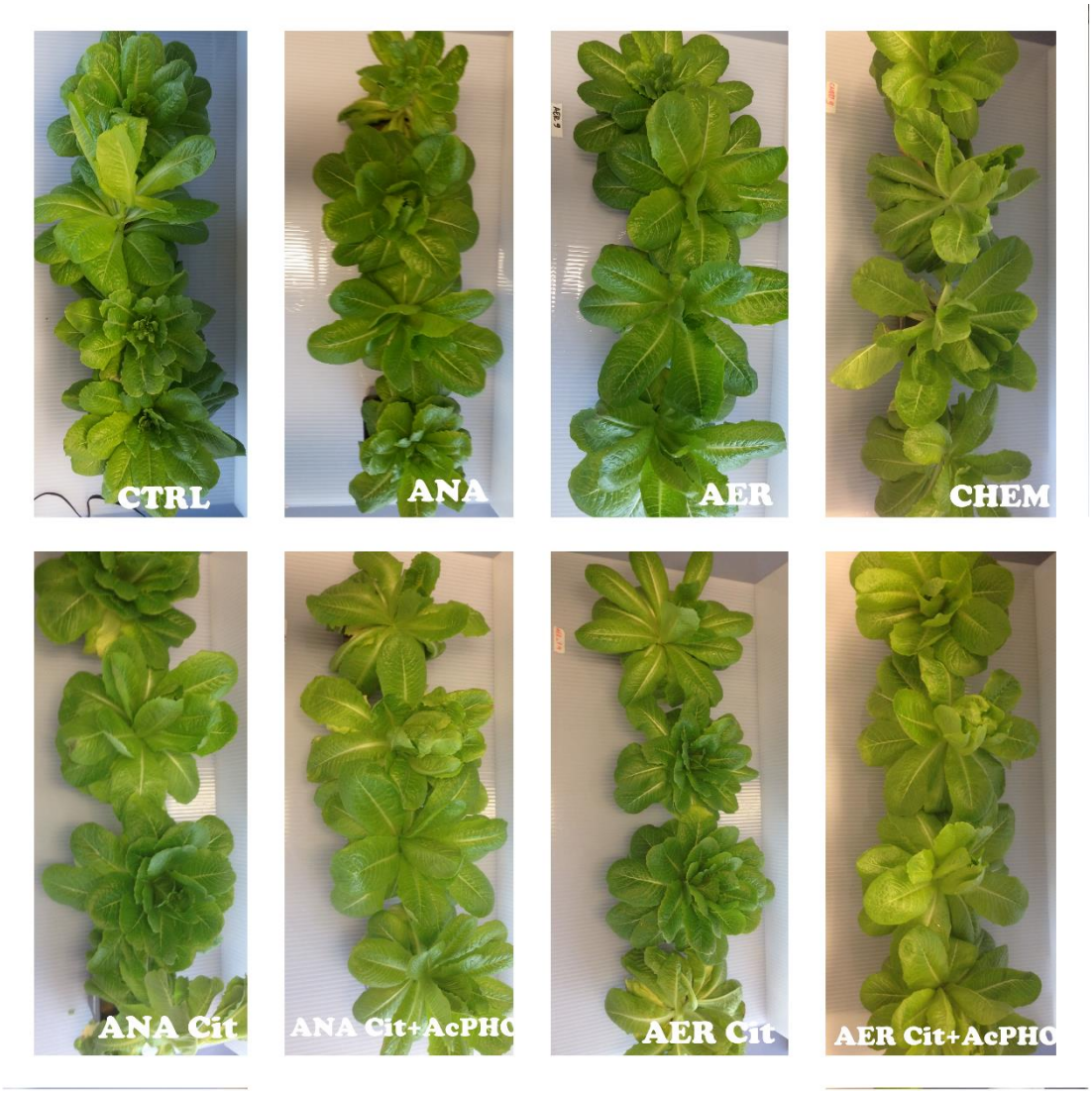
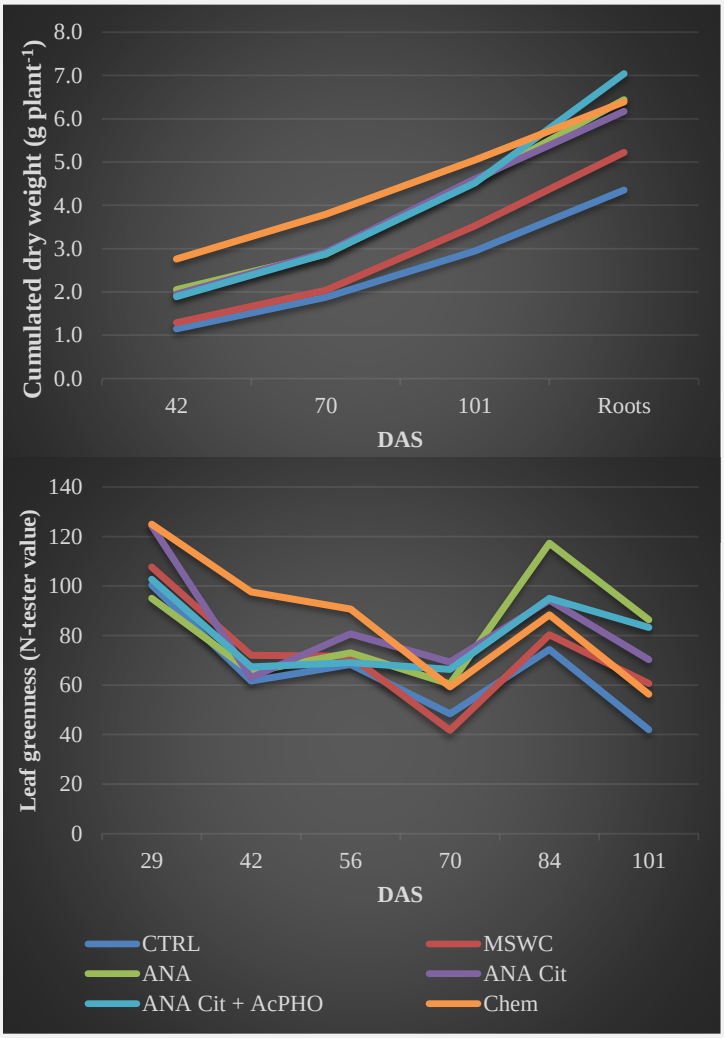


Figure 5.14: Plants of different treatments at 120 DAS before destructive sampling.

Box 5.1: Exhausted sludge as soil conditioners

Exhausted anaerobic sludge after hydrolysis (ANA Cit and ANA Cit+AcPHO) were tested as soil conditioners compared to the raw sludge (ANA). As reference, unamended soil (CTRL), a compost (MSWC) (previously described in chap. 2) and a chemical fertilizer (CHEM) (NH_4NO_3), were chosen. They were added to sandy soil, with low N content, in a dose of 150 mg N kg^{-1}. A soil test was performed to measured soil microbial C and soil respiration. Afterwards, the exhausted sludge and the raw sludge were tested in a plant pot trial with cutting lettuce. On lettuce, leaf greenness (N-tester value) was measured non-destructively every two weeks. Every 4 weeks (at 42, 79 and 101 days after sowing (DAS)), the plants were cut, dried and weighted. At 101 DAS also roots were harvested and washed, dried and weighted. Samples of dried leaves and roots were analysed for metals, nitrate and ammonium content. After soil test, the exhausted sludge resulted comparable to raw sludge in term of respiration and microbial biomass growth.



Results of plant biomass (Fig. 5.24) showed a higher growth in plant treated with the chemical references. However, the two sludge residues provided a plant growth similar to the raw sludge conditioners. Raw and exhausted sludge conditioners provided a leaves growth in the last cut even higher than the chemical reference. ANA Cit+AcPHO residual sludge showed a higher root formation, which make the total dry weight higher than the chemical reference. The root proliferation was probably due to a low P content of the hydrolysed sludge (due to high P recovery of hydrolysis).

Fig. 5.15: Cumulated dry weight (above) at different cuts and leaf greenness (below) at different time.

Box 1 continue: Exhausted sludge as soil conditioners

Chemical reference plants showed higher values also for leaf greenness (Fig. 5.15) up to the second leaf cut (70 DAS), afterwards ANA and the exhausted sludge showed higher values, while control and compost treated plants resulted always lower. Thus, no high differences were found between raw and exhausted sludge. Higher plant growth and higher leaf greenness in the last cut suggested that nutrients release is only postponed in the sludge as compared to the chemical nitrogen. The nitrate content (Table 5.4) was extremely higher in the chemical reference in the first cut, with values doubled of the raw and exhausted sludge and even 10-fold as compared to control and compost. In the second cut nitrate dropped in all the treatments, chemical fertilizer remained the highest, but similar to ANA Cit+AcPHO. In the third cut, all the content resulted low and similar and almost no NO_3 was found in roots. NH_4 content resulted similar in all the treatments (data not shown).

Table 5.4: Nitrate content in different treatments at different cuts.

N-NO_3 (mg N kg^{-1})				
DAS	**42**	**70**	**101**	**Roots**
CTRL	256.5	26.2	21.3	3.7
MSWC	160.2	20.5	40.5	1.7
ANA	906.0	58.3	22.2	0.0
ANA Cit	937.5	52.3	8.7	0.0
ANA Cit + AcPHO	1050.8	137.7	22.3	0.0
Chem	2469.7	154.0	49.0	0.0

Only Chem at 42 DAS was close to the maximum levels of NO_3 fixed by UE Commission (EU Reg. 1258/2011), which are 4000 mg NO_3 kg^{-1} for summer crops under cover.

Regarding plant metal content, exhausted sludge showed higher Ca and Al content as compared to ANA (data not shown). Exhausted sludge showed lower Cd as compared to raw sludge. Cr, Cu, Ni, Pb, Zn was lower in raw and exhausted sludge as compared to control and MSWC plants. In ANA Cit+AcPHO high level of P, Al, Ca, Fe and Mn were found in the first cuts leaves (data not shown), showing very high solubilisation of metal, probably caused by the hydrolysis. Based on the toxic limits for plants (White and Brown, 2010) only no toxic limits were reached for Cd, B, Fe, Mn, Cu, Ni, Mo, Na, Se and Co in every cuts and treatments. Zn content were found always slightly more than the toxic levels (probably it is a lettuce specific features), while Al was found above toxic limits in the last cut in all the treatments. Pb levels, on the other hand, were above the toxic limit for plants in ANA and Chem at 42 DAS and in Ctrl and MSWC at 101 DAS. In exhausted sludge samples, some nutrients, among which P, probably were in a too low amount due to the high recovery of the hydrolysis. Thus, the plants treated with these conditioner had a higher root proliferation, probably to increase the soil volume exploration and the root surface for nutrient assimilation.

In conclusion, exhausted sludge resulted perfectly comparable to the raw sludge when used as soil conditioner or nitrogen fertilizer. The sludge (raw or exhausted) provided a growth that is similar to the chemical fertilizer and significantly higher than the reference compost. Furthermore, the exhausted sludge treated plants uptake less Cd and Pb, avoiding the problems of heavy metal accumulation, generally accounted for sludge fertilization.

Discussion

Can a liquid fertilizer for phosphate plant nutrition derived from sewage sludge be an alternative solution of phosphate rock derived fertilizers? In previous chapters the hydrolysis of two different sewage sludge samples has been studied and the combination of citric acid and acid phosphatase resulted in high P recovery and limited heavy metal concentration in the liquid phase resulting from the hydrolysis (chap. 3). The fertilizers derived from the hydrolysis of an anaerobic sludge, with a high concentration in Fe has been also tested in a soilless cultivation of lettuce (chap. 4). In this chapter, the liquid fertilizer was tested on a soil-plant system, with the addition of the aerobic sludge hydrolysates. Contrasting the results of chap. 3 on the P recovery in aerobic sludge hydrolysates, the PO_4 measured in this experiment resulted very similar between the two sludge hydrolyses. Probably, in the aerobic sludge hydrolysis there were some components which had interfered with the spectrophotometric analysis, bonding the Mo in the molybdenum blue reaction, among which an excess of organic acids (citric acid is one of this), salts or surfactants (*Nagul et al.*, 2015).

The problems previously occurred in the soilless cultivation was an increase of pH in the nutrient solutions with a consequent metal and nutrient precipitation and a strong Al toxicity with consequent low root developments (chap. 4). These problems were overcome with the addition of soil in the system. The soil incubation test revealed a pH decreased with the addition of the hydrolysates in soil. The differences between the soilless and the soil system is the starting pH, which was 5.5 in the nutrient solutions of the floating system and almost 8 in the calcareous soil. In addition, the pH control is difficult in soil, thus, the addition of an acid source (such as the hydrolysates) decreased the pH at the beginning, but this returned to the original pH already after 40 days. The effect of the soil pH and mostly the solubility of P in the hydrolysates make the analysis of available P very promising for the hydrolysates. The P added remained highly available in soil and the plants were able to uptake higher amount of P, even if at the beginning of the experiment, the chemical reference resulted higher in P available. Analysing plant P concentration, P concentration were higher in the chemical reference in the first sampling date. However, the P available in chemical references soil decreased faster than in hydrolysates, concurring to the very low P uptake of the plants samples at 80 and 120 DAS. The highest P concentration were found in leaves of plants treated with ANA Cit+AcPHO and AER Cit+AcPHO. The organic matter mineralizing during the experiment could have influenced the microbial biomass estimation. The microbial C remained higher in the raw sludge while it was quickly mineralised in the hydrolysates, mostly the one deriving from the anaerobic sludge. The hydrolysates showed an evident increase in the phosphatase activity, probably due to the more acid environment. The enzymatic activity (phosphatase

included) increased also in a study on soil after the addition of a solution derived from the enzymatic hydrolysis (*Tejada et al.*, 2013). The phosphatase activity remained always high in the plants treated with the hydrolysates of aerobic sludge with citric acid and acid phosphatase (AER Cit+AcPHO). The high phosphatase activity can also explain the high P uptake of such plants and the reduced P precipitation. However, although the highest P uptake, AER Cit+AcPHO plants did not performed the best between the hydrolysates treatments. The highest growth was reached from the plants treated with hydrolysates of only citric acid (ANA Cit and AER Cit). Between these two treatments, ANA Cit showed a lower shoot:root ratio as compared to the other hydrolysates treatments. The low shoot:root ratio can be a marker of a possible nutrient deficient environment: the microbial biomass which was slightly increasing in this treatment between 40 and 120 DAS, even if did not compete for P uptake with plants can compete for N uptake. Indeed, the ANA Cit treatment showed the highest nitrogen sequestered by the microbial biomass (data not shown). However, the plants did not show lower value of leaf greenness which is normally decreased in N deficient plants (*Gianquinto et al.*, 2011).

Contrasting the previous finding on Al toxicity for roots developments (*Marschner and Marschner*, 2012) and on the higher concentration of Al in AER hydrolysates (chap. 3), the anaerobic treatments showed higher Al concentration in leaves at 120 DAS. The hydrolysates treatments showed also the highest Mg and Ca concentration, showing that the hydrolysates decreased the precipitation of P with Ca and Mg, which otherwise would have been occurred in a calcareous soil (*Hinsinger*, 2001). Furthermore, the plants grown with hydrolysates fertilizers did not show higher toxic metal accumulation: they had similar Cr, C, Ni and Zn, even lower Cd than control and lower Pb than the raw sludge samples.

Comparing the metal concentration of the leaves at 120 DAS to the critical leaf concentration of elements (*White and Brown*, 2010), it can be recognizable that meso and micro nutrients were found upper the sufficiency limits in all the treatments, with the exception of Mo which was above the lower limits only in ANA Cit+AcPHO treated plants. The macronutrient K, on the other hand, was below the sufficient level (5 mg g^{-1}) in control plants, plants treated with ANA and AER and with ANA Cit and AER Cit+AcPHO. Thus, only ANA Cit+Ac PHO and AER Cit fertilizer can be comparable to Chem (which contained K, beside P) in term of potassium fertilization. Therefore, the fertilization with hydrolysates of lettuce should be implemented with more potassium and molybdenum. Among the toxic element critic levels, Fe and Na, but also Cr and Al, were found above the toxic limit in all the treatments (also control and chemical reference), showing that lettuce has an upper critical limits for this elements. Cu concentration can be considered toxic in plants treated with ANA, ANA Cit+AcPHO, AER Cit and AER Cit+AcPHO, while Pb reached the toxic limits only

in control plants and ANA treated plants. Although, the hydrolysates did not accumulated in leaves toxic levels of Pb and Cd, which are the most dangerous, the Cu toxicity symptoms should be monitored.

Actually, the hydrolysates performed much better in every trait as compared to the raw sludge conditioners, which provided less P available, but also less phosphatase activity. A comparison with other waste derived fertilizers should be made. However, in recent studies struvite had the best crop growth (*Duboc et al.*, 2017; *Wollmann et al.*, 2017) among different recycled fertilizers. We have tested struvite in chap. 2 and its P availability in our calcareous soil decreased quicker than the hydrolysates, thus we can hypothesize that our hydrolysates would result better fertilizers for lettuce growth and P uptake.

In addition, a pot trial with the residual sludge after the hydrolysis was carried out in order to understand if the sludge can still be disposed in field with some fertilizing or amending characteristics (Box 1). The results were above the expectations, with exhausted sludge resulting perfectly comparable to the raw sludge in term of plant growth and nutrition, with the addition of a less toxic metal uptake.

For the comparison between the hydrolysates, different parameters should be taken into account: the hydrolysates with acid phosphatase increased the phosphatase activity and the P availability in the soil but considering the plant biomass the citric acid hydrolysates performed the best (ANA Cit and AER Cit), with the anaerobic one compartmentalising much biomass in roots. The aerobic hydrolysates (AER Cit and AER Cit+AcPHO) instead performed very well for growth and P availability, resulting the best in apparent P recovery.

In conclusion, the hydrolysates were a much valid alternative to the mineral fertilizers as a source of P: they increased the P availability for plants and protected P from precipitation with Ca and Mg, without showing any problematic. However, further studies are needed to evaluate the best option, trying to understand the P use efficiency of the hydrolysates in a P balance conception.

References

Achat, D.L., Bakker, M.R., Zeller, B., Pellerin, S., Bienaimé, S., Morel, C. (2010): Long-term organic phosphorus mineralization in Spodosols under forests and its relation to carbon and nitrogen mineralization. *Soil Biol. Biochem.* 42, 1479–1490.

Adam, C., Peplinski, B., Michaelis, M., Kley, G., Simon, F.-G. (2008): Thermochemical treatment of sewage sludge ashes for phosphorus recovery. *Waste Manag.* 29, 1122–1128.

Adesemoye, A.O., Kloepper, J.W. (2009): Plant–microbes interactions in enhanced fertilizer-use efficiency. *Appl. Microbiol. Biotechnol.* 85, 1–12.

AEEP (2017): Phosphorus: Key to Plant Life [WWW Document]. URL http://aeep.eu/phosphorus/ (accessed 11.29.17).

Ajiboye, B., Akinremi, O.O., Hu, Y., Flaten, D.N. (2007): Phosphorus Speciation of Sequential Extracts of Organic Amendments Using Nuclear Magnetic Resonance and X-ray Absorption Near-Edge Structure Spectroscopies. *J. Environ. Qual.* 36, 1563.

Alborés, A.F., Cid, B.P., Gómez, E.F., López, E.F. (2000): Comparison between sequential extraction procedures and single extractions for metal partitioning in sewage sludge samples. *Analyst* 125, 1353–1357.

Alvarenga, P., Mourinha, C., Farto, M., Santos, T., Palma, P., Sengo, J., Morais, M.-C., Cunha-Queda, C. (2015): Sewage sludge, compost and other representative organic wastes as agricultural soil amendments: Benefits versus limiting factors.

Alvarez, A.E., Mochon, C.M., Sanchez, J.C., Rodriguez, T.M. (2002): Heavy metal extractable forms in sludge from wastewater treatment plants. *Chemosphere* 47, 765–775.

Andersen, M.S. (2007): An introductory note on the environmental economics of the circular economy. *Sustain. Sci.* 2, 133–140.

Ayaga, G., Todd, A., Brookes, P.C. (2006): Enhanced biological cycling of phosphorus increases its availability to crops in low-input sub-Saharan farming systems. *Soil Biol. Biochem.* 38, 81–90.

Baek, D., Kim, M.C., Chun, H.J., Kang, S., Park, H.C., Shin, G., Park, J., Shen, M., Hong, H., Kim, W.-Y., Kim, D.H., Lee, S.Y., Bressan, R.A., Bohnert, H.J., Yun, D.-J. (2013): Regulation of miR399f Transcription by AtMYB2 Affects Phosphate Starvation Responses in Arabidopsis. *Plant Physiol.* 161, 362–373.

Baldwin, J.C., Karthikeyan, A.S., Raghothama, K.G. (2001): LEPS2, a phosphorus starvation-induced novel acid phosphatase from tomato. *Plant Physiol.* 125, 728–37.

Bates, T.R., Lynch, J.P. (1996): Stimulation of root hair elongation in Arabidopsis thaliana by low phosphorus availability. *Plant, Cell Environ.* 19, 529–538.

Becana, M., Moran, J.F., Iturbe-Ormaetxe, I. (1998): Iron-dependent oxygen free radical generation in plants subjected to environmental stress: Toxicity and antioxidant protection. *Plant Soil* 201, 137–147.

Becker, M., Asch, F. (2005): Iron toxicity in rice-conditions and management concepts. *J. Plant Nutr. Soil Sci.* 168, 558–573.

Bernal, M.P., Alburquerque, J.A., Moral, R. (2009): Composting of animal manures and chemical criteria for compost maturity assessment. A review. *Bioresour. Technol.* 100, 5444–5453.

Bhadoria, P.S., Steingrobe, B., Claassen, N., Liebersbach, H. (2002): Phosphorus efficiency of wheat and sugar beet seedlings grown in soils with mainly calcium, or iron and aluminium phosphate. *Plant Soil* 246, 41–52.

Biswas, B.K., Inoue, K., Harada, H., Ohto, K., Kawakita, H. (2009): Leaching of phosphorus from incinerated sewage sludge ash by means of acid extraction followed by adsorption on orange waste gel. *J. Environ. Sci.* 21, 1753–1760.

Blöcher, C., Niewersch, C., Melin, T. (2012): Phosphorus recovery from sewage sludge with a hybrid process of low pressure wet oxidation and nanofiltration. *Water Res.* 46, 2009–2019.

Bolan, N.S. (1991): A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. *Plant Soil*.

Breeuwsma, A., Reijerink, J.G.A., Schoumans, O.F. (1995): Impact of manure on accumulation and leaching of phosphate in areas of intensive livestock farming, in: Animal Waste and the Land-Water Interface. pp. 239–249.

Brod, E., Øgaard, A.F., Hansen, E., Wragg, D., Haraldsen, T.K., Krogstad, T. (2015): Waste products as alternative phosphorus fertilisers part I: inorganic P species affect fertilisation effects depending on soil pH. *Nutr. Cycl. Agroecosystems* 103, 167–185.

Brookes, P., Beyaert, R., Voroney, R. (2007): Soil Microbial Biomass C, N, P, and S, in: Soil Sampling and Methods of Analysis, Second Edition. CRC Press.

Brookes, P.C., Powlson, D.S., Jenkinson, D.S. (1982): Measurement of microbial biomass phosphorus in soil. *Soil Biol. Biochem.* 14, 319–329.

Brown, T.A., Shrift, A. (1982): SELENIUM: TOXICITY AND TOLERANCE IN HIGHER PLANTS. *Biol. Rev.* 57, 59–84.

Bünemann, E.K. (2015): Assessment of gross and net mineralization rates of soil organic phosphorus – A review. *Soil Biol. Biochem.* 89, 82–98.

Burleigh, S.H., Cavagnaro, T., Jakobsen, I. (2002): Functional diversity of arbuscular mycorrhizas extends to the expression of plant genes involved in P nutrition. *J. Exp. Bot.* 53, 1593–1601.

Cakmak, I., Hengeler, C., Marschner, H. (1994): Partitioning of shoot and root dry matter and carbohydrates in bean plants suffering from phosphorus, potassium and magnesium deficiency. *J. Exp. Bot.* 45, 1245–1250.

CCIA (2016): Prezzi - News — Camera di Commercio di Modena [WWW Document]. URL http://www.mo.camcom.it/tutela-del-mercato/ufficio-prezzi (accessed 11.9.16).

Chen, C.R., Condron, L.M., Davis, M.R., Sherlock, R.R. (2002): Phosphorus dynamics in the rhizosphere of perennial ryegrass (Lolium perenne L.) and radiata pine (Pinus Radiata D. Don.). *Soil Biol. Biochem.* 34, 487–499.

Childers, D.L., Corman, J., Edwards, M., Elser, J.J. (2011): Sustainability Challenges of Phosphorus and Food: Solutions from Closing the Human Phosphorus Cycle. *Bioscience* 61, 117–124.

Christodoulou, A., Stamatelatou, K. (2016): Overview of legislation on sewage sludge management in developed countries worldwide. *Water Sci. Technol.* 73.

Cieslik, B., Konieczka, P. (2017): A review of phosphorus recovery methods at various steps of wastewater treatment and sewage sludge management. The concept of " no solid waste generation " and analytical methods. *J. Clean. Prod.* 142, 1728–1740.

Clarkson, D.T., Scattergood, C.B. (1982): Growth and Phosphate Transport in Barley

and Tomato Plants During the Development of, and Recovery from, Phosphate-stress. *J. Exp. Bot.* 33, 865–875.

Cogliatti, D.H., Santa Marìa, G.E. (1990): Influx and Efflux of Phosphorus in Roots of Wheat Plants in Non-Growth-Limiting Concentrations of Phosphorus. *J. Exp. Bot.* 41, 601–607.

Cooper, J., Lombardi, R., Boardman, D., Carliell-Marquet, C. (2011): The future distribution and production of global phosphate rock reserves. *Resour. Conserv. Recycl.* 57, 78–86.

Cordell, D., Drangert, J., White, S. (2009): The story of phosphorus: global food security and food for thought. *Glob. Environ. Chang.*

Cordell, D., Rosemarin, A., Schroder, J.J., Smit, A.L. (2011): Towards global phosphorus security: A systems framework for phosphorus recovery and reuse options. *Chemosphere* 84, 747–758.

Corominas, L., Foley, J., Guest, J.S., Hospido, A., Larsen, H.F., Morera, S., Shaw, A. (2013): Life cycle assessment applied to wastewater treatment: State of the art. *Water Res.* 47, 5480–5492.

Da Ros, C., Cavinato, C., Pavan, P., Bolzonella, D. (2016): Mesophilic and thermophilic anaerobic co-digestion of winery wastewater sludge and wine lees: An integrated approach for sustainable wine production. *J. Environ. Manage.*

Dakora, F.D., Phillips, D.A. (2002): Root exudates as mediators of mineral acquisition in low-nutrient environments. *Plant Soil* 245, 35–47.

Delhaize, E., Randall, P.J., Laurie, S., Phillips, R., Ratcliffe, R., Stewart, G. (1995): Characterization of a Phosphate-Accumulator Mutant of Arabidopsis thaliana. *Plant Physiol.* 107, 207–213.

Direzione Generale Ambiente, D. del suolo e della costa-D.A.R.E.-R. (2004): Linee di Intervento in materia di utilizzo dei fanghi di depurazione in agricoltura. Bologna.

Domenech, C.E., Lisa, T.A., Salvano, M.A., Garrido, M.N. (2015): Selective release of phosphorus and nitrogen from waste activated sludge with combined thermal and alkali treatment. *Bioresour. Technol.* 190, 522–528.

Donatello, S., Tong, D., Cheeseman, C.R. (2010): Production of technical grade phosphoric acid from incinerator sewage sludge ash (ISSA). *Waste Manag.* 30, 1634–1642.

Doyle, J.D., Parsons, S.A. (2002): Struvite formation, control and recovery. *Water Res.* 36, 3925–3940.

Duboc, O., Santner, J., Fard, A.G., Zehetner, F., Tacconi, J., Wenzel, W.W. (2017): Predicting phosphorus availability from chemically diverse conventional and recycling fertilizers. *Sci. Total Environ.* 599–600, 1160–1170.

Easlon, H.M., Bloom, A.J. (2014): Easy Leaf Area: Automated Digital Image Analysis for Rapid and Accurate Measurement of Leaf Area. *Appl. Plant Sci.* 2, 1400033.

Eghball, B. (2002): Soil Properties as Influenced by Phosphorus-and Nitrogen-Based Manure and Compost Applications WASTE MANAGEMENT Soil Properties as Influenced by Phosphorus-and Nitrogen-Based Manure and Compost Applications. *Waste Manag.* 94, 128–135.

Eghball, B., Power, J.F. (1999): Phosphorus- and Nitrogen-Based Manure and Compost Applications: Corn Production and Soil Phosphorus. *Soil Sci. Soc. Am. J.* 63, 895–901.

Ellis, D.R., Salt, D.E. (2003): Plants, selenium and human health. *Curr. Opin. Plant Biol.*

Eneji, A.E., Honna, T., Yamamoto, S., Masuda, T., Endo, T., Irshad, M. (2003): Changes in Humic Substances and Phosphorus Fractions During Composting. *Commun. Soil Sci. Plant Anal.* 34, 2303–2314.

European Biogas Association (2015): EBA Biomethane & Biogas Report 2015 [WWW Document]. URL http://european-biogas.eu/2015/12/16/biogasreport2015/ (accessed 11.9.16).

Eurostat (2016): Eurostat [WWW Document]. URL http://ec.europa.eu/eurostat/web/main/home (accessed 11.9.16).

Ewa Sroga, G. (1987): Plant regeneration of two Lupinus spp. from callus cultures via organogenesis. *Plant Sci.* 51, 245–249.

FAO (2016): FAOSTAT [WWW Document]. URL http://www.fao.org/faostat/en/#home (accessed 12.2.17).

Feng, R., Wei, C., Tu, S. (2013): The roles of selenium in protecting plants against abiotic stresses. *Environ. Exp. Bot.*

Fertilisers Working Group Meeting (2014): Essential safety and quality requirements for fertilising materials.

Foehse, D., Jungk, A. (1983): Influence of phosphate and nitrate supply on root hair formation of rape, spinach and tomato plants. *Plant Soil* 74, 359–368.

Fredeen, A., Rao, I., Terry, N. (1989): Influence of phosphorus nutrition on growth and carbon partitioning in Glycine max. *Plant Physiol.*

Frossard, E., Condron, L.M., Oberson, A., Sinaj, S., Fardeau, J.C. (2000): Processes Governing Phosphorus Availability in Temperate Soils. *J. Environ. Qual.* 29, 15.

Frossard, E., Sinaj, S., Zhang, L.-M., Morel, J.L. (1996): The Fate of Sludge Phosphorus in Soil-Plant Systems. *Soil Sci. Soc. Am. J.* 60, 1248.

Fuentes, B., Bolan, N., Naidu, R., de la Luz Mora, M., Australia, A. (2006): PHOSPHORUS IN ORGANIC WASTE-SOIL SYSTEMS Fósforo en los sistemas suelo – residuos orgánicos. *J.Soil Sc. Plant. Nutr* 66, 64–8364.

García-Albacete, M., Martín, A., Cartagena, M.C. (2012): Fractionation of phosphorus biowastes: Characterisation and environmental risk. *Waste Manag.* 32, 1061–1068.

Gianquinto, G., Orsini, F., Sambo, P., D'Urzo, M.P. (2011): The Use of Diagnostic Optical Tools to Assess Nitrogen Status and to Guide Fertilization of Vegetables. *Horttechnology* 21, 287–292.

Gonzalez-Ponce, R., López-de-Sá, E.G., Plaza, C. (2009): Lettuce Response to Phosphorus Fertilization with Struvite Recovered from Municipal Wastewater. *HortScience* 44, 426–430.

Grigatti, M., Boanini, E., Cavani, L., Ciavatta, C., Marzadori, C. (2015): Phosphorus in Digestate-Based Compost: Chemical Speciation and Plant-Availability. *Waste and Biomass Valorization* 6, 481–493.

Grigatti, M., Boanini, E., Mancarella, S., Simoni, A., Centemero, M., Veeken, A.H.M. (2017): Phosphorous extractability and ryegrass availability from bio-waste composts in a calcareous soil. *Chemosphere* 174, 722–731.

Grigatti, M., Di Girolamo, G., Chincarini, R., Ciavatta, C., Barbanti, L. (2011): Potential nitrogen mineralization, plant utilization efficiency and soil CO2 emissions following the addition of anaerobic digested slurries. *Biomass and Bioenergy* 35, 4619–4629.

Grusak, M. a., DellaPenna, D. (1999): Improving the Nutrient Composition of Plants To Enhance Human Nutrition and Health1. *Annu. Rev. Plant Physiol. Plant Mol.*

Biol. 50, 133–161.

Guppy, C., Menzies, N., Moody, P., Blamey, F. (2005): Competitive sorption reactions between phosphorus and organic matter in soil: a review. *Soil Res.*

Haug, R.T. (1993): The practical handbook of compost engineering. Lewis Publishers.

Heimersson, S., Harder, R., Peters, G.M., Svanström, M. (2014): Including pathogen risk in life cycle assessment of wastewater management. 2. Quantitative comparison of pathogen risk to other impacts on human health. *Environ. Sci. Technol.* 48, 9446–9453.

Herzel, H., Kruger, O., Hermann, L., Adam, C. (2016): Sewage sludge ash - A promising secondary phosphorus source for fertilizer production. *Sci. Total Environ.* 542, 1136–1143.

Hinsinger, P. (2001): Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. *Plant Soil* 237, 173–195.

Hooda, P.S., Truesdale, V.W., Edwards, A.C., Withers, P.J.A., Aitken, M.N., Miller, A., Rendell, A.R. (2001): Manuring and fertilization effects on phosphorus accumulation in soils and potential environmental implications. *Adv. Environ. Res.* 5, 13–21.

Hospido, A., Moreira, M.T., Feijoo, G. (2008): A comparison of municipal wastewater treatment plants for big centres of population in Galicia (Spain). *Int. J. Life Cycle Assess.* 13, 57–64.

Hukari, S., Hermann, L., Nättorp, A. (2016): From wastewater to fertilisers — Technical overview and critical review of European legislation governing phosphorus recycling. *Sci. Total Environ.* 542, 1127–1135.

Hunter, P.J., Teakle, G.R., Bending, G.D. (2014): Root traits and microbial community interactions in relation to phosphorus availability and acquisition, with particular reference to Brassica. *Front. Plant Sci.* 5, 27.

Indexmundi (2016): DAP fertilizer - Monthly Price - Commodity Prices - Price Charts, Data, and News - IndexMundi [WWW Document]. URL http://www.indexmundi.com/commodities/?commodity=dap-fertilizer&months=180 (accessed 11.9.16).

Jaffer, Y., Clark, T.A., Pearce, P., Parsons, S.A. (2002): Potential phosphorus recovery by struvite formation. *Water Res.* 36, 1834–1842.

Jeng, A.S., Haraldsen, T.K., Grønlund, A., Pedersen, P.A. (2006): Meat and bone meal as nitrogen and phosphorus fertilizer to cereals and rye grass. *Nutr. Cycl. Agroecosystems* 76, 183–191.

Johri, A.K., Oelmüller, R., Dua, M., Yadav, V., Kumar, M., Tuteja, N., Varma, A., Bonfante, P., Persson, B.L., Stroud, R.M. (2015): Fungal association and utilization of phosphate by plants: success, limitations, and future prospects. *Front. Microbiol.* 6, 984.

Jones, D.L. (1998): Organic acids in the rhizosphere – a critical review. *Plant Soil* 205, 25–44.

Kabata-Pendias, A. (2011): Trace elements in soils and plants. Fourth Edition, CRC Press.

Kataki, S., West, H., Clarke, M., Baruah, D.C. (2016): Phosphorus recovery as struvite: Recent concerns for use of seed, alternative Mg source, nitrogen conservation and fertilizer potential. *Resour. Conserv. Recycl.* 107, 142–156.

Khan, A., Jilani, G., Akhtar, M.S., Naqvi, S.M.S., Rasheed, M., Khan, A.A., Jilani, G., Akhtar, M.S., Muhammad, S., Naqvi, S., Rasheed, M. (2009): Phosphorus

Solubilizing Bacteria: Occurrence, Mechanisms and their Role in Crop Production. *Publ. J. agric. biol. sci* 1, 48–58.

Khan, K.S., Joergensen, R.G. (2012a): Relationships between P fractions and the microbial biomass in soils under different land use management. *Geoderma* 173, 274–281.

Khan, K.S., Joergensen, R.G. (2012b): Compost and phosphorus amendments for stimulating microorganisms and growth of ryegrass in a Ferralsol and a Luvisol. *J. Plant Nutr. Soil Sci.* 175, 108–114.

Kidd, P.S., Domínguez-Rodríguez, M.J., Díez, J., Monterroso, C. (2007): Bioavailability and plant accumulation of heavy metals and phosphorus in agricultural soils amended by long-term application of sewage sludge. *Chemosphere* 66, 1458–1467.

Kim, M., Han, D.W., Kim, D.J. (2015): Selective release of phosphorus and nitrogen from waste activated sludge with combined thermal and alkali treatment. *Bioresour. Technol.*

Kleinman, P.J.A., Sharpley, A.N., Wolf, A.M., Beegle, D.B., Moore, P.A. (2002): Measuring Water-Extractable Phosphorus in Manure as an Indicator of Phosphorus in Runoff. *Soil Sci. Soc. Am. J.* 66, 2009.

Kochian, L. V (1995): Cellular Mechanisms of Aluminum Toxicity and Resistance in Plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 46, 237–260.

Koppelaar, R.H.E.M., Weikard, H.P. (2013): Assessing phosphate rock depletion and phosphorus recycling options. *Glob. Environ. Chang.* 23, 1454–1466.

Krüger, O., Adam, C. (2015): Recovery potential of German sewage sludge ash. *Waste Manag.* 45, 400–406.

Kumar, V., Gilkes, R.J., Bolland, M.D.A. (1992): A comparison of seven soil P tests for plant species with different external P requirements grown on soils containing rock phosphate and superphosphate residues. *Fertil. Res.* 33, 35–45.

Lambers, H., Brundrett, M.C., Raven, J.A., Hopper, S.D. (2011): Plant mineral nutrition in ancient landscapes: high plant species diversity on infertile soils is linked to functional diversity for nutritional strategies. *Plant Soil* 348, 7–27.

Laturnus, F., von Arnold, K., Grøn, C. (2007): Organic Contaminants from Sewage Sludge Applied to Agricultural Soils. False Alarm Regarding Possible Problems for Food Safety? (8 pp). *Environ. Sci. Pollut. Res. Int.* 14 Suppl 1, 53–60.

Leite, W.R.M., Gottardo, M., Pavan, P., Belli Filho, P., Bolzonella, D. (2016): Performance and energy aspects of single and two phase thermophilic anaerobic digestion of waste activated sludge. *Renew. Energy* 86, 1324–1331.

Leytem, A.B., Westermann, D.T. (2003): PHOSPHATE SORPTION BY PACIFIC NORTHWEST CALCAREOUS SOILS. *Soil Sci.* 168.

Leytem, A.B., Westermann, D.T. (2005): PHOSPHORUS AVAILABILITY TO BARLEY FROM MANURES AND FERTILIZERS ON A CALCAREOUS SOIL. *Soil* 170, 401–412.

Liang, Y., Sun, W., Zhu, Y.G., Christie, P. (2007): Mechanisms of silicon-mediated alleviation of abiotic stresses in higher plants: A review. *Environ. Pollut.* 147, 422–428.

López-Arredondo, D.L., Leyva-González, M.A., Alatorre-Cobos, F., Herrera-Estrella, L. (2013): Biotechnology of nutrient uptake and assimilation in plants. *Int. J. Dev. Biol.* 57, 595–610.

Lynch, J., Läuchli, A., Epstein, E. (1991): Vegetative Growth of the Common Bean in

Response to Phosphorus Nutrition. *Crop Sci.* 31, 380.

Ma, J.F. (2004): Role of silicon in enhancing the resistance of plants to biotic and abiotic stresses. *Soil Sci. Plant Nutr.* 50, 11–18.

Ma, Z., Bielenberg, D.G., Brown, K.M., Lynch, J.P. (2001): Regulation of root hair density by phosphorus availability in Arabidopsis thaliana. *Plant, Cell Environ.* 24, 459–467.

Malik, M., Khan, K., Marschner, P. (2013): Microbial biomass, nutrient availability and nutrient uptake by wheat in two soils with organic amendments. *J. Soil Sci. Plant Nutr.* 13, 955–966.

Mamani, I., Averanga, Kattia, Boris, E., Terrazas, E. (2014): Establecimiento del sistema de generación in vitro de callos de Lupinus mutabilis sweet (tarwi) a partir de hipocótilos. *Rev. CON-CIENCIA* 2.

Marchetti, R., Castelli, F., Orsi, A., Sghedoni, L., Bochicchio, D. (2012): Biochar from swine manure solids: influence on carbon sequestration and Olsen phosphorus and mineral nitrogen dynamics in soil with and without digestate incorporation. *Ital. J. Agron.* 7, 26.

Marschner, H., Kirkby, E.A., Cakmak, I. (1996): Effect of mineral nutritional status on shoot-root partitioning of photoassimilates and cycling of mineral nutrients. *J. Exp. Bot.* 47, 1255–1263.

Marschner, H., Marschner, P. (2012): Marschner's mineral nutrition of higher plants. Academic Press.

Mattenberger, H., Fraissler, G., Brunner, T., Herk, P., Hermann, L., Obernberger, I. (2008): Sewage sludge ash to phosphorus fertiliser: Variables influencing heavy metal removal during thermochemical treatment. *Waste Manag.* 28, 2709–2722.

Mayer, B.K., Baker, L.A., Boyer, T.H., Drechsel, P., Gifford, M., Hanjra, M.A., Parameswaran, P., Stoltzfus, J., Westerhoff, P., Rittmann, B.E. (2016): Total Value of Phosphorus Recovery. *Environ. Sci. Technol.* 50, 6606–6620.

McBride, M., Sauve, S., Hendershot, W. (1997): Solubility control of Cu, Zn, Cd and Pb in contaminated soils. *Eur. J. Soil Sci.* 48, 337–346.

McCoy, J.L., Sikora, L.J., Weil, R.R. (1986): Plant Availability of Phosphorus in Sewage Sludge Compost1. *J. Environ. Qual.* 15, 403.

Møller, J., Boldrin, A., Christensen, T.H. (2009): Anaerobic digestion and digestate use: accounting of greenhouse gases and global warming contribution. *Waste Manag. Res.* 27, 813–824.

Möller, K., Müller, T. (2012): Effects of anaerobic digestion on digestate nutrient availability and crop growth: A review. *Eng. Life Sci.* 12, 242–257.

Mosier, N.S., Ladisch, C.M., Ladisch, M.R. (2002): Characterization of acid catalytic domains for cellulose hydrolysis and glucose degradation. *Biotechnol. Bioeng.* 79, 610–618.

Muhammad, S., Müller, T., Joergensen, R.G. (2006): Decomposition of pea and maize straw in Pakistani soils along a gradient in salinity. *Biol. Fertil. Soils* 43, 93–101.

Murphy, J., Riley, J.P. (1962): A modified single solution method for the determination of phosphate in natural waters. *Anal. Chim. Acta* 27, 31–36.

Nagul, E.A., McKelvie, I.D., Worsfold, P., Kolev, S.D. (2015): The molybdenum blue reaction for the determination of orthophosphate revisited: Opening the black box. *Anal. Chim. Acta*.

Nannipieri, P., Giagnoni, L., Landi, L., Renella, G. (2011): Role of Phosphatase Enzymes in Soil, in: Phosphorus in Action. Springer, Berlin, Heidelberg, pp. 215–

243.

Neumann, G. (2010): Mining for nutrients - regulatory aspects of cluster root function and development. *New Phytol.* 187, 879–882.

Neumann, G. (2016): The Role of Ethylene in Plant Adaptations for Phosphate Acquisition in Soils – A Review. *Front. Plant Sci.* 6, 1224.

Neumann, G., Martinoia, E. (2002): Cluster roots – an underground adaptation for survival in extreme environments. *Trends Plant Sci.* 7, 162–167.

Nkebiwe, P.M., Weinmann, M., Bar-Tal, A., Müller, T. (2016): Fertilizer placement to improve crop nutrient acquisition and yield: A review and meta-analysis. *F. Crop. Res.* 196, 389–401.

Øgaard, A.F. (1996): Effect of Phosphorus Fertilization on the Concentration of Total and Algal-available Phosphorus in Different Particle-size Fractions in Norwegian Agricultural Soils. *Acta Agric. Scand. Sect. B - Soil Plant Sci.* 46, 24–29.

Olsen, S.R. (1954): Estimation Of Available Phosphorus In Soils By Extraction With Sodium Bicarbonate.

Ottosen, L.M., Kirkelund, G.M., Jensen, P.E. (2013): Extracting phosphorous from incinerated sewage sludge ash rich in iron or aluminum. *Chemosphere* 91, 963–969.

Pant, H.K., Warman, P.R. (2000): Enzymatic hydrolysis of soil organic phosphorus by immobilized phosphatases. *Biol. Fertil. Soils* 30, 306–311.

Pasqualino, J.C., Meneses, M., Abella, M., Castells, F. (2009): LCA as a Decision Support Tool for the Environmental Improvement of the Operation of a Municipal Wastewater Treatment Plant. *Environ. Sci. Technol.* 43, 3300–3307.

Pastor, L., Marti, N., Bouzas, A., Seco, A. (2008): Sewage sludge management for phosphorus recovery as struvite in EBPR wastewater treatment plants. *Bioresour. Technol.* 99, 4817–4824.

Petronici, C. (1989): Gli elementi nutritivi, in: Paolo Sequi (ed.): Chimica Del Suolo. Patron Editore, Bologna, pp. 357–380.

Pniewski, T., Kapusta, J., Płucienniczak, A. (2006): Agrobacterium-mediated transformation of yellow lupin to generate callus tissue producing HBV surface antigen in a long-term culture. *J. Appl. Genet.* 47, 309–318.

Polowick, P.L., Loukanina, N.N., Doshi, K.M. (2014): Agrobacterium-mediated transformation of tarwi (Lupinus mutabilis Sweet), a potential platform for the production of plant-made proteins. *Vitr. Cell. Dev. Biol. - Plant* 50, 401–411.

Pote, D.H., Daniel, T.C., Sharpley, A.N., Moore, P.A., Edwards, D.R., Nichols, D.J. (1996): Relating Extractable Soil Phosphorus to Phosphorus Losses in Runoff t'. *Soil Sci. Soc. Am. J.* 60, 855–859.

Raghothama, K.G. (1999): PHOSPHATE ACQUISITION. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 50, 665–693.

Rahman, M.M., Salleh, M.A.M., Rashid, U., Ahsan, A., Hossain, M.M., Ra, C.S. (2014): Production of slow release crystal fertilizer from wastewaters through struvite crystallization – A review. *Arab. J. Chem.* 7, 139–155.

Reijnders, L. (2014): Phosphorus resources, their depletion and conservation, a review. *Resour. Conserv. Recycl.*

Richardson, A.E., Simpson, R.J. (2011): Soil microorganisms mediating phosphorus availability update on microbial phosphorus. *Plant Physiol.* 156, 989–96.

Russo, A. (2012): ANALISI E MIGLIORAMENTO DELL'IMPIANTO DI

DEPURAZIONE DI LEGNAGO. Università degli Studi di Padova.

Shannon, M.C., Grieve, C.M. (1999): Tolerance of vegetable crops to salinity. *Sci. Hortic. (Amsterdam).* 78, 5–38.

Sharpley, A., Moyer, B. (2000): Phosphorus Forms in Manure and Compost and Their Release during Simulated Rainfall. *J. Environ. Qual.* 29, 1462.

Sharpley, A.N., Chapra, S.C., Wedepohl, R., Sims, J.T., Daniel, T.C., Reddy, K.R. (1994): Managing Agricultural Phosphorus for Protection of Surface Waters: Issues and Options. *J. Environ. Qual.* 23, 437.

Shen, J., Yuan, L., Zhang, J., Li, H., Bai, Z., Chen, X., Zhang, W., Zhang, F. (2011): Phosphorus dynamics: from soil to plant. *Plant Physiol.* 156, 997–1005.

Shenoy, V. V., Kalagudi, G.M. (2005): Enhancing plant phosphorus use efficiency for sustainable cropping. *Biotechnol. Adv.* 23, 501–513.

Siddique, M.T., Robinson, J.S. (2003): Phosphorus Sorption and Availability in Soils Amended with Animal Manures and Sewage Sludge. *J. Environ. Qual.* 32, 1114.

Simpson, M., McLenaghen, R.D., Chirino-Valle, I., Condron, L.M. (2012): Effects of long-term grassland management on the chemical nature and bioavailability of soil phosphorus. *Biol. Fertil. Soils* 48, 607–611.

Sims, J.T., Simard, R.R., Joern, B.C. (1998): Phosphorus Loss in Agricultural Drainage: Historical Perspective and Current Research. *J. Environ. Qual.* 27, 277.

Smith, K.A., Van Dijk, T.A. (1987): Utilisation of phosphorus and potassium from animal manures on grassland and forage crops, in: Animal Manure on Grassland and Fodder Crops. Fertilizer or Waste? Springer Netherlands, Dordrecht, pp. 87–102.

Smith, S.R. (2009): A critical review of the bioavailability and impacts of heavy metals in municipal solid waste composts compared to sewage sludge. *Environ. Int.* 35, 142–156.

Sonneveld, C., Straver, N. (1994): Nutrient solutions for vegetables and flowers grown in water or substrates, Tenth. ed.

Stewart, J.W.B., Tiessen, H. (1987): Dynamics of soil organic phosphorus. *Biogeochemistry* 4, 41–60.

Suh, Y.J., Rousseaux, P. (2002): An LCA of alternative wastewater sludge treatment scenarios. *Resour. Conserv. Recycl.* 35, 191–200.

Syers, D.J., Johnston, A. (2008): Efficiency of soil and fertilizer phosphorus use Reconciling changing concepts of soil phosphorus behaviour with agronomic information. *FAO Fertil. Plant Nutr. Bull.*

Tabatabai, M.A., Bremner, J.M. (1969): Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biol. Biochem.* 1, 301–307.

Takahashi, M., Kato, S., Shima, H., Sarai, E., Ichioka, T., Hatyakawa, S., Miyajiri, H. (2001): Technology for recovering phosphorus from incinerated wastewater treatment sludge, in: Chemosphere. pp. 23–29.

Takahashi, S. (2013): Phosphorus characterization of manure composts and combined organic fertilizers by a sequential-fractionation method. *J. Plant Nutr. Soil Sci.* 176, 494–496.

Talboys, P.J., Heppell, J., Roose, T., Healey, J.R., Jones, D.L., Withers, P.J.A. (2016): Struvite: a slow-release fertiliser for sustainable phosphorus management? *Plant Soil* 401, 109–123.

Tang, X., Li, J., Ma, Y., Hao, X., Li, X. (2008): Phosphorus efficiency in long-term (15

years) wheat–maize cropping systems with various soil and climate conditions. *F. Crop. Res.* 108, 231–237.

Tarayre, C., De Clercq, L., Charlier, R., Michels, E., Meers, E., Camargo-Valero, M., Delvigne, F. (2016): New perspectives for the design of sustainable bioprocesses for phosphorus recovery from waste. *Bioresour. Technol.*

Tejada, M., García-Martínez, A.M., Rodríguez-Morgado, B., Carballo, M., García-Antras, D., Aragón, C., Parrado, J. (2013): Obtaining biostimulant products for land application from the sewage sludge of small populations. *Ecol. Eng.* 50, 31–36.

Toor, G.S., Peak, J.D., Sims, J.T. (2005): Phosphorus Speciation in Broiler Litter and Turkey Manure Produced from Modified Diets. *J. Environ. Qual.* 34, 687.

Torri, S.I., Correa, R.S., Renella, G. (2017): Biosolid Application to Agricultural Land—a Contribution to Global Phosphorus Recycle: A Review. *Pedosphere* 27, 1–16.

Turner, B.L., Cade-Menun, B.J., Condron, L.M., Newman, S. (2005): Extraction of soil organic phosphorus. *Talanta* 66, 294–306.

Turner, B.L., McKelvie, I.D., Haygarth, P.M. (2002): Characterisation of water-extractable soil organic phosphorus by phosphatase hydrolysis. *Soil Biol. Biochem.* 34, 27–35.

Uhde-Stone, C., Liu, J., Zinn, K.E., Allan, D.L., Vance, C.P. (2005): Transgenic proteoid roots of white lupin: a vehicle for characterizing and silencing root genes involved in adaptation to P stress. *Plant J.* 44, 840–853.

Uysal, A., Dilsad Yilmazel, Y., Demirer, G.N. (2010): The determination of fertilizer quality of the formed struvite from effluent of a sewage sludge anaerobic digester. *J. Hazard. Mater.* 181, 248–254.

Vance, C.P. (2001): Symbiotic Nitrogen Fixation and Phosphorus Acquisition. Plant Nutrition in a World of Declining Renewable Resources. *Plant Physiol.* 127.

Vance, C.P., Uhde-Stone, C., Allan, D.L. (2003): Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. *New Phytol.* 157, 423–447.

Veneklaas, E.J., Lambers, H., Bragg, J., Finnegan, P.M., Lovelock, C.E., Plaxton, W.C., Price, C.A., Scheible, W.-R., Shane, M.W., White, P.J., Raven, J.A. (2012): Opportunities for improving phosphorus-use efficiency in crop plants. *New Phytol.* 195, 306–320.

Walan, P., Davidsson, S., Johansson, S., Höök, M. (2014): Phosphate rock production and depletion: Regional disaggregated modeling and global implications. *Resour. Conserv. Recycl.* 93, 178–187.

Wang, Z., Straub, D., Yang, H., Kania, A., Shen, J., Ludewig, U., Neumann, G. (2014): The regulatory network of cluster-root function and development in phosphate-deficient white lupin (*Lupinus albus*) identified by transcriptome sequencing. *Physiol. Plant.* 151, 323–338.

Wellinger, A., Murphy, J., Baxter, D. (2013): The biogas handbook : science, production and applications.

Wheeler, B.D., Al-Farraj, M.M., Cook, R.E.D. (1985): Iron toxicity to plants in base-rich wetlands: comparative effects on the distribution and growth of Epilobium hirsutum l. and Juncus subnodulosus Schrank. *New Phytol.* 100, 653–669.

White, P.J., Brown, P.H. (2010): Plant nutrition for sustainable development and global health. *Ann. Bot.* 105, 1073–1080.

Wollmann, I., Gauro, A., Müller, T., Möller, K. (2017): Phosphorus bioavailability of sewage sludge-based recycled fertilizers. *J. Plant Nutr. Soil Sci.*

Xu, H., He, P., Gu, W., Wang, G., Shao, L. (2012): Recovery of phosphorus as struvite from sewage sludge ash. *J. Environ. Sci. (China)* 24, 1533–1538.

Ye, Y., Ngo, H.H., Guo, W., Liu, Y., Li, J., Liu, Y., Zhang, X., Jia, H., Ngo, H.H. (2016): Insight into chemical phosphate recovery from municipal wastewater. *Sci. Total Environ.* 576, 159–171.

Zang, Y., Li, Y., Wang, C., Zhang, W., Xiong, W. (2015): Towards more accurate life cycle assessment of biological wastewater treatment plants: A review. *J. Clean. Prod.*

Zhu, J., Li, M., Whelan, M., Gan, J. (2017): Phosphorus activators contribute to legacy phosphorus availability in agricultural soils: A review. *Sci. Total Environ.* 612, 522–537.

Chapter 6

Phosphorus balance: the fate of P from sewage sludge to plants, through acid and enzymatic hydrolysates fertilization.

Abstract

The P solubility and the P use efficiency of different organic waste has proved to be very low. Here the assessment of the P use efficiency of the hydrolysates as compared to the same amount of sludge used for the hydrolysis is described. Hydrolysates of Fe-rich anaerobic sludge and Al-rich aerobic sludge with citric acid and the combination of citric acid and acid phosphatase were compared to the original raw sludge and to the exhausted sludge after the hydrolysis. In the soil trial, the hydrolysates were tested in term of soil P availability, phosphatase activity, soil pH and microbial biomass, while in the pot trial cutting lettuce plants were monitored for growth and P and metals uptake. The results were positive for hydrolysates, which had promoted higher biomass growth maintaining similar P concentration as compared to the chemical P reference, but mostly an higher P uptake and a lower toxic element uptake as compared to the deriving raw sludge and the exhausted sludge. The hydrolysates with their high P use efficiency are the best choice for fast growing and high demanding crops as lettuce, while raw sludge showed a potential for the long-term fertilization, due to their slow release of P and other nutrients. The exhausted sludge with their low P concentration and high metal concentration, behave as a sink of P, showing a lower P uptake then not amended plants.

Introduction

The intensive cultivation has led to an over-fertilization of the arable lands, particularly of phosphate, mostly due to the difficult P uptake from plants (*Hooda et al.*, 2001). The need to reduce P fertilization and consequently to increase P use efficiency by plants is thus, not only a request driven by the mineral P scarcity, but also by the need to limit the soil P accumulation (*Frossard et al.*, 2000). The high amount of P in high-fertile soil caused a P leaching and a consequent water basins pollution, which can cause eutrophication (*Sims et al.*, 1998). Besides genetic and physiological advances (*Shenoy and Kalagudi*, 2005; *Veneklaas et al.*, 2012) and mycorrhizal symbiosis studies (*Bolan*, 1991), P use efficiency can be improved also using P fertilizers more efficiently (*Frossard et al.*, 2000).

Organic waste products, which have been extensively studied as a source of P for plant nutrition, had caused high accumulation in the countries where livestock farms are widespread (*Breeuwsma et al.*, 1995). According to the previous results of this study, the P plant availability of bio-waste products (chap. 2) and sewage sludge (chap. 3) is often scarce. The P solubility was improved using an acid and enzymatic hydrolysis of sewage sludge (chap. 3) which was tested as a liquid fertilizer for lettuce fertilization (chap. 4 and 5). However, the increase of P solubility (and consequently the P use efficiency) in the different material going from sewage sludge to hydrolysates was not tested. Previously, in the fertilization trial, we have used a fixed dose of P, but to compare the raw material to the new fertilizers the same dose of products should be compared.

The conventional method of establish the fate of P in the soil-plant cycle is the use of isotopes (*Frossard et al.*, 1996). Due to the radioactivity of the P isotopes, the test is not easy to conduct. Therefore, a pot test with cutting lettuce and a soil test was used to predict the fate of P in the different products, all derived from the same raw sludge, at different time after the addition of the fertilizers.

Therefore, the aim of this experiment was to assess the P balance starting from the first raw material, the sludge, and arriving to the plants passing through the hydrolysates. In order to reach the aim, we have fixed the amount of raw sludge instead of the dose of P. Thus, the amount of liquid hydrolysates, which has extracted P from that amount of sludge, and finally the same amount of exhausted sludge (after the hydrolysis) were added to the soil. In this way, the solubility of the different products was measured and the fate of the P was described in a plant-soil system.

Material and Methods

Sludge and hydrolysates treatments

The amount of hydrolysates was definite differently: in order to add 30 mg P kg^{-1} of soil it should be add 1.5 g of dry raw sludge (with a P concentration of 20 mg g^{-1}). The hydrolysis were done with a ratio of 1:20, so we have used 30 ml of solution for hydrolysing 1.5 g of sludge, thus the amount of hydrolysates solution to add were defined as 30 ml kg^{-1} of soil. Furthermore it was used also the sludge exhausted after the hydrolysis at the same amount of 1.5 g kg^{-1}.

The products were the anaerobic and aerobic sludge (ANA and AER), the hydrolysates with citric acid (ANA Cit and AER Cit) and with the combination of citric acid and acid phosphatase (ANA Cit+AcPHo and AER Cit+AcPHO) and the residual exhausted sludge after the hydrolysis (Res ANA Cit, Res ANA Cit+AcPHO, Res AER Cit, Res AER Cit+AcPHO).

The P concentration and the P added in the soil for the different products is reported in Table 6.1.

To sum up the treatments were:

- **CTRL**: control, soil without the addition of P sources;
- **ANA**: soil with the addition of ANA dried raw sludge at the dose of 1.5 g kg^{-1} to add 30 mg P kg^{-1} of soil;
- **AER**: soil with the addition of AER dried raw sludge at the dose of 1.5 g kg^{-1} to add 30 mg P kg^{-1} of soil;
- **ANA Cit**: soil with the addition of the hydrolysates of ANA with citric acid at the volume of 30 ml kg^{-1}, which was the volume to hydrolyse 1.5 g of sludge;
- **ANA Cit+AcPHO**: soil with the addition of the hydrolysates of ANA with citric acid and acid phosphatase at the volume of 30 ml kg^{-1}, which was the volume to hydrolyse 1.5 g of sludge;
- **AER Cit**: soil with the addition of the hydrolysates of AER with citric acid at the volume of 30 ml kg^{-1}, which was the volume to hydrolyse 1.5 g of sludge;
- **AER Cit+AcPHO**: soil with the addition of the hydrolysates of AER with citric acid and acid phosphatase at the volume of 30 ml kg^{-1}, which was the volume to hydrolyse 1.5 g of sludge;
- **Res ANA Cit:** soil with the addition of ANA residual sludge of the hydrolysis with citric acid at the dose of 1.5 g kg^{-1};
- **Res ANA Cit+AcPHO:** soil with the addition of ANA residual sludge of the hydrolysis with citric acid and acid phosphatase at the dose of 1.5 g kg^{-1};

- **Res AER Cit:** soil with the addition of AER residual sludge of the hydrolysis with citric acid at the dose of 1.5 g kg^{-1};
- **Res AER Cit+AcPHO:** soil with the addition of AER residual sludge of the hydrolysis with citric acid and acid phosphatase at the dose of 1.5 g kg^{-1};
- **CHEM**: chemical reference, soil with the addition of KH_2PO_4 in the proportion to add 30 mg P kg^{-1} of soil.

Table 6.1: P concentration and P added in soil for different products

	P concentration		Sludge (g kg^{-1})	Hydrolysates (ml kg^{-1})	P added (mg kg^{-1})
ANA	mg P kg^{-1}	20039.8	1.5		30.0
AER	mg P kg^{-1}	20322.7	1.5		30.0
ANA Cit	mg P l^{-1}	871.0		30	26.1
ANA Cit+AcPHO	mg P l^{-1}	860.7		30	25.8
AER Cit	mg P l^{-1}	592.4		30	17.8
AER Cit+AcPHO	mg P l^{-1}	715.9		30	21.5
Res ANA Cit	mg P kg^{-1}	2579.6	1.5		3.9
Res ANA Cit+AcPHO	mg P kg^{-1}	2785.4	1.5		4.2
Res AER Cit	mg P kg^{-1}	8152.2	1.5		12.2
Res AER Cit+AcPHO	mg P kg^{-1}	5682.9	1.5		8.5

Soil incubation test and soil analysis

Soil incubation test was performed with the procedure previously described in chap. 5, with the same incubation conditions. The soil was amended with the raw sludge samples and the residual sludge samples before dividing the replicates in 3 boxes, while the liquid hydrolysates and the chemical reference were distributed after the soil distribution.

pH, available P, phosphatase activity and microbial biomass C was performed as described in chap. 5, at 0, 20, 40, 80 and 120 days after treatment (DAT).

Plant test

Plant growth

Seed of cutting lettuce (or baby leaf lettuce), variety *Foglia di quercia* (Blumen Group S.P.A, Bologna, Italy), were sown in a dose of 1 g pot^{-1} in pots with 2 L volume and 14 cm Ø. The bottom of the pots were filled with expanded clay. Above the expanded clay, 500 mg of soil amended with the abovementioned products at the same dose of the incubation test, filled the pots. A thin layer of sand covered the seed to avoid water loss and to enhance germination. 4 pots per treatments in a complete randomized

design was used. The plants were grown in a growth chamber with 25/18°C of day/night temperature and a photoperiod of 16 h. The light was ensure with Master Tld 58 W-840 tubes (Philips, Amsterdam, The Netherlands). The dried sludge was mixed by hand to the soil before potting, while the hydrolysates and the chemical reference were uniformly distributed after sowing. Urea at the dose of 100 mg N kg^{-1} and KNO_3 at the dose of 50 mg kg^{-1} were distributed in every pots at the sowing, while every 40 days a solution of KNO_3 at the dose of 50 mg N kg^{-1} was applied in order to avoid N and K limiting environment.

Leaf greenness

At 40, 80 and 120 DAS, the plants were measured for leaf greenness with N-tester (Yara International ASA, Oslo, Norway).

Plant growth

At 40 days after sowing (DAS) the first leaves cut was done, cutting the plants at 1 cm above the soil. The cutting was repeated at 40, 80 and 120 DAS. At 120 DAS, roots were gently washed from soil and dried. Leaves and roots were dried in an oven at 65°C for 72°C after each cut, and weighted.

Plant P and metal concentration

At 40 and 80 DAS dried leaves and at 120 DAS dried leaves and roots were finely grinded. Then, 0.25 g of sample was digested with 6 ml of HNO_3 and 2 ml of H_2O_2 in a microwave for 40 minutes. The resulting solutions were diluted and analysed with ARCOS ICP-OES (Spectro Analytical Instruments, Kleve, Germany).

Plant P uptake, apparent recovery fraction and P use efficiency

Apparent recovery fraction (ARF) was calculated as previously described in chap. 2 with the different P added as reported in Table 6.1. In order to overcome the problems of different P addition and high root development of not amended treatment, plant P uptake was also calculated as the product of plant dry weight and plant P concentration in mg pot^{-1}. The P uptake was, then, divided by the P added in order to have the P use efficiency calculated with the balance method (*Syers and Johnston*, 2008).

The use efficiency of the hydrolysis vs the raw sludge

In order to compare the P use efficiency of the raw sludge and the use efficiency of the hydrolysis the P accumulation of hydrolysates treated plants were summed to the P accumulation of the plants treated with the equivalent residual sludge. These values were then divided by the P contained initially in the raw sludge

Statistical analysis

Statistical analysis was performed with IBM SPSS (IBM, Armonk, North Castle, New York, USA) on plants and soil data. Normality of population was assessed with Kolmogorov-Smirnov test and equality of variance with Levene test. Two-way ANOVA was performed in traits with different sampling time on treatments and time as fixed factors and the interaction between factors. Later, One-way ANOVA and Tukey test with $P \leq 0.05$ were performed within time and in one-factor traits.

Results

Soil pH

Soil pH (Fig. 6.1) significantly decreased after the addition of sewage sludge hydrolysates to the soil at 0 DAT (-5.3% as an average of ANA Cit, AER Cit and AER Cit+AcPHO as compared to control), while raw sludge treatments, residual sludge treatments, chemical treatment and ANA Cit+AcPHO did not decrease the soil pH. AER sludge showed the highest pH (+0.36% as compared to control).

After 40 DAT, ANA hydrolysates and AER Cit+AcPHO showed an increased pH, while AER Cit and the residual sludge treatments resulted lower in pH as compared to the other treatment (-2.52% in AER Cit, -3.32% in Res ANA Cit, -2.91% in Res ANA Cit+AcPHO, -2.78% in Res AER Cit and -1.59% in Res AER Cit+AcPHO, as compared to control). ANA Cit+AcPHO showed the highest pH (+0.81% as compared to control).

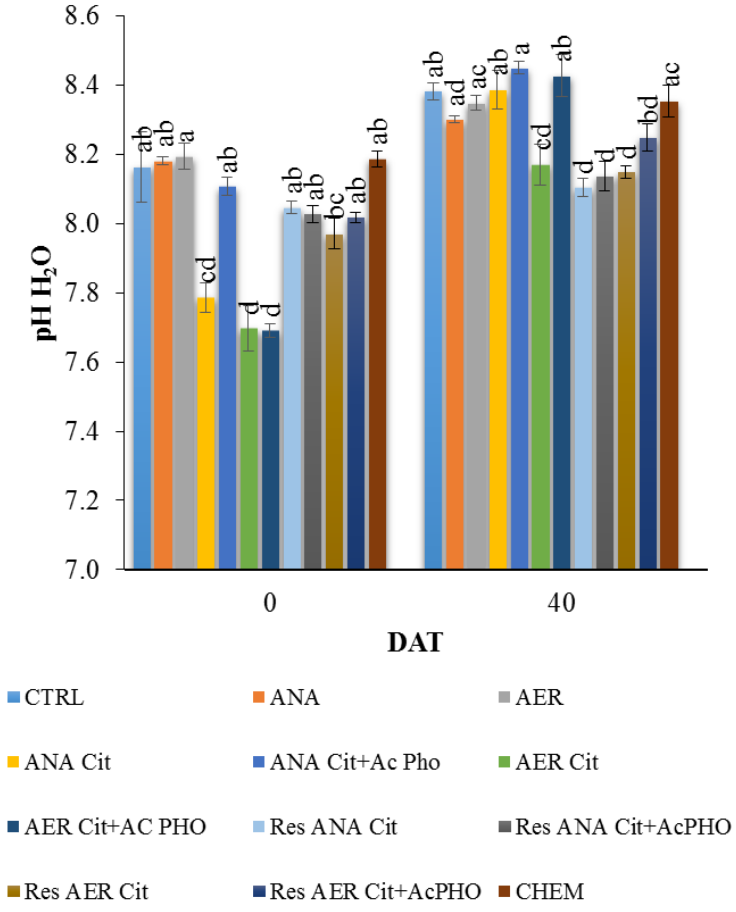


Figure 6.1: pH at 0 and 40 DAT of the different treatments on soil. Error bars represent standard error (n=3) and different letters represent different group with Tukey test (P≤0.05). In two-way ANOVA treatments, time and the interaction (treatment*time) were highly significant (P≤0.001).

Soil P availability

Available P (P Olsen) showed differences between treatments in every sampling, from 0 to 120 DAT (Fig. 6.2). The chemical reference showed always high P available (+180% at 0 DAT. +146% at 20 DAT, +88% at 40 DAT, +76% at 80 DAT and +57% at 120 DAT as compared to control). At 0 DAT, hydrolysates treatments showed the second highest P available, below Chem (+71% as an average of the hydrolysates as compared to control). At 20 DAT AER Cit+AcPHO and Res AER Cit showed a P Olsen similar to Chem (+135% for AER Cit+AcPHO and +137% in Res AER Cit, as compared to control). At 40 DAT the highest available P resulted in ANA Cit+AcPHO (+127% as compared to control), followed by Chem and AER Cit+AcPHO (+64% as compared to control). At 80 DAT Chem showed the highest P available, while all the other treatments resulted similar to control. At 120 DAT, however, the highest P Olsen was found in AER Cit+AcPHO (+60% as compared to control) and Chem.

Soil phosphatase activity

Soil phosphatase activity (Fig. 6.3) showed differences at 0, 20 and 120 DAT. The trend was an higher activity at 0 DAT of the hydrolysates and residual sludge (+62% as an average of the hydrolysates and +44% as an average of the residual sludge, as compared to control), with a consistent decrease of the same treatments after 20 DAT (-47% as an average of the hydrolysates and -46% as an average of the residual sludge, as compared to control). Chem remained, instead, constantly lower (-13% at 0 DAT and -27% at 20 DAT as compared to control). At 40 DAT, the differences thinned and at 80 DAT all the treatments resulted similar, with a control showing a very low phosphatase activity (even though not statistically different). At 120 DAT, the hydrolysates returned higher with ANA Cit showing the highest activity (+138% as compared to control).

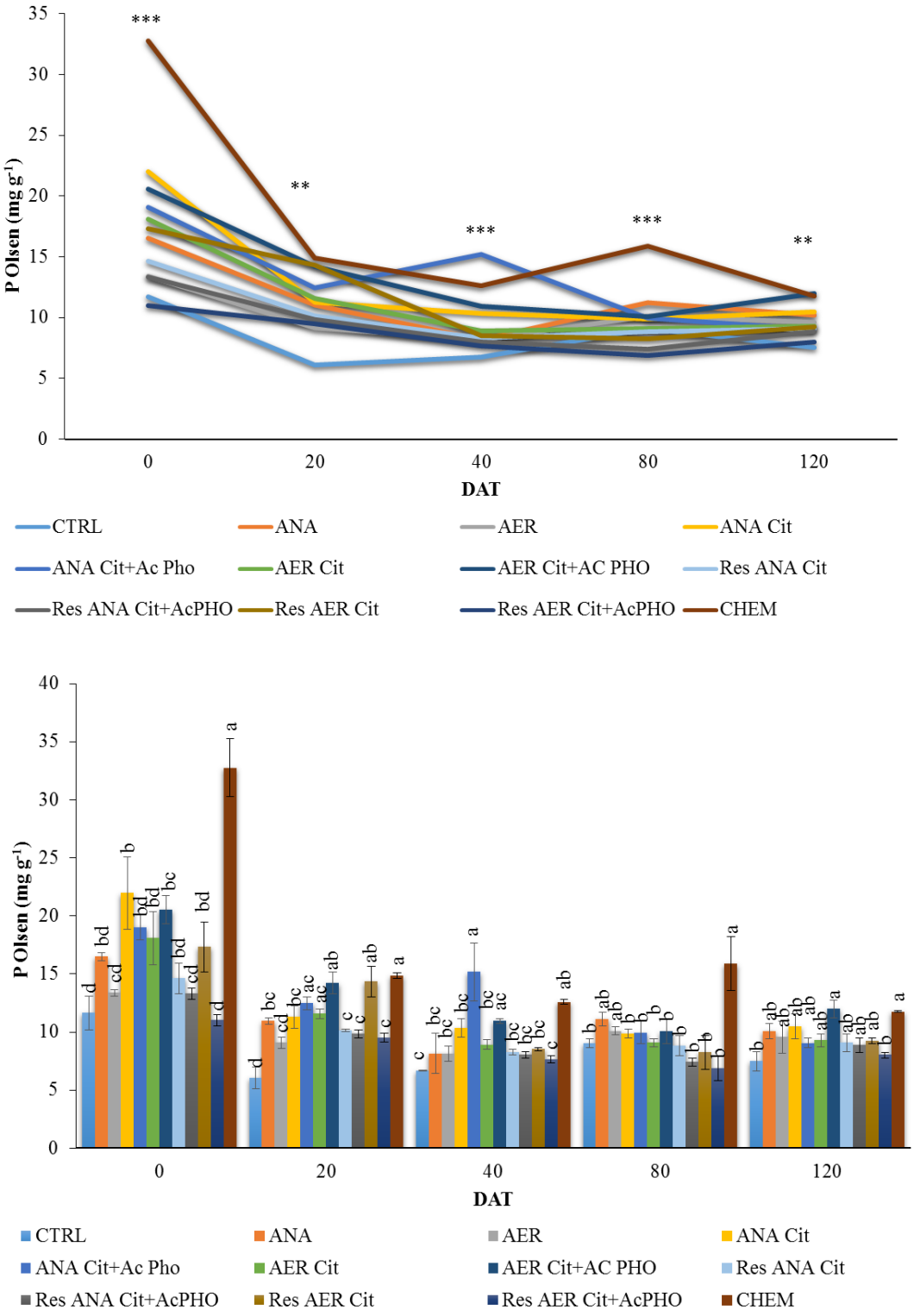


Figure 6.2: Available P (P Olsen) of the different treatments on soil at 0, 20, 40, 80 and 120 DAT. In the above picture One-way ANOVA within time is shown as ***= P≤0.001; **= P≤0.01, *= P≤0.05 and ns= P>0.05. In the bottom picture error bars represent standard error (n=3) and different letters represent different group with Tukey test (P≤0.05). In two-way ANOVA treatments, time and the interaction (treatment*time) were highly significant (P≤0.001).

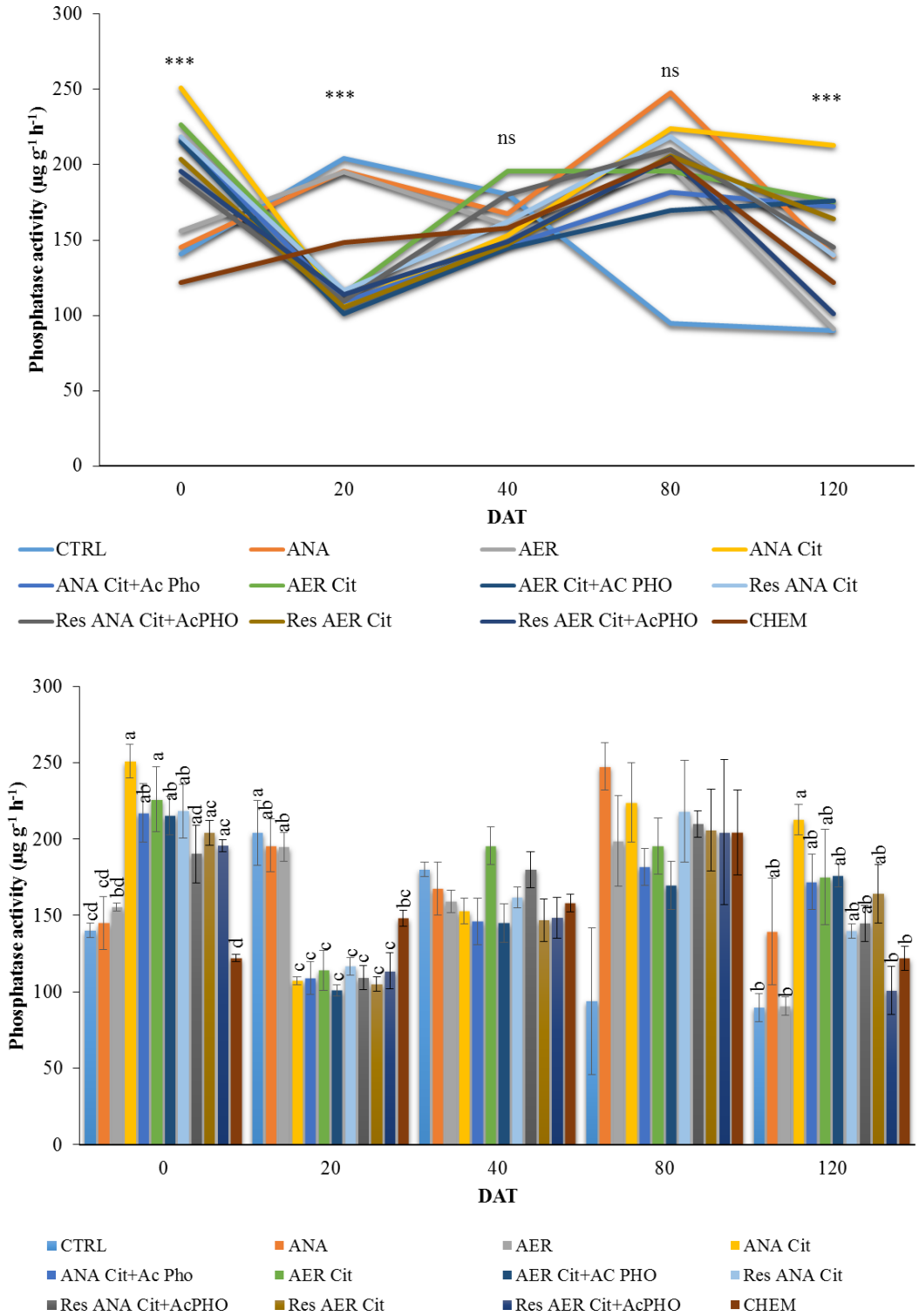


Figure 6.3: Soil phosphatase activity of the different treatments on soil at 0, 20, 40, 80 and 120 DAT. In the above picture One-way ANOVA within time is shown as ***= P≤0.001; **= P≤0.01, *= P≤0.05 and ns= P>0.05. In the bottom picture error bars represent standard error (n=3) and different letters represent different group with Tukey test (P≤0.05). In two-way ANOVA treatments (P≤0.01), time and the interaction (treatment*time) were significant (P≤0.001).

Soil microbial biomass

Microbial biomass C (C_{mic}) (Fig. 6.4) resulted different between time ($P \leq 0.001$), treatments ($P \leq 0.05$) and interaction treatments *time ($P \leq 0.001$). The C_{mic} generally decrease with time, with a considerable decrease at 80 DAT in all the treatments. Within different sampling time the only significant differences was found between treatments at 40 and 80 DAT. The hydrolysates, even though had showed high microbial C at 0 and 20 DAT, showed a lower C_{mic} at 40 DAT as compared to control and the raw sludge (-14% as an average of ANA Cit+AcPHo, AER Cit and AER Cit+AcPHO, as compared to control). Only ANA Cit and Chem showed similar microbial C, while the residual sludge also resulted lower (-25% average of the residual sludge as compared to control). At 80 DAT, the difference were not significant with the statistical results compromised from the very low value of Res AER Cit+AcPHO. However, the ANA hydrolysates resulted lower than the other treatments (-42% as an average of ANA Cit and ANA Cit+AcPHO, as compared to control). At 120 DAT, no significant differences were found.

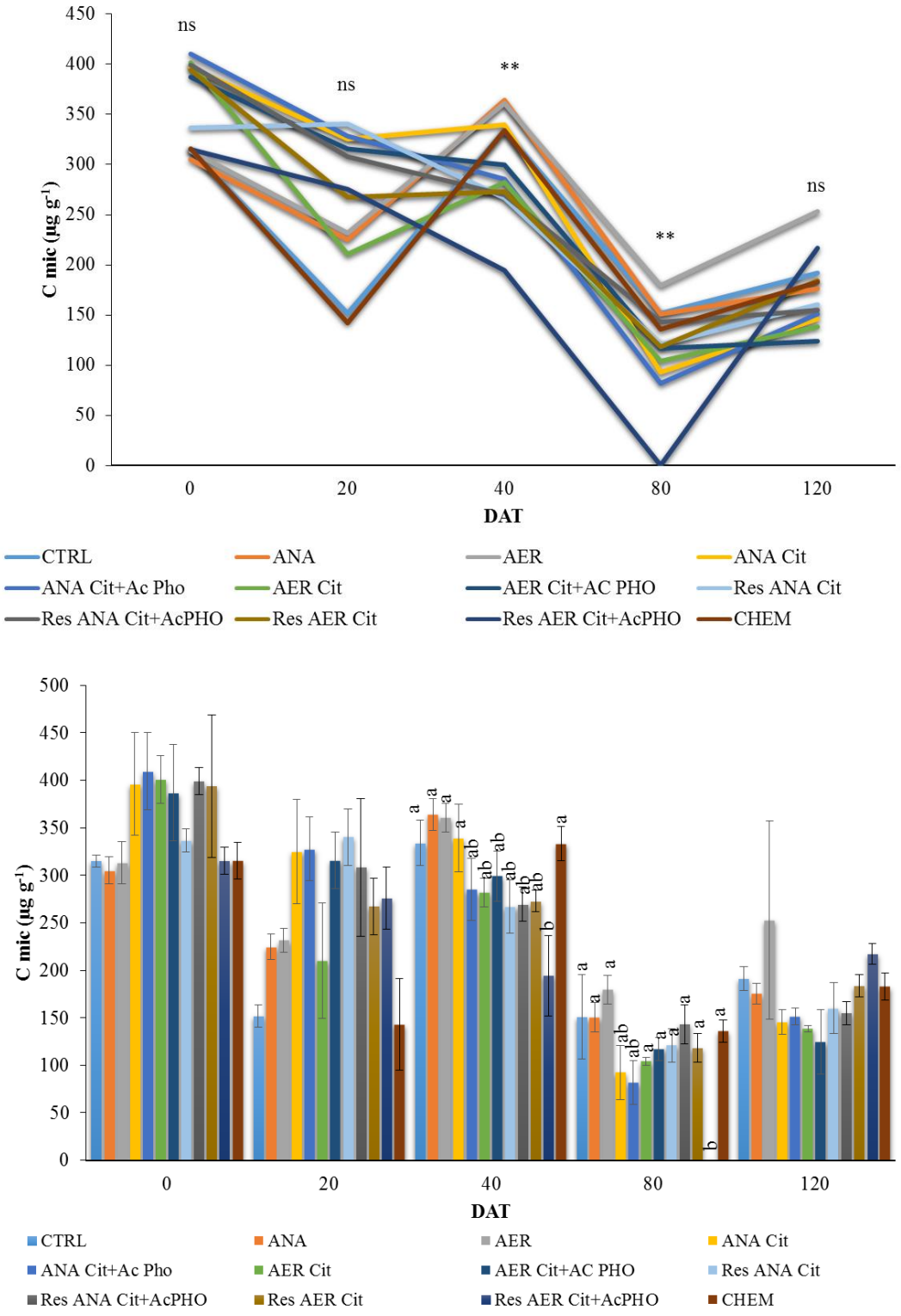


Figure 6.4: Soil microbial C of the different treatments on soil at 0, 20, 40, 80 and 120 DAT. In the above picture One-way ANOVA within time is shown as ***= P≤0.001; **= P≤0.01, *= P≤0.05 and ns= P>0.05. In the bottom picture error bars represent standard error (n=3) and different letters represent different group with Tukey test (P≤0.05). In two-way ANOVA treatments (P≤0.05), time and the interaction (treatment*time) were significant (P≤0.001)

Plant growth

Plant dry weight (Fig. 6.5) resulted significant with Two-way ANOVA for treatments, time ($P \leq 0.001$) and interaction ($P \leq 0.01$). Within sampling times, leaves dry weight resulted significantly different between treatments, while no differences were found in roots. At 40 DAS leaves dry weight resulted higher in AER Cit+AcPHO (+107% as compared to control), followed by ANA Cit+AcPHO (+92% as compared to control). Lower plant biomass resulted in residual sludge treatments, with the lowest value of Res ANA Cit (-42% as compared to control). At 80 DAS the highest leaf biomass was found in ANA Cit+AcPHO (+37% as compared to control), followed by AER Cit+AcPHO (+34% as compared to control). At 120 DAS, the highest leaf biomass was produced, again, by the plants treated with AER Cit+AcPHO (+85% as compared to control).

The cumulated plant biomass (Fig. 6.6) showed that the hydrolysates treatments were always higher than control and also than chemical reference. With AER Cit+AcPHO accumulated more biomass with a higher slope. The consequent total plant dry weight (Fig. 6.7) showed an obvious higher value for AER Cit+AcPHO (+31% vs control, +33% vs AER raw sludge and +57% vs its residual sludge), followed by ANA Cit (+17% vs control, +52% vs ANA sludge and +40% vs its residual sludge). Control, AER, ANA Cit+AcPHO, AER Cit, Res AER Cit and Chem resulted similar; while ANA, and the other residual sludge resulted lower than control (mostly due to the highest root biomass of control plants) and lower than chemical reference.

The shoot:root ratio (Fig. 6.8) showed no significant differences. However, it confirmed the very high roots development of the control plants and a very low roots biomass as compared to shoots for Res AER Cit+AcPHO.

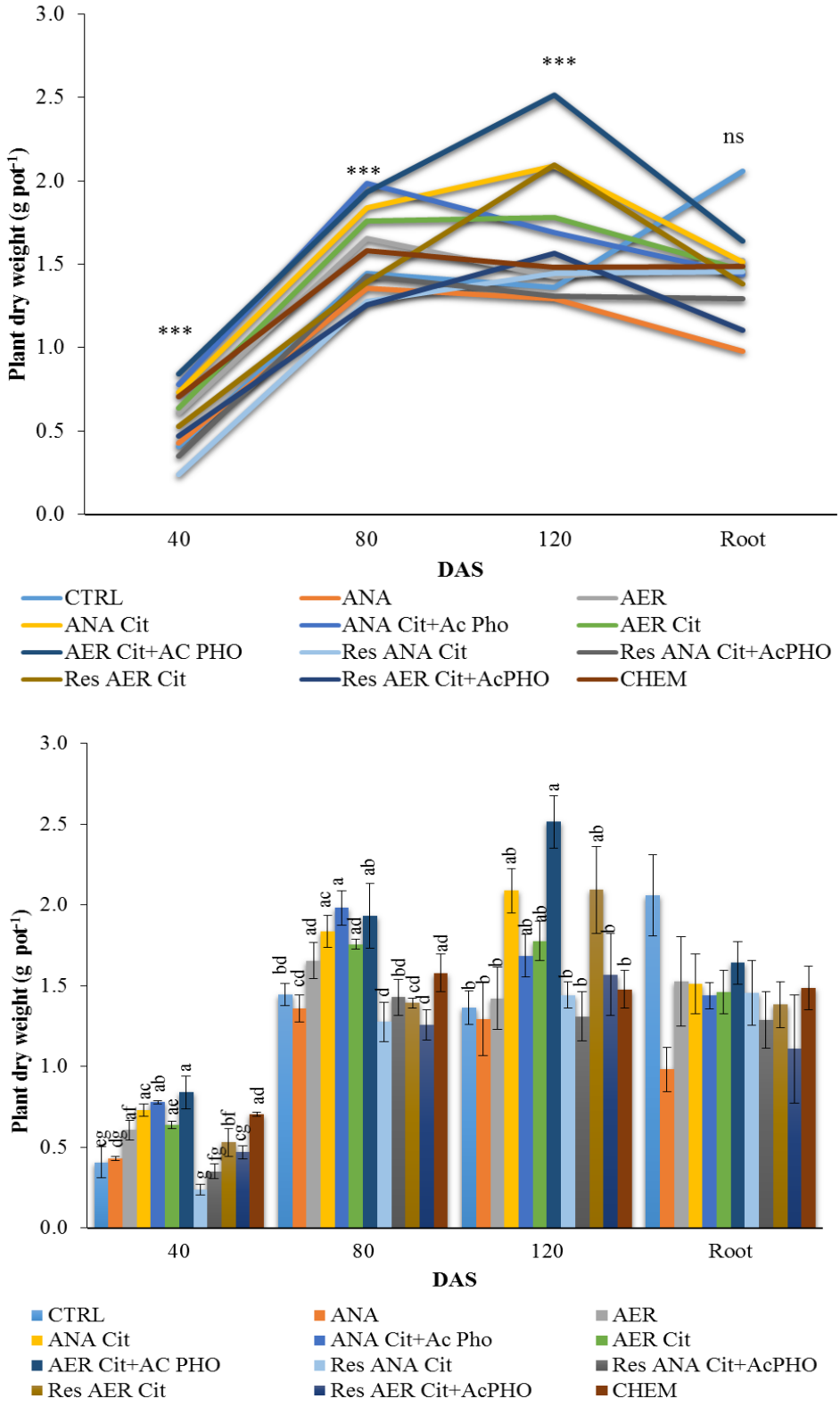


Figure 6.5: Leaves dry weight at 40, 80 and 120 DAS and roots at 120 DAS of the different treatments. In the above picture One-way ANOVA within time is shown as ***= P≤0.001; **= P≤0.01, *= P≤0.05 and ns= P>0.05. In the bottom picture error bars represent standard error (n=4) and different letters represent different group with Tukey test (P≤0.05). In Two-way ANOVA treatments, time and treatment*time were significant (P≤0.001).

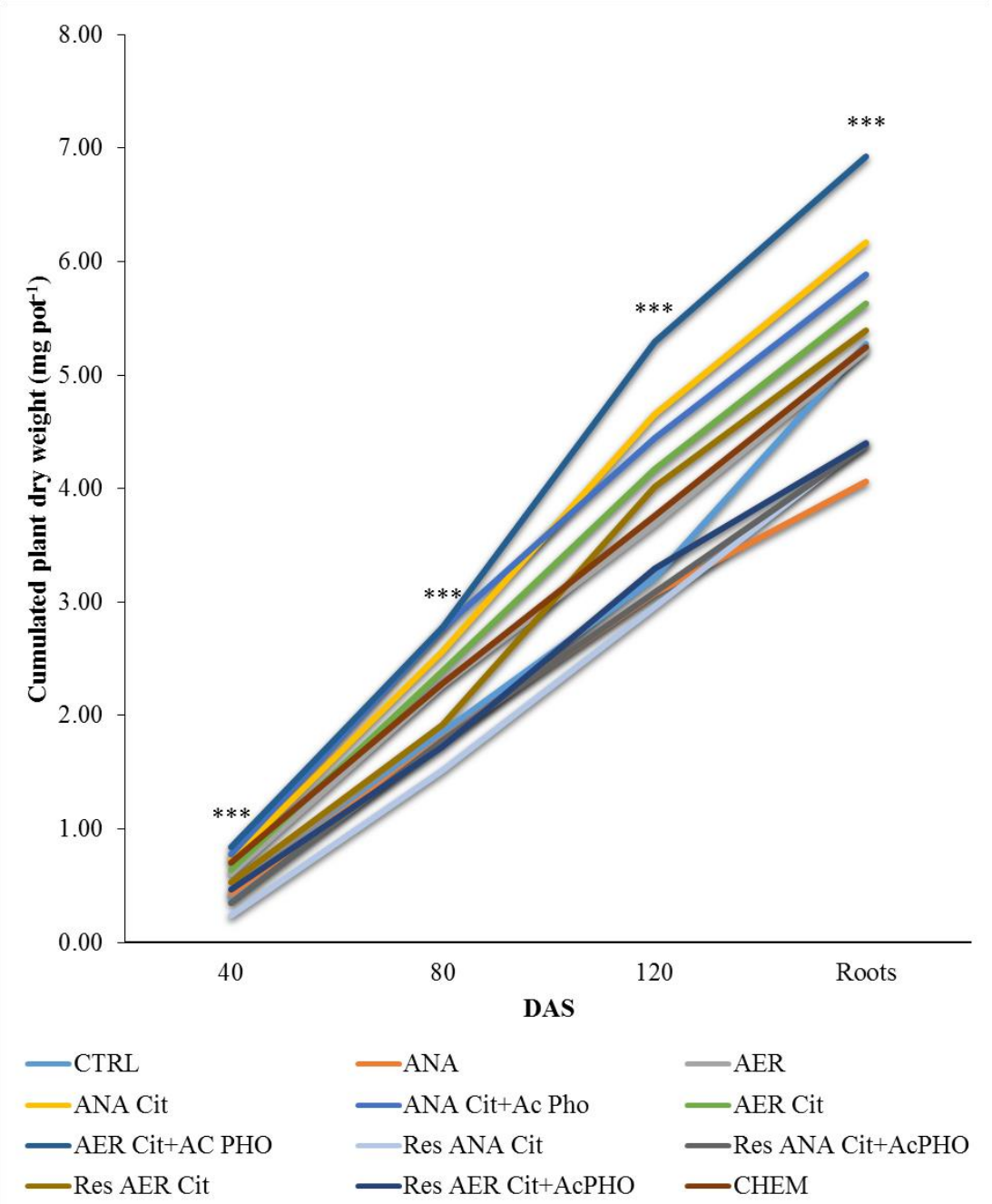


Figure 6.6: Cumulated leaves dry weight at 40, 80 and 120 DAS and roots at 120 DAS of the different treatments. One-way ANOVA within time is shown as ***= P≤0.001; **= P≤0.01, *= P≤0.05 and ns= P>0.05. In Two-way ANOVA treatments, time and treatment*time were significant (P≤0.001).

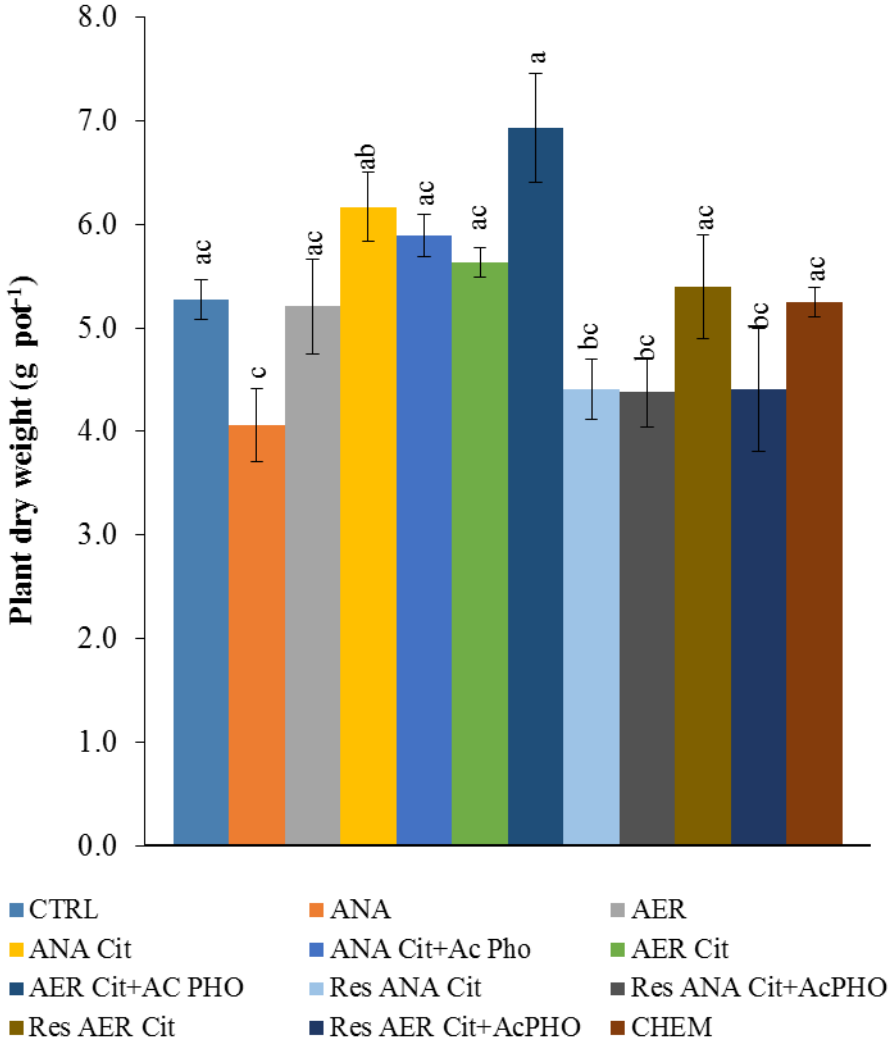


Figure 6.7: Total plant dry weight of the different treatments. Error bars represent standard error (n=4) and different letters represent different group with Tukey test (P≤0.05).

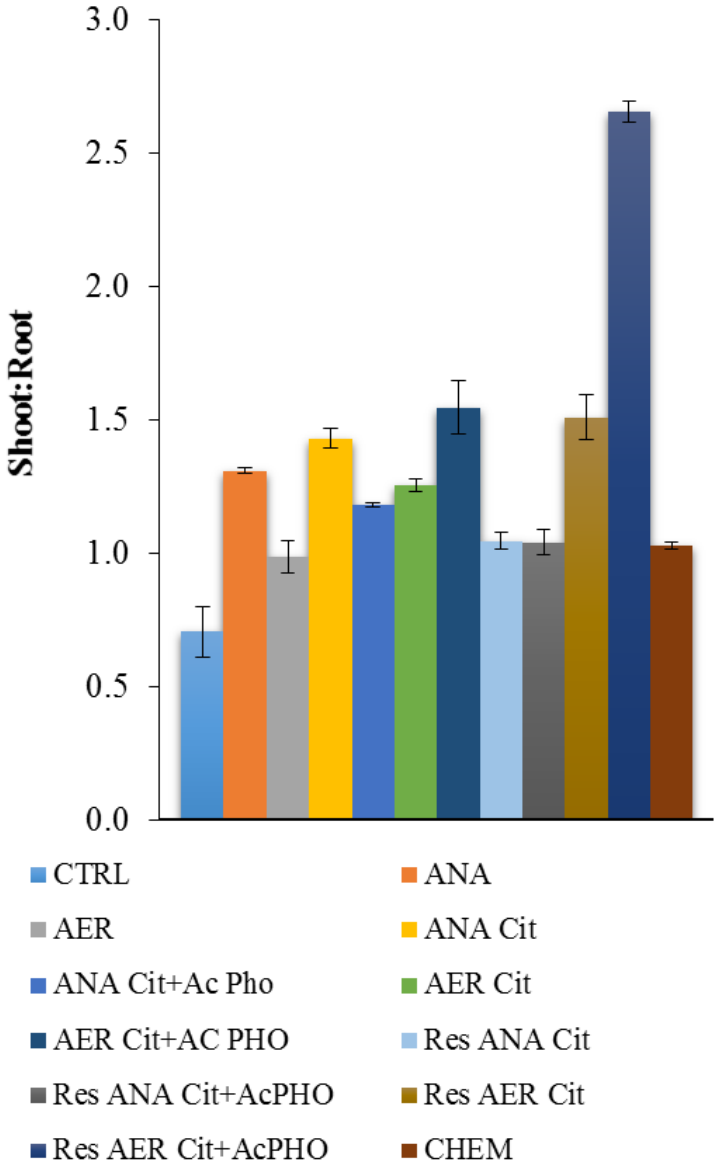


Figure 6.8: Shoot:root ratio of the different treatments. Error bars represent standard error (n=4). No significant differences were found with One-way ANOVA (P>0.05).

Leaf greenness

Significant differences in leaf greenness (Fig. 6.9) were found with two-way ANOVA only in the time factor (P≤0.01), but non-significant differences was detectable between treatments with two-way ANOVA and within the time sampling with One-way ANOVA. The general pattern of the leaf greenness was a slight increase during time, with a more marked increase in Res ANA Cit.

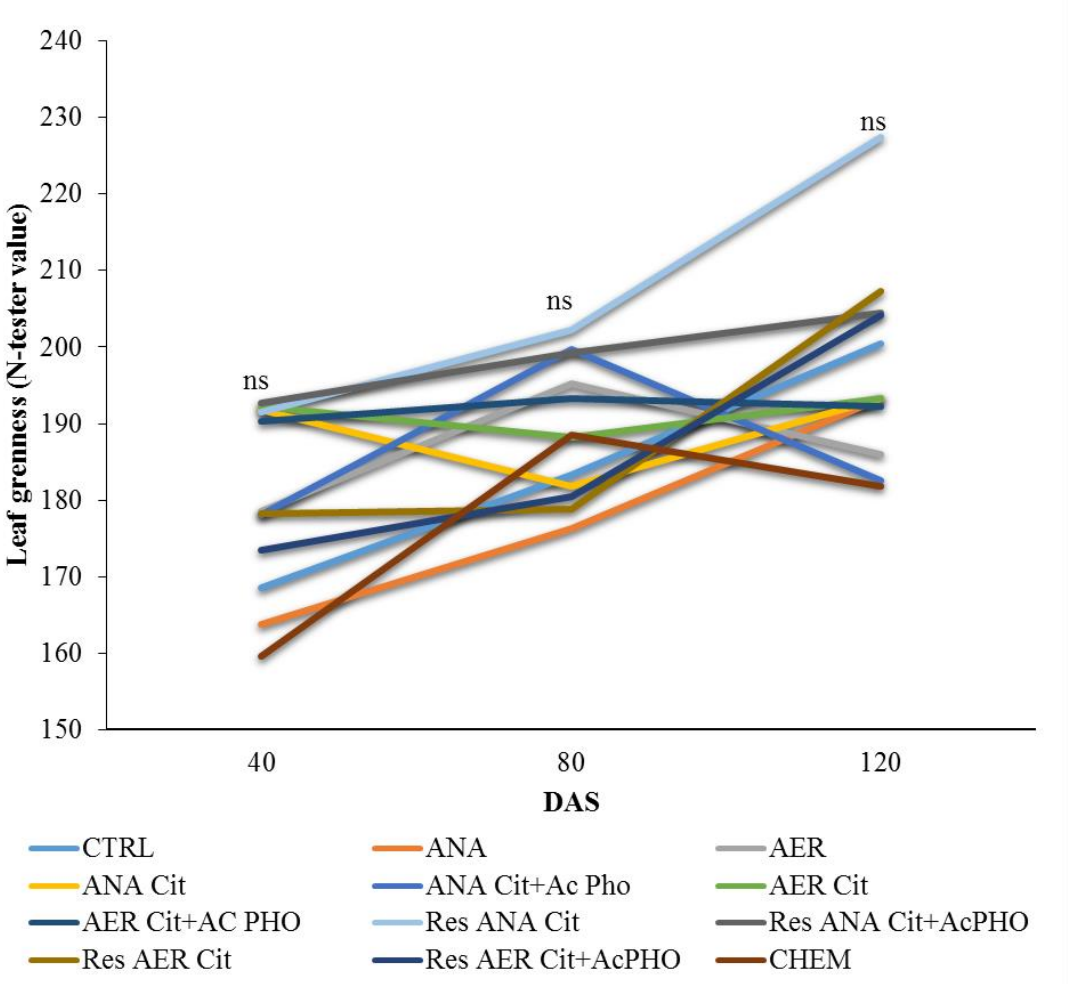


Figure 6.9: Leaf greenness (N-tester value) of plants ratio of the different treatments. No significant differences were found with One-way ANOVA (P>0.05). Two way ANOVA resulted significant only for the factor time (P≤0.01).

Plant metals concentration

Plant P concentration (Fig. 6.10) was significantly different in treatment and time ($P \leq 0.001$), but not in the interaction. Within the sampling time, the leaf P concentration was not different between treatments at 40, 80 and 120 DAS. While, roots P concentration showed significant differences, with Chem showing the highest P concentration (+29% as compared to control) and AER Cit resulted the lowest with Control. All the residual sludge treatments showed low P concentration in roots.

Ca concentration (Fig. 6.11) results were significant between treatments ($P \leq 0.05$), time and interaction ($P \leq 0.001$). The general patterns showed a high increase of Ca concentration in leaves at 120 DAS, where though the differences were not significant. At 40 DAS Res ANA Cit showed the highest Ca concentration (+10% as compared to control), followed by Chem, ANA and Res AER Cit. ANA Cit and AER Cit+AcPHO showed the lowed Ca concentration (-13% and -15%, respectively, as compared to control). At 80 DAS Control showed the lowest value, with all the other treatments being significantly higher. While Res AER Cit, followed by ANA and ANA Cit showed the highest value (+18%, +17% and +17%, respectively, as compared to control). On the other hand, control showed the highest Ca concentration in roots, followed by AER and Chem. Res AER Cit, instead, showed the lowest Ca concentration value (-34% as compared to control). ANA Cit+AcPHO, AER Cit and Res ANA Cit+AcPHO also showed very low value (-29%, -33% and -33%, respectively, as compared to control).

Mg concentration (Fig. 6.12) were significantly different between treatment ($P \leq 0.05$), time ($P \leq 0.001$), but not for the interaction. Within sampling time, the One-way ANOVA resulted significant at 80 DAS and Roots. The general outline, as the Ca pattern, showed an increase from 40 to 120 DAS. At 80 DAS, Chem and Res ANA Cit resulted the treatments with higher Mg concentration in leaves (+8% and +7% as compared to control). On the other hand, ANA Cit+AcPHO and AER Cit+AcPHO showed the lowest Mg leaf concentration (-8% and -7%, respectively, as compared to control). Regarding the roots Mg concentration, AER and ANA Cit showed the highest values (+5% and +2% as compared to control). However, control showed a higher value compared to all the other treatments, with Res ANA Cit+AcPHO being the lowest (-20% as compared to control). ANA Cit+AcPHO and AER Cit+AcPHO resulted similar to Chem.

Al leaf concentration (Fig. 6.13) were significantly different between treatments and time ($P \leq 0.001$), but not for the interaction. Within time, there were significant differences only at 40 DAS. The general outline showed a sharp decrease of the Al concentration from 40 to 80 DAS and a successive rapid increase. At 40 DAS Ctrl showed the highest concentration followed by Res ANA Cit, while Chem, AER, ANA

Cit, ANA Cit+AcPHO, Res AER Cit and Res AER Cit+AcPHO showed similar amount. Al roots concentration (Fig. 6.15) showed also higher concentration in Ctrl plants, followed by AER.

Fe leaves concentration (Fig. 6.14) was significantly different only between times ($P \leq 0.001$). Thus, within time, the differences between treatments were significant only at 40 DAS. Briefly, the general pattern of Fe retraced the Al concentration behaviour, with a rapid decrease at 80 DAS and the next exponential increase. As Fe, also Al was higher in Ctrl and Res ANA Cit, while lower in ANA Cit, ANA Cit+AcPHO, Res AER Cit and Chem (-56%, -32%, -59% and -21%, respectively, as compared to control). Roots Fe concentration (Fig. 6.15), also had similar results to Al concentration, with Control plants showing the highest amount.

In Table 6.2 and 6.3 metal concentration of leaves and roots are reported. Ag, As, Be, Co, Mo, Sb, Se and Tl were found under detection in every sampling limits in leaves. Ag, Be, Se, Tl and Sn was found under detection limits in roots. K was found under detection limits at 40 and 80 DAS in all the treatments, while Sn was found under detection limits at 120 DAS. In two-way ANOVA on leaves samples, time was significant in all the elements, with the exception of V, while the treatment was significant in Ca, K, Na, P, B, Cd, Cu, Li, Mn, Pb, Sn, Sr and Zn. The interaction, however, was significant only in Ca, Na, Si, B, Ba, Cd, Cu, Mn, Ni, Pb, Sn and Sr.

K was found higher in the sludge ANA and AER (+270% and +310% as compared to control) in leaves at 120 DAS, while in roots ANA and Ctrl showed the highest K concentrations.

Na was higher in ANA Cit+Ac PHO and AER Cit+AcPHO at 40 DAS (+49% and +51% as compared to control) and at 120 DAS (+30% and +30% as compared to control), while at 80 DAS the highest Na concentration was found in Ctrl and ANA Cit.

S had significant differences only at 80 DAS when the highest values was found in Res ANA Cit and Res ANA Cit+AcPHO (+8% and +6% as compared to control) and the lowest in AER Cit+AcPHO (-12% as compared to control).

B had significant differences at 80 DAS, where the lowest values was found in control plants and the highest in AER (+16% vs control), and in roots, where the highest values was found in AER (+16% vs control) and the lowest in AER Cit (-9% vs control) and AER Cit+AcPHO (-9% vs control). Residual sludge generally showed lower values.

Ba was higher in control at 40 DAS and in roots, while ANA Cit + AcPHO resulted the highest at 80 DAS. Residual sludge showed always lower values.

Cd resulted always higher in sludge (+29% at 40 DAS, +42% at 120 DAS and +8% in roots as the average of ANA and AER vs control), and lower in hydrolysates. The hydrolysates concentration was lower as compared to control (-36% at 40 DAS, -24%

at 120 DAS and -36% in roots as the average of hydrolysates vs control) but even more compared to the original sludge (-50% at 40 DAS, -47% at 120 DAS and -40% in roots as the average of hydrolysates vs the average of ANA and AER).

Cu concentration resulted higher in control and the raw sludge, but also in the residual sludge. The lowest concentration was found in Chem and hydrolysates treatments.

Mn was significantly different between treatments only at 40 DAS, where the highest concentration was found in ANA Cit, followed by ANA and Ctrl.

Pb concentration was found higher in the ANA residual sludge in all the leaves sampling (+79% at 40 DAS, +144% at 80 DAS and +31% at 120 DAS Res ANA Cit+AcPHO vs control and +100% at 40 DAS Res ANA Cit vs control). In roots, however, the highest concentration was found in control plants followed by raw sludge treatments. The hydrolysates and the chemical reference showed generally a lower value.

On the other hand, Sn concentration, was higher in ANA hydrolysates at 40 DAS (+140% as the average of ANA Cit and ANA Cit+AcPHO), while at 80 DAS all the treatments resulted similar, with the exception of Res ANA Cit+AcPHO which showed a higher value (+266% vs control).

Sr resulted always higher in chemical reference and raw sludge, while the hydrolysates and the residual sludge results generally lower.

Zn concentration was significantly different only at 40 DAS, where the hydrolysates showed the lowest value (-31% as the average of the hydrolysates vs control), while residual and raw sludge showed higher concentration.

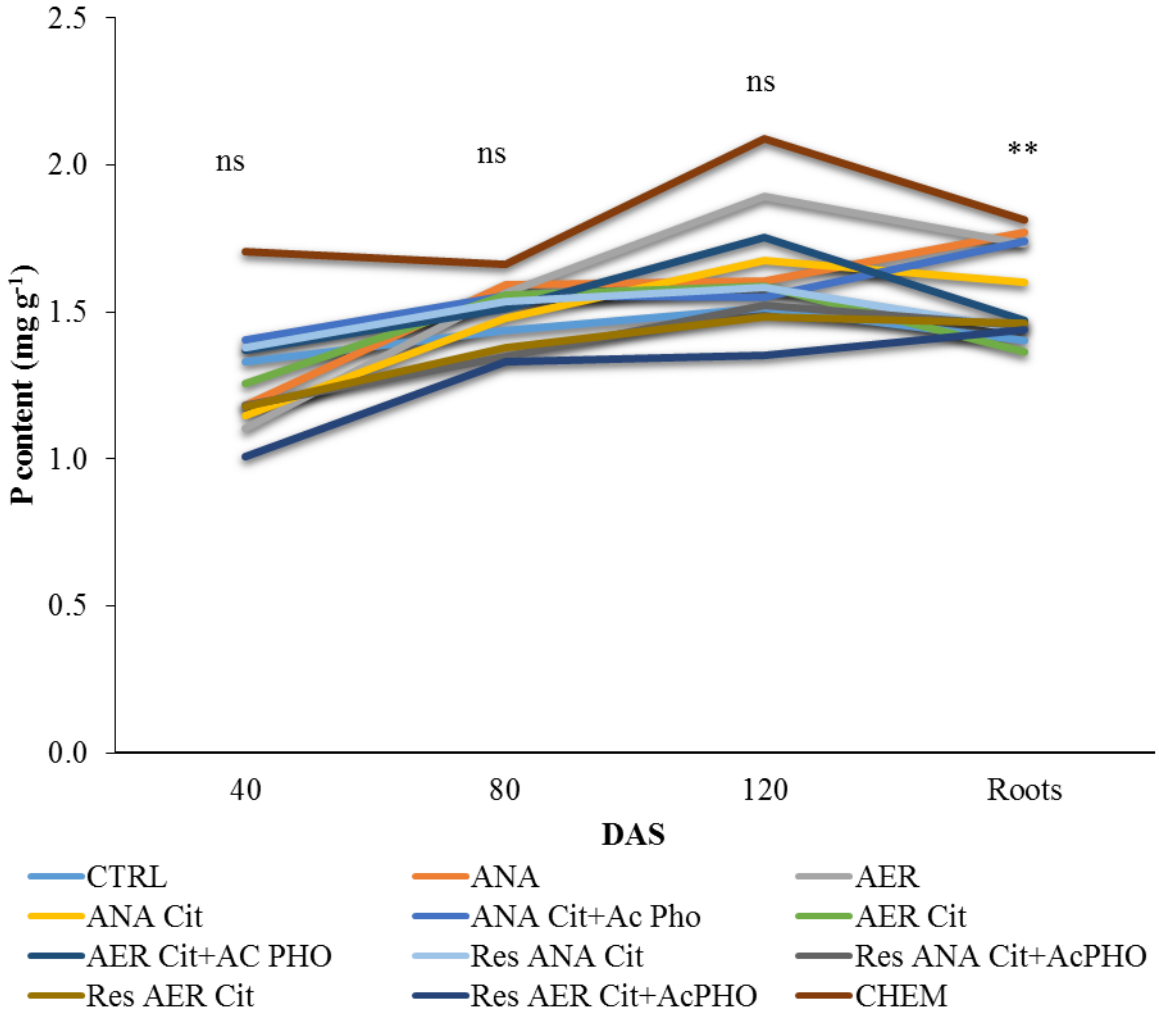


Figure 6.10: Leaves P concentration (mg g^{-1}) at 40, 80 and 120 DAS and roots at 120 DAS of the different treatments. One-way ANOVA within time is shown as ***= P≤0.001; **= P≤0.01, *= P≤0.05 and ns= P>0.05. In Two-way ANOVA, treatments and time were significant (P≤0.001).

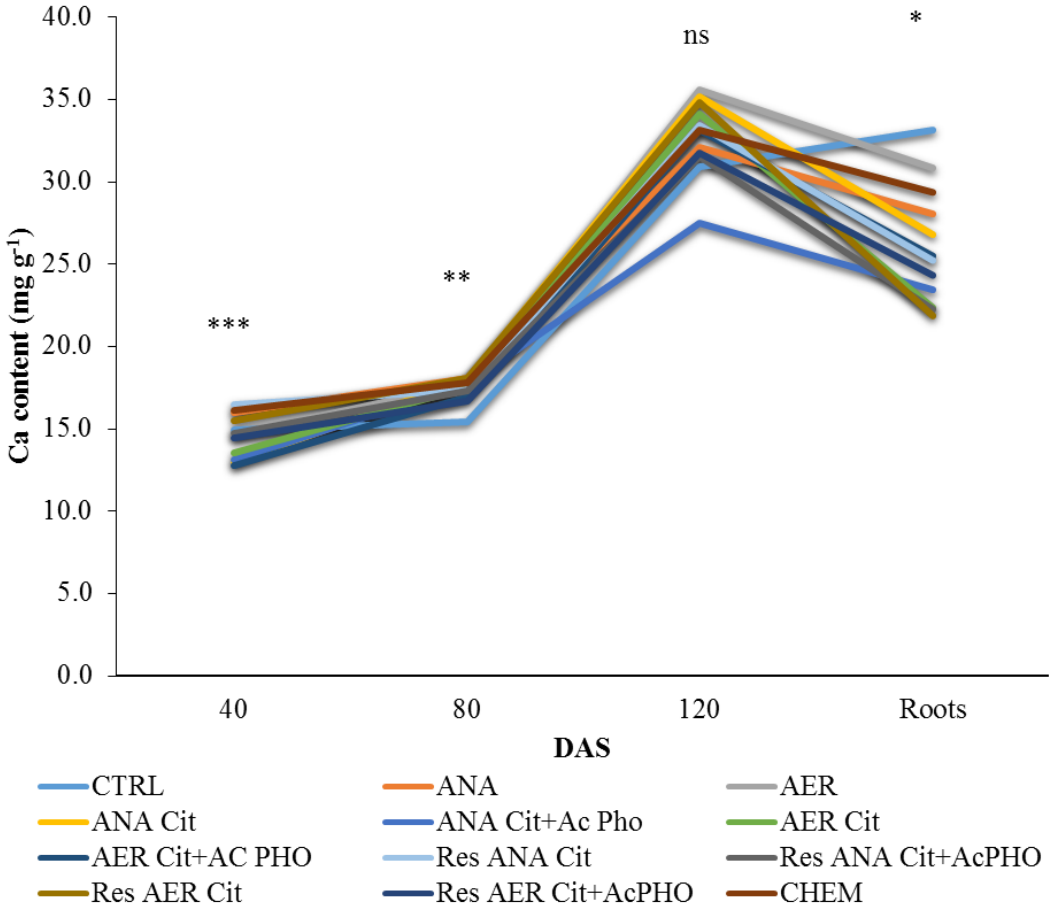


Figure 6.11: Leaves Ca concentration (mg g^{-1}) at 40, 80 and 120 DAS and roots at 120 DAS of the different treatments. One-way ANOVA within time is shown as ***= P≤0.001; **= P≤0.01, *= P≤0.05 and ns= P>0.05. In Two-way ANOVA treatments (P≤0.05), time (P≤0.001) and treatment*time (P≤0.001) were significant.

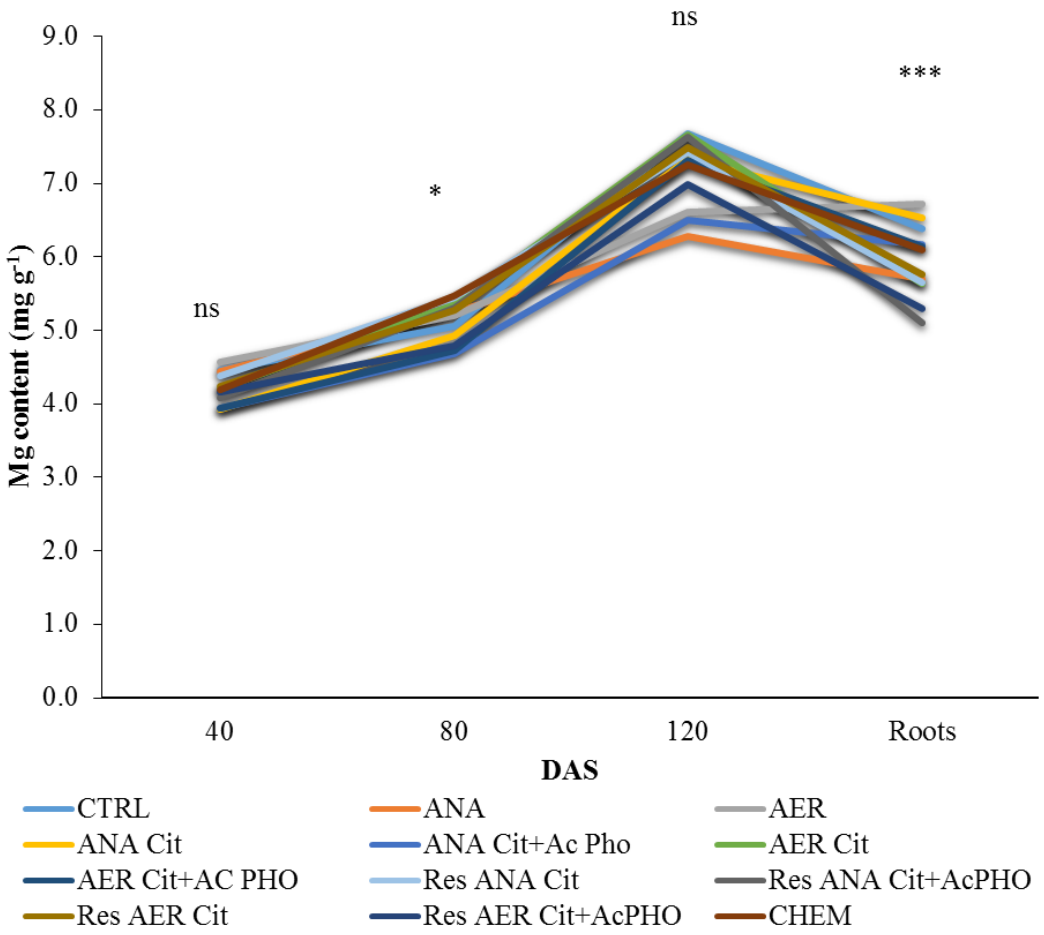


Figure 6.12: Leaves Mg concentration (mg g^{-1}) at 40, 80 and 120 DAS and roots at 120 DAS of the different treatments. One-way ANOVA within time is shown as ***= P≤0.001; **= P≤0.01, *= P≤0.05 and ns= P>0.05. In Two-way ANOVA treatments (P≤0.01), time (P≤0.001) and treatment*time (P≤0.05) were significant.

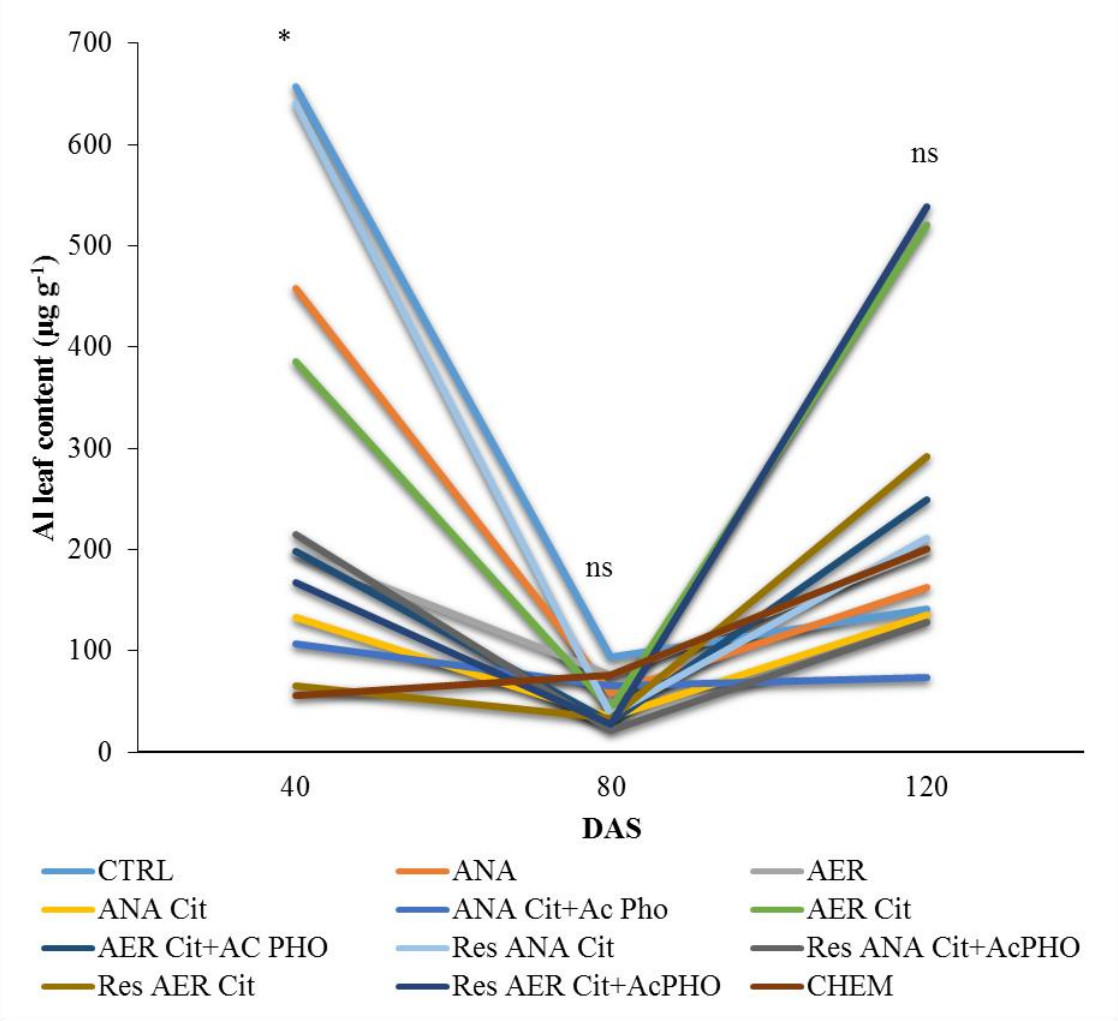


Figure 6.13: Leaves Al concentration (µg g^{-1}) at 40, 80 and 120 DAS of the different treatments. One-way ANOVA within time is shown as ***= P≤0.001; **= P≤0.01, *= P≤0.05 and ns= P>0.05. In Two-way ANOVA only time (P≤0.001) was significant.

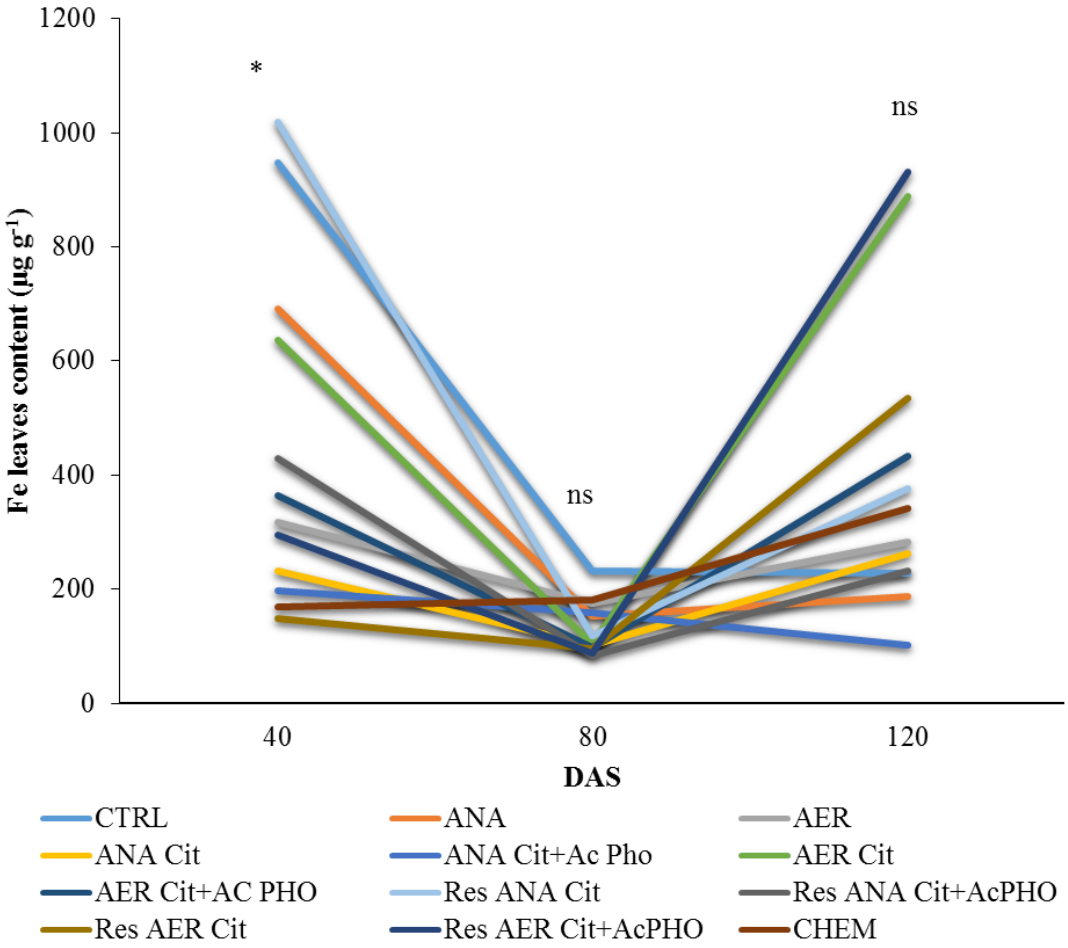


Figure 6.14: Leaves Fe concentration (µg g^{-1}) at 40, 80 and 120 DAS of the different treatments. One-way ANOVA within time is shown as ***= P≤0.001; **= P≤0.01, *= P≤0.05 and ns= P>0.05. In Two-way ANOVA treatments only time (P≤0.001) was significant.

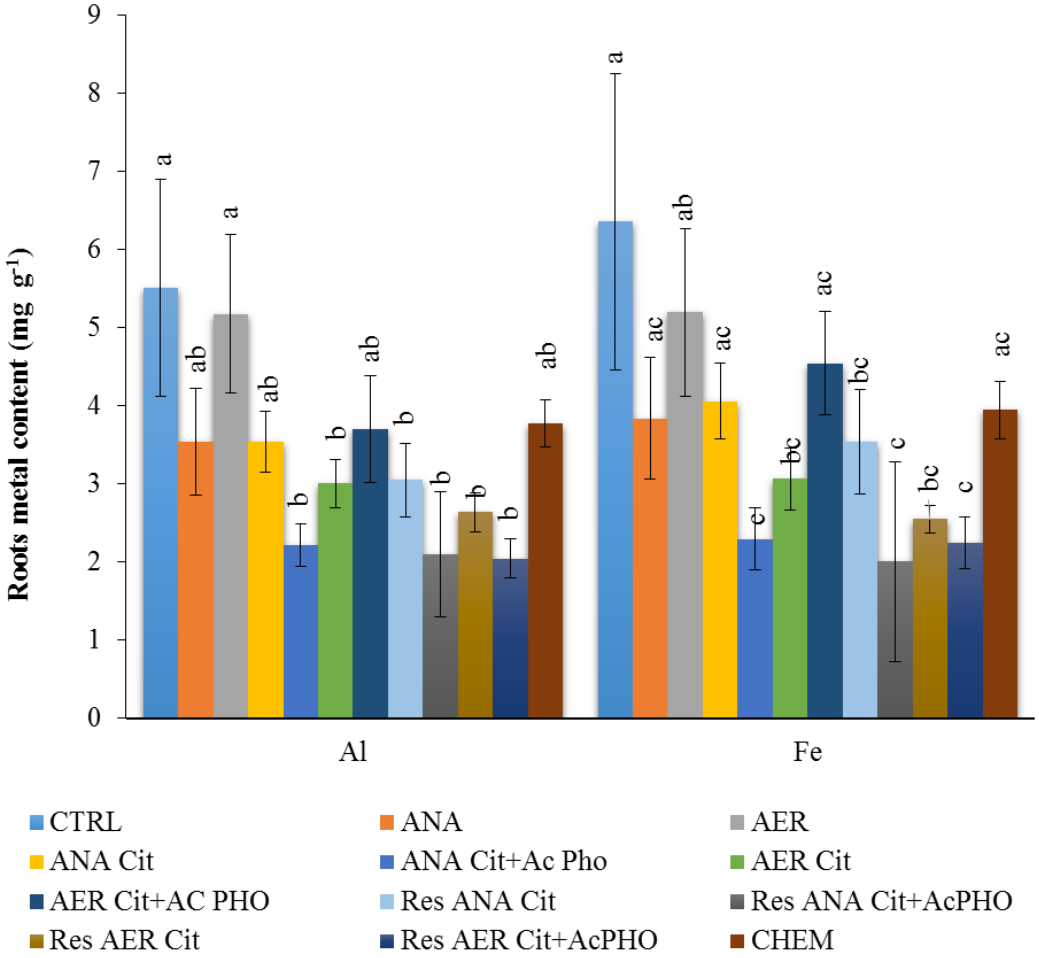


Figure 6.15: Roots Al and Fe concentration at 120 DAS of the different treatments. Error bars represent standard error (n=4) and different letters represent different group with Tukey test ($P \leq 0.05$).

Table 6.2: Elements leaves concentration at different sampling time (DAS).

	DAS	Ca	K	Mg	Na	P	S	Si	Al	B	Ba	Cd	Cr	Cu	Fe	Li	Mn	Ni	Pb	Sn	Sr	Ti	V	Zn
Treatments		**mg g^{-1}**							**µg g^{-1}**															
CTRL	40	14.9	<DL	4.5	4.2	1.3	2.1	0.1	657	25.8	13.7	1.2	8.9	13.4	947	4.9	91.2	6.6	3.3	1.1	68.5	27.1	2.3	127
ANA		15.9	<DL	4.4	4.8	1.2	2.2	0.1	458	29.3	11.9	1.6	3.8	14.4	692	5.1	94.1	4.1	4.7	2.1	68.3	9.3	3.0	157
AER		14.6	<DL	4.6	5.1	1.1	2.2	0.1	196	30.7	9.6	1.4	2.7	13.0	317	4.5	79.5	2.7	3.5	1.7	60.7	5.6	<DL	130
ANA Cit		13.0	<DL	3.9	4.8	1.1	2.0	0.1	133	28.8	8.1	0.8	3.0	12.5	232	3.5	64.9	3.2	5.2	2.9	51.4	4.3	<DL	81
ANA Cit+Ac PHO		13.1	<DL	3.9	6.3	1.4	2.2	0.1	106	29.2	9.1	0.7	2.5	13.3	198	4.6	66.8	4.3	4.7	2.5	52.8	4.0	<DL	91
AER Cit		13.5	<DL	4.2	4.7	1.3	2.1	0.1	385	29.7	10.2	0.9	8.3	13.8	637	5.2	74.2	5.4	4.0	1.6	53.6	11.1	1.4	93
AER Cit+AC PHO		12.7	<DL	3.9	6.4	1.4	2.0	0.1	198	27.8	8.6	0.6	3.4	12.5	364	4.8	63.6	3.2	4.8	2.1	50.4	9.1	0.8	86
Res ANA Cit		16.4	<DL	4.4	4.9	1.4	2.2	0.1	641	25.8	12.3	1.5	7.6	14.1	1018	5.2	96.6	6.7	6.6	2.2	72.6	16.6	1.7	141
Res ANA Cit+AcPHO		14.7	<DL	4.1	5.4	1.2	2.1	0.1	215	27.6	9.6	1.4	5.0	13.0	430	4.7	77.2	4.3	5.9	2.6	62.7	9.6	0.8	139
Res AER Cit		15.5	<DL	4.2	5.1	1.2	2.2	0.1	65.8	29.8	9.3	1.4	2.0	12.9	149	4.0	79.3	2.1	2.7	1.0	60.7	2.8	<DL	134
Res AER Cit+AcPHO		14.4	<DL	4.2	5.4	1.0	2.0	0.1	167	29.3	9.1	1.4	2.3	12.4	296	4.6	76.9	2.4	2.1	0.6	57.8	7.1	0.7	136
CHEM		16.1	<DL	4.2	5.4	1.7	2.5	0.1	55.7	29.2	10.8	0.7	1.2	12.1	169	4.2	69.1	2.3	3.5	1.8	64.4	3.1	<DL	117
CTRL	80	15.4	<DL	5.1	6.2	1.4	3.2	0.1	93.6	37.2	7.2	0.3	2.7	17.0	231	4.8	58.6	2.2	2.3	0.7	65.1	4.6	0.7	108
ANA		18.0	<DL	5.2	5.6	1.6	3.2	0.1	57.7	42.4	7.2	0.3	1.0	16.7	153	4.5	55.2	2.3	2.3	0.8	73.3	3.6	<DL	120
AER		17.6	<DL	5.2	5.6	1.6	3.1	0.1	75.8	43.0	7.2	0.3	0.8	16.7	175	4.5	51.9	2.3	2.3	0.9	71.7	3.8	<DL	113
ANA Cit		18.0	<DL	4.9	6.4	1.5	3.0	0.1	34.7	42.4	7.6	<DL	<DL	13.7	102	4.2	49.7	2.6	2.2	0.8	72.3	2.1	<DL	99
ANA Cit+Ac PHO		17.7	<DL	4.7	4.3	1.6	2.9	0.1	64.9	41.4	8.9	0.3	0.6	15.7	158	5.0	53.3	2.1	<DL	0.8	70.1	3.4	<DL	100
AER Cit		17.6	<DL	5.3	4.7	1.6	3.2	0.1	42.7	41.9	7.4	<DL	<DL	16.5	103	5.6	48.5	1.7	<DL	0.7	74.5	2.7	<DL	123
AER Cit+AC PHO		16.9	<DL	4.7	3.5	1.5	2.8	0.1	29.6	41.8	6.8	<DL	0.3	12.8	97	5.4	47.4	1.5	2.3	0.7	68.7	2.3	<DL	96
Res ANA Cit		17.3	<DL	5.4	5.1	1.5	3.5	0.1	36.5	39.2	7.0	0.4	0.3	18.3	117	4.7	57.2	1.4	<DL	0.8	66.3	2.2	<DL	113
Res ANA Cit+AcPHO		17.3	<DL	5.3	3.9	1.3	3.4	0.1	21.3	39.4	7.1	0.3	0.9	16.9	84	5.5	52.2	1.6	5.6	2.5	70.3	1.7	<DL	126
Res AER Cit		18.1	<DL	5.3	5.6	1.4	3.1	0.1	33.6	40.8	7.7	0.3	0.5	14.8	96	4.4	52.3	1.5	2.5	0.9	70.7	2.2	<DL	106
Res AER Cit+AcPHO		16.7	<DL	4.8	4.7	1.3	3.1	0.1	27.4	38.9	6.6	0.3	0.4	17.4	88	4.7	47.3	2.0	2.3	0.8	63.7	1.9	<DL	108
CHEM		17.8	<DL	5.5	5.2	1.7	3.1	0.1	75.6	41.0	8.0	0.4	1.3	13.4	182	5.6	57.0	2.2	2.2	0.8	76.4	5.2	<DL	99

Treatments	DAS	Ca	K	Mg	Na	P	S	Si	Al	B	Ba	Cd	Cr	Cu	Fe	Li	Mn	Ni	Pb	Sn	Sr	Ti	V	Zn
		mg g^{-1}							µg g^{-1}															
CTRL	120	30.9	1.7	7.7	8.6	1.5	5.3	0.1	141	42.8	15.1	0.4	1.9	17.4	228	5.0	62.0	2.2	1.1	<DL	126	3.8	0.3	194
ANA		32.0	6.5	6.3	8.7	1.6	4.9	0.1	163	40.2	15.3	0.6	0.5	15.8	187	3.8	56.3	1.9	1.2	<DL	144	3.3	<DL	260
AER		35.5	7.2	6.6	8.9	1.9	5.3	0.1	199	41.6	15.5	0.6	1.2	15.9	282	3.8	57.5	2.3	1.4	<DL	143	5.1	<DL	228
ANA Cit		35.1	6.2	7.3	9.7	1.7	5.3	0.1	136	46.0	15.8	0.4	1.7	13.3	262	4.8	61.7	1.9	1.5	<DL	140	5.3	0.2	217
ANA Cit+Ac PHO		27.5	4.9	6.5	11.2	1.6	6.0	0.1	73.9	42.8	13.0	0.3	0.2	10.4	102	5.0	46.0	1.0	1.1	<DL	113	2.7	<DL	170
AER Cit		34.1	5.5	7.6	9.3	1.6	5.1	0.1	521	44.9	17.0	0.3	8.3	14.9	888	6.2	74.1	5.5	1.0	<DL	144	17.2	1.8	247
AER Cit+AC PHO		33.1	6.1	7.3	11.3	1.8	5.0	0.1	250	47.0	15.0	0.2	3.1	12.7	433	6.1	58.1	2.7	0.9	<DL	141	8.8	0.6	243
Res ANA Cit		33.4	5.8	7.4	9.4	1.6	5.5	0.1	212	44.0	13.4	0.4	2.7	14.9	375	4.6	64.3	3.3	0.9	<DL	128	6.5	0.8	238
Res ANA Cit+AcPHO		31.5	5.5	7.6	9.6	1.5	5.6	0.1	128	45.8	13.9	0.5	1.0	17.1	232	4.9	60.7	1.9	1.5	<DL	129	3.4	0.4	254
Res AER Cit		34.8	5.8	7.5	9.5	1.5	5.1	0.1	292	46.3	16.1	0.6	3.0	14.2	534	5.0	69.4	6.0	1.3	<DL	142	10.0	0.6	273
Res AER Cit+AcPHO		31.7	6.3	7.0	8.9	1.4	4.7	0.1	539	40.6	15.8	0.4	10.1	14.4	932	5.3	74.6	8.3	1.4	<DL	129	17.5	1.9	237
CHEM		33.1	3.7	7.2	10.8	2.1	5.3	0.1	201	43.3	16.1	0.3	2.4	14.2	342	6.3	67.2	7.5	1.1	<DL	147	7.0	0.3	166
		Ca	**K**	**Mg**	**Na**	**P**	**S**	**Si**	**Al**	**B**	**Ba**	**Cd**	**Cr**	**Cu**	**Fe**	**Li**	**Mn**	**Ni**	**Pb**	**Sn**	**Sr**	**Ti**	**V**	**Zn**
ANOVA																								
Treatments		0.03	0.00	0.01	0.04	0.00	0.23	0.97	0.13	0.00	0.25	0.00	0.10	0.00	0.22	0.00	0.01	0.47	0.00	0.00	0.01	0.22	0.53	0.00
Time		0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.62	0.00
*Treatments*Time*		0.00		0.05	0.00	0.88	0.52	0.05	0.06	0.03	0.02	0.00	0.48	0.02	0.09	0.08	0.03	0.01	0.00	0.00	0.02	0.05	0.43	0.07
ANOVA One-way																								
within time	*40*	0.00		0.19	0.00	0.08	0.40	0.32	0.03	0.24	0.00	0.00	0.21	0.12	0.05	0.36	0.00	0.07	0.01	0.00	0.00	0.04	0.23	0.00
within time	*80*	0.00		0.05	0.02	0.31	0.02	0.75	0.37	0.00	0.00	0.66	0.48	0.00	0.33	0.01	0.10	0.10	0.00	0.00	0.01	0.04		0.55
within time	*120*	0.31	0.00	0.05	0.00	0.18	0.52	0.23	0.49	0.09	0.70	0.00	0.19	0.00	0.43	0.00	0.37	0.12	0.33		0.16	0.47	0.64	0.08

<DL: concentration under detection limits

Table 6.3: Roots elements concentration.

	Al	Ca	Fe	K	Mg	Na	P	S	Si	As	B	Ba	Cd	Co	Cr	Cu	Li	Mn	Mo	Ni	Pb	Sb	Sr	Ti	V	Zn
Treatments	**mg g-1**									**µg g-1**																
CTRL	5.5	33.1	6.4	7.8	6.4	10.0	1.4	3.1	0.1	1.9	25.1	64.9	0.7	4.8	18.3	95.1	16.2	218	1.6	21.4	8.8	0.8	150	40.3	15.7	266
ANA	3.5	28.0	3.8	7.9	5.7	10.5	1.8	3.4	0.1	1.1	24.5	48.5	0.8	3.4	12.9	97.0	11.3	122	1.0	14.9	5.8	0.8	137	29.8	10.9	351
AER	5.2	30.8	5.2	7.6	6.7	10.7	1.7	3.4	0.0	1.5	29.0	57.2	0.8	4.3	18.1	91.1	14.0	148	1.2	18.3	7.6	0.9	147	39.6	13.7	321
ANA Cit	3.5	26.8	4.1	7.8	6.5	11.3	1.6	3.8	0.1	1.2	24.0	47.3	0.5	3.8	14.0	86.7	12.2	119	0.8	17.7	6.5	0.8	131	26.9	12.7	335
ANA Cit+Ac PHO	2.2	23.4	2.3	7.8	6.2	11.9	1.7	4.2	0.1	1.0	25.0	40.4	0.6	2.9	10.3	86.8	9.9	65.2	0.7	13.0	4.4	0.7	129	20.5	9.5	354
AER Cit	3.0	22.3	3.1	7.6	5.6	11.3	1.4	3.5	0.0	1.1	22.8	40.7	0.5	3.0	13.5	87.0	10.7	87.6	0.7	15.3	5.1	0.6	122	28.9	10.3	355
AER Cit+AC PHO	3.7	25.5	4.5	7.3	6.1	11.4	1.5	3.4	0.1	1.5	22.9	51.9	0.3	3.8	13.4	71.8	12.6	134	1.0	18.6	6.3	0.7	129	32.2	12.8	301
Res ANA Cit	3.1	25.2	3.5	7.7	5.6	11.1	1.4	3.7	0.1	2.0	24.2	44.5	0.7	3.6	12.6	99.3	10.6	112	0.8	14.2	5.2	0.8	121	27.1	9.3	314
Res ANA Cit+AcPHO	2.1	22.2	2.0	7.6	5.1	11.4	1.5	3.6	0.1	0.6	24.0	36.1	0.9	2.7	9.0	129	8.7	65.0	0.9	11.2	4.2	0.8	115	21.0	7.3	375
Res AER Cit	2.6	21.9	2.6	7.7	5.8	11.8	1.5	3.9	0.1	0.9	23.9	35.0	0.6	3.5	9.6	99.0	10.0	74.2	0.8	14.4	4.4	0.6	117	25.4	8.7	347
Res AER Cit+AcPHO	2.0	24.3	2.2	7.7	5.3	11.2	1.4	3.5	0.1	0.8	25.5	34.3	0.9	3.1	8.5	110	8.6	75.2	0.8	12.7	3.7	0.6	127	21.6	7.2	439
CHEM	3.8	29.3	3.9	7.8	6.1	10.7	1.8	3.7	0.1	1.3	26.3	60.5	0.5	3.4	12.4	90.4	12.2	127	1.0	14.3	6.3	0.7	143	31.9	12.5	401
ANOVA One-way	0.01	0.02	0.03	0.01	0.00	0.14	0.01	0.17	0.01	0.07	0.00	0.01	0.00	0.07	0.12	0.00	0.02	0.06	0.02	0.06	0.00	0.00	0.00	0.16	0.00	0.07

<DL: concentration under detection limits

Plant P uptake, apparent recovery fraction and P use efficiency

Cumulated P uptake (Fig. 6.16), calculated as the product of plant dry weight and P concentration, in mg of P, showed a fast and steady increase in AER Cit+AcPHO, which had a total value significantly higher compared to the other treatments (+37% as compared to control and +13% as compared to Chem). On the other hand, residual sludge assimilated less P compared to control (-18% as an average of the residual sludge compared to control). Similarly, also ANA sludge treated plants had a P uptake smaller than control (-18%). ANA Cit and ANA Cit+AcPHO had a behavior very similar to the chemical references (+19% of the average of ANA Cit and ANA Cit+AcPHO and +22% of Chem, as compared to control), while AER Cit resulted similar to the AER raw sludge (+6% in AER Cit and +7% in AER, as compared to control).

Apparent recovery fraction (ARF) results (Fig. 6.17) were highly compromised by the P added to the soil, which was different between treatments. Moreover, often the P uptake of control plants was similar to the uptake of the treated plants. This was easily detectable in roots, where control plants had a dry weight value very high, thus increasing the P uptake. The Two-way ANOVA results for ARF resulted significant for treatments and time ($P \leq 0.001$), but not the interaction. One-way ANOVA within time were significant at 40, 80 DAS and roots. At 40 DAS AER Cit+AcPHO resulted the highest value (2.8%), while residual sludge (except for Res AER Cit) resulted with a negative ARF. At 80 DAS, ANA Cit+AcPHO showed the highest ARF (3.9%), while all the residual sludge treated plants showed negative values. At 120 DAS, leaves ARF results were not significantly different. On the other hand, roots ARF resulted negative in all the treatments, with lower values in the residual sludge.

The P use efficiency calculated with the balance method (Fig. 6.18) showed a very high use efficiency of the residual sludge, with total recovery of more than 100%, due to the very low P added with the sludge. When comparing only raw sludge treatments and hydrolysates with chemical reference (Fig. 6.19), a very high P use efficiency of the AER hydrolysates was found (100% in AER Cit+AcPHo and 94% in AER Cit), while ANA hydrolysates showed values similar to Chem (72% ANA Cit, 71% ANA Cit+AcPHO and 64% Chem).

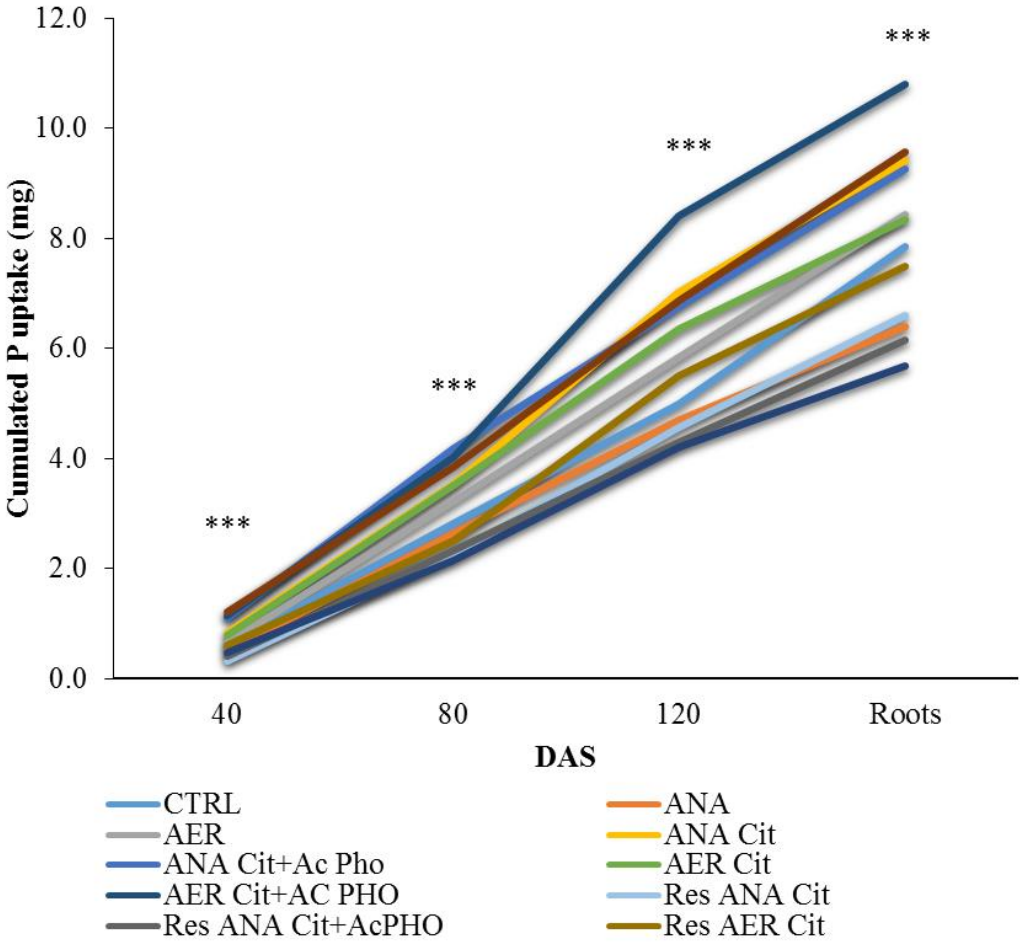


Figure 6.16: Cumulated plants P uptake (mg) of the different treatments. One-way ANOVA within time is shown as ***= P≤0.001; **= P≤0.01, *= P≤0.05 and ns= P>0.05. In Two-way ANOVA treatments, time and treatment*time were significant (P≤0.001).

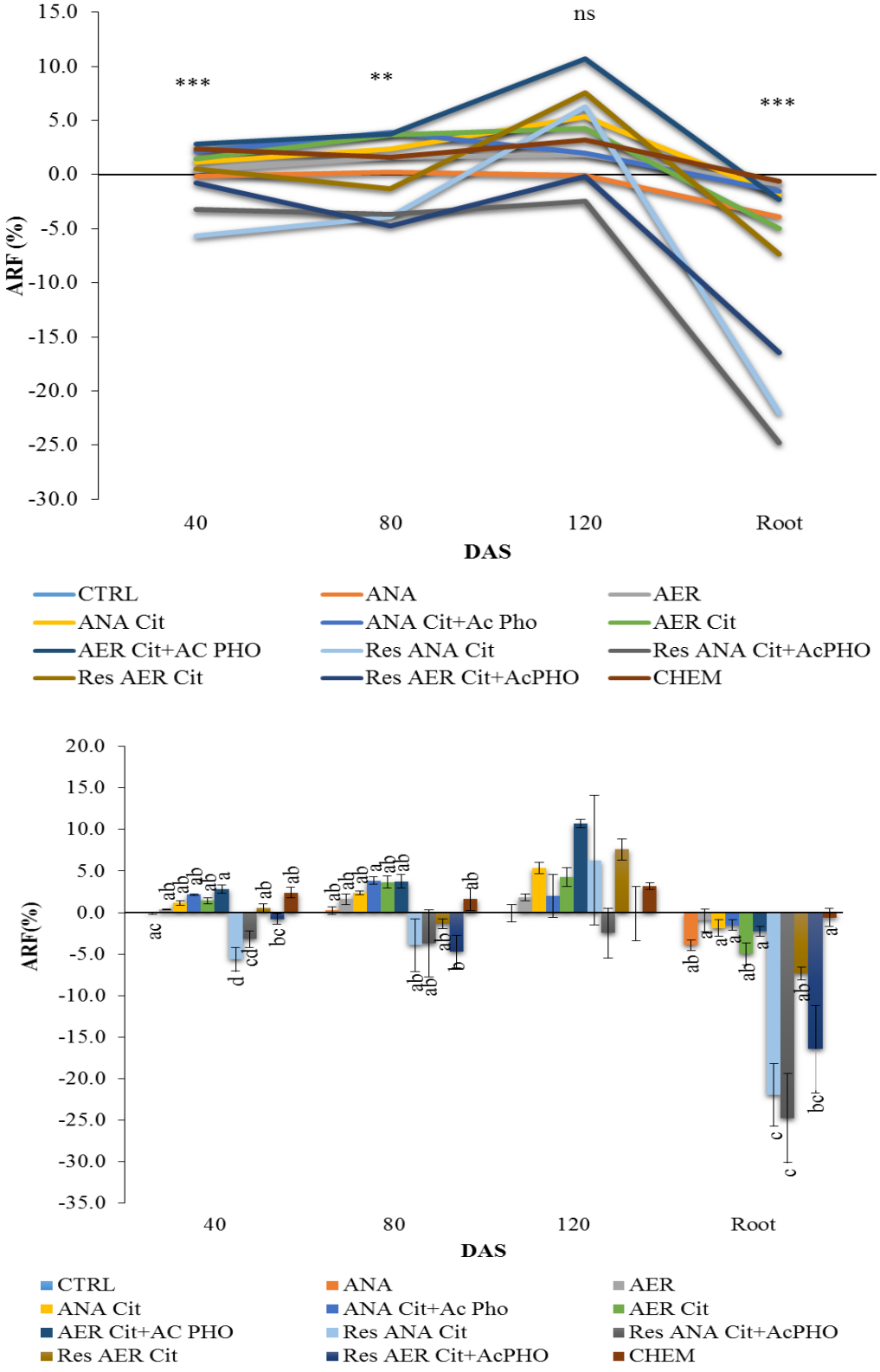


Figure 6.17: Leaves and root apparent recovery fraction (%) of the different treatments. In the above picture One-way ANOVA within time is shown as ***= P≤0.001; **= P≤0.01, *= P≤0.05 and ns= P>0.05. In the bottom picture error bars represent standard error (n=4) and different letters represent different group with Tukey test (P≤0.05). In Two-way ANOVA, treatments and time were significant (P≤0.001).

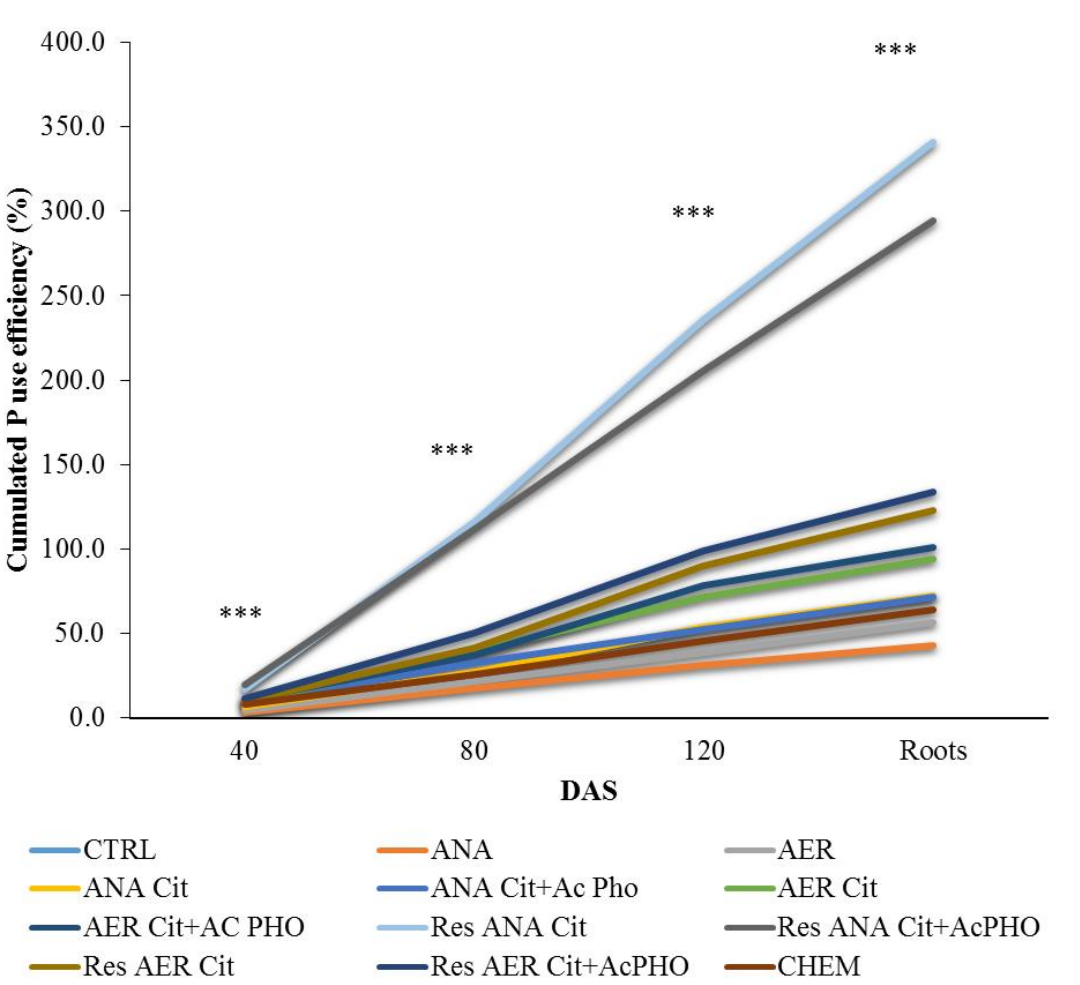


Figure 6.18: Cumulated P use efficiency (%) calculated with balance method. One-way ANOVA within time is shown as ***= P≤0.001; **= P≤0.01, *= P≤0.05 and ns= P>0.05. In Two-way ANOVA treatments, time and treatment*time were significant (P≤0.001).

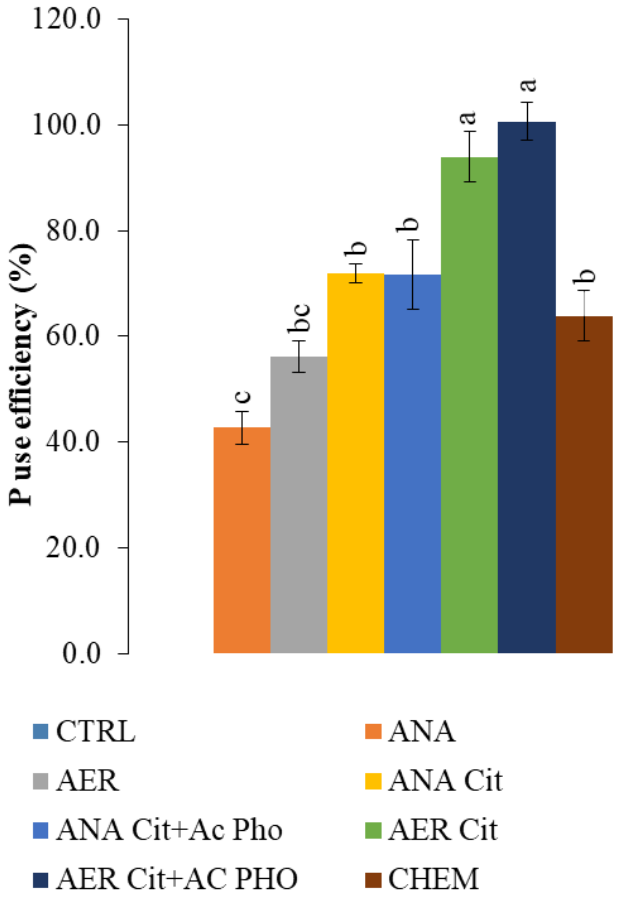


Figure 6.19: Total P use efficiency of the raw sludge and hydrolysates treatments with balance method. Error bars represent standard errors and different letters represent different groups with Tukey test (P≤0.05).

Use efficiency of the hydrolysis vs the raw sludge

The use efficiency of the hydrolysis (Fig. 6.20) showed the high increase of efficiency between the raw anaerobic sludge treated plants to the deriving hydrolysate treatment (+60.0% in ANA Cit and Res ANA Cit and +58.4% in ANA Cit+AcPHO and Res ANA Cit+AcPHO as compared to ANA). Similar increase was found between the raw aerobic sludge treated plants to the deriving hydrolysate treatment (+46.8% in AER Cit and Res AER Cit and +48.9% in AER Cit+AcPHO and Res AER Cit+AcPHO as compared to AER). In anaerobic hydrolysates a large percentage of efficiency derived from the residual sludge; while in the aerobic hydrolysates only a small fraction of the efficiency came from the residual sludge.

Considering the percentage increase of the hydrolysates and the residual sludge compared to the raw sludge from which they were derived during the different harvest (Fig. 6.21), it is clear that the higher P uptake in the hydrolysates decrease in time, with ANA hydrolysates showing the highest increments. While in the residual sludge, the P uptake was always lower than the original sludge, with also the ANA residual increasing slightly in roots uptake.

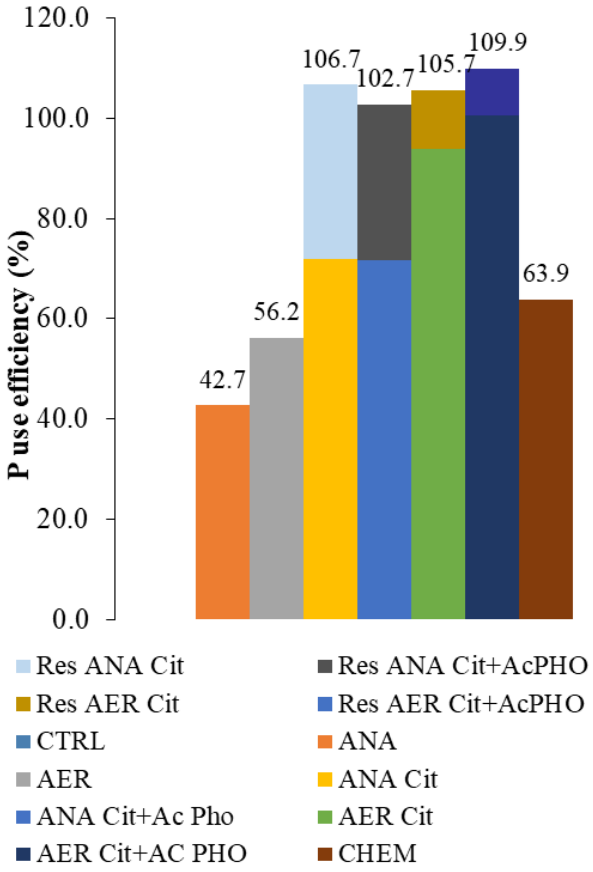


Figure 6.20: Total P use efficiency of the raw sludge and hydrolysates treatments summed with the residual sludge treatment correspondent. The residual sludge treatments efficiency are piled on top of their hydrolysates treatment. Numbers on the bars represent the values of the sum between hydrolysates and residual sludge treatments.

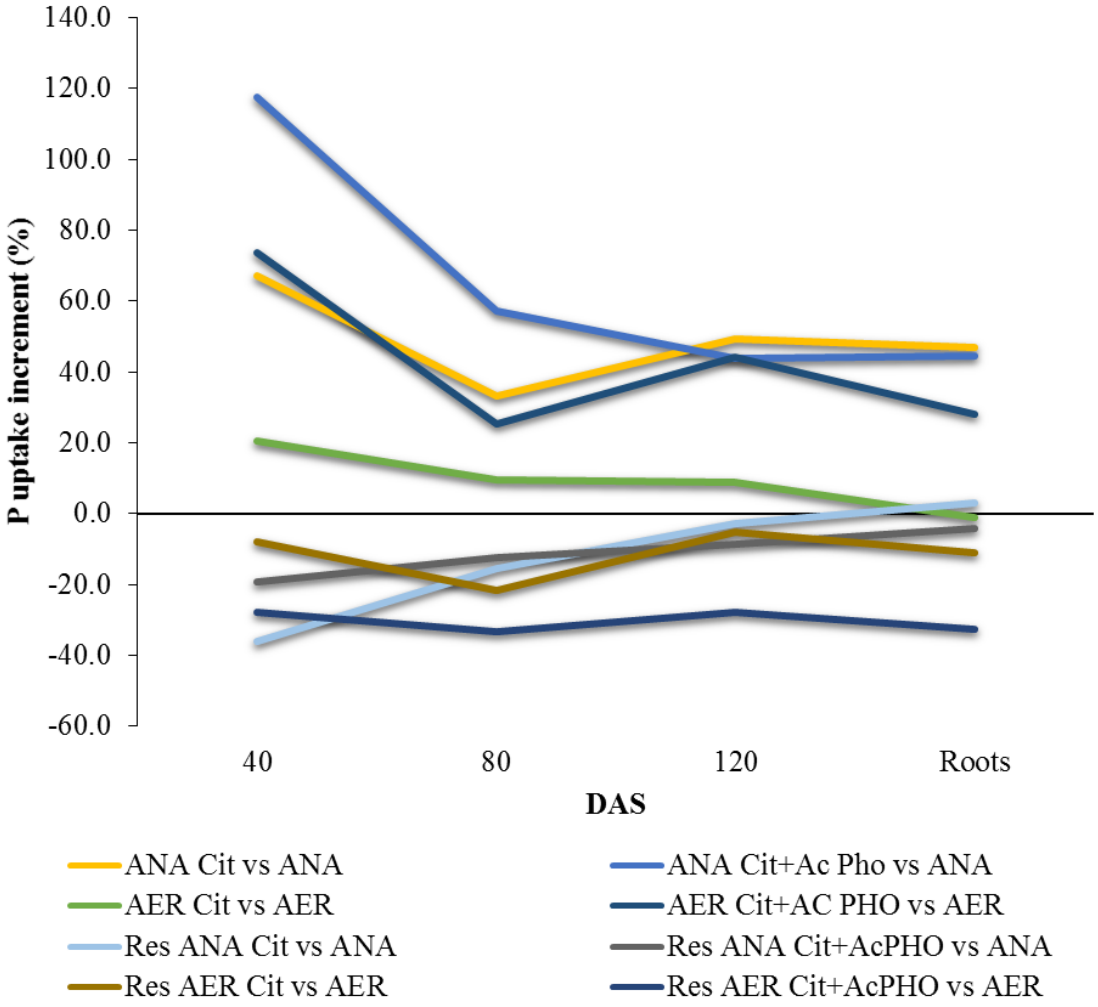


Figure 6.21: P uptake increment, calculated as the P uptake of the hydrolysates treatments and the residual sludge treatments vs the raw sludge from which they derived.

Mass balance

A summary of the mass balance experiment is shown in Fig. 6.22, which showed that Ctrl plants and all the residual treatments plants accumulated more P than the added amount, showing a positive balance. On the other hand, Chem and the hydrolysates treatments showed a negative balance, with plants that accumulated less P than the added one. The highest amount of P remained in soil was found in Chem treatment, followed by AER Cit and ANA Cit and ANA Cit+AcPHO. The only hydrolysates treatment which showed a very equilibrated balance was found in AER Cit+AcPHO, which has accumulated all the P added without losing any nutrient in the soil.

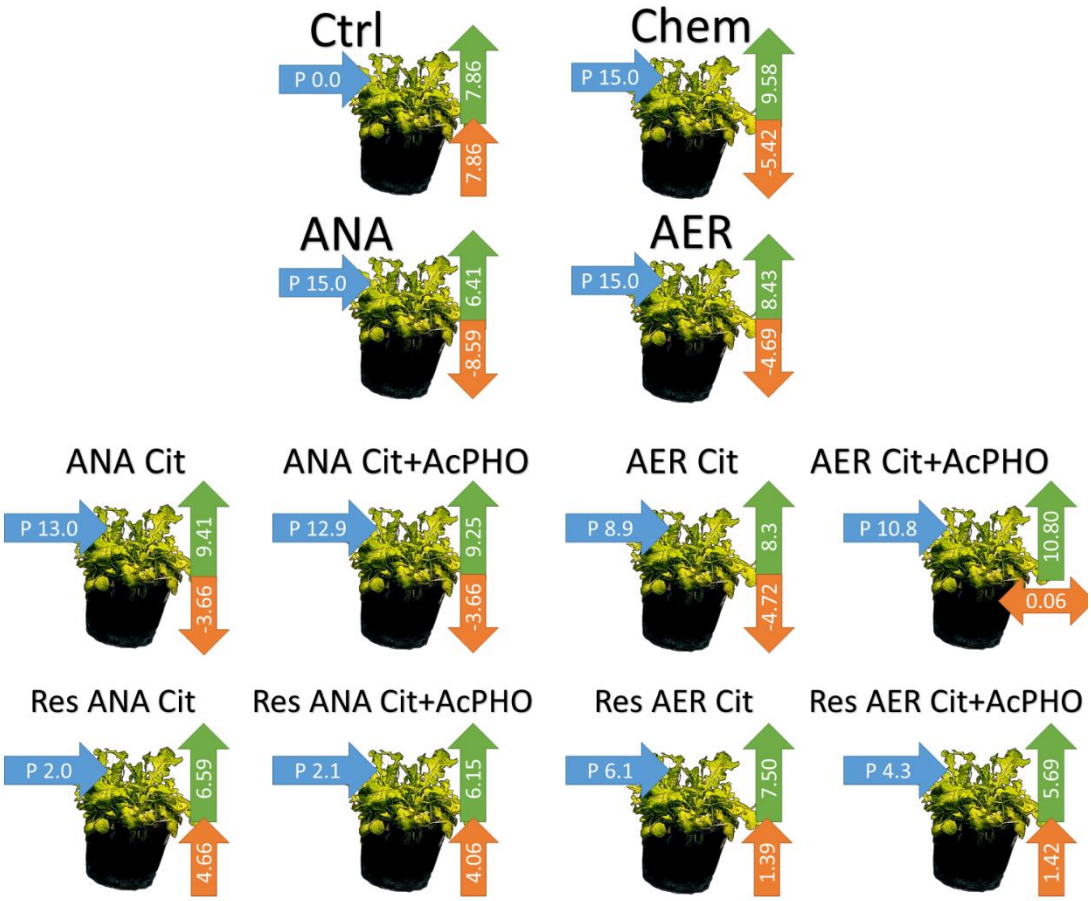


Figure 6.22: Mass balance summary. Blue arrows indicate the P added to the pot, while the green arrows the P accumulated in the plant tissue during the whole experiment. In the orange arrows are indicated the P that theoretically was assimilated from the soil (or which remained in the soil in case of negative values). All the values in the arrows are in mg pot^{-1}.

Plant visual comparison

In Fig. 6.23, 6.24 and 6.25 there were pictures of plants at 80 DAS (Fig. 6.24) and at the final destructive sampling, 120 DAS (Fig. 6.23 and 6.25). It is possible to note that the plants treated with raw sludge and residual sludge had a worse growth compared to plants in the chemical reference and in the hydrolysates treatments. The plants grew thinner and with a narrow leaf blades.

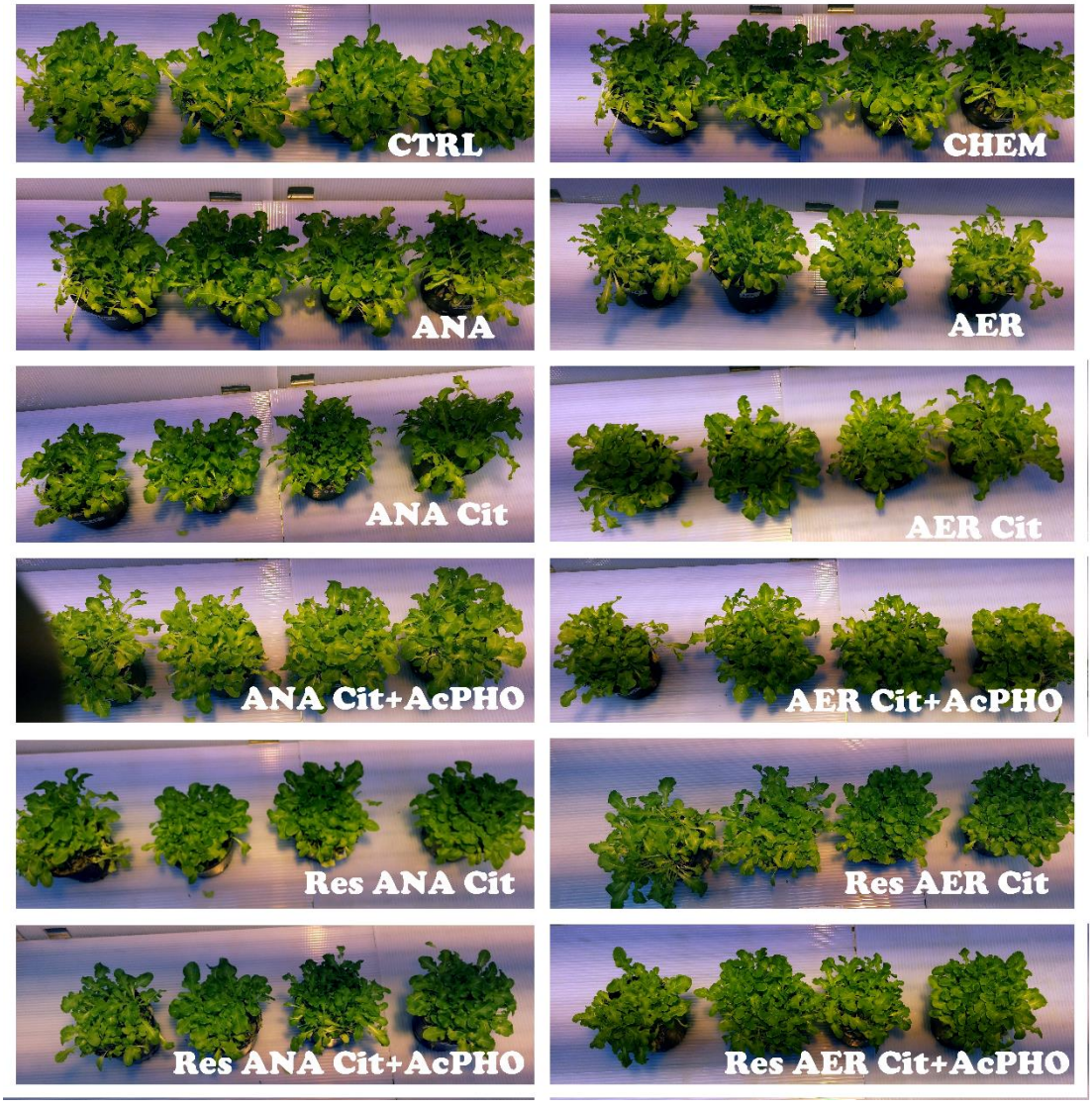


Figure 6.23: Plants of different treatments in comparison at 120 DAS.

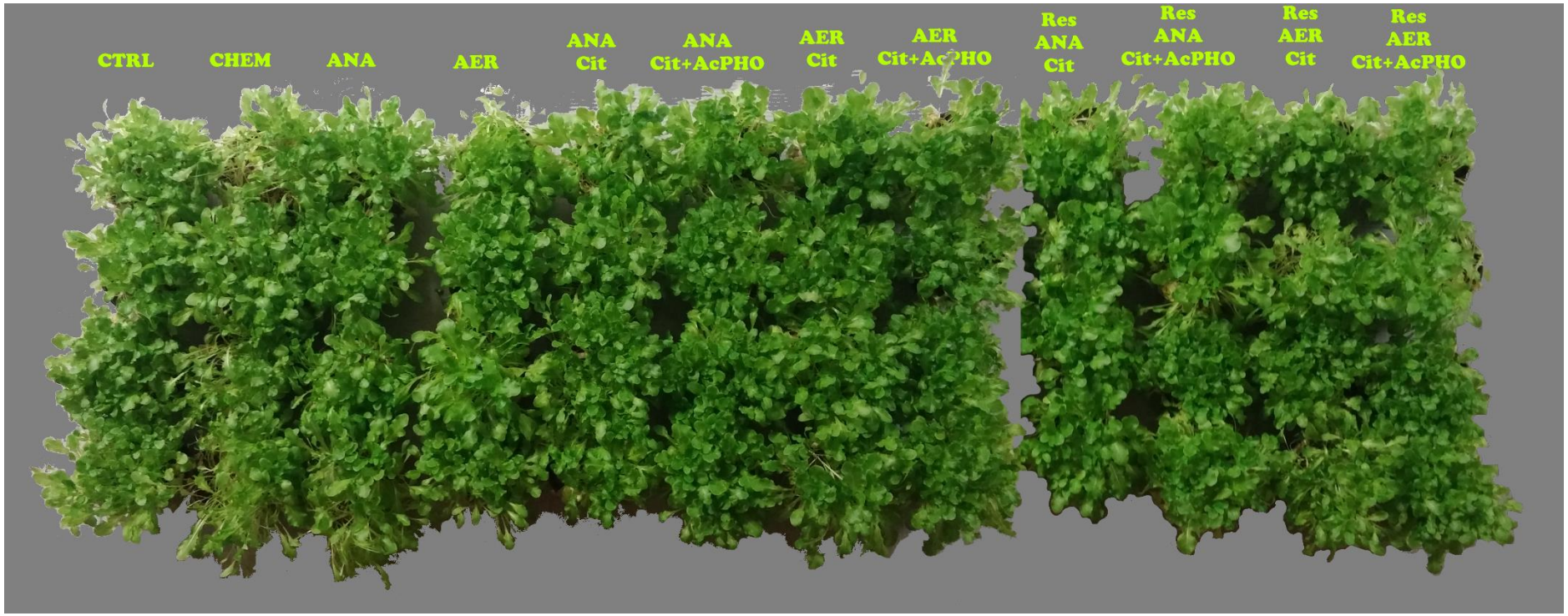


Figure 6.24: Plants of different treatments in comparison at 80 DAS.

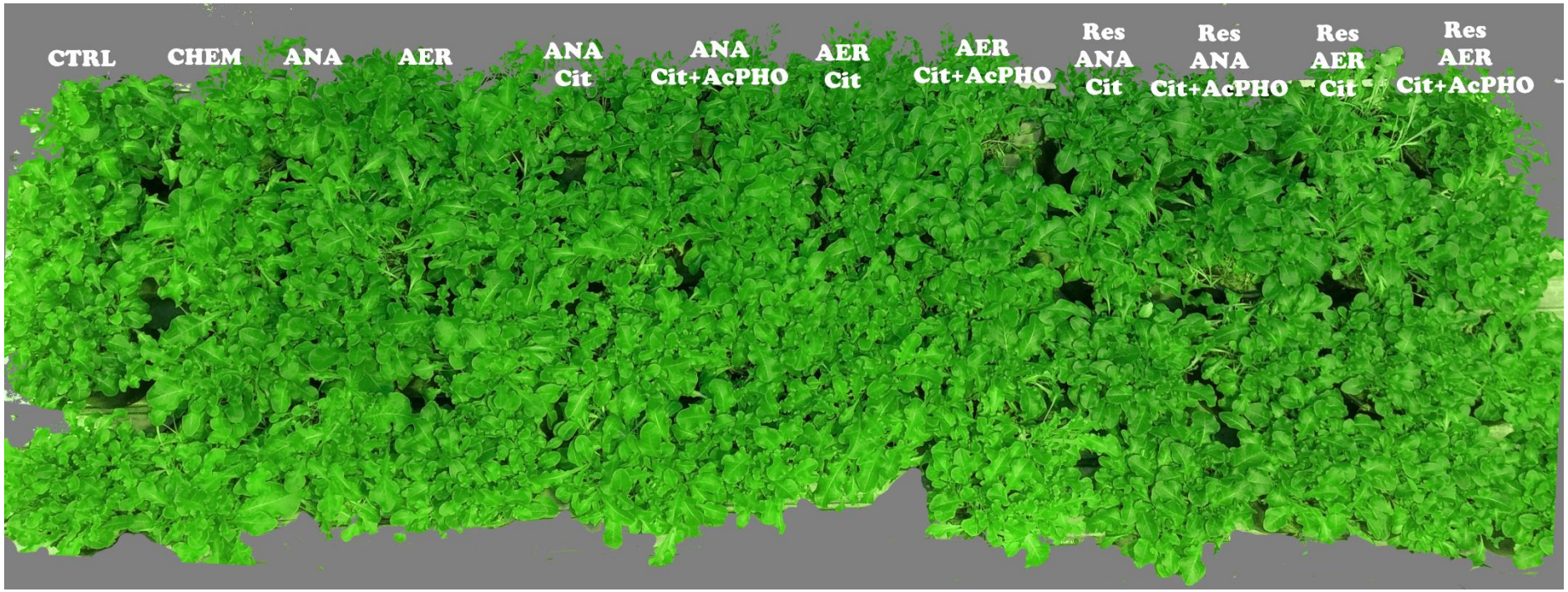


Figure 6.25: Plants of different treatments in comparison at 120 DAS.

Discussion

Bio-waste products are a real possibility to replace the mineral P source for plant fertilization (*Alvarenga et al.*, 2015; *Cordell et al.*, 2009; *Fuentes et al.*, 2006; *Grigatti et al.*, 2015). We have extensively discussed the effect of bio-waste in soil for plant nutrition and the possibility of creating a liquid fertilizer derived from sewage sludge. However, we have broadly shown that most of the bio-waste do not contain high soluble phosphorus. Up to now, though, although the effectiveness of the hydrolysates as fertilizers were proved, the better choice between the different hydrolysates was not always clear. From our experience, the solubility is the major problem of phosphate plant nutrition and the recommended dosage are overestimated (*Sharpley et al.*, 1994; *Vance*, 2001). After this experiment, in which a fixed dose of sewage sludge, instead of a fixed dose of P, evident differences came up between the different products tested. Furthermore, the addition of the residual sludge after the hydrolysis improved the research and gave useful information.

In this experiment, the soil P availability resulted always higher in the chemical reference. Nevertheless, it should be considered than the dose of P added was different between the chemical reference and the hydrolysates. The hydrolysates available P was though always high and in ANA Cit and AER Cit+AcPHO treated soil remained more constant in time. Furthermore, the hydrolysates had increased the phosphatase activity, with some oscillation, but stably at the end of the experiment, even if they showed a slightly lower microbial growth as compared to the deriving raw sludge.

According to the plant growth results, the anaerobic sludge tested and all the residual sludge were not able to increase the plant biomass in respect to the plants grown in not amended soil. Rather, these treatments did not enhance the roots developments as occurred in the control plants. The plants grown without any soil conditioners or fertilizers (except for K and N), had developed a dense network of roots in order to explore more volume of soil in search of nutrients (*Marschner et al.*, 1996). On the other hand, hydrolysates treatments ameliorated the growth conditions and improved the plant biomass accumulation. The P use efficiency of the hydrolysates was thus, significantly higher as compared to the raw sludge, showing that also a lower total P addition can support a better growth, when P is more available for plants. The highest growth was reached in the plants treated with the hydrolysates of citric acid and acid phosphatase derived from aerobic sludge (AER Cit+AcPHO). Good plant growth was also detectable in plants grown with the citric acid hydrolysates derived from the anaerobic sludge (ANA Cit). The growth increase in the hydrolysates plants was even more visible considering only the leaf biomass, due to the non-limiting environments created by the fertilizers and the consequent low roots developments. The plants grown in soil with the residual sludge of AER Cit+AcPHO had low growth and consistently

reduced roots developments. So why did not the residual sludge promote the roots growth? In the soilless experiments, we had very low root growth and we supposed that could have been Al toxicity effects (chap. 3). In this case, the Al concentration in the plants grown in the residual sludge treated soil were always lower as compared to the raw sludge. Thus, soil has buffered the Al toxicity and there should be another reason. It is know that phosphorus is needed in plants mostly nearly after the sowing to promote root growth (*Bates and Lynch*, 1996; *Foehse and Jungk*, 1983; *Ma et al.*, 2001). In the residual sludge, the P concentration was very low, due to the high P recovery obtained with the hydrolysis. This was more marked in the Res AER Cit+AcPHO, where the hydrolysis had the highest P recovery. However, this cannot explain alone the lower biomass and the lower roots developments in residual sludge treated plant than in the control ones. Often the apparent recovery fraction of the residual sludge treated plants but also the available P in the residual sludge soil were lower than in the unamended soil. We can suppose, thus, than the residual sludge behaved as a sink for P in soil, decreasing the P availability. This can be explained with the high metal concentration of these sludge products, such as Fe and Al, that the hydrolysis has solubilized making them more reactive in creating P-bonds.

We have previously seen that bio-waste with high metal concentration have a slow release of P (chap. 2). In this experiment P uptake in plants were higher in hydrolysates treatments as compared to the deriving sludge, however, these increments had decreasing with time. This can be another proof of the slow P release of bio-waste rich in Al and Fe, which should be taken into account when projecting a basal fertilization of long cycle crops. On the other hand, hydrolysates were perfect for very short and demanding crop such as lettuce (*Kumar et al.*, 1992), with their fast P release, which make them preferable to raw organic products, and the soluble micronutrients, such as B and Fe, which make them preferable to the chemical fertilizer.

Mg and Ca was slowly released from all the products, as they increased in the last cut and in roots. However, hydrolysates plants showed some differences. The hydrolysates derived from the aerobic sludge (AER Cit and AER Cit+AcPHO) showed always low Ca concentration; while the hydrolysates with citric acid and acid phosphatase (ANA Cit+AcPHO and AER Cit+AcPHO) showed always low Mg. On the contrary, the raw sludge ANA and AER, showed low P concentration in leaves, but high concentration in Al, Ca, Fe and Mg. Thus, they should really be taken into account in a long-term fertilization.

On the other hand, plants grown in raw sludge amended soil accumulated more heavy metals. Hydrolysates plants, instead, accumulated less Cd and Zn as compared to control plants and raw sludge, less Cu than control plants, raw sludge and residual sludge plants, less Ni than the raw sludge and less Pb than residual sludge (and similar

to the raw sludge). In roots, hydrolysates accumulated also less As, Mo and Sb than the raw sludge.

Although the Na accumulation in plants grown with hydrolysates treated soil resulted higher than the other treatments, the plants did not show salt stress effect. Being lettuce moderately sensitive to salt stress (*Shannon and Grieve*, 1999), the Na concentration in the hydrolysates can be considered below the toxic level and even below the first symptoms level.

The P use efficiency had very different results if calculated as apparent recovery fraction or with balance method. *Syers and Johnston* (2008) suggested using the balance method for the P use efficiency calculation, because of the residual P generally accumulated in soil. The ARF resulted lower than the PUE calculated with balance method, but both had a similar conclusion: the plants were able to uptake a high amount of P even in the not amended soil, mostly due to the high root development and the thin layer of soil in the pots, which was used for the specific reason of a complete root exploration. However, both ARF and balance method, showed that the AER hydrolysates had the highest P use efficiency, with value that reached almost 100% of the added P (in the balance method). The comparison of the raw sludge plants P accumulation to the hydrolysates treatment plants evidenced that the hydrolysis drastically increased the efficiency of the fertilizers. If we sum the P accumulated in hydrolysates treated plants to the corresponding residual sludge plants we can detect an uptake higher than the P originally contained in the raw sludge.

To sum up, raw sewage sludge and even more the residual sludge after the P hydrolysis, when used to amend a calcareous soil with low P concentration, can react as a sink of phosphorus, bonding the P available in the soil solution. In raw sludge, possibility of slow P release is supposed and the suitable as a long-term P fertilizers have to be tested. The residual sludge, though, cannot be used as a P fertilizer; however, the suitability of those sludge samples to be used as soil conditioners or N fertilizers has been positively tested previously in chap. 5. The hydrolysates, however, in soil behaved as a source with very fast release and high solubility, showing a higher P use efficiency when compared to the raw sludge and the chemical reference.

In conclusion, the best choice for P fertilization, using sewage sludge, was the hydrolysates with the combination of citric acid and acid phosphatase derived from the aerobic sludge rich in Al, which has promoted the best plant growth, the highest P available and the highest P uptake. This choice has also evidenced a perfect balance between the added P and the plant P uptake, revealing the possibility to reduce to 0 the soil P accumulation and the possibility of P leaching. However, also the other treatments with hydrolysates were very effective, with the citric acid hydrolysates derived from the anaerobic sludge rich in Fe that can be considered the most

convenient choice. This treatment also resulted in a good plant growth and P uptake without the addition to the hydrolysates of the enzymes, which can increase exponentially the cost of the hydrolysis.

Reference

Achat, D.L., Bakker, M.R., Zeller, B., Pellerin, S., Bienaimé, S., Morel, C. (2010): Long-term organic phosphorus mineralization in Spodosols under forests and its relation to carbon and nitrogen mineralization. *Soil Biol. Biochem.* 42, 1479–1490.

Adam, C., Peplinski, B., Michaelis, M., Kley, G., Simon, F.-G. (2008): Thermochemical treatment of sewage sludge ashes for phosphorus recovery. *Waste Manag.* 29, 1122–1128.

Adesemoye, A.O., Kloepper, J.W. (2009): Plant–microbes interactions in enhanced fertilizer-use efficiency. *Appl. Microbiol. Biotechnol.* 85, 1–12.

AEEP (2017): Phosphorus: Key to Plant Life [WWW Document]. URL http://aeep.eu/phosphorus/ (accessed 11.29.17).

Ajiboye, B., Akinremi, O.O., Hu, Y., Flaten, D.N. (2007): Phosphorus Speciation of Sequential Extracts of Organic Amendments Using Nuclear Magnetic Resonance and X-ray Absorption Near-Edge Structure Spectroscopies. *J. Environ. Qual.* 36, 1563.

Alborés, A.F., Cid, B.P., Gómez, E.F., López, E.F. (2000): Comparison between sequential extraction procedures and single extractions for metal partitioning in sewage sludge samples. *Analyst* 125, 1353–1357.

Alvarenga, P., Mourinha, C., Farto, M., Santos, T., Palma, P., Sengo, J., Morais, M.-C., Cunha-Queda, C. (2015): Sewage sludge, compost and other representative organic wastes as agricultural soil amendments: Benefits versus limiting factors.

Alvarez, A.E., Mochon, C.M., Sanchez, J.C., Rodriguez, T.M. (2002): Heavy metal extractable forms in sludge from wastewater treatment plants. *Chemosphere* 47, 765–775.

Andersen, M.S. (2007): An introductory note on the environmental economics of the circular economy. *Sustain. Sci.* 2, 133–140.

Ayaga, G., Todd, A., Brookes, P.C. (2006): Enhanced biological cycling of phosphorus increases its availability to crops in low-input sub-Saharan farming systems. *Soil Biol. Biochem.* 38, 81–90.

Baek, D., Kim, M.C., Chun, H.J., Kang, S., Park, H.C., Shin, G., Park, J., Shen, M., Hong, H., Kim, W.-Y., Kim, D.H., Lee, S.Y., Bressan, R.A., Bohnert, H.J., Yun, D.-J. (2013): Regulation of miR399f Transcription by AtMYB2 Affects Phosphate Starvation Responses in Arabidopsis. *Plant Physiol.* 161, 362–373.

Baldwin, J.C., Karthikeyan, A.S., Raghothama, K.G. (2001): LEPS2, a phosphorus starvation-induced novel acid phosphatase from tomato. *Plant Physiol.* 125, 728–37.

Bates, T.R., Lynch, J.P. (1996): Stimulation of root hair elongation in Arabidopsis thaliana by low phosphorus availability. *Plant, Cell Environ.* 19, 529–538.

Becana, M., Moran, J.F., Iturbe-Ormaetxe, I. (1998): Iron-dependent oxygen free radical generation in plants subjected to environmental stress: Toxicity and antioxidant protection. *Plant Soil* 201, 137–147.

Becker, M., Asch, F. (2005): Iron toxicity in rice-conditions and management concepts.

J. Plant Nutr. Soil Sci. 168, 558–573.

Bernal, M.P., Alburquerque, J.A., Moral, R. (2009): Composting of animal manures and chemical criteria for compost maturity assessment. A review. *Bioresour. Technol.* 100, 5444–5453.

Bhadoria, P.S., Steingrobe, B., Claassen, N., Liebersbach, H. (2002): Phosphorus efficiency of wheat and sugar beet seedlings grown in soils with mainly calcium, or iron and aluminium phosphate. *Plant Soil* 246, 41–52.

Biswas, B.K., Inoue, K., Harada, H., Ohto, K., Kawakita, H. (2009): Leaching of phosphorus from incinerated sewage sludge ash by means of acid extraction followed by adsorption on orange waste gel. *J. Environ. Sci.* 21, 1753–1760.

Blöcher, C., Niewersch, C., Melin, T. (2012): Phosphorus recovery from sewage sludge with a hybrid process of low pressure wet oxidation and nanofiltration. *Water Res.* 46, 2009–2019.

Bolan, N.S. (1991): A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. *Plant Soil*.

Breeuwsma, A., Reijerink, J.G.A., Schoumans, O.F. (1995): Impact of manure on accumulation and leaching of phosphate in areas of intensive livestock farming, in: Animal Waste and the Land-Water Interface. pp. 239–249.

Brod, E., Øgaard, A.F., Hansen, E., Wragg, D., Haraldsen, T.K., Krogstad, T. (2015): Waste products as alternative phosphorus fertilisers part I: inorganic P species affect fertilisation effects depending on soil pH. *Nutr. Cycl. Agroecosystems* 103, 167–185.

Brookes, P., Beyaert, R., Voroney, R. (2007): Soil Microbial Biomass C, N, P, and S, in: Soil Sampling and Methods of Analysis, Second Edition. CRC Press.

Brookes, P.C., Powlson, D.S., Jenkinson, D.S. (1982): Measurement of microbial biomass phosphorus in soil. *Soil Biol. Biochem.* 14, 319–329.

Brown, T.A., Shrift, A. (1982): SELENIUM: TOXICITY AND TOLERANCE IN HIGHER PLANTS. *Biol. Rev.* 57, 59–84.

Bünemann, E.K. (2015): Assessment of gross and net mineralization rates of soil organic phosphorus – A review. *Soil Biol. Biochem.* 89, 82–98.

Burleigh, S.H., Cavagnaro, T., Jakobsen, I. (2002): Functional diversity of arbuscular mycorrhizas extends to the expression of plant genes involved in P nutrition. *J. Exp. Bot.* 53, 1593–1601.

Cakmak, I., Hengeler, C., Marschner, H. (1994): Partitioning of shoot and root dry matter and carbohydrates in bean plants suffering from phosphorus, potassium and magnesium deficiency. *J. Exp. Bot.* 45, 1245–1250.

CCIA (2016): Prezzi - News — Camera di Commercio di Modena [WWW Document]. URL http://www.mo.camcom.it/tutela-del-mercato/ufficio-prezzi (accessed 11.9.16).

Chen, C.R., Condron, L.M., Davis, M.R., Sherlock, R.R. (2002): Phosphorus dynamics in the rhizosphere of perennial ryegrass (Lolium perenne L.) and radiata pine (Pinus Radiata D. Don.). *Soil Biol. Biochem.* 34, 487–499.

Childers, D.L., Corman, J., Edwards, M., Elser, J.J. (2011): Sustainability Challenges

of Phosphorus and Food: Solutions from Closing the Human Phosphorus Cycle. *Bioscience* 61, 117–124.

Christodoulou, A., Stamatelatou, K. (2016): Overview of legislation on sewage sludge management in developed countries worldwide. *Water Sci. Technol.* 73.

Cieslik, B., Konieczka, P. (2017): A review of phosphorus recovery methods at various steps of wastewater treatment and sewage sludge management. The concept of " no solid waste generation " and analytical methods. *J. Clean. Prod.* 142, 1728–1740.

Clarkson, D.T., Scattergood, C.B. (1982): Growth and Phosphate Transport in Barley and Tomato Plants During the Development of, and Recovery from, Phosphate-stress. *J. Exp. Bot.* 33, 865–875.

Cogliatti, D.H., Santa Marìa, G.E. (1990): Influx and Efflux of Phosphorus in Roots of Wheat Plants in Non-Growth-Limiting Concentrations of Phosphorus. *J. Exp. Bot.* 41, 601–607.

Cooper, J., Lombardi, R., Boardman, D., Carliell-Marquet, C. (2011): The future distribution and production of global phosphate rock reserves. *Resour. Conserv. Recycl.* 57, 78–86.

Cordell, D., Drangert, J., White, S. (2009): The story of phosphorus: global food security and food for thought. *Glob. Environ. Chang.*

Cordell, D., Rosemarin, A., Schroder, J.J., Smit, A.L. (2011): Towards global phosphorus security: A systems framework for phosphorus recovery and reuse options. *Chemosphere* 84, 747–758.

Corominas, L., Foley, J., Guest, J.S., Hospido, A., Larsen, H.F., Morera, S., Shaw, A. (2013): Life cycle assessment applied to wastewater treatment: State of the art. *Water Res.* 47, 5480–5492.

Da Ros, C., Cavinato, C., Pavan, P., Bolzonella, D. (2016): Mesophilic and thermophilic anaerobic co-digestion of winery wastewater sludge and wine lees: An integrated approach for sustainable wine production. *J. Environ. Manage.*

Dakora, F.D., Phillips, D.A. (2002): Root exudates as mediators of mineral acquisition in low-nutrient environments. *Plant Soil* 245, 35–47.

Delhaize, E., Randall, P.J., Laurie, S., Phillips, R., Ratcliffe, R., Stewart, G. (1995): Characterization of a Phosphate-Accumulator Mutant of Arabidopsis thaliana. *Plant Physiol.* 107, 207–213.

Direzione Generale Ambiente, D. del suolo e della costa-D.A.R.E.-R. (2004): Linee di Intervento in materia di utilizzo dei fanghi di depurazione in agricoltura. Bologna.

Domenech, C.E., Lisa, T.A., Salvano, M.A., Garrido, M.N. (2015): Selective release of phosphorus and nitrogen from waste activated sludge with combined thermal and alkali treatment. *Bioresour. Technol.* 190, 522–528.

Donatello, S., Tong, D., Cheeseman, C.R. (2010): Production of technical grade phosphoric acid from incinerator sewage sludge ash (ISSA). *Waste Manag.* 30, 1634–1642.

Doyle, J.D., Parsons, S.A. (2002): Struvite formation, control and recovery. *Water Res.* 36, 3925–3940.

Duboc, O., Santner, J., Fard, A.G., Zehetner, F., Tacconi, J., Wenzel, W.W. (2017): Predicting phosphorus availability from chemically diverse conventional and recycling fertilizers. *Sci. Total Environ.* 599–600, 1160–1170.

Easlon, H.M., Bloom, A.J. (2014): Easy Leaf Area: Automated Digital Image Analysis for Rapid and Accurate Measurement of Leaf Area. *Appl. Plant Sci.* 2, 1400033.

Eghball, B. (2002): Soil Properties as Influenced by Phosphorus-and Nitrogen-Based Manure and Compost Applications WASTE MANAGEMENT Soil Properties as Influenced by Phosphorus-and Nitrogen-Based Manure and Compost Applications. *Waste Manag.* 94, 128–135.

Eghball, B., Power, J.F. (1999): Phosphorus- and Nitrogen-Based Manure and Compost Applications: Corn Production and Soil Phosphorus. *Soil Sci. Soc. Am. J.* 63, 895–901.

Ellis, D.R., Salt, D.E. (2003): Plants, selenium and human health. *Curr. Opin. Plant Biol.*

Eneji, A.E., Honna, T., Yamamoto, S., Masuda, T., Endo, T., Irshad, M. (2003): Changes in Humic Substances and Phosphorus Fractions During Composting. *Commun. Soil Sci. Plant Anal.* 34, 2303–2314.

European Biogas Association (2015): EBA Biomethane & Biogas Report 2015 [WWW Document]. URL http://european-biogas.eu/2015/12/16/biogasreport2015/ (accessed 11.9.16).

Eurostat (2016): Eurostat [WWW Document]. URL http://ec.europa.eu/eurostat/web/main/home (accessed 11.9.16).

Ewa Sroga, G. (1987): Plant regeneration of two Lupinus spp. from callus cultures via organogenesis. *Plant Sci.* 51, 245–249.

FAO (2016): FAOSTAT [WWW Document]. URL http://www.fao.org/faostat/en/#home (accessed 12.2.17).

Feng, R., Wei, C., Tu, S. (2013): The roles of selenium in protecting plants against abiotic stresses. *Environ. Exp. Bot.*

Fertilisers Working Group Meeting (2014): Essential safety and quality requirements for fertilising materials.

Foehse, D., Jungk, A. (1983): Influence of phosphate and nitrate supply on root hair formation of rape, spinach and tomato plants. *Plant Soil* 74, 359–368.

Fredeen, A., Rao, I., Terry, N. (1989): Influence of phosphorus nutrition on growth and carbon partitioning in Glycine max. *Plant Physiol.*

Frossard, E., Condron, L.M., Oberson, A., Sinaj, S., Fardeau, J.C. (2000): Processes Governing Phosphorus Availability in Temperate Soils. *J. Environ. Qual.* 29, 15.

Frossard, E., Sinaj, S., Zhang, L.-M., Morel, J.L. (1996): The Fate of Sludge Phosphorus in Soil-Plant Systems. *Soil Sci. Soc. Am. J.* 60, 1248.

Fuentes, B., Bolan, N., Naidu, R., de la Luz Mora, M., Australia, A. (2006): PHOSPHORUS IN ORGANIC WASTE-SOIL SYSTEMS Fósforo en los sistemas suelo – residuos orgánicos. *J.Soil Sc. Plant. Nutr* 66, 64–8364.

García-Albacete, M., Martín, A., Cartagena, M.C. (2012): Fractionation of phosphorus biowastes: Characterisation and environmental risk. *Waste Manag.*

32, 1061–1068.

Gianquinto, G., Orsini, F., Sambo, P., D'Urzo, M.P. (2011): The Use of Diagnostic Optical Tools to Assess Nitrogen Status and to Guide Fertilization of Vegetables. *Horttechnology* 21, 287–292.

Gonzalez-Ponce, R., López-de-Sá, E.G., Plaza, C. (2009): Lettuce Response to Phosphorus Fertilization with Struvite Recovered from Municipal Wastewater. *HortScience* 44, 426–430.

Grigatti, M., Boanini, E., Cavani, L., Ciavatta, C., Marzadori, C. (2015): Phosphorus in Digestate-Based Compost: Chemical Speciation and Plant-Availability. *Waste and Biomass Valorization* 6, 481–493.

Grigatti, M., Boanini, E., Mancarella, S., Simoni, A., Centemero, M., Veeken, A.H.M. (2017): Phosphorous extractability and ryegrass availability from bio-waste composts in a calcareous soil. *Chemosphere* 174, 722–731.

Grigatti, M., Di Girolamo, G., Chincarini, R., Ciavatta, C., Barbanti, L. (2011): Potential nitrogen mineralization, plant utilization efficiency and soil CO2 emissions following the addition of anaerobic digested slurries. *Biomass and Bioenergy* 35, 4619–4629.

Grusak, M. a., DellaPenna, D. (1999): Improving the Nutrient Composition of Plants To Enhance Human Nutrition and Health1. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 50, 133–161.

Guppy, C., Menzies, N., Moody, P., Blamey, F. (2005): Competitive sorption reactions between phosphorus and organic matter in soil: a review. *Soil Res.*

Haug, R.T. (1993): The practical handbook of compost engineering. Lewis Publishers.

Heimersson, S., Harder, R., Peters, G.M., Svanström, M. (2014): Including pathogen risk in life cycle assessment of wastewater management. 2. Quantitative comparison of pathogen risk to other impacts on human health. *Environ. Sci. Technol.* 48, 9446–9453.

Herzel, H., Kruger, O., Hermann, L., Adam, C. (2016): Sewage sludge ash - A promising secondary phosphorus source for fertilizer production. *Sci. Total Environ.* 542, 1136–1143.

Hinsinger, P. (2001): Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. *Plant Soil* 237, 173–195.

Hooda, P.S., Truesdale, V.W., Edwards, A.C., Withers, P.J.A., Aitken, M.N., Miller, A., Rendell, A.R. (2001): Manuring and fertilization effects on phosphorus accumulation in soils and potential environmental implications. *Adv. Environ. Res.* 5, 13–21.

Hospido, A., Moreira, M.T., Feijoo, G. (2008): A comparison of municipal wastewater treatment plants for big centres of population in Galicia (Spain). *Int. J. Life Cycle Assess.* 13, 57–64.

Hukari, S., Hermann, L., Nättorp, A. (2016): From wastewater to fertilisers — Technical overview and critical review of European legislation governing phosphorus recycling. *Sci. Total Environ.* 542, 1127–1135.

Hunter, P.J., Teakle, G.R., Bending, G.D. (2014): Root traits and microbial community interactions in relation to phosphorus availability and acquisition, with particular

reference to Brassica. *Front. Plant Sci.* 5, 27.

Indexmundi (2016): DAP fertilizer - Monthly Price - Commodity Prices - Price Charts, Data, and News - IndexMundi [WWW Document]. URL http://www.indexmundi.com/commodities/?commodity=dap-fertilizer&months=180 (accessed 11.9.16).

Jaffer, Y., Clark, T.A., Pearce, P., Parsons, S.A. (2002): Potential phosphorus recovery by struvite formation. *Water Res.* 36, 1834–1842.

Jeng, A.S., Haraldsen, T.K., Grønlund, A., Pedersen, P.A. (2006): Meat and bone meal as nitrogen and phosphorus fertilizer to cereals and rye grass. *Nutr. Cycl. Agroecosystems* 76, 183–191.

Johri, A.K., Oelmüller, R., Dua, M., Yadav, V., Kumar, M., Tuteja, N., Varma, A., Bonfante, P., Persson, B.L., Stroud, R.M. (2015): Fungal association and utilization of phosphate by plants: success, limitations, and future prospects. *Front. Microbiol.* 6, 984.

Jones, D.L. (1998): Organic acids in the rhizosphere – a critical review. *Plant Soil* 205, 25–44.

Kabata-Pendias, A. (2011): Trace elements in soils and plants. Fourth Edition, CRC Press.

Kataki, S., West, H., Clarke, M., Baruah, D.C. (2016): Phosphorus recovery as struvite: Recent concerns for use of seed, alternative Mg source, nitrogen conservation and fertilizer potential. *Resour. Conserv. Recycl.* 107, 142–156.

Khan, A., Jilani, G., Akhtar, M.S., Naqvi, S.M.S., Rasheed, M., Khan, A.A., Jilani, G., Akhtar, M.S., Muhammad, S., Naqvi, S., Rasheed, M. (2009): Phosphorus Solubilizing Bacteria: Occurrence, Mechanisms and their Role in Crop Production. *Publ. J. agric. biol. sci* 1, 48–58.

Khan, K.S., Joergensen, R.G. (2012a): Relationships between P fractions and the microbial biomass in soils under different land use management. *Geoderma* 173, 274–281.

Khan, K.S., Joergensen, R.G. (2012b): Compost and phosphorus amendments for stimulating microorganisms and growth of ryegrass in a Ferralsol and a Luvisol. *J. Plant Nutr. Soil Sci.* 175, 108–114.

Kidd, P.S., Domínguez-Rodríguez, M.J., Díez, J., Monterroso, C. (2007): Bioavailability and plant accumulation of heavy metals and phosphorus in agricultural soils amended by long-term application of sewage sludge. *Chemosphere* 66, 1458–1467.

Kim, M., Han, D.W., Kim, D.J. (2015): Selective release of phosphorus and nitrogen from waste activated sludge with combined thermal and alkali treatment. *Bioresour. Technol.*

Kleinman, P.J.A., Sharpley, A.N., Wolf, A.M., Beegle, D.B., Moore, P.A. (2002): Measuring Water-Extractable Phosphorus in Manure as an Indicator of Phosphorus in Runoff. *Soil Sci. Soc. Am. J.* 66, 2009.

Kochian, L. V (1995): Cellular Mechanisms of Aluminum Toxicity and Resistance in Plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 46, 237–260.

Koppelaar, R.H.E.M., Weikard, H.P. (2013): Assessing phosphate rock depletion and

phosphorus recycling options. *Glob. Environ. Chang.* 23, 1454–1466.

Krüger, O., Adam, C. (2015): Recovery potential of German sewage sludge ash. *Waste Manag.* 45, 400–406.

Kumar, V., Gilkes, R.J., Bolland, M.D.A. (1992): A comparison of seven soil P tests for plant species with different external P requirements grown on soils containing rock phosphate and superphosphate residues. *Fertil. Res.* 33, 35–45.

Lambers, H., Brundrett, M.C., Raven, J.A., Hopper, S.D. (2011): Plant mineral nutrition in ancient landscapes: high plant species diversity on infertile soils is linked to functional diversity for nutritional strategies. *Plant Soil* 348, 7–27.

Laturnus, F., von Arnold, K., Grøn, C. (2007): Organic Contaminants from Sewage Sludge Applied to Agricultural Soils. False Alarm Regarding Possible Problems for Food Safety? (8 pp). *Environ. Sci. Pollut. Res. Int.* 14 Suppl 1, 53–60.

Leite, W.R.M., Gottardo, M., Pavan, P., Belli Filho, P., Bolzonella, D. (2016): Performance and energy aspects of single and two phase thermophilic anaerobic digestion of waste activated sludge. *Renew. Energy* 86, 1324–1331.

Leytem, A.B., Westermann, D.T. (2003): PHOSPHATE SORPTION BY PACIFIC NORTHWEST CALCAREOUS SOILS. *Soil Sci.* 168.

Leytem, A.B., Westermann, D.T. (2005): PHOSPHORUS AVAILABILITY TO BARLEY FROM MANURES AND FERTILIZERS ON A CALCAREOUS SOIL. *Soil* 170, 401–412.

Liang, Y., Sun, W., Zhu, Y.G., Christie, P. (2007): Mechanisms of silicon-mediated alleviation of abiotic stresses in higher plants: A review. *Environ. Pollut.* 147, 422–428.

López-Arredondo, D.L., Leyva-González, M.A., Alatorre-Cobos, F., Herrera-Estrella, L. (2013): Biotechnology of nutrient uptake and assimilation in plants. *Int. J. Dev. Biol.* 57, 595–610.

Lynch, J., Läuchli, A., Epstein, E. (1991): Vegetative Growth of the Common Bean in Response to Phosphorus Nutrition. *Crop Sci.* 31, 380.

Ma, J.F. (2004): Role of silicon in enhancing the resistance of plants to biotic and abiotic stresses. *Soil Sci. Plant Nutr.* 50, 11–18.

Ma, Z., Bielenberg, D.G., Brown, K.M., Lynch, J.P. (2001): Regulation of root hair density by phosphorus availability in Arabidopsis thaliana. *Plant, Cell Environ.* 24, 459–467.

Malik, M., Khan, K., Marschner, P. (2013): Microbial biomass, nutrient availability and nutrient uptake by wheat in two soils with organic amendments. *J. Soil Sci. Plant Nutr.* 13, 955–966.

Mamani, I., Averanga, Kattia, Boris, E., Terrazas, E. (2014): Establecimiento del sistema de generación in vitro de callos de Lupinus mutabilis sweet (tarwi) a partir de hipocótilos. *Rev. CON-CIENCIA* 2.

Marchetti, R., Castelli, F., Orsi, A., Sghedoni, L., Bochicchio, D. (2012): Biochar from swine manure solids: influence on carbon sequestration and Olsen phosphorus and mineral nitrogen dynamics in soil with and without digestate incorporation. *Ital. J. Agron.* 7, 26.

Marschner, H., Kirkby, E.A., Cakmak, I. (1996): Effect of mineral nutritional status on shoot-root partitioning of photoassimilates and cycling of mineral nutrients. *J. Exp. Bot.* 47, 1255–1263.

Marschner, H., Marschner, P. (2012): Marschner's mineral nutrition of higher plants. Academic Press.

Mattenberger, H., Fraissler, G., Brunner, T., Herk, P., Hermann, L., Obernberger, I. (2008): Sewage sludge ash to phosphorus fertiliser: Variables influencing heavy metal removal during thermochemical treatment. *Waste Manag.* 28, 2709–2722.

Mayer, B.K., Baker, L.A., Boyer, T.H., Drechsel, P., Gifford, M., Hanjra, M.A., Parameswaran, P., Stoltzfus, J., Westerhoff, P., Rittmann, B.E. (2016): Total Value of Phosphorus Recovery. *Environ. Sci. Technol.* 50, 6606–6620.

McBride, M., Sauve, S., Hendershot, W. (1997): Solubility control of Cu, Zn, Cd and Pb in contaminated soils. *Eur. J. Soil Sci.* 48, 337–346.

McCoy, J.L., Sikora, L.J., Weil, R.R. (1986): Plant Availability of Phosphorus in Sewage Sludge Compost1. *J. Environ. Qual.* 15, 403.

Møller, J., Boldrin, A., Christensen, T.H. (2009): Anaerobic digestion and digestate use: accounting of greenhouse gases and global warming contribution. *Waste Manag. Res.* 27, 813–824.

Möller, K., Müller, T. (2012): Effects of anaerobic digestion on digestate nutrient availability and crop growth: A review. *Eng. Life Sci.* 12, 242–257.

Mosier, N.S., Ladisch, C.M., Ladisch, M.R. (2002): Characterization of acid catalytic domains for cellulose hydrolysis and glucose degradation. *Biotechnol. Bioeng.* 79, 610–618.

Muhammad, S., Müller, T., Joergensen, R.G. (2006): Decomposition of pea and maize straw in Pakistani soils along a gradient in salinity. *Biol. Fertil. Soils* 43, 93–101.

Murphy, J., Riley, J.P. (1962): A modified single solution method for the determination of phosphate in natural waters. *Anal. Chim. Acta* 27, 31–36.

Nagul, E.A., McKelvie, I.D., Worsfold, P., Kolev, S.D. (2015): The molybdenum blue reaction for the determination of orthophosphate revisited: Opening the black box. *Anal. Chim. Acta*.

Nannipieri, P., Giagnoni, L., Landi, L., Renella, G. (2011): Role of Phosphatase Enzymes in Soil, in: Phosphorus in Action. Springer, Berlin, Heidelberg, pp. 215–243.

Neumann, G. (2010): Mining for nutrients - regulatory aspects of cluster root function and development. *New Phytol.* 187, 879–882.

Neumann, G. (2016): The Role of Ethylene in Plant Adaptations for Phosphate Acquisition in Soils – A Review. *Front. Plant Sci.* 6, 1224.

Neumann, G., Martinoia, E. (2002): Cluster roots – an underground adaptation for survival in extreme environments. *Trends Plant Sci.* 7, 162–167.

Nkebiwe, P.M., Weinmann, M., Bar-Tal, A., Müller, T. (2016): Fertilizer placement to improve crop nutrient acquisition and yield: A review and meta-analysis. *F. Crop. Res.* 196, 389–401.

Øgaard, A.F. (1996): Effect of Phosphorus Fertilization on the Concentration of Total

and Algal-available Phosphorus in Different Particle-size Fractions in Norwegian Agricultural Soils. *Acta Agric. Scand. Sect. B - Soil Plant Sci.* 46, 24–29.

Olsen, S.R. (1954): Estimation Of Available Phosphorus In Soils By Extraction With Sodium Bicarbonate.

Ottosen, L.M., Kirkelund, G.M., Jensen, P.E. (2013): Extracting phosphorous from incinerated sewage sludge ash rich in iron or aluminum. *Chemosphere* 91, 963–969.

Pant, H.K., Warman, P.R. (2000): Enzymatic hydrolysis of soil organic phosphorus by immobilized phosphatases. *Biol. Fertil. Soils* 30, 306–311.

Pasqualino, J.C., Meneses, M., Abella, M., Castells, F. (2009): LCA as a Decision Support Tool for the Environmental Improvement of the Operation of a Municipal Wastewater Treatment Plant. *Environ. Sci. Technol.* 43, 3300–3307.

Pastor, L., Marti, N., Bouzas, A., Seco, A. (2008): Sewage sludge management for phosphorus recovery as struvite in EBPR wastewater treatment plants. *Bioresour. Technol.* 99, 4817–4824.

Petronici, C. (1989): Gli elementi nutritivi, in: Paolo Sequi (ed.): Chimica Del Suolo. Patron Editore, Bologna, pp. 357–380.

Pniewski, T., Kapusta, J., Płucienniczak, A. (2006): Agrobacterium-mediated transformation of yellow lupin to generate callus tissue producing HBV surface antigen in a long-term culture. *J. Appl. Genet.* 47, 309–318.

Polowick, P.L., Loukanina, N.N., Doshi, K.M. (2014): Agrobacterium-mediated transformation of tarwi (Lupinus mutabilis Sweet), a potential platform for the production of plant-made proteins. *Vitr. Cell. Dev. Biol. - Plant* 50, 401–411.

Pote, D.H., Daniel, T.C., Sharpley, A.N., Moore, P.A., Edwards, D.R., Nichols, D.J. (1996): Relating Extractable Soil Phosphorus to Phosphorus Losses in Runoff t'. *Soil Sci. Soc. Am. J.* 60, 855–859.

Raghothama, K.G. (1999): PHOSPHATE ACQUISITION. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 50, 665–693.

Rahman, M.M., Salleh, M.A.M., Rashid, U., Ahsan, A., Hossain, M.M., Ra, C.S. (2014): Production of slow release crystal fertilizer from wastewaters through struvite crystallization – A review. *Arab. J. Chem.* 7, 139–155.

Reijnders, L. (2014): Phosphorus resources, their depletion and conservation, a review. *Resour. Conserv. Recycl.*

Richardson, A.E., Simpson, R.J. (2011): Soil microorganisms mediating phosphorus availability update on microbial phosphorus. *Plant Physiol.* 156, 989–96.

Russo, A. (2012): ANALISI E MIGLIORAMENTO DELL'IMPIANTO DI DEPURAZIONE DI LEGNAGO. Università degli Studi di Padova.

Shannon, M.C., Grieve, C.M. (1999): Tolerance of vegetable crops to salinity. *Sci. Hortic. (Amsterdam).* 78, 5–38.

Sharpley, A., Moyer, B. (2000): Phosphorus Forms in Manure and Compost and Their Release during Simulated Rainfall. *J. Environ. Qual.* 29, 1462.

Sharpley, A.N., Chapra, S.C., Wedepohl, R., Sims, J.T., Daniel, T.C., Reddy, K.R. (1994): Managing Agricultural Phosphorus for Protection of Surface Waters:

Issues and Options. *J. Environ. Qual.* 23, 437.

Shen, J., Yuan, L., Zhang, J., Li, H., Bai, Z., Chen, X., Zhang, W., Zhang, F. (2011): Phosphorus dynamics: from soil to plant. *Plant Physiol.* 156, 997–1005.

Shenoy, V. V., Kalagudi, G.M. (2005): Enhancing plant phosphorus use efficiency for sustainable cropping. *Biotechnol. Adv.* 23, 501–513.

Siddique, M.T., Robinson, J.S. (2003): Phosphorus Sorption and Availability in Soils Amended with Animal Manures and Sewage Sludge. *J. Environ. Qual.* 32, 1114.

Simpson, M., McLenaghen, R.D., Chirino-Valle, I., Condron, L.M. (2012): Effects of long-term grassland management on the chemical nature and bioavailability of soil phosphorus. *Biol. Fertil. Soils* 48, 607–611.

Sims, J.T., Simard, R.R., Joern, B.C. (1998): Phosphorus Loss in Agricultural Drainage: Historical Perspective and Current Research. *J. Environ. Qual.* 27, 277.

Smith, K.A., Van Dijk, T.A. (1987): Utilisation of phosphorus and potassium from animal manures on grassland and forage crops, in: Animal Manure on Grassland and Fodder Crops. Fertilizer or Waste? Springer Netherlands, Dordrecht, pp. 87–102.

Smith, S.R. (2009): A critical review of the bioavailability and impacts of heavy metals in municipal solid waste composts compared to sewage sludge. *Environ. Int.* 35, 142–156.

Sonneveld, C., Straver, N. (1994): Nutrient solutions for vegetables and flowers grown in water or substrates, Tenth. ed.

Stewart, J.W.B., Tiessen, H. (1987): Dynamics of soil organic phosphorus. *Biogeochemistry* 4, 41–60.

Suh, Y.J., Rousseaux, P. (2002): An LCA of alternative wastewater sludge treatment scenarios. *Resour. Conserv. Recycl.* 35, 191–200.

Syers, D.J., Johnston, A. (2008): Efficiency of soil and fertilizer phosphorus use Reconciling changing concepts of soil phosphorus behaviour with agronomic information. *FAO Fertil. Plant Nutr. Bull.*

Tabatabai, M.A., Bremner, J.M. (1969): Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biol. Biochem.* 1, 301–307.

Takahashi, M., Kato, S., Shima, H., Sarai, E., Ichioka, T., Hatyakawa, S., Miyajiri, H. (2001): Technology for recovering phosphorus from incinerated wastewater treatment sludge, in: Chemosphere. pp. 23–29.

Takahashi, S. (2013): Phosphorus characterization of manure composts and combined organic fertilizers by a sequential-fractionation method. *J. Plant Nutr. Soil Sci.* 176, 494–496.

Talboys, P.J., Heppell, J., Roose, T., Healey, J.R., Jones, D.L., Withers, P.J.A. (2016): Struvite: a slow-release fertiliser for sustainable phosphorus management? *Plant Soil* 401, 109–123.

Tang, X., Li, J., Ma, Y., Hao, X., Li, X. (2008): Phosphorus efficiency in long-term (15 years) wheat–maize cropping systems with various soil and climate conditions. *F. Crop. Res.* 108, 231–237.

Tarayre, C., De Clercq, L., Charlier, R., Michels, E., Meers, E., Camargo-Valero, M.,

Delvigne, F. (2016): New perspectives for the design of sustainable bioprocesses for phosphorus recovery from waste. *Bioresour. Technol.*

Tejada, M., García-Martínez, A.M., Rodríguez-Morgado, B., Carballo, M., García-Antras, D., Aragón, C., Parrado, J. (2013): Obtaining biostimulant products for land application from the sewage sludge of small populations. *Ecol. Eng.* 50, 31–36.

Toor, G.S., Peak, J.D., Sims, J.T. (2005): Phosphorus Speciation in Broiler Litter and Turkey Manure Produced from Modified Diets. *J. Environ. Qual.* 34, 687.

Torri, S.I., Correa, R.S., Renella, G. (2017): Biosolid Application to Agricultural Land—a Contribution to Global Phosphorus Recycle: A Review. *Pedosphere* 27, 1–16.

Turner, B.L., Cade-Menun, B.J., Condron, L.M., Newman, S. (2005): Extraction of soil organic phosphorus. *Talanta* 66, 294–306.

Turner, B.L., McKelvie, I.D., Haygarth, P.M. (2002): Characterisation of water-extractable soil organic phosphorus by phosphatase hydrolysis. *Soil Biol. Biochem.* 34, 27–35.

Uhde-Stone, C., Liu, J., Zinn, K.E., Allan, D.L., Vance, C.P. (2005): Transgenic proteoid roots of white lupin: a vehicle for characterizing and silencing root genes involved in adaptation to P stress. *Plant J.* 44, 840–853.

Uysal, A., Dilsad Yilmazel, Y., Demirer, G.N. (2010): The determination of fertilizer quality of the formed struvite from effluent of a sewage sludge anaerobic digester. *J. Hazard. Mater.* 181, 248–254.

Vance, C.P. (2001): Symbiotic Nitrogen Fixation and Phosphorus Acquisition. Plant Nutrition in a World of Declining Renewable Resources. *Plant Physiol.* 127.

Vance, C.P., Uhde-Stone, C., Allan, D.L. (2003): Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. *New Phytol.* 157, 423–447.

Veneklaas, E.J., Lambers, H., Bragg, J., Finnegan, P.M., Lovelock, C.E., Plaxton, W.C., Price, C.A., Scheible, W.-R., Shane, M.W., White, P.J., Raven, J.A. (2012): Opportunities for improving phosphorus-use efficiency in crop plants. *New Phytol.* 195, 306–320.

Walan, P., Davidsson, S., Johansson, S., Höök, M. (2014): Phosphate rock production and depletion: Regional disaggregated modeling and global implications. *Resour. Conserv. Recycl.* 93, 178–187.

Wang, Z., Straub, D., Yang, H., Kania, A., Shen, J., Ludewig, U., Neumann, G. (2014): The regulatory network of cluster-root function and development in phosphate-deficient white lupin (*Lupinus albus*) identified by transcriptome sequencing. *Physiol. Plant.* 151, 323–338.

Wellinger, A., Murphy, J., Baxter, D. (2013): The biogas handbook : science, production and applications.

Wheeler, B.D., Al-Farraj, M.M., Cook, R.E.D. (1985): Iron toxicity to plants in base-rich wetlands: comparative effects on the distribution and growth of Epilobium hirsutum l. and Juncus subnodulosus Schrank. *New Phytol.* 100, 653–669.

White, P.J., Brown, P.H. (2010): Plant nutrition for sustainable development and

global health. *Ann. Bot.* 105, 1073–1080.

Wollmann, I., Gauro, A., Müller, T., Möller, K. (2017): Phosphorus bioavailability of sewage sludge-based recycled fertilizers. *J. Plant Nutr. Soil Sci.*

Xu, H., He, P., Gu, W., Wang, G., Shao, L. (2012): Recovery of phosphorus as struvite from sewage sludge ash. *J. Environ. Sci. (China)* 24, 1533–1538.

Ye, Y., Ngo, H.H., Guo, W., Liu, Y., Li, J., Liu, Y., Zhang, X., Jia, H., Ngo, H.H. (2016): Insight into chemical phosphate recovery from municipal wastewater. *Sci. Total Environ.* 576, 159–171.

Zang, Y., Li, Y., Wang, C., Zhang, W., Xiong, W. (2015): Towards more accurate life cycle assessment of biological wastewater treatment plants: A review. *J. Clean. Prod.*

Zhu, J., Li, M., Whelan, M., Gan, J. (2017): Phosphorus activators contribute to legacy phosphorus availability in agricultural soils: A review. *Sci. Total Environ.* 612, 522–537.

Chapter 7

Conclusions

Nowadays, the cycle of phosphorus is open with high inputs for agriculture from mineral sources and high losses, in P leaching from soil erosion, in agricultural residues, in food waste and ultimately, but probably the most affecting for the communities, in the civil wastewater and animal excreta. Circular economy strategy suggest reusing the waste as the primary input. In the case of P, this is possible, due to the high availability of P-rich waste, and recommended, because mineral source of P, phosphate rocks, is a non-renewable and limited resource. Furthermore, the P fertilizers have generally a very low P use efficiency, because phosphorus is quickly ligated to the soil components when applied to the field. The soil pH and the metal concentration can change the P availability in soil: in acid soil P is easily bonded with Al and Fe, while in alkaline soil it can precipitate with carbonates (Ca) or Mg. Organic waste, or bio-waste, can be naturally rich in P or, in case of the sewage sludge, artificially enriched due to the need to precipitate P from wastewater to avoid the P contamination of the water basins and the consequent eutrophication. Diverse possibilities for an alternative P fertilization to phosphate rocks had been tested. Between the bio-waste, which are animal manure, digestates and compost the P concentration but mostly the P solubility vary highly. In chap. 2 the solubility and the suitability of different bio-waste as P fertilizer has been tried on soil and on ryegrass. Many are the product characteristics that can be useful to predict the P solubility. The easiest and cheaper is the metals, mostly Al and Fe, concentration. The highest is the Al:P, Fe:P or Ca:P ratio the lowest will be the P solubility. Between the tested products, thus, the most soluble were the swine slurries and the biodigestate (deriving from swine slurries). However, although the products had very different concentration in Al and Fe, those were not reflected in the plant metal content. This occurred because the calcareous soil used in our experiment was the first and the strongest sink of these metals. Microbial biomass can immobilize P, but we have seen that generally there is no competition between plants and microbial biomass for P uptake. Rather, the microbial biomass is able to uptake less soluble P and protect it from soil precipitation and, in the long term, to release it for plant assimilation. Thus, also the high organic matter of the product can be useful for the P solubilisation, because it improve the microbial growth and the consequent microbial protection of P. In addition, the organic matter can complexed the P and also avoid the P precipitation with Ca. In addition, the metal content can protect P from Ca precipitation, in calcareous soils, and release them gradually but constantly. Thus, for the short-term fertilization, products with low Al,

Fe and Ca, such as swine slurries or derived swine slurries products, are desirable, but for long-term fertilization Al and Fe-rich products, such as compost, or even better, products with high organic C, such as bovine manure, which can improve microbial P immobilization and postpone gradual mineralization, are recommended.

Later, our research focused on sewage sludge. Their massive production, high P concentration and difficult disposal make them a very good candidate for the circular economy strategy. Many studies are focused on the use of sewage ash or the P recovery through struvite crystallization. However, in many countries, such as Italy, sludge incineration is prohibited and struvite precipitation is not widespread. Struvite, also, did not performed well in our calcareous soil, with a very fast decline in P availability and consequent low P uptake. Therefore, the need of a different solution for recycling P from sludge has increased. Many different types of extraction has been tested on two different sludge types, an anaerobic and Fe-rich sludge and an aerobic and Al-rich sludge (chap. 3). Chemical and enzymatic extraction has been used for P solubilisation from the sludge, with different results. High P recovery was found in the sequence of maleic acid at 120°C and acid phosphatase, in the combination of citric acid and acid phosphatase, and in the combination of sulfuric acid and acid phosphatase. However, the maleic acid and acid phosphatase treatment was discarded because of the high temperature needed and the sulphuric acid mixed with acid phosphatase was not chosen, besides its very high P recovery, because of the high heavy metal release in the solution. Thus, the citric acid and acid phosphatase resulted as the best choice for our sludge samples. However, in these first extractions, aerobic sludge performed always very poorly as a source of P for hydrolysis, except for the sulphuric acid treatments. On the other hand, when P was measured as total P, rather than PO_4 with colorimetric method, the results were comparable to the anaerobic sludge. The hypothesis was, thus, that some elements, that could be the excess of Al or organic acids, can complexes P or interfere with the blue molybdenum method. Lately, when the hydrolysates were prepared for the plant fertilization, in higher amount and bigger tubes, this problem was overcome and both the sludge samples resulted very suitable for a high P recovery.

In the first plant test, though, the hypothesis were not yet formulated and only the anaerobic sludge was used. In order to avoid the interaction with soil, a soilless trial with lettuce in a floating system was carried out (chap. 4). The hydrolysates were used as liquid fertilizers as a source of P, with the addition of a standard solution, or as a source of P and meso and microelements, with the addition of only N and K to the water. Nevertheless, the hydrolysates did not performed perfectly in the soilless system, mostly due the pH increase they provided, which caused nutrients precipitation and a probable Al toxicity that affected the roots developments. The pH increase had caused the precipitation of P, but also of NO_3, Mn, Cu, Zn and Pb, and simultaneously

the solubilisation of the dangerous As and Se. Besides these problematic, the hydrolysates ensured a good plant growth, with the good addition of a comparable effect when used only with N and K. This highlighted the high amount of micro and meso nutrients, such as Fe, Mg, S and B, present in the hydrolysates.

The pH increase and the Al toxicity was easily overcome with the addition of a calcareous soil to the system. The same variety of lettuce was sown in soil and treated with hydrolysates (for this experiment also the aerobic one) and the raw sludge from which they derived (chap. 5). The high amount of Fe and Al in the hydrolysates were quickly adsorbed by the calcareous soil and the plants did not show any toxicity effects. Contrary to the previous results, the hydrolysates with only citric acid of both sludge types (ANA Cit and AER Cit), provided the best plant growth. However, the anaerobic sludge hydrolysates promoted more the root developments, while the aerobic one enhanced the shoot biomass. Furthermore, the apparent recovery fraction (one of the possible way of calculate the P use efficiency of the fertilizer) were higher in the hydrolysates of aerobic sludge (AER Cit and AER Cit+AcPHO), mostly due to the higher concentration of P in the plants treated with these solutions. The hydrolysates were also able to decrease slightly the pH of the soil. These led to the solubilisation of Ca and Mg, beside P, that were found in higher amount in the plants treated with hydrolysates. Therefore, it was clear that the hydrolysates performed better than the raw sludge, from which they were derived, but they were also better than the chemical reference, because they had a more constant P availability in soil and provided protection against P precipitation with their organic acids concentration. Furthermore, the residual sludge after the hydrolysis were tested as soil conditioner or N fertilizer compared to the raw sludge or compost. They resulted similar in term of plant growth to the raw sludge, but with lower heavy metal accumulation in soil and plants.

The increased P availability going from the raw sludge to the hydrolysates were then assessed with an input-output balance method (chap. 6). We have tested the hydrolysates at the dose used to hydrolyse a fixed amount of raw sludge, which was then used to fertilize the soil. Additionally, we tested that specific amount of exhausted sludge after the hydrolysis. In this way, we could measure how the P solubility of the sludge has increased with the hydrolysis and we could hypothesize the fate of P from the raw to the exhausted sludge, passing from the hydrolysates, the soil and the plant. The P use efficiency (calculated with the balance method, without subtracting the not fertilized plants uptake) of the hydrolysates reached very high levels, with the hydrolysates of aerobic sludge with citric acid and acid phosphatase (AER Cit+AcPHO) reaching a plant P uptake that was 100% of the P added. This result is really promising because show a possible solution to have the best crop growth and null P accumulation in soil avoiding possible P leaching.

The residual sludge fertilizers, in this experiment, decreased the P uptake in respect of the not amended plants, probably acting as a sink of P from the soil solution, due to the high concentration and high solubility of the metals and the very low concentration of P. Hence, they should not be used in a P deficient soil, but they can be used as soil conditioner (as already explained) or in soils with a high level of P that is bonded to the constituents, due their content in organic acids and their low pH.

In conclusion, in calcareous soil, where struvite is not effective, the sewage sludge hydrolysates are a real alternative to the fertilizers derived from phosphate rocks. In case of anaerobic wastewater treatment plants with Fe precipitation, which are more widespread and usually in higher scale, the citric acid hydrolysis can be a cheaper way to provide a good plant growth. In case of small-scale aerobic treatment plants, with Al precipitation, citric acid in combination of acid phosphatase can grant a highly efficient P fertilizer, with the addition of meso and microelements.

Further developments of this research should involve a microbial health test of the products, with an assessment of the fate of organic pollutants and antibiotics derived from sludge. Moreover, a costs analysis should be carried out and a life cycle assessments (LCA) of the products can exacerbate their environmental benefits. The LCA analysis could also point out the different possibilities for solution concentration to decrease the volume and the hypothetical cost of transport. In order to decrease even more the inputs in the cycle, we have though to use other organic waste as a source for citric acid production or even better to use microorganisms, such as *Aspergillus niger*, able to solubilise P from an organic matter, directly on sludge to increase the solubility of the organic waste without creating a liquid fertilizer.

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