Università degli Studi di Sassari

Corso di Dottorato di Ricerca in Scienze Agrarie

DIPARTIMENTO DI AGRARIA

CURRICULUM " MONITORAGGIO E CONTROLLO DEGLI ECOSISTEMI AGRARI E FORESTALI IN AMBIENTE MEDITERRANEO"

XXXII CICLO

Soil remediation of Lebanese contaminated soils using organic sorbents

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Anno Accademico 2016/2019

Declaration

By submitting this thesis, I hereby declare that this PhD thesis entitled “Soil remediation of Lebanese contaminated soils using organic sorbents” was carried out by myself for the degree of Doctor of Philosophy in Agricultural Science, *Curriculum* Monitoring and control of agricultural and forest ecosystems in the Mediterranean environment under the guidance and supervision of Prof. Paola Castaldi and Dr. Giovanni Garau at the University of Sassari, Italy and Dr. Nadine Nassif at the Lebanese University.

This thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; to the best of my knowledge it does not contain any materials previously published or written by another person except where due reference is made in the text; and all substantive contributions by others to the work presented, including jointly authored publications, is clearly acknowledged.

Chapter 1 consists in a general introduction about the PhD project. Chapters 2, 3 and 4 describe the experimental work carried out. Chapters 2 and 3 have been published in peer-reviewed journal (the permission to publication is attached at the end of this thesis) and it appear in their original form. Chapter 5 consists in a general conclusion about the experimental work carried out in this project.

PhD Candidate

Sassari, the 5th of November 2019

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Abstract

The studies on the influence of organic-based sorbents on the mobility of potentially toxic elements (PTE) in contaminated soils have been constantly growing in the last decades. However, given the variety of PTE and pollution status, as well as the diversity of factors governing the fate of PTE in soil, to date it is still hard to foresee the amendment effectiveness in a specific soil environment. The aim of this PhD thesis was therefore to assess the influence of biochar, compost and their combination on the mobility, (phyto)toxicity and bioavailability of several PTE (i.e. Sb, As, Ni, Cr and Zn) present in different polluted soils of Lebanon.

The addition of compost, biochar and their combination generally reduced labile PTE pools (i.e. readily soluble) and increased their residual (non-extractable) fractions. The amendments also changed the C-source utilization potential of the soil microbial community as revealed by the Biolog community level physiological profile. At the same time, soil dehydrogenase, β-glucosidase and urease were significantly enhanced in amended soils while the growth of *Capsicum annuum* L. and *Triticum aestivum* L. (in a selected polluted soil) was significantly enhanced and PTE uptake significantly reduced.

Overall, the results obtained indicated that biochar and compost could be useful to stabilise PTE in contaminated soils, to stimulate soil microbial activity and functionality and to improve plant growth.

Acknowledgements

I am sincerely grateful to Prof. Paola Castaldi and Dr. Giovanni Garau whose expertise were invaluable in the research topic and for their guidance, advices, comments, and knowledge during this project. Their exceptional patience and dedication are sincerely noted.

I would like to appreciate Prof. Nadine Nassif for her valuable support and insightful comments during these three years. Her contribution in stimulating suggestions and encouragement helped me to coordinate my work.

I also would like to appreciate Dr. Georges Hassoun who gave me this golden opportunity to take part of this project.

A special appreciation goes to Dr. Talal Darwish for sharing with me his experiences, comments, help and knowledge.

In addition, I would like to thank my family for their support and wise council. Finally, I thank my partner Ralph, who was of great support since the beginning and in every step, as well as providing happy distraction to rest my mind outside of my research.

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Chapter 1

General introduction

Soil pollution by potentially toxic trace elements (PTE)

Soil pollution is defined by the presence of an unfamiliar substance exceeding the normal concentration that might have harmful effect on any living organisms (Food And Agriculture Organization of the United Nation, 2018). Lead (Pb), zinc (Zn), nickel (Ni), cadmium (Cd), etc., are potentially toxic elements (PTE) which naturally occur at trace levels in soils due to their presence in the earth's crust (Mele et al., 2015; Smedley and Kinniburgh, 2002). Elevated concentrations of PTE in soil can be due to geochemical processes, such as the weathering of the underlying parent rock (Smedley and Kinniburgh, 2002). Also anthropogenic activities can be additional sources of PTE in the environment, e.g., industrial and mining activities, fossil fuels use, agrochemicals production and application (Adriano et al., 2001; Boruvka et al., 2005). As a consequence, the concentration of PTE in soil can reach long lasting alarming levels, since PTE unlike organic pollutants are not biodegradable (Adriano et al., 2004; Debela et al., 2011; Garau et al., 2007; Manzano et al., 2016). In the last decades, soil pollution by PTE has been attracting a wide research interest due to an increased awareness on their toxicity and ability to negatively affect the environment and human health, e.g. soil microbial community and plant growth, groundwater quality, soil physico-chemical properties and food chain, (Castaldi et al., 2009; Chabukdhara and Nema, 2013; Chaza et al., 2018; Darwish et al, 2011; He et al., 2018; Mehmood et al., 2017; Renella et al., 2005).

Sources of soil pollution by PTE

Contamination of soils with PTE is a serious problem which involves environmental and human health implications because of the adverse effects that PTE may have on plant growth (among the others) and food quality. Adding to this, it is difficult to specify one source of soil pollution, because different sources can contribute to it (Bundschuh et al., 2012; Luo et al., 2009). These sources can be categorized as follow (Mishra et al., 2016; Wuana and Okieimen, 2011):

- Natural source: some PTE are endogenous and this implies that they are inherited from the parent material. Such PTE derive from pedological alteration processes of the source rock and they can spread in soil by pedogenetic processes. These PTE are generally present at levels that are considered as trace (<1g kg^{-1} d.m.) and sometimes might be very important such as Ni, Co and Cr on serpentine (Koleli et al., 2014). However, they are rarely toxic to natural levels (Laghlimi et al., 2015).
- Anthropogenic source: exogenous PTE are provided by various human activities [i.e., industries, mining activities, and urban expansion into arable lands, agriculture (farming, fertilizers, herbicides, solid waste disposal, and sewage sludge). Exogenous PTE can often reach critical levels becoming dangerous and not tolerated by the environment (Adriano, 2001; Gowd et al., 2010; Mishra et al., 2016; Yaylalı-Abanuz, 2011; Zhao et al., 2010).

PTE speciation and mobility in contaminated soils

In general, the soil can contain a wide range of PTE at different concentrations depending on the geological environment and surrounding human activities (Dube et al., 2000). These PTE can be present either in solution, e.g. in the form of aqua-ions or soluble complexes, or adsorbed to different components of the soil solid phase, e.g. associated to organic and/or inorganic surfaces (Adriano et al., 2005; Marques et al., 2009; Morris, 2005; Sparks, 2002).

The PTE in soil, depending on their chemical form, can be absorbed by plants or soil microorganisms, leached from the surface to deeper layers, adsorbed by different soil colloids or precipitated. Moreover, microbial communities can influence PTE transport and favour their mobility by a variety of processes, e.g. metabolite complexation, and can affect their toxicity by methylation (which refers to the transfer of methyl groups to PTE), demethylation and oxidation–reduction reactions. For example, methylated Hg is more toxic than its inorganic form and will bio accumulate in the organisms (Adriano et al., 2005; Morris, 2005; Sparks, 2002).

Adsorption is the major process responsible for PTE immobilization in soil (Bradl 2004). PTE are concentrated in the solid fraction of the soil and distributed in different mineral and organic fractions (Perrono, 1999). The most important adsorbents are:

- Clays - where a large part of PTE are found in the silicate crystal structures or adsorbed on internal and external surfaces;
- Metal oxides and hydroxides - iron, aluminum and manganese oxides and hydroxides are able to retain potentially toxic metals and metalloids by inner- and outer-sphere complexation;
- Carbonates - in calcareous soils, carbonate and bicarbonate anions can contribute to PTE immobilization by the formation of insoluble compounds;
- Organic matter - humic substances are important PTE adsorbents (Bradl, 2004).

Many parameters control the adsorption of PTE and their partitioning into the different soil components (Bradl, 2004):

- Soil pH is considered the most influent parameter in affecting PTE mobility. For instance, a decrease of soil pH can enhance the mobility of trace metals (i.e., by dissolving the metal salts), while a progressive increase of soil pH can lead to the formation of trace metal precipitates. However, certain PTE such as boron, molybdenum, arsenic, antimony and selenium does not follow strictly this type of behavior. These latter PTE, that are present in soil mainly as oxy-anionic compounds, show an opposite behavior, since they become more mobile when the pH increases (Chibuike and Obiora, 2014; Perrono, 1999).

- Temperature and humidity of the soil. High temperature can improve the soil biological activity and enhance the formation of complexing compounds derived from the biodegradation of the organic matter. This can favour trace metals fixation. However, moisture can increase PTE solubility (Perrono, 1999).

However, humus compounds and inorganic minerals can adsorb and control the mobility and bioavailability of metals, such as Pb, Cu, Ni, Cd, Ca, K, Cr and Zn, in soils through physical and chemical processes, as follow (Ge et al., 2000; Silvetti et al., 2017; Sundman et al., 2015; Wang and Mulligan, 2009):

- Chemical processes refer to the formation of inner-sphere complexes between metal cations (i.e. Pb, Cu, Fe, Al, and Mn) or anions with organic compounds (e.g., humic substances) or with clay minerals or Fe, Al, Mn oxides and hydroxides, which can complex different PTE (Ge et al., 2000; Silvetti et al., 2017; Sundman et al., 2015; Wang and Mulligan, 2009):
- Physical processes involve ion exchange (outer-sphere complexes) and partitioning forces (van der Waals forces) between cationic and anionic metals and humus compounds or clay minerals or Fe, Al, Mn oxides and hydroxides (Sparks, 2002).

Additionally, the pollutant immobilization in soil is related to the PTE species and their physico-chemical properties. For instance, Cd, Cu, Pb, Hg and Zn are bound in the surface and remain there. However, a change of the redox parameters might mobilize the metals adsorbed to the oxides and transport them to deeper horizon (Namieśnik and Rabajczyk, 2010). Additionally, As can reach deeper horizons between 30-60 cm (Fodor and Szabó, 2004). The most important physical and chemical properties of some PTE are listed below (Fodor and Szabó, 2004; Kabata and Pendias, 2001; Perrono, 1990):

- Arsenic (As)

In soil pore water, arsenic can be mainly found as arsenate oxyanions ($H_2AsO_4^{-}$, $HAsO_4^{2-}$ and AsO_4^{3-}), as arsenious acid (H_3AsO_3) and arsenite (H_2AsO^{3-}) in a wide pH range, i.e. between 2.0 and 11. Arsenate and arsenite can be sorbed by the soil mineral surfaces, especially by iron and aluminum oxi–hydroxides, to which, therefore, has been attributed an important role in limiting arsenic toxicity in soil. Moreover, soil microbial community play an essential role in accelerating the oxidation of arsenites to arsenates and the methylation and alkylation of As. Under anaerobic conditions and microbiological processes, As compounds such as arsenate can be reduced to oxidized trivalent methylated arsenicals such as arsine (AsH_3) or arsenite (AsO_3^{3-}) form, which refers to the total depletion of oxygen level such as in hydromorphic soils (i.e., rice soil). The reduced forms have a greater mobility than arsenate in soil and can move easily into deeper horizons (Kabata and Pendias, 2001).

- Cadmium (Cd)

In soil solution, ionic Cd^{2+} species are the predominant, and it can also form complex ions ($CdOH^{+}$, $CdCl^{+}$, $CdCl_3^{-}$, $CdHCO_3^{+}$, $Cd(OH)_3^{-}$ and $CdCl_4^{2-}$) and organic chelates (Kabata and Pendias, 2001). Adding to this, cadmium cannot be easily retained by clays. The mobility of this PTE is greatly decreased starting from a pH higher than 7.5 (Kabata and Pendias, 2001). Moreover, the formation of sparingly soluble sulphides can also reduce strongly the cadmium mobility.

- Chromium (Cr)

Chromium is present in cationic or anionic form such as trivalent form, i.e., Cr^{3+} or hexavalent, i.e., CrO_4^{2-}. This latter, resulting from industrial activities, can be adsorbed to iron oxides or remain mobile in the anionic form. Moreover, it can be reduced to Cr^{3+} at pH <5 or in the presence of organic matter and sulfur. Nevertheless, trivalent chromium is more stable and is dominant in precipitated form such as hydrated oxides (included or associated in insoluble organometallic complexes and iron oxides).

- Nickel (Ni)

Ni^{2+} is relatively stable in aqueous solutions and can migrate over long distances in soil. However, Nickel can coprecipitate mainly with Mn and Fe oxides (Kabata and Pendias, 2001). It can also bind to carbonates in calcareous soils and to organic matter particularly on the surface in loamy soils.

- Lead (Pb)

Lead is retained firmly by clay and organic matter. This PTE is considered as the least mobile in soil. Pb^{2+} and $Pb(OH)^+$ ions predominate in acidic or neutral environment. These ions can be replaced by $Pb(OH)_4^{2-}$, $Pb(OH)_3^-$ and $Pb(OH)_2$ when pH increases. Lead is strongly less mobile in the 5 - 9 pH range.

- Zinc (Zn)

Zn^{2+} is the most predominant chemical species of zinc. It is also the most mobile form and can be adsorbed by organic compounds and minerals such as clays and aluminum or iron hydroxides. However, zinc is strongly soluble and mobile compared to other PTEs in soil, particularly in acidic soils.

Consequences of metals exposure on plants

Some PTE such as As, Cd, Hg, Pb, Sb and Se are not essential for plants and animals and are highly toxic. However, other trace elements, such as Cu, Mn, Mo, Ni, Cr and Zn are essential elements for plant growth and health. Nevertheless, if PTE concentration in soil exceeds the maximum tolerable limit for plants, their uptake can cause severe toxicity to plants and possible accumulation in the food chain (Dembitsky and Rezanka, 2003; Duruibe et al., 2007; Jordão et al., 2006; Kumar Sharma et al., 2007; Sprynskyy et al., 2007). The dangerousness of PTE is related to their chemical forms, time, and dose exposure (Alissa and Ferns, 2011). The accumulation of PTE in plant tissues relies on numerous variables such as plant species, temperature, humidity, soil pH and nutrient availability. The highest metal uptake by plant is during the summer season due to the high rate of plant transpiration, high temperature and low moisture (Kumar Sharma et al., 2007). High concentrations of PTE in soil can lead to symptoms of toxicity and growth inhibition in most plants (e.g. Root and Das, 2002). PTE can cause toxicity by damaging the cell structure of the plants due to the inhibition of a number of cytoplasm enzymes (Laghlimi et al., 2015; Van Assche and Clijsters, 1990;). Trace metals (i.e., Cd, Cu, Ni, Zn, Cr and Pb) can cause harmful consequences on plant growth such as (Laghlimi et al., 2015):

1. Disturbance of photosynthesis, plant growth and reproductive processes;
2. Membrane damage, chlorosis and damage in the root (Laghlimi et al., 2015).
3. Reduction of seed germination, protein production, chlorophyll, enzyme production, dry mass accumulation and lipid content.

Consequences of metals exposure on humans

To maintain a healthy life, a small amount of PTE is required in the daily life. Once this amount is exceeded (commonly through food intake), PTE can become dangerous for humans (Wu et al., 2016). Several symptoms can be provoked by PTE such as damaging the functions of the brain, kidney, liver, blood composition and other essential organs. Adding to this, the exposition to PTE for a long period of time can cause cancer and can show muscular dystrophy, neurological, muscular and physical problems such as Alzheimer, Parkinson and sclerosis diseases (Jaishankar et al., 2014; Jarup, 2003). Some PTE might become toxic even when they are slightly exceeding the background level in soils. For this reason, a strong knowledge of the PTE toxicity is essential in order to know the defensive measures toward their excessive contact (Ferner, 2001; Jaishankar et al., 2014). The consequences of these exposure are numerous, and differ from one element to another (Table 1) (Duruibe et al., 2007).

Table 1. Toxic effects of some PTE on human health adapted from Duruibe et al. (2007) and Mahurpawar (2015)

PTE	Effect on human health
Arsenic	Lung damage, skin cancer, abdominal pain
Cadmium	kidney damage and hypertension
Chromium	Lung damage
Manganese	Nervous system damage
Lead	Nervous system damage, kidney damage
Nickel	lung, liver and kidney damage
Mercury	spontaneous abortion, congenital malformation and gastrointestinal disorders
Aluminum	Alzheimer's disease and Parkinson's disease

Consequences of PTE exposure on soil microbial communities

PTE can affect soil microbial communities, in particular their structure and abundance. Additionally, microbial communities may change the mobility of PTE, through immobilization, chelation and change of their oxidation state. For instance, microbial communities can help in transforming PTE into a complexed form and making them less mobile. This means, the structure of cells in microorganisms can trap PTE ions and sorb them consequently onto the binding sites of the cells. This process is called biosorption (Malik, 2003; Pérez De Mora et al., 2006).

Some of the PTE are essential for physiological activities, however they can become harmful once exposure reaches a certain level, causing toxic effects to living cells. For instance, PTE such as Pb might affect soil microbial activity such as respiration, which, this latter is a process governed by the enzyme activities (Sobolev and Begonia, 2008). This means that PTE can alter the enzyme activities, influencing their activities indirectly, i.e. through a toxic effect on soil microbial community, as well as directly, i.e. by reacting with the enzyme-substrate complex, and/or causing the enzyme denaturation or its inactivation. Several studies showed that high levels of PTE in soil determine a lower number of microorganisms and decrease their activity, compared to uncontaminated soils (Sobolev and Begonia, 2008).

Status of soils in Lebanon and geology

Lebanon is located on the eastern coast of the Mediterranean Sea in an active tectonic area with three dominant faults such as Roum, Yammouneh and Serghaya (Stephan, 2010). Moreover, carbonated rocks constitute more than 66 % of the Lebanese mountains. According to NRCS (2006), most of the agricultural plains in Lebanon are on calcareous and red soils, e.g. in Bekaa, Akkar, Koura, Sour, Saida, Rachaya and Hasbaya regions, while Halba region (Northern Lebanon) is derived from sandstone and basalts (Darwish et al., 2006).

Many of such soils are known as being very fragile with low depth on hill slopes. This type of soil can lead into soil degradation and erosion. However, human intervention can worsen the situation remarkably and expand the degradation phenomena to a higher level (Stephan, 2011). Particularly, this could be due to the excessive application of pesticides and fertilizers and especially to the irrigation with wastewater as previously mentioned in Nsouli et al. (2004).

Central plain of Bekaa agricultural area

Lebanon has two main mountains: Mount Lebanon located on the coastal range and Anti Lebanon located in the inner range. The first predominant region with the major agricultural land in Lebanon is located between these two mountains with an average altitude of 900 m, known by the Central plain of Bekaa (Figure 1) (Darwish et al., 2008). The Bekaa plain is 12,753 ha, it is located between 33 47' 00" and 33 54' 00" latitude and 35 50' 08" and 35 56' 38" longitude, and it is situated on the foothills of Anti-Lebanon mountain chains and crossed mainly by the Litani River. According to the ICARDA station located in Terbol, the climate in the Central plain of Bekaa is Mediterranean sub-humid and benefits from an average annual precipitation of 600 mm with an average lowest temperature of 2˚ C in January and an average highest temperature of 31˚ C in August. (Darwish et al., 2008; Darwish et al., 2011)

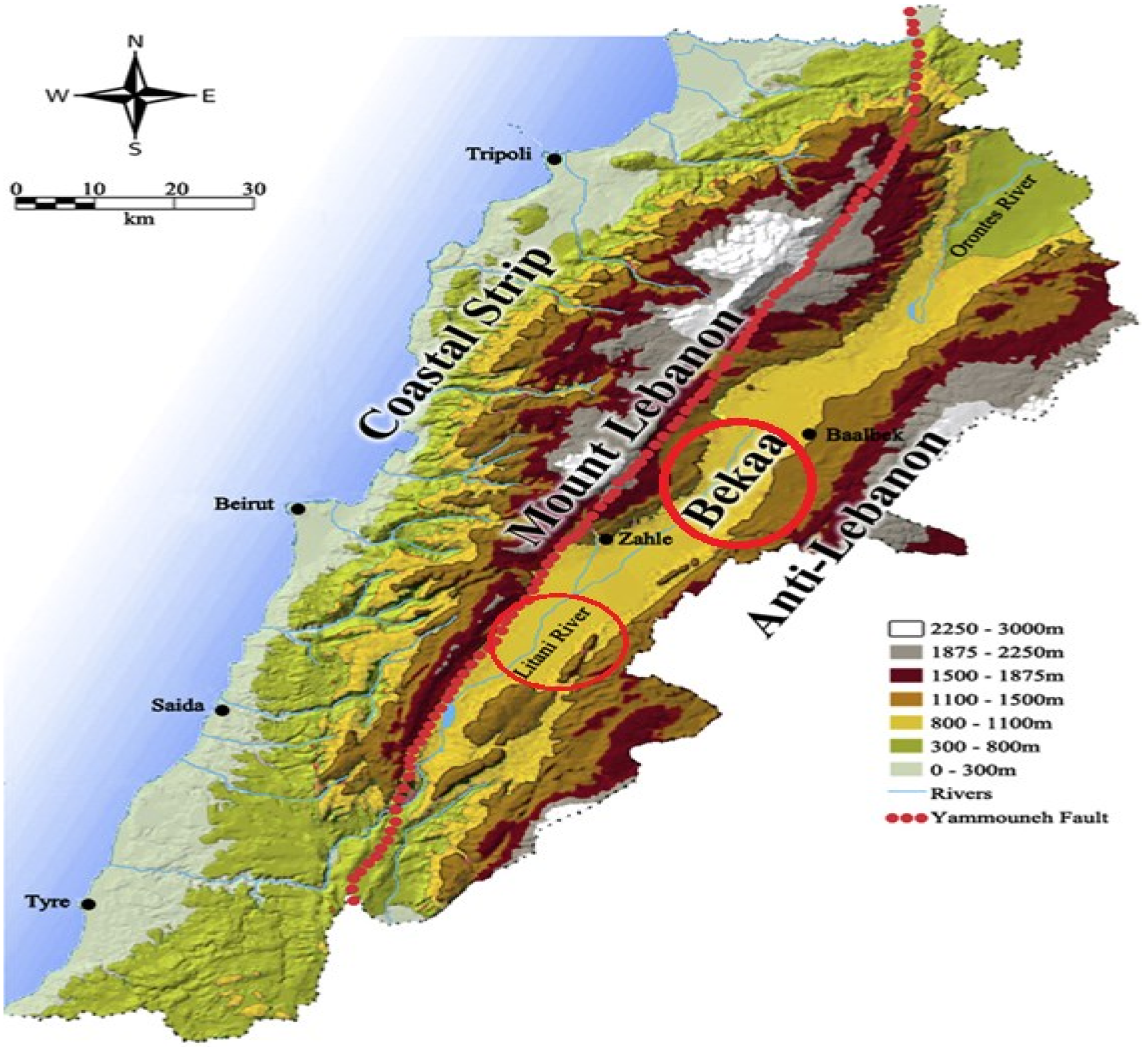

Figure 1. Map showing the Bekaa area located between Mount Lebanon and Anti-Lebanon mountains and crossed by the Litani River (El Zaatari, 2017)

In the Central plain of Bekaa there are several fertile soils (such as Terbol, Arab Faour, Ryak, Taanayel, etc…) that have been cultured intensively with sugar beet, fruit trees, vegetables and wheat, essentially, in the previous decades using a good rotation system (Figure 2) (Darwish et al., 2008).

Figure 2. An overview of an agricultural land in the Central plain of Bekaa, particularly in Terbol region

This agricultural sector consumes up to 70 % of the available water resources. The cultivated lands in this area are mostly irrigated by groundwater and Litani River. This latter is considered as the most important and largest water resource in Lebanon (Assaf et al., 2008; Korfali et al., 2014). However, Litani River is facing a massive environmental problem due to the dumping in this river of waste coming from several pollution sources such as intensive agriculture, urban and industrial activities (BAMAS; 2005; Darwish et al., 2008). Adding to this, farmers are enforced to irrigate with this contaminated water, particularly during the peak crop demand (dry season) to compensate the shortage of water (Darwish et al., 2008).

Furthermore, the overuse of pesticides such as fungicides, insecticides, acaricides, herbicides and nematicides (MoE, 2005), the excessive use of fertilizers such as nitrogen, phosphorus, and potassium and also "micronutrients", such as zinc and other metals for plant growth (FAO, 2014) containing excessive contaminants contributed to the contamination of these areas (Korfaly et al., 2014; Stephan, 2011). For these reasons, in Bekaa region some PTE were found exceeding the permissible concentration levels according to the German legislative reference (ACSAD, BGR and CNRS-CRS, Arab-German Cooperation project 1997- 2003). Particularly, elevated concentrations of nickel and chromium were detected in the Central plain of Bekaa (Figure 3) (Darwish, 2008).

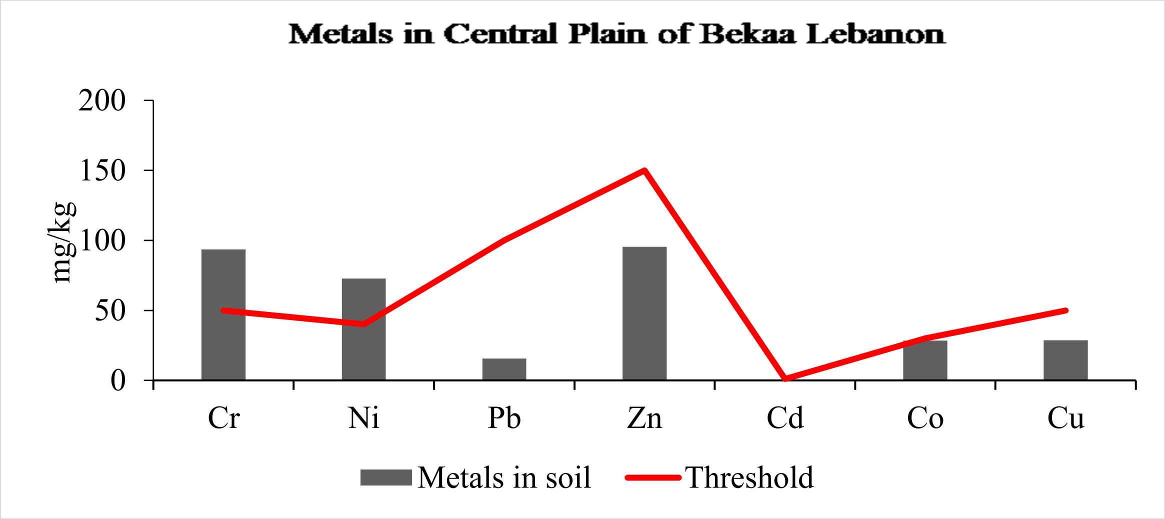

Figure 3. Concentration of some PTE in the soil of the Central plain of Bekaa (Lebanon) compared to their respective thresholds (Darwish et al., 2008)

North of Lebanon agricultural area

The second main region in Lebanon with major agricultural land is located in North of Lebanon with a total area of 2,024 km^2 (Figure 4). The agricultural soil within this area is bordered by mountain lands, particularly from the east. This area is influenced by the Mediterranean climate with an average annual rainfall of 800 mm taking place from October to March. The average mean annual temperature is around 18 °C, with an average lowest temperature of 11˚ C taking place in January and the average highest temperature reached is in August (26˚ C) (Kassir et al., 2012).

Figure 4. Map showing the location of the two predominant regions in Lebanon with major agricultural land (Agenzia Italiana per la Cooperazione allo Sviluppo - Ministero degli Affari Esteri. 2018 – 2019)

The main crops in the north area are olive trees (Figure 5) (Atlas Climatique du Liban, 1977; Kassir et al., 2012). The agricultural land occupies 47 % of the total lands in North of Lebanon, while industrial and commercial sites are limited (Kassir et al., 2012).

Figure 5. An overview of a land cultivated with olive trees in North of Lebanon

Nsouli et al. (2004) reported that north of Lebanon, known as the second main agriculture region of the country, was polluted with nickel, copper and zinc, particularly in Halba region (Figure 6). As mentioned before, anthropogenic activities might have substantially contributed to soil contamination. In particular, the excessive application of fertilizers and agrochemicals (MoE, 2005) and overall the use of wastewater and sewage sludge in agriculture are the likely contamination sources of such agricultural soils (Nsouli et al., 2004).

However, it should be also noted that high background concentrations of Ni (i.e., high levels of Ni in the earth crust) were previously reported in these agricultural soils of Northern Lebanon, and attributed to the soil parent material and not only to anthropogenic activities (Figure 7) (Nsouli et al., 2004).

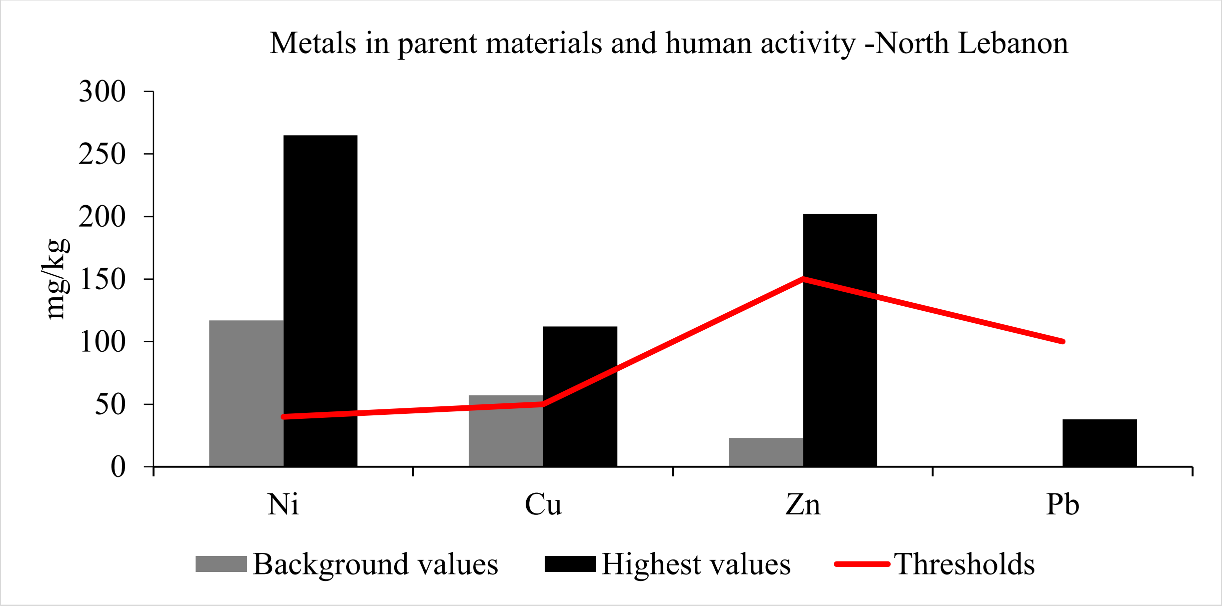

Figure 6. PTE concentration in soil of Northern Lebanon (parent materials, i.e. background values, and highest values) compared to their respective thresholds (Nsouli et al., 2004)

Sources of pollution and their consequences on Lebanese soils

Various sources might be the cause of PTE accumulation in the Lebanese agricultural soils (Darwish et al., 2008; Kassir et al., 2012), such as natural pollution (Nsouli et al., 2004), which was pointed out in the previous paragraphs, and different anthropogenic activities including (among the others) crop irrigation with contaminated water from the Litani river (BAMAS., 2005; Darwish et al., 2008) and the excessive use of pesticides (Bhattacharyya et al., 2008; FAO, 2012; Stephan, 2011). It is important to underline that chemical fertilizers such as soil organic and inorganic fertilization (i.e., phosphate fertilizers and/or sewage sludge) and pesticides such as fungicides, insecticides and herbicides are the most imported and overused in Lebanon (Bhattacharyya et al., 2008; FAO, 2012; MoE, 2005; Stephan, 2011). For instance, nitrate and phosphate fertilizers, farmyard manure, herbicides and fungicides revealed the presence of As, Cr, Cd, Ni, Pb, Zn, Mn and Fe (Gimeno-Garcia et al., 1996; Gray et al., 1999), with the concentration of As, Cr and Ni in nitrate and phosphate fertilizers and farmyard manure can range from 2 to 1200 mg kg^{-1} for As, 1.1 to 410 mg kg^{-1} for Cr and 0.9 to 279 mg kg^{-1} for Ni (Gray et al., 1999). Adding to this, the application of pesticide in a cumulative way such as lead arsenate insecticide, can lead into having about 20 g elemental As m^{-2} in soil (Robinson and Ayuso, 2004). The antimony concentration in plant protection products is 1.5 mg kg^{-1} in lead arsenate insecticide (Tschan et al., 2009; Wagner et al., 2003), and 100 mg kg^{-1} in fertilizers such as superphosphates (Edwards et al., 1995) and 44 mg kg^{-1} in sewage sludge (Edwards et al., 1995) which has led to large-scale contamination of agricultural lands (Wagner et al., 2003). These sources are likely responsible of the current contamination status of Lebanese soils (Table 2).

Table 2. Sources of pollution and their consequences on Lebanese soil (FAO, 2012)

Sources of soil pollution		**Consequences on Lebanese soils**
Natural metals in parent material		Weak rocks
Anthropogenic	Industrial and municipal practices	Land degradation caused by chemicals released into the soil (i.e., Jieh electrical plants, Nehme landfill)
	Agricultural practices	Erosion High nitrate and PTE content in soil Adsorption of pesticides by soil particles Salinity on the southern coastal strip caused by the absence of crop rotation, excessive use of fertilizer and irrigation with waste water
	Urban expansion	Deforestation Crusting of the soil Soil desertification Drought

Threshold values for PTE in soil

Soils have the ability to immobilize contaminants through adsorption, reduction or oxidation and precipitation reactions. However, once this capacity is exceeded, PTE can be leached into groundwater or absorbed by plant roots (Nsouli et al., 2004; Su et al., 2014). For this reason, the pollution of soil and water resources is considered one of the most serious environmental problems on a world scale (Korfali et al., 2014).

The first step toward the evaluation of the level of soil contamination is to know the natural concentrations of PTE in soil, in order to know the PTE fraction which differs from that present in the geological origin material (Nsouli et al., 2004).

In Lebanon, there is no law set up to define the values of the maximum permissible PTE levels, or PTE threshold values. Nonetheless, several researches showed that PTE concentration of the agricultural soils of Bekaa and Northern Lebanon (Darwish et al., 2008; Nsouli et al., 2004) was largely exceeding the threshold values defined by international regulatory bodies such as the Food and Agricultural Organization (FAO) as well as the Canadian standards and the Finnish legislation (Government Decree on the Assessment of Soil Contamination and Remediation, Needs 214/2007). This latter can be considered a good approximation of the mean values of a wide range of several countries (Table 3 and 4) (Carlon et al., 2007; Darwish et al., 2008; Korfali et al., 2014; Nsouli et al., 2004; Tóth et al., 2016).

Table 3. Threshold and guideline values for selected PTE in soils (Chiroma et al., 2014; Korfali et al., 2014; Tóth et al., 2016)

Trace metal thresholds	Finnish legislation mg/kg	FAO mg/kg	Canadian standards mg/kg
As	5	20	12
Cd	1	3	1.4
Co	20	50	40
Cu	100	100	63
Pb	60	100	70
Ni	50	50	50
Cr	100	100	64
Zn	200	300	200

Table 4. Thresholds and guideline values for selected PTE in vegetables (Chiroma et al., 2014)

Trace metal thresholds	FAO mg/kg
As	0.0
Cd	0.1
Co	50
Cu	73
Pb	0.3
Ni	67
Cr	0.0
Zn	100

Remediation of soil polluted by PTE using organic and inorganic sorbents

Recently, remediation techniques of soils contaminated with PTE were attracting global researchers concern (Garau et al., 2007; Kassir et al., 2012; Manzano et al., 2016). Remediation technologies can be divided in two main types: *ex situ* and *in situ* methods. *Ex situ* methods refer to techniques which require removal of contaminated soil, followed by a treatment either on site or off site, and in some cases returning the treated soil to the restored site. *In situ* methods provide for the treatment of contaminated soil without removing it (Table 5) (Anup and Kalu., 2015). *In situ* techniques may be favoured with respect to *ex-situ* techniques for different reasons such as cost reduction by minimizing or eliminating site excavation, transportation and disposal costs and reduced health impacts on the public or remediation crews, by decreasing exposure to contaminants during excavation and/or treatment process (Anup and Kalu., 2015; Liu et al., 2018). Between the *in situ* techniques, most of these deals with the immobilization of pollutants in the soil in order to improve soil quality and fertility.

Table 5. Remediation techniques (Liu et al., 2018)

Mechanisms	Remediation technique	Applicability	Proceeding technique
Physical mechanisms	Surface capping	In situ	Containment
	Encapsulation	In situ	
	Landfilling	Ex situ	
Electrical mechanisms	Vitrification	In situ and ex situ	Solidification and/or stabilization
Chemical mechanisms	Immobilization/stabilization	In situ	
	Solidification	In situ and ex situ	
	Soil flushing	In situ	Extraction/Removal
	Soil washing	Ex situ	
Biological mechanisms	Phytoremediation	In situ	
	Bioremediation	In situ	

Immobilization and stabilization techniques

Most of the technologies used for the remediation of soil contaminated with PTE are very expensive and not eco-friendly. However, one of more promising remediation techniques is based on the immobilization and/or precipitation of the contaminant. This technology is receiving great interest by the research community due to its ability in reducing PTE availability and hence toxicity, its low cost, its rapidity and facility in reaching high public acceptability (Castaldi et al., 2005; Derakhshan; Nejad et al., 2017; Garau et al., 2007).

Immobilization and stabilization are *in situ* techniques based on the addition of organic (e.g., compost and biochar) or inorganic (e.g., zeolite, red mud, lime, phosphate, iron rich water treatment residuals) amendments to polluted soil in order to reduce the mobility and leaching of contaminants through adsorption and/or precipitation reactions (Garau et al., 2014; Mele et al., 2015). For instance, compost, which is a good way for recycling urban organic wastes, contains high concentrations of humified organic matter which can diminish the mobility of PTE in soil, and accordingly, allowing the functional recovery of polluted soils (Castaldi and Melis, 2004; Castaldi et al., 2005).

Moreover, also inorganic sorbents showed substantial PTE-immobilizing abilities with a significant effect on soil microbial communities and plant growth (Castaldi et al., 2005; Garau et al., 2007; Garau et al., 2014; Komarek et al., 2013; Qiao et al., 2018; Zanuzzi et al., 2013). For example, zeolites (a class of porous alumino-silicates) are able to fix PTE in soil, i.e. their negative charge can be neutralized by introducing trace metal cations such as Pb, Cd or Zn in the exchanged sites of the zeolite (Castaldi et al., 2005). Other immobilizing inorganic materials are red muds, which are derived from the digestion of crushed bauxite using caustic soda. These materials, considered a by-product of the alumina industry, showed promising results in reducing PTE mobility in contaminated soils (Garau et al., 2007). Similarly, lime addition to contaminated soils can increase the pH and trigger the precipitation of PTE (trace metals in particular) as metal carbonates, oxides and hydroxides so decreasing their solubility as previously reported (Garau et al., 2007).

Nevertheless, it is quite challengeable to find an effective sorbent that can immobilize simultaneously different PTE present in cationic and anionic form (Fang et al., 2012; Filella et al., 2009; Garau et al., 2017; Mele et al., 2015; Okkenhaug et al., 2013). In this context, the addition of sorbents containing organic matter, such as biochar and/or compost, can represent a good solution to decrease the mobility of co-occurring anionic and cationic PTE in polluted soils (Manzano et al., 2016) as well as to promote their functional recovery.

Organic amendments

Organic-based amendments, such as compost and biochar, derived from food and/or green waste or sludge, manure and/or municipal solid waste (Fang et al., 2016; Manzano et al., 2016) can be used for the remediation, through stabilization of PTE-contaminated soils (Fang et al., 2016; Manzano et al., 2016). Importantly, these organic amendments showed a certain ability in the immobilization of PTE present as cations (e.g. Pb, Cu and Zn) or anions (e.g. $Sb(OH)_6^-$) species (Fang et al., 2012; Filella et al., 2009; Garau et al., 2017; Mele et al., 2015;.Okkenhaug et al., 2013).

- Biochar

Biochar is a carbonaceous product derived from the pyrolysis of organic solid waste at low oxygen conditions (Xu et al., 2016). Organic solid waste used for the biochar production include animal manures, sewage sludges, crop residues, municipal solid wastes and woodchips, etc. (Tang et al., 2013). The type of organic solid waste chosen and the time and temperature of pyrolysis (starting from 300˚C till 1000˚C) can affect the physical and chemical properties of biochar (Lee et al., 2014; Xu et al., 2016). For instance, during the pyrolysis process, chemical bonds are broken and rearranged giving rise to a product characterized by a variety of functional groups such as amino, hydroxyl, ester, nitro and carboxyl (Xu et al., 2016). Adding to this, the increase of pyrolysis temperature can affect the specific surface area of biochar and decrease its acidity due to its impact to the physical and chemical properties of biochar such as the reduction of carboxylic group (Jindo et al., 2014; Lehmen et al., 2007; Lehmen et al., 2011;.Xu et al., 2016).

Biochar has an alkaline pH (typically between 7.5 and 12), it is rich in aromatic carbon, and has a large number of micro, meso and macro pores distributed in its structure (Jindo et al., 2014; Xu et al., 2016). Biochar can persist in soil for long periods of time (Sebastian Meyer, 2017); it is considered as an innovative, environmentally friendly and cost-effective amendment for the recovery of soils polluted with PTE, due to its ability to decrease the PTE mobility and uptake by plants (Arabyarmohammadi et al., 2017; Bandara et al., 2016; Derakhshan Nejad et al., 2017; Fang et al., 2016; Hmid et al., 2014; Mehmood et al., 2017; Mohan et al., 2014; Ok et al., 2015; Qiao et al., 2018; Zhang et al., 2016). However, some findings showed that the addition of biochar into soils may cause negative effects (i.e., inhibition and decrease) on plant growth and soil microorganism activity due to the presence of polycyclic aromatic hydrocarbons (PAHs) and heavy metals found within biochar and produced during the pyrolysis process (Zheng et al., 2018).

Other researches, such as Qiao et al. (2018), reported that biochar was able to reduce the mobility of arsenic and cadmium present in a polluted soil. Moreover, biochar also showed a great capacity in decreasing trace metals (e.g. Pb and Cd) mobility in co-contaminated soils (Tooth et al., 2016). This ability is due to its microporous structure, high specific surface area and cation exchange capacity (Bandara et al., 2016; Mehmood et al., 2017; Qiao et al., 2018; Xu et al.; 2013). In addition, oxygenated functional groups such as carboxyl, alcoholic hydroxyl or phenolic hydroxyl groups can be involved in the formation of inner-sphere complexes with trace metals (Beesley et al., 2015; Qiao et al., 2018).

Other interactions can occur between biochar and PTE in cationic form, such as electrostatic interactions between carboxylate functional groups of biochar and trace metals, which can also occur as the result of exchange reactions between H^+ or other cations adsorbed on biochar surface (e.g. Ca^{2+}, Mg^{2+}, Na^{2+}) as well as π-coordination between trace metals (TM) and electrons involved in biochar C=C bounds (Lu et al., 2012; Qiao et al., 2018; Zhang et al., 2013). Adding to this, the alkaline nature of biochar can increase the pH of contaminated soils. This can favour the precipitation of PTE and the formation of strong complexes between the PTE and functional groups negatively charged of biochar (Lu et al., 2012; Fang et al., 2016; Zhang et al., 2013).

The ability of biochar in reducing the mobility of oxy-anionic species in soil, such as As(V) or Sb(V), was not broadly investigated (Tooth et al., 2016). However, several researches showed that biochar can immobilize anionic specie in soil (i.e., arsenate) due to the presence of amorphous Al or/and Fe oxy-hydroxides, containing $-OH_2$ or -OH groups, capable to bind the metalloids through ligand exchange processes (Qiao et al., 2018; Tooth et al., 2016; Zhang et al., 2015a; Zhang et al., 2015b; Zhang et al., 2016). Furthermore, biochar revealed a strong capability at increasing the soil nutrient status and its water retention, reducing the need of fertilizers, irrigation and the emission of greenhouse gases and storing large amount of carbon (Bandara et al., 2015; Lehmann et al., 2011; Li et al., 2017; Zheng et al., 2013). Particularly, biochar addition to soil can increase the nutrients supply and accordingly, it can enhance significantly plant growth (Rawat et al., 2019).

Finally, biochar can be viewed as a perfect place for soil microorganisms because of its pore system. For instance, Thies and Rillig (2009) reported that biochar could have a positive impact on soil microbial community due to its ability to give a habitat to escape from predators, as well as to provide C substrates, energy, and nutrients to soil microorganisms (Thies and Rillig, 2009; Xu et al., 2016).

- Compost

Compost is an organic sorbent derived from bio-oxidation of the organic fraction arising from different waste matrices such as municipal waste (i.e., organic waste and food waste), agricultural and crop waste (i.e., crop, garden and fruit waste, animal manure and waste from abattoirs) (Muralikrishna and Manickam, 2017).

Compost contains a high organic content with a total organic carbon between 16 and 24 % and a total organic matter between 28 and 41 %**.** The pH generally ranges between 6 and 8, EC ranges between 2 and 4 dS m^{-1}, and the total nitrogen values range between 0.95 to 1.7 % approximately. Moreover, the moisture and the water holding capacity showed a range between 23 - 32 % and 3.50 - 4.40 g water/g dry compost, respectively (Khater, 2012).

The addition of sorbents containing organic matter such as compost to PTE-polluted soils can decrease the mobility of contaminants and improve the physico-chemical and biological properties of soils. In particular, the humic substances of compost can react with trace metals (e.g. Pb, Cd, Cu and Zn) to form complexes of different strengths. This is due to a high surface charge density of humic substances and to the presence of ligands or functional groups such as carboxyl, phenolic, hydroxyl, carbonyl, and sulfhydryl functional groups, which are able to form metal–organic complexes (Silvetti et al., 2017). The nature of the binding between PTE and the functional groups of humic substances ranges from purely electrostatic, that is, non-specific interaction of the metal cation with the negative charges present on the surface of the humic molecules, to specific binding, that is, formation of inner-sphere complexes and chelates involving the functional groups of humic substances and PTE (Garau et al., 2017; Manzano et al., 206).

Finally, the capacity of compost to immobilize anionic PTE is rather questionable and, in this regard, consistent results are lacking (Garau et al., 2017; Manzano et al., 2016). Moreover, a release of anionic PTE in soil was often noticed when compost was added to the contaminated soil. This is likely because As and Sb bound to soil particles could be involved in competition phenomena with soluble organic or inorganic anions within compost (e.g. sulphate, phosphate, humic and fulvic acids), leading to As and Sb release in solution (Garau et al., 2017; Manzano et al., 2016).

Aims and objectives

The aim of this thesis was to evaluate:

- The influence of compost derived from municipal solid waste and green waste composting (MSWC) on the mobility of PTE, i.e. metals and metalloids, in two different contaminated soils, as well as to determine the amendment effect on selected soil biochemical and microbial features (i.e. dehydrogenase, urease and β-glucosidase activities and Biolog community level physiological profile);

- The influence of a biochar from organic municipal solid waste (Bio), a compost derived from municipal solid waste and green waste composting (MSWC), and their combination (Bio+MSWC) on the mobility of PTE, i.e. metals and metalloids, in two middle-eastern contaminated soils, as well as to determine the effect of amendments on selected soil biochemical and microbial features;

- The influence of a biochar derived from organic municipal solid waste (Bio), a municipal solid waste compost (MSWC) and their combination (Bio+MSWC), on the growth of *Capsicum annuum* L. and *Triticum aestivum* L. in a PTE contaminated soil. A special emphasis was given to the study of metal(loid)s uptake by the plants and their partitioning into roots and shoots. The PTE mobility in soil before plant growth, and the amendment influence on selected soil microbial and biochemical features, was also considered.

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Chapter 2

Metal(loid)s immobilization in soils of Lebanon using municipal solid waste compost: Microbial and biochemical impact

Abou Jaoude[a], L., Garau[b], G., Nassif[a], N., Darwish[c], T., Castaldi[d], P., 2019. Metal(loid)s immobilization in soils of Lebanon using municipal solid waste compost: Microbial and biochemical impact. Applied Soil Ecology 143 (2019) 134–143.
DOI: https://doi.org/10.1016/j.apsoil.2019.06.011

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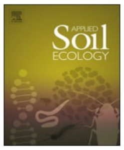
Applied Soil Ecology 143 (2019) 134–143

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil



Metal(loid)s immobilization in soils of Lebanon using municipal solid waste compost: Microbial and biochemical impact

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ARTICLE INFO

Keywords:
Municipal solid waste compost
Polluted soils
Sequential extraction
Enzyme activities
Biolog community level physiological profile

ABSTRACT

Organic based amendments can be used to reduce metal(loid)s mobility in polluted soils and alleviate possible toxicity phenomena towards soil microbiota. Therefore, the objectives of this study were to evaluate the influence of municipal solid waste compost (MSWC) on the mobility of metal(loid)s in different contaminated soils and to determine the amendment effect on selected soil biochemical and microbial features. To this aim, MSWC was added at two rates (1 and 3% w/w) to different soils sampled in distinct regions of Lebanon (Halba and Terbol, i.e. HS and TS respectively) polluted with metal(loid)s such as As (total concentrations between 31 and 54 $mg \cdot kg^{-1}$) and Sb (between 93 and 120 $mg \cdot kg^{-1}$), and trace metals such as Ni, Cr and Zn. After two months of soil-amendment contact, compost addition at both rates significantly increased the non-extractable fractions of As, Sb, Ni, Cr and Zn (e.g. +25% of residual Sb in TS-MSWC 3% with respect to untreated TS), while decreased the environmentally-relevant water soluble and exchangeable fractions of all the metal(loid)s. This effect was more relevant for both soils when 3% MSWC was applied. The amendment addition similarly affected the community level physiological profile (Biolog CLPP) of the respective soil culturable microbial communities, which however showed an opposite trend in the two soils. In particular, compost addition increased the metabolic potential and catabolic versatility of the HS microbial community (compared to the untreated control), while the same proxies were significantly reduced in the amended TS soil. Differently, soil dehydrogenase (DHG), β-glucosidase (β-GLU) and urease (URE) activities were significantly enhanced in both soils treated with compost (e.g. + 17, 45 and 27% for DHG, URE and β-GLU in TS-MSWC 3% with respect to untreated TS). The results obtained showed that the addition of MSWC, particularly at the 3% rate, was effective at fixing the metal(loid)s present in both soils, had a substantial influence on the structure of the soil microbial communities and a positive effect on soil functionality. Taken together the results presented suggest that MSWC can be used as an alternative and environmental friendly amendment for the in situ remediation of metal(loid)s-polluted soils.

1. Introduction

Accumulation in soil of trace metals (TM) and metalloids above critical thresholds has become a relevant environmental problem, with serious impact on ecosystem biodiversity and functionality (Adriano, 2001; Yaylalı-Abanuz, 2011). Soil metal(loid)s might accumulate in crops and animals and in the long term they might enter the food cycle threatening also public health (Yaylalı-Abanuz, 2011).

Calcareous red soils are very common in Lebanon, e.g. in the Bekaa, Akkar, Sour, Saida, Rachaya and Hasbaya regions, as well as soils derived from sandstone and basalts which are prevailing in the Halba region (Northern Lebanon) (Darwish et al., 2006). Many of such soils are recognized as extremely delicate with slight profundity on hill slopes, which contributes to erosion and soil degradation. In addition, anthropogenic pollution can significantly contribute to worsening such degradation phenomena (Stephan, 2011). In this sense, the discovery of several TM-rich hot spots in agricultural soils of Northern Lebanon was particularly worrying (Nsouli et al., 2004). Moreover, also the agricultural soils located in the Central plain of Bekaa and irrigated by the Litani river were characterized by high levels of TM contamination (Korfali et al., 2014). Various sources might be the cause of metal(loid)s occurrence and accumulation in such Lebanese agricultural soils (Darwish et al., 2008; Kassir et al., 2012). These include irrigation with contaminated water (e.g. municipal or industrial waste water) as well

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https://doi.org/10.1016/j.apsoil.2019.06.011
Received 3 October 2018; Received in revised form 13 June 2019; Accepted 19 June 2019

as excessive and continued use of pesticides and chemical fertilizers (Bhattacharyya et al., 2008; Stephan, 2011).

In the last 20 years sustainable approaches for the in situ remediation of metal(loid)s-polluted sites generated considerable interest in the scientific community (e.g. Garau et al., 2007; Kassir et al., 2012; Mele et al., 2015; Manzano et al., 2016). One such methodology is based on the addition of amendments to polluted soils in order to decrease the mobility of contaminants through sorption and/or precipitation reactions (Garau et al., 2014; Mele et al., 2015). Nevertheless, trace metals such as Pb, Ni, Zn, and Cd can be found in the soil solution as complexed or free hydrated cations, while metalloids such as As and Sb are mainly found as arsenate ($H_2AsO_4^-$, $HAsO_4^{2-}$) and antimonate ($Sb(OH)_6^-$) anions in a wide pH range (i.e. 4.0–8.0) (Garau et al., 2017; Mele et al., 2015; Okkenhaug et al., 2013). For this reason, the choice of a remediation treatment able to retain both trace metals and metalloids is quite challenging (Filella et al., 2009; Mele et al., 2015). Indeed, sorbents containing organic matter, such as compost, have good potentialities to reduce metal(loid)s mobility in soil, while improving at the same time its microbial abundance and/or activity (Garau et al., 2017; Manzano et al., 2016; Castaldi et al., 2018). This is likely due to the presence in compost of a variety of functional groups (e.g. carboxylic, phenolic, thiophenolic and sulfhydrylic), which are negatively charged in a wide pH range (i.e. 4.0 8.0) and/or able to form strong binding (i.e. inner sphere complexes) with metal cations (Ge et al., 2000; Silvetti et al., 2017a; Wang and Mulligan, 2009). Moreover, it was shown that humic substances extracted from compost are able to form As-metal-organic-complexes where divalent or trivalent metal cations (e.g. Ca, Mg, Fe, Pb, Al, and Mn) act as bridging elements between humic substances and As (Lin et al., 2004). Importantly, this was supported by some recent findings (e.g. Mikutta and Kretzschmar, 2011; Silvetti et al., 2017b) and a similar binding mechanism was also reported for humic acids with respect to Sb (Filella and Williams, 2012; Tella and Pokrovski, 2012). In addition, the ability of Sb and As to form strong complexes with compost poly-functional organic ligands, such as hydroxyl-carboxylic acids and polyols, was also highlighted (Tella and Pokrovski, 2012; Wenzel, 2013; Silvetti et al., 2017b).

Nonetheless, although compost addition to TM-polluted soils can result in a significant immobilization of trace metals, in many instances researches reported increased TM solubility (Beesley and Dickinson, 2009) and extractability (Pardo et al., 2011). Likewise, the impact of compost on Sb and As mobility in soil is disputable because of the anionic nature of such metalloids in aerated soils, which should limit their interactions with the negatively charged sites of the organic matter (OM) (Filella and Williams, 2012). Moreover, As and Sb bound to soil particles could be involved in competition phenomena with soluble organic or inorganic anions within compost (e.g. sulphate, phosphate, humic and fulvic acids), leading to As and Sb release in solution (Wang and Mulligan, 2009). The general lack of agreement regarding these issues, likely due to the complexity and heterogeneity of the experiments carried out, highlights the importance of further studies on the interactions between TM, metalloids and organic amendments such as compost. Moreover, the effectiveness of municipal solid waste compost (MSWC) at fixing As, Sb and TM co-occurring in the same contaminated soil was poorly (or not at all) addressed.

The aim of this work was therefore to evaluate the influence of MSWC on the mobility of metal(loid)s in two different contaminated soils, as well as to determine the amendment effect on selected soil biochemical and microbial features (i.e. dehydrogenase, urease and β-glucosidase activities and Biolog community level physiological profile).

2. Materials and methods

2.1. Study area and soil sampling

Single core soil samples (20 cm depth) were randomly collected from Halba in the Akkar Governorate (Northern Lebanon; 34.533 latitude, 36.207 longitude) and from Terbol in the Central plain of Bekaa (Eastern Lebanon; 33.807 latitude, 35.995 longitude) at spring time in May 2017. These soils were chosen because they are located in the two mains agricultural areas of Lebanon, the first in the Central plain of Bekaa (Terbol soil: TS) and the second in North of Lebanon (Halba soil: HS) (Darwish et al., 2006). HS was a Halba Clay Typic Rhodoxeralf while TS was a Terbol Clay Typic Eutrochrept (Soil Survey Staff, 2010). Particle size determined with the pipette method (Tan, 1996) allowed classifying HS and TS as clay soils (USDA classification), with 26% sand, 18% silt and 56% clay for HS and 30% sand, 14% silt and 56% for TS. The main mineral of TS soil was montmorillonite while minor minerals were illite, vermiculite, calcite and kaolinite (Darwish et al., 2008). Quartz and kaolinite were the major mineral of HS while other minor minerals present were illite, montmorillonite, and vermiculite (Darwish et al., 2001, 2006). The Terbol region benefits from 600 mm of annual rainfall taking place from October to March and 16 °C of mean annual temperature (Darwish et al., 2006). The pH of Terbol soil (TS) is weakly alkaline and the main crops cultivated are radish vegetables (Darwish et al., 2006). The Halba region benefits from 800 mm of annual rainfall (from October to March) and mean annual temperature is around 18 °C. The pH of Halba soil (HS) is neutral and wheat is the main crop cultivated in this area (Darwish et al., 2006).

The soil cores collected from TS were pulled together, as well as the ones from HS soil, and sieved to < 2 mm to obtain representative composite soil samples from each site. These latter were immediately used to set up different experimental mesocosms (see next paragraph).

2.2. Experimental design and mesocosms set up

The experiment had a completely randomized block design with three replicates for each mesocosm (a total of 18 mesocosm were prepared) that had the following treatments: control soil (HS and TS); soil treated with 1% (w/w dry basis) MSWC (HS- and TS-MSWC 1%); soil treated with 3% (w/w dry basis) MSWC (HS- and TS-MSWC 3%). These rates were selected based on the specific metal(loid)s immobilizing capabilities of MSWC which were highlighted in previous studies (Manzano et al., 2016; Silvetti et al., 2017a). In addition, the percentages chosen are reproducible at the field scale and feasible from an economic and environmental point of view.

In particular, each HS and TS composite soil was used to set up a total of 9 soil mesocosms (approximately 8 kg each) to which the above mentioned treatments were randomly imposed.

The compost used in the study was provided by the Facility Plant Secit S.p.A. Consorzio Zir (Chilivani Ozieri–Sardegna, Italy) and derived from green and food waste. The physico-chemical features of the compost used are reported in Table 1. Before addition to soil mesocosms, compost was finely ground and sieved to < 2 mm. Afterwards, all soil mesocosm were (independently) mixed and their moisture content raised to 40% of their water holding capacity (WHC), which was equal to 33.33 and 26.67% for HS and TS soils respectively. All mesocosms were left to equilibrate for 2 months at 22 °C (until physico-chemical, microbiological and biochemical analyses) during which they were mixed two times per week and their water content maintained constant by weighing the mesocosms twice a week and adding water when needed.

2.3. Physico-chemical characterization of HS and TS soils and MSWC

After the equilibration period, soil mesocosms (and MSWC), were chemically characterized with respect to pH, electric conductivity (EC), total organic C (TOC) and N (TON), cation exchange capacity (CEC), dissolved organic carbon (DOC), total concentration of metalloids and trace metals. In particular, soil pH (in 1:2.5 soil:water suspension), EC, TOC and TON, CEC were determined according to the Italian standard guidelines (Gazzetta Ufficiale, 1992) (Tables 1 and 2). DOC was

Table 1
Characterization of HS and TS soils[a] and municipal solid waste compost (MSW-C). Mean ± SE.

Chemical parameters	HS soil	TS soil	MSW-C
pH $_{H2O}$	7.31 ± 0.03	7.38 ± 0.01	8.84 ± 0.02
EC (mS·cm^{-1})	0.29 ± 0.00	0.34 ± 0.01	15.58 ± 0.69
Total organic Carbon (TOC, % d.m.)	2.39 ± 0.01	2.20 ± 0.03	24.50 ± 3.70
Total N (% d.m.)	0.25 ± 0.02	0.21 ± 0.01	1.80 ± 0.36
Organic matter (% d.m.)	4.11 ± 0.01	3.80 ± 0.04	42.14 ± 3.70
Cationic exchange capacity (CEC, cmol$_{(+)}$·kg^{-1})	22.03 ± 0.06	22.43 ± 0.01	87.10 ± 1.60
Dissolved organic carbon (DOC, mg·kg^{-1} d.m.)	42.25 ± 0.01	74.13 ± 0.02	1944 ± 0.02
Total Cd (mg·kg^{-1} d.m.)	1.23 ± 0.50	0.22 ± 0.06	0.42 ± 0.04
Total Ni (mg·kg^{-1} d.m.)	113.50 ± 1.27	168.40 ± 3.96	15.10 ± 3.00
Total Cr (mg·kg^{-1} d.m.)	183.15 ± 4.74	202.15 ± 0.50	< 0.50
Total Pb (mg·kg^{-1} d.m.)	17.61 ± 1.03	22.74 ± 1.92	85.00 ± 17.00
Total Zn (mg·kg^{-1} d.m.)	209.00 ± 1.41	156.50 ± 3.54	209.00 ± 63.00
Total As (mg·kg^{-1} d.m.)	30.77 ± 4.68	53.96 ± 1.87	–
Total Sb (mg·kg^{-1} d.m.)	119.50 ± 27.58	93.00 ± 1.41	–

[a] HS: Halba soil; TS: Terbol soil.

determined in soil (and MSWC) samples treated with distilled water (1:10, *w/v* ratio) and agitated for 24 h. The samples were then centrifuged for 10 min at 3000 rpm and the liquid phase filtered using 0.2 μm pore size nylon filters. The DOC values were finally derived from the absorbance readings at 254 nm as previously described (Silvetti et al., 2014). The total concentration of metalloids and trace metals in soil (and MSWC) was quantified by using a Perkin Elmer Analyst 600 atomic absorption spectrometer (HGA-600 graphite furnace), after digestion of soils and MSWC with HNO_3 and HCl mixture (1:3 v/v ratio) in a Microwave Milestone MLS 1200.

All the analyses were carried out on triplicate independent MSWC and soil samples from each mesocosm. Mean values ± standard deviations were reported in Tables 1 and 2.

2.4. Metalloids mobility in HS and TS soils

Arsenic and antimony mobility was determined in duplicate soil samples from each mesocosm (amended with MSWC and unamended) using the sequential extraction procedure described by Wenzel et al. (2001). In particular, to determine the non-specifically sorbed As and Sb (Fraction 1), 1 g of soil sample was shaken for 4 h at 20 °C with 25 mL of 0.05 M $(NH_4)_2SO_4$. After separating the liquid from the solid phase (i.e. by centrifuging for 15 min at 3000 rpm and filtering using Whatman 41 filters), this latter was shaken for 16 h with 25 mL of 0.05 M $NH_4H_2PO_4$ to extract the specifically sorbed As and Sb (Fraction 2). After separating the liquid phase as early mentioned, the solid sample was shaken for 4 h with 25 mL of 0.2 M NH_4^+-oxalate buffer to extract the As and Sb associated with amorphous and poorly crystalline Fe and Al hydrous oxides (Fraction 3). Finally, As and Sb linked with well crystallized Fe and Al hydrous oxides were extracted treating the solid sample with 25 mL of 0.2 M NH_4^+-oxalate buffer + 0.1 M ascorbic acid and shaking for half an hour at 96 °C (Fraction 4). The concentration of As and Sb in each of the liquid phase recovered during the extraction procedure was determined using a Perkin Elmer Analyst 600 atomic absorption spectrometer (HGA-600 graphite furnace). After the last step of the sequential extraction procedure, metalloids in the remaining solid phases (residual fraction) were determined, after digestion with HNO_3 and HCl mixture (1:3 v/v ratio) in a Microwave Milestone MLS 1200, as previously described.

2.5. Trace metals mobility in HS and TS soils

Trace metals mobility was quantified in duplicate soil samples from each mesocosm (amended with MSWC and unamended) using the sequential extraction procedure of Basta and Gradwohl (2000). In particular, the first fraction (i.e. the Me-exchangeable pool) was extracted by adding 1 g of soil sample to 25 mL of 0.5 M $Ca(NO_3)_2$ and shaking for 16 h at 25 °C (Fraction 1). In step 2, the same soil samples were shaken for 5 h with 25 mL of 1 M NaOAc at pH 5.0 to separate Me(II) forming weak surface complexes (Fraction 2). In the last fraction, surface complexes and precipitated metals were extracted treating the soil samples with 25 mL of 0.1 M Na_2EDTA solution and shaking for 6 h (Fraction 3).

After each extraction step samples were centrifuged (15 min, 3000 rpm) and filtered (Whatman 41 filter paper) to separate the solid from the liquid phase. Trace metal concentrations in the liquid phase of each extraction step was determined as for As and Sb. After the last step of each sequential extraction procedure, trace metals in the remaining solid phases (residual fraction) were determined, after digestion with HNO_3 and HCl mixture (1:3 v/v ratio) in a Microwave Milestone MLS 1200, as previously described.

2.6. Soil enzyme activities in HS and TS soils

Selected enzyme activities were determined in duplicate soil samples from each mesocosm (amended with MSWC for two months and unamended). The dehydrogenase (DHG) activity was determined by quantifying (colorimetrically) the concentration of triphenylformazan produced after incubation of the soil samples with triphenyltetrazolium chloride (Alef and Nannipieri, 1995). The urease (URE) activity was determined by the colorimetric determination of the ammonia released following soil samples incubation with urea (Alef and Nannipieri, 1995). The β-glucosidase (GLU) activity was estimated by quantifying the *p*-nitrophenol released following the incubation of soil samples with *p*-nitrophenyl glucoside (Alef and Nannipieri, 1995).

2.7. Community level physiological profile of HS and TS soils

The community level physiological profile (CLPP) was determined

Table 2
Characteristics of HS and TS soils amended with MSW-C 1% and MSW-C 3% (w/w) after two months incubation.[a] Mean ± SE.

Chemical parameters	HS soil		TS soil	
	1% MSW-C	3% MSW-C	1% MSW-C	3% MSW-C
pH $_{H2O}$	8.07 ± 0.01	8.11 ± 0.01	8.19 ± 0.05	8.30 ± 0.01
EC (mS·cm^{-1})	0.35 ± 0.01	0.54 ± 0.00	0.40 ± 0.00	0.49 ± 0.00
Total organic Carbon (TOC, % d.m.)	2.62 ± 0.03	2.95 ± 0.01	1.94 ± 0.00	2.39 ± 0.00
Total N (% d.m.)	0.28 ± 0.01	0.32 ± 0.01	0.22 ± 0.01	0.26 ± 0.01
Organic matter (% d.m.)	4.50 ± 0.03	5.10 ± 0.01	3.34 ± 0.00	4.11 ± 0.00
Cationic exchange capacity (cmol$_{(+)}$·kg^{-1})	23.24 ± 0.30	23.85 ± 0.28	23.26 ± 0.53	24.76 ± 0.25
Dissolved organic carbon (mg·kg^{-1} d.m.)	60.03 ± 0.01	100.15 ± 0.01	80.27 ± 0.01	140.4 ± 0.01

[a] HS: Halba soil; TS: Terbol soil.

in duplicate soil samples from each mesocosm (amended with MSWC for two months and unamended) using Biolog EcoPlates (Biolog Inc., Hayward, CA). CLPPs relative to each mesocosm were obtained according to the protocol described by Garau et al. (2011). Briefly, soil samples (10 g) were diluted with 90 mL of sodium pyrophosphate ($2\,g\,L^{-1}$) solution and shaken at 150 rpm for 30 min at 28 °C to release resident microorganisms. Afterwards, soil suspensions were 10 fold diluted using saline solution (0.89% w/v NaCl), centrifuged at 2000 rpm for 5 min (Muñiz et al., 2014; Zhai et al., 2017), filtered with sterile (autoclaved) Whatman 1 filter paper and finally used to inoculate the Biolog Ecoplate wells (120 µL in each well). The Biolog Ecoplate is a 96-wells microtiter plate containing in its wells a replicated ($n = 3$) set of 31 carbon sources (and a control well with no carbon) of soil/rhizosphere relevance (Insam, 1997). Carbon source utilization by the microbial communities is revealed by the reduction of the redox dye tetrazolium violet (i.e. purple color formation) which is contained in each well. After inoculation, Biolog Ecoplates were incubated in the dark for 96 h at 28 °C and carbon source utilization was recorded in each well every 24 h by measuring the absorbance at 590 nm (OD_{590}) with a Biolog MicroStation™ reader (Garau et al., 2007). Biolog Ecoplate data (i.e. raw OD_{590} readings) were processed as follows: all the OD_{590} readings were subtracted with those recorded at time zero. The resulting OD_{590} values were then subtracted by the respective control well values and used to determine the Average Well Color Development (AWCD; Garland, 1997), quantifying the general metabolic activity of the culturable microbial community. The use of different carbon source guilds (i.e. sugar and sugar derivates, carboxylic acids and amino acids) was also obtained. Principal components analysis (PCA) was performed (using the correlation matrix) on normalized OD_{590} values (Garland, 1997) to reduce multidimensional data and allow a more straightforward interpretation of C source utilization data. All the Biolog derived data presented refer to the 96 h incubation time as this time point allowed the best discrimination among microbial communities.

2.8. Statistical analysis of data

For each soil, sequential extraction data (i.e. each of the fractions recovered), soil enzyme activities and Biolog-derived indexes were analyzed by one-way analysis of variance (one-way ANOVA) to identify possible differences between treatments. When significant P values were obtained (i.e. $P < 0.05$), differences between means were investigated using the post hoc Fisher's least significant difference test ($P < 0.05$). All data were analyzed using the Minitab 17 software for windows.

3. Results and discussion

3.1. Soils properties and pollution

Both soils showed very similar values of pH, EC, TOC, TON and CEC (Table 1). Despite the similar content of organic matter (~4.0%), TS showed a higher DOC content ($74\,mg{\cdot}kg^{-1}$) compared to HS ($42\,mg{\cdot}kg^{-1}$) (Table 1). Moreover, also the content of trace metals and metalloids was different among soils. Total Sb concentration was substantially higher in HS soil, i.e. > 28% compared to TS, while total As concentration in TS soil was 75% higher compared to HS. The TS soil showed higher Ni and Cr concentrations compared to HS, i.e. > 49 and 10% respectively, whereas this latter soil was characterized by higher Zn content, i.e. > 34% compared to TS.

Although in Lebanon threshold values for potentially toxic metalloids in soil are not set up by the law, nonetheless the As and Sb concentrations detected in HS and TS soils were largely exceeding the threshold values defined by the Finnish legislation (Government Decree on the Assessment of Soil Contamination and Remediation, Needs 214/2007) (i.e. 2 and $5\,mg{\cdot}kg^{-1}$ soil for Sb and As respectively), which can be considered a good approximation of the mean values of different European countries (Carlon et al., 2007; Tóth et al., 2016). Based on such guidelines, the metalloids contamination in HS and TS soils presented both ecological and health relevance (Tóth et al., 2016). Similarly, total concentrations of Ni, Cr and Zn in HS soil were exceeding the above mentioned thresholds (i.e. 50, 100 and $200\,mg{\cdot}kg^{-1}$ respectively) while the same was true for Ni and Cr in TS soil (Table 1). As for As and Sb, the concentrations of Ni and Cr in both soils can be considered relevant from an environmental point of view (Tóth et al., 2016). It should be also noted that high background concentrations of Ni in agricultural soils of Northern Lebanon were previously reported (i.e. 52–117 $mg{\cdot}kg^{-1}$) and attributed to the soil parent material (Nsouli et al., 2004). However, the detection in HS and TS soils (note that TS soil is located in the central plain of Bekaa, far away from Northern Lebanon) of high concentrations of other trace metals such as Cr, and especially metalloids such as As and Sb, suggests that anthropogenic activities could have substantially contributed to soil contamination. (Nsouli et al., 2004). In particular, inorganic and organic fertilization of soils, e.g. using sewage sludge and/or phosphate fertilizers, may be responsible for trace elements accumulation. For example, Gray et al. (1999) reported that the content of As, Cr and Ni in phosphate and nitrate fertilizers, farmyard manure and composted refuse was between 2 and $1200\,mg\,kg^{-1}$ for As, 1.1–410 $mg\,kg^{-1}$ for Cr and 0.9–279 $mg\,kg^{-1}$ for Ni. Moreover, phosphate rock can contain up to $485\,mg\,kg^{-1}$ of Cr and $64\,mg\,kg^{-1}$ of Ni (Adriano, 2001). Robinson Jr and Ayuso (2004) reported that cumulative pesticide application rates, i.e. lead arsenate, may result in as much as 22.4 g elemental As m^{-2} soil. Finally, in the last decades, the presence of Sb in plant protection products (such as in lead arsenate insecticide, up to $1.5\,mg\,kg^{-1}$; Wagner et al., 2003; Tschan et al., 2009), fertilizers (such as superphosphates, up to $100\,mg\,kg^{-1}$; Edwards et al., 1995) and sewage sludge (up to $44\,mg\,kg^{-1}$; Edwards et al., 1995) has led to large-scale contamination of agricultural lands (Wagner et al., 2003).

Moreover, the irrigation of soils with waters originating from contaminated rivers (this is the case of the Litani river which, in the dry season, is used for the irrigation of the soils of the Bekaa valley) has been identified as an additional source of metal(loid) contamination of Lebanese soils (Korfali et al., 2014). In particular, amounts of Cu, Pb, As and Ni largely exceeding the range of environmental limit values for surface waters (MoE Decision 8/1; MoE, 2001) (e.g. ~5.83, 0.16, 0.26 and $0.75\,mg\,L^{-1}$ respectively) have been recently detected in the Litani river (Abi Saab et al., 2018).

In order to limit the mobility of metal(loid)s and their potential bioavailability in HS and TS soils, MSWC was applied at two rates (1 and 3%) to the contaminated soils. After 2 months of soil-amendment contact, the addition of MSWC caused some changes of the soil chemical properties with respect to those of the unamended soils which were characterized at the same time-point (i.e. after 2 months). In particular, MSWC addition led to an increase of the soil pH (e.g. ~ one unit in TS-MSWC 3%, pH = 8.3), changing the soils from circum-neutral (HS) and sub-alkaline (TS) to alkaline (Table 2). The pH increase observed, in agreement with previous studies (e.g. Tandy et al., 2009), was proportional to the rate of compost applied, and was attributable to the alkaline nature of the compost used (pH = 8.84). Compost addition, especially at 3% rate, also improved some fertility attributes of the soils such as CEC (> 8.3 and 10.4% in HS- and TS- MSWC 3%), OM (> 24.1 and 8.2% in HS- and TS-MSWC 3%), and TON content (> 28.0 and 23.8% in HS- and TS-MSWC 3%) (Table 2). Also DOC increased remarkably in both soils treated with compost, e.g. 2.4 and 1.9 fold higher in HS- and TS-MSWC 3% than respective controls (Table 2).

3.2. Influence of MSWC on metalloids mobility

The mobility and potential bioavailability of As and Sb were determined by the sequential extraction procedure of Wenzel et al. (2001). The amount of metalloids extracted from the first and the

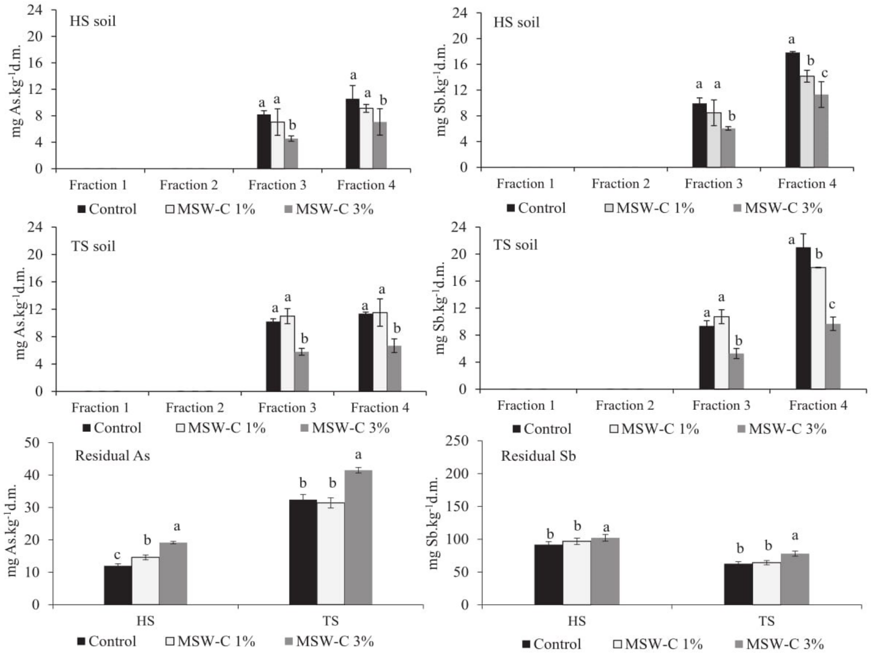

Fig. 1. Arsenic and antimony released after sequential extraction. For each soil and each fraction, bars with the same letter are not statistically different at $P < 0.05$.

second step (i.e. Fractions 1 and 2) were very low in both soils treated and untreated with MSWC (Fig. 1). This is important as these fractions represent the water-soluble and easily exchangeable Sb and As (Fraction 1) and those forming inner-sphere complexes with the soil components (Fraction 2). As such, these metalloid pools are expected to be those more labile in soil and potentially bioavailable (Garau et al., 2017). Importantly, compost addition had no obvious effect on such As and Sb pools and, differently from other studies, no mobilization effect was recorded (Manzano et al., 2016). The As and Sb extracted in step 3 (Fraction 3), which accounted for the metalloids associated with amorphous Fe and Al (hydr)oxides, decreased significantly in both soils treated with 3% MSWC (Fig. 1). In particular, As and Sb extracted in Fraction 3 from HS-MSWC 3% decreased of ~45 and 39% respectively compared to the untreated HS soil; while As and Sb extracted from TS-MSWC 3% decreased of ~44% (Fig. 1). This could be explained by a competitive exchange of As and Sb bound to amorphous Fe- and Al-oxides surface sites by inorganic (e.g. phosphate ions) and/or organic compounds within compost (e.g. low molecular weight organic anions) as reported by several researchers (e.g. Garau et al., 2017; Renella et al., 2008). Alternatively, a partial dissolution of amorphous Fe and/or Al phases, with the consequent release of bound As and/or Sb, could be also occurred due the action of fulvic and/or humic acids within compost (Chen et al., 2016; Garau et al., 2017).

The addition of MSWC reduced significantly also the As and Sb extracted in step 4 (Fraction 4), which accounted for the metalloids associated with well crystallized Fe and Al (hydr)oxides (Fig. 1). However, MSWC decreased significantly the As content recovered in this extraction step only when added at the 3% rate, i.e. < 33 and 41% with respect to the untreated HS and TS soils respectively (Fig. 1). In the case of Sb, significant reductions of the metalloid were recorded after MSWC addition at both rates. In particular, MSWC at 1% rate reduced the Sb recovered in step 4 by 20 and 14% in HS and TS soils respectively, compared to the untreated controls. On the other hand, Sb reduction after 3% MSWC addition was approximately 36 and 54% in HS and TS soils respectively, compared to the untreated controls (Fig. 1). As for the metalloids extracted in step 3, ligand exchange and/or ligand-promoted and/or microbially-induced dissolution of the crystalline phases could be the main processes involved in the reduction of the As and Sb recovered in Fraction 4 (Garau et al., 2017).

The residual As and Sb, i.e. the fractions strongly retained in soil and hardly bioavailable, increased significantly in both soils treated with MSWC. The addition of compost at 3% was most effective at immobilizing As and Sb (> 59 and 28% for As and > 11 and 26% for Sb, in HS- and TS-soil compared to respective untreated controls) (Fig. 1). The increase of residual arsenic and antimony suggests that the metalloids released from amorphous and crystalline Fe and Al (hydr) oxides after compost addition (Fig. 1) were incorporated in poorly soluble compounds and/or became occluded by the mineral and/or organic soil components, which agrees with the results reported by several authors (e.g. Garau et al., 2017; Manzano et al., 2016). In particular, the precipitation of antimonate and arsenate with soluble Ca^{2+}, which is abundantly available within both soils and MSWC could have contributed to increase the residual Sb and As pools in treated soils (Garau et al., 2017).

Overall, these results showed that the addition of MSWC at 1 and 3% rates lowered the environmental impact of the metalloids and also provided a contribution on the not well defined role of the organic matter as immobilizing agent of arsenic and antimony in soil (Pardo et al., 2011).

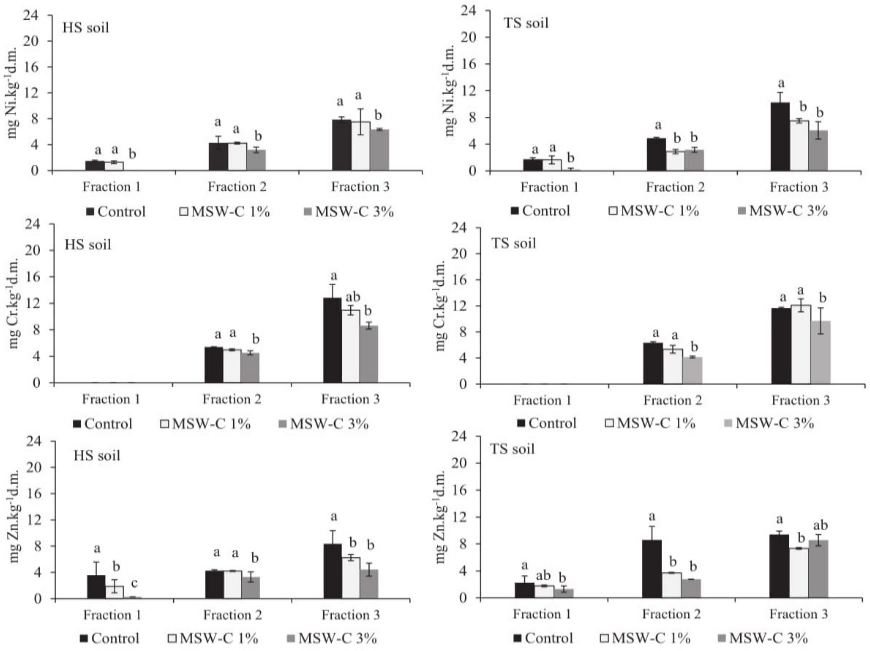

Fig. 2. Trace metals released after sequential extraction. For each soil and each fraction, bars with the same letter are not statistically different at $P < 0.05$.

3.3. Influence of MSWC on trace metals mobility

The mobility and potential bioavailability of trace metals (i.e. Ni, Cr and Zn) in treated and untreated soils were determined using the sequential extraction procedure described by Basta and Gradwohl (2000). In the first step of this procedure (Fraction 1), the MSWC addition (especially at 3% rate) decreased significantly the water-soluble and readily exchangeable Zn and Ni in both soils (e.g. by ~2 and 17-fold for Zn; and by ~1.2 and 1.5-fold for Ni, in HS-MSWC 1% and 3% respectively compared to controls) while no Cr was detected in this fraction (Fig. 2). These results could be mainly explained by the capacity of the MSWC organic phase to strongly bind water-soluble and easily exchangeable metals as well as by the involvement of the MSWC water-soluble fraction in the formation of poorly soluble Me-precipitates (Castaldi et al., 2017; Garau et al., 2014; Park et al., 2011; Silvetti et al., 2015). In this sense, it was recently shown that phosphate, chloride and sulphate anions within municipal solid waste composts were involved in the formation of Pb(II) and Cu(II) precipitates when these latter TM were present in solution (Castaldi et al., 2017). Importantly, and differently from other studies (e.g. Manzano et al., 2016), the MSWC addition did not lead to an increase of the water soluble and exchangeable Zn.

In agreement with the results reported by other authors (e.g. Manzano et al., 2016), the percentage of Me extracted in the second step (Fraction 2), which represented the TM bound to the weak acid-soluble phase and TM co(precipitated) as Me-carbonates, decreased after MSWC amendment (e.g. < 25 and 36% for Ni; < 16 and 35% for Cr; < 23 and 68% for Zn, in HS- and TS-MSWC 3% respectively, compared to untreated soils; Fig. 2).

The last step of the sequential extraction (Fraction 3) quantified the TM extracted with EDTA, which represented the TM fraction forming strong inner-sphere complexes with the soil components. EDTA-extractable Ni, Cr and Zn decreased greatly in treated soils, particularly after the addition of 3% MSWC (Fig. 2). These results are in contrast with those reported by other authors who observed an increase of EDTA-extractable metals in soils treated with compost (e.g. Manzano et al., 2016). The observed reduction of TM extracted with EDTA (< 19, 32 and 47% and < 41, 17 and 10% of Ni, Cr and Zn in HS- and TS-MSWC 3% respectively, compared to the untreated soils), could be due to increased metal precipitation as stable insoluble salts, e.g. as Me (II)-phosphates (phosphates were largely present in the compost used), as previously highlighted (Clemente et al., 2006; Silvetti et al., 2017a). Indeed, the residual fraction of Ni, Cr and Zn (i.e. the fraction strongly retained by soil, which accounts for the metal fractions very insoluble and/or occluded metal pools) increased with the addition of compost in both soils, and the highest increase was recorded in HS- and TS-MSWC 3% (> 3.7, 3.0 and 4.2% and > 5.0, 4.0 and 5.0% for Ni, Cr and Zn respectively, in HS- and TS-MSWC 3% compared to the respective controls) (Fig. 3). These results highlight an increase of inaccessible and immobilized TM in the MSWC-treated soils (Ge et al., 2000; Wang and Mulligan, 2009).

3.4. Influence of MSWC on soil enzyme activities

Trace metals and metalloids have the ability to decrease soil enzyme activities due to their i) interaction with the enzyme-substrate complex, ii) enzyme denaturation capacity, and iii) interaction with protein active groups (Bhattacharyya et al., 2008; Garau et al., 2011). In this sense, the analysis of soil enzyme activities can be helpful in the evaluation of the metal(loid)s fixing capabilities of an amendment, as well as to establish its role on the recovery of soil biological activity and functionality (Garau et al., 2017). That is why such kind of analyses,

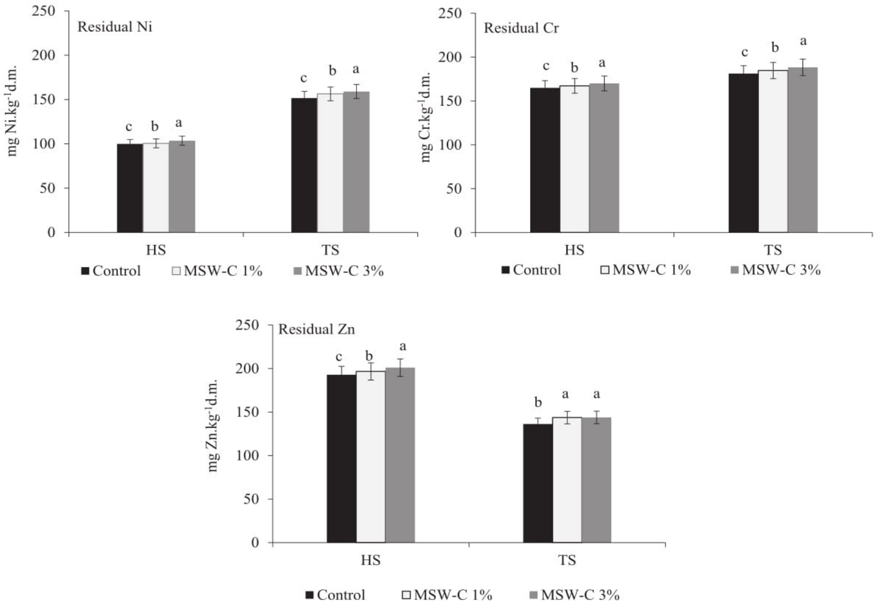

Fig. 3. Residual trace metals. For each soil, bars with the same letter are not statistically different at $P < 0.05$.

which are often overlooked, should always accompany any chemical assessment to get a more complete understanding of the amendment effects.

In this study the influence of MSWC on the activity of selected enzyme such as dehydrogenase (DHG), urease (URE) and β-glucosidase (GLU), was evaluated. Dehydrogenase activity reflects the ability of a group of intracellular enzymes to oxidize organic molecules. As such, DHG activity provides an estimation of the total oxidative activity in soil and to some extent reflects the stress level faced by soil microbial communities (Fernandez et al., 2005; Garau et al., 2011). The DHG values of treated HS and TS soils were higher than those recorded in untreated soils (e.g. ~76 and 17% higher in HS and TS soils treated with 3% MSWC) (Fig. 4). On one hand, this could be due to the higher concentration of labile contaminants (especially trace metals) in control soils which exerted a negative effect towards soil microbial abundance and activities (Fernandez et al., 2005; Garau et al., 2011). On the other hand, the higher DHG values in amended soils indicated enhanced oxidative capacities of the microbial community which could be due to enhanced microbial growth and/or enhanced specific metabolic activity (Garau et al., 2011, 2014, 2017). Similarly, urease activity, which can provide useful information on the rate of urea hydrolysis in soil (i.e. a specific step of N cycling), increased in both soils treated with 3% MSWC (Fig. 4). This suggests that metal(loid)s contamination can have an impact on soil N cycle and that MSWC can be helpful to alleviate and/or reverse such negative effects (Bhattacharyya et al., 2008).

As for DHG and URE, β-glucosidases activity (carried out by extracellular enzymes that cleave β 1 → 4 bonds linking two glucose or glucose substituted molecules) was significantly enhanced in both soils treated with MSWC (e.g. 12 and 27% higher in HS and TS soils treated with 3% MSWC compared with respective controls; Fig. 4). Differently from other studies (e.g. Garau et al., 2011), β-glucosidase showed the same trend of DHG and URE indicating that MSWC can also have a role in the improvement of specific steps of C cycling in metal(loid)s

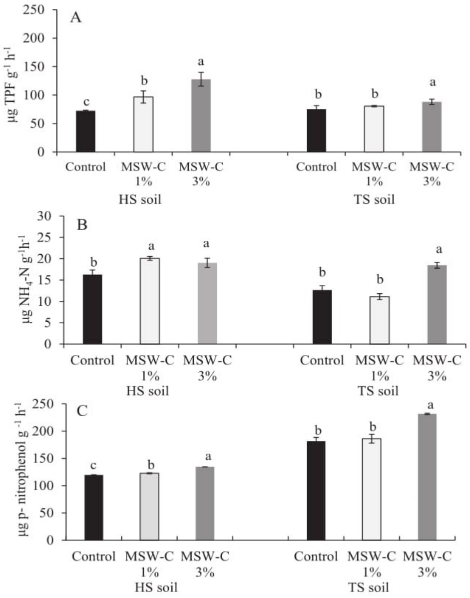

Fig. 4. Selected enzyme activities in HS and TS untreated and amended soils: dehydrogenase (A), urease (B), and β-glucosidase (C). For each soil, bars with the same letter are not statistically different at $P < 0.05$.

polluted soils (Nannipieri et al., 2018).

3.5. Influence of MSWC on microbial community level physiological profile

The Biolog community level physiological profile (CLPP) is a useful tool for distinguishing the culturable fraction of the microbial communities based on their carbon source utilization profile (Garland, 1997). In the last 20 years, this approach was successfully applied to investigate the influence of TM and metalloids on soil microbial communities (e.g. Ellis et al., 2002; Chaerun et al., 2011; Xie et al., 2016), as well as the amendment effects on soil microbiota (e.g. Garau et al., 2011, 2014, 2017).

The CLPP data clearly distinguished HS and TS microbial communities and this pointed out to the presence of distinct microbial populations inhabiting the two soils. This could be somehow expected given the different mineralogical characteristics of HS and TS soils. As already mentioned, TS is a clay soil developed from limestone where the dominant mineral is montmorillonite and illite, vermiculite, calcite and kaolinite occur as minor minerals (Darwish et al., 2001, 2006). Similarly to TS, also HS is a clay soil developed from limestone but kaolinite and quartz are the major minerals present and illite, montmorillonite, and vermiculite occur as minor minerals (Darwish et al., 2001, 2006). In HS soil treated with MSWC, microbial communities showed higher carbon source utilization (AWCD) compared to control (~2-fold higher) (Fig. 5). This was mainly due to a significantly higher utilization of sugar and sugar derivates, carboxylic acids and amino acids in HS-treated soils compared to control (Fig. 5). This suggested an improved C metabolism by the HS microbial community which could be explained with an increased size of the microbial population and/or with a change of the community structure. All these possibilities are compatible with a reduced environmental pressure in HS amended soils (Ellis et al., 2002; Garau et al., 2017), i.e. a reduced concentrations of labile metal(loid)s. Furthermore, the higher content of TOC and TON in treated HS soils could have contributed to the AWCD increase (Garau et al., 2011). The identification of a number of carbon sources exclusively catabolized by the microbial communities of treated HS soils (i.e. Tween 80, D-Cellobiose, α-D-Lactose, β-Methyl-D-Glucoside, γ-Hydroxybutyric acid, Itaconic acid, L-Thereonine, Glycogen, D-Glucosaminic Acid, Glucose-1-Phosphate and D,L-α-Glycerol Phosphate) also suggested an influence of MSWC on the overall soil microbial diversity (Garau et al., 2017).

By contrast, microbial communities of TS control soil showed the highest carbon source utilization (i.e. AWCD) compared to the amended soils (< 40 and 20% for TS-MSWC 1 and 3% respectively, compared to control; Fig. 5). This was complemented by a reduced utilization of sugar and sugar derivates, carboxylic acids and amino acids by the amended soils (Fig. 5). These results are conflicting with those reported in other studies where a reduction of labile metal(loid)s in soil, after compost addition, was accompanied by enhanced AWCD values (e.g. Garau et al., 2017). However, in this latter case MSWC was mixed with water treatment residuals and therefore its influence on the microbial community could not be ascertained. Moreover, other studies reported that substrate utilization in the Biolog plates was mostly due to Gram negative bacteria while Gram positive ones hardly participate to the Biolog pattern (Garau et al., 2007; Preston-Mafham et al., 2002; Smalla et al., 1998). Based on this consideration, the Biolog data not necessarily implied a decline of functionality of TS-amended soils, but they might reflect a strong influence of MSWC on the structure of the culturable resident microbial communities. Apparently, this seems supported by the enzyme activity data, which indicated a positive MSWC effect on soil functionality. Moreover, principal component analysis (PCA) of C source utilization data showed significant modification of the culturable fraction of the bacterial communities after compost addition (Fig. 6). In particular, PCA analysis, which explained approx. 66% of the total variance in the first two components (PC1 and PC2), showed that microbial communities from HS amended soils (especially HS MSWC-3%) were clustered well apart from untreated HS

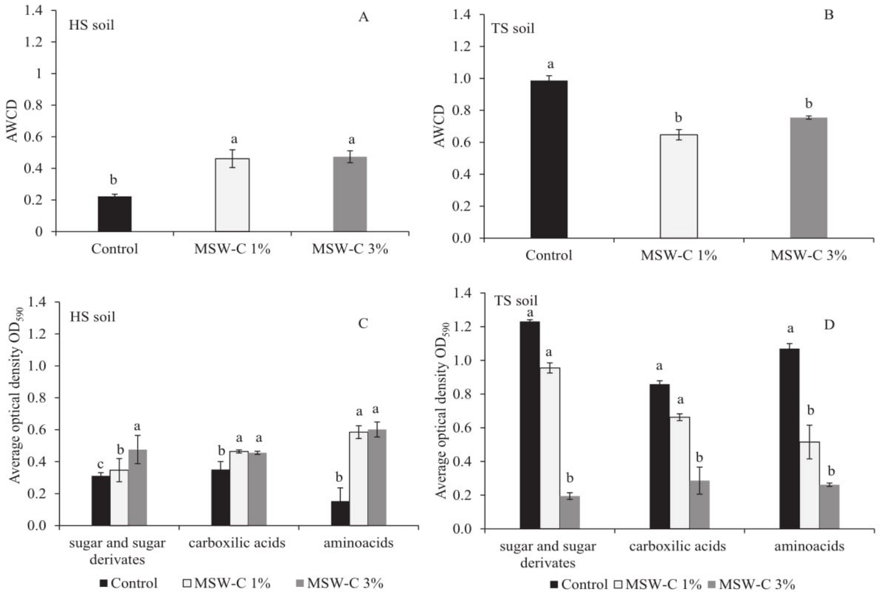

Fig. 5. Average carbon source utilization (AWCD) (A, B) and utilization of specific C sources (C, D) by microbial communities of HS and TS soils. For each soil and Biolog index, bars with the same letter are not statistically different at $P < 0.05$.

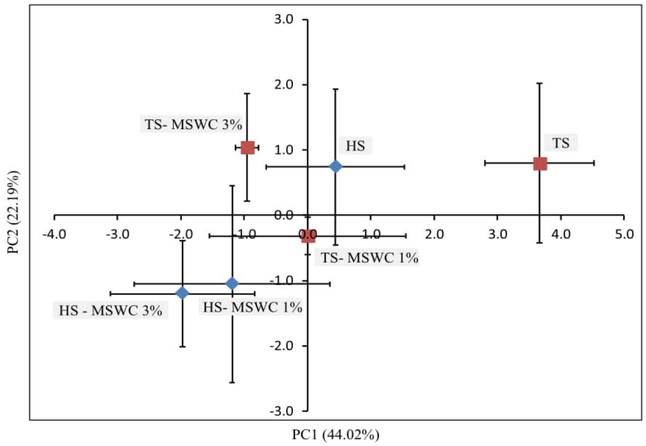

Fig. 6. Principal Components Analysis (PCA) applied to normalized carbon source utilization data (i.e. OD_{590} values).

soil indicating a clear impact of compost on the microbial community structure (Fig. 6). This also applies to TS soil in which the amendment impact on the microbial communities appeared even clearer: treated and untreated TS soils were all well separated by the PCA analysis implying a substantial influence of compost, and its addition rate, on the microbial community (Fig. 6).

4. Conclusions

Municipal solid waste compost revealed effective at fixing TM and metalloids co-occurring in two circum neutral and sub alkaline Lebanese soils. Overall, the addition of MSWC, especially when applied at 3% rate, favored the reduction of labile TM pools in soils (Ni and Zn in particular) and of the As and Sb associated to Fe and Al (hydr)oxides, while increasing at the same time the residual metal(loid) fractions. This had a relevant impact on soil enzyme activities such as DHG, URE and GLU, which appeared significantly increased in soils amended with 3% MSWC. Biolog data also indicated that MSWC clearly influenced the structure of microbial communities. Taken together, these results suggest that MSWC, especially at the 3% rate, could be used to limit the mobility and bioavailability of metal(loid)s in similar Eastern Mediterranean soils and promote the recovery of soil functionality. Given the large availability of MSWC worldwide, and its limited costs, the use of compost for the in situ remediation of contaminated soils can be considered as a sustainable and environmental friendly approach which should be considered when soil remediation interventions are needed. Furthermore, the lack of threshold limits for metal(loid)s in Lebanese soils makes urgently necessary the proposal of a specific project of law.

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Chapter 3

Biochar and compost as gentle remediation options for the recovery of trace elements contaminated soils

Lena Abou Jaoude[a], Paola Castaldi[b]*, Nadine Nassif[a], Maria Vittoria Pinna[c], Giovanni Garau[c]*, 2019. Biochar and compost as gentle remediation options for the recovery of trace elements contaminated soils. Science of the Total Environment
DOI: https://doi.org/10.1016/j.scitotenv.2019.134511

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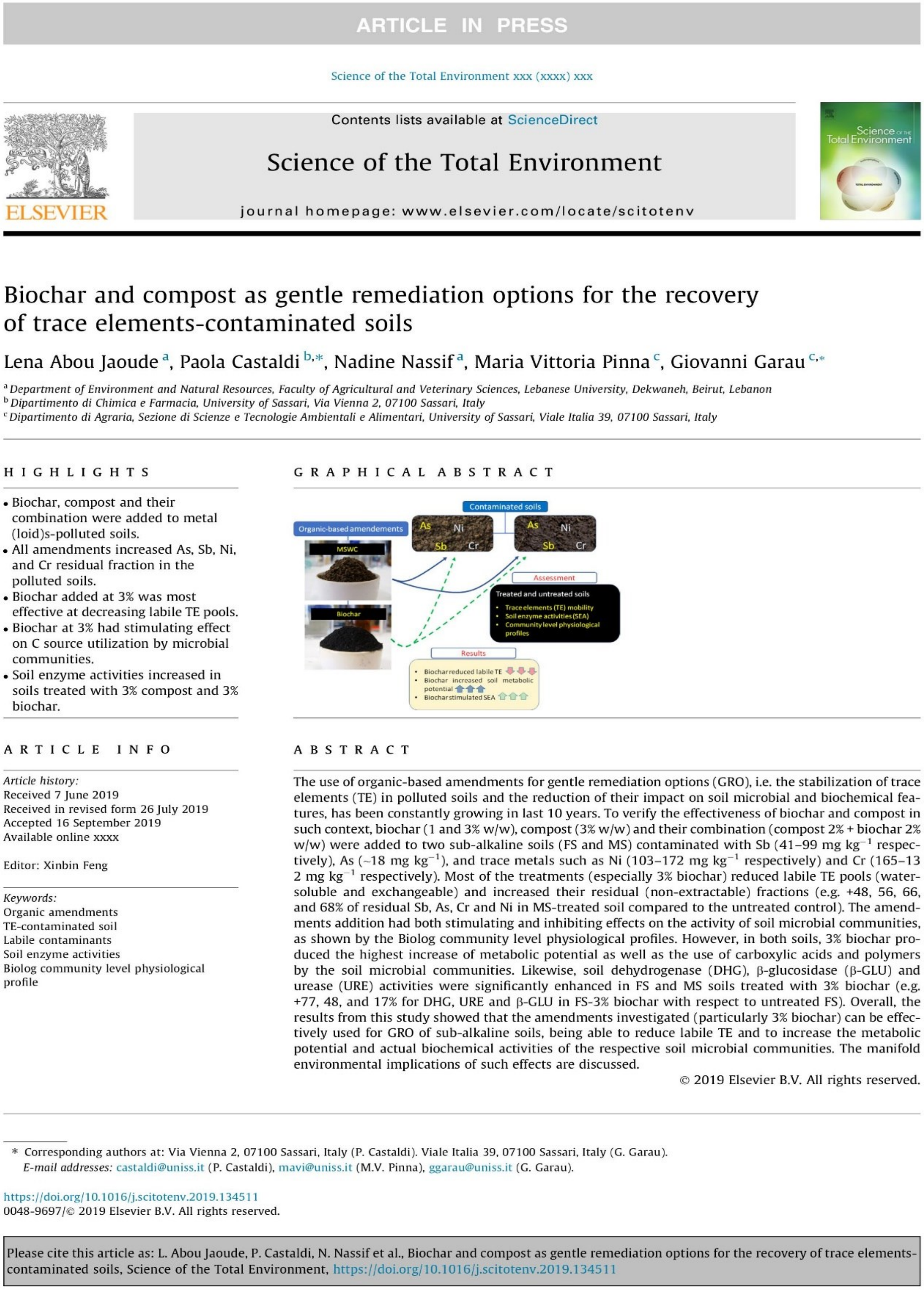

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Science of the Total Environment xxx (xxxx) xxx

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Biochar and compost as gentle remediation options for the recovery of trace elements-contaminated soils

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HIGHLIGHTS

- Biochar, compost and their combination were added to metal (loid)s-polluted soils.
- All amendments increased As, Sb, Ni, and Cr residual fraction in the polluted soils.
- Biochar added at 3% was most effective at decreasing labile TE pools.
- Biochar at 3% had stimulating effect on C source utilization by microbial communities.
- Soil enzyme activities increased in soils treated with 3% compost and 3% biochar.

GRAPHICAL ABSTRACT

ARTICLE INFO

Article history:
Received 7 June 2019
Received in revised form 26 July 2019
Accepted 16 September 2019
Available online xxxx

Editor: Xinbin Feng

Keywords:
Organic amendments
TE-contaminated soil
Labile contaminants
Soil enzyme activities
Biolog community level physiological profile

ABSTRACT

The use of organic-based amendments for gentle remediation options (GRO), i.e. the stabilization of trace elements (TE) in polluted soils and the reduction of their impact on soil microbial and biochemical features, has been constantly growing in last 10 years. To verify the effectiveness of biochar and compost in such context, biochar (1 and 3% w/w), compost (3% w/w) and their combination (compost 2% + biochar 2% w/w) were added to two sub-alkaline soils (FS and MS) contaminated with Sb (41–99 mg kg^{-1} respectively), As (~18 mg kg^{-1}), and trace metals such as Ni (103–172 mg kg^{-1} respectively) and Cr (165–132 mg kg^{-1} respectively). Most of the treatments (especially 3% biochar) reduced labile TE pools (water-soluble and exchangeable) and increased their residual (non-extractable) fractions (e.g. +48, 56, 66, and 68% of residual Sb, As, Cr and Ni in MS-treated soil compared to the untreated control). The amendments addition had both stimulating and inhibiting effects on the activity of soil microbial communities, as shown by the Biolog community level physiological profiles. However, in both soils, 3% biochar produced the highest increase of metabolic potential as well as the use of carboxylic acids and polymers by the soil microbial communities. Likewise, soil dehydrogenase (DHG), β-glucosidase (β-GLU) and urease (URE) activities were significantly enhanced in FS and MS soils treated with 3% biochar (e.g. +77, 48, and 17% for DHG, URE and β-GLU in FS-3% biochar with respect to untreated FS). Overall, the results from this study showed that the amendments investigated (particularly 3% biochar) can be effectively used for GRO of sub-alkaline soils, being able to reduce labile TE and to increase the metabolic potential and actual biochemical activities of the respective soil microbial communities. The manifold environmental implications of such effects are discussed.

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https://doi.org/10.1016/j.scitotenv.2019.134511
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Please cite this article as: L. Abou Jaoude, P. Castaldi, N. Nassif et al., Biochar and compost as gentle remediation options for the recovery of trace elements-contaminated soils, Science of the Total Environment, https://doi.org/10.1016/j.scitotenv.2019.134511

1. Introduction

Soil contamination by trace elements (TE, e.g. Pb, Cd, Zn, As and Sb) is attracting global concern due to their toxicity, persistency and ability to negatively affect soil chemical, microbial and biochemical properties, plant growth, groundwater and food quality, as well as animal and human health (He et al., 2018; Mehmood et al., 2018; Renella et al., 2005). For these reasons, reclamation of TE-contaminated soils should be considered as a priority objective worldwide. Gentle soil remediation options (GRO) can be exploited to achieve soil recovery while limiting at the same time the environmental and economic impact of the interventions. One of such options consists in the use of sorbents (or amendments) elaborated with recycled materials able to reduce the mobility and bioavailability of TE in contaminated soils (i.e. *in-situ* stabilization) and to promote the recovery of soil functioning (Castaldi et al., 2018; Garau et al., 2017, 2019a). A number of organic and inorganic amendments have been tested for this aim such as zeolite, red mud, lime, compost, zero-valent Fe, Fe/Al oxides and water treatment residuals among the others (e.g. Garau et al., 2014, 2019a; Komarek et al., 2013; Manzano et al., 2016; Qiao et al., 2018; Silvetti et al., 2014). Some of these treatments revealed effective at reducing TE mobility, through sorption or precipitation reactions, as well as at enhancing the microbiological and biochemical features of the contaminated soils. However, the simultaneous presence of potentially-toxic cationic and anionic TE in soil can make the search for an efficient sorbent very challenging, as recently highlighted (Garau et al., 2017; Mele et al., 2015).

In this context, biochar, i.e. the carbonaceous material originated from the pyrolysis of different organic wastes in low-oxygen conditions, can represent a potentially effective and environmental-friendly solution. During the past few years, the use of biochar as a GRO for the recovery of TE-polluted soils has been attracting growing research interest (Bandara et al., 2016; Fang et al., 2016; Mehmood et al., 2018; Mohan et al., 2014; Qiao et al., 2018). Biochar is an alkaline sorbent rich in aromatic carbon (that confers a substantial chemical and biological stability to the material), characterized by a porous structure encompassing micro, meso and macro pores (Jindo et al., 2014; Xu et al., 2016). The pyrolysis conditions (i.e. temperature and time) influence the physical and chemical properties of biochar, e.g. high temperatures decrease biochar acidity and increase its specific surface area (Jindo et al., 2014; Xu et al., 2016). During the pyrolysis process, chemical bonds are broken and rearranged giving rise to a product characterized by a variety of functional groups such as amino, hydroxyl, ester, nitro, carboxyl and carbonyl (Xu et al., 2016). Other than increasing soil fertility status (Li et al., 2017; Zhang et al., 2013) and stimulating soil microbial abundance and activity (Gómez et al., 2014; Xu et al., 2016), biochar can have a role in decreasing the mobility of trace metal cations such as Pb(II), Ni (II) and Cd(II) (Fang et al., 2016). Overall, this is due to its microporous structure and high surface area as well as to the presence of different functional groups able to retain positively and negatively-charged TE species (Bandara et al., 2016; Mehmood et al., 2018; Qiao et al., 2018; Xu et al.; 2013). In particular, the presence of oxygenated functional groups (e.g. phenolic, hydroxyl and carboxyl groups) can give rise to the formation of inner-sphere complexes with trace metal cations (Beesley et al., 2015; Qiao et al., 2018). Moreover, electrostatic interactions between carboxylate functional groups of biochar and trace metals can also occur as the result of exchange reactions between H^+ or other cations adsorbed on biochar surface (e.g. Ca^{2+}, Mg^{2+}, Na^{2+}) as well as π-coordination between trace metals (TM) and electrons involved in biochar C═C bonds (Lu et al., 2012; Qiao et al., 2018; Zhang et al., 2013). Besides, the addition of biochar to polluted soils may promote the precipitation of metal cations, e.g. by increasing soil pH (Fang et al., 2016; Lu et al., 2012; Zhang et al., 2013).

The effectiveness of biochar in immobilizing oxy-anionic species in soil, such as As(V), was previously reported, even if a limited number of reports addressed this issue (Fang et al., 2016; Qiao et al., 2018). Some studies showed that biochar could immobilize anionic species in soil due to its mineral oxides content (e.g. amorphous Fe or Al oxy-hydroxides). This likely occurred through ligand exchange reactions where the surface —OH groups of the oxide were exchanged by the metalloid (Fang et al., 2016; Qiao et al., 2018). However, Beesley et al. (2014) and Derakhshan Nejad et al. (2017) reported that biochar applied to a multi-contaminated soil, determined the immobilization of metal cations and a significant increase of the available arsenic, due to the increase of soil pH. The same was found by Qiao et al. (2018) when biochar was applied to a soil contaminated by Cd and As. Taken together, these studies suggest that biochar can be a promising candidate for GRO of TE-contaminated soils even if, due to the variety of the starting organic feedstock and pyrolysis conditions, its actual capabilities should be experimentally proven, especially when negatively-charged TE are present in soil. The same applies to other organic-based amendments such as compost, whose fixing abilities towards TE were widely proved for cationic species but still remain controversial for negatively-charged elements (Garau et al., 2017; Manzano et al., 2016; Silvetti et al., 2017; Sundman et al., 2015). Overall, this highlights the need of further researches on the effectiveness of organic-based amendments, such as biochar and compost, as resources for GRO of TE-contaminated soils such as trace metal cations and negatively-charged metalloids. The aim of this work was therefore to evaluate the influence of a biochar from organic municipal solid waste (Bio), a compost derived from municipal solid waste and green waste composting (MSWC), and their combination (Bio + MSWC) on the mobility of trace elements in two middle-eastern contaminated soils, as well as to determine the amendments effect on selected soil biochemical and microbial features.

2. Materials and methods

2.1. Study area and soil sampling

Soil samples were randomly collected (between 5 and 30 cm depth) from two agricultural regions of Lebanon, where fertilizers and pesticides were used excessively (Korfali et al., 2014): Arab Faour in the Central plain of Bekaa [Faour soil (FS): Eastern Lebanon; 33°47.590′ latitude, 35°58.597′ longitude] and Mazerih Balde in Akkar Governate [Mazerih soil (MS): Northern Lebanon; 34 33′36.0″ latitude, 36 08′56.4″ longitude] at spring time in April 2018. The former soil was a Calcic Vertisols (Word Reference Base, WRB, Soil Survey Staff 2014), while the latter was an Areno-Eutric Leptosols (WRB, Soil Survey Staff, 2014).

The main crops cultivated in the Arab Faour area were *Lactuca sativa* L. (lectuce) and *Allium fistulosum L.* (green onion) (Darwish et al., 2008, 2011), while *Olea europaea* L. (olive) trees are mainly grown in Mazerih (Kassir et al., 2012).

The soil samples collected from each region (n = 100; approx. 1.5 kg each) were bulked together in the laboratory, sieved to <2 mm and used to set up different soil mesocosms.

2.2. Experimental design and mesocosms set up

The experiment was a completely randomized design with five treatments and three replicates. A total of 30 mesocosms, each consisting of approx. 8 kg soil, were prepared (2 soils × 5

Please cite this article as: L. Abou Jaoude, P. Castaldi, N. Nassif et al., Biochar and compost as gentle remediation options for the recovery of trace elements-contaminated soils, Science of the Total Environment, https://doi.org/10.1016/j.scitotenv.2019.134511

treatments × 3 replicates = 30). The following treatments were applied to FS and MS soil mesocosms: 100% soil (control), 1% (w/w) biochar (1% Bio), 3% (w/w) biochar (3% Bio), 3% (w/w) municipal solid waste compost (3% MSWC), 2% (w/w) biochar + 2% (w/w) municipal solid waste compost (2% Bio + 2% MSWC).

The biochar, obtained by pyrolysis of an organic municipal solid waste from South of Lebanon at 400 °C for two hours, was provided by the Intaj S.A.L pyrolysis plant in Zrarieh (Saida - South Lebanon). The municipal solid waste compost (MSWC) was provided by the Secit Facility Plant S.p.A. Consorzio Zir (Chilivani Ozieri - Sardegna, Italy) and derived from food and green waste.

Before use, compost and biochar were finely ground, sieved to <2 mm (Table 1) and then applied to soil mesocosms. These were equilibrated at 25 °C for a period of 2 months during which they were mixed twice a week and maintained at 30% of their water holding capacity.

2.3. Physico-chemical characterization of soil mesocosms, compost and biochar

After the incubation period, triplicate soil samples from the different mesocosms were characterized from a physico-chemical point of view according to the Italian standard guidelines (Gazzetta Ufficiale, 1992). Particularly, pH and electric conductivity (EC) were determined using 1:2.5 w/v soil:water supsensions, total organic C (TOC) and total N were determined using a CHN analyzer (Leco CHN 628) with Oat meal Leco part n° 502–276 as calibration sample, dissolved organic carbon (DOC) was determined in 1:10 w/v soil:water suspensions (Silvetti et al., 2014). Cation exchange capacity (CEC) was measured using the $BaCl_2$ and triethanolamine method. Total concentrations of trace elements in soil, biochar and compost were quantified after digestion with HNO_3 and HCl (1:3 v/v ratio) in a Microwave Milestone MLS 1200 using an Analytik Jena AG atomic absorption spectrometer (ZEEnit-650P graphite furnace). For each parameter, mean values ± standard deviations were reported.

2.4. Arsenic and antimony mobility in soil mesocosms

The mobility of As and Sb, which are mainly present in soil as anionic species [i.e. arsenate As(V), and antimonate Sb(V)], was determined in triplicate independent soil samples collected from each mesocosm using the sequential extraction procedure of Wenzel et al. (2001). Briefly, the non-specifically and specifically-sorbed Sb and As were determined after extraction with 0.05 M $(NH_4)_2SO_4$ (fraction 1) and 0.05 M $NH_4H_2PO_4$ (fraction 2) respectively. Sb and As bound to amorphous and well crystallized Fe and Al hydroxides were assessed after extraction with 0.2 M NH_4^+-oxalate buffer (fraction 3) and 0.2 M NH_4^+-oxalate buffer + 0.1 M ascorbic acid (fraction 4) respectively. Following each step of the sequential extraction, samples were centrifuged for 15 min at 3000 rpm and filtered using Whatman 41 filters to separate the liquid and the solid phases. Soluble As and Sb were quantified in the liquid phase collected after each step of the sequential extraction using an Analytik Jena AG atomic absorption spectrometer (ZEEnit-650P graphite furnace). After the fourth step, the residual solid phase was dried at 105 °C overnight and digested using an HNO_3 and HCl mixture (1:3 v/v ratio) in a Microwave Milestone MLS 1200. As and Sb were then quantified as previously described and their concentrations were reported as mean values ± standard deviations.

Table 1
Characterization of biochar (Bio) and municipal solid waste compost (MSWC) (dry matter basis).

Chemical parameters	Bio	MSWC
pH	8.85 ± 0.01	8.84 ± 0.02
Ash (%)	31.86 ± 0.48	42.05 ± 0.73
EC (mS cm^{-1})	11.67 ± 0.04	15.58 ± 0.69
Total organic Carbon (TOC, %)	41.52 ± 0.65	24.56 ± 3.7
Total N (%)	1.34 ± 0.07	1.80 ± 0.36
Organic matter (%)	71.38 ± 0.65	42.14 ± 3.70
Cationic exchange capacity (CEC, $cmol_{(+)} \cdot kg^{-1}$)	105.1 ± 0.1	87.10 ± 1.60
Dissolved organic carbon (DOC, mg kg^{-1})	2317 ± 0.02	1944 ± 0.02
Total trace elements (mg kg^{-1})		
Cd	n.d.	n.d.
Ni	40.12 ± 0.03	15.13 ± 3.02
Cr	1.33 ± 0.02	0.35 ± 0.06
Pb	0.34 ± 0.06	3.72 ± 0.15
Zn	2.50 ± 0.02	30.52 ± 3.20
As	n.d.	n.d.
Sb	n.d.	n.d.

n.d. not detected.

2.5. Trace metals mobility in soil mesocosms

The mobility of trace metals, specifically Ni and Cr, was determined in triplicate independent soil samples collected from each mesocosm using the sequential extraction procedure of Basta and Gradwohl (2000). Briefly, the concentration of soluble and exchangeable Ni and Cr was determined after extraction with 0.5 M $Ca(NO_3)_2$ (fraction 1). The metals forming weak surface complexes were quantified with 1 M NaOAc (fraction 2). Finally, 0.1 M Na_2EDTA was used to quantify strongly surface-complexed and precipitated metals. Following each step of the sequential extraction, samples were centrifuged for 15 min at 3000 rpm and filtered using Whatman 41 filters to separate the liquid and the solid phases. Soluble Ni and Cr were quantified in the liquid phase collected after each step using an Analytik Jena AG atomic absorption spectrometer (ZEEnit-650P graphite furnace). After the third step, the residual solid phase was dried at 105 °C overnight and digested using HNO_3 and HCl mixture (1:3 v/v ratio) in a Microwave Milestone MLS 1200. Ni and Cr were then quantified as previously described. Trace metal concentrations were reported as mean values ± standard deviations.

2.6. Soil enzyme activities and Biolog community level physiological profiles

Selected enzyme activities were determined in triplicate independent soil samples collected from each mesocosm following the methodology described by Alef and Nannipieri (1995). In particular, the dehydrogenase activity (DHG) was determined colorimetrically by quantifying the concentration of triphenylformazan after incubation of the soil samples with triphenyltetrazolium chloride. The urease activity (URE) was determined colorimetrically by quantifying the ammonia released from soil samples incubated with urea. The β-glucosidase activity (GLU) was determined colorimetrically by quantifying the concentration of *p*-nitrophenol released from soil samples incubated with *p*-nitrophenyl glucoside. Enzyme activity values were reported as mean values ± standard deviations.

The community level physiological profile (CLPP) was determined in triplicate independent soil samples collected from each mesocosm using the Biolog EcoPlates (Biolog Inc., Hayward, CA) as previously described (Garau et al., 2011, 2014). Briefly, CLPP analysis started with the recovery of soil microbial communities which was carried out by diluting 10 g of soil in 90 mL of sodium pyrophosphate (2 g L^{-1}) and shaking at 28 °C for 30 min at 150 rpm. The suspension was then 1:10 diluted in NaCl solution (0.89% w/v), centrifuged for 5 min at 2000 rpm and filtered using Whatman 1 filter paper. Afterwards, 120 μL of the filtrate were inoculated in each of the Biolog Ecoplate wells and all the Biolog

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plates were incubated in the dark at 26 °C for 4 days. The Biolog Ecoplate is a 96-wells microtiter plate which contains a triplicate set of 31 carbon sources and a control well (with no carbon) (Insam, 1997). Carbon source utilization in each well was measured at time zero, and then every 24 h for 4 days, by recording the absorbance at 590 nm (OD_{590}) using a Biolog MicroStation™ reader. Biolog Ecoplate data (i.e. OD_{590} values) were then processed as previously reported (Garau et al., 2019b). Briefly, raw OD_{590} values were first blanked against the time zero readings and the resulting data were further blanked against the respective control well values. These data were finally used to determine the potential metabolic activity of each soil microbial community, i.e. the Average Well Colour Development (AWCD). This was calculated as the average OD_{590} value over 31 substrates as previously described (Garland, 1997). Likewise, the use of selected substrate guilds (i.e., carboxylic acids, amino acids and polymers) was also quantified (Garland, 1997) along with the number of carbon sources catabolized by each microbial community, i.e richness values ($OD_{590} > 0.15$). AWCD, richness and carbon source utilization data were reported as mean values ± standard deviations.

2.7. Statistical analysis of data

All analyses were performed in triplicate, with data reported as mean values. Differences between means were analyzed using Minitab 17 software for windows. One-way ANOVA followed by post hoc Fisher's least significant difference test were applied for mean separation ($P < 0.05$). When significant P values were obtained (i.e. $P < 0.05$), differences between individual means were investigated using the post hoc Fisher's least significant difference test ($P < 0.05$).

The relationship between some chemical soil parameters and TE extracted in fractions 1 and 2 and residual TE was evaluated by Pearson correlation analysis (Tables S1 and S2). Statistical analyses were carried out using the NCSS 2007 Data Analysis software (v. 07.1.21; Kaysville, Utah).

3. Results and discussion

3.1. Soils characteristics and pollution status

FS and MS soils had sub-alkaline pH values and similar EC, total nitrogen and cation exchange capacity (Table 2). MS had a higher TOC compared to FS, and this was in line with the higher DOC concentration detected in the former soil (~10% higher in MS compared to FS; Table 2). These soil characteristics suggest a fertility status suitable for different agricultural activities which are indeed practiced in both sampling areas, i.e. olive production in the north and potatoes, lettuce, wheat, radish, onions and industrial crops (e.g. grapevine, sugar beet and tobacco) in Bekaa area (Darwish et al., 2006).

Concerning trace elements concentration in both soils, it should be noticed that reference or threshold limits are not defined by the Lebanese legislation. Accordingly, risks related to the presence of potentially toxic trace elements in soil can only be identified using approaches developed by other countries. The threshold limits established by the Finnish legislation can be useful in this case (Ministry of the Environment – MEF, Finland, 2007), as they represent a mean of the different standards set by EU countries and India (Awasthi, 2000; Carlon et al., 2007). The same approach has been recently used by Tóth et al. (2016) to assess the trace elements contamination status and remediation needs in agricultural soils of the European Union. According to these standards, the total concentration of Sb and Ni, in FM and MS soils (Table 3), exceeded the lower and/or the higher guideline limits, posing significant eco-

Table 2
Characterization of FS and MS unamended and amended with biochar (Bio), compost (MSWC) and their combination (Bio + MSWC) (dry matter basis). For each parameter and each soil, mean values with the same letter are not statistically different at $P < 0.05$ (Fisher's LSD test).

Chemical parameters	FS soil					MS soil				
	Untreated	1% Bio	3% Bio	3% MSWC	2% Bio + 2% MSWC	Untreated	1% Bio	3% Bio	3% MSWC	2% Bio + 2% MSWC
Texture	Sandy Clay Loam	–	–	–	–	Sandy Clay Loam	–	–	–	–
Sand %	58.19 ± 2.95	–	–	–	–	64.16 ± 3.14	–	–	–	–
Silt %	11.66 ± 0.77	–	–	–	–	11.95 ± 2.95	–	–	–	–
Clay %	30.15 ± 1.46	–	–	–	–	23.87 ± 1.32	–	–	–	–
pH	8.00 ± 0.05^{a}	8.10 ± 0.01^{b}	8.17 ± 0.01^{c}	8.12 ± 0.01^{b}	8.11 ± 0.05^{bc}	7.91 ± 0.01^{a}	8.16 ± 0.00^{b}	8.18 ± 0.02^{b}	8.16 ± 0.01^{b}	8.10 ± 0.06^{b}
EC ($mS \cdot cm^{-1}$)	0.20 ± 0.07^{a}	0.22 ± 0.01^{a}	0.36 ± 0.02^{c}	0.30 ± 0.01^{b}	0.34 ± 0.04^{bc}	0.19 ± 0.01^{a}	0.23 ± 0.02^{b}	0.25 ± 0.01^{b}	0.25 ± 0.03^{b}	0.27 ± 0.01^{b}
TOC (%)	1.60 ± 0.13^{a}	1.79 ± 0.17^{b}	2.26 ± 0.15^{c}	1.89 ± 0.10^{b}	2.15 ± 0.12^{c}	1.87 ± 0.08^{a}	2.06 ± 0.11^{b}	2.48 ± 0.12^{c}	2.09 ± 0.08^{b}	2.09 ± 0.16^{b}
Total N (%)	0.17 ± 0.01^{a}	0.19 ± 0.04^{a}	0.23 ± 0.01^{a}	0.22 ± 0.01^{a}	0.24 ± 0.13^{a}	0.19 ± 0.02^{a}	0.21 ± 0.05^{a}	0.24 ± 0.02^{a}	0.25 ± 0.08^{a}	0.24 ± 0.01^{a}
Organic matter (%)	2.75 ± 0.15^{a}	3.08 ± 0.12^{b}	3.89 ± 0.11^{c}	3.25 ± 0.13^{b}	3.70 ± 0.12^{c}	3.22 ± 0.11^{a}	3.54 ± 0.15^{b}	4.27 ± 0.13^{c}	3.59 ± 0.11^{b}	3.59 ± 0.18^{b}
CEC ($cmol_{(+)} \cdot kg^{-1}$)	18.17 ± 0.17^{a}	18.36 ± 0.02^{a}	19.46 ± 0.03^{b}	18.43 ± 0.14^{a}	19.41 ± 0.06^{b}	19.44 ± 0.14^{a}	19.77 ± 0.11^{b}	21.96 ± 0.07^{e}	20.67 ± 0.15^{c}	21.46 ± 0.11^{d}
DOC ($mg \cdot kg^{-1}$)	35.42 ± 0.52^{a}	46.92 ± 0.15^{b}	86.06 ± 0.36^{e}	61.59 ± 0.47^{c}	76.62 ± 0.29^{d}	38.77 ± 0.27^{a}	45.08 ± 0.30^{b}	82.02 ± 0.40^{d}	80.72 ± 0.66^{c}	79.27 ± 0.79^{c}

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Table 3
Trace elements content in FS and MS soils. Threshold and guideline values for TE in soils are also reported (extract; MEF, 2007).

Total trace elements (mg kg^{-1} d.m.)	FS soil	MS soil	Threshold value	Lower guideline values	Higher guideline values
Antimony (Sb)	41.01 ± 5.66*	99.24 ± 14.1*	2	10	50
Arsenic (As)	18.20 ± 0.85*	18.63 ± 2.50*	5	50	100
Cadmium (Cd)	0.69 ± 0.13	0.12 ± 0.03	1	10	20
Chromium (Cr)	164.7 ± 3.22*	132.0 ± 0.23*	100	200	300
Lead (Pb)	10.73 ± 0.68	18.26 ± 0.25	60	200	750
Nickel (Ni)	103.3 ± 4.10*	172.1 ± 1.57*	50	100	150
Zinc (Zn)	172.5 ± 3.54	123.5 ± 2.12	200	250	400

TE exceeding the threshold values defined by the Finnish legislation are marked by an (*) (Government Decree on the Assessment of Soil Contamination and Remediation, Needs 214/2007).

logical and/or health risks (Tóth et al., 2016). As and Cr largely exceeded the threshold values, but not the lower guideline limits, thus requiring further assessment (Tóth et al., 2016). FS and MS soils showed the same As content and similar Cr level, while Sb and Ni concentrations were ~ 2.4- and 1.6-fold higher in MS compared to FS soil (Table 3). Overall, the co-occurrence of such concentrations of TE in both soils might be due to different causes such as the soil parent material (this could be the case of Ni; Nsouli et al., 2004), the excessive use of agrochemicals as well as the use of wastewater or contaminated water (this is the case of the Litani river) for crop irrigation. It should be noted that MS and FS soils are located in the first and the second most important agricultural area of Lebanon respectively (Darwish et al., 2008; Korfali et al., 2014; Nsouli et al., 2004), i.e. the North Lebanon-Akkar and the Central plain of Bekaa. Moreover, a number of other contaminated sites have been previously identified within these areas and, together with our results, this overall indicates a diffuse TE pollution of such agricultural soils (Darwish et al., 2008; Korfali et al., 2014). In this sense, the recorded soil contamination status confirms a worrying situation that can affect food quality and can have a serious impact on human health. For these reasons, the remediation of such soils should be a prompt priority and the reduction of the TE mobility and potential bioavailability the main aim to achieve.

3.2. Influence of compost, biochar and their combination on soil features and TE mobility

Biochar, compost, and their combination generally determined a significant increase of soil pH (Table 2). Moreover, the amendments addition clearly improved the soil CEC and its OM content, e.g. CEC increased up to 7.1 and 13% in FS and MS soils treated with 3% Bio with respect to untreated soils, while OM increased up to 41 and 33% respectively. Also, DOC content significantly increased in all treated soils, with the higher DOC content recorded in MS and FS soils treated with 3% Bio (Table 2). Overall, these results indicate that, in the medium term, the amendments selected had a positive impact on some of the main soil attributes regulating plant growth. As such they could have a stimulating effect on plant yield in TE-contaminated soils which however should be experimentally proved (e.g. Agegnehu et al., 2016; Gong et al., 2019).

To evaluate the amendment effects on As and Sb chemical fractionation in soil, sequential extraction procedures were carried out. The results obtained by the sequential extraction procedure highlighted relatively low concentrations (0.85–1.72 mg kg^{-1} soil) of water-soluble and easily exchangeable Sb and As in both untreated soils (fraction 1; Fig. 1). These contaminant pools are considered as the most potentially bioavailable, i.e. those having the greatest impact on soil microorganisms and soil biota in general (Garau et al., 2019a). As such they are also expected to affect soil functionality, e.g. element cycling and/or soil organic matter turn-over (Garau et al., 2017; Manzano et al., 2016). In this sense, these low Sb and As concentrations can be possibly deemed as not so critical from an environmental point of view, even if their precise impact should be determined against different biological endpoints. The water-soluble and easily exchangeable As (fraction 1) decreased significantly in all the treated soils, with the exception of FS-3% MSWC. The highest reductions were observed in 3% Bio (<48.2 and 47.6% in FS- and MS-3% Bio soils respectively, compared to untreated soils; Fig. 1). Similarly, the Sb extracted in fraction 1 (Fig. 1) significantly decreased in both treated soils (<33.1 and 20.9% in FS- and MS-3% Bio soils respectively, compared to untreated soils; Fig. 1). The decrease of such labile fractions overall suggests a strong binding affinity of both TE with the amendments, biochar in particular. This might be due to the presence of amorphous metal oxides (e.g. amorphous Fe or Al oxy-hydroxides) within the biochar and to their well-known affinity towards anionic species in soil (Castaldi et al., 2012, 2014; Fang et al., 2016; Qiao et al., 2018). Moreover, the presence of such metal oxides within the pores and cages of the biochar, and their possible occlusion after metalloid binding, may be responsible for the observed results. This supports some previous findings (e.g. Beesley et al., 2014) in which a reduction of soluble As in soil was noticed after biochar addition. The most labile fractions of As and Sb (i.e. fraction 1) showed significant negative correlations with selected soil chemical parameters, such as pH, organic matter, CEC and DOC (no correlation was detected between labile As and DOC in FS soil) (Tables S1 and S2), thus highlighting the role of the amendments (and their organic matter content in particular) in the immobilization of the TE considered.

The As and Sb extracted in fraction 2 represent the TE fractions that can be mobilized (i.e. becoming bioavailable) following a pH change (Wenzel et al., 2001), e.g. induced by the root and/or microbial activity, or an increase of phosphate concentration, e.g. due to organic matter degradation in soil hot spots. The added amendments did not show a clear and consistent effect on the TE extracted in this fraction (and no correlation between As and Sb and chemical soil parameters were found; Tables S1 and S2), even if, in most cases, the addition of biochar at 3% induced a decrease of these TE extracted (Fig. 1).

The Sb (except for FS-2% Bio + 2% MSWC soil) and As fractions associated with amorphous Fe- and Al-(hydr)oxides (fraction 3) decreased after soil amendment, nonetheless 3% Bio treatment was the most effective at reducing this fraction (Fig. 1). Likewise, the As and Sb extracted in fraction 4, i.e. the TE associated with well crystallized Fe- and Al-(hydr)oxides, decreased after amendment, with 3% Bio showing the highest decrease (Fig. 1). Residual As and Sb, which represent the strongly bound and hardly (bio) available fractions, significantly increased in all treated soils, and 3% Bio revealed the most effective treatment (>136 and 80% of residual As and >76 and 24% of residual Sb, in FS- and MS-3% Bio with respect to controls; Fig. 1).

These findings indicated that biochar, MSWC and their combination were able to modify the As and Sb partitioning in both soils, i.e. mostly reducing the water-soluble and exchangeable fractions of these TE and increasing the hardly (bio)available ones. This

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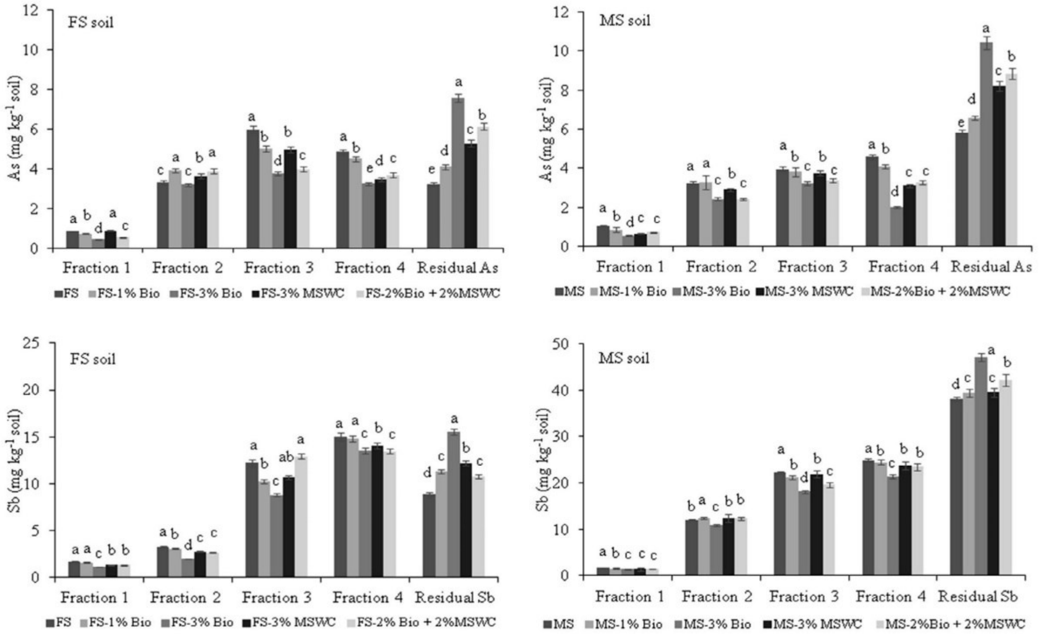

Fig. 1. Arsenic (As) and antimony (Sb) released after sequential extraction. For each soil and for each fraction of the sequential extraction, mean values followed by different letters denote statistically significant differences according to the Fisher's Least Significant Difference (LSD) test ($P < 0.05$).

can be the result of the amendments influence on the soils chemical properties, in particular the OM and CEC increase (Table 2), as supported by the correlations analysis (Tables S1 and S2). The amendments addition probably favored the formation of (insoluble) ternary complexes involving Sb(V) or As(V), polyvalent metal cations (e.g., Fe, Al, and Ca) and the negatively charged carboxylic groups of the biochar or compost. This interaction was demonstrated for both TE by several authors (e.g. Filella and Williams, 2012; Silvetti et al., 2017). Finally, also the co-precipitation of Sb or As with metals present in both the amendments (Ca in particular) could have contributed to increase the residual fraction of Sb and As (e.g. Castaldi et al., 2012, 2014; Diquattro et al., 2018; Manzano et al., 2016). Amongst the treatments, 3% Bio showed the most remarkable effects on metalloid immobilization in agreement with previous reports (Fang et al., 2016; Qiao et al., 2018). However, it should be noted that these latter studies did not address the simultaneous presence of different metalloids in soil. In this sense, this is likely the first report showing a biochar effectiveness at fixing different co-occurring metalloids. Importantly, the only report addressing a similar situation, i.e. the concomitant presence of both As and Sb in a contaminated soil, showed that biochar addition produced a significant increase of the metalloid's solubility (Lomaglio et al., 2017). Overall, our results support the use of biochar at 3% as GRO for the recovery of metalloid contaminated soils.

3.3. Influence of biochar, compost and their combination on TM mobility in soil

The sequential extraction data showed limited amounts of water soluble and exchangeable Ni and Cr in MS and FS soils (fraction 1, Fig. 2), which mostly decreased significantly after amendment addition (Fig. 2). Overall, 3% Bio and 2% Bio + 2% MSWC revealed the most effective treatments. The observed decrease of Ni and Cr labile pools in treated soils could be mainly explained by the increase of pH, OM and CEC, which could have favored the immobilization of the TE considered (i.e. Bandara et al., 2016; Beesley et al., 2014; Manzano et al., 2016). This was also supported by the negative correlations detected in both soils between Ni and Cr extracted in fraction 1 and selected soil chemical parameters (e.g. pH, organic matter and CEC in particular; Tables S1 and S2). The capacity of biochar to reduce the mobility of labile trace metals could be also due to its high specific surface area (Beesley et al., 2015; Qiao et al., 2018).

The amount of Cr bound to carbonates, or forming weak surface complexes (fraction 2), decreased significantly in both soils only after 3% Bio addition (Fig. 2), while Ni extracted in fraction 2 decreased only after MSWC addition in MS soil, while remained unchanged in FS soil (Fig. 2). No correlation between Ni and Cr extracted in fraction 2 and the chemical parameters considered in both soils was detected (Tables S1 and S2). The metals extracted with EDTA (fraction 3), i.e. TM involved in the formation of strong inner-sphere surface complexes and/or precipitates, decreased significantly after the addition of 3% Bio (this is the case of both Cr and Ni in both soils) and of MSWC (this is the case of Ni in FS soil) (Fig. 2).

Residual Ni and Cr, which account for the hardly retained fractions in soil, were approx. 54 and 61% of total Ni and 60 and 47% of total Cr in untreated FS and MS soil, respectively. These fractions increased in both soils following the addition of biochar, compost and their combination indicating a certain effectiveness of all the treatments in reducing the environmental mobility of Ni and Cr.

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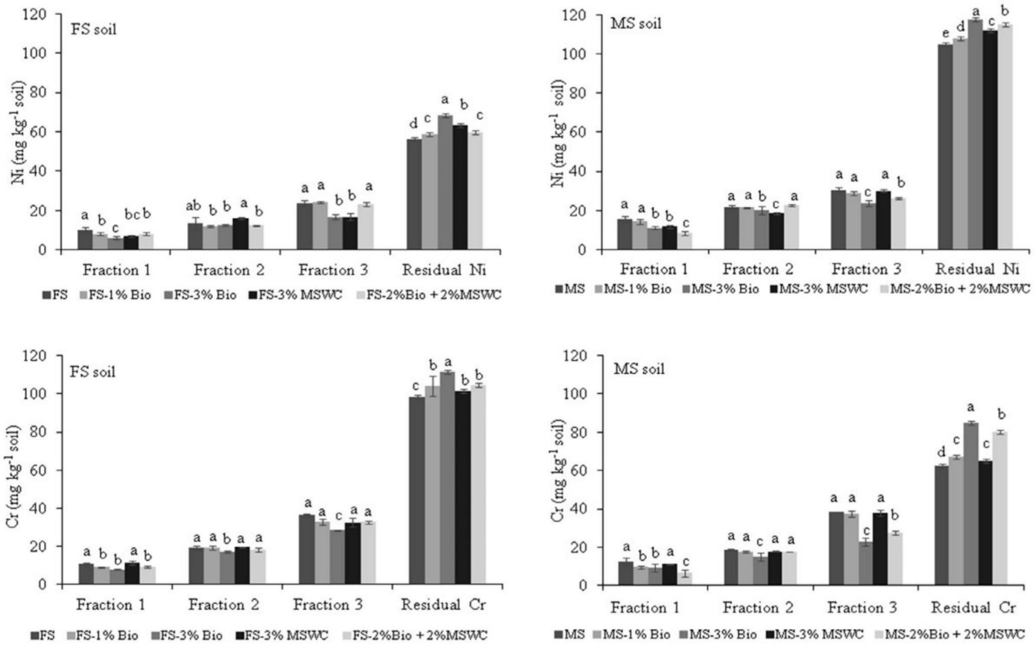

Fig. 2. Chromium (Cr) and Nickel (Ni) released after sequential extraction. For each soil and for each fraction of the sequential extraction, mean values followed by different letters denote statistically significant differences according to the Fisher's Least Significant Difference (LSD) test ($P < 0.05$).

The highest increases of residual TE were recorded in soils treated with 3% Bio. This could be ascribed to the significant changes of the soil chemical properties determined by the addition of this adsorbent (i.e. an increase of pH, OM, and CEC). In particular, such changes could have favored the formation of stable inner sphere complexes between TE and the carboxylic and/or phenolic surface groups of biochar, as well as TE fixation via intraparticle diffusion in meso- and micropores of biochar (Beesley et al., 2015; Fang et al., 2016; Qiao et al., 2018). In addition, the liming effect of all the amendments could have favored metal precipitation, thus contributing to reduce the TM labile pools and increase their residual fraction, as highlighted by the correlation analyses (Table S1 and S2).

3.4. Influence of biochar, compost and their combination on soil enzyme activities

Trace elements above certain thresholds can denature enzymes, interact with their active site or with the enzyme-substrate complex, leading nevertheless to a reduction of the enzymatic activity in contaminated soils (Garau et al., 2019a; Oliveira and Pampulha, 2006). Hence, soil enzyme activities are increasingly considered as sensible proxies of soil ecotoxicity and/or disturbance phenomena occurring in contaminated sites (Garau et al., 2014, 2017, 2019a).

DHG, which reflects the activity of a group of intracellular enzymes to oxidize organic molecules, can be used to infer the total oxidative activity of a soil microbial community (Nannipieri et al., 2018; Oliveira and Pampulha, 2006). Overall, DHG increased in the amended soils particularly after 3% Bio and 2% Bio + 2% MSWC addition, which induced a significant DHG increase compared to control soils (Fig. 3). For instance, DHG increased up to 49% in MS treated with 3% Bio compared to the untreated MS soil (Fig. 3), while in FS soil treated with 3% Bio and 2% Bio + 2% MSWC, DHG increased up to ~77 and 40%, respectively. These results clearly indicate an increase of the oxidative metabolism of microbial communities within the amended soils. This could be due to the observed reduction of labile TE in treated soils as well as to the increase of SOM and DOC content which in turn could have enhanced enzyme activity and/or stimulated microbial growth and multiplication in soil (Garau et al., 2017, 2019a).

Likewise, URE activity, which accounts for the hydrolysis of urea and the release of NH_4^+ ions, increased significantly in treated soils, with 3% Bio being the most effective treatment (>48.3 and 51.6% in FS- and MS-3 %Bio compared to respective controls) (Fig. 3). These results suggest that the amendments addition (biochar in particular) was able to positively affect soil N cycle likely by limiting the concentration of labile TE and/or by increasing the number of urease-producing microbial strains. However, this deserves further investigation as URE catalyzes a specific step of the N cycle and its measurement cannot simply reflect the total N status of the soil as recently highlighted (Nannipieri et al., 2018).

GLU activity, which is due to extracellular enzymes that cleave β 1→4 bonds linking two glucose or glucose substituted molecules, increased in soils treated with 3% Bio, MSWC and Bio + MSWC. Particularly, 3% Bio and 2% Bio + 2% MSWC were the most effective treatments for FS soil (approx. > 16.7% compared to the untreated control), while 3% MSWC performed better in MS soil (>42.7% compared to the untreated control) (Fig. 3). As for URE, the addition of the amendments likely enhanced soil C cycling and this could be supported by the higher DOC content recorded in treated soils.

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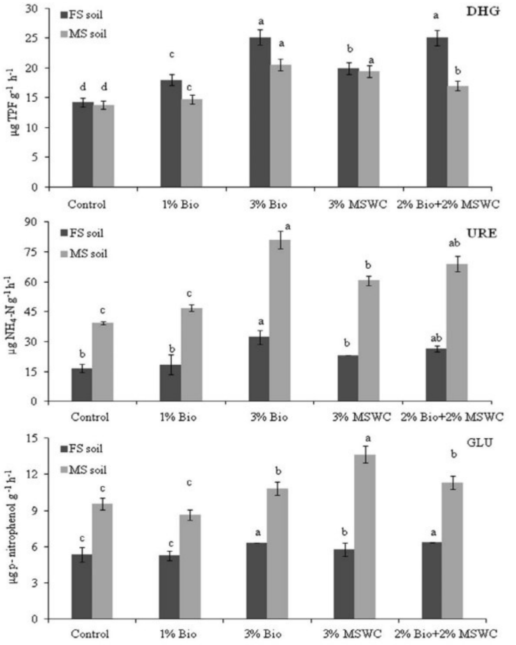

Fig. 3. Dehydrogenase (DHG), urease (URE), and β-glucosidase (GLU) activities in amended and unamended soils. For each soil mean values followed by different letters denote statistically significant differences according to the Fisher's Least Significant Difference (LSD) test ($P < 0.05$).

Overall, the results obtained suggest an active role of biochar and MSWC in the functional recovery of the contaminated soils.

3.5. Influence of biochar, compost and their combination on the microbial community level physiological profile

The Biolog CLPP indicated a clear influence of the tested amendments on soil microbial communities of both soils. In particular, FS soils amended with 3% Bio showed a significant increase of potential carbon source utilization (AWCD) with respect to control soil and similar results were obtained for MS soil (Fig. 4). However, in this latter soil also 1% Bio and 3% MSWC (and not 2% Bio + 2% MSWC) performed well (Fig. 4). Overall, this indicated a positive impact of the amendments (in particular biochar) on the potential catabolic activity of soil microbial populations which was only partially reflected by the total number of carbon sources oxidized (richness) (Fig. 4) which is not uncommon (e.g. Garau et al., 2019a). This might be explained by a larger size of the microbial communities in amended soils (possibly due reduced TE bioavailability and larger availability of C and N sources) and/or by the amendment influence on the structure of the microbial communities as previously pointed out (Ellis et al., 2002; Garau et al., 2017, 2019a). In this sense, it should be noted that the different usage of different carbon source guilds, i.e. carboxylic acids, aminoacids and polymers (Fig. 5), supported an impact of the amendments on the structure and function of soil microbial communities (Garau et al., 2017, 2019a). Overall, these results indicate that biochar added at 3% was able to enhance the potential catabolic activity of microbial

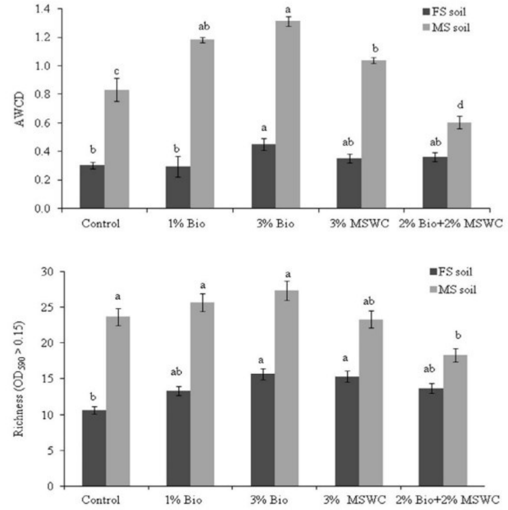

Fig. 4. Average carbon source utilization (AWCD) and richness values of microbial communities extracted from FS and MS soils. For each soil, mean values followed by different letters denote statistically significant differences according to the Fisher's Least Significant Difference (LSD) test ($P < 0.05$).

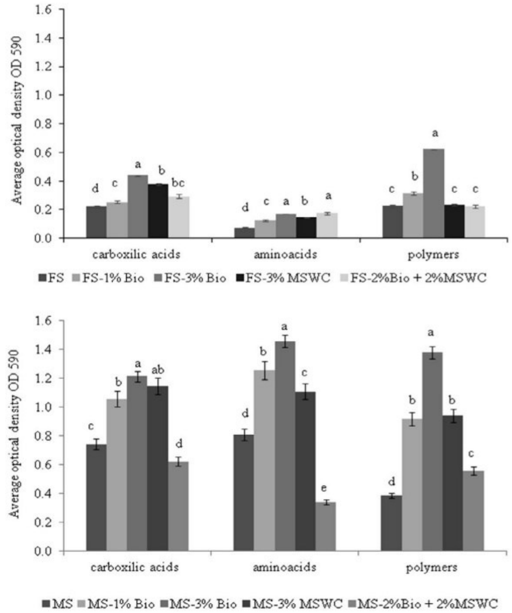

Fig. 5. Utilization of specific carbon source guilds (average OD_{590} values) by microbial communities extracted from FS and MS soils. For each soil, mean values followed by different letters denote statistically significant differences according to the Fisher's Least Significant Difference (LSD) test ($P < 0.05$).

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community of both contaminated soils while the influence of biochar at 1% and MSWC at 3% was dependent on the soil type. Moreover, all the amendments likely had an influence on the structure of the respective soil microbial communities as revealed by the analysis of carbon source guilds consumption.

4. Conclusions

In this study, biochar and compost were added, singly and combined, to two different agricultural soils contaminated by several TE, with the aim to investigate their potential suitability as GRO for the in-situ recovery of such soils.

All the amendments tested revealed effective at increasing the non-extractable (and hardly bioavailable) fraction of the TE present in two agricultural soils, while biochat added at 3% was also particularly successful at reducing the labile fractions of the contaminants, i.e. those more mobile and bioavailable. This was deemed as relevant given the very different chemical behavior of the TE addressed. Most of the treatments enhanced soil biochemical functioning, notably DHG, URE and GLU activities, thus suggesting a positive impact of biochar and compost on soil microbial abundance and/or functioning as well as soil fertility status. The Biolog community level physiological profile also indicated an influence of the amendments on the structure and function of soil microbial communities. Taken together, our results indicate that biochar (especially at 3% rate) and compost can be used in the mid-term as GRO to reduce the mobility and the biological impact of TE occurring in multi-contaminated soils. However, further studies are needed to evaluate the long-term effectiveness of these amendments especially under natural conditions.

Declaration of Competing Interest

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.134511.

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Please cite this article as: L. Abou Jaoude, P. Castaldi, N. Nassif et al., Biochar and compost as gentle remediation options for the recovery of trace elements-contaminated soils, Science of the Total Environment, https://doi.org/10.1016/j.scitotenv.2019.134511

Chapter 4

Influence of biochar on green bell pepper and wheat growth in polluted amended soils

Abstract

The aim of this study was to assess the influence of a biochar added at 3% w/w (3% Bio) and its combination with a municipal solid waste compost, i.e. 2% biochar+2% MSWC (2% Bio-MSWC), on the mobility, potential bioavailability and toxicity of several metal(loid)s, i.e., arsenic (55 mg·kg^{-1}), antimony (84 mg·kg^{-1}), Ni (176 mg kg^{-1}) and Cr (214 mg kg^{-1}), present in a Lebanese contaminated soil (TS). Metal(loid)s uptake by green bell pepper (*Capsicum annuum* L.*)* and wheat (*Triticum aestivum* L.*)* grown on the amended and unamended soils was also investigated in a pot experiment. Biochar addition at 3% rate decreased the labile fractions of metal(loid)s and at the same time increased remarkably their residual fraction compared to the untreated soil (i.e. + 57.5, 56.9, 84.2 and 90.1 % for Sb, As, Ni and Cr respectively). The amendments addition also favoured an increase of the metabolic potential and catabolic versatility of the soil microbial community, as well as soil dehydrogenase, β-glucosidase and urease activities. All the treatments enhanced plant yield significantly, decreased Sb and Cr uptake by roots of green bell pepper and wheat and reduced metal(loid)s transfer from root to shoot. Overall, the results obtained revealed that biochar addition at 3% rate can be used as an environmental friendly amendment for the *in situ* remediation of metal(loid)s-polluted Eastern Mediterranean soils.

Keywords: Metal(loid)s-polluted soils; Biochar; Enzyme activities; Community level physiological profile; Plant growth; transfer factor.

Introduction

The industrial revolution, including the consumption of non-renewable energy sources, mining, massive use of pesticides and fertilizers in agriculture, etc., increased rapidly in the last century to meet the needs of a growing industrial society. This was accompanied by the diffusion of potentially toxic elements, prompting several environmental issues (Callender 2003; Hmid et al., 2014). For instance, high concentration of metal(loid)s in soils such as chromium, nickel, arsenic and antimony can have harmful consequences on plant growth and soil microbiota, can deteriorate the food chain and cause severe problems to human health (Castaldi et al., 2009; Hmid et al., 2014; United States Environmental Protection Agency 1997). In order to estimate the potential ecological problems posed by metal(loid)s in soil, their potentially-bioavailable concentrations should be considered instead of their total amount (Adriano et al., 2004; Castaldi et al., 2009). Indeed, metal(loid)s bioavailability mainly depends on soil physical and chemical properties: for example, soil pH, dissolved organic carbon (DOC), organic matter (OM) and Fe/Al oxy-hydroxides among the others can influence metal(loid)s solubility and bioavailability without affecting their total amount (Castaldi et al., 2009; Lombi et al., 2002). Unfortunately, legal threshold limits established by governments around the world to define a soil as polluted (or potentially polluted) still consider the total amount of contaminants rather than their bioavailable fractions (Adriano et al., 2004).

To remediate metal(loid)-polluted soils, ex-situ techniques were frequently applied in the past (Anup and Kalu., 2015; Liu et al., 2018). Nevertheless, these latter were gradually replaced by in-situ strategies characterized by low cost, simplicity, limited environmental impact and high public acceptability (Castaldi et al., 2005; Derakhshan Nejad et al., 2017; Garau et al., 2007; Hmid et al., 2014). In-situ remediation techniques are mostly based on the addition of different organic (e.g. biochar or compost) or inorganic (e.g. zeolite, red mud, lime, phosphate among the others) amendments to the polluted soil in order to reduce the bioavailability of contaminants, thereby reducing the exposure to living organisms, and their leaching to groundwater (Basta and McGowen, 2004; Castaldi et al., 2009).The immobilization of contaminants is commonly based on adsorption and/or precipitation reactions triggered by the amendments or sorbents added (Castaldi et al., 2005; Castaldi et al., 2009; Garau et al., 2014; Mele et al., 2015).

Among the potential amendments that can be used for the in-situ recovery of metal(loid)-contaminated soils, biochar, a carbonaceous product derived from the pyrolysis of several organic wastes at low-oxygen conditions (e.g. from green and/or food waste or sludge, manure and/or other animal waste), is attracting substantial research interest as it appears an environmental-friendly and cost effective material (Bandara et al., 2016; Fang et al., 2016; Mehmood et al., 2018; Mohan et al., 2014; Ok et al., 2015; Qiao et al., 2018). Most importantly, in many instances biochar revealed effectiveness in the stabilization of metals and metalloids co-occurring in soil (Fang et al., 2012, 2016; Zhang et al., 2017). The immobilization of metal(oid)s by the biochar mainly relies on the presence on this latter of several functional groups (such as phenolic, hydroxyl and carboxyl groups) able to bound negatively and positively-charged chemical species by means of the formation of outer and inner-sphere complexes with trace metal cations and immobilizing anionic species in soil due to the biochar mineral oxides content (e.g. amorphous Fe or Al oxy-hydroxides) (Bandara et al., 2016; Mehmood et al., 2018; Qiao et al., 2018; Sparks, 2005; Xu et al., 2013; Xu et al., 2016). Previous studies showed that biochar was effective in the reduction of soluble metal cations, such as Ni^{2+}, Pb^{2+} and Cd^{2+}, and oxy-anionic species such as $H_2AsO_4^{-}$ and $HAsO_4^{2-}$, which can occur through ligand exchange reactions where the surface -OH groups of the oxide found in biochar were exchanged by the metalloid (Fang et al., 2016; Qiao et al., 2018). Nevertheless, Beesley et al. (2014) and Nejad et al. (2017) showed that the biochar added to a multi-contaminated soil effectively immobilized metal cations but significantly increased the concentration of available arsenic likely due to the increase of soil pH. Moreover, biochar was found to be effective at increasing soil fertility (Li et al., 2017;

Zhang et al., 2013), soil microbial abundance and activity (Gómez et al., 2013; Xu et al., 2016).

Despite the addition of amendments to contaminated soils, large metal(loid)-polluted areas, or heavily contaminated ones, might still pose substantial environmental threats due to presence of residual bioavailable contaminants. This is particularly relevant for contaminated areas devoted (or to be devoted) to agriculture where excessive metal(loid)s uptake by plants can compromise (or significantly reduce) crop yield and, most importantly, can threat human health. Metal(loid)s uptake by the roots and their translocation to shoots can lead to significant alteration of enzyme functionality and metabolic pathways which eventually ends up into several detectable symptoms such as chlorosis, leaf epinasty and stunted growth (Hmid et al., 2014). On the other hand, the contaminants uptake and their immobilization in roots and/or shoots could contribute to further reduce the amount of labile (readily soluble) metal(loid)s in soil (Castaldi et al., 2009; Cheng et al.; 2004). In this regard, the knowledge of the metal(loid)s partitioning between shoots and roots becomes fundamental in order to select potential crops to be safely grown in such remediated areas. Therefore, plant growth experiments appear crucial in view of a thorough evaluation of the amendment effectiveness in contaminated areas and this seems particularly urgent for areas where agriculture could be potentially practiced.

In previous chapters we evaluated the influence of two organic-based amendments such as compost and biochar on the mobility of Ni, Cr, Zn, As and Sb in contaminated soils collected from different agricultural areas in Lebanon (Abou Jaoude et al., 2019). In the same studies, the impact of the amendment on selected soil microbiological properties and soil enzyme activities was also considered. Here we mainly tested the hypothesis that biochar, compost or a combination of these two, can be used to increase plant growth and reduce metal(loid)s uptake in polluted soils in view of their potential use in the remediation of large agricultural areas of Lebanon contaminated by metal(loid)s.

More in depth, this work addressed the influence of a biochar derived from organic municipal solid waste, a municipal solid waste compost and their combination, on the growth of green bell pepper (*Capsicum annuum* L.) and wheat (*Triticum aestivum* L.). Special emphasis was given to metal(loid)s uptake by the plants and their partitioning into roots and shoots. The metal(loid)s mobility in soil before plant growth, and the amendment influence on selected soil microbial and biochemical features, were also considered.

Materials and methods

Study area and soil sampling

Soil samples were collected randomly from 5 to 30 cm depth from the Terbol area in the Central plain of Bekaa (Eastern Lebanon; 33.807 latitude, 35.995 longitude). One of the soil used in Chapter 2 (TS) was also collected in the same area. This site is located in one of the most important agricultural areas of Lebanon (Central plain of Bekaa). As previously mentioned (see Chapter 2), the soil was a Terbol Clay Typic Eutrochrept (Soil Survey Staff, 2010). The parent material of this soil is limestone (Darwish et al., 2006) while the main crops cultivated in the area are radish vegetables (Darwish et al., 2006), green bell pepper and wheat (Darwish et al., 2006). The soil samples collected were bulked in the laboratory and sieved to < 2 mm to obtain a representative composite soil sample (TS soil). This latter was used to set up soil mesocosms, which were treated with different amendments, and subsequently used for plant growth experiments.

Experimental design and mesocosms set up

The experiment had a completely randomized block design with three replicates for each of the treatments applied (n = 3). A total of 9 mesocosms (approx. 10 kg each) were prepared, i.e., TS soil x 3 treatments x 3 replicates = 9. The treatments/amendments applied (to TS soil) were the following: 0% (control TS soil, no amendment added); 3% (w/w) biochar (3% Bio) and 2% (w/w) biochar + 2% (w/w) municipal solid waste compost (2% Bio-MSWC). The percentages selected were in line with those used in previous studies for organic-based amendments (Rizwan et al., 2016; Zahedifar, 2017) and, most importantly, they are applicable from a practical, economic and environmental point of view.

The biochar was provided by the Intaj S.A.L pyrolysis plant in Zrarieh (Saida - South Lebanon) and was the same and characterized in chapter 3. The municipal solid waste compost (MSWC), obtained from food and green waste, was the same described (and characterized) in Chapter 2. It was provided by the Secit Facility Plant S.p.A. Consorzio Zir (Chilivani Ozieri - Sardegna, Italy).

Before addition to the soil mesocosms, compost and biochar were finely ground and sieved to < 2 mm. TS control, TS-3% Bio and TS-2% Bio-MSWC were left in contact at 25 °C for a period of 2 months during which they were periodically mixed (approx. two times per week) and maintained at 30 % of their water holding capacity (WHC). After this time, soil physico-chemical, biochemical and biological analyses (including plant growth) were carried out on soil samples from the different mesocosms.

Physico-chemical characterization of soil mesocosms and amendments

After two months of equilibration, soil samples were chemically and physically characterized: pH and electric conductivity (EC) were determined in 1:2.5 w/v soil:water suspensions, total organic C (TOC) and total N were measured using a CHN analyzer (Leco CHN 628) with Oat meal Leco part n° 502-276 as calibration sample, dissolved organic carbon (DOC) was determined in 1:10 w/v soil:water systems as previously described (Silvetti et al., 2014), cation exchange capacity (CEC) was measured using the $BaCl_2$ and triethanolamine method. All physico-chemical analysis (unless otherwise stated) were determined according to the Italian standard guidelines (Gazzetta Ufficiale, 1992). Total concentrations of metal(loid)s in soil were quantified using an Analytik Jena AG atomic absorption spectrometer (ZEEnit-650 P graphite furnace) after digestion with HNO_3 and HCl (1:3 v/v ratio) in a Microwave Milestone MLS 1200. Mean values ± standard deviations were reported in Table 1 and 2 for each parameter. Table 1 also presents the main characteristics of the MSWC and biochar used in this study. Note that these amendments were the same used in Chapter 2 and 3 respectively and that their main characteristics were already mentioned in those chapters.

Table 1

Physico-chemical characteristics of TS soil, biochar and compost. Mean ± SD

Chemical parameters	TS soil	Biochar
Texture	Clay	-
Sand %	29.86	-
Silt %	14.22	-
Clay %	55.92	-
Humidity %	24.36	25.92
Ash %	11.74±0.01	31.86±0.00
pH $_{H2O}$	7.34±0.01	8.85±0.01
EC (mS·cm^{-1})	0.34±0.06	11.67 ± 0.04
Total organic Carbon (TOC, % d.m.)	2.21±0.03	41.58 ± 0.65
Total N (% d.m.)	0.19±0.03	1.39±0.07
Organic matter (OM, % d.m.)	3.80±0.10	71.38±0.65
Cationic exchange capacity (cmol$_{(+)}$·kg^{-1})	22.13±0.10	105.10±0.10
Dissolved organic carbon (DOC, mg·kg^{-1}d.m.)	83.02±0.52	2317±0.00
Available potassium (K, mg·kg^{-1}d.m.)	242.19±0.00	-
Available phosporus (P, mg·kg^{-1}d.m.)	110.67±0.00	-
Total Cd (mg·kg^{-1} d.m.)	0.29±0.10	2.45±0.00
Total Ni (mg·kg^{-1} d.m.)	176.59±10.60	40.73±0.00
Total Cr (mg·kg^{-1} d.m.)	214.37±20.10	1.35±0.00
Total Pb (mg·kg^{-1} d.m.)	22.55±0.69	0.34±0.00
Total Zn (mg·kg^{-1} d.m.)	165.04±7.07	2.52±0.00
Total As (mg·kg^{-1} d.m.)	55.92±1.41	-
Total Sb (mg·kg^{-1} d.m.)	84.63±1.41	-

Metalloids mobility in soil mesocosms

The mobility of As and Sb (which were exceeding the threshold values for metalloids in soil according to the Italian legislation and Toth et al., 2016) was determined, after the contact time, in soil samples from each mesocosm using the sequential extraction procedure described by Wenzel et al. (2001). Particularly, labile (water soluble and exchangeable) and surface-bound Sb and As were quantified after extraction with 0.05 M $(NH_4)_2SO_4$ (fraction 1) and 0.05 M $NH_4H_2PO_4$ (fraction 2) respectively, while Sb and As linked to amorphous and crystallized Al and Fe hydroxides were quantified after extraction with 0.2 M NH_4^+-oxalate buffer (fraction 3) and 0.2 M NH_4^+-oxalate buffer + 0.1 M ascorbic acid (fraction 4) respectively. After each step of the sequential extraction, the soil suspensions were centrifuged for 15 min at 3000 rpm, then the liquid phase was filtered with Whatman 41 filters. The concentration of As and Sb in the liquid phase of each fraction was determined using an Analytik Jena AG atomic absorption spectrometer (ZEEnit-650 P graphite furnace). The residual As and Sb in the remaining solid phase were quantified after digestion with HNO_3 and HCl mixture (1:3 v/v ratio) in a Microwave Milestone MLS 1200. Metalloid concentrations were reported as mean values ± standard deviations.

Trace metals mobility in soil mesocosms

The mobility of Ni and Cr (which were exceeding the threshold values for metals in soil according to the Italian legislation and Toth et al., 2016) in soil samples from the different mesocosms was determined, after the contact time, using the sequential extraction procedure described by Basta and Gradwohl (2000). Particularly, exchangeable and readily soluble Ni and Cr were extracted by 0.5 M $Ca(NO_3)_2$ (Fraction 1), acid-soluble metals and weak surface complexes were determined with 1 M NaOAc (Fraction 2) while in the last fraction, precipitated metals and surface complexes were quantified by 0.1 M Na_2EDTA (Fraction 3). After each step of the sequential extraction, the soil suspensions were centrifuged for 15 min at 3000 rpm, then the liquid phase was filtered with Whatman 41 filters. The concentration of Ni and Cr in the liquid phase was quantified using an Analytik Jena AG atomic absorption spectrometer (ZEEnit-650 P graphite furnace). The residual Ni and Cr in the remaining solid phase were quantified after digestion with HNO_3 and HCl mixture (1:3 v/v ratio) in a Microwave Milestone MLS 1200. Trace metal concentrations were reported as mean values ± standard deviations.

Soil enzyme activities in soil mesocosms

Selected enzyme activities, i.e., dehydrogenase (DHG), β-glucosidase (GLU) and urease (URE) were determined, after the contact time, in soil samples from each mesocosms following Alef and Nannipieri (1995). Briefly, the dehydrogenase activity was analyzed by determining the triphenylformazan concentration following the soil samples incubation for 24 h at 30 °C with triphenyltetrazolium chloride. The urease activity was quantified by quantifying the ammonia released from the soil samples incubated for 2 h at 37 °C with urea. The β-glucosidase was measured by determining the *p*-nitrophenol released from soil samples incubated for 2 h at 37 °C with *p*-nitrophenyl glucoside. Enzyme activity values were reported as mean values ± standard deviations.

Biolog community level physiological profiles in soil mesocosms

After the contact time, the community level physiological profile (CLPP) was determined in each mesocosm using the Biolog EcoPlate approach (Biolog Inc., Hayward, CA) as previously described (Garau et al., 2014, 2019b). Particularly, CLPP analysis was achieved by extracting the microbial communities from the respective soil samples (through 1:100 soil dilution with 0.89 % NaCl solution), then inoculating aliquots of the microbial suspension (120 µL) into each of the 96 wells of the Biolog Ecoplate. The Biolog Ecoplate contains in each of its wells a sole C source and a redox dye able to reveal the oxidative catabolism of the C source. Overall, the Biolog Ecoplate contains a triplicate set of 31 carbon sources and three respective control wells (with no carbon) (Insam, 1997). After inoculation, Biolog plates were incubated in the dark at 26 °C for 4 days and carbon source utilization in each well was recorded daily by measuring the absorbance at 590 nm (OD_{590}) using a Biolog MicroStation™ reader.

Biolog Ecoplate data (i.e. raw OD_{590} values) were processed as previously described (Garau et al., 2019b) to obtain selected Biolog-derived indexes such as the Average Well Colour Development (AWCD), the Richness (i.e., the number of carbon sources catabolized by each microbial community) and the utilization of selected substrate guilds (i.e., carboxylic acids, polymers and amino acids). AWCD, richness and carbon source utilization data were reported as mean values ± standard deviations. A detailed description of the procedure employed for CLPP analysis is reported in Chapter 2.

Pot experiment and plant analysis

After the contact period, soil samples from the different mesocosms were used to fill plastic pots (approximately 1 kg soil in each pot), in which one seed of green bell pepper and four seeds of wheat were planted after overnight imbibition with water. We selected these plant species as they are extensively cultivated in the Central plain of Bekaa, where Terbol is located. A total of 18 pots were prepared, i.e. 1 soil x 3 treatments (0% amendment, 3% Bio, 2% Bio-MSWC) x 2 plant species (green bell pepper and wheat) x 3 replicates. Plants were grown for eight weeks under controlled conditions (temperature 25 °C, relative humidity 60–70 %) and watered when required. After the growth period, no plant mortality was recorded, and plants were harvested while separating the shoots (leaves plus stem) from the roots. Then, the aerial part and the roots were carefully washed with distilled water and oven-dried for 72 h at 70 °C to quantify their dry weight. The growth of wheat and green bell pepper in each pot was assessed by measuring several parameters, such as shoot and root length and shoot and root biomasses. The concentration of Sb and Cr were measured due to their high and direct effect on plant growth inhibition (Baek et al., 2014; Laghlimi et al., 2015). Cr and Sb concentration in roots and aerial part were determined by dissolving 0.5 g of ash, obtained after ashing the organic matter in a muffle furnace, in 6 ml of 2N HNO_3 (Castaldi et al., 2005). The solutions obtained were analyzed using an Analytik Jena AG atomic absorption spectrometer (ZEEnit-650 P graphite furnace), and metal(loid) concentrations were expressed as mg kg^{-1} of plant dry matter.

Statistical analysis of data

The sequential extraction, soil enzyme activities, Biolog analysis and plant growth data were analyzed by one-way analysis of variance (one-way ANOVA, $P < 0.05$) to identify statistical differences among mean values and by the post hoc Fisher's least significant difference test for mean separation ($P < 0.05$). All data were investigated using the Minitab 17 software for windows.

Results and discussion

TS soil characteristics

TS soil had neutral pH value (7.34) and an EC of 0.34 mS·cm^{-1} (Table 1). Total N, TOC and OM were about 0.19, 2.21 and 3.80 % and CEC was ~22.13 $cmol_{(+)}$·kg^{-1} (Table 1). The available K and P concentration were about 242.19 and 110.67 mg·kg^{-1}d.m.The DOC concentration detected in the TS soil was ~51.25 mg·kg^{-1}d.m. These soil characteristics, which are very similar to those of the Terbol soil used in Chapter 2, indicate a fertility status suitable for different agricultural activities which are indeed practiced in the Bekaa area such as the cultivation of wheat and radish as well as industrial crops, i.e. sugar beet and tobacco.

The TS soil showed a high concentration of Cr, Ni, Sb and As (Table 1). As previously mentioned in Chapter 2 (Abou Jaoude et al., 2019), Lebanon does not have any legal threshold limits regarding metal(loid)s concentration in soil. Most importantly (and worryingly), this also applies to agricultural soils. Therefore, threshold limits set by other countries legislation can be used to infer the pollution status of Lebanese soils. For instance, the threshold limits set by the Finnish legislation, that represents a mean of the different standards set by EU countries and India (Awasthi, 2000; Carlon et al., 2007; Tóth et al., 2016), can be helpful in this sense. Based on the Finnish legislation, the total concentration of Sb and Ni in TS soil (Table 1) were exceeding the lower and the higher guideline limits (e.g. 10 and 50 $mg \cdot kg^{-1}$ soil respectively for Sb) and pose both environmental and health risks (Tóth et al., 2016). Moreover, the concentrations of Cr and As were exceeding the lower guideline values (i.e. 200 and 50 $mg \cdot kg^{-1}$ soil for Cr and As respectively) generating remarkable ecological risks (Tóth et al., 2016) (Table 1). Such soil pollution status is certainly worrying given that the TS soil is located in the Central plain of Bekaa which is the major agricultural area in Lebanon (Darwish et al., 2008; Korfali et al., 2014; Nsouli et al., 2004). The presence of a high concentration of contaminants in such area might be explained, as previously mentioned (see Chapter 2), by an excessive and/or prolonged use of pesticides and fertilizers as well as to the use of wastewater for crop irrigation (Darwish et al., 2008; Korfali et al., 2014; Nsouli et al., 2004). Also, the use of water from the Litani River for crop irrigation can be partly responsible for soil pollution given the indiscriminate discharge of wastewater into the river. It should be noted that the pollution status of the Litani River, the main river of Lebanon, has been recently defined as “a national catastrophe” by the Lebanese Minister of Industry (http://www.asianews.it/news-en/Lebanese-minister:-Litani-river-pollution-a-national-catastrophe-47591.html).

Overall, the metal(loid)s concentration found in TS soil is alarming as it can affect plant growth and deteriorate the food chain, thus potentially generating harmful consequences on human health. This also implies the urgent need to remediate such soil, in order to decrease the metal(loid)s mobility and their potential bioavailability.

Influence of biochar and its combination with MSWC on soil properties and metalloids mobility

An increase of pH of about one unit was noted for amended TS soils compared to control (Table 2). Additionally, 3% Bio and 2% Bio-MSWC enhanced the soil OM and the DOC content by approximately 30 and 14 % (OM) and 84 and 75 % (DOC) respectively compared to the untreated soil. Similarly, total N increased in treated soils, especially when 3% Bio was added, i.e. up to 0.29 % (Table 2). In general, these results indicate that the amendments addition improved, or did not change, the main soil properties somehow ruling out the possibility of a detrimental effect for plant growth and/or soil (micro) biota which, however, should be experimentally proved.

Table 2. Chemical characteristics of TS soil amended with 3% biochar (3% Bio) and 2% Biochar +2% MSWC (2% Bio-MSWC). Mean ± SD

	TS soil	
Chemical parameters	3% Bio	2% Bio-MSWC
Humidity %	23.4	22.9
Ash %	12.21±0.02	12.20±0.05
pH $_{H2O}$	8.38±0.01	8.10±0.01
EC ($mS \cdot cm^{-1}$)	0.35±0.00	0.32±0.00
Total organic Carbon TOC (% d.m.)	2.85±0.04	2.53±0.02
Total N (% d.m.)	0.29±0.01	0.24±0.04
Organic matter (% d.m.)	4.90±0.04	4.34±0.01
Cationic exchange capacity($cmol_{(+)} \cdot kg^{-1}$)	24.64±0.12	24.36±0.56
Dissolved organic carbon DOC ($mg \cdot kg^{-1}$d.m.)	152.54±0.50	145.12±0.58

The As and Sb mobility in TS treated and untreated soils was assessed using the sequential extraction procedure of Wenzel et al. (2001). The labile As and Sb (fraction 1), and those forming inner-sphere complexes with the soil components (fraction 2), were relatively low in the TS untreated soil, e.g. 0.58 and 2.89 mg kg^{-1} soil of As and Sb respectively in fraction 1. Moreover, As and Sb extracted in fraction 1 and 2 decreased significantly in all the amended soils compared to control, with no recorded extraction of As and Sb in TS-3% Bio and 2% Bio-MSWC (Fig. 1). The labile As and Sb are considered as potentially bioavailable pools, and as such they can have a significant influence on soil biota (Garau et al., 2019a). In this sense, their decrease after amendment addition should be considered as positive from an environmental point of view. A decrease of the concentration of easily exchangeable As in soil was previously supported by Beesley et al. (2014) after biochar addition. However, these results suggest that amorphous metal oxides, (e.g.,amorphous Fe or Al oxy-hydroxides), might be present within the biochar that can bind the anionic species in soil and lead into strong adsorption of As and Sb to its solid phases (Fang et al., 2016; Zhang et al., 2015a, 2015b).

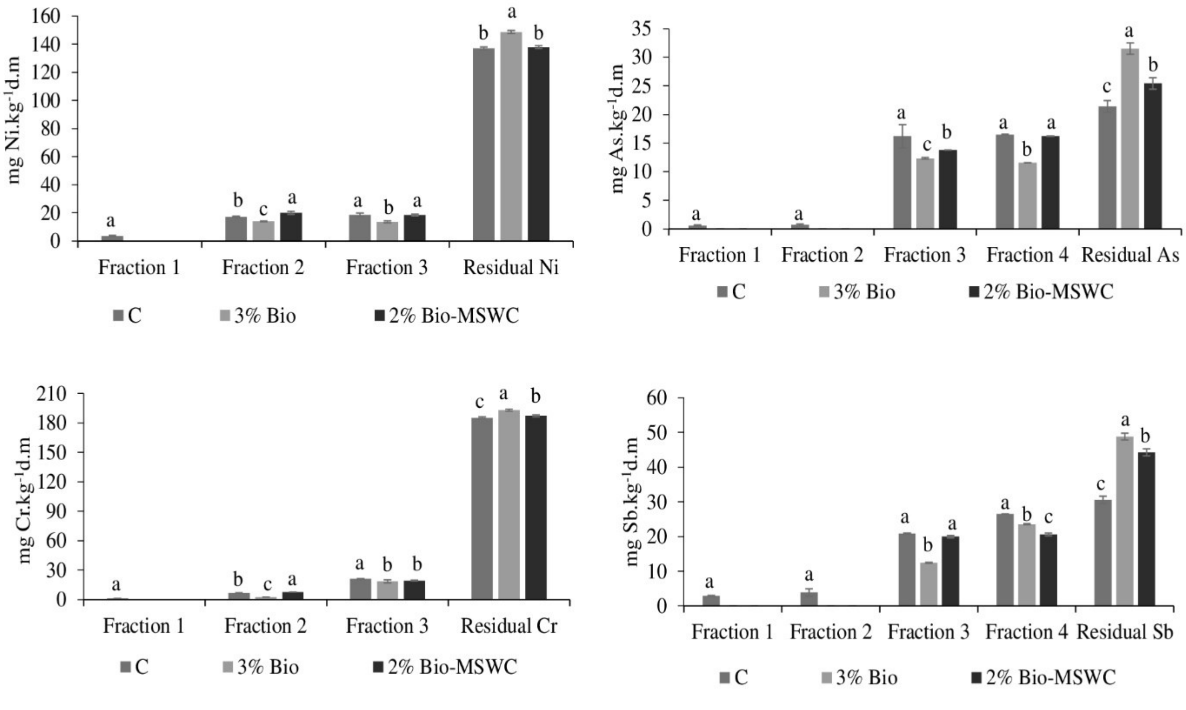

Fig. 1. Arsenic (As), antimony (Sb), Chromium (Cr) and Nickel (Ni) released after sequential extraction. For each step of the sequential extraction, mean values followed by different letters denote statistically significant differences according to the Fisher's Least Significant Difference (LSD) test ($P < 0.05$).

The As and Sb fractions associated with amorphous Fe- and Al-(hydr)oxides (fraction 3) decreased in treated soils, particularly with the addition of 3% Bio, i.e. by an approx. 25 and 40 % for As and Sb respectively (Fig. 1). Similarly, the As and Sb extracted in fraction 4, referring to the metalloids associated with well crystallized Fe- and Al-(hydr)oxides, decreased after amendment with 3% Bio (Fig. 1). Moreover, 2% Bio-MSWC decreased significantly Sb in fraction 4, i.e., by an approx. 23 % but not As (Fig. 1). Apparently, this decrease is conflicting with the suggestion provided for the reduction of metal(loid)s in fraction 2. However, this can be explained by the fact that Al/Fe oxy-hydroxides might have become inaccessible following their dissolution in the micro- and mesopores inside the porous structure of biochar. These metal oxides diffusion can form an occlusion within the biochar pores. Overall, the addition of 3% Bio consistently reduced the metalloids fractions linked to amorphous and well crystalized Fe- and Al-(hydr)oxides, while the influence of 2% Bio-MSWC appeared rather variable. Residual As and Sb, referring to the strongly bonded and hardly retained fractions, significantly increased in all treated soils, particularly in soils treated with 3% Bio which revealed the most effective treatment (>47 and 60 % of residual As and Sb respectively compared to control soil; Fig.1). These results revealed that biochar and its combination with compost was adequate in decreasing the labile (water-soluble and exchangeable) fractions of As and Sb and increasing at the same time the residual ones. These findings can be due to As and Sb immobilization by mineral oxides within biochar (i.e., amorphous Fe or Al oxy-hydroxides), where metal oxides might have been shifted directly to the hardly extractable fraction (the residual fraction) following their diffusion within the micro- and mesopores of biochar (Agegnehu et al., 2016; Diquattro et al., 2018; Fang et al., 2016; Manzano et al., 2016; Qiao et al., 2018). The 3% Bio highlighted a significant impact on As and Sb stabilization. These findings came in agreement with the results of Fang et al. (2016) and Qiao et al. (2018) reporting that As was immobilized by biochar. Particularly, Qiao et al. (2018) revealed that the reason of As stabilization might be due to its diffusion into micropores of biochar leading into As sorption in the presence of biochar. Overall, these results suggest the suitability of 3% Bio and its combination with compost for metalloids stabilization in contaminated soils.

Influence of biochar and its combination with MSWC on trace metals mobility in soil

The assessment of the biochar impact and its combination with compost on Cr and Ni mobility in TS soil was carried out by using the sequential extraction procedure developed by Basta and Gradwohl (2000). This procedure revealed very restricted amounts of easily exchangeable and water-soluble Cr and Ni in the untreated soil, with up to ~3.67 mg kg^{-1} of Ni and 1.31 mg kg^{-1} of Cr (fraction 1, Fig. 1). These amounts decreased mostly and became negligible with the addition of both amendments (Fig. 1). This can be explained by the ability of inorganic and organic components of biochar to adsorb labile metals as supported by its high cation exchange capacity and specific surface area (Table 1; Beesley et al., 2015; Qiao et al., 2018). For instance, the oxides/hydroxides within biochar could be involved in inner- and outer-sphere complexation of the water-soluble and easily exchangeable metals as previously shown (Manzano et al., 2016; Silvetti et al., 2015). Likewise, carboxylic and phenolic groups could contribute to metal fixation as previously shown (Bandara et al., 2016; Garau et al., 2015; Silvetti et al., 2017).

The extraction of Ni in the second fraction was highly reduced with 3% Bio (i.e. approx. 17 % compared to the untreated soil) while 2% Bio-MSWC did not show any influence (Fig. 1). During the extraction of metals with EDTA (fraction 3), which refers to trace metals forming precipitates and/or strong inner-sphere surface complexes, Ni and Cr were reduced in soils treated with 3% Bio, i.e. approx. 27 and 14 % decrease respectively compared to control soil (Fig. 1).

Residual Ni and Cr, referring to the strongly adsorbed and hardly retained fractions, were around 77.63 % of total Ni and 86.41 % of total Cr in untreated TS soil. Following the addition of biochar and its combination with MSWC, the residual Ni and Cr concentrations increased in TS soil, especially when it was treated with 3% Bio. This can be due to trace metals fixation via intraparticle diffusion in meso- and micropores of biochar, as well as to inner-sphere Me complexation by carboxylic and/or phenolic surface groups of biochar followed by organic matter stabilization (Beesley et al., 2015; Fang et al., 2016; Qiao et al., 2018). Finally, the formation of metal precipitates could have contributed to the increase of the residual fraction of Cr and Ni.

Influence of biochar and its combination with MSWC on soil enzyme activities

Dehydrogenase (DHG), urease (URE) and β-glucosidase (GLU) activities were quantified in treated and untreated soils to evaluate the impact of the amendments on selected soil biochemical activities. Excessive amounts of metal(loid)s in soil can lead to decreased enzyme activities due to a denaturation of the enzyme, or to its inactivation due to metal binding with the enzyme-substrate complex or to the enzyme active site (Bhattacharyya et al., 2008; Oliveira and Pampulha, 2006).

DHG activity, referring to the activity of an intracellular group of enzymes to oxidize organic molecules, can be used to infer the oxidative activity of the soil microbial community (Fernandez et al., 2005; Oliveira and Pampulha, 2006). In general, DHG increased after 3% Bio and 2% Bio-MSWC addition (e.g. approx. by 45 % in TS-3% Bio compared to control) (Fig. 2) supporting a role of the amendments on the reduction of labile metal(loid)s in soil. Moreover, the increase of DHG activity can be partly due to the significant increase of DOC and OM content in treated soils (Table 2). All this could have stimulated microbial growth and induced an increase of the oxidative metabolism of microbial communities in treated soils (Garau et al., 2017, 2019a). GLU activity, referring to the extracellular enzymes that cleave β 1→4 bonds occurring within two glucose or glucose substituted molecules, had a significant increase in TS soil treated with 3% Bio and 2% Bio-MSWC (approx. >21 % in both soils compared to control) (Fig. 2). These results suggest that the addition of biochar and its combination with compost could have a positive influence on soil C cycling in metal(loid)s-polluted soils. However, it should be noted that many other enzymes can be involved in soil C cycle and general conclusions about the efficiency of this latter cannot be drawn only based on GLU activity.

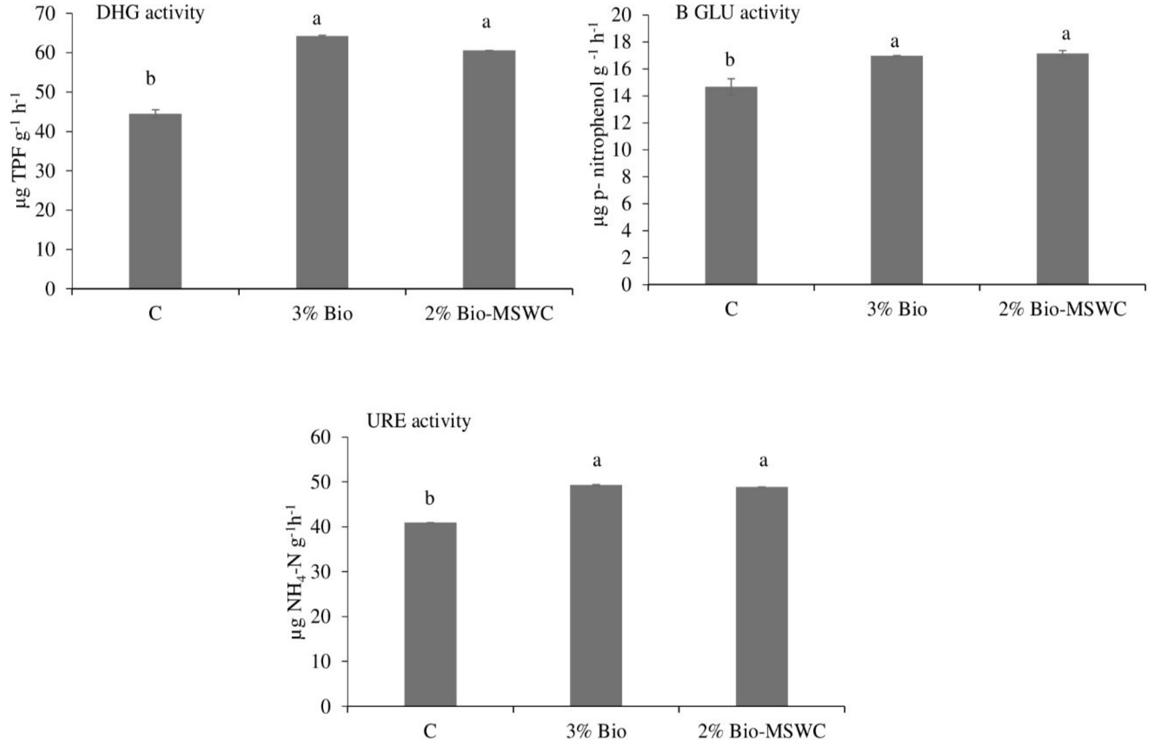

Fig. 2. Dehydrogenase (DHG), urease (URE), and β-glucosidase (GLU) activities in amended and unamended soils. For each enzyme activity mean values followed by different letters denote statistically significant differences according to the Fisher's Least Significant Difference (LSD) test ($P < 0.05$).

Likewise, URE activity, referring to the urea hydrolysis and the release of NH_4^+ ions, increased notably in treated soils, i.e. approx. >22 % in both 3% Bio and 2% Bio-MSWC soils (Fig. 2). These results indicate that biochar addition and its combination with compost had a positive effect on one important step of the soil N cycle, and this likely occurred by the reduction of labile trace metals and metalloids in amended soils. Overall, the results obtained demonstrate a positive impact of biochar, and its combination with MSWC, on the activity of selected intracellular and extracellular enzymes. Potentially, this could have a positive impact in the recovery of such metal(loid)s polluted soils, e.g. by stimulating plant growth through an improved organic matter turnover and higher nutrients availability.

Impact of biochar and its combination with MSWC on the microbial community level physiological profile

The Biolog CLPP was identified as a good indicator in assessing the influence of metal(loid)s on soil microbial communities (Garau et al., 2017). For this reason, Biolog CLPP was determined to monitor the impact of the amendments on the potential metabolic activity of microbial communities within treated TS soils. In this sense, biochar and its combination with compost were found to have a great impact on the potential catabolic activity of the soil microbial communities. Particularly, TS soils treated with 3% Bio and 2% Bio-MSWC showed a 5.8- and 5.2-fold increase (compared to control soil) of the average well color development (AWCD), that is a measure of the potential carbon source utilization of the soil microbial community (Fig. 3). Overall, this can be explained by an increased microbial abundance in treated soils, which might be induced by the decreased metal(loid) bioavailability in such soils and a larger availability of N and C sources as previously reported (Garau et al., 2017, 2019a). Moreover, the different usage of different carbon source guilds, such as sugar and sugar derivate, carboxylic acids and amino acids (Fig. 3), could also indicate an influence of the amendments on the structure of soil microbial communities (Garau et al., 2017, 2019a). However, this could be merely due to an influence of the amendments on the size of the soil microbial community, with 3% Bio being the most effective treatment in this sense.

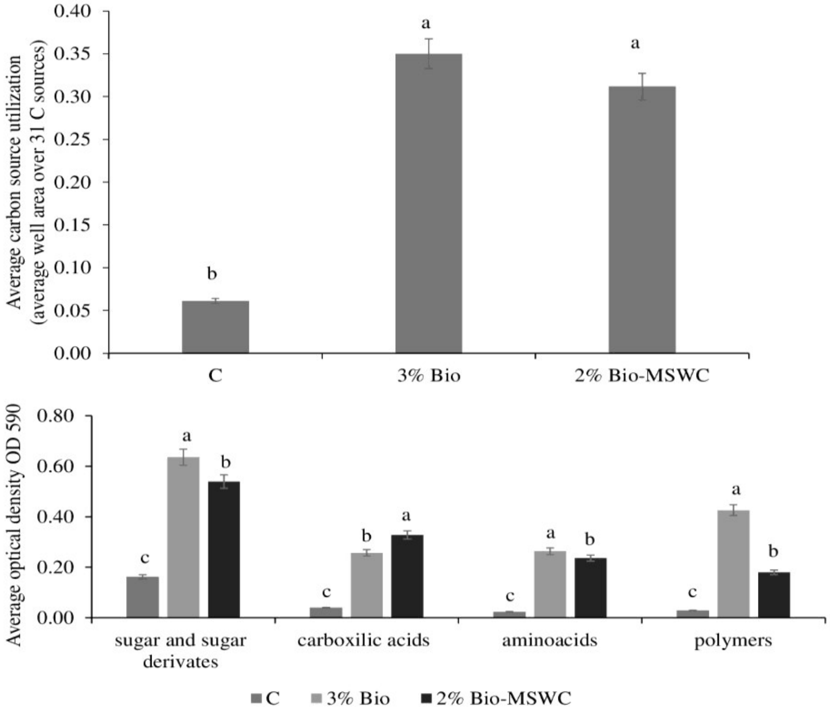

Fig. 3. Average carbon source utilization (AWCD) and utilization of specific carbon source guilds (average OD_{590} values) by microbial communities extracted from TS soils. Mean values were followed by different letters denoting statistically significant differences according to the Fisher's Least Significant Difference (LSD) test ($P < 0.05$).

Growth performance of green bell pepper and wheat grown on treated and untreated TS soils

Root and shoot growth of green bell pepper and wheat were significantly reduced in TS untreated contaminated soil compared to amended ones (Table 3). The best growth performance was recorded for plants grown on 3% Bio followed by 2% Bio-MSWC and control soil. Particularly, the shoot length of green bell pepper and wheat grown on 3% Bio increased respectively by a factor of 1.8 and 1.2 compared to TS control plants (Table 3; Fig. 6-7). Similarly, root growth increased by a factor of 1.6 and 1.3 respectively for plants amended with 3% Bio compared to control plants (Table 3; Fig. 6-7). Although the combined application of compost and biochar to the polluted soil was less efficient in root growth compared to 3% Bio. The shoots of green bell pepper and wheat increased by a factor of 1.5

and 1.1, respectively, compared to control plants (Table 3; Fig. 6-7). Likewise, shoot and root dry weight of both plants significantly increased in the presence of 3% Bio, e.g. by 2.1 and 2.2 factor respectively for shoot dry weight of green pepper bell and wheat compared to TS control soil (Table 3; Fig. 6-7). These (stimulating) plant-growth effects might be due to the amendments capability to stabilize metal(loid)s in soil, i.e. to reduce their labile contents (as shown in this study) and alleviate metal(loid)s phytoxicity (Chen et al., 2000). Moreover, also the increase of the soil microbial abundance (and possibly diversity) triggered by the amendments, as indicated by the significant increase of the Biolog-derived indexes and DHG activity, could be partly responsible for the higher plant yield in treated soils.

Table 3

Shoot and root length and yield of green bell pepper and wheat plants grown on amended and unamended TS soil.

Plant growth	Green bell pepper			Wheat		
	C	3% Bio	2% Bio-MSWC	C	3% Bio	2% Bio-MSWC
Shoots (cm)	20.35± 1.03^c	36.70± 0.78^a	29.56± 1.07^b	30.39± 1.02^c	36.50± 2.60^a	34.24± 1.06^b
Roots (cm)	6.86± 0.71^b	10.74± 1.23^a	8.06± 0.52^b	6.66± 0.88^b	8.43± 1.35^a	7.59± 0.87^a
Dry shoot weight (g/plant)	4.76± 0.34^c	9.88± 0.64^a	8.13± 0.64^b	0.16± 0.01^c	0.35± 0.03^a	0.26± 0.01^b
Dry root weight (g/plant)	1.18± 0.09^b	2.25± 0.10^a	1.36± 0.13^b	0.02± 0.01^b	0.03± 0.00^a	0.02± 0.01^b

Fig. 6. Effect of 3% Bio, 3% MSWC and 2% Bio-MSWC on Green bell pepper compared to control (from left to right: Control, 3% Bio, 3% MSWC, 2% Bio-MSWC).

Fig. 7. Effect of 3% Bio, 3% MSWC and 2% Bio-MSWC on Wheat compared to control (from left to right: Control, 3% Bio, 3% MSWC, 2% Bio-MSWC).

Metal(loid)s uptake by wheat and green bell pepper plants grown on treated and untreated TS soils

Metal uptake by both plants grown on 3% Bio and 2% Bio-MSWC was significantly reduced compared to plant grown on control soil. In particular, the lowest concentrations of Cr and Sb were found in shoots and roots of wheat plants grown on 3% Bio (i.e., 41.9 and 67.8 % of Cr and Sb decrease in shoots of wheat plants grown on 3% Bio compared to TS control soil, respectively) (Fig. 4). However, also the Cr content in shoots of green bell pepper plants grown on amended soils significantly decreased compared to untreated soil, i.e. by approx. 60 % (Figure 5). A significant reduction of metal(loid)s uptake by the roots of green bell pepper was also observed for plants grown on treated soils (Fig. 5). Nevertheless, 3% Bio was the most effective treatment in decreasing Sb in shoots and roots of green bell pepper with 68.6 and 23.9 % decrease respectively (Fig. 5).

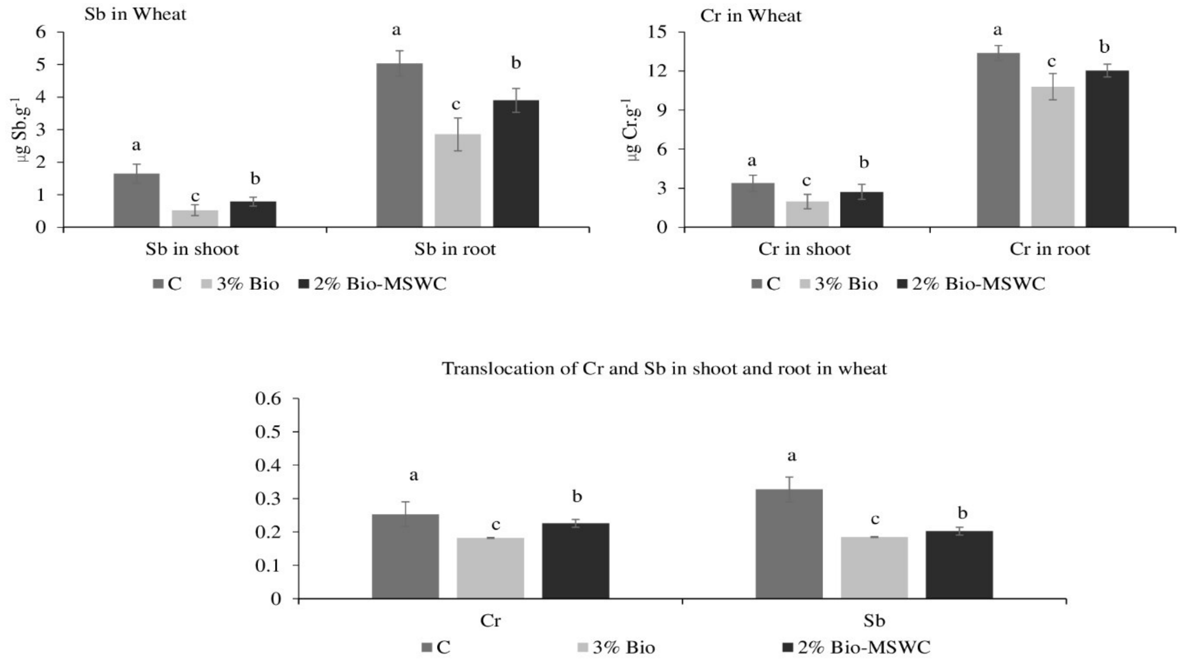

Fig. 4. Shoots and roots uptake of Cr and Sb concentrations in wheat plants and their translocation mean (Shoot/root ratio). Mean values were followed by different letters denoting statistically significant differences according to the Fisher's Least Significant Difference (LSD) test ($P < 0.05$)

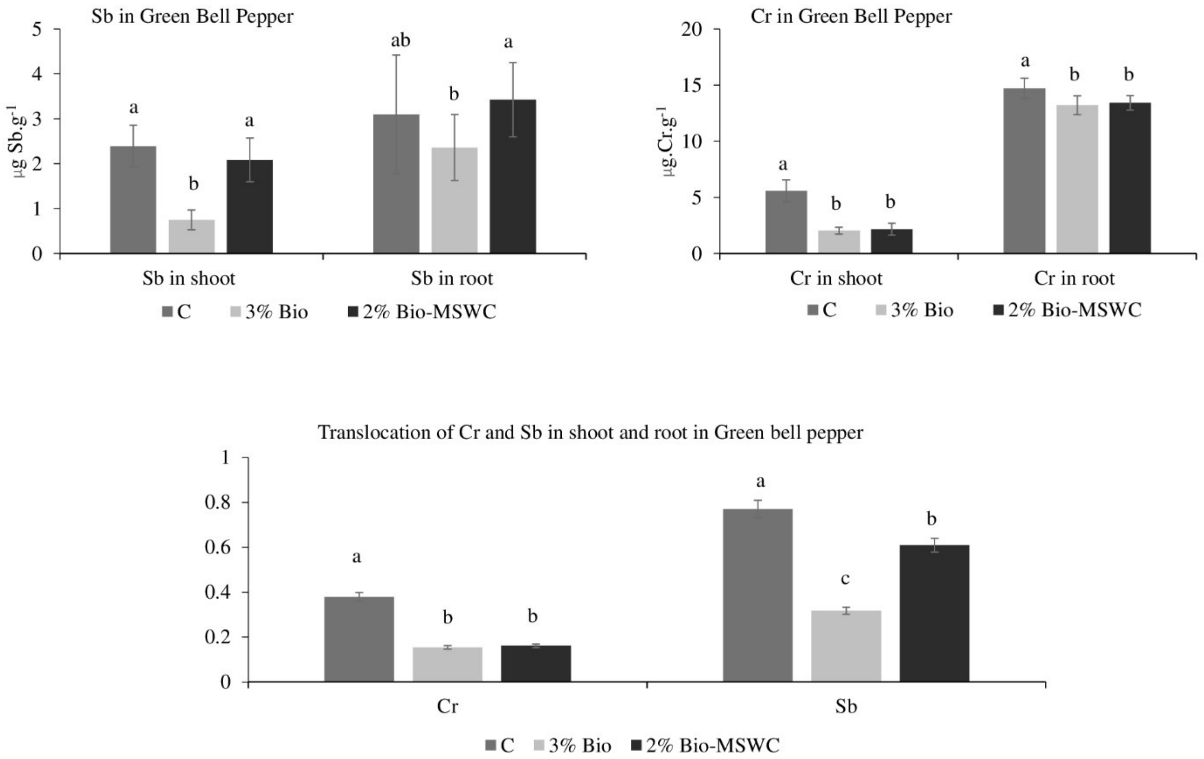

Fig. 5. Shoots and roots uptake of Cr and Sb concentrations in green bell pepper plants and their translocation mean (Shoot/root ratio). Mean values were followed by different letters denoting statistically significant differences according to the Fisher's Least Significant Difference (LSD) test ($P < 0.05$).

Overall, the amendments addition marked a substantial reduction of Sb and Cr in plant tissues compared to control soil, even though Sb and Cr in shoots of green bell pepper and wheat grown on amended soils were still exceeding the average levels commonly found in plants growing in uncontaminated soils (Kabata-Pendias, 2011), knowing that the permissible levels of Cr and Sb in wheat are between 0.28 and 0.62 μg.kg^{-1} (Cr) (Wang et al., 2015) and around 0.073 μg.kg^{-1} (Sb) (Bermudez et al., 2011). However, the maximum permissible levels in vegetables (i.e., green bell pepper) of Cr ad Sb is 0 μg.kg^{-1} (Cr) (Chiroma et al., 2014) and 1 μg.kg^{-1} (Sb) (Choy YY, 2011). All these ranges are considered safe when the concentration levels in Cr and Sb are not exceeding their thresholds. Nevertheless, Cr and Sb levels obtained in shoots in green bell pepper were around 5 and 2 μg.kg^{-1} in control, respectively, and 2 μg.kg^{-1} in treated soils, respectively, except for Sb-3%

Bio with a concentration of 0.75 $\mu g.kg^{-1}$. Similarly, Cr and Sb levels obtained in shoots in wheat were around 3.39 and 1.65 $\mu g.kg^{-1}$ in control, respectively, and around 2 $\mu g.kg^{-1}$ of Cr and 0.60 $\mu g.kg^{-1}$ of Sb in treated soils (Fig. 4-5). This means that the treated soils reduced the concentration levels of Cr and Sb in both plants, however, this reduction was still exceeding the thresholds with Cr (i.e., 2 $\mu g.kg^{-1} > 0$ $\mu g.kg^{-1}$). The reduction of Sb concentration was remarkable with 3% Bio, which has led to reduce its level below the permissible levels in vegetables (0.60 $\mu g.kg^{-1} < 1$ $\mu g.kg^{-1}$). Moreover, since wheat and green bell pepper were mostly exceeding the thresholds with Cr and Sb, even after a significant reduction with 3% Bio, these concentrations remain unsafe to be consumed by the humans, due to the large interval between the threshold and the values found, particularly with 3% Bio, and since vegetables and wheat are considered staple foods in the humans diet, their excessive presence in the body will cause harmful health consequences.

Besides, the availability to plants of Cr and Sb seemed widely dependent on the concentration of labile metal(loid)s in soil even if, other specific soil characteristics such as the organic matter content (Gerriste and van Driel, 1984) and cation exchange capacity (Buchter et al., 1989) could have eventually contributed to influence the metal(loid)s uptake by the plants. Plant roots of both species accumulated more Cr and Sb than shoots and this especially true for wheat. Similar findings were reported for wheat plants grown on differently-amended soils (Bose and Bhattacharyya, 2008; Bose et al., 2008; Castaldi et al., 2009). For green bell pepper and wheat, Cr and Sb translocation from roots to shoots, expressed by the translocation factor (TF: Me shoot/Me root ratio) followed the order: control soil ≥ 2% Bio-MSWC ≥ 3% Bio (Fig. 4-5). Similar findings were reported by Castaldi et al. (2009) showing that heavy metals translocation in wheat plants was directly correlated to the concentration of metals in the soil solution. Moreover, metal translocation was highly crop dependent, with translocation values for Sb and Cr being higher in green bell pepper plants than wheat (Fig. 4-5). This suggests that, beside the great role of soil properties on the availability of heavy metals to plants, plant species with their root physiology can play a significant role in the regulation of metal(loid) uptake and translocation (Castaldi et al., 2009; Gray et al., 2006). In this study green bell pepper plants showed higher metal(loid)s uptake and translocation than wheat, which possibly implies that growing wheat in such soils, and using 3% Bio as amendment, could decrease the risk of spreading metals in the environment and limit the risk of health damages to a certain level. However a higher % of biochar might be recommended in order to reduce the metals concentrations below the permissible levels allowed in wheat and vegetables. A higher % of biochar could lead to a safer consumption of these basic food elements and reduce partially or completely the heavy metals toxic impacts on human health.

Conclusions

Biochar and its combination with compost showed a significant efficiency at reducing the labile As, Sb, Cr and Ni present in a polluted Lebanese soil and increasing their residual fractions. Both treatments increased selected soil enzyme activities, notably DHG, URE and GLU, and had a strong (positive) influence on the potential catabolic activity of soil microbial communities as highlighted by the Biolog CLPP. This was also accompanied by an enhanced growth of green bell pepper and wheat in the amended soils. Metal(loid)s uptake by wheat and green bell pepper, and their translocation factor (TF: Me shoot/Me root ratio), was reduced for plant grown on amended soils and followed the order: control soil ≥ 2% Bio-MSWC ≥ 3% Bio. Biochar addition could be a new strategy to remediate such metal(loid)s-contaminated soils while limiting the contaminants uptake by the plant and reducing the possible risks of health damages. However, further studies are needed to evaluate the long-term effectiveness of the biochar, especially under natural conditions, and the transfer (and possible accumulation) of plant metal(loid)s along the food or feed chain.

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Chapter 5

General Conclusion

The aim of the present PhD thesis was to provide new insights concerning the impact of compost (MSWC) and biochar (Bio) on the physico-chemical, microbial and enzymatic characteristics of different Lebanese soils contaminated by PTE. The impact of the amendments on plant growth and PTE uptake was also addressed in this thesis. Overall, our results indicate that the biochar and compost used were able to fix the trace metals and metalloids co-occurring in neutral and sub alkaline Lebanese soils. Both the organic and inorganic components of these organic sorbents might have influenced the sorption of PTE.

Importantly, sequential extractions showed that the addition of MSWC at 1 and 3% rates lowered the environmental impact of metal(loid)s present in HS and TS soils due to the remarkable decrease of water-soluble and exchangeable As and Sb fractions. Moreover, 3% Bio and 2% Bio-MSWC were effective at reducing the water soluble and exchangeable As and Sb in TS soil. Likewise, biochar, MSWC and their combination added to FS and MS soils were able to reduce the water-soluble and exchangeable As and Sb and increasing the residual ones. This was explained the formation of stable inner-sphere complexes between the metalloids and the surface functional groups of the amendments, or the metalloid precipitation and/or occlusion within the compost and biochar pores. Concerning trace elements, i.e. Ni, Cr and Zn, results showed an increase of inaccessible and immobilized TM following the addition of biochar, compost and their combination indicating that all the treatments were effective in reducing the environmental mobility of Ni and Cr. The highest increases of residual TM were recorded in FS, MS and TS soils treated with 3% Bio and in HS and TS soils treated with 3% MSWC. This was attributed to the formation of inner-sphere complexes between trace metals and the carboxylic and/or phenolic surface groups of biochar and MSWC.

Sequential extractions also indicated that most of the PTE were recovered in the residual fraction, particularly after the addition of 3% Bio and 3% MSWC. This apparently explained the significant increase of DHG, URE, -GLU and Biolog AWCD values in amended soils and helped to explain the enhanced plant growth in the presence of Bio and MSWC. Moreover, the physico-chemical analyses of the different contaminated soils indicated that the amendments had a positive impact on some of the main soil attributes which could partly explained the enhanced plant growth in the amended soil.

In summary, the amendments addition had a clear positive impact on soil fertility, microbial and biochemical properties whilst promoting plant growth and reducing metal(loid)s uptake. Overall, the results arising from this PhD project highlight that compost and biochar can be effectively used as PTE-immobilizing materials to decrease the mobility and toxicity of PTE in contaminated soils and to alleviate the metal(loid) impact on the environment.

Appendix

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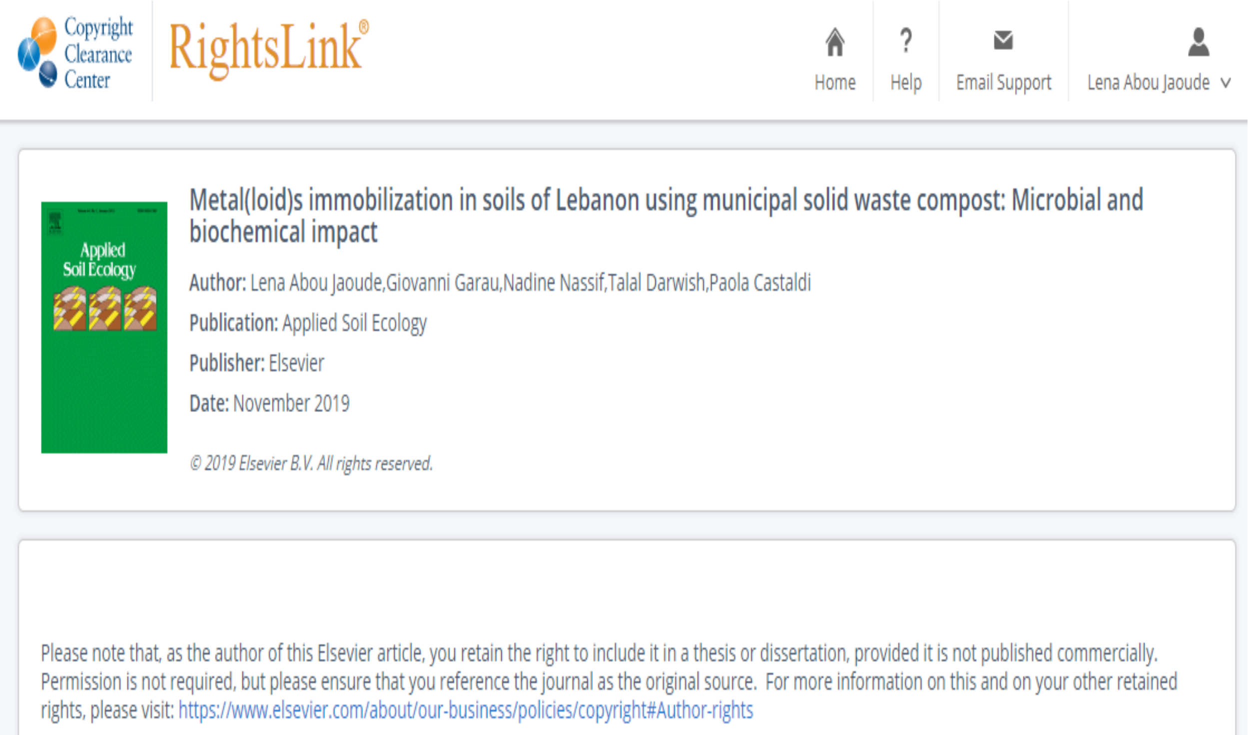

Metal(loid)s immobilization in soils of Lebanon using municipal solid waste compost: Microbial and biochemical impact

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Publication: Applied Soil Ecology

Publisher: Elsevier

Date: November 2019

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Biochar and compost as gentle remediation options for the recovery of trace elements-contaminated soils

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Publication: Science of The Total Environment

Publisher: Elsevier

Date: Available online 22 November 2019

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