Università degli Studi di Sassari

Corso di Dottorato di Ricerca in Scienze Agrarie

DIPARTIMENTO DI AGRARIA

CURRICULUM "BIOTECNOLOGIE MICROBICHE AGROALIMENTARI"

XXXI CICLO

Chemical and biological recovery of soils contaminated with antimony using eco-friendly materials

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A mio marito, con amore

Declaration

By submitting this thesis, I hereby declare that this PhD thesis entitled "Chemical and biological recovery of soils contaminated with antimony using eco-friendly materials" was carried out by myself for the degree of Doctor of Philosophy in Agricultural Science, *Curriculum* Agricultural and food microbial biotechnology under the guidance and supervision of Dr. Giovanni Garau and Prof. Severino Zara of the University of Sassari, Italy.

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, 4 and 5 describe the experimental work carried out. Chapters 2, 3, and 5 have been published in peer-reviewed journals (the permission to publication is attached at the end of this thesis) and they appear in their original form. Chapter 4 is will be soon submitted to a peer-reviewed journal for publication. Figures and tables of the published chapters are inserted in the text near first referencing and are therefore not listed in the Table of Contents.

Sassari, the 5th of November 2018

PhD Candidate

Stefania Diquattro

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Abstract

Antimony (Sb), a non-essential plant element, can be present in soils and waters in very high concentrations often due to a variety of anthropogenic activities. Though antimony is classified as a pollutant of priority interest, very little is known about its environmental behaviour and soil speciation.

In recent years, different sorbents for the in-situ remediation of metalloid-polluted soils were proposed and tested with variable success. In a search for alternative, low-cost and environmental friendly Sb sorbents, drinking water treatment residuals (WTRs), the waste material resulting from the treatment of surface or ground water, and municipal solid waste compost (MSWC) were identified as potential good candidates.

In this PhD thesis we showed that the Sb mobility, and its impact on the microbial and biochemical features of two soils artificially contaminated, were highly influenced by selected soil properties such as pH and organic matter content. Both WTRs and MSWC were able to act as effective Sb(V)-immobilizing agents in aqueous solutions especially at acidic pH (i.e. 4.5). Moreover, when added to a contaminated mine soil, the combination of both sorbents significantly reduced the mobility of antimony, alleviated its impact on soil microbiota and promoted a recovery of soil biochemical functioning. Plant growth (*Helichrysum italicum L.*) in the amended soil was finally enhanced while the Sb uptake was definitively reduced.

Chapter 1

General introduction

History and application

Antimony (Sb) is an important metalloid already known in the ancient world (it was used in Egypt between 2500 and 2200 BC to cover with a thin layer copper objects, while antimony objects dating back to the first millennium were found in Mesopotamia). The papyrus of Leyden, among others, gives a recipe “to confer on objects of copper the appearance of gold” by heating them in the presence of substances among which antimony compounds must have played a prominent role [Dufrenoy and Dufrenoy, 1950]. Antimony was also considered as the most suitable starting material from Alchemist and became a medicine thanks to Paracelsus (as published in Leipzig in 1604). Stibnite (Sb_2S_3) was used both as a medicine and for eye make-up. Finds dating back to the 4th millennium BC have been found [Hansell, 2015]. As regards the name, Plinio called it *stibium*, while around 800 AD the name *antimonium* was more used and the two names were used both for the element and for its sulphide. The English word antimony, is derived from the Latin *antimonium*, which is assumedly derived from the arabic *al ithmid* [Schelenz, 1909]. Also the Greek word ἀντίμόνος (*antimonos*), which means against aloneness, could be related to the origin of the name. Indeed, Sb is rarely found naturally in its metallic state [Hansell, 2015]. Resistant Cu-Sb alloys have been prepared for several thousand years, and in our days, patents are still granted for such alloys [Dufrenoy and Dufrenoy, 1950; Li and Thornton, 1993; Hansell, 2015]. Antimony and its compounds still represent an important source for the world economy as they are used for many purposes [Schwarz and Schampera, 2014; Hadjikakou *et al.,* 2015; Haldar *et al.,* 2011; Sharma *et al.,* 2008]. Antimony trioxide (Sb_2O_3) is used as a catalyst in the production of polyethylene terephthalate (PET) and as a flame retardant in the production of plastics, textiles and rubber [Reimann *et al.,* 2010].

Antimony is mainly consumed as flame retardants (about 60%) or it is employed in alloys (about 20%) [Biswas *et al.,* 2009]. Indeed, it is used in alloys because it improves the hardness and other properties of other metal: i.e. in alloy with lead (3-5% of antimony) it is used in the electrodes of car batteries grids and for semiconductor components; also the brake linings alloys used for bearings and power transmission equipment are tin-antimony or lead-antimony alloys. More applications involved antimony like sheet and pipe and in pigments for paints [Grund *et al.,* 2006]. It is also applied as addictive in glassware and ceramics, or as an active ingredient in the treatment of Leishmaniasis disease [Amarasiriwardena and Wu, 2011; Badaro *et al.,* 1990; Kato *et al.,* 2018] or antitumoral activity [Tiekink, 2002; Sharma *et al.,* 2008] and, as elemental Sb, in bullets [Guo *et al.,* 2009]. As a consequence, antimony which terrestrial abundance is usually low (0.7 mg kg^{-1} dm) [Filella *et al.,* 2002a], has been increased until its concentrations in soil became toxic for the environment as well as for human health [Tella and Pokrovsky, 2008; Wilson *et al.,* 2010; Martínez-Lladó *et al.,* 2011; Tella and Pokrovsky, 2012].

Chemical properties of antimony

Antimony is a trace element of the Group 15 (one of the elements called pnictogens) with atomic number 51, atomic mass 121.76 and atomic radius 153 pm. It has chalcophilic properties, an electronegativity of 2.05. The most important minerals are the sulfide Sb_2S_3 (antimonite or stibnite), Sb_2O_4 (cervantite) and Sb_4O_6 (valentinite and senarmontite), in addition to native antimony. It is also a substitute for As in several minerals such as pyrargyrite (Ag_3SbS_3) and bournonite ($PbSCu_2SSb_2S_3$). It also easily forms soluble anionic thiocomplexes, such as SbS_2^{2-} and SbS_4^{3-} [Kabata-Pendias, 2010]. Other sulphide ores are NiSbS (ullmannite), $HgSb_4S_8$ (livingstonite), Cu_3SbS_3 (tetrahedrite), $CuSbS_2$ (wolfsbergite) and $FePb_4Sb_6Si_4$ (jamesonite) [Smith, 2016].

Antimony is stable in air at 25 °C but reacts with oxygen if heated to produce antimony trioxide, Sb_2O_3. In its stable form, it is a lustrous gray metalloid with a Mohs scale hardness of 3. This rhombohedral antimony has the appearance of a silvery, fragile white metal. Its semi-metal character (given its position in the periodic system) is denounced by the rather low values of electrical and thermal conductivity, 2.88×10^6 m·Ω and 24.3 W· $m^{-1} \cdot K^{-1}$ respectively.

Elemental antimony adopts a layered structure (space group R3m No. 166) in which layers consist of fused, ruffled, six-membered rings. The nearest and next-nearest neighbours form an irregular octahedral complex, with the three atoms in each double layer slightly closer than the three atoms in the next. This relatively close packing leads to a high density of 6.697 g cm^{-3}, but the weak bonding between the layers leads to the low hardness and brittleness of antimony [Wiberg, 2001].

Antimony exists in different allotropic forms. Four allotropes of antimony are known: a stable metallic form and three metastable forms (explosive, black and yellow). Elemental antimony is a brittle, silver-white shiny metalloid. When slowly cooled, molten antimony crystallizes in a trigonal cell, isomorphic with the gray allotrope of arsenic. A rare explosive form of antimony can be formed from the electrolysis of antimony trichloride. When scratched with a sharp implement, an exothermic reaction occurs and white fumes are given off as metallic antimony forms; when rubbed with a pestle in a mortar, a strong detonation occurs. Black antimony is formed upon rapid cooling of antimony vapor. It has the same crystal structure as red phosphorus and black arsenic, it oxidizes in air and may ignite spontaneously. At 100 °C, it gradually transforms into the stable form. The yellow allotrope of antimony is the most unstable. It has only been generated by oxidation of stibine (SbH_3) at −90 °C. Above this temperature and in ambient light, this metastable allotrope transforms into the more stable black allotrope [Norman, 1997].

There are generally two oxidation states in soil: trivalent and pentavalent. Sb(III) is typical of anoxic environments while Sb(V) is the main (if not exclusive) specie in well-aerated soils [Johnson *et al.,* 2005; Filella *et al.,* 2009; Filella *et al.,* 2002b; Buschmann and Sigg, 2005; Steely *et al.,* 2007; Wilson *et al.,* 2010; Dousova *et al.,* 2015]. In aqueous systems across a wide pH range (i.e. pH 4-10) the inorganic antimonite [$Sb(OH)_3$] and antimonate [$Sb(OH)_6^-$] are the dominant species [Okkenhaug *et al.,* 2013; Filella *et al.,* 2002b; Herath *et al.,* 2017].

In soils Sb mainly exists as the oxidized species Sb(V), which displays a high affinity to amorphous and crystalline (hydr)oxide and other soil minerals [Johnson *et al.,* 2005; Guo *et al.,* 2014]. In the pH range of the majority of soils (i.e. pH 5-9), such interactions are particularly favoured by the high point of zero charge of amorphous and crystalline Fe-(hydr) oxides (e.g. pH_{PZC} 7.5-9.0 for goethite, 8.5 for hematite, 7.0 and 9.0 for ferrihydrite, 9.5 and 10 for akaganeite) [Strawn *et al.,* 2015]. Overall, data about the environmental chemistry and speciation of antimony in natural environments are quite limited, despite their relevance for the understanding of the element bioavailability and toxicity in soils and waters [Wilson *et al.,* 2010].

Trace element concentration in any environmental compartment is dependent on several factors, including the solubility of the solid phases it may form. Several simple Eh–pH and related diagrams for antimony in various oxidation states and involving oxide phases and aqueous or hydrolysed species have been reported in an effort to explain phase and solubility relationships in metallurgical and geochemical processes (Figure 1) [Filella *et al.,* 2009].

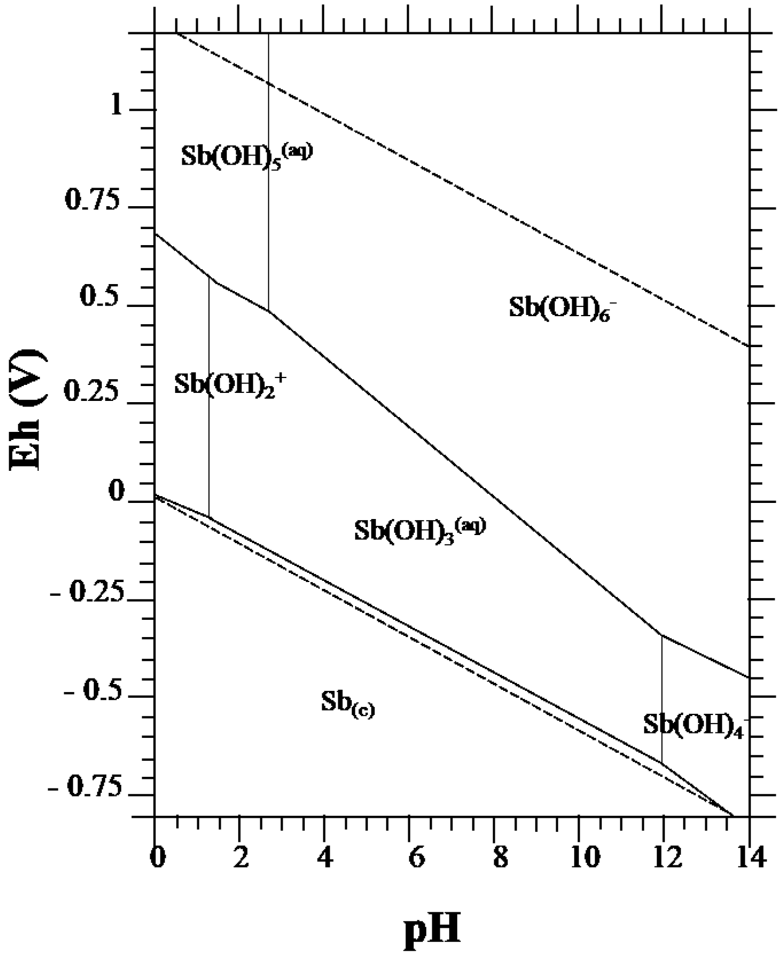

Figure 1. Eh-pH diagram on Sb species in H_2O.

Antimony in the environment

Antimony concentration in earth crust is around 0.2-0.5 mg kg^{-1}. Indeed, mean background Sb levels in soils range from 0.25 to 1.4 mg kg^{-1} [Kabata-Pendias, 2011]. Antimony distribution in rocks is associated with minerals such as sulfur minerals like stibnite (Sb_2S_3), or valentinite (Sb_2O_3) which is an oxidation product of stibnite [Kabata-Pendias, 2010; Filella *et al.,* 2002b; Wilson *et al.,* 2010]. Generally high concentrations of antimony (> 3 mg kg^{-1}) are typically found close to hydrothermal deposits of galena and sfalerite [Wedepohl, 1978; Salminen *et al.,* 2005], while the concentration in soils is between 0.1-1.9 mg kg^{-1} [Oorts *et al.,* 2008]. High antimony concentrations in soil present a high risk for soil biological activity and biota [Ahmad *et al.,* 2012a]. Such high values are reached in different sites like in proximity of mining areas and mineral processing facilities [Telford *et al.,* 2009; Li *et al.,* 2014; Wang *et al.,* 2010, Wang *et al.,* 2018] but also in army firing range soils [Lewińska *et al.,* 2017], where Sb reaches high levels in associations with others toxic metals, such as lead (Pb) and copper (Cu), due to bullets and their fragments [Cao *et al.,* 2003; Okkenhaug *et al.,* 2018]. High concentrations of antimony were also registered close to main streets due to the road traffic [Ceriotti and Amarasiriwardena, 2009; Ettler *et al.,* 2010; Foldi *et al.,* 2018]. In recent years, heavily Sb-polluted sites have been identified all around Europe, e.g. in Italy (Tuscany ~15000 mg kg^{-1} soil; Sardinia ~4400 mg kg^{-1} soil), Germany (~500 mg kg^{-1} soil), Switzerland (~17,000 mg kg^{-1} soil), France (5700 mg kg^{-1} soil), Spain (~5200 mg kg^{-1} soil) [Tschan *et al.,* 2009; Murciego *et al.,* 2007; Cidu *et al.,* 2013; Cidu *et al.,* 2014; Contini *et al.,* 2012] as well as worldwide e.g., in Iran, Canada, and China with up to ~ 640, ~ 1000 and ~ 7300 mg kg^{-1} soil [Rafiei *et al.,* 2010; Fawcett *et al.,* 2015; Wang *et al.,* 2010; Sanderson *et al.,* 2014]. These sites, often characterised by critical concentrations of co-occurring metal(loid)s [Wang *et al.,* 2010, 2011; Okkenhaug *et al.,* 2013; Sanderson *et al.,* 2014], can be of particular environmental concern since they represent hazardous multi-element contamination sources for neighbouring soils and water bodies and constitute a threat for soil functionality and fertility.

Abandoned mining sites in Italy figure out about 3000 sites [ISPRA, 2017]. Sardinia holds some of the largest mining areas in Europe. Indeed, some metals such as lead, zinc, copper and silver, antimony, aluminium and gold were extracted between 1900 and 1960, thanks to the high demand for raw materials and the low cost of workers [Brigaglia, 2002]. So mining represented the mainly economic and industrial market for a long time based on the natural wealth of the Sardinian subsoil, which was systematically exploited from the second half of the nineteenth century [Mezzolani and Simoncini, 2001]. The exploitation of mineral deposits, which almost everywhere closed at the end of the last century, left a strong imprint.

In most cases, these have been delivered to the territory a compromised environmental situation**,** due to the high levels of contamination linked to the mismanagement of the mine tailings and flotation sludge containing critical quantities of trace metals and metalloids; consequently, a slow and progressive degradation of these areas was observed [Rassu, 2004; Cidu *et al.*, 2013; Cidu *et al.,* 2014].

Some important district for antimony were located for instance at Su Suergiu (SE Sardinia, Italy) where Sb deposits were exploited since 1880 until mine closure in 1960. A foundry was active from 1882 to 1987. The most abundant minerals were antimonite, scheelite, arsenopyrite, pyrite, with calcite and quartz in the gangue. Slag and tailings (87% of dumped materials) and waste rocks were dumped nearby the mine plant. Mining and processing residues are estimated at about 66,000 m^3. Another important mining site polluted by antimony is Argentiera (NW Sardinia) where Pb, Ag and Zn were extracted for about one century from silver-rich galena [(Pb, Ag)S] and sphalerite (ZnS). High levels of antimony in this area have been found as antimony was an impurity leaved after the extraction of Ag from pyrargyrite. This deposit was used between 1860 and 1963.

Redox conditions and pH are the main parameters controlling Sb speciation in soils, which in turn is one of the key factors regulating Sb mobility and toxicity [Wilson *et al.,* 2004; Scheinost *et al.,* 2006; Clemente *et al.,* 2008; Nakamaru and

Altansuvd, 2014]. Sb(III) has been reported to be much more toxic (approx. 10-fold) and less stable than Sb(V) oxo-anionic species [Filella *et al.,* 2002b; Buschmann and Sigg, 2005; Steely *et al.,* 2007; Dousova *et al.,* 2015], but it was also recognized as less mobile and available to plants and soil microorganisms compared to Sb(V) [Nakamaru and Altansuvd, 2014; Herath *et al.,* 2017]. Median lethal dose (LD_{50}) for the oral uptake of Sb(V) in the form of potassium antimony tartrate in animals is nearly 115 mg kg^{-1} of body weight in rabbits and rats to 600 mg kg^{-1} of body weight in mice, while Sb_2O_3 was known as practically non-toxic ($LD_{50} > 20000$ mg kg^{-1} of body weight), which is due to its very low solubility in water [Gebel, 1999]. However, this does not rule out the possibility of Sb(III) uptake by the plant [Mathews *et al.,* 2011; Ren *et al.,* 2014; Ji *et al.,* 2017]. The pH strongly influences the Sb sorption in soil, particularly sorption maxima for Sb(V) were reported at low pH values, i.e., below pH 6, while abrupt decreases were recorded at higher pH values [Filella and Williams, 2012]. This behaviour can be explained considering that Sb(V) is primarily present in the soil solution as anionic $Sb(OH)_6^-$ (the acid form has a pKa = 2.55) and its affinity for soil colloids gradually decreases as the net negative charges increase [Filella and Williams, 2012]. This behaviour is quite different in comparison with others trace metals, i.e. Pb, Cd and Zn. Indeed, these trace metals are commonly present in soil aqueous solution like divalent and/or monovalent (hydroxylated) cations at acidic or neutral pH while it resembles that of metalloids forming anionic species, such as arsenic [Kabata- Pendias, 2011].

Antimony risk assessment

Due to the increasing use of antimony in many industrial applications, in the last decades there has been a marked increase in the concentrations of soil Sb which often exceeded the critical thresholds of toxicity for the flora, fauna and human health [ATSDR, 2017]. Occupational exposure to antimony occurs mainly in workers involved in industries producing antimony and antimony trioxide, metal mining, smelting and refining, coal-fired power plants, refuse incineration, or those working in indoor firing ranges. Health effects in both humans and animals have been observed following inhalational exposure to several antimony compounds e.g., antimony trioxide, stibine (antimony hydride), antimony trisulfide, antimony pentoxide, antimony trichloride, antimony pentasulfide, metallic antimony. The absorption of antimony from the respiratory tract is a function of particle size. Aerosols containing small particles composed of antimony compounds with low water solubility (e.g particles of antimony oxides) are retained in the lungs for a longer period of time than those containing larger particles with high water solubility (e.g., particles of antimony tartrate) [Sundar and Chakravarty, 2010]. These studies predominantly assessed the toxicity of trivalent antimony compounds, particularly antimony trioxide and/or pentoxide dust (8.87 mg antimony/m^3 or greater). Adverse health effects have also been observed in humans and animals following oral or dermal exposure to antimony and antimony compounds. The lung is the primary target of toxicity within the respiratory tract, and effects are observed following acute-, intermediate-, and chronic-duration exposure. In antimony workers, pneumoconiosis and clinical signs such as coughing and laryngitis have been reported [ATSDR, 2017]. In laboratory animals, the lung effects include the accumulation of antimony particles in the lungs, increases in alveolar/intra-alveolar macrophages, decreases in antimony lung clearance times, chronic interstitial inflammation, and interstitial fibrosis. Lung effects have been found in rats, mice, and rabbits following exposure to antimony trioxide, antimony trisulfide, and antimony ore; lung effects have also been observed in laboratory animals following exposure to stibine gas. Intermediate-and chronic-duration studies demonstrated that pulmonary damage can occur post exposure due to the persistence of the antimony trioxide in the lung [ATSDR, 2017]. Other respiratory effects that have been observed in some studies include squamous metaplasia of the epiglottis and hyperplasia of the nasal respiratory epithelium, chronic bronchitis, chronic emphysema, inactive tuberculosis, pleural adhesions, and respiratory irritation (characterized by chronic coughing, wheezing and upper airway inflammation. The lowest lowest-observed-adverse-effect levels (LOAELs) for respiratory tract effects following acute-, intermediate-, and chronic-duration exposures are 12 mg Sb/m^3 as antimony trioxide, 4.11 mg Sb/m^3 as antimony trioxide, and 1.6 mg Sb/m^3 as antimony trioxide, respectively. Also cardiovascular effects, especially myocardial damage and alterations in EKGs, have been observed in humans and animals exposed to antimony. Historically, antimony has been known for its emetic properties. Repeated prolonged exposure to airborne antimony trichloride, antimony trisulfide or antimony oxide was seen to cause gastrointestinal tract irritation as abdominal pain, vomiting, diarrhea, nausea, and ulcers have been observed in antimony workers. The development toxicity of antimony has not been extensively evaluated in humans or animals. Moreover, antimony and its compound may cause disturbances in menstruation and problem on conception. Decrease in growth have been reported in the infants of female antimony workers. Other health effects that have been observed in animals orally exposed to higher doses of antimony include hepatocellular vacuolization, hematological alterations including decreases in red blood cell counts and hemoglobin levels, and histological alterations in the thyroid. Dermatosis and ocular irritation have been reported in workers exposed to airborne antimony. Two occupational exposure studies examining carcinogenicity of antimony have found increases in lung cancer deaths. Mixed results have been found in chronic inhalation studies in rats. Increases in lung neoplasms were observed in rats exposed to 4.2 or 36 mg Sb/m^3 as antimony trioxide for approximately 1 year. The International Agency for Research on Cancer categorized antimony trioxide in group 2B (possibly carcinogenic to humans) and antimony trisulfide in group 3 (not classifiable as to its carcinogenicity to humans) [ATSDR, 2017].

Biological effect of antimony

Antimony is a non-essential element for plants, microorganism and human, and it naturally occurs in soil at low concentrations. Bioavailable Sb and co-occurring metal(loid)s can severely compromise soil functionality by affecting the size, composition and activity of the resident microbial communities [Garau *et al.*, 2011, 2014; Wang *et al.*, 2011; Garau *et al.*, 2014; Wei *et al.*, 2015] as well as plant growth [Kabata-Pendias, 2011; Pan *et al.*, 2011]. Several studies report that this trace element can be bioaccumulated via food chain and assimilated by human involving health risks. For instance, Agah *et al.* [2009] evaluated Sb accumulation in different species of fishes. Comparing the element levels in the muscles and liver it appears that the hepatic tissues bio accumulated Sb.

Plants may alter soil biogeochemical properties, especially in rhizosphere, and may consequently influence metal species transformation in soils [Ahmad *et al.*, 2014]. Ji *et al.* [2017] used XANES spectroscopy to analyse the speciation of Sb in roots and shoots of rye grass (*Lolium perenne L.*). Sb(V) was the dominant species in root. He *et al.* [1999] evaluated the effects of Sb(V) on rice. The results indicated that Sb affect the growth of root and rice sprout (the length of root and sprout decreased from approximately 21.4 to 100% and 5.7 to 64.1 %, respectively), and at high Sb concentration (200-1000 mg L^{-1}) reduced the transformation ratio of dry matter during the germination period of rice seed. Sb(V) also, affected the activity of α-amylase, this latter was increased until 50 mg L^{-1} or inhibited at higher concentration, and the growth of rice. The root systems were few and short and there were more red-brown root fibrils. A reduction in yield and an increase of antimony in rice were significantly related to Sb application rates to soils. Other studies conducted by Hammel *et al.* [2000] reported that leaves of spinach grown under controlled conditions in soils could accumulate up to 399 mg Sb kg^{-1} dry mass when soil antimony contamination was around 1000 mg kg^{-1}. Baroni *et al.* [2000] also indicated some species like *Achillea ageratum*, *Plantago lanceol*ata and *Silene vulgaris* as bioindicators for antimony in as they strongly accumulated antimony when its extractable fraction in the soil is high (139-793 mg kg^{-1}). *A. ageratum* grew in mining soil accumulated in basal leaves (1367 mg kg^{-1}) and in inflorescences (1105 mg kg^{-1}), *P. lanceolata* in roots (1150 mg kg^{-1}) and *S. vulgaris* in shoots (1164 mg kg^{-1}).

More studies reported reduced growth and biomass of both grasses and legumes under Sb and/or heavy metal(loid)s stress [Pan *et al.*, 2011] as well as decreased abundance of culturable soil microbial populations and reduced soil enzyme activities [Wang *et al.*, 2011; Garau *et al.,* 2014]. Moreover, it was recently shown that elevated Sb reduced the diversity of arbuscular mycorrhizal fungi [Wei *et al.*, 2015] and increased the distribution, diversity and abundance in soil bacterial populations of selected genes involved in Sb detoxification [Luo *et al.,* 2014].

Threshold limits

Anthropogenic processes continuously increase the amount of trace metals in the environment [Agah *et al.,* 2009]. In recent years, community has shown a growing interest about pollution. In Europe the Sixth Action Plan for the Environment and the communication of the European Commission to the Council and the European Parliament, to the Economic and Social Committee and to the Committee of the Regions (COM No. 179 of 2002), is an evidence. The plan entitled: "Towards a thematic strategy for soil protection" is a relevant instance. The paper addresses various issues including soil protection against pollution, erosion risk, waterproofing, compaction, organic matter and biological activity reduction and salinization [European Commission, 2002]. Indeed, soil is an essential constituent of the environment and it regulates productivity. Therefore, all the disruption processes, increased by anthropic activity, can affect its fertility and functionality [Mendes *et al.,* 2016; Hinojosa *et al.,* 2005; Filella *et al.,* 2007]. In these view soil contamination by trace metals results one of the main aspect to consider. Because of its high toxicity and no known biological role [Filella *et al.,* 2002a, 2002b; Tella and Pokrovsky, 2008; Dousova *et al.,* 2015] antimony and its compounds are considered high priority pollutants by the United States Environmental Protection Agency and the European Union [CEC, 1998; USEPA, 2009]. In Italy, the current national legislation on land reclamation refers to the Decreto Legislativo, 03/04/2006 n. 152, G.U. 14/04/2006 and subsequent modifications of the Testo Unico 156/2006. According to the environmental regulations (Annex 4/14 - Annex 5 Title V of Part Four - Limit concentration values acceptable in soil and subsoil referred to the specific intended use of the sites to be reclaimed), the antimony limit concentration in sites destined for "public, private and residential green use" is 10 mg kg^{-1} soil, while in sites destined for "commercial and industrial use" is 30 mg kg^{-1} soil. As regards drinking water, the Environmental Protection Agency of the United States [USEPA] established 6 $\mu g\ L^{-1}$ as the maximum contaminant level (MCL) for Sb [USEPA, 2009], while the European Union fixed a maximum admissible concentration of 5 $\mu g\ L^{-1}$ [CEC, 1998].

Amendments and bioremediation

In recent years, several studies have been carried out for the development of new technologies for remediation of soil contaminated by trace metals, both *in situ* and *ex situ* approach. However, many of the *ex situ* methods are difficult to apply because of i) the extent of polluted areas; ii) the complexity of the ecosystem; iii) expensive cost. Opposite, some *in situ* methodologies, based on trace metal stabilization through its inactivation and/or chemical immobilization by amendments (both organic and inorganic) addition to soil, showed good results [Bagherifam *et al.,* 2014]. Amendments can adsorb and/or precipitate the antimony present in mobile form, so they represent a sustainable and environmental-friendly strategy for the recovery of polluted soils [Dousova *et al.,* 2015; Rodríguez *et al.,* 2016]. The objective of such remediation technologies is to reduce metal bioavailability and/or toxicity to plants and animals in soils [Ahmad *et al.,* 2014].

The consideration of amendment cost is also critically required. For example, Guo *et al.,* 2006 reported that the cost for metal fixation by soil amendments is \$90–200 compared to \$100–400 and \$250–500 for landfilling and soil extraction technologies, respectively, to remediate one ton of soil. The primary mechanisms of metal immobilization by soil amendments are adsorption, cation-exchange, precipitation, and surface complexation [Ok *et al.,* 2010; Jung *et al.,* 2011; Kim *et al.,* 2012a, b]. The amendments most likely transform the existing bioavailable metal species in soil into geochemically more stable forms [Traina and Laperche, 1999].

The waste materials/by products generated from industries may provide important resources as soil amendment materials. In the last decades, various waste materials have been used as potential sorbent materials for the in-situ remediation of metal(loid) polluted soils, such as red mud [Lee *et al.,* 2009; Garau *et al.,* 2011; Castaldi *et al.,* 2010; Liu *et al.,* 2011; Hua *et al.,* 2017], fly ash [Ciccu *et al.,* 2003; Lim *et al.,* 2012; Moon *et al.,* 2013b], furnace slag [Lee *et al.,* 2009], poultry waste [Lim *et al.,* 2013b], oyster shells [Moon *et al.,* 2011, 2013a; Lim *et al.,* 2013a; Ok *et al.,* 2010, 2011c], eggshells [Ok *et al.,* 2011a,b], plant residue [Ok *et al.,* 2011d], biochar [Awad *et al.,* 2012, 2013; Ahmad *et al.,* 2014; Mohan *et al.,* 2012; Uchimiya *et al.,* 2012; Beesley *et al.,* 2011], mussel shell and cow bone [Ahmad *et al.,* 2014; Sanderson *et al.,* 2015], and municipal solid waste compost [Manzano *et al.,* 2016; Silvetti *et al.,* 2017a; Silvetti *et al.,* 2017b; Castaldi *et al.,* 2017].

However, the number of studies addressing the remediation of antimony contaminated soils is very limited. So it is important to evaluate remediation strategies in those soils where Sb and additional metal(loid)s reach critical concentrations, in order to decrease the potential hazard for soil and ecosystem functioning. To date, only a few amendments, mostly based on Fe- and Al-containing materials/ minerals, have been tested with variable success as Sb immobilising agents. For instance, adding olivine and hematite to a contaminated soil was found to have no effect on pore water Sb concentration [Okkenhaug *et al.,* 2012], while red mud (alumina industry residues high in Fe-oxides) addition reduced pore water Sb only in some cases [Sanderson *et al.,* 2015]. The addition of organic amendments (e.g. mussel shell, cow bone, chicken manure, sodium humate) have mostly been deemed ineffective at fixing Sb [Conesa *et al.,* 2010; Ahmad *et al.,* 2014; Shtangeeva *et al.,* 2014].

Other research has shown that a 2% commercial Fe-oxyhydroxide, and very high rates of amorphous Fe- and Al-oxyhydroxides, could effectively stabilise the antimony in different polluted soils [Alvarez-Ayuso *et al.,* 2013; Okkenhaug *et al.,* 2013, 2016; Tandy *et al.,* 2017]. However, the mobilisation of co-occurring metals (e.g. Pb, Cu, and Zn) was detected after the addition of Fe based materials to Sb-contaminated soils [Okkenhaug *et al.,* 2013]. Amorphous and crystalline Fe, Al, Mn-(hydr)oxides, and humic substances display a high affinity for antimony [Johnson *et al.,* 2005; Tighe *et al.,* 2005; Scheinost *et al.,* 2006; Martínez-Lladó *et al.,* 2011; Van Vleek *et al.,* 2011; Nakamaru and Altansuvd, 2014] and their occurrence in soil seems to control Sb mobility and bioavailability [Tighe *et al.,* 2005; Clemente *et al.,* 2008; Martínez-Lladó *et al.,* 2011; Filella andWilliams, 2012; Tella and Pokrovsky, 2012; Nakamaru and Altansuvd, 2014].

Eco-friendly materials

Antimony pollution, interest many areas around the world therefore new technologies for remediation are needed. Studies are based on the search for alternative, low cost and environmental friendly materials to use for bioremediation. Research is focused to evaluate new sorbents for antimony, in order to limit its mobility in the soil. Dousova *et al.* [2015] observed that the addition of sorbents containing organic matter (OM), e.g. compost, could be a sustainable strategy for decreasing the mobility of the metalloid and improving the physico-chemical and biological properties of the soil. Indeed, it was proved that antimony(III) can interact with both soft and hard OM ligands (e.g. thiol and COOH functional groups respectively) [Buschmann and Sigg, 2005; Tighe *et al.,* 2005; Sh *et al.,* 2012]. However, few studies have been done regarding Sb(V) complexation by OM and results are often contradictory [Tella and Pokrovsky, 2008; Filella, 2011; Filella and Williams, 2012; Nakamaru and Altansuvd, 2014]. Indeed, Sb(V) in soil is mostly present in its anionic form (i.e. $Sb(OH)_6^-$), which limits the interaction with the negatively charged sites of the OM [Filella, 2011; Filella and Williams, 2012; Dorjee *et al.,* 2014]. On the other hand, some researchers reported a significant Sb(V) complexation by OM and/or humic substances [Tighe *et al.,* 2005; Steely *et al.,* 2007; Tella and Pokrovsky, 2008; Ceriotti and Amarasiriwardena, 2009; Van Vleek *et al.*, 2011; Dousova *et al.,* 2015]. Tella and Pokrovski [2008] provided a first direct evidence that poly-functional organic ligands like oxy-carboxylic acids and polyols were able to form complexes with Sb(V) in aqueous solution. Moreover, other mechanisms, such as ternary complexation in which polyvalent metal cations (e.g. Fe, Al and Ca) act as a bridge between the negatively charged functional groups of OM and the antimony oxyacid (as demonstrated for phosphate and arsenate), could be involved in Sb(V) binding by OM [Filella and Williams, 2012]. Among the environmental friendly sorbents for antimony the drinking-water treatment residuals (WTRs), the waste material resulting from the treatment of surface, or groundwater with Al and Fe salts, can also be identified as a potential good candidate [Nagar *et al.,* 2010]. In the conventional coagulation-filtration treatment process, suspended solids, natural organic matter, dyes, contaminants, etc., are removed from the raw water supply by the addition of aluminum, and/or iron and/or calcium salts as coagulants, resulting in the production of WTRs [Babatunde *et al.,* 2009]. Such residuals generally contain activated carbon, natural organic matter (especially humic substances), $CaCO_3$ and, above all, Fe and/or Al (oxy)-hydroxides [Babatunde *et al.,* 2009]. The simultaneous presence of these constituents makes WTRs as potential good candidates for reducing the Sb(V) mobility in polluted soil and waters. Many studies showed that WTRs are able to accumulate As(V) and P(V) anions and that such ability is closely related to the pH of the system [Babatunde *et al*., 2009; Nagar *et al.*, 2010; Castaldi *et al.*, 2014].

These potential sorbents have been never tested before at the best of our knowledge with respect to Sb(V). Nevertheless, data suggest that compost can have an influence on Sb(V) mobility in soil and WTRs can be a useful sorbent for Sb(V) in polluted soils.

Aim and objectives

The aim of the PhD thesis is to give a contribution for improving the knowledge about the antimony speciation and toxicity in polluted soils, as well as the suitability of two low-cost and sustainable materials (i.e. drinking-water treatment residuals and municipal solid waste compost) as amendments for the chemical and biological recovery of a degraded soil contaminated with Sb. The objectives of this research therefore include: i) to determine the ability of selected WTRs and compost as immobilizing agents towards Sb(V) in aqueous solutions; ii) to evaluate the speciation of antimonate, added to two different soils, its mobility, as well as its impact on selected soil microbial and biochemical features; iii) to evaluate the suitability of the combined application of selected WTR and compost for the chemical and biological recovery of a degraded alkaline soil contaminated with Sb and other trace metals.

The originally approach given to this study can provide important and new information on Sb mobility and toxicity in soil, as well as on the efficiency of low-cost materials as immobilizing agents for antimony. The results obtained could represent fundamental knowledge for the chemical and biological remediation of soil polluted by antimony.

Organization of the thesis and experimental approaches

Chapter 1 provides a comprehensive review of the current research status regarding antimony, including a brief introduction on the chemistry of antimony and its toxic effects for human health, as well as a brief introduction on consequences for the environment. The main part of this chapter summarizes the strategies and experimental approaches and the thesis tasks.

Chapter 2 describes the interaction between Sb(V) and two municipal solid waste composts (MSW-Cs) in aqueous solution at acidic pH (pH 4.5). The aim of the present chapter was therefore to evaluate the ability of MSW-Cs to block antimony(V) and to determine the role of humic acids extracted from compost in

Sb(V) sorption. Moreover, the different Sb(V) pools bound to the MSW-Cs were determined through specific extraction procedures, and the capacity of low molecular weight organic acids (LMWOAs) commonly present in soils to mobilize the metalloid sorbed by the MSW-Cs was studied.

The aim of Chapter 3 was similar to the previous one, and it gives information on interaction between Sb(V) and residues from the treatment of drinking water (WTRs) in aqueous solution at different pH. Laboratory work was focused on the quantification of different Sb(V) pools bound to the WTRs through specific extraction procedures, and the evaluation of the capacity of citric and malic acids to mobilize the metalloid sorbed by the WTRs was carried out.

The other two chapters are focused on mobility, toxicity and bioavailability of antimony in artificially or naturally contaminated soils. Indeed the chapter 4 gives results regarding the antimony mobility in two soils artificially contaminated, as well as the impact of contamination on the microbial and biochemical properties of the soils. The suitability of the combined application of WTR and compost for the chemical and biological recovery of a degraded alkaline soil contaminated by antimony and other trace metals was evaluated in the chapter 5.

A summary of the outcome of this work and perspectives is provided in the final chapter, which is presented in the form of general conclusion of the PhD project.

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Chapter 2

Municipal solid waste compost as a novel sorbent for antimony(V): adsorption and release trials at acidic pH

Diquattro[1], S., Garau[1], G., Lauro[1], G. P., Silvetti[1], M., Deiana[1], S., and Castaldi[1], P., 2018. Municipal solid waste compost as a novel sorbent for antimony (V): adsorption and release trials at acidic pH. Environmental Science and Pollution Research, 25(6), 5603-5615. DOI: https://doi.org/10.1007/s11356-017-0933-y.

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RESEARCH ARTICLE

Municipal solid waste compost as a novel sorbent for antimony(V): adsorption and release trials at acidic pH

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Abstract

The ability of two municipal solid waste composts (MSW-Cs) to sorb antimony(V) in acidic conditions (pH 4.5) was investigated. Sorption isotherms and kinetics showed that both MSW-Cs could sorb antimony(V), even if in different amounts (~ 0.18 and 0.24 mmol g^{-1} of Sb(V) by MSW-C1 and MSW-C2, respectively). These differences were ascribed to the chemical composition of composts, as well as to the total acidity of their humic substances. The Sb(V) sorption by both MSW-Cs followed a pseudo-second-order kinetic model, while the sorption isotherms data fitted the Freundlich model better than the Langmuir one. The humic acids extracted from composts contributed to 4.26 and 8.24% of Sb(V) sorption by MSW-C1 and MSW-C2 respectively. SEM-EDX spectra of the MSW-C+Sb(V) systems showed a certain association of Ca(II) with Sb(V), while sequential extraction procedures indicated that more than 80% of the Sb(V) sorbed was strongly retained by MSW-Cs. On the other hand, treatment with oxalic acid at pH 4.5 favored the release of more than 98 and 65% of the Sb(V) sorbed by MSW-C1 and MSW-C2 respectively, supporting a possible role of calcium in Sb(V) retention. The results from this study suggest that MSW-Cs could be used as amendments for the in-situ immobilization of Sb(V) in acidic-polluted soils.

Keywords Antimony(V) · Municipal solid waste compost · Humic acids · Sorption isotherms · Sb(V) release

Introduction

Antimony (Sb) and its compounds are considered as priority pollutants by the international environmental protection agencies (CEC 1998; USEPA 2009), because of their high toxicity and no known biological role (Filella et al. 2002a, 2002b; Tella and Pokrovsky 2009; Dousova et al. 2015). The Environmental Protection Agency of the United States (USEPA) established 6 μg l^{-1} as the maximum contaminant level (MCL) for Sb in drinking water (USEPA 2009), while the European Union fixed a maximum admissible concentration of 5 μg l^{-1} (CEC 1998). On the other hand, mean background Sb levels in soils range from 0.25 to 1.04 mg kg^{-1} (Kabata-Pendias 2010). However, the metalloid can be found in much higher concentrations where Sb-rich ores are present in the pedogenic material and/or as a consequence of anthropogenic activities (Tella and Pokrovsky 2009; Wilson et al. 2010; Martínez-Lladó et al. 2011; Tella and Pokrovsky 2012). In particular, antimony can mainly occur as stibnite (Sb_2S_3), the primary Sb ore, and valentinite (Sb_2O_3), which is an oxidation product of stibnite (Filella et al. 2002b; Kabata-Pendias 2010; Wilson et al. 2010). Anthropogenic sources of antimony include mining activity and industrial emissions; however, plastic waste, road traffic, and shooting ranges have become the dominant Sb sources in the last decades (Dousova et al. 2015; Ceriotti and Amarasiriwardena 2009). As a result, heavily Sb-polluted sites have been recently found in Europe (e.g., in Italy, Germany, and Switzerland with up to ~ 100, ~ 500 and ~ 17,000 mg kg^{-1}) (Tschan et al. 2009; Garau et al. 2017) as well as worldwide (e.g., in Iran, Canada, and China with up to ~ 640, ~ 1000 and ~ 7300 mg kg^{-1}) (Rafiei et al. 2010; Wang et al. 2010; Fawcett et al. 2015).

Antimony, according to thermodynamic equilibrium estimates, can occur in most natural systems in two oxidation

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states, + 3 and + 5, with the inorganic antimonite [$Sb(OH)_3$] and antimonate [$Sb(OH)_6^-$] being the dominant species in aqueous systems in a wide pH range (i.e., pH 4–10) (Filella et al. 2002b; Herath et al. 2017). Pentavalent Sb species are prevailing in oxygenated natural waters and aerated soils, at most environmentally relevant pH values, but it can also be present under reducing conditions, where the trivalent Sb is the thermodynamically stable form (Filella et al. 2002b; Buschmann and Sigg 2005; Steely et al. 2007; Wilson et al. 2010; Dousova et al. 2015).

Redox conditions and pH are the main parameters controlling Sb speciation in soils, which in turn is one of the key factors regulating Sb mobility and toxicity (Wilson et al. 2004; Scheinost et al. 2006; Clemente et al. 2008; Nakamaru and Altansuvd 2014). Sb(III) has been reported to be much more toxic (approx. 10×) and less stable than Sb(V) (Filella et al. 2002b; Buschmann and Sigg 2005; Steely et al. 2007; Dousova et al. 2015), but it was also recognized as less mobile and available to plants and soil microorganisms compared to Sb(V) (Nakamaru and Altansuvd 2014; Herath et al. 2017). However, this does not rule out the possibility of Sb(III) uptake by the plant (Mathews et al. 2011; Ren et al. 2014; Ji et al. 2017). The pH strongly influences the Sb sorption in soil, particularly sorption maxima for Sb(V) were reported at low pH values, i.e., below pH 6, while abrupt decreases were recorded at higher pH values (Filella and Williams 2012). This behavior can be explained considering that Sb(V) is primarily present in the soil solution as anionic $Sb(OH)_6^-$ (the acidic form has a pKa = 2.55) and its affinity for soil colloids gradually decreases as the net negative charges increase (Filella and Williams 2012).

Amorphous and crystalline Fe, Al, Mn-(hydr)oxides, and humic substances display a high affinity for antimony (Johnson et al. 2005; Tighe et al. 2005; Scheinost et al. 2006; Martínez-Lladó et al. 2011; Van Vleek et al. 2011; Nakamaru and Altansuvd 2014) and their occurrence in soil seems to control Sb mobility and bioavailability (Tighe et al. 2005; Clemente et al. 2008; Martínez-Lladó et al. 2011; Filella and Williams 2012; Tella and Pokrovsky 2012; Nakamaru and Altansuvd 2014).

Although substantial progress has been recently made on the comprehension of factors governing Sb mobility in soil (Wilson et al. 2010; Filella and Williams 2012; Garau et al. 2017), the remediation of Sb-polluted sites still remains a challenging task. Iron-based materials are among the most investigated and (so far) effective Sb sorbents (Tandy et al. 2017; Garau et al. 2017). However, the addition to Sb-polluted soils of sorbents containing organic matter (OM), e.g., compost, could be an alternative and more sustainable strategy for decreasing the mobility of the metalloid and improving the physico-chemical and biological properties of the soil (Dousova et al. 2015; Garau et al. 2017). There are some evidences supporting this point. It was proved that antimony(III) can interact with both soft and hard OM ligands (e.g., thiol and COOH functional groups, respectively) (Buschmann and Sigg 2005; Tighe et al. 2005; Sh et al. 2012). Moreover, few reports suggest the Sb(V) binding to OM even if this point has not been clarified yet, and the results in literature are often controversial (Tella and Pokrovsky 2008; Filella 2011; Filella and Williams 2012; Nakamaru and Altansuvd 2014). The weak affinity of Sb(V) to soil organic matter, observed by some researchers, was explained with the anionic nature of Sb(V) in soil (i.e., $Sb(OH)_6^-$), which limits the interaction with the negatively charged sites of the OM (Filella 2011; Filella and Williams 2012; Dorjee et al. 2014). Nevertheless, some researchers reported a significant Sb(V) complexation by OM and/or humic substances (Tighe et al. 2005; Steely et al. 2007; Tella and Pokrovsky 2008; Ceriotti and Amarasiriwardena 2009; Van Vleek et al. 2011; Dousova et al. 2015). Tella and Pokrovski (2008) provided a first direct evidence that poly-functional organic ligands like oxy-carboxylic acids and polyols were able to form complexes with Sb(V) in aqueous solution. Moreover, other mechanisms, such as ternary complexation in which polyvalent metal cations (e.g., Fe, Al, and Ca) act as a bridge between the negatively charged functional groups of OM and the antimony oxyacid (as demonstrated for phosphate and arsenate), could be involved in Sb(V) binding by OM (Filella and Williams 2012).

Taken together, these data suggest that OM can have an influence on Sb(V) mobility in soil and that OM-based sorbents, such as compost, could be used as alternative and sustainable amendments for the remediation of Sb-polluted soils. However, the Sb(V)-immobilizing capabilities of composts were never investigated and should be experimentally proved.

The aims of the present study were therefore (i) to evaluate the sorption capacity of two municipal solid waste composts (MSW-Cs) for Sb(V) in aqueous solutions at pH 4.5; (ii) to estimate the role of the MSW-C humic acids in Sb(V) sorption; (iii) to quantify the different Sb(V) pools bound to the MSW-Cs through specific extraction procedures; and (iv) to evaluate the capacity of low molecular weight organic acids (LMWOAs) commonly present in soils, i.e., citric and oxalic acids, to mobilize the metalloid sorbed by the MSW-Cs.

Materials and methods

Municipal solid waste composts (MSW-Cs)

Two different municipal solid waste composts (MSW-Cs), deriving from mixed green and food waste, were investigated: the first compost (MSW-C1) came from the Secit S.p.A. facility plant of the Consorzio ZIR (Sassari, Italy), while the second (MSW-C2) came from Verde Vita S.r.l. (Sassari, Italy).

The composts were dried at 25 °C, then ground and sieved to < 2 mm for chemical analyses which were performed according to Chefetz et al. (1996) and Castaldi et al. (2008) (Table 1).

The total concentration of selected heavy metals was determined on dried (105 °C) composts using a Perkin Elmer Analyst 600 flame atomic absorption spectrometer after microwave (Milestone MLS 1200) digestion of the samples with aqua regia (HNO_3/HCl, 1:3).

For Sb(V) sorption/desorption trials, compost samples were brought to pH 4.5 with either 0.01 or 0.1 M HNO_3 solutions. The chemical characteristics of the untreated composts, and of MSW-Cs brought to pH 4.5, are reported in Table 1. Each determination was carried out on triplicate compost samples and mean values ± standard deviations are reported.

Sorption isotherms of Sb(V) on MSW-Cs at pH 4.5

MSW-C1 and MSW-C2 samples brought at pH 4.5 were artificially polluted with solutions containing increasing concentrations of Sb(V) (pH 4.5) derived from $KSb(OH)_6 \cdot 7H_2O$. Chemicals were of analytical grade and used without further purification. At the pH selected for this trial, and at the highest metalloid concentrations tested (i.e., 6 mM), more than 99% of Sb(V) was present in solution after 48 h, thus ruling out the formation of Sb(V) precipitates. Polyethylene bottles containing 1.0 g

Table 1 Chemical characteristics of the MSW-Cs investigated

Chemical parameters	MSW-C1		MSW-C2	
	Untreated	pH 4.5	Untreated	pH 4.5
pH	7.93 ± 0.06	4.5	8.60 ± 0.02	4.5
EC (mS cm^{-1})	3.26 ± 0.03	–	4.59 ± 0.01	–
Ashes (% d.m.)	42.05 ± 3.21	38.88 ± 2.61	46.42 ± 2.84	31.05 ± 0.49
Humidity %	9.75 ± 0.53	5.78 ± 0.44	5.02 ± 0.68	5.99 ± 0.14
Total organic carbon (TOC, % d.m.)	27.34 ± 0.61	26.98 ± 0.28	25.48 ± 0.33	24.86 ± 0.45
Dissolved organic carbon (DOC; mg kg^{-1} d.m.)	0.817 ± 0.04	0.573 ± 0.04	0.603 ± 0.04	0.405 ± 0.03
Cation exchange capacity (cmol$_{(+)}$ kg^{-1})	92.30 ± 0.83	62.50 ± 0.19	77.75 ± 0.21	59.28 ± 0.13
Total phosphorus (% d.m.)	0.71 ± 0.11	0.57 ± 0.02	1.30 ± 0.10	0.78 ± 0.08
Elemental composition C (% d.m.)	27.46 ± 0.37	26.70 ± 0.28	26.38 ± 0.39	25.58 ± 0.57
Elemental composition N (% d.m.)	2.18 ± 0.04	2.60 ± 0.06	2.80 ± 0.07	3.72 ± 0.06
Elemental composition H (% d.m.)	3.17 ± 0.01	4.67 ± 0.05	3.20 ± 0.01	5.07 ± 0.05
Total extractable carbon (TEC: % d.m.)	16.35 ± 0.17	17.82 ± 0.23	13.34 ± 0.58	16.44 ± 0.15
Humic acids (HA: % d.m.)	14.24 ± 0.11	12.06 ± 0.06	11.18 ± 0.25	10.85 ± 0.04
Total Acidity (meq g^{-1} HA)	8.71 ± 0.24	8.48 ± 0.66	9.43 ± 0.44	9.39 ± 0.31
COOH (meq g^{-1} HA)	5.19 ± 0.84	5.06 ± 0.53	5.86 ± 0.53	5.94 ± 0.88
Ar-OH (meq g^{-1} HA)	3.52 ± 0.34	3.42 ± 0.69	3.57 ± 0.39	3.45 ± 0.55
Fulvic acids (FA: % d.m.)	1.10 ± 0.21	0.75 ± 0.05	1.01 ± 0.13	0.69 ± 0.07
Total Acidity (meq g^{-1} FA)	13.29 ± 1.44	12.86 ± 1.59	13.41 ± 1.53	12.68 ± 1.69
COOH (meq g^{-1} FA)	11.56 ± 1.59	12.24 ± 1.60	11.61 ± 1.42	12.45 ± 1.46
Ar-OH (meq g^{-1} FA)	1.75 ± 0.09	0.62 ± 0.02	1.81 ± 0.07	0.23 ± 0.00
Metals (mg kg^{-1} d.m.)				
Pb	3.72 ± 0.15	0.16 ± 0.00	n.d.	n.d.
Zn	30.52 ± 3.20	17.46 ± 1.49	26.91 ± 0.89	21.70 ± 2.07
Cd	n.d.	n.d.	n.d.	n.d.
Cu	19.24 ± 1.74	4.68 ± 0.39	n.d.	n.d.
Fe	5587 ± 88	6498 ± 65	5494 ± 66	7456 ± 54
Mn	140.5 ± 5.7	123.7 ± 4.8	147.9 ± 6.2	100.4 ± 4.9
Na	993.3 ± 24.2	369.9 ± 15.5	2534 ± 28.5	928.6 ± 18.7
K	1709 ± 21	372.3 ± 17.9	2780 ± 24	696.7 ± 19.1
Mg	5403 ± 135	1453 ± 169	4504 ± 121	1407 ± 60.8
Ca	63,444 ± 130	22,599 ± 105	80,113 ± 122	32,339 ± 245

of MSW-C1 or MSW-C2 were filled with 100 mL solutions of Sb(V) at pH 4.5. Nine different Sb(V) concentrations were used [from 0.0 to 6.0 mM]. A background electrolyte of 1.0 mM $NaNO_3$ was used for all batch experiments. The final mixtures were shaken for 24 h at constant temperature (20 ± 1 °C). Afterwards, the samples were centrifuged at 8000 rpm for 15 min and filtered using a 0.2-μm filter paper. Antimony(V) in the supernatant was measured by ion chromatography using an IonPac AS9-HC Analytical Column equipped with an IonPac AG9-HC Guard Column, 4 mm (Castaldi et al. 2014; Silvetti et al. 2017a). Sodium bicarbonate (10 mM) was employed as the eluent at a flow rate of 1.0 mL min^{-1}. The sample loop was 10 μL. Each point of the sorption isotherms derived from Sb(V) quantification in three independent samples and mean values ± standard deviations are reported.

To investigate the role of humic acids (HA) in Sb(V) sorption, HA were extracted from composts as described by Ciavatta et al. (1990) and brought at pH 4.5. Polyethylene bottles containing 250 mg of the extracted HA were filled with 25 mL of 3.0 or 6.0 mM Sb(V) solutions (1:100 *w/v* ratio). The mixtures were shaken for 24 h at constant temperature (20 ± 1 °C) and then centrifuged and filtered, and Sb(V) in the supernatant was quantified as described above.

Sorption kinetics of Sb(V) on MSW-Cs at pH 4.5

For kinetic studies, polyethylene bottles containing 1.0 g of each compost brought at pH 4.5 were filled with 100 mL of a solution containing 0.6 mmol of Sb(V) derived from $KSb(OH)_6 \cdot 7H_2O$. The final mixtures were shaken at constant temperature (20 ± 1 °C) for different times, i.e., 5, 10, 30, 60, 180, 360, 720, 1080, 1440 min. The samples were then centrifuged at 8000 rpm for 15 min and filtered. Supernatant aliquots were taken and Sb(V) in solution was quantified as previously described. Each point of the sorption kinetics derived from Sb(V) quantification in three independent samples and mean values ± standard deviations are reported.

Scanning electron microscopy and energy-dispersive X-ray spectroscopy analysis

SEM-EDX analyses of untreated MSW-Cs and of MSW-Cs-Sb(V) systems at pH 4.5 [i.e., saturated with 6.0 mM $KSb(OH)_6 \cdot 7H_2O$] were carried out using an environment electron microscope ESEM-Zeiss EVO LS10 (Oberkochen-Germany). Compost samples were observed with the backscattered electron detector (BSD) and chemically analyzed with a microanalysis X-ray energy-dispersive device (EDS) INCAx-ACT (Oxford Instruments). The whole processes occurred at low vacuum mode and a pressure of 10 Pa.

Sequential extraction of Sb(V) by the MSW-Cs-Sb(V) systems

The different Sb(V) pools bound to the MSW-Cs doped with 3.0 or 6.0 mM $KSb(OH)_6 \cdot 7H_2O$ at pH 4.5 were quantified through the sequential extraction procedure (originally designed for As(V)) of Wenzel et al. (2001) with minor modifications: essentially, an additional step was added to estimate the water-soluble Sb(V), while the step designed to extract Sb(V) fractions bound to crystalline Al- and Fe-oxyhydroxides was not applied given the essentially organic and amorphous nature of composts.

MSW-C samples (1.0 g) brought at pH 4.5 and doped with 3.0 or 6.0 mM Sb(V) solutions (i.e., deriving from the sixth and the last point of the sorption isotherms) were added with 100 mL of distilled water (pH 4.5) and shaken for 2 h at 20 °C to extract the water soluble Sb(V) (step 1). Solid samples were then treated with 100 mL of 0.05 M $(NH_4)_2SO_4$ to extract the non-specifically sorbed Sb(V) (step 2). Solid samples were subsequently treated with 100 mL of 0.05 M $NH_4H_2PO_4$, to extract the specifically sorbed Sb(V) (step 3), while the Sb associated to amorphous Al- and Fe-oxyhydroxides was determined after extraction with 0.2 M NH_4-oxalate pH 3.25 (step 4). After each step of the extraction process, MSW-Cs were centrifuged at 8000 rpm for 15 min and filtered using a 0.2-μm filter paper. The concentrations of Sb(V) in solution were determined after each extraction step as previously described. Each step of the sequential extraction procedure was carried out on three independent samples and mean values ± standard deviations are reported.

Influence of organic acids on the release of Sb(V) by MSW-Cs-Sb(V) systems

Polyethylene bottles containing 1.0 g of each MSW-C+ Sb(V) brought at pH 4.5 and doped with 3.0 or 6.0 mM Sb(V) solutions were separately filled with 100 mL of 1.5 mM citric (CA) and oxalic (Ox) acids or EDTA solutions (all brought to pH 4.5). The mixtures were shaken for 24 h at constant temperature (20 ± 1 °C), centrifuged at 8000 rpm for 15 min, and filtered using the 0.2-μm filter paper. Supernatant aliquots were taken and Sb(V) quantified as described above. Each trial was carried out on three independent samples and mean values ± standard deviations are reported.

Results and discussion

The first aim of this research was to evaluate the capacity of two MSW-Cs to act as sequestering agents for Sb(V) in acidic conditions, i.e., at pH 4.5. This pH value was selected since most of the mining soils, where trace metals and metal(loid)s are present as sulfide ores, are generally strongly acidic (pH 3.5–5.0) (Tandy et al. 2009; Manzano et al. 2016) and since preliminary trials, carried out in our laboratory, did not show any Sb-immobilizing capacity of the MSW-Cs at neutral and sub-alkaline pH.

Chemico-physical properties of the MSW-Cs

Table 1 reports the physico-chemical features of the untreated MSW-C1 and MSW-C2, and those of the same composts brought to pH 4.5. Notably, the same composts were recently investigated for their ability to bind Pb, Cu, Cd, and Zn ions from aqueous solutions at acidic pH (Silvetti et al. 2017a, 2017b). When composts were brought to pH 4.5, a decrease of DOC, CEC, total P, humic, fulvic acids and of the content of the metals detected (with the exception of Fe) was recorded (Table 1). This was due to metal solubilization at acidic pH and to protonation processes involving the organic and inorganic phases. Fe, Mg, and, above all, Ca were the most abundant elements, with Ca and Fe being higher in MSW-C2 (Table 1). In both composts at pH 4.5, the stabilized organic matter, i.e., humic + fulvic acids (12.81 and 11.54% d.m. in MSW-C1 and MSW-C2, respectively), represented more than 40% of the total organic carbon.

Sorption isotherms of Sb(V) by MSW-C at pH 4.5

The two composts showed a different ability to bind the metalloid, i.e., 0.177 and 0.243 mmol g^{-1} were the maximum Sb(V) sorption capacities of MSW-C1 and MSW-C2 respectively in the presence of 6.0 mM Sb(V) (Fig. 1a). However, the isotherm shapes were similar for the two MSW-Cs and

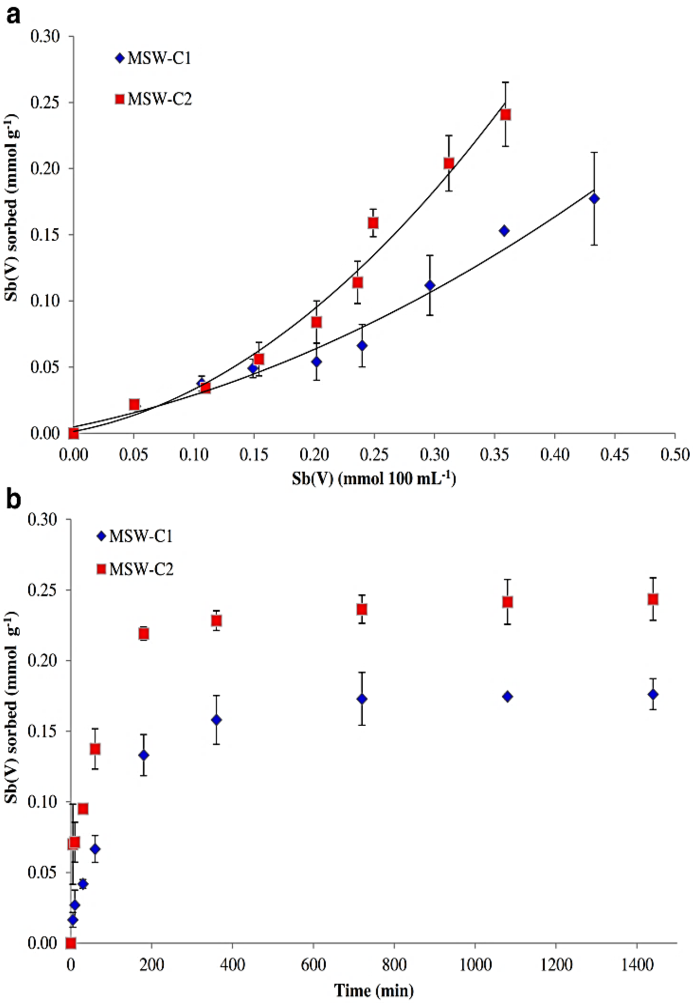


Fig. 1 Sorption isotherms (**a**) and sorption kinetics (**b**) of Sb(V) by MSW-Cs at pH 4.5

typical of adsorption phenomena directly proportional to sorbate concentration. Both isotherms did not reach a defined plateau (Me_{ads}/Me_{added} ratio increasing) as the Sb(V) concentrations increased, indicating that the saturation of the sites involved in the Sb(V) sorption was not completed (Fig. 1a) (Santona et al. 2006). However, higher Sb(V) concentrations, i.e., > 6.0 mM, could not be employed as the metalloid started to precipitate. These results highlight a substantial affinity of both composts towards Sb(V), in line with some studies reporting a strong association between antimony(V) and soil organic matter (Clemente et al. 2008; Ceriotti and Amarasiriwardena 2009; Wilson et al. 2010).

Given the high chemical complexity of composts, it is reasonable to assume that different mechanisms could be involved in the Sb(V) sorption. In particular, the formation of stable complexes between pentavalent antimony and polyfunctional organic ligands, displaying aromatic or aliphatic hydroxyl and carboxyl functional groups, could partly explain the observed results. In this case, Sb(V) sorption by MSW-Cs could have occurred via the establishment of pentagonal or hexagonal rings through Sb-O-C bonds (Tella and Pokrovsky 2012). The non-specific Sb(V) binding to anion-exchange sites on MSW-Cs, e.g., protonated amino functional groups, may have also contributed to Sb(V) sorption (Thanabalasingam and Pickering 1986). Moreover, the Sb(V) interaction with cationic metals within composts [e.g., Fe(III), Mg(II), and Ca(II)], in which the metals act as bridging elements between Sb(V) and other negatively charged functional groups of compost (humic substances in particular), could further explain the observed results (Tighe et al. 2005). In addition, the likely presence in the MSW-Cs of metal-hydroxides physically and/or chemically bound to the organic components (Brown et al. 2012; Zeng et al. 2014) could have contributed to metalloid sorption. Finally, the co-precipitation of Sb(V) with metals could not be excluded. For instance, the Ca(II) involved in outer-sphere complexes (Ca(II) is abundantly present in both MSW-Cs) could be a good candidate for Sb(V) (co)precipitation. In this regard, Johnson et al. (2005) and Okkenhaug et al. (2011) found that the solid $Ca(Sb(OH)_6)_2$ strongly controls the concentration of dissolved Sb(V) in soil. In the light of these considerations, the higher Sb(V) sorption capacity of MSW-C2 with respect to MSW-C1 could be attributed to the higher content of Ca, N, and Fe in the former compost as well as to a greater total acidity of C2-HA. This could have favored the formation of (i) ternary complexes; (ii) stable complexes with amino functional groups and/or with Fe-idroxide surfaces; and (iii) mono and di-esters bonds involving the $Sb(OH)_6^-$ anion and compost components.

In order to better understand the Sb(V) sorption by the MSW-Cs, two types of models, i.e., the Langmuir and Freundlich isotherms, were applied to sorption data.

The Langmuir model assumes homogeneous and monolayer sorption and it is described by Eq. (1):

$$\frac{x}{m} = \frac{KbC}{1+KC} \tag{1}$$

where b is the maximum monolayer coverage capacity of the sorbent (mg g^{-1}) and K (dm^3 mg^{-1}) is a constant related to the energy of sorption which quantitatively reflects the affinity between the sorbent and the sorbate.

The Freundlich model is an empirical equation employed to describe heterogeneous systems. The Freundlich isotherm can be expressed as:

$$\frac{x}{m} = KFC\ 1/\mathrm{n} \tag{2}$$

where K_F and n are the Freundlich constants related to the uptake capacity and intensity, respectively.

Table 2 indicates that the Freundlich model described better the equilibrium sorption data of Sb(V) by MSW-C1 and MSW-C2 ($R^2 = 0.968$ and 0.944, respectively) than the Langmuir equation did ($R^2 = 0.939$ and 0.927, respectively), suggesting that these sorbents had heterogeneous adsorption sites characterized by different Sb(V) adsorption energies.

Table 2 Langmuir and Freundlich and kinetic model parameters related to Sb(V) sorption by MSW-Cs at pH 4.5

	Langmuir parameters				Freundlich parameters			
	b (mmol g^{-1})	K (L mmol^{-1})	R^2		K_F (mmol g^{-1})	n	R^2	
MSW-C1	0.484	2.066	0.939		0.455	1.008	0.968	
MSW-C2	0.975	1.026	0.927		1.206	0.760	0.944	
	Pseudo-first-order equation			Pseudo-second-order equation		Elovich equation		
	$q = q_{max} - \exp.(\ln(q_{max}) - kt)$			$q = q_{max} + q_{max} / (kq_{max}t–1)$		$y = a + k\ln(t)$		
	q_{max} (mmol/g)	k (mmol/g h)	R^2	q_{max} (mmol/g)	k (mmol/g h)	R^2	k (mmol/g h)	R^2
MSW-C1	0.186	0.544	0.612	0.191	0.064	0.993	0.348	0.970
MSW-C2	0.243	0.712	0.704	0.246	0.131	0.998	0.188	0.950

Sorption kinetics of Sb(V) by MSW-Cs at pH 4.5

In order to get additional insight into the process of Sb(V) sorption by MSW-Cs, sorption kinetics data were acquired. The kinetic curves were clearly biphasic, highlighting an initially fast sorption step (first 180 min), followed by a slower sorption rate (Fig. 1b); indeed, after 3 hours, more than 75 and 90% of Sb(V) were sorbed by MSW-C1 and MSW-C2, respectively. Subsequently, the adsorption rate slowed down significantly, and during the 3–24 h contact time, the increase in Sb(V) sorption was only 23 and 7.8% for MSW-C1 and MSW-C2, respectively. This suggested that the initial fast rate could be due to the retention of the metalloid on easily accessible (surface) sorption sites. Moreover, this fast step was likely characterized by strong coulombic attraction and/or coprecipitation reactions with accessible cations within composts (e.g., Fe(III), Ca(II), Al(III)) (Mohan et al. 2007; Ahmad et al. 2014). On the other hand, the slow sorption step may be attributed to specific (chemo-) and irreversible sorption (Ahmad et al. 2014; Wang et al. 2015) or to Sb(V) binding with MSW-C sites not easily accessible.

Different kinetic models were used to evaluate the main mechanism involved in Sb(V) sorption by the MSW-Cs, and the fitted kinetic parameters were shown in Table 2. As indicated by the R^2 values, the pseudo-second-order model was the best at describing the Sb(V) adsorption by MSW-C1 and MSW-C2 ($R^2 = 0.993$ and 0.998 respectively), suggesting that the rate-limiting step is a chemical sorption between the Sb(V) and both MSW-C (Mohan et al. 2007; Martínez-Lladó et al. 2011; Xu et al. 2011).

Role of MSW-C humic acids on Sb(V) sorption at pH 4.5

In order to assess the contribution of humic substances to the sorption of Sb(V) by MSW-Cs, sorption experiments were carried out using the HA extracted from both composts. The Sb(V) sorption by HA followed the same trends reported for the sorption and kinetic isotherms of the composts, being equal to ~ 0.017 and 0.059 mmol g^{-1} for HA-C1 and ~ 0.036 and 0.183 mmol g^{-1} for HA-C2, in the presence of 3.0 and 6.0 mM Sb(V), respectively (Fig. 2). The contribution of HA on the overall sorption of Sb(V) was equal to approximately 3.42 and 4.26% for MSW-C1 and 6.98 and 8.24% for MSW-C2, in the presence of 3.0 and 6.0 mM Sb(V), respectively.

These results clearly show that HA can have a role in Sb(V) sorption by MSW-Cs in an acidic environment, and that the extent of the sorption can be quite different depending on the HA considered. For instance, the HA extracted from MSW-C2 showed an approx. 70% higher Sb(V) sorption capacity compared with those extracted from MSW-C1 (Fig. 2). The Sb(V) binding to HA could be explained by electrostatic interactions involving the $Sb(OH)_6^-$ anion and protonated amino functional groups and or by specific chemisorption (e.g., mono and di-esters bonds between $Sb(OH)_6^-$ and hydroxyl functional groups of HA) as previously reported (Thanabalasingam and Pickering 1986; Tighe et al. 2005; Tella and Pokrovsky 2012). In this context, the higher total acidity (in particular of COOH groups) of MSW-C2 HA could partly explain its higher sorption with respect to HA from MSW-C1 (Table 1). Nevertheless, the results obtained highlight the limited role of HA in Sb(V) sorption and indicate that other organic (e.g., non-humic), or inorganic components (metal-hydroxides and cations such iron and calcium) of MSW-Cs are involved in Sb(V) sorption.

SEM-EDX analysis of MSW-Cs-Sb(V) systems

Representative EDX spectra of untreated MSW-Cs and of composts doped with Sb(V) at pH 4.5 (point analyses) were shown in Fig. 3. The spectra of the Sb(V)-doped MSWCs always showed a distinct peak at 3.620 KeV which supported the Sb(V) sorption by the MSW-Cs. This peak is due to the combination of the Sb-Lα (3.605 KeV) and the Ca-Kα (3.691 V) emissions (Russ 1977; Suzuki and Sugi 1978; Suzuki and Sugi 1989) and indicated a certain association of Ca(II) and Sb(V) in both MSWCs. This supported the view that Sb(V) sorption by MSW-Cs could involve, to some extent, the formation of ternary complexes where Ca(II) acted as a bridging element between the negatively charged functional groups of MSW-Cs and the $Sb(OH)_6^-$ anion and/or the formation of solid $Ca(Sb(OH)_6)_2$ as previously shown (Johnson et al. 2005; Okkenhaug et al. 2011).

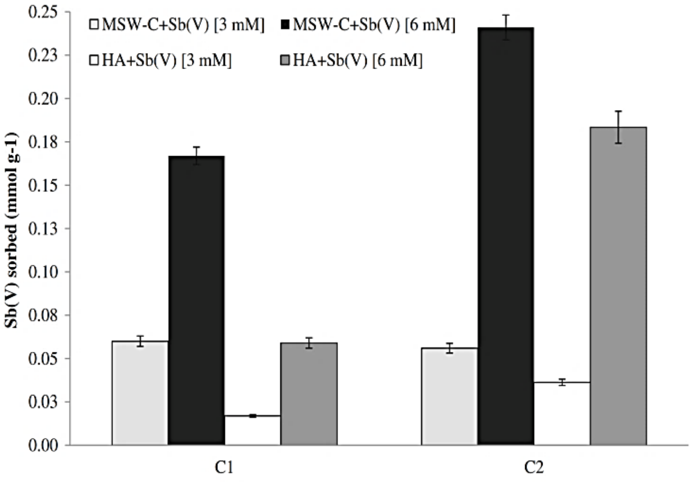
Sequential extraction of Sb(V) sorbed by MSW-Cs at pH 4.5

MSW-Cs doped with Sb(V) were subjected to selected steps of the sequential extraction procedure of Wenzel et al. (2001) to investigate the different Sb(V) pools retained by composts.

The water-soluble Sb(V) fractions (step 1) were negligible in both MSW-Cs when 3 mM Sb(V) was added, while they are accounted for ~ 4.53 and 1.85% of the total Sb(V) sorbed in MSW-C1 and MSW-C2, respectively, when 6 mM Sb(V) was added (Fig. 4). This can be relevant from an environmental point of view since it suggested a substantial stability of the interactions between MSW-Cs and Sb(V) and a limited risk of Sb(V) release from compost to surface and groundwater (Filella 2011).

The amounts of antimonate extracted from both MSW-Cs treated with $(NH_4)_2SO_4$ (step 2) were also very low (< 6.5% of the total Sb(V) sorbed) (Fig. 4). Such negligible effect of sulfate on Sb(V) desorption supported the prevalence of specific (inner sphere) binding between Sb(V) and the MSW-C surfaces, while sulfate can only exchange anions forming weaker

Fig. 2 Sorption of Sb(V) by MSW-Cs and respective HA at pH 4.5



(outer-sphere) complexes with positively charged compost functional groups (e.g., protonated amino-groups) (Nagar et al. 2010). The amount of Sb(V) extracted with $NH_4H_2PO_4$ (step 3), which represented the Sb(V) specifically sorbed, was similar to that extracted in the previous step being equal for both composts to ~7.0% of the total antimonate sorbed, at the two concentrations tested (3.0 or 6.0 mM of Sb(V)). Finally, the Sb(V) fraction released in step 4 (designed

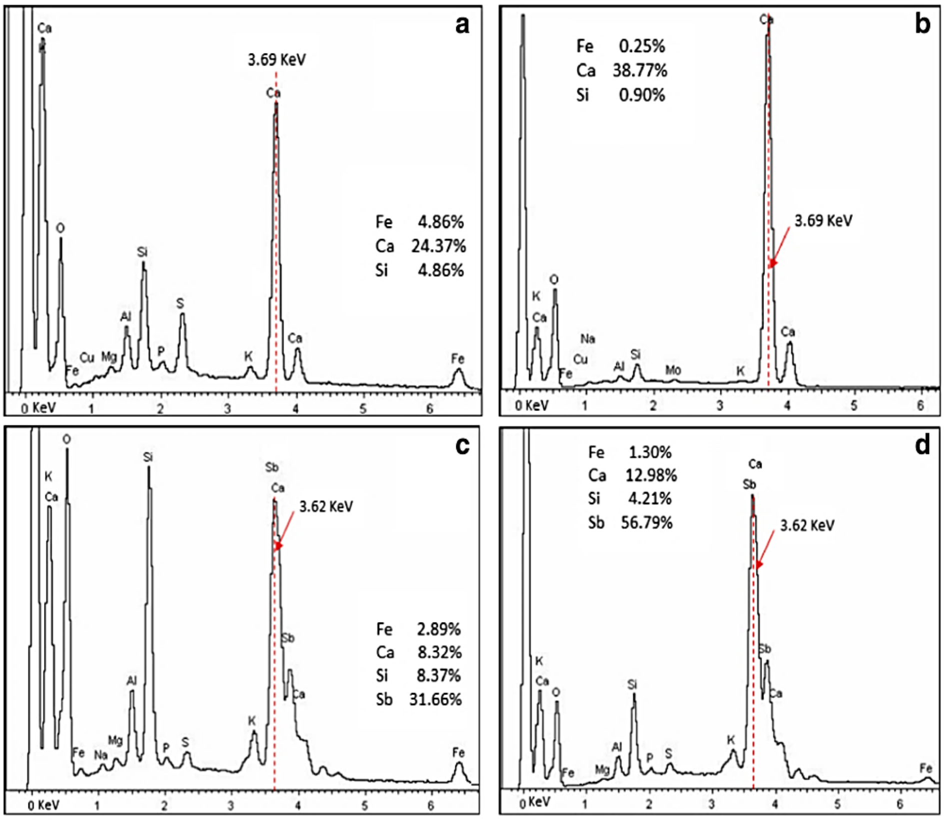


Fig. 3 Representative SEM-EDX spectra of untreated MSW-Cs (**a**, **b**) and of composts doped with Sb(V) (**c**, **d**) at pH 4.5 (point analyses) Note the peak at 3.69 KeV in untreated composts due to Ca-Kα emission (**a**, **b**) and that at 3.62 KeV in composts doped with Sb(V) (**c**, **d**) due to the combination of the Sb-Lα (3.605 KeV) and the Ca-Kα (3.691 V) emissions.

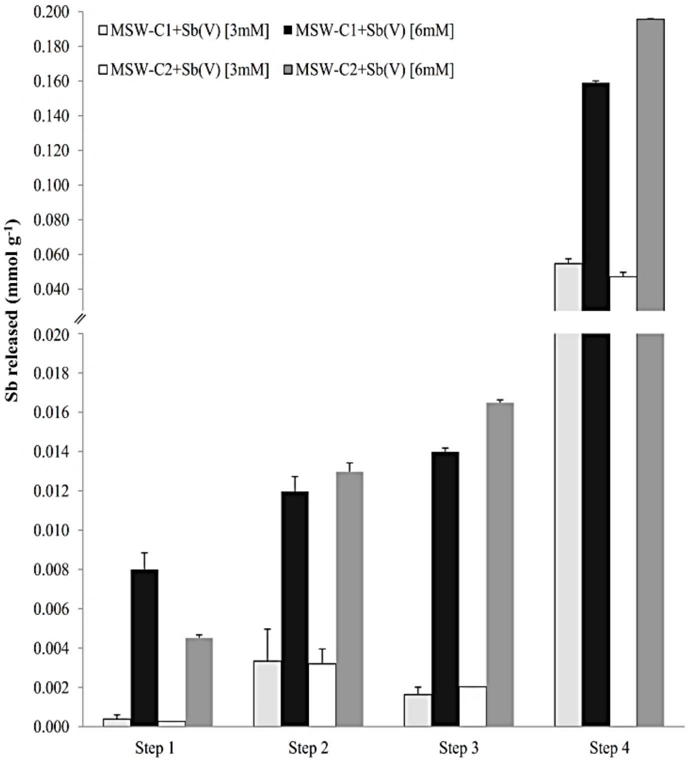


Fig. 4 Sb(V) extracted with H_2O (step 1), $(NH_4)_2SO_4$ (step 2), $NH_4H_2PO_4$ (step 3), and NH_4-oxalate (step 4) from MSW-Cs doped with Sb(V) [0.3 and 0.6 mM] at pH 4.5

to detect Sb(V) associated with amorphous Al- and Fe-oxides) was higher than 80% in both MSW-Cs (Fig. 4). The residual Sb(V) fraction (i.e., Sb(V) not extracted) was lower than 0.2% of total antimonate sorbed (data not shown).

These results indicated that very low amounts of antimony(V) could be extracted from the MSW-Cs-Sb(V) systems by using mild extractants. This also implies strong antimony(V) binding to compost solid phases and/or possible Sb co-precipitation with metal cations [e.g., Ca(II)] as suggested by the SEM-EDX results. In this case, the Sb(V) released in step 4 could be partly attributed to the dissolution of Sb/Ca containing precipitates (e.g., $Ca(Sb(OH)_6)_2$) other than amorphous Al/Fe-oxides.

Effect of organic acids on the release of Sb(V) by MSW-C-Sb(V) systems at pH 4.5

In order to better clarify the potential mobility and bioavailability in soil of the Sb(V) sorbed by the composts, MSW-C samples doped with 3 and 6 mM Sb(V) were treated with citric (CA) and oxalic (OX) acids at pH 4.5 and the metalloid released was quantified after 24 h contact (Fig. 5). Citric and oxalic acids are abundantly present in the rhizosphere as products of the biological activity of plants and microorganisms (Stevenson 1994). As such, the Sb(V) release after CA and/or OX treatment has certain environmental implications. The concentration of the two organic anions (i.e., 1.5 mM) was chosen considering that their concentration in the soil solution can vary from $1{\cdot}10^{-5}$ to $1{\cdot}10^{-3}$ mol L^{-1} (Stevenson 1994) and that in selected microenvironments, such as rhizosphere and soil sites where decomposing plant residues is enhanced; it can be higher than 10^{-3} mol L^{-1} (Stevenson 1994). EDTA, which is probably the most efficient metal chelating agent, was selected as a control chemical in order to complex metal cations such as Fe, Al, and Ca, to which Sb(V) could be bounded. In principle, the formation of such EDTA-Me complexes should favor the release of antimonate bounded to the metals.

At pH 4.5, the organic acids added, which are common and abundant compounds in soil and rhizosphere (Stevenson 1994), were in the form of organic anions ($pK_1 = 3.09$ and 1.23 for CA and OX, respectively). Citrate and oxalate could be involved in competition phenomena with Sb(V) for the same adsorbing sites on the MSW-Cs, thus causing the release of Sb(V) in solution by means of common anion exchange phenomena. At the same time, these low molecular weight organic acids (LMWOAc) could cause the partial dissolution of compost phases containing Fe(III) oxy-hydroxides or Ca(II) cations and the concurrent release of the Sb(V) bonded to iron nuclei or calcium (Onireti and Lin 2016).

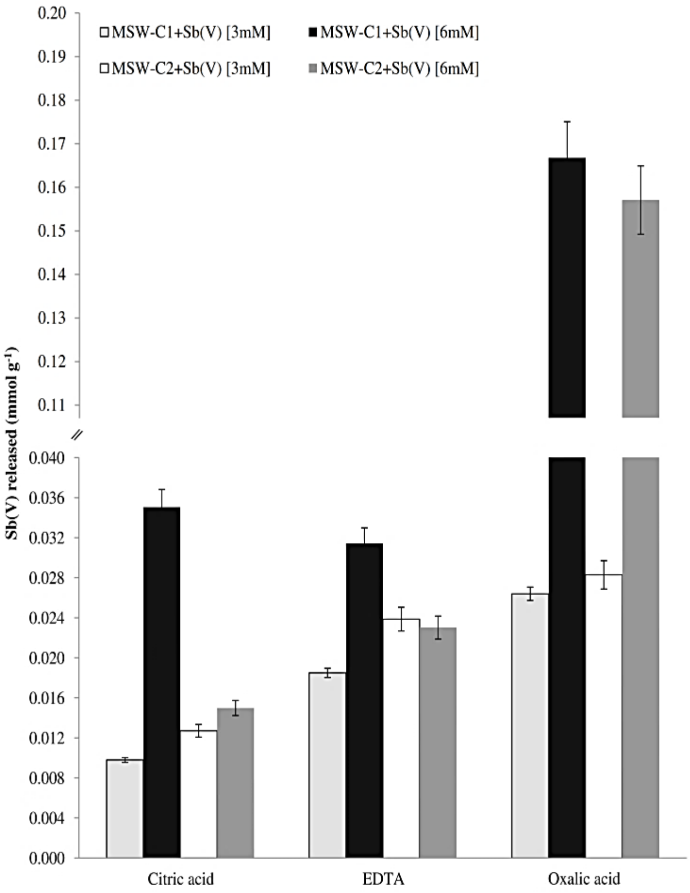


Fig. 5 Sb(V) released from MSW-Cs doped with Sb(V) [0.3 and 0.6 mM] at pH 4.5 after citric and oxalic acids and EDTA treatment

The data obtained highlighted a different capability of the organic molecules to mobilize the Sb(V) sorbed by the MSW-Cs, regardless of the compost type (Fig. 5). Oxalate released 43 and 98% of total Sb(V) sorbed by MSW-C1 and 18 and 65% of total Sb(V) sorbed by MSW-C2, when 3 and 6 mM of Sb(V) were added, respectively. This indicated that MSW-C2 not only was able to adsorb more Sb(V), but it was also capable of a stronger metalloid retention. EDTA and CA showed a lower efficacy, being able to extract < 20% of total antimony(V) sorbed in both MSW-Cs when 6 mM of Sb(V) was added. In particular, EDTA gave very low yields of Sb(V), and this is in agreement with several studies which reported low percentages (< 4%) of total soil antimony extracted with EDTA (Conesa et al. 2010; Wilson et al. 2010; Filella 2011). However, it should be mentioned that the complexing capacities of EDTA increase significantly from acidic to alkaline pH and this could explain the low amounts of antimony(V) released at pH 4.5.

The higher release of Sb(V) following OX treatment may be attributed to the stronger acidity of oxalic acid, as compared to citric acid, as well as to the strong capacity of OX to solubilize inorganic phases containing iron and calcium through non-reductive and reductive pathways (Onireti and Lin 2016). This in turn could have caused the indirect release of the Sb(V) bound to such inorganic phases.

The results obtained suggest that the different LMWOAs tested mobilized different Sb(V) pools bound to the MSW-Cs. In particular, CA likely mobilized the Sb(V) involved in the formation of inner or outher-sphere complexes with the MSW-C organic molecules; on the other hand, OX likely mobilized the former Sb(V) pools as well as those more strongly retained by the MSW-Cs, i.e., the Sb(V) (co-)precipitated or bound with calcium as well as that bound to iron oxy-hydroxides.

Conclusions

The results from this study indicate that the MSW-Cs investigated are able to act as Sb(V) accumulators in acidic conditions, such as those characterizing many polluted sites. Different processes, most likely interrelated, and different organic and inorganic components of composts affected the sorption of Sb(V). Irrespective of the compost type, HA had a role in Sb(V) sorption even if it was quantitatively limited.

Importantly, sequential extractions indicated that the majority of the Sb(V) sorbed (approx. 80%) was tightly bound to MSW-Cs through inner-sphere surface complexes and/or immobilized through co-precipitation reactions. This implies that such Sb(V) is not expected to be readily released under natural conditions.

SEM-EDX spectra revealed a certain association of calcium with antimony suggesting the occurrence of $Ca(Sb(OH)_6)_2$ precipitates and/or the involvement of Ca(II) in the formation of stable ternary complexes with Sb(V) and compost functional groups. At the same time, this could suggest a minor role of amorphous Fe/Al oxides in Sb(V) binding in the presence of abundant Ca.

Treatment with citric and oxalic acids (commonly occurring plant and/or microbial metabolites) mobilized very different amounts of sorbed Sb(V) indicating the presence of different interaction types between Sb(V) and MSW-Cs, and supporting a possible role of calcium in the sorption processes. Treatment with OX, which has a marked and well-known affinity for calcium, released approx. 65 and 98% of the Sb(V) bound to MSW-C2 and MSW-C1, respectively.

The results presented indicate that the selected MSW-Cs should be tested as alternative and sustainable amendment for the remediation of acidic Sb(V)-contaminated soils.

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Chapter 3

Water Treatment Residuals as a Resource for the Recovery of Soil and Water Polluted with Sb(V): Sorption and Desorption Trials at Different pH Values

Castaldi[1], P., Diquattro[2], S., Lauro[2], G. P., Marceddu[3], S., Garau[2], G., 2018. Water Treatment Residuals as a Resource for the Recovery of Soil and Water Polluted with Sb (V): Sorption and Desorption Trials at Different pH Values. Water, Air, & Soil Pollution, 229(6), 174. DOI: https://doi.org/10.1007/s11270-018-3830-1.

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Water Treatment Residuals as a Resource for the Recovery of Soil and Water Polluted with Sb(V): Sorption and Desorption Trials at Different pH Values

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Abstract In this study, the ability of two different water treatment residuals (Fe- and Al-WTRs) to accumulate antimony(V) from an aqueous solution was investigated at different pH values (pH 4.5 and 6.5). Both WTRs showed a maximum Sb(V) sorption capacity of approx. 0.22 mmol g^{-1} at pH 4.5 which declined at pH 6.5, particularly for Fe-WTR (i.e., 0.059 and 0.163 mmol g^{-1} of Sb(V) sorbed by Fe- and Al-WTRs respectively). The greater capacity of WTRs to accumulate antimonate at pH 4.5 seemed to be linked to their chemical properties, such as the pH_{PZC} and the specific surface area. At both pH values, the Sb(V) sorption by Al- and Fe-WTRs followed a pseudo-second-order kinetic model, while the sorption isotherms data fitted the Freundlich model better than the Langmuir one, suggesting the presence of heterogeneous Sb(V) adsorption sites. The sequential extraction of WTR-Sb(V) systems showed that a significant amount of Sb(V) was retained by WTRs through chemical interactions, i.e., through the formation of inner sphere surface complexes [e.g., Fe/Al–O–Sb(V)]). This was particularly relevant at higher pH values (pH 6.5) where more than 60 and 50% of the Sb(V) sorbed by Fe- and Al-WTRs respectively was retained by specific chemical bonding. The residual Sb(V) was higher for the Al-WTR at both pH values, and the highest amount of residual Sb(V) was recorded at pH 4.5 [> 65% of the total Sb(V) sorbed]. SEM-EDX analysis of the WTR-Sb(V) systems showed that antimony was mainly associated with Fe and Al, thus supporting the Sb(V) affinity for Al/Fe oxy-hydroxides. Treatment of WTR-Sb(V) systems with citric and malic acids, at concentrations relevant in the rhizosphere, indicated that Sb(V) could be released by both acids, with 4.5 mM citric acid favoring the highest Sb(V) release in both WTRs. The results from this study suggest that WTRs could be used as alternative amendments for the in situ immobilization of Sb(V) in acidic or circumneutral polluted soils.

Novelty Statement In this study, we showed that two different water treatment residuals (Fe- and Al-WTRs) are able to act as effective Sb(V) accumulators at different pH values (pH 4.5 and 6.5), i.e., those characterizing many polluted sites.
We believe this is quite significant since, to our knowledge, this is the first report addressing the sorption and desorption capacity of water treatment residuals with respect to Sb(V). In this sense, the submitted paper is definitely novel.

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1 Introduction

Antimony (Sb) and its compounds are pollutants of emerging concern and, because of their reported toxicological effects, the European Union (Council of the European Communities, CEC 1998) and the Environmental Protection Agency of the USA (USEPA, 2009) have established maximum Sb contaminant levels (MCLs) in drinking water of 5 and 6 μg L^{-1} respectively. Sb occurs naturally as trace element in soil, its main source being the weathering of soil parent materials (Wilson et al. 2010). In general, the natural Sb abundance is low, 0.2–2 μg g^{-1} in sediment rocks, < 1 μg L^{-1} in natural waters, and few μg g^{-1} in soils and sediments (Filella et al. 2002a). However, elevated Sb concentrations can be found in the proximity of mining and smelting sites, in areas where sewage sludge and fertilizers are widely used, at shooting ranges, and along roadsides (Ilgen et al. 2012; Vithanage et al. 2013; Cai et al. 2015; van Genuchten and Peña 2016; Garau et al. 2017).

In most natural systems, antimony mainly exists in inorganic form, primarily in the trivalent Sb(III) or pentavalent Sb(V) state (Wilson et al. 2010; Doherty et al. 2017). Antimonate [$Sb(OH)_6^-$], which results from the hydrolysis of antimonic acid [$HSb(OH)_6$), $pK_1 = 2.85$], is the predominant and thermodynamically stable Sb species in oxic and suboxic environments, while under reducing conditions, the most abundant Sb(III) species is the uncharged antimonous acid $Sb(OH)_3$ ($pK_a = 11.9$) (Wilson et al. 2010; Ilgen et al. 2012; Cai et al. 2015). Although Sb(III) compounds can be more toxic than Sb(V) ones, the mobility and solubility of Sb(V) is greater than Sb(III) (Filella et al. 2002b; Steely et al. 2007; Rakshit et al. 2011).

Sorption on soil surfaces is one of the most important Sb retention mechanisms when the metalloid enters the soil system (Leuz et al. 2006; Wilson et al. 2010; Rakshit et al. 2011). Many factors can affect the Sb sorption, including its speciation and soil characteristics such as pH and redox conditions, content and type of organic matter, and the presence of Fe-, Al-, and Mn-oxy-hydroxides (Wilson et al. 2010; Vithanage et al. 2013; van Genuchten and Peña 2016; Doherty et al. 2017). In particular, the anionic species $Sb(OH)_6^-$, which is prevalent (if not exclusive) in aerated soils, displays a high affinity to amorphous and crystalline Fe-, Al-, and Mn- oxy-hydroxides, with which it can establish stable interactions especially at low pH (Xu et al. 2001; Tighe et al. 2005; Leuz et al. 2006; Wilson et al. 2010; Rakshit et al. 2011; Ilgen et al. 2012; Cai et al. 2015). Electrostatic interactions and specific chemical bonding (i.e., inner sphere complexes where the metal cations coordinate Sb(V) anions) appear to be the main mechanisms of Sb(V) adsorption onto Fe-, Al-, and Mn-(hydr)oxides (Wang et al. 2015; Shangguan et al. 2016). By contrast, the interactions between the soil organic matter (SOM) and Sb(V) are little known. Some researchers observed a weak affinity of Sb(V) to soil organic matter, which was explained with the anionic nature of Sb(V) in soil (i.e., $Sb(OH)_6^-$), which limits the interaction with the negatively charged sites of SOM (Filella and Williams 2012; Dorjee et al. 2014). Nevertheless, other researchers reported a significant Sb(V) complexation/sorption by SOM and/or purified humic substances (Tighe et al. 2005; Steely et al. 2007; Tella and Pokrovsky 2008; Diquattro et al. 2018). For example, Tella and Pokrovski (2008) provided a first direct evidence that poly-functional organic ligands like carboxylic acids and polyols were able to form complexes with Sb(V) in aqueous solution.

Although substantial progress has been recently made on the comprehension of factors governing the Sb mobility in soil, effective and reliable approaches for the remediation of Sb-polluted sites are currently missing. To date, only a few amendments, mostly Fe- and Al- minerals, have been tested with variable success as Sb-immobilizing agents (Alvarez-Ayuso et al. 2013; Okkenhaug et al. 2013, 2016; Garau et al. 2017).

In a search for alternative, low-cost and environmental friendly sorbents for antimony, we identified the drinking water treatment residuals (WTRs), the waste material resulting from the treatment of surface, or groundwater with Al and Fe salts, as a potential good candidate (Nagar et al. 2010). In the conventional coagulation-filtration treatment process, suspended solids, natural organic matter, dyes, contaminants, etc., are removed from the raw water supply by the addition of aluminum, and/or iron and/or calcium salts as coagulants, resulting in the production of WTRs (Babatunde et al. 2009). Such residuals generally contain activated carbon, natural organic matter (especially humic substances), $CaCO_3$, and, above all, Fe and/or Al (oxy)-hydroxides (Babatunde et al. 2009). The simultaneous presence of these constituents makes WTRs as potential good candidates for reducing the Sb(V) mobility in polluted soil and waters. Many studies showed that WTRs are able to accumulate As(V) and P(V) anions and that such ability is closely related to the pH of the system (Babatunde et al. 2009; Nagar et al. 2010; Castaldi et al. 2014). Moreover, it was recently shown that WTRs can limit the mobility of soil antimony at polluted sites even if their precise role could not be ascertained since they were added to soil together with compost (Garau et al. 2017). To the best of our knowledge, the sorption capabilities of WTRs with respect to Sb(V) were never addressed before.

The aim of this paper was therefore (i) to determine the ability of selected Fe- and Al-WTRs to accumulate Sb(V) at pH 4.5 and 6.5; (ii) to quantify the different Sb(V) pools bound to the WTRs through specific extraction procedures; and iii) to evaluate the capacity of low molecular weight organic acids (LMWOAs), commonly present in soil and rhizosphere (i.e., citric and malic acids) to mobilize the metalloid sorbed by the WTRs.

2 Materials and Methods

2.1 WTRs Origin and Physico-chemical Features

Two different water treatment residues (WTRs) were studied: one, identified as Al-WTR, was obtained from the drinking water treatment plant of Abbanoa industry located in Truncu Reale (Sassari, Italy), whereas the other, identified as Fe-WTR, was derived from the Bidighinzu plant (Sassari, Italy) of the same industry. Both WTRs were directly sampled from stockpiles that were formed within 1 year of production. WTR samples were air-dried overnight at 105 °C and finely ground. The pH and electric conductivity (EC) were determined in 1:2.5 ratio of WTR/distilled water. The specific surface area was determined by applying the BET model to the N_2 sorption results obtained from a Sorptomatic instrument (Carlo Erba). The pH_{PZC} of WTRs was measured by laser Doppler velocimetry coupled with photon correlation spectrometry using a Coulter Delsa 440 spectrometer equipped with a 5-mW He–Ne laser (632.8 nm). Total organic matter in WTRs was determined using the method of Walkley and Black previously described (Castaldi et al. 2014). The content of dissolved organic carbon (DOC) in Fe- and Al-WTRs was determined according to Brandstetter et al. (1996). To determine the content of humic (HA) and fulvic (FA) acids, the method reported by Ciavatta et al. (1990) was used. The total concentration of selected metals in Fe- and Al-WTR was determined on dried (105 °C) WTRs after digestion with a mixture of nitric acid and hydrochloric acid (HNO_3/HCl, 1:3 ratio) in a microwave Milestone MLS 1200. The metal concentrations were determined using a Perkin Elmer Analyst 600 flame atomic absorption spectrometer (FAAS) equipped with an HGA graphite furnace.

For sorption trials, Fe- and Al-WTR samples were brought at pH 4.5 and 6.5 with either 0.01 or 0.1 M HCl solutions. The chemical characteristics of the untreated and treated WTRs are reported in Table 1.

Analytical determinations were carried out on triplicate independent samples and mean values ± standard deviations are reported.

2.2 Sorption Isotherms of Sb(V) on WTRs at pH 4.5 and 6.5

All chemicals were of analytical grade and used without further purification. Fe- and Al-WTR samples brought to pH 4.5 and 6.5 were artificially and separately enriched with solutions containing increasing concentrations of Sb(V) (brought to pH 4.5 or 6.5) derived from $KSb(OH)_6 \cdot 7H_2O$ to obtain sorption isotherms. Two batch experiments were prepared for Fe- and Al-WTRs at two pH values (4.5 and 6.5) and at constant temperature (20 ± 1 °C). Polyethylene bottles containing 1.0 g of WTRs were filled with 25 mL of antimonate solutions. Nine different concentrations of $KSb(OH)_6 \cdot 7H_2O$ varying from 0.0 to 6.0 mM were used to determine the sorption isotherms. A background electrolyte of 1.0 M NaCl was used as diluents for all batch experiments. If needed, the pH of the mixtures WTR/polluting solution was adjusted to 4.5 or 6.5 with 0.01 or 0.1 M HCl solutions. The mixtures (1:25 *w/v* ratio of WTR-Sb(V) solution) were shaken for 24 h at constant temperature (20 ± 1 °C). Afterwards, the samples were centrifuged at 8000 rpm for 15 min and filtered using a 0.2-μm filter paper. The antimony(V) in the supernatant was measured by ion chromatography using an IonPac AS9-HC Analytical Column (Thermo Fischer Scientific) equipped with an IonPac AG9-HC Guard Column, 4 mm (Thermo Fischer Scientific). Sodium bicarbonate (10 mM) was employed as the eluent at a flow rate of 1.0 mL min^{-1}. The sample loop was 10 μL (Diquattro et al. 2018). Each point of the sorption isotherms derived from Sb(V) quantification in three independent samples and mean values ± standard deviations are reported.

Table 1 Properties of the Fe- and Al-WTR samples used in the study

Chemical parameters	Fe-WTR			Al-WTR		
	Untreated	pH 6.5	pH 4.5	Untreated	pH 6.5	pH 4.5
pH	7.15 ± 0.06	–	–	6.81 ± 0.01	–	–
pH$_{PZC}$	5.70 ± 0.11	5.68 ± 0.07	5.66 ± 0.06	6.38 ± 0.13	6.38 ± 0.10	6.35 ± 0.05
Electrical conductivity (mS cm^{-1})	1.24 ± 0.08	–	–	1.33 ± 0.06	–	–
SBET (m^2 g^{-1})	38	38	40	44	44	47
CEC (cmol$_{(+)}$kg^{-1} d.m.)	73.12 ± 1.22	72.84 ± 1.16	65.38 ± 1.97	70.11 ± 1.16	68.25 ± 1.94	59.32 ± 1.83
Organic matter (% d.m.)	14.51 ± 0.13	13.82 ± 0.12	12.94 ± 0.15	24.13 ± 0.16	23.54 ± 0.17	22.76 ± 0.16
Dissolved organic carbon (mg g^{-1} d.m.)	0.21 ± 0.00	0.26 ± 0.00	0.38 ± 0.03	0.22 ± 0.03	0.29 ± 0.03	0.39 ± 0.00
Humic and fulvic acids (HA + FA) (% d.m.)	2.65 ± 0.14	2.16 ± 0.12	2.02 ± 0.08	2.27 ± 0.20	2.24 ± 0.08	2.18 ± 0.11
Total Fe (mg kg^{-1} d.m.)	20,948 ± 952	20,883 ± 812	20,828 ± 841	6373 ± 341	6324 ± 312	6275 ± 286
Total Al (mg kg^{-1} d.m.)	8158 ± 129	8068 ± 102	6863 ± 111	98,320 ± 837	98,243 ± 756	98,015 ± 736
Total Mn (mg kg^{-1} d.m.)	6786 ± 279	6124 ± 233	5831 ± 174	8700 ± 451	8632 ± 411	8593 ± 393
Total Ca (mg kg^{-1} d.m.)	689.42 ± 12.3	433.42 ± 18.4	386.42 ± 12.44	11,346 ± 253	10,269 ± 221	845.36 ± 84.2
Total Mg (mg kk^{-1} d.m.)	69.83 ± 7.34	59.21 ± 9.53	42.35 ± 8.72	2064 ± 14.3	2036 ± 12.3	1873 ± 9.76
Total K (mg kg^{-1} d.m.)	100.5 ± 3.43	87.46 ± 2.91	67.54 ± 2.15	837.2 ± 8.34	782.7 ± 6.57	659.6 ± 5.71
Total Na (mg kg^{-1} d.m.)	30.56 ± 4.27	18.52 ± 4.03	10.43 ± 1.15	87.16 ± 6.43	77.23 ± 4.43	38.42 ± 2.36

2.3 Sorption Kinetics of Sb(V) on WTRs at pH 4.5 and 6.5

For the kinetic studies, polyethylene bottles containing 1.0 g of each WTR brought at pH 4.5 or 6.5 were filled with 25 mL of 6.0 mM $KSb(OH)_6 \cdot 7H_2O$ while 1.0 M NaCl was used as background electrolyte. The final mixtures were shaken at constant temperature (20 ± 1 °C) for different times, i.e., 5, 10, 30, 60, 180, 360, 720, 1080, and 1440 min. The samples were then centrifuged at 8000 rpm for 15 min and filtered as previously mentioned. Supernatant aliquots were taken and the Sb(V) in solution was quantified as previously described. Each point of the sorption kinetics derived from Sb(V) quantification in three independent samples and mean values ± standard deviations are reported.

2.4 Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray Spectroscopy (EDX) Analysis

SEM-EDX analyses of untreated and Sb(V)-treated [i.e., saturated with 6.0 mM $KSb(OH)_6 \cdot 7H_2O$] Fe- and Al-WTRs systems at pH 4.5 and 6.5 were carried out using an environmental scanning electron microscope ESEM-Zeiss EVO LS10 (Oberkochen-Germany). WTR samples were observed with the backscattered electron detector (BSD) and chemically analyzed with a micro-analysis X-ray energy dispersive device (EDS) INCAx-ACT (Oxford Instruments). The whole processes occurred at low vacuum mode and a pressure of 10 Pa.

2.5 Sequential Extraction of Sb(V) from WTR-Sb(V) Systems at pH 4.5 and 6.5

The different antimonate pools bound to WTRs were determined using the sequential extraction procedure of Wenzel et al. (2001) in order to better understand the interaction mechanisms which regulate the Sb(V) sorption by the WTRs. WTR samples (1.0 g) brought at pH 4.5 and 6.5, and doped with 6.0 mM Sb(V) solutions (i.e., the samples deriving from the last point of the sorption isotherms), were added with 25 mL of 0.05 M $(NH_4)_2SO_4$ and shaken for 4 h at 20 °C to extract the non-specifically sorbed antimonate (step 1). The same WTR samples were treated with 25 mL of 0.05 M $NH_4H_2PO_4$ and then shaken for 16 h at 20 °C to extract the specifically sorbed antimonate (step 2). The Sb(V) associated to amorphous Al- and Fe-(hydr)oxides was determined after extraction with 0.2 M NH_4-oxalate in the dark, pH 3.25, for 4 h at 20 °C (step 3).

After each step of the extraction process, the WTR samples were centrifuged at 8000 rpm for 15 min and filtered using a 0.2-μm filter paper to separate the liquid and solid phases. The Sb(V) concentration was determined in the liquid phase by ionic chromatography as previously described.

Each step of the sequential extraction procedure was carried out on three independent samples and mean values ± standard deviations are reported.

2.6 Influence of Environmentally Relevant Organic Acids on the Release of Sb(V) by WTR-Sb(V) Systems at pH 4.5 and 6.5

Polyethylene bottles containing 1.0 g of each WTR-Sb(V) system brought at pH 4.5 and 6.5 and (i.e., WTR samples deriving from the last point of the sorption isotherms) were separately filled with 25 mL of 1.5 and 4.5 mM citric (CA) and malic (MA) acid solutions previously brought at pH 4.5 and 6.5 with 0.01 or 0.1 M NaOH solutions. Both organic acids are commonly occurring in soil/rhizosphere in the concentration range employed (Stevenson 1994). The mixtures were shaken for 24 h at constant temperature (20 ± 1 °C), then centrifuged at 8000 rpm for 15 min and filtered using a 0.2-μm filter paper. Supernatant aliquots were taken and Sb(V) quantified as described above. Each trial was carried out on three independent samples and mean values ± standard deviations are reported.

3 Results and Discussion

The main goal of this research was to determine the ability of Fe- and Al-WTRs to act as sequestering agents of Sb(V) in view of their possible use as alternative, low-cost, and environmental friendly amendments for Sb-polluted soils and waters. Water treatment residuals have been widely investigated during these years showing promising adsorption capabilities towards different potentially toxic cations [e.g., Cd(II), Zn(II), Cu(II), Pb(II), Hg(II)] and anions (e.g., phosphate and arsenate) in solution (Castaldi et al. 2014; Castaldi et al. 2015; Silvetti et al. 2015; Deliz Quiñones et al. 2016). They also were promising as metal(loid) sorbents when added to contaminated soils (e.g., Nagar et al. 2015; Manzano et al. 2016). However, their sorption capabilities towards Sb(V) were never tested before despite a recent study suggested a role of WTR in the retention of the metalloid in contaminated soils (Garau et al. 2017). The assessment of such capabilities are of outmost importance since they could allow the selection of WTRs as versatile, low-cost, and sustainable amendments for the immobilization of heavy metals and/or metalloids such as Sb in contaminated water/soil environments.

In this context, as a first step, we studied the sorption isotherms and kinetics of antimonate ions in aqueous solution by Fe- and Al-WTRs at pH 4.5 and 6.5. Subsequently, we attempted to elucidate the mechanisms which regulate the sorption of the metalloid by WTRs through a sequential extraction procedure and SEM-EDX investigation. Finally, we evaluated the contribution of environmentally relevant organic acids (i.e., citric and malic acids, commonly present in the soil/rizosphere) on the release of Sb(V) sorbed by WTRs. Understanding the main mechanisms controlling the antimony(V) retention by WTRs, and its possible release in the environment, is an essential prerequisite for further evaluations of these sorbents as amendments for polluted soils and waters.

3.1 pH Influence on the Physico-chemical Properties of Fe- and Al-WTRs

The chemical and physical properties of the untreated Fe- and Al-WTRs, together with those relative to the same sorbents brought to pH 4.5 and 6.5, are reported in Table 1.

Fe- and Al-WTRs were both neutral (pH 7.15 and 6.81 respectively), they had a similar pH_{PZC} (5.70 and 6.38) and electrical conductivity (1.24 and 1.33 mS cm^{-1} respectively) (Table 1). The untreated Al-WTR showed a higher content of organic matter (+ 66%) and specific surface area (+ 16%) compared to Fe-WTR. The stabilized organic matter, i.e., humic + fulvic acids (2.65 and 2.27% in Fe- and Al-WTRs, respectively) represented 18.2 and 9.4% of the total organic carbon, while the dissolved organic carbon (DOC) was ~ 0.22 mg g^{-1} in both WTRs. Finally, 20,948 and 6373 mg kg^{-1} of Fe, 8158 and 98,320 mg kg^{-1} of Al, 6786 and 8700 mg kg^{-1} of Mn, and 689 and 11,346 mg kg^{-1} of Ca were found in Fe- and Al-WTRs, respectively. In addition, X-ray diffraction analysis revealed no crystalline Al, Fe, Mn, or Ca solid phases, indicating that both materials were amorphous and particles poorly ordered.

Both WTRs brought at pH 6.5 and 4.5 showed a progressive CEC and organic matter decrease and an increase of the DOC (Table 1). Moreover, also the Fe, Al, and Mn content (especially in the case of Fe-WTR) and that of the alkaline and alkaline earth metals decreased along with the pH (Table 1). This was mainly due to protonation processes involving the organic matter adsorbed on mineral surfaces as well as the surface OH groups coordinated by the Fe and/or Al nuclei. This lead to a reduced affinity of the organic matter towards the surface sites of the Fe and Al oxy-hydroxides as well as the solubilization of low molecular weight organic molecules (previously involved in organo-chemical complexes). Moreover, the increased H^+ concentration also led to an enhanced solubilization of metals, e.g., Fe, Al, Mn, and Ca, which get lost when the solid phase was separated from the liquid one.

3.2 Sorption Ability of WTRs Towards Sb(V)

Sorption isotherms, carried out by dispersing 1.0 g of each sorbent in solutions containing Sb(V) at concentrations ranging from 0 to 6.0 mM, showed that the sorption of antimonate by Fe- and Al-WTRs decreased as the pH increased from 4.5 to 6.5 (Fig. 1). In particular, the maximum amount of Sb(V) sorbed by Fe-WTR at pH 4.5 [0.22 mmol g^{-1}] was about 3.7 times higher than that recorded at pH 6.5, while that sorbed by Al-WTR [0.23 mmol g^{-1} at pH 4.5] was about 1.41 times higher than that recorded at pH 6.5. Further, a similar affinity of Sb(V) towards Fe- and Al-WTRs was noticed at pH 4.5 (Fig. 1), while at pH 6.5, the amount of Sb(V) sorbed by the Al-WTR was 2.76 times higher than that sorbed by Fe-WTR (Fig. 1).

The decrease of Sb(V) sorption capacity by both WTRs at the higher pH value is consistent with previous studies on iron and aluminum oxides (Tighe et al. 2005; Wilson et al. 2010; Rakshit et al. 2011; Filella and Williams 2012; Ritchie et al. 2013; Vithanage et al. 2013; Cai et al. 2015; Shangguan et al. 2016; van Genuchten and Peña 2016). For instance, Tighe and Lockwood (2007) reported that Sb(V) sorption by a non-crystalline Fe hydroxide was maximum at pH 4.0 while, decreasing at higher pH values. Sorption of Sb(V) by hematite and gibbsite was found to be pH dependent, with maximum sorption values again in the 2.0–4.0 pH range (Wilson et al. 2010; Rakshit et al. 2011). Antimony(V) behaves as a weak monoprotic acid (pK_a 2.85) in very acidic environments (i.e., pH 1.0–3.5) while in the 4.5–6.5 pH range (i.e., that considered in this study), it is present as $Sb(OH)_6^-$ oxyanion (e.g., Cai et al. 2015). The point of zero charge (pH_{PZC}) of Fe- and Al-WTRs brought to pH 6.5 and 4.5 was equal to 5.68 and 6.38, and 5.66 and 6.35 respectively (Table 1). At pH values below the pH_{PZC}, the WTR surfaces possessed a net positive charge which facilitated the sorption of the antimonate anion through electrostatic attraction. On the contrary, at pH values above the pH_{PZC} the net surface charge of WTRs became negative increasing the electrostatic repulsion between antimonate anions and WTRs. This can explain the higher Sb(V) sorption capabilities of both WTRs at pH 4.5 rather than 6.5 and also the lower Sb(V) sorption of Fe-WTR compared to Al-WTR at pH 6.5. These results clearly indicate that the selected WTRs are able to retain the Sb(V) anion and highlight the importance of the surface charge of WTRs on Sb(V) sorption. Moreover, the results also suggest that the chemical differences among WTRs likely played a minor role in Sb(V) sorption, as indicated by the very similar amounts of Sb(V) sorbed by Fe- and Al-WTR at pH 4.5.

The maximum sorption capacity of the WTRs tested at pH 4.5 (~0.23 mmol g^{-1}) was lower compared to some pure Fe-, Al-, or Mn-oxides (Leuz et al. 2006; Xu et al. 2011; Ilgen and Trainor 2012; Cai et al. 2015; van Genuchten and Peña 2016), but quite similar to that observed by Rakshit et al. (2011) on gibbsite and higher than that detected by Bullough et al. (2010) working with activated alumina (i.e., 0.072 mmol g^{-1}). The low sorption capacity of WTRs compared to pure Fe-, Al-, or Mn(oxy)-hydroxides could be due to the low surface area of WTRs (between 38 and 47 vs. 70 and 261 m^2 g^{-1} for pure oxy-hydroxides) (Xu et al. 2011; Ilgen and Trainor 2012); nevertheless, the amount of Sb(V) sorbed was relevant with respect to the environmentally available Sb(V) concentrations in soil pore water (Filella et al. 2002a, b; Rakshit et al. 2011).

Given the high chemical complexity of WTRs, it is reasonable to assume that different mechanisms could be involved in the Sb(V) sorption. In particular, as shown in several studies, pentavalent antimony might be retained by the surface Fe–O or Al–O groups of WTRs through specific and non-specific adsorption mechanisms, i.e., through the formation of inner and outer sphere complexes respectively (Leuz et al. 2006; Scheinost et al. 2006; Rakshit et al. 2011; Cai et al. 2015). Besides, since both WTRs contain organic matter (and approx. 2% of humic and fulvic acids), the formation of stable complexes between Sb(V) and

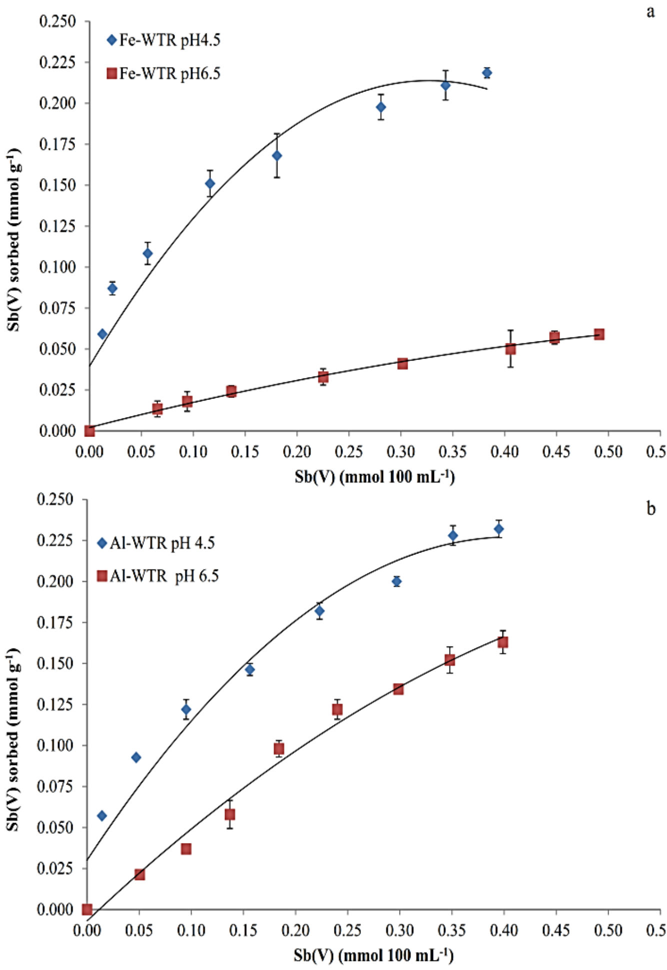


Fig. 1 Sorption isotherms of Sb(V) on Fe- (**a**) and Al-WTR (**b**) at pH 4.5 and pH 6.5

poly-functional organic ligands, displaying aromatic or aliphatic hydroxyl and carboxyl functional groups, could have contributed to the antimony retention. In this sense, the Sb(V) sorption by humic substances via the establishment of pentagonal or hexagonal rings through Sb–O–C bonds has been shown by Tella and Pokrovski (2012).

In order to better understand the Sb(V) sorption by the WTRs, two types of models, i.e., the Langmuir and Freundlich isotherms, were applied to sorption data.

The Langmuir model assumes homogeneous and monolayer adsorption by an adsorbent surface and can be described by the following equation:

$$\frac{x}{m} = \frac{KbC}{1 + KC} \qquad (1)$$

where b is the maximum monolayer coverage capacity of the sorbent at equilibrium (mmol g^{-1}) and K (L mmol^{-1}) is a constant related to the energy of sorption which quantitatively reflects the affinity between the sorbent and the sorbate.

The Freundlich model is an empirical equation employed to describe heterogeneous systems and can be expressed by the following equation:

$$\frac{x}{m} = KFC\ 1/n \qquad (2)$$

where K_F (mmol g^{-}1) and n are the Freundlich constants related to the uptake capacity and intensity, respectively.

Parameters and goodness-of-fit values for the two sorbents are summarized in Table 2. Although both Freundlich and Langmuir equations predicted well the Sb(V) sorption by WTRs, the Freundlich model showed a significantly better agreement with the experimental data as indicated by the higher R^2 values compared to the Langmuir model. This suggests that the two

Table 2 The Langmuir and Freundlich parameters relating to Sb(V) sorption into Fe- and Al-WTRs samples

Model	Parameters					
Langmuir isotherm	*b* (mmol g^{-1})		*K* (L $mmol^{-1}$)		R^2	
	pH 4.5	pH 6.5	pH 4.5	pH 6.5	pH 4.5	pH 6.5
Fe-WTR	0.203	0.062	4.92	9.33	0.970	0.907
Al-WTR	0.242	0.107	4.94	16.06	0.938	0.851
Freundlich isotherm	K_F (mmol g^{-1})		*n*		R^2	
	pH 4.5	pH 6.5	pH 4.5	pH 6.5	pH 4.5	pH 6.5
Fe-WTR	0.314	0.099	0.362	0.725	0.988	0.998
Al-WTR	0.340	0.237	0.425	1.371	0.996	0.916

adsorbents were characterized by heterogeneous surface adsorption sites with different adsorption energies for Sb(V). The values of the Freundlich constant *n*, when comprised between 0 and 1 indicate favorable adsorption (Dou et al. 2011; Ahmad et al. 2012; Vithanage et al. 2013). The increase of *n* with the solution pH (that means a decrease of sorption) shows that protonation–deprotonation reactions have a great impact on the affinity and heterogeneity of Sb(V) binding sites of both WTR surfaces (Cai et al. 2015). Furthermore, the distribution coefficient K_F decreased from 0.314 and 0.340 at pH 4.5 to 0.099 and 0.237 at pH 6.5, for Fe- and Al-WTRs respectively. This is consistent with the significant decrease of Langmuir sorption maxima (*b*) from 0.203 and 0.242 mmol g^{-1} at pH 4.5 to 0.062 and 0.107 mmol g^{-1} at pH 6.5 for Fe- and Al-WTRs respectively. Overall, the sorption models showed that Sb(V) sorption by both WTRs was more significantly favored under acidic conditions than under neutral ones, in agreement with our experimental results and with previous studies (e.g., Cai et al. 2015).

3.3 Sorption Kinetics of Sb(V) by WTRs at pH 4.5 and 6.5

The results of kinetic experiments, carried out at pH 4.5 and 6.5, clearly showed that Sb(V) sorption by both WTRs was fast in the first 60 min, followed by a slower sorption rate of the metalloid. This is consistent with previous studies where it was shown that Sb(V) sorption by goethite and iron oxide was initially rapid followed by a much slower retention rate (Fig. 2) (Leuz et al. 2006; Martinez-Llado et al. 2011; Cai et al. 2015).

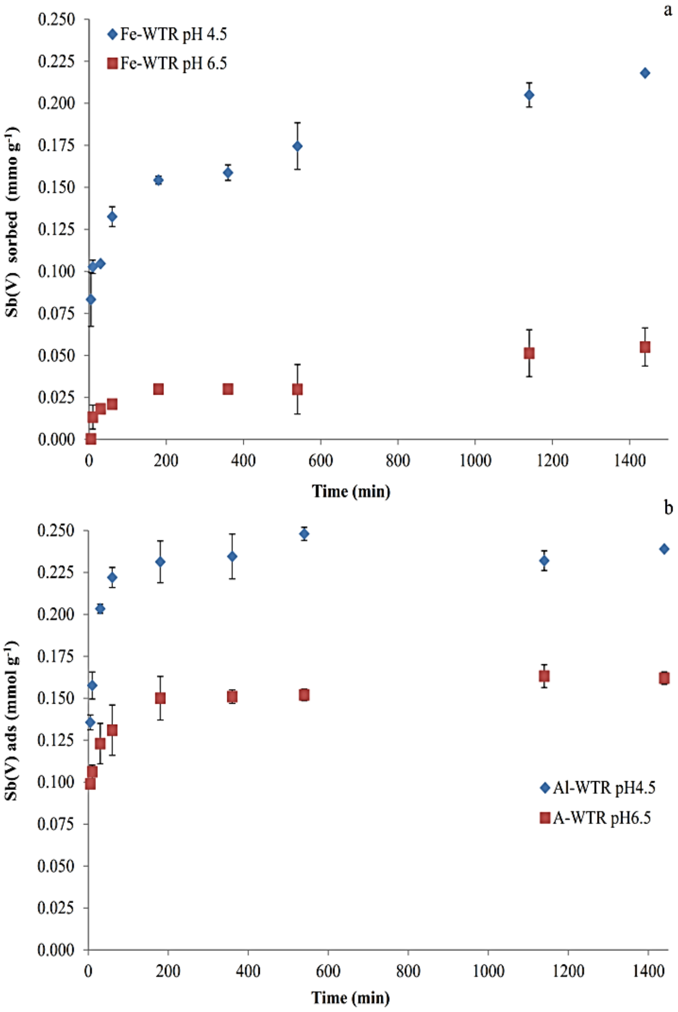
In the first 180 min, the sorption rate was faster for Al-WTR, where almost 97 and 92% of the Sb(V) was sorbed at pH 4.5 and 6.5 respectively, while 71 and 54% of Sb(V) was sorbed by Fe-WTR. This also highlights a slower adsorption kinetic by both sorbents at increasing of pH values. The rapid sorption can be ascribed to the retention of the metalloid on easily accessible (surface) sorption sites. Moreover, this fast step was likely characterized by a strong coulombic attraction (Mohan et al. 2007; Ahmad et al. 2014). On the other hand, a slow intra-particle diffusion process likely dominated the antimonate(V) sorption in the next (slower) step together with the formation of specific (chemo-) and irreversible binding (Mohan et al. 2007; Ahmad et al. 2014) also involving not easily accessible sites.

Different kinetic models were used to evaluate the dominant mechanism involved in Sb(V) adsorption by the WTRs and the fitted kinetic parameters are shown in Table 3. As indicated by the R^2 values, the pseudo-second-order model best described the Sb(V) sorption by both WTRs at pH 4.5 and 6.5 ($R^2 > 0.921$). This also suggests that the rate-limiting step is a chemical sorption between the Sb(V) and both WTRs (Mohan et al. 2007; Martínez-Lladó et al. 2011; Xu et al. 2011; Filella and Williams 2012).

3.4 SEM-EDX Analysis of WTR-Sb(V) Systems

SEM images at varying magnifications, EDX spectra of different point analyses, and maps of untreated and Sb(V)-treated WTRs at pH 4.5 and 6.5 were recorded. Only the results related to Al- and Fe-WTRs doped with Sb(V) at pH 4.5 are reported in Figs. 3 and 4, as those relative to pH 6.5 were very similar. While antimony was not present in the spectra of untreated Al- and Fe-WTRs (not shown), the metalloid was consistently

Fig. 2 Kinetic sorption of Sb(V) on Fe- (**a**) and Al-WTR (**b**) at pH 4.5 and pH 6.5



detected in the EDX spectra of the Sb(V)-doped WTRs (Fig. 3) supporting its sorption by the sorbents. The spectra also confirmed the substantial presence of Fe and Al in both WTRs, which were recognized as good candidate for Sb retention (Rakshit et al. 2011; Vithanage et al. 2013).

Elemental maps based on SEM-EDX analyses of Al- and Fe-WTR-Sb(V) systems showed a diffuse

Table 3 The kinetic model parameters for the adsorption of Sb(V) by Fe-WTR and Al-WTR at pH 4.5 and 6.5

	Pseudo-first-order equation			Pseudo-second-order equation			Elovich equation	
	$q = q_{\max} - \exp.(\ln(q_{\max}) - kt)$			$q = q_{\max} + q_{\max} / (kq_{\max}t - 1)$			$y = a + k\ln(t)$	
	$q_{\max}$ (mmol g^{-1})	k (mmol g^{-1} h^{-1})	R^2	$q_{\max}$ (mmol g^{-1})	k (mmol g^{-1} h^{-1})	R^2	k (mmol g^{-1} h^{-1})	R^2
Fe-WTR pH 4.5	1.00	2.11	0.733	0.217	0.085	0.991	0.335	0.954
Fe-WTR pH 6.5	1.01	4.73	0.283	0.057	0.104	0.921	0.069	0.900
Al-WTR pH 4.5	1.00	1.66	0.296	0.238	1.562	0.999	0.318	0.822
Al-WTR pH 6.5	1.00	2.11	0.550	0.164	0.350	0.999	0.054	0.977

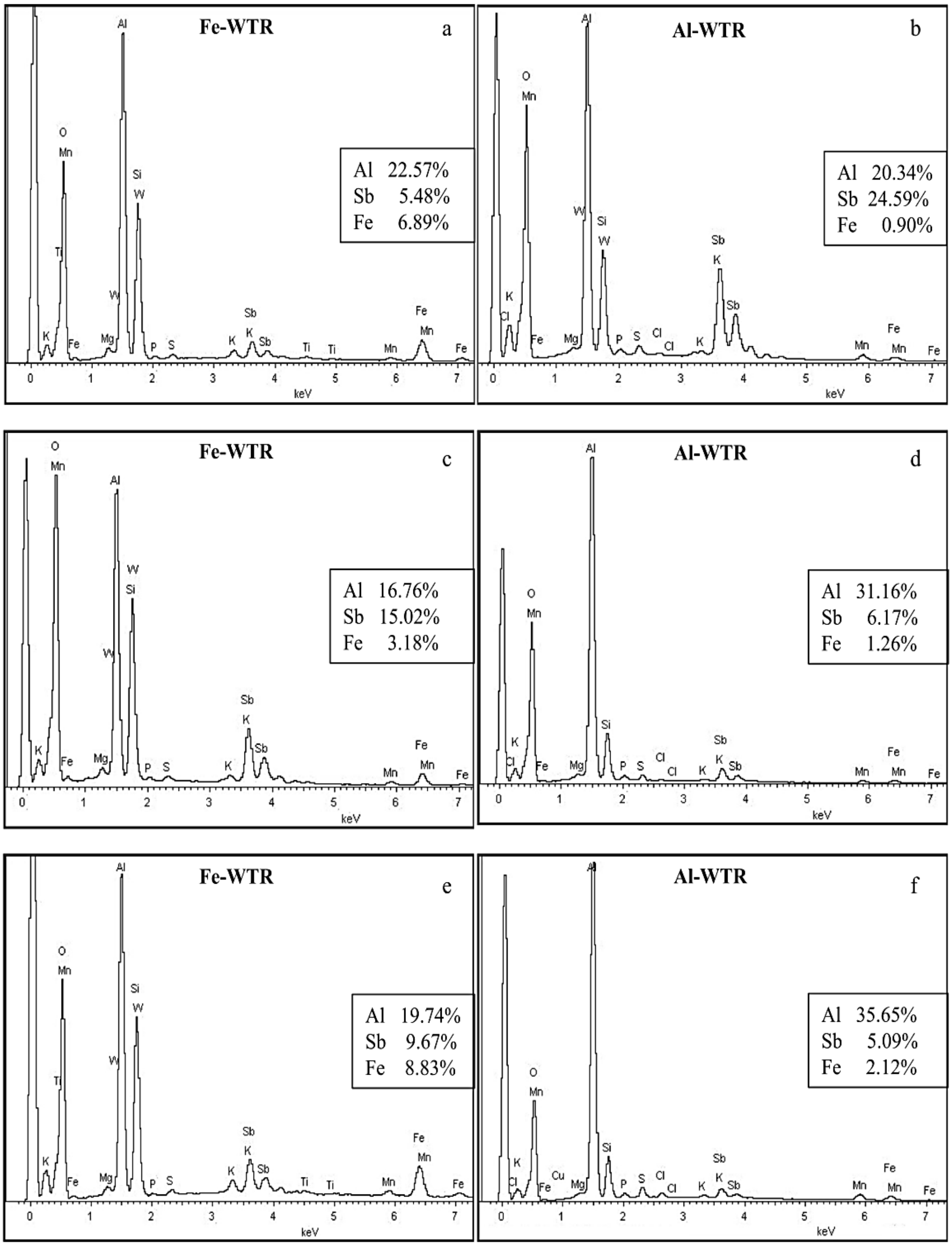


Fig. 3 Representative EDX spectra of Fe- (**a**, **c**, **e**) and Al-WTRs (**b**, **d**, **f**) at pH 4.5 after Sb(V) sorption. Each spectrum refers to a single point analysis. Semi-quantitative element composition is also reported

distribution of Sb which appeared mainly associated with Fe in Fe-WTR and with Fe and Al in Al-WTR (Fig. 4). This supported the view that the Sb(V) sorption by WTRs mainly occurred through the formation of Fe(Al)–O–Sb bond, confirming the high affinity of Sb(V) for metal (hydr)oxides (Wilson et al. 2010).

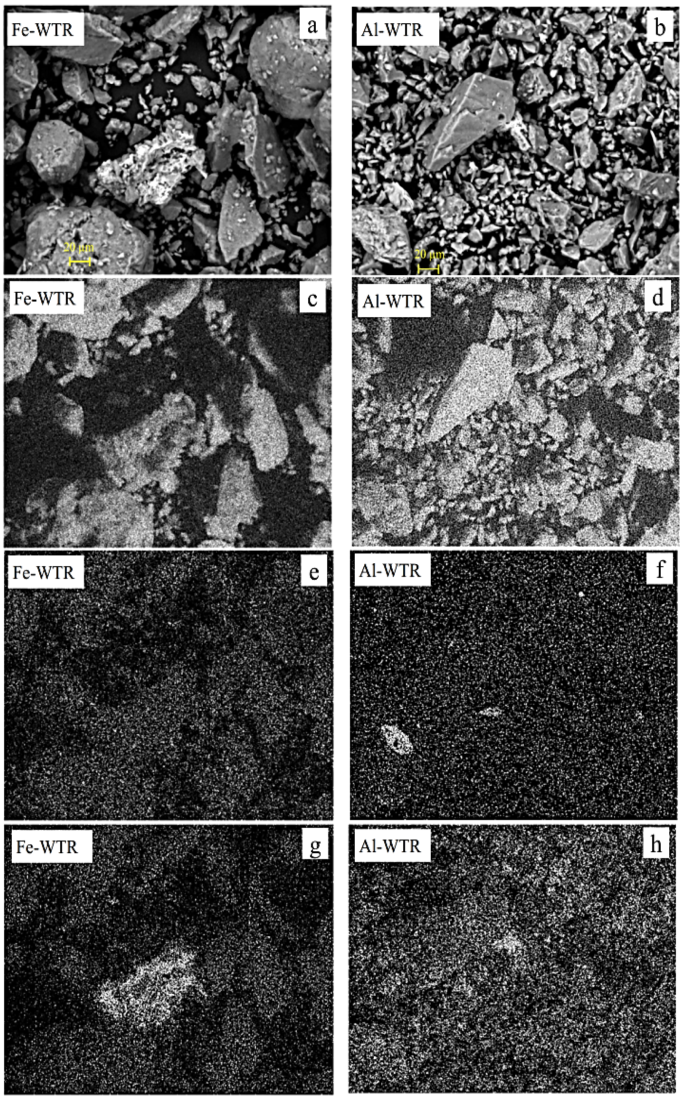


Fig. 4 Representative SEM images of Fe-WTR (**a**) and Al-WTR (**b**) treated with Sb(V) at pH 4.5 and X-ray maps of Al (**c**, **d**), Fe (**e**, **f**) and Sb (**g**, **h**) in the same Fe- and Al-WTR samples

However, especially in the case of Al-WTR, the Sb(V) association with Fe and Al was not so clear thus implying that other WTR components (e.g., organic matter) likely played a role in Sb(V) retention.

3.5 Sequential Extraction of WTRs Doped with Sb(V) at pH 4.5 and 6.5

Fe- and Al-WTR samples doped with antimonate and brought at pH 4.5 and 6.5 were treated with solutions of increasing extraction strength (Wenzel et al. 2001), in order to determine the type of interactions which regulate the Sb(V) retention by WTRs. This procedure, originally designed for As(V) extraction, was successfully applied to the Sb(V) present in contaminated soils as well as bound to different sorbents such as compost (e.g., Ngo et al. 2016; Garau et al. 2017; Földi et al., 2018; Diquattro et al. 2018).

The percentage of antimonate extracted with $(NH_4)_2SO_4$, (step 1, i.e., the relatively labile and exchangeable fraction) was substantially higher for Fe-WTR with respect to Al-WTR at both pH values (Fig. 5). Moreover, after $(NH_4)_2SO_4$ treatment, the percentage of Sb(V) extracted at pH 6.5 from both WTRs was consistently higher compared to that extracted at acidic pH (Fig. 5). This fraction accounts for the Sb(V) exchanged by the sulfate and retained by WTRs through weak outer sphere complexes. A similar trend was

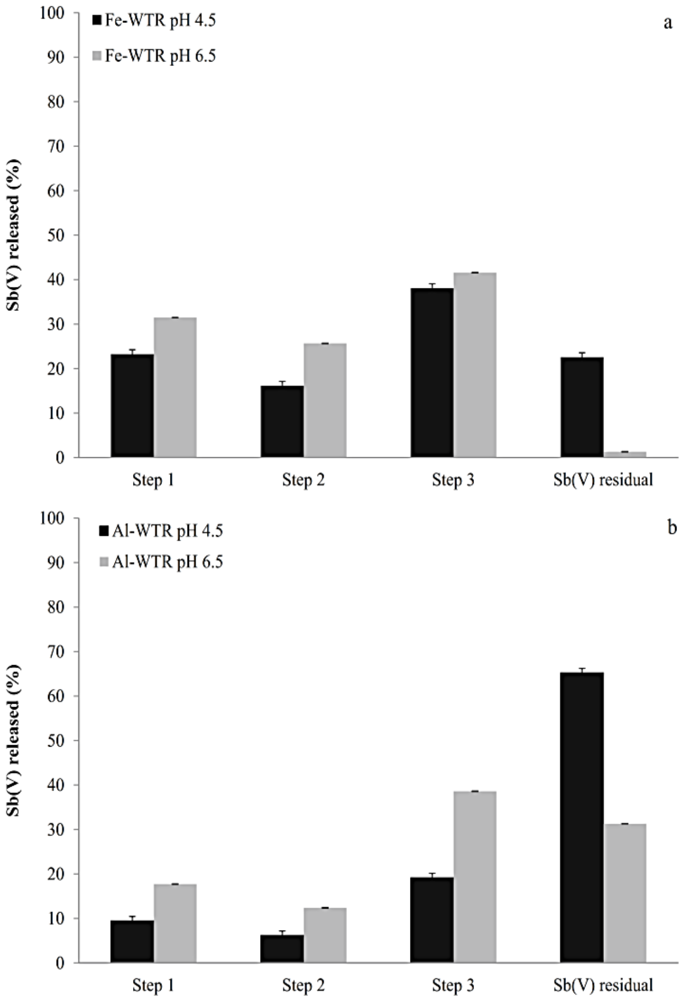


Fig. 5 Percentage of Sb(V) extracted with $(NH_4)_2SO_4$ (step 1), $NH_4H_2PO_4$ (step 2), NH_4-oxalate (step 3), and not extracted from Fe- (**a**) and Al-WTRs (**b**) at pH 4.5 and 6.5

observed for both WTRs after steps 2 and 3 of the sequential extraction (Fig. 5). Concerning the Sb(V) released after $NH_4H_2PO_4$ treatment (i.e., the Sb(V) specifically sorbed and exchanged by phosphate; step 2), it was 6.2 and 12.4% of the total Sb(V) sorbed for Al-WTR and 16.1 and 25.7% for Fe-WTR at pH 4.5 and 6.5 respectively (Fig. 5). The Sb(V) fraction released in step 3 after NH_4^+-oxalate treatment (i.e., the Sb(V) associated with amorphous Al- and Fe-oxides) was approx. 40% of the total Sb(V) sorbed for Fe-WTR, at both pH values, and for Al-WTR at pH 6.5. As for the previous steps, the lowest releases were detected for the Al-WTR at pH 4.5 (approx. $< 20\%$ of the total Sb(V) sorbed). The higher percentages of Sb(V) extracted with NH_4^+-oxalate (compared to the previous steps) were in agreement with the results obtained by other researchers, which confirmed the relevance of non-crystalline oxide and hydroxide minerals for Sb(V) sorption (Tighe and Lockwood 2007; Wilson et al. 2010). The residual Sb(V) fraction (i.e., the Sb(V) not extracted) was lower than 2% of the total antimonate sorbed in the case of Fe-WTR at pH 6.5 and higher than 20% for Fe- and Al-WTRs at pH 4.5 and Al-WTR at pH 6.5. The residual Sb(V) in both WTRs was significantly higher at pH 4.5 rather than 6.5 and it was higher for Al-WTR (65 and 31% of the total antimonate sorbed at pH 4.5 and 6.5 respectively) rather than Fe-WTR (22.5 and 1.3% of the total antimonate sorbed at pH 4.5 and 6.5 respectively) (Fig. 5). This is relevant as this Sb(V) fraction represents the very insoluble and/or occluded metalloid which can be hardly mobilized (Garau et al. 2017; Diquattro et al. 2018).

The results of the sequential extraction procedure showed that (i) the distribution of the Sb(V) pools within the WTR surfaces was largely influenced by the pH, i.e., the residual antimony was significantly higher at acidic pH compared to pH 6.5, while the opposite was true for the labile Sb(V) (step 1), for the chemically bound antimony (step 2) and for the one associated to amorphous metal hydroxides (step 3) and (ii) a relatively large amount of Sb(V) was sorbed by the amorphous metal oxy-hydroxide within WTRs and this led us to speculate that the antimonate anion was retained by WTRs through stable inner sphere complexes. All this seems relevant from an environmental point of view, as the most part of the Sb(V) sorbed by both WTRs (i.e., that extracted in step 3 and the residual Sb) is not expected to be released in the short term and does not constitute a threat for living organisms (Garau et al. 2017).

3.6 Effect of Organic Acids on the Release of Sb(V) by WTRs at pH 4.5 and 6.5

To investigate the potential mobility and bioavailability in the soil/rhizosphere environments of the Sb(V) sorbed by WTRs, both sorbents doped with 6 mM Sb(V) (i.e., the samples deriving from the last point of the sorption isotherms) were treated with two different concentrations of citric (CA) and malic (MA) acids at pH 4.5 and 6.5 (Fig. 6). At these pH values, the organic acids added, which are common and abundant compounds in soil and rhizosphere (Stevenson 1994), were in the form of organic anions (pK_a values of 3.13, 4.76, and 6.40 for CA and 3.40 and 5.11 for MA).

Citrate and malate could be involved in competition phenomena with Sb(V) for the same adsorbing sites on WTRs, thus causing the release of Sb(V) in solution by means of an anion exchange mechanism (Jones, 1998; Goh et al. 2008, Goh and Lim, 2010). At the same time, these low molecular weight organic acids could cause the dissolution of WTR phases containing amorphous Al and/or Fe oxy-hydroxides. In particular, citrate and malate are strong complexants of Fe and Al and this can cause a progressive ligand-promoted dissolution of Fe and Al oxy-hydroxide phases leading to the formation of soluble Fe/Al-citrate or malate complexes (Jones, 1998). Concurrently, this can also cause the release of the Sb(V) bonded to the metal nuclei (Onireti and Lin 2016; Diquattro et al. 2018). In addition, pentavalent antimony can directly form stable aqueous complexes with the aliphatic hydroxyl and carboxyl functional groups of citric and malic acids via the establishment of pentagonal rings that incorporate two Sb–O–C bonds (Tella and Pokrovski 2012).

The data obtained highlighted a different capability of the organic molecules to mobilize the Sb(V) sorbed by the WTRs, which was highly dependent on the WTR type and on the concentration of the organic acids (Fig. 6). In general, both organic acids released higher percentages of Sb(V) from Fe- rather than Al-WTR, with the only exception of CA at 4.5 mM concentration, which mobilized more Sb(V) from Al-WTR rather than Fe-WTR (Fig. 6). Moreover, while CA (at 1.5 mM) and MA (at 1.5 and 4.5 mM) released similar percentages of Sb(V) from Fe-WTR at each pH value, a growing trend of Sb(V) release was noticed in the case of Al-WTR (i.e., MA 1.5 mM < CA 1.5 mM < MA 4.5 mM; Fig. 6). In both WTRs, CA added at 4.5 mM caused a release of Sb(V) higher than 69%. These results indicated that only the highest concentrations of CA were able to efficiently remove the Sb(V) sorbed, regardless of the sorbent type and the pH tested. MA showed a comparatively lower efficacy, being able to extract approx. 30–35% of the total antimony(V) sorbed by both WTRs at both pH values when a concentration of 4.5 mM was used.

The higher release of Sb(V) following CA treatment (compared to MA) may be due to the stronger complexation of Al^{3+} and Fe^{3+} by citric acid as highlighted by the respective complexation constants (Jones, 1998). Likewise, the higher release of Sb(V) from Fe-WTR, rather than Al-WTR (but with the exception of CA treatment at 4.5 mM) could be justified by the higher stability constants of both organic acids with Fe^{3+} compared to Al^{3+} (Jones, 1998). However, as earlier mentioned, an opposite trend was noticed after CA treatment at 4.5 mM, i.e., a higher Sb(V) release was recorded for Al-WTR rather than Fe-WTR (Fig. 6). This could be explained by a substantial Fe solubilization in Fe-WTR, after 4.5 mM CA addition, followed by a subsequent partial co-precipitation of Sb(V) with newly formed Fe oxy-hydroxides similarly to what reported for As(V) (Henke and Hutchison 2009).

Overall, the results obtained indicated that some organic anions (citrate in particular) common in soil and rhizosphere could substantially enhance the mobility of Sb(V) sorbed by WTRs, but only at relatively high concentrations (i.e., 4.5 mM). Such a release seemed slightly less effective at lower pH values, in agreement with the results of the sequential extraction, which highlighted a generally higher affinity of Sb(V) towards the functional groups of WTRs at lowest pH values.

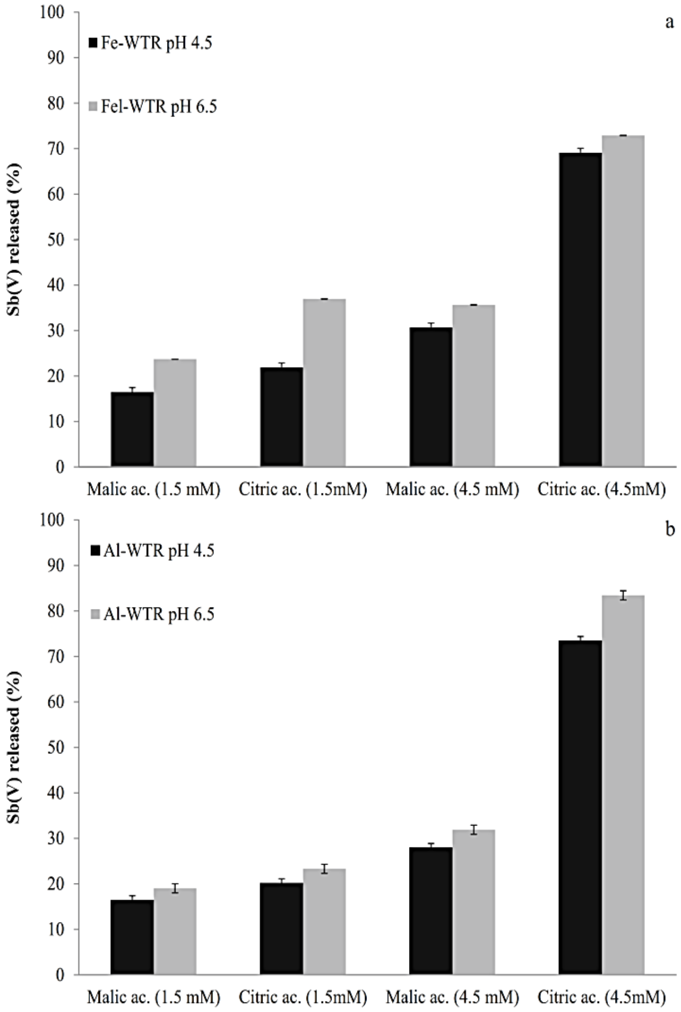


Fig. 6 Percentage of Sb(V) extracted with citric and malic acids from Fe- (**a**) and Al-WTR (**b**) at pH 4.5 and 6.5

4 Conclusions

The obtained results showed that the WTRs investigated were able to act as Sb(V) accumulators at different pH values and that such ability was closely related to the pH of the system, which influenced the surface charge of the sorbents. Accordingly, Al-WTR, with an higher pH_{PZC}, revealed a better sorbent compared to Fe-WTR, particularly at pH 6.5. The Sb(V) sorption by both WTRs was likely governed by (i) coulombic attraction between the Sb(V) anion and the positively charged functional groups of WTRs (e.g., protonated –OH groups of inorganic phases and/or protonated amino groups of the organic matter); (ii) ligand exchange where the surface OH and/or OH_2 groups are exchanged by antimonate leading to the formation of strong inner sphere complexes; and (iii) the formation of stable complexes between Sb(V) and poly-functional organic ligands of WTRs.

The sequential extractions indicated that the most part of the antimonate sorbed by both WTRs [i.e., that extracted in step 3 and the residual Sb(V)] was not expected to be released in the short term, while SEM-EDX mapping revealed that Fe and Al were substantially associated with antimony in likely Fe/Al–O–Sb(V) structures. Treatment with citric and malic acids (commonly occurring plant and/or microbial metabolites) mobilized very different amounts of sorbed Sb(V) and this was depending on the type and concentration of the organic acid used.

Globally, the results obtained showed for the first time that Fe- and Al-WTRs can act as sequestering agents towards Sb(V) and indicate that these sorbents could be used as alternative, low-cost, and environmental friendly amendments for Sb-polluted soils and waters.

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Chapter 4

Antimony (Sb) mobility in soil and its effect on soil microbial and biochemical features

Abstract

Antimony (Sb) and its compounds, are emerging priority pollutants, which represent a serious threat to the environment and human health. The aim of this study was to evaluate the fate of antimonate added to an alkaline soil (S1, pH 8.2) and to an acidic soil (S2, pH 4.9), as respect to its mobility, as well as its impact on the soil microbial and biochemical features. S1 was a sandy-clay-loam, whereas S2 was a loamy-coarse-sand. A high cation exchange capacity (CEC) was determined for S1, opposite to a low-medium one detected for S2. Besides, S2 showed a higher organic matter (2.19% d.m.) compared to S1 (1.75% d.m.). Microcosms with different concentrations of $KSb(OH)_6$ (100 and 1000 mg kg^{-1}) were set up for each soil and compared with non-spiked soil. Sequential extraction data indicated a higher percentage of water-soluble antimony in Sb-spiked S1 soils rather than S2 (i.e. ~13% in S1-100 and -1000 and 10.1 and 4.1% in S2-100 and -1000 respectively). A similar trend was observed for the antimony physically and chemically bonded (Sb extracted with $(NH_4)_2SO_4$ and $NH_4H_2PO_4$ respectively). Residual Sb was lower in Sb-spiked S1 soils (e.g. 14% in S1-1000) than in S2 (43% in S2-1000). This could be mainly ascribed to the lower pH value (i.e. 4.9) detected in the latter soil, which probably favoured the formation of stable precipitates of Sb(V) and Al^{3+} or Fe^{3+} in solution, such as ferric and aluminium antimonate. Microbiological and biochemical data showed a decreased number of culturable heterotrophic bacteria and *Bacillus* spp in Sb-spiked S1, while the number of fungi increased. Opposite results were obtained for S2 soil, where the Sb(V) addition favoured an increase of the bacterial number and a decrease of fungi and actinomycetes. The Biolog AWCD values of the Sb-spiked S1 soils were reduced compared to control (i.e. ~30% and 50% for Sb-100 and 1000 respectively), while a significant increase was recorded for the Sb-spiked S2 soils (2-fold for S2-1000). The dehydrogenase activity significantly decreased in S1 soil contaminated with antimonate (by approx. <15 and 11% for S1-100 and S1-1000 respectively, compared with control), while it increased significantly in contaminated S2 soil (approx. 2- and 2.6-fold increase for S2-100 and S2-1000).

Overall, the results of this study suggested that the Sb mobility and toxicity were different in the two soils, being related to the soil physico-chemical properties, such as pH, clay and organic matter content.

Introduction

Antimony (Sb) and its compounds, listed as priority pollutants by the European Union and the United States environmental protection agencies (CEC, 1998; USEPA, 2009), are becoming increasingly widespread in the environment mainly due to anthropogenic activities [Tandy *et al.,* 2017; Hockmann and Schulin, 2012].

Antimony (Sb) is a shiny white metal usually combined in alloys with metals such as lead, zinc and copper [Hansell, 2015]. Sb alloys are commonly used as lead storage batteries, solder, sheet and pipe metal, bearings, castings, type metal, ammunition, pewter, flame retardants, pigments, plastic stabilizers and like a fireproof for ceramic and glass production [Grund *et al.,* 2006]. Antimony is emitted by combustion of coal and waste incineration and It is often associated with the fine particulates fraction of stack emissions [Filella *et al.,* 2002; Ainsworth and Cooke, 1990]. High antimony levels in soil can be due to the presence of high vehicular traffic and shooting areas [Okkenhaug *et al.,* 2018; Földi *et al.,* 2018]. More in general, Sb is a widely exploited element, and as such its growing presence in the environment is becoming critical because of its high toxicity [Filella *et al.,* 2002b; Tella and Pokrovsky 2008; Dousova *et al.,* 2015]. Indeed, antimony, usually detected as trace element in the earth's crust (0.2–0.3 grams per metric ton) [Wilson *et al.,* 2010; Filella *et al.,* 2002] and water (less than 1 µg L^{-1}) [Filella *et al.,* 2002], reached, in the last decades, worrying levels of contamination in different areas of the world [Casiot *et al.,* 2007; Anawar *et al.,* 2011; Cidu *et al.,* 2013; Garau *et al.,* 2017].

Antimony exists in different oxidation states (-III, 0, III, V). Naturally, Sb mainly occurs in aqueous systems as trivalent Sb(III) or pentavalent Sb(V) inorganic species [Lintschinger *et al.,* 1998] and antimonite [$Sb(OH)_3^-$] and antimoniate [$Sb(OH)_6^-$] are the most common Sb compounds in a wide pH range [Filella *et al.,* 2002b;

Herath *et al.,* 2017]. Regarding soils, antimony oxidation state and its reactivity are largely dependent on the pH, redox conditions, occurrence and concentration of co-occurring reducing and oxidising agents [Wilson *et al.,* 2010; Doherty *et al.*, 2017]. Sb(V) dominates in oxygenated natural waters and aerated soils and seems less toxic, but more mobile than Sb(III) [Herath *et al.,* 2017]. Sb(V) mobility is strongly associated with pH [Nakamaru *et al.,* 2006]. Sb(V) mainly occurs as octahedral antimonate ion, i.e. $Sb(OH)_6^-$, which is also the prevailing form occurring in polluted soils near smelters and shooting ranges [Okkenhaug *et al.,* 2011].

In the last decade, the Sb mobility in soil has been investigated by several studies. In particular, sequential extraction and spiking experiments indicated, that soil Sb tends to be associated with hydrous oxides of Fe, Al, and Mn or adsorbed by humic acids [Garau *et al.,* 2017; Diquattro *et al.,* 2018; Bagherifam *et al.,* 2014; Steely *et al.,* 2007].

From an environmental point of view, Sb mobility in soil is of utmost importance since this element can have a strong negative impact on soil microbiota which in turn regulates soil fertility and functionality. In particular, the mobile (or labile) Sb in soil, which represents the bioavailable Sb pool, can have a negative influence on the main biogeochemical cycles governed by soil microbes, soil enzyme activities and microbial community structure and function [Luo *et al.,* 2014; Garau *et al.,* 2017; Kataoka *et al.,* 2018]. Hence, for a thorough assessment of trace metals and metal(loid)s impact in polluted soils, microbial and biochemical analyses targeting the abundance and functionality of soil microbial communities should always accompany chemical determinations. For instance, soil dehydrogenase activities can be of help in the assessment of the environmental effect of trace metals and metalloids in polluted soil [Roger and Li, 1985]. Likewise, it was shown in many instances that the size of culturable heterotrophic microbial populations, bacteria in particular, was very sensitive to soil disturbance phenomena including critical amounts of metal(loid)s in soil [Wang *et al.,* 2011; Wei *et al.,* 2015; Courtin-Nomade *et al.,* 2012]. Moreover, high concentrations of Sb in soil can also influence the structure of soil microbial community and this can have relevant effects on the resilience of such communities to external changes [Guillamot *et al.,* 2014; Li *et al.,* 2016]. Despite the relevance of such aspects, few studies are available on these issues [An and Kim, 2009; Wang *et al.,* 2011] and the fate of Sb in contaminated soils is often studied only by a chemical point of view whilst the impact on soil microbial component and biochemical status is rarely addressed [Wang *et al.,* 2011; Wang *et al.,* 2018; Courtin-Nomade *et al.,* 2012].

The aim of the present work was therefore to evaluate the fate of antimonate, added to two different soils, as respect to its mobility, as well as its impact on selected soil microbial and biochemical features. In particular, the speciation of antimony(V) was evaluated in the two soils spiked with different Sb(V) concentrations through sequential extractions, while its impact on soil biochemical and microbial features was addressed by monitoring the size of culturable microbial populations and quantifying soil dehydrogenase activities in the same polluted (and unpolluted – control) soils. Finally, the microbial community structure in spiked and unspiked (control) soils was addressed through the Biolog community level physiological profile.

Materials and methods

Soil characteristics and microcosms set up

Topsoil samples (0-20 cm depth) were collected from two different sites in Sardinia (Italy). The first (S1) was located in north-west Sardinia, loc. Saccheddu (40°43′32.77″N 8°24′48.6″E). The second one (S2) was located in north Sardinia, loc. Azzagulta (40°56'15.7"N 8°53'30.4"E). According to particle-size analysis, carried out using the pipette method [Day, 1965], the soil in S1 was a sandy clay loam (USDA classification); while S2 was a loamy-coarse sand (USDA classification).

The collected soil samples were separately bulked and sieved to <2 mm before being employed for the set-up of different soil microcosms, each consisting of approx. 5 kg soil (dry weight equivalent).

Soils were subjected to the following physico-chemical analysis, data are reported in Table 1: electric conductivity (EC) and pH were both determined in soil suspensions with a 1:2.5 soil: deionised water ratio (Gazzetta Ufficiale, 2002). Total and active carbonates were determined by acid dissolution (volumetric calcimeter method) and by reaction of soil with 0.1 M ammonium oxalate respectively (Gazzetta Ufficiale, 1999, Gazzetta Ufficiale, 2002). Elemental CHN analysis of soils, provided information on total organic carbon and nitrogen in soil. Total P were determined spectrophotometrically (ascorbic acid method) after soil mineralisation (Gazzetta Ufficiale, 2002). Estimation of available P in soil was determined by extraction with sodium bicarbonate (Olsen method, Gazzetta Ufficiale, 2002). The dissolved organic carbon (DOC) was estimated as described by Brandstetter *et al.* [1996] while the point of zero charge (pH_{PZC}) has been determined by potentiometric titration as described in Appel *et al.* [2003]. The method reported by Ciavatta *et al.* [1990] was used to determine the content of humic substances (i.e. humic and fulvic acids). Soil cation exchange capacity (CEC) was determined using the $BaCl_2$ and triethanolamine method (Gazzetta Ufficiale, 1999). Trace metals and metalloids in soils were quantified using a flame atomic absorption spectrometer equipped with a HGA 600 graphite furnace (Perkin Elmer Analyst 600, Software AA-WinLab32) after soil cold digestion with aqua regia reverse solution (HNO_3/HCl 3:1 ratio) and mineralisation through microwave digestor Milestone MLS1200.

Spiking experiments

Three soil microcosms (5 kg each) were prepared for each soil type and treatments (in total 18 microcosms were set up). In particular, the following treatments were applied to each soil type: control soil [no Sb(V) added]; S-100 [soil+100 mg kg^{-1} Sb(V)]; S-1000 [soil+1000 mg kg^{-1} Sb(V)]. Soil spiking was carried out using Sb(V) solutions deriving from $KSb(OH)_6 \cdot 7\ H_2O$, which were added to soils to reach a final contamination level of 100 and 1000 mg kg^{-1} Sb(V). All soils were then brought to 60% of their water holding capacity. Before addition to a soil, each Sb(V) solution was brought to the pH of the same soil using [0.1 M] NaOH/HCl solutions. After spiking, treated and untreated (control) soils were left for three months at 25 °C during which they were periodically mixed (once every two weeks) and the water content maintained to 60% of their water holding capacity.

Sequential extraction of Sb in spiked soils

After the equilibration period the Sb mobility in spiked soils was evaluated through the sequential extraction procedure proposed by Wenzel *et al.* [2001] and originally designed for As, with minor modifications. Triplicate soil samples (1 g) from each microcosm were treated with 25 mL of extraction reagents added sequentially. After each step, tubes containing soil and solution (i.e. the extraction reagent) were centrifuged at 3000 rpm for 10 min and filtered through 0.45 µm cellulose acetate filter paper. Antimony in filtered solutions was quantified using a flame atomic absorption spectrometer equipped with a HGA 600 graphite furnace (Perkin Elmer Analyst 600, Software AA-WinLab32). Soil samples were sequentially treated with water (Step 0 additional step with respect to the original extraction procedure), to estimate the water- soluble Sb; then with 0.05 M $(NH_4)_2SO_4$ solution (4h, 130 rpm), to assess the non-specifically adsorbed Sb fraction (Step 1); with $(NH_4)H_2PO_4$ 0.05 M (16h, 130 rpm) to extract the Sb pool specifically-sorbed (Step 2); then with 0.2 M NH_4-oxalate pH 3.25 (4h shaking 130 rpm in the dark, Step 3) followed by 0.2 M NH_4-oxalate solution+0.1 M ascorbic acid pH 3.25 (30 min in a water basin at 96°C, Step 4) to extract the Sb associated to amorphous and crystalline Al- and Fe-oxides respectively. The residual Sb was quantified by subtracting all the Sb fractions extracted from total Sb in soil.

Soil microbial and biochemical analyses

To evaluate the impact of Sb on culturable heterotrophic populations, total bacteria and fungi, actinomycetes, *Bacillus* spp. and *Pseudomonas* spp. were enumerated in triplicate soil samples from the different microcosms. The common ten-fold serial dilution and spread plate method was used [Pinna *et al.,* 2012]. The growth media used to detect and count the different microbial populations were as follow: 1:10 TSB (Tryptic Soy Broth, Microbiol, Cagliari, Italy) + agar (1.5%) for soil heterotrophic bacteria; GYEP pH 4.0 (Glucose Yeast Extract Peptone medium;

Garau *et al.,* 2007) for soil fungi; AIA (Actinomycete Isolation Agar, Difco, Milan, Italy) for actinomycetes; PSA (Pseudomonas Selective Agar, Microbiol, Cagliari, Italy) for *Pseudomonas* spp.. Soil samples (10 g) were dispersed in 90 mL sodium pyrophosphate solutions (2 g L^{-1}) and serial ten-fold dilutions were obtained using 0.89% NaCl solution. Agar plates were then inoculated with 100 μL from the different ten-fold dilutions and incubated at 28°C for 3 days until colony count. The microbial abundance in each soil was expressed as Log_{10} CFU (Colony Forming Unit) g^{-1} soil (dry weight basis).

The microbial community structure of spiked and control soils was investigated through the Biolog community level physiological profile using Biolog Ecoplates (Biolog Inc., Hayward, CA). Microbial communities were extracted from triplicate soil samples (10 g each) deriving from respective microcosms (treated and untreated) as previously described [Pinna *et al.,* 2012]. Briefly, 30 mL of the 100-fold dilution used for the enumeration of culturable microorganisms were centrifuged for 8 min at 2600 rpm and the clear supernatant was used to inoculate the wells of the Biolog Ecoplate (120 μL per well). The Biolog Ecoplates are ready to use 96 wells microtiter plates containing in each well a different carbon source of soil/environmental relevance [Insam *et al.,* 1997]. A total of 31 different carbon sources and a control well with no carbon (all replicated three times) are present in each Biolog ecoplate. A tetrazolium dye is also incorporated in the plate wells and reveals oxidative catabolism (i.e. carbon source utilisation) as purple colour formation. Inoculated plates were incubated at 28 °C for 7 days and purple colour formation in each well was recorded daily by measuring the absorbance at 590 nm (OD_{590}), using an automatic Biolog MicroStation^{TM} reader. The OD_{590} values recorded at all incubation times (24-168 hours) were first blanked against the respective time 0 readings and further subtracted against the respective control well (with no carbon source). Finally, they were processed to obtain a measurement of the potential catabolic activity of the microbial community, i.e. the average well- colour development (AWCD). This latter was calculated as follow:

$$AWCD = [\Sigma (R-C)] / 31$$

where C represents the absorbance value of control wells, R is the mean absorbance of the response wells, and n is the number of carbon substrates (i.e. 31).

The richness value, or the number of substrates catabolised by each microbial community, was also determined as the number of wells with OD_{590} > than 0.15 [Garau *et al.,* 2007].

Soil dehydrogenase activity (DHG) was determined colorimetrically in duplicate soil samples collected from each microcosm. Soil samples (10 g) were treated with a triphenyltetrazolium chloride solution and leaved for 24 h at 30 °C as described by Alef and Nannipieri [1995]. The DHG activity was quantified spectrophotometrically in the liquid phase (OD_{480} nm) and expressed as triphenyl formazan (TPF) formed per g^{-1} soil (dry weight basis).

Results and Discussion

Soil characterisation and sequential extractions of Sb from contaminated soils

The main aim of this study was to evaluate the antimony mobility in different type of soils doped with two concentrations of Sb(V) (i.e. 100 and 1000 mg kg^{-1}). In this context, we firstly characterised the two unpolluted soils. As previously reported, S1 was a sandy-clay-loam (USDA classification) with ~62% sand, ~17% silt, and ~21% clay, while S2 was a loamy-coarse-sand, characterised by a high sand content ~88% and low clay 10% and silt 2%. S1 was an alkaline soil (pH 8.2), whereas S2 was acidic (pH 4.9); S1 had a high cation exchange capacity (CEC) as opposed to S2 which showed, a low-medium CEC (Table 1). The soil characterisation also revealed high concentrations of total Fe and Al in both soils and higher total Ca (62500 mg kg^{-1}) in S1 with respect to S2 (48 mg kg^{-1}).

As mentioned, the mobility of antimony in soil samples was determined by the sequential extraction procedure of Wenzel *et al.* [2001] modified according Castaldi *et al.* [2010]. This method was originally designed for arsenic, however many studies demonstrated its efficacy when employed for antimony [Ngo *et al.,* 2016; Garau *et* *al.,* 2017; Földi *et al.,* 2018; Diquattro *et al.,* 2018]. The percentage of water-soluble antimony (Step 0), extracted from S1 polluted soils (~13.4 and ~13% in S1-100 and -1000 respectively) was higher than that recorded in S2 polluted ones (10.1 and 4.1% of Sb was extracted in S2-100 and -1000 respectively) (Fig. 1). Mykolenko *et al.* [2018] recorded similar results for water soluble antimony (~16% of total Sb) in an alkaline soil (pH 8.39) contaminated with 112 mg kg^{-1} of Sb. This could be mainly due to the higher competition between hydroxide (OH^-) and Sb(V) anions for retention sites in S1 than in S2 soil [Rakshit *et al.,* 2015]. The Sb extracted in this step represents the metalloid pools more labile and potentially bioavailable [Garau *et al.,* 2017; Agah *et al.,* 2009]. In this sense, the relevant amount of water-soluble antimony recorded at pH 8.2 in S1 soil may cause adverse environmental effects [Li *et al.,* 2014; Sun *et al.,* 2017]. Also the antimony extracted with $(NH_4)_2SO_4$ (Step 1), which indicates the relatively labile and exchangeable fraction, was significantly higher in S1 (7.9 and 12.5% of Sb extracted in S1-100 and -1000 respectively) than in S2 (7.4 and 1.9% of Sb extracted in S2-100 and -1000 respectively) (Fig. 1) even if the opposite could be expected. Indeed, even if S1 is an alkaline soil, and this generally limits the electrostatic interaction of antimonate (and sometimes antimonite) with the surface of soil colloids, it contains a higher quantity of clay and CEC with respect to S2 soil. This could imply the higher occurrence in S1 soil of ternary complexation in which polyvalent metal cations (e.g., Ca, Fe and Al) act as a bridge between the negatively charged functional groups of clays and the antimony oxyacid, as demonstrated for phosphate and arsenate [Filella and Williams, 2012; Diquattro *et al.,* 2018]. Moreover, given the higher content of total Fe and Al in S1 soil, a higher number of positively-charged exchange sites on Fe and Al compounds characterised by high values of point of zero charge (i.e. > 8.2) could favour the formation of electrostatic bonds with Sb(V). A similar trend was also detected for the Sb extracted in Step 2 (using $NH_4H_2PO_4$), which quantifies the specifically sorbed Sb, forming inner-sphere complexes with the soil components. The Sb amount extracted in Step 2 was 13.5 and 20.2% for S1-100 and -1000, and 7.8 and 3.7% in S2-100 and -1000 respectively.

As regards the extraction step with NH_4^+-oxalate (Step 3) and with NH_4^+-oxalate + ascorbic acid (Step 4), more than 40% of total antimony was extracted in the S1 and S2 soil. These steps account for the Sb fractions retained by adsorption at surfaces of amorphous and crystalline Fe/Al oxides and oxy-hydroxides. However, it is important to underline that in S1 soil, most of the antimony was extracted in Step 3, while in S2 the highest amount of antimony was extracted in Step 4, thus highlighting a different soil mineral composition. The residual antimony, i.e. the fraction strongly retained in soil and hardly bioavailable, was higher in S2 (22 and 43% in S2-100 and -1000 respectively) than in S1 (20 and 14% in S1-100 and -1000 respectively). Generally, this suggests that a large fraction of Sb (particularly in S2 soil) was strongly retained (and not expected to be readily released), and most likely bounded to the solid phases and/or involved in the formation of stable precipitates, as highlighted by several studies [Tighe *et al.,* 2005; Wilson *et al.,* 2010; Rakshit *et al.,* 2015]. In particular, the higher amounts of residual antimony recorded in S2 soil could be ascribed to the lower pH value (i.e. 4.9) detected in this soil. The acidic environment could have promoted the formation of stable precipitates between Sb(V) and Al^{3+} or Fe^{3+} in solution, such as ferric and aluminium antimonate ($FeSbO_4$ and $AlSbO_4$) [Fan *et al.,* 2016; Vandenbohede *et al.,* 2019; Dousova *et al.,* 2018; Herath *et al.,* 2017]. Fe/Al antimonate are shown to be highly insoluble even under acidic conditions and may play an important role in antimony immobilisation processes [Okkenhaug *et al.,* 2013].

In addition, the higher content of organic matter in S2, could have favoured the formation of stable complexes between Sb(V) and poly-functional organic ligands like oxy-carboxylic acids and polyols [Tella and Pokrovski, 2008]. In our previous study [Diquattro *et al.,* 2018] we reported the important role of organic matter (humic acids in particular) as sorbent for Sb(V) at acidic condition.

Overall, the results of the sequential extraction procedure showed that the distribution of the Sb(V) pools in S1 and S2 was largely influenced by the physico-chemical properties of the soils, such as texture, pH, Al and Fe in solution and organic matter content, reflecting the high complexity of the soil system.

Influence of antimony on soil microbial abundance, dehydrogenase activity and community level physiological profile

Sb(V) addition (100 and 1000 mg kg^{-1}) significantly affected soil microbial populations. However, the antimony impact on S1 and S2 soils was different. Total bacterial number decreased significantly in S1 soil treated with Sb(V) (~1.9 and 3% lower on a Log_{10} basis in S1-100 and -1000 respectively compared to control S1), while an opposite trend was recorded for S2 soil (~3.2 and 3% higher on a Log_{10} basis in S2-100 and -1000 respectively compared to control S2) (Fig. 2). Contrasting trends were also noticed for fungi, whose number increased in S1 soil treated with Sb(V) (~7.5 and 9.2% on a Log_{10} basis in S1-100 and -1000 respectively) whilst decreased in S2 (~3 and 2.5% on a Log_{10} basis in S2-100 and -1000 respectively) (Fig. 2). The number of *Bacillus* spp. slightly decreased in S1-100 and -1000 (~0.9 and 2.1% on a Log_{10} basis respectively) while in S2 remained unaffected (Fig. 2). Counts of total actinomycetes indicated an increase in S1 soil (~3.3 and 1.4% on a Log_{10} basis in S1-100 and -1000 respectively) while a significant decrease was noticed in S2-1000 (3.1% on a Log_{10} basis).

The higher concentration of labile antimony in S1 could explain the decrease of total bacterial number and *Bacillus* spp. in this soil. Such decrease may be due to a toxic effect of Sb against soil bacteria as was previously reported [Wang *et al.,* 2011] and, interestingly, it was not influenced by the Sb concentration, i.e. the bacterial number in S1-100 and -1000 was not statistically different. On the other hand, the increase of fungi in the presence of Sb suggested that this microbial group took advantage by the bacterial decrease. This was likely due to a substantial competitive pressure exerted by bacteria against fungi as pointed out by Rousk *et al.* [2008]. Total actinomycetes were apparently unaffected by Sb (e.g. their number in control S1 and S1-1000 was not dissimilar; Fig. 2) even if Wang *et al.* [2011] reported a significant increase of this soil microbial group after Sb addition up to 500 and 2000 mg kg^{-1}.

A completely different picture was observed in S2 soil. In particular, the significant increase of readily culturable bacteria in the Sb-spiked soils (+ 1.04 in S2-100 and -1000) was difficult to explain, since soil microorganisms, bacteria in particular, are commonly negatively affected by critical levels of metal(loid)s [Luo *et al.,* 2014; Garau *et al.,* 2017; Kataoka *et al.,* 2018; Jiang *et al.,* 2019]. However, these data are in line with those reported by Guillamot *et al.* [2014] where a significant increase of substrate-induced respiration (SIR), which is well correlated with active soil microbial biomass [Anderson and Domsch, 1978], was reported after the addition of 50, 500 and 5000 mg kg^{-1} of Sb_2O_3 to different soils. Moreover, Wang *et al.* [2011] evaluated the effects of Sb contamination, both Sb(III) and Sb(V), on culturable soil microbial populations and enzyme activities in slightly acidic soils, which were quantified during different incubation times (after 7, 14, 21, 28 and 35 days). Unexpectedly, Wang *et al.* [2011] reported a significant decrease of the inhibition rate of microbial populations (%) for Sb ~<150% and 100% for 500 and 2000 mg kg^{-1} of Sb(V) added and <150% for 10 mg kg^{-1} Sb(III). These researchers hypothesized the stimulation of actinomycetes populations as a possible consequence of Sb-resistant actinomycetes groups selection in the spiked soil. A similar adaptation could be referred to data reported here, where Sb-resistant soil bacterial communities able to use in a more effective way (compared to the original microbial community) the available “food” resources in soil could be reproduced at the expense of the others bacterial communities decreased because of Sb toxicity. Rosen [2002] also supported that in the presence of As(III) and Sb(III), the microbial communities reacted with different resistance mechanism which involve efflux proteins (e.g. GlpF aquaglyeroporins), ATPase pumps (ArsAB efflux pump), chemo-osmotic antiports (Acr3p), and intracellular storage (Ycf1p transports $As(GS)_3$ into the vacuole). In addition, Li *et al.* [2016] reported information about bacterial antimony transformations. Li *et al.* [2016] considered Sb(V) reduction, the main resistance mechanism against antimony in the environment. In particular, Sb(V) reduction was combined to a dissimilatory bacterial respiratory pathway, which utilized acetate or lactate as the electron donor. However, enzymes involved in this reaction have not yet been identified. Wang *et al.* [2013] observed that sulfate-reducing bacteria (SRB) were employed to remove Sb(V) from Sb mine drainage. Moreover, it should be also mentioned that the concentration of labile Sb fractions in the more acidic S2 soil was significantly lower compared to S1 (especially for S2-1000), and this could explain the response of the S2 soil bacterial community after Sb addition, i.e. an easier adaptation mechanism due to a lower environmental/contaminant pressure. Additionally, it should be also pointed out that microbial communities of S1 and S2 soils were likely different as highlighted by the size of the microbial groups considered and by the different community level physiological profiles (see next paragraph). According Oliveira and Pampulha, [2006] different species of microorganisms can show considerable differences in their sensitivity to metal(loid) toxicity. Therefore, this could be one of the possible explanations of the opposite Sb impact on the two microbial communities observed in this study.

The Biolog Community level physiological profile (CLPP) of S1 and S2 soils showed a significantly different trend. In particular, the microbial population extracted from S1 polluted soils displayed a significant decrease of AWCD values, i.e. ~30 and 50% for S1-100 and 1000, respectively (Fig. 3). The same trend was recorded for richness values, or the number of carbon sources metabolised by the microbial community (Fig. 3). On the other hand, a significant increase of AWCD (2-fold) but not for richness, was recorded in S2 soils treated with 1000 mg kg^{-1} Sb(V) compared to control soil. The general AWCD trend followed the number of heterotrophic bacteria, especially in S1 soil. This correlation was previously highlighted [e.g Garau *et al.*, 2017] and was mainly due to the fact that fast-growing culturable bacteria are recognised as the most relevant contributors of colour development in Biolog Ecoplates [Garau *et al.,* 2007; Preston-Mafham *et al.,* 2002]. If this is our case, the different AWCD and richness values of unpolluted S1 and S2 microbial communities should indicate a different composition of the bacterial community of S1 and S2 soils. This could also explain why Sb addition had contrasting effects on the two microbial communities.

Microbial dehydrogenase (DHG) are involved in many redox processes occurring within active cells, e.g. they are involved in the biological oxidation of natural soil organic matter, in the synthesis of humus, in the degradation of xenobiotics [Wolińska and Stępniewska, 2012]. Consequently, DHG have been often used as indicators of soil microbial activity [Nannipieri *et al.,* 2003; Garcia *et al.,* 1997]. DHG was strongly affected by Sb(V) contamination (Fig.4). Tukey multiple comparison analysis indicated significant ($P < 0.05$) differences in S1 and S2 soils. In particular, DHG values significantly decreased in S1 soil contaminated with antimony (by approx. -15 and -11% for S1-100 and S1-1000 respectively, compared with control), while they increased significantly in contaminated S2 soil (approx. 2- and 2.6-fold increase for S2-100 and S2-1000 respectively). Generally, trace elements could reduce the enzyme activity by their interaction with the enzyme-substrate complex, denaturation of the enzyme protein or interaction with the protein-active groups [Nannipieri, 1994]; however, they could also affect the enzyme synthesis and abundance which is in turn dependent on the microbial number and activity [Pan and Yu, 2011]. In our case, this latter seems the most likely hypothesis as DHG values were essentially following the number of soil heterotrophic bacteria. This also implies that heterotrophic bacteria are significantly contributing to soil microbial activity and functionality [Garau *et al.*, 2017]. The DHG activity in S1 and S2 soils seems therefore governed by the Sb impact on the respective culturable bacterial populations which is in turn governed by the antimony speciation and the physico-chemical properties of the respective soils. However, studies on the effects of antimony pollution on enzymatic activity are very limited and do not agree with each other, making the interpretation of our data more complicated. For instance, Murata *et al.* [2005] reported that Sb addition to soil (25 and 250 μM) did not affect soil dehydrogenase activity. Wang *et al.* [2011], reported a 20% increase of acid phosphatase activity after the addition of 1000 mg kg^{-1} Sb(V).

Conclusions

The results obtained indicated that the behaviour of Sb (in particular its mobility and toxicity) was significantly affected by the physico-chemical properties of soil, such as pH, clay and organic matter content, as well as Al and Fe content.

Sequential extractions data indicated that, regardless of the concentration of antimonate added, a higher amounts of exchangeable and chemically bound Sb was recorded in the alkaline soil (S1), as consequence of its physico-chemical properties and mineral composition. A large fraction of Sb added to the acidic soil (S2) was strongly retained by the solid phases through different mechanisms. In particular, the acidic conditions of S2 soil could have promoted the formation of stable precipitates involving the antimonate and Al^{3+} or Fe^{3+} in solution), such as $FeSbO_4$ and $AlSbO_4$. Moreover, the higher content of organic matter in S2, could have favoured the formation of stable complexes between Sb(V) and poly-functional organic ligands like oxy-carboxylic acids and polyols. The results of Sb speciation were confirmed by the Biolog AWCD and DHG activity values, which resulted significantly decreased in S1 soil doped with Sb(V) with respect to the control. On the other hand, the increase of heterotrophic bacterial numbers, AWCD values and DHG activities in S2 soil was likely due to the selection of Sb-resistant soil microbial communities able to use, in a more effective way, the soil carbon sources.

The results obtained suggest that risk assessment for soil polluted with antimony should be a priority especially for alkaline soils where the high mobility of the anionic $Sb(OH)_6^-$ can represent a serious threat for soil functionality and human health.

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Table 1 Selected physico-chemical characteristics of uncontaminated soils used in this study.

Physico-chemical parameters	***S1***	***S2***
pH_{H2O}	8.22 ± 0.14	4.91 ± 0.26
EC ($\mu S\cdot cm^{-1}$)	378 ± 8.20	327 ± 4.46
DOC ($mg\cdot g^{-1}$)	0.17 ± 0.00	0.39 ± 0.03
Organic matter (%)	1.75 ± 0.13	2.19 ± 0.18
Total Carbonate ($g\cdot Kg^{-1}$)	300 ± 1.27	12 ± 0.28
Active Carbonate ($g\cdot Kg^{-1}$)	82 ± 0.91	11 ± 0.44
Total P ($mg\cdot Kg^{-1}$)	330.1 ± 0.00	272.7 ± 34.20
Available P ($mg\cdot Kg^{-1}$)	31 ± 2.61	56 ± 1.42
CEC ($cmol\cdot Kg^{-1}$)	21.1± 0.88	12.9± 2.51
pH_{PCZ}	5.7	2.6
USDA Texture	sandy clay loam	loamy coarse sand
Total metals ($mg\cdot Kg^{-1}$)		
Fe	16350 ± 1061	5650 ± 71
Mn	174 ± 16	87 ± 2
Na	2150 ± 636	750 ± 71
K	7100 ± 707	1500 ± 1
Mg	3100 ± 283	770 ± 14
Ca	62500 ± 2828	48 ± 5
Al	19930 ± 1018	3924 ± 749
Sb	n.d.*	n.d.*

* n.d., not detected

Figure 1 Sequential extraction of antimony from soils contaminated with Sb(V) (100 and 1000 mg Kg^{-1}). Within each categories (each separatly soil and Sb concentration), values which share a common letter do not differ significantly (Tukey Kramer multiple comparison test, $P < 0.05$). Within each step for each Sb concentration and both soils, values whith «*» are significantly (Tukey Kramer multiple comparison test, $P < 0.05$) higher than the same values for the same step in the other soil.

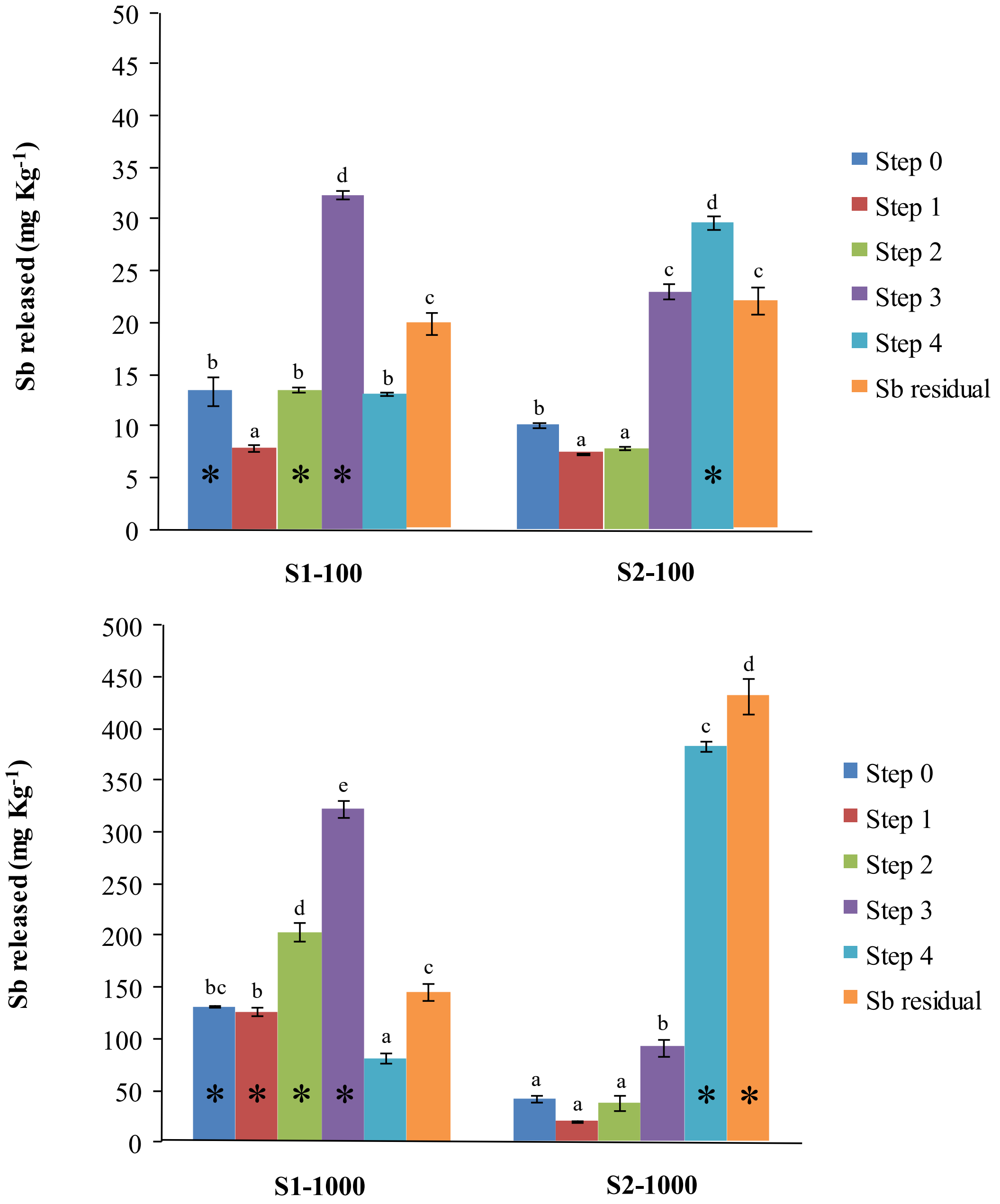


Figure 2 Counts of heterotrophic microorganisms in S1 (up) and S2 (at the bottom) of uncontaminated and contamineted soils (100-1000 mg Kg^{-1}). Within each categories, values which share a common letter do not differ significantly (Tukey Kramer multiple comparison test, $P < 0.05$).

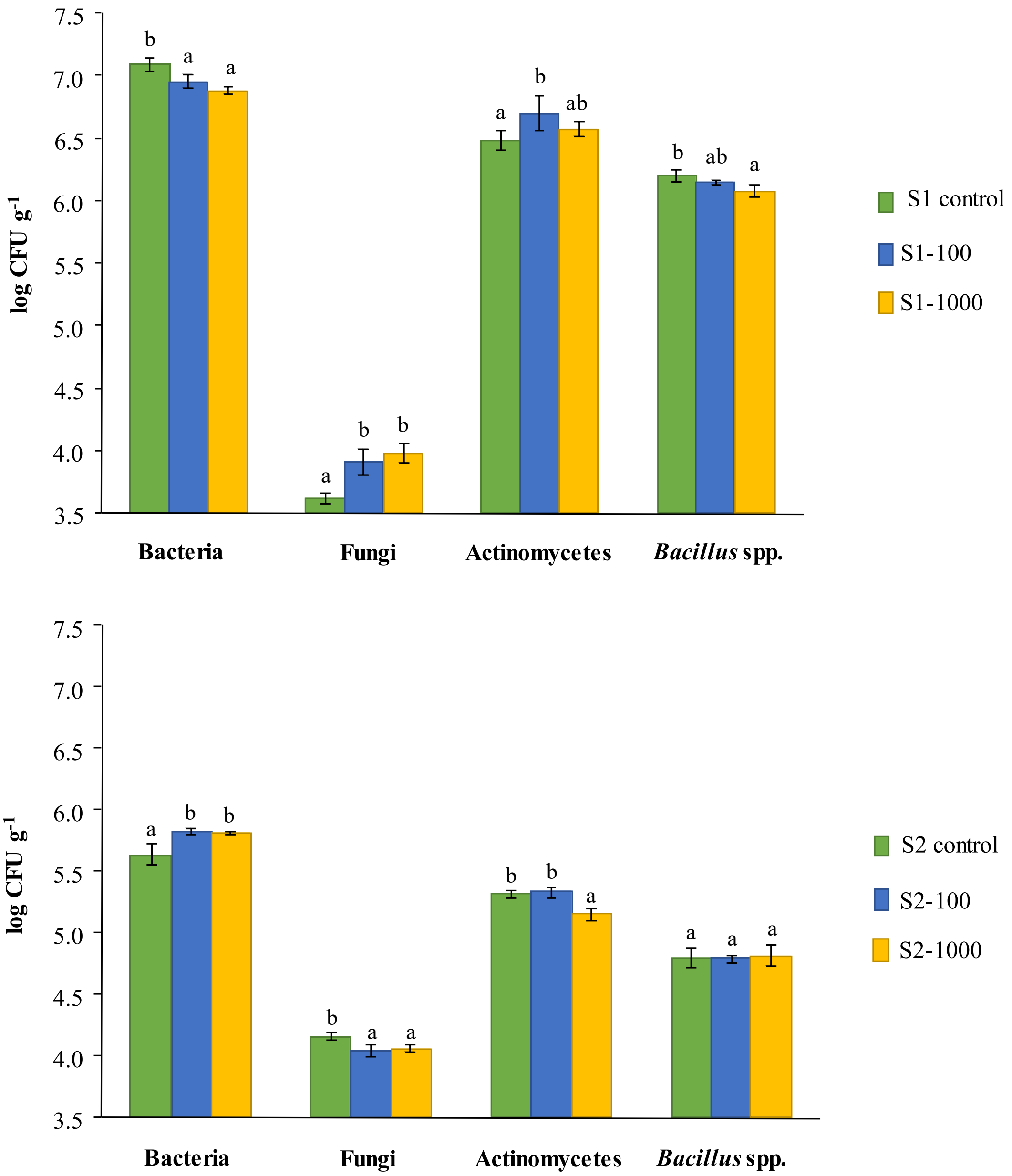


Figure 3 Average carbon source utilisation and richness values of microbial communities extracted from S1 (up) and S2 (at the bottom of the figure). Within each histogram, values which share a common letter do not differ significantly (ANOVA, Tukey Pairwise comparisons test, $P < 0.05$).

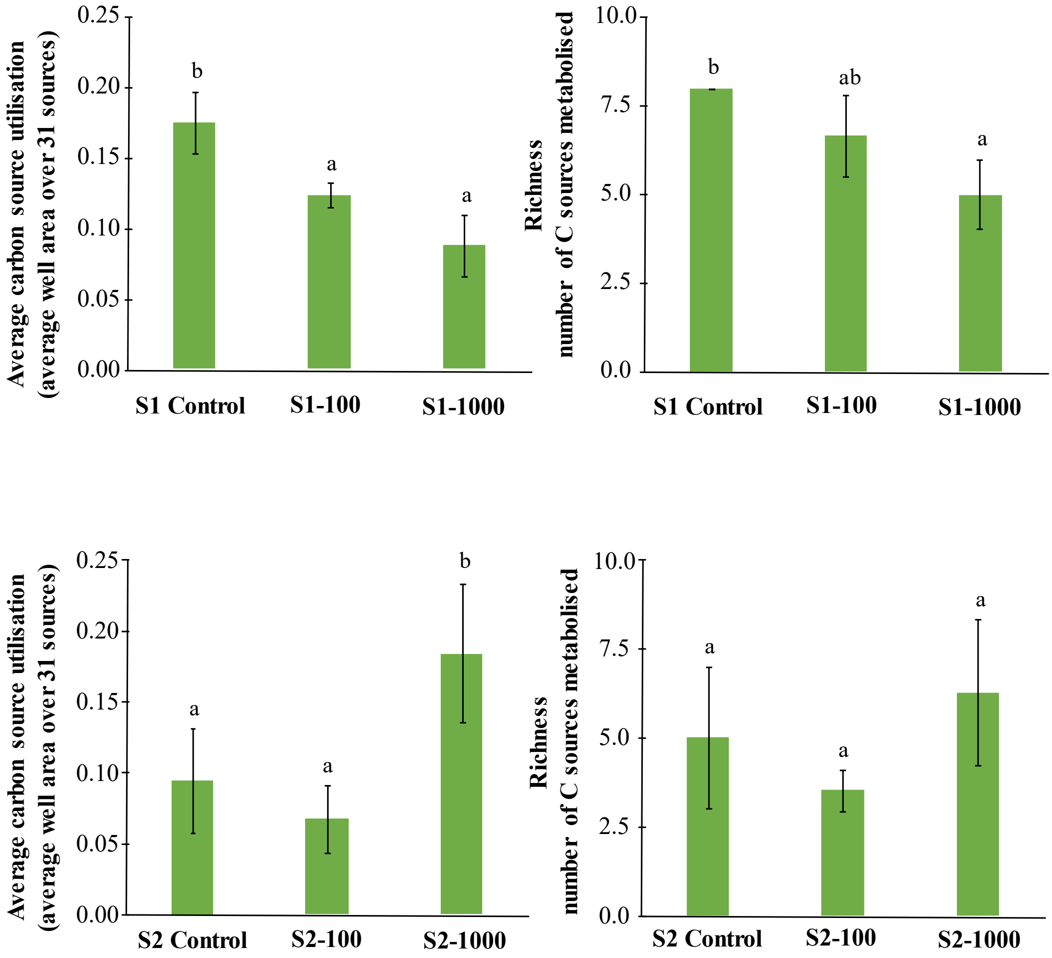
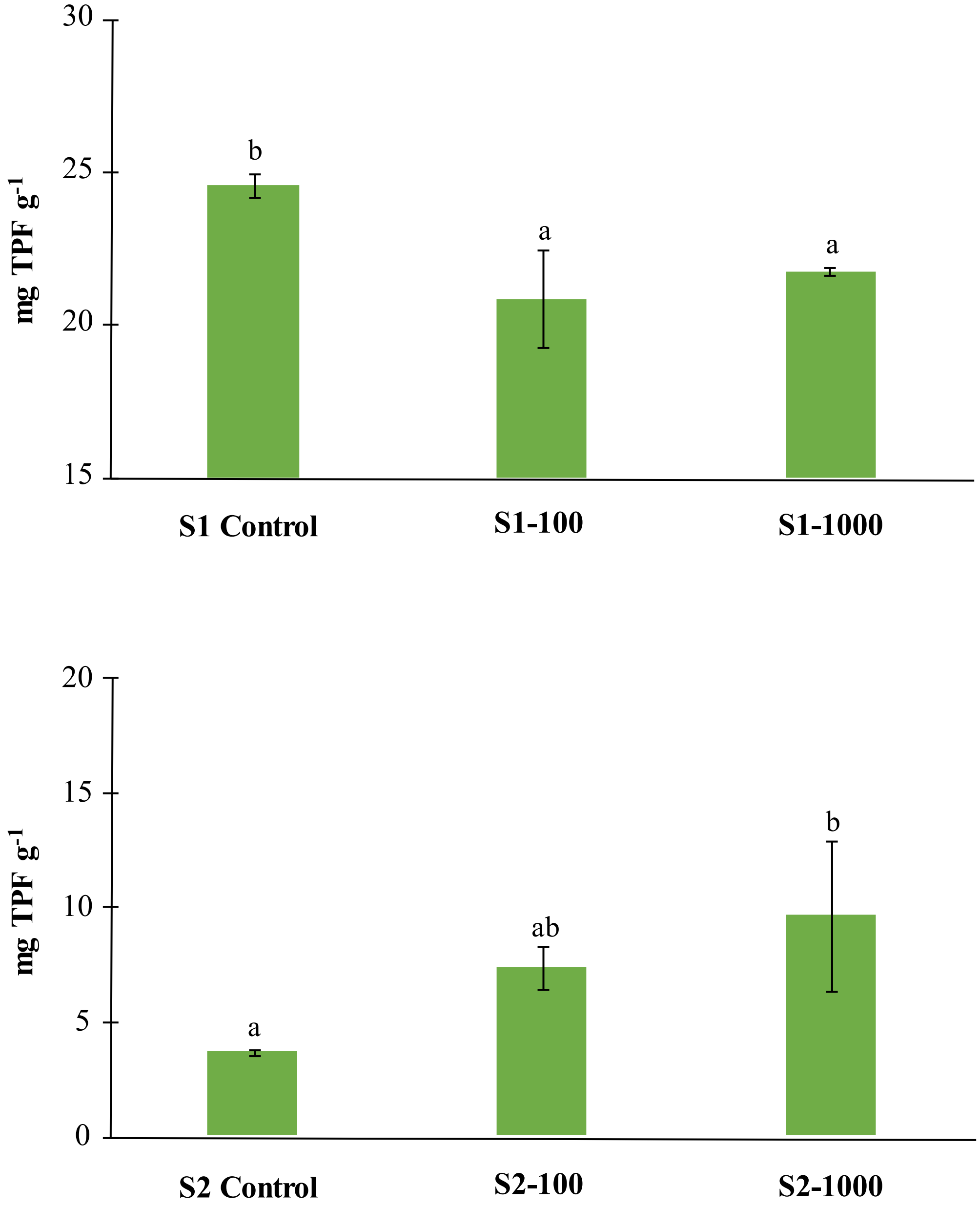


Figure 4 Dehydrogenase (DHG) activity in control and contaminated soils (100-1000 mg Kg^{-1}). S1 values on top and S2 values down. Within each soil, values which share a common letter do not differ significantly (ANOVA, $P < 0.05$).



Chapter 5

Use of municipal solid wastes for chemical and microbiological recovery of soil contaminated with metal(loid)s

Garau[1], G., Silvetti[1], M., Vasileiadis[2], S., Donner[2], E., Diquattro[1], S., Deiana[1], S., Lombi[2], E., Castaldi[1], P., 2017. Use of municipal solid wastes for chemical and microbiological recovery of soils contaminated with metal (loid) s. Soil Biology and Biochemistry, 111, 25-35.

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Use of municipal solid wastes for chemical and microbiological recovery of soils contaminated with metal(loid)s

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ABSTRACT

Iron-rich water treatment residues (Fe-WTRs) and municipal solid waste compost (MSWC) were added together at two different total rates (i.e. 0.5% Fe-WTRs+0.5% MSWC and 1% Fe-WTRs+1% MSWC) to a degraded sub-alkaline soil (pH 8.0) contaminated with Sb (~110 mg kg^{-1} soil), Pb (~1200 mg kg^{-1}), Cd (~23 mg kg^{-1}), and Zn (~5400 mg kg^{-1}). A large number of chemical and biological endpoints were evaluated to assess the efficacy of the treatments after five months of incubation. Both treatments significantly reduced the labile fractions of the metal(loid)s in soil, especially Sb, while increasing the abundance of culturable heterotrophic bacteria, actinomycetes and fungi (i.e. up to 6.3-, 1.6- and 4.1-fold higher than control respectively). Soil enzyme activities, i.e. dehydrogenase, β-glucosidase and urease, were also significantly enhanced in the treated soils (i.e. up to ~12-, 3- and 2-fold higher than control respectively). The amendment addition affected the structure of the soil microbial community as highlighted by the higher metabolic potential and catabolic versatility of treated soils (Biolog CLPP) and by the significantly higher α-diversity values based on high throughput partial 16S rRNA gene sequencing. Moreover, analysis of the dominant operational taxonomic units (OTUs) showed differences in the microbial communities of untreated and treated soils. Plant growth (*Helichrysum italicum*) in the treated soils was greatly stimulated while metal(loid)s uptake was significantly reduced. Overall, the results indicated that the applied treatment could be ideal for the chemical and (micro)biological recovery of sub-alkaline soils contaminated with Sb and co-occurring metals, and *H. italicum* appears to be a promising plant species for aided phytostabilisation of such soils.

1. Introduction

Antimony (Sb) is a non-essential plant element which occurs naturally in soil, with its main source being the weathering of soil parent materials containing minerals such as stibnite (Sb_2S_3) and valentinite (Sb_2O_3) (Kabata-Pendias, 2011). While natural background concentrations of Sb in soils range from 0.25 to 1.4 mg kg^{-1} (Kabata-Pendias, 2011), much higher concentrations can be found in the proximity of mining sites, mineral processing facilities and/or shooting ranges (due to human activities). In recent years, heavily Sb-polluted sites have been identified all around Europe, e.g. in Italy (~15,000 mg kg^{-1} soil), Germany (~500 mg kg^{-1} soil), Switzerland (~17,000 mg kg^{-1} soil), France (~5700 mg kg^{-1} soil) (Tschan et al., 2009) as well as worldwide (Wang et al., 2010; Sanderson et al., 2014). These sites, often characterised by critical concentrations of co-occurring metal(loid)s (Wang et al., 2010, 2011; Okkenhaug et al., 2013; Sanderson et al., 2014), can be of particular environmental concern since they represent hazardous multi-element contamination sources for neighbouring soils and water bodies and constitute a threat for soil functionality and fertility.

Bioavailable Sb and co-occurring metal(loid)s can severely compromise soil functionality by affecting the size, composition and activity of the resident microbial communities (Garau et al., 2011, 2014; Wang et al., 2011; Garau et al., 2014; Wei et al., 2015) as well as plant growth (Castaldi et al., 2009; Kabata-Pendias, 2011; Pan et al., 2011). For instance, previous studies have reported reduced growth and biomass of both grasses and legumes under Sb

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and/or heavy metal(loid)s stress (Castaldi et al., 2009; Pan et al., 2011; Garau et al., 2014) as well as decreased abundance of culturable soil microbial populations and reduced soil enzyme activities (Garau et al., 2007; Wang et al., 2011; Garau et al., 2014). Moreover, it was recently shown that elevated Sb reduced the diversity of arbuscular mycorrhizal fungi (Wei et al., 2015) and increased the distribution, diversity and abundance in soil bacterial populations of selected genes involved in Sb detoxification (Luo et al., 2014).

Although the co-occurrence of critical concentrations of Sb and additional metal(loid)s represents a substantial threat for soil and ecosystem functioning, effective and reliable approaches for the remediation of these polluted sites are currently lacking. In particular, the different speciation, mobility and bioavailability of Sb with respect to selected co-occurring metal(loid)s (e.g. trace metal cations) makes the identification of suitable amendments a very challenging task.

Antimony is commonly present in the environment in the trivalent (III) and pentavalent (V) states with the inorganic antimonite [$Sb(OH)_3$] and antimonate [$Sb(OH)_6^-$] being the dominant species in aqueous systems across a wide pH range (i.e. pH 4–10) (Okkenhaug et al., 2013). In aerated soils, the anionic species $Sb(OH)_6^-$ is prevalent (if not exclusive) (Johnson et al., 2005; Filella et al., 2009) and displays a high affinity to amorphous and crystalline Fe-(hydr)oxides with which it can form stable bidentate inner-sphere complexes (Guo et al., 2014). In the pH range of the majority of soils (i.e. pH 5–9), such interactions are particularly favoured by the high point of zero charge of amorphous and crystalline Fe-(hydr)oxides (e.g. pH_{PZC} 7.5–9.0 for goethite, 8.5 for hematite, 7.0–9.0 for ferrihydrite, 9.5–10 for akaganeite (Strawn et al., 2015)). However, in the same pH range (i.e. pH 5–9), heavy metals such as Pb, Cd and Zn behave quite differently, being commonly present in the soil solution as divalent and/or monovalent (hydroxylated) cations at acidic and circumneutral pH or as soluble SOM-metal(II) complexes at higher pH values (Kabata-Pendias, 2011). Moreover, at neutral and/or alkaline pH substantial amounts of heavy metals are immobilised as Me-hydroxides, -carbonates and/or -hydroxycarbonates. Due to their cationic nature, and in contrast to Sb(V), soluble heavy metals show a limited affinity for positively charged soil surfaces but interact more strongly with negatively charged components (e.g. soil organic matter and clay minerals).

In the last decades, a variety of potential sorbent materials for the in-situ remediation of metal(loid) polluted soils have been proposed and tested (Castaldi et al., 2009; Garau et al., 2011, 2014), however Sb-contaminated (or co-contaminated) sites have been largely overlooked and neglected until recently. To date, only a few amendments, mostly based on Fe- and Al-containing materials/minerals, have been tested with variable success as Sb-immobilising agents. For instance, adding olivine and hematite to a contaminated soil was found to have no effect on porewater Sb concentration (Okkenhaug et al., 2012), while red mud (alumina industry residues high in Fe-oxides) addition reduced porewater Sb only in some cases (Sanderson et al., 2015). The addition of organic amendments (e.g. mussel shell, cow bone, chicken manure, sodium humate) have mostly been deemed ineffective at fixing Sb (Conesa et al., 2010; Ahmad et al., 2014; Shtangeeva et al., 2014), although mussel shell and cow bone, and also red muds, reduced the labile co-occurring Pb from the soil solution (Ahmd et al., 2014; Sanderson et al., 2015). Other research has shown that a 2% commercial Fe-oxyhydroxide, and very high rates of amorphous Fe- and Al-oxyhydroxides, could effectively stabilise the antimony in different polluted soils (Alvarez-Ayuso et al., 2013; Okkenhaug et al., 2013, 2016). However, the mobilisation of co-occurring metals (e.g. Pb, Cu, and Zn) was detected after the addition of Fe-based materials to Sb-contaminated soils (Okkenhaug et al., 2013). These findings, together with the limited number of studies addressing the remediation of Sb-contaminated sites, highlight the need to deepen our knowledge on the physico-chemical factors governing the mobility of Sb (and co-occurring metal(loid)s) in soil and to further select for sorbents with ideal Sb-immobilising capabilities. Such sorbents, other than reducing the mobility and phytoavailability of Sb and co-occurring metal(loid)s, should also ideally improve soil fertility as well as soil microbial abundance, diversity and functionality.

In this context, the aim of this work was to evaluate the suitability of the combined application of two low-cost and sustainable amendments for the chemical and biological recovery of a degraded alkaline soil contaminated with Sb, Pb, Cd and Zn. Iron-rich drinking-water treatment residuals (Fe-WTRs) and municipal solid waste compost (MSWC) were the selected amendments.

The presence in Fe-WTRs of an abundant mineral component rich in Fe-(hydr)oxides makes these residues potentially effective for the immobilisation of Sb in our alkaline soil (Guo et al., 2014), while earlier studies also suggest their suitability as metal-fixing agents (Castaldi et al., 2015). On the other hand, the application of MSWC together with Fe-WTRs was expected to contribute to soil recovery mainly by improving its nutritional and functional status.

The effectiveness of the combined application of Fe-WTRs and MSWC in a 1:1 ratio, at two different total rates (i.e. 1 and 2%), was assessed in this study using a comprehensive approach that spanned chemical investigation of metal(loid)-mobility, plant growth and pollutant phytoavailability, soil microbial abundance and bacterial diversity, community level physiological profiling, and soil enzyme activity.

2. Materials and methods

2.1. Soil and amendment origins, characteristics and microcosm set up

Sixty topsoil samples (0–20 cm depth; ~2 kg each) were collected from an area of approximately 1 ha located in the abandoned mining site of Argentiera (N40°44′11″, E8°8′54″) in north-western Sardinia (Italy), where Pb, Ag and Zn were extracted for about one century (1867–1963) from silver-rich galena [(Pb, Ag)S] and sphalerite (ZnS). The site is characterized by the presence of Sb-rich minerals such as boulangerite ($Pb_5Sb_4S_{11}$), tetrahedrite [$(Cu,Fe)_{12}Sb_4S_{13}$], freibergite [$(Ag,Cu,Fe)_{12}(Sb,As)_4S_{13}$], bournonite ($PbCuSbS_3$) and pyrargyrite ($Ag_3SbS_3$) (Pirri, 1996). According to particle-size analysis, carried out using the pipette method (Day, 1965), the soil in the sampled area was a sandy-loam (USDA classification) with 68% coarse sand, 15% fine sand and 17% silt.

The collected soil samples were bulked together, air dried and sieved to <2 mm before being employed for the set-up of different soil microcosms, each consisting of approx. 10 kg soil. Triplicate microcosms were amended with 0.5% (w/w) Fe-WTRs + 0.5% (w/w) MSWC (1% total amendment rate) or 1% (w/w) Fe-WTRs + 1% (w/w) MSWC (2% total amendment rate) or left untreated as controls. These rates were selected based on the specific metal(loid)s immobilising capabilities of Fe-WTRs and MSWC highlighted in previous studies (Manzano et al., 2016; Garau et al., 2014). Before addition to the microcosms, Fe-WTRs (provided by the Bidighinzu plant of the Abbanoa industry, Sassari, Italy) and MSWC (provided by the Secit S.p.A. facility plant of the Consorzio ZIR, Chilivani-Ozieri, Sassari, Italy) were oven dried at 60 °C for 48 h and sieved to <2 mm.

After amendment, treated (and untreated) soils were carefully mixed, brought to 60% of their water holding capacity and equilibrated for 5 months at 20 °C. During this period, soils were mixed twice a week and maintained at 60–70% relative humidity.

2.2. Amendments and soil chemical analyses

Selected physico-chemical characteristics of the treated and untreated soils after equilibration, and of the amendments used, are reported in Tables 1 and 2. The pH and electric conductivity (EC) were determined using a 1:2.5 soil/amendment:deionised water ratio according to national standard guidelines (Gazzetta Ufficiale, 1999, 2002). The total organic matter and N were determined according to the Walkley-Black and to the Kjeldhal method respectively (Gazzetta Ufficiale, 1999, 2002) while total P was determined spectrophotometrically (ascorbic acid method) (Gazzetta Ufficiale, 1999, 2002). The dissolved organic carbon (DOC) was estimated as described by Brandstetter et al. (1996) while the point of zero charge (pH_{PZC}) has been determined by potentiometric titration as described in Appel et al. (2003). The method reported by Ciavatta et al. (1990) was used to determine the content of humic substances (i.e. humic and fulvic acids) while the specific surface area was determined by applying the BET model to the N_2 adsorption results obtained from a Carlo Erba Sorptomatic instrument (Milan, Italy) (Castaldi et al., 2010). Total and active carbonates were determined by acid dissolution (volumetric calcimeter method) and by reaction of soil with 0.1 M ammonium oxalate respectively (Gazzetta Ufficiale, 1999, 2002). Soil cation exchange capacity (CEC) was determined using the $BaCl_2$ and triethanolamine method (Gazzetta Ufficiale, 1999).

Total concentration of metal(loid)s in soils and amendments was determined after digestion with an HNO_3/HCl mixture (1:3 v/v ratio) using a flame atomic absorption spectrometer equipped with a HGA-600 graphite furnace (Perkin Elmer Analyst 600). The NIST-SRM 2710 was used as standard reference material for quality assurance and quality control.

The Sb mobility in soil was assessed through the sequential extraction procedures of Wenzel et al. (2001) (originally designed for soil As fractionation) while the protocol of Basta and Gradwohl (2000) was used to assess the mobility of Cd, Zn and Pb. Briefly, labile and surface-bound Sb in soil was assessed after extraction with 0.05 M $(NH_4)_2SO_4$ (Step 1) and 0.05 M $(NH_4)H_2PO_4$ (Step 2) respectively, while the Sb associated to amorphous and crystalline Al- and Fe-oxides was determined after extraction with 0.2 M NH_4-oxalate (Step 3) and 0.2 M NH_4-oxalate solution + 0.1 M ascorbic acid (Step 4) respectively (Wenzel et al., 2001). Exchangeable and readily soluble Cd, Zn and Pb in soil were determined after extraction with 0.5 M $Ca(NO_3)_2$ while acid-soluble metals and weak surface complexes were determined after extraction with 1 M NaOAc (pH 5) (Basta and Gradwohl, 2000). Surface complexes and metal precipitates were determined after 0.1 M Na_2EDTA extraction. After each step of the respective extraction procedures, the samples were centrifuged at 8000 rpm for 10 min, filtered to completely separate the liquid and solid phases and metal(loid)s in the supernatant determined using a flame atomic absorption spectrometer equipped with a HGA-600 graphite furnace (Perkin Elmer Analyst 600). The residual Sb in soil as well as the very insoluble and/or occluded metals were determined after microwave digestion with an HNO_3/HCl mixture as previously mentioned.

Table 1
Selected physico-chemical characteristics of the amendments used in this study (dry matter basis).

Physico-chemical parameters	Fe-WTR	MSWC
$pH(H_2O)$	7.88 ± 0.04	7.93 ± 0.06
EC ($mS \cdot cm^{-1}$)	3.01 ± 0.02	3.26 ± 0.03
S_{BET} ($m^2 \cdot g^{-1}$)	35.00	2.78
pH_{PZC}	7.80	4.53
Organic matter ($g \cdot kg^{-1}$)	244.71 ± 2.79	420.19 ± 3.44
Fulvic and Humic acids ($g \cdot kg^{-1}$)	24.34 ± 1.05	153.24 ± 2.76
DOC ($mg \cdot g^{-1}$)	0.101 ± 0.00	0.573 ± 0.04
Total N ($g \cdot kg^{-1}$)	8.04 ± 0.28	21.84 ± 0.42
Total P ($g \cdot kg^{-1}$)	0.68 ± 0.03	7.16 ± 0.41
Total metals ($mg \cdot kg^{-1}$)		
Fe	245,480 ± 1350	5587 ± 88
Mn	31,636 ± 764	141 ± 6
Ca	89 ± 2	63,444 ± 130
Mg	69 ± 2	5403 ± 135
Na	32 ± 1	993 ± 24
K	99 ± 3	1709 ± 21
Pb	12 ± 0.3	3.7 ± 0.1
Zn	246 ± 7	30 ± 3
Cd	0.2 ± 0.0	n.d.
Cu	29 ± 1	19 ± 2

n.d., not detected.

2.3. Heterotrophic microorganisms, community level physiological profiles and soil enzyme activities

Fast-growing culturable bacteria, fungi and actinomycetes were enumerated in soil microcosms (after equilibration) using the conventional ten-fold serial dilution and spread plate method. Solidified (15 g L^{-1} agar) 1:10 strength TSA (Tryptic Soy Agar, Microbiol, Cagliari, Italy), GYEP at pH 4.5 (Glucose Yeast Extract Peptone medium) and AIA (Actinomycete Isolation Agar, Difco, Milan, Italy) were used as growth media for the enumeration of culturable bacteria, actinomycetes and fungi respectively (Pinna et al., 2012). Microbial counts were conducted after incubation of the plates for 72 h at 25 °C.

The Community Level Physiological Profile (CLPP) of microbial communities was determined using Biolog Ecoplates (Biolog Inc., Hayward, CA). Microbial communities were extracted from the respective soils and separately inoculated in each of the 96 microtiter wells of a Biolog Ecoplate as previously described (Pinna et al., 2012). The Biolog Ecoplate contains a triplicate set of 31 carbon sources of soil/environmental relevance, each present in a separate well, and three control wells with no carbon. A tetrazolium dye incorporated in the plate wells reveals oxidative catabolism as purple colour formation. This latter was recorded for each well every 15 min for 96 h using a Biolog Omnilog unit (i.e. a charge-coupled-device camera; Biolog, Hayward, CA) to obtain a colour vs. time curve. For each well/substrate, the area under this curve was control-subtracted and the potential catabolic activity of each microbial community was calculated as the average well area over the 31 carbon sources (AWCD; Garau et al., 2007, 2014). Richness values (i.e. the number of carbon sources metabolised by each microbial community) were determined as the number of wells with (control-subtracted) areas higher than 1000 (Biondi et al., 2009).

Selected enzyme activities, i.e. dehydrogenase (DHG), β-glucosidase (GLU) and urease (URE), were determined (colorimetrically) in duplicate soil samples collected from each microcosm as previously described (Garau et al., 2007). Briefly, DHG activity was quantified by determining the concentration of triphenyl formazan produced after incubation of soil samples (5 g each) with triphenyltetrazolium chloride for 24 h at 30 °C. GLU activity was determined as *p*-nitrophenol released after incubation of soil samples (1 g each) with *p*-nitrophenyl glucoside for 2 h at 37 °C. URE activity was determined as ammonia released after incubation of soil samples (5 g each) with urea for 2 h at 37 °C.

Table 2
Physico-chemical characteristics of the treated (amended) and untreated contaminated soils (dry matter basis). Shaded values are exceeding the national (Italian) threshold values for private, residential and public green areas and for commercial and industrial sites (see Table S2 in SI for details).

Physico-chemical parameters	Untreated soil	Amended soils	
		Fe-WTR + MSWC (1.0%)	Fe-WTR + MSWC (2.0%)
pH(H_2O)	8.01 ± 0.08	7.97 ± 0.02	7.92 ± 0.03
EC (mS·cm^{-1})	1.43 ± 0.23	1.65 ± 0.09	1.69 ± 0.10
Total carbonate (g·kg$^{-1)}$)	174.0 ± 5.0	185.7 ± 3.4	199.3 ± 2.9
Active carbonate (g·kg^{-1})	64.0 ± 9.6	79.2 ± 3.8	83.3 ± 1.4
CEC (cmol$_{(+)}$·kg^{-1})	16.92 ± 0.17	21.31 ± 1.24	33.46 ± 2.43
pH$_{PCZ}$	6.90	7.42	7.49
Organic matter (g·kg^{-1})	3.89 ± 0.03	5.93 ± 0.03	7.48 ± 0.06
DOC (mg·g^{-1})	0.029 ± 0.001	0.123 ± 0.002	0.213 ± 0.006
Total N (g·kg^{-1})	0.70 ± 0.03	0.81 ± 0.06	1.02 ± 0.06
Total metal(loid)s (mg·kg^{-1})			
Fe	40,533 ± 1197	43,382 ± 470	43,972 ± 698
Mn	1758 ± 34	1837 ± 315	2054 ± 68
Ca	50,132 ± 2301	49,162 ± 467	50,653 ± 728
K	4269 ± 362	4114 ± 394	4331 ± 321
Sb	108.86 ± 1.74	106.46 ± 0.84	105.85 ± 0.97
As	7.67 ± 0.76	7.44 ± 0.25	7.42 ± 0.21
Pb	1214 ± 31	1184 ± 4	1146 ± 25
Zn	5400 ± 70	5468 ± 482	5385 ± 137
Cd	23.17 ± 2.87	22.38 ± 1.24	24.86 ± 4.54
Cu	48.86 ± 2.48	45.65 ± 3.62	46.86 ± 2.62

2.4. Molecular analysis of soil bacterial communities and bioinformatics

Total DNA was extracted from soil samples (~500 mg) of each microcosm using the FastDNA™ SPIN Kit and FastPrep® Instrument (MP Biomedicals, Santa Ana, CA) according to the manufacturer's instructions with the homogenization step performed 3 times at maximum speed and time (3 × 6.5 m s^{-1} × 60 s). DNA extracts were quantified using the Qubit® dsDNA HS Assay kit (Invitrogen, Waltham, Massachusetts, USA) with the Qubit® v2.0 Fluorometer (Invitrogen, ThermoFisher-Scientific, Waltham, Massachusetts, USA) and stored at −20 °C prior to downstream analyses. The V3-4 hypervariable region of the bacterial small ribosomal subunit coding gene (SSU) was amplified via polymerase chain reaction (PCR) performed on 0.2 ng of the DNA extracts using the 343SF forward primer (5′-TACGGGAGGCAGCAG-3′; modified version of the S-D-Bact-0343-a-S-15 (Vasileiadis et al., 2012)) and a modified version of the 802R reverse primer (5′-TACNVGGGTWTCTAATCC-3′; version of S-D-Bact-0785-b-A-18 (Claesson et al., 2009)) in 20 μl reactions. The PCR reactions were performed in two steps to restrict amplification and high throughput sequencing indexing related biases as described previously (Berry et al., 2011). The primers mentioned above were used in the first step while the indexed primer versions (for multiplexing prior to sequencing) provided in Table S1 were used in the second step. Briefly, 2 μl of DNA extracts diluted to 0.1 ng μl^{-1} were added as templates to 18 μl mixtures containing 10 μl Phusion Flash High-Fidelity PCR Master mix (Finnzymes, ThermoFisher-Scientific, Waltham, Massachusetts, USA), 1 μl of each primer solution (10 μM), 0.4 μl of bovine serum albumin (BSA – ThermoFisher-Sientific – Waltham, Massachusetts, USA – 20 mg ml^{-1}) and 5.6 μl PCR grade water. The resulting 20 μl reaction mixtures were subject to the first PCR comprising: an initial step of 3 min at 95 °C for enzyme activation/DNA-denaturation; followed by 28 cycles of 95 °C for 15 s for DNA denaturation, 50 °C for 15 s for primer annealing and 72 °C for 15 s for DNA elongation; followed by 10 min at 72 °C for the final elongation step. Two microliters of the resulting amplification products were used as DNA template in the second PCR cycle (same mixture as above; 7 cycles only) to add indexed primers (Table S1) in preparation for sequencing. Equimolar amounts of the PCR products were then multiplexed and sequenced with the Illumina HiSeq 2500 platform for 300 cycles using the paired-end reads module by Fasteris SA (Geneva, Switzerland).

Output sequences were analysed with the LotuS v1.44 software (Hildebrand et al., 2014) as follows: reads were de-multiplexed and quality controlled with the LotuS native simple de-multiplexer (smd) v1.26 using the default parameters with the exception of a minimum good quality sequence length of 170 bp; read pairs were then merged with FLASH v1.2.8 (Magoč and Salzberg, 2011); clustering of reads into 97% identity operational taxonomic units (OTUS) was performed with the USEARCH/UPARSE v8.0.1623 algorithm (Edgar, 2013); chimeric OTUs were removed with UCHIME v4.2 (Edgar et al., 2011) and the ribosomal database project (RDP) gold database version; and representative OTU sequences were classified into known taxa using the RDP naïve Bayesian classifier v2.11 (Wang et al., 2007). Selected OTU sequences were further analysed to obtain phylogenetic information according to a maximum likelihood approach, whereby closely related sequences were obtained from the National Center for Biotechnology Information (NCBI) 16S rRNA gene type strain collection using the basic local alignment search tool (BLAST) (Altschul et al., 1990). The best BLAST hits were clustered with Cdhit v4.6 (Li et al., 2001) to reduce sequence redundancy and the cluster representative sequences were aligned with Muscle v3.8.31 (Edgar, 2004). Non-properly aligned and uninformative alignment blocks were removed using Gblocks v0.91b (Talavera and Castresana, 2007) and the remaining concatenated alignment blocks were subjected to maximum likelihood phylogenies with the RAxML software v8.1.24 (Stamatakis, 2014) using the general time-reversible (GTR) model for gamma rate heterogeneity and considering invariable sites using 1000 bootstrap replicates. The sequences obtained were submitted to the NCBI (NCBI - https://www.ncbi.nlm.nih.gov) Sequence Read Archive (SRA - https://www.ncbi.nlm.nih.gov/sra/) and are currently available under the accession numbers from SRR4424247 to SRR4424255.

2.5. Plant growth experiment and plant analysis

At the end of the 5 months incubation, soils from each microcosm were used to grow *Helichrysum italicum* (Roth) G.Don plantlets (approx. height 5 cm) in plastic pots [23 cm (L) × 15 cm (H) × 15 cm (D)] filled with ~3 kg of soil. We selected this plant species as it was growing abundantly at the site investigated suggesting an apparent tolerance to the metal(loid)s present and good adaptability to the low fertility conditions which characterised the soil. A total of thirty-six *H. italicum* plantlets were grown on each treated and untreated soil in 6 pots (6 plants x pot; 2 pots x microcosm) for 16 weeks under controlled conditions (20 °C; 60–70% relative humidity). At harvest, shoots and roots were separated, thoroughly washed with distilled water, and oven-dried at 70 °C for 96 h for dry weight determination.

Metal(loid)s in shoots and roots were determined, after mineralization of the plant material (EPA Method 3052), using a flame atomic absorption spectrometer equipped with a HGA-600 graphite furnace (Perkin Elmer Analyst 600). Peach leaf was used as standard reference material (NIST-SRM 1515).

2.6. Data analysis

Chemical (i.e. sequential extraction) and (micro)biological data were subjected to One-way analysis of variance and when significant *P* values were obtained ($P < 0.05$) differences between individual means were compared with the post-hoc Tukey Kramer multiple comparison test ($P < 0.05$) using the NCSS software (Keysville, Utah). The 16S rRNA gene sequence data were analysed using the R v3.2.3 software (R Core Team, 2015). Tests performed included: Hellinger transformation of the OTU matrix as suggested by Legendre and Gallagher (2001) followed by hierarchical clustering using the unweighted pair group method with arithmetic mean (UPGMA) and principal component analysis with modelling of the physical/chemical variables with the Vegan package v2.4-0 (Oksanen et al., 2016). Treatment group related OTU differential abundances were assessed with Fisher's exact tests as implemented by the Edge R v3.12.0 package using the Holm correction for multiple hypothesis testing (Robinson et al., 2010).

3. Results and discussion

3.1. Influence of the amendments on physico-chemical soil features and metal(loid) mobility in soil

In this study we evaluated the combined application of Fe-WTRs and MSWC, at two different rates (i.e. at 1 and 2%), as potential amendments for the remediation of an alkaline (pH 8.0) degraded soil polluted by Sb and co-occurring metals. The soil was characterized by a significant content of sand (~83% coarse and fine sand), a medium cation exchange capacity (CEC, ~17 $cmol_{(+)} \cdot kg^{-1}$ soil) and very low organic matter content (OM, ~0.4%) (Table 2). Moreover, the total concentrations of Sb, Cd, Zn and Pb all exceeded the national (Italian) threshold values for private, residential and public green areas and for commercial and industrial sites (Tables 2 and S2).

The combined addition of Fe-WTRs and MSWC clearly improved some fertility characteristics of the soil such as the CEC (>25 and 97% increase at 1 and 2% amendment rates, respectively), OM (>50 and 90%), and N content (>15 and 45%) (Table 2). DOC also increased significantly after soil amendment (>400 and 700% at 1 and 2% amendment rates, respectively) while pH, EC, total and active carbonate were little affected (Table 2).

The amendment additions did not change the total concentrations of metal(loid)s in the soil (Table 2). On the other hand, the sequential extraction data indicated a relatively low content of readily labile and surface-bound Sb in the untreated polluted soil, i.e. the water-soluble and easily exchangeable Sb extracted in Step 1 and the Sb forming inner-sphere complexes extracted in Step 2 respectively (Fig. 1). This may be encouraging from an environmental point of view since these Sb fractions can be considered to have the greatest impact on soil biota (Wenzel et al., 2001; Garau et al., 2014). Most of the Sb in the polluted soil (~85%) was instead associated with amorphous and especially crystalline Al- and/or Fe-oxides as highlighted in Step 3 and 4 respectively (Fig. 1).

The added amendments had no obvious effects on the readily labile Sb but significantly reduced the amount of metalloid extracted in Step 2 (Fig. 1). This is relevant as this Sb fraction can be simply mobilised (i.e. becoming bioavailable) following a pH change (e.g. due to plant and/or microbial activity) or phosphate increase (Wenzel et al., 2001). The Sb fraction associated with amorphous Al- and Fe-oxides (Step 3) increased significantly after soil amendment while the opposite was true for the metalloid fraction associated to crystalline Al- and Fe-oxides (Step 4; Fig. 1). Finally, the residual Sb fraction (i.e. that is strongly retained and not expected to be readily released) increased by approximately 3- and 4-fold in the soils amended at 1 and 2% rate respectively.

Overall, these data show that the combined addition of Fe-WTRs and MSWC induced a significant redistribution of Sb in soil, i.e. a reduction of its more labile and bioavailable forms and an increase of the residual (i.e. not readily bioavailable) forms. This can be the result of different mechanisms induced by the amendments, likely including Sb mobilisation from soil and its redistribution within the soil/amendment components. In particular, Sb mobilisation in the treated soils could have occurred through competitive exchange phenomena involving organic and inorganic components of both amendments, but also through partial dissolution of Sb-containing Al and Fe-(hydr)oxides (amorphous and crystalline) due to solubilisation of Fe/Al nuclei. As recently highlighted (Chen et al., 2016), fulvic acids within Fe-WTRs and MSWC may have played an important role in this (Table 1). On the other hand, the formation of soluble complexes between the organic matter within Fe-WTRs/MSWC and Sb is not expected to be relevant at the pH value of this soil (Filella and Williams, 2012).

As supported by the sequential extraction data (Step 3; Fig. 1), sorption of Sb in the amended soils may have involved existing amorphous Al- and Fe-(hydr)oxides in soil and, above all, the amorphous Al- and Fe-(hydr)oxides added with Fe-WTRs (Table 1). This could explain the significant increase of the Sb detected in Step 3 and the reduction of that extracted in Step 4 (Fig. 1). Moreover, the precipitation of part of the released $Sb(OH)_6^-$ with soluble Ca^{2+} (abundantly present within MSWC; Table 1) may also have contributed to the increase of the residual Sb pool in the treated

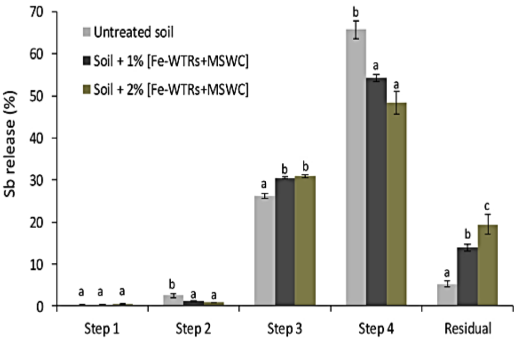

Fig. 1. Sequential extraction of Sb from untreated polluted and amended soils.

soils (Johnson et al., 2005; Conesa et al., 2010).

The sequential extraction of Basta and Gradwohl (2000) showed a limited amount of water soluble and exchangeable Cd, Zn and Pb in the contaminated soil (Fig. 2). This was somewhat expected in this alkaline (carbonate-rich) soil where a substantial amount of the metals are likely to be precipitated as metal carbonates and/or as metal (hydr)oxides (Kabata-Pendias, 2011). Accordingly, approx. 40, 50 and 66% of total Zn, Cd and Pb respectively were extracted with EDTA (Fig. 2) suggesting that these relatively immobile (and not readily bioavailable) metal fractions were likely involved in inner-sphere surface complexes and/or were in the form of precipitates (Basta and Gradwohl, 2000). The residual metal fractions in the contaminated soil, i.e. very insoluble and/or occluded metal pools, were approx. 30, 45 and 50% of total Pb, Cd and Zn respectively (Fig. 2). The most important amendment effect was the significant reduction of water soluble and exchangeable Cd, Zn and Pb, i.e. the more mobile and bioavailable fractions. This could be mainly explained with the capacity of the organic and inorganic components of both the amendments to sorb the metals considered as previously highlighted (Castaldi et al., 2015; Silvetti et al., 2015; Manzano et al., 2016). A reduction of the acid-soluble and/or weakly complexed Pb (extracted with NaOAc) was also observed after amendment addition together with a significant increase of the residual Cd (Fig. 2).

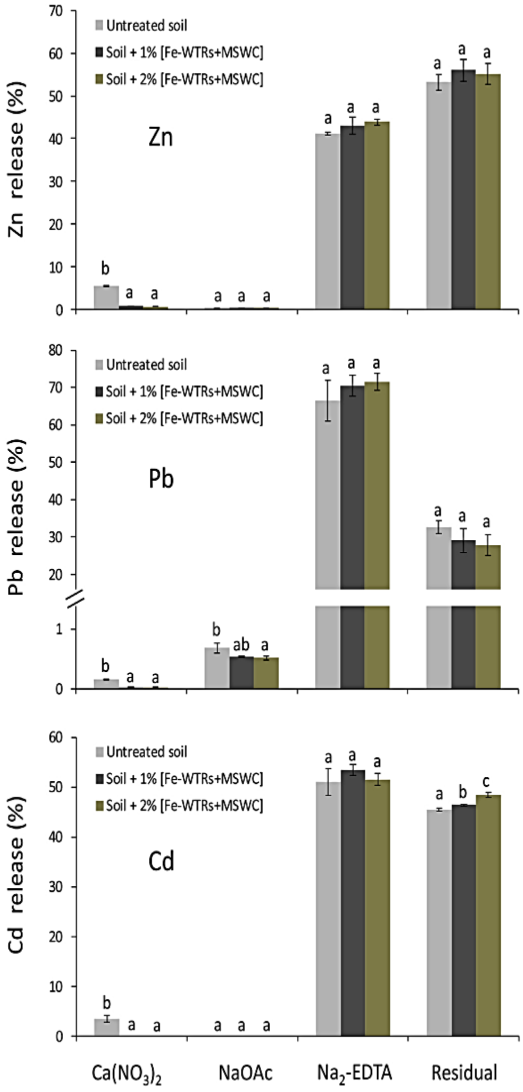

Fig. 2. Sequential extraction of metals from untreated polluted and amended soils.

Overall, the addition of Fe-WTRs and MSWC to the contaminated soil contributed to lower the environmental impact of the metal(loid)s present by limiting the amount of their soluble and exchangeable forms and in some cases (especially for Sb and Cd) by substantially increasing their residual fraction. As previously pointed out, this is expected to have relevant positive effects on the soil microbial component and its functionality (Garau et al., 2007, 2011; Garau et al., 2014).

3.2. *Influence of the amendments on the soil microbial abundance, enzyme activities and community level physiological profile*

The combined addition of Fe-WTRs and MSWC induced significant increases in all targeted soil microbial groups. In the case of total culturable heterotrophic bacteria and actinomycetes (but not fungi) the increase in CFUs (colony forming units) g^{-1} soil was also positively correlated with the amendment rate (Table 3). This increase of readily culturable microorganisms in the treated soils was likely due to the presence of additional easily metabolisable carbon sources deriving from the OM within MSWC and Fe-WTRs (Garau et al., 2011). This was supported by the significant increase of the DOC and OM in the amended soils (Table 2). The reduction of labile metal(loid) fractions in the treated soils (Figs. 1–2) could have also contributed to microbial growth by increasing the metabolic energy dedicated by microorganisms to cell growth (and multiplication) instead of high-energy demanding metal detoxification processes (Garau et al., 2011). The number of readily culturable bacteria and fungi can provide an estimate of the potentially active microbial populations in soil, i.e. microbial populations involved in the prompt degradation of soil organic matter and the release (and cycling) of mineral elements essential for plant growth, e.g. N and P (Timms-Wilson et al., 2006). In this sense, the increase of these microbial populations in the amended soils can be considered as positive as it suggests an improved fertility status while confirming a reduced environmental impact of the contaminants.

Enzyme activity data indicated functional improvements in the treated soils. In particular, higher DHG values were recorded in amended soils compared to the control (approx. 6 and 12-fold higher at 1 and 2% amendment rate). Also URE and GLU significantly increased after 2% amendment (2.17 and 2.9-fold higher respectively compared to controls) (Table 3). All this indicated a higher microbial activity in the amended soils and possibly an improved C and N turnover. Pearson correlation analysis indicated significant ($P < 0.05$) positive correlations between the number of culturable microorganisms and all the enzyme activities (r = 0.69–0.94; Table S3). This may support the view that culturable microorganisms may play a role in soil functioning and nutrient turnover. However, it should be noted that the relationship between soil functioning and microbial community is only partially explored by culturable techniques and it cannot be excluded that unculturable microorganisms also play a very important role.

The Biolog Community level physiological profile (CLPP) showed significant differences between untreated and treated soils. In particular, the microbial populations extracted from treated soils displayed a significantly higher potential carbon source utilisation compared to those extracted from control soil (Fig. 3). This increase also appeared proportional to the amendment rate. The same was recorded for richness values, or the number of carbon sources metabolised by the microbial communities (Fig. 3). As pointed out in earlier studies, fast-growing culturable bacteria are the most

Table 3
Counts of heterotrophic microorganisms and selected enzyme activities in the untreated polluted (UP) and amended soils. All values are expressed on a soil dry matter basis. DHG, dehydrogenase; GLU, β-glucosidase; URE, urease. Within each column, values which share a common letter do not differ significantly (Tukey Kramer multiple comparison test, $P < 0.05$).

	Microbial counts (log CFU·g^{-1} soil)			Enzyme activities		
	Bacteria	Fungi	Actinomycetes	DHG (μg TPF·g^{-1} soil)	GLU (μg ρ-nitrophenol·g^{-1} soil)	URE (μg NH_4-N·g^{-1} soil)
UP soil	5.81 ± 0.07^a	4.79 ± 0.09^a	5.57 ± 0.03^a	2.93 ± 0.29^a	70.67 ± 10.06^a	2.37 ± 0.36^a
UP soil+1% amendment	6.36 ± 0.07^b	4.89 ± 0.03^a	5.97 ± 0.01^b	18.86 ± 1.72^b	95.06 ± 1.79^a	3.25 ± 0.06^a
UP soil+2% amendment	6.61 ± 0.01^c	5.00 ± 0.05^b	6.19 ± 0.02^c	37.41 ± 6.72^c	208.65 ± 16.07^b	4.96 ± 0.44^b

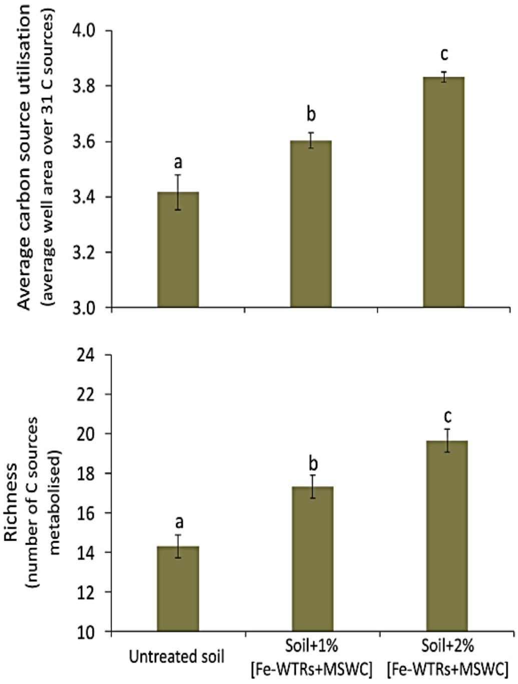

Fig. 3. Average carbon source utilisation and richness values of microbial communities extracted from untreated polluted and amended soils.

relevant contributors of colour development in Biolog Ecoplates (Pinna et al., 2012; Garau et al., 2014) and in this sense, Biolog data support the higher bacterial counts recorded in the treated soils (Table 3). Moreover, CLPP analysis also indicates that the amendment addition had a positive effect on the catabolic versatility of the microbial communities as indicated by the richness values (Fig. 3). For instance, only the microbial communities of treated soils were able to catabolise i-erythritol, ʟ-serine and D-glucosaminic and 4-hydroxy benzoic acids (other than the substrates catabolised by the control soil microbial community) while glycogen and itaconic acid were exclusively metabolised by the microbial populations extracted from the 2% amended soil. The appearance in the amended soils of microbial communities with higher and more diverse catabolic potentials could be partly the result of reduced environmental pressure (i.e. reduced metal(loid) bioavailability) on microbial taxa that were poorly represented in the contaminated soil (e.g. because they are more sensitive to contaminants). All this combined with the larger availability in the treated soils of easily metabolisable carbon sources could have sustained the growth of these rare taxa and allowed for the appearance of the new phenotypes.

3.3. Amendment impact on soil bacterial diversity

Most often, the selection of an amendment for the in-situ treatment of contaminated soils is primarily based on its ability to reduce the concentration of labile metal(loid)s. By contrast, additional effects such as the impact of the amendment on the soil microbial abundance and soil functionality are often neglected as well as the impact of the amendment on the soil microbial diversity. Nowadays it is widely accepted that soil microbial diversity affects many key ecosystem processes such as the biogeochemical cycle of plant nutrients as well as the evolutionary dynamics in plant communities. Since soil microbial diversity allows for the preservation of such functional traits, even in the face of external perturbations (Fitter et al., 2005), its maintenance and/or improvement following amendment addition should be of primary importance. Accordingly, soil bacterial diversity in the treated soils was evaluated by high throughput partial 16S rRNA gene sequencing.

The sequencing effort was suitable for screening the vast majority of the existing bacterial diversity as indicated by the Good's coverage estimate (Good, 1953) with a range of 98.4–99.4% of the soil bacterial SSU diversity being uncovered (Table 4). Both inverse Simpson (1/λ) and Gini-Simpson (1-λ) indexes were significantly higher in the treated soils while the other indexes did not reveal significant differences (Table 4). In this regard, it should be noted that the Simpson's index and its derivatives, also known as 2nd order diversity indexes, have been shown to better capture the diversity of dominant OTUs in environmental samples (Mendes et al., 2008; Gihring et al., 2011) as opposed to other indexes like the Fisher's α diversity (Fisher et al., 1943), the effective number of OTUs based on a generalised Tsallis entropy (Tsallis, 1988) as implemented by Mendes et al. (2008) and, of course, the observed richness (*S*) or richness estimates (Chao, 1984; Chao and Lee, 1992). PCA of the Hellinger transformed OTU matrix using OTUs with relative participation of 1% or above (13 OTUs in total), and grouping the rest OTUs as a single group of "rare" OTUs, showed a treatment-wise grouping of samples (Fig. 4a). Furthermore, modelling of selected soil physico-chemical parameters showed strong correlations of the community with the pH_{PZC}, DOC and OM, CEC and the concentration of the more labile fractions of Sb, Pb, Cd and Zn (Fig. 4a). Three OTUs, namely OTU 2, 4 and 8 were dominantly associated with the treated soils as shown in Fig. 4b. These were classified as *Bacilli* (OTUs 2 and 4) and *Acidobacteria* (OTU 8) according to the RDP classifier with the maximum likelihood phylogenies showing evolutionary associations with halo-philic/-tolerant or metal resistant *Bacilli* for OTUs 2 and 4. OTU 2 was placed next to the *Bacillus persicus* B48 and *B. luteolus* YiM93174 strains (Fig. S1a), previously shown to be able to thrive or tolerate NaCl of up to 10% (w/v) (Shi et al., 2011; Didari et al., 2013). OTU 4 was placed next to *B. beringiensis* BR035 (Fig. S1b), a psychrotolerant isolate of the Bering Sea (Yu et al., 2011), and species like *B. megaterium* which encompasses several metal resistant isolates (Yu et al., 2011; Fierros-Romero et al., 2016). Maximum likelihood

Table 4
Number of partial 16S rRNA gene sequences investigated and related diversity indexes. For each diversity index different letters denote significant differences between treatments (ANOVA F and P values are reported).

Sample[a]	Seq# used	Good's Coverage	Gini-Simpson 1-λ	ANOVA: F = 118, P = 1.51e-05	Inverse Simpson 1/λ	ANOVA: F = 35.26, P = 4.82e-04	Fisher's α	ANOVA: F = 4.156, P = 0.073	Effective number of OTUs at q*	ANOVA: F = 4.405, P = 0.066	Observed S	ANOVA: F = 1.864, P = 0.235	Chao 1	ANOVA: F = 2.401, P = 0.171
S1	22889	0.988	0.977	a	43.8	a	384	a	674	a	1615	a	1814	a
S2	21849	0.992	0.977		42.7		307		602		1339		1452	
S3	20865	0.990	0.979		46.6		360		688		1509		1646	
S4	21092	0.990	0.988	b	82.8	b	376	a	790	a	1561	a	1693	a
S5	17760	0.987	0.989		92.5		378		801		1495		1664	
S6	14921	0.984	0.987		75.8		353		704		1359		1508	
S7	22258	0.994	0.991	b	117.0	b	293	a	725	a	1295	a	1384	a
S8	22520	0.993	0.989		93.2		329		758		1422		1511	
S9	18541	0.990	0.990		99.0		303		684		1272		1412	

[a] S1-S3, untreated soils; S4-S6, soils amended at 1% (Fe-WTR + MSWC) rate; S7-S9, soils amended at 2% (Fe-WTR + MSWC) rate.

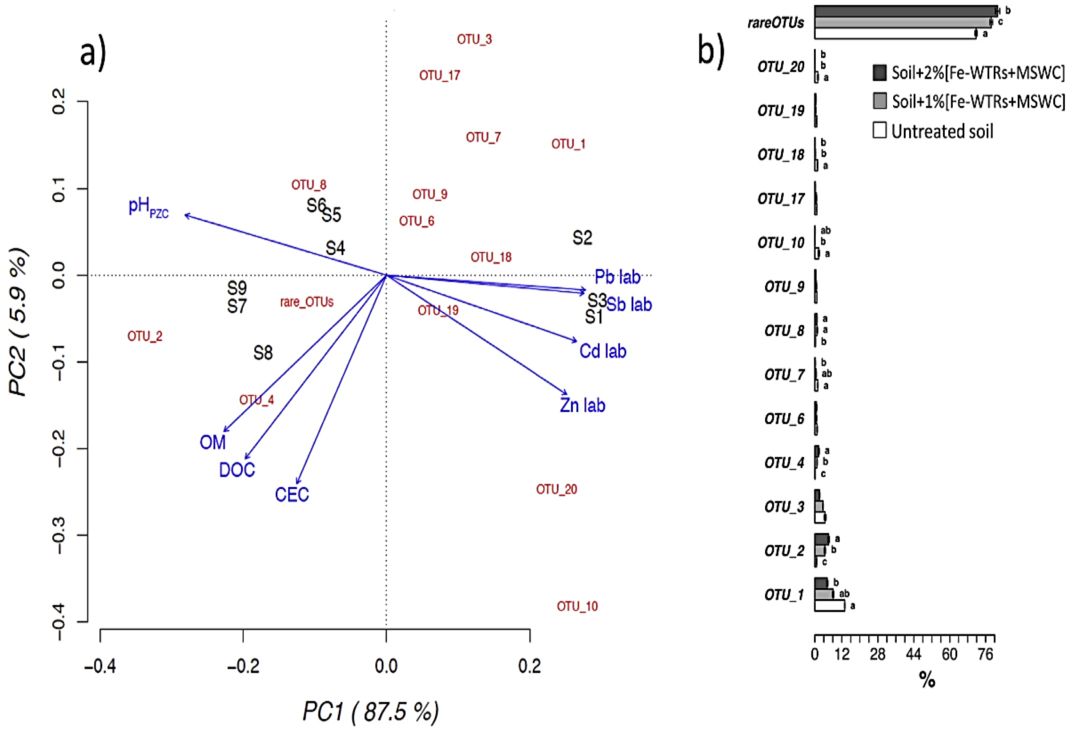

Fig. 4. Triplot of the PCA scores of the S1-9 soil samples (black characters; percentage of variance explained by each axis is also provided) according to their composition in dominant OTUs using the Hellinger transformed values, the scores of the dominant OTUs (red characters) and the fitting of selected soil physico-chemical parameters (blue characters and arrows; highly significant fits with $P \leq 0.01$) (panel a), and differential abundance of the dominant OTUs (different letters denote significant differences for $\alpha \leq 0.05$ using the Holm correction – panel b). Rare OTUs refers to the group of sequences with relative participation of less than 1%. S1-3, untreated soils; S4-6, soils +1% amendment; S7-9, soils +2% amendment; OM, organic matter; DOC, dissolved organic carbon; CEC, cation exchange capacity; pH_{PZC}, point of zero charge; Sb, Pb, Cd and Zn lab, concentrations of metal(loid) labile fractions (i.e. Sb extracted in Step1+2 and Pb, Cd and Zn extracted with $Ca(NO_3)_2$ + NaOAc).

phylogeny showed relation with a versatile *Acidobacterium* named *Holophaga foetida* TMBS4 (Fig. S1c) able to degrade aromatic compounds, which, however, did not show tolerance to high NaCl concentrations (up to 1.5%) (Anderson et al., 2012). Nevertheless, lack of a sufficient number of isolates and the poor 16S rRNA marker gene evolutionary signal of *Acidobacteria* (as shown by the elimination of most acidobacterial representative sequences via the Cd-hit clustering) suggest that deeper investigations are necessary to gain further insights about the niche of the OTU 8 related strains.

Taken together these data highlight a clear impact of the amendments on the bacterial diversity, with amended soils showing significantly higher α-diversity indices (i.e. inverse Simpson and Gini-Simpson indexes; Table 4). At the same time, β-diversity was also affected by soil treatment as pointed out by the significant differences in the bacterial community composition of treated and untreated soils (Fig. 4a,b). This is likely due to a combination of reduced metal(loid) mobility and enhanced availability of C sources in the amended soils which eventually lead to the increase of rare taxa (possibly more sensitive to metal(loid)s in soil). Moreover, this can also explain the appearance of new metabolic potentials (i.e. higher Biolog richness values) in the microbial communities of treated soils (Fig. 3).

3.4. Influence of the amendments on plant growth and metal(loid) uptake

In order to obtain a comprehensive overview of the treatment suitability we carried out a pot experiment to evaluate the combined impact of Fe-WTRs and MSWC on the growth and metal(loid)s uptake of *Helichrysum italicum* plants.

Adding Fe-WTRs and MSWC to the polluted soil had a positive impact on plant growth. Root dry weight increased by approx. 45 and 73% after amendment addition at 1 and 2% rate respectively and very similar increases were recorded for shoot dry weight (Fig. 5a). This was apparently due to the improved fertility of the amended soils (Table 1) and also to the reduced metal(loid) bioavailability, as indicated by the sequential extraction data (Figs. 1–2). Plants grown in the amended soils showed approximately 50% reduction in Sb concentration in roots at both the amendment rates while Sb concentration in shoots (i.e. stem + leaves) was reduced by 40 and 60% at 1 and 2% amendment rate respectively (Fig. 5b).

Significant reductions in Cd, Pb and Zn uptake were also recorded for amended soils (Fig. 5c). Irrespective of the amendment rate, metal concentrations in roots were consistently reduced (by more than 50% compared to the untreated soil on average) while the addition of the 2% amendment brought the concentration of Cd in plant shoots below the instrumental detection limit (i.e. <0.2 μg kg^{-1}; Fig. 5c). Moreover, the amendment addition did not significantly change the translocation factor of Sb (i.e. the roots to shoots metal(loid) concentration ratio) which remained close to 0.1 while decreased significantly that relative to Cd (from 0.50 to 0.14). On the other hand, an increase of Zn translocation factor was recorded at the 2% amendment rate (from 0.14 to 0.28). Taken together, these data highlight the overall effectiveness of the applied amendments for reducing the mobility and bioavailability of both Sb and selected co-occurring metals and improving soil fertility. Moreover, they also indicated the potential use of *H. italicum* for aided phytostabilisation of metal(loid) contaminated soils.

4. Conclusions

This study showed that Fe-WTRs and MSWC can be used as amendments for the chemical and micro-biological recovery of degraded subalkaline soils contaminated with Sb and co-occurring metals such as Pb, Cd and Zn. The amendments addition had a clear positive impact on the soil fertility attributes and reduced the labile fractions of the metal(loid)s in soil, especially Sb. All this likely had a substantial influence on the microbial community, and functionality, of the amended soils. Culturable microorganisms were more abundant in the treated soils and dehydrogenase, β-glucosidase and urease activities were also significantly enhanced.

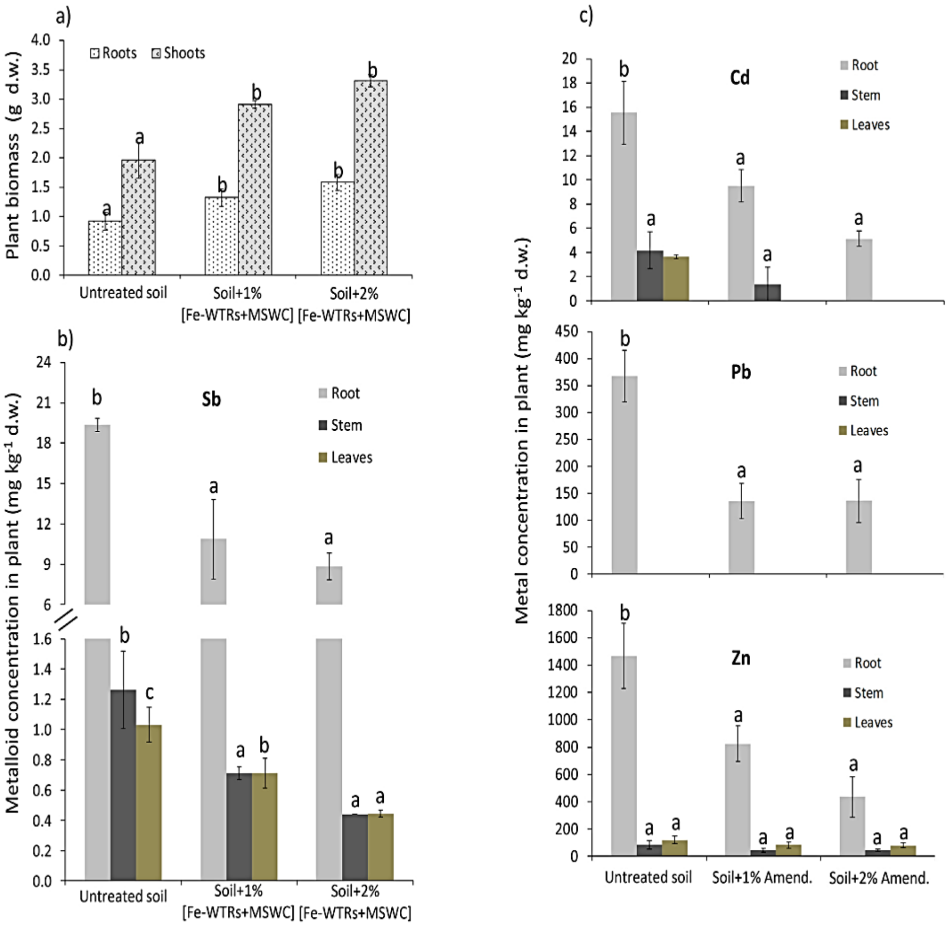

Fig. 5. Biomass (a), Sb (b) and metal uptake (c) of *H. italicum* plants grown in the untreated polluted and amended soils.

Moreover, microbial communities extracted from the amended soils showed higher metabolic potential and catabolic versatility as well as greater α-diversity values based on high throughput partial 16S rRNA gene sequencing. Plant growth in the treated soils was also greatly stimulated while metal(loid)s uptake was significantly reduced. These results suggest that Fe-WTRs and MSWC can be effectively used to alleviate the impact of inorganic xenobiotics on soil microbiota and promote a recovery of ecosystem functioning.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2017.03.014.

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Chapter 6

General Conclusion

The aim of the present PhD thesis was to provide new insights concerning: i) the affinity of different soil amendments (i.e. compost and WTRs) with respect to antimony(V), ii) the impact of Sb on the chemico-physical, microbial and enzymatic characteristics of two soils artificially contaminated, iii) the effectiveness of compost and WTR in the immobilization of Sb and trace metals present in a polluted soil.

Our results indicate that the MSW-Cs investigated were able to act as Sb(V) accumulators in acidic conditions, such as those characterizing many polluted sites. In particular, compost composition was really important and both organic and inorganic components of composts could affect the sorption of Sb(V). Importantly, sequential extractions indicated that the majority of the Sb sorbed (approx. 80%) was tightly bound to MSW-Cs through inner-sphere surface complexes and/or immobilized through co-precipitation reactions. This implies that such Sb is not expected to be readily released under natural conditions.

Likewise, the WTRs investigated were able to act as Sb(V) sorbents at different pH values. The extent of sorption was closely related to the pH of the system, which influenced the surface charge of the sorbents. Accordingly, Al-WTR, with a higher pH_{PZC}, revealed a better sorbent compared to Fe-WTR, particularly at pH 6.5. The Sb(V) sorption by both WTRs was likely governed by (i) coulombic attraction between the Sb(V) anion and the positively charged functional groups of WTRs (e.g., protonated –OH groups of inorganic phases and/or protonated amino groups of the organic matter); (ii) ligand exchange where the surface OH and/ or OH_2 groups are exchanged by antimonate leading to the formation of strong inner sphere complexes; and (iii) the formation of stable complexes between Sb(V) and poly-functional organic ligands of WTRs. The sequential extractions indicated that the most part of the antimonate sorbed by both WTRs was not expected to be released in the short term.

Concerning fate of antimony, added at two different soils, as respect to its mobility as well as its impact on the soil microbial and biochemical features, our results showed that Sb mobility and toxicity was related to soil physico-chemical features, e.g. pH, clay and organic matter content. Sequential extractions indicated that soluble or exchangeable Sb was significantly higher in the alkaline soil compared to the acidic one where most of the antimony was recovered in the residual fraction. This apparently explained the significant decrease of the bacterial numbers, DHG and Biolog AWCD values in the alkaline S1 and help to explain the opposite trend of all such microbial and biochemical parameters in the acidic S2 soil.

Concerning the immobilisation ability of compost (MSWC) and WTRs towards, Sb and other trace metals in a polluted soil, our results showed that the combined application of both sorbents effectively reduced the metal(loid)s mobility and improved some fertility characteristics of the soil such as the CEC, OM, N content and DOC. The amendments addition had a clear positive impact on soil microbial and biochemical features. whilst promoting plant growth and reducing metal(loid)s uptake.

Overall, the results arising from this PhD project highlight that Fe-WTRs and MSWC can be effectively used as Sb-immobilising materials to decrease the mobility and toxicity of antimony in contaminated soils and to alleviate the metalloid impact on the environment.

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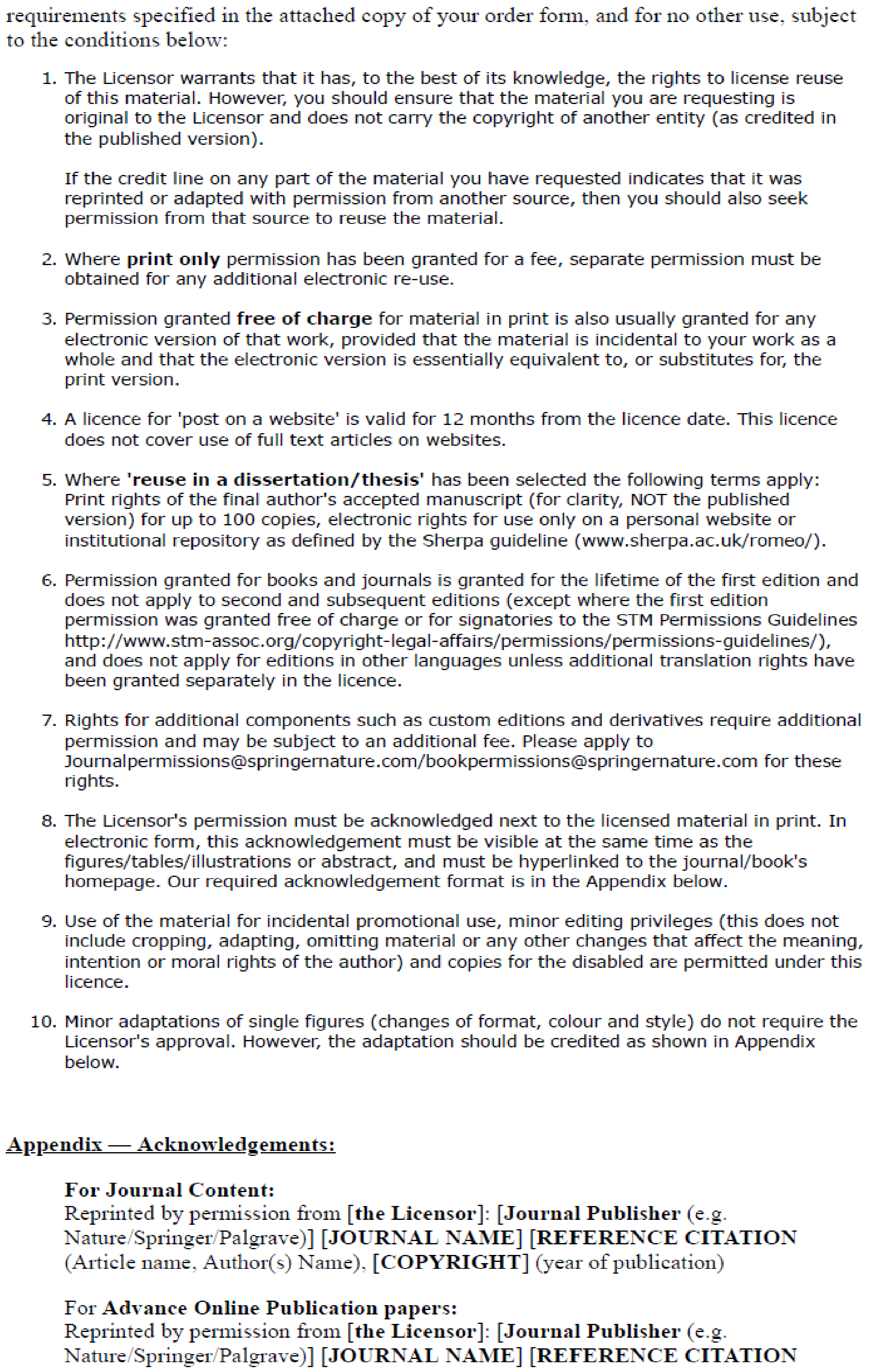

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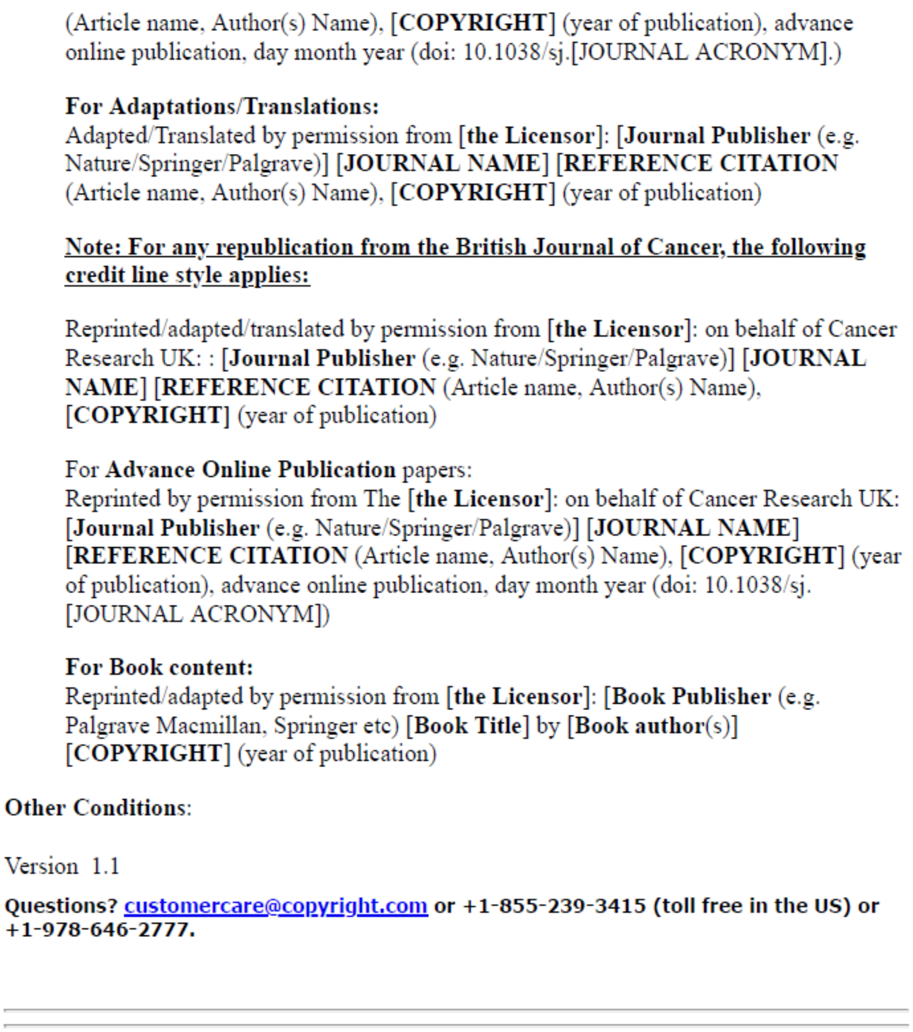

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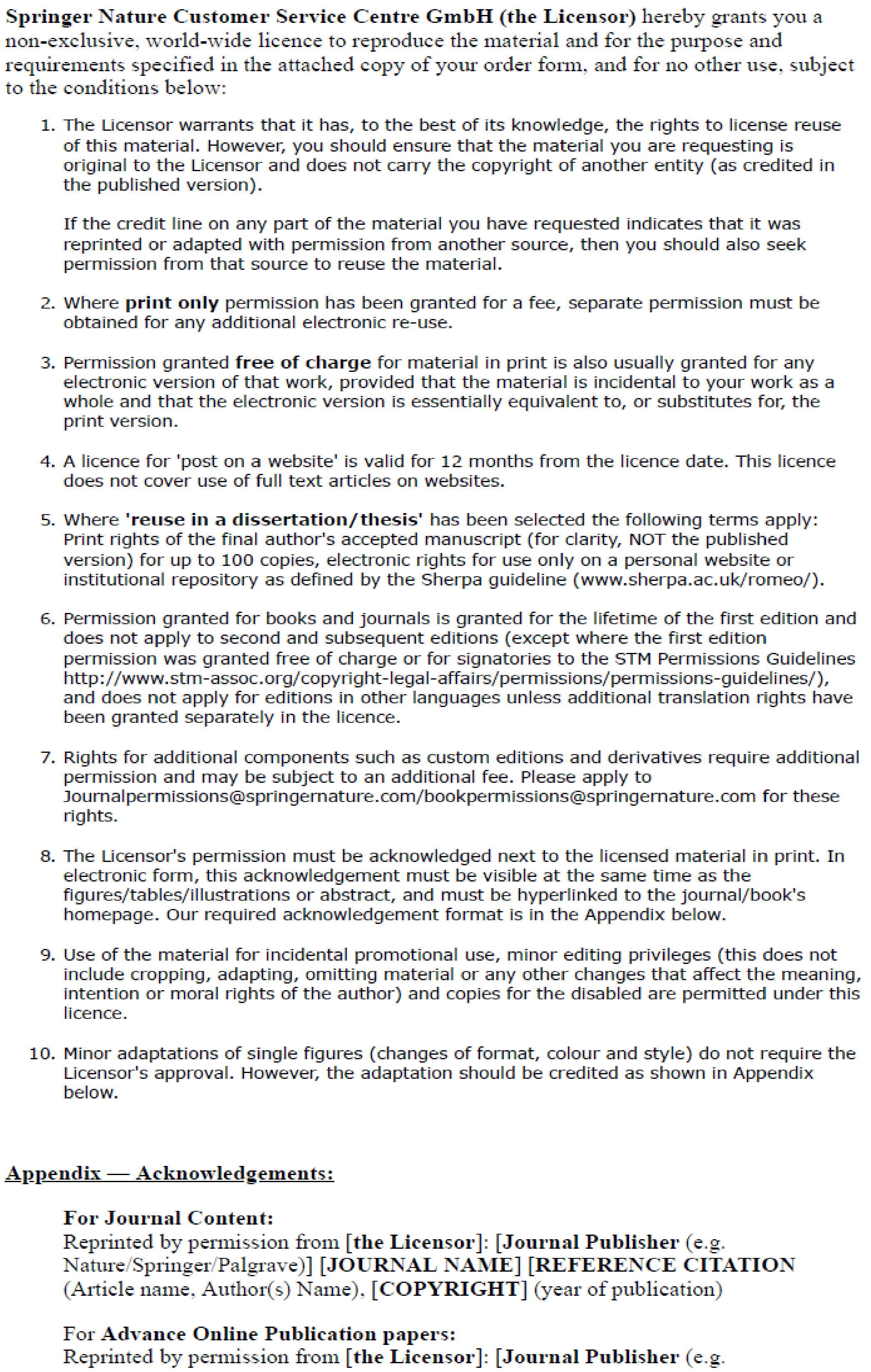

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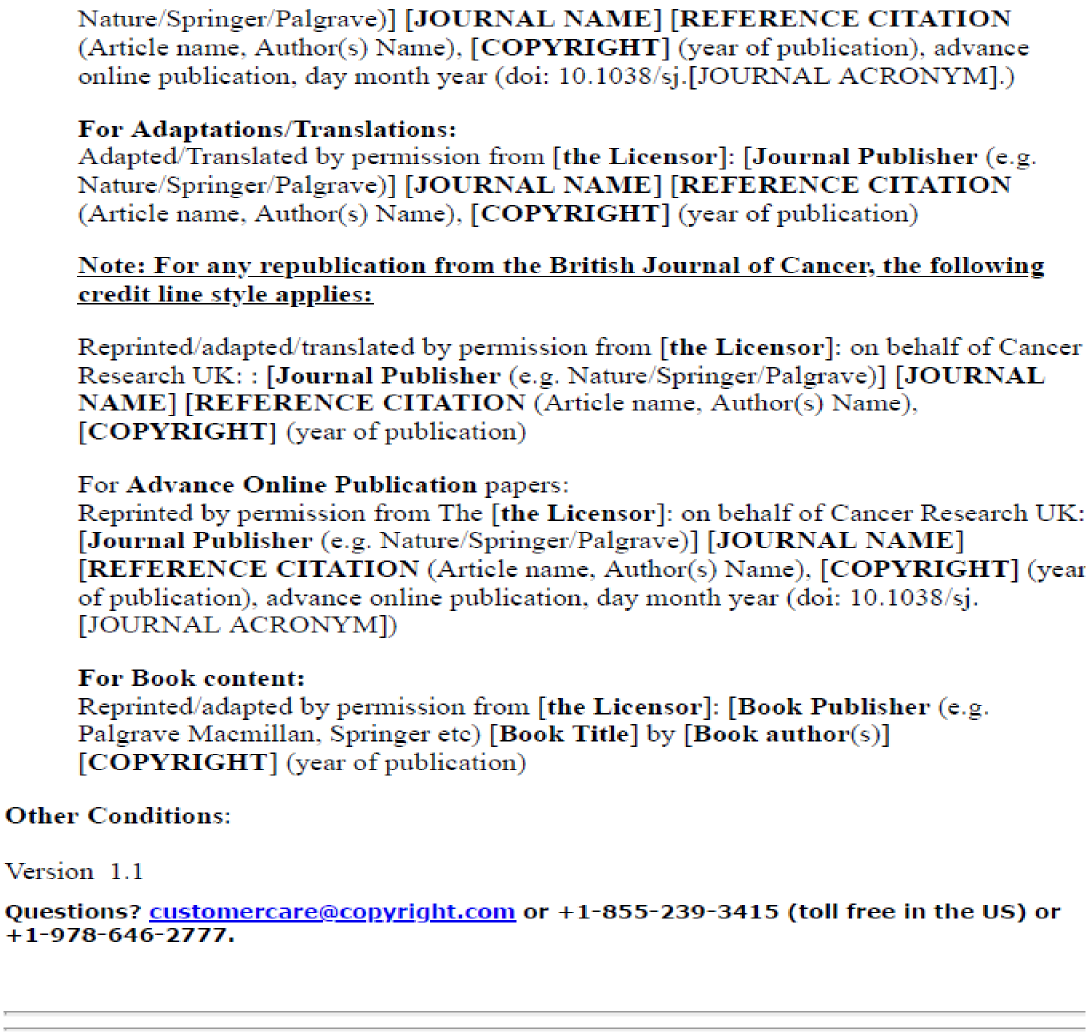

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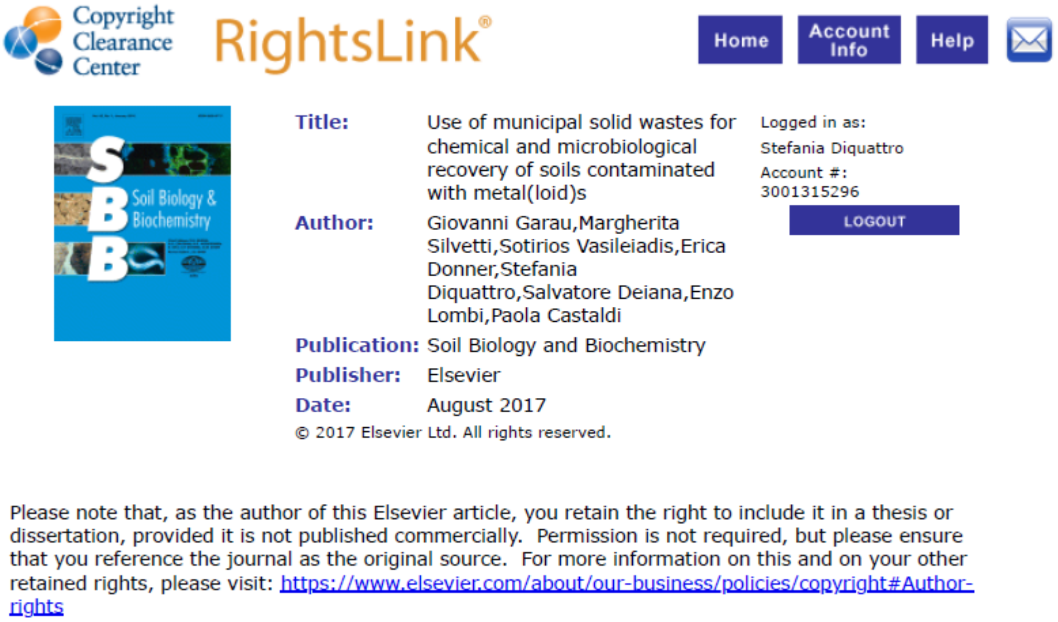

Title:	Use of municipal solid wastes for chemical and microbiological recovery of soils contaminated with metal(loid)s
Author:	Giovanni Garau,Margherita Silvetti,Sotirios Vasileiadis,Erica Donner,Stefania Diquattro,Salvatore Deiana,Enzo Lombi,Paola Castaldi
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