Journal of Environmental Management 139 (2014) 146–153

Contents lists available at ScienceDirect

Journal of Environmental Management

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

Stabilising metal(loid)s in soil with iron and aluminium-based products: Microbial, biochemical and plant growth impact

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ARTICLE INFO

Article history:
Received 2 August 2013
Received in revised form
24 January 2014
Accepted 23 February 2014
Available online

Keywords:
Arsenic-polluted soil
Iron and aluminium-based products
Sequential extraction
Soil microbial community
Soil enzyme activity
Plant growth

ABSTRACT

Four iron and aluminium-based products, including red mud (RM), hematite (Fe_2O_3), an iron-rich water treatment residual (Fe-WTR) and amorphous Al hydroxide (Al-OH), were evaluated for their effectiveness at stabilising As and heavy metals (i.e. Cd, Cu, Pb, Zn) in a circumneutral contaminated soil [As (2105 mg kg^{-1}), Cd (18 mg kg^{-1}), Cu (264 mg kg^{-1}), Pb (710 mg kg^{-1}), Zn (522 mg kg^{-1})]. Treatment impacts on soil microbial and biochemical features (i.e. microbial biomass-C, microbial counts, 16S rRNA PCR-TTGE of culturable bacteria, dehydrogenase, urease and β-glucosidase activity, Biolog derived parameters-AWCD and richness) as well as bean (*Phaseolus vulgaris*) and wheat (*Triticum vulgare*) growth were also assessed.

After 6 months equilibration, all the amendments (application rate 3% w/w) but RM reduced labile As while only Al-OH reduced the concentration of water-soluble heavy metals. Despite the highest bioavailability of contaminants, most of the soil microbial and biochemical features monitored (i.e. microbial biomass-C, total bacterial counts, dehydrogenase activity and AWCD) were significantly higher in the RM-soil. Bean germination was completely inhibited in RM-soil while wheat growth was similar to that of the control. The Al-OH treatment was best overall, promoting microbial abundance, diversity and activity while increasing bean and wheat growth and reducing As accumulated in plant shoots. Results suggest that Al-OH is a suitable candidate for field evaluations while the use of RM in the remediation of circumneutral or subalkaline contaminated soils should be reconsidered.

1. Introduction

High concentrations of metal(loid)s have a detrimental influence on microbial abundance, diversity and activity (Baath, 1989; Giller et al., 2009; de Santiago-Martín et al., 2014). In turn, this can have substantial negative implications on plant growth which is usually stimulated by an abundant, diverse and active rhizospheric microbiome (Berendsen et al., 2012). In this sense, soil microbes and their activities should be considered as primary targets, together with plant growth, in the assessment of the effectiveness of any interventions aimed at soil or ecosystem recovery (Castaldi et al., 2009).

Different conventional methods are available to remediate metal(loid) contaminated soils, most of which are highly invasive and very expensive such as thermal processes, physical separation, electrochemical methods, washing, stabilisation/solidification and burial (Mulligan et al., 2001). Alternative, low cost and more sustainable approaches have been proposed during the last 10 years for *in situ* remediation of polluted soils. These are mainly based on the use of organic and/or inorganic amendments able to reduce the bioavailable fraction of the contaminants hence alleviating the environmental and biological risks of these latter (Zhou et al., 2012). This is the case of aluminium(Al) and iron(Fe)-oxyhydroxides as well as Al and Fe-based products such as the drinking water treatment residuals (WTRs) and red muds (RMs) (Komárek et al., 2013).

Iron and aluminium oxides and hydroxides can be very effective at decreasing metal(loid)s bioavailability in soils and waters due to their high specific surface area and reactive surficial functional groups (Violante et al., 2010; Komárek et al., 2013). Also WTRs (waste materials generated during the drinking-water treatment

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http://dx.doi.org/10.1016/j.jenvman.2014.02.024
0301-4797/

process), mainly composed by Fe/Al (hydr)oxides, are other potentially effective amendments for metal(loid) polluted soils (Ippolito et al., 2011).

RMs (a fine-textured alkaline residue deriving from the digestion of bauxite during the Bayer process) were similarly effective at decreasing metal(loid) bioavailability in soil pore water (Garau et al., 2007, 2011; Lee et al., 2011) and the number of studies on RM remediation capability have been increasing (Liu et al., 2011).

Although the potential of such amendments has been shown, their effectiveness for *in situ* remediation of soils containing both metals and metalloids is poorly understood. Moreover, the influence of these amendments on soil microbial community structure and function and plant growth was rarely addressed. Importantly, this can lead to an overestimation of the amendment capabilities and to the selection of suboptimal treatments for soil remediation.

Therefore the objective of this study was (i) to compare the influence of hematite [iron(III) oxide (Fe_2O_3)], amorphous Al-hydroxide (Al-OH), Fe-WTR and RM on the mobility of As and different heavy metals (Cd, Cu, Pb, Zn) in a circumneutral contaminated soil and to determine their impacts on (ii) plant growth and metal(loid)s accumulation by bean and wheat plants and (iii) soil microbial community structure and function.

2. Materials and methods

2.1. Soil origin, characteristics and microcosms set up

The soil was collected in the vicinity of the abandoned mining site of Baccu Locci (Villaputzu, Italy; N39°32′36″, E9°31′36″) where lead and arsenic have been extracted from galena and arsenopyrite for about one century (1873–1965) (Frau et al., 2012). Approximately 150 kg of soil (15 samples, 0–30 cm depth) were collected from an area of about 1 ha, bulked together in the laboratory, air dried and sieved to <2 mm. The soil within the sampling area was a sandy clay loam (36% coarse sand, 22% fine sand, 17% silt, 25% clay) with quartz (45 wt.%), illite (30 wt.%), albite (15 wt.%), hematite (6.5 wt.%) and jarosite (3.5 wt.%) as the soil mineral constituents (Garau et al., 2011). Approximately 20 wt % of the soil was comprised of amorphous phases and the XRD analysis did not show the presence of crystallised arsenic mineral phases.

Microcosms, each consisting of approx. 10 kg soil (10 cm depth), were separately treated with one of the following amendments: RM, Fe-WTR, crystallised Fe_2O_3 and amorphous Al–HO. All treatments were applied to triplicate microcosms at the 3% w/w basis and 3 additional microcosms were kept untreated ($P_{(olluted)}$-soil). Before addition to soil, the RM (originating from the "Eurallumina" plant located in the industrial area of Portoscuso–Portovesme, Sardinia, Italy) was oven dried at 60 °C for 48 h, finely ground and sieved to <0.02 mm (Table 1).

Similarly, Fe-WTR (dried overnight at 105 °C), Fe_2O_3 and Al-OH (both from Sigma Aldrich, Milan, Italy) were finely ground and sieved to <0.02 mm before addition to soil. XRD analysis confirmed the crystalline and amorphous structure of the Fe_2O_3 and Al-OH employed respectively (not shown).

The Fe-WTR (Table 1) was provided by the Public limited company Abbanoa S.p.A. (Sardinia, Italy) and derived from the drinking-water treatment plant in Bidighinzu (Sassari, Italy) where the raw water was added with $Fe_2(SO_4)_3$ as coagulant.

Following amendment, treated and untreated soils were mixed and moisture content increased to 40% of their water holding capacity. Soils were then equilibrated for 6 months at 20 °C (60–70% of relative humidity), mixed twice a week and their water content maintained at a 40–50% level. Table 1 could be inserted here.

Table 1
Main characteristics of the red mud (RM) and Fe-WTR used in the study.

Chemical parameters	RM[a]	Fe-WTR
pH	11.12	7.78
EC (mS cm^{-1})	8.70	3.01
S_{BET} (m^2 g^{-1})	19.50	35
Organic matter (% d.m.)	0.60	24.47
Al (% d.m.)	9.65	1.93
Ca (% d.m.)	1.04	0.009
Fe (% d.m.)	30.35	24.51
Na (% d.m.)	5.17	n.d.

[a] Data from Garau et al. (2011).

2.2. Chemical analysis

After equilibration, the pH, electric conductivity, total organic carbon and nitrogen were determined for treated and untreated soils following the national standard guidelines (Gazzetta Ufficiale, 1992). Water-soluble carbon (WSC) was determined on 20 g of dried (65 °C) and sieved (<2 mm) soil samples as described by Castaldi et al. (2008a,b). The total concentration of As, Cd, Cu, Pb and Zn in soils was determined after digestion with HNO_3 and HCl mixture (1:3 v/v ratio) using a Perkin Elmer Analyst 600 atomic absorption spectrometer (HGA-600 graphite furnace).

The sequential extraction procedure of Wenzel et al. (2001) was used to determine the mobility of arsenic in soils while Cd, Cu, Pb and Zn mobility was determined using the sequential extraction procedure of Basta and Gradwohl (2000).

2.3. Microbial biomass-C and count of fast-growing heterotrophic microorganisms in soils

After equilibration, microbial biomass-C was determined in duplicate soil samples (35 g each) from each microcosm using the rapid chloroform-fumigation extraction method (Witt et al., 2000).

Culturable fast-growing heterotrophic bacteria, fungi and actinomycetes were enumerated in triplicate soil samples (10 g each) from each treated and untreated soil using the conventional serial dilution and spread plate method and the microbiological media described by Pinna et al. (2012). Microbial counts were expressed as average Log CFUs (Colony Forming Units)±standard deviation per gram of soil dry weight.

2.3.1. 16S rRNA PCR-TTGE of the predominant soil culturable bacteria

To perform 16S rRNA PCR-TTGE of the dominant culturable fast-growing heterotrophic bacteria, colonies grown on TSA plates inoculated with the 10^{-5} soil dilutions were washed off with 5 ml of sterile peptone water (Microbiol, Cagliari, Italy). The bacterial suspension was brought to an optical density at 600 nm (OD_{600}) equal to 2.3 using peptone water and 1.5 ml of the suspension was used for DNA extraction as in Parayre et al. (2007). The DNA was then purified using the Dneasy Blood and Tissue kit (Qiagen, Milan Italy) as described in the manufacturer's instructions.

Bacterial 16S rRNA gene fragments were amplified using the universal primers 968F-GC (5′-CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG CCC CCG CCC GAA CGC GAA GAA CCT TAC-3′) holding a GC clamp (underlined) and 1401R (5′-CGG TGT GTA CAA GAC CC-3′) (Felske et al., 1998). PCR conditions were as reported previously (Cebron et al., 2009). 16S rRNA PCR-TTGE analysis was performed on a DCode system (Bio-Rad, Italy) by using vertical poliacrylamide gel (8% wt/vol acrylamide, 7 M urea, 2% vol/vol glycerol) in 1.25× TAE buffer (40 mM Tris-acetate, 20 mM acetic acid and 1 mM EDTA pH 8). After electrophoresis at 120 V for 6 h and 70 °C constant temperature, gels were stained with SYBR safe (1:10,000 final dilution, Invitrogen) and analysed on a Chemi Doc transilluminator