

University of South Australia

The Library
www.library.unisa.edu.au

Educating Professionals, Creating and Applying Knowledge, Engaging our Communities

NOTICE: This is the accepted author manuscript (AAM) or post-print which was accepted for publication in *Geoderma*. Changes resulting from the publishing process, such as peer review, editing, corrections, structural formatting, and other quality control mechanisms are reflected in this document. A definitive version was subsequently published in *Geoderma*, vol. 214, pp. 204-212, 2014, http://dx.doi.org/10.1016/j.geoderma.2013.09.010

Elsevier Editorial System(tm) for Geoderma
Manuscript Draft

Manuscript Number: GEODER8032

Title: LEACHABILITY, BIOACCESSIBILITY AND PLANT AVAILABILITY OF TRACE ELEMENTS IN CONTAMINATED SOILS TREATED WITH INDUSTRIAL BY-PRODUCTS AND SUBJECTED TO OXIDATIVE/REDUCTIVE CONDITIONS.

Article Type: Research Paper

Section/Category: Soil Chemistry and Biogeochemistry

Keywords: In situ remediation; Red mud; Water treatment residue; Trace element; Arsenic

Corresponding Author: Mrs. Margherita Silvetti, Ph.D.

Corresponding Author's Institution: University of Sassari

First Author: Margherita Silvetti, Ph.D

Order of Authors: Margherita Silvetti, Ph.D; Margherita Silvetti, Ph.D.; Paola Castaldi, Ph.D; Peter E Holm, Ph.D; Salvatore A Deiana, Professor; Enzo Lombi, Ph.D

Abstract: Two industrial by products, red mud (RM) and aluminium-rich water treatment residue (Al-WTR), were used for the immobilization of As, Cu and Pb in two polluted soils. Two different redox conditions (aerobic and 3 repeated reduction/oxidation cycles) were also applied. The immobilization of trace elements and their bioaccessibility were evaluated through a leaching test and simplified bioaccessibility extraction test (SBRC) respectively, whereas phytoavailability was assessed in pot trials with Lolium perenne.
The RM addition increased the solubility of As and Pb in Soil-1, under both redox conditions, with respect to untreated soil. In Soil-2 Al-WTR was the amendment most efficient at decreasing As mobility. The high correlations detected between trace element mobility and dissolved organic carbon (DOC) suggests that As, Cu and Pb formed soluble organic complexes. The SBRC results suggested that this test was mainly affected by physical and chemical characteristics of soils, whereas trial pot showed that the treatments with RM and Al-WTR were able to decrease the plant uptake of As, Cu and Pb in both polluted soils.
The results of this study show that the efficacy of the soil amendments is a complex function of soil conditions, source of contamination, and perhaps most importantly the environmental endpoint considered.

*Highlights (for review)
Click here to download Highlights (for review): Highlights.docx

Research highlights

- Red mud and water treatment residues reduced As and Cu leachability in sandy soil
- As, Cu and Pb phytoavailability decreased in treated-soils
- Difference between treatments were negligible in bioaccessibility test
- Redox cycles did not decrease leachability, bioaccessibility and phytoavailability

*Abstract
Click here to download Abstract: Abstract.docx

Abstract

Two industrial by products, red mud (RM) and aluminium-rich water treatment residue (Al-WTR), were used for the immobilization of As, Cu and Pb in two polluted soils. Two different redox conditions (aerobic and 3 repeated reduction/oxidation cycles) were also applied. The immobilization of trace elements and their bioaccessibility were evaluated through a leaching test and simplified bioaccessibility extraction test (SBRC) respectively, whereas phytoavailability was assessed in pot trials with *Lolium perenne.*

The RM addition increased the solubility of As and Pb in Soil-1, under both redox conditions, with respect to untreated soil. In Soil-2 Al-WTR was the amendment most efficient at decreasing As mobility. The high correlations detected between trace element mobility and dissolved organic carbon (DOC) suggests that As, Cu and Pb formed soluble organic complexes. The SBRC results suggested that this test was mainly affected by physical and chemical characteristics of soils, whereas trial pot showed that the treatments with RM and Al-WTR were able to decrease the plant uptake of As, Cu and Pb in both polluted soils.

The results of this study show that the efficacy of the soil amendments is a complex function of soil conditions, source of contamination, and perhaps most importantly the environmental endpoint considered.

*Manuscript
Click here to download Manuscript: Manuscript.docx Click here to view linked References

LEACHABILITY, BIOACCESSIBILITY AND PLANT AVAILABILITY OF TRACE ELEMENTS IN CONTAMINATED SOILS TREATED WITH INDUSTRIAL BY-PRODUCTS AND SUBJECTED TO OXIDATIVE/REDUCTIVE CONDITIONS.

Authors:

Margherita Silvetti[a*], Paola Castaldi[a], Peter E. Holm[b], Salvatore A. Deiana[a], Enzo Lombi[c]

a Dipartimento di Agraria, Sezione di Scienze e Tecnologie Ambientali e Alimentari, University of Sassari, Viale Italia 39, 07100 Sassari, Italy

b Department of Plant and Environmental Sciences Faculty of Science, University of Copenhagen, Frederiksberg C, Denmark

c Centre CERAR, University of South Australia, Mawson Lakes Campus, Mawson Lakes, SA-5095, Australia

*Corresponding author. Tel.: +39079229213; fax: +39079229276. E-mail address: silvetti@uniss.it (M. Silvetti)

Abstract

Two industrial by products, red mud (RM) and aluminium-rich water treatment residue (Al-WTR), were used for the immobilization of As, Cu and Pb in two polluted soils. Two different redox conditions (aerobic and 3 repeated reduction/oxidation cycles) were also applied. The immobilization of trace elements and their bioaccessibility were evaluated through a leaching test and simplified bioaccessibility extraction test (SBRC) respectively, whereas phytoavailability was assessed in pot trials with *Lolium perenne.*

The RM addition increased the solubility of As and Pb in Soil-1, under both redox conditions, with respect to untreated soil. In Soil-2 Al-WTR was the amendment most efficient at decreasing As mobility. The high correlations detected between trace element mobility and dissolved organic carbon (DOC) suggests that As, Cu and Pb formed soluble organic complexes. The SBRC results suggested that this test was mainly affected by physical and chemical characteristics of soils, whereas trial pot showed that the treatments with RM and Al-WTR were able to decrease the plant uptake of As, Cu and Pb in both polluted soils.

The results of this study show that the efficacy of the soil amendments is a complex function of soil conditions, source of contamination, and perhaps most importantly the environmental endpoint considered.

1. Introduction

Trace elements are ubiquitous, generally in small concentrations, in natural components of the environment. They are also emitted into the environment from different sources, i.e. transportations, industrial and mining activities, fossil fuels, pesticides manufacturing and their applications, and other human activities [Wong et al., 2002; Boruvka et al. 2005]. Toxic trace elements of concern include metalloids such as arsenic and heavy metals, e.g., cadmium, lead, and mercury.

In many areas of the world, soils are contaminated with toxic trace elements; however human and environmental risk posed by these potential toxic substances is related not only to their total concentrations but also their leachability and bioavailability [Schmidt, 1997]. Particularly, trace metals mobility depends on soil characteristics: soil pH and texture, the type and quantity of the oxy–hydroxides present, the content of organic matter, carbonates, phosphates and clays, which are the main soil constituents, responsible for heavy metals and metalloids sorption [e.g. Holm et al. 2003; Marcussen et al. 2009].

Many technologies have been proposed for the remediation of soils polluted by trace elements, including physical, chemical and biological treatments. Among these approaches, *in-situ* stabilisation with soil amendments has attracted attention for its low cost and ease of implementation [e.g. Sarkar et al. 2007]. These remediation methods are based on the reduction of trace elements mobility and bioavailability by using of amendments, which promote adsorption and/or precipitation reactions [e.g. Lombi et al., 2002a-b; Lombi et al., 2010]. Many amendments used for the *in-situ* remediation techniques, are rich in Fe/Al/Mn amorphous and/or crystalline oxy-hydroxides that can reduce the trace elements bioavailability through sorption reactions [Violante et al, 2008]. The retention mechanisms of these sorbents consist of adsorption of metals on external surface, metal binding, fixation inside mineral particles and promotion of surface precipitation reactions [Castaldi et al., 2008].

Some *in-situ* technologies proposed to remediate As and heavy metals polluted soils include the use of industrial by-products such as bauxite residues (Red Mud, RM) or drinking–water treatments

residues (WTR) [Lombi et al., 2002a-b; Lombi et al., 2004; Makris et al., 2006; Santona et al., 2006; Garau et al., 2007; Sakar et al., 2007; Castaldi et al., 2008, Castaldi et al., 2011].

Red mud (RM) is a waste material derived from the digestion of bauxite during the Bayer process, but it might also be considered as a secondary raw material for the fixation of (cationic) contaminants, due to its alkaline pH (10.0–12.5) and sorptive capacity. The combined presence in RM of ferric, aluminium, and tectosilicate-like compounds is expected to be particularly effective in relation to the removal and immobilization of heavy metals from waste waters and polluted soils [Santona et al., 2006; Castaldi et al, 2010a-b; Garau et al 2011] or in the reduction of leaching of soil nutrients [Phillips, 1998].

WTRs are by-products resulting from the industrial process of drinking-water treatment, where suspended solids, organic matter, color, contaminants, etc., are removed from the raw water supply by addition of aluminum, iron or calcium salts as coagulants [Nagar et al., 2010]. WTRs contain Fe or Al oxides and hydroxides generally amorphous in nature [Lombi et al., 2004; Makris et al., 2006; Sakar et al., 2007]. Recent literature has shown that WTRs are cost-effective amendments that reduce both excess of soluble P concentration [Agyin-Birikorang and O'Connor, 2007] and As concentration in soil and water [Lombi et al.,2004; Makris et al., 2006; Sakar et al., 2007; Cederkvist et al., 2010;].

The success of a remediation treatment is also affected by chemical, physical and biological soil properties, such as pH and redox potential [Charlatchka and Cambeier, 2000], CEC, salt content, soil constituents and microbiological activity. In nature, soil constituents are subject to variation in oxidation–reduction state status which can affect the speciation of elements such as Fe, Mn, S but also as As, Cu and Cr. High Eh and pH values can favour the co-precipitation of Fe oxy-hydroxides with metals, while reducing conditions can cause the dissolution of Mn and Fe oxy-hydroxides and their co-precipitated elements can be released into the soil solution [Charlatchka and Cambeier, 2000]. The possibility of fixing toxic elements in Fe-(hydro)oxides by inducing *in-situ* cycles of reduction/oxidation has been suggested and studied by Contin et al. [2007]. Their study is based on

the partial reductive dissolution of Fe(III) oxy-hydroxides which cause an increase of their specific surface area and of their temporary sorption capacity. The re-precipitation of re-oxidized Fe can also occlude the sorbed elements within the mineral [Bartlett and James, 1993].

In literature some researches have shown the negative impact of reductive conditions on the mobility of heavy metals bounded to Fe and Mn oxy-hydroxides [Davranche and Bollinger, 2000] or to industrial wastes [Davranche et al., 2003]. Nevertheless few studies have assessed both the mobility and bioavailability of trace elements in polluted soils, treated with sorbents and subjected to aerobic and anaerobic conditions. The latter represents a clear knowledge gap as those conditions are likely to occur at many sites and have the potential to modify the longevity of the treatments.

The aim of this research was therefore to test industrial residuals such as RMs and Al-WTR as amendment to decrease the mobility of toxic trace element such as As, Cu and Pb in soils. These industrial by-products were added to two contaminated soils and solubility and bioavailability of As, Cu and Pb were investigated by inducing repeated cycles of reduction and oxidation. We hypothesised that redox cycles would have a more marked effect on RM treated soils, due to Fe redox reactivity, rather than to the Al-based WTR one. The leachability of metal(oids)s was assessed by leaching test [European Method EN 12457-2] while bioaccessibility of As and Pb was evaluated by SBRC (gastric phase) [Juhasz et al., 2009]. Moreover the effect of these industrial by-products and of redox cycles on As, Cu and Pb bioavailability was tested through ryegrass (*Lolium perenne*) growth in a trial pot, which was selected for its resistance to heavy metals [i.e. Bidar et al, 2007].

2. Materials and methods

2.1. Soils and Amendments

Two arsenic contaminated soils were used in this study. A soil sample from southeast Sardinia (Soil-1) was collected near the Baccu Locci mine, Villaputzu (CA), where the principal As-ore extracted was arsenopyrite. Particle size analysis of Soil-1 classified the soil as sandy clay loam (USDA classification) (Table 1). The second soil (Soil-2) was collected from a chromated copper arsenate (CCA) contaminated site named Collstrup, a 6 hectare site, situated in northern Zealand, Denmark. The soil is a glacio-fluvial deposit consisting of a mixture of clay, silt and sand and it was classified as sandy loam (USDA classification) (Table 1). From 1936 to 1976 the area was used for impregnation of wood. Soil samples were air-dried and passed through a 2 mm sieve and their chemical characteristics were determined following standard methods (Table 1) [Castaldi et al. 2010a; Garau et al., 2011].

The total concentration of As, Pb, Cu, Fe and Al was determined in both soils after digestion with HNO_3 and HCl mixture (1:3 v/v ratio) using a Microwave Milestone MLS 1200. The element concentrations presented in Table 1 were determined using a flame atomic absorption spectrometer PerkinElmer Analyzer 600, equipped with a HGA-600 graphite furnace.

The industrial by-products used as sorbents in this study were red muds (RMs) and drinking-water treatment residuals (WTRs).

The RM, obtained from the Ex-Eurallumina plant in Portovesme, SW Sardinia, Italy, was dried overnight at 60 °C and finely grounded with a mortar. X-ray powder diffraction analysis of RM, carried out with a Rigaku D/MAX diffractometer (CuKα) equipped with a graphite monochromator in the diffracted beam, identified hematite (44 wt.%), sodalite (20 wt.%) and boehmite (12 wt.%) as the crystalline phases, as previously described by Castaldi et al. [2010a-b] (Table 2). RM samples were neutralized with seawater (RM_{sw}) or with acid treatment (RM_a). RM_{sw} was prepared by suspending 200 g of RM in 100 mL of synthetic seawater solution [Ferreira et al., 2002] (solid to liquid ratio 2:1 w/w); the mixture was shaked for 1 h and after centrifugation the liquid was discarded; this treatment was repeated three times until an equilibrium pH of 9.1 was reached. RM_a

was prepared by washing RM with 0.05 M HCl (RM/HCl = 1:25 w/w) in a stirred batch system with an equilibrium pH of 9.3 [Santona et al., 2006]. After equilibration both red muds were rinsed with deionized water and dried overnight at 60 °C. Untreated red mud is highly caustic (the pH was 11.5), due to the use of NaOH during bauxite digestion. This high alkalinity is environmentally hazardous and, therefore, red mud needs to be neutralized before being used for soils treatment; for these reasons, as suggested by some researchers [Ciccu et al. 2003; Genç-Fuhrman et al., 2004], we first treated the red mud with both seawater and HCl, to low the sodium concentration and to reduce the pH to environmentally acceptable levels.

The WTR used in this study was Al-based (Al-WTR), and it was obtained from the drinking-water treatment plant in Truncu Reale, Abbanoa Industry, North Sardinia, Italy, where $Al_2(SO_4)_3$ was used to purify water. Before its use it was dried overnight at 60 °C and finely grounded with a mortar. X-ray powder diffraction analysis did not reveal crystalline Al component, suggesting that this material was amorphous.

RM_{sw}, RM_a, and Al-WTR were oven dried at 60 °C for 48 h and finely grounded with a mortar before being added to the soils. Their main chemical characteristics are summarized in Table 2.

2.2. Soil Treatments

Samples of Soil-1 and 2 (~ 2.0 kg for each test) were separately mixed with RM_{sw}, RM_a or WTR (2 % w/w). Soil samples were also amended with lime (0.12 % and 0.08 % w/w were added to Soil-1 and Soil-2 respectively) to obtain a pH similar to that of the RM_{sw} treated soils. Untreated control soils and soils amended with RM_{sw}, RM_a, WTR and lime were carefully mixed with deionised water to 60% and 80% of water holding capacity of Soil-1 and Soil-2, respectively. The soils were then allowed to incubate for 2 weeks and kept at approximately 20 °C and 60–70% of relative humidity. After the incubation period the soil samples were divided into two subsamples, one of these was used for the redox cycles treatments and the other was maintained under aerobic

conditions. For the redox cycles 260 g of each subsample was placed in a desiccator and the air was replaced by nitrogen. These soil subsamples were then incubated under anoxic conditions at room temperature for 20 days. Each treatment was carried out in triplicate. At the end of the incubation period the desiccators were opened and the soils were placed in air at room temperature for 3 days (23 days per cycle in total). This cycle was repeated 3 times. After the third cycle, aliquots of soil samples both subjected or not to redox treatments were sampled for the leaching test and SBRC analysis.

All the analyses were performed in triplicate.

2.3. Leaching of toxic trace elements

Soil samples were assessed for their leaching properties by the European Method EN 12457-2. Five g of each soil sample were placed in bottles and 50 ml of leachant (deionised water) were added. The mixtures were shaked for 24 h and after centrifugation the supernatant was filtered through a 0.2 μm membrane filter. The pH and conductivity of the filtered solutions were measured (data not shown) and As, Cu and Pb concentrations were determined by ICP-MS after acidification with HNO_3. Dissolved organic carbon (DOC) analysis of the leachates was carried out according to Brandstetter et al, [1996]. Aliquots of samples solutions deriving from the leaching test (~10 mL) were taken for immediate UV analysis. The UV measurements were carried out at 254 nm with an UV-1700 Pharmaspec Shimazu spectrofotometer.

2.4. The in vitro arsenic and lead bioaccessibility

As, Cu and Pb bioaccessibility (As and Pb in Soil-1, As and Cu in Soil-2) was assessed using the SBRC gastric phase method [Juhasz et al., 2009]. This *in vitro* method is a simplified test, if compared to the Physiologically Based Extraction Test (PBET) [Ruby et al, 1996]; only the gastric

phase was determined for the assessment of As and Pb bioacessibility. Aliquots (0.5 g) of each amended and control soil sample, finely grounded and crushed to <250 µm, were mixed with 50 ml of gastric solution (30.03 $g \cdot L^{-1}$ glycine adjusted to pH 1.5 with concentrated HCl) in 60 mL polyethylene screw cap flasks. The pH was noted and the flasks were incubated at 37 °C on a rotary shaker (120 rpm). After 1 hour of incubation, the pH was determined; when the fluid pH was not within ± 0.5 unit of the starting pH (pH 1.5), the test was discarded. After centrifugation an aliquot of gastric phase sample (15 mL) was directly taken from each bottle, passed through a 0,2 µm filter and analyzed by ICP-OES. The *in vitro* bioaccessibility was calculated as the percentage of As and Pb extractable relatively to the total soil As and Pb concentrations, respectively.

2.6. Pot experiment and plant analysis

The pot trials were conducted for all treated and untreated polluted soils both subjected or not to redox cycles treatments. Sub-samples of soil were placed in free-draining plastic pots (containing approximately 200 g of soil) in which ryegrass (*Lolium perenne*) seeds (30 seeds of ryegrass in each pot) were planted after overnight imbibition. A total of 60 pots were prepared [3 pots for each treated (*n*=4, amendments) and untreated (*n*=1, control) soil samples per two soil type (*n*=2, Soil-1 and Soil-2)]. The ryegrass (*Lolium perenne*) was grown for 60 days with a day temperature of 18 °C and a night temperature of 15 °C.

Before planting, the soil samples were fertilized with a nutrient solution as reported by Lombi et al. (2002b). After planting, each pot was watered: Soil-1 samples reached the 60% and Soil-2 samples the 80% of their water holding capacity. Subsequently each pot was weighted and the pots were watered every day to maintain the same weight.

After 60 days shoots were separated from roots, thoroughly washed with distilled water and fresh and dry (70°C for 24 h) weights were recorded (data not reported). Dry shoots (0.25 g) were weighed in microwave teflon-tubes; 5 mL of 65% HNO_3 and an 1:1 mixture of H_2O and 30% H_2O_2

were added in each tube. The samples were heated at 220 °C and digested in a Microwave Milestone MLS 1200. The As and Pb in shoots of Soil-1 and As and Cu in shoots of Soil-2 were analysed by ICP-OES.

2.7. Statistical analysis

All the determinations were performed in triplicate and the data reported in the Tables and Figures refer to mean values. One-way analysis of variance (One-way ANOVA) was carried out to compare all the means of the different treatments. Where significant P-values ($P < 0.05$) were obtained, differences between individual means were compared using a post-hoc Fisher's least significant difference test (LSD, $P < 0.05$). Two-way analysis of variance (Two-way ANOVA) was performed to evaluate the influence of treatments (RMsw, RMa, WTR, Lime and Control-Soil) and redox condition (aerobic and redox cycles conditions) on leachability, DOC, bioaccessibility and phytoavailability. All data were analyzed using the Minitab statistical software for Windows.

3. Results and Discussion

3.1. Characteristics of polluted soil samples

The reported physical and chemical characteristics of the two soils (Table 1) show that Soil-1 and Soil-2 were both weakly acidic with a total organic carbon of 3.5 % in Soil-1 and 1.9 % in Soil-2. The total concentrations of As (2428 and 860 $mg \cdot kg^{-1}$ in Soil-1 and Soil-2 respectively), Cu (1500 $mg \cdot kg^{-1}$ in Soil-2) and Pb (559 $mg \cdot kg^{-1}$ in Soil-1) in the unamended soils (Table 1) were higher compared to the normal background values for unpolluted soils [Alloway, 1997]. In particular, the total concentration of As in both soils exceeded substantially 20 $mg \cdot kg^{-1}$, which is the guideline

limit for the As concentration in agricultural soil proposed by the European Community [Oliveira and Pampulha, 2006].

3.2. The leaching of trace elements

The leached metal concentrations (As in both soils, Pb in Soil-1 and Cu in Soil-2) differed between treatments (RM_{sw}, RM_a, Al-WTR, lime and control) and oxidation conditions (aerobic and redox cycles conditions) (Fig. 1).

Two-way ANOVA highlighted a significant influence ($P < 0.05$) of the treatments and redox conditions on leachability of As, Pb and Cu in both soils (Table 3). Moreover, As in Soil-1 and Cu in Soil-2 leachability were significantly influenced by treatments, redox conditions and by their interactions, while As in Soil-2 and Pb in Soil-1 leachability were influenced by both the treatments and redox conditions but not by their interactions (Table 3).

Relatively low amounts of leached As and Pb, compared to their overall concentrations in soils, were detected in the control Soil-1 (i.e. 1.56% and 0.21% of the total As and Pb respectively in control Soil-1 kept under aerobic condition) (Fig. 1 A and B). The RM_{sw} and RM_a addition increased the As and Pb leached concentrations with respect to the control soil, under both oxidative conditions (aerobic and redox cycles). Particularly, As leaching in the RM_{sw} treatment was 2.6 and 3.2 times higher compared to the control under aerobic and redox cycles conditions, respectively (Fig. 1A). Similarly, Pb leaching, in the RM_{sw} treatment, was 2.8 and 2.3 times higher than in the control under aerobic and redox cycles conditions (Fig. 1B). The increase in As and Pb leaching in the samples treated with both RMs could be due to the increase of the dissolved organic carbon (DOC) detected following the addition of RM_{sw} and RM_a (Fig. 2). Two-way ANOVA, indicated that the DOC were mostly influenced by treatment in Soil-1 while, in Soil-2, DOC was significantly influenced ($P<0.001$) by either the treatments, the redox conditions and their interactions (Table 3). The increase in DOC in RM_{sw} and RM_a Soil-1 is most likely caused by an increase of soil pH and/or of salt (Na mainly) concentrations (data not shown). Similar results were reported by Garau

et al. (2011), which found that the combined effect of the RM alkalinity and its high Na content enhanced the release of water soluble carbon from the stabilized soil organic matter. The coefficients of correlation between DOC and As and Pb leached in soils treated with RM_{sw} and RM_a, in aerobic and in redox cycle conditions, were always positive and higher than 0.93 ($P<0.05$), showing a strong relationship between the DOC content and As and Pb solubility. These results were in agreement with those reported by Lombi et al. (2002a) who found that application of red muds increased the concentration of metals in soil solution in comparison with the untreated soil probably due to the significant increase of dissolved organic matter, which formed soluble complexes with metals in the red mud treated soil. An enhanced mobilization of Pb, as dissolved organic complexes, has been observed by several authors [Alvim Ferraz and Lourenço, 2000; Charlatchka and Cambier 2000]. Other studies show an increase in As mobility in presence of high DOC concentrations, due to the formation of soluble complexes between As and dissolved organic matter in presence of metal ions such as Al and Fe which act as bridge [Dobran and Zagury, 2006; Mohapatra et al. 2007]. Furthermore, mechanisms of competition between DOC and arsenate for the same positively charged sites should not be excluded [Grafe et al., 2002].

In the context of this study, it is important to underline the differences found between soil samples incubated under aerobic conditions and those subjected to redox cycles. Particularly, the data showed an increase in As and Pb mobility in soils treated with both the RMs and subjected to 3 repeated redox cycles in comparison to the same treatment kept under aerobic conditions. The different As and Pb mobility in RMs-soil samples subject to different oxidative conditions could be due to a reductive dissolution of the Fe phases of RM (i.e. hematite and goethite) under anaerobic conditions [Ghosh et al., 2006, Pique et al., 2010]. The dissolution of the Fe phases could have caused the release of the As previously bounded, with the concomitant increase of its solubility, as reported by several authors [e.g. Ghosh et al., 2006, Pique et al., 2010]. The short time elapsed between the anaerobic and aerobic cycles (3 days) was probably not enough to allow the formation of secondary phases of Fe, that allow the soluble As to be re-incorporated on the newly forming

solids, as suggested by Fendorf et al. [2010]. Furthermore, since an analogous increase of leached Pb in the RM_{sw} and RM_a in Soil-1, following the redox cycle, was not detected, another possible explanation could be the reduction of As(V) to As(III) in the anaerobic cycle treatment [Genç-Fuhrman et al., 2004]. Arsenite at pH values between 4.0-9.5 is mainly present as arsenious acid (H_3AsO_3), which is more mobile and soluble than As(V) [Deiana et al. 2009]. However, if this was the case, we would have expected to see an increase in As solubility also in the other treatments subjected to redox cycles.

No significant difference was observed between the As and Pb leached in Soil-1 samples treated with WTR and Lime, with respect to the control, under both oxidation conditions (Fig. 1A and B).

Leached As and Cu decreased in all treated Soil-2 samples and in both soil oxidation condition, with respect to the control Soil-2 (Fig. 1C and D).

Similarly to Soil-1 an increase (> 10 %) of the As leached from Soil-2 was observed in the soil samples treated with RM_{sw} and RM_a subjected to the redox cycles conditions in comparison to the same treatment kept under aerobic conditions (Fig. 1C). As previously reported, the difference could be due to the dissolution of RM phase containing ferric iron or to the reduction of As(V) in As(III).

WTR was the most effective amendment at decreasing the leaching of As in both aerobic and redox cycles conditions in Soil-2. The As mobility in WTR-Soil-2 was about twice lower compared to the untreated control soil (this trend was also observed in Soil-1, even if the decrease of leached As in the WTR-Soil-1 was not statistically significant). The decrease of As mobility in WTR-soil samples could be due to the presence, in WTR used, of amorphous Al phases and stable organic matter (more than 20%) which have a strong affinity towards As [Wang and Mulligan, 2006]. The organic matter of WTR is mainly constituted by humic substances as highlighted by several authors [Makris et al., 2009; Babatunde et al., 2009]. The presence of stable organic matter could have favoured the formation of insoluble complexes between the As, the humic substances and cations such as Fe^{3+} and Al(III) which acted as bridge [Lin et al., 2004; Wang and Mulligan, 2006]. Wang and Mulligan

(2006) reported also that functional groups containing N- and S- have the potential to influence As sorption.

The highest concentration of leached Cu was found in control Soil-2 after three redox cycles (8.62 $mg \cdot kg^{-1}$) while the lowest one was found in lime amended soil (4.91 $mg \cdot kg^{-1}$ d.m.) kept under aerobic condition. In general, Cu mobility was statistically higher in soils (treated and untreated) subjected to three redox cycles than the same soil samples incubated under aerobic conditions (Fig. 1D). In particular, the concentration of Cu leached was 1.3 times higher in control Soil-2 subjected to redox cycles in comparison to the aerobic control. The increased Cu leaching in Soil-2 subjected to redox cycles was correlated to an increase in DOC (Fig. 2). The formation of soluble complexes between the Cu and DOC, which lead to the increase in Cu mobility, is well known [e.g. Neagoe et al., 2012]. In fact, in Soil-2 a significant correlation between the leached Cu and DOC was observed [R^2= 0.89 and 0.75 ($P<0.05$) under aerobic and redox cycles conditions respectively].

The decrease in As and Cu leaching in the treated Soil-2 samples, in compariosn to control, could be due to the changes in physical and chemical properties of the samples following the addition of sorbents [Kabata-Pendias, 2000]. In contrast to Soil-1, Soil-2 is a sandy loam soil with the 80% of sand; the addition of amendments could have improved the soil texture, increasing the finer fraction and the content of Al and Fe (particularly in the soil samples treated with WTR and RMs), so promoting the formation of stable surface complexes between the soil solid phase and soluble Cu and As [Qian et al., 1996; Hartley and Lepp, 2008].

3.2. In Vitro Arsenic and Lead Bioaccessibility

Bioaccessibility of a contaminant is defined as the soluble fraction in the gastrointestinal tract which is available for absorption [Juhasz et al, 2007]. Indeed even if drinking water and food are the primary routes of exposure to arsenic in the world, accidental ingestion of contaminated soil can be a significant exposure pathway for children because of their important hand to mouth activity. It has

been documented that accidental ingestion of As-contaminated soil is a major concern and that exposure to arsenic by dermal absorption and inhalation is considered negligible compared to ingestion [Mitchell and Barr, 1995].

The two-way ANOVA showed that bioaccessibility in Soil-1 was significantly influenced by either the treatments, the redox conditions and their interactions while in Soil-2 the bioaccessibility of As was mostly influenced by redox conditions (Table 3).

As and Pb bioaccessibility was slightly, but significantly, lower in RM_{sw}, RM_a and WTR treated Soil-1 left under aerobic condition, or subjected to redox cycles with respect to the control (Fig. 3 A and B).

Similarly, As in-vitro bioaccessibility slighty decreased in all the treated Soil-2 samples, in comparison to the untreated soil, under both aerobic conditions following the 3 redox cycles (Fig. 3C).

It is important to underline the different As bioaccessibility between Soil-1 and Soil-2. Whereas total As concentration in Soil-1 was 2428 mg kg^{-1}, the highest percentage of bioacessibility, detected in lime treated soil under aerobic condition, was 25% of total As. By contrast, in Soil-2, total As concentration was 860 mg·kg^{-1}, but the As bioaccessibility exceeded in each soil samples and redox conditions the 70% of total As. These differences are most likely due to the different origin of As in the two soils: geogenic in Soil-1 and as a result of CCA application in Soil-2. In addition, the higher fraction of coarse soil particles in Soil-2 than Soil-1, the lower number of sorption sites and the lower Fe and Al content are also likely to play a role in the differences between the two soils [Yang et al., 2002; Juhasz et al. 2007].

The results of the bioaccessibility tests showed that in the case of As the differences between the two soils, in terms of % bioaccessibility, was much larger than the reduction in bioaccessibility caused by the amendments. Nevertheless, sorbent additions caused a small but significant decrease in As and Pb bioaccessibility.

3.3. Metal uptake by ryegrass grown on different treated soils

Ryegrass was chosen for the pot experiments because it shows high tolerance to metal(oid)s such as As, Cu, and Pb [Dong et al., 2008; Karami et al. 2011] and to low soil fertility [Fan et al. 2012].

With very few exceptions, the two-way ANOVA indicated that the phytoavailabilty of the trace elements studied, was significantly influenced by either the treatments, the redox conditions and their interactions; only the availability of Cu was influenced by the treatments ($P<0.001$) but it was not influenced ($P>0.05$) by the redox conditions and by the interactions between the treatments and redox conditions (Table 3).

The ryegrass growth data showed no significant differences in terms of plant biomass production, between the treatments and the different oxidative conditions of the soils (data not shown).

By contrast, significant differences in the uptake of trace elements were found. Arsenic uptake significantly decreased in the ryegrass plants grown in treated Soils 1 and 2 when compared to control soils (from 1.5 to 7 times lower in plants grown in treated soil samples compared with untreated soils) (Fig. 4 A and C). In particular, lower amounts of As in shoots were detected in plants grown in soil samples 1 and 2 treated with RM_{sw}, RM_a, and WTR. Redox cycles generally increased As concentrations in plants.

The content of Pb in ryegrass grown on Soil-1, under both redox conditions, showed a trend similar to that of As (Fig. 4 B). The Pb uptake decreased in the treated soils in comparison to the control, and this decrease was always higher than 1.5 times in the aerobic treatments (Fig. 4 B). The RMs treatments were the most effective in reducing Pb uptake.

Although the amount of leached As and Pb in RMs treated Soil-1 was higher than the Control Soil-1 ones, the content of As and Pb in shoot plants grown in RMs Soil-1 followed a different trend. The results above reported showed that the addition of RMs in Soil-1 led to an increase of the soluble fractions of As and Pb, but their bioavailability decreased with RM application; this is in agreement with the results reported by Castaldi et al.[2010a].

The uptake of As by plants grown on Soil-2 showed a trend similar to that observed in the Soil-1. A significant and marked decrease in Cu uptake was detected in all the plants grown in treated Soils-2; no difference between the treatments and the redox conditions was observed (Fig. 4 D).

Differences between the concentration of As in plants of ryegrass grown in the untreated Soil-1 and -2 were found. The amount of As in shoots was higher in plants grown on untreated Soil-2 under both redox conditions despite the higher total concentration of As in Soil-1 (Table 1; Fig. 4 A and B). The different chemical and physical characteristics of the soils (particularly the different soil texture, the content of Fe, Al and organic matter) strongly affected the As phytoavailability. In particular, the higher sand content in Soil-2 compared to Soil-1 (80% vs 59%) could be a possible reason of the higher As uptake in plants grown in Soil-2 with respect to plants grown in Soil-1, in agreement with the results reported by several authors [i.e. O'Neill, 1995; Hartley and Lepp, 2008].

The results above reported showed that all the treatments were able to decrease the uptake of As, Cu and Pb in the Soils 1 and 2, although plant growth was similar between ryegrasses grown in treated and untreated soils.

4. Conclusions

The results of these experiments show that the effect of various soil amendments is a complex function of soil conditions, source of contamination, and perhaps, most importantly, the environmental endpoint considered.

Contamination in Soil-1 is due to mining activity, while Soil-2 was contaminated by CCA. Soil-1 is also more clayey and contains more organic C than Soil-2, which is also slightly more acidic. As a consequence, the relative leachability of As in the control treatment of Soil-1 is lower than in Soil-2. Similarly, relative As bioaccessibility and phytoavailability in Soil-2 is much higher than in Soil-1 where, most likely, As is strongly retained in mineral phases.

Soil amendments did not reduce the leachability of As and Pb in Soil-1. In fact, both As and Pb leachability increased when RMs were applied; most likely as a result of increase in DOC. In the

more sandy and acidic Soil-2, the amendment used had a more positive effect in reducing As and Cu leachability.

When bioaccessibility is considered, small differences were found between treatments in both soils. This indicates that if new phases of As and Pb were formed in the treated soils, these were largely unstable under the strongly acidic conditions used to simulate gastric conditions. The results also confirm the importance of contamination origin in controlling bioaccessibility.

The effect of soil amendments was more evident when metal uptake by plants was considered. In this case, RMs and Al-WTR were the most effective treatments able to significantly reduce As and metal uptake.

The results indicate that redox cycles is not generally able to decrease the leachability, bioaccessibility and phytoavailability of As and metals. On the contrary, some endpoints were negative affected by alternating aerobic/anaerobic cycles.

Overall, the results indicate a positive effect of the soil amendments, used but also highlighted the fact that some environmental endpoints, such as metal leachability, may be at least temporarily negatively affected by perturbation of the soil system upon application of amendments. Furthermore some environmental endpoints, such as bioaccessibility, seem to be more difficult to modify the phytoavailability. Consequently, when designing a remediation strategy based on the application of soil amendments a thorough knowledge, and ranking, of the most relevant environmental endpoint is essential in order to identify the most appropriate material to be used.

Acknowledgements

The Financial support of the Ministero dell'Istruzione, dell'Università e della Ricerca (PRIN 2008), of Regione Sardegna (L.R. 7/2007 Progetti di ricerca di base- Bando 2008) and of Fondazione Banco di Sardegna, is gratefully acknowledged.

Reference:

Alvim Ferraz, M.C.M., Lourenço, J.C.N., 2000. The influence of organic matter content of contaminated soils on the leaching rate of heavy metals. Environmental Progress 19, 53–58.

Alloway, B.J., 1997. Soil processes and the behaviour of metals. In BJ Alloway editor: Heavy Metals in Soils, 2nd edition. Chapter 2, Blackie Academic & professional, 11–37.

Babatunde, A.O., Zhao Y.Q., Burke A.M., Morris M.A., Hanrahan J.P., 2009. Characterization of aluminium-based water treatment residual for potential phosphorus removal in engineered wetlands. Environmental Pollution 157, 2830–2836.

Bartlett, R.J. James, B.R., 1993. Redox chemistry of soils. In Spark DL Editor: Advances in Agronomy. Academic Press, San Diego, CA. 50, 151–208.

Bidar, G., Garcon, G., Pruvot, C., Dewaele, D., Cazier, F., Douay, F., Shirali, P., 2007. Behavior of Trifolium repens and Lolium perenne growing in a heavy metal contaminated field: plant metal concentration and phytotoxicity. Environmental Pollution 147, 546–553.

Boruvka, L., Vacek, O., Jehlicka, J., 2005. Principal component analysis as a tool to indicate the origin of potentially toxic elements in soils. Geoderma 128, 289–300.

Brandstetter, A., Sletten, R.S., Mentler, A., Wenzel, W.W., 1996. Estimating dissolved organic carbon in natural waters by UV absorbance (254 nm). Z. Pflanzenernähr. Bodenk 159, 605–607.

Charlatchka, R., Cambier P., 2000. Influence of reducing conditions on solubility of trace metals in contaminated soils. Water, Air and Soil Pollution 118, 143–167.

Castaldi, P., Silvetti, M., Santona, L., Enzo, S., Melis, P., 2008. XRD, FT-IR, and thermal analysis of bauxite ore-processing waste (red mud) exchanged with heavy metals. Clays and Clay Minerals 56, 461–469.

Castaldi, P., Silvetti, M., Enzo, S., Melis, P., 2010a. Study of sorption processes and FT-IR analysis of arsenate sorbed onto red muds (a bauxite ore processing waste. Journal of Hazardous Materials 175, 172–178.

Castaldi, P., Silvetti, M., Garau, G., Deiana, S., 2010b. Influence of the pH on the accumulation of phosphate by red mud (a bauxite ore processing waste). Journal of Hazardous Materials 182, 266–272.

Castaldi, P., Silvetti, M., Enzo, S., Deiana, S., 2011. X-ray diffraction and thermal analysis of bauxite ore-processing waste (red mud) exchanged with arsenate and phosphate. Clays and Clay Minerals 59, 189–199.

Cederkvist, K., Holm, P.E., Jensen, M.B., 2010. Full scale removal of arsenate and chromate from water using a limestone and ochreous sludge mixture as low-cost sorbent material. Water Environment Research 82, 401–408.

Ciccu, R., Ghiani, M., Serci, A., Fadda, S., Peretti, R., Zucca, A., 2003. Heavy metal immobilization in the mining-contaminated soils using various industrial wastes. Minerals Engineering 16, 187–192.

Contin, M., Mondini, C., Leita, L., De Nobili, M., 2007. Enhanced soil toxic metal fixation in iron (hydr)oxides by redox cycles. Geoderma 140, 164–175.

Davranche, M., Bollinger, J.C., 2000. Heavy Metals Desorption from Synthesized and Natural Iron and Manganese Oxyhydroxides: Effect of Reductive Conditions. Journal of Colloid and Interface Science 227, 531–539.

Davranche, M., Bollinger, J.C., Bril, H., 2003. Effect of reductive conditions on metal mobility from wasteland solids: an example from the Mortagne-du-Nord site (France). Applied Geochemistry 18, 383–394.

Deiana, S., Deiana, L., Premoli, A., Senette, C., 2009. Accumulation and mobilization of arsenate by Fe(III) polyions trapped in a Ca-polygalacturonate network. Plant Physiology and Biochemistry 47, 615–622.

Dobran, S., Zagury, G.J., 2006. As speciation and mobilization in CCA-contaminated soils: influence of organic matter content. Science of the Total Environment 364, 239–250.

Dong, Y., Zhu, Y-G., Smith, F.A., Wang, Y., Chen, B., 2008. Arbuscular mycorrhiza enhanced arsenic resistance of both white clover (Trifolium repens Linn.) and ryegrass (Lolium perenne L.) plants in an arsenic-contaminated soil. Environtal Pollution 155, 174–181.

European Standard Method, Ref. No. EN 12457-2:2002 E

Fan, J., Zhenli, H.Z., Ma, L.Q., Nogueira, T.A.R., Wang, Y., Liang, Z., Stoffella, P.J., 2012. Calcium water treatment residue reduces copper phytotoxicity in contaminated sandy soils. Journal of Hazardous Materials 382, 199–200.

Fendorf, S., Noco, P.S., Kocar, B.D., Masue, Y., Tufano, K.J., 2010. Arsenic Chemistry in Soils and Sediments. In: Developments in Soil Science 34, 357–374.

Ferreira, S.L.C., Queiroz, A.S., Fernandes, M.S., dos Santos, H.C., 2002. Application of factorial designs and Doehlert matrix in optimization of experimental variables associated with the preconcentration and determination of vanadium and copper in seawater by inductively coupled plasma optical emission spectrometry. Spectrochimica Acta Part B, 1939–1950.

Garau, G., Castaldi, P., Santona, L., Deiana, P., Melis, P., 2007. Influence of red mud, zeolite and lime on heavy metal immobilization, culturable heterotrophic microbial populations and enzyme activities in a contaminated soil. Geoderma 142, 47–57.

Garau, G., Silvetti, M., Deiana, S., Deiana, P., Castaldi, P., 2011. Long-term influence of red mud on As mobility and soil physico-chemical and microbial parameters in a polluted sub-acidic soil. Journal of Hazardous Materials 185, 1241–1248.

Genç-Fuhrman, H., Tjell, J.C., McConchie, D., 2004. Increasing the arsenate adsorption capacity of neutralized red mud (Bauxol). Journal of Colloid and Interface Science 271, 313 – 320.

Ghosh, A., Mukiibi, M., Saez, A.E., Ela, W.P., 2006. Leaching of arsenic from granular ferric hydroxide residuals under mature landfill conditions. Environmental Science and Technology 40, 6060–6075.

Grafe, M., Eick, M.J., Grossl, P.R., Saunders, A.M., 2002. Adsorption of arsenate and arsenite on ferrihydrite in the presence and absence of dissolved organic carbon. Journal of Environmental Quality 31, 1115–1123.

Hartley, W., Lepp, N.W., 2008. Effect of in situ soil amendments on arsenic uptake in successive harvests of ryegrass (Lolium perenne cv Elka) grown in amended As-polluted soils. Environmental Pollution 156, 1030–1040.

Holm, P.E., Rootzén, H., Borggaard, O.K., Møberg, J.P., 2003. Christensen TH. Correlation of cadmium distribution coefficients to soil characteristics. Journal of Environmental Quality 32, 138–145.

Juhasz, A.L., Smith, E., Weber, J., Rees, M., Rofe, A., Kuchel, T., Sansom, L., Naidu, R., 2007. In vitro assessment of arsenic bioaccessibility in contaminated (anthropogenic and geogenic) soil. Chemiosphere 69, 69 – 78.

Juhasz, A.L., Weber, J., Smith, E., Naidu, R., Marschner, B., Rees, M., Rofe, A., Kuchel, T., Sansom, L., 2009. Evaluation of SBRC-Gastric and SBRC-Intestinal Methods for the Prediction of In Vivo Relative Lead Bioavailability in Contaminated Soils. Environmental Science and Technology 43, 4503–4509.

Kabata-Pendias, A., 2000. Trace elements in Soils and Plants, third edition: Soils and Soil processes. CRC Press Boca Raton. 27–48.

Karami, N., Clemente, R., Moreno-Jiménez, E., Lepp, N.W., Beesley, L., 2011. Efficiency of green waste compost and biochar soil amendments for reducing lead and copper mobility and uptake to ryegrass. Journal of Hazardous Materials 191, 41–48.

Lin, H.T., Wang, M.C., Li, G.C., 2004. Complexation of arsenate with humic substance in water extract of compost. Chemosphere 56, 1105–1112.

Lombi, E., Zhao, F.J., Zhang, G., Sun, B., Fitza, W., Zhang, H., McGrath, S.P., 2002a. In situ fixation of metals in soils using bauxite residue: chemical assessment. Environmental Pollution 118, 435–443.

Lombi, E., Zhao, F.J., Wieshammer, G., Zhang, G., McGrath, S.P., 2002b. In situ fixation of metals in soils using bauxite residue: biological effects. Environmental Pollution 118, 445–452.

Lombi, E., Hamon, R., Wieshammer, G., McLaughlin, M.J., McGrath, S.P., 2004. Assessment of the use of industrial by-products to remediate a copper- and arsenic-contaminated soil. Journal of Environmental Quality 33, 902–910.

Lombi, E., Stevens, D.P., McLaughlin, M.J., 2010. Effect of water treatment residuals on soil phosphorus, copper and aluminium availability and toxicity. Environmental Pollution 158, 2110–2116.

Makris, K.C., Sakar, D., Datta, R., 2006. Evaluating a drinking-water waste by-product as a novel sorbent for arsenic. Chemosphere 64, 730–741.

Makris, K.C., Sakar, D., Parsonsc, J.G., Datta, R., Gardea-Torresdey, J.L., 2009. X-ray absorption spectroscopy as a tool investigating arsenic(III) and arsenic(V) sorption by an aluminum-based drinking-water treatment residual. Journal of Hazardous Materials 171, 980–986.

Marcussen, H., Holm, P.E., Strobel, B.W., Hansen, H.C.B., 2009. Nickel sorption to goethite and montmorillionite in presence of citrate. Environmental Science and Technology, 1122–1127.

Mitchell, P., Barr, D., 1995. The nature and significance of public exposure to arsenic: a review of its relevance to South West England. Environmental geochemistry and health 17, 57–82.

Mohapatra, M., Sahoo, R.K., Rout, K., Das, R.P., Anand, S., 2007. Completion report on utilization of goethite for effluent treatment. Prepared for Ministry of Environment and Forests, New Delhi, India, 634.

Neagoe, A., Iordache, V., Fărcăşanu, I.C., 2012. The role of organic matter in the mobility of metals in contaminated catchments. In Kothe and Varma Editors: Bio-geo-interactions in metal contaminated soils. Soil Biology 31, 297–325.

Oliveira, A., Pampulha, M.E., 2006. Effects of long-term heavy metal contamination on soil microbial characteristics. Journal of Bioscience and Bioengineering 102, 157–161.

O'Neill, P., 1995. Arsenic. In Alloway BJ editor: Heavy Metals in Soils. 105–121.

Pique, A., Grandia, F., Canals, A. 2010. Processes releasing arsenic to groundwater in the Caldes de Malavella geothermal area, NE Spain. Water Research 44, 5618–5630.

Phillips, I.R., 1998. Use of soil amendments to reduce nitrogen, phosphorus and heavy metal availability. Journal of Soil Contamination 7, 191–212.

Qian, J., Shan, X.Q., Wang, Z.J., Tu, Q., 1996. Distribution and plant availability of heavy metals in different particle-size fractions of soil. Science of the Total Environment 187, 131–141.

Ruby, M.V., Davis, A., Schoof, R., Eberle, S., Sellstone, C.M., 1996. Estimation of lead and arsenic bioavalability using a physiologically based extraction test. Environmental Science and Technology 33, 422–430.

Sakar, D., Makris, K.C., Vandanapu, V., Datta, R.. 2007. Arsenic immobilization in soil amended with drinking-water treatment residuals. Environmental Pollution 146, 414–419.

Santona, L., Castaldi, P., Melis, P., 2006. Evaluation of the interaction mechanisms between red muds and heavy metals. Journal of Hazardous Materials B136, 324–329.

Schmidt, J.P., 1997. Understanding phytoxicity thresholds for trace elements in land-applied sewage sludge. Journal of Environmental Quality 26, 4–10.

Violante, A., Krishnamurti, G.S.R., Pigna, M., 2008. Factor affecting the sorption-desorption of trace elements in soil environments. In Violante A, Huang P, Gadd GM editors: Biophysico-chemical processes of heavy metals and metalloids in soil environment. Wiley and Sons, inc., 169–213

Wang, S., Mulligan, C., 2006. Occurrence of arsenic contamination in Canada: Sources, behavior and distribution. Science of the Total Environment 366, 701–721.

Wong, S.C., Li, X.D., Zhang, G., Qi, S.H., Min, Y.S., 2002. Heavy metals in agricultural soils of the Pearl river Delta, south China. Environmental Pollution 119, 33–44.

Yang, J.K., Barnett, M., Jardine, P., Basta, N., Casteel, S., 2002. Adsorption, Sequestration, and Bioaccessibility of As(V) in Soils. Environmetals Science and Technology 36, 4562–4569.

Table 1. Properties of Soil-1 and Soil-2

Chemical parameters	**Soil-1**	**Soil-2**
pH	6.22	5.45
Electric conductivity (mS cm^{-1})	0.41	0.86
Texture composition:		
Coarse sand (%)	36.62	34.00
Fine Sand (%)	22.33	46.00
Silt (%)	16.63	4.00
Clay (%)	24.41	16.00
Total organic C (g kg^{-1} d.m.)	34.9	18.9
Total Fe (mg kg^{-1} d.m.)	25600	7238
Total Al (mg kg^{-1} d.m.)	15340	11744
Concentrations of total As Pb Cu		
As (mg kg^{-1} d.m.)	2428.0	860
Pb (mg kg^{-1} d.m.)	558.59	---
Cu (mg kg^{-1} d.m.)	234.46	1500

Table 2. Main properties of the RM and Al-WTR samples used in this study

Chemical parameters	**RM**	**Al-WTR**
pH	11.10	6.45
EC ($mS \cdot cm^{-1}$)	8.70	5.69
SBET	19.50	28.00
pH_{PCZ}	4.77	6.85
Organic matter (% d.m.)	0.60	23.17
Al (% d.m.)	9.65	13.85
Ca (% d.m.)	1.04	1.10
Fe (% d.m.)	30.35	1.74
Na (% d.m.)	5.17	15.65
Concentrations of total As and heavy metals		
As ($mg\ kg^{-1}$ d.m.)	n.d	n.d
Cd ($mg\ kg^{-1}$ d.m.)	1.46	0.30
Cu ($mg\ kg^{-1}$ d.m.)	5.70	9.70
Pb ($mg\ kg^{-1}$ d.m.)	48.50	21.17
Zn ($mg\ kg^{-1}$ d.m.)	n.d.	38.59
Crystalline phases (wt.%)		
Cancrinite [$Na_6Ca_{1.5}Al_6Si_6O_{24}(CO_3)_{1.6}$]	4.0	n.d
Sodalite [$Na_8(Cl,OH)_2\|Al_6Si_6O_{24}$]	20.0	n.d
Hematite [Fe_2O_3]	44.0	n.d
Boehmite [AlO(OH)]	12.0	n.d
Gibbsite [$Al(OH)_3$]	4.0	n.d
Anatase [TiO_2]	4.5	n.d
Andradite [Ca–Fe–Al–Si oxide]	5.5	n.d
Quartz [SiO_2]	6.0	n.d

*Table 3. Two-way ANOVA of the leachability, bioaccessibility and phytoavailability of As and Pb in Soil-1; As and Cu in Soil-2; two-way ANOVA of the DOC in Soil-1 and Soil-2. Treatment : RMsw, RMa, WTR, Lime, Control; Condition: Aerobic condition, Redox Cycles. All values are F values with significance level P as indicated by asterisks. *, P < 0.05; **, P < 0.01; ***, P < 0.001.*

Leachability	**Soil-1**		**Soil-2**	
	As	**Pb**	**As**	**Cu**
Treatment	100.58***	28.79***	134.45***	18.50***
Condition	62.06***	3.71*	8.48**	94.43***
Treatment*Condition	7.05**	0.49	2.11	5.08**
DOC	**Soil-1**		**Soil-2**	
Treatment	52.59***		114.99***	
Condition	4.84*		338.44***	
Treatment*Condition	0.75		57.49***	
Bioaccessibility	**Soil-1**		**Soil-2**	
	As	**Pb**	**As**	
Treatment	9.57***	24.45***	5.97**	
Condition	9.89**	73.80***	36.26***	
Treatment* Condition	3.44*	53.91***	1.04	
Phytoavailability	**Soil-1**		**Soil-2**	
	As	**Pb**	**As**	**Cu**
Treatment	34.64***	44.41***	605.58***	63.36***
Condition	45.49***	24.62***	9.18**	2.49
Treatment*Condition	7.78**	7.29**	15.32***	2.72

Figure Captions

Fig. 1 Arsenic leached from Soil-1 (A), Pb leached from Soil-1 (B), As leached from Soil-2 (C), Cu leached from Soil-2 (D) after different soil amendments and oxidative conditions (aerobic condition and redox cycles). RM_{sw} = seawater treated red mud; RM_a = acid treated red mud; WTR = water treatment sludge; Lime = addition of lime; Control = untreated soil. Means values followed by the same letter, within the same soil and metal, are not significantly different at $P \leq 0.05$

Fig. 2 Dissolved Organic Carbon (DOC) in Soil-1 (A) and Soil-2 (B) after different soil amendments and oxidative conditions (aerobic condition and redox cycles). RM_{sw} = seawater treated red mud; RM_a = acid treated red mud; WTR = water treatment sludge; Lime = addition of lime; Control = untreated soil. Means values followed by the same letter, within the same soil and metal, are not significantly different at $P \leq 0.05$

Fig. 3 *In-vitro* bioaccessibility of As from Soil-1 (A), Pb from Soil-1 (B), As from Soil-2 (C) after different soil amendments and oxidative conditions (aerobic condition and redox cycles). RM_{sw} = seawater treated red mud; RM_a = acid treated red mud; WTR = water treatment sludge; Lime = addition of lime; Control = untreated soil. Means values followed by the same letter, within the same soil and metal, are not significantly different at $P \leq 0.05$

Fig. 4 Arsenic (A) and Pb (B) concentrations in plants grown in Soil-1, As (C) and Cu (D) concentrations of plants grown in Soil-2. RM_{sw} = seawater treated red mud; RM_a = acid treated red mud; WTR = water treatment sludge; Lime = addition of lime; Control = untreated soil. Means values followed by the same letter, within the same soil and metal, are not significantly different at $P \leq 0.05$

Figure 1

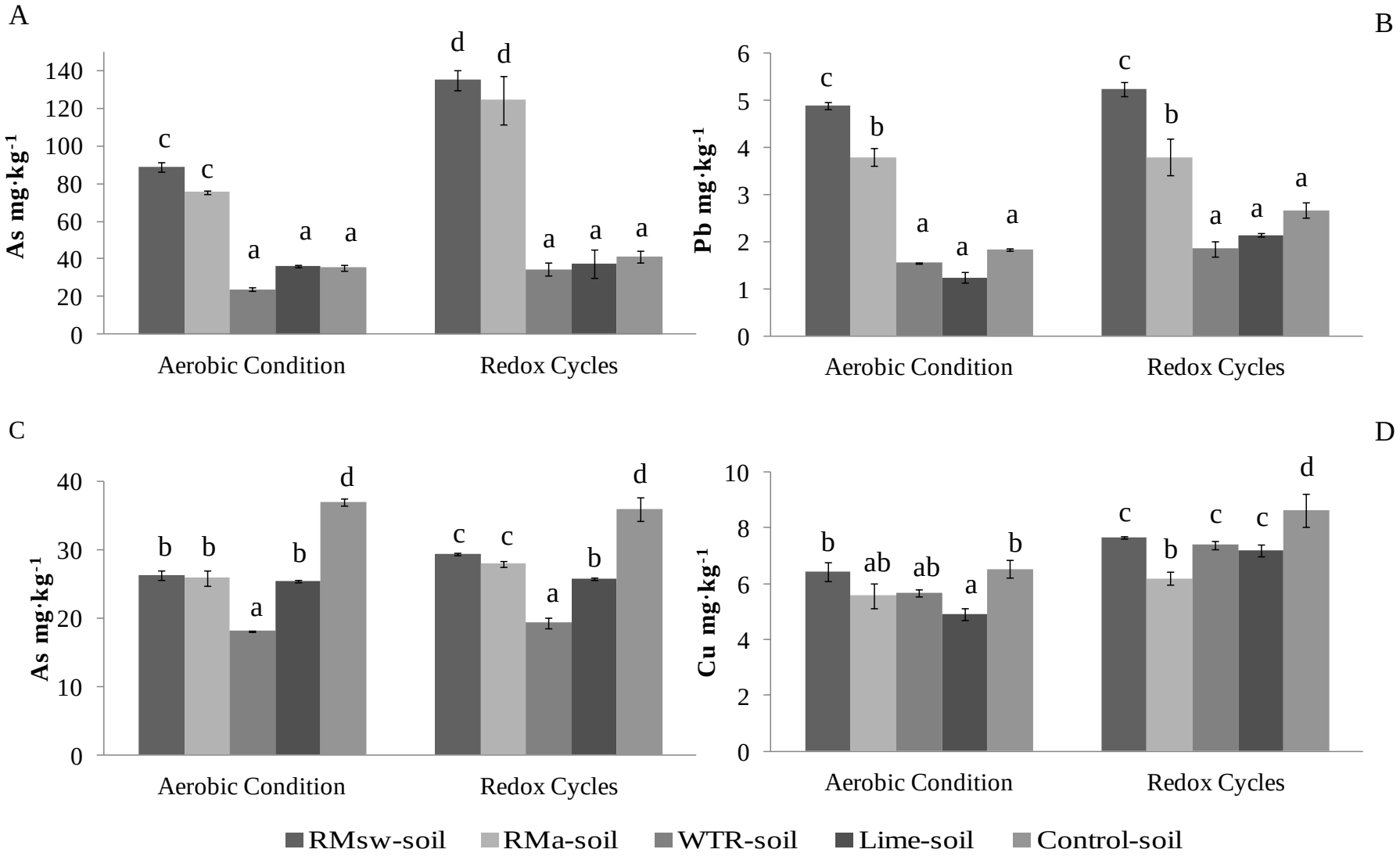

A
As mg·kg-1
140
120
100
80
60
40
20
0
c
c
a
a
a
d
d
a
a
a
Aerobic Condition
Redox Cycles
B
Pb mg·kg-1
6
5
4
3
2
1
0
c
b
a
a
a
c
b
a
a
a
Aerobic Condition
Redox Cycles
C
As mg·kg-1
40
30
20
10
0
b
b
a
b
d
c
c
a
b
d
Aerobic Condition
Redox Cycles
D
Cu mg·kg-1
10
8
6
4
2
0
b
ab
ab
a
b
c
b
c
c
d
Aerobic Condition
Redox Cycles
RMsw-soil
RMa-soil
WTR-soil
Lime-soil
Control-soil

Figure 2

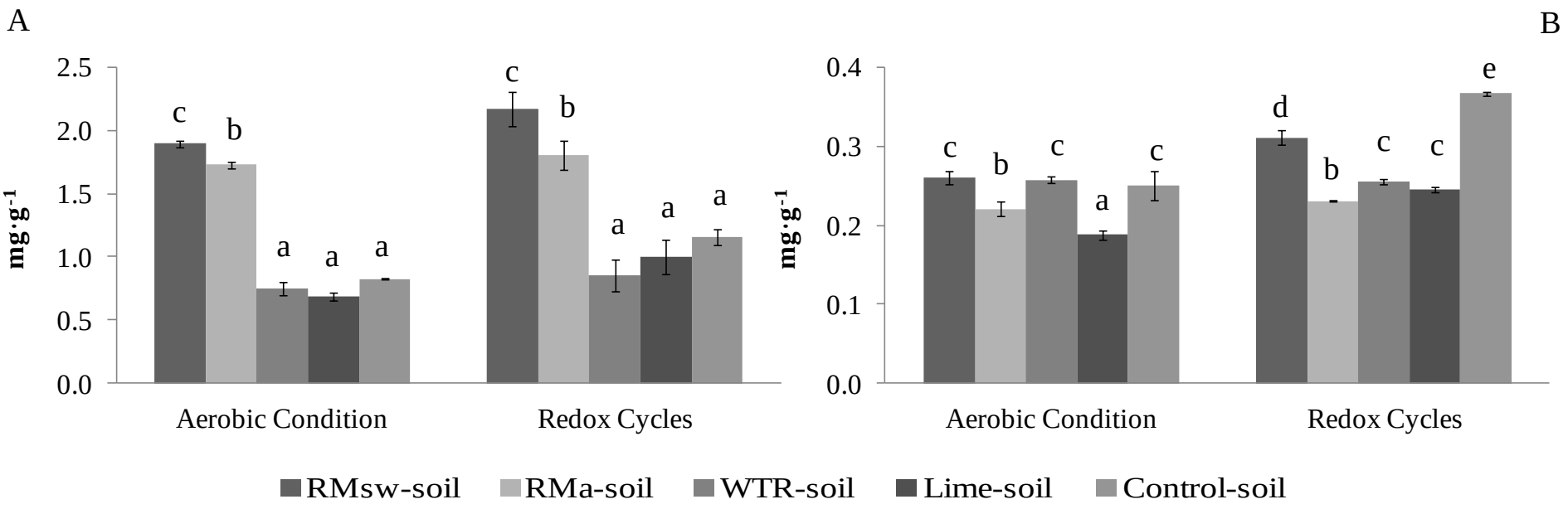

A
B
mg·g-1
2.5
2.0
1.5
1.0
0.5
0.0
c
b
a
a
a
c
b
a
a
a
Aerobic Condition
Redox Cycles
mg·g-1
0.4
0.3
0.2
0.1
0.0
c
b
c
a
c
d
b
c
c
e
Aerobic Condition
Redox Cycles
RMsw-soil
RMa-soil
WTR-soil
Lime-soil
Control-soil

Figure 3

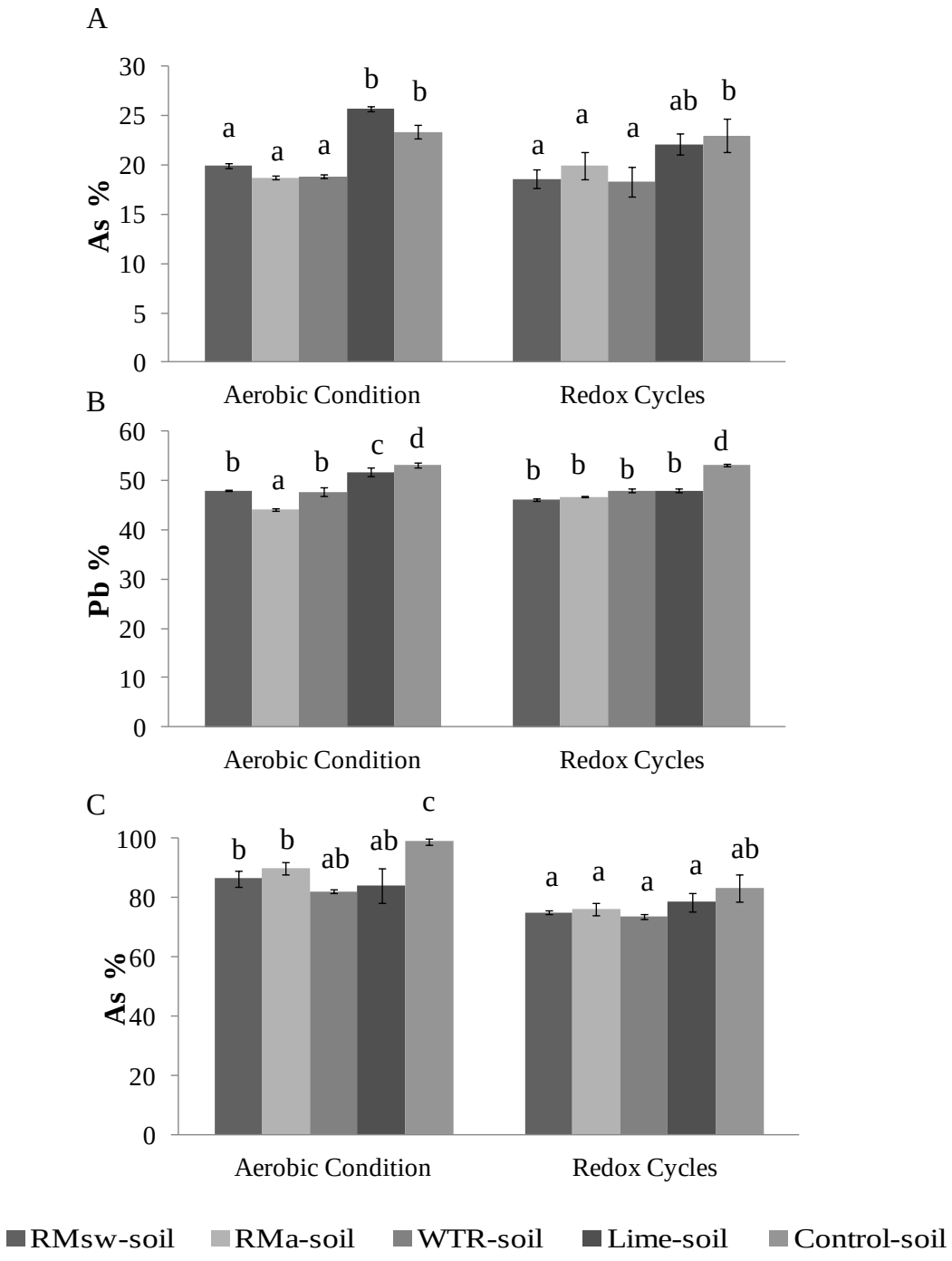
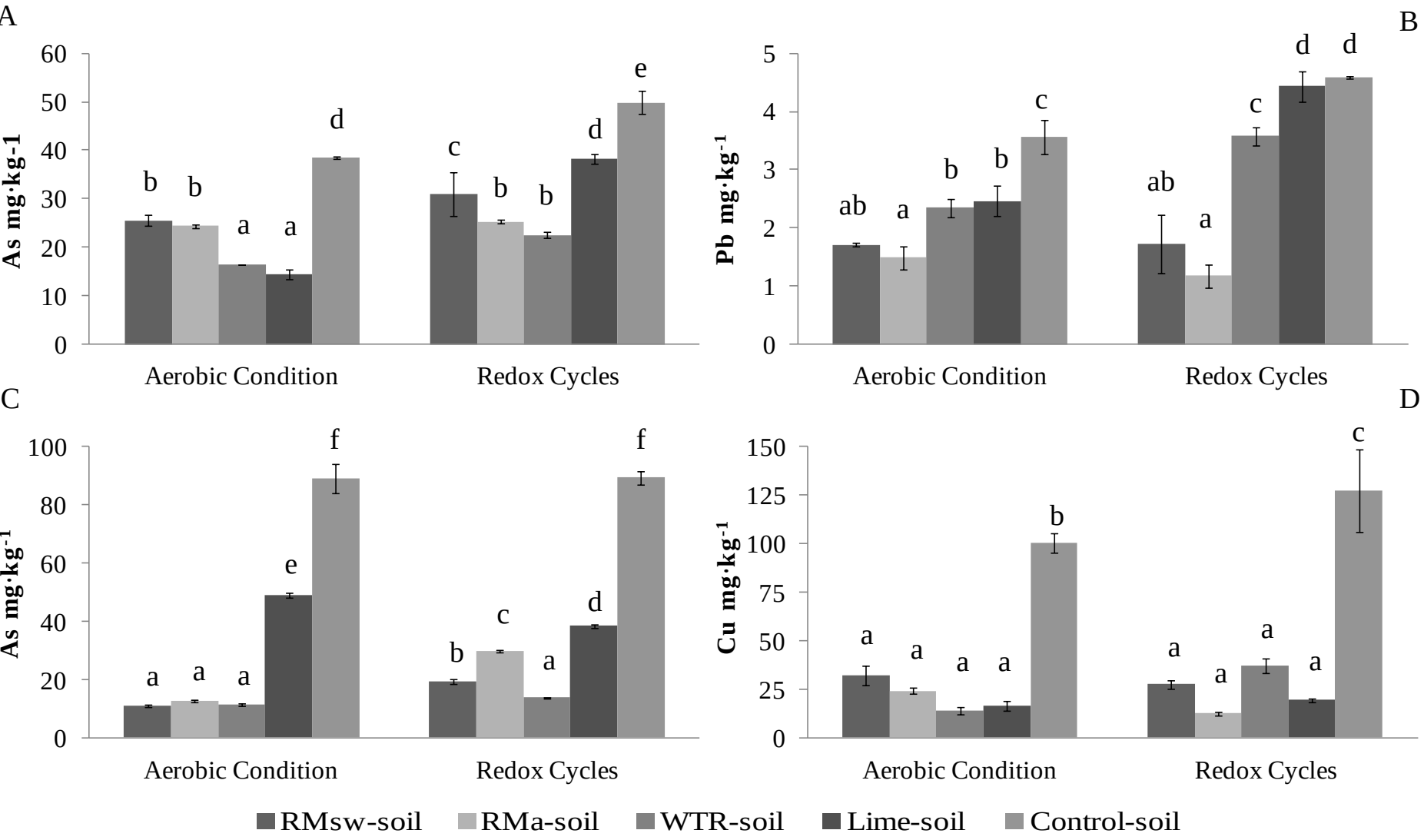

A
As %
30
25
20
15
10
5
0
a a a b b
a a a ab b
Aerobic Condition
Redox Cycles
B
Pb %
60
50
40
30
20
10
0
b a b c d
b b b b d
Aerobic Condition
Redox Cycles
C
As %
100
80
60
40
20
0
b b ab ab c
a a a a ab
Aerobic Condition
Redox Cycles
RMsw-soil
RMa-soil
WTR-soil
Lime-soil
Control-soil

Figure 4


A
As mg·kg-1
60
50
40
30
20
10
0
b b a a d
c b b d e
Aerobic Condition
Redox Cycles
B
Pb mg·kg-1
5
4
3
2
1
0
ab a b b c
ab a c d d
Aerobic Condition
Redox Cycles
C
As mg·kg-1
100
80
60
40
20
0
a a a e f
b c a d f
Aerobic Condition
Redox Cycles
D
Cu mg·kg-1
150
125
100
75
50
25
0
a a a a b
a a a a c
Aerobic Condition
Redox Cycles
RMsw-soil
RMa-soil
WTR-soil
Lime-soil
Control-soil