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In Situ Fixation of Metal(loid)s in Contaminated Soils: A Comparison of Conventional, Opportunistic, and Engineered Soil Amendments

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Elena Mele[a], Erica Donner[b,c], Albert L. Juhasz[b,c], Gianluca Brunetti[b,c], Euan Smith[b,c], Aaron R. Betts[d,e], Paola Castaldi[a], Salvatore Deiana[a], Kirk G. Scheckel[f], Enzo Lombi[b*].

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

[b] Centre for Environmental Risk Assessment and Remediation, University of South Australia, Building X, Mawson Lakes Campus, South Australia 5095, Australia

[c] Cooperative Research Centre for Contamination Assessment and Remediation of the Environment (CRC CARE), PO Box 486, Salisbury, South Australia 5106, Australia

[d] United States Environmental Protection Agency, Office of Research and Development, National Risk Management Research Laboratory, 26 West Martin Luther King Drive, Cincinnati, OH 45268, USA

[e] Oak Ridge Institute for Science and Education, P.O. Box 117, Oak Ridge, TN 37831, USA

[f] National Risk Management Research Laboratory, US Environmental Protection Agency, 5995 Centre Hill Avenue, Cincinnati, Ohio 45224, USA

* Corresponding author: Enzo Lombi, enzo.lombi@unisa.edu.au; Phone +61 8 8302 6267; Fax +61 8 8302 3057.

ABSTRACT: This study aimed at assessing and comparing the *in vitro* and *in vivo* bioaccessibility/bioavailability of As and Pb in a mining contaminated soil (As 2267 mg kg^{-1}, Pb 1126 mg·kg^{-1}), after the addition of conventional (phosphate), by product (water treatment residues: WTRs), and engineered (nano- and micro-scale zero valent iron (ZVI)) amendments.

Phosphoric acid was the only amendment able to significantly decrease Pb bioaccessibility with respect to untreated soil (41 and 47% in the gastric phase, 2.1 and 8.1% in the intestinal phase respectively), giving a Treatment Effect Ratio (TER: the bioaccessibility in the amended soil divided by the bioaccessibility in the untreated soil) of 0.25 and 0.87 in the gastric and intestinal phase respectively. *In vivo* bioavailability of Pb decreased in the phosphate treatment with respect to the untreated soil (6.0 and 24% respectively), but also in the Fe-WTR 2% (12%) and in nZVI-2 (13%) treatments. The ZVI amendments caused a decrease in As bioaccessibility, with the greatest decrease for the nZVI2-treated soil (TER of 0.59 and 0.64 in the gastric and intestinal phases, respectively). Based on arsenic K-edge XANES most of As in the untreated soil was present as As(V) associated with Fe-mineral phases. For the treated soil the proportion of arsenosiderite increased. Arsenite was present only as a minor species in the treated soils, with the exception of an nZVI treatment (~14% of As(III)), suggesting a partial reduction of As(V) to As(III) caused by nZVI oxidation.

INTRODUCTION

Arsenic (As) and lead (Pb), ubiquitous elements in the environment, are generally only present at trace levels in soil (ppb range to less than 10 ppm in various environmental matrices). [1,2] Their presence at heightened concentrations can be caused by both geochemical and anthropogenic processes, and is of concern due to their negative long-term impacts on environmental receptors [3]. Among anthropogenic sources, mining and smelting of metal(loid) sulphide ores are major sources of As and Pb contamination [4]. Human and animal exposure to As and Pb may occur through the consumption of contaminated drinking water and food, inhalation of particulate matter, and the ingestion of soil or dust. The latter, especially in the case of children and animals, is of concern due to the fact that many former mining and industrial areas have been developed into residential property or used for grazing animals in different areas of Southern Europe (Sardinia in particular) [5].

In many countries, regulatory limits for As and Pb in soils are based on their total concentration. However, due to mineralogical differences and the influence of soil properties, contaminant absorption following incidental soil ingestion is frequently less that the default value of 100%. However exposure parameters maybe refined through the assessment of relative contaminant bioavailability (RBA); i.e. the fraction of the ingested dose that is absorbed into the systemic circulation. Contaminant RBA can be measured using *in vivo* assays and model organisms, but cost and ethical considerations limit their routine use. As a result, *in vitro* gastrointestinal methods have been developed as surrogate assays for estimating contaminant RBA. In this context, bioaccessibility refers to the fraction of a contaminant that is soluble in gastrointestinal fluid and therefore potentially available for absorption into systemic circulation. [6, 7].

Metal/(loid) RBA and bioaccessibility depend on the physicochemical properties of the soil and the chemical characteristics of the metal(oid)s (e.g. mineral forms, solubility, and redox state) [8]. Remediation strategies that promote the formation of stable forms of As and Pb (e.g. in situ immobilization) have been proposed for the remediation/management of contaminated soils [9]. One such technology is based on the use of soil amendments which can immobilize contaminants through sorption and/or precipitation reactions. [10] However, remediation of As and Pb co-contaminated soils using soil amendments is challenging, as Pb is present as a divalent cation and As may be found as arsenate oxyanions, arsenious acid and arsenite, at soil pH values from 4 to 9.5 9. Nevertheless, a range of materials have been proposed for the in situ immobilization of these contaminants. Some traditional materials, such as phosphate, are particularly useful for metal cations and Pb in particular. [5,11-14] However, in the case of soil contaminated by both metals and As, phosphate may induce As mobilisation through exchange reactions. In this case, iron, aluminium and manganese (hydr)oxide amendments may provide a better solution, as they play a significant role in the retention, mobility and bioaccessibility of both metals and metalloids in soil [9,15]. Various Fe/Al-rich industrial by-products have thus been proposed as potential sorbents capable of immobilizing Pb and As *in situ* [11, 16-18]

In particular, Fe, (Al)-hydroxides are the dominant components of water treatment residues (WTRs) [19, 20]. These materials are derived from the use of Fe^{3+} salts or Al^{3+} salts to flocculate particulate and dissolved constituents from water during drinking water treatment. They are characterised by a neutral pH, low contaminant content, and have been tested for the *in situ* treatment of metal and metalloid contaminated soils, as a result of their low-cost and lack of recycling options. [10,15, 19].

The development of nanomaterials specifically designed for soil and water remediation has attracted significant attention [21] The most widely studied of these new materials is nanoscale Zero Valent Iron (nZVI), a smaller-scale, more reactive form of the more traditional ZVI material typically used in permeable reactive barriers [22]. Aside from its potential to remediate organochloride contaminated groundwater, nZVI has also been proposed as a treatment for As, chromium, nickel and Pb contaminated water and soil [3, 23-26, 27]. In addition, some impurities such as Mn may contribute to Fe/Mn (hydr)oxides with better sorption capacity. Both of these characteristics can be used to influence metal/loid immobilization. Although some previous studies have evaluated the potential use of these amendments for *in situ* remediation of metal(loid)-polluted soils, studies evaluating the effectiveness of conventional, by-product and engineered amendments on As and Pb co-contaminated soils are lacking.

Therefore, the objective of this study was to evaluate the ability of different amendments, added to a metal(oid) polluted soil, to decrease the risk to human health or environment, through *in vivo* and *in vitro* bioaccessibility/bioavailability tests. In particular Pb and As bioaccessibility in a mining soil was compared pre- and post-amendment with conventional (phosphate), by product (WTRs), and engineered (nZVI) remediation products. Pb RBA was also investigated in the treated and untreated soil using an *in vivo* mouse model. In addition, As speciation before and after treatment was assessed using X-ray absorption near-edge spectroscopy (XANES) to elucidate the specific mechanisms underlying the reductions in bioaccessibility.

MATERIALS AND METHODS

Soil and amendment characterisation. Arsenic and Pb contaminated soil was

collected in the vicinity of the Baccu Locci abandoned mining site in Sardinia (Italy; 39°31'25"N, 9°35'23"E) [18,28]. 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).[29].

Approximately 150 kg of soil was collected from an rural area, where the most representative vegetation is the Mediterranean maquis, of about 1 ha (15 sampling points, 0-30 cm depth), mixed together in the laboratory, air dried and sieved to < 2 mm. The main chemical characteristics were determined by standard methods [29]

In addition, soil mineralogy was determined on finely ground samples by X-ray diffraction (XRD; PANalytical Empyrean X1-39). The XRD spectra were collected in the 2θ range from 4° to 70°. Soil particles were investigated using a Field Emission-Scanning Electron Microscope (FE- SEM; FEI: Quanta 450FEG) equipped with an Energy Dispersive (X-Ray) Spectrometer (TEAM™ EDS Analysis System, AMETEK Materials Analysis Division, NJ - USA).

Pseudo-total concentrations of metal(loid)s in the soil were determined by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES; Perkin Elmer, Optima 5300V) after digestion of the <250 µm size fraction (also used for bioaccessibility/bioavailability tests), with aqua regia (HNO_3 and HCl mixture 1:3 v/v ratio, USEPA Method 3051A) in a microwave CEM Discover SP-D. Samples were digested in triplicate and a standard reference material (NIST-SRM 2711) was included for quality assurance and quality control. One Al-based and one Fe-based WTR were used. Both were provided by Abbanoa S.p.A., Italy.

The Al-WTR was obtained from the drinking-water treatment plant in Truncu Reale, Sassari, where raw water is treated with $Al_2(SO_4)_3$, while the Fe-WTR was provided by

the Bidighinzu plant in Sassari, where $Fe_2(SO_4)_3$ is used as the coagulant.

The WTR samples were dried overnight at 60°C, then ground and sieved to < 2 mm. The pH and electric conductivity (EC) values were determined using a 1:2.5 ratio of WTR: deionised water. Total organic matter in the WTRs was determined using the Walkley and Black method [30]. Humic (HA) and fulvic (FA) acid content was determined using the method reported by Ciavatta et al.[31], and total N was determined using the Kjeldhal method [32].

The pH_{PZC} of the WTRs was measured by Laser Doppler Velocimetry coupled with Photon Correlation Spectrometry using a Coulter Delta 440 spectrometer equipped with a 5 mW He-Ne laser (632.8 nm).

The total concentration of selected metal(loid)s in Fe-WTR and Al-WTR was determined on dried WTRs (105°C) after digestion as described above. Two commercial nZVI products (one from Nanoamor; and Nanofer 25S from Nanoiron), and a micron-size ZVI powder (H200 Plus Iron, Hepure Metals) were used in this project. These products are referred to as nZVI-1, nZVI-2 and μZVI, respectively.

Soil treatments. Soil treatments were prepared by combining 20 g of soil (2 mm sieved) with either phosphoric acid, Fe-WTR or Al-WTR, or (n)ZVI commercial products. All amendments were prepared in triplicate on a 1% (w/w) (~18 t/ha for a clay-silt loam soil) basis and, in the case of the WTRs, a 2% (w/w) (~36 t/ha for a clay-silt loam soil) amendment treatment was also assessed; these amendment rates have previously been used by other authors (e.g.[16, 33]) Unamended soil served as a control. Following the addition of amendments, treated and untreated soils were thoroughly mixed with a spatula, manually shaken, moistened with MilliQ water to 60% of their water holding capacity (WHC) and incubated under controlled conditions (temperature

20°C and 60-70% relative humidity) for two months. During this period, all samples were mixed every three days and the moisture maintained through gravimetric determination. pH was measured in all soils after two weeks and at the end of the incubation period. After two weeks, the pH of the soils treated with phosphoric acid (pH=3.94) was readjusted with calcium oxide (CaO 0.12 g for 20 g of soil) to reinstate the original (unamended) pH condition.

Assessment of As, Pb and Fe bioaccessibility. Two months into the incubation experiment, As, Pb and Fe bioaccessibility were determined using the Solubility Bioaccessibility Research Consortium in vitro assay (SBRC) incorporating both gastric (SBRC-G) and intestinal phases (SBRC-I),[34, 35]. For in vitro analysis, the ≤ 250 µm particle size fraction was used as this is the size fraction that adheres to fingers and is available for incidental ingestion, especially by children [6]. Soil samples (0.4 g) were combined with 40 mL of gastric solution (30.03 $g \cdot L^{-1}$ glycine adjusted to pH 1.5 with concentrated HCl) and incubated at 37°C on an end-over-end shaker (30 rpm). After 1 h of incubation, samples were centrifuged (2621 g for 5 min) and 10 mL was collected and filtered (0.45 µm Ash- free filter) prior to ICP- OES analysis. For SBRCG-I analysis, the gastric phase was modified to the intestinal phase after 1 h of incubation by adjusting the pH to 7.0 with NaOH (5 and 50%) and adding 70 mg of bile (Fluka Analytical, St. Louis, MO) and 20 mg of pancreatin (MerckKGaA, Germany) [36]. After an additional 4 h, samples were likewise centrifuged (2621 g for 5 min) and 10 mL was collected and filtered (0.45 µm) for ICP-OES analysis.

During intestinal phase extraction, the pH was monitored each hour and adjusted with NaOH or HCl as required. In vitro As, Pb and Fe bioaccessibility was calculated by dividing the SBRC-G or SBRC-I extractable As, Pb and Fe by the total soil As, Pb and

Fe concentration.

Assessment of in vivo Pb relative bioavailability (RBA). In vivo studies were conducted with adult male (Balb/c) mice (20 to 25 g) as detailed by Smith et al [37]. Briefly, animals were housed in groups of 4 mice and received a 12/12 light/dark cycle and access to water adlibitum. Animal care was in compliance with the Standard Operating Procedures of the South Australian Health and Medical Research Institute, Adelaide, Australia. Lead relative bioavailability studies were approved and conducted according to application 16/11 of the Institute of Medical and Veterinary Science Animal Ethics Committee (Adelaide, South Australia). When Pb RBA was assessed, a single dose of soil suspension (0.25 g of soil in 0.5 mL MilliQ water) or Pb acetate was administered via gavage to fasting animals.

A total of 5 treatments were assessed (each treatment was carried out in triplicate):

1. Untreated contaminated soil
2. Contaminated soil treated with phosphoric acid (1%)
3. Contaminated soil treated with nZVI-2 (1%)
4. Contaminated soil treated with Fe WTR (2%)
5. Contaminated soil treated with Al WTR (2%)

Following soil/ Pb acetate administration, replicate mice (n = 3) were sacrificed over a 48 h per with blood analysis used to construct blood-Pb time curves. Samples (0.5 ml) were stored in 7.5 mL EDTA collection tubes at -20 ºC prior to Pb analysis.

In order to quantify blood Pb concentrations, blood (1 mL) was digested in a CEM Mars 6 microwave with hydrogen peroxide (2 mL; 30 %) and nitric acid (2 mL; 70 %) [38] according to CEM's blood digestion application note. Digested samples were then diluted with MilliQ water and analysed by ICP-MS. During the determination of Pb

concentration, reagent blanks, duplicate analysis, spiked sample recoveries and check values were also included. Lead bioavailability was assessed using pharmacokinetic analysis encompassing areas under the blood concentration (AUC) time curves following zero correction and dose normalisation. Lead RBA was calculated according to equation 1. When calculating Pb RBA, the AUC for the Pb acetate oral treatment was used for comparison.

$$\text{Pb RBA, \%} = \left(\frac{AUC_{\text{Oral-Soil}}}{AUC_{\text{Oral-Pb acetate}}} * \frac{DR_{\text{Oral-Pb acetate}}}{DR_{\text{Oral-Soil}}} \right) * 100$$

Where:

$AUC_{\text{Oral-Soil}}$ = area under the Pb blood concentration versus time curve for an oral Pb-contaminated soil dose.

$AUC_{\text{Oral-Pb}}$ = area under the Pb blood concentration versus time curve for an oral dose of lead acetate.

$DR_{\text{Oral-Soil}}$ = dose of orally administered soil (mg kg^{-1}).

$DR_{\text{Oral-Pb}}$ = dose of orally administered lead acetate (mg kg^{-1}).

Arsenic K-edge XANES. Arsenic speciation was investigated in the untreated and treated soils after two months of incubation from the bioaccessibility and bioavailability protocols. Lead XANES was not feasible in this instance due to interference between the As Kα line and the Pb L3 edge, as the samples had much higher molar As concentrations than Pb concentrations.

In addition to the treated and untreated soils, As K-edge XANES analysis was also performed on subsamples after SBRC-G and SBRCG-I in order to assess whether a particular mineral phase was removed during the bioaccessibility extractions or whether new phases were formed during the extraction procedures. The As K- edge XANES

were collected at the Materials Research Collaborative Access Team's (MRCAT) beamline 10-BM at the Advanced Photon Source (APS), Argonne National Laboratory (ANL), Argonne, IL,[39] USA. The electron storage ring operated at 7 GeV in top-up mode. A liquid nitrogen cooled double crystal Si(111) monochromator was used to select incident photon energies and a platinum-coated mirror was used for harmonic rejection. The transmission signal from a sodium arsenate standard was collected congruently with each sample scan in order to correct for energy drifts. Samples were pressed into pellets and three XANES scans were collected for each sample in transmission mode and fluorescence mode using a four element silicon drift detector.

A total of 22 As standards were analysed in order to facilitate linear combination fitting (LCF) of the sample spectra to investigate the likely As speciation in the samples. Principal Component Analysis (PCA) and Target Transformation (TT) were done using Athena [40] as described in Gräfe et al [41], i.e. to determine which standards to include in the LCF procedure and the maximum combination of standards to allow per fit). PCA indicated that four components (standards) were sufficient to explain the majority of the variance in the system and the following standards were selected based on their SPOIL values [42] : beudantite, arsenosiderite, scorodite, arsenopyrite, As(V) sorbed by hematite and As(V) or As(III) sorbed by ferrihydrite.

Statistical Analysis. All analyses were performed in triplicate, with data reported as mean values. Data was subjected to analysis of variance (One-way ANOVA) to assess the effect of different treatments on the bioaccessibility of As, Pb and Fe. Where significant P-values ($P<0.05$) were obtained, differences between individual means were compared using the post-hoc test (LSD, $P<0.05$). A Pearson correlation 2 matrix (i.e. Pearson product-moment correlation coefficient) was also calculated to assess the

possible significant correlation among Fe and As/Pb bioaccessibility following treatments.

RESULTS AND DISCUSSION

Soil and amendment properties. The total concentration of As (2267 $mg \cdot kg^{-1}$) and Pb (1126 $mg \cdot kg^{-1}$) in the untreated soil was much higher than background values for the surrounding area (Table 1) [43].·Particle size analysis identified the soil as a sandy clay loam (USDA; Table 1), and XRD analysis showed that the soil contained quartz (58 wt.%), muscovite/illite (32 wt.%) and clinochlore (32 wt.%) (see Supporting Information, Figure S1).

SEM-EDX analysis confirmed Frau et al's results [38], showing the presence of hydroniumjarosite [$(H_3O)Fe_3(SO_4)_2(OH)_6$] ,muscovite [$KAl_2(Si_3Al)O_{10}(OH,F)_2$], galena [PbS], lead jarosite [$Pb_{0.5}Fe_3^{3+}(SO_4)_2(OH)_6$] and beudantite [As and Pb sulphate mineral $PbFe_3(AsO_4)(SO_4)(OH)_6$]; the latter resulting from the process of weathering of galena (PbS) and pyrite (FeS_2) (Figure S2).

The phosphoric acid treatment caused a decrease in pH to 3.94 after two weeks of incubation respect to the untreated soil (pH=6.15), while the addition of µZVI and nZVI-2 produced an increase of pH (pH 6.57 and 6.28 respectively) (Table S1) Calcium oxide was used to readjust the pH towards the original value. After two months of incubation, the difference in pH across all treatments was less than 1 pH unit (Table S1).

The Fe- and Al-WTRs had approximately neutral pH (7.2 and 6.8 respectively), with electrical conductivity of 1.2 and 1.3 mS cm^{-1}, pHpzc of 6.7 and 6.6, and organic matter contents of 14.5 and 24.1% (w/w), respectively.

Humic and fulvic acids accounted for 2.7 and 2.3% (w/w) in Fe and Al-WTR, respectively (Table 1). The Fe and Al contents in both WTRs reflect the type of coagulant used during the water treatment process (Table1).

XRD analysis revealed no crystalline components, suggesting that amorphous Al-, Fe or Mn-hydroxides are the principal components of both WTRs.

The manufacturer's specification indicate that the Nanoamor product had an average particle size of 25 nm and was partially (approx. 10%) passivated; Nanofer 25S was supplied as a slurry containing nZVI particle with an average size of 50 nm; H200 Plus Iron was largely (>99%) comprised of particles less than 250 µm in size (vendor supplies value). These materials were characterised by Chekli et al.[44], who showed, using XAS and XRD, that ZVI was the dominant form of Fe in these products.

Bioaccessibility and relative bioavailability of Pb. The bioaccessibility of Pb in untreated soil was 47.3±1.8% and 8.1±1.2% in the gastric and intestinal phases, respectively, illustrating that the bioaccessibility of this element is strongly controlled by the pH of the extractant solution (Figure 1). The acidic pH of the gastric phase extraction resulted in the solubilisation of mineral phases and the release of sorbed Pb. At the pH value of the intestinal phase (pH 7.0), a portion of solubilised Pb was removed from solution by precipitation and adsorption reactions with the soil components [45], [46], [47], [48]. To better compare the effect of amendments on the Pb bioaccessibility and bioavailability we used the Treatment Effect Ratio (TER), i.e. the bioaccessibility in the amended soil divided by the bioaccessibility in the untreated soil (Table S2).

Phosphoric acid was the only amendment able to significantly decrease Pb bioaccessibility following gastric and intestinal phase extraction, giving TERs of 0.25 and 0.87 respectively. However, Pb bioaccessibility in the SBRC-G phase decreased

by only 13% (compared to the untreated soil), whereas a decrease of 75% was observed in the intestinal phase.

The reduction in Pb bioaccessibility in the gastric phase could be the result of in situ pyromorphite formation or, alternatively, it could be due to in vitro formation of pyromorphite (as the conditions in the SBRC- G are favourable for pyromorphite formation) [49, 50]. In the intestinal phase, the increase in pH from 1.5 to 7.0 may reduce Pb bioaccessibility through the sorption of Pb onto soil mineral phases [42] or precipitation of Pb-phosphate mineral phases. The reduction in Pb bioaccessibility in the intestinal phase concurs very well with the 75% reduction in bioavailability measured in the animal study (Table 2).

The nZVI-2 and Fe-WTR treatments also reduced *in vivo* Pb RBA, however this reduction was not reflected in the bioaccessibility data. A possible cause of these contrasting results could be due to the formation of nanoscale Pb-Fe coprecipitates that may have passed through the SBRC-I filtration procedure.

The presence of non-labile metal associated with soil colloids less than 0.45µm has been demonstrated previously [51 52]. This hypothesis is supported by the enhanced Fe extractability in the nZVI-2 treatment (Figure S3), but is not supported by the Fe-WTR data. Alternatively, as a consequence of the increased concentration of soluble Fe following amendment with nZVI and Fe- WTR, [53] the decrease in Pb RBA may have been due to Pb co-precipitation with Fe.

This would be facilitated by the increase in gastrointestinal pH upon transition of fluids from the stomach to small intestines. In any case, the processes that led to the decrease in Pb absorption in vivo were not reflected in this particular in vitro assay.

Bioaccessibility of As and Fe. Arsenic bioaccessibility in the untreated soil was 29.5 ±

1 % and 28.8 ± 0.8 % following gastric and intestinal phase extraction respectively (Figure 2), showing that the pH of the two extractant solutions play a lesser role in controlling As bioaccessibility in comparison to Pb [8, 54].

The ZVI treatments favoured a decrease in As bioaccessibility in both gastric and intestinal phase although the reduction varied depending on the type of ZVI treatment used (Table S2). In particular the As bioaccessibility was 22.3±0.7 % and 18.7±0.7 % in μZVI and nZVI-2 treatments in the SBRC-G phase respectively and 19.2±1.1% and 17.1±1.2% in the SBRC-I phase (Figure 2).

The reduction in As bioaccessibility can be attributed to the increase in Fe or Al mineral phases in the soils which control the solubility and mobility of As, through the formation of surface precipitates and strong chemical bonds (inner-sphere complexes) [55]. In particular, As bioaccessibility (18.7±0.2% in the SBRC-G phase and 17.1±1.2% in the SBRC-I phase) was reduced to the greatest extent in soils treated with the Nanoiron Nanofer 25S (nZVI-2, Figure 2). Due to its small particle size, this material possesses a large surface area and high reactivity [24, 56], which probably results in greater absorption, and hence lower As bioaccessibility. Unlike Pb bioaccessibility, a small but significant increase in As bioaccessibility, with respect to untreated soil, was observed in the phosphoric acid treated soil for the gastric phase assays. This increase in As bioaccessibility could be explained through the exchange of As(V) sorbed by the soil components with phosphate [57], with a consequent increase of As(V) in solution. This exchange process is due to the analogous chemical nature of phosphate and arsenate (similar tetrahedral geometry, electronegativity, and atomic radii), as both may compete for the same anion sorption sites [15].

Fe bioaccessibility in the untreated soil was low (3.3 ± 0.1 % and 1.8 ± 0.3 % in the

gastric and intestinal phase respectively) (Figure S3).

This may be in part due to the presence of hydroniumjarosite, a mineral with high stability at low pH [58, 59]. As expected, the low pH value in the gastric phase favoured an higher solubilisation of Fe compounds. Iron bioaccessibility in the SBRC-G increased following the amendment addition, reaching the highest values in the soil samples treated with nZVI-2 (13.0 ± 0.8 %), followed by μZVI and nZVI-1 (7.0 ± 0.1 and 6.9 ± 0.5 % respectively).

A similar trend is observed in the intestinal phase extraction, where the iron bioaccessibility of soil samples treated with nZVI-2, μZVI and nZVI-1 (4.9 ± 0.6, 3.1 ± 0.3 and 2.7± 0.2 % respectively) is greater than the untreated soil(Figure S3).

The increase of Fe bioaccessibility in the soil samples treated with nZVI-2 and μZVI, following both gastric and intestinal phases, may result from the lack of removal of Fe nanoparticles during the filtration step prior to ICP-OES analysis. In contrast, Fe bioaccessibility in the gastric and intestinal phases of soils treated with Fe-WTR (1- 2%) did not change with respect to the untreated soil.

Arsenic speciation by XANES analysis. Arsenic K-edge XANES of unamended soil showed that the large majority of As was present as As(V) associated to Fe minerals (Table 3; Figure S4a). The results of the LCF analysis confirmed the presence of beudantite (observed by SEM; Figure S2) [60], arsenosiderite (possibly originating from the oxidation of arsenopyrite or scorodite) and As(V) sorbed on a hematite-like material. XANES spectra of the soil treated with the various amendments after 2 month incubation were similar to the spectra from the untreated soil.

However, decreased amounts of As were associated with beudantite and hematite with a concomitant increase in As resembling arsenosiderite. This supports the suggestion

that Fe minerals play a significant role in binding As in a stable manner, which is difficult to modify / enhance through the application of soil amendments [60, 61]. Reduced As was only present in minor proportions as detailed in Table 3. However, small proportions such as this should be considered with caution due to the uncertainty in determining minor species by XANES analysis in complex environmental samples [41]. The only exception was in the nZVI-2 treatment where As(III) contributed to 14% of the total As as evidenced by the spectral shoulder at approximately 11,871eV (Figure S4a) and confirmed by the LCF results (Table 3). This may have resulted from the partial reduction of As(V) to As(III) corresponding with the oxidation of nZVI [62]. Additionally, the acidic conditions of the SBRC gastric phase may have resulted in the appearance of reduced forms of As in the unamended soil and in the soil treated with nZVI-2 (Table 3 and Figure S4b). In particular, arsenopyrite accounted for approximately 30 and 14 % of the total As in the untreated and nZVI-2 treated soil following SBRC-G extraction. As the total amount of As extracted by the SBRC-G among the different treatments accounted for 20-30% of the total As, the difference in the XANES spectra are attributable to a change in speciation in the unamended and nZVI-2 treated soils rather than to a selective removal of oxidised As species. Reduced forms of As are more favoured at lower pH values in oxidised soils [63]. In all soil residues collected after the SBRC-I phase, the dominant form of As was identified as As(V) associated with Fe mineral phases (Table 3 and Figure S4c). Similar results have been reported by [64] indicating sorption of As by Fe mineral phases which is a common phenomenon controlling As bioaccessibility.

Implication for in situ immobilization of As-Pb contaminated soils. The *in vitro* and *in vivo* bioaccessibility/bioavailability assays were performed in order to evaluate

the potential human exposure to metal(loids) in contaminated sites and consequently to identify possible remediation strategies or safety measures for these sites.

The addition of amendments containing Fe, such as the nZVI, resulted in a decrease in As bioaccessibility. The addition of the Nanoiron 25Slurry (N25S) was particularly effective in decreasing As bioaccessibility compared to the other treatments. These results are of great practical importance, since As bioaccessibility represents one of the more significant predictors of As RBA and consequently drives exposure and risk outcomes.

A different trend was observed for Pb, with phosphoric acid identified as the only amendment capable of decreasing Pb bioaccessibility. However, the addition of this amendment had a negative effect on As bioaccessibility. Although not reflected in the in vitro data, the addition of nZVI and WTRs resulted in a decrease in Pb RBA. As noted by the USEPA [65, 66], in vitro assays such as SBRC-G may not be appropriate for predicting Pb RBA in phosphate amended soils due to potential artefacts associated with assay conditions (i.e. low pH which may facilitate the formation of pyromorphite). Similarly, in vitro assays may not be able to accurately predict Pb RBA when other amendments are utilized (e.g. nZVI) as illustrated by the discrepancy in in vivo and in vitro results.

At the same way, while in vitro results suggest that the addition of phosphate amendments may decrease have a negative impact of As bioaccessibility (and therefore predicted As RBA), conceivably this may not be reflected in vitro data due to preferential absorption of phosphate [66]. In order to accurately assess the in situ fixation efficacy of amendment strategies, As and Pb RBA should be assessed in concert with secondary evidence provided through spectroscopic assessment.

ASSOCIATED CONTENT

Supporting Information. Soil pH after two weeks and at the end of the incubation period (Table S1), efficacy of amendments for reducing As and Pb bioaccessibility in contaminated soil (Table S2). XRD Spectra of Baccu Locci soil (Figure S1) and SEM images of Pb (Beudantite) phase resulting from natural soil weathering processes (Figure S2). Fe bioaccessibility in untreated (control) and treated soils (Figure S3) and Arsenic K-edge XANES spectra of untreated (control) and treated soils before (a) and after SBRC-G (b) and SBRC-I (c) extractions (Figure S4).

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this article are solely those of the authors and do not necessarily reflect or represent EPA's views or policies.

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Table1. Characteristics of the soil and WTRs used[a]

Characteristics	Soil	Fe WTR	Al WTR
pH	6.08±0.02[a]	7.15±0.06[c]	6.81±0.01[b]
Electrical conductivity (mS cm^{-1})	0.41±0.06[a]	1.24±0.10[b]	1.33±0.13[b]
pHpzc	6.68±0.10[a]	6.70±0.11[a]	6.58±0.13[a]
Organic matter (% d.m.)	39.34±0.14[c]	14.51±0.13[a]	24.13±0.16[b]
Humic and Fulvic acids (% d.m.)	n.d.	2.65±0.14[a]	2.27±0.20[a]
Total N ($g \cdot kg^{-1}$ d.m.)	1.53±0.70[a]	6.08±0.64[b]	8.80±0.58[c]
Coarse sand (%)	36.6	-	-
Fine Sand (%)	22.3	-	-
Silt (%)	16.6	-	-
Clay (%)	24.4	-	-
Total Fe (mg kg^{-1} d.m.)	36,674.8±1397[c]	20,948±952[b]	6,373±341[a]
Total As (mg kg^{-1} d.m.)	2,267.4±67.8[c]	15.8±1.0[a]	49.5±2.7[b]
Total Pb (mg kg^{-1} d.m.)	1,126.2±34.4[c]	9.1±0.5[b]	4.9±0.4[a]
Total Al (mg kg^{-1} d.m.)	61,301±757[b]	34,158±129[a]	98,320±837[c]
Total Mn (mg kg^{-1} d.m.)	642.1±19.9[a]	6,786.2±279[b]	8,699.5±451[c]
Total Cu (mg kg^{-1} d.m.)	132.1±49.6[c]	27.1±0.8[b]	18.6±0.6[a]

[a] Values represent the mean and standard deviation of triplicate analyses
Mean values with the same letter within a row do not differ significantly according to the Tukey Kramer test ($P < 0.05$).

Table 2. Lead relative bioavailability (RBA) in untreated and treated soils

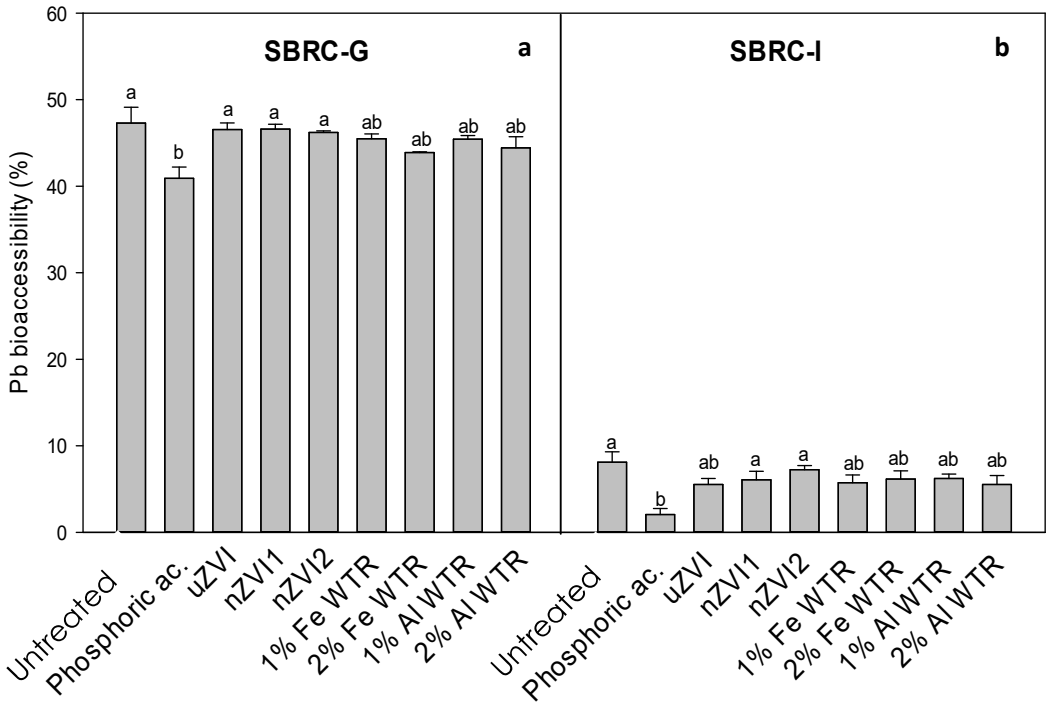
Treatment	Pb RBA (%)[a]	SE
Untreated	23.7	0.8
Phosphoric acid	6.0**	0.4
nZVI-2	12.9*	2.1
Fe WTR 2%	12.4*	0.3
Al WTR 2%	17.6*	4.1

[a]Asterisks indicate a significant reduction in Pb relative bioavailability (compared to untreated soil) at the $p < 0.05$ (*) and < 0.01 (**) level, according to the Tukey test.

Table 3: Linear Combination Fitting (LCF)-XANES Analysis of soil samples

Treatment	Beudantite	Arsenosiderite	Arsenopyrite	As(V)-hematite	As(III)-ferrihydrite	R-factor
Untreated						
Before SBRC	17	51		31	5	0.0037
After SBRC-G	19	25	32	23		0.0113
After SBRC-I	19	54		22	6	0.0079
Phosphoric acid						
Before SBRC	16	71		11	3	0.0060
After SBRC-G	12	68		19	3	0.0114
After SBRC-I	15	62		21	6	0.0060
μZVI						
Before SBRC	13	63		18	6	0.0051
After SBRC-G	12	81		9	5	0.0071
After SBRC-I	11	81		5	7	0.0036
nZVI-1						
Before SBRC	18	62		20	4	0.0152
After SBRC-G	11	75		14	4	0.0087
After SBRC-I	13	81		4	5	0.0071
nZVI-2						
Before SBRC	16	61		12	14	0.0060
After SBRC-G	12	70	14		6	0.0114
After SBRC-I	13	71		6	10	0.0060
1% Fe WTR						
Before SBRC	11	75		11	4	0.0062
After SBRC-G	9	78		11	4	0.0085
After SBRC-I	19	61		18	6	0.0051
2% Fe WTR						
Before SBRC	9	77		10	5	0.0034
After SBRC-G	8	77		17	5	0.0047
After SBRC-I	16	72		10	5	0.0055
1% Al WTR						
Before SBRC	10	78		9	5	0.0047
After SBRC-G	9	77		15	4	0.0079
After SBRC-I	11	79		6	5	0.0039
2% Al WTR						
Before SBRC	8	83		8	5	0.0023
After SBRC-G	9	82		13	5	0.0053
After SBRC-I	9	86		4	4	0.0043

4 **Figure 1. Pb bioaccessibility in untreated and treated soils following gastric (SBRC-G) a**
5 **and intestinal (SBRC-I) b phase extraction.**

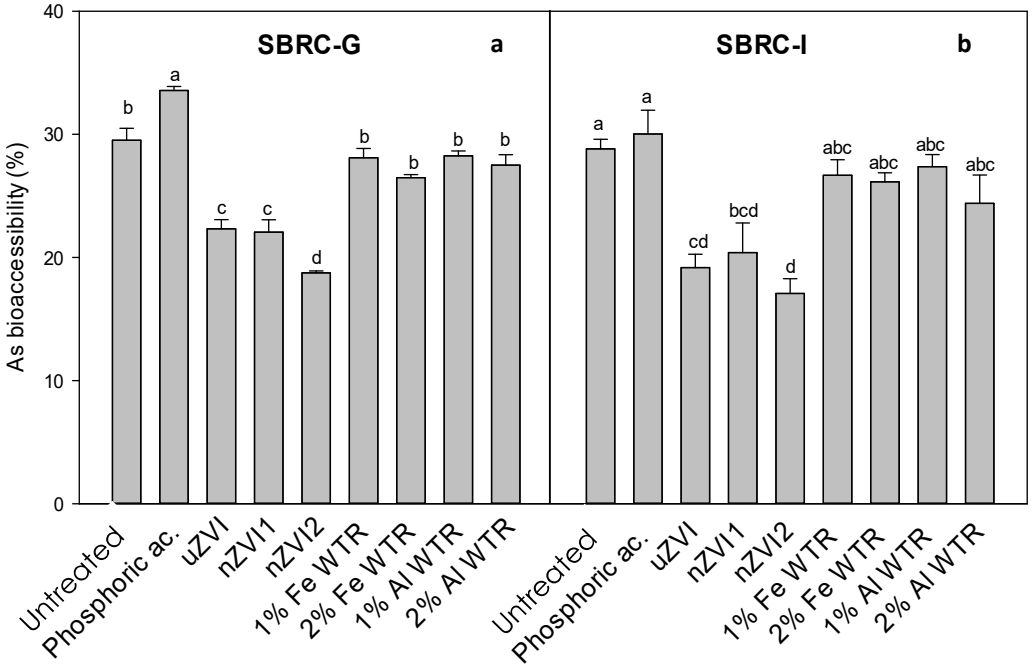


6

7 For each in vitro bioaccessibility, mean values followed by different letters denote statistically significant
8 differences according to the post-hoc test (LSD, $P<0.05$).
9

10

Figure 2. As bioaccessibility in untreated and treated soils following gastric (SBRC-G) a and intestinal (SBRC-I) b phase extraction.



For each in vitro bioaccessibility, mean values followed by different letters denote statistically significant differences according to the post-hoc test (LSD, $P<0.05$).