

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

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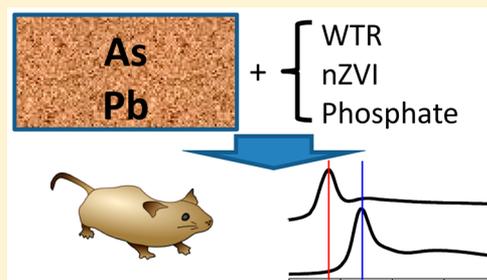
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## S Supporting Information

**ABSTRACT:** This study aimed to assess and compare the *in vitro* and *in vivo* bioaccessibility/bioavailability of As and Pb in a mining contaminated soil (As, 2267 mg kg<sup>-1</sup>; Pb, 1126 mg kg<sup>-1</sup>), after the addition of conventional (phosphoric acid), opportunistic [water treatment residues (WTRs)], and engineered [nano- and microscale zero valent iron (ZVI)] amendments. Phosphoric acid was the only amendment that could significantly decrease Pb bioaccessibility with respect to untreated soil (41 and 47% in the gastric phase and 2.1 and 8.1% in the intestinal phases, respectively), giving treatment effect ratios (TERs, 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. The *in vivo* bioavailability of Pb decreased in the phosphate treatment relative to the untreated soil (6 and 24%, respectively), and also in the Fe WTR 2% (12%) and nZVI-2 (13%) treatments. The ZVI amendments caused a decrease in As bioaccessibility, with the greatest decrease in the nZVI2-treated soil (TERs of 0.59 and 0.64 in the gastric and intestinal phases, respectively). Arsenic X-ray absorption near-edge spectroscopy analysis indicated that most of the As in the untreated soil was present as As(V) associated with Fe mineral phases, whereas in the treated soil, the proportion of arsenosiderite increased. Arsenite was present only as a minor species (3–5%) 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 (typically ranging from the microgram per kilogram range to <10 mg/kg in environmental matrices).<sup>1,2</sup> Elevated soil concentrations can be caused by both geochemical and anthropogenic processes and are of concern because of their potentially negative impacts on environmental receptors.<sup>3</sup> Among anthropogenic sources, mining and smelting of metal(loid) sulfide ores are major sources of As and Pb contamination.<sup>4</sup> Human and animal exposure to As and Pb may occur through the consumption of contaminated drinking water and food, inhalation of particulate matter, and ingestion of soil or dust. The latter, especially in the case of children and animals, is of concern because many former mining and

industrial areas have been redeveloped into residential property or are now used for grazing animals (this is common in some areas of Southern Europe and in Sardinia in particular).<sup>5</sup>

In many countries, regulatory limits for As and Pb in soils are based on their total concentrations. However, because of mineralogical differences and the influence of soil properties, contaminant absorption following incidental soil ingestion is frequently less than the default value of 100%. Importantly, exposure parameters may be refined through the assessment of contaminant relative bioavailability (RBA), i.e., the fraction of

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Table 1. Characteristics of the Soil and WTRs Used<sup>a</sup>

	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 <sup>-1</sup> )	0.41 ± 0.06 a	1.24 ± 0.10 b	1.33 ± 0.13 b
pH <sub>PZC</sub>	6.68 ± 0.10 a	6.70 ± 0.11 a	6.58 ± 0.13 a
organic matter [% dry matter (d.m.)]	39.3 ± 0.14 c	14.51 ± 0.13 a	24.1 ± 0.16 b
humic and fulvic acids (% d.m.)	n.d.	2.65 ± 0.14 a	2.27 ± 0.20 a
total N [g (kg of d.m.) <sup>-1</sup> ]	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 of d.m.) <sup>-1</sup> ]	36674 ± 1397 c	20948 ± 952 b	6373 ± 341 a
total As [mg (kg of d.m.) <sup>-1</sup> ]	2267 ± 67.8 c	15.8 ± 1.0 a	49.5 ± 2.7 b
total Pb [mg (kg of d.m.) <sup>-1</sup> ]	1126.2 ± 34.4 c	9.1 ± 0.5 b	4.9 ± 0.4 a
total Al [mg (kg of d.m.) <sup>-1</sup> ]	61301 ± 757 b	34158 ± 129 a	98320 ± 837 c
total Mn [mg (kg of d.m.) <sup>-1</sup> ]	642 ± 19.9 a	6786 ± 279 b	8699 ± 451 c
total Cu [mg (kg of d.m.) <sup>-1</sup> ]	132 ± 49.6 c	27.1 ± 0.8 b	18.6 ± 0.6 a

<sup>a</sup>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 ( $P < 0.05$ ).

the ingested dose that is absorbed into the systemic circulation of the organism in question. 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.<sup>6,7</sup>

Metal/(loid) RBA and bioaccessibility depend on the physicochemical properties of the soil and the chemical characteristics of the element considered.<sup>8</sup> Remediation strategies that promote the formation of stable forms of As and Pb (e.g., *in situ* immobilization) have been proposed for the remediation and/or management of contaminated soils.<sup>9</sup> One such technology is based on the use of soil amendments that can immobilize contaminants through sorption and/or precipitation reactions.<sup>10</sup> However, remediation of As- and Pb-cocontaminated soils using soil amendments is challenging as Pb is present as a divalent cation while As may be found either as arsenate or as arsenite at soil pH values from 4 to 9.5. Nevertheless, a range of materials have been proposed for the *in situ* immobilization of these contaminants. Some traditional remediation materials, such as phosphate, are particularly useful for metal cations and Pb in particular.<sup>5,11–14</sup> However, in the case of soil contaminated by both metals and As, phosphate may induce As mobilization through exchange reactions. In this case, iron (Fe), aluminum, 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.<sup>9,15</sup> Various Fe/Al-rich industrial byproducts and wastes, which are often available locally, have thus been proposed as potential sorbents capable of immobilizing Pb and As *in situ*.<sup>11,16–18</sup> Because of their low cost and local availability, we define them here as “opportunistic”.

Iron and aluminum hydroxides are the dominant components of water treatment residues (WTRs),<sup>19,20</sup> another form of amendment that was tested in this study. These materials are derived from the use of Fe<sup>3+</sup> salts or Al<sup>3+</sup> salts to flocculate particulate and dissolved constituents from water during

drinking water treatment. They typically have neutral pH values and low contaminant contents, and they have been tested for the *in situ* treatment of metal- and metalloid-contaminated soils because of their low cost and a lack of other recycling options.<sup>10,15,19</sup>

The development of nanomaterials specifically designed for soil and water remediation has also attracted significant attention<sup>21</sup> in recent years, and some of these engineered remediation materials have also been included in this study. 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.<sup>22</sup> 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.<sup>3,23–27</sup> We note that some impurities in the ZVI materials such as Fe/Mn (hydr)oxides may also contribute to metal immobilization by enhancing metal sorption capacity. 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, byproduct, and engineered amendments on As- and Pb-co-contaminated soils are lacking.

The objective of this study was to evaluate the ability of a range of amendments added to a metal(loid)-polluted soil to decrease the associated risks to human health and the environment. Pb and As bioaccessibility in a mining soil was compared pre- and postamendment with conventional (phosphate), opportunistic [water treatment residuals (WTRs)], and engineered (nZVI) remediation products. Lead 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 observed reductions in bioaccessibility.

## ■ MATERIALS AND METHODS

**Soil and Amendment Characterization.** 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).<sup>18,28</sup> The soil is a glacio-fluvial deposit consisting of a mixture of clay, silt, and sand and is classified as sandy loam [U.S. Department of Agriculture classification (Table 1)].<sup>29</sup>

Approximately 150 kg of soil was collected from an area, where the most representative vegetation is the Mediterranean maquis, of ~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.<sup>29</sup> Soil mineralogy was determined on finely ground samples by X-ray diffraction (XRD) (PANalytical Empyrean X1-39). The XRD spectra were collected in the  $2\theta$  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).

Pseudototal concentrations of metal(loid)s in the soil were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES) (PerkinElmer, Optima 5300V) after digestion of the <250  $\mu\text{m}$  size fraction (also used for bioaccessibility/bioavailability tests) with aqua regia [1:3 (v/v)  $\text{HNO}_3/\text{HCl}$  mixture, U.S. EPA Method 3051A] in a CEM Discover SP-D microwave. Samples were digested in triplicate, and a standard reference material (NIST-SRM 2711) was included for quality assurance and quality control.

The WTR amendments included both Al-based and Fe-based WTR materials. Both were provided by Abbanoa S.p.A. The Al WTR was obtained from the drinking water treatment plant in Truncu Reale, Sassari, where raw water is treated with  $\text{Al}_2(\text{SO}_4)_3$ , while the Fe WTR was provided by the Bidighinzu plant in Sassari, where  $\text{Fe}_2(\text{SO}_4)_3$  is used as the coagulant. The WTR samples were dried overnight at 60 °C, ground, and sieved to <2 mm. The pH and electric conductivity (EC) values were determined using a 1:2.5 WTR:deionized water ratio. The total organic matter in the WTRs was determined using the Walkley and Black method.<sup>30</sup> The humic (HA) and fulvic (FA) acid content was determined using the method reported by Ciavatta et al.,<sup>31</sup> and total N was determined using the Kjeldhal method.<sup>32</sup> The  $\text{pH}_{\text{PZC}}$  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 micrometer-sized ZVI powder (H200 Plus Iron, Hepure Metals) were used in this project. These products are termed nZVI-1, nZVI-2, and  $\mu\text{ZVI}$ , respectively.

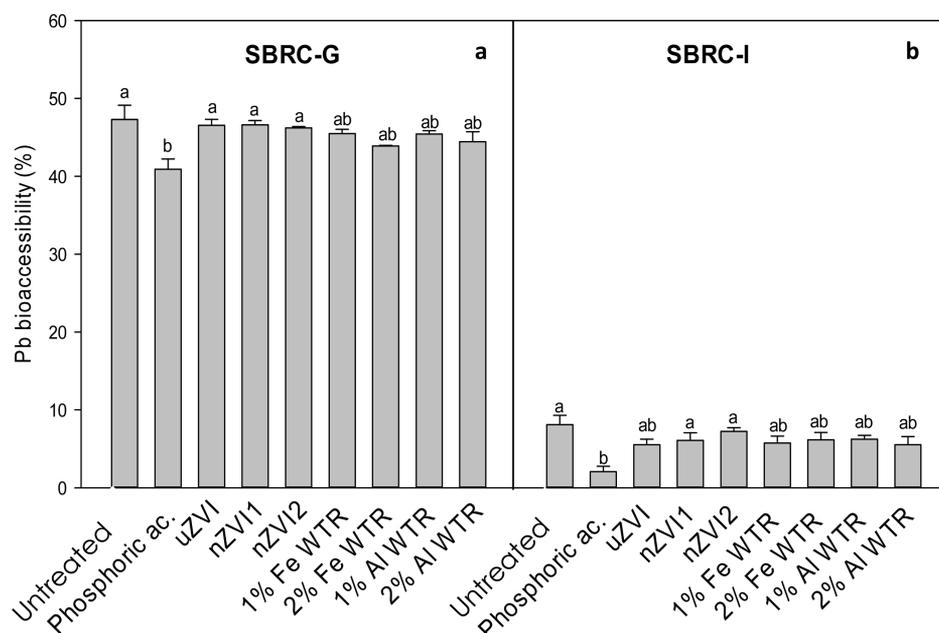
**Soil Treatments.** Soil treatments were prepared by combining 20 g of soil (<2 mm sieved fraction) with either phosphoric acid, Fe WTR or Al WTR, or (n)ZVI commercial products. All amendments were prepared in triplicate on a 1% (w/w) basis (equating to ~18 t/ha for a clay-silt loam soil), and in the case of the WTRs, a 2% (w/w) (~36 t/ha) treatment was also assessed; these amendment rates have previously been used by other researchers (e.g., refs 16 and 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 Milli-Q water to 60% of their water holding capacity (WHC), and incubated under controlled conditions (20 °C and 60–70% relative humidity) for 2 months. During this period, all samples were mixed every

3 days and the moisture was maintained through gravimetric determination. The pH was measured in all soils after 2 weeks and at the end of the incubation period. After 2 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 bioaccessibilities were determined using the Solubility Bioaccessibility Research Consortium *in vitro* assay (SBRC) incorporating both gastric (SBRC-G) and intestinal phases (SBRC-I).<sup>34,35</sup> For *in vitro* analysis, the  $\leq 250 \mu\text{m}$  particle size fraction was used as this is the size fraction that adheres to fingers and is available for incidental ingestion, especially by children.<sup>6</sup> Soil samples (0.4 g) were combined with 40 mL of gastric solution (30.03 g  $\text{L}^{-1}$  glycine adjusted to pH 1.5 with concentrated HCl) and incubated at 37 °C on an end-over-end shaker (30 rpm). After incubation for 1 h, the samples were centrifuged (2621g for 5 min) and 10 mL was collected and filtered (0.45  $\mu\text{m}$  ash-free filter) prior to ICP-OES analysis. For the SBRC bioaccessibility assessment, the gastric phase was modified to form the intestinal phase after incubation for 1 h 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 (Merck KGaA).<sup>36</sup> After an additional 4 h, samples were centrifuged (2621g) for 5 min and 10 mL was collected and filtered (0.45  $\mu\text{m}$ ) 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 values were calculated by dividing the SBRC-G or SBRC-I extractable As, Pb, and Fe by the total soil As, Pb, and Fe concentration, respectively.

**Assessment of *in Vivo* Pb Relative Bioavailability (RBA).** *In vivo* studies were conducted with adult male (Balb/c) mice (20–25 g) as detailed by Smith et al.<sup>37</sup> Briefly, animals were housed in groups of four mice and received a 12/12 light/dark cycle and access to water ad libitum. 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 of Milli-Q water) or Pb acetate was administered via gavage to fasting animals.

A total of five treatments were assessed (each treatment conducted 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%), and (5) contaminated soil treated with Al WTR (2%). Following soil/Pb acetate administration, replicate mice ( $n = 3$ ) were sacrificed over a 48 h period 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 °C prior to Pb analysis. 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%),<sup>38</sup> according to the CEM blood digestion application note. Digested samples were then diluted with Milli-Q water and analyzed by ICP-MS. During the determination of the Pb concentration, reagent blanks, duplicate analyses, and spiked sample recoveries were also



**Figure 1.** Pb bioaccessibility in untreated and treated soils following (a) gastric (SBRC-G) and (b) intestinal (SBRC-I) phase extraction. For each *in vitro* bioaccessibility, mean values followed by different letters denote statistically significant differences according to the post hoc test (LSD;  $P < 0.05$ ).

included. Lead bioavailability was assessed using pharmacokinetic analysis encompassing areas under the blood concentration (AUC)–time curves following zero correction and dose normalization. Lead RBA was calculated according to eq 1. For calculation of Pb RBA, the AUC for the Pb acetate oral treatment was used for comparison.

$$\text{Pb RBA (\%)} = \left( \frac{\text{AUC}_{\text{oral-soil}}}{\text{AUC}_{\text{oral-Pb acetate}}} \times \frac{\text{DR}_{\text{oral-Pb acetate}}}{\text{DR}_{\text{oral-soil}}} \right) \times 100 \quad (1)$$

where  $\text{AUC}_{\text{oral-soil}}$  is the area under the Pb blood concentration versus time curve for an oral Pb-contaminated soil dose,  $\text{AUC}_{\text{oral-Pb}}$  is the area under the Pb blood concentration versus time curve for an oral dose of lead acetate,  $\text{DR}_{\text{oral-soil}}$  is the dose of orally administered soil (milligrams per kilogram), and  $\text{DR}_{\text{oral-Pb}}$  is the dose of orally administered lead acetate (milligrams per kilogram).

**Arsenic K-Edge XANES.** Arsenic speciation was investigated in the untreated and treated soils from the bioaccessibility and bioavailability tests after incubation for 2 months. Lead XANES was not feasible in this instance because of interference between the As  $K\alpha$  line and the Pb L3 edge, as the samples had molar As concentrations much higher than the 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 extraction to assess whether a particular mineral phase was removed or whether new phases were formed during the bioaccessibility extraction procedures. The As K-edge XANES spectra were recorded at Materials Research Collaborative Access Team's (MRCAT) beamline 10-BM at the Advanced Photon Source (APS), Argonne National Laboratory (ANL), Argonne, IL.<sup>39</sup> 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 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 analyzed 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 conducted using Athena<sup>40</sup> as described by Gräfe et al.,<sup>41</sup> i.e., to determine which standards to include in the LCF procedure and the maximal 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 on the basis of their SPOIL values:<sup>42</sup> 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 were subjected to analysis of variance (one-way ANOVA) testing to assess the effect of different treatments on As, Pb, and Fe bioaccessibility. 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 concentrations of As ( $2267 \text{ mg kg}^{-1}$ ) and Pb ( $1126 \text{ mg kg}^{-1}$ ) in the untreated soil were much higher than background values for the surrounding area (Table 1).<sup>43</sup> Particle size analysis identified the soil as a sandy clay loam [U.S. Department of Agriculture (Table 1)], and XRD analysis showed that the soil contained quartz (58 wt %), muscovite/illite (32 wt %), and clinocllore (32 wt %) (see Figure S1 of the Supporting Information).

SEM-EDX analysis showed the presence of hydroniumjarosite  $[(\text{H}_3\text{O})\text{Fe}_3(\text{SO}_4)_2(\text{OH})_6]$ , muscovite  $[\text{KAl}_2(\text{Si}_3\text{Al})\text{O}_{10}(\text{OH},\text{F})_2]$ , galena (PbS), lead jarosite  $[\text{Pb}_{0.5}\text{Fe}_3^{3+}(\text{SO}_4)_2(\text{OH})_6]$ , and beudantite  $[\text{As} \text{ and } \text{Pb} \text{ sulfate mineral } \text{PbFe}_3(\text{AsO}_4)(\text{SO}_4)(\text{OH})_6]$ , the latter resulting from the process of weathering of galena (PbS) and pyrite ( $\text{FeS}_2$ ) (Figure S2). These results are similar to those reported by Frau et al.<sup>28</sup>

The phosphoric acid treatment caused a decrease in pH to 3.94 after incubation for 2 weeks with respect to the untreated soil (pH 6.15), while the addition of  $\mu\text{ZVI}$  and  $\text{nZVI-2}$  increased the pH to 6.57 and 6.28, respectively (Table S1). Calcium oxide was used to readjust the phosphoric acid-treated soil to the original value. No pH adjustment was undertaken for the ZVI treatments. After incubation for 2 months, the difference in pH across all treatments was <1 pH unit (Table S1).

The Fe and Al WTR amendments had approximately neutral pH (7.2 and 6.8, respectively), with electrical conductivities of 1.2 and 1.3  $\text{mS cm}^{-1}$ ,  $\text{pH}_{\text{PZC}}$  values 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 (Table 1). 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 indicated that the Nano-amor product had an average particle size of 25 nm and was partially (approximately 10%) passivated. Nanofer 25S was supplied as a slurry containing  $\text{nZVI}$  particles with an average primary particle size of 50 nm. H200 Plus Iron was largely (>99%) comprised of particles <250  $\mu\text{m}$  in size (vendor supply value). These materials were characterized by Chekli et al.,<sup>44</sup> 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  $47.3 \pm 1.8$  and  $8.1 \pm 1.2\%$  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 solubilization of mineral phases and the release of sorbed Pb. In the intestinal phase (pH 7.0), a portion of solubilized Pb was removed from the solution by precipitation and adsorption reactions with the soil components.<sup>45–48</sup> To better compare the effect of the amendments on 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 that could significantly decrease Pb bioaccessibility following gastric and intestinal phase extraction, giving TERs of 0.87 and 0.25, respectively. However, Pb bioaccessibility in the SBRC-G phase decreased by only 13% (compared to that of 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, *in vitro* formation of pyromorphite (as the conditions in SBRC-G are favorable for pyromorphite formation).<sup>49,50</sup> In the intestinal phase, the increase in pH from 1.5 to 7.0 may also reduce Pb bioaccessibility through the sorption of Pb onto soil mineral phases<sup>42</sup> or precipitation of Pb

phosphate mineral phases. The reduction in Pb bioaccessibility in the intestinal phase concurs very well with the 75% reduction in RBA measured in the animal study (Table 2).

**Table 2. Lead Relative Bioavailabilities (RBA) in Untreated and Treated Soils**

treatment	Pb RBA (%) <sup>a</sup>	standard error
untreated	23.7	0.8
phosphoric acid	6.0**	0.4
$\text{nZVI-2}$	12.9*	2.1
Fe WTR 2%	12.4*	0.3
Al WTR 2%	17.6	4.1

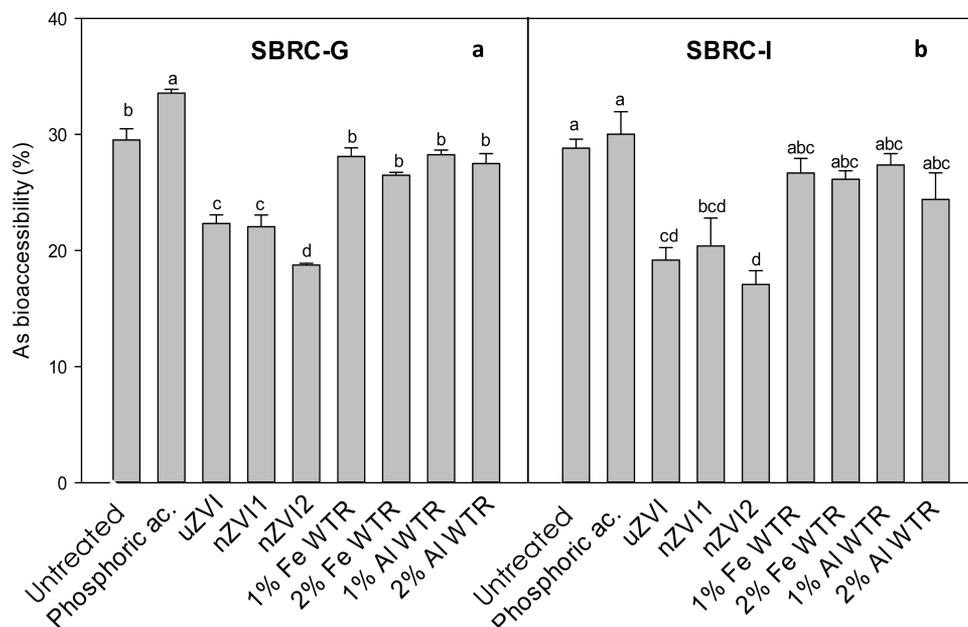
<sup>a</sup>Asterisks indicate a significant reduction in Pb relative bioavailability (compared to that of untreated soil) at the  $P < 0.05$  (one asterisk) and  $P < 0.01$  (two asterisks) levels, according to the Tukey test.

The  $\text{nZVI-2}$  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 the formation of nanoscale Pb–Fe coprecipitates that may have been subjected to the SBRC-I filtration procedure during the collection of the supernatant. The presence of nonlabile metal associated with soil colloids <0.45  $\mu\text{m}$  in size has been demonstrated previously.<sup>51,52</sup> This hypothesis is supported by the enhanced Fe extractability in the  $\text{nZVI-2}$  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  $\text{nZVI}$  and Fe WTR,<sup>53</sup> the decrease in Pb RBA may have been due to coprecipitation of Pb 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 bioaccessibilities in the untreated soil were  $29.5 \pm 1$  and  $28.8 \pm 0.8\%$  following gastric and intestinal phase extraction, respectively (Figure 2), showing that the pH of the two extractant solutions plays a lesser role in controlling As bioaccessibility in comparison to Pb.<sup>8,54</sup>

The ZVI treatments favored a decrease in As bioaccessibility in both gastric and intestinal phases, although the reduction varied depending on the type of ZVI treatment used (Table S2). In particular, As bioaccessibilities were  $22.3 \pm 0.7$  and  $18.7 \pm 0.7\%$  in  $\mu\text{ZVI}$  and  $\text{nZVI-2}$  treatments in the SBRC-G phase, respectively, and  $19.2 \pm 1.1$  and  $17.1 \pm 1.2\%$  in the SBRC-I phase, respectively (Figure 2).

These reductions in As bioaccessibility could be attributed to the increase in Fe or Al mineral phases in the soils as these control the solubility and mobility of As through the formation of surface precipitates and strong chemical bonds (inner-sphere complexes).<sup>55</sup> In particular, As bioaccessibility ( $18.7 \pm 0.2\%$  in the SBRC-G phase and  $17.1 \pm 1.2\%$  in the SBRC-I phase) was reduced to the greatest extent in soils treated with the Nanoiron Nanofer 25S [ $\text{nZVI-2}$  (Figure 2)]. Because of its small particle size, this material possesses a large surface area and high reactivity,<sup>24,56</sup> 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



**Figure 2.** As bioaccessibility in untreated and treated soils following (a) gastric (SBRC-G) and (b) intestinal (SBRC-I) phase extraction. For each *in vitro* bioaccessibility, mean values followed by different letters denote statistically significant differences according to the post hoc test (LSD;  $P < 0.05$ ).

the exchange of As(V) sorbed by the soil components with phosphate,<sup>57</sup> with a consequent increase in 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.<sup>15</sup>

Fe bioaccessibility in the untreated soil was low ( $3.3 \pm 0.1$  and  $1.8 \pm 0.3\%$  in the gastric and intestinal phases, respectively) (Figure S3). This may be partly due to the presence of hydroniumjarosite, a mineral with high stability at low pH.<sup>58,59</sup> As expected, the low pH value in the gastric phase favored a higher degree of solubilization of Fe compounds. Iron bioaccessibility in the SBRC-G phase increased following the addition of amendments, reaching the highest values in the soil samples treated with nZVI-2 ( $13.0 \pm 0.8\%$ ), followed by  $\mu$ ZVI and nZVI-1 ( $7.0 \pm 0.1$  and  $6.9 \pm 0.5\%$ , respectively). A similar trend was observed in the intestinal phase extraction, where the Fe bioaccessibilities of soil samples treated with nZVI-2,  $\mu$ ZVI, and nZVI-1 ( $4.9 \pm 0.6$ ,  $3.1 \pm 0.3$ , and  $2.7 \pm 0.2\%$ , respectively) were greater than in the untreated soil (Figure S3).

The increase in Fe bioaccessibility in the soil samples treated with nZVI-2 and  $\mu$ ZVI, 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 with Fe minerals (Table 3 and Figure S4a). The results of the LCF analysis confirmed the presence of beudantite [observed by SEM (Figure S2)],<sup>60</sup> 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 incubation for 2 months were similar to the spectra from the untreated soil; however, decreased amounts of

As were associated with beudantite and hematite, coupled 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, and this is difficult to modify and/or enhance through the application of soil amendments.<sup>60,61</sup> Reduced As was only present in minor proportions as detailed in Table 3. However, small proportions such as this should be considered with caution because of the uncertainty in determining minor species by XANES analysis in complex environmental samples.<sup>41</sup> The only exception was in the nZVI-2 treatment in which As(III) contributed to 14% of the total As as evidenced by the spectral shoulder at approximately 11871 eV (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.<sup>62</sup> 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, respectively, following SBRC-G phase extraction. As the total amount of As extracted by the SBRC-G phase among the different treatments accounted for 20–30% of the total As, the difference in the XANES spectra can be attributed to a change in speciation in the unamended and nZVI-2-treated soils rather than to a selective removal of oxidized As species. Reduced forms of As are more favored at lower pH values in oxidized soils.<sup>63</sup> 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 in ref 64, indicating sorption of As by Fe mineral phases, which is a common phenomenon controlling As bioaccessibility.

**Implication for *in Situ* Immobilization of As- and Pb-Contaminated Soils.** The *in vitro* and *in vivo* bioaccessibility/bioavailability assays were performed to evaluate the potential human exposure to metal(oids) in contaminated sites and

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
			$\mu$ ZVI			
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

consequently to identify possible remediation strategies or safety measures for these sites. The addition of amendments containing Fe, such as nZVI, resulted in a decrease in As bioaccessibility. The addition of the Nanoiron 25 Slurry (N25S) was particularly effective in decreasing As bioaccessibility compared to the other treatments. These results are of great practical importance, because 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 U.S. EPA,<sup>65,66</sup> *in vitro* assays such as SBRC-G may not be appropriate for predicting Pb RBA in phosphate-amended soils because of potential artifacts associated with assay conditions (i.e., low pH that 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 between *in vivo* and *in vitro* results.

While the *in vitro* results suggest that the addition of phosphate amendments may have a negative impact of As bioaccessibility (and therefore predicted As RBA), conceivably this may not be reflected by *in vitro* data because of preferential absorption of phosphate.<sup>66</sup> 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

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b01356.

Soil pH after 2 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 Bacca Locci soil (Figure S1) and SEM images of the 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) (PDF)

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### Notes

The authors declare no competing financial interest.

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