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Influence of compost addition on lead and arsenic bioavailability in reclaimed orchard soil assessed using *Porcellio scaber* bioaccumulation test

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ABSTRACT

Long-term application of lead arsenate in orchards has led to a significant accumulation of Pb and As in the topsoil. Reclamation of old orchards for agricultural purposes entails the exposure of humans to Pb and As, which can be reduced by adequate remediation actions. In this study, we assessed the remediation efficiency of compost addition, commonly used as a sustainable agricultural practice, in decreasing the human exposure Pb and As by direct ingestion. The remediation was evaluated based on Pb and As bioavailability, assessed by means of a selective non-exhaustive chemical extraction (modified Morgan extraction, MME), with a physiologically based extraction test (PBET) for the assessment of Pb and As bioavailability in ingested soils and with a novel *in vivo* bioaccumulation test with isopods (*Porcellio scaber*). All the tests showed that compost addition consistently reduced Pb, but increased As potential bioavailability. The bioaccumulation test with *P. scaber* was sensitive to changes in Pb and As bioavailability in test soils. However, the results indicate that the bioavailability of As could be under- or overestimated using solely chemical extraction tests. Indirect assessment of trace metal bioavailability with bioaccumulation in isopods can be used as complementary source of data to the existing *in vitro* chemical extraction test approach for the estimation of human exposure to trace elements in polluted and remediated soil. This is the first report on the use of As accumulation in *P. scaber* as a tool for the assessment of As bioavailability in contaminated orchard soil.

1. Introduction

Trace metals such as lead (Pb) and arsenic (As) in soil are not biodegraded by natural processes and they therefore persist in soil, leading to long-term effects. Agricultural inputs in the past were one of the major source of As in soils, due to the wide use of inorganic substances as pesticides or plant defoliants. Lead arsenate ($PbAsO_4$) was the most extensively used of the arsenical insecticides from the 1890s until the introduction of organic pesticides in the 1940s, resulting in heavy As and Pb soil contamination [1]. Although lead arsenate is no longer used in orchards, these soils are often converted to other uses, such as the production of vegetables, which could affect the bioavailability of Pb and As in soil. Composts, commonly used in vegetable production, when added to contaminated soil can affect the bioavailability and mobility of trace metals, either increasing or decreasing them, depending on the element [2–4].

It has become recognized, that the total soil concentrations of trace metals is not an appropriate predictor of their environmental impact, since their chemical forms are rarely 100% bioavailable. The assessment of the trace metal bioavailability in soil is mainly based on the simpler assessment of their potential bioavailability, i.e. the maximum amount of trace metals available for absorption into living organisms [5]. Various non-exhaustive selective chemical extractions have been designed, such as sequential extractions and simple one-step extractions [6–10]. These extractions are generally used for the assessment of the potential bioavailability to all living organisms [11]. More elaborate *in vitro* digestion models have been developed for a more targeted assessment of the trace metal potential bioavailability for humans through accidental soil ingestion [12–14], such as the two-step physiologically based extraction test (PBET) designed by Ruby et al. [14–16]. However, past studies have shown that chemical soil extractions are not suitable for describing the bioavailable fraction of trace metals, due to the dynamic and complex nature of metal–soil and metal–organism interactions [16]. Instead, bioassays in which accumulated trace metals are determined can be used for assessing trace metal bioavailability in soil. Terrestrial isopods accumulate metals from their environment

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in proportion to their bioavailable concentration in the soil [17] and they therefore appear to be very suitable as indicators of the metal bioavailable fraction in polluted soil and leaf litter [18,19]. A simple *in vivo* test with isopods as accumulating indicator organisms has been proposed as a supplement to chemical extractions, providing a more complete and relevant picture of the bioavailability of Pb, Zn and Cd in soil to organisms [19], but no study has so far been done on As accumulation in isopods exposed to polluted soil.

The aim of this study was to assess the efficiency of compost addition as a remediation method for modifying Pb and As bioavailability in historically polluted orchard soils. The novelty of the study was the use of the isopod *Porcellio scaber* as a metal bioaccumulation assay. This is the first report on the use of an isopod short-term bioaccumulation test as a tool for assessing the bioavailability of As in contaminated and remediated soils.

2. Experimental

2.1. Soil and soil analysis

Soils (silty clay loam soils, Hudson series) were sampled at Dilmun Hill Student Organic Farm in Ithaca, NY (USA), a former apple orchard, which was extensively treated in the past with lead arsenate. Three locations (A, B, C) where chosen within the orchard with different Pb and As soil concentrations. An experimental plot was setup at each location in June 2010, as part of a larger study on the effect of compost on the uptake of As and Pb in vegetables. Each experimental plot was composed of orchard soil without amendments and orchard soil with different amendments. One of the treatments involved mixing household/garden compost into the orchard soil to the depth of 15 cm. The compost used in this study is produced at the Cornell University in Ithaca, NY (USA), using a turned windrow system. The windrows are about 2 m tall and 80 m long, which allows temperature to be maintained in the 50–65 °C temperature range. The complete composting process is about 6–9 months. The feedstock for the compost are mainly food scraps and biodegradable plastic and paper serviceware from the cafeterias, discarded greenhouse soil mix (mostly peat moss) and wood-based animal bedding. The compost was analyzed for total element content before its application [20], as described in Section 2.4; the total Pb, As and P contents were 16.1, 0.97 and 8238 $mg\,kg^{-1}$, respectively. In September 2010, several samples of topsoil were randomly collected on each experimental plot without compost (i.e. A, B and C; hereinafter: non-amended soils) and with 10% (w/w) added compost (i.e. A + 10%, B + 10% and C + 10%; hereinafter: amended soils) and bulked to get one sample per experimental plot for further analyses. Soil from a nearby managed field at Caldwell Field (Ithaca, NY) was sampled as control, uncontaminated soil. The sampled soils were air-dried, homogenized and sieved as required. Soil pH was measured in a 1/2 (w/v) 0.01 M $CaCl_2$ suspension. Soil samples were analyzed for total carbon content (C%) by modified Walkley–Black titration [21].

2.2. Potential bioavailability of Pb and As

The potential bioavailability of Pb and As was assessed with the single-step modified Morgan extraction procedure (MME) and with a two-step physiologically based extraction test (PBET).

MME was initially developed for the assessment of the biological availability of macronutrients in soil, but has been shown to be useful in evaluating trace element availability [9]. It was performed by extracting 10 g of sieved (2 mm) air-dried soil for 15 min on an orbital shaker (180 rpm) in 50 mL of extraction solution containing 0.65 M NH_4OH and 1.25 M CH_3COOH buffered to pH 4.8 ± 0.05 [22]. The suspension was paper-filtered (Whatman 42) and As and Pb concentrations determined in the filtrate by ICP-AES (described below). All the extractions were conducted in triplicate.

The PBET used is based on the method of Ruby et al. [14], designed to approximate the gastrointestinal tract parameters of 2–3-year-old children and is used to assess the potential bioavailability of trace metals in soil to humans [23]. Pediatric physiological characteristics were chosen for the method, since children ingest more soil and dust particles than adults, mainly due to their mouthing behavior, and are thus more exposed to soil pollutants [24]. The PBET involves a first step extraction at pH 2.5, which simulates soil digestion in the stomach (stomach phase), and a second step extraction at pH 7.0, which simulates the small intestine phase. For the stomach phase, 0.5 g of sieved soil sample (<250 mm) was extracted in a 250 mL Erlenmeyer flask in an incubator orbital shaker at 180 rpm for 2 h at constant temperature (37 °C) in simulated gastric fluid (50 mL). The gastric fluid was prepared to contain 1.25 g of pepsin (porcine, Sigma), 0.50 g of citrate, 0.50 g of malate, 420 mL of lactic acid and 500 mL of acetic acid per liter, adjusted to pH 2.50 ± 0.05. The pH of the reaction mixture was checked every 30 min and adjusted with 1 M HCl as necessary to keep it at a value of 2.50 ± 0.05. After 2 h, the reaction mixture was titrated to pH 7 with saturated $NaHCO_3$ solution, followed by the addition of 175 mg of bile salts (porcine, Sigma) and 50 mg of pancreatin (porcine, Sigma), thus simulating small intestine conditions. After 2 h, the reaction solutions were centrifuged at $1600 \times g$ for 10 min. The liquid fraction was decanted and analyzed for As and Pb by ICP-AES (see below) as the small intestine fraction.

The PBET was conducted in triplicate. Only As and Pb concentrations in the intestinal fraction were measured, since the metals are absorbed into the blood system from the intestine [21].

2.3. As and Pb accumulation in P. scaber

Adult specimens of a terrestrial isopod *P. scaber* were purchased from Carolina Science (Burlington, NC), kept in laboratory at constant temperature (24 °C) and fed with hay pellets (Hertz, USA). For the experiment, 6 adult specimens of 30–110 mg fresh weight were exposed for 14 days to 200 g of air dried experimental soil (control soil, non-amended and amended soils) in plastic vessels (16.5 cm × 16.5 cm, 379 mL) with perforated plastic lids. Four replicate vessels were used for each soil treatment. The experimental soils in plastic vessels were moistened daily with deionized water in one corner of the experimental vessels, in order to achieve a soil moisture gradient. No other food than soil was presented to the animals. After exposure, the animals were removed from the vessels and fed with hay pellets for 24 h to remove metals from their digestive systems. They were then frozen and lyophilized [19,25]. For digestion, the six animals in each of the four experimental vessels were pooled; there were therefore 4 replicates for each soil treatment. We assumed that by pooling the animals, we would average the variability among individuals, thus simplifying the test. The animals were then completely digested in a nitric/perchloric acid mixture (volume ratio 7:1) by progressively heating (steps of 20 °C/h, starting at 40 °C) the samples to 185 °C, where they were heated for 14 h. Dry residues were resuspended in 1 M HNO_3 and total Pb and As concentrations in whole animals were determined by ICP AES at the US Agricultural Research Service Laboratory in Ithaca, NY (Robert W. Holley Center for Agriculture and Health, Cornell University). Bioaccumulation factors (BAFs) were used to express Pb and As accumulation in the animals. They were calculated as the ratio of total Pb and As in the animals to their pseudototal concentration measured in the soil (see below).

2.4. Trace metal analysis

Most contaminant metals in soils are not structural in minerals; a "pseudototal" analysis using strong acid digest without the necessity of dissolution of silicates by hydrofluoric acids is therefore sufficient to estimate total metals [26,27]. Air-dried samples of non-amended, amended and control soils (3 g) were ground in an agate mill, sieved (<150 μm), digested in 18 mL of nitric–hydrochloric acid mixture (volume ratio 1:7) and diluted with deionized water up to 100 mL. Pseudototal Pb, As and P concentrations were analyzed by inductively coupled plasma atomic emission spectrometry (ICP-AES) (SPECTRO CIROS CCD – ICP Spectrophotometer) at the Cornell Nutrient Analysis Laboratory. Pb and As in PBET and Modified Morgan's extracts were determined by ICP-AES directly. Standard reference material from the National Institute for Standards and Technology (NIST SRM 2702) was used in the digestion and analyses as part of the QA/QC protocol. The recovery percentages were 83, 71, and 70% for Pb, As and P, respectively. Reagent blanks were used to ensure accuracy of the analysis and standards matrix was matched according to the measured samples.

2.5. Data analysis

The significance of the measured treatment differences in soil characteristics (pH, total organic carbon), Pb, As and P pseudototal concentrations, Pb and As potential bioavailability and Pb and As accumulation in *P. scaber* in non-amended and amended soils were determined by the *t*-test at a 95% confidence level ($p < 0.05$). Linear regression analysis was performed to assess correlations between Pb and As concentrations in animals, Pb and As in soil and their potentially bioavailable concentration in soil. Statgraphics software (Statgraphics Plus for Windows 4.0, Statistical Graphics, Herndon, VA, USA) and Microsoft Office Excel 2007 were used.

3. Results and discussion

3.1. Soil amendments

Soil properties assessed for control, non-amended and amended soils are presented in Table 1. The non-amended soils used in the study were weakly to strongly acidic, with pH values ranging from 4.6–4.7 to 6.3–6.5. After 10% (w/w) addition of compost, the pH significantly increased ($p < 0.05$) in soils A, B and C. Pb and As pseudototal concentrations in the experimental control and non-amended soils formed a gradient ranging from 18 to 820 mg kg^{-1} for Pb and from 5.3 to 172 mg kg^{-1} for As (Table 1). The generally small but significant ($p < 0.05$) differences in Pb and As pseudototal concentrations between non-amended and amended soils could be attributed to the soil dilution with compost and to the difficulty to homogeneously mix the compost into the soil due to the cloddy consistence of the fresh compost applied in June 2010. By adding compost, the pseudototal concentration of phosphorus and sulfur and the total organic carbon content in soils significantly ($p < 0.05$) increased by factors up to 3, 2.9 and 4.1, respectively (Table 1).

3.2. Pb and As potential bioavailability

The results of the selective chemical extraction methods used in this study showed that compost is an effective stabilizing agent for the limitation of Pb potential bioavailability in polluted soil (Table 1). The MME showed a 12.3, 2.5 and 1.7-fold significant decrease ($p < 0.05$) of Pb potential bioavailability in A, B and C soils, respectively. The very large reduction in extractable Pb from soil A is probably the result of the initially very low pH of this soil (4.6–4.7) and the fact that compost addition raised the pH up to 6.9–7.1. The compost amendment in soil A decreased the potential Pb bioavailability assessed with PBET below the limit of detection, while in soils B and C it decreased by factors of 2.7 and 1.3, respectively ($p < 0.05$). The soil pH is likely to be the most important factor controlling the conversions of trace metal chemical forms in soil [1,4]. The solubility of Pb is known to be inversely proportional to the soil pH, phosphate compounds content and humic materials. Humic materials are introduced into the soil by adding compost and seem to be the main sites of Pb sorption in soil by strong complexation, while Pb–phosphate compounds are likely to precipitate [28,29]. Conversely, As potential bioavailability increased by factors up to 3.1 and 2.2 after compost addition, as assessed with MME and PBET, respectively. As tends to become more soluble and mobile at higher soil pH values, since chemisorption of anions on minerals is less favorable at higher pH. Humic acids, by which As may be adsorbed, tend to dissolve at higher soil pH values, resulting in As mobilization [30–33]. Moreover, the potential bioavailability of As assessed with MME was significantly positively correlated with pseudototal phosphorus ($r = 0.4818$, $p < 0.01$). The compost contained high levels of phosphorous (Table 1), which could lead to As mobilization by competitive displacement by PO_4^{3-} [2,34,35]. Literature data on the effect of compost amendments on trace metals in soil report both increased and decreased potential bioavailability, depending on soil properties, chemical characteristics of trace metals [4,36,37], compost composition and its stabilization in time [38,39]. Moreover, compost application could have opposite effects on the bioavailability of trace metals with different chemical characteristics, as shown in the present study. Similarly, Tandy et al. [40] reported increased As, but decreased Pb soil pore concentration after compost application. This stresses the importance of a complete assessment of trace metals in soil along with their chemical properties, when choosing the remediation method, in order to detect and counteract side effects of remediation actions on different trace metals present in soil.

3.3. Pb and As accumulation in P. scaber

The majority of isopods (95%) survived the 14-day long exposure to the experimental soil. The duration of exposure was determined on the basis of preliminary studies, in which the authors concluded that 14 days of exposure under suboptimal condition does not severely affect the animals. However, this is long enough for them to accumulate substantial amounts of bioavailable metals [25]. No data have to date been available for As accumulation in *P. scaber* in a short-term exposure test, so no comparisons could be made. The animals in this study accumulated measurable Pb and As amounts in their bodies, without experiencing considerable stress, since their body weights did not significantly ($p > 0.05$) change during the exposure time (data not shown).

The increasing concentrations of Pb and As in soil were reflected in the accumulated Pb and As in animals (Table 2). As expected, the lowest Pb and As accumulation was measured in animals exposed to soil A and the highest in animals exposed to soil C. The results are in accordance with reports of trace metals accumulated in isopods exposed to remediated soils [19], in isopods collected in differently polluted areas [18,41], or exposed to food spiked with increasing concentrations of trace metals [42,43]. The opposite effects of compost addition on Pb and As bioavailability in soil, already predicted by the chemical extraction tests, were also reflected in the accumulated amounts in *P. scaber*. The BAFs for Pb decreased below the limit of quantification in soils A and B and by a factor of 12.5 in soil C, while for As, the BAFs increased by factors up to 1.8 (Table 2).

The potential bioavailability of Pb and As assessed with chemical extraction tests was significantly correlated ($p < 0.05$) with the accumulated Pb and As in animals (Fig. 1), suggesting that the

Table 1
Selected soil properties, Pb, As and P pseudototal concentration (assessed with *aqua regia* digestion) and their potential bioavailability assessed with the modified Morgan extraction procedure and with the physiologically based extraction test (PBET; only the concentrations in the intestinal phase are shown) in control soil (CO) and in low, medium and highly polluted soil, with and without the addition of 10% (w/w) compost (A, A + 10%, B, B + 10%, C, C + 10%, respectively). Results are presented as means of three replicates ± SD. LOQ, below the limit of quantification.

	CO	A	A + 10%	B	B + 10%	C	C + 10%
pH	5.1–5.3	4.6–4.7	*6.9–7.1	5.8–6.0	*7.3–7.5	6.3–6.5	*7.7–7.8
Total C (%)	0.22 ± 0.04	5.1 ± 0.2	*20.4 ± 0.3	5.1 ± 0.2	*15.4 ± 3.2	3.3 ± 0.4	*13.6 ± 0.5
Pseudototal concentration							
Pb (mg kg^{-1})	18.4 ± 0.3	64.9 ± 0.9	*35.9 ± 15.5	317.5 ± 2.8	*296.8 ± 3.6	647.5 ± 17.5	*820.4 ± 9.5
As (mg kg^{-1})	5.3 ± 0.1	31.7 ± 0.5	*11.3 ± 0.1	84.1 ± 0.6	*76.2 ± 0.6	171.7 ± 4.3	171.4 ± 1.9
P (mg kg^{-1})	647 ± 13	522 ± 8.5	*1417 ± 12	827 ± 6.7	*2450 ± 48	1142 ± 15	*2690 ± 115
Modified Morgan extraction							
Pb (mg kg^{-1})	0.4 ± 0.02	10.9 ± 0.1	*0.89 ± 0.06	35.2 ± 2	*14.1 ± 0.3	79.2 ± 3.2	*46.2 ± 3.3
As (mg kg^{-1})	0.13 ± 0.01	0.31 ± 0.02	*0.37 ± 0.04	1.03 ± 0.03	*3.18 ± 0.07	6.08 ± 0.08	*12.61 ± 0.13
PBET (intestinal phase)							
Pb (mg kg^{-1})	LOQ	11.2 ± 1.7	LOQ	54.8 ± 2.5	*20.2 ± 3.7	119.3 ± 9.2	*89.2 ± 4
As (mg kg^{-1})	LOQ	5.8 ± 0.3	*1.8 ± 1.6	18.8 ± 1	20.7 ± 1.6	65.7 ± 1.2	*71.5 ± 1.3

* Significant difference (LSD, $p < 0.05$) between soil without and soil with the addition of 10% (w/w) compost.

Table 2
Pb and As concentrations and Pb and As bioaccumulation factors (BAFs) for *Porcellio scaber* (whole bodies) after 14-days exposure to control soil (CO), three non-amended soils (A, B and C) and respective soils amended with 10% (w/w) compost (A + 10%, B + 10% and C + 10%). Results are presented as the average of 4 samples, each consisting in 6 pooled animals, with respective standard deviations. * Significant difference between animals exposed to non-amended and amended soil (*t*-test, $p < 0.05$). LOQ, below the limit of quantification.

	Concentration in *P. scaber* (μg g^{-1})		BAF	
	Pb	As	Pb	As
CO	LOQ	LOQ	LOQ	LOQ
A	0.75 ± 0.2	LOQ	0.012 ± 0.003	LOQ
A + 10%	LOQ	LOQ	LOQ	LOQ
B	5.4 ± 2.2	2.6 ± 0.7	0.017 ± 0.007	0.031 ± 0.008
B + 10%	LOQ	5.5 ± 2.9	LOQ	*0.055 ± 0.017
C	17.7 ± 5.1	12 ± 1.8	0.028 ± 0.008	0.071 ± 0.01
C + 10%	*1.8 ± 1.1	*22 ± 5.5	*0.002 ± 0.001	*0.127 ± 0.032

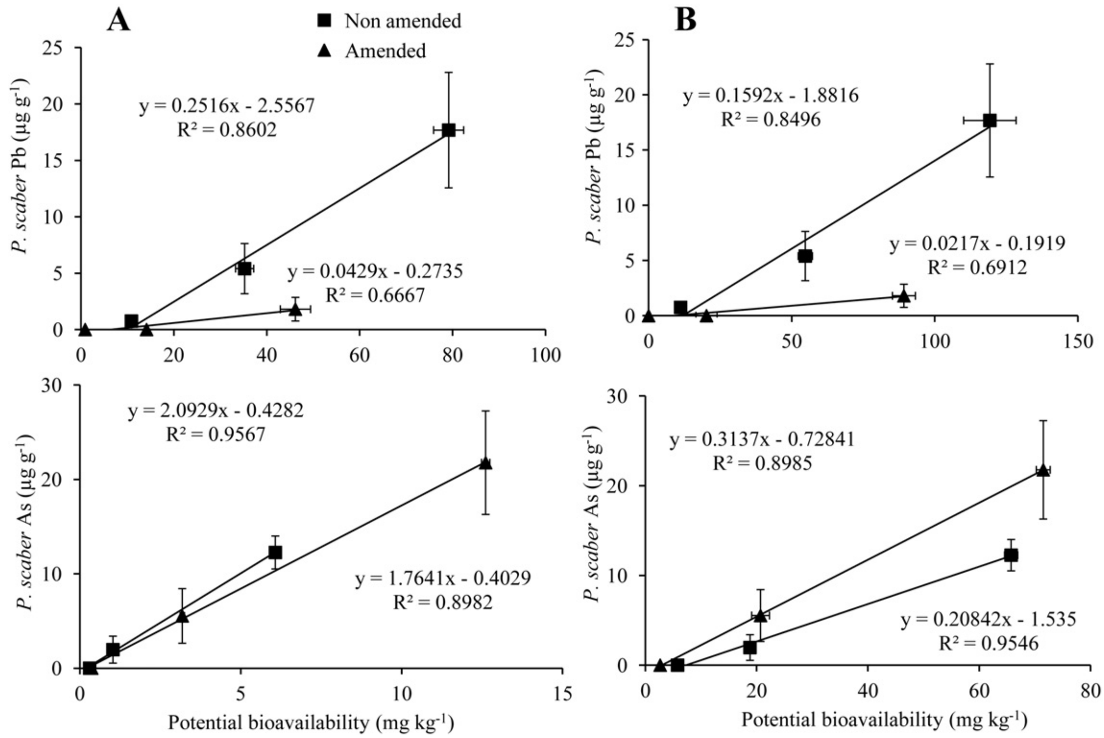

Fig. 1. Linear relationships between accumulated Pb and As in *Porcellio scaber* exposed to non-amended soils and soils amended with 10% (w/w) compost and Pb and As potential bioavailability in respective soils, as assessed by the modified Morgan extraction procedure (A) and by a physiologically based extraction test (PBET) (B). Data are presented as averages ± STDEV.

fractions of soil Pb and As dissolved using the chemical extraction tests in this study are proportional to the fractions of Pb and As available to soil organisms. This indicates that accumulation in *P. scaber* is a reflection of bioavailability and not of the pseudo-total concentration of trace metals in soil, as reported by Godet et al. [44]. Similarly, in their study on the relationship between Pb, Zn, Cd and Cu soil concentration and accumulation in soil animals [41], they reported that the accumulation Cd and Pb in isopods was independent of the total soil concentrations. The relationship between bioaccumulated and potentially bioavailable Pb and As in non-amended soils are however different from that in the amended soils, supporting the conclusion of other authors, that no simple universal relationship exists between soil and biota concentrations [45]. Additional studies should be performed on this topic to better discuss the differences.

The importance of a holistic approach in understanding the risk of soil pollution and the effects of its remediation is rapidly gaining importance [45,46]. Assessing the bioavailability of trace metals solely on the basis of chemical extraction tests might lead to misleading results. In a study on trace metal dynamics in the soil amended with compost, increased As and Cu concentration in soil pore water after the amendment indicated their increased potential bioavailability. Conversely, wheat shoot concentrations were lowered [40]. In our study, the Pb potentially bioavailable fraction assessed with MME and PBET overestimated the bioaccumulated amount in *P. scaber*, while for As, the accumulation was underestimated by the MME (Table 2). Similarly, studies in which chemical extraction tests, including the PBET, are compared to animal models give contrasting conclusions. Ruby et al. [14] compared the results of the PBET with a New Zealand White rabbit model and a *Cynomologus* primate model, and found the PBET to be over-predictive for As. Conversely, Rodriguez and Basta [15] reported that the PBET underestimates the bioavailability of As in calcine materials, compared to an *in vivo* immature swine model. This indicates that Pb and As bioavailability could be under- or overestimated using solely chemical extraction tests, so that it would be difficult to recognize a general trend. Indirect measures of trace metals' bioavailability, such as bioaccumulation in isopods, could be therefore used as complementary source of data to reinforce the existing *in vitro* chemical extraction test approach for the estimation of human exposure to trace elements in polluted and remediated soil.

4. Conclusions

The following conclusions can be drawn from this study:

- All the tests showed that compost addition efficiently reduced Pb bioavailability in orchard soil but increased As bioavailability. Soil and contaminant chemical properties should be therefore carefully considered before applying compost with the purpose of soil remediation.
- The bioaccumulation test with *P. scaber* was sensitive to changes in Pb and As bioavailability in experimental soils and the animals accumulated measurable amounts of Pb and As.
- Chemical extraction tests overestimated Pb accumulation in *P. scaber*, while for As, MME and PBET over- and underestimated As accumulation, respectively. This indicates the possibility of acquiring misleading assessments of trace metal bioavailability in soil based solely on chemical extractions.
- The novel bioaccumulation test with *P. scaber*, here used for the first time to assess the bioavailability of As in soil, is proposed as a tool in addition to chemical extraction tests for the assessment of trace metal bioavailability in polluted and remediated soils.

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