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Predictive capabilities of in vitro assays for estimating Pb relative bioavailability in phosphate amended soils

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Keywords

chloropyromorphite; in vitro, in vivo; lead bioaccessibility; lead relative bioavailability; phosphoric acid; rock phosphate

Abstract

In this study, the in vitro bioaccessibility (IVBA) of lead (Pb) in phosphate-amended Pb-contaminated soil was assessed using a variety of IVBA assays with an overarching aim of determining whether changes in Pb IVBA were congruent to those observed for Pb relative bioavailability (RBA) determined using an in vivo mouse assay. Amending soil with

phosphoric acid or rock phosphate resulted in changes in Pb speciation, however, varying Pb IVBA results were obtained depending on the methodology utilised. In addition, IVBA assays influenced Pb speciation as a consequence of interactions between dissolved Pb and unreacted phosphate arising from the amendment or from assay constituents. When the relationship between Pb RBA and IVBA was assessed, a comparison of treatment effect ratios (Pb RBA or IVBA in treated soil divided by Pb RBA or IVBA for untreated soil) provided the best in vivo-in vitro correlation particular for SBRC ($r^2 = 0.83$) and IVG ($r^2 = 0.89$) intestinal extraction. For these assays, the slope of the lines of best fit were close to 1 (1.12, 0.82; SBRC, IVG intestinal extraction respectively) with small y-intercepts (0.09, 0.08 respectively) indicating that the efficacy of phosphate amendments for reducing Pb RBA may be predicted using IVBA assays.

Introduction

Children's exposure to lead (Pb) is a significant concern due to associated adverse health impacts, particularly on neurological and cognitive development.[1,2] Exposure may occur from a variety of sources, however, a significant source is via incidental soil ingestion.[3-5] In order to reduce exposure to incidental soil-Pb ingestion, phosphate treatment of Pb-contaminated soil has been proposed. Various phosphate amendments have been shown to facilitate the formation of Pb-phosphate minerals with low solubility products[6] resulting in a reduction in Pb relative bioavailability (RBA) following ingestion. For example, Hettiarachchi et al.[7] observed a decrease in Pb RBA when smelter-impacted soil was treated with rock phosphate (RP) or triple super phosphate (TSP). Treatment effect ratios (TER; ratio of Pb RBA in treated soil divided by Pb RBA in untreated soil) ranged from 0.59 (RP) to 0.65 (TSP). Similarly, Juhasz et al.[8] observed TER of 0.39-0.67, 0.48-0.90 and 0.03-0.19 for smelter, non-ferrous slag and shooting range impacted soils, respectively using phosphoric acid (PA) and RP. X-ray absorption spectroscopy (XAS) determined that the decrease in Pb RBA was attributable to the formation of poorly soluble Pb-phosphates including chloropyromorphite, hydroxypyromorphite and tertiary Pb-phosphate.[8]

Although a limited number of studies have determined the efficacy of phosphate amendments in reducing Pb RBA,[7,8] in vitro methodologies have been utilised more extensively for assessing changes in Pb bioaccessibility (IVBA) as a result of soil amendments. Methodologies including the Solubility Bioaccessibility Research Consortium (SBRC) assay, [9] In Vitro Gastrointestinal extraction method (IVG),[10,11] Physiologically Based Extraction Test (PBET),[3] Unified Bioaccessibility Research Group of Europe Method (UBM)[12] and Deutsches Institut für Normunge.V. (DIN)[13] have been utilised to overcome cost and ethical constraints associated with in vivo models. As detailed by Scheckel et al.[6] and others,[14-17]

TER vary depending on Pb source, phosphate amendment, application rate and IVBA assay utilised. While sequential extractions have been shown to induced pyromorphite formation in Pb-P systems,[18] Scheckel et al.[19] determined that 'artefacts' in IVBA assays may also occur whereby Pb IVBA assessment may overestimate treatment efficacy. As a consequence, the use of IVBA assays for predicting Pb RBA in phosphate-amended soil is not recommended due to pH effects and the potential of excess phosphate interfering with IVBA results.[20]

Although the assessment of phosphate-amended soil using IVBA assays may result in lower than expected TER as a consequence of in vitro pyromorphite formation,[6,19] a recent study highlighted that in vivo processes (i.e. gastrointestinal tract interactions) may also facilitate the formation of poorly soluble Pb-phosphates which decrease Pb absorption.[8] Juhasz et al.[8] reported similar decreases in Pb RBA when phosphate-amended soil (treated with PA or RP) or untreated soil plus phosphate amendments were administered to mice supported by XAS analysis showing in vivo changes in Pb speciation through the gastrointestinal tract. While it has been suggested that excess soil phosphate may interfere with Pb IVBA results making it unsuitable for predicting Pb RBA in phosphate-amended soils,[20] conceivably in vivo interactions between excess phosphate and Pb may also influence Pb RBA outcomes.

In this study, amended and unamended soils from Juhasz et al.[8] were utilised to determine whether changes in Pb IVBA, as a consequence of phosphate amendments, were congruent to those observed in Pb RBA assessments. XAS analysis was also undertaken on selected samples following IVBA assessment (i.e. residual soil-Pb) to determine whether parameters associated with IVBA assays (i.e. pH or solution constituents) influenced IVBA outcomes and Pb RBA predictions.

Materials and Methods

Pb-contaminated soils and phosphate amendments

Previously, the effect of phosphate amendments (PA and RP) on Pb RBA was assessed in soils impacted by Pb smelting (PP2), non-ferrous slag application (SH15) and shooting range activities (SR01).[8] In the current study, these soils were utilised to determine whether IVBA assays can predict Pb RBA in phosphate-amended soil. A detailed description of Pb-contaminated soils, amendment protocols and the in vivo mouse bioassay can be found in Juhasz et al.[8] and the Supporting Information. The elemental composition of soils was determined after sieving and recovering the < 250 µm particle size fraction.[21] The concentrations of major and minor elements were quantified using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) following soil digestion (USEPA 3051)[22] using a CEM Mars6 microwave. Table S1 outlines selected properties for soils used in this study.

The accuracy of aqua-regia digests was confirmed by a quantitative average Pb recovery of 1128 ± 55 mg kg^{-1} (n = 6) from NIST 2711 (1162 ± 31 mg Pb kg^{-1}, certified). During the determination of Pb concentration in soil digests and in vitro extractions, duplicate analysis, spiked sample recoveries and check values were included. The average deviation between duplicate samples (n = 6) was 2.4% (0.9-8.4%), the average recovery from spiked samples (n = 6) was 96.4% (95.2-98.6%) whereas Pb standard solution (50 µg l^{-1}) recoveries (n = 16) ranged from 93.0-101.5% (96.7% average recovery).

Assessment of Pb bioaccessibility

In order to determine phosphate amendment treatment efficacy, Pb IVBA was assessed using a number of assays incorporating both gastric and intestinal phases. IVBA assays were

conducted according to Kelley et al. (SBRC),[9] Rodriguez et al. (IVG),[10] Ruby et al. (PBET),[3] DIN[13] and Wragg et al. (UBM) [12] with Pb IVBA calculated according to equation 1.

$$in\ vitro\ Pb\ bioaccessibility\ (\%) = \left(\frac{in\ vitro\ Pb}{total\ Pb} \right)\ x\ 100 \qquad \text{Equation 1}$$

Where:

In vitro Pb = Pb extracted during the in vitro assay

Total Pb = Amount of Pb in the contaminated soil used for bioaccessibility determination

Lead IVBA was determined prior to the addition of PA or RP and following treatment and ageing in order to calculate TER according to equation 2.

$$treatment\ effect\ ratio\ (TER) = \left(\frac{Pb\ bioaccessibility_{\ treated\ soil}}{Pb\ bioaccessibility_{untreated\ soil}} \right) \qquad \text{Equation 2}$$

Where:

Pb bioaccessibility$_{\text{treated soil}}$ = the IVBA of Pb in contaminated soil following the addition of phosphate amendments (and ageing).

Pb bioaccessibility$_{\text{untreated soil}}$ = the IVBA of Pb in contaminated soil without phosphate amendments.

A TER of 1 indicates that the treatment had no effect on Pb IVBA while a value < 1 indicates a decrease in Pb IVBA as a result of the treatment.

Pb speciation in soils pre- and post-bioaccessibility assessment

Following IVBA extraction, residual Pb speciation was determined in selected soils by XAS. Soil was recovered from gastrointestinal extractions by centrifugation (3000 g for 10 minutes) followed by residual soil washing (triplicate washes) with MilliQ water adjusted to the pH of the corresponding in vitro solution. XAS data were collected at the MRCAT beamline 10-ID, Sector 10, at the Advanced Photon Source of the Argonne National Laboratory, U.S.[23] Details of the XAS methodology can be found in the Supporting Information while Figure S1 details XAS data of Pb reference samples.

Correlation between Pb bioaccessibility and Pb relative bioavailability in phosphate-amended soils

Soil used in this study was previously assessed for Pb RBA (pre- and post-phosphate amendment) using an in vivo mouse model.[8] Data from Juhasz et al.[8] was utilised to determine the capacity of IVBA assays to predict Pb RBA in phosphate-amended soils. The strength of the relationship between Pb RBA and IVBA was assessed for each assay and phase using simple linear regression while differences between Pb bioaccessibility data for each assay was determined using the Tukey-Kramer multiple comparison test (at the $p < 0.05$ significance level).

Results and Discussion

Assessment of Pb bioaccessibility in unamended soil using gastric extraction

Figure 1 illustrates the variability in Pb IVBA when different assays were utilised for the assessment of unamended soils. In PP2 (Figure 1a) and SH15 (Figure 1b), Pb IVBA following gastric extraction ranged from <32 to >80% and <4 to >32%, respectively. The large range in Pb IVBA values was attributable to differences in gastric extraction pH (1.5-

2.5) and its influence on the dissolution of Pb and phases (e.g. Fe oxides and organic matter) to which Pb may be sorbed.[3,4,24] In unamended PP2, Pb was present as mineral sorbed (55%) and organic bound (45%) species while lower Pb IVBA in SH15 was attributed to the low solubility of Pb present in the non-ferrous slag[3,25] (i.e. Pb-phosphate and hydroxypyromorphite) in addition to the elevated phosphate concentration (as a result of agricultural practices). In contrast, Pb IVBA in SR01 ranged from 78.6 to 93.0%, however, there was no significant difference ($p = 0.59$) in IVBA when different in vitro assays were utilised (Figure 1c). Previous studies with Pb-impacted shooting range soils have reported Pb IVBA to be > 60%[5,14,26,28] as a result of highly soluble Pb bullet weathering products including hydrocerussite ($Pb_3[CO_3]_2[OH]_2$), cerussite ($PbCO_3$) and massicot (PbO).[29-31] XAS analysis of unamended SR01 identified the presence of similar Pb bullet weathering products as previously reported (PbO: 77%; $PbCO_3$: 11%; mineral sorbed Pb: 13%).

XAS analysis of Pb remaining in soil post-gastric extraction revealed that Pb speciation may be influenced by the assay which may impact IVBA outcomes (Figures 2-4, 4). While some operational parameters are unique to the DIN assay (i.e. solid-to-solution ratio of 1:50 and gastric extraction time of 2 h), the extracting fluid contains phosphate (0.27 g l^{-1} of KH_2PO_4) which, at the low gastric pH, may interact with soluble Pb to facilitate the formation of Pb-phosphate minerals.[32] XAS analysis of residual soil-Pb post-DIN gastric extraction revealed significant changes in Pb speciation in untreated PP2 (Figure 2) and SR01 (Figure 4). Residual Pb in PP2 was present as mineral sorbed (53%) and organic bound (28%) species, however, chloropyromorphite (12%) and Pb-phosphate (7%) were also present. In SR01 post-DIN gastric extraction, chloropyromorphite (37%) was also observed. Presumably, the combination of low pH, to facilitate Pb dissolution, and ~2.0 mM P (in excess of the theoretical P:Pb molar ratio to facilitate pyromorphite formation) resulted in the formation of

Pb-phosphate minerals as an artefact of the assay. In contrast, following SBRC gastric extraction, Pb-phosphates were not detected in either soil due to the absence of phosphate and the presence of glycine (0.4M)[33], however, changes in the proportion of mineral sorbed, organic bound (PP2 and SR01), $PbCO_3$ and PbO (SR01) were observed.

XAS analysis of residual Pb in SH15 post-gastric extraction also revealed that speciation was influenced by the assay (Figure 3). Following SBRC gastric extraction, although the percentage of hydroxypyromorphite was unchanged, the proportion of Pb-phosphate (18%) and mineral sorbed Pb (25%) decreased while an increase in organic bound Pb (15%) and chloropyromorphite (28%) was observed. Similarly, assessment of SH15 following UBM gastric extraction resulted in hydroxypyromorphite removal, reduction in Pb-phosphate (15%) and mineral sorbed Pb (36%) and the appearance of organic bound Pb (18%) and chloropyromorphite (30%). As detailed by Mavropoulos et al.,[34] the stability of hydroxypyromorphite is dependent on its calcium content with lower stability observed for higher calcium concentrations. As a result, it is conceivable that high calcium content hydroxypyromorphite underwent dissolution during UBM gastric extraction, and in the presence of elevated chlorine ions, precipitated as the more stable chloropyromorphite structure.[34]

Assessment of Pb bioaccessibility in unamended soil using intestinal extraction

When IVBA assays were modified to reflect intestinal conditions (Figure 1), a significant decrease ($p < 0.05$) in Pb IVBA was observed in untreated soil for all assays. Lead IVBA decreased up to 207-fold compared to values determined using gastric extraction with IVBA ranging from 2.3 to 42.3% (PP2), 0.1 to 3.8% (SH15) and 0.4 to 56.0% (SR01). The

212 decrease in Pb IVBA was similar to previous studies whereby the increase in solution pH
213 facilitated the re-adsorption of Pb onto soil particles and/or Pb/Pb-Fe (co)precipitation.[25,35-40]
214
215 When the speciation of residual Pb in PP2 was assessed post-SBRC intestinal extraction
216 (Figure 2), a change in the distribution of mineral sorbed (72%) and organic bound Pb (28%)
217 was observed illustrating the influence of Fe in reducing soluble Pb. In contrast, following
218 DIN intestinal extraction, an increase in the proportion of Pb associated with
219 chloropyromorphite (22%) and Pb-phosphate (9%) was observed with a significant decrease
220 ($p < 0.05$) in mineral sorbed Pb (53 to 44%) compared to gastric extraction residuals. For
221 SH15 (Figure 3), Pb speciation was not influenced by transitioning gastric to intestinal phases
222 although small differences in the weighted percentage of Pb species were observed
223 particularly post-UBM assessment. XAS analysis of Pb remaining in SR01 post-intestinal
224 extraction revealed significant differences in Pb speciation between assays (Figure 4).
225 Following SBRC intestinal extraction, residual Pb was distributed between mineral sorbed
226 (51%), organic bound (27%), PbO (17%) and $PbCO_3$ (5%). In contrast, following DIN
227 intestinal extraction, a significant proportion of residual Pb was present as
228 chloropyromorphite (47%) and Pb-phosphate species (14%) indicating the influence of in
229 vitro solution constituents on Pb IVBA outcomes.
230
231 ***Impact of phosphate treatment on gastric phase Pb bioaccessibility***
232 Following treatment of Pb-contaminated soil with PA or RP, soil was aged (14 d) then
233 reassessed for changes in Pb IVBA and speciation. Neither amendment strategy had a
234 significant effect ($p < 0.05$) on reducing Pb IVBA in PP2 when IVG and UBM gastric phases
235 were used (TER = 0.98 to 1.14) (Figure 1D). Although treatment of PP2 with PA resulted in
236 a small (TER = 0.84 ± 0.04) but significant decrease ($p < 0.05$) in Pb IVBA following SBRC

gastric extraction, no reduction in Pb IVBA was observed in RP treated soils (TER = 0.98 ± 0.02). In contrast, an increase in Pb IVBA was observed in phosphate treated PP2 when assessed using the PBET (PA treatment; TER = 1.47 ± 0.13) and DIN gastric extraction (RP treatment: TER = 1.53 ± 0.17) (Figure 1D). Similarly, neither amendment strategy had a significant effect ($p < 0.05$) on reducing Pb IVBA in SH15 following assessment using SBRC and IVG gastric extraction (TER = 0.93 to 1.14; Figure 1E). Although treatment of SH15 with RP resulted in a significant decrease ($p < 0.05$) in Pb IVBA when assessed using the UBM gastric extraction (TER = 0.24 ± 0.04), no reduction in Pb IVBA was observed in PA treated soils (TER = 1.19 ± 0.26). Similar to PP2, an increase in Pb IVBA was observed in PA treated SH15 when assessed using the PBET (TER = 1.39 ± 0.03) compared to the untreated soil (Figure 1E).

Although changes in Pb speciation occurred as a consequence of phosphate amendments (Tables S2-S3), significant reductions in Pb IVBA were not evident in PP2 and SH15 following gastric extraction. While the form of Pb in soil plays a significant role in Pb IVBA, speciation alone does not dictate the extent of IVBA with soil characteristics such as pH, elemental composition, and reaction time requiring consideration.[41,42] In addition, the pH of the gastric extraction will influence Pb-phosphate stability as values below 2.12 (pK_{a1}) may result in the protonation of phosphate and its release from the structure.[6]

In contrast, assessment of phosphate-amended SR01 resulted in a significant reduction ($p < 0.05$) in Pb IVBA when evaluated using gastric extractions (Figure 1F). Pb IVBA ranged from 0.6 to 33.8% with TER of 0.007 (PBET) to 0.32 (SBRC). Following treatment of SR01 with RP, gastric Pb IVBA was also reduced compared to untreated soil, however, the reduction in Pb IVBA was not as significant (TER = 0.5-0.6). SR01 contained the highest

concentration of Pb, but also lacked high concentrations of Al, Ca, Fe, Mg, Mn, and Zn that could compete with Pb for available P. Previous studies investigating the effect of phosphate treatments on Pb IVBA have reported TER to range from 0.08-0.98 based on gastric IVBA results.[7,14-17,43-47] Treatment efficacy may be influenced by the phosphate amendment, Pb speciation, presence and concentration of ions that may compete for phosphate in addition to properties of the IVBA assay utilised (e.g. pH, dissolved organic content).[19,28,33,48,49]

Impact of phosphate treatment on intestinal phase Pb bioaccessibility

When assays were modified to reflect intestinal conditions, Pb IVBA decreased compared to values derived using gastric extraction. TER of 0.30-0.84 and 0.29-0.82 were derived for PP2 (Figure 1D) and SH15 (Figure 1E) when assessed using intestinal extractions with the exception of PA treated soils assessed using the DIN assay where there was no significant decrease ($p > 0.05$) in Pb IVBA compared to untreated soil. In SR01 treated with PA, Pb IVBA was < 0.5% for all assays when assessed using intestinal extraction (TER < 0.03). When RP treated soils were assessed, Pb IVBA was < 0.5% for all assays with the exception of the PBET (4.4 ± 0.3%) with TER ranging from < 0.001 (SBRC) to 0.15 (PBET) (Figure 1F). While TER have been determined for a variety of Pb-contaminated soils and phosphate amendments,[7,14-17,43-47] few studies have reported TER following IVBA assessment using intestinal extraction. The reason for this may reflect the preferred use of gastric extractions for predicting Pb RBA,[21] although an intestinal approach was proposed by Smith et al.[5] which accounted for soil property influences on Pb IVBA. It is well established that transitioning from gastric to intestinal conditions will reduce Pb IVBA due to Pb precipitation and re-sorption effects.[4,25,38-40] While these effects will be observed in both unamended and amended soils, the increase in pH may also facilitate Pb-phosphate formation due to the pH-dependent formation of pyromorphite.[19]

Changes in residual Pb speciation following bioaccessibility assessment

Several studies have explored changes in Pb speciation resulting from phosphate amendments to Pb-contaminated soils as a means to sequester Pb,[50-52] however, few studies have assessed speciation changes following the assessment of Pb IVBA. As detailed by Scheckel et al.,[19] the pH- and phosphate-dependent formation of pyromorphite in vitro may influence IVBA measurement and therefore TER.

XAS analysis of Pb remaining in phosphate treated soil post-in vitro extraction revealed that there were changes in Pb speciation compared to the starting material although speciation changes varied depending on the assay utilised. The phases identified in the post-extraction solids may be characterized as non-bioaccessible fractions as well as redistributed extraction components undergoing adsorption/re-precipitation during IVBA. Figures 2-4 and Tables S2-S3 show the influence of amendment strategy and gastro-intestinal extraction (for selected assays) on residual Pb speciation. Following SBRC, DIN or UBM gastric extraction, hydroxypyromorphite was not detected in PA treated PP2 (Figure 2) or SH15 (Figure 3), however, chloropyromorphite was detected with varying proportions depending on the assay utilised. Although transitioning from gastric to intestinal conditions did not influence residual Pb speciation in PP2 and SH15 (Figures 2-3), percentages varied with increasing proportions of chloropyromorphite. Changes in Pb speciation were also observed in PA treated SR01 pre- and post-IVBA assessment (Figure 4), in particular increasing proportions of chloropyromorphite following DIN gastric and intestinal extraction. Changes in residual Pb speciation were also evident in RP amended soil (Figures 2-4). In PP2, apatite sorbed Pb was present following SBRC and DIN assessment, although chloropyromorphite was also detected (in increasing proportions) following DIN gastric and intestinal extraction (Figure 2). Following SBRC and UBM assessment of RP amended SH15, hydroxypyromorphite was

not present in residual soil, however, apatite sorbed Pb and chloropyromorphite were present (Figure 3). In SR01, changes in Pb speciation were also observed in pre- and post-IVBA assessed soil (i.e. appearance of chloropyromorphite and organic bound Pb) in addition to speciation differences between IVBA phases (SBRC gastric versus intestinal) and assays (SBRC versus DIN) (Figure 4). These results illustrate that IVBA assays may influence Pb speciation in vitro as a consequence of interactions of dissolved Pb with unreacted phosphate from amendment strategies[19,34] or reaction of dissolved Pb with assay constituents.

Relationship between Pb relative bioavailability and bioaccessibility in phosphate amended soils

When Pb IVBA was assessed in amended and unamended soil, different results were obtained depending on the assay utilised (Figure 1). As a consequence, if IVBA assays are used as surrogate measures for predicting Pb RBA, the appropriateness of IVBA assays is dependent on the strength of the in vivo-in vitro correlation (IVIVC). USEPA method 9200.2-86,[20] the standard operating procedure for predicting Pb RBA in soil based on the SBRC assay, offers a strong IVIVC for non-amended soils, however, the assay is not recommended for the assessment of phosphate-amended soil as excess phosphate may interfere with Pb IVBA results. However, Juhasz et al.[8] demonstrated that a similar phenomenon may occur in vivo whereby phosphate and Pb react in the gastrointestinal tract resulting in a decrease in Pb RBA as a consequence of in vivo chloropyromorphite formation. Conceivably, artefacts observed during IVBA assessment may also occur during in vivo Pb RBA assessment. However, similar to results observed for different IVBA methodologies, the in vivo approach for assessing phosphate-amended soils may influence Pb RBA outcomes. For example, incorporating phosphate-amended soil into standard AIN93G rodent diet (containing 6.9 g KH_2PO_4 kg^{-1}) may result in different bioavailability measurements

compared to a strategy using a single dose via gavage[5] as a result of additional phosphate inputs.

Previously, Pb RBA was assessed in PA and RP amended PP2, SH15 and SR01 using a mouse bioassay incorporating a single gavage dose strategy.[8] As the soils assessed in Juhasz et al.[8] were identical to those used in this study, data were compared to determine whether Pb RBA may be predicted from Pb IVBA measurement (Figure 5). Although a limited number of paired in vivo-in vitro measurements were available for comparison (n = 6), with a limited range in Pb RBA, Figure 5 and Table S4 illustrate the varying strengths (r^2 = 0.23-0.84) of IVIVC. While the goodness of fit is a strong indicator of IVIVC strength, it has been suggested that other criteria (e.g. slope close to one, y-intercept near zero) should be considered when assessing RBA predictive models.[12] For IVIVC utilising SBRC and PBET gastric values, strong linear relationships were observed (r^2 = 0.84, 0.79 respectively) although the slope of the line of best fit was small (0.16, 0.23 respectively). This indicates that gastric extraction solubilised a significantly greater proportion of Pb from phosphate-amended soil compared to that which was absorbed during the mouse bioassay. Although the strength of IVIVC was poor for IVG (r^2 = 0.58), UBM (r^2 = 0.37) and DIN (r^2 = 0.55) gastric extraction, small slopes (0.05-0.14) were also observed for these assays (Table S4). In contrast, when intestinal Pb IVBA values were utilised for IVIVC, only the PBET (r^2 = 0.75) resulted in a goodness of fit > 0.4. The slope of the in vivo-PBET intestinal model was 1.71 indicating that a significantly higher proportion of Pb from phosphate-amended soil was absorbed into the systemic circulation compared to the proportion remaining in solution after extraction.

An alternative strategy for assessing the capacity of IVBA assays for predicting Pb RBA in phosphate-amended soil is to compare TER for (paired) in vivo-in vitro measurements. As illustrated in Figure 5 and Table S4, this approached provided stronger IVIVC for SBRC (r^2 = 0.83) and IVG (r^2 = 0.89) intestinal extraction. In addition, the slopes of the line of best fit were close to 1 (1.12, 0.82 for SBRC, IVG respectively) with small y-intercepts (0.09, 0.08 respectively). However, IVIVC utilising the TER approach did not improve the goodness of fit for PBET, UBM and DIN assays.

Conceivably, the TER approach could be utilised for predicting the efficacy of phosphate treatments for reducing Pb RBA in contaminated soils. Pb IVBA TER could be calculated following the in vitro assessment of soil pre- and post-phosphate treatment (using the SBRC intestinal phase). Lead IVBA TER would then be utilised to predict Pb RBA TER (using the IVIVC) with subsequent values used to calculate the reduction in Pb RBA as a consequence of the amendment strategy. While this approach seems feasible, the accuracy of predicting the efficacy of phosphate amendments in reducing Pb RBA is dependent on the strength of the TER IVIVC. The robustness of this model is currently lacking (due to the small data set, limited range in Pb RBA values, limited soil types assessed), however, the approach provides a strategy for further progressing the assessment of phosphate amendment efficacy for minimising soil-Pb exposure.

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Supporting Information

Details of physicochemical properties (Table S1), Pb speciation in contaminated soil (Tables S2 and S3), linear regression models describing the relationship between Pb RBA and IVBA in phosphate-amended soil (Table S4) and XAS spectra of Pb reference samples (Figure S1) is available free of charge via the Internet at http://pubs.acs.org

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559 **List of Figures**

560

561 **Figure 1**. Lead bioaccessibility in PP2 (A), SH15 (B) and SR01 (C) contaminated soils
562 determined using gastric and intestinal phases of the SBRC, IVG, PBET, UBM and DIN in
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568

569 **Figure 2**. Lead speciation in PP2 prior to and following treatment with phosphoric acid (PA)
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583

584 **Figure 5**. Relationship between Pb relative bioavailability, determined using an in vivo
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586 of the SBRC, IVG, PBET, UBM and DIN assays for Pb contaminated soils amended with
587 phosphoric acid or rock phosphate. The in vivo-in vitro relationship is also illustrated using
588 Pb relative bioavailability and bioaccessibility treatment effect ratios. Dotted lines represent
589 regression lines for in vivo-in vitro relationships, the solid red line represents a 1:1
590 relationship for comparison while error bars represent the standard deviation of triplicate
591 analysis.

Figure 1.

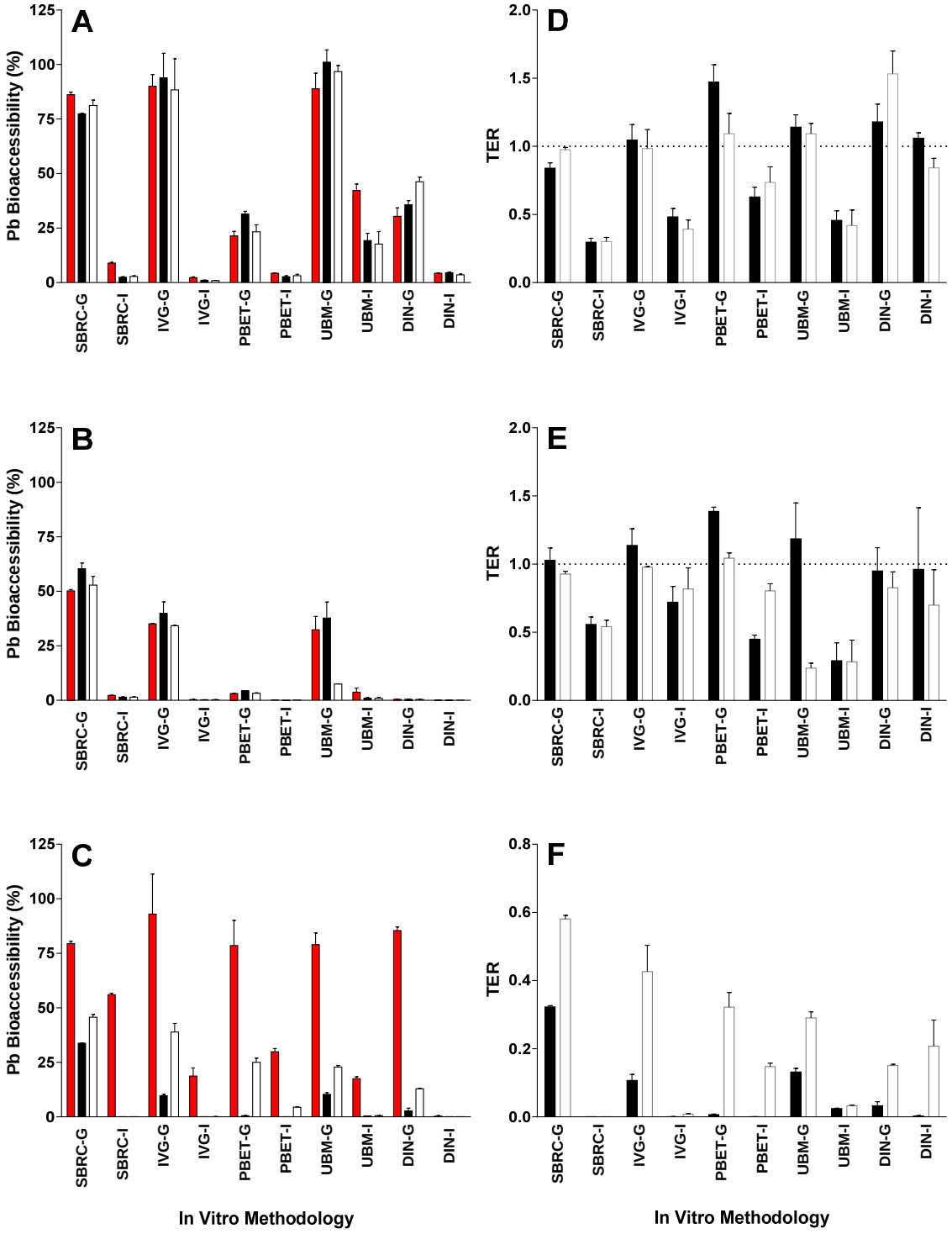

A
B
C
D
E
F
Pb Bioaccessibility (%)
TER
SBRC-G
SBRC-I
IVG-G
IVG-I
PBET-G
PBET-I
UBM-G
UBM-I
DIN-G
DIN-I
In Vitro Methodology

Figure 2.

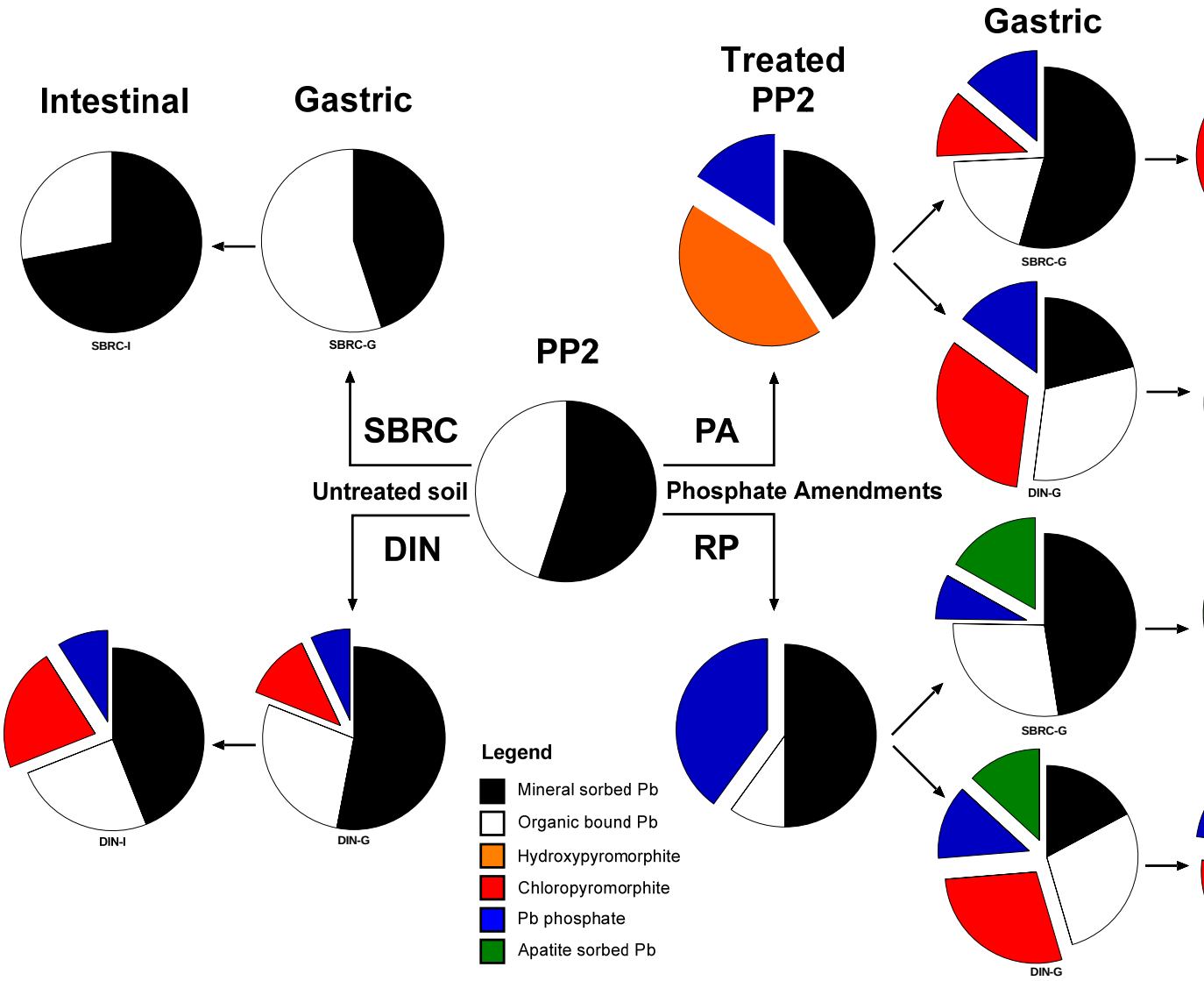

Intestinal
Gastric
Treated PP2
Gastric
SBRC-I
SBRC-G
SBRC-G
DIN-G
PP2
SBRC
PA
Untreated soil
Phosphate Amendments
DIN
RP
DIN-I
DIN-G
SBRC-G
DIN-G
Legend
Mineral sorbed Pb
Organic bound Pb
Hydroxypyromorphite
Chloropyromorphite
Pb phosphate
Apatite sorbed Pb

Figure 3.

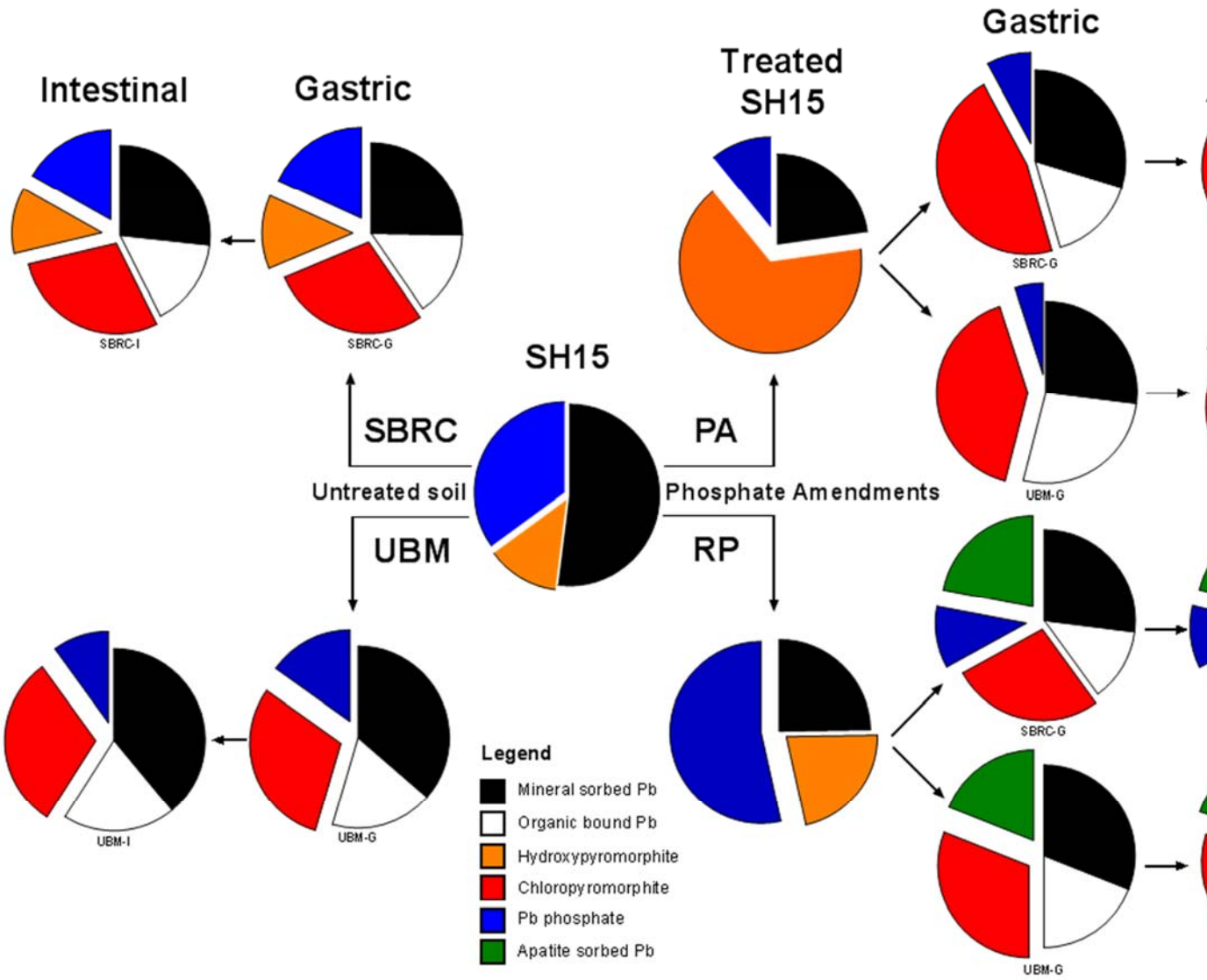

Intestinal
Gastric
SBRC-I
SBRC-G
SH15
SBRC
Untreated soil
UBM
PA
Phosphate Amendments
RP
Treated SH15
Gastric
SBRC-G
UBM-G
UBM-I
UBM-G
SBRC-G
UBM-G
Legend
Mineral sorbed Pb
Organic bound Pb
Hydroxypyromorphite
Chloropyromorphite
Pb phosphate
Apatite sorbed Pb

Figure 4

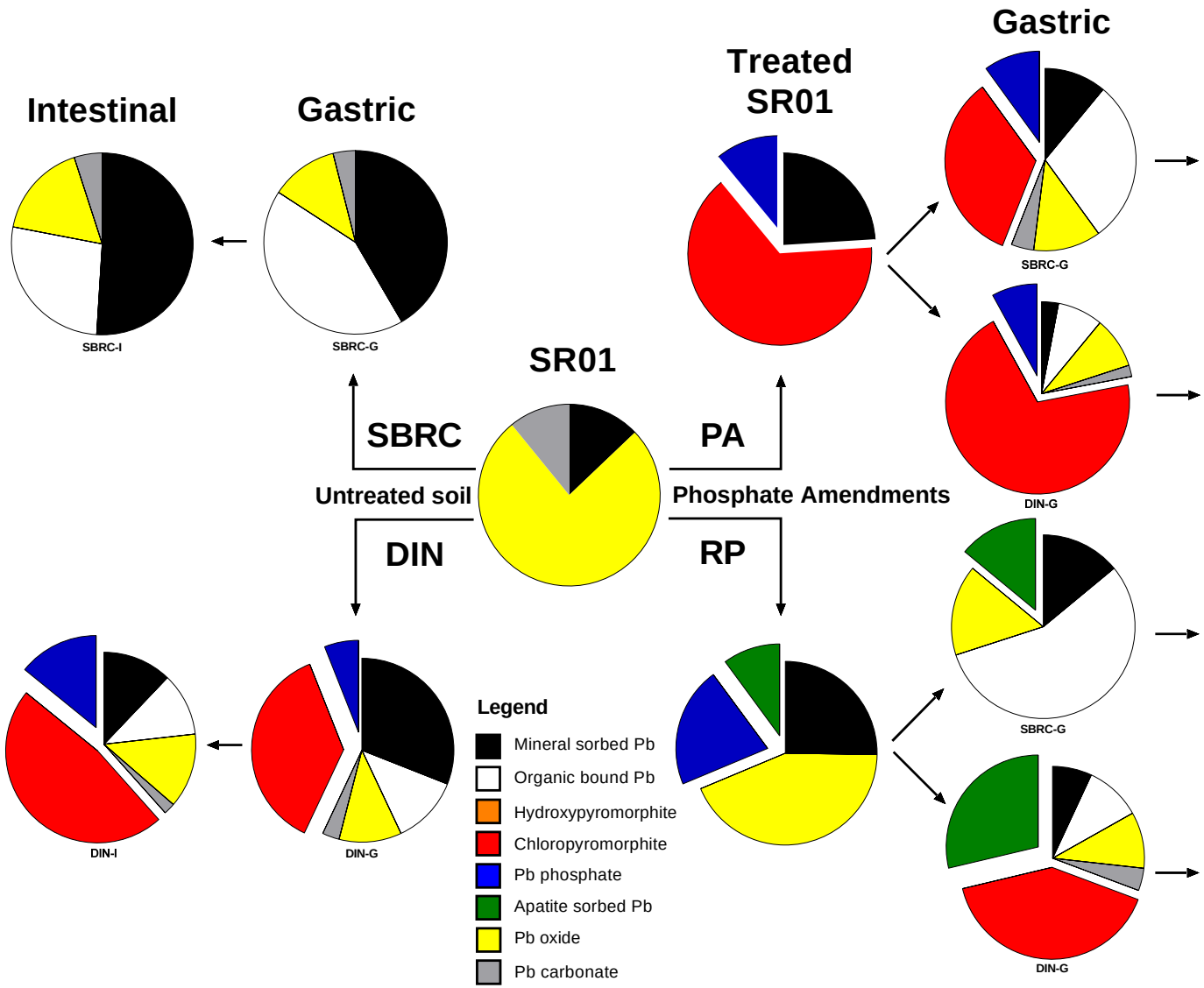
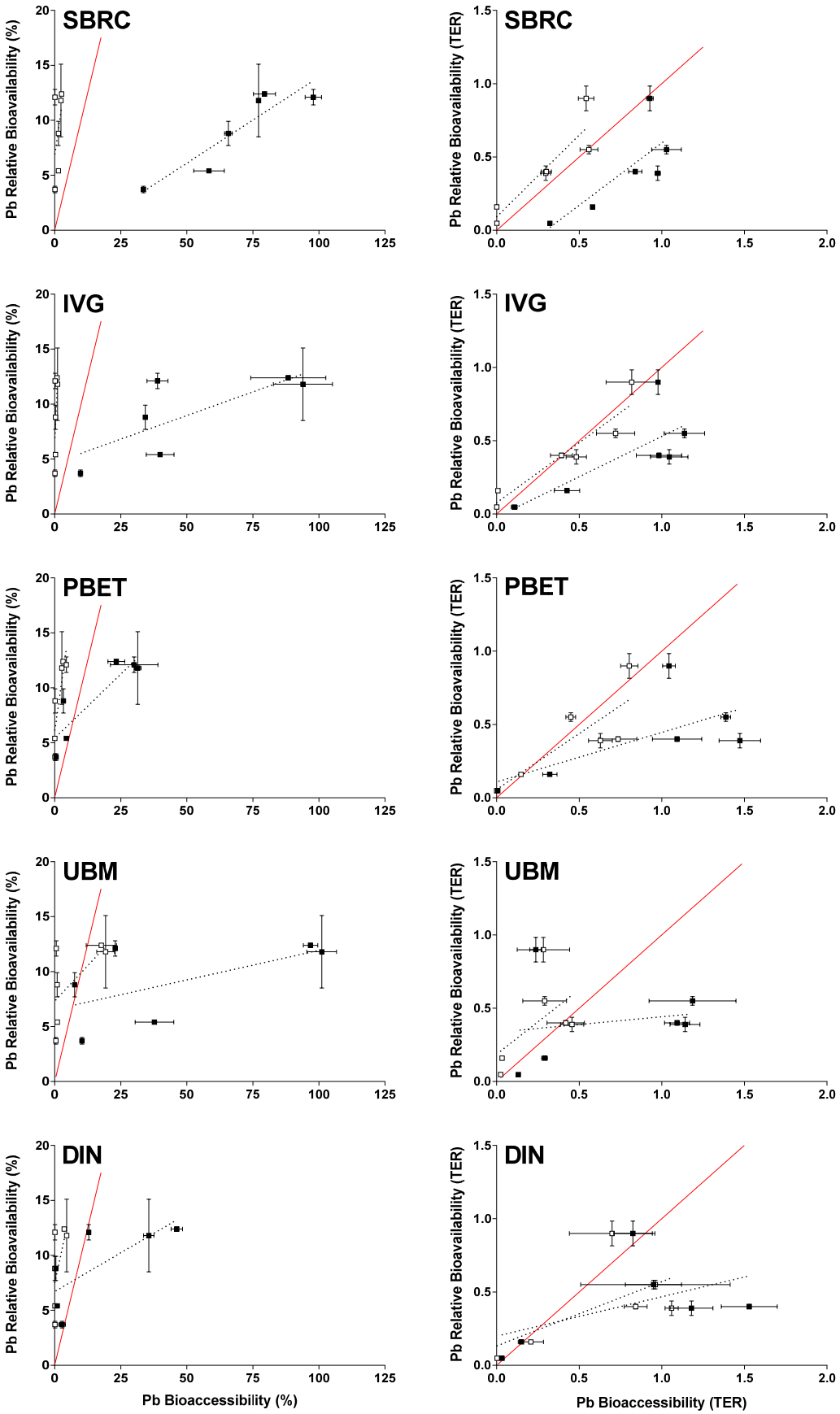
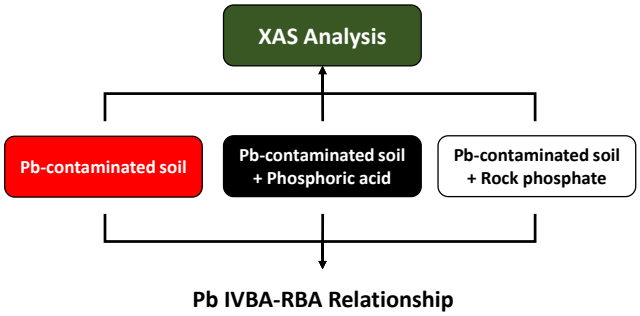

Intestinal
Gastric
SBRC-I
SBRC-G
SR01
SBRC
Untreated soil
DIN
PA
Phosphate Amendments
RP
Treated SR01
Gastric
SBRC-G
DIN-G
SBRC-G
DIN-G
DIN-I
DIN-G
Legend
Mineral sorbed Pb
Organic bound Pb
Hydroxypyromorphite
Chloropyromorphite
Pb phosphate
Apatite sorbed Pb
Pb oxide
Pb carbonate

Figure 5.


SBRC
Pb Relative Bioavailability (%)
Pb Relative Bioavailability (TER)
IVG
PBET
UBM
DIN
Pb Bioaccessibility (%)
Pb Bioaccessibility (TER)

TOC art


XAS Analysis
Pb-contaminated soil
Pb-contaminated soil + Phosphoric acid
Pb-contaminated soil + Rock phosphate
Pb IVBA-RBA Relationship