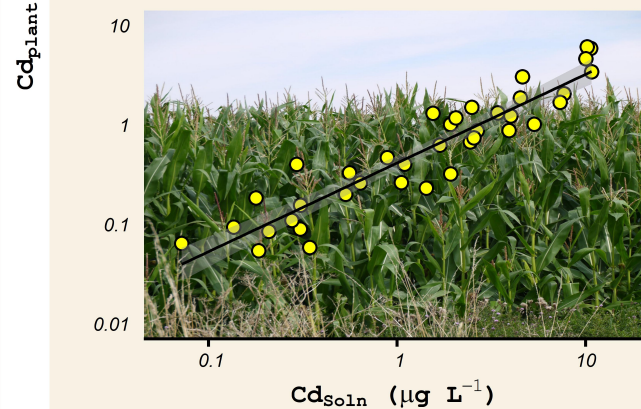# THE CONSEQUENCES OF LONG-TERM APPLICATION OF BIOSOLIDS TO ARABLE SOILS
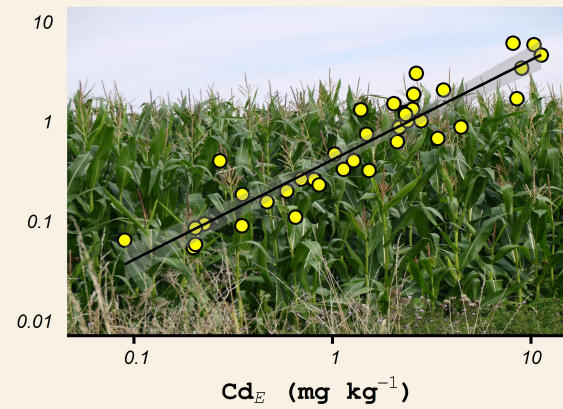

Metal-specific effects of biosolids on soil metal lability

Both *decreasing* lability and *increasing* lability observed
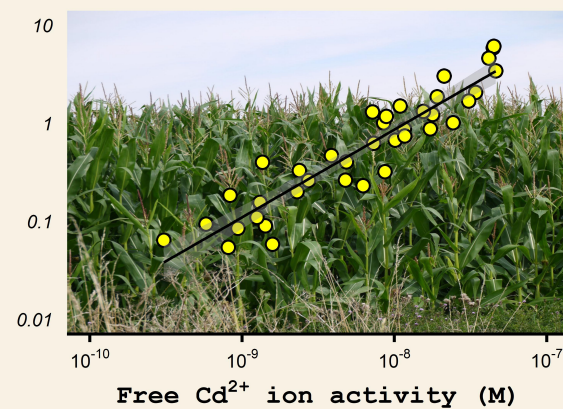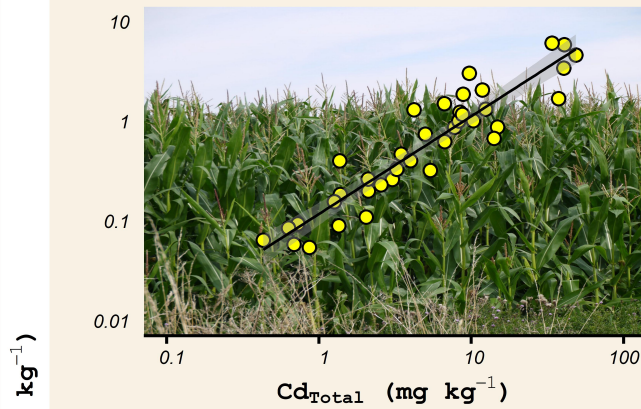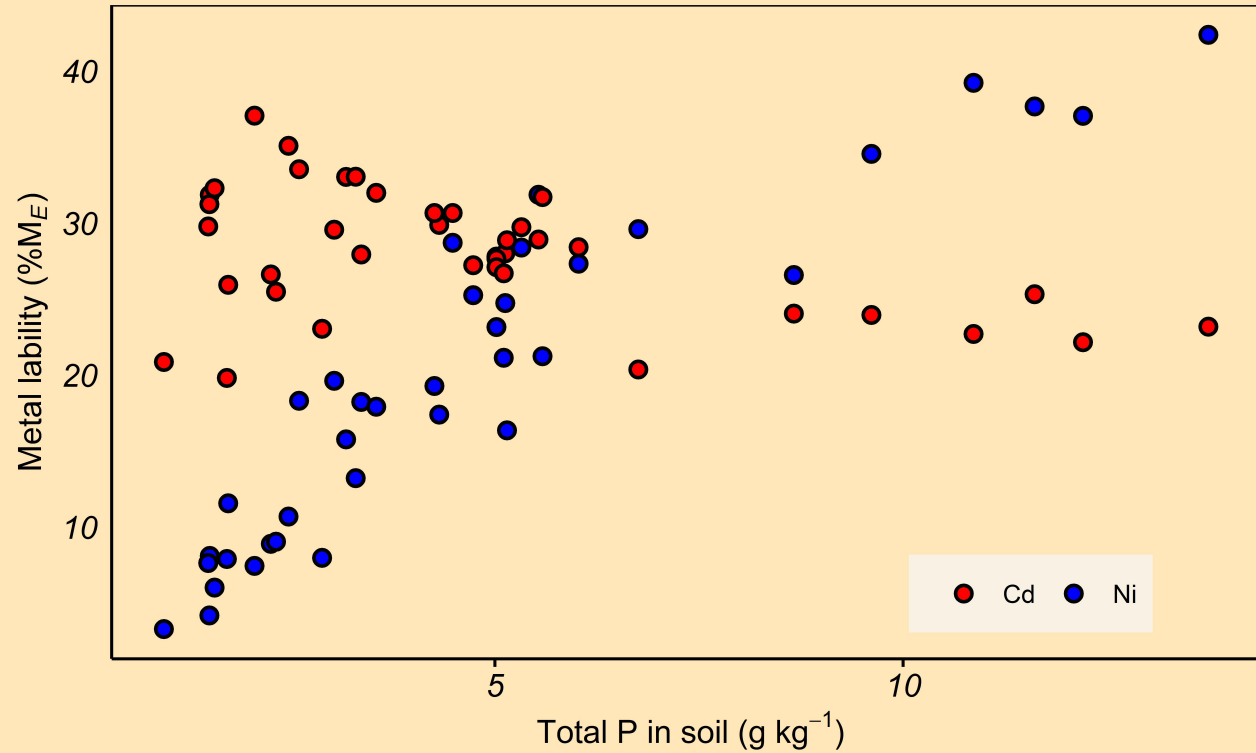
Metal lability (%M_E) vs Total P in soil (g kg^{-1})

Legend: Cd, Ni

Cd_{plant} (mg kg^{-1}) vs Cd_{Total} (mg kg^{-1})

Cd_{plant} (mg kg^{-1}) vs Cd_E (mg kg^{-1})

Cd_{plant} (mg kg^{-1}) vs Cd_{Soln} (µg L^{-1})

Cd_{plant} (mg kg^{-1}) vs Free Cd^{2+} ion activity (M)

The impact of long-term biosolids application (>100 years) on soil metal dynamics

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1 **The impact of long-term biosolids application (>100 years) on soil metal**

2 **dynamics**

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7 **Abstract**

8 Biosolids application to arable land is a common, and cost-effective, practice but the impact of

9 prolonged disposal remains uncertain. We evaluated the dynamics of potentially toxic elements

10 (PTEs) at a long-established 'dedicated' sewage treatment farm. Soil metal concentrations

11 exceeded regulations governing application of biosolids to non-dedicated arable land.

12 However, measurement of isotopic exchangeability of Ni, Cu, Zn, Cd and Pb demonstrated

13 support for the 'protection hypothesis' in which biosolids constituents help immobilise

14 potential toxic metals (PTMs). Metal concentrations in a maize crop were strongly, and almost

15 equally, correlated with all 'capacity-based' and 'intensity-based' estimates of soil metal

16 bioavailability. This was attributable to high correlations between soil factors controlling

17 bioavailability (organic matter, phosphate etc.) on a site receiving a single source of PTMs.

18 Isotopic analysis of the maize crop suggested contributions to foliar Pb from soil dust

19 originating from neighbouring fields. There was also clear evidence of metal-specific effects

20 of biosolids on soil metal lability. With increasing metal concentrations there was both

21 *decreasing* lability of Cd and Pb, due to interaction with increasing phosphate concentrations,

22 and *increasing* lability of Ni, Cu and Zn due to weaker soil binding. Such different responses

23 to prolonged biosolids disposal to arable soil should be considered when setting regulatory

24 limits.

1. Introduction

Application of biosolids to arable soil is currently the preferred disposal method. This is partly because it is cost effective compared with alternative methods: the cost is approximately 30-50% lower than landfilling or incineration (Antille et al., 2017). Moreover, recycling plant nutrients and organic matter make the use of biosolids in agriculture an environmentally favourable option in comparison to other management options (Mamindy-Pajany et al., 2014; Samaras et al., 2008; Wang et al., 2008). However, contamination of sewerage systems with potentially toxic elements (PTEs), such as Cd, Zn, Cu and Pb, diminishes the beneficial impact of biosolids and presents a significant environmental burden (Hernandez-Soriano and Jimenez-Lopez, 2012; Roig et al., 2012; Singh and Agrawal, 2008).

Ample research has indicated that a variety of soil properties alter metal bioavailability (Rosenfeld et al., 2018). Therefore, assessment of metal contamination based solely on total soil metal concentration is a poor indicator of environmental impact (Meers et al., 2007). This is particularly relevant in biosolid-amended soils where contaminants are introduced into soils already in association with adsorptive organic and inorganic phases. This limits the environmental risks of these contaminants and forms the basis of the "protection hypothesis" (Mamindy-Pajany et al., 2014). On the other hand, organic matter mineralization (post application) could result in the release of contaminants into more bioavailable forms over long periods of biosolids application – a concept loosely labelled the "time bomb hypothesis" (Stietiya and Wang, 2011).

Some studies suggest that the free ion activity (FIA) of divalent metal cations in the soil solution is the best indicator of PTE bioavailability (Hooda, 2010; Tye et al., 2003). The FIA will depend on the soil characteristics which control the solid$\rightleftharpoons$solution equilibrium of labile PTEs (Groenenberg et al., 2017). Thus, estimating the labile pool in soil may enable a more

50 accurate assessment of toxicity and risk from PTEs. The 'labile' pool of metal is the fraction

51 of soil metal that forms an equilibrium between the solid and solution phases within a short

52 time scale; see for example Groenenberg et al., (2017). Whilst chemical extractions have been

53 traditionally used to quantify available metal (Kim et al., 2015), it is acknowledged that these

54 extractions are operationally defined and can be poorly selective (Young et al., 2005). Other

55 limitations include re-adsorption processes during extraction or overestimation of lability due

56 to the dissolution of mineral surfaces thereby releasing non-labile metal (Marzouk et al., 2013a;

57 Young et al., 2005). The isotopic dilution technique has been recognised as a more robust and

58 mechanistically based method that reliably quantifies the labile reservoir of metal in soil

59 (Hamon et al., 2008; Stacey et al., 2001). The technique is based on the premise that when a

60 known amount of a tracer isotope of a metal is introduced into a soil suspension, its

61 solid:solution ratio mirrors that of the native reactive metal. Thus, the ratio of the tracer isotope

62 to that of the indigenous isotope in solution can be used to calculate the size of the soil metal

63 labile pool (also termed the E-value or isotopically exchangeable fraction) (Hamon et al., 2008;

64 Stacey et al., 2001).

65 The aim of this study was to assess the consequences of long-term application of biosolids to

66 arable soils. The specific objectives were to determine: (i) solubility of PTEs, (ii) the

67 'reactivity' of soil PTE reservoir and (iii) transfer of PTEs to agricultural crops at a long-

68 established sewage treatment farm in the UK.

69 **2. Materials and methods**

70 **2.1. Location and sample collection.**

71 Soil and plant samples (n = 38) were collected on 7th and 8th of August 2013 from 31 fields of

72 a sewage processing farm (c. 700 ha) in the East Midlands of England that has been in use for

73 over 100 years. At the time of sampling (n = 31 fields), the site was used to grow feedstock

74 (nine varieties of fodder maize; Table S1) for biogas production. The practice is to ensile the

75 maize prior to use for gas production and residues are returned to the arable fields. No produce

76 from the farm is used for human or animal consumption; the facility operates under a U.K.

77 DEFRA (Department for Environment, Food & Rural Affairs) licence as a 'dedicated site'. It

78 is worth noting that the site is a fully operational disposal site with more than 60 individual

79 fields, each of which carries the imprint of urban biosolids disposal, and the facility has been

80 used for more than a century. Therefore, even though there are no audits of biosolids

81 composition or disposal rate, the range of biosolids application across the site is evident from

82 the range of metal concentrations in soil, for example the concentration of Zn ranges from near

83 background concentration (122 mg kg^{-1}) to heavily contaminated (> 2000 mg kg^{-1}). The

84 variation in soil organic matter content, phosphate concentration and metal concentrations

85 undoubtedly represent the historical imprint of past biosolids applications and provide unique

86 evidence for testing the two confounding hypotheses surrounding the use of biosolids in

87 agricultural soils. The invaluable advantage of using the site is its longevity – a feature which

88 is key to examining the long term effects and which provides considerable advantages over

89 short-term (but controlled) trials with biosolids disposal to soil.

90 Four maize plants were cut 3-4 cm above ground level from within one square metre in each

91 field. Four soil samples (0 – 20 cm) were taken within rows inside the same square metre area

92 and mixed into one composite sample. Leaves were removed from the maize stems and both

93 leaves and stems were washed with tap water, followed by deionised water, and then oven-

94 dried at 60 °C for four days. Stems and leaves were ground and combined into one composite

95 sample using a cutting mill (Retsch, Model SM 100) and ultra-centrifugal mill with titanium

96 screen (Retsch, Model ZM 200), prior to analysis. Soil samples were sieved to <4 mm, air-

97 dried and a subsample (c. 20 g) was agate ball-milled (Retsch, Model PM 400) prior to analysis

98 of carbon, free oxides and total elemental concentrations.

2.2.   Plant material digestion and analysis

A portion of finely ground plant material (c. 200 mg) was digested with 6 mL of concentrated *Primar* grade HNO_3 using a Multiwave PRO Anton Paar microwave reaction system, with heating at 140°C for 20 minutes. Elemental analysis was by ICP-MS (Model iCapQ, Thermo Scientific, Bremen). To assess the accuracy and precision of the digestion and analysis, a standard reference material, NIST 1573a *Tomato Leaves* (National Institute of Standards and Technology), and 3 operational blanks, were included in each sample batch. The elemental recoveries for the certified reference material were 120% ± 0.61%, 96.1% ± 5.05%, 99.3% ± 4.50%, and 103% ± 7.21% for Ni, Cu, Zn, and Cd respectively.

2.3.   Soil characterization

Soil pH was determined in a suspension with Milli-Q water (18.2 MΩ cm; 1:2.5 m/v) after shaking for 30 minutes on an end-over-end shaker; the measurement was repeated in 0.01 M $Ca(NO_3)_2$ (1:10 m/v) suspensions. Soil organic matter was estimated from loss on ignition (% LOI): approximately 5 g of oven-dried soil (< 4 mm) was ignited in a muffle furnace at 550 °C for 4 hours. Available P (Olsen-P) in soil was estimated using the bicarbonate extraction method described by Olsen et al., (1954) followed by colorimetric analysis adapted from Murphy and Riley, (1962). Approximately 2.0 g of soil was weighed into 50 mL centrifuge tubes with approximately 0.3 g of low phosphate charcoal and 30 mL of 0.5 M sodium bicarbonate. Samples were shaken end-over-end shaken for 30 minutes before centrifuging at 2500 g for 15 minutes. An aliquot of the extracted supernatant was added to 2 mL 3 M H_2SO_4 to neutralise the $NaHCO_3$, followed by 4 mL of acid molybdate reagent and 4 mL of ascorbic acid as a reducing agent; the final volume was made up to 50 mL with Milli-Q water. The colour was allowed to develop for 20 minutes and absorbance read at 880 nm in a spectrophotometer (Model Ce1011, Cecil Instruments, Cambridge, UK). Samples were run in triplicates. Estimates of total Al, Fe and Mn oxides were determined using the Dithionite–

124 Citrate–Bicarbonate (DCB) extraction method (Anschutz et al., 1998). Finely ground soil

125 (0.250 mg) was shaken with 20 mL DCB reagent at 20 °C and the concentrations of Al, Mn,

126 Fe in solution were determined by ICP-MS after centrifuging and syringe filtration (<0.22 µm).

127 *2.3.1.   Total elemental concentrations in soil (M_{Total})*

128 Approximately 200 mg of finely ground soil was digested in a mixture of HNO_3, $HClO_4$, and

129 HF acids in a heating block digester prior to analysis by ICP-MS. Approximately 200 mg of

130 finely ground soil were digested with 2 mL of HNO_3 (70% trace element grade) and 1 mL of

131 $HClO_4$ (70% analytical grade) at 80 °C for 8 h followed by 2 h of heating at 100 °C. This was

132 followed by addition of 2.5 mL of HF (40% trace element grade) and heating at 120 °C for 8 h

133 to dryness. A further 2.5 mL of HNO_3 and 2.5 mL of Milli-Q water were then added to the

134 dried residue and the vessels were heated at 50 °C for 30 min. After the digestion was complete

135 the final volume was made up to 50 mL using Milli-Q water. The reference material NIST

136 2711a *Montana soil*, (National Institute of Standards and Technology) was used to ensure QA

137 and 10 operational blank digestions were used to estimate limits of detection (LOD). The

138 recoveries for the certified reference material were 86.7% ± 1.72%, 105 ± 1.90%, 102% ±

139 1.38%, 114% ± 2.09%, and 107% ± 2.82% for Ni, Cu, Zn, Cd and Pb respectively.

140 *2.3.2.   Soluble metal concentrations (M_{soln})*

141 Dissolved trace and major metallic elements were determined on soil suspensions in 0.01 M

142 $Ca(NO_3)_2$ (1:10 soil:solution ratio) following 3 days equilibration time on an end-over-end

143 shaker. Following measurement of suspension pH, the soil aqueous phase was isolated by

144 centrifugation (2200 g) and filtration (0.22 µm syringe filters) prior to elemental analysis by

145 ICP-MS and determination of dissolved organic and inorganic carbon using a Shimadzu TOC

146 –Vcp analyser.

147 *2.3.3.   Isotopically exchangeable metal (M_E):*

148   The concentrations of isotopically exchangeable Ni, Cu, Zn, Cd and Pb were determined as

149   described in Mossa et al., (2017). Briefly, soils were suspended in 0.01 M $Ca(NO_3)_2$ (3 g: 30

150   mL) and pre-equilibrated on an end-over-end shaker for 2 days. Suspensions were then spiked

151   with five enriched stable isotopes, $^{62}Ni^{2+}$, $^{65}Cu^{2+}$, $^{70}Zn^{2+}$, $^{108}Cd^{2+}$ and $^{204}Pb^{2+}$, and equilibrated

152   for a further three days. The solution phase was isolated by centrifuging (2200 g) for 15 min

153   and syringe-filtration (<0.22 μm) prior to determination of isotopic ratios ($^{62}Ni/^{60}Ni$, $^{65}Cu/^{63}Cu$,

154    $^{70}Zn/^{66}Zn$, $^{108}Cd/^{111}Cd$, $^{204}Pb/^{206}Pb$, $^{204}Pb/^{207}Pb$, and $^{204}Pb/^{208}Pb$) by Q-ICP-MS (Model iCapQ,

155   Thermo Scientific, Bremen, Germany). To reduce polyatomic interference, the instrument was

156   operated in 'collision cell with kinetic energy discrimination (KED) mode'. To minimize

157   plasma flicker noise, multiple runs (c. 10×100 sweeps) and short dwell times were employed.

158   To minimise mass bias, a correction factor was estimated at a regular interval throughout the

159   analytical session (every 15 samples). The correction factor was defined as the ratio between

160   the true isotopic ratio (expected) and the isotopic ratio in standard reference material NIST

161   SRM-981 (lead wire). The correction factors were used to correct for mass bias and thereby

162   convert intensity (CPS) ratios to true isotope ratios and calculate isotopic abundances of the

163   spike isotopes. Each measurement was replicated twice, and the relative standard deviation was

164   mostly less than 5%. As the relative abundance of Pb isotope varies according to its sources,

165   Two unspiked samples were used determine Pb isotopic ratio instead of relying on the relative

166   abundance of naturally occurring isotopes. The concentrations of isotopically exchangeable

167   Ni, Cu, Zn, Cd, and Pb, (E- values), were calculated from Equation 1 (Gäbler et al., 1999).

168    $$M_E = \left(\frac{M_{soil}}{W}\right)\left(\frac{C_{spike}V_{spike}}{M_{spike}}\right)\frac{(^{Iso1}IA_{Spike} - {}^{Iso2}IA_{Spike}R_{SS})}{(^{Iso2}IA_{Soil}R_{SS} - {}^{Iso1}IA_{Soil})} \qquad\qquad 1$$

169   Where M_{soil} and M_{spike} are the average atomic mass metal in soils and spike solutions

170   respectively, W is the mass of the soil (kg), C_{spike} is the gravimetric concentration (mg L^{-1}) of

171   the metal in the spike solution, V_{spike} is the volume of spike added (L), IA is the isotopic

172   abundance, R_{ss} is the ratio of isotopic abundances for the two isotopes in the spiked soil

173   solution. The same experimental procedure was repeated for all soils using 10^{-5} M Na_2EDTA

174   as the suspending solution instead of 0.01 M $Ca(NO_3)_2$. This was in response to the extremely

175   low solubility of Pb in the soils (Atkinson et al., 2011); the Pb data (Pb_E) presented include

176   only *E*-values obtained using EDTA as the soil suspension matrix.

177   *2.3.4.   Soil solution speciation*

178   The geochemical model, WHAM VII (Tipping, 1994), was used to speciate Ni, Cu, Zn, Cd and

179   Pb in the solution phase of the 0.01 M $Ca(NO_3)_2$ soil suspension that was used to determine *E*-

180   values. Inputs to the model included cation and anion concentrations and pH in the $Ca(NO_3)_2$

181   suspension. The dissolved organic carbon (DOC) was converted to fulvic acid (FA)

182   concentration by assuming (i) a carbon content of 50% and (ii) that FA constituted 65% of

183   DOC (Buekers et al., 2008; Lofts et al., 2008; Marzouk et al., 2013b). Partial pressure of CO_2

184   (P_{CO2}) was set to 0.004 atm and the temperature was set to 25 °C.

185   *2.3.5.   Lead isotopic composition analysis in soil and plant samples*

186   Abundances of the Pb isotopes ^{204}Pb, ^{206}Pb, ^{207}Pb and ^{208}Pb were determined in soil and plant

187   acid digests by ICP-MS. Instrument parameters were 6000 quadrupole sweeps with a dwell

188   time of 10 ms and a dead time correction factor of 34.7 ns. To ensure that the detector remained

189   within the range of pulse-counting mode, all soil digests were diluted to a uniform Pb

190   concentration of 10 µg L^{-1} with Milli-Q water. This was not possible for plant digests, which

191   were simply diluted to a HNO_3 concentration of 3% using Milli-Q water. Isobaric interference

192   from ^{204}Hg was corrected by measuring ^{202}Hg. Thallium (^{203}Tl, ^{205}Tl) was used as an internal

193   mass bias correction (MBC) and NIST-981 was then used to refine the MBC, as described by

194   Usman et al. (Usman et al., 2018). The NIST-981 standard was run after every 10 samples.

195   **2.4.   Data analysis**

196 Multiple regression analysis was utilised to explore the effect of soil properties on metal

197 solubility and lability. All variables, apart from pH, included in the multiple regression were

198 tested for normality and log-transformed when required. All statistical analysis were performed

199 using R (R Core Team, 2018).

3. Results and discussion

3.1. General characterization of the site

202 General characteristics of soils used in the study are given in Table S2. Soil pH_{Ca}, pH measured

203 in the solution phase of the 0.01 M $Ca(NO_3)_2$ suspensions, showed near-neutral values with

204 very little variation across the soil dataset (5.95-6.94). Loss on ignition (LOI), covered a broad

205 range 3.52-23.4% (Table 1). As expected in biosolids-amended soils, total P concentrations

206 were positively correlated with LOI (r = 0.98; p<0.001) and varied widely from 0.945 to 13.7

207 g kg^{-1}. The concentration of total free oxides in soil (DCB extraction) were variable, with

208 ranges of 1.49-7.29 g kg^{-1}, 0.24-2.82 g kg^{-1}, and 10.9-36.3 mg kg^{-1} for Al, Mn and Fe

209 respectively. DOC concentration, in the solution phase of 0.01 M $Ca(NO_3)_2$ suspensions, varied

210 between 3.10 – 40.9 mg L^{-1}, reflecting different histories of biosolids application.

3.2. Enrichment factors

212 An enrichment factor (EF) was used to reflect the status and the degree of pollution in soil.

213 Values of EF were determined after normalizing against Al following Eq. 2 (Bing et al., 2016;

214 Izquierdo et al., 2013):

215 $$EF = \frac{(\frac{Me}{Al})sample}{(\frac{Me}{Al})background} \qquad (2)$$

216 Where $(Me/Al)_{sample}$ is the concentration (mg kg^{-1}) of an element relative to Al concentration

217 normalised to a similar ratio with the local background ratio $(Me/Al)_{background}$. Background

218 concentrations used for comparison were median values for topsoils derived from the Triassic

219 Mercian Mudstone of the UK (British Geological Survey, 2006; Izquierdo et al., 2013).

220 Sutherland. (2000) identified five contamination categories based on the enrichment factor:

221 EF<2 depletion to minimal enrichment, EF=2-5: moderate enrichment, EF=5-20: significant

222 enrichment, EF=20-40: very high enrichment, EF>40 extremely high enrichment. The majority

223 of elements had a mean EF greater than 5 reflecting significant enrichment (Fig S1).

224 **3.3.   Total metal concentrations in soils**

225 Total metal concentrations in soil (M_{Total}) were strongly correlated with LOI (r = 0.93–0.98, *p*

226 < 0.001, Table S3), suggesting a common source of contamination, consistent across all fields.

227 Samples showed a wide range of M_{Total}, with Zn being the most abundant metal, with

228 concentration spanning over one order of magnitude (122–2050 mg kg^{-1}); Cu ranged from 25.3

229 to 766 mg kg^{-1}, Cd from 0.43 to 48.6 mg kg^{-1}, Cr from 43.2 to 1670 mg kg^{-1}, and Pb from 68.6

230 to 688 mg kg^{-1} (Fig. 1 and Table 1). The large variation in total soil metal concentration reflects

231 variable biosolids application rates throughout the sampling area and the length of time that

232 each field has been used for biosolids disposal. Values of M_{Total} in most fields exceeded the

233 maximum permissible concentrations of metals in normal arable soils permitted by current

234 regulations (The Sludge Use in Agriculture Regulations, 1989). However, it should be noted

235 that the site is operated under licence from the Department of Environment Food & Rural

236 Affairs (DEFRA, U.K) and does not produce crops for human or animal consumption.

237 Figure 2 shows ratios between Zn and several metals (Cr, Cu, Rb, and Cd) in soils as a function

238 of total soil P concentration (P_{Total}), which can be used as a reasonable proxy for biosolids

239 loading. Rubidium was selected as a fingerprint for the presence of clays and therefore as an

240 indication of native soil. Rubidium was used as a proxy for clay minerals based on (i) the fact

241 that it is a common constituent of clay minerals (Kabata-Pendias and Pendias, 2001) and (ii)

242 the assumption that the parent material is similar across the site. This is evidenced by the strong

243 correlation between Rb and Al (r = 0.84) and Cs (r = 0.88) in these soils. Also biosolids are an

244 unlikely source of Rb, as indicated by the weakly negative correlation between LOI and Rb

245 concentration in soil (r = - 0.37). Biosolids composition, leaching, and plant uptake are the

246 main factors that most likely control the ratios between different metals in biosolids-amended

247 soils. An attempt was made to estimate the annual loss through leaching and offtake by crop

248 plants (Table 2). For metal loss through leaching, it was assumed that half of the annual rainfall

249 (c. 600 mm) was leached below the surface soil (20 cm). Metal concentration measured in 0.01

250 M $Ca(NO_3)_2$, M_{Soln}, was used as a proxy of metal concentration in the soil solution. Metal loss

251 through crop harvest was based on metal concentration in the maize crop (above-ground

252 biomass) assuming an annual yield of 10 t ha^{-1}.

253 Notwithstanding the very crude assumptions underpinning the estimates in Table 2 it seems

254 reasonable to conclude that metal losses through plant uptake and leaching are probably

255 negligible and metals added with the sludge are largely conserved. Thus the relationships

256 shown in Fig. 2 suggest that metal ratios in soil are mainly the result of differences in the ratios

257 of the two end members – the native soil and the biosolids. The positive correlation between

258 Zn:Rb ratio and P_{Total} (Fig. 2A) indicates that biosolids application caused (i) increased Zn

259 contents in soil and (ii) a dilution effect of *native* soil through increasing organic matter content,

260 thus shifting Zn:Rb to greater values. This is evidenced by a range of 0.76 – 1.55 EFs for Rb,

261 indicating depletion to minimal enrichment. By contrast, the trends in Fig. 2(B-D) show that

262 the ratios decrease towards an asymptote reflecting the composition of the applied sludge

263 integrated over time.

264 **3.4.   Changes in metal lability with sludge loading**

265 The *E*-values (M_E) of the studied metals spanned over two orders of magnitude (Table 3). M_{Total}

266 was strongly correlated with M_E ($r \geq 0.99$ for Ni, Cu, Zn, and Cd; r = 0.72 for Pb). This is

267 probably the result of co-variance of the soil properties that are likely to affect metal lability

268 (e.g. soil organic matter, pH) with biosolid application, coupled with the restricted range of pH

269   across the site (Table 1). The ranges of metal lability ($\%M_E$) are shown in Table 3. Copper was

270   the most labile metal and the lability decreased in the order $Cu > Cd > Zn \approx Ni > Pb$. With the

271   exception of Ni, this order is in accordance with the findings of Garforth et al., (2016) who

272   determined E-values for a single soil from the same site. In the present study $\%Ni_E$ exhibited a

273   large variation (from 3.30 to 42.4 %); Pb was the least labile metal with an average $\%Pb_E$ of

274   5.3 (Table 3).

275   Figure 3 shows metal lability ($\%M_E$) as a function of P_{Total}. Nickel, Cu, and Cd largely exhibited

276   a single trend in which $\%M_E$ increased with P_{Total} (i.e. metal loading from biosolids). Given the

277   strong correlation between M_{Total} and P_{Total} (Table S3), and M_{Total} and M_E, a clear trend in $\%M_E$

278   with P_{Total} was not anticipated. However, Figs 3A-3C indicate that the interaction of metals

279   with sludge-amended soil have a strong effect on metal lability; in the case of Ni, Cu, and Zn

280   lability ($\%M_E$) increased with metal loading. This may be due to progressive occupancy of

281   weaker binding sites on soil humus and metal oxides as metal concentration increases. By

282   contrast, there was a clear decreasing trend with metal loading in the case of $\%Cd_E$ and $\%Pb_E$.

283   This is likely due to the high content of P on the site (up to 13,700 mg kg^{-1}); correlations (r

284   values) between P_{Total} and $\%Cd_E$ and $\%Pb_E$ were -0.45 ($P = 0.005$) and -0.47 ($P = 0.003$) for

285   Cd and Pb respectively. Large soil P concentrations may promote the precipitation of Cd and

286   Pb as insoluble P compounds in soil (Atkinson et al., 2011; Fang et al., 2016; Garforth et al.,

287   2016; McBride, 2016; Paltseva et al., 2018). It is worth noting, however, particularly in the

288   case of $\%Cd_E$, that there was an initial increase in metal lability with P_{Total}, followed by a

289   downward trend. This may indicate that initially biosolids application has mobilising effects

290   through increased organic matter, dissolved organic matter and the progressive occupancy of

291   weaker metal adsorption sites with metal loading. This trend is then reversed when the

292   immobilizing effect of phosphate becomes the predominant factor, for Cd and Pb.

293   **3.5.   Solubility of metals**

294   Soluble metal concentrations (M_{Soln}) in soil were estimated by equilibration with 0.01 M

295    $Ca(NO_3)_2$; results are reported as supplementary material (Table S4). Values of M_{Soln} were less

296   than 1% of M_{Total} for most elements; mean values were 0.78%, 0.25%, 0.13%, 0.31% and

297   0.001% for Ni, Cu, Zn, Cd, and Pb respectively. Distribution coefficients (K_d) were calculated

298   for each element as the ratio between the *adsorbed* isotopically exchangeable form ($M_E - M_{Soln}$;

299   mg kg^{-1}) and the free ion activity (M^{2+}; mg L^{-1}) in soil solution, determined from M_{Soln} in 0.01

300   M $Ca(NO_3)_2$ following speciation using the geochemical model WHAM VII. Values of

301    $Log_{10}(K_d)$ for Ni, Zn and Cd were strongly correlated ($r = 0.79$, 0.93, and 0.79 respectively)

302   with soil pH, while no distinctive trend was observed for Cu and Pb ($r = 0.43$ and 0.44

303   respectively).

304   To relate metal partitioning between soil solid and solution phases to the variations in soil

305   properties, multiple regression analysis was used. The regression models were based on Sauvé

306   et al., (2000) where M_{Total}, LOI and soil pH were considered as major explanatory variables

307   controlling K_d. Concentrations of total soil metal (M_{Total}) were excluded from the regression

308   analysis because of the strong correlation between M_{Total}, M_E, and LOI indicating that they

309   provide redundant information which was confirmed by high variance inflation factors (VIF >

310   10). The results of the regression models are reported in Table 4. Most of the variation in K_d

311   for Ni, Zn, and Cd was described by the model (Table 4). However, only 25% and 22% of the

312   variation in K_d were explained for Cu and Pb, respectively, indicating that other soil factors

313   control the partitioning of these two metals. As expected, pH had positive coefficients in the

314   regression models reflecting the dual role of pH in controlling metal solubility through

315   increasing proton competition for binding sites and decreasing pH-dependent surface charges

316   with decreasing pH (Sauvé et al., 2003; Shaheen et al., 2013). Most of the variation in K_d for

317   Ni, Zn, and Cd was explained by soil pH only in the regression equations (Table 4).

318   Surprisingly, the variation in K_d explained by LOI was minor but this may have arisen because

319   LOI was negatively correlated with pH which may mask any independent effect of LOI on K_d.

320   Inclusion of other soil properties in the regression model was also explored. Only total Mn

321   concentration in soil was significant in the case of Ni, Zn, Cd, and Pb; including it in the

322   regression model increased the variation in K_d values explained to 65%, 92%, 85%, and 57%

323   for Ni, Zn, Cd, and Pb respectively.

324   *3.5.1.   Metal speciation in solution.*

325   Metal speciation in the solution phase of the 0.01 M $Ca(NO_3)_2$ suspensions was calculated

326   using WHAM VII. Cadmium, Ni, and Zn were largely (>91% on average) present as free ionic

327   species, with minor proportions (<10%) bound to DOC. Similarly, Ivezić et al., (2012) reported

328   that only 9% and 7% of Cd and Zn, respectively, were associated with FA in the soil solution

329   and Zhang et al., (2015) found that including DOC as a factor did not improve the prediction

330   of dissolved Ni using WHAM. By contrast, on average, 41% and 58% of dissolved Pb and Cu

331   were complexed with DOC, respectively, reflecting their high affinity for soil organic matter.

332   Again, this in agreement with Ivezić et al., (2012) who reported that 66% of Cu and Pb were

333   bound to FA in the soil solution.

334   It has been well established that metal 'intensity', rather than 'quantity', is the most relevant

335   representation of metal bioavailability in assessing the ecological consequence of metals in soil

336   (Golui et al., 2020; Hamels et al., 2014; McBride and Cai, 2015; Mossa et al., 2020). Using

337   total metal concentration in soil overestimates the 'quantity' of available metal as only a portion

338   of that is reactive. It is therefore important to use the reactive (labile) portion of soil metal as

339   input for WHAM in order to estimate the free ion activity. Thus it is evident from Fig. 4 that

340   using total metal concentrations as input to WHAM largely overestimated the free ion activities

341   determined from solution data alone. Conversely, using the reactive metal concentrations (E-

342 values) substantially improved the prediction of the free ion activity, particularly for Ni and

343 Zn. The only exception was for Pb where no reasonable predictions were obtained irrespective

344 of which pool was used as input. This is consistent with the findings of several other authors

345 who reported poor predictions of Pb using WHAM (Bonten et al., 2008; Marzouk et al., 2013b;

346 Rennert et al., 2017). The poor prediction of the free ion activity of Pb could be due to the

347 formation of ternary complexes and poorly insoluble compounds with P (Marzouk et al.,

348 2013b). It is clear from Fig. 4 that reasonable prediction can be obtained using only E-values

349 and selected characteristics of the solid phase in soil.

350 **3.6.   Lead isotope ratios**

351 To assess the contribution of Pb sources to plant uptake, Pb isotope ratios in soil and the maize

352 crop were determined. Two dominant Pb sources in the UK were used as end members in a

353 simple binary mixing model: (i) Pb from leaded petrol with isotope ratios $^{206}Pb/^{207}Pb$ and

354 $^{208}Pb/^{207}Pb$ estimated as 1.067 ± 0.007 and 2.340 ± 0.011 respectively (Sugden et al., 1993);

355 and (ii) Pb ore from the Southern Pennine ore-field (U.K.) with values of $^{206}Pb/^{207}Pb$ and

356 $^{208}Pb/^{207}Pb$ of 1.182 ± 0.004 and 2.458 ± 0.002 respectively (Rohl, 1996). The relative

357 contribution of petrol-derived Pb to the total soil and plant Pb concentrations ($\%Pb_{Petrol}$) was

358 estimated using Eq. 3 (Farmer et al., 2005).

359 $$\%Pb_{Petrol} = 100 \left(\frac{IR_{Ore} - IR_{Sample}}{IR_{Ore} - IR_{Petrol}} \right) \qquad (3)$$

360 The IR indicates the isotopic ratio ($^{206}Pb/^{207}Pb$) and subscripts indicate the medium (Ore,

361 Petrol, Sample). Soil and plant samples had a range of isotope ratios extending between the

362 two end members (Pennine Pb and petrol) (Fig. 5A). Soils with low Pb concentrations (i.e. low

363 biosolids inputs) showed a greater influence from local ore or coal, whereas the contribution

364 from petrol-derived Pb was more apparent in the soils with high Pb content (Fig. 5B and C).

365 The slope in Fig. 5C suggests that petrol-derived Pb constitutes about 52% of the Pb in

366   biosolids on a time-averaged basis. The binary mixing model demonstrates a range of Pb

367   sources in the maize crop; however, crucially, the plant samples showed a narrower range of

368   Pb isotope ratios than the soil samples (Fig. 5A). Figure 5D compares petrol-derived Pb in soil

369   with that in the maize crop. At high levels of petrol-derived Pb in soil, the isotopic signature of

370   Pb in soil is similar to that in plant samples. However, the ratios are increasingly divergent at

371   low proportions of petrol-derived Pb (i.e. high geogenic source). Two trends in Fig. 5D might

372   suggest that the petrol-derived Pb is more accessible to plants than geogenic Pb, possibly due

373   to differences in contact time. This would be consistent with Izquierdo et al., (2012) who found

374   that petrol-Pb was enriched in the bioavailable pools of Pb for alluvial soils within the same

375   catchment as the current study. Alternatively, it is likely that most of the Pb measured in plant

376   samples was the result of physical contamination from soil dust (despite sample washing).

377   Thus, a small amount of dust from a neighbouring field with a large soil Pb concentration, and

378   therefore a higher proportion of petrol-derived Pb, will significantly affect the isotopic

379   signature of plants growing on a soil with a low Pb concentration. The converse of course is

380   not true – dust from a soil with low Pb concentration (i.e. mainly geogenic sources) will have

381   a negligible effect on the isotopic signature of plants growing on a soil with a high Pb

382   concentration. The net result is that, inevitably, the range of isotopic signatures in the plant

383   samples is narrower than in the soils and shifted towards petrol-derived Pb (Fig. 5A).

384   **3.7.   Plant uptake of metals**

385   Plant uptake of metals (Ni, Cu, Zn, Cd, Pb) was evaluated using different estimates of metal

386   bioavailability in soil: M_{Total}, M_E, M_{Soln} and (M^{2+}) (Fig. 6 and Figs S2 – S5 in the supplementary

387   material). All correlations were significant ($P < 0.01$) for the five trace metals and the four

388   estimates of metal bioavailability explained more than 70% of the variation in Ni, Cu, Zn and

389   Cd concentrations in the maize crop. For Pb_{Plant} (Fig. S5), only a maximum of 34% of the

390   variation in Pb_{Plant} concentration could be explained by Pb concentration in soil – probably due

391 to surface contamination of plant samples by soil dust and very limited systemic uptake of Pb

392 by the maize plants (Degryse et al., 2009; Nolan et al., 2005; Nordløkken et al., 2015). The

393 latter route is probably further limited by low Pb solubility (McBride et al., 2014; Schreck et

394 al., 2012), as is the case of the sludge-amended soils, due to high phosphate concentrations.

395 In general, soil total metal concentration is of little use of assessing metal bioavailability and

396 plant uptake (Ciadamidaro et al., 2017; McBride et al., 2009) whereas concentration or activity

397 in the soil solution, regarded as an '*intensity*' factor, provide the best predictors for plant uptake

398 and toxicity (Black et al., 2011; Zhang et al., 2015). However, in the present study, as seen in

399 Fig. 6 (and Fig S2-S5), there was hardly any difference amongst the contrasting estimates of

400 soil metal availability. This lack of discrimination is likely to arise where a single soil type is

401 in receipt of variable amounts of a single contaminant source and is subject to a fairly uniform

402 management regime. Thus, it is reasonable to assume that all the factors that are likely to affect

403 metal availability in soils receiving biosolids (pH, organic matter concentration, phosphate

404 concentration, metal loading) will co-vary with sludge loading (Table S3). This coupled with

405 the narrow pH range (6 - 6.9) controlled by management may explain the similar ability of soil

406 *capacity variables* (i.e. metal reservoir; M_{soil}, M_E) and *intensity variables* (M_{soln}, M^{2+}) to predict

407 M_{Plant}.

4. Conclusions

409 The consequences of long-term application of biosolids to arable soils on metal dynamics were

410 investigated. The study was based on a site that had been utilised for biosolids disposal to arable

411 land for well over a century. Several important conclusions emerged.

412 o Despite the large concentration of metals, low levels of lability (%) were observed for

413   some metals (19.9 ± 10.7, 36.0 ± 5.16, 21.5 ± 7.59, 28.0 ± 4.21, 5.19 ± 2.56 for Ni, Cu,

414   Zn, Cd, and Pb respectively). These observations support the 'protection hypotheses'

415   regarding biosolids application to soil in which constituents of biosolids, such as organic

416   matter and phosphate, accumulate in the soil concurrently with metals and thereby reduce

417   metal reactivity. Thus, the potential risks arising from biosolids application for over 100

418   years may be low.

419   o   Two distinct trends of metal lability with P concentration in soil were observed. The %

420   lability of Cd and Pb declined as soil P concentration increased suggesting a direct

421   interaction. By contrast the lability of Ni, Zn, and Cu increased with soil P. This may be

422   due to the strong correlation between P and total metal content and progressive occupancy

423   of weaker binding sites on soil humus and metal oxides as metal concentration increases.

424   Thus, there is a case for metal specific behaviour to be considered when devising

425   regulatory limits governing the use of biosolids in agriculture.

426   o   Average mole ratios of Zn to Ni, Cu, Cd and Pb in soil were 4.4, 2.4, 69, and 8.4, reflecting

427   disposal through household sewage and industrial wastewater from the city of Nottingham.

428   o   Analysis of the Pb isotopic composition in the maize crop revealed a greater proportion of

429   petrol-derived Pb, compared to the underlying topsoil, at low crop Pb concentrations. This

430   may indicate the influence of soil dust originating from more highly contaminated nearby

431   fields. This was consistent with a relatively poor relationship between soil and plant Pb

432   concentrations compared to the other metals studied.

433   o   The European Parliament (Directive 2002/32/EC, 2002) set limits of As, Cd, Hg and Pb in

434   animal feed of 2, 1, 0.1, and 30 mg kg^{-1} respectively. Cadmium exceeded these limits in

435   15 fields, with mean and maximum Cd concentrations of 1.2 and 6.1 mg kg^{-1} respectively.

436   However, the maximum concentrations of As, Hg, and Pb were 0.35, 0.06, and 0.335

437   respectively - considerably below the EU limits of 2, 0.1, and 30 respectively despite high

438   levels of enrichment in the soils on site.

439 o High correlation between all measures of metal availability in soil in addition to the limited

440 soil pH range obscured any advantage in using soluble or labile metal concentration over

441 total soil metal concentration in predicting metal bioavailability, determined as

442 concentration in a fodder maize crop.

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Table captions

Table 1. Soil organic matter (LOI), pH and elemental concentrations (mg kg^{-1}) in soil

Table 2. Estimates of average (n = 38) annual metal loss through leaching and offtake by crop. Numbers between brackets denote standard deviations

Table 3. E-values (mg kg^{-1}) and 'lability' (E-value as a % of M$_{Total}$)

Table 4. Coefficients of the multiple regression equation used for predicting distribution coefficient K$_d$. Superscripts 'NS' indicate that the coefficient was not significant ($P < 0.05$).

Table 1. Soil organic matter (LOI), pH and elemental concentration (mg kg^{-1}) in soil

	Mean	Minimum	Maximum	Std. deviation
pH	6.38	5.95	6.94	0.35
LOI (%)	10.2	3.52	23.4	4.96
(mg kg^{-1})				
P	4750	945	13700	3230
Ni	114	24.4	415	107
Cu	198	25.3	766	211
Zn	565	122	2050	532
Cd	9.89	0.43	48.6	12.7
Pb	246	68.6	688	194

Table 2. Estimates of average (n = 38) annual metal loss through leaching and offtake by plant. Numbers between brackets denote standard deviations

LOSS (%)	Cr	Cu	Zn	Cd	Rb
Plant offtake	0.00156 (± 0.00444)	0.0221 (± 0.0144)	0.0727 (± 0.0278)	0.0570 (± 0.0318)	0.0406 (± 0.0332)
Leaching	0.00159 (± 0.000686)	0.0343 (± 0.00858)	0.0178 (± 0.0142)	0.0424 (± 0.0184)	0.00464 (± 0.00416)

Table 3. E-values (mg kg^{-1}) and 'lability' (E-value as a % of M_{Total})

	E-values (mg kg^{-1})				Lability (%M_E)			
	Mean	Median	Min	Max	Mean	Median	Min	Max
Ni	32.1	17.3	1.1	176	19.9	18.8	3.30	42.4
Cu	76.5	45.3	5.8	304	36.0	37.0	22.9	44.2
Zn	149	81.1	12.0	679	21.5	22.1	7.6	33.2
Cd	2.5	1.5	0.1	11.3	28.0	28.3	19.7	37.3
Pb	10.9	9.4	1.3	36.4	5.30	4.80	1.50	12.6

Table 4. Coefficients of the multiple regression equation used for predicting distribution coefficient K_d. Superscripts 'NS' indicate that the coefficient was not significant ($P < 0.05$)

	Regression equation				Variance explained (%)		
	Intercept	pH	LOI	R^2	pH	LOI	Residual
Ni	-1.53^{NS}	0.70	-0.09^{NS}	0.62	61.7	0.38	37.9
Cu	-1.24^{NS}	0.89	0.67^{NS}	0.25	18.4	7.12	74.5
Zn	-4.73	12.8	0.27	0.88	86.5	1.84	11.5
Cd	-1.36	0.67	0.4	0.76	62.2	13.5	24.3
Pb	3.57	0.43	-0.30	0.22	19.2	2.57	78.2

Figure captions

Figure 1. Box and whisker diagram of total elemental concentrations in topsoil from 38 arable field locations at a dedicated sewage sludge processing facility.

Figure 2. Ratios of Zn to Rb, Cr, Cu and Cd as a function of total P concentration in soil.

Figure 3. Variation in $\%M_E$ with P_{Total} for Ni, Cu, Zn, Cd and Pb.

Figure 4. Comparison between free ion activities calculated by WHAM using only solution data (X-axis) and the free ion activities (Y-axis) calculated using either total metal concentration in soil (diamond; ◆) or E-values (circle; ●) as WHAM inputs. The solid line represents the 1:1 relationship.

Figure 5. (A) Lead isotope signatures in soil and plant samples. Petrol-Pb and geogenic-Pb signatures are shown as end members; the dashed line is the mixing line between them; (B) $^{206}Pb/^{207}Pb$ plotted against soil total metal content; (C) the relationship between petrol-derived Pb and Pb_{Total}; (D) $\%Pb_{Petrol}$ in soil and the maize crop. Symbol size in B and D is proportional to total Pb concentration in soil.

Figure 6. Relationships between Cd concentrations in the maize crop and soil. Potential drivers for Cd uptake included (A) Cd_{Total}, (B) Cd_E, (C) Cd_{Soln}, (D) Cd^{2+} ion activity. The solid lines represent a linear regression fit; the grey shaded areas represent 95% confidence interval around fitted values.
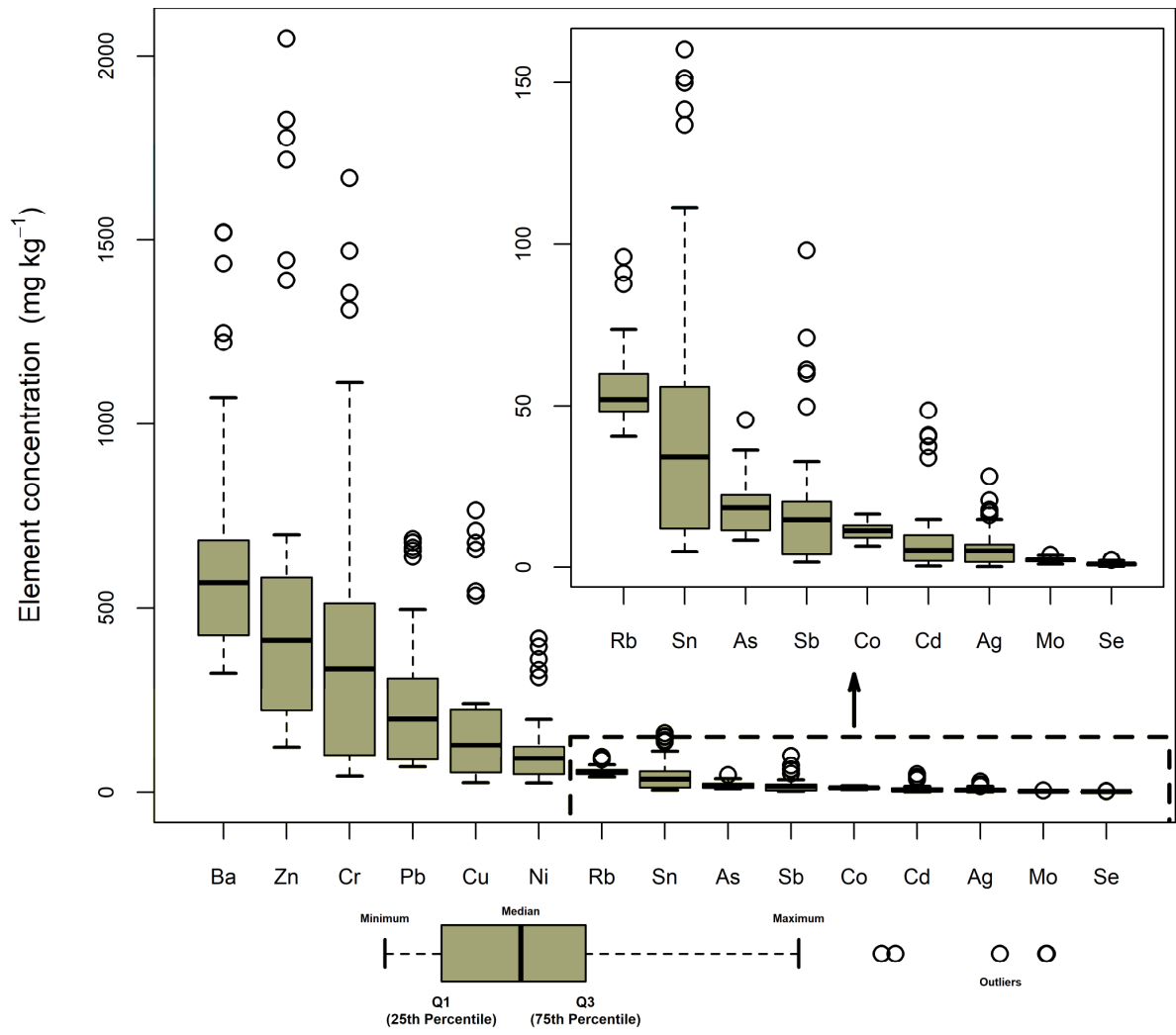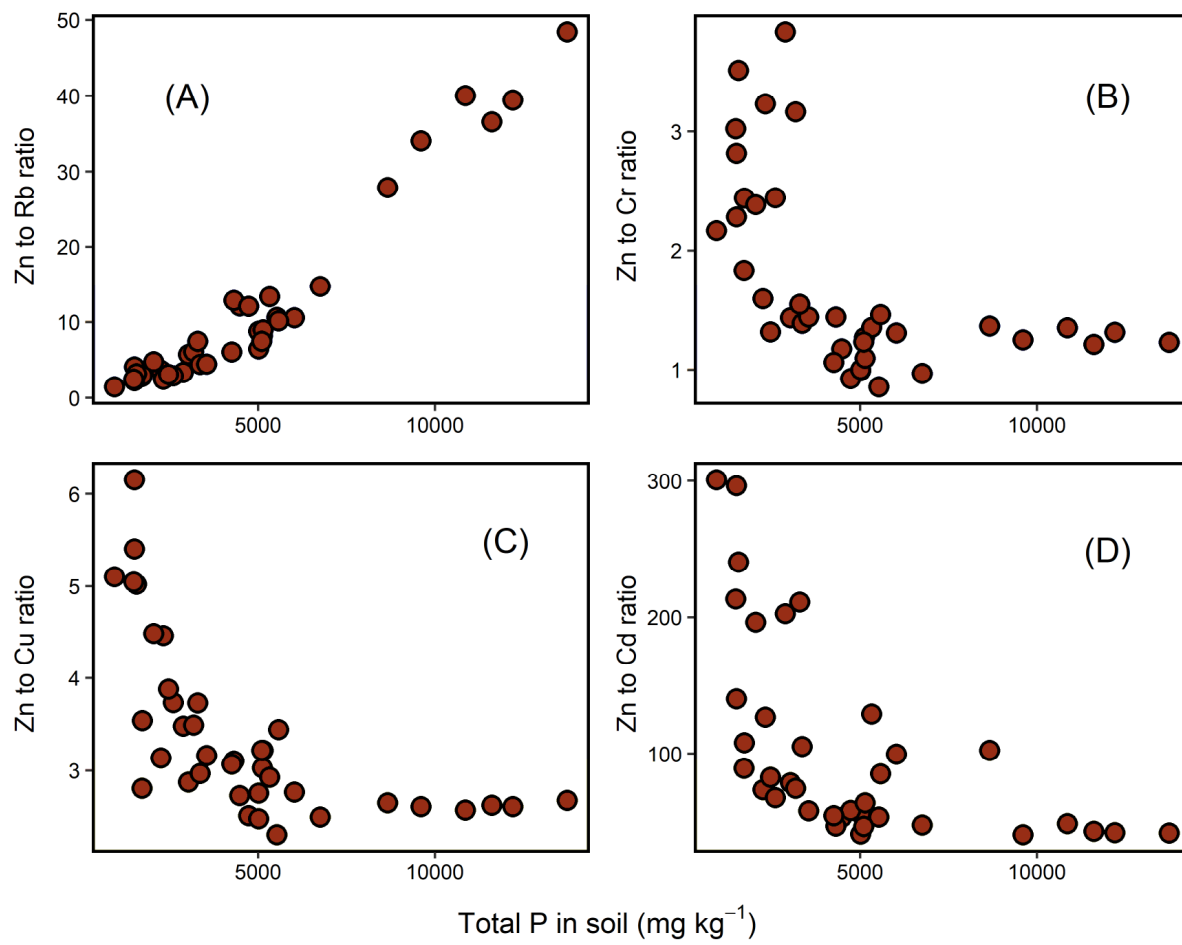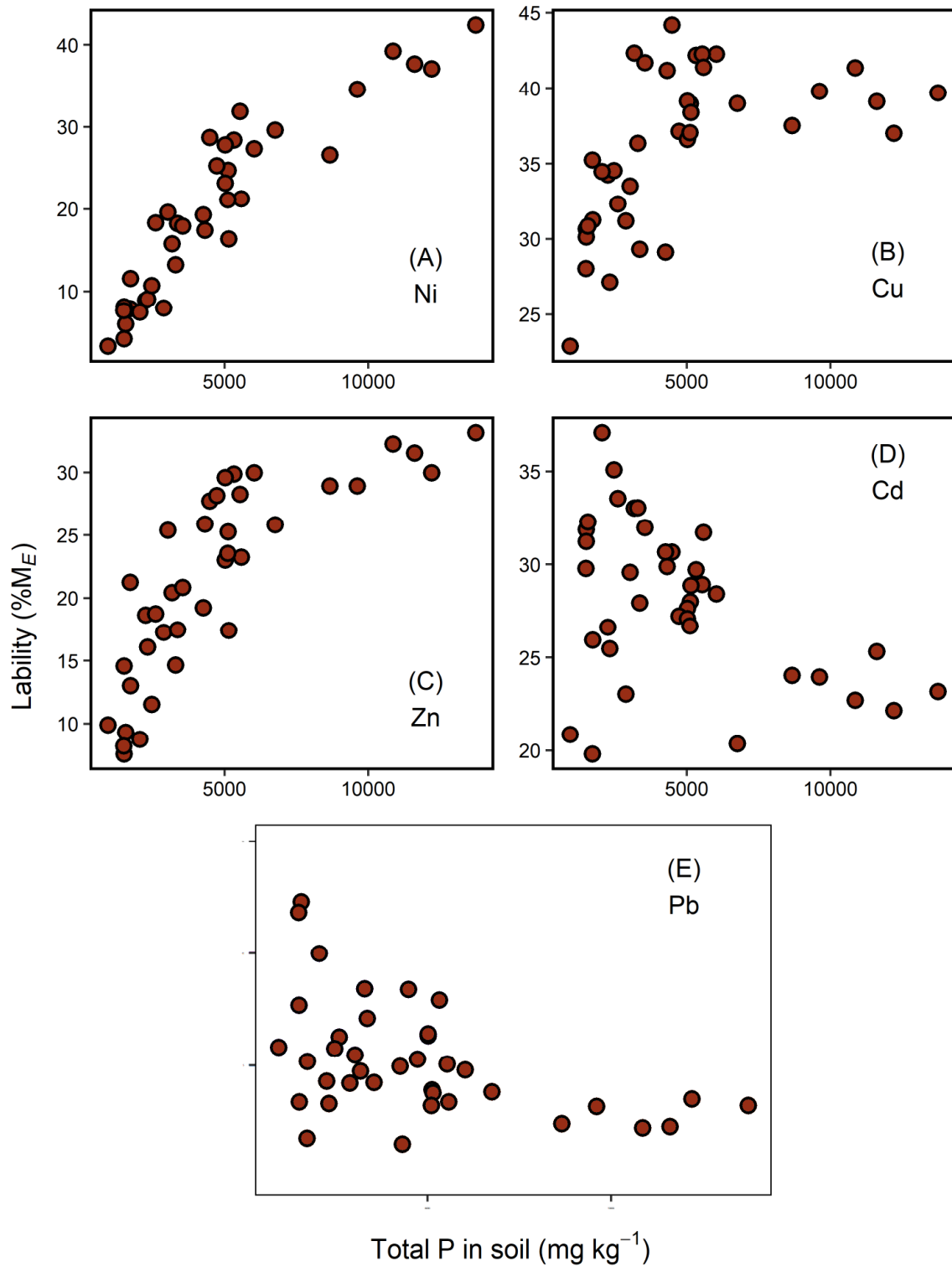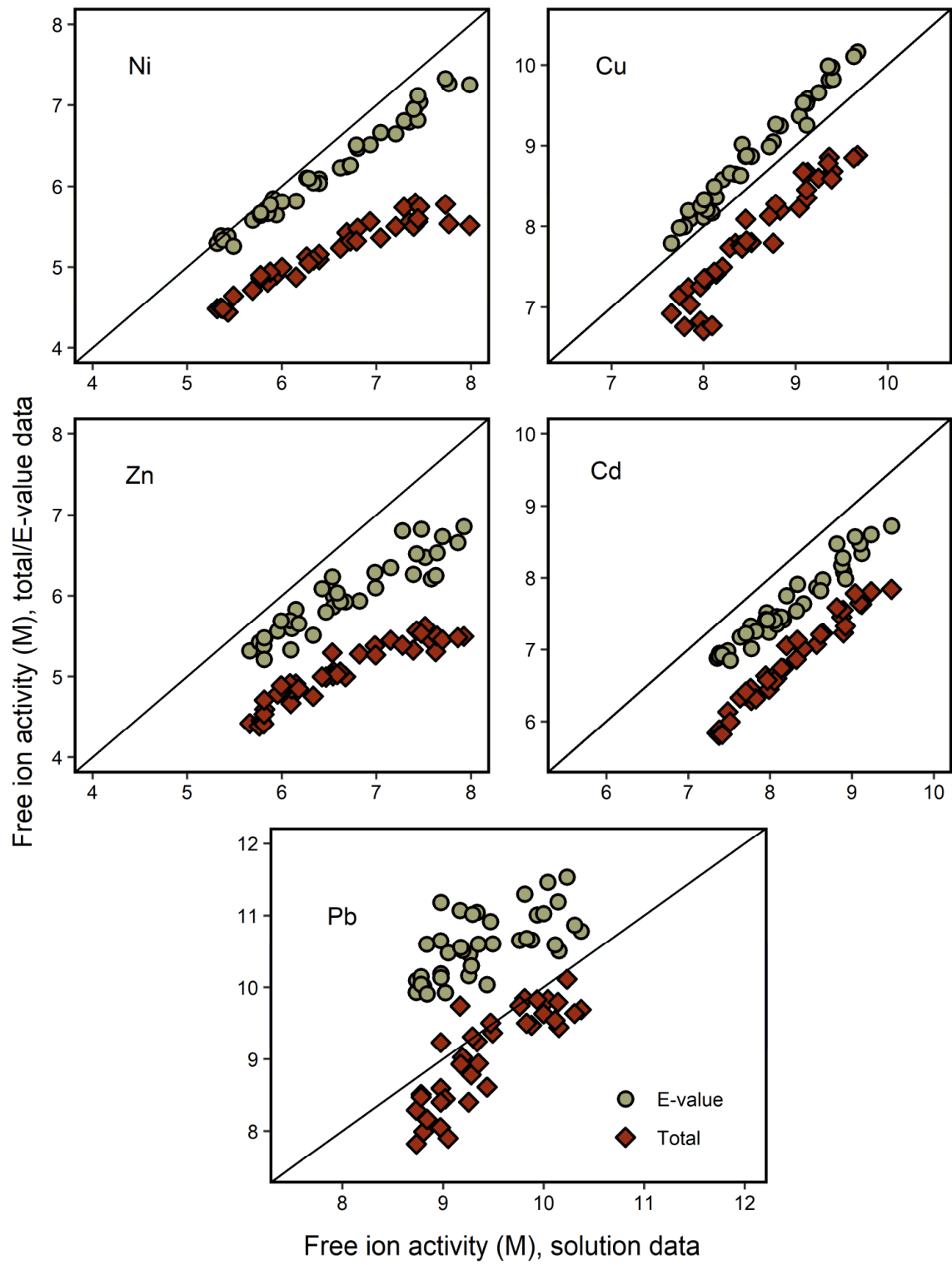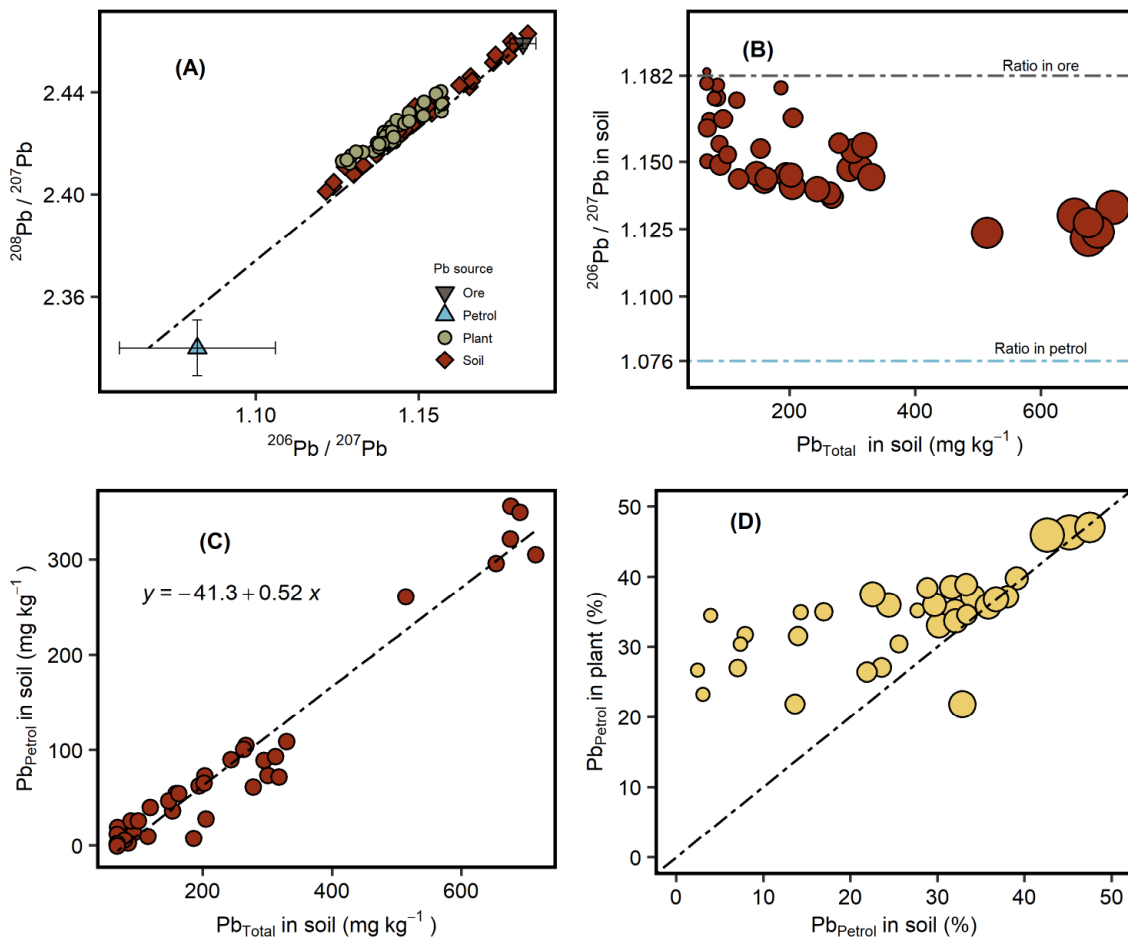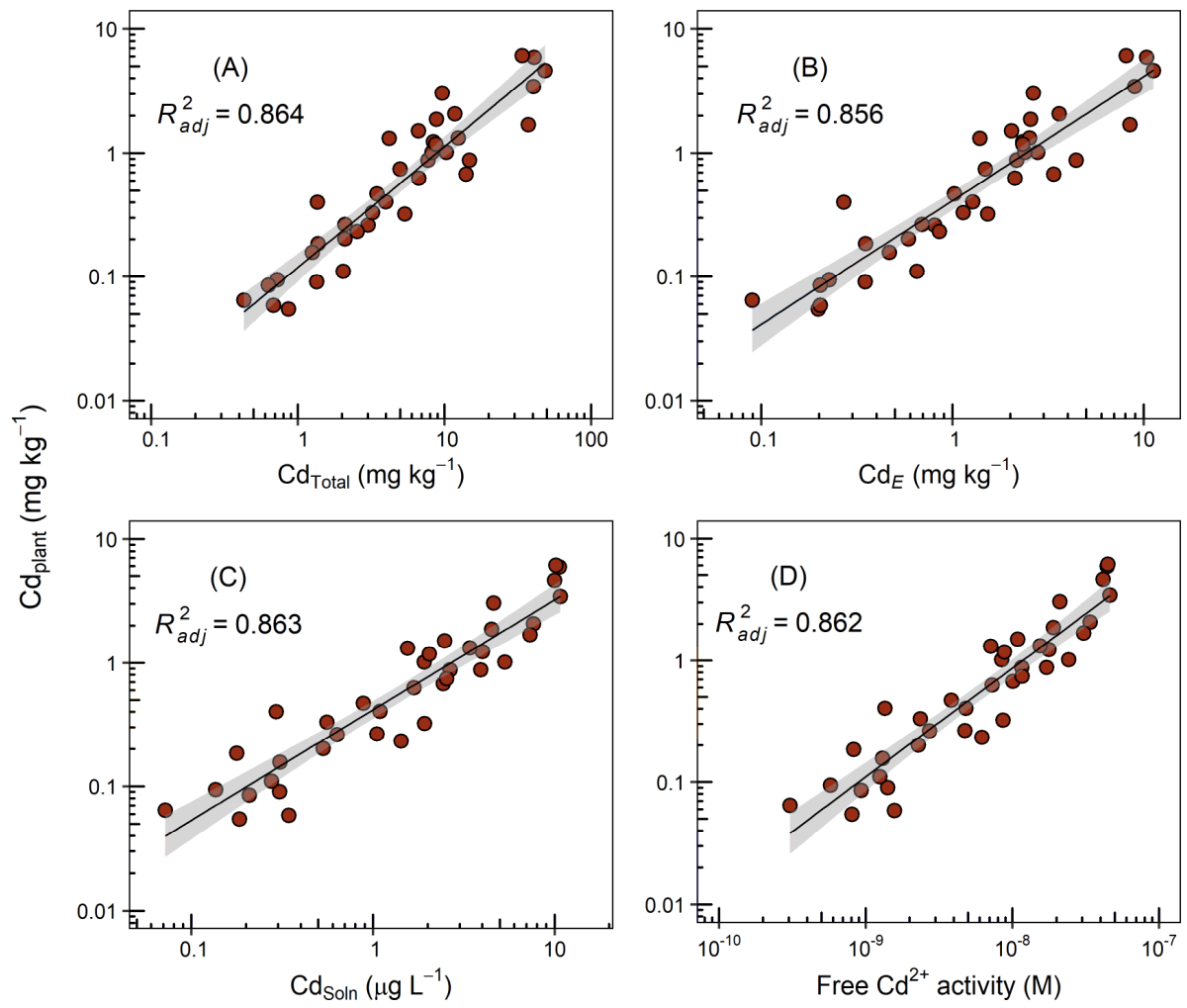
Figure 1

Figure 2

Figure 3

Fig 4

Figure 5

Figure 6

The impact of long-term biosolids application (> 100 years) on soil metal dynamics

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Study site:

The study site is a dedicated sewage disposal site, run by a water company. The area of the site is 900 ha. The majority of the farm is grown with nine varieties of maize (Table S1); the crop silage is used for biogas production (Anaerobic Digestion).

Table S1. Maize varieties that are cropped in each field

Variety	No. of fields
Barros	3
P7892	4
Ambrosini	7
Anjou 277	2
Torres	7
Valve	1
Cassilas	2
Ronaldinio	4
P7905	1

Supplementary Tables

Soil chemical properties are shown in Table S2. A broad range of soil properties and total element concentration have been observed. The large variation in total elemental concentration in soil indicates variable biosolids application rates throughout the sampling area and over the time period that each field has been used for biosolids disposal.

Table S2. Soil organic matter (LOI), pH and element concentration (mg kg^{-1}) in soil

	Mean	Minimum	Maximum	Std. deviation
pH	6.38	5.95	6.94	0.35
LOI (%)	10.2	3.52	23.4	4.96
		(mg kg^{-1})		
Mg	6980	3160	21500	4260
K	14400	4940	31000	5530
Ca	12700	3830	41800	9290
Al	1560	514	2500	521
V	53.4	33.9	109	17.0
Cr	433	43.2	1670	440
Mn	565	152	1790	339
Fe	14100	6880	22800	3470
Co	11.4	6.72	16.5	2.47
As	18.7	8.59	45.6	8.21
Se	1.07	0.32	2.53	0.56
Rb	55.8	40.5	96.1	13.3
Sr	74.1	46.6	136	22.9
Mo	2.33	1.02	4.04	0.70
Ag	6.57	0.25	28.2	6.45
Sn	46.5	5.02	160	45.8
Sb	18.7	1.65	98.1	21.8
Cs	3.55	1.89	9.43	1.69
Ba	648	322	1520	332
Th	7.99	5.55	11.1	1.38
U	2.61	1.39	3.42	0.39

Total metal concentrations in soil strongly correlated with soil organic matter content (LOI) (Table S3) suggesting a common source of contamination.

Table S3. Pearson correlation matrix for M_{Total}, LOI and soil pH. Bold numbers are **not** significant at $p < 0.05$.

pH	LOI	Total C	P	Cr	Co	Ni	Cu	Zn	As	Se	Mo	Cd	Ba	Pb
pH														
-0.58	LOI													
-0.59	0.93	Total C												
-0.69	0.95	0.89	P											
-0.65	0.96	0.91	0.97	Cr										
0.13	0.39	0.29	**0.21**	**0.26**	Co									
-0.63	0.95	0.88	0.96	0.98	**0.31**	Ni								
-0.62	0.97	0.92	0.97	0.99	**0.29**	0.98	Cu							
-0.60	0.97	0.92	0.96	0.98	0.34	0.98	1.00	Zn						
-0.49	0.85	0.80	0.81	0.85	**0.31**	0.76	0.83	0.84	As					
-0.61	0.93	0.83	0.90	0.92	0.44	0.90	0.90	0.91	0.87	Se				
-0.69	0.86	0.79	0.90	0.85	**0.33**	0.85	0.85	0.86	0.73	0.87	Mo			
-0.62	0.92	0.87	0.95	0.96	**0.26**	0.99	0.96	0.96	0.69	0.86	0.84	Cd		
-0.50	0.94	0.89	0.89	0.94	0.40	0.90	0.95	0.96	0.91	0.91	0.79	0.87	Ba	
-0.60	0.95	0.89	0.94	0.96	**0.32**	0.93	0.97	0.97	0.89	0.92	0.83	0.90	0.96	Pb

Table S4 shows element concentrations in the solution phase of 0.01 M $(CaNO_3)_2$ extractions. This extraction estimates the readily bioavailable portion of an element of interest.

Table 4. Elemental concentrations ($\mu g\ L^{-1}$) in the solution phase of 0.01 M $Ca(NO_3)_2$

	Min.	Max.	Median	Mean
V	0.36	13.1	5.48	6.22
Cr	0.25	12.9	3.98	4.19
Co	0.08	0.72	0.26	0.3
Fe	2.97	48.9	15.4	16.8
Al	1.18	33.6	6.56	9.43
Mo	0.5	14.5	4.01	4.85
Cd	0.07	10.8	1.8	2.9
As	0.95	25	5.82	8.4
Mn	1.61	164	14.8	36.1
Ni	1.33	661	52.5	136
Cu	2.58	196	36.4	52.1
Zn	1.93	378	42	92.8
Sb	1.26	157	34.6	44.6
Ba	53.2	472	120	155
Sr	225	764	301	339
Se	0.55	2.86	1.16	1.25
Rb	0.1	4.94	1.52	1.68
Pb	0.02	1.31	0.08	0.19

Supplementary figures

Values of enrichment factor for different elements are shown in Figure S1. The enrichment factor was calculated as a ratio to the concentration of Al in soil and was normalised to the ratio in the regional background.
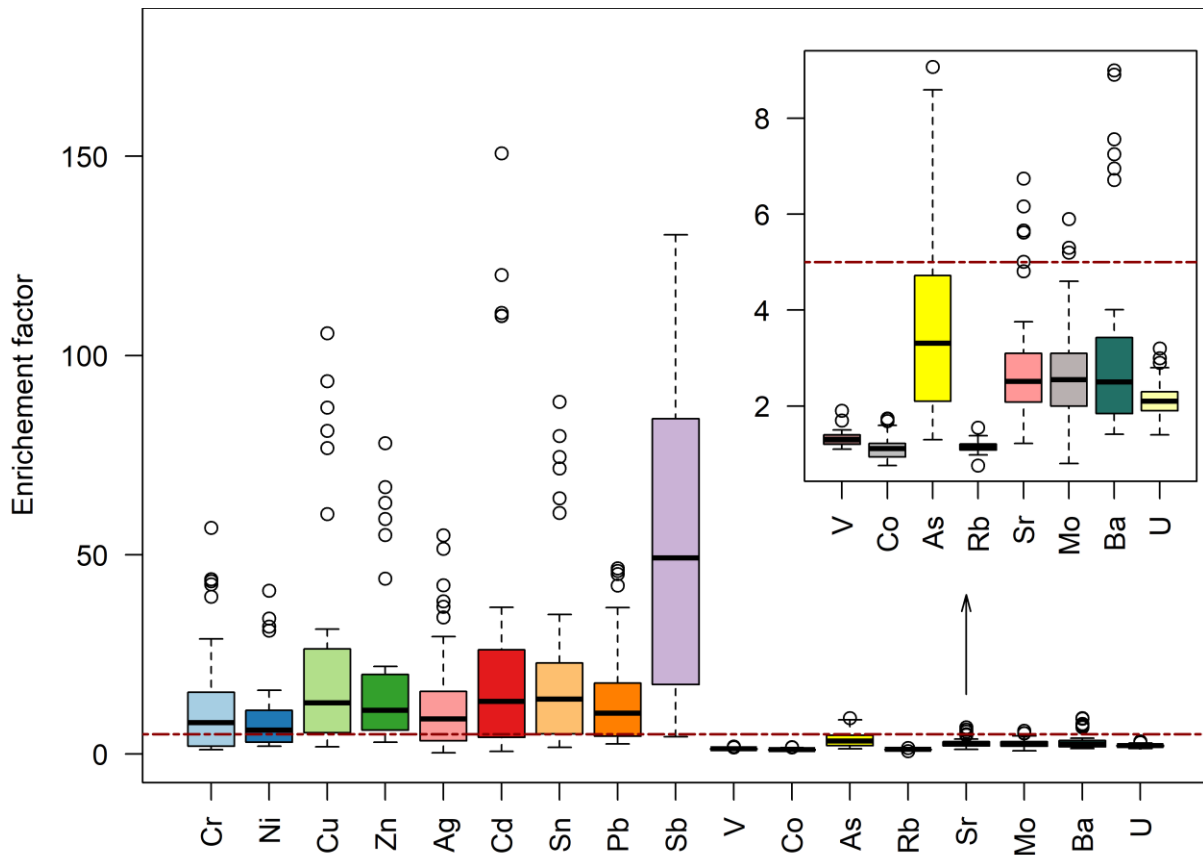

Figure S1. Boxplots of enrichment factors in the studied soils. Horizontal dashed lines represent EF of 5

Figures S2-S5 show the relationships between metal concentration in plant and the metal concentration in soil measured using different estimates of metal bioavailability in soil. All metal bioavailability indices produced good predictions of Ni, Cu, and Zn concentration in plant, explaining more that 70% of the variation in the concentration in plant (Figs S2-S4).
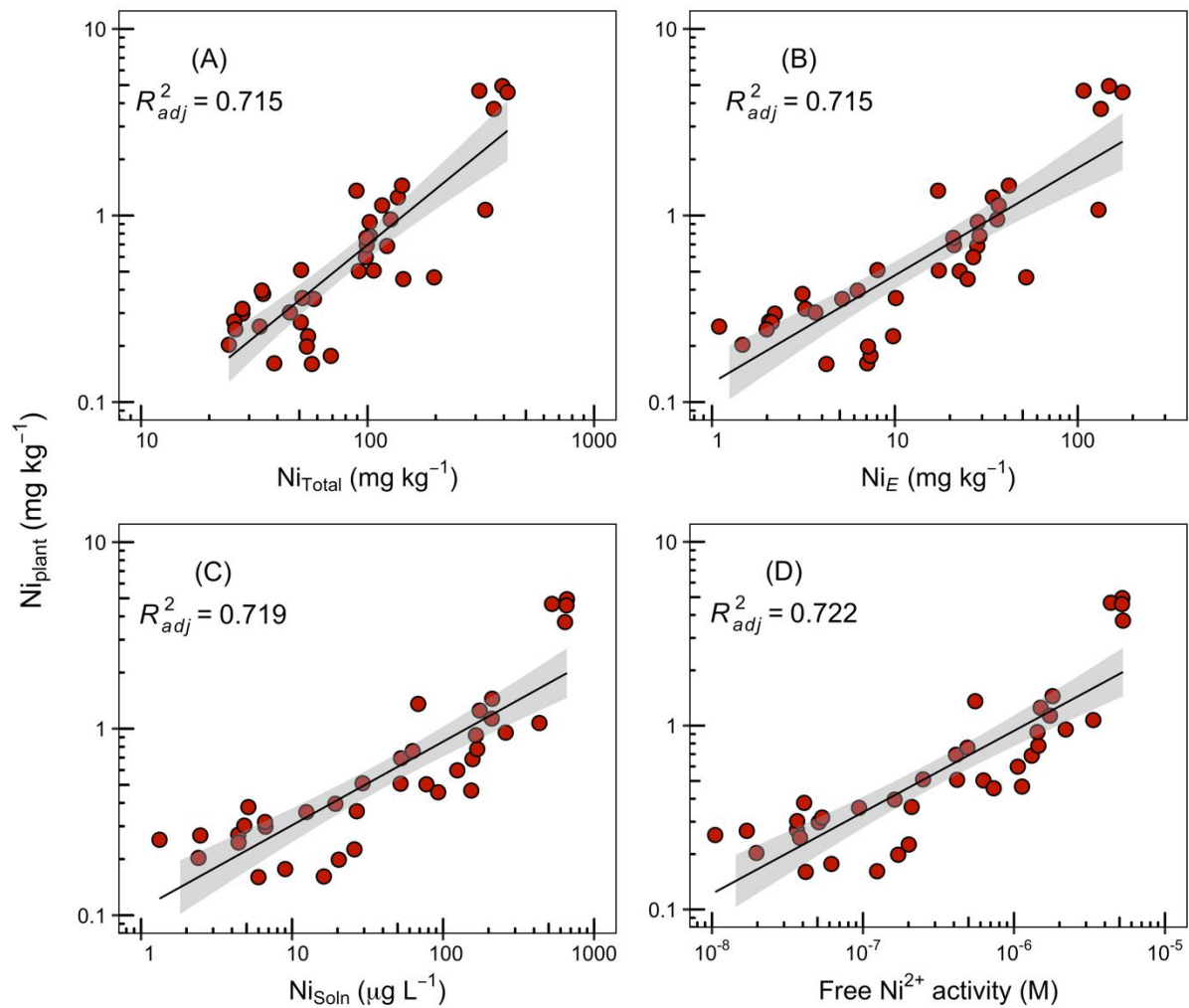

Figure S2. Relationships between Ni concentration in maize plants and Ni concentration in soil. The latter included (A) total Ni_{Total}, (B) labile Ni_E, (C) Ni_{Soln}, (D) Ni^{2+} activity. The solid lines represent a linear regression fit. Grey shaded areas represent 95% confidence interval around fitted values.
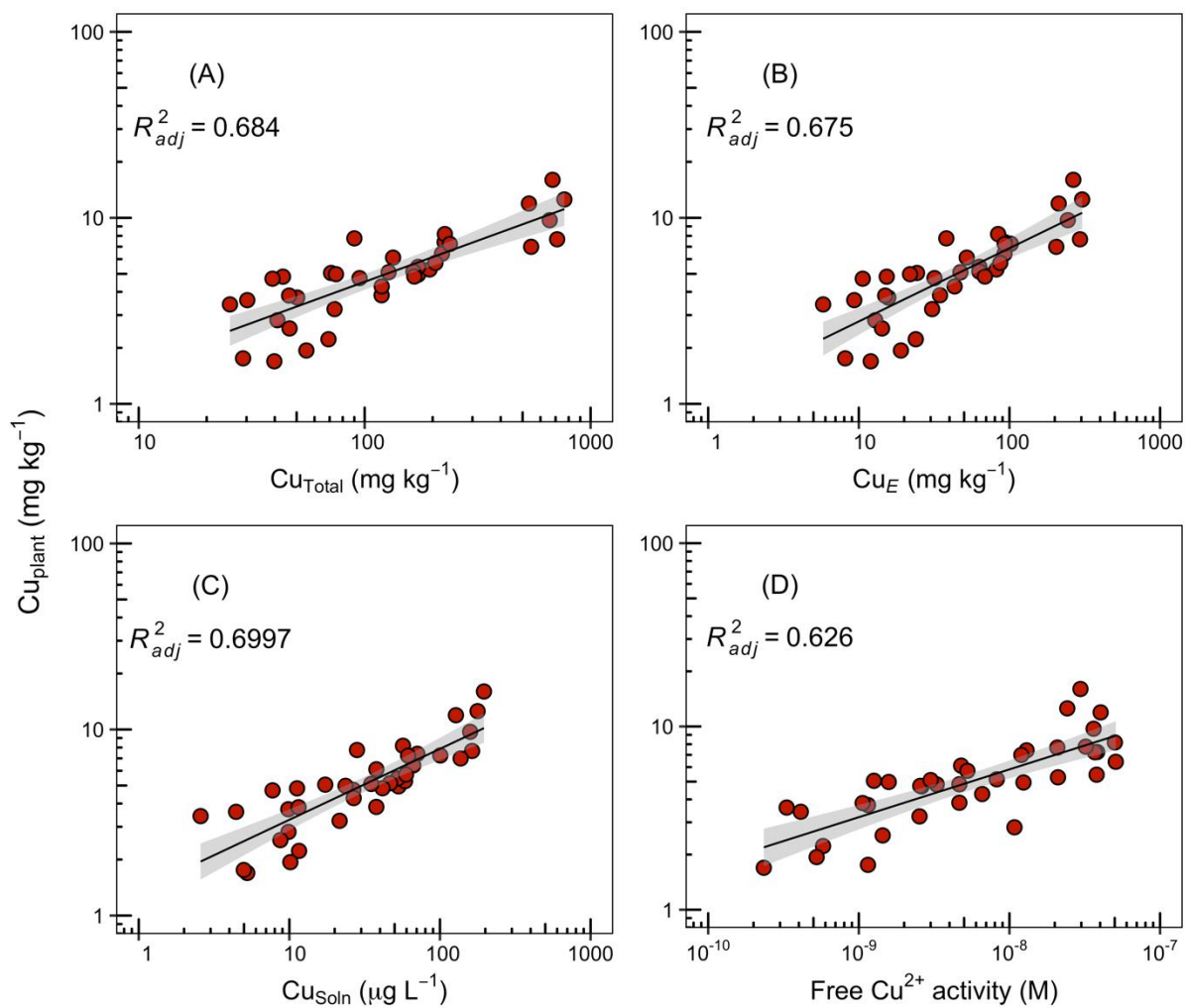
Figure S3. Relationships between Cu concentration in maize plants and Cu concentration in soil. The latter included (A) total Cu_{Total}, (B) labile Cu_E, Cusoln, (D) Cu^{2+} activity. The solid lines represent a linear regression fit. Grey shaded areas represent 95% confidence interval around fitted values.
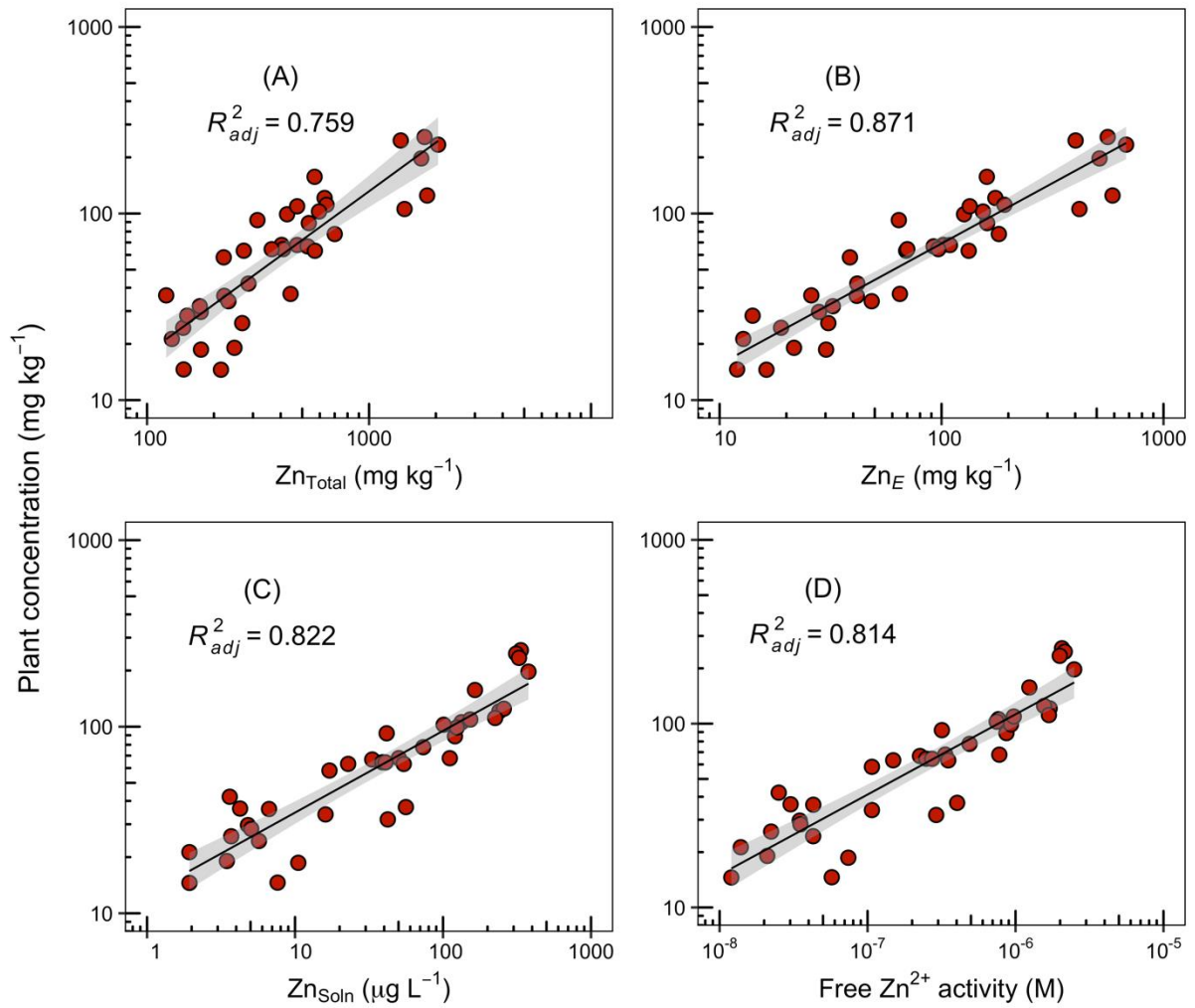
Figure S4. Relationships between Zn concentration in maize plants and Zn concentration in soil. The latter included (A) total Zn_{Total}, (B) labile Zn_E, (C) Zn_{Soln}, (D) Zn^{2+} activity. The solid lines represent a linear regression fit. Grey shaded areas represent 95% confidence interval around fitted values.

In the case of Pb (Fig. S5) however, all metal bioavailability measures resulted in weak relations, albeit significant, with Pb concentration in maize plants, where only a maximum of 34% of the variation was accounted for. Contamination of the crop by soil-derived dust probably accounted for most of the Pb measured in the plant.
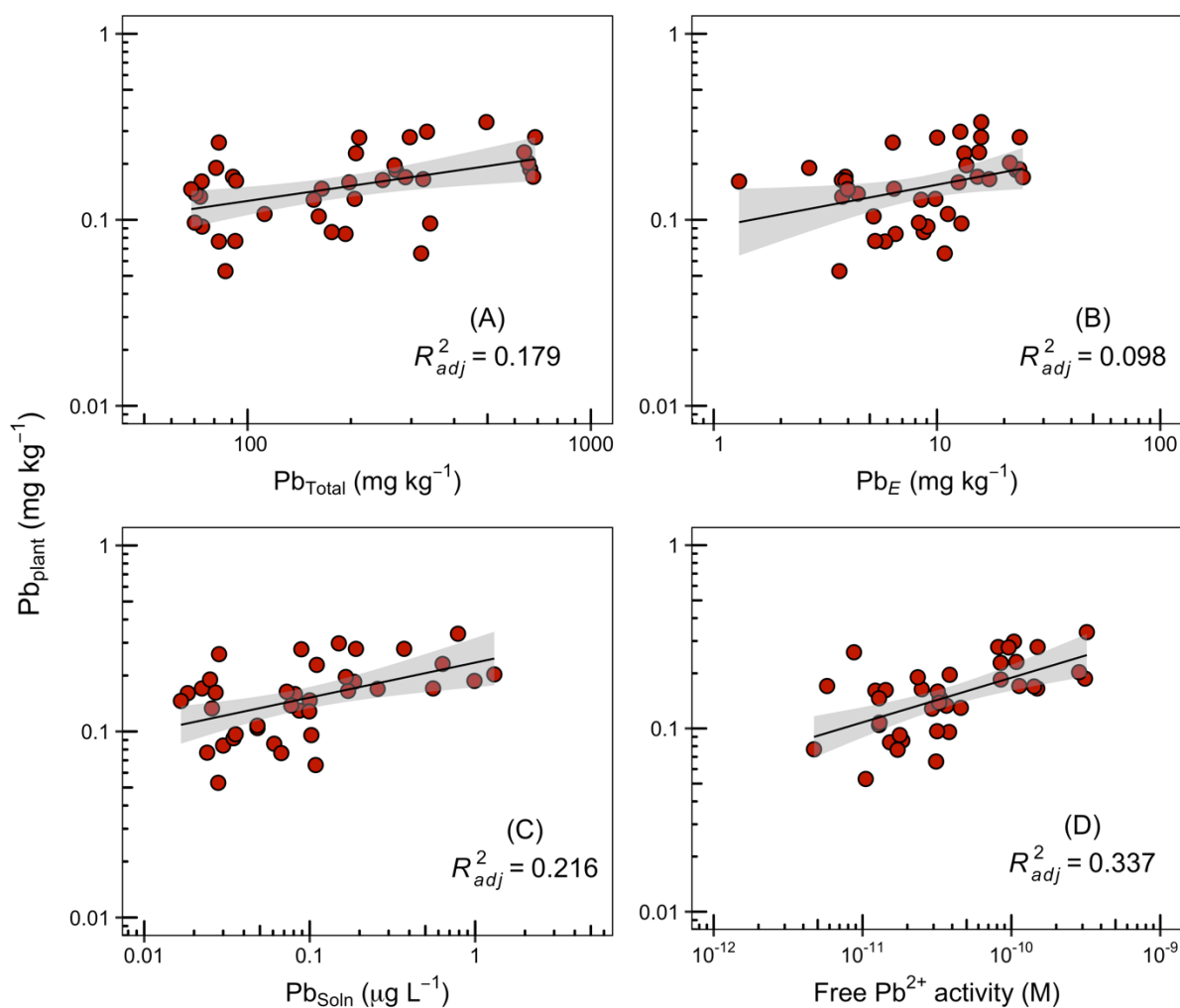

Figure S5. Relationships between Pb concentration in maize plants and Pb concentration in soil. The latter included (A) total Pb_{Total}, (B) labile Pb_E, (C) Pb_{Soln}, (D) Pb^{2+} activity. The solid lines represent a linear regression fit. Grey shaded areas represent 95% confidence interval around fitted values.