Mechanisms of metal-phosphates formation in the rhizosphere soils of pea and tomato: environmental and sanitary consequences

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Abstract

Purpose At the global scale, soil contamination with persistent metals such as lead (Pb), zinc (Zn), and copper (Cu) induces a serious threat of entering the human food chain. In the recent past, different natural and synthetic compounds have been used to immobilize metals in soil environments. However, the mechanisms involved in amendment-induced immobilization of metals in soil remained unclear. The objective of the present work was therefore to determine the mechanisms involved in metal-phosphates formation in the rhizospheric soils of pea and tomato currently cultivated in kitchen gardens.

Materials and methods Pea and tomato were cultivated on a soil polluted by past industrial activities with Pb and Zn under two kinds of phosphate (P) amendments: (1) solid hydroxy-apatite and (2) KH_2PO_4. The nature and quantities of metal-P formed in the rhizospheric soils were studied by using the selective chemical extractions and employing the combination of X-ray fluorescence micro-spectroscopy, scanning electron microscopy, and electron microprobe methods. Moreover, the influence of soil pH and organic acids excreted by plant roots on metal-P complexes formation was studied.

Results and discussion Our results demonstrated that P amendments have no effect on metal-P complex formation in the absence of plants. But, in the presence of plants, P amendments cause Pb and Zn immobilization by forming metal-P complexes. Higher amounts of metal-P were formed in the pea rhizosphere compared to the tomato rhizosphere and in the case of soluble P compared to the solid amendment. The increase in soil-metal contact time enhanced metal-P formation.

Conclusions The different forms of metal-P formed for the different plants under two kinds of P amendments indicate that several mechanisms are involved in metal immobilization. Metal-P complex formation in the contaminated soil depends on the type of P amendment added, duration of soil-plant contact, type of plant species, and excretion of organic acids by the plant roots in the rhizosphere.

Keywords Low molecular weight organic acids · Metal immobilization · Phosphates · Rhizosphere · Speciation

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1 Introduction

The contamination of the human food chain with toxic metals such as Pb, Cd, Cu, Ni, Zn,… has become inevitable owing to industrialization and the application of modern technologies (Radwan and Salama 2006; Austruy et al. 2013; Shahid et al. 2013a). The uptake of toxic metals by vegetables represents the main source of human exposure to environmental pollutants (Niazi et al. 2011a). As vegetables and fruits are the major components of human diet, their quality was studied in

relation with soil (Uzu et al. 2009) or air pollution (Schreck et al. 2013). According to Sharma et al. (2008) or Foucault et al. (2013), the determination of the mechanisms involved in the soil-plant-human transfers of hazardous metals constitutes an important scientific goal. At the global scale, numerous soils are polluted with persistent metals with consequences on environmental and human health (Arshad et al. 2008; Shahid et al. 2012a; Pourrut et al. 2013). For example, in the USA, more than 60 % of the contaminated sites from the National Priority List are polluted by metals and lead (Pb) is reported to be the most common metal (present at 15 % of the sites) (Suzuki et al. 2014). In Japan, according to the Ministry of Environment Government (2007), more than 40 % of the contaminated sites are polluted by Pb.

To contend with this problem, environmentally sustainable management of polluted sites is crucial. Among different remediation techniques, the in situ metal immobilization using chemical amendment together with plants has gained particular interest owing to its less disruptive nature and cost-effectiveness (Lopareva-Pohu et al. 2011; Austruy 2012). The formation of metal-P complexes after the application of P amendments is considered as a major in situ stabilization approach for Pb and Zn polluted soils (Jiang et al. 2012; Mignardi et al. 2012). Certain Pb-P and Zn-P minerals are highly stable with limited solubility in soils (Cao et al. 2009; Waterlot et al. 2011). Phosphorus-induced immobilization of metals in soil is recommended by the US Environmental Protection Agency as one of the best management practices for metal pollutants in soils (Chrysochoou et al. 2007).

Despite the useful effect of P amendments on Pb and Zn immobilization, the approach has some uncertainties as well, which need to be addressed before field scale application. One major drawback is the partial formation of stable metal-P complexes after P amendment. Although, the formation of metal-P complex after P addition to metal polluted soil is well established, the phenomenon is rather complex and generally incomplete. Several recent studies reported that more than 50 % of Pb present in soil was not transformed into Pb-P complex after P amendment (Hashimoto et al. 2009; Debela et al. 2013). The reduced formation of Pb-P complexes is due to limited availability of free P and metals. In this way, the addition of P amendments at high rates may result in an excess of available P, which can cause eutrophication of ground or surface waters (Park et al. 2011). This implies that P-induced metal immobilization in soil may endure economic and environmental consequences. The enhanced formation and thermodynamic stability of metal-P complexes particularly depends on physico-chemical soil characteristics such as mineral composition, pH, organic contents, microbial activity, etc. (Lopareva-Pohu et al. 2011; Park et al. 2011; Mignardi et al. 2013). The increased solubilization and/or dissolution of metals in soils are generally related to changes in pH and/or complexing effects of organic acids (Shahid et al. 2012b,

2014a, b). Organic acids are also known to enhance metal solubilization and thus may favor the formation of metal-P complexes (Debela et al. 2010). Moreover, formation of metal-P complexes is also influenced by the presence of high amounts of Fe and Mn oxides or multi-metal (Pb, Zn, Cd,…) contaminated soils.

In terms of transfers and ecotoxicity, the impact of metal(loid)s on terrestrial ecosystems is strongly influenced by their compartmentalization and speciation and on-site variability (in bulk soil as well as in rhizosphere) (Niazi et al. 2011b; Schreck et al. 2011; Leveque et al. 2013; Shahid et al. 2012c, 2013b). Changes in metal speciation can occur under the action of plants and application of organic or inorganic amendments (Mignardi et al. 2012; Shahid et al. 2014c). For example, P amendments may change metal speciation in soil and in turn, its bioavailability. A few studies concern the quantification of phosphate phases formed and the mechanisms involved. Therefore, a good understanding of the underlying mechanisms is required in order to predict, model, and optimize metal immobilization in treated polluted soils. The objectives of the present work were to: (1) locate Pb and Zn in the root system and in the rhizosphere of pea and tomato by X-ray fluorescence micro-spectroscopy (μ-XRF), (2) identify (chemical analysis) metal-P formed under the action of the plant and/or in the presence of P amendments (solid or soluble), by electron microprobe and scanning electron microscopy interfaced with an energy-dispersive spectrometer (SEM-EDS), (3) quantify metal-P formed in the different samples by SEM-EDS and automatic statistical treatment, and (4) check the influence of low molecular weight organic acids released by plant roots and pH on P-metal complex formation.

2 Materials and methods

2.1 Study area

A highly contaminated top soil (0–5 cm) under poplar trees was sampled for micro-culture experiments in the laboratory (see the chapter later for details). The sampled soil under those trees has not been disturbed for the last 50 years and therefore represents the possible minimum total metal accumulation in soil during the last five decades. The site is located 500 m NE from the former Metaleurop Nord smelter in Northern France. Metaleurop was one of the largest European Pb- and Zn-smelter during 1884–2004. In 2002, the atmospheric releases recorded from this smelter were 1, 17, and 31 tons, respectively for Cd, Pb, and Zn (Fritsch et al. 2010). The atmospheric releases from the smelter has contaminated over tens of km^2 of nearby areas (Douay et al. 2009; Shahid et al. 2013c). The highly contaminated loamy clayed top soils classified as pseudogley brown leached soils developed over quaternary clays (0.6–1.5 m) covering the Ostrincourt sand in which the

water table is 1 m below ground level (Dumat et al. 2001). The physico-chemical properties of soils are: pH 6, OM 104 mg kg^{-1}, and CEC 21.3 cmol$^{(+)}$ kg^{-1}. Soil concentrations of Pb, Cd, Zn, and Cu are higher than the legal French limits except for Cu (100, 300, 2, and 100 ppm, respectively for Pb, Zn, Cd, and Cu). In this top soil, Pb and Zn are mainly associated with Fe and Mn oxides and organic matters (Dumat et al. 2001). The concentrations of Fe, Mn, Al, Si, and P, respectively are: 27,246 mg kg^{-1}±2.8 %, 498 mg kg^{-1} ±3.1 %, 41,900 mg kg^{-1}±4 %, 33,420 mg kg^{-1}±3.2 %, and 330 mg kg^{-1}±3.4 % (Table 1).

2.2 Culture experiments

Seeds of pea (*Pisum sativumpea* L.) and tomato (*Lycopersicon esculentum* L.) were germinated in an aerated solution of CaCl$_2$ (600 μM)+H$_3$BO$_3$ (2 μM) after disinfection with H$_2$O$_2$ (6 %). After 1 week, the uniform and healthy seedlings were transplanted to an aerated nutrient solution for 14 days of pre-culture. The composition of the nutrient solution was: 1 mM Ca(NO$_3$)$_2$, 1 mM KNO$_3$, 1 mM KCl, 1 mM MgSO$_4$, 0.5 mM NaH$_2$PO$_4$, 6 μM FeEDDHA (C$_{18}$H$_{20}$O$_6$N$_2$, ethylene diamine di (o-hydroxyphenylacetic) acid), 10 μM H$_3$BO$_3$, 2 μM MnCl$_2$, 1 μM ZnCl$_2$, 0.2 μM CuCl$_2$, and 0.05 μM (NH$_4$)$_6$Mo$_7$O$_{24}$. The nutrient solution was renewed on every 4th day to keep its composition and pH constant.

Three-week-old pea and tomato plants were exposed for seven days to contaminated soil (sampled from Metaleurop Nord smelter) and mixed with the following two types of P amendments: (1) water-soluble KH$_2$PO$_4$ and (2) solid hydroxyapatite, Ca$_5$(PO$_4$)$_3$OH. Culture experiments were performed using a biotest first described by Niebes et al. (1993) and then adapted by Chaignon and Hinsinger (2003). A small PVC cylinder was closed by a polyamide net inserted into a larger cylinder, itself closed by a finer polyamide mesh. A space of 3 mm was left between the net and the finer mesh, where the roots could develop as a mat. This biotest device presents two main advantages: (1) the roots are physically separated from the soil which enables total recovery of the shoots, roots, and soil (2) the thickness of the soil layer used enables it to be considered as rhizospheric soil and provides enough rhizospheric material to evaluate root-induced changes in metal speciation.

The P amendments were added at 9,000 mg P kg^{-1} of soil for Ca$_5$(PO$_4$)$_3$OH and 120 mg P kg^{-1} of soil for KH$_2$PO$_4$. Metal content analysis using inductively coupled plasma atomic emission spectrometry (ICP-AES) showed that both the P amendments did not contain detectable Cd, Pb, or Zn. Five replicates for each experimental condition were performed under controlled laboratory conditions (T=26 °C and humidity=80 %). After the soil-plant contact period (i.e., 7 days), plants and rhizospheric soils were carefully removed from the devices.

2.3 Analysis of metals distribution within plant roots

In order to investigate the distribution of accumulated Pb and Zn within plant tissues, X-ray fluorescence microspectroscopy (μ-XRF) analysis was performed on plant roots. During the analysis, the samples were fixed and kept frozen using a Peltier freezing system. Experiments were carried out using a microscope (XGT7000, Horiba Jobin Yvon) equipped with an X-ray guide tube producing a finely focused beam with a 10 mm or 100 mm spot size (Rh X-ray tube, 30 kV, 1 mA equipped with an EDX detector), as described by Lapied et al. (2011).

2.4 Analysis of metal-P in rhizospheric soil of pea

To identify the formation of metal-P in the rhizosphere, the soil samples obtained after culture of pea and tomato were analyzed using SEM-EDS. The chemical composition of metal-P observed by SEM-EDS was determined by electronic micro probe. We focused on the pea rhizosphere for this analysis because the phases detected for tomato were more difficult to analyze due to their small size (nanometers).

2.5 Quantification of metal-P in rhizospheric soil of pea and tomato

In order to quantify the metal-P phases, energy-dispersive X-ray (EDX) analysis was carried out on the rhizospheric soil of both the plants after P amendments. Counting a mineral carrier and Pb-P phases was carried out using EDX which classifies the chemical composition of minerals according to criteria defined by Kotula et al. (2003). The software (PGT ® Autochem Class) was used to quantify Pb/P ratio for the classification of metal-P minerals. For Pb, the phases are classified as follows: Pb/P=1.5 for pyromorphite, 0.9<Pb/P <1.5 for Ca-pyromorphite, and Pb/P≤0.9 for Pb-apatite (Eighmy et al. 1997). Two uncultivated control soils were also

Table 1 Total trace metal and mineral element concentrations in studied soil (milligrams per kilogram)

Trace metals	Soil concentrations
Cd	32±9
Cu	83±6
Mn	498±15
Pb	4,151±270
Zn	2,036±58
Fe	27,246±763
Al	41,900±1,676
Si	33,420±1,069
P	330±11

analyzed to identify the effect of plants and the effect of phosphate type. The approach has also been applied to Zn to classify different forms of Zn-P as following: Zn/P=1.5 for $Zn_5(PO_4)_3OH$); $0.9<Zn/P<1.5$ for $CaZn_3(PO_4)_3OH$ et Zn/P$\leq$ 0.9 for $ZnCa_4(PO_4)_3OH$.

2.6 Measurements of soil pH and low molecular weight organic acids (LMWOA)

The influence of soil pH and LMWOA excreted by plant roots was observed on the formation of metal-P complexes in rhizospheric soil of pea and tomato. Soil pH was determined before and after culture in 1:2.5 soil/water suspensions after 0.5 h with a combination pH electrode. Organic acids were identified and quantified by capillary electrophoresis for different experimental conditions (rhizospheric soil of pea and tomato for both types of P amendments). Electrophoresis (PACE MDQ, Beckman) was equipped with an UV detector filters (μ=214 nm) to detect the presence of the LMWOA in the rhizosphere. Selected conditions to analyze the organic acids were: (1) capillary untreated silica; 75 μm$\times$375 μm (diameter internal/external) 70–80 cm (effective length/actual), T=17 °C; (2) organic acid buffer used without dilution (Interchim 5 mMol L^{-1} o-potassium hydrogenophtalate (a) $HOOCC_6H_4COOK$, 0.5 mMol L^{-1} of CTAB* N-hexadecyl-N, N, N trimethylammonium bromide (b) in water stored at 40 °C); (3) rinse TAO 20 psi_3 min; (4) injection 0.5 psi_5 s; and (5) separation-15 kV_13 min_2.7 μA.

2.7 Statistical analysis

We performed one-way analysis of variance (ANOVA) to evaluate any interaction effects between different treatments. Statistica Edition'98 (StatSoft Inc., Tulsa, OK, USA) was used for all statistical analysis. The results of chemical analysis correspond to the mean of five replicates. For each bioassay, mean values with different letters are significantly different ($p<0.05$) as measured by LSD Fisher test.

3 Results and discussion

3.1 Pb and Zn phytoavailability and spatial distribution in the plants roots

Figure 1 shows the effect of P amendments on Pb and Zn uptake by pea and tomato plants. Addition of soluble P amendment significantly increased Pb contents in the roots of pea (32 %) and tomato (10 %) and Zn content (14 %) in the pea roots. In tomato roots, Zn contents were the opposite trend (decreased by 54 %). In case of solid P amendments, both the
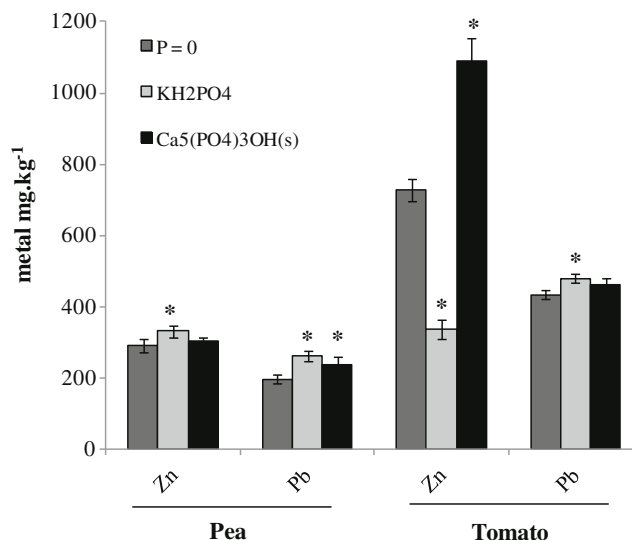
Fig. 1 Lead and zinc concentrations (milligrams per kilogram dry plant) in the roots of tomato and pea cultivated in soils with the various P conditions (P=0 or P as KH_2PO_4 or $Ca_5(PO_4)_3OH(s)$). Data are means ±SD of five replicates. Significant differences between treatments at P< 0.05 are indicated with an *asterisk* as measured by a LSD fisher test

Pb and Zn levels increased in the roots of pea and tomato but the effect was significant only for Pb (19 %) in pea and Zn (49 %) in tomato. Generally, application of P amendments induces a decrease in metal uptake by plants due to formation of Pb-P complexes. In our study, the increase in Pb and Zn uptake by pea and tomato in the presence of P can be due to increased plant biomass (Huang et al. 2012; Shahid et al. 2013d).

The accumulation of both the metals was higher in tomato roots compared to pea roots. One hypothesis to explain that phenomenon is the preferential formation of metal-P in the rhizosphere of pea limiting the phytoabsorption of the metals (Shahid et al. 2013d). Several previous studies reported the different effects of the plant on biogeochemical behaviors (mobility and uptake) of metals, which are generally attributed to root exudation of organic acids of various type and at different levels (Shahid et al. 2012d). These organic acids are well known to affect metal mobility in soil and uptake by plant roots. However, the plant capacity to secrete organic acids with respect to type and amount varies under metal stress conditions; certain plants are capable to exudate relatively higher levels of organic acids and macromolecular to promote metal dissolution in soil (Jiang et al. 2012). Moreover, Zn was taken up in higher amount than Pb by both plants. Generally, Pb-P is effectively less soluble and phytoavailable than Zn-P and the affinity of phosphorus for Pb is greater compared to Zn (Panfili et al. 2005). The total metal analysis in the plant roots performed by μ-XRF verified our results that the absorption of Zn was higher than Pb for both pea and tomato plants after 1 week of soil-plant contact with soluble KH_2PO_4 (Fig. 2).
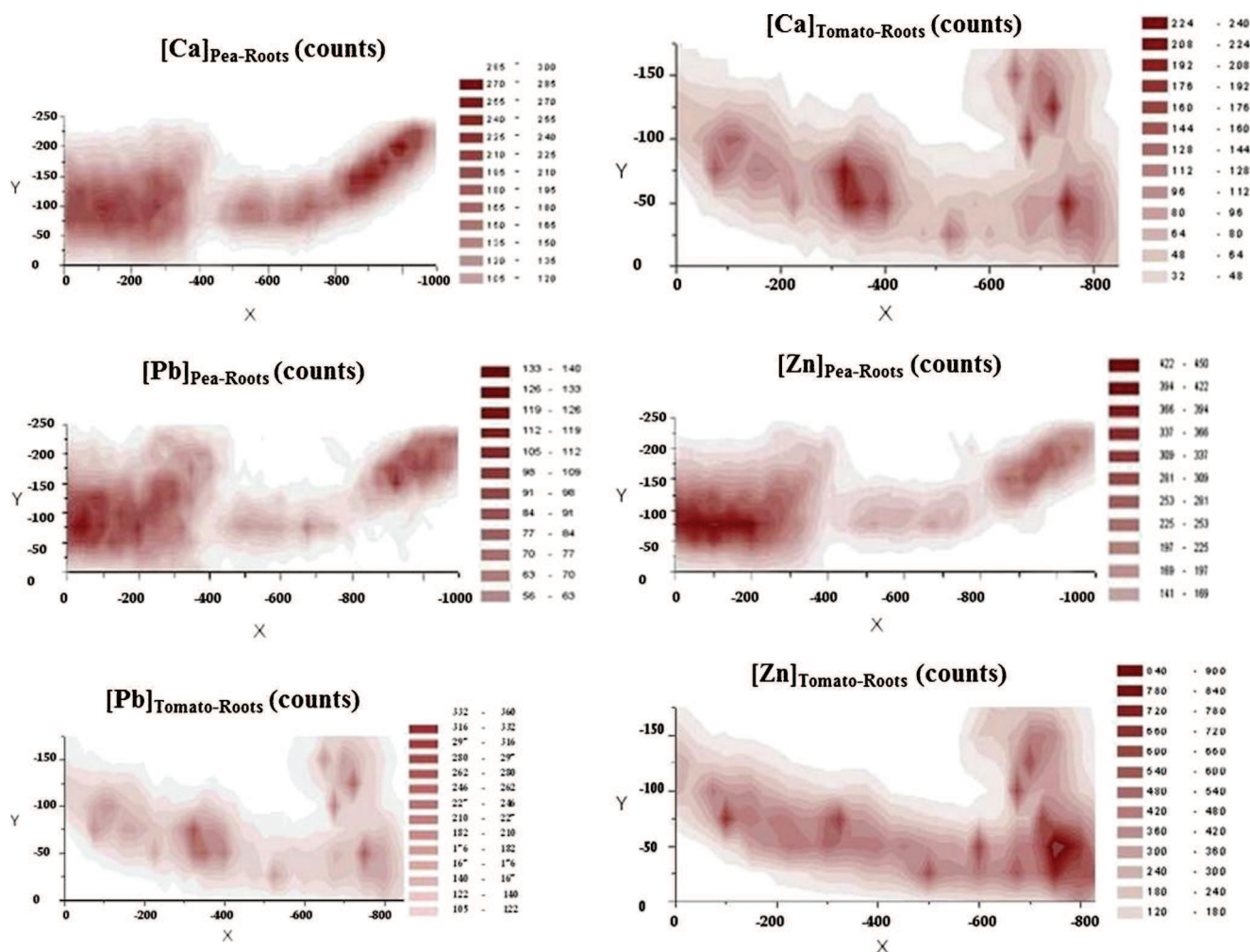
Fig. 2 Spatial distribution of Pb and Zn in the root systems of pea and tomato determined by µFX after plant cultures on the polluted soil amended with KH$_2$PO$_4$

3.2 Observations, chemical analysis, and quantification of metal-P compounds

Formation of metal-P in P-treated soils was further supported by SEM-EDS analysis (Fig. 3). Micrometer-sized minerals crystallize in large quantities in the pea rhizosphere were observed for soluble P. The electron microprobe analysis of minerals observed by SEM-EDS clarified their chemical composition as hydroxyl-pyromorphite (Pb$_5$(PO$_4$)$_3$(OH)) (Fig. 3a). For solid P amendments, various Pb-Ca-P associations were observed by SEM-EDS (Fig. 3b) probably due to formation of Pb-substituted Ca-P mineral or Pb sorption on the Ca-P mineral (Cao et al. 2009). Using SEM-EDS technique, several previous studies also reported the formation of pyromorphite-like minerals in Pb-contaminated soils in the presence of P amendments (Debela et al. 2013; Mignardi et al. 2013).

Figure 4 presents the data on the distribution of mineral metal-P phases obtained by microprobe analysis. These results are completed by the P amendments effects on quantification

of metal-P complexes (pyromorphite, apatite, and other metal-P complexes) in the presence and absence of plants (Table 2). The results for non-rhizospheric soil treated and untreated with soluble P indicate that the chemical composition of minerals is close to the Fe+Mn pole (Fig. 4a, b). This finding is consistent with a common association of Pb with amorphous Fe and Mn oxides (Pourrut et al. 2011). Actually, application of P amendments to soils without plant culture has no effect on the formation of pyromorphite or apatite of Pb (Table 2). The effect of the P amendment alone on metal-P formation is, therefore, negligible. In the absence of plants, soluble P amendment altered Pb speciation by increasing Ca-pyromorphite (5 % against 3.1 % for the untreated soil). The presence of solid P in non-rhizospheric soil results in the formation of Pb-hydroxyapatite (close to Ca+P pole). The results suggest mobilization of its initial Pb-bearing phases (oxides of Fe, Mn) to a phosphate phase thus confirming the observations made by SEM imaging. These results were confirmed by the quantification of metal-P complexes (Table 2) which highlights that addition of solid
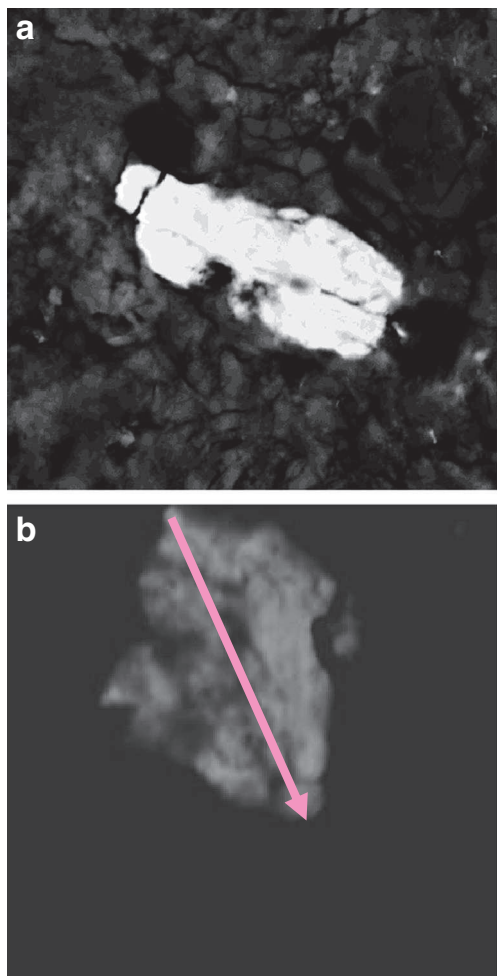
Fig. 3 Observation of Pb-phosphates in pea rhizosphere by SEM image and EDS analysis. **a** for soluble KH_2PO_4 amendment, $Pb_5(PO_4)_3(OH)_2$ (Hydroxypyromorphite) was observed. **b** for solid hydroxyapatite, the following Pb-Ca-P associations were observed: (1) $Ca_{4.5}Pb_{0.5}(PO_4)_3OH$, (2) $Ca_{4.0}Pb_{0.9}(PO_4)_3OH$, (3) $Ca_{3.3}Pb_{1.7}(PO_4)_3OH$, and (4) $Ca_{3.0}Pb_{2.0}(PO_4)_3OH$

Fig. 4 Distribution of mineral phases in a ternary diagram: (Mn+Fe), ▶ (Ca+P), and Pb or Zn, determined by microprobe analyzes. The center of the gray form locates point average composition. *Rays* indicate the standard deviation of the composition. **a** Case of soluble P addition for Pb, **b** Case of solid P addition for Pb, **c** Case of soluble P addition for Zn, and **d** Case of solid P addition for Zn

P amendments to non-rhizospheric soil significantly enhanced Pb-apatite formation.

The chemical composition of the mineral phases in the pea rhizosphere treated with soluble P is close to Ca+P pole indicating a mixture of Pb and Ca+P. This suggests the formation of complex phosphates: Ca-P, Pb-P and/or a mixed Pb-Ca-P. Likewise, addition of solid P to pea rhizosphere resulted in chemical composition of minerals further close to the Ca+P pole compared to non-rhizospheric soil, indicating the increased formation of Pb-P phases. Indeed, the quantification of metal-P complexes (Table 2) shows P amendments changed Pb speciation in favor of the Pb-apatite in rhizospheric soil (>90 % against 0.4 % for the untreated soil); the addition of P amendments also affected the formation and quantification of other Pb-P complexes (decreased from >90 to <1.1 %). The effect of solid P amendment was more intense compared to soluble P in the soils with or without plant
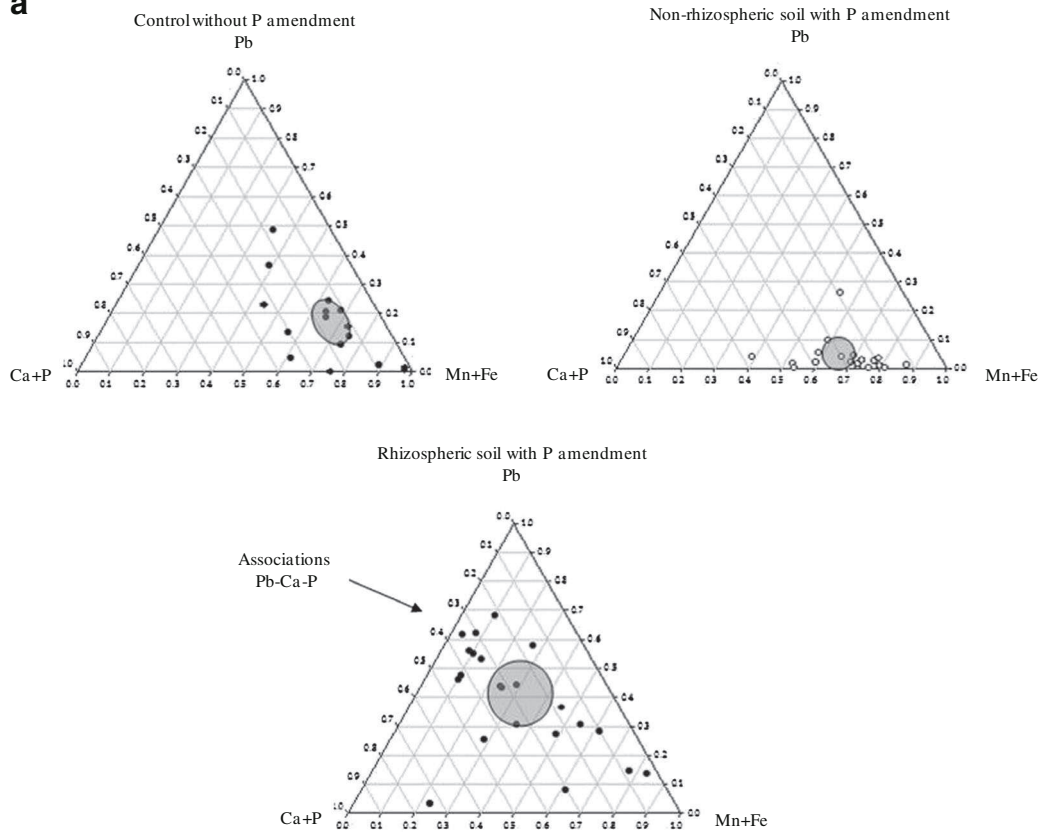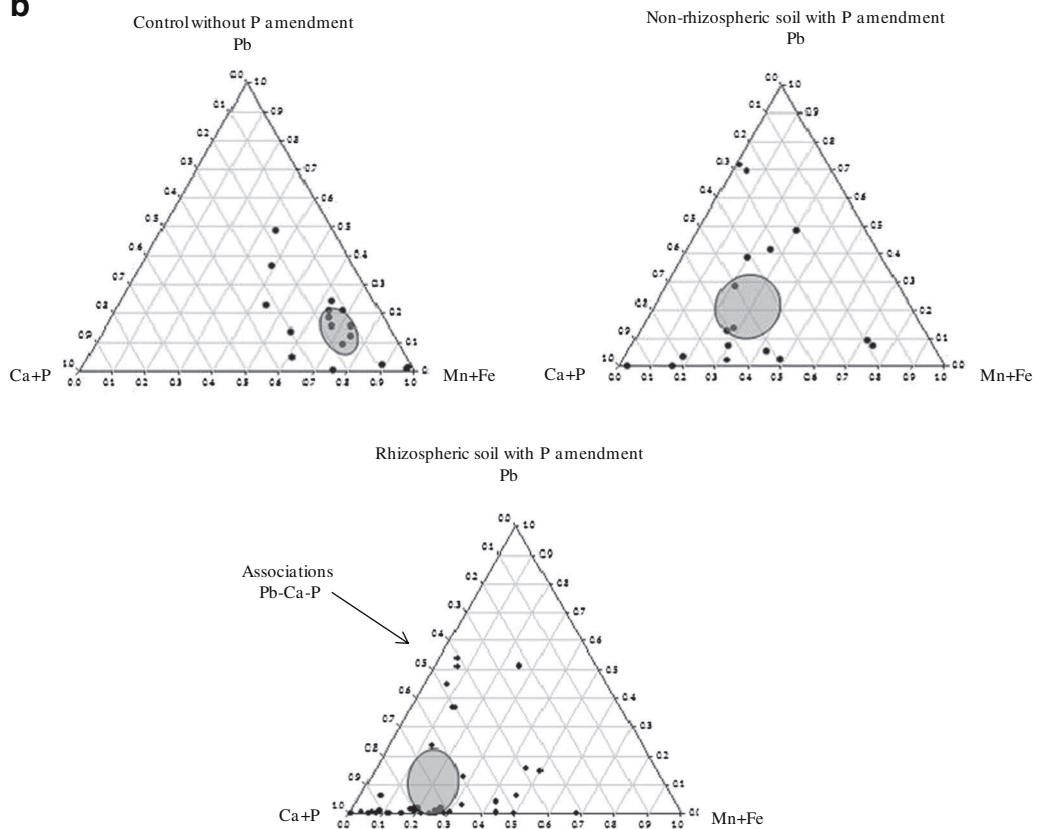
culture. Soluble P amendment enhanced the formation of Pb-pyromorphite whereas solid P amendment favored Ca-pyromorphite formation in rhizospheric soil of both the plants.
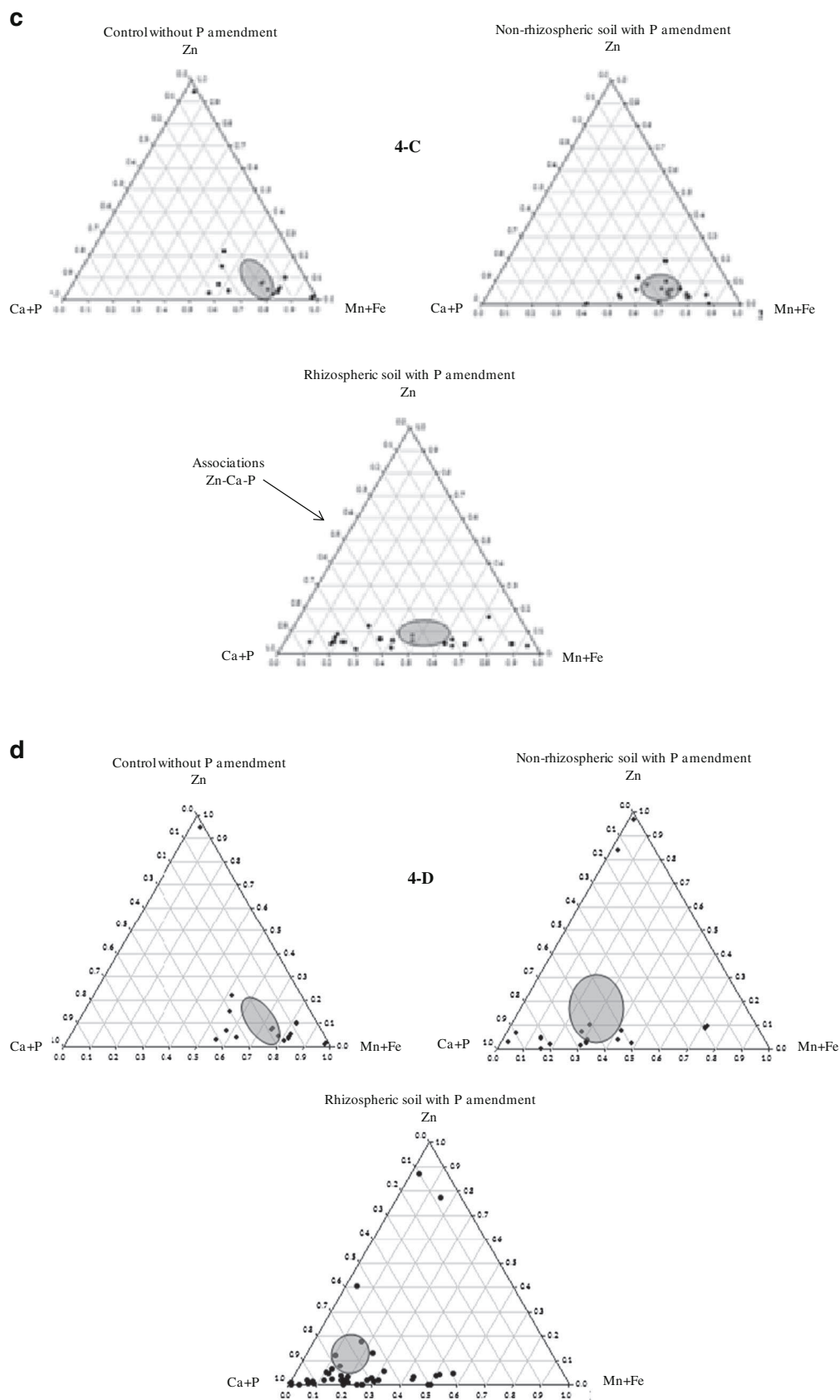
In the case of Zn, non-rhizospheric soil samples (untreated or treated with soluble P) have a distribution similar to that observed for Pb phases (Fig. 4c, d). Both the amendments have no effect on Zn speciation in soils in the absence of plants (Table 2). Moreover, mineral phases in the pea rhizosphere are close to Fe+Mn pole in the presence of soluble P compared to solid P amendment. This shows that pea plants have a low effect on the formation of Zn-P complexes in the presence of soluble P amendment. Addition of solid P resulted in the composition close to the pole (Ca+P), indicating the mobilization of Zn primary phases to phosphates. Indeed, the P amendments allowed to significantly increase the amount of Zn-apatite more abundant than other types of Zn-P complexes (>90 %). These results confirmed our above mentioned findings that plants favor the formation of metal-P complexes.

Our results indicate that the formation of Pb-P depends on applied form of P amendments. The solid P amendment was more effective in forming Pb-P complexes than soluble P amendment. The differences observed between soluble and solid P amendments towards metal-P formation were related to the availability of P and / or Ca, if we assume a liquid phase for the formation of metal-P associations (Shahid et al. 2013d). This could be due to inability of solid P amendments to provide free phosphate because of its low dissolution / solubility in soil (Debela et al. 2013). In contrast, soluble P amendments provide high concentration of available P for the formation of Pb-P minerals. However, it is not always wise to prefer soluble P amendments over solid P amendments because leaching of an excess of available P in the soil amended by soluble P may cause eutrophication of groundwater (Park et al. 2011; Tang and Yang 2012).

Moreover, the presence of plants favors the formation of Pb-P complexes in soil after P amendments. The electron microprobe analysis of phases observed by SEM indicates that phosphates can be pure or mixed with Ca depending on the type of amendments. A number of researchers reported that formation of mineral phases containing Pb or Zn, Ca, and P involves different successive chemical reactions (Jiang et al. 2012; Mignardi et al. 2012). The results presented in the literature clearly support a mechanism of dissolution and precipitation for Pb-P formation in soil after P application (Mignardi et al. 2013; Debela et al. 2013). Phosphate

a

Control without P amendment
Pb

Non-rhizospheric soil with P amendment
Pb

Rhizospheric soil with P amendment
Pb

Associations
Pb-Ca-P

b

Control without P amendment
Pb

Non-rhizospheric soil with P amendment
Pb

Rhizospheric soil with P amendment
Pb

Associations
Pb-Ca-P

c

Control without P amendment
Zn

4-C

Ca+P　　　　　　　　Mn+Fe

Non-rhizospheric soil with P amendment
Zn

Ca+P　　　　　　　　Mn+Fe

Rhizospheric soil with P amendment
Zn

Associations
Zn-Ca-P

Ca+P　　　　　　　　Mn+Fe

d

Control without P amendment
Zn

4-D

Ca+P　　　　　　　　Mn+Fe

Non-rhizospheric soil with P amendment
Zn

Ca+P　　　　　　　　Mn+Fe

Rhizospheric soil with P amendment
Zn

Ca+P　　　　　　　　Mn+Fe

Fig. 4 (continued)

Table 2 Percentage of Pb-phosphates [($Pb_5(PO_4)_3OH$, Ca-pyromorphite and Pb-apatite] and Zn phosphates [($Zn_3(PO_4)_2$ or $Zn_5(PO_4)_3OH$, $CaZn_3(PO_4)_3(OH)$ and $ZnCa_4(PO_4)_3(OH)$] determined by MEB-EDS analysis

Metal-phosphates	Controls			Tomato (1 week)		Pea (1 week)		Pea (3 weeks)	
	Untreated-P control	Soluble P control	Solid P control	Soluble P tomato	Solid P tomato	Soluble P pea	Solid P pea	Soluble P pea	Solid P pea
Pb									
Pb5(PO4)3OH)	0.7 (a)	0.9 (a)	0.3 (a)	2.9 (ab)	0.2 (a)	7.2 (b)	3 (ab)	14 (c)	5 (a)
Ca-pyromorphite	3.1 (ab)	5 (b)	4 (ab)	2 (a)	6 (b)	2 (a)	4 (ab)	8 (c)	6 (b)
Pb-apatite	0.4 (a)	0.2 (a)	6 (b)	94 (c)	93 (c)	90.8 (c)	92 (c)	78 (c)	89 (c)
Other Pb-P	95.8 (d)	93.9 (d)	89.7 (d)	1.1 (a)	0.8 (a)	0	1 (a)	0	0
Zn									
Zn5(PO4)3OH	0	0	0	3 (a)	1.3 (a)	7.4 (c)	2 (a)	18 (b)	4 (a)
CaZn3(PO4)3(OH)	0.4 (a)	0.3 (a)	0.3 (a)	2.3 (a)	6 (b)	1.6 (a)	3 (a)	14 (b)	4 (a)
Zn-apatite	9.2 (b)	4.7 (b)	8.8 (b)	95 (c)	93.2 (c)	92 (c)	95 (c)	68 (c)	92 (c)
Other Zn-P	90.4 (d)	95 (d)	90.9 (d)	0	0	0	0	0	0

One week and 3 weeks indicate exposure duration of P amendment; Rhizospheres (pea or tomato), after P amendment (soluble or solid), contact time 1 week, are compared with non-rhizospheric soil treated or untreated by P amendments. Unclassified phosphate forms: if Pb/P>1.5 and Zn/P>1.5. Moreover, for pea, data are also given after 3 weeks of soil-plant contact (Pea-3w) in addition to 1 week data. The different letters identify a significant difference at $p < 0.05$

amendments also participate in the immobilization of Zn to form Zn-P (Raicevic et al. 2009). In particular, the fixation mechanism involves the formation of Zn-P on the surface of the amendment grains, followed by the diffusion of Zn ions into the P structure. The co-precipitation of these metals to form polymetallic phosphate was also observed by Crannell et al. (2000).

3.3 Effect of soil-plant contact time on metal-P formation

The influence of soil-plant contact time for P amendments was only tested in the case of pea plant only. The percentages of Zn and Pb-phosphates formed after 3 weeks of contact time were compared to the shorter period (1 week). Addition of soluble P amendment increased the formation of Pb-pyromorphite (from 7 to 14 %) and Ca-pyromorphite (from 2 to 8 %) after 3 weeks compared to 1 week contact time. Similarly, solid P

amendment also increased Pb-pyromorphite from 3 to 5 % and Ca-pyromorphite from 4 to 6 %. Moreover, the fraction of Pb-apatite decreased from 91 to 78 % for soluble and from 92 to 89 % for solid P amendment. For Zn, the trend is similar but less effective to that observed for Pb. These results indicate that a longer soil-plant contact time promotes the formation of stable Pb-P. Cao et al. (2009) also reported that water solubility of metals decreased with soil-metal contact time (aging) due to formation of stable and insoluble metal-P minerals. The key factors involved in the formation of stable metal-P minerals are the availability of free P and metals in soil solution in addition to soil physico-chemical characteristics (Debela et al. 2013). It is proposed that plants favor the formation of stable metal-P association by secreting organic acids. The high efficiency of soluble P amendment than solid P amendment to promote metal-P association with time could be probably due to availability of more soluble phosphate owing to its high dissolution (Shahid et al. 2013d).

Table 3 The pH ($CaCl_2$) measured for different types (solid and soluble) and amounts (0, P, 3P) intake of phosphate in the rhizosphere soils (peas and tomato) and controls (P=0, non-rhizosphere soils

Amendments	Pea rhizosphere	Tomato rhizosphere
P=0	5.5±0.8 (a)	5.5±0.8 (a)
P soluble	5.4±0.9 (a)	5.4±0.7 (a)
3P soluble	5.1±0.9 (a)	5.7±0.7 (a)
P solid	5.5±0.6 (a)	5.1±0.8 (a)
3P solid	4.3±1.0 (b)	4.7±0.6 (ab)

Different letters indicate that pH values are significantly different at $p < 0.05$ according to the LSD test

Table 4 Percentage of P released from the apatite (after soluble P addition) in the supernatant under the effect of citric or tartaric acids (at concentrations observed in rhizospheres of pea and tomato)

Sample	Apatite+ water	Apatite+ CA Pea	Apatite+ CA Tomato	Apatite+ TA Pea	Apatite+ TA Tomato
P release (%)	0.2	12	6	32	25

The results obtained for apatite mixed with deionized water (control) are also presented

Table 5 Percentages of P, Pb, and Zn released from the polluted soil+ soluble P+citric acid in representative quantities of pea or tomato

Sample	Soil, soluble P	Soil, soluble P +CA-pea	Soil, soluble P +CA-tomato
P release (%)	0.1 (a)	15 (c)	12 (b)
Pb release (%)	0.1 (a)	34 (c)	26 (b)
Zn release (%)	0.05 (a)	21 (c)	18 (b)

The results obtained for the polluted soil+soluble P and deionized water (control) are also presented. Different letters indicate that pH values are significantly different at $p < 0.05$ according to the LSD test
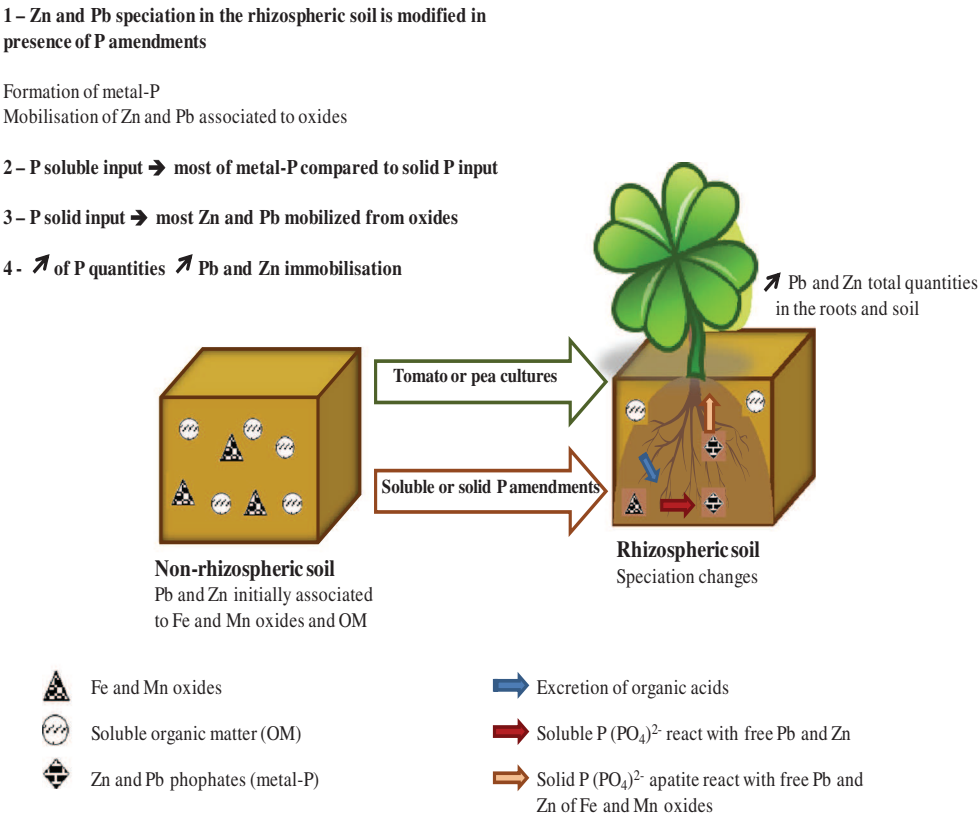
3.4 Effect of P amendments on soil pH

Table 3 presents the change in the rhizospheric pH of pea and tomato after the addition of P amendments at two levels (9,000 and 27,000 mg P kg^{-1} of soil for $Ca_5(PO_4)_3OH$ and 120 and 360 mg P kg^{-1} of soil for KH_2PO_4). The results show that neither the type nor the concentration of P amendments affects the pH significantly, except for the rhizospheric soil of pea after 2,700 mg P kg^{-1} (3P) of solid amendments where pH was significantly decreased. The absence of significant differences in pH for pea and tomato invalidates the hypothesis of an effect of pH on the formation of metal-P. Valsami et al. (1998) showed that the dissolution of apatite

([P]=9,000 mg kg^{-1}) in the presence of Pb (450 mg kg^{-1}) first decreased soil pH from 5.5 to 4.4 and then increased to 5. They explained this behavior by the formation of Pb-phosphates hydroxypyromorphite. In our case, the pH did not change for solid and soluble P addition which may indicate the presence of a soil buffering effect. Recently, Jiang et al. (2012) reported that variation in soil solution pH from 2.0 to 5.0 has no effect on metal-P complex formation. Decrease in soil pH after the addition of solid phosphate at a concentration 27,000 mg kg^{-1} (3P), can be explained by the competition between Pb^{2+}, Ca^{2+}, and H^+ on the adsorption sites which results in the release of H^+ in the soil solution (Mavropoulos et al. 2002).

3.5 Role of the pea and tomato plants in the change of metal speciation

The amounts of low molecular weight organic acids excreted by the two plants species were measured for various experimental conditions. The pea plants excreted more citric and tartaric acids in comparison to tomatoes, 0.715 and 0.184 μM of citric acid and 2.747 and 0.056 μM of tartaric acid, respectively, for rhizospheric soils of pea and tomato. Other acids such as lactate, malonate, malate, and glycolate aconate were excreted in minor amounts. The effect of citric and tartaric acids, at quantities excreted by pea or tomato rhizospheres, on

Fig. 5 Schematic overview of the main soil-plant interactions observed

1 – Zn and Pb speciation in the rhizospheric soil is modified in presence of P amendments

Formation of metal-P
Mobilisation of Zn and Pb associated to oxides

2 – P soluble input ➜ most of metal-P compared to solid P input

3 – P solid input ➜ most Zn and Pb mobilized from oxides

4 - ↗ of P quantities ↗ Pb and Zn immobilisation

↗ Pb and Zn total quantities in the roots and soil

Tomato or pea cultures

Soluble or solid P amendments

Non-rhizospheric soil
Pb and Zn initially associated to Fe and Mn oxides and OM

Rhizospheric soil
Speciation changes

▲ Fe and Mn oxides

⊘ Soluble organic matter (OM)

⊕ Zn and Pb phophates (metal-P)

➡ Excretion of organic acids

➡ Soluble P $(PO_4)^{2-}$ react with free Pb and Zn

➡ Solid P $(PO_4)^{2-}$ apatite react with free Pb and Zn of Fe and Mn oxides

the release of P was tested in unpolluted control and metal-contaminated soil (Tables 4 and 5).

In unpolluted soil, citric acid significantly ($p<0.05$) enhanced the amount of P released compared to control (12 and 6 %, respectively, for pea and tomato compared to 0.2 % for control) (Table 4). The effect of tartaric acid on P release (32 and 25 %, respectively, for pea and tomato) was more significant compare to citric acid. In polluted soil, the release of P, Pb, and Zn increased from 0.1, 0.1, and 0.05 % to respectively 15, 34, and 21 % for pea and 12, 26, and 18 % for tomato. The results suggest that citric acid caused the dissolution of apatite which results in the release of P. Organic acids are reported to enhance metal mobilization (Shahid et al. 2011; Niazi et al. 2011a) and mineral weathering by supplying both the protons and metal complexing anions (Fig. 5). Weng et al. (2002) reported release of P after mixing apatite and citric acid. The amounts of phosphorus released into solution by the apatite can then react with Pb and Zn to form metal-P complexes (Jiang et al. 2012). The proposed mechanism can be responsible for the enhanced formation of metal-P complexes by pea than tomato due to increased release of organic acids by pea (Fig. 5). There are at least two mechanisms by which phosphorus can be released under the influence of organic acids (Bolan et al. 1994). The first is the direct exchange of ligands on the mineral surface (e.g., on the surface of $Al(OH)_3$, $Fe(OH)_3$, the organic acid replaces P adsorbed on the surface). The second is the complexation of the metal cation in the solid phosphate phase implying P mobilization (e.g., Ca^{2+} in phosphate rock and precipitation of Ca-citrate or Fe^{3+} to $Fe(OH)_3$). Generally, these two mechanisms occur simultaneously. Desorption and release of phosphorus however highly depends on soil type and concentration of organic acids (Bolan et al. 1994).

4 Conclusions

The coupling of chemical and spectroscopic methods allowed us to identify the metal-P formation based on their size and chemical composition in the rhizospheric soil. The maps produced by μ-XRF, indicate that the soil-root transfer of Zn is larger than that of Pb. The metal-P associations are mainly Pb-Zn-apatite or apatite, Ca-pyromorphite or similar for Zn and phosphate pyromorphite or pure Zn. The different forms of metal-P formed indicate that several mechanisms are involved in metal immobilization. Presence of plants significantly increases the formation of stable metal-P complexes by secreting the LMWOAs. The type and nature of metal-P complexes changed with the soil-metal contact time. We observed biggest metal-P complexes with increased soil-metal contact time. It is proposed that the contact time effect was also due to potential excretion of organic acids in the soil to promote the formation of stable and coarser metal-P solid phases.

With the development of gardening activities on strongly anthropogenic urban soils and Pb being often a major pollutant, the development of cultivation techniques that guarantee the quality of the harvest is an important research pursuit. The data obtained in this study provides avenues of research to reduce transfers of pollutants to plants commonly consumed in the gardens and reduces in consequence the human exposure to metals.

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