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Mobility and bioavailability of selected trace elements in Mediterranean red soil amended with phosphate fertilizers: Experimental study

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ABSTRACT

Trace element (TE) distribution and mobility were monitored in a typical profile of Mediterranean red soil massively amended with phosphate fertilizers (PFs), over a period of 15 months. Samples of soil (across a depth of 55 cm) and entire plants (roots and shoots), collected at different points in time, in addition to PF samples, were analyzed for TEs (Cu, Cd, Zn, and Pb) concentrations, sequentially extracted, and examined under a Scanning Electron Microscopy (SEM) coupled with an Energy Dispersive X-ray Spectrometer (EDX). Results showed peaks in TE mean concentrations (Cu: 43.13, Cd: 0.52, Zn: 116.36, and Pb: 14.92 mg kg^{-1}) in the soil profile 4 months following PF application, but they did not reach harmful levels. Sequential extractions revealed that the studied TEs were transferred from residual to exchangeable fractions in the amended soil profile (except for Cd), which may consequently lead to their transfer to the saturated zone. The order of TE mobility was: Zn > Cd > Cu > Pb. Amended-soil plants, *Cichorium intybus* L, accumulated higher TE concentrations (Cu: 9.67, Cd: 0.37, Zn: 13.81, and Pb: 2.58 mg kg^{-1}) than the reference plants, but they remained within normal reported levels for plants (Cu: 3–20, Cd: 0.5–1.0, Zn: 15–150, and Pb: 2–5 mg kg^{-1}). Soil–plant transfer factor was notably affected by PF application, with highest acquired values being for Cd. Evidence of fluorine presence was detected by SEM in the amended soil, which should be a matter of concern in PF application.

1. Introduction

Large application of fertilizers has been practiced on arable lands, to improve crop production and recover inherent and induced soil nutrient deficiency. Contamination of agricultural soils with trace elements (TEs), such as Cd, Pb, Zn and Cu, and with fluorine can occur as these elements are transferred during manufacturing from phosphate rock to phosphate fertilizers (PFs) (Camelo et al., 1997). Cd and Pb are unessential elements, having no specific biological functions. They are considered toxic elements, whereas Zn and Cu are micronutrients that could become toxic when exceeding certain limits. Concentrations of Zn, Cu, Cd and Pb can vary in the ranges of 50–600; 10–60; 9–100; 0.5–40 mg kg^{-1} in PFs (Adler, 2001), depending on the origin of phosphate rock. Generally, sedimentary phosphate rocks (e.g. apatite) contain higher concentrations of TEs than igneous rocks (Van Kauwenbergh, 2002).

The effect of continuous application of PFs has been assessed in long term experiments conducted in many studies worldwide (Adriano, 1986; Guttormsen et al., 1995; Jeng and Singh, 1995; Loganathan et al., 1995; Malavolta, 1994; McLaughlin et al., 1996; Morvedt, 2005). Cd has been the most concerned element in PF application, since it can accumulate in relatively large amounts in soil and plants, which could be harmful to human health (Kirkham, 2006; McLaughlin et al., 1996). Lambert et al. (2007) studied the solubility of Cd and Zn, following the application of PFs in different rates and with different concentrations of these two elements. They found that when both the application rate of PFs and Cd concentration in PFs increased, the content of Cd in soil extracts increased, whereas such relationship was not confirmed for Zn (i.e., increasing the PF application rate and using PF with higher Zn concentration did not result in increased Zn level in soil). Furthermore, a competition between Zn and Cd was reported, leading to Cd increase in the soil extracts. Morvedt (2005) found that a minimal increase of Cd total concentration in soil occurred in short term, leading to higher absorption of this element by leaf vegetables compared to maize or wheat. A study in Norway established that Cd tend to accumulate in the soil after long term (70 years) and abundant treatment with PFs (Jeng and Singh, 1995). Applying PFs at a rate inducing a Cd input of

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0.07–28 g ha^{-1} y^{-1} in an experimental field, increased Cd uptake by cultivated plants (Guttormsen et al., 1995)–for locally manufactured PF containing Cd in a concentration of 6.18 mg kg^{-1}, the equivalent PF rate is 11.32–4350 kg ha^{-1}y^{-1}. On the other hand, different studies showed that applying PF with a Cd input of 0.3–1.2 g ha^{-1} y^{-1}(equivalent to locally made PF rate of 48.50–194.20 kg ha^{-1}y^{-1}) did not lead to such result (Singh and Myhr, 1997). The average use of fertilizers on arable lands in Lebanon is1860 kg ha^{-1}y^{-1}(Farajalla et al., 2010).

Earlier studies showed that PF application in recommended rates on Brazilian soils did not increase TE concentrations to hazardous levels, in short and medium terms (De Conceicao and Bonotto, 2006; Malavolta, 1994). These studies pointed out that the cause of such “safe” increase could not be related to PF application.

On the other hand, a recent study carried by Kassir et al. (2011a) revealed that Cd was the most susceptible element in Mediterranean red soil and associated Chicory plants, when soil was amended with phosphogypsum by-product obtained from PF processing. In addition, a study on the impact of phosphate fertilizer industry on local soil contamination showed that deposited contaminants contained in the industry's emissions were potentially hazardous to plants and ground waters (Kassir et al., 2011b).

To evaluate the potential risk of TEs in PF-amended soils, quantification of different forms and phases of TEs, referring to their speciation, must be performed, in order to assess their mobility in the soil profile, influencing their availability to plants and their transport to groundwater, and thus to food and ecological chains. Extraction procedure (or sequential extraction) has been commonly used in the literature, whereby ascending aggressive reagents are applied to solubilize five different chemical forms (exchangeable, acid-soluble, reducible, oxidizable and residual) (Tessier et al., 1979; Ure et al., 1993). The release or the seizing of TE in a defined phase depends on soil properties such as pH and other soil properties (Kabala and Singh, 2001).

The main purpose of this work is to assess the effects of PF amendment on Mediterranean red soil, in order to reveal any associated environmental hazards, and thus possible contingency measures could be established. Therefore, in relation to soil mineralogical composition, four objectives were set: (1) to determine the total concentrations of TEs in the amended-soil profile, and their variation with time, (2) to evaluate TE mobility in amended soil using sequential chemical fractionation (3) to determine the TE concentration variation in the amended-soil plant roots and shoots, and (4) to carry out microscopic analyses of soil samples in attempts to spot TE mineralogical species and other suspected minerals of environmental concern, such as fluorine, in the amended soil matrix.

2. Materials and method

2.1. Field survey and soil sampling

The study area, where the experimental site was selected, is located off the east Mediterranean coast, in north Lebanon (Fig. 1), and it extends over 100 km^2 of agricultural lands. It is almost surrounded by mountainous land, notably from the east. The region has a Mediterranean climate with intensive precipitations between January and May (600–900 mm). Meteorological data for the sampling period (May 2008–November 2009) were obtained from the Meteorological Department at Beirut airport. Residential, commercial or industrial sites are rare in this area; while, agricultural land (47% of the study area) is more widespread than natural vegetation. Agriculture is mainly dominated by plantation of olive trees, which is the main crop of the study area. According to Darwish et al. (2005), the studied region is dominated by well drained red soil classified in association with Gleyic and Vertic Luvisols. The texture of the soil is clayey (sand 24%, silt 20%, and clay 56%) with a calcium carbonate content reaching 22%. Despite its loamy texture, the Mediterranean red soil is distinguished by a solid granular surface structure and porosity promoting intermediate and high soil permeability. Drainage course is improved by dominant gravel content and the type of underlying rocks, which are known for fissures and karsts (Darwish and Zurayk, 1997). Soil profile is constituted of three horizons (A, B and C). The soil is relatively deep with a thickness of A and B horizons reaching 55 cm. Stoniness is common with 30 to 40%. Medium to coarse subangular and angular blocky aggregates represent the strong structure of the soil. The organic matter content is relatively low (1.3%). Common fine to medium roots are also found. The common plants growing between olive trees were of *Cichorium intybus* species.

The experimental site consisted of a land of 256-m^2 area. A study plot (7 m × 7 m) was divided into 24 individual parcels of 1 m^2 each, separated by distances of 75 cm. Each parcel was delineated with a wooden frame of 1 × 1 × 0.15 m (length × width × height). The plot was fenced and gated as to restrict its accessibility, and prevent possible cattle intrusion.

To simulate the potential source of pollution originating from PF-amended soils, an opulent amount of 2 kg of PF, collected from the Lebanese fertilizer plant, was uniformly scattered on the soil surface

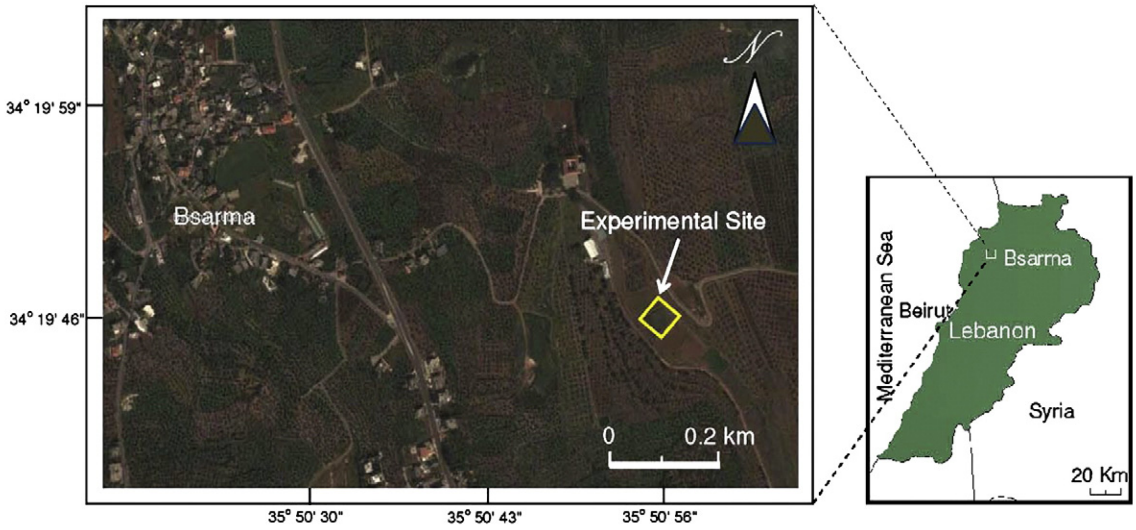

Fig. 1. Experimental site localization map.

in every parcel over a surface of 0.5 m × 0.5 m without mixing with the soil. This is equivalent to applying PF at an intensity of 8 kg m^{-2} (80,000 kg ha^{-1}), about forty times higher than the fertilizer yearly use of 1860 kg ha^{-1} on arable soils in Lebanon (Farajalla et al., 2010). The purpose of this abundant application was to subject the soil to a leveraged state of influence induced by PF application, so as extreme ecological responses to such stimulus could be reached and measured. The initial PF application was done in October 2008. A reference parcel was left without amendment.

Coring was carried out to a depth of 60 cm, using a high power (2500 W) motor of type Cobra TT. The core shaft used was 100-cm long, 6-cm in diameter. The first soil sample was cored from the reference parcel (i.e., not amended with PFs) labeled as R. Then, sample coring had been successively performed in three different parcels labeled as P1, P2 and P3 at three different subsequent dates: T1, T2, and T3—four, eleven, and fifteen months, respectively, after the initial application and sampling from the reference plot R at T0, allowing a full plant growing cycle. Sampling times with corresponding weather data (temperature, humidity and rain) for each coring campaign are indicated in Table 1. Samples were collected from the cored cylindrical soil blocks at 5 cm intervals to a depth of 55 cm. Plant roots (extended to a depth of about 20 cm) and leaves were also collected simultaneously with soil samplings, from each parcel.

2.2. Sample preparation and analytical methods

Soil samples were cleaned from roots and stones, oven-dried at 50 °C, sieved to pass a 2 mm sieve, and ground to a fine powder using an agate pestle and mortar, while, vegetation samples were washed in ultra-pure water, dried at 60 °C and ground to a fine powder using an agate pestle and mortar.

2.2.1. Soil pH

Soil pH was determined in a ratio of 1:5 (soil:ionized water suspension) according to AFNOR X 31-103.

2.2.2. Soil and PF total concentrations

TEs in soil and PF samples were measured using an Iris Advantage ERS Inductively Coupled Plasma Mass Spectroscopy (ICP-MS X7) “ThermoFischer”, after melting the samples with lithium metaborate, and dissolution of the melt in diluted nitric acid. Analysis was conducted in an accredited laboratory SARM (CRPG, Nancy).

2.2.3. Vegetation analysis

Vegetation samples were accurately weighed to 0.25 g directly in microwave PTFE vessels, and then subjected to 1 mL H2O2 (30%) and 9 mL concentrated HNO3 (65%). The digestion program itself consisted of a 10 min gradual increase in temperature to 200 °C, a 15 min step at 200 °C (1000 W; 106 Pa) and then a stage of ventilation cooling. The digests were filtered through a 0.45 μm and analyzed using an Atomic Absorption Spectroscopy (AAS) “Analytical Gena Zeenit 700” to determine the content of Pb, Zn, Cu and Cd in roots and plant leaves.

2.2.4. Speciation of soil and PF samples

A five-stage sequential extraction method was performed on soil and PF samples using modified Tessier sequential extraction (Tessier et al., 1979). Soil samples from each of 0–20 cm, 20–35 cm, and 35–55 cm depth intervals were mixed and homogenized. The extractable contents of trace elements, from an initial 5 g aliquot, were determined; five TE fractions were separated in five steps as follows:

(1) Exchangeable fraction (F1) was extracted with 40 mL of 1 M $Mg(NO_3)_2$ (Shuman, 1979);
(2) acid soluble fraction (F2) with 40 mL of 1 M CH_3COONa adjusted to pH 5 with CH_3COOH;
(3) reducible fraction (F3) with 20 mL Na-citrate (78,4 g L^{-1}) and 20 mL $NaHCO_3$(9.82 g L^{-1}), then adding 1 g of Na_2S2O_4 (80 °C for 4 h) (Mehra and Jackson, 1960);
(4) oxidizable fraction (F4) by adding 8 mL HNO_3 (0.02 M) and 20 mL of H_2O_2 (35%) in small aliquots, then 12 mL of 3.2 M CH_3COONH_4 in nitric acid (85 °C for 2 h); and
(5) residual fraction (F5) was extracted with 40 mL HNO_3 (65%) (Hudson-Edwards, 1997; Ribeiro and Mexia, 1997).

Each extraction step was followed by centrifugation of the mixtures at 5000 rpm for 30 min, and the solutions were separated from the solid residues. Liquors were first filtered using a membrane filter of ester cellulose of 0.45 μm porosity. All the solutions were stored in polyethylene vials at 4 °C pending subsequent analyses.

Table 1
Sampling times and weather data during periods between samplings.

Time	Temperature (°C)		Relative humidity (%)		Wind maximal		Rain	Cumulative rain
	Min	Max	Min	Max	dd (°)	ff (m/s)	(mm)	(mm)
Amendment								
Oct. 08	14.1	35.8	27	91	280	13	75.7	429.2
Nov. 08	9.8	26.9	18	92	340	13	85.1	
Dec. 08	4.1	23.5	32	94	340	23	167.9	
Jan. 09	2.9	21.3	27	94	120	13	100.5	
P1 samples								
Feb. 09	6.8	22.1	14	99	300	23	183.4	342.8
Mar. 09	6	26.5	21	98	90	22	115.8	
Apr. 09	8.4	32.2	13	99	240	22	39.6	
Mai 09	12.8	36.5	10	98	240	26	2.4	
Jun. 09	16.2	36.3	24	99	230	14	1.6	
Jul. 09	19.8	36.7	16	96	260	17	0	
Aug.09	22.1	34.4	34	88	240	11	0	
P2 samples								
Sep. 09	19.8	32.4	37	87	280	17	2	327.4
Oct. 09	15.3	36.6	28	88	300	15	72	
Nov. 09	10.1	26.3	17	94	320	14	81.2	
Dec. 09	3.9	22.7	35	91	380	18	172.2	
P3 samples								
Jan. 10	3	20.4	29	93	180	12	118.3	

Analyses were carried out by AAS to determine the content of Pb, Zn, Cu and Cd in each extracted fraction.

2.2.5. Quality control

In order to monitor the accuracy and precision of the used analytical methods, reference materials SRM2711 (soil), CMI7004 (soil), and NCS DC73349 (bush branches and leaves) were analyzed for Cu, Pb, Zn and Cd total concentrations, using the same described methods for soil and vegetation sample analyses. Results were within −9.57% to +10.95% of the reference mean values.

The accuracy of the sequential extraction procedure was assessed by comparing the sum of the five TE fractions obtained from the procedure with the total TE concentration. The percent recovery of the sequential extraction procedure was calculated as follows:

$$Recovery\% = (F1 + F2 + F3 + F4 + F5/Total\ element\ analysis) \times 100, \text{where F is the TE fraction.}$$

2.3. Mineralogical analysis

X-ray Diffractograms (XRD) were recorded using a D8-Brucker diffractometer (cobalt radiation source, $\lambda = 1.788965$ Å). The diffractometer is equipped with a (θ, 2θ) goniometer and a position sensitive detector. Reflexions were collected in ambient conditions within the [3–65°] 2θ range, with a step width of 0.036° and a 3 s collecting time. About 500 mg of homogenized phosphogypsum, ground reference soil samples were deposited as sub-compacted powder within a thin layer of 1 mm thickness onto a plexiglass disc 2 cm in diameter.

Electron microscopy observations were performed with a S-2500 Hitachi SEM (Scan Electron Microscopy) equipped with a Kevex 4850-S EDX (energy dispersive X-ray Spectrometer). Trace elements carriers were identified from elemental analysis of individual particles. Stoichiometric ratios were first calculated from atomic percentages given by EDX spectra, and then compared with known mineralogical compositions. For SEM imaging and microanalysis, the powder sample was re-suspended in ethanol under ultrasonication, and a drop of suspension was evaporated on a carbon-coated copper grid (EuroMEDEX, Mesh200).

Samples for SEM-EDX examination were sprinkled onto 2 cm^2 plates and carbon coated. Backscattered Electron Imaging (BEI) was used to identify the particles of interest. In that mode, brightness is related to the average atomic number of materials, and the mineral particles appear as bright spots within the matrix of sediments. The relative abundance of a given heavy metal carrier can then be assessed by conducting systematic microanalysis of bright spots. It should nevertheless be noted that this procedure largely overlooks mineral phases with low atomic number elements. In order to increase the emission of backscattered electrons, the SEM microscope was generally operated with a beam current of 3 pA and an accelerating voltage of 20 keV.

2.4. Statistical analysis

ANOVA tests were performed on the samples data of trace and major element concentrations and pH values obtained for the soil profile in all parcels. The data was distributed in different levels of two categories; time (T0, T1, T2, and T3, corresponding to parcels R, P1, P2, and P3) and depth (horizons 0–5 cm, 5–10 cm, etc.). When significant differences were found, a multiple comparison of mean values was carried out by the Walker-Duncan test ($P<0.05$). Normality of variances was examined by the Shapiro–Wilk test, before running ANOVA. Correlation analysis (Pearson r) was carried out between trace and major elements, TE concentrations and soil characteristics. All statistical analyses were performed using SPSS Version 17.

3. Results and discussion

3.1. Soil background values: reference parcel

The mineralogical results (XRD) showed that the background soil contained quartz (SiO_2), calcite ($CaCO_3$), clay minerals (montmorillonite, kaolinite), anatase (TiO_2) and hematite (Fe_2O_3). The soil pH showed alkaline range (8.23 ± 0.12), and the cation exchange capacity (CEC) exhibited relatively high values (31.06 ± 0.5 cmol kg^{-1} of dry weight) with a dominance of calcium ions at the exchange sites.

The average background soil contained relatively low Cd concentration (0.30 ± 0.02 mg kg^{-1}) and high Zn concentration (103.35 ± 6.72 mg kg^{-1}). As for Cu and Pb, their average concentrations were found to be 35.84 ± 2.33 mg kg^{-1} and 11.50 ± 0.75 mg kg^{-1}, respectively. No significant differences were found in TE distribution with depth (till 55 cm), except in layer (30–55 cm) where Cu and Pb concentrations decreased with respect to upper layers (Table 2).

It follows that, the study area presented elevated Zn total concentrations and slightly elevated Cu concentration when compared to the world agriculture soils (20–30 mg kg^{-1} and 50 mg kg^{-1} for Cu and Zn, respectively) (Alloway, 1995). Pb and Cd concentrations were within the range of agricultural and normal soils (10–30 mg kg^{-1} and 0.2–1 mg kg^{-1} for Pb and Cd; respectively) (Alloway, 1995; Baize, 1997). Moreover, the total Zn and Cu concentrations exceeded the background values (12 ± 2 and 64 ± 2 mg kg^{-1} for Cu and Zn, respectively) reported for the arable soils of North Lebanon (Nsouli et al., 2004). In general, the natural occurrence and concentration of the studied elements were homogenous with depth. However, Cu and Pb concentrations decreased in the deeper layer (30–55 cm).

Chemical speciation of the studied TEs in the reference soil showed that they were mainly associated with Fe and Mn oxides and hydroxides (reducible fraction F3) (64, 45, 43 and 13% for Cd, Pb, Zn and Cu respectively) and bound to the structure of clays (residual fraction F5) (63, 56, 23 and 11% for Cu, Zn, Pb and Cd respectively). This partition of the studied elements is due to the soil nature (Luvisol) with a dominance of swelling–shrinking clays and iron oxides, which agrees with the findings of previous works (Darwish et al., 1988; Lamouroux et al., 1968; Sayegh et al., 1990). In addition, Cu was accumulated in the oxidizable fraction (F4) (20%), in agreement with other findings reported in the literature (Kuo et al., 1983; Zaccone et al., 2007).

3.2. Characterization of PF amendment

XRD results of PF showed that the main peak recorded in the diffractogram was calcium phosphate ($Ca(H_2PO_4)_2.H_2O$) and to less extent anhydrite ($CaSO_4$). The presence of $CaSO_4$ in PF is the result of the acidulation of phosphate rock with H_2O and H_2SO_4. The PF sample showed an acidic pH value of 2.63 ± 0.8.

TEM elemental mapping of PF showed particles of phosphorus, sulfur and calcium. Ca was associated with sulfur ($Ca/S = 1$) and with phosphorus ($Ca/P = 0.5$). Particles of gypsum ($CaSO_4$) and acidulated P-fertilizers ($Ca(H_2PO_4)_2$) were thus identified.

The total concentration of the studied elements (Cu, Zn, Pb and Cd) in the Lebanese manufactured PF showed that Zn was the most abundant TE (199.3 mg kg^{-1}), with the lowest concentration being for Pb (2.66 mg kg^{-1}). Copper and cadmium concentrations were found to be 16.62 and 6.18 mg kg^{-1}, respectively. These results were generally within the average metal concentrations analyzed from a total of 196 European PFs (Cd, 7.4; Zn, 166; Pb, 2.9 mg kg^{-1}) (Nziguheba and Smolders, 2008).

Results of PF speciation showed that the studied metals (Zn, Cd, Cu and Pb) were mainly present in the exchangeable fraction (F1) (60%,

Table 2
Trace element concentrations and pH values in soil parcels R, P1, P2, and P3 at different depth intervals with ANOVA test for concentration and pH variations (p is for significant difference with time and p′ with depth).

	Depth (cm)	R (mg/kg)	±SD	P1 (mg/kg)	±SD	P2 (mg/kg)	±SD	P3 (mg/kg)	±SD	p (ANOVA)	Parcel average	±SD	p′ (ANOVA)
Cu	**0–5**	38.29	±2.49	40.54	±2.64	19.08	±1.24	36.08	±2.35	**0.008****	33.50	±2.18	0.144
	5–10	37.05	±2.41	39.36	±2.56	34.44	±2.24	29.95	±1.95	0.153	35.20	±2.29	
	10–15	36.03	±2.34	36.81	±2.39	35.74	±2.32	37.89	±2.46	0.916	36.62	±2.38	
	15–20	36.88	±2.40	48.45	±3.15	36.24	±2.36	37.57	±2.44	0.078	39.79	±2.59	
	20–25	38.47	±2.50	48.45	±3.15	39.37	±2.56	38.47	±2.50	0.142	41.19	±2.68	
	25–30	38.47	±2.50	44.78	±2.91	37.89	±2.46	38.65	±2.51	0.333	39.95	±2.60	
	30–40	30.77	±2.00	44.78	±2.91	36.35	±2.36	42.65	±2.77	0.054*	38.64	±2.51	
	40–55	30.77	±2.00	41.88	±2.72	41.17	±2.68	43.30	±2.81	0.078	39.28	±2.55	
	Depth average	35.84	±2.33	43.13	±2.80	35.04	±2.28	38.07	±2.47	**0.000****	38.02	±2.47	
Cd	**0–5**	0.30	±0.02	0.41	±0.03	0.83	±0.05	0.59	±0.04	**0.002****	0.54	±0.03	**0.000****
	5–10	0.31	±0.02	1.05	±0.07	0.40	±0.03	0.52	±0.03	**0.001****	0.57	±0.04	
	10–15	0.31	±0.02	0.86	±0.06	1.28	±0.08	0.95	±0.06	**0.002****	0.85	±0.06	
	15–20	0.30	±0.02	0.37	±0.02	0.32	±0.02	0.31	±0.02	0.218	0.32	±0.02	
	20–25	0.32	±0.02	0.37	±0.02	0.28	±0.02	0.27	±0.02	0.073*	0.31	±0.02	
	25–30	0.32	±0.02	0.39	±0.03	0.30	±0.02	0.34	±0.02	0.156	0.34	±0.02	
	30–40	0.27	±0.02	0.39	±0.03	0.26	±0.02	0.30	±0.02	**0.032****	0.30	±0.02	
	40–55	0.27	±0.02	0.31	±0.02	0.36	±0.02	0.27	±0.02	0.080*	0.30	±0.02	
	Depth average	0.30	±0.02	0.52	±0.03	0.50	±0.03	0.44	±0.03	0.066*	0.44	±0.03	
Zn	**0–5**	99.46	±6.46	108.40	±7.05	52.73	±3.43	105.90	±6.88	**0.009****	91.62	±5.96	**0.030****
	5–10	99.84	±6.49	134.00	±8.71	94.59	±6.15	83.43	±5.42	**0.024****	102.97	±6.69	
	10–15	106.20	±6.90	122.70	±7.98	131.80	±8.57	124.40	±8.09	0.276	121.28	±7.88	
	15–20	107.50	±6.99	112.90	±7.34	98.13	±6.38	97.73	±6.35	0.417	104.07	±6.76	
	20–25	101.60	±6.60	112.90	±7.34	99.35	±6.46	99.60	±6.47	0.509	103.36	±6.72	
	25–30	101.60	±6.60	115.20	±7.49	100.60	±6.54	101.10	±6.57	0.454	104.63	±6.80	
	30–40	105.30	±6.84	115.20	±7.49	94.61	±6.15	107.30	±6.97	0.339	105.60	*±6.86*	
	40–55	105.30	±6.84	109.60	±7.12	105.40	±6.85	109.60	±7.12	0.942	107.48	±6.99	
	Depth average	103.35	±6.72	116.36	±7.56	97.15	±6.31	103.63	±6.74	**0.004****	105.12	±6.83	
Pb	**0–5**	13.22	±0.86	13.49	±0.88	7.11	±0.46	12.56	±0.82	**0.012****	11.60	±0.75	**0.022****
	5–10	12.82	±0.83	13.45	±0.87	12.48	±0.81	10.83	±0.70	0.269	12.39	±0.81	
	10–15	11.87	±0.77	12.64	±0.82	17.23	±1.12	13.20	±0.86	**0.045****	13.73	±0.89	
	15–20	11.76	±0.76	21.24	1.38	13.83	±0.90	13.84	±0.90	**0.010****	15.17	±0.99	
	20–25	11.79	±0.77	21.24	±1.38	14.12	±0.92	13.72	±0.89	**0.010****	15.22	±0.99	
	25–30	11.79	±0.77	12.86	±0.84	13.33	±0.87	12.95	±0.84	0.631	12.73	±0.83	
	30–40	9.38	±0.61	12.86	±0.84	13.06	±0.85	12.53	±0.81	0.079*	11.96	±0.78	
	40–55	9.38	±0.61	11.61	±0.75	13.28	±0.86	12.00	±0.78	0.087*	11.57	±0.75	
	Depth average	11.50	±0.75	14.92	±0.97	13.06	±0.85	12.70	±0.83	**0.006****	13.05	±0.85	
pH	**0–5**	8.23	±0.53	7.40	±0.48	6.98	±0.45	7.01	±0.46	0.352	7.41	±0.48	0.116
	5–10	8.23	±0.53	7.40	±0.48	6.98	±0.45	7.01	±0.46	0.352	7.41	±0.48	
	10–15	8.27	±0.54	7.11	±0.46	7.10	±0.46	7.37	±0.48	0.392	7.46	±0.49	
	15–20	8.20	±0.53	6.74	±0.44	6.60	±0.43	7.10	±0.46	0.208	7.16	±0.47	
	20–25	8.20	±0.53	7.74	±0.50	7.10	±0.46	7.76	±0.50	0.548	7.70	±0.50	
	25–30	8.23	±0.53	8.10	±0.53	7.57	±0.49	8.00	±0.52	0.822	7.98	±0.52	
	30–40	8.23	±0.53	8.10	±0.53	7.57	±0.49	8.00	±0.52	0.822	7.98	±0.52	
	40–55	8.25	±0.54	7.95	±0.52	7.98	±0.52	7.90	±0.51	0.961	8.02	±0.52	
	Depth average	8.23	±0.53	7.57	±0.49	7.24	±0.47	7.52	±0.49	**0.000****	7.64	±0.50	

* Significant difference at the 0.10 level.

** Significant difference at the 0.05 level.

41.9%, 40.7% and 38% respectively). They were also found in the acid-soluble fraction (F2) (27.7%, 10.4%, 19% and 34.7%; respectively). Moreover, part of these TE mass (27% and 23.6% for Pb and Cd; respectively) was bound with reducible fraction (F3).

3.3. Variation of total concentrations in the soil profile

Table 2 shows the results of total concentrations and pH measurements obtained for the soil profile in all parcels, along with the statistical analyses performed on the samples data, including the mean and standard deviation for each set of samples, and the p-value for the parcel data collected for each soil horizon.

To analyze the PF-originated TE transfer in soil with time, the concentration of Cd, Zn, Pb, and Cu was plotted against time for the reference parcel R at T0 and incubated parcels P1, P2 and P3 (corresponding to T1 = 4-, T2 = 11- and T3 = 15-month periods) (Fig. 2)—to simplify the tracking of the profile variations, the eight layers in each parcel were grouped in three depth zones: upper (0–20 cm), intermediate (20–35 cm), and lower zone (35–55 cm); zone TE concentration was taken as the average content of TE in the zone layers—the time axis was started at the date when the PF was just applied on the study parcels; the corresponding metal concentrations at the different depths represent the soil background concentrations.

Generally, a similar trend was observed for the studied TEs. For the overall profile, peak concentrations of TEs (Cu: 43.13, Cd: 0.52, Zn: 116.36, and Pb: 14.92 mg kg^{-1}) occurred 4 months after amendment (in January, parcel P1). Then, TE average total concentration decreased between parcels P1 and P2 (February–August) to finally reach near background values in parcel P3 (December) (Fig. 2). However, Cd average concentration remained relatively constant between parcels P1–P3 (0.44 to 0.52 mg kg^{-1}). Changes in Cd concentration between parcels occurred only in the upper layers (0–20 cm) (0.31 to 0.71 mg kg^{-1}), thus not reaching deeper layers (Fig. 2a), indicating possible in situ release and recovery. A soil enrichment of about 0.30 mg/kg (~100% enrichment) in Cd remained in P3 (11 months after PF application) in the upper zone (0–20 cm). Layer 35–55 cm showed an increase in Pb content between P1 and P2 (February–August) (Fig. 2c). Practically, with the exception of Cd, slight variations in TE total concentrations occurred between P2 and P3 (August–December), where the TE content in the soil approached the background levels, indicating that the direct effect of PF on the soil profile took place mainly during around a year following PF

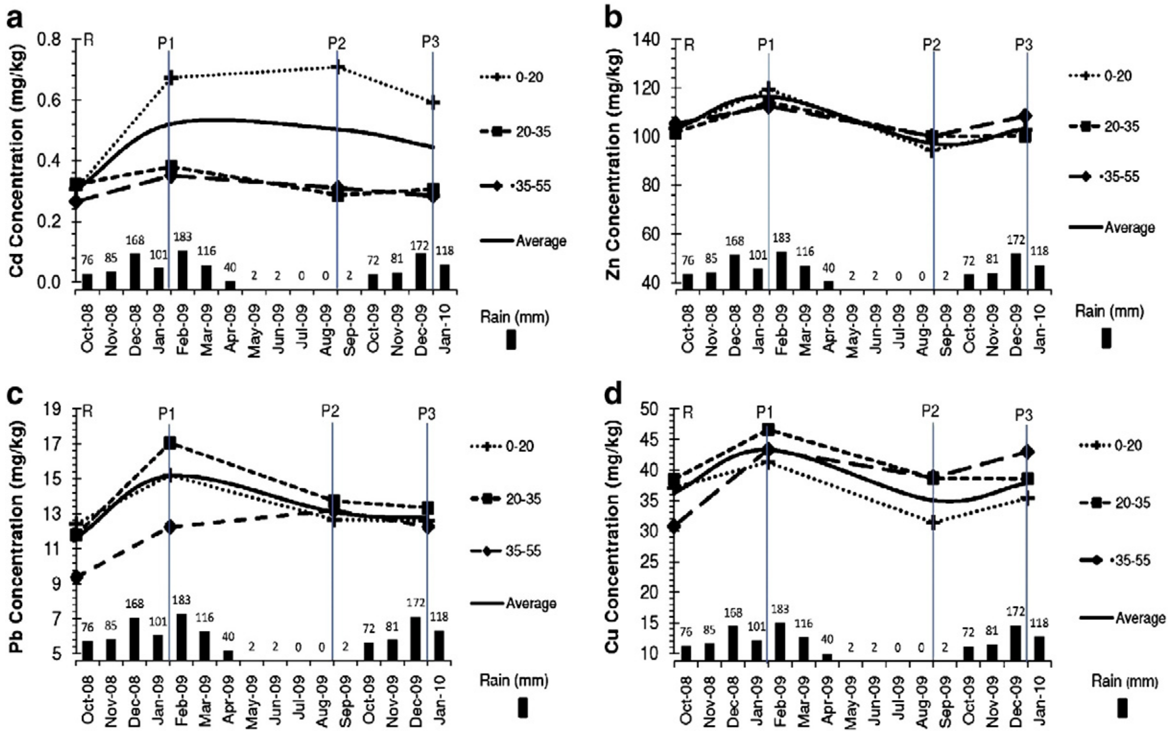

Fig. 2. Time-variation of TE concentrations in the soil profile — reference parcel R (T = 0); PF-amended parcels P1 (T1 = 4 months); P2 (T2 = 11 months); and P3 (T3 = 15 months).

application. No evidence of released TE accumulation in the soil was observed by the end of the sampling period (15 months after PF application)—except for Cd which was somewhat accumulated in the upper layers (0–20 cm). However, Cd content in this layer was decreasing between P2 and P3, indicating it would eventually reach the background level.

A decrease of soil pH following the PF amendment was detected from the surface (0–5 cm) till layer 15–20 cm that showed the lowest value with slightly acidic pH (6.6 ± 0.2) in parcels P1 and P2 (Table 2).

Statistically, the fluctuation of TE concentration in all depths with time proved a significant difference in the average concentration between parcels for Cu, Zn and Pb. The upper layers of the parcels (down to 25 cm) showed a significant difference with time for Cd (Table 2).

As a result of local and temporary pH decrease after PF amendment, and heavy rainfall during October–January, TE average concentrations increased in the soil profile to reach their highest values in parcel P1 (Fig. 2). However, despite the relatively massive quantity of PF amendment, their concentration remained below the permissible limits of TEs in agricultural soils (Cu: 63, Cd: 1.4, Zn: 200, and Pb: 70 mg kg^{-1}) (CCME, 1999). TEs were transferred from PF and moved downward through the soil profile with incoming rain water creating temporary leaching conditions. Generally, the continuous rainfall between parcels P1 and P2 (particularly between January and April) caused TE dilution, and thus a significant decrease in Zn, Cu, and Pb average concentrations was observed in the soil profile, and this trend persisted in the period between April and August, due to TE uptake by growing plants. However, Cd nearly constant average concentration in the soil profile between parcels P1 and P2 indicated its accumulation in the soil matrix (Fig. 2a). In the period between September and December (P2–P3), the drying–wetting cycle of the soil caused the re-augmentation of Zn and Cu average concentrations in the soil profile. However, a different behavior was observed for Pb and Cd that showed a slight decrease of their average concentration between P2 and P3 (13.06 and 12.70 mg kg^{-1} for Pb, 0.50 and 0.44 mg kg^{-1} for Cd in P2 and P3, respectively) (Table 2),indicating possible uptake by plants or leaching toward the saturated zone.

3.4. Metals mobility in soil

In order to assess the chemical form of TEs, and thus their mobility and potential risk on the environment, speciation of Cu, Cd, Zn and Pb was performed in all parcels (R, P1, P2 and P3). TE speciation results for soil samples taken from parcel P1 are presented in Table 3, with replicates, averages, and standard deviations. TE recovery, measured total concentration, and percent recovery are also shown in Table 3.

The TE content obtained by adding the sequentially extracted fractions (TE recovery) was to some extent lower than the actual value found by TE total concentration analysis. Significantly low recoveries were found only for Pb and Cd (62% and 66%) in parcel P1. The difference between recovered and actual TE total concentrations is due to the fact that the used sequential speciation method is subject to some inherent drawbacks. In fact, the re-adsorption of metals during the sequential extractions has been widely reported in other works that used sequential extractions for soil (Harrison et al., 1981; Ramos et al., 1994), and this process together with re-precipitation could take place during the extractions resulting in lower recovery values. A number of authors have established that the dissolution of iron oxides was incomplete during the reductive step of Tessier's scheme, leading to an overestimation of the residual fraction (Gleyzes et al., 2002; Kheboian and Bauer, 1987).

The results of TE chemical fractions (F1, F2, F3, F4 and F5) concentrations in the different parcels, at the different study layers (0–20 cm, 20–35 cm, and 35–55 cm), were plotted on separate charts for each of the considered metals (Cd, Pb, Zn and Cu), as shown in Fig. 3.

In the reference plot, most of the studied elements were stable and associated with the reducible and residual fractions (F3 and F5). Moreover, in the reference parcel (R), Cd and Pb were bound to acid-soluble fraction (F2). Previous studies showed that in alkaline soil, Pb was fixed to the iron and manganese oxide and carbonate fractions (Jaradat et al., 2006; Sheppard and Thibaut, 1992; Tuins and Tels, 1990). Copper appeared mainly in the iron and manganese oxide and residual fraction (Castillo-Carrion et al., 2007).

Table 3
Fractionated TE concentrations, recovery, and measured total concentration in the soil profile of parcel P1. (Two replicates with average and standard deviation (SD) are shown for each fraction).

Horizon	F1		F2		F3		F4		F5		Recovery	SD	Measured total conc.	SD	% Recovery
	Rep.1	Rep.2	Rep.1	Rep.2	Rep.1	Rep.2	Rep.1	Rep.2	Rep.1	Rep.2					
	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD					
Cd — P1 (T = 4 months)															
0–20 cm	<D.L.[a]	<D.L.	0.19	0.27	0.12	0.16	0.06	0.09	<D.L.	<D.L.	0.44	0.07	0.67	0.04	66.27
	<0.005	–	0.23	0.04	0.14	0.02	0.07	0.01	<0.005	–					
20–35	<D.L.	<D.L.	0.11	0.15	0.08	0.06	0.07	0.06	<D.L.	<D.L.	0.27	0.03	0.38	0.02	69.74
	<0.005	–	0.13	0.02	0.07	0.01	0.06	0.01	<0.005	–					
35–55	<D.L.	<D.L.	<D.L.	<D.L.	0.09	0.06	0.26	0.17	<D.L.	<D.L.	0.29	0.06	0.35	0.03	83.71
	<0.005	–	<0.005	–	0.08	0.01	0.216	0.04	<0.005	–					
Pb — P1 (T = 4 months)															
0–20 cm	1.72	2.22	1.32	1.73	1.82	2.53	2.34	3.47	4.33	5.44	13.46	1.93	15.20	0.99	88.50
	1.97	0.25	1.52	0.20	2.18	0.36	2.90	0.56	4.88	0.55					
20–35	1.10	1.53	0.54	0.72	3.12	3.95	3.75	4.59	0.97	0.81	10.55	1.22	17.05	1.11	61.86
	1.32	0.22	0.63	0.09	3.54	0.42	4.17	0.42	0.89	0.08					
35–55	<D.L.	<D.L.	2.73	2.28	2.71	2.20	2.33	2.86	2.22	1.72	9.52	1.00	12.24	0.80	77.81
	<0.005	–	2.50	0.23	2.46	0.25	2.59	0.26	1.97	0.25					
Zn — P1 (T = 4 months)															
0–20 cm	4.01	2.93	<D.L.	<D.L.	42.32	32.94	45.22	36.27	17.45	12.67	96.91	12.09	119.50	7.77	81.09
	3.47	0.54	<0.005	–	37.63	4.69	40.74	4.48	15.06	2.39					
20–35	<D.L.	<D.L.	0.21	0.16	22.45	16.72	78.33	62.95	4.98	3.13	94.46	11.51	114.05	7.41	82.83
	<0.005	–	0.19	0.02	19.58	2.87	70.64	7.69	4.05	0.93					
35–55	<D.L.	<D.L.	<D.L.	<D.L.	74.43	62.27	9.03	7.03	15.03	11.95	89.87	8.62	112.40	7.31	79.96
	<0.005	–	<0.005	–	68.35	6.08	8.03	1.00	13.49	1.54					
Cu — P1 (T = 4 months)															
0–20 cm	4.52	3.42	7.22	9.70	10.77	7.83	6.76	5.31	10.02	14.89	40.22	6.42	41.29	2.68	97.41
	3.97	0.55	8.46	1.24	9.30	1.47	6.03	0.73	12.46	2.44					
20–35	11.23	8.28	3.55	2.83	5.22	4.14	5.88	4.70	12.03	16.05	36.96	4.97	46.62	3.03	79.28
	9.75	1.48	3.19	0.36	4.68	0.54	5.29	0.59	14.04	2.01					
35–55	6.67	5.04	2.65	3.75	6.98	8.58	10.55	7.55	18.26	13.18	41.60	6.20	43.33	2.82	96.01
	5.86	0.82	3.20	0.55	7.78	0.80	9.05	1.50	15.72	2.54					

[a] <D.L.: value is below the AAS detection limit (0.001–0.005 depending on the studied element).

3.4.1. Cd speciation

At T0, Cd was associated with the acid-soluble and reducible fractions (F2 and F3) in the reference parcel at all depths. Exchangeable, oxidizable and residual fractions (F1, F4 and F5) were generally negligible in soil profile (Fig. 3a).

At T1 (4 months after PF application), Cd amounts were doubled (0.23 mg kg^{-1}) in the acid-soluble fraction F2 (Fig. 3b) in layer 0–20 cm. Furthermore, Cd related to the oxidizable fraction (F4) appeared in all depths of the soil profile of parcel P1, or at T1 (0.07, 0.06 and 0.22 mg kg^{-1} at 0–20; 20–35 and 35–55 cm, respectively).

At T2 (11 months after PF application), in parcel P2, Cd was found associated mainly with the exchangeable fraction (F1) (0.33 mg kg^{-1}—65%) at layer 0—20 cm, and to oxidizable fraction (F4) in layers 20–35 (0.20 mg kg^{-1}—87%) and 35–55 cm (0.20 mg kg^{-1}—69%) (Fig. 3c). Thus, Cd concentration increased in the mobile fraction (exchangeable) at 0–20 cm and in the oxidizable fraction at 20–35 and 35–55 cm.

At T3 (15 months after PF amendment), the reducible and residual fractions were dominant (F3:96% at 35–55 cm, and F5: 73% at 0–20 cm) (Fig. 3d).

The change in Cd speciation over the study period can be attributed to phosphate fertilizer application decreasing soil pH in parcels P1 and P2 at the surface layer (0–20 cm). It is well reported that under acid conditions, Cd was found accumulated in the exchangeable fraction and acid soluble fraction (F1 and F2) (Castillo-Carrion et al., 2007; Li and Thornton, 2001). In the end of our study, under alkaline soil conditions, Cd was adsorbed to iron and manganese oxides, organic matter, and clay structure.

3.4.2. Pb speciation

Pb was mainly combined to carbonates (F2), iron and manganese oxides and hydroxides (F3) and to clays (F5) in the reference parcel (R) in all depths. At natural abundance, the exchangeable (F1) and oxidizable fractions (F4) could be considered insignificant in all depths (Fig. 3e).

At T1 (5 months after PF application), a change of Pb speciation was observed in P1 with the appearance of the exchangeable (F1) and oxidizable (F4) Pb fractions (1.97 mg kg^{-1}—15% and 2.90 mg kg^{-1}—22% at 0–20 cm; 1.32 mg kg^{-1}—12% and 4.17 mg kg^{-1}—40% at 20–35 cm; <1 mg kg^{-1}—<1% and 2.59 mg kg^{-1}—27% at 35–55 cm, in F1 and F4, respectively) (Fig. 3f). Layer 35–55 cm of parcel P1 contained Pb in a concentration of 2.50 mg kg^{-1} (26.30%) in the acid-soluble fraction (F2). Pb revealed an increase of 15% and 13% at 0–20 and 20–35 cm, respectively, in the exchangeable fraction F1 of parcel P1.

At T2 (11 months after PF application), the reducible and oxidizable fractions (F3 and F4) in parcel P2 were dominant (Fig. 3g).

At T3 (15 months after PF amendment), Pb was associated with the reducible and alumino-silicates fractions (F3, 1.74 mg kg^{-1}–17% and F5, 8.78 mg kg^{-1}–84%) in the surface layer (0–20 m), and mainly to acid-soluble (F2) (8.44 mg kg^{-1}—81%) and exchangeable fractions (F1) (8.67 mg kg^{-1}—88%) in layers 20–35 and 35–55 cm, respectively (Fig. 3h). Hence, Pb solubility increased in layers 20–35 and 35–55 cm.

The appearance of Pb in the exchangeable fraction F1 in parcels P1 and P2 (acid pH) is in agreement with different studies (Castillo-Carrion et al., 2007). It can be ascribed to the input of phosphate fertilizer with speciation results showing that 38% of Pb occurred in the exchangeable fraction F1. Pb binding to oxidizable fraction F4 has been reported in the literature (Chaney et al., 1988), generally in the detriment of the residual fraction (F5). The presence of complexing and organic ligands and competing cations, decreasing Pb sorption, could substantially enhance its mobility (Kotuby-Amacher

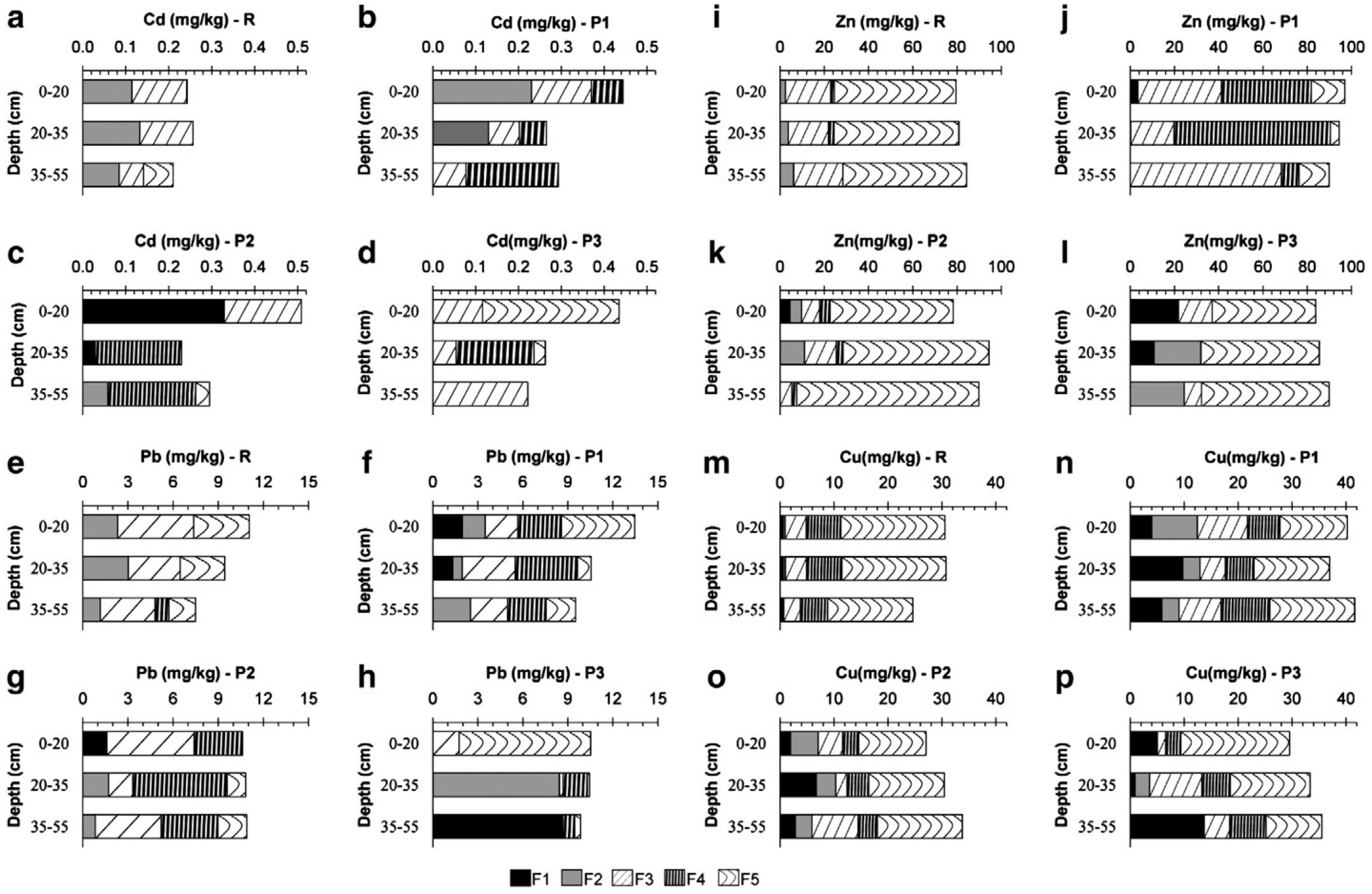

Fig. 3. Time-variation of TE geochemical fraction concentrations in the soil profile — reference parcel R (T = 0); PF-amended parcels P1 (T1 = 4 months); P2 (T2 = 11 months); and P3 (T3 = 15 months). F1—exchangeable; F2—acid soluble; F3—reducible; F4—oxidizable; and F5—residual fraction.

and Gambrell, 1988; Puls et al., 1991), which may lead to reinstating, to some extent, the exchangeable fraction F1 (P3 layer 35–55 cm).

3.4.3. *Zn speciation*

Zn reducible and residual fractions (F3 and F5) were dominant in the reference parcel R throughout the soil profile (Fig. 3i). F1, F2 and F4 were negligible in all soil horizons (<6 mg kg^{-1}).

At T1 (parcel P1), more oxidizable zinc fraction (F4) accumulated in all layers, particularly in the upper and intermediate layers (40.74 mg kg^{-1}—42% and 70.64 mg kg^{-1}—75% in layers 0–20 and 20–35 cm, respectively) (Fig. 3j).

At T2 (parcel P2), Zn was found mostly associated with residual fractions (F5) in all layers (55.59 mg kg^{-1}—71%, 66.01 mg kg^{-1}—70%, and 82.40 mg kg^{-1}—92%, in layers 0–20, 20–35 and 35–55 cm, respectively) (Fig. 3k). Zn was sorbed mainly in clay fraction.

At T3 (parcel P3), Zn mobility was modified, where Zn in the exchangeable and acid soluble fractions (F1 and F2) was in relatively higher concentrations of 21.90 mg kg^{-1}—26%, 31.94 mg kg^{-1}—37%, and 24.40 mg kg^{-1}—27% at 0–20, 20–35 and 35–55 cm, respectively (Fig. 3l). Zn solubility increased, comparing with parcel P2, in the detriment of residual fraction (at 0–20 and 35–55 cm) and reducible fraction (at 20–35 cm) (net shifts of 14%, 15% and 27% at 0–20, 20–35 and 35–55 cm, respectively, were observed from Fe/Mn oxides and clay fractions to the mobile fractions (F1 and F2).

Phosphate fertilizer application varied Zn speciation in the soil horizons where the greatest proportion of Zn resided in the oxidizable fraction (F4) in parcel P1, in the detriment of the residual fraction (F5). This can be related to the soil organic matter's affinity for soluble Zn (Kabata-Pendias and Pendias, 1984), which was released into soil solution from the applied PF containing high proportion (60%) of the metal's exchangeable fraction F1. The increase of fractions F1 and F2 in P3 may be related to the formation of zinc complexes with inorganic and organic ligands, affecting Zn adsorption reactions with the clay minerals by altering its chemical species (McLean and Bledsoe, 1992).

3.4.4. *Cu speciation*

At T0, copper was mainly partitioned between reducible (F3), oxidizable (F4), and residual fractions (F5) in all layers of the reference soil (parcel R). It is obvious that the greatest quantity of copper was detected in the residual fraction at all depths (19.27 mg kg^{-1}—63%, 19 mg kg^{-1}—63% and 15.81 mg kg^{-1}—64% in 0–20 cm, 20–35 cm, and 35–55 cm, respectively). The exchangeable (F1) and the acid-soluble fractions (F2) were negligible (<1 mg kg^{-1} and <4 mg kg^{-1}, respectively) (Fig. 3m).

At T1 (parcel P1), these two fractions (F1 and F2) held a larger quantity of copper at all depths (12.43, 12.97 and 9.06 mg kg^{-1} in 0–20 cm, 20–35 cm, and 35–55 cm respectively) (Fig. 3n). A mobilization of Cu in the soil profile of parcel P1 was therefore induced (3% to 32%, 3% to 35%, and 3% to 23% increase in mobile fractions (F1 and F2) at 0–20, 20–35 and 35–55 cm, respectively, comparing R to P1), with a decrease occurring mainly in the fraction associated with clays (F5) (63% to 32%; 63% to 35%; 64% to 36% at 0–20, 20–35 and 35–55 cm, respectively).

At T2 (P2), Cu quantities in fractions F1 and F2 decreased in the soil profile (12.43 mg kg^{-1}—31% to 7.05 mg kg^{-1}—26%, 12.95 mg kg^{-1}—35% to 10.33 mg kg^{-1}—34%, and 9.05 mg kg^{-1}—22% to 5.91 mg kg^{-1}—18%, in layers 0–20, 20–35, and 35–55, respectively (Fig. 3o).

At the end of the experiment in P3, Cu was partitioned in the reducible, oxidizable and alumino-silicate fractions of the soil (Fig. 3p).

However, 17% and 39% of Cu content was present in the exchangeable fraction (F1) in layers 0–20 and 35–55 cm.

The development of Cu exchangeable fraction F1 in the soil horizons was the immediate result of the application of PF carrying this Cu species in abundance (40.7%) relative to the background (~0%). Fraction F2 could be formed by the adsorption of Cu to soil carbonate (CaCO3) surfaces (Dudley et al., 1991). The general increase in the reducible fraction could be attributed to the binding of some Cu in soluble form to Fe Mn oxides. Mclaren and Crawford (1973) reported that manganese oxides played a crucial role in binding copper in soil. Cu mobility in soils may be enhanced by forming complexes with soluble organic ligands (McLean and Bledsoe, 1992).

3.4.5. Cu, Pb and Zn versus Cd

Fe/Mn oxides and clays were the most susceptible to changes that resulted in an increase of Pb, Zn and Cu solubility in soil profile. The presence of soluble phosphates and sulfates in PF amendment (at least 85–90% of the total P in PF is water-soluble) (Chien et al., 2011) may have had a significant impact on Pb, Zn and Cu solubility. After 15 months of amendment (parcel P3), comparing to the reference plot, the mobile fractions and mainly the exchangeable fraction (F1) showed increments in Cu, Pb and Zn amounts relative to the reference (14%, 7.7%, 35.5% for Cu; 23%, 32.9%, 20% for Zn at 0–20, 20–35 and 35–55 cm respectively; and 48%, 72.4% for Pb at 20–35 and 35–55 cm respectively), which could mobilize these TEs in soil profile, imposing a threat to the ground water. On the contrary, Cd was sorbed in soil particles (clays and Fe/Mn oxides and hydroxides) over the study period. These results were opposite to those of phosphogypsum-amended soil where Cd, after the study period, was mostly in the exchangeable fraction, whereas Pb, Zn and Cu were sorbed in soil particles (Kassir et al., 2011a).

3.5. TEs concentration in plants

Concentrations of the studied elements in the entire plant, *C. intybus* L, collected from reference and amended parcels were presented in Table 4, along with the respective TE concentrations in the soil plant's roots zone (0–20 cm) and the resulting soil–plant transfer factors (concentration in plant/concentration in soil). Fig. 4 illustrates the transfer factor time-variation for the different TEs.

The TE transfer factor (TF) is a measure of its availability to plant uptake. There are many factors that can influence the TF, including soil properties and TE chemical phases. The exchangeable fraction (F1) is readily available and considered to be the primary nutrient source for plants (Narwal and Singh, 1998). The fraction associated with carbonate (F2) is potentially available for plant uptake, depending on soil pH conditions, whereas TE in the other chemical fractions (oxides-F3, organic-F4, and residual-F5) with minimal solubility and very low mobility are not involved with the plants biological activities (Xian, 1989). Thus, during the experiment course, the main changing factors affecting TE bioavailability, hence their TFs, are the soil moisture content (associated with rainfall), soil pH (influenced by PF acidity), and the respective TE leaching and available fraction concentrations. Cd exhibited the highest TF (0.32 ± 0.04–0.52 ± 0.05) among the other TEs in all parcels, followed by Cu (0.16 ± 0.02–0.27 ± 0.03). Pb and Zn had comparable TFs (0.08 ± 0.01–0.17 ± 0.02) in all parcels, except in P3 where it increased to 0.20 ± 0.03 for Pb, while remained in the same range for Zn (0.11 ± 0.01) (Table 4).

Table 4
TE concentration in whole plants and soil root zone, and the respective transfer factor, for the different parcels.

Parcel	Cu (mg/kg)	±SD	Pb (mg/kg)	±SD	Zn (mg/kg)	±SD	Cd (mg/kg)	±SD
	Plants		Plants		Plants		Plants	
	Soil (0–20 cm)		Soil (0–20 cm)		Soil (0–20 cm)		Soil (0–20 cm)	
	Transfer	Factor	Transfer	Factor	Transfer	Factor	Transfer	Factor
R	6.07	0.15	1.33	0.10	8.73	0.09	0.09	0.01
	37.06	2.41	12.42	0.81	103.25	6.71	0.31	0.02
	0.16	0.01	0.11	0.02	0.08	0.01	0.29	0.05
P1	9.61	0.11	2.54	0.11	13.81	0.31	0.29	0.02
	41.29	2.68	15.20	0.99	119.50	7.77	0.67	0.04
	0.23	0.02	0.17	0.02	0.12	0.01	0.43	0.06
P2	5.08	0.14	1.04	0.08	9.89	0.24	0.37	0.02
	31.38	2.04	12.66	0.82	94.31	6.13	0.71	0.05
	0.16	0.02	0.08	0.01	0.10	0.01	0.52	0.05
P3	9.67	0.45	2.58	0.24	10.83	0.61	0.19	0.01
	35.37	2.30	12.61	0.82	102.87	6.69	0.59	0.04
	0.27	0.03	0.20	0.03	0.11	0.01	0.32	0.04

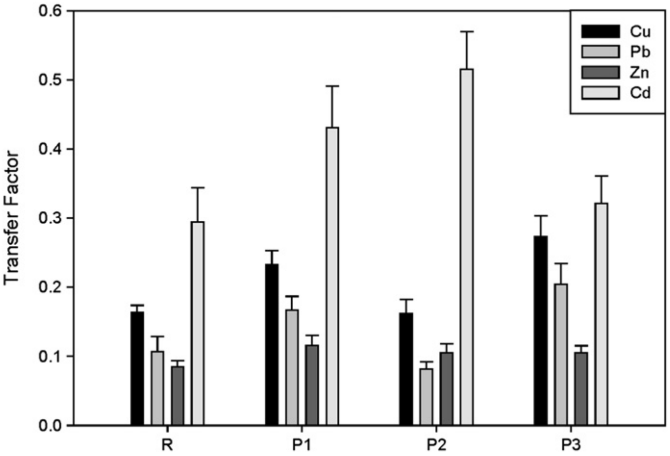

Fig. 4. Time-variation of the transfer factor of TEs in the plants — reference parcel R (T = 0); PF-amended parcels P1 (T1 = 4 months); P2 (T2 = 11 months); and P3 (T3 = 15 months).

In comparison with the reference parcel, it can be seen from Fig. 4 that the TF of Cd, thus its bioavailability, increased in P1 and P2 by about 49% and 78%, respectively, while it dropped back to near its reference value in P3. This variation in Cd bioavailability is in line with its chemical fraction changes depicted in Fig. 3. In fact, in the root zone (0–20 cm), the Cd pH-susceptible mobile fraction F2 increased from 0.102 mg/kg in parcel R to 0.203 mg/kg in P1 (Fig. 3a and b), simultaneously with a drop in the layer average pH from 8.23 ± 0.54 to 6.92 ± 0.45 (Table 2). This was followed by a phase transformation yielding 0.303 mg/kg of Cd exchangeable fraction (F1) in P2, and the depletion of the mobile fractions in P3 (Fig. 3c and d).

The copper TF went through an increase in P1 by about 44% relative to the reference, declined to the reference value in P2, then increased again by about 71% in P3 (Fig. 4). If we examine Fig. 3m–p for Cu fractions in layer 0–20 cm, we find a similar fluctuating pattern in the mobile fractions (F1 and F2) concentrations, with the pH factor favoring the availability of the carbonate fraction (F2) to plant uptake.

Similarly, the Pb TF went through an increase of nearly 52% from R to P1, a drop in P2, followed by an increase of about 86% in P3 (Fig. 4). However, this increase in bioavailability of Pb in P3 is contradicted with the prevailing insoluble and residual Pb fractions in layer 0–20 cm (Fig. 3h). This could be explained by the idea that some plant roots were extended to deeper layer (20–35 cm), where the Pb plant available fraction F2 prevailed in P3 (Fig. 3h).

As for Zn, plant available fractions (F1 and F2) barely existed in parcels R, P1 and P2, with bio-available fraction F1 appearing in parcel P3 with a concentration of about 20 mg/kg in layer 0–20 cm (Fig. 3i–l). Zn plant availability, however, remained the same in P3 as in the other parcels, with relatively low TF (0.11 ± 0.01). This could possibly be due to the existence of competing available Cu (Simon, 2000; Tani and Barrington, 2005) in P3 layer 0–20 cm (Fig. 3p).

There was little evidence of significant elevated uptake of TEs in soil extensively amended with PFs. Pb, Zn, Cu and Cd concentrations were within the normal levels reported by Chaney (1989) (2–5 mg kg^{-1} for Pb; 15–150 mg kg^{-1} for Zn; 3–20 mg kg^{-1} for Cu; 0.5–1 mg kg^{-1} for Cd) though they are considerably higher than the reference, particularly for Cd. This phenomenon could be attributed to the formation of TE phosphate compounds which are non absorbable. In fact, it is recognized that high levels of phosphate fertilization decrease Zn concentrations in plant tissues (Lindsay, 1972; Loneragan and Webb, 1993; Moraghan, 1984), which was attributed to the interaction of P with Zn in soil (Grant and Bailey, 1997; McLaughlin et al., 1995). Cd availability in plants from PFs depends on many factors such as soil texture, plant species, Cd concentration, and type of fertilizers used (Singh and Myhr, 1997).

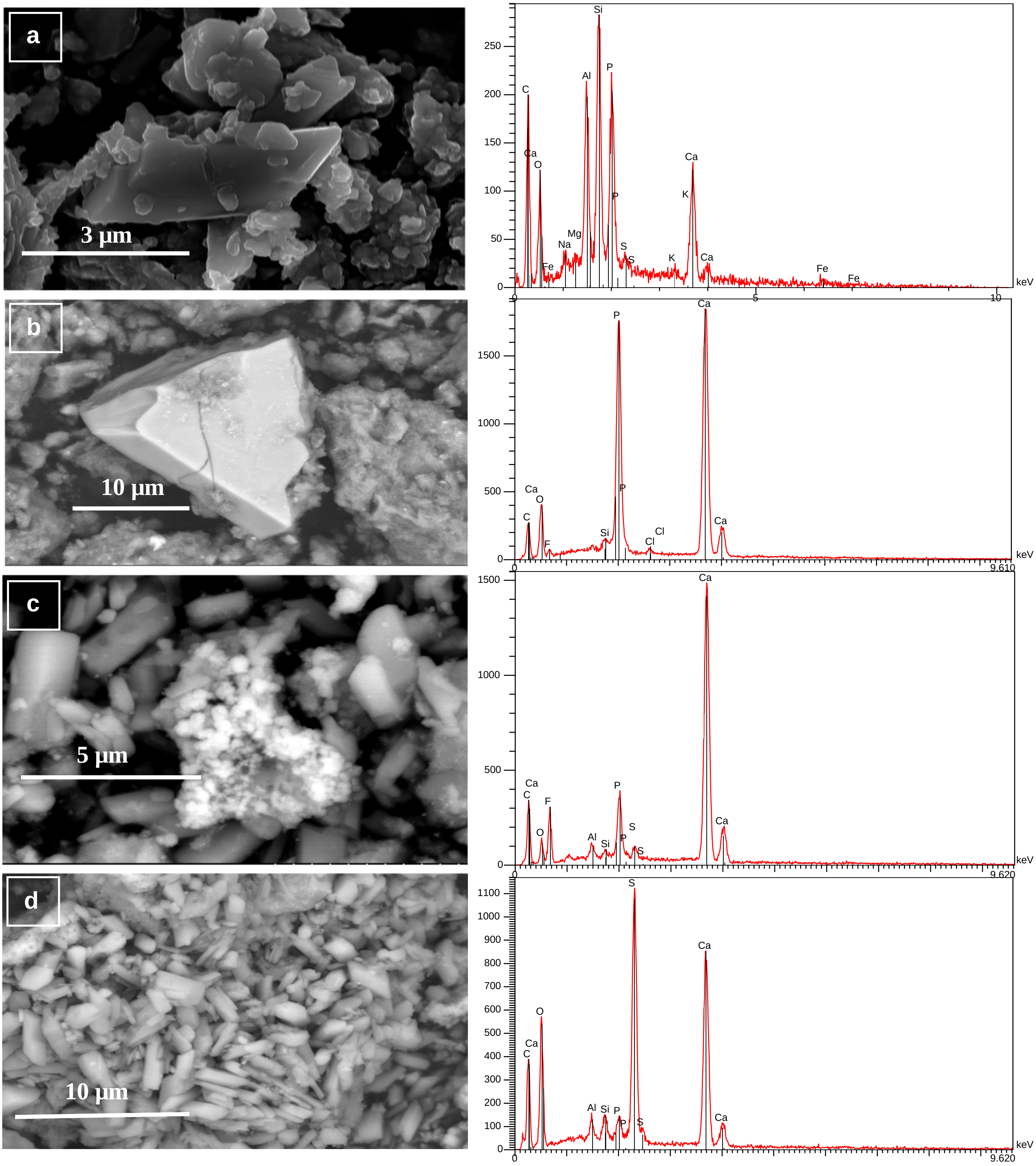

Fig. 5. Images of SEM and EDX microanalyses of parcels P1 and P3: (a) SSP; (b) tricalcic phosphate with fluorine (c) fluorine; (d) gypsum ($CaSO_4$).

3.6. TE mobility comparison

TE overall mobility during the study period could be compared by assessing the following mobility factor (MF), derived on the basis of the ratio of displaced (not retained) TE from a soil control volume—to its input to the control volume (Kassir et al., 2011a):

$$MF = 1–(C_s–C_r)/C_{pf}$$

Where,

C_s = TE average concentration in the control volume of studied soil profile (mg kg^{-1}) for all layers in parcels P1, P2 and P3

C_r = TE average concentration in reference soil for all layers (mg kg^{-1})

C_{pf} = TE concentration in PF (mg kg^{-1})

Comparing MF for the studied TEs, the order of their mobility was found to be: Zn (0.99) > Cd (0.98) > Cu (0.87) > Pb (0.42). This pattern is in accordance with data available in the literature of some mobility trends of TEs in soils (Carrillo-Gonzalez et al., 2006). It should be noted that this assessment incorporates the transport of TEs in the soil by different mechanisms, including colloidal mobility that facilitates such transport (BIN et al., 2011).

3.7. Mineral phases

Samples of amended soil at different depths (from surface till 55 cm) and time (T1, T2 and T3) were examined by electron microscopy. Typical electron micrographs and corresponding EDX spectra for different detected mineral phases were illustrated in Fig. 5. Particles of gypsum in rectangular and rounded shape were associated with PFs having a molar ratio Ca/P = 0.5 or 1 (Fig. 5a). In parcel P2, this ratio (Ca/P) became 1.6 (Fig. 5b) suggesting that a P transfer reaction occurred. Indeed, with heavy precipitations in parcel P1, P in the soil solution of parcel P2 can precipitate as Ca–P compounds (Chien et al., 2011). Furthermore, fluor was found associated with these compounds leading to the formation of fluorine at depth 36–40 cm of parcel P2 (Fig. 5c). In fact, PFs manufactured from phosphate rocks may contain fluorine (Camelo et al., 1997; Mirlean et al., 2001) that can be responsible for polluting freshwater and groundwater (Mirlean et al., 2001). In fact, 1 g of fluorine may pollute 714 m^3 of water, with tolerable concentration being 1.4 mg L^{-1} (CONAMA, 2005). Tubular forms of gypsum remained at depth 11–15 cm of parcel P3 (15 months after amendment) (Fig. 5d), indicating that its threat to the soil and groundwater quality persisted after the study period.

4. Conclusion

PF amendment increased the TE concentration in the soil profile shortly (4 months) after application, and caused a variation of the studied element speciation through time. Precipitation, pH variation, and the drying–wetting cycle were the main factors controlling the accumulation and mobility of TEs in the soil profile.

The excessive addition of PFs (about forty times the locally used rate) increased TE concentration in soil profile, but not to harmful levels. However, fluorine may be of major concern. Pb, Zn and Cu may as well reach the saturated zone, being steadily transferred to exchangeable fractions.

Since the studied TEs were found mainly in the exchangeable fraction of PF, they were transferred gradually to the soil matrix over the study period, except for Cd that appeared in its exchangeable fraction only after 11 months (in the 0–20 cm layer) following the amendment application, with an increment of 64% in percentage compared to the reference, to be finally adsorbed, 4 months later, by soil particles. Zn exchangeable fraction exhibited an increase of 26% and 13% in the 0–20 and 20–35 cm layers, respectively, Pb of 84% in 35–55 cm, and Cu of 16% and 36% in 0–20 cm and 35–55 cm layers, indicating some risks to the food chain and groundwater ecology.

Soil–plant transfer factor was notably affected by PF application, with highest acquired values being for Cd, followed by Cu, Pb, and Zn. The TE transfer factor varied in accordance with the plant available fraction concentration alteration in the root layer. Although plants accumulated TEs in higher concentrations than the reference, they didn't exceed the normal levels reported in the literature, which could be attributed to the formation of metal-phosphate in soil solution.

The current experimental study indicated that the risk of PFs amendment could be attributed to two reasons: (1) the presence of fluorine in the soil profile, imposing a threat to the saturated zone, and (2) the increment in mobile fractions of Pb, Zn and Cu in the deep soil profile layer, 35–55 cm, over a 15-month period may intrude as well a threat to the saturated zone, when long term soil exposures to PFs are considered.

To comprehensively evaluate the effects of PF application, further investigations related to extended and seasonally repetitive use of PFs in the amendments of agricultural soils, with quantitative emphasis on soil contamination with fluorine, should be undertaken, based on similar analytical methodologies employed in this study.

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