Potentially toxic element phytoavailibility assessment in Technosols from former smelting and mining areas

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Abstract

This study reports the chemical bioavailability of several potentially toxic elements (Zn, Pb, Cd, As and Sb) in contaminated technosols from two former smelting and mining areas. Though these elements have long been recognized as a potentially harmful elements, understanding of their toxicity and environmental behavior in technosols developed on former mining and smelting sites are more limited, particularly for As and Sb. Surface soils were sampled from metallophyte grassland contaminated with Zn, Pb and Cd located at Mortagne –du –Nord (MDN) (North France) and from a former mining settling basin contaminated with As, Pb and Sb located at la Petite Faye (LPF) (Limoges, France). Various selective single extraction procedures ($CaCl_2$, $NaNO_3$, NH_4NO_3, DTPA and EDTA) were used together with germination tests with dwarf beans whose shoots were analyzed for their potentially toxic element concentrations after 21 days of growth. The extraction capacity of the potentially toxic elements followed the order: EDTA>DTPA>NH_4NO_3>$CaCl_2$> $NaNO_3$ for both studied areas. Pearson's correlation coefficient analysis between the concentrations of potentially toxic elements accumulated in bean primary leaves or their mineral mass with their extractable concentrations showed a positive significant correlation with dilute $CaCl_2$ and nitrate solutions extraction procedures. In contrast, for all studied elements, except Pb, the complexing and chelating extractants (EDTA and DTPA) exhibited poor correlation with the dwarf bean leaves concentrations. Moreover results showed that the 0.01M $CaCl_2$ extraction

procedure was the most suitable and provided the most useful indications of metal phytoavailibility for studied elements.
Keywords: Potentially toxic element, Partial extraction, Bioavailability, Mining and smelting activities, Technosol

Abbreviations

EC Electrical conductivity

LPF La Petite Faye

MDN Mortagne-du-Nord

Ng No growth

Ns No significant correlation

TOC Total organic carbon

1 Introduction

High metal and metalloid concentrations in soils have negative effects on terrestrial ecosystems and generate potential health risks (Brown and Calas, 2011). Mining and smelting activities are the major sources of metals contamination by release of a huge amount of potentially toxic elements (PTE) into the environment through wind and water runoff erosion (Razo et al. 2004; Navarro et al. 2008). The accurate estimation of PTE bioavailability in soils is becoming more important to improve the management of contaminated land. Thus, information about accumulation of different PTE in different vegetable species, as well as soil factors that control their bioavailability are very important issues (Xu et al. 2013).

Due to different and complex distribution patterns of PTE among various chemical species and solid phases, their total concentration in soils is not a good indicator of their availability (Abollino et al. 2002). It appears therefore that the knowledge of PTE availability and ecotoxicity is more suitable than their total concentration for predicting their transfer from the soil to plants and may provide useful information to improve environment risk assessment (McLaughlin et al. 2000; Menzies et al. 2007; Plaza et al. 2010).

There is no ideal definition of the bioavailable fraction of PTE in soil. The International Standards Organization's (ISO) defined bioavailability as the degree to which chemicals

present in the soil may be absorbed or metabolized by human or ecological receptors or are available for interaction with biological systems (ISO 11074 (2005)). The bioavailability of PTE in soils depends on the PTE species and their partitioning between the solid phase and the soil pore water. In the past few decades, many studies have been realized to evaluate PTE phytoavailability and related their uptake by plants with their extractable fractions by different chemical agents (Houba et al. 1990; Gupta and Aten, 1993; Pascual et al. 2004).

A wide range of single and sequential extraction schemes have been designed in order to assess the different binding forms of PTE in soils. The mobility and toxicity of PTE in soils were assessed depending on their total concentration in the soil, as well as their specific chemical form, physico-chemical properties, their binding state and soil properties like pH and organic matter content (Nyamangara 1998). Water-soluble, exchangeable, carbonate associated, Fe/Mn oxide-associated, organic-associated and residual forms are often the geochemical forms in which PTE are believed to accumulate in soils. Water-soluble and exchangeable fractions are considered to be bioavailable while the mineral fraction is mainly not available to either plants or microorganisms (Rodrıguez et al. 2009; He et al. 2005). However, the lack of selectivity of the extraction reagents and artifacts such as coprecipitation, reprecipitation and readsorption of previously released PTE are the main limitations of such procedures (Cornu and Clozel 2000; David et al. 2002).

PTE extracted by single extractants are usually considered as indicators of the availability in soils (Black et al. 2011; Chojnacka et al. 2005; McBride et al. 2003). In fact, techniques to estimate the bioavailability of PTE have focused on selecting suitable extractants and developing extraction procedures with different experimental conditions (Black et al. 2011; Anjos et al. 2012). The advantages of a single extractant test compared with a bioassay to determine bioavailability include the simplicity and reproducibility, reduced costs and reduced time frame (Conder et al. 2001). Several authors (Li et al. 1996; Lin et al. 1998) consider that a more correct approach to the distribution of species in natural environment could be obtained by studying the kinetics of the extraction with a single reagent.

Moreover combination of data from extraction procedures and the PTE quantities that accumulated in plants growing on contaminated sites can provides good information for identifying their input into the environment (Wang et al. 2009; Meers et al. 2005; Fernandez et al. 2002).

The most widely used extractants in single extraction methods include: i) chelating, complexing or reducing agents (such as EDTA and DTPA extractants) which are considered as suitable for the prediction of plant uptake by extracting a large portion or the entire total PTE from the solid phase, ii) acid extractants (such as 0.1M HCl because low pH favors the

dissociation of the existing complexes) and iii) neutral salt extractants based on un-buffered salt solutions, which mainly dissolve the cation exchangeable fraction (such as 0.01M $CaCl_2$, 0.1M $NaNO_3$ and 1M NH_4NO_3) which are often called 'mild' extractants. ((Ure et al. 1993; Brun et al. 1998; Negrel et al. 2000; Sutherland 2002; McCready et al. 2003; Feng et al. 2005; Gupta and Sinha 2007; Fang et al. 2007; Menzies et al. 2007; Kubova et al. 2008; Black et al. 2011).

The aim of this study was thus to assess the chemical availability and phytoavailibility of PTE, respectively Zn, Pb and Cd, in contaminated surface technosols from metallophyte grassland located at (Mortagne-du-Nord, France) and As, Pb and Sb from a former mining site (La Petite Faye, Limoges, France) using various chemical single extraction procedures and germination tests with dwarf beans. Results of the chemical extraction tests were compared with the PTE concentrations of primary dwarf bean leaves and mineral masses in order to gain information about the plant availability and mobility.

2 Materials and methods

2.1 Soil sampling

Two contaminated Technosols were selected for this study. The first one is metallophyte grassland contaminated with Zn, Pb and Cd located at Mortagne-du-Nord (MDN) in Northern France. This area is adjacent to the former metallurgical site occupied for over 60 years by a Zn smelter unit linked to a sulfuric acid production unit and a Pb smelting unit (Thiry and van Oort 1999). The second is a former mine tailing of La Petite Faye (LPF) located 60 km North-East of Limoges (France), where about 35000 t wastes from a former gold mine are stored for over 45 years. These mining wastes were characterized by high total concentrations in PTEs such as As, Pb and Sb. (Néel et al. 2003).

Three locations from each site named (MDN1, MDN2, MDN3) and (LPF1, LPF2, LPF3) respectively, were selected for this study according to the level of PTE concentrations and spatial distribution of the vegetation cover. In the MDN site the vegetation cover essentially consists of *Arabidopseshallari* and *Avena sativa* L., whereas, in LPF site, *Graminea, Equisetum telmateia, Betulapendula* and *Pteridiumaquilinum* were present with large horsetails of black alder and poplar (Wanat et al. 2014).

Surface soils (0-20 cm) were sampled from each location with a stainless steel spade. All soil samples collected were carefully transferred to clean polyethylene bags before transport to the laboratory. Collected soil samples were oven-dried at a constant temperature of 40°C, manually homogenized and then quartered. Two equivalent fractions were obtained from each

quartered sample. One fraction was used for the determination of the soil physico-chemical properties whereas the other fraction was used for chemical analysis of PTE. Samples for chemical analysis were sieved first using a 2mm mesh sieve, to remove gross plant matter and subsequently crushed to pass through a 250μm sieve (Brooks 1983).

2.2 Reagents and standards

All the reagents used to prepare the extracting solutions were products of analytical-grade quality (Merck pro-analysis, Darmstadt, Germany). All solutions and dilutions were prepared using doubly deionized water ($18.2 M\Omega cm^{-1}$) (Thermo Scientific Barnstead Easy pure II systems). Standard stock solutions of $1000 mg.l^{-1}$ of different elements were prepared from metal wires or salts of purity higher than 99.998% (VWR international, BDH Prolabo ICP Standards, Belgium). Diluted standard working solutions were prepared from these on a daily basis. All laboratory glassware and plastic ware were rinsed three times with double deionized water after being soaked in a HNO_3 (10%, v/v) batch for 24h.

2.3 Aqua regia / hydrofluoric acid digestion

The Pseudo-total element concentrations were determined by aqua regia / hydrofluoric acid digestion (Zhang et al. 2008). Approximately 1 g of the sample was accurately weighed into a 100 ml teflon beaker. 20 ml of aqua regia (HNO_3: HCl, 1:3v/v) and 20 mL of concentrated hydrofluoric acid were added to the beaker. The beaker was then placed on a hot plate that had been adjusted to 130–150°C. After the solution was evaporated to near dryness, the beaker walls were rinsed with double deionized water and this solution was heated to dryness. One milliliter of concentrated nitric acid and a further 20 ml of double deionized water were added after the beaker was removed from the hot plate and cooled to room temperature. The beaker was heated again on a hot plate at 90–100°C, most of the sample being digested into solution after 1 h. Cooled to room temperature; the solution was filtered through a 0.45μm cellulose nitrate filter and then diluted to volume with double deionized water in a 100 ml volumetric flask. The PTE concentrations in the solutions were determined by inductively coupled plasma mass spectrometry (ICP–MS, Finnigan Element XR,Thermo Electron, Germany). Blank extractions (without sample) were carried out throughout the complete procedure.

2.4 Single extraction methods

Two categories of extractants were used in this study: (i) $CaCl_2$, $NaNO_3$ and NH_4NO_3 as salts or cationic exchange extractants and (ii) EDTA and DTPA as chelating or reducing agents.

For $CaCl_2$ extraction, 10 ml of 0.01M $CaCl_2$ were added to 1.0 g of soil in a 25 ml polypropylene centrifugation tube for each soil sample, shaken for 2 h at 20°C (Uzu et al. 2009). The liquid to solid ratio of 10 is high enough to avoid sample heterogeneities (Houba et al. 1996). For $NaNO_3$, 20 ml of 0.1M $NaNO_3$ were added to 8.0 g of air-dry soil, shaken for 2 h at 20°C (Gupta and Aten 1993). For NH_4NO_3 extraction, in 100 ml polypropylene centrifugation tube, 50 ml of 1M NH_4NO_3 were added to 10 g of air-dry soil, shaken for 2 h at 20°C (Gupta and Sinha 2007). In the case of EDTA extraction, 25 ml of 0.05 M EDTA were added to 5.0 g of air-dry soil, shaken for 1 h at 20°C (Quevauviller et al. 1997). For DTPA extraction, 20 ml of extractant consists of 0.005M DTPA (diethylenetriaminepentaacetic acid), 0.1M triethanolamine, and 0.01M $CaCl_2$, with a pH of 7.3 were added to 10 g of air-dry soil, shaken for 2 h at 20°C (Lindsay and Norvell 1978). For all above procedures, after agitation, samples were subsequently centrifuged and the samples were filtered through a 0.45µm filter paper then acidified with a few drops of concentrated HNO_3 to prevent adsorption to the polyethylene of the storage vessel. The acidified supernatant was collected in polyethylene bottles and stored at 4°C until analysis. PTE concentrations were determined by ICP-MS (Finnigan Element XR, Thermo Electron, Germany). Five replicates were performed for each sample and each procedure. Blanks from extracting reagents described above were measured in parallel for each batch of analysis. ICP–MS measurements were carried out by diluting 1:10 $CaCl_2$, NaNO3, NH_4NO_3, EDTA and DTPA extracts (in 1% HNO_3) to minimize interferences from calcium and sodium present in the extracts (Vaughan et al. 1986).

2.5 Germination tests

Pot experiments were conducted with contaminated technosols from the MDN and LPF sites. Approximately 1kg of soil for each sub-location was potted in plastic plant pots after homogenizing and sieving the soil to pass a 2mm mesh. 4 seeds of dwarf bean were sown in each pots and cultivated for 21 days in controlled conditions (16h light/8h darkness regime, 25°C/21°C). The photoperiod was set at 16h day with an approximate photon flux of 150 µE $m^{-2}s^{-1}$. The soil water holding capacity was kept between 55% and 65% of the potential water holding capacity by daily measuring weight of each pot. It was adjusted with deionized water throughout the experimental period.

2.6 Plant analysis

Plants were harvested 21 days after sowing, the above ground plant tissues (primary leaves) were cut and rinsed thoroughly with double deionized water. Washed leaves were dried at

80°C for 72 h before recording the dry matter yield. Dried plant leaves were ground with a laboratory grinder and digested with a pressurized closed-vessel microwave system (Multiwave 3000, Anton Paar GmbH, Germany). Microwave polyfluoroacetylene (PFA)-teflon vessels were cleaned before each digestion using 10ml of aqua regia (HNO_3/HCl, 1:3v/v), heated for 20min. at 200°C and then rinsed with double deionized water. After the microwave-assisted digestion procedure was optimized, the following conditions were employed: samples were accurately weighed to 200mg (±0.1mg) in microwave vessels. The digestion program consisted of a 15min gradual increase to 200°C, a 15min digestion step at 200°C and 1200w and then a cooling stage. After cooling to room temperature, the samples were filtered through a 0.45μm filter paper and diluted to 50 ml with ultra-pure water (18 $M\Omega cm^{-1}$). Blanks were processed in a method identical to the samples. ICP–MS measurements were carried out by diluting 1:10 the plant digests to determine the primary dwarf bean leaves PTE concentrations. Mineral masses of PTE related to one dwarf bean were calculated based on the bean dry weight and PTE concentration in the bean leaves.

2.7 Statistical analysis

All tests were performed in five replicates and results are presented as mean ± standard deviation. The data were statistically analyzed using Excel and Statistica 10 package software. Statistical analysis including Pearson's correlation coefficient were calculated to determine the relationships between the PTE concentrations in the above parts of dwarf bean (primary leaves), the PTE mineral masses and extractable PTE concentrations in soils by the various single extraction methods. The correlations were examined based on plant concentrations on dry weight (DW) basis. The level of significance was set at ($p< 0.05$).

3 Results and discussion

3.1 Physico-chemical properties of the studied soils

The main physico- chemical properties of the soil samples are summarized in Table 1. pH of MDN samples was slightly acid and ranged from 6.14 to 6.92, whilst, pH of LPF samples was acidic to slightly acid ranged from 3.65 to 5.75. Organic carbon ranged from 3.35 to 6.45% and 0.3 to 1.67% for both MDN and LPF samples respectively. Cation exchange capacity varied from 6.7 to 8.5 $cmol(+)kg^{-1}$ and 18.3 to 39.7 $cmol(+)kg^{-1}$ for MDN and LPF respectively. Pseudo total PTE contents for both MDN and LPF soil samples are presented in Table 2.

3.2 PTE extractability

The results of Zn, Cd, Pb, As and Sb extractabilities in $mg.kg^{-1}$ obtained with the five extraction procedures on the studied polluted samples are presented in Table 3 and Table 4 for the MDN and LPF samples respectively. To help compare the metals extracted by different methods, the relative metals extractabilities expressed in percent are presented in Fig. 1. The $CaCl_2$, $NaNO_3$, NH_4NO_3, EDTA and DTPA soil extractable fractions were different from sample to sample for both studied sites.

The highest extracted amounts of Zn were observed for the highly contaminated soil (MDN1). Zn extractability obtained with the EDTA extraction was higher compared with that obtained with the DTPA and the other extraction procedures for all MDN selected locations. The extracted Zn decreased successively for extraction with DTPA to $NaNO_3$, but exhibited a significantly higher extracted amount for the EDTA solution. $NaNO_3$ exhibits the lowest extraction efficiency in all samples. Overall, the sequence of Zn extractability in all studied samples being: EDTA>DTPA>NH_4NO_3> $CaCl_2$>$NaNO_3$, except, for MDN2, extractable-Zn was found higher with $CaCl_2$ than that of NH_4NO_3. It can be seen that dilute $CaCl_2$ and nitrate extracting media does not extract high amount of PTE as compared to the complexing extracting media such as EDTA. These results are in concordance with previous studies, where EDTA was found the best extractant for phytoavailability of metals and solubilizes solid phase minerals giving a potential to over-estimate the bioavailable fraction of metals in soils (Singh, 2007). McLaughlin et al. (2000) also suggested that complexing extractants may provide a better estimate of potential hazards from future mobilization of metals than neutral salt extractions.

Similarly to Zn, the highest amount of extracted Cd was also observed for MDN1 with the EDTA extract. The amount of Cd extracted with EDTA and DTPA procedures was always higher than with the other solutions, the sequence of extractability in all samples being: EDTA>DTPA> $CaCl_2$>NH_4NO_3 > $NaNO_3$. Gupta and Sinha (2007) also reported that the complexing agents such as EDTA and DTPA were extracted higher Cd content in tannery waste contaminated soil. Indeed, in a comparison of the effectiveness of the neutral salt solutions, NH_4NO_3 extractability was in general higher than that obtained with $CaCl_2$ and $NaNO_3$. For MDN2, the extraction efficiency ($CaCl_2$> NH_4NO_3) for both Cd and Zn is consistent with previous findings of Novozamskyet al. (1993); Száková et al. (2001) and Pueyo et al. (2004) which that due to the combined effect of complexation by chloride, $CaCl_2$ in some soils can extract a higher metal amount than that by NH_4NO_3.

For Pb, similar extraction efficiencies for all studied samples were obtained. Again, the extractability with EDTA was higher in comparison with the other extractants. In contrast to

the EDTA, other methods yielded 10 times lower extracted amounts. The extraction efficiency in all samples decreased with the order: EDTA>DTPA>NH_4NO_3 > $CaCl_2$>$NaNO_3$. This result is consistent with the findings of Menzies et al. (2007); Gupta and Sinha (2007). Due to fact that many precipitation processes (e.g. hydroxides, carbonate and phosphates) affect the Pb extract content in soils, Lebourg et al. (1998b) referred to that these processes are limiting the estimation of lead bioavailability in soils by using the mild extraction solutions. In recent study, Gupta and Aten, (1993); Hornburg et al. (1995) have also referred to that the using of these extraction procedures have exhibits low Pb extractability.

Among all the extractants, EDTA extraction showed the better extractability of As and Sb in all the tested contaminated soil samples. Overall, extraction capacity was as follows: EDTA>DTPA >NH_4NO_3> $CaCl_2$>$NaNO_3$. These results are consistent with that reported by Etteler et al. (2007a). It can be seen from the results, the concentration of the EDTA-extractable Sb was in the range of 11.1 – 11.8 %. These results are relatively similar to that reported by He (2007) and Serafimovska et al. (2012) for the phytoavailable fraction (2.1 – 11.8 %) of Sb in contaminated soils. The relatively high phytoavailable As and Sb can represent a significant proportion in highly contaminated soils, but actual uptake will depend on plant species (Pérez-Sirvent et al. 2011).

In this study, it can be observed that neither dilute $CaCl_2$ extracting media nor nitrate solutions extract very high amount of elements as compared to EDTA and to total metal contents especially for LPF metals As, Pb and Sb. On the other hand, Zn, Pb and Cd in MDN site showed a better extractability in comparison to both total content and to LPF site metals. This is due to the fact that LPF site PTE were strongly associated with the residual fraction in all studied samples, whereas, a good percentage of PTE in the MDN site were found to associated with acid soluble fraction (Qasim and Motelica-Heino, 2014). This findings matched with that of (Lintschinger et al. 1998; Tighe et al. 2005; Denys et al. 2008; Wanat et al. 2013; 2014) which the low soluble contents can be explained by the association of As and Sb to stable phases, as iron oxides and residual fraction.

3.3 Evaluation of single extraction efficiency

From the results obtained during our work (Table 3 and Table 4) it can be seen that the EDTA was found to be the most efficient solution due to extract both carbonate and organically bound fractions of PTE which may be due to its low pH (Sahuquillo et al. 2003). EDTA extractants use organic ligands capable of forming strong complexes with metals as the basis of the extraction process (Menzies et al. 2007). Moreover, Dermont et al. (2008b) in

reviewing soil washing for metal removal, considers that EDTA is effective because it is recoverable and reusable (low biodegradability).

DTPA was also found to be an efficient extraction solution for soils with pH >5 to neutral pH conditions, but it exhibits low efficient in highly acidic soil for some PTE (in the case of As), where DTPA extracted amounts significantly lower than that of other extractants. This is probably due to the re-adsorption of negatively charged metal-DTPA complexes. Furthermore, Norvell (1984) and Ettler et al. (2007b) also reported that the pH buffering capacity of the DTPA method fails in some highly acidic soils.

In general, complexing agents form soluble complexes with metals in solution, thus reducing their activity and causing additional metal ions to desorb from the soil (Singh, 2007). Moreover, complexing agents are aggressive and solubilize solid phase minerals giving a potential to over-estimate the bioavailable fraction of metals in soils.

Besides, the NH_4NO_3 extraction solution yielded higher values for the extracted concentrations for all the studied PTE than that of $CaCl_2$, except for As and Sb in some soils. Lebourg et al. (1998a); Pueyo et al. (2004) referred to that the high Cd and Zn extractability obtained with the NH_4NO_3 procedure in comparison with the other neutral salt solutions might be due to the higher salt concentration of the NH_4NO_3 solution and the possible complexation of these elements by NH_3.

The use of neutral salt solution such as $CaCl_2$ solution as extractant is advocated on the assumption that phytoavailable PTE are mostly located on mineral surfaces and can be displaced by other cations (Meers et al. 2007a). Hornburg et al. (1995); Lopez-Sanchez et al. (2002) referred to that as due to the combined effect of complexation by chloride; $CaCl_2$ can extract higher amounts of both Zn and Cd than the other neutral extractants. Also, van der Sloot et al. (1996) reported that the divalent cation Ca^{2+} can remove them more easily than monovalent cations, such as Na^+ and NH_4^+ by the fact that these elements are adsorbed in cation-exchange positions.

Weak salts are favored because of their role in releasing elements from particular soil phases with which they are bound or associated especially $CaCl_2$. The use of $CaCl_2$ solution in this experiment might be due to that several studies have shown its ability to predict soil-solution concentrations for metals (e.g. Cd, Zn), and also, the extracted metals are not re-adsorbed on the solid soil constituents during the extraction, which are present in the extracts as free ionic forms or positively charged chloro-complexes (Pueyo et al. 2004; Meers et al. 2007b; Ettler et al. 2007b).

3.4 PTE bioavailability

The pot experiment of dwarf beans grown in both MDN and LPF soils for 21 days showed an accumulation of varying amounts of PTE in the plant primary leaves. Among all tested elements, the accumulation of Zn was found maximum, whereas, Sb was found to be minimum in the plant leaves. On average, the total areal biomass after 21 days varied between 0.06 and 0.16 g/plant dry weight. In the present study, the range and distribution of observed primary leaves concentrations for all PTE were broad (Table 5).

In general, dwarf bean plants growing in the MDN soils were found to have high Zn concentration (240 – 372 mg.kg^{-1} DW). Foliar Zn concentration in MDN1 plants was 1.5 times higher compared to both MDN2 and MDN3. The bean leaves presented Zn levels generally above the considered normal levels of Zn in plant tissues (10 to 100 mg kg^{-1}) according to Frisberg et al. (1986), which may indicate that these plant species growing on the contaminated site were tolerant to Zn. The high level of Zn concentrations in bean leaves may be due to the fact that Zn have a high mobility, and particularly in the Zn-rich soils, Zn might be translocate from the plant roots (which contain high Zn levels) and accumulated in the top parts of the plant.

Foliar Cd concentrations varied from 3.12 – 6.30 mg.kg^{-1}DW, this value being much higher than that reported by Feeney et al. 1984 in bean (0.13 mg.kg^{-1}) and MacLean 1976 (0.29 mg.kg^{-1}). A foliar cadmium concentration in plants from uncontaminated soils was about 0.044 mg.kg^{-1} (Hattab 2013). The long-term availability of Cd in plants is related to several soil properties, the presence of other ions in the soil solution, and the plant species. Due to the competition of Zn and Cd for -SH groups of proteins and enzymes in plants, Chaney (1974) proposed that zinc-cadmium interactions reduce the amount of Cd taken up by plants when the concentration of Cd is less than 1% of the zinc content in the sludge.

Foliar Pb concentrations ranged between (16 – 29 mg.kg^{-1}) and (5.7 – 8.6 mg.kg^{-1} DW) in both MDN and LPF soils respectively and were above the mean lead content in bean as reported by Theodoratos et al. (2002) (0.1 – 5 mg.kg^{-1} DW). Foliar As and Sb concentrations in bean leaves were ranged from (46 – 72 mg.kg^{-1}) and (1.48 – 1.52 mg. kg^{-1}) respectively. Concentrations of As in plants grown on uncontaminated soils vary from 0.009 to 1.5 mg.kg-1DW, (Kabata- Pendias and Pendias, 2000). It has also been reported that foliar As concentrations (mg As kg^{-1}) described in beans grown on control soils were 5.2 ± 0.2 (Carbonell-Barrachina et al., 1997), 0.38 (Mench et al. 2006), and 1- 4 (Macnicol and Beckett, 1985). In this study foliar As concentrations were many times higher than those reported by above studies. Overall, the total PTE accumulation was following the order: Zn>As>Pb>Cd>Sb. Meers et al. (2007b) also reported that high concentration of Zn was

accumulated in the aerial parts of *Phaseolus vulgaris* grown in different soil textures in comparison to other metals such as Cd, Cu, Pb and Mn. Wanat et al. (2014) showed that As concentrations in various organs of plants from the natural vegetation cover were much higher than that of both Pb and Sb.

Liphadzi and Kirkham (2005); Gupta and Sinha (2007a) shown that the process of metal accumulation in plants depends on the concentration of available metals in soils and their mobility and the plant species growing on these soils. The PTE considered readily available for plant uptake are those that exist as soluble components in the soil solution or that are easily desorbed or solubilized by root exudates, representing often only a small portion of the total content of the soil (Blaylock and Huang, 2000), as was found in the present study.

For MDN site, foliar Zn, Pb and Cd mass varied from (25.2 to 59.5), (1.68 to 4.64) and (0.32 to 1.008) mg per plant respectively (Fig. 2a, b, c). Moreover, for LPF site, foliar As, Pb and Sb mass varied from (3.34 to 5.08), (0.53 to 0.57) and (0.08 to 0.12) mg per plant respectively (Fig. 2d, e, f).

3.5 Correlation of soil single extraction and bioavailability

Results of the Pearson's correlation tests used to establish possible relationships between the extractable PTE contents and the concentration of PTE measured in dwarf bean primary leaves and foliar PTE mass are presented in Table 6 and Table 7 respectively. EDTA and DTPA represented the highest Zn extractability (Table 3), while they have present a poor prediction of Zn phytoavailibility for both foliar Zn concentrations and foliar Zn mass. In contrast, the other extractions based on diluted chloride solution 0.01M $CaCl_2$ and nitrate solutions $NaNO_3$ and NH_4NO_3 provided significant positive correlations ($P< 0.05$) with Zn accumulation and foliar Zn mass. These results correspond to those reporting that neutral salt solutions are more effective in estimating plant availability than the more aggressive tests such as EDTA and DTPA (Gupta and Aten, 1993; Lebourg et al., 1996; Sauerbeck and Styperek, 1985). Sauerbeck and Styperek (1983) also observed that the neutral salt solution is a useful extractant to predict the availability of trace metals in soil, and to be a good predictor of Zn in green beans (Houba et al. 1990).

In the case of Cd, although both EDTA and DTPA exhibited the highest Cd extractability in comparison to other neutral solutions; neither EDTA nor DTPA exhibited significant correlations with Cd levels in plant leaves and foliar mass. The best predictors for accumulated Cd and Cd mass were found with the results of unbuffered salt solutions $CaCl_2$, $NaNO_3$ and NH_4NO_3. Again, these weak extraction procedures were all correlated

significantly ($P< 0.05$) with bean leaves Cd accumulation. Krishnamurti et al. (2000) found that EDTA provided the poorest prediction of Cd availability. This apparent conflict in the reported effectiveness of complexing extractants may be due, at least partially, to differences between studies in the soils organic matter content, soil pH, the amount, source and form of the metal contaminant, and also the age of contaminant. Whitten and Richie (1991); Novozamsky et al. (1992) also reported a good correlation between extracted Cd by 0.01 M $CaCl_2$ and accumulated Cd in subterranean clover, lettuce and endive plants. Smild et al. (1992) also found high correlations between $CaCl_2$ extractable Zn and Cd and their concentrations in maize plant.

In a comparison of the effectiveness of the five different extractants for the prediction of Cd availability, Novozamsky et al. (1993) proposed using of 0.01M $CaCl_2$ as extraction reagent for estimation of metals and nutrients in air dry soil samples.

All the five extraction procedures showed significant correlations ($P< 0.05$) with accumulated Pb in dwarf bean leaves for both MDN and LPF soils but not for foliar Pb mass in LPF samples. Similar to findings for both Zn and Cd, the weaker extractions based on neutral salt solutions exhibited the best correlations between chemical extractions and plant PTE accumulation but not for Pb mass in LPF site. Even if many authors (Baxter et al. 1983; Cajuste et al. 2000; Miner et al. 1997; Sistani et al. 1995) concluded that complexing reagents give poor correlation to plant uptake, Gupta and Sinha (2007) reported that EDTA has shown best correlation with metals accumulated in the Sesamum indicum grown on different amendment of tannery sludge. Meers et al. (2007b) also found significant interactions of DTPA extractable Pb with plant uptake in the assessment of metals phytoavailibility.

In the current data set, good correlations were also observed between bean leaves accumulation and weaker extractants based on neutral salt solutions ($CaCl_2$, $NaNO_3$ and NH_4NO_3) and extractable As. Again, neither EDTA nor DTPA extraction procedures, aimed at ascertaining soil solution levels yielded consistently significant correlations with plant uptake and foliar As mass. These results are in accordance with findings of Martínez-Sánchez et al. (2011).

In contrast to As, $NaNO_3$ did not exhibit a significant correlation in the case of Sb, whilst, $CaCl_2$ and NH_4NO_3 presented positive significant correlation between extractable Sb and its level in the dwarf bean leaves and foliar Sb mass. In comparison to complexing agent, the extraction carried out with DTPA and EDTA cannot be considered appropriate for studying Sb mobility since they were not well correlated with plant Sb accumulation.

In general, distinct differences can be observed for the various PTE as significant correlations between the extraction procedures and PTE accumulation in the dwarf bean leaves in both

MDN and LPF samples. For all studied PTE the weaker extractants based on neutral salt solution exhibited good correlations with the PTE plant content. Except for Pb, the complexing agents resulted in higher correlations. The best correlation was that of 0.01M $CaCl_2$ in all studied samples. An important advantage is that the ionic strength of this extraction solution is similar to that of soil solution. In addition, Ca is generally the most abundant cation present in the soil solution.

4. Conclusion

Different extractants were used to evaluate the best extractant for bioavailability of metals to dwarf bean leaves in two former mining and smelting Technosols. The highest PTE (Zn, Cd, Pb, As and Sb) extractabilities were observed for the EDTA solution whereas other extraction media yielded lower extractabilities in comparison to EDTA. The results of Pearson's correlation coefficient analysis showed significant positive correlations between PTE accumulation in bean leaves and extractable metals by the neutral solutions ($CaCl_2$, $NaNO_3$ and NH_4NO_3). Poor correlation was found with both EDTA and DTPA extractable metals, except for Pb in both studied sites. In MDN site, Zn, Pb and Cd can be considered the most mobile elements and potentially available to the biosphere, representing therefore a more significant environmental risk than Pb, Sb and As in LPF samples.

In general, the $CaCl_2$ extraction procedure proved to be a suitable method for the assessing of metal mobility in soils and as it provided a good indication of phytoavailability of all metals under investigation. In accordance with other findings (Novozamsky et al. 1993; Pueyo et al. 2004; Meers et al. 2007b), the use of this procedure is therefore supported for evaluation of plant available PTE in the soil. In investigations dealing with soil ecosystems, it is necessary to take into consideration the content of soluble forms of metals in the soil solution, i.e. susceptible to transfer to the trophic chain, irrespective of the total PTE content.

Acknowledgements

The authors wish to acknowledge the Ministry of Higher Education and Scientific Research (Baghdad, Iraq) for financial support, Dr. Domenico Morabito (Université d'Orléans) for microwave digestion and Dr. Cécile Grosbois (Université de Tours) for granulometric analysis.

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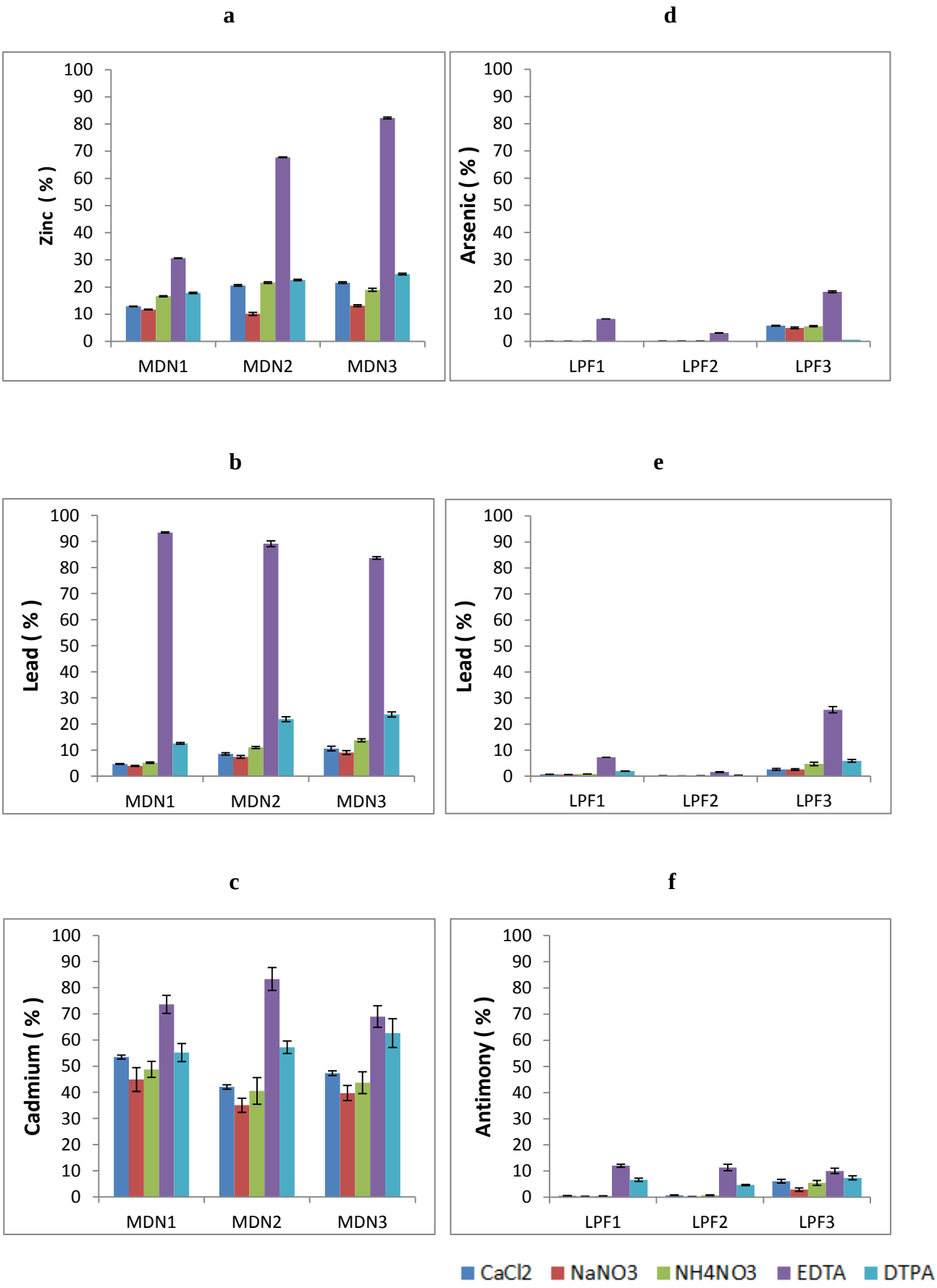


Fig. 1 Extractable potentially toxic elements (Zn, Cd, Pb, As and Sb) (%) by various single extractants ($CaCl_2$, $NaNO_3$, NH_4NO_3, EDTA and DTPA) for MDN site (a, b and c) and LPF site (d, e and f).

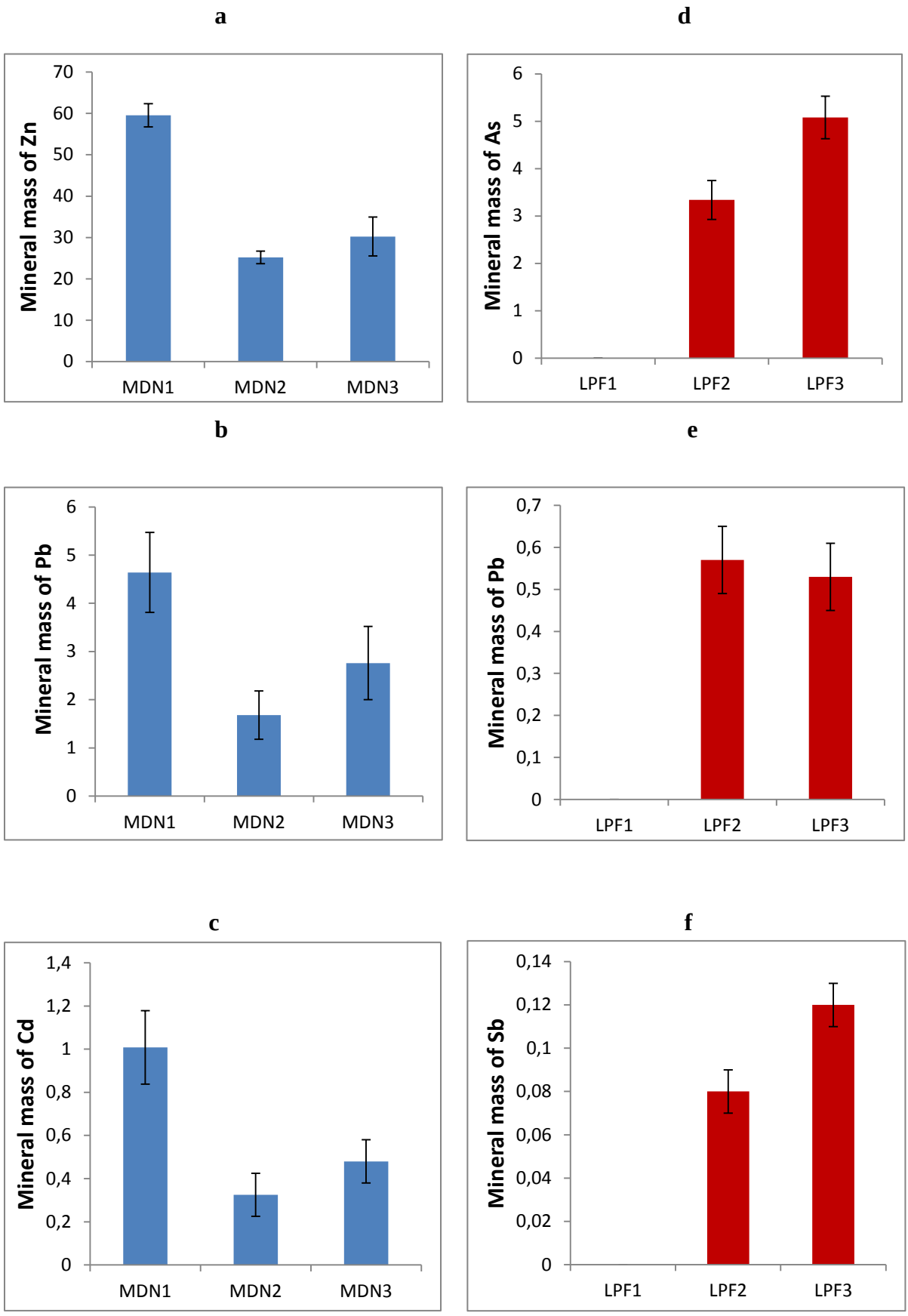


Figure 2 Mineral masses of Zn, Pb, Cd, As and Sb (mg/plant) in the dwarf bean primary leaves for MDN site (a, b and c) and LPF* site (d, e and f).

* The plants in LPF1 were not growth.

Table 1: Physico-chemical characteristics of the selected samples from Mortagne-du-Nord (MDN) and La Petite Faye (LPF) (n=3; ± standard deviation)

	MDN samples			LPF samples		
Parameters	MDN1	MDN2	MDN3	LPF1	LPF2	LPF3
pH-H_2O	6.92± 0.12	6.35± 0.34	6.14 ± 0.17	3.65 ± 0.05	4.93 ± 0.4	5.75 ±0.1
pH-KCl	6.58 ± 0.2	6.02 ± 0.2	5.89 ± 0.7	3.42 ± 0.1	4.57 ± 0.3	5.61 ± 0.1
EC	112 ±3	112 ±7	113 ±1	258 ± 4	200 ± 3	150 ± 3
OM %	8.26 ± 2.3	10.28 ±1.4	13.84 ±0.7	6.46 ±3.4	5.27 ±1.1	6.83 ±0.9
CEC	7.2 ±0.7	8.5 ± 0.2	6.7 ± 1.2	18.3 ± 0.7	29.1 ± 0.2	39.7 ± 0.3
TOC %	3.35 ±0.9	4.39 ±0.1	6.45 ±0.1	0.3 ± 0.8	0.47 ± 0.4	1.67 ± 0.8
Clay %	0.95± 0.24	0.86±0.01	0.9±0.24	3.79±0.34	1.07±1.03	2.02±0.41
Silt %	22.3±0.35	22.16±0.41	22.13±0.34	48.09±0.54	19.81±0.24	39.14±0.23
Sand %	75.45±0.12	76.84±1.03	76.66±0.28	48.12±0.1	78.6±0.64	58.8±0.03

Table 2: Pseudo total potentially toxic element content ($mg.kg^{-1}$) for the selected samples from Mortagne-du-Nord (MDN) and La Petite Faye (LPF) (n=3; ± standard deviation)

Sample	**Elements**		
	Zn	**Pb**	**Cd**
MDN 1	7726±12	3551±10	72±11
MDN 2	3114±11	881±8	64±5
MDN 3	3127±9	874±5	51±6
	As	**Pb**	**Sb**
LPF 1	83941± 11	16109±13	863±8
LPF 2	37462±8	8541±7	493±7
LPF 3	2672±4	367±4	107±9

Table 3: Extractable potentially toxic element content in the selected (**MDN**) samples (mg.kg^{-1}) (n=3; ± standard deviation)

Element	MDN 1	MDN 2	MDN 3
Zn			
$CaCl_2$	993.4±7	642.8±9	674.5±3
$NaNO_3$	906.6±2	315.6±4	409.2±1
NH_4NO_3	1082.4±4	674.6±0.3	591.6±1
EDTA	2366±3	2113.2±7	2564±0.9
DTPA	1375.3±1	611.6±2	772±0.2
Pb			
$CaCl_2$	166.1±4	74.78±3	93.2±1
$NaNO_3$	137.5±1	64.14±0.4	80.64±4
NH_4NO_3	182.5±0.6	96.28±4	120.6±0.1
EDTA	3318±2	780±1	731±7
DTPA	446.2±7	191.2±3	206.3±5
Cd			
$CaCl_2$	35.8±0.9	25.24±1	27.4±1.6
$NaNO_3$	30.08±2.3	21.22±0.6	23.2±2.5
NH_4NO_3	32.8±1.7	24.2±1	25.4±2
EDTA	49.2±2	50±3	40±0.8
DTPA	37±2.4	34.6±0.3	36.2±2

Table 4: Extractable potentially toxic element content in the selected (**LPF**) samples (mg.kg^{-1}) (n=3; ± standard deviation)

Element	LPF1	LPF2	LPF3
As			
$CaCl_2$	43.07±4	62.62±0.9	153.22±1
$NaNO_3$	20.59±1	43.04±1.1	132.52±0.6
NH_4NO_3	36.72±0.6	51.48±0.2	148.02±1.3
EDTA	6931.2±2	1151±1	486.8±2.1
DTPA	5.17±0.7	14.1±0.8	15.3±3.3
Pb			
$CaCl_2$	117.8±2	17.48±2.4	9.52±1.7
$NaNO_3$	101.34±0.8	11.3±1	9.4±2.1
NH_4NO_3	137.32±4	19.7±0.01	17.3±0.4
EDTA	1167±0.2	137.2±3	93.4±0.03
DTPA	314.5±5	25.42±6	21.5±1.4
Sb			
$CaCl_2$	4.6±2.7	3.7±1.3	6.96±0.4
$NaNO_3$	2.5±0.6	1.04±2.9	3.3±0.7
NH_4NO_3	3.8±2.4	3.4±4	6.24±2
EDTA	103.6±4	55.2±3	11.4±1
DTPA	57±1.1	22.7±2.7	8.4±0.6

Table 5: Total potentially toxic element content in primary bean leaves (DW) after 21 days in the selected (**MDN**) and (**LPF**) samples (mg.kg^{-1} DW) (n=3; ± standard deviation)

Sample	**Element**		
	Zn	**Pb**	**Cd**
MDN1	372.5±2.8	29.24±2	6.30±0.4
MDN2	240±1.5	16 ±1	3.12±0.1
MDN3	252.7±4.7	23.3±0.5	4.02±1
	As	**Pb**	**Sb**
LPF1	Ng	Ng	Ng
LPF2	55.70±4	9.6±1	1.48±1.2
LPF3	72.76±2.8	6.76±3	2.52±1.8

Ng, no growth

Table 6: Pearson's correlation coefficients between potentially toxic element concentrations in primary bean leaves and their extractable concentrations by different single extraction procedures for **MDN** and **LPF** samples.

	Primary bean leaves after 21days (DW)					
	MDN samples			LPF samples		
Extraction	Zn	Pb	Cd	As	Sb	Pb
$CaCl_2$	0.99**	0.90**	0.94**	0.86*	0.88*	0.91*
$NaNO_3$	0.99**	0.89**	0.80**	0.92**	0.64 ^{NS}	0.69 ^{NS}
NH_4NO_3	0.97**	0.93**	0.84*	0.87*	0.78*	0.88*
EDTA	0.14 ^{NS}	0.81*	0.01 ^{NS}	0.28 ^{NS}	0.44 ^{NS}	0.89*
DTPA	0.67 ^{NS}	0.85**	0.23 ^{NS}	0.42 ^{NS}	- 0.78 ^{NS}	0.90*

** Correlation is significant at the 0.01 level; * correlation is significant at the 0.05 level; NS, no significant correlation.

Table 7: Pearson's correlation coefficients between foliar potentially toxic element mineral mass and their extractable concentrations by different single extraction procedures for **MDN** and **LPF** samples.

	Primary bean leaves after 21days (DW)					
	MDN samples			LPF samples		
Extraction	Zn	Pb	Cd	As	Sb	Pb
$CaCl_2$	0.99**	0.87*	0.95**	0.80*	0.6 ^{NS}	0.28 ^{NS}
$NaNO_3$	0.99**	0.86*	0.93**	0.83*	0.21	0.67 ^{NS}
NH_4NO_3	0.96**	0.91**	0.81**	0.81**	0.65*	0.03 ^{NS}
EDTA	0.2 ^{NS}	0.83**	0.2 ^{NS}	- 0.76 ^{NS}	- 0.48 ^{NS}	- 0.15 ^{NS}
DTPA	0.49 ^{NS}	0.86**	0.04 ^{NS}	0.51 ^{NS}	- 0.58 ^{NS}	- 0.24 ^{NS}

** Correlation is significant at the 0.01 level; * correlation is significant at the 0.05 level; NS, no significant correlation.