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Environmental Science and Pollution Research

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--Manuscript Draft--

Manuscript Number:	ESPR-D-15-04475R1
Full Title:	Potentially toxic element contamination in soil and accumulation in maize plants in a smelter area in Kosovo
Article Type:	Research Article
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Funding Information:	
Abstract:	A biogeochemical field study was carried out in the industrial area of Kosovska Mitrovica in northern Kosovo, where agricultural soils were contaminated by potentially toxic elements due to smelting activity. Total and bioavailable contents of As, Cd, Co, Cu, Pb, Sb, U and Zn in soil and their concentrations in maize roots and grains were determined. Soil contamination by As, Cd, Cu, Pb, Sb and Zn was variable from slightly to highly contaminated soils, and influenced both the bioavailable fraction and accumulation of these potentially toxic elements in maize tissues. The comparison between potentially toxic element concentrations in roots and grains indicated that maize is able to limit the transfer of non-essential elements to edible parts. The plant-to-soil bioconcentration indices suggested that the transfer of potentially toxic elements from soil to plant was predicted better by bioavailable concentrations than by the total contents. These indices further identified some competitions and interactions among these elements in root uptake and root-to-grain translocation.
Response to Reviewers:	Ref.: Ms. No. ESPR-D-15-04475 Potentially toxic element contamination in soil and accumulation in maize plants in a smelter area in Kosovo Reviewers' comments Reviewer #1: The manuscript Number: ESPR-D-15-04475 "Heavy element contamination in soil and accumulation in maize plants in a smelter area in Kosovo" submitted for Research Article in Environmental Science and Pollution Research presents an interesting field study on soil-plant transfers of various inorganic elements potentially toxic. Actually, the question of produce (human) safe vegetables in polluted areas is increasingly asked at the global scale. The biogeochemical field studies are needed to better understand the transfers of metal(loi)ds in agricultural soils. However, several changes are needed before the manuscript could be accepted.

Potentially toxic element contamination in soil and accumulation in maize plants in a smelter area in Kosovo

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ABSTRACT

A biogeochemical field study was carried out in the industrial area of Kosovska Mitrovica in northern Kosovo, where agricultural soils were contaminated by potentially toxic elements due to smelting activity. Total and bioavailable contents of As, Cd, Co, Cu, Pb, Sb, U and Zn in soil and their concentrations in maize roots and grains were determined. Soil contamination by As, Cd, Cu, Pb, Sb and Zn was variable from slightly to highly contaminated soils, and influenced both the bioavailable fraction and accumulation of these potentially toxic elements in maize tissues. The comparison between potentially toxic element concentrations in roots and grains indicated that maize is able to limit the transfer of non-essential elements to edible parts. The plant-to-soil bioconcentration indices suggested that the transfer of potentially toxic elements from soil to plant was predicted better by bioavailable concentrations than by the total contents. These indices further identified some competitions and interactions among these elements in root uptake and root-to-grain translocation.

Keywords: Potentially toxic elements; Soil; *Zea mays* L.; Smelter; Bioavailability; Kosovo.

1. Introduction

Smelting activity is an important source of potentially toxic elements (PTEs) and cause of adverse environmental impacts. The emission of PTEs from smelters in form of particulate matter and gases frequently produced soil contamination (Adriano 2001; Cappuyns et al. 2006; Li et al. 2006; Navarro et al. 2008; Razo et al. 2004). The PTEs released into the atmosphere by smelting activities may accumulate in topsoil and be transferred to plants through root uptake, involving a contamination risk of the food chain.

Contamination of agricultural soils around smelting plants is a major environmental concern as it can cause accumulation of PTEs in crops affecting plant productivity, food quality and human health (Dudka and Miller 1999; Gupta and Gupta 1998; Kachenko and Singh 2006; Lim et al. 2008; Pruvot et al. 2006; Roy and McDonald 2013; Wcisło et al. 2002; Yang et al., 2011; Zheng et al. 2007).

Maize (*Zea mays* L.) is one of most widely and intensively cultivated cereal worldwide, and it constitutes a staple food for humans and animals. For the global production, utilization and consumption of maize, the transfer of PTEs through the soil-maize system may represent an important pathway of human exposure, especially in contaminated areas (Rosas-Castor et al. 2014). Contamination of agricultural soils by PTEs may result in an increase of their uptake by maize with negative effects on the quality of crops and a potential risk for human health. For these reasons, several studies were carried out to determine levels and distribution of PTEs in maize plants grown in soils contaminated by industrial, smelting and mining activities (Awokunmi et al. 2015; Bi et al. 2009; Bini et al. 2013; Liu et al. 2005; Marwa et al. 2012; Wahsha et al. 2014) or cultivated using different tillage systems (Brennan et al. 2014; Carbonell et al. 2011; Garcia-Marco et al. 2014; Lavado et al. 2007; Lu et al. 2015; Muchuweti et al. 2006). These experimental and field studies aimed to assess the impact of soil contamination and agricultural practices on uptake, translocation and accumulation of PTEs in maize tissues (roots, leaves and grains).

The Kosovska Mitrovica area in northern Kosovo represents a zone of interest for studies concerning the contamination of agricultural soils by PTEs caused by smelter emissions, and their transfer and accumulation in cultivated plants. This area was one of the main industrial sites in the former Yugoslavia and an important mining district in Europe. Industrial activity focused on the Zvecan Pb-Zn smelter and the Trepča factory of batteries and accumulators, caused an intense and widespread soil contamination by PTEs such as As, Cd, Cu, Pb, Sb and Zn (Borgna et al. 2009; Šajn et al. 2013). Soil contamination

involved a wide agricultural area extending in the Ibar and Sitnica alluvial plains where maize is the most cultivated crop.

A field biogeochemical research was carried out in the K. Mitrovica area in order to define the influence of soil contamination due to smelting activity on uptake, translocation and accumulation of potentially toxic elements in maize tissues. The total contents and the bioavailable concentrations of As, Cd, Co, Cu, Pb, Sb, U and Zn in soil samples as well as their concentrations in maize roots and grains were determined.

This research is a contribution to the knowledge about the soil-to-plant transfer of PTEs in smelter-contaminated areas. The main objectives of the study were: i) to establish a relationship between soil contamination and bioavailability of PTEs in soil; ii) to evaluate the influence of soil contamination on PTE transfer from soil to maize; iii) to define the behaviour of PTEs in uptake, translocation and accumulation in maize roots and grains grown in soils affected by variable contamination levels.

Our research considered both the total content and bioavailable fraction of PTEs in soil as chemical pools that may rule the soil-to-maize transfer of these elements, and measured the levels and distribution of Sb and U in the soil-maize system. To the best of our knowledge, few field studies used the bioavailable concentrations of PTEs in soil for these purposes (Garcia-Marco et al. 2014; Lavado et al. 2007), and focused on uptake, translocation and accumulation of Sb and U in maize tissues.

2. Materials and methods

2.1. Study area

The study area is located in northern Kosovo and includes the Trepča mining and industrial complex centred on the town of Kosovka Mitrovica. The mines of the Trepča complex exploited skarn-type ore bodies mainly consisting of base-metal (Pb-Zn) massive sulphides. Industrial activity was focused on the Zvecan Pb-Zn smelter and the Trepča factory (Fig. 1).

The Zvecan smelter worked from 1939 to 2000 to extract metals such as Ag, Au, Bi, Pb and Zn from the crude ore of Trepča mines. Smelting processes released into the atmosphere huge quantities of contaminants (mainly SOx and potentially toxic elements) as gas and particulate matter, and produced waste disposed in large open dumps close to

the smelter. The Trepča factory produced battery and accumulators from 1967 to 1999. An open dump of industrial waste is present in the factory site.

In the study area soil was intensively and extensively contaminated by potentially toxic elements (As, Cd, Cu, Pb, Sb and Zn) due to the emissions from the Zvecan smelter and Trepča factory and, to a lesser degree, the airborne dispersion of mining and industrial waste (Borgna et al. 2009; Nannoni et al. 2011; Šajn et al. 2013). Contamination affected the upper part of soil profile (usually up to 50-60 cm deep) and extended up to 22 km north and south of the smelting plant (Borgna et al. 2009). The concentrations of As, Cd, Cu, Pb, Sb and Zn in soil progressively decreased with distance from the Zvecan smelter, and were influenced by the dominant wind directions (mainly from NNW). Soil contamination involved a wide agricultural zone extending mainly in the Sitnica alluvial plain south of the K. Mitrovica town (Fig. 1).

The landscape of the study area is characterized by high hills usually from 600 to 1000 m a.s.l. These reliefs are arranged in two NW-SE ridges surrounding the valleys of the Ibar and Sitnica Rivers. The Sitnica River originates about 40 km south-east of K. Mitrovica and flows north-east in a wide asymmetrical valley. Near the town of K. Mitrovica the Sitnica River merges into the Ibar River that flows northward in a narrow valley.

2.2. Sampling strategy

Topsoil samples (20 cm deep) and maize plants were collected in 24 cultivated sites located in the alluvial plains of the Ibar and Sitnica Rivers at variable distances from the Zvecan smelter (from 2.2 to 20.7 km; Fig. 1).

Each topsoil sample was a composite sample consisting of three sub-samples collected a few metres apart in three subsites. Soil sub-samples were collected using a stainless steel spade. This tool was utilized to extract a 20x20x20 cm soil block containing the roots of a maize plant. By using this sampling procedure, in each sampling site three plants of maize were collected just before the harvest, and mixed to form a composite sample.

2.3. Laboratory treatment

Soil samples were dried at +40 °C and manually sieved through a 2 mm mesh. Soil fraction <2 mm was homogenized by quartering and pulverization. Soil samples were solubilized adding 1 mL HF, 2 mL HNO_3, 2 mL HCl and 1 mL $HClO_4$ to about 200 mg of powdered soil. To determine the total content of As, chlorine-free chemical reagents were used and soil digestion was carried out adding 2 mL HF, 2 mL HNO_3, and 1 mL H_2O_2 to about 200

mg of powdered soil. Both acid digestion procedures were performed in Teflon® bombs using a Milestone Ethos 900 microwave lab station. Ultrapure trace-grade reagents were used for soil laboratory treatment.

The maize plants were stored at +4 °C and roots and grains separated. Roots were preliminarily washed with running tap water and then immersed in an ultrasonic bath for 30 min and rinsed with deionized water. Grains were washed with running tap water and rinsed with deionized water. Both roots and grains were dried at +30 °C and then finely powdered before elemental analysis. The roots and grains were solubilized by acid digestion adding in Teflon® bombs 6 mL HNO_3 and 1 mL H_2O_2 (ultrapure reagents) to about 500 mg of powdered sample. Acid digestion was performed using a Milestone Ethos 900 microwave lab station.

2.4. Analytical determinations

The hydrometer method was used to determine the size distribution of soil particles expressed as percent content of the sandy, silty and clayey fractions. Soil pH (pH_{H_2O}) and the effective cation exchange capacity (CECe) were measured following the EPA method 9045/D and the procedure of Hendershot and Duquette (1986), respectively. The method of Walkley and Black (1934) was employed to determine the organic carbon content, and De Astis calcimeter to measure the carbonate content (as % $CaCO_3$).

Inductively coupled plasma-mass spectrometry (Perkin Elmer Sciex Elan 6100 spectrometer) was employed to measure the total contents and bioavailable concentrations of As, Cd, Co, Cu, Pb, Sb, U and Zn in soil samples as well as the concentrations in maize roots and grains.

The analytical accuracy of the total contents of the analyzed elements was established using the NIST 2709 (San Joaquin Soil) and NIST 2710 (Montana Soil) reference materials. The analytical precision was evaluated by five replicate analyses of each soil sample and expressed as percent relative standard deviation (% RSD).

A chemical extraction procedure was used to determine the concentrations of As, Cd, Co, Cu, Pb, Sb, U and Zn in the bioavailable (extractable) fraction of soil samples. This extraction based on the protocol (step A) of the Standards, Measurements and Testing Programme (formerly BCR, Bureau Community of Reference) of the European Commission, was performed adding 40 mL of a 0.11M CH_3COOH (acetic acid) solution to 1 g of powdered soil sample. The mixture was shaken for 16 h at room temperature and

centrifuged. To validate the analytical results of extraction procedure, the concentrations of Cd, Cr, Cu, Ni, Pb and Zn were determined in the BCR-701 reference material.

The GBW 07603 (Bush branches and leaves) and NIST RM 8413 (Corn Kernel-Zea mays) reference materials were utilized to check the accuracy of analytical determinations in maize samples. The analytical pprecision was determined by means of five replicate analyses of each maize sample.

2.5. Geochemical and biological indices

To assess the overall soil contamination level, the Pollution Load Index (PLI) developed by Tomlinson et al. (1980), was employed. The value of PLI was calculated by the *n*-root of the product of the Concentration Factor (CF) of the *n* chemical elements considered: PLI = $n\sqrt{}$(CF1 x CF2 x CF3 x …. CF*n*). The CF of a chemical element was given by $[C]_{element}/[C]_{background\ value}$, where: $[C]_{element}$ = element concentration in soil, $[C]_{background\ value}$ = average value of local geochemical background of the element in soil. According to Singh et al. (2003), PLI values vary as follows: PLI = 0 background concentration; 0< PLI ≤1 uncontaminated; 1< PLI ≤2 moderately to uncontaminated; 2< PLI ≤3 moderately contaminated; 3< PLI ≤4 moderately to highly contaminated; 4< PLI ≤5 highly contaminated; PLI >5 very highly contaminated.

The mobility in soil of the analyzed chemical elements was assessed using the Mobility Factor Index (MFI; Kabala and Singh 2001; Narwal et al. 1999) calculated as the ratio between the bioavailable concentration of the element and its total content in soil.

In the current scientific literature, the plant-to-soil bioconcentration ratios are indices used to evaluate the transfer of a chemical element from soil to plant, comparing concentrations in vegetation parts to concentrations in soil. In this study the Biological Concentration Factor (BCF; Yoon et al. 2006) was calculated as the ratio of the element concentration in maize roots to its total content (BCF_{tot}) and bioavailable concentration (BCF_{bio}) in soil. Similarly, the Biological Accumulation Coefficient (BAC; Cui et al. 2007; Li et al. 2007) was determined as the ratio of the element concentration in maize grains to its total content (BAC_{tot}) and bioavailable concentration (BAC_{bio}) in soil.

The Translocation Factor (TF) as the ratio of the element concentration in plant shoots to that in roots (Cui et al. 2007; Li et al. 2007), was used to evaluate the effectiveness of the maize plants in translocating As, Cd, Co, Cu, Pb, Sb, U and Zn from roots to grains.

2.6. Statistical analysis

The analytical data were treated statistically using the software Statistica 8 (Statsoft, Padova). The Shapiro-Wilk W normality test was used to check the normal distribution of the element contents in soil and maize samples. The test results showed that most of data had a non normal distribution (p<0.05). The non-parametric Mann-Whitney U test was used to check the difference of the median values of the analyzed chemical elements between the two groups of soils and maize plants defined according to soil contamination level. The Spearman's correlation coefficient was employed to measure the correlation between the variables. A multivariate statistical approach was applied to verify the geochemical characterization of soil samples. Principal Component Analysis (PCA) was applied to the standardized data of element concentrations (total and bioavailable) in soil sample. Soil samples were clustered based on the case coordinates in respect to the principal axis (components 1 and 2) of PCA analysis. The distances between objects were defined by the Euclidean distances and the objects were linked together by Ward's method.

3. Results and discussion

3.1. Potentially toxic elements in soil

The sampled soils were loam soils formed from the Quaternary alluvial sediments of the Ibar and Sitnica Rivers, and, to a lesser extent, clay loam soils derived from the Pliocene sediments cropping out in the Sitnica valley. Soil pH usually ranged between 6.9 and 7.9 (neutral to alkaline soils) and carbonate content between 0.5 and 2% $CaCO_3$ (slightly calcareous soils). Soil samples had a very high organic carbon content (C_{org} >2.2%) and a high cation exchange capacity (CECe usually from 24 to 42 $cmol_{(+)}kg^{-1}$). The cation exchange capacity increased as the organic carbon content and clayey fraction increased.

In order to define the contaminants and the contamination levels in soils of K. Mitrovica area, the total contents of As, Cd, Co, Cu, Pb, Sb, U and Zn in soil samples were first compared with the respective local natural variability in soil (geochemical background) using the Concentration Factor (CF). The geochemical background of As, Cd, Co, Cu, Pb, Sb, U and Zn in soils of the study area (alluvial plains of the Ibar and Sitnica Rivers) was estimated using the total contents of these PTEs in uncontaminated subsoil samples (below 90 cm depth) derived from the alluvial sediments of the Ibar and Sitnica Rivers (Borgna et al. 2009). This approach was applied as soil contamination by PTEs extended for tens of

kilometers involving the topsoil of the study area, and affected soil profiles usually up to 50-60 cm deep (Borgna et al. 2009; Šajn et al. 2013).

Using the CF scale proposed by Sutherland (2000), Cd, Pb and Sb were moderately to significantly enriched elements in soil as the CF values were usually above 2 (0.8 to 17.5 for Cd, 0.9 to 20.4 for Pb, 0.4 to 7.5 for Sb). The CFs of As (0.4-21.3), Cu (0.7-2.7) and Zn (0.4-7.5) indicated geogenic abundances to moderate enrichments. Co and U were not enriched in soil samples as CF values were below 2 (0.6 to 2.2 for Co, 0.4 to 1.4 for U).

The highest enrichments of As, Cu, Pb and Sb were found in soils collected around the Zvecan smelter within a distance of about 5 km from the plant. Cd and Zn were more enriched in soils close the Trepča factory. As a general trend, the enrichment levels of As, Cd, Cu, Pb, Sb and Zn in soil decreased as the distance from the industrial district of K. Mitrovica (Zvecan smelter and Trepča factory) increased.

According to the scale proposed by Singh et al. (2003), the values of Pollution Load Index (PLI) pointed out that soil samples were usually contaminated by PTEs (mainly Cd, Pb and Sb), and contamination levels were variable from slightly to highly contaminated soils. Based on the PLI values, it was possible to divide soil samples in two groups: the first group included uncontaminated to moderately contaminated soils ($0< PLI \leq 2$), hereafter named slightly contaminated soils (n=14); the second group included moderately to highly contaminated soils ($2< PLI <5$), hereafter named contaminated soils (n=10).

Table 1 reports the geochemical features of both slightly contaminated and contaminated soils expressed by min, max, mean and median of As, Cd, Co, Cu, Pb, Sb, U and Zn total contents. Statistically significant differences between the median of PTE total contents in soils of two groups ($p<0.05$) were found.

Multivariate statistical techniques such as Principal Component Analysis (PCA) and cluster analysis were also applied for soil characterization. PCA indicated that the first factor was defined by the soil contaminants as the following order: Sb>Pb>Cd>Cu>Zn>As (Fig. 2a). Co and U described the second factor suggesting a geogenic origin of these elements. Soil samples (cases) coordinates were used as variables for soil classification by cluster analysis. The results confirmed the above described division of soil samples in slightly contaminated and contaminated soils (Fig. 2b).

As shown in Table 1, slightly contaminated and contaminated soils had also distinctive bioavailable concentrations of the analyzed PTEs. The differences were statistically significant ($p<0.05$) for the contaminants (As, Cd, Cu, Pb, Sb and Zn). On the contrary, the

concentrations of the geogenic elements (Co and U) were rather uniform in both soil groups.

The multivariate statistical approach (PCA and cluster analyses) applied to the bioavailable concentrations of As, Cd, Co, Cu, Pb, Sb, U and Zn confirmed the same grouping of soil samples in slightly contaminated and contaminated soils obtained using the total contents of these PTEs.

The bioavailable fraction of PTEs was constantly higher in contaminated soils than in the slightly contaminated ones. The most significant difference was found for Zn, whose bioavailable percentage amount in contaminated soils was on average double than in slightly contaminated soils (13% vs 6.5%).

On the basis of Mobility Factor Index (MFI), the order of mobility of the potentially toxic elements in contaminated soils was: Cd>>Zn>Co>Pb>As≈Cu>Sb>U, similar to that in slightly contaminated soils: Cd>>Co>Zn>Pb>As≈Cu>Sb>U. In both groups of soils Cd was by far the most bioavailable PTE.

3.2. Potentially toxic elements in maize

Table 2 reports the main statistical indices of As, Cd, Co, Cu, Pb, Sb, U and Zn concentrations in roots and grains of maize specimens grouped according to the contamination level of soil. Analytical data indicated that the analyzed PTEs had the highest concentrations in the roots of maize plants grown in contaminated soils. Nevertheless, statistical analysis by means the Mann-Whitney U test revealed that significant differences (p<0.05) were only between the concentrations of contaminants (As, Cd, Cu, Pb, Sb and Zn) in maize roots from slightly contaminated and contaminated soils. In maize roots grown in slightly contaminated soils, the order of PTE concentrations (Zn>>Cu>Pb>>Co>As>Cd>U>Sb) partly differed from that of maize plants from contaminated soils (Zn>>Pb>Cu>As>>Co>Cd>Sb>U). These findings suggested that soil contamination caused a substantial increase of root uptake of PTEs resulting in root reservoir for toxic metals, especially As, Cd, Pb and Sb.

No statistical difference (p<0.05) was found for the concentrations of PTEs in grains of maize plants cultivated in slightly contaminated and contaminated soils (Table 2). However, the average concentrations of contaminants were higher in maize grains from contaminated sites.

In all maize plants the concentrations of As, Cd, Co, Pb, Sb and U in grains were significantly lower than in roots, suggesting that maize was able to limit the transfer of

these non-essential elements from root to edible part (Table 2). Conversely, Cu and Zn concentrations were comparable in maize grains and roots likely because these elements are plant micronutrients.

The concentrations of As, Cd and Pb in maize grains were compared with the maximum permissible levels set by the European Commission Regulation for foodstuff (European Commission 2006) and feedstuff (European Commission 2002). European Commission established the maximum permissible level in cereals for human consumption at 0.1 mg kg^{-1} for Cd and 0.2 mg kg^{-1} for Pb (fresh weight). The maize grains sampled in this study had always Pb concentrations over the legislation limit of 0.2 mg kg^{-1}, while 36% of Cd concentrations in grains from slightly contaminated soils and 50% from contaminated soils exceeded the maximum permissible level of 0.1 mg kg^{-1}.

European limits in products for animal feed are given for As (2 mg kg^{-1}), Cd (1 mg kg^{-1}) and Pb (10 mg kg^{-1}). As these values are referred to foods with 12% humidity, As, Cd and Pb concentrations in maize grains were normalized to this level of humidity to allow a correct comparison. Analytical data showed that no grain samples exceeded As, Cd and Pb European limits for feedstuff.

As suggested by Andersson and Pettersson (1981) and Carbonell et al. (2011), the concentration of Zn in maize grains should be less than 34 mg kg^{-1} to be fit for human consumption. Zn exceeded this indicative threshold in 50% of grains from contaminated soils.

3.3. Potentially toxic elements from soil to maize

The values of the Biological Concentration Factor (BCF) and Biological Accumulation Coefficient (BAC) showed a large variability depending on the chemical element, maize part, use of total or bioavailable concentration and soil contamination level (Tab. 3). Nevertheless, it has to be noted that the BCF_{tot} and BCF_{bio} values of the analyzed PTEs (except for Zn) were higher than the respective BAC_{tot} and BAC_{bio}. This finding confirmed an important restriction of the internal transport of PTEs from roots to grains in maize plants, likely due to the metal-binding capacity of root system (Carbonell et al. 2011; Singh and Agrawal 2007).

There was no statistically significant difference between the BCF_{tot} and BCF_{bio} of As, Cd, Co, Cu, Pb, Sb and U in maize roots grown in slightly contaminated and contaminated soils. The only exception is given by Zn, as the BCF_{tot} and BCF_{bio} values of this element were significantly higher in maize plants from slightly contaminated soils (Tab. 3). A

possible explanation is that the high concentrations of Cd and Cu in contaminated soils competitively inhibited the uptake of Zn by roots (Choudhary et al. 1995; Hart et al. 2002; Kabata-Pendias and Pendias 1984; Mullins and Sommers 1986; Wang et al. 2007).

This study confirmed that the bioavailable concentrations and BCF_{bio} predict the transfer of PTEs from soil to plant better than the total content and BCF_{tot}. Statistical analysis indicated significant inverse correlations ($p<0.05$) between BCF_{bio} and bioavailable concentration in soil for the analyzed PTEs (except for Cd), while correlations between the BCF_{tot} and total concentration in soil were found only for As, Cu, U and Zn. The lack of significant correlation for Cd could be due to its high geochemical mobility in soil and the ability of cultivated plants to tolerate high concentrations of the element without any symptom of toxicity (Dudka et al. 1995). Likewise, BAC_{bio} values were negatively correlated with bioavailable concentration in soils for all the analyzed PTEs.

As a whole, the Translocation Factor (TF) of PTEs was comparable in maize plants from slightly contaminated and contaminated soils (Tab. 3). Only the TF values of Cu and Pb were statistically different and higher in maize grown in slightly contaminated sites (Tab. 3). This evidence could be attributed to greater competition among PTEs during translocation from roots to grains at high concentrations (Greger 1999).

The TF values in maize plants from slightly contaminated and contaminated soils varied as the following order: Zn>Cu>Cd>U>Sb>Pb>Co>As. FTs were usually <0.5 and only Zn had values about 1. These results highlighted that maize plants are able to store higher amounts of PTEs (except Zn) in roots than in grains.

As immobilization of PTEs in roots is mainly related to exclusion strategy (Baker 1981; Bose and Bhattacharyya 2008), the highest TF values of Cu and Zn can be attributed to their essential functions in plant tissues. Cu is necessary for carbohydrate and nitrogen metabolism, and Zn plays a key role as structural constituent or regulatory cofactor of a wide range of different enzymes and proteins in many important biochemical pathways. Among the non-essential elements, Cd showed the highest TFs as more than 40% of Cd taken up by maize roots was translocated to grains. Translocation of Cd in maize plants was significantly greater than for the other non-essential elements. This fact could be explained by the chemical similarity of Cd and Zn which can lead to their interaction in root uptake, transport from roots to aboveground parts, and accumulation in edible parts (Das et al. 1997). The lowest TFs of As proved that the movement of this element along the conductive system of maize was strongly limited. This evidence is likely related to the presence of a self-detoxification mechanism in maize plant which prevents As

translocation to the aboveground parts of plant. Baig et al. (2010) reported that As translocation in different tissues of maize was in the order roots>shoots>grains.

4. Conclusions

This biogeochemical field study carried out in the industrial area of Kosovska Mitrovica highlighted that soil contamination influenced uptake, translocation and accumulation of potentially toxic elements in maize plants.

In the study area agricultural soil was contaminated by potentially toxic elements (As, Cd, Cu, Pb, Sb and Zn) mainly due to the emissions from industrial plants such as the Zvecan smelter and Trepča factory. Soil contamination by As, Cd, Cu, Pb, Sb and Zn was variable from slightly to highly contaminated soils, and influenced both the bioavailable fraction and accumulation of these PTEs in maize roots and grains.

The comparison of PTE concentrations in roots and grains suggested that maize was able to limit the translocation of non-essential elements (As, Cd, Pb and Sb) to edible parts, likely due to the metal-binding capacity of plant roots. Conversely, the levels of micronutrients (Cu and Zn) were comparable in maize roots and grains.

The plant-to-soil bioconcentration indices suggested that the bioavailable concentrations predict better the transfer of the potentially toxic elements from soil to plant, and identified some competitions and interactions among these elements in root uptake and root-to-grain translocation.

In the study area contamination of agricultural soils by potentially toxic elements affected the quality of maize crops: Cd and Pb concentrations in maize grains exceeded the maximum permissible limits for human consumption established by the European Commission Regulation for foodstuff.

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FIGURE CAPTIONS

Fig. 1. Sketch map of study area. Figure reports the geographic distribution of sampling sites (grey circle), as well as the location of Zvecan smelter, Trepča factory and urban area of Kosovska Mitrovica

Fig. 2. Soil grouping by multivariate statistical approach. (a) PCA biplot shows a projection of the variables (potentially toxic elements) onto the scatter plot (projection of soil samples on the factorial plain); (b) Tree-clustering of soil samples based on the case coordinates in respect to the principal axis (components 1 and 2) of PCA analysis. The distances between objects were defined by the Euclidean distances and the objects were linked together by Ward's method

Figure_1

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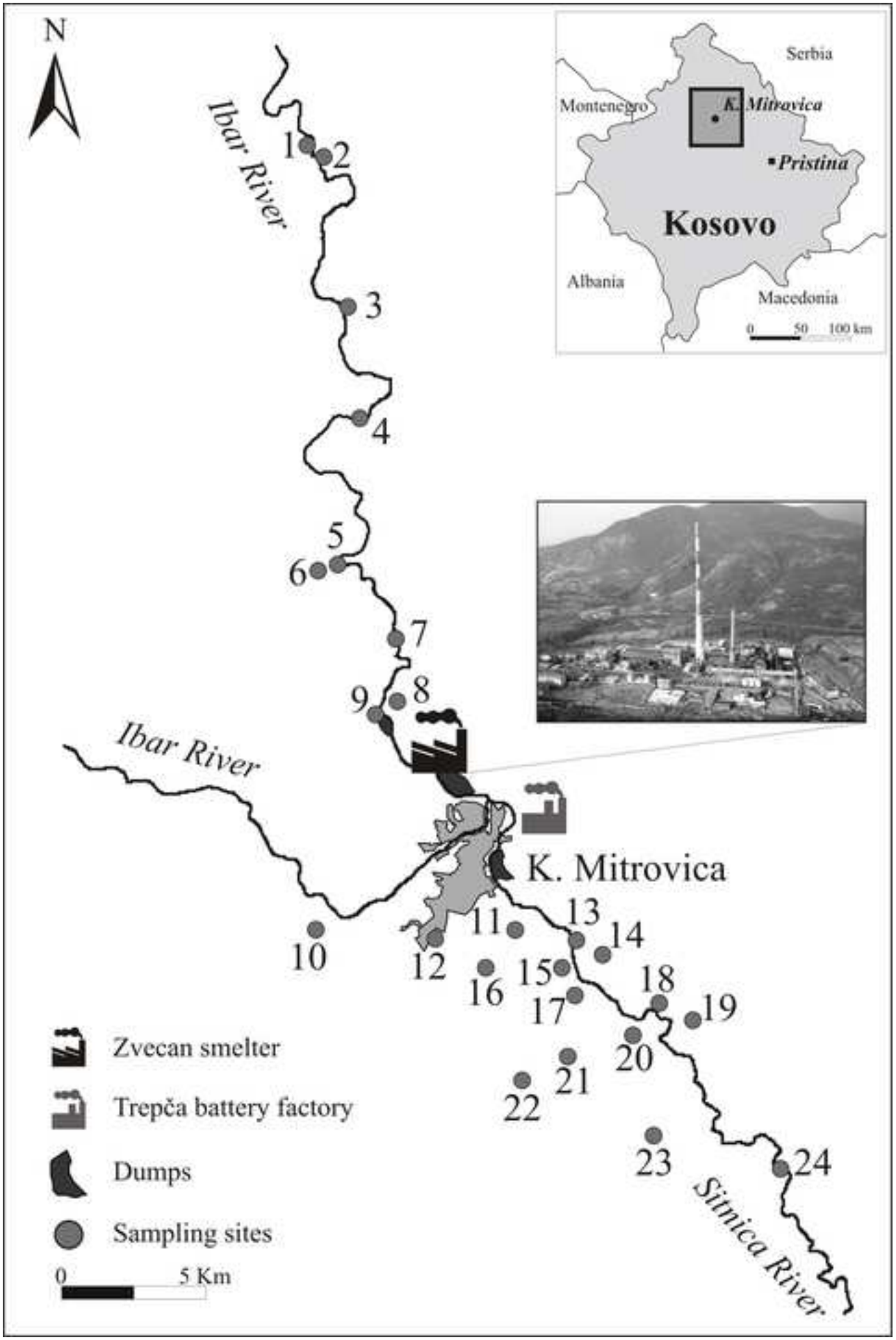

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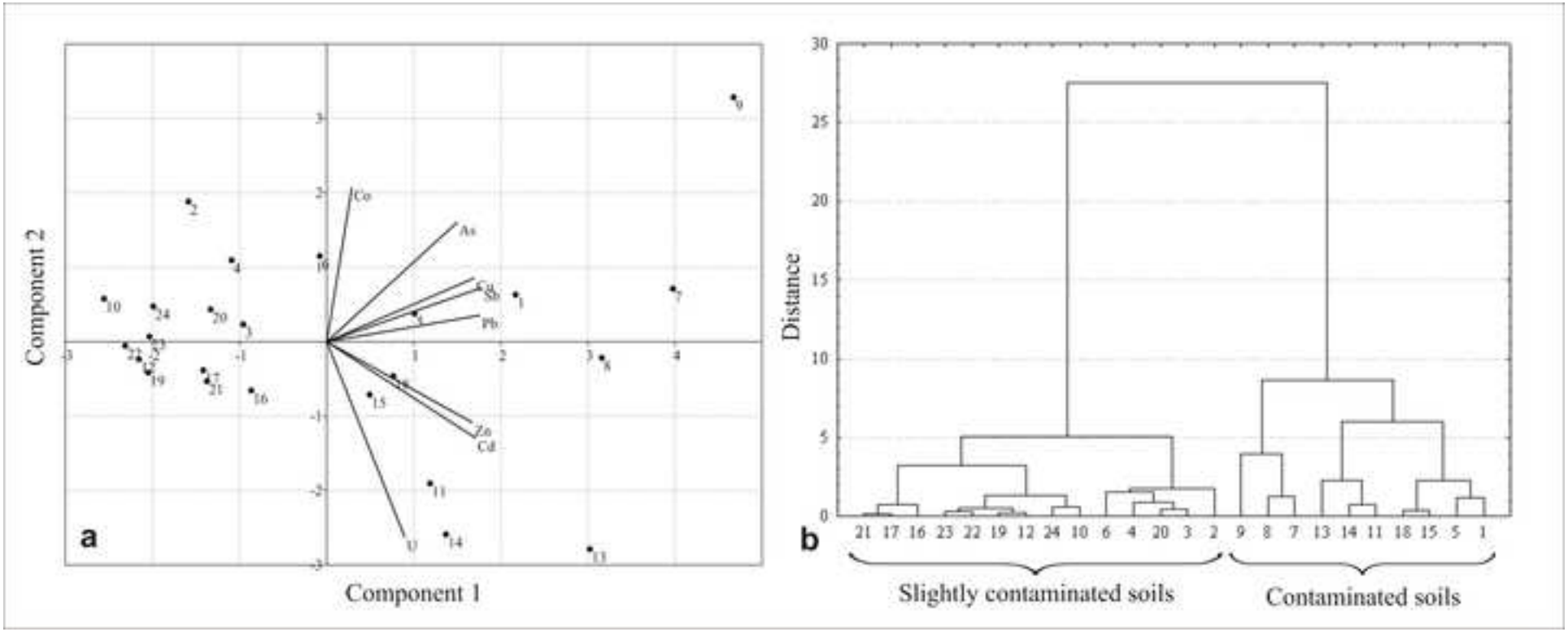

Table 1 Descriptive statistic of As, Co, Cd, Cu, Pb, Sb, U and Zn total contents and bioavailable concentrations in soil samples grouped according to the Pollution Load Index (data in mg/kg^{-1}; *n* = number of soil samples)

Element		Slightly contaminated soils (0< PLI ≤ ; *n* = 14)					Contaminated soils (2< PLI <5; *n* = 10)				
		Minimum	Maximum	Mean	Median	St. Dev.	Minimum	Maximum	Mean	Median	St. Dev.
As	tot	10.2	54.9	26	23.4 *	11.8	30	501	148	84.5 *	162
	bio	0.05	1.2	0.38	0.27 *	0.33	0.37	2.4	1.6	1.7 *	0.6
Cd	tot	0.37	1.8	1.1	1.2 *	0.45	2.9	8.2	4.5	4.3 *	1.4
	bio	0.11	0.68	0.36	0.35 *	0.18	0.76	2.6	1.5	1.5 *	0.59
Co	tot	12.8	51.6	25.9	20.7	11.7	14.2	43.5	25.9	20.6	11.5
	bio	0.4	4	1.8	1.6	1.2	0.1	5	1.8	1.2	1.6
Cu	tot	22.3	53.8	34.5	32.8 *	10.7	42.7	79.3	60.8	59.3 *	12.9
	bio	0.26	0.54	0.36	0.37 *	0.08	0.52	2.9	1.2	0.81 *	0.89
Pb	tot	72.1	698	293	220 *	182	512	1677	915	756 *	425
	bio	0.49	22.3	7.6	4.2 *	7.8	2.9	200	44.6	22.4 *	59.4
Sb	tot	1.5	8.9	4.8	4 *	2	7.4	26.2	13.4	10.5 *	6.9
	bio	0.01	0.16	0.05	0.04 *	0.04	0.05	0.63	0.24	0.17 *	0.22
U	tot	1	2.4	1.9	1.8 *	0.37	1.6	3.4	2.5	2.4 *	0.61
	bio	0.005	0.03	0.01	0.008	0.007	0.005	0.02	0.01	0.01	0.007
Zn	tot	66.6	377	233	240 *	77.3	473	1131	711	647 *	218
	bio	1.3	41.6	15.5	12.6 *	12.1	31	230	99.3	85.8 *	60.6

tot: total potentially toxic element contents in soil.

bio: bioavailable potentially toxic element concentrations in soil.

***** Significant differences between the median contents of potentially toxic elements in slightly contaminated and contaminated soils ($p<0.05$).

Table 2 Descriptive statistic of As, Co, Cd, Cu, Pb, Sb, U and Zn concentrations in roots and grains of maize plants grown in slightly contaminated and contaminated soils (data in mg kg^{-1} fresh weight; *n* = number of soil samples)

Element		Slightly contaminated soils (0 < PLI ≤ 2; *n* = 14)					Contaminated soils (2 < PLI < 5; *n* = 10)				
		Minimum	Maximum	Mean	Median	St. Dev.	Minimum	Maximum	Mean	Median	St. Dev.
As	roots	0.23	1.3	0.51	0.43 *	0.28	0.44	4.3	1.8	1.4 *	1.4
	grains	0.005	0.05	0.02	0.02	0.01	0.01	0.28	0.06	0.03	0.08
Cd	roots	0.11	1.2	0.32	0.23 *	0.32	0.14	2.5	0.83	0.47 *	0.81
	grains	0.02	0.25	0.1	0.07	0.08	0.04	0.3	0.13	0.1	0.08
Co	roots	0.24	1.4	0.78	0.74	0.32	0.36	1.7	0.93	0.83	0.44
	grains	0.02	0.31	0.06	0.04	0.08	0.02	0.11	0.06	0.06	0.04
Cu	roots	1	11.3	4.2	3.9 *	2.3	3.9	10.8	6.3	5.4 *	2.3
	grains	1.4	3.3	2.3	2.2	0.57	1.4	4.6	2.6	2.3	1.1
Pb	roots	1.4	11.8	3.7	2.6 *	2.9	3.0	25.3	9.7	8.3 *	6.9
	grains	0.22	0.65	0.44	0.39	0.13	0.25	1.5	0.58	0.5	0.36
Sb	roots	0.03	0.13	0.06	0.05 *	0.03	0.07	0.48	0.22	0.15 *	0.17
	grains	0.001	0.04	0.01	0.01	0.01	0.003	0.06	0.02	0.01	0.02
U	roots	0.06	0.33	0.11	0.08	0.08	0.07	0.16	0.11	0.09	0.03
	grains	0.02	0.04	0.03	0.03	0.01	0.02	0.03	0.03	0.03	0.002
Zn	roots	12.8	55	26.7	23.6 *	12.3	22.2	56.4	36	32.6 *	12.2
	grains	14.7	38.7	26.9	26.8	5.5	21.7	59.7	33.9	30.8	12.2

***** Significant differences between the median contents of potentially toxic elements in roots and grains of maize plants grown in slightly contaminated and contaminated soils ($p<0.05$).

Table 3 Bioconcentration, accumulation and transfer of potentially toxic elements from soil to roots and grains of maize plants from slightly contaminated and contaminated soils

Maize from:		BCFs Slightly contaminated soils	BCFs Contaminated soils	BACs Slightly contaminated soils	BACs Contaminated soils	TFs Slightly contaminated soils	TFs Contaminated soils
As	tot	0.02	0.02	0.001	0.001	0.06	0.06
	bio	2.7	1	0.14	0.04		
Cd	tot	0.28	0.17	0.09	0.03	0.43	0.45
	bio	0.92	0.53	0.35	0.09		
Co	tot	0.03	0.04	0.003	0.003	0.09	0.09
	bio	0.76	1.3	0.05	0.16		
Cu	tot	0.13	0.11	0.07	0.05	0.67 *	0.44 *
	bio	12.3	7.3	6.5	3.3		
Pb	tot	0.02	0.01	0.002	0.001	0.18 *	0.09 *
	bio	1.5 *	0.51 *	0.22	0.03		
Sb	tot	0.02	0.01	0.003	0.002	0.28	0.16
	bio	1.7	1.1	0.41	0.16		
U	tot	0.06	0.04	0.02	0.01	0.35	0.3
	bio	14.1	12.6	3.8	3.6		
Zn	tot	0.14 *	0.05 *	0.14	0.05	1.1	0.96
	bio	3.5 *	0.48 *	3.8	0.45		

Bioconcentration, accumulation and transfer of potentially toxic elements from soil to maize were evaluated in terms of Biological Concentration Factor (BCF), Bioaccumulation Coefficient (BAC) and Translocation Factor (TF).

$BCF = [element]_{root}/[element]_{soil}$

$BAC = [element]_{grain}/[element]_{soil}$

$TF = [element]_{grain}/[element]_{root}$

tot: BCF_{tot} and BAC_{tot} calculated using the total potentially toxic element contents in soil.

bio: BCF_{bio} and BAC_{bio} calculated using the bioavailable potentially toxic element concentrations in soil.

***** Significant differences between BCFs, BACs and TFs values of maize plants grown in slightly contaminated and contaminated soils ($p<0.05$).