Phytoavailability of potentially toxic elements from industrially contaminated soils to wild grass

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ABSTRACT

Topsoil and grass samples from 14 sites located in different distances from three copper mining factories and a copper smelter were collected in Srednogorie, Bulgaria. The paper discusses results of studies on the mobility of potentially toxic elements (As, Cd, Cr, Cu, Mn, Ni, Pb, Zn) from contaminated soils to wild grass *Cynodon dactylon* in terms of transfer (TF: soil to plant) and phytoavailability (PF: EDTA (ethylenediaminetetraacetic acid)-soluble to plant) factors. Soils located close to mine and smelter factories were heavily contaminated by As and Cu reaching levels up to 500 mg/kg and 2400 mg/kg, respectively for As and Cu. At seven sites arsenic and copper in grass were over the normal levels (1 mg/kg As; 20 mg/kg Cu). The content of Cu in grass averaged 63 mg/kg exceeding the maximum allowable for sheep. Relationship between PF and TF values was found for As, Cd, Cu, Ni, Pb, Zn but not for Mn and Cr. To some extent the soil pH, TOM (total organic matter) and $CaCO_3$ content are related to the phytoavailability of As, Cd and Cu since for Zn and Pb this relation is attributed to Al- and Fe-content of soil. Statistically significant correlation coefficients were determined between the EDTA-soluble fraction of soil and content in grass in the case of As, Cd, Cr, Cu, Mn and Pb indicating the suitability of the short procedure for phytoavailability studies of those elements. The application of hierarchical cluster analysis and self-organizing maps of Kohonen made it possible to reveal specific hidden relationships between the soil variables and transfer factors as well as between the sampling locations. This additional information helps in more detailed interpretation of phytoavailability and transfer processes in the region of interest.

The novelty in this study is achieved by careful consideration of the possibility of using EDTA extracts of the toxic metals in investigating of the phytoavailability and transfer processes soil/plant. Additionally, chemometric expertise used makes it possible to differentiate the behavior of each toxic metal in the processes studied. The combination of easy option for rapid extraction and intelligent data analysis gives a new perspective for contributions in explanation of the complex interactions between soils and plants when assessing pollution events in a certain environment.

1. Introduction

World primary production of Cu has increased from 9.2 Mt in 1990–13.7 Mt in 2003 (USGS, 2004). In the 20th century its utilization increased rapidly and now the mining and refining of Cu takes place on all six continents, as an important by-product from non-ferrous Cu ores is Au (Kabata-Pendias and Mukherjee, 2007).

The largest Bulgarian companies for mining and processing of copper, gold-containing ores and copper concentrate are located on the southern flank of the Balkan ranges, Central Sredna Gora Mountain, central-western Bulgaria. All the leading copper mining companies and the biggest in South-Eastern Europe facility for smelting and refining of copper are located approximately between the 60th and 90th kilometers east of the capital Sofia. The country basic industries of copper mining and smelting are concentrated on a relative small territory. The factories are located in the suburbs of cities and villages surrounded of a grassland used for animal feeding. The activities of all these factories for mining and smelting of copper ores may contribute to elevated levels of heavy metals in the environment, their accumulation in soils and plants, followed by their transport in terrestrial food chains (Kloke et al., 1984). The high concentration levels of elements as As, Cd, Cu, Pb,

Fig. 1. Map of the study area and location of the sampling sites.

Table 1

Selected soil characteristics (for Al and Fe: mean (SD), n = 3).

Site	Description	pH	CaCO$_3$ (%)	TOM (%)	Al (%)	Fe (%)	Soil texture	Clay content (%)
1	Elatsite—0.6 km from the mine	6.6	1.2	0.4	1.11(0.08)	7.0(0.3)	Sandy loam	13
2	Elatsite—2.2 km from the mine	7.7	5.4	2.6	1.89(0.09)	2.7(0.2)	Sandy loam	11
3	Chelopech—0.3 km from the mine	6.7	3.3	4.2	1.38(0.04)	7.1(0.4)	Sandy clay loam	29
4	Chelopech—0.95 km from the mine	6.7	2.7	6.4	2.01(0.06)	7.8(0.4)	Sandy loam	15
5	Chelopech—4 km from the mine	5.6	1.4	1.7	2.20(0.07)	7.2(0.3)	Clay loam	38
6	Zlatitsa—0.6 km from the smelter	5.9	0.6	5.8	1.52(0.08)	3.4(0.2)	Loam	22
7	Zlatitsa—1.5 km from the smelter	6.0	0.6	6.7	1.58(0.06)	3.5(0.3)	Loam	20
8	Pirdop—0.7 km from the smelter	6.5	2.4	7.5	1.87(0.06)	3.2(0.2)	Loam	25
9	Pirdop—1.3 km from the smelter	5.2	0.7	6.9	1.76(0.08)	3.0(0.2)	Loam	22
10	Asarel—0.7 km from the mine	5.1	0.6	6.2	1.08(0.04)	6.4(0.4)	Sandy loam	11
11	Asarel—4 km from the mine	5.8	1.8	2.6	1.45(0.05)	2.8(0.2)	Sandy loam	10
12	Asarel—6 km from the mine	5.4	1.1	3.2	1.36(0.04)	2.7(0.2)	Loamy sand	6
13	Poibrene Bridge at the Topolnitsa river	7.2	4.0	0.4	2.30(0.08)	2.8(0.2)	Sandy loam	12
14	Topolnitsa dam	6.8	3.5	5.5	1.2	6.8(0.4)	Loamy sand	8

Table 2

Soil–plant transfer (TF) and phytoavailability (PF) factors for *Cynodon dactylon* and reported ranges for TF for plants.

Element	TF this study	PF this study	TF (Uchida et al., 2007)	TF (Jolly et al., 2013)	TF (IAEA TRS-472, 2010)
As	0.01–0.1	0.5–35	0.0002–0.025	0.001–0.002	
Cd	0.25–1.1	0.44–1.6	0.02–2.6	0.19–1.2	0.08–6.5
Cr	0.04–0.33	4–15	0.0002–0.05	0.008–0.03	0.0002–0.002[a]
Cu	0.03–0.11	0.06–0.41	0.004–3.8	0.07–0.13	0.8
Ni	0.14–0.35	0.23–0.67	0.005–0.4	0.04	0.1–2.7[a]
Mn	0.03–0.16	0.28–0.53	0.002–0.1	0.04	0.02–0.6[a]
Pb	0.01–0.13	0.05–0.51	0.0003–0.06	0.008–0.065	0.002–1.0[a]
Zn	0.11–0.93	0.65–10	0.07–1.3	0.26–1.15	0.05–3.2

[a] TF for Grasses/Pasture.

Zn observed in areas of close proximity to copper factories could cause serious environmental problems (Kuo et al., 1983; Karszewska et al., 1998; Maiz et al., 2000; Kabala and Singh, 2001; Burt et al., 2003; Pope et al., 2005; Yan et al., 2007; Ge and Zhang, 2015). Although the currently strong control on the managing and handling the industrial wastes (due to tight legislation implementation), pollution due to previous activities connected with deposition of potentially toxic elements (PTE) in soil over very long periods still exist. The long-term persistence of heavy metals in the environment presents potential human health risk. The risk is associated with plant consumption or direct soil dust inhalation by inhabitants. PTE-related concerns have not to be overlooked despite the lower levels of industrial pollution in the last

years (Antoniadis et al., 2017). A continuous monitoring of toxic element uptake and allocation in the selected plants must be carried out in order to avoid a consistent transfer of these elements along terrestrial food chains (Madejón et al., 2002). In order to enhance the effectiveness of the analytical work for regular quality control of the contaminated areas, considerably reducing the experimental task, procedures for fast and simple estimation of the available, mobile and mobilizable fraction of PTE have to be applied (Alvarez et al., 2006). Short single extraction procedures for assessing the plant available forms in soils have been proposed (Quevauviller et al., 1996; Pueyo et al., 2001; Chojnacka et al., 2005; Alvarez et al., 2006). Despite the multitude of attempts to elaborate a standard extraction procedure that would rep-

Table 3

Correlation coefficients in total soil–EDTA soluble soil, total soil–plant and EDTA soluble soil–plant systems (significant correlation coefficients are marked in bold).

Element	Soil$_{total}$–Soil$_{EDTA}$	Soil$_{total}$–Plant	Soil$_{EDTA}$–Plant
As	**0.85**	**0.97**	**0.83**
Cd	**0.94**	**0.76**	**0.73**
Cr	− 0.09	− 0.17	**0.62**
Cu	**0.95**	**0.92**	**0.96**
Mn	0.10	0.19	**0.96**
Ni	**0.95**	0.39	0.36
Pb	**0.98**	**0.66**	**0.69**
Zn	**0.93**	0.24	0.02

resent the content of PTE available to plants, there is still a need to obtain new additional information for a particular soil and plant genotype (Alvarez et al., 2006). The prediction of phytoavailability of potentially toxic elements is very difficult for contaminated environments (Kabata-Pendias, 2004). There are several models that can be used to predict the phytoavailability of microelements (Rodrigues et al., 2012; Liu et al., 2015; Chen et al., 2016; Lim et al., 2016). But these models are limited to a given plant and local soil conditions. It means that environmental control requires comprehensive separate study of specific contaminated sites. Native plants are tolerant to toxic elements and of-

ten better in terms of survival and growth under environmental stress (Yoon et al., 2006). Therefore, the wild grass *Cynodon dactylon* (Bermuda grass) was used in the present study for investigation of contaminated sites around the copper mines and the copper smelter.

The objectives of this research were as follows:

- to evaluate the soil pollution around three copper mines and a copper smelter;
- to find the availability of potentially toxic elements (As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn) from contaminated soil to grass (*Cynodon dactylon*) and to interpret the relationships between soil-plant transfer processes and basic soil characteristics using multivariate techniques;
- to evaluate the potential of EDTA soil extraction procedure for rapid regular monitoring of PTEs uptake and food chain risk assessment.

2. Materials and methods

2.1. Study area

Grass samples together with the associated soil samples were collected in September of 2016. A total of 14 topsoil (0–20 cm) samples together with the wild grass *Cynodon dactylon* growing on this soils were selected: a) eight samples along the three Bulgaria's leading cop-
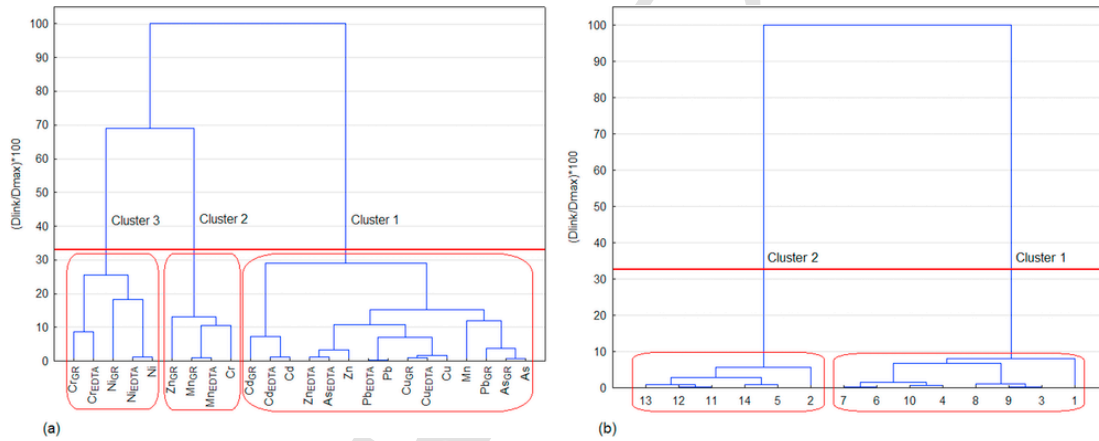

Fig. 2. Hierarchical dendrograms for variables (a) and locations (b).
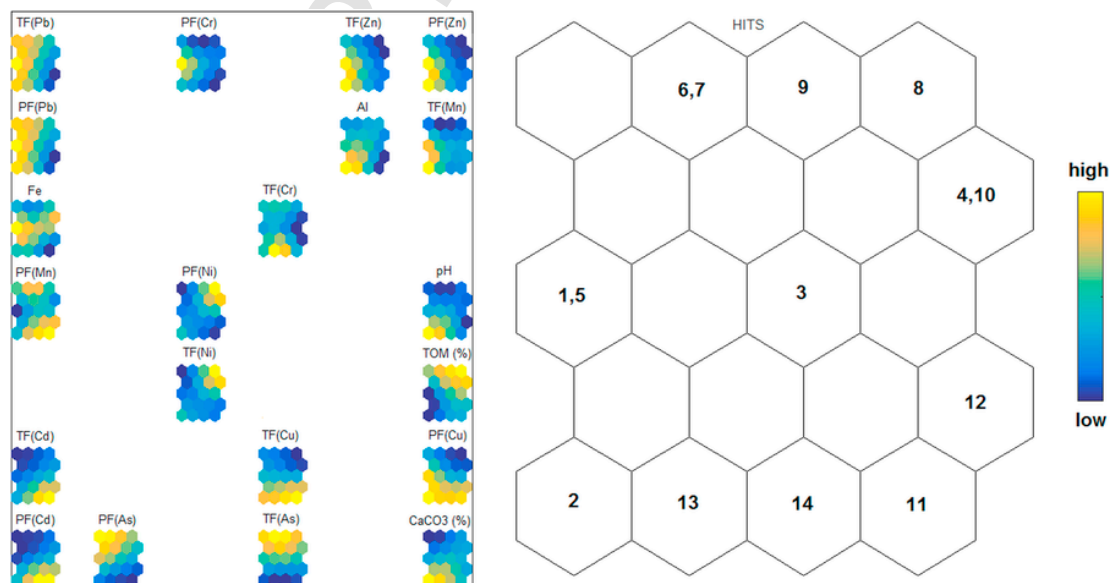

Fig. 3. Ordering of PTE transfer coefficient and specific soil feature planes and hit diagram for all locations.

per ore mining and processing companies Elatsite Med (flotation factory near the village of Mirkovo, 60 km east of Sofia) – sampling sites 1, 2; Chelopech Mining (processing of copper-bearing massive sulphide and porphyry copper deposits, in the village of Chelopech, 70 km east of Sofia) – sampling sites 3–5; and Asarel-Medet (open pit mining and copper ore processing factory located at an altitude of about 1000 m in the Sashtinska Sredna Gora Mountain, near to the city of Panagyurishte, 90 km east of capital Sofia) – sampling sites 10–12; b) four sampling sites around the copper smelter and refinery, situated between the towns of Zlatitsa and Pirdop (located seven kilometers east of Chelopech) – sampling sites 6–9; c) two sampling sites at the two ends of a Topolnitsa dam, serving as collector of waste waters from the copper industry activities in the study area – sampling sites 13, 14 (Fig. 1). The sites and the general characteristics of the soil samples are specified in Table 1. The pH was measured in a 1:5 suspension of soil in pure water (ISO 10390:2005). Equivalent calcium carbonate (%) and total organic matter content (TOM %) were determined according to ISO 10693:1995 and ISO 10694:1995, respectively. The content of Al and Fe was determined in *aqua regia* soil extracts by ICP-OES (inductively coupled optical emission spectrometry).

2.2. Sample preparation and chemical analysis

The digestion of soil samples was performed by adopted method of ISO 11466:1995. A 1.0 g of sample was weighed and transferred into a tall form conical beaker. To each sample 30 ml of *aqua regia* was added. The beakers were covered with appropriate small glass funnels and left overnight. Then the mixture was heated on a hot plate at 100 °C and evaporated to about 5 ml. After cooling to room temperature 4 ml 30% H_2O_2 were added and heated again near to dryness. The residue was dissolved in 10 ml 20% HNO_3. The solution was filtered and transferred into a 50 ml volumetric flask and diluted to the mark. Three repetitions were weighed for each sample and procedural blanks were run during the procedure. The treatment of soil samples is schematically presented in Supplementary Fig. 1. The *aqua regia* soluble concentration of all the analytes was measured by inductively coupled optical emission spectrometry (Perkin Elmer ICP-OES 6000). For accuracy checking two certified reference materials Stream Sediments STSD-1 and STSD-3 were digested in parallel. The obtained values for analytical recovery varied between 95% and 112% (the data are presented in Supplementary Table 1) which was considered as satisfactory. EDTA soil extracts (2.0 g soil + 20 ml 0.05 mol/l EDTA, one hour shaking time, room temperature) were prepared according to harmonized BCR protocols (Pueyo et al., 2001). The element concentrations in EDTA soil leachates were measured using flame (Perkin Elmer AAnalyst 400) and electrothermal atomic absorption spectrometry (ETAAS) (Zeeman Perkin Elmer 3030/HGA-600). The reliability of the results was controlled by the standard reference material BCR-700. The recoveries were higher than 95% (range 95–109%) as can be seen in Supplementary Table 2.

The aerial part of the grass samples was gently washed with Milli-Q water for approximately 3 min to remove soil and dust particles adhered to the plants. After washing, grass samples were air-dried at room temperature for 15 days and then thoroughly ground, milled, mixed and uniformed in order to obtain representative samples. Plant samples were digested with 65% HNO_3 and 30% H_2O_2 (USGS Test Method B-9001-95) (Hoffman, 1996). The content of elements was determined by ICP-OES and by ETAAS. The standard reference material ERM-CD 281 RYE GRASS was used to check the reliability of the results. The measured concentrations (refer to Supplementary Tables 3 and 4) were in very good agreement with the certified values (recoveries between 93% and 105%).

All reagents used were of analytical-reagent grade (p.a. Merck, Darmstadt, Germany). Milli-Q water (Millipore, Bedford, MA, USA) was used throughout.

2.3. Data analysis methods

2.3.1. Hierarchical Cluster analysis (HCA)

Hierarchical Cluster analysis (HCA) is a well-known and widely used unsupervised multivariate statistical method for interpretation of large data sets (Massart and Kaufman, 1983). The main goal of the hierarchical clustering is to find out and visualize any structure of the data, mainly similarity between the objects or the variables that characterize them. Usually, the HCA procedure includes three steps. The first step is normalization of the raw input data (e.g., auto scaling or z-transformation) in order to eliminate the scale and variance dependence of the original values. Secondly, the distance between the objects (or the variables) is determined, very often by using the Euclidean distance or squared Euclidean distance as a measure of similarity. The final step is to link together the similar variables (PTEs) or objects (sampling points) and to present the results of the clustering as graph, normally a treelike scheme with hierarchical structure called dendrogram. There are various methods for linkage of similar objects into clusters – the single linkage, the complete linkage, the average linkage methods but Ward's method is dominantly used because is offering a very stable procedure of clustering. The statistical significance of the clusters formed is checked by the Sneath's criterion ($\frac{1}{3}D_{max}$ or $\frac{2}{3}D_{max}$, where D_{max} is the maximal linkage distance in the dendrogram).

2.3.2. Self-organizing maps (SOM)

Self-organizing maps (SOM), described by Kohonen (2001), is a neural network based model used to visualize and interpret large high-dimensional data sets. It is a competitive learning algorithm based on unsupervised learning process. SOM aimed at nonlinear projection from multidimensional space input data into a two-dimensional array of neurons, also called nodes. Each node in the map is initialized as a random unit vector in the multidimensional space of variables. Each object from the data set is considered as an n-dimensional input vector, where n is the number of variables. Kohonen clustering algorithm is based on the "winner-takes-all" rule, where the "winner" is the node, which vector is the most similar to input sample. The winning neuron adjusts its vector weights in order to correspond to the input vector, while neighbors of the "winner" nodes are modified to be less similar to the input vector. In such a way the self-organizing maps algorithm recognizes vectors similar to themselves and locates them in one and the same node.

When already trained, the map is graphically presented on 2D planes for each variable indicating their distribution pattern with various colors. On the right side of the plane there is a color bar scale that indicates the variable value. Plane ordering is performed by absolute correlation coefficients between the planes and reveals the relationships between variables. SOM also provides a hit diagram with location of sampling sites on the map. This simultaneous presentation makes SOM a suitable technique for detecting and identifying similar groups between objects and between variables, as well. SOM calculations in this study were performed by a free SOM toolbox 2.0 for Matlab (Vesanto, 1999).

3. Results and discussion

3.1. Soil pollution

3.1.1. Aqua regia extractable PTEs in soils

"Total" concentration values (extracted with *aqua regia*) of Cr, Mn, Ni, Pb and Zn were found to be far below the maximum permissible

levels for grassland according to Regulation No. 3/2008 (see Supplementary Table 1). Cadmium content in sites with lower pH values (pH < 6.0/6.5) was found to be close to the maximum permissible level of 2.0/2.5 mg/kg Cd, but below the intervention values.

There can be distincted two pollution levels for arsenic: (1) the soils which are not close to the mining factories are "non-polluted" with As; (2) the soils in the close proximity to the copper ore processing mines (up to around one kilometer from the contamination source) and the copper smelter factory (up to ca. 2 km from the factory) are "highly polluted". The arsenic concentration is from seven to sixteen times higher than the maximum permitted level of 30 mg As /kg. It exceeds even the values of 90 mg As /kg requiring intervention.

Expectedly, the main soil contamination was caused by copper. Only for three sites studied the concentration of Cu was found to be below 80 mg Cu /kg (maximum permissible level at pH < 6.0). In all the other studied sites the copper levels were 2- to 10-fold higher reaching values of 2400 mg/kg Cu. Seven sites need intervention as soil remediation activities. The total amount measurements should be complemented with the measurements of the phytoavailable fraction because only soluble and mobilizable element species in the soils could be available for plants (Kabata-Pendias, 1993).

3.1.2. EDTA extractable PTEs in soils

EDTA tends to release the soluble, exchangeable, weakly absorbed and mobilizable element species from soil (Schramel et al., 2000; Alvarez et al., 2006). At pH 7.0 (BCR procedure) EDTA can dissolve elements which are bound to soil humic substances as well. Due to very high stability of the element-EDTA complexes (elements with oxidation state higher than one) EDTA could dissolve also elements which are present in the soil as water insoluble carbonates. It means that the extractable with EDTA soil elements represent to high extent their phytoavailable content. Other reasons to select EDTA as extractant were the simplicity of the procedure and the existence of Certified Reference Material CRM 700, which enabled quality control of the analytical procedure.

Only 0.18–5.0% of "total" As were found to be soluble in EDTA (see Supplementary Table 2). Arsenic does not form stable complexes with EDTA (Pribil, 1982) which could explain the low extractability of soil arsenic species in 0.05 mol/l EDTA. Only the soluble at pH 7 (pH value of the extractant) chemical forms of arsenic in soils were probably found in the soil leachates. In similar way behaves chromium. Despite the relative high "total" soil Cr, the available forms are only 0.29–4.1% of the total chromium content. The trivalent chromium species are the dominant form of this element in the soils. Cr(III) forms very stable complexes with EDTA, but this complex is characterized with its high inertness (Christian, 2003). At room temperature the formation of Cr(III)-EDTA complex is practically not possible. For the elements Cd, Cu, Ni, Pb and Zn higher values for potential phytoavailability were registered. Averaged the available content of the microelements constitute 58% for Cd (range 41–71%), 37% for copper (range 24–54%), 46% for nickel (range 39–55%), 25% for lead (range 21–29%), 17% for Zn (range 9–30%). The Mn extracted with EDTA varied in wide range from 6.5% to 54% depending fairly on the total amount. This agrees with the results reported by other authors (Alvarez et al., 2006; Madejón et al., 2009). Manganese presents a relatively high content in soils distributed in fractions with different solubility and this could probably explain the absence of any relationships between total and available Mn.

In the case of copper a remarkable trend for its potential phytoavailability was observed. For six of the studied soils (sites 2, 5, 11–14) with "total" Cu content less or around the regulatory permissible levels (80–200 mg/kg Cu, Supplementary Table 1), the average available copper was found to be 26 ± 2% of its *aqua regia* soluble content. For the highly contaminated sites the availability of copper enhanced

twice to 52 ± 3%. It shows different mechanism of Cu binding to soil fractions in the polluted sites. This confirms the reported observations by other authors that in contaminated soils up to 60% of heavy metals can occur in easily labile forms (Kuo et al., 1983; Karszewska et al., 1998; Kabala and Singh, 2001).

3.2. Effects on grass micronutrients

The Cu, Mn and Zn elements are essential micronutrients for plants. However, their excessive content in plant tissue may induce toxicity in plants and may cause toxic effects in animals feeding on them. The obtained results (refer to Supplementary Table 3) and the comparison with the phytotoxicity levels (Kabata-Pendias, 2001) and the maximum tolerable contents by some animals (ACAF, 2000; Directive 2002/32/EC, 2002; Madejón et al., 2002; Commission Regulation (EC) No 479/2006, 2006) indicate: (1) the contents of Mn and Zn in wild grass grown on all studied sites are within the normal levels; (2) the content of copper exceeds the normal levels of 3–20 mg/kg Cu at 7 sites located in close proximity to smelter/mines (Asarel, Chelopech) and phytotoxic levels of 25–40 mg/kg Cu in four sites located around the copper smelter and refinery factory between the towns of Zlatitsa and Pirdop. The content of Cu in *Cynodon* harvested in this region averaged 63 mg/kg exceeding the maximum level allowable for sheep (Madejón et al., 2002). In addition the extremely high copper content in these soils (Supplementary Table 1) means that the unwashed Bermuda grass with adhered soil on plant surfaces (as they would be ingested by animals) could contain copper at levels much higher than the tolerated limit.

3.3. Trace elements in grasses

The contents of Cd, Ni and Pb in grass were within the normal levels (0.1–1 mg/kg Cd, 0.1–5 mg/kg Ni, 2–5 mg/kg Pb) (Kloke et al., 1984) and far below the phytotoxic levels (5–30 mg/kg Cr, 5–30 mg/kg Cd, 10–100 mg/kg Ni, 30–300 mg/kg Pb; Kabata-Pendias, 2001) for plants for all the areas studied (see Supplementary Table 4). Chromium content was found to be in the range 3.2–8.9 mg/kg Cr, which is within the range reported by Rodrigues et al. (2012) for Ryegrass (0.8–11 mg/ kg Cr). Arsenic content was 12–33 times higher than the normal levels (0.01–0.1 mg/kg As) (Kloke et al., 1984) for wild grass grown in the vicinity of mining factories Elatsite, Chelopech, Asarel and of the copper smelter between the towns Zlatitsa and Pirdop. At these sites arsenic was over the phytotoxic levels (3–10 mg/kg As) (Kabata-Pendias, 2001), but lower than the maximum levels allowable for sheep and chicken (50 mg/kg As) (Madejón et al., 2002). A visible signs of toxicity in these areas are the yellowing (chlorosis) of the foliage, reduction in grass growth and yield.

3.3.1. Transfer ability of PTEs

The uptake of micronutrients and of trace elements by wild grass *Cynodon dactylon* was evaluated by two transfer factors: factor defined as element concentration ratio of grass aerial part to "total" soil (TF) and phytoavailability factor (PF) defined as ratio of microelements content in plant to their EDTA extractable soil concentration. The calculated in the present work values for TF and PF and the reported in the literature ranges for soil-plant transfer coefficients (Uchida et al., 2007; IAEA TRS-472, 2010; Jolly et al., 2013) are given in Table 2.

The comparison between the TFs obtained in the present study to values reported in other articles and IAEA TRC 472 (2010) indicates that TFs for *Cynodon dactylon* are in general within the intervals for crops in the literature. The transfer factors in the Bermuda grass samples were similar to the results reported by Maiz et al. (2000), Cui et al. (2004) and Yan et al. (2007). Only the TFs for As and Cr were higher than the expected values. The TFs for As found in the present study

confirm the results reported by Madejón et al. (2002) for *Cynodon*. Wild grass seems able to enhance the mobility of arsenic from roots to its tissues. The PFs for As at highly polluted sampling sites (1, 3, 6–10) lied in the range 10–35. Published TF values for Cr for *Cynodon dactylon* were not found. Our investigations showed that the only soluble Cr species are the complexes of Cr(III) with soil organic matter (Voyslavov et al., 2018). The mechanism of chromium uptake by the wild grass consist probably in absorpion and translocation of Cr(III)-complexes with soil humic substances. This could be the reason for higher Cr contents in *Cynodon* tissues than in other crops.

In the case of copper, the sites could be divided again in two groups: for sites with total Cu less than 200 mg/kg Cu (EDTA soluble part less than 50 mg/kg) the transfer factors (TF) lied in the range 0.09–0.11. For the highly contaminated sites TF were within 0.03–0.05. The reason for lower levels of TF at higher soil copper content could be the limited accumulation capacity of the Bermuda grass. Despite the higher total and phytoavailable copper, the wild grass probably has some mechanism to regulate the internal content of mineral nutrients in its tissues (Madejón et al., 2002) and cannot accumulate Cu over determinately levels. This could be a defense mechanism of the plant, accumulating Cu in the roots and restricting its translocation to the aerial part. This protective mechanism, however, can operate until biochemical resistance of plant cells exists (Kabata-Pendias, 2004).

3.3.2. Correlation analysis

In Table 3 the correlation coefficients for all PTEs for different experimental conditions are shown. It is found that:

- strong positive correlation between the total (*aqua regia* soluble) and the EDTA-soluble fraction of soil for all the studied elements with exception of Cr and Mn;
- significant positive correlation between the EDTA-soluble soil fraction and content in grass in the case of all the elements studied, except Ni and Zn.

The knowledge of EDTA-soluble content would allow fast prediction of the ecologic risk of soil contamination and of accumulation in wild grass in the study area. It has to be noted that for other soil parameters, other plants, elements and climate factors, the use of EDTA as a bioavailability and health risk predictor needs a preliminary detailed investigations.

3.4. Chemometric analysis

In the first run of the chemometric data interpretation analytical data for total content of PTEs (As, Cd, Cr, Cu, Mn, Ni, Pb and Zn), for element concentration after extraction by EDTA and metal content in grass for all 14 sampling sites (data matrix 14 × 24) were considered. The EDTA and grass PTE contents are denoted by the appropriate subscripts: EDTA and GR, respectively. The goal was to find out if EDTA is an appropriate extraction agent for assessing the elements total amount in soil and, further, to check the relationships between the biotransfer "soil—plant" and some basic soil characteristics.

Using hierarchical cluster analysis (HCA) for detecting patterns of similarity between 24 variables described above following clustering (z-transform of the input data, squared Euclidean distances as measure of similarity, Ward's method of linkage and Sneath's criterion for cluster significance) was obtained (Fig. 2a) as well as the clustering of 14 sampling locations (Fig. 2b).

Three major clusters of variables are identified as follows:

K1 (As, As_{GR}, Pb_{GR}, Mn, Cu, Cu_{EDTA}, Cu_{GR}, Pb, Pb_{EDTA}, Zn, As_{EDTA}, Zn_{EDTA}, Cd, Cd_{EDTA}, Cd_{GR})
K2 (Cr, Mn_{EDTA}, Mn_{GR}, Zn_{GR})

K3 (Ni, Ni_{EDTA}, Ni_{GR}, Cr_{EDTA}, Cr_{GR})

In the biggest cluster one observes complete linkage for the three cases of As, Cd, Cu and Pb; K3 represents the complete linkage for Ni. For the other three elements analyzed (Cr, Mn and Zn) only partial linkage between the three forms (total, EDTA extracted and content in grass) is detected.

It could be concluded that the results of correlation analysis is in good agreement with cluster analysis - for 5 (As, Cd, Cu, Ni, Pb) out of all 8 elements tested there is a resemblance in the amount found as total concentration, EDTA extract and content in grass. It should be noticed that the three forms of Ni are in separate cluster from the 4 other elements, because the correlation between the total and grass content and between the EDTA and grass content is not so strong (Table 3). For Cr and Mn the resemblance is partial and confirms the strong relation between EDTA extract concentrations of both metals and their content in grass. The Zn resemblance (total concentration and EDTA extract) is a confirmation for lack of relationship between the total and EDTA extract concentrations, from one side, and grass content on the other.

Two major clusters are obtained for the sampling locations (Fig. 2b):

K1 (1, 3, 9, 8, 4, 10, 6, 7)
K2 (2, 5, 14, 11, 12, 13)

This separation corresponds entirely to the specificity of the sampling locations (see Fig. 1). To K1 belong locations close to anthropogenic sources of pollution (smelters, mines) since the other locations are more distant from possible pollution impacts. Obviously, the separation with respect to sampling locations is acceptable.

The SOM confirms convincingly the results found by hierarchical cluster analysis. The self-organizing map of Kohonen for the PTE total content distributions in the sampling locations is presented in Supplementary Fig. 2. The distribution of the total concentrations of the elements monitored proves that highest concentrations of As, Cd, Cu, Pb and Zn are found at locations with anthropogenic pollution impact (smelter, mining activity) like locations with numbers 6, 7, 8, 3, 4. For Ni and Mn locations 6 and 7 are still strongly contaminated since Cr reveals the opposite situation - lowest concentrations at locations 6 and 7. For the locations from the upper part of the SOM (5, 13, 14, 12, 2, 11) one observes the lowest levels of all elements. Locations 9, 10, 1 are intermediate with respect to the PTE concentrations.

Again, the results found by hierarchical clustering are generally confirmed by SOM analysis of PTE EDTA extract and grass contents (see Supplementary Fig. 3). For As, Cd, Cu, Mn and Pb a very good correlation between concentrations found in soil samples after EDTA extraction and contents in grass samples is shown. It might mean that the simple and easy extraction with EDTA could replace the traditionally complicated determination of total amount of elements in soils. For Cr, Ni and Zn, however, the correlation is not so well expressed. If the special distribution of the elements (in soil after EDTA extraction and in grass) is considered the anthropogenically impacted locations (3, 4, 6, 7, 8, 9, 10) indicated the highest levels of As, Cd, Cu, Pb since the other locations are characterized by high levels of Cr and expectedly low levels of the rest of the elements.

In Fig. 3a comparison of the specific soil quality properties ($CaCO_3$ content, pH, TOM, Fe-content, Al-content) and PF and TF values for each of the monitored elements at each sampling location is presented. Relationship between PF and TF values is found for As, Cd, Cu, Ni, Pb, Zn but not for Cr and Mn. To some extent the soil characteristics like pH and $CaCO_3$ content are related to the phytoavailability of Cd and Cu since for Pb and Zn this relation is attributed to Fe-and Al-content of soil, respectively. The positive correlation between the specific soil feature planes for transfer coefficients (TF and PF) of Zn and Al is an

evidence that soil-plant transfer processes of Zn are controlled by the Al content of soil samples. This fact could explain the absence of relationship between the total and EDTA extract concentrations of Zn and their content in grass.

It should be mentioned the opposite "behavior" of Cu and As transfer coefficients. Total and phytoavailability transfers of Cu are positively correlated with soil pH and negatively with TOM of soil samples. Such relationships could be explained by increasing stability of Cu(II)-EDTA complex at higher pH values and binding ability of organic soil fraction. The reason for positive correlation between As phytoavailability transfer coefficient and TOM could be found in dissolution of As which is bound to soil humic substances at pH of the EDTA leaching procedure.

4. Conclusions

The main contaminants of soils around the copper mining and smelter factories were As and Cu. As long as the content of Cd, Cr, Mn, Ni, Pb and Zn in grass was within the normal range for plants, As and Cu exceeded the upper limits on their normal levels. Serious risk for the food chain could present the transfer of contaminants from the adhered on the grass fine soil dust with high content of As and Cu.

Statistically significant relationships were registered between i) the *aqua regia* soluble and EDTA extractable concentrations of As, Cd, Cu, Pb, Zn in soil; ii) the EDTA-soluble fraction of soil and content in grass in the case of As, Cd, Cr, Cu, Mn and Pb. Single extraction with EDTA reduces considerably the experimental task of estimating the available and mobilizable fraction of soil elements.

Relationship between phytoavailability (PF) and transfer (TF) values was found for As, Cd, Cu, Ni, Pb, Zn but not for Cr and Mn. The relationship between PTE transfer coefficients and basic soil characteristics resembles the factor controlling PTE transfer in the investigated region. To some extent the soil pH, TOM and CaCO$_3$ are related to the phytoavailability of As, Cd and Cu since for Pb and Zn this relation is attributed to Al-and Fe-content of soil.

The approach used in the present study (simplification of the metal extraction procedure and intelligent data analysis) offers a new way of assessing the phytoavailability and transfer processes in the complex relationship soil/plant. Additionally, it reveals options for reliables risk assessment and pollution management in a region considered as polluted by industrial activity. Based on the results of this study rapid and reliable EDTA soil extraction procedure should be applied for assessment of phytoavailable soil fraction for all investigated PTEs excluding Ni and Zn. It will be of substantial help in evaluation of the food chain risk assessment and undertaking of proper remediation activities.

Uncited references

European Commission (2006), European Parliament, Council of the European Union (2002), Ministry of Environment and Water, Ministry of Health-Care, Ministry of Agriculture and Forestry (2008).

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2018.07.077.

References

ACAF, 2000. (Advisory Committee on Animal Feedingstuffs), 2000. Copper poisoning in Sheep, Information paper ACAF/00/55; Seventh ACAF Meeting 30 November.

Alvarez, J.M., Lopez-Valdivia, L.M., Novillo, J., Obrador, A., Rico, M.I., 2006. Comparison of EDTA and sequential extraction tests for phytoavailability prediction of manganese and zinc in alkaline soils. Geoderma 132 (3–4), 450–463.

Antoniadis, V., Shaheen, S.M., Boersch, J., Frohne, T., Laing, G.D., Rinklebe, J., 2017. Bioavailability and risk assessment of potentially toxic elements in garden edible vegetables and soil around a highly contaminated former mining area in Germany. J. Environ. Manag. 186 (Pt 2), 192–200.

Burt, R., Wilson, M.A., Keck, T.J., Dougherty, B.D., Strom, D.E., Lindahl, J.A., 2003. Trace element speciation in selected smelter-contaminated soils in Anaconda and Deer Lodge Valley, Montana, USA. Adv. Environ. Res. 8 (1), 51–67.

Chen, H., Yuan, X., Li, T., Hu, S., Ji, J., Wang, C., 2016. Characteristics of heavy metal transfer and their influencing factors in different soil-crop systems of the industrialization region, China. Ecotoxicol. Environ. Saf. 126, 193–201.

Chojnacka, K., Chojnaski, H., Górecka, H., Górecki, H., 2005. Bioavailability of heavy metals from polluted soils to plants. Sci. Total Environ. 337 (1–3), 175–182.

Christian, G., 2003. Analytical Chemistry, sixth ed Wiley, Hoboken, 294–313.

Cui, Y.J., Zhu, Y.G., Zhai, R.H., Chen, D.Y., Huang, Y.Z., Qiu, Y., Liang, J.Z., 2004. Transfer of metals from soil to vegetables in an area near a smelter in Nanning, China. Environ. Int. 30 (6), 785–791.

European Commission, 2006. Commission Regulation (EC) No 479/2006 of 23 March 2006 as regards the authorisation of certain additives belonging to the group compounds of trace elements.

European Parliament, Council of the European Union, 2002. Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed - Council statement.

Ge, Y., Zhang, J., 2015. Heavy metal contamination and accumulation in soil and plant species from the Xinqiao copper deposit, Anhui Province, China. Anal. Lett. 48 (3), 541–552.

Hoffman, G.L., 1996. Methods of analysis by the U.S. Geological Survey National Water Quality Laboratory - preparation procedure for aquatic biological material determined for trace metals, Open-File Report 96-362; U.S. Geological Survey.

IAEA TRS-472, 2010. International Atomic Energy Agency, Handbook of Parameter Values for the Prediction of Radionuclide Transfer in Temperate Environments, Technical Report Series 472, Vienna.

ISO 10390, 2005. Soil quality - Determination of pH.

ISO 10693, 1995. Soil quality - Determination of carbonate content - Volumetric method. 1995.

ISO 10694, 1995. Soil quality - Determination of organic and total carbon after dry combustion (elementary analysis). 1995.

ISO 11466, 1995. 1995, Soil quality – Extraction of trace elements soluble in aqua regia.

Jolly, Y.N., Islam, A., Akbar, S., 2013. Transfer of metals from soil to vegetables and possible health risk assessment. Springerplus 2 (1), 385–392.

Kabala, C., Singh, B.R., 2001. Fractionation and mobility of copper, lead and zink in soil profiles in the vicinity of copper smelter. J. Environ. Qual. 30 (2), 485–492.

Kabata-Pendias, A., 1993. Behavioural properties of trace metals in soils. Appl. Geochem. 8 (S2), 3–9.

Kabata-Pendias, A., 2001. Trace Elements in Soils and Plants, third ed CRC Press LLC, Boca Raton.

Kabata-Pendias, A., 2004. Soil-plant transfer of trace elements – an environmental issue. Geoderma 122 (2–4), 143–149.

Kabata-Pendias, A., Mukherjee, A.B., 2007. Trace Elements From Soils to Humans. Springer-Verlag, Berlin Heidelberg.

Karszewska, A., Szerszen, L., Kabala, C., 1998. Forms of selected heavy metals and their transformation in soils polluted by the emissions from copper smelters. Adv. Geo-Ecol. 31, 705–712.

Kloke, A., Sauerbeck, D.R., Vetter, H., 1984. The contamination of plants and soils with heavy metals and the transport of metals in terrestrial food chains. In: Nriagu, J.O. (Ed.), Changing metal Cycles and Human Health, Dahlem Workshop Reports, Life Sciences Research Report 28, Springer-Verlag, Berlin, Heidelberg, New York, Tokyo, pp. 113–141.

Kohonen, T., 2001. Organizing Maps, third ed Springer, Berlin.

Kuo, S., Helman, P.E., Baker, A.S., 1983. Distribution and forms of copper, zinc, cadmium, iron, and manganese in soils near a copper smelter. Soil Sci. 135, 101–109.

Lim, G.H., Seo, B.H., Kim, K.H., Kim, H.S., Kim, W.L., Owens, G., Kim, K.R., 2016. Transfer functions for estimating phytoavailable Cd and Pb in metal contaminated paddy and upland soils: implications for phytoavailability based land management. Geoderma 270, 89–97.

Liu, K., Lv, J., He, W., Zhang, H., Cao, Y., Dai, Y., 2015. Major factors influencing cadmium uptake from the soil into wheat plants. Ecotoxol. Environ. Saf. 113, 207–213.

Madejón, P., Murillo, J.M., Marañón, T., Cabrera, F., López, R., 2002. Bioaccumulation of As, Cd, Cu, Fe and Pb in wild grasses affected by the Aznalcor llar mine spill (SW Spain). Sci. Total Environ. 290, 105–120.

Madejón, P., Burgos, P., Murillo, J.M., Cabrera, F., Madejón, E., 2009. Bioavailability and accumulation of trace elements in soils and plants of a highly contaminated estuary (Domingo Rubio tidal channel, SW Spain). Environ. Geochem. Health 31 (6), 629–642.

Maiz, I., Arambarri, I., Garcia, R., Millán, E., 2000. Evaluation of heavy metal availability in polluted soils by two sequential extraction procedures using factor analysis. Environ. Pollut. 110 (1), 3–9.

Massart, D.L., Kaufman, L., 1983. The Interpretation of Analytical Chemical Data by the Use of Cluster Analysis. Wiley Interscience, New York.

Ministry of Environment and Water, Ministry of Health-Care, Ministry of Agriculture and Forestry, 2008. Regulation No3/01Aug2008 on the permissible limits of toxic substances in the soils, Bulgarian Official Gazette.

Pope, J.M., Farago, M.E., Thornton, I., Cordos, E., 2005. Metal enrichment in Zlatna, a Romanian copper smelting town. Water Air Soil Pollut. 162 (1–4), 1–18.

Pribil, R., 1982. Applied Complexometry. Pergamon Press Ltd, Oxford, 91–222.

Pueyo, M., Rauret, G., Bacon, J.R., Gomez, A., Muntau, H., Quevauviller, P., Lopez-Sanchez, J.F., 2001. A new organic-rich soil reference matterial certified for its EDTA and acetic acid extractable contents of Cd, Cr, Cu, Ni, Pb and Zn, following collaboratively tested and harmonised procedures. J. Environ. Monit. 3 (2), 238–242.

Quevauviller, P., Lachica, M., Barahona, E., Rauret, G., Ure, A., Gomez, A., Muntau, H., 1996. Interlaboratory comparison of EDTA and DTPA procedures prior to certification of extractable trace elements in calcareous soil. Sci. Total Environ. 178 (1–3), 127–132.

Rodrigues, S.M., Pereira, E., Duarte, A.C., Römkens, P.F., 2012. Derivation of soil to plant transfer functions for metals and metalloids: impact of contaminant's availability. Plant Soil. 361 (1–2), 329–341.

Schramel, O., Michalke, B., Kettrup, A., 2000. Study of the copper distribution in contaminated soils of hop fields by single and sequential extraction procedures. Sci. Total Environ. 263 (1–3), 11–22.

Uchida, S., Tagami, K., Hirai, I., 2007. Soil-to-plant transfer factors of stable elements and naturally occurring radionuclides (1) Upland field crops collected in Japan. J. Nucl. Sci. Technol. 44 (4), 628–640.

USGS, 2004. (United States Geological Survey). Mineral commodity summary. USGS, Reston VA.

Vesanto, J., 1999. SOM-based data visualization methods. Intell. Data Anal. 3 (2), 111–126.

Voyslavov, Ts, Tsakovski, S., Simeonov, V., Arpadjan, S., 2018. The effect of iron and manganese oxyhydroxide soil fraction on occurrence of Cr(VI). Bulg. Chem. Commun. 50 (3).

Yan, S., Ling, Q.C., Bao, Z.Y., 2007. Metals contamination in soils and vegetables in metal smelter contaminated sites in Huangshi, China. Bull. Environ. Contam. Toxicol. 79 (4), 361–366.

Yoon, J., Cao, X., Zhou, Q., Ma, L.Q., 2006. Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. Sci. Total Environ. 368 (2–3), 456–464.