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Comparison of the ability of different plant species to reflect environmental pollution in Bulgaria

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Abstract

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The purpose of this study was to assess the heavy metals (cadmium, lead, zinc, copper, iron and manganese) pollution in three regions in Bulgaria characterized with different type of pollution sources and to compare their bioaccumulation in selected agricultural plant species – perennial (apple and pear) and annual (corn and sunflower). The bioaccumulative ability of different plant parts was also investigated. The bioaccumulation factors of the analyzed heavy metals were evaluated by comparison of the determined concentrations in the plant parts from polluted regions to corresponding metal concentrations in the same plant species collected from a background region. A strong dependence of the accumulation of the studied plants on the specific pollutant was established as well as decreased transfer factors at increased heavy metals concentrations in the soils.

Keywords: heavy metals; accumulation factor; transfer factors; annual; perennial; plants; ICP-MS

Introduction

Heavy metals are some of the most serious pollutants of the environment due to their toxicity, long-term persistence in nature and possible bioaccumulation and biomagnification (Demirak et al., 2006; Wijaya et al., 2012).

Numerous studies have been performed to study the behavior of heavy metals in contaminated environment (Djingova et al., 1993; 2004; Djingova and Kuleff, 1999; Järup, 2003; Markert et al., 2008; Mihaylova et al., 2013, Lyubomirova et al., 2014; 2015). Estimation of environmental pollution using the chemical composition of plants is most often used for monitoring the environment. Plants can accumulate heavy metals in or on their tissues due to their great ability to adapt to variable chemical properties of the environment. Studying variabilities in the chemical compositions between plants from pristine and polluted environments is a way to identify the influence of pollution sources (Hu et al., 2014). The assessment of the environmental metal pollution is usually performed by analyses of naturally occurring plant species (Djingova et al., 1993, Djingova and Kuleff, 1999, Lyubomirova et al., 2014; Ozturk et al., 2017).

One of the basic environmental problems is the heavy metals contamination of agricultural land (Chenery et al., 2012) and subsequently their accumulation in agricultural plants. Accumulation of toxic elements in leaves, crops, vegetables, and fruits may pose a significant risk to animal and human health. Dietary intake of plant-derived food represents a major fraction of potentially health-threatening human exposure (Clements and Ma, 2016).

The aim of the present work is to (i) determine the concentration of several heavy metals in agricultural plant leaves, fruits and grains used for animal and human feed; (ii) compare the accumulation abilities of different plant parts – fruits vs. leaves and grains vs. leaves (iii) assess the dependence of the transfer and accumulation factors on the plant species and the concentration of the corresponding element in the soil.

Materials and Methods

Soil and plant samples were collected from four sampling places. Sampling place S1 is a background region, in the vicinity of the town Dzhebel, situated in the Rhodope Mountains in Southern Bulgaria. The other samples (S2-S4) were collected from immediate vicinity to different sources of anthropogenic pollution. Sampling site 2 (Sedlovina, S2) is close (5 km) to the Pb-Zn smelter in Kardzhali – South Bulgaria. Sampling site 3 (Parvomay, S3) is located closely to Plovdiv. The sampling is performed from an area near former mines. Sampling site S4 is close to a chemical fertilizer plant and cement industry in Dimitrovgrad, situated in South Bulgaria.

To study the response of plant species to different anthropogenic pollution in the autumn (at the end of September) of 2015, leaves of perennial (apple and pear) and annul (corn and sunflower) plant species were taken. Additionally, apple fruits and corn grains from all the sampling places were collected.

The sampling of the tree species was performed at an average height of the tree crown on all sides of the tree. Only medium large, healthy leaves were selected and taken from 6 individual apple and pear trees. The minimum number of leaves from each individual tree was 50.

Samples of corn and sunflower were collected from 10-20 m^2 area. Only healthy leaves from third to seventh leaf were taken for analysis.

Immediately after sampling, the foliage was washed thoroughly under tap water and rinsed with distilled water. The plant samples were air dried in a clean room for 4–5 days. After air drying, the samples were put in an oven for 4 h at 85°C, ground in a polytetrafluoroethylene (PTFE) ball mill to fine powder and subjected to digestion.

The fruit samples were washed with tap and distilled water, then cut into small pieces and ground to pulp. The pulp was frozen at a temperature of liquid nitrogen (-196°C) and the sample was lyophilized under reduced pressure using a Freeze Dryer 3 (LABCONTO) lyophilizer. The lyophilized material was homogenized in a PTFE ball mill and stored at stored at 4°C. The samples of corn grain were milled using a rotor mill, homogenized and stored at 4°C.

The soil sampling was performed according to ISO 10381-1:2002 and ISO 10381-2:2002. The samples were collected from the surface 20 cm soil layer from an area of min 10 m^2. Sampling was performed with polytetrafluoroethylene (PTFE) blades. The minimum quantity of the soil samples was 2 kg.

Sample preparation included air drying, sieving through 2-mm PTFE sieve, grinding, and homogenization in a Teflon ball mill (ISO 11464:2006). pH of the soils was determined according to ISO 10390:2005 and CEC according to ISO 23470:2007. The quantity of the total and inorganic carbon was determined using a Shimadzu 5000 TOC analyzer, in solid sample module (furnace temperature TC-900°C, IC-200°C).

Evaluation of the response of the plant species to heavy metal pollution was made by calculation of the accumulation factors (AF – concentration of the element in the polluted region to its concentration in the background region) of the studied plant leaves.

To reveal the dependence of the metal concentrations in the plant on their total concentrations in the adjacent soil, transfer factors (TF, ratio between the concentration of the element in the plant to its concentration in the soil) of the studied metals were calculated.

Reagents

Suprapur chemicals (HNO_3, H_2O_2) and double deionized water (MilliQ) were used for preparing all solutions. Working standard solutions were prepared from single element calibration solutions (Merck) by appropriate dilution.

The accuracy of the method was evaluated by analysis four certified/standard reference materials: NIST 1547 (Peach leaves, National Institute of Standards and Technology, USA), INCT-MPH-2 (mixed Polish herbs, Institute of Nuclear Chemistry and Technology, Warsaw – Poland), IAEA Soil-7 and STSD-1 (reference stream sediment, Canada Center for Mineral and Energy Technology, Geological Survey of Canada).

Instrumentation

For microwave digestion of the samples, Microwave Reaction System (Anton Paar, Multiwave 3000) was used.

Analysis of the plant and soil samples was carried out by using a Perkin Elmer SCIEX DRC-e ICP-MS. The spectrometer was equipped with a dynamic reaction cell (DRC) for removal of multi-element interferences, using methane as a reaction gas. ICP-MS analysis of the plant and soil samples was performed, as described in Mihaylova et al. (2013) and Lyubomirova et al. (2015).

Sample preparation for total element determination

To 0.25 g of the plant samples 8 mL HNO_3 and 3 mL H_2O_2 were added and microwave digestion was performed for 25 min. The digested samples were quantitatively transferred and diluted to 50 mL with double deionized water. The digestion of the soil samples was performed as described in Lyubomirova et al., 2015.

Aqua regia digestion of the soil samples was performed according US-EPA 3051a. Afterwards, the supernatant was decanted and diluted to 50 mL with double deionized water. Before analysis, 1 mL of the sample was taken and diluted to 10 mL with double deionized water.

Results and Discussions

Table 1 presents the analytical data for the investigated elements determined in the leaves of the investigated plant species, in the respective soils and the calculated transfer factors (TFs) of the elements. The determined heavy metal concentrations in the soil sample from sampling site S1 fall within the concentration intervals given for background soils, collected from 12 background sampling sites (Lyubomirova et al., 2015) which give reason to assume that the area, although not so far (20 km away) from the Pb-Zn smelter is not affected from anthropogenic activity and can be considered as a background region.

The comparative examination of the concentrations of the studied elements in the polluted soils (S2-S4) showed increase of the concentrations in the polluted soils in comparison to the background (S1), the most significant was in sampling site S2. The increase of Cd was between 115 and 320, of Pb between 70 and 340 and of Zn between 70 and 110 times in the order S2> S4>S3. Elevated concentrations

Table 1. Concentrations and TFs of Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn (in µg g^{-1}) in the studied soils and plant leaves, collected from background and polluted regions in Bulgaria

		Cd	Co	Cr	Cu	Mn	Ni	Pb	Zn
S1	apple	0.29±0.01 (1.65)	0.058±0.001 (0.004)	0.25±0.01 (0.017)	3.7±0.1 (0.07)	29.9±0.9 (0.027)	0.41±0.01 (0.010)	1.73±0.05 (0.085)	14.2±0.4 (0.14)
	pear	0.09±0.01 (0.51)	0.098±0.004 (0.007)	0.33±0.01 (0.022)	7.2±0.3 (0.13)	31.7±1.3 (0.029)	0.38±0.02 (0.009)	1.42±0.06 (0.070)	12.5±0.5 (0.12)
	corn	0.38±0.02 (2.16)	0.033±0.002 (0.002)	0.95±0.05 (0.063)	11.6±0.6 (0.21)	39±2 (0.035)	0.81±0.04 (0.020)	0.53±0.03 (0.026)	50±2 (0.50)
	sunflower	0.12±0.01 (0.68)	0.45±0.01 (0.034)	0.61±0.02 (0.041)	18.7±0.6 (0.35)	79±2 (0.072)	1.90±0.06 (0.046)	2.70±0.08 (0.13)	66±2 (0.65)
	soil	0.18±0.02	13.4±0.4	15.0±1.3	54±2	1100±45	41±3	20.3±0.8	102±7
S2	apple	0.56±0.03 (0.028)	0.15±0.01 (0.006)	0.53±0.03 (0.007)	7.9±0.4 (0.020)	64.5±1.2 (0.061)	0.74±0.04 (0.027)	17.3±0.7 (0.002)	87±3 (0.007)
	pear	1.34±0.03 (0.067)	0.21±0.01 (0.009)	0.69±0.01 (0.009)	15.3±0.3 (0.039)	67.1±1.3 (0.064)	0.89±0.02 (0.033)	49±1 (0.007)	217±4 (0.019)
	corn	11.7±0.4 (0.58)	0.87±0.03 (0.037)	3.3±0.1 (0.041)	24.1±0.7 (0.061)	79±2 (0.075)	3.6±0.1 (0.13)	72±2 (0.010)	324±8 (0.028)
	sunflower	8.4±0.3 (0.42)	1.14±0.04 (0.049)	3.0±0.1 (0.038)	38.1±1.5 (0.096)	98±4 (0.093)	2.8±0.1 (0.103)	85±3 (0.012)	1028±39 (0.088)
	soil	20±1	23.4±0.9	81±3	395±17	1054±48	27.3±1.4	6980±260	11700±400
S3	apple	0.21±0.01 (0.004)	0.64±0.03 (0.038)	0.23±0.01 (0.001)	7.9±0.4 (0.037)	180±9 (0.18)	0.29±0.01 (0.018)	3.4±0.1 (0.001)	6.8±0.3 (0.001)
	pear	0.18±0.01 (0.003)	0.10±0.01 (0.006)	0.78±0.03 (0.003)	13.1±0.5 (0.062)	170±6 (0.17)	0.45±0.02 (0.028)	5.8±0.2 (0.002)	33±1 (0.004)
	corn	0.29±0.01 (0.005)	0.19±0.01 (0.011)	3.63±0.05 (0.012)	22.9±0.7 (0.11)	172±5 (0.18)	1.94±0.06 (0.12)	7.6±0.2 (0.003)	41±1 (0.006)
	sunflower	1.10±0.02 (0.020)	0.46±0.01 (0.027)	2.55±0.05 (0.009)	38±1 (0.18)	163±3 (0.17)	1.04±0.02 (0.065)	8.5±0.2 (0.003)	56±2 (0.008)
	soil	56±3	17±1	294±15	211±13	982±40	16±1	2874±115	7445±280
S4	apple	0.20±0.01 (0.004)	0.60±0.02 (0.019)	2.21±0.07 (0.006)	9.12±0.27 (0.021)	127±4 (0.098)	0.43±0.01 (0.011)	3.3±0.1 (0.002)	7.5±0.6 (0.001)
	pear	0.28±0.01 (0.005)	0.16±0.01 (0.005)	2.04±0.06 (0.005)	12.8±0.4 (0.029)	71±2 (0.055)	0.64±0.02 (0.017)	10.4±0.3 (0.008)	24.1±0.7 (0.003)
	corn	0.77±0.02 (0.015)	0.24±0.01 (0.008)	4.63±0.14 (0.012)	24.5±0.7 (0.056)	136±4 (0.11)	1.25±0.04 (0.033)	7.9±0.2 (0.006)	32±1 (0.004)
	sunflower	1.70±0.05 (0.033)	0.83±0.02 (0.026)	4.11±0.12 (0.010)	32±1 (0.073)	151±5 (0.12)	1.51±0.05 (0.040)	9.5±0.3 (0.007)	60±2 (0.008)
	soil	51±3	32±2	396±12	440±16	1299±48	38±2	1369±51	7977±310

() – transfer factor

were also found for Cr (between 5 and 20 times) and Cu (between 4 and 8 times). Data on contamination of the area around the smelter has been published by many researchers (Stoyanov, 1999). Very similar concentrations for lead, zinc and cadmium were also measured by Zheleva et al., 2012 in soil samples, taken from an arable land which is at least twice a remote area from the smelter compared to sampling place S2. The comparison of the present soil concentrations for the major pollutants in S2 with data obtained for the period 2008-2010 (Chilingirova et al., 2011) show a tendency for increasing by factors of 10 (for Pb and Cd) and 30 (for Zn). A trend of concentration increase for Zn and Pb in the soil near the smelter over time was also found by Staykova and Naydenova, 2008 for the period 1988-2006.

The evaluation of the data for the plant samples shows that at background levels (sampling place S1), most of the elements (Cd, Co, Cr, Mn and Ni) were present in similar concentrations in the studied plant leaves. Higher natural concentrations were found for Zn and Cd in the leaves of both annual plants, while the concentration of Pb was lower in the corn leaves than in the other plant species. In the polluted areas sunflower has the highest concentrations of Cr, Cu, Pb and Zn in all regions. Corn has similar concentrations of Cr and higher Ni. Generally the concentrations of perennial plants are lower than of annual.

The influence of the total heavy metal concentrations in the soils on their concentrations in the plants was assessed using the element transfer factors (TFs). The calculated TFs of the plant species from the background and the polluted regions are presented in Table 1 (the data in brackets).

The review of the obtained results shows that there is no significant difference in the TFs for the different plant species collected from the background region.

The increased concentration of the studied elements in the polluted soils showed the following effects on the TFs compared to the clean region:

– Lower TFs for Cd, Cr, Cu, Pb and Zn, irrespective of the plant species and the type of pollution. Similar tendency was earlier established for the TFs of Cd, Cu, and Zn for *Taraxacum officinale*, collected from 12 background regions in Bulgaria (Lyubomirova et al., 2015).

– No significant difference was observed for the rest of the elements.

In a previous study factor analysis was used to investigate the dependence of transfer factors for 52 elements on the soil characteristics – total soil concentration, pH, CEC, organic C, and concentration of the element in the soil fractions) in the system soil – *T.O.* (Lyubomirova et al., 2015). The results proved that the TFs of the studied elements depend mainly on the organic matter content (Cd, Cr, Ni, Pb, and Zn) or on the total soil concentration and organic matter (Cu).

Since the soils studied in the present work do not differ in soil type (all of them are leached cinnamonic forest), and soil characteristics (neutral or slightly alkaline with pH values between 7.3 and 7.8, organic carbon content varies between 0.38 and 0.64 and CEC in the range 6 and 8) the calculated TFs are an indication for the dependance of the transfer factor on the total concentration in the soil.

Unlike the data for the soils showing increased concentrations in all polluted areas, the AFs (Fig.1), indicated that high degree of accumulation of these elements was observed only in the leave samples from S2. Furthermore, a strong dependence on the plant species was established. The comparison of the obtained AF values demonstrate higher accumulation of Pb and Cd in the annual plants, e.g. AF(Cd) is 1.9 in the apple and 70 in the sunflower, AF(Pb) is 10 in the apple and 135 in the corn, while the highest accumulation of Zn is observed in the pear (AF 17.4). Significantly lower AFs values for Cd, Pb and Zn were obtained for the leave samples from S3 and S4, despite the increase of their concentrations in the soils. These results and the lower TFs (Table 1) are most probably due to the chemical species of the heavy metals in the respective soils. Obviously, due to the high level of constant pollution in the region of the smelter, the elements are present mostly in bioavailable species. Probably in the other regions, the high metal concentrations in the soils are due to residual contamination (especially in S3) and during the long stay in the soil, transformations to immobilized chemical species occurred. Despite the low values of AFs, the trend for the highest accumulation of Pb by the corn, Cd by the sunflower and Zn by the pear is confirmed.

In order to investigate variations in the elemental concentrations in individual plant parts, samples of apple fruits and corn grains were also analyzed. Table 2 presents the calculated element concentration ratios in the apple fruit/leaves and grain/leaves from the studied regions. The calculated values for both plant species are less than 1, indicating that irrespective of the plant species and the type of pollution, the leaves have higher accumulative ability than the fruits, resp. grains.

The comparison of the data for the apple and the corn from the background and the polluted regions demonstrate more significant change in the behavior of the corn organs under pollution. The data show that at higher metal concentration, the differences between the corn leaves and grain concentrations increase. The highest differences are observed in the sample from S2 for Cd, Pb and Zn, corresponding to the main pollutants of the regions. The substantial decrease in the ratios of these elements can be explained by the

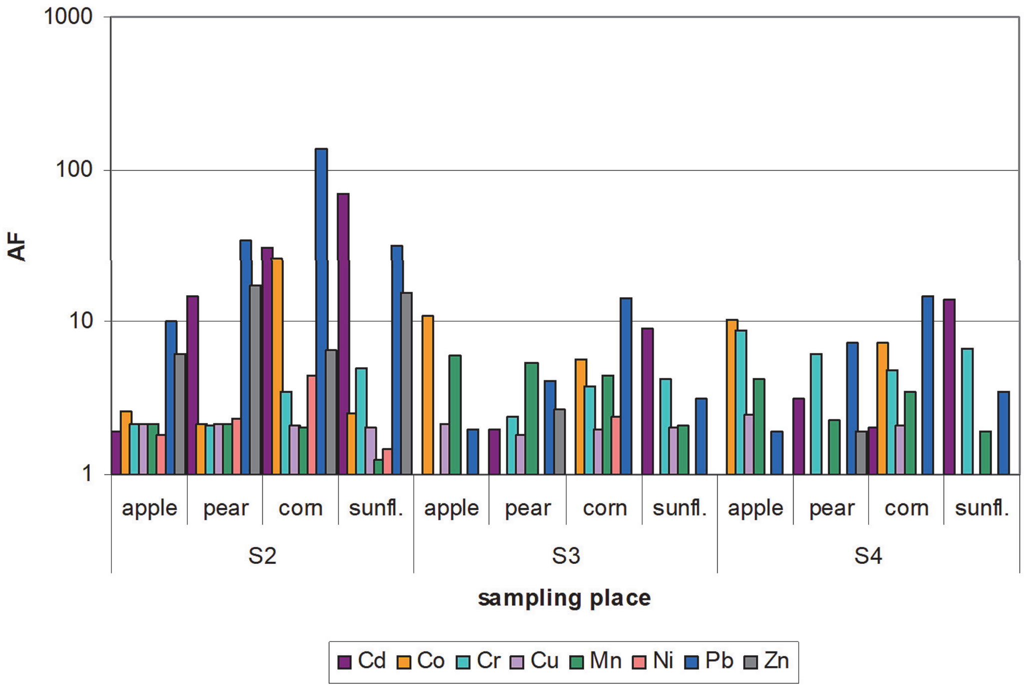

Fig. 1. Accumulation factors of apple, pear, corn and sunflower leaves collected from the polluted areas

Table 2. Concentration ratios of the studied elements in apple fruits/leaves and corn grains/leaves

	S1		S2		S3		S4	
	apple	corn	apple	corn	apple	corn	apple	corn
Cd	0.22	0.04	0.17	0.002	0.16	0.21	0.68	0.06
Co	0.33	0.06	0.81	0.04	0.25	0.13	0.33	0.05
Cr	0.37	0.30	0.67	0.13	1.06	0.20	0.24	0.07
Cu	0.95	0.03	0.59	0.02	1.15	0.01	0.14	0.02
Mn	0.34	0.25	0.71	0.20	0.73	0.14	0.35	0.17
Ni	0.15	0.07	0.18	0.07	0.79	0.07	0.16	0.33
Pb	0.19	0.13	0.26	0.01	0.07	0.17	0.22	0.11
Zn	0.38	0.15	0.16	0.01	0.13	0.07	0.43	0.14

protective function of the leaf sheath against direct exposure to atmospheric deposition on the corn cobs. The close values for the apple, collected from the clean and the polluted regions are an indication that if there is no protection, the plant parts have a similar response to the pollution – at higher levels of pollution the concentrations in the plant parts increase but the ratio is kept constant.

The comparison of the concentrations obtained in the present work with published literature data from a large scale monitoring of the region of the smelter (Zheleva et al., 2012) shows comparable or increased concentration of the main pollutants over time both in the leaves and in the fruits. Monitoring of lead content in cereals, fruits and vegetables, collected from the regions of the two largest smelters in Bulgaria (Pb-Zn smelters near Kardzhali and Plovdiv) and the Fe metallurgical plant in Kremikovtsi showed that the pollution of these products is strongest in the region of the smelter in Kardzhali, followed by the smelter in Plovdiv and then in Kremikovtsi. It has been found that the concentration of lead decreases with distance and is reduced to a background concentration in the vegetables at a distance of 10 km and 20 km for the fruits. Investigations for cadmium content of soil samples, cereals, fruits and vegetables from the region of the smelter in Kardzhali also showed elevated concentrations as the highest degree of accumulation is established within a radius of 3 km (Staykova & Naydenova, 2008; National Action Plan "Food and Nutrition, 2005-2010).

The elevated concentrations of the potentially toxic elements in the soil and plant samples, including fruits and grains are an indication for a serious pollution of the region around the smelter and the need for a remediation activity or change of the use of the agricultural lands.

Conclusions

The comparison of the accumulation ability of four agricultural plants for heavy metals indicates selective accumulation of the main pollutants – Pb, Cd and Zn in the plants. The comparison of the metals concentration in the plant parts (apple leaves vs. fruits, resp. corn leaves vs. grains) demonstrates lower accumulation by the fruits and grain than the respective leaves, which is an indication for the existence of plant protection mechanisms. The presence of regulatory mechanism of the polluted environment is also confirmed by the observed decrease of the TFs for the main pollutants, irrespective of the plant species.

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