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Original article

Transfer Factors for Endocrine Disrupting Metals in a Rural Mining Area, NW Romania

MICLEAN Mirela[1]*, Oana CADAR[1], Cecilia ROMAN[1], Laura PARLAPAN[2], I. Şt. GROZA[2]

[1]INCDO-INOE 2000, Research Institute for Analytical Instrumentation, Donath St., No. 67, 400293 Cluj-Napoca, Romania
[2]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Mănăştur St., No. 3 - 5, 400372 Cluj-Napoca, Romania

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Abstract

In this study, concentrations of Mn, Co, Ni, Cu, Zn, As, Cd, Hg, Pb in vegetables (carrots and potatoes) and adjacent soil samples were determined, using Inductively Coupled Plasma Mass Spectrometry, subsequently appropriate wet acid digestions. Samples were collected from the private gardens in the villages situated near sedimentation ponds, in Baia Mare mining area, NW Romania. In order to assess the bio-available fraction the DTPA extractable metals content was analyzed. Transfer factors were calculated and Pearson correlations were performed, revealing a common pollution source of vegetables and soils with heavy metals.

Keywords: endocrine disrupting metals, transfer factor, ICP-MS

1. Introduction

Environmental contamination and exposure to heavy metals is a serious growing problem throughout the world, which has risen dramatically in the last 50 years as a result of an exponential increase in the use of heavy metals in industrial processes and products. Some metals are naturally found in the body and are essential to human health, being necessary for a proper metabolism (Cu, Zn, Mn, Co), but become toxic at high concentrations. Other elements, such as mercury, cadmium, arsenic, nickel and lead are highly toxic and can cause damaging effects even at very low concentrations.

They tend to accumulate in the food chain and in the body and can be stored in soft (e.g., kidney, liver, brain) and hard tissues (e.g., bone) and are systemic toxins with specific neurotoxic, nephrotoxic, fetotoxic and teratogenic effects [1, 4, 7].

Heavy metals can alter a vast array of metabolic processes and disrupt natural systems, including endocrine system. During the last decade, the studies about the potential of heavy metals to interfere with endocrine system have increased significantly, introducing these elements as endocrine disrupting metals (EDMs) [14]. Among various mechanisms of action, metals are suspected of exerting estrogenic activity in human and wildlife [3]. Choe et al. (2003) found high oestrogenicity for Cd, followed by Co, Pb, Cr and Cu, hence considered these metals as a new class of nonsteroidal environmental oestrogen [2]. Martin et al. [12] had found that Co, Cu, Ni, Pb, Hg, or Cr,

* Corresponding author.
Tel.: 0264420590; Fax: 0264420667
e-mail: mirelamiclean@yahoo.com

decreased the concentration of the oestrogen receptor protein and an induced expression of the oestrogen-regulated genes for the progesterone receptor was observed [6, 9, 13].

The objective of this study was to investigate the levels of Mn, Co, Ni, Cu, Zn, As, Cd, Hg, and Pb contamination in soil and vegetables in the vicinity of tailing deposits from Baia Mare mining area and to assess the availability of these metals to vegetables.

The transfer characteristics of investigated metals from soils to the edible parts of vegetables were determined by calculating the soil-to-plant transfer factors.

2. Site description and sampling

The studied villages (Tautii Magherausi, Sasar, Bozanta Mare) are located near three tailing deposits: one in preservation for 20 years and the other two still in use to collect the wastewaters from ore processing plants of Baia Mare area. The studied area is shown in figure 1.

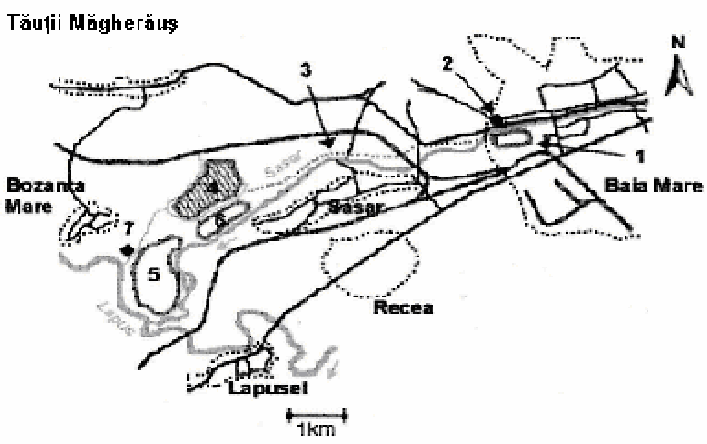

Figure 1. Sampling area of soils and vegetables

In the autumn of 2009 a number of 9 potato tubers (*Solanum tuberosum*), 9 carrot taproots (*Daucus carota sativus*) and adjacent soil (18) samples were collected from private gardens of residents from the three villages, three types of each vegetable in each village. The studied vegetable species are commonly grown and consumed in the area and were collected just before shedding to assure maximum metal accumulation.

The soil samples were collected from 0 - 20 cm depth, using a stainless steel shovel. All samples were stored in clean, labeled, polyethylene bags, closed tightly to avoid contamination during transportation to the laboratory.

3. Material and Method

Soil samples were air dried to constant weight and sieved through the 2 mm sieve. The fraction < 2 mm was split in two parts. One part was stored in polyethylene bags for the determination of DTPA-extractable metal contents. The other part was ground to a fine powder in a tungsten-carbide swing mill for 3 min and sieved through 250 μm shieve, homogenized and stored in polyethylene bags until the *aqua regia* extraction. The *aqua regia* extractable metals were determined according to ISO 11466:1995. An amount of 1 g sample (< 250 mm) was weighted, introduced into the reaction flask and maintained at room temperature for 16 h with 21 mL of 12 M HCl and 7 mL of 15.8 M HNO_3. The mixture was then heated under reflux conditions for 2 h. The solution was filtered and diluted to 100 mL with 0.5 M HNO_3. The available metal contents of soils were extracted in DTPA (diethylenetriaminepentaacetic acid) according to ISO 14870:2001. An amount of 10 g of soil sample (< 2 mm) was weighted into a 125 mL Erlenmeyer flask, then 20 mL of DTPA extracting solution was added and shaken for 2 h at room temperature using a magnetic stirrer. The solution was filtered and diluted to 100 mL with ultrapure water.

Vegetable samples were washed with tap water, then with distilled water to remove superficial dust, oven-dried at 80 - 90^{o}C for 15-30 min and at 65^{o}C for 12 - 24 h [16]. The dried samples were ground with a stainless steel mill to pass a 0.5 mm sieve. A portion of plant material (about 0.5 g) was accurately weighed into a Teflon vessel followed by the addition of 6 mL HNO_3 (65%) and 2 mL H_2O_2 (30%) using a method in three stages of pressure and times with a microwave digestion system, OI Analytical (Texas, SUA). The metal contents in vegetables and in soil (aqua regia and DTPA extractable) samples were measured by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) using the ELAN DRC II – Perkin Elmer (Toronto, Canada), after appropriate dilutions.

The quantification was performed using an external calibration with multielemental Merck standard solution.

Blank extractions were carried out for each set of analyses. Analytical grade or suprapure quality reagents (Merck, Darmstadt, Germany) as well as ultrapure water obtained from a Milipore Direct Q 3 purifier system were used to prepare solutions.

4. Results and Discussions

The DTPA extraction method was initially designed to predict micronutrient deficiencies in neutral to calcareous soils, but it has been also employed for the estimation of metal availability for plants [11]. Minimum, maximum levels, mean, median and standard deviation of mean for aqua regia and DTPA extractable Mn, Co, Ni, Cu, Zn, As, Cd, Hg and Pb contents in soils and vegetables are presented in tables 1- 4.

Table 1. Basic statistics regarding concentrations of endocrine disrupting metals in soil samples, extracted in aqua regia, µg kg^{-1}

No.	Element	Minimum	Maximum	Mean	Median	Std. Dev.
1	Mn	67955	1184833	462351	406003	320120
2	Co	937	4619	2784	2443	1272
3	Ni	1650	8645	4667	4354	2561
4	Cu	4699	73778	25365	19154	21314
5	Zn	16320	385123	94212	50086	113954
6	As	2737	32259	12550	12856	9452
7	Cd	339	3842	1103	610	1148
8	Hg	52,23	176	120	122	51,33
9	Pb	79153	270742	155374	137233	56631

Aqua regia extractable average metal contents decreased in the following order: Mn>Pb>Zn>Cu>As>Ni>Co>Cd>Hg.

Aqua regia extractable Pb contents exceeded the alert level (50 mg kg^{-1}) in all soil samples and average and median concentrations exceeded the intervention level (100 mg kg^{-1}), for sensitive soils, according to Romanian legislation (Ministerial Order 756/1997). Regarding Cd, As and Zn concentrations, 2 soil samples exceeded alert levels, respectively. Average and median Zn concentrations were higher than alert level. The concentrations of Mn, Co, Ni, Cu and Hg extractable in aqua regia were below alert levels in all soil samples.

Table 2. Basic statistics regarding concentrations of endocrine disrupting metals in soil samples, extracted in DTPA, µg kg^{-1}

No.	Element	Minimum	Maximum	Mean	Median	Std. Dev.
1	Mn	6159	25271	15997	15765	6356
2	Co	9.74	58.55	29.65	25.33	17.32
3	Ni	163	1081	402	369	276
4	Cu	2636	13762	6463	5906	3454
5	Zn	5534	197025	36639	15651	60615
6	As	61.69	521	182	160	150
7	Cd	281	1536	672	569	362
8	Hg	ldl	0.49	0.11	0.03	0.16
9	Pb	70382	254516	141078	136466	49610

ldl = lower than detection limit

The concentration limit of 20 mg kg^{-1}, advocated for DTPA extractable Pb in soil to avoid health risk [15], was exceeded for all soil samples.

A relatively high fraction of aqua regia extractable metal content was extracted in DTPA, especially for Zn (mean value 35.2%, std. dev. 16.0%), Pb (mean value 54.2%, std. dev. 17.5%) and Cd (mean value 97.4%, std. dev. 57.9.0%).

In potatoes samples, the average metals contents decreased in the following order: Zn > Mn > Cu > Cd > Ni > As > Co > Pb > Hg. Average Cd concentration exceeded maximum admitted level in potatoes samples, according to Commission Regulation (EC) No. 1881/2006, setting maximum levels for certain contaminants in foodstuffs (100 µg kg^{-1} wet weight).

Table 3. Basic statistics regarding concentrations of endocrine disrupting metals in potatoes samples, µg kg^{-1}

No.	Element	Minimum	Maximum	Mean	Median	Std. Dev.
1	Mn	455	15004	3483	1286	5694
2	Co	1.17	18.46	9.42	9.96	6.57
3	Ni	1.47	123.43	69.71	85.74	59.31
4	Cu	152	3087	1691	1707	1066
5	Zn	1972	15751	6304	5537	4960
6	As	3.36	68.81	37.07	40.62	24.21
7	Cd	27.76	162	99.43	99.01	50.29
8	Hg	ldl	4.55	0.98	0.23	1.77
9	Pb	22.77	82.78	7.93	17.99	45.50

Table 4. Basic statistics regarding concentrations of endocrine disrupting metals in carrots samples, µg kg^{-1}

No.	Element	Minimum	Maximum	Mean	Median	Std. Dev.
1	Mn	753	10828	3521	2913	3202
2	Co	1046	18233	6416	5481	5701
3	Ni	7802	157147	47576	35637	47613
4	Cu	7.88	85.91	33.56	24.12	24.77
5	Zn	13.24	355	185	168	136
6	As	9.61	80.38	44.19	39.73	23.29
7	Cd	0.09	0.63	0.26	0.22	0.17
8	Hg	0.06	0.36	0.23	0.26	0.09
9	Pb	ldl	7.48	3.12	1.65	3.02

In carrots samples, the average metals contents decreased in the following order: Ni > Co > Mn > Zn > As > Cu > Pb > Cd > Hg.

Mean concentrations of Co, Ni and As were higher in carrots than in potatoes samples. The mean concentrations of Mn were comparable.

4.1. Pearson correlations

Pearson correlations were performed among concentrations of studied endocrine disrupting metals in vegetables and soil extracted in aqua regia and DTPA, respectively, in order to determine the interrelationships between metals. Pearson correlations were carried out using the software XLSTAT (statistics and data analysis program for Microsoft Excel) and represent significant values for a confidence level of 95% and observation number, n = 9, being considered the sampling sites of vegetable and adjacent soil samples.

The following significations were used: Letter "T" referred to concentration extracted in aqua regia and "DTPA" concentration extracted in DTPA; letters "C" and "P" referred to concentrations of EDMs in Carrots and Potatoes, respectively. Principal Component Analysis (PCA) revealed the following significant correlations (at the level of significance alpha = 0.050) among EDM's concentrations extracted in aqua regia and DTPA, respectively:

Ni DTPA correlated with: Cu DTPA, Zn DTPA, Cd DTPA, Hg DTPA, Pb DTPA, Ni T, Cu T, Zn T, Pb T;
Cu DTPA correlated with: Ni DTPA, Zn DTPA, Cd DTPA, Hg DTPA, Pb DTPA, Co T, Ni T, Cu T, Zn T, Pb T;
Zn DTPA correlated with: Ni DTPA, Cd DTPA, Hg DTPA, Pb DTPA, Cu T, Zn T, Pb T;
As DTPA correlated with: Co T, Ni T;
Cd DTPA correlated with: Ni DTPA, Cu DTPA, Zn DTPA, Pb DTPA, Cu T, Zn T, Pb T;
Hg DTPA correlated with: Ni DTPA, Cu DTPA, Zn DTPA, Pb DTPA, Cu T, Zn T, Pb T;
Pb DTPA correlated with: Ni DTPA, Cu DTPA, Zn DTPA, Cd DTPA, Hg DTPA, Cu T, Zn T, Pb T.

Concentrations of Mn DTPA, Co DTPA, Mn T were not correlated with any investigated EDM's concentration.

PCA analysis revealed the following significant correlations (at the level of significance alpha = 0.050) among EDM's concentrations in vegetables and soil extracted in aqua regia and DTPA:

Mn C correlated with: Co C, Zn C, As C, Cd C, Hg C, As DTPA;
Co C correlated with: Mn C, Zn C, As C, Cd C, Hg C, As DTPA;
Cu C correlated with: As C;

Zn C correlated with: Mn C, Co C, As C, Cd C, Pb C, As DTPA;
As C correlated with: Mn C, Co C, Cu C, Zn C, Cd C, Pb C, As DTPA;
Cd C correlated with: Mn C, Co C, Zn C, As C, Pb C;
Hg C correlated with: Mn C, Co C;
Pb C correlated with: As T, Zn C, Cd C, As C.

Nickel concentration in carrots was not significant correlated with any EDM's in soil extracted in aqua regia or DTPA.

Any EDM's concentrations in carrots samples were not significant correlated with EDM's in soil extracted in aqua regia.

Mn P correlated with: Zn P;
Co P correlated with: Ni P, Mn DTPA;
Ni P correlated with: Co P, Mn DTPA;
Cu P correlated with: Zn P, As P;
Zn P correlated with: Mn P, Cu P, As P;
As P correlated with: Cu P, Zn P.

Cadmium, Hg and Pb concentration in potatoes were not significant correlated with any EDM's in soil extracted in aqua regia or DTPA.

Any EDM's concentrations in potatoes samples were not correlated with EDM's in soil extracted in aqua regia.

These findings suggest that the interaction between Mn, Co, Ni, Cu, Zn, As, Cd, Hg, Pb in both vegetables and soil is important, in relation to the known geochemical association between the studied metals. The soil metal concentrations appear to influence the uptake of Mn, Co, Cu, Zn, As, Cd, Hg, Pb in vegetables [8]. The correlation analysis of aqua regia-extractable metals in soil samples revealed a significant correlation between the investigated EDMs (except for Mn and Co), indicating similar sources of contamination.

4.2. Transfer factors

The relationship between contaminant concentrations in soil and edible plant material is highly specific to the plant species. The relationship between contaminant concentration in vegetables and the concentration in soil is described using Soil-to-Plant Transfer Factor (TF), which is defined as follows:

TF = Concentration in plant (μg kg^{-1})/ Concentration in soil (μg kg^{-1}) [5].

The TF values quantify the relative differences in bioavailability of metals to plants and identify the efficiency of a plant species to accumulate a given metal [10].

Minimum, maximum, mean, median and standard deviation of mean for transfer factors of Mn, Co, Ni, Cu, Zn, As, Cd, Hg and Pb from soil to potatoes and carrots samples are presented in table 5 and table 6, respectively.

The lowest mean value of TF for potatoes was registered for Pb (6.99 x 10^{-4}) and the highest for Cd (2929 x 10^{-4}). The mean values for transfer factors of EDMs in potatoes samples decreased in the following order: Cd > Zn > Cu > Hg > Ni > As > Mn > Co > Pb.

Table 5. Basic statistics regarding transfer factors of endocrine disrupting metals in potatoes samples (x 10^{-4})

No.	Element	Minimum	Maximum	Mean	Median	Std. Dev.
1	Mn	12.41	409.67	95.09	35.11	155.45
2	Co	6.27	98.53	50.28	53.17	35.08
3	Ni	2.83	238	134	166	115
4	Cu	22.00	446	244	247	154
5	Zn	394	3145	1259	1106	990
6	As	9.11	187	101	110	65.67
7	Cd	818	4772	2929	2916	1481
8	Hg	ldl	871	187	43.53	339
9	Pb	ldl	39.66	6.99	ldl	16.03

Table 6. Basic statistics regarding transfer factors of endocrine disrupting metals in carrots samples (x 10^{-4})

No.	Element	Minimum	Maximum	Mean	Median	Std. Dev.
1	Mn	28,55	498	179	150	156
2	Co	42,05	459	175	129	132
3	Ni	25,57	686	359	325	264
4	Cu	379	1918	933	741	531
5	Zn	395	8610	2826	2455	2497
6	As	216	3947	1015	633	1205
7	Cd	3306	34381	12843	8553	11340
8	Hg	ldl	1432	598	316	577
9	Pb	16.72	601	187	120	184

The mean values for transfer factors of EDMs in carrots samples decreased in the following order: Cd > Zn > As > Cu > Hg > Ni > Mn > Co > Pb similarly with potatoes samples (except for As). The mean values of transfer factors for carrots varied greatly, over three orders of magnitude: 175 x 10^{-4} for Co to 12843 x 10^{-4} for Cd.

The transfer factors were significantly higher for carrot samples than potato samples, regarding all the investigated elements.

The most easily mobilized metals from soil to plants were Cd, Zn, As, Cu, the most dangerous being Cd and As. Lead, present in high amounts in soil samples, had the lowest uptake by the two investigated vegetables.

5. Conclusions

The results showed that metal concentrations of polluted soils varied widely, in most cases exceeding the corresponding alert levels. The high percentages of DTPA extractable metals indicate an anthropogenic pollution. Element concentrations differed from one vegetable to another as the result of differences in element selectivity and accumulation from soil solution. The risk is especially high for Cd, As, Hg and Ni, extremely toxic elements. Due to the high heavy metal content in the studied area the metal accumulation in vegetables grown in the vicinity of industrial sites represents a potential risk for public health, because the ingestion of contaminated food is one of the main routs through which endocrine disrupting metals enter the human body.

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