BIOACCUMULATION OF METALS IN DIFFERENT SPECIES OF MULBERRY

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The concentrations of Zn, Mn, Fe, Pb, Ni, Cu and Cd in soil, leaves and mulberry fruits (*Morus alba* L. and *Morus nigra* L.) from southeast Serbia were determined by atomic absorption spectroscopy. Metal translocations from soil to fruit were calculated, as well as their oral intake and health risk indices. Positive correlations were found among metal concentrations in soil, leaves and fruit.

Keywords: mulberry fruits, soil metal, leaves metal, plant metal

Introduction

Metals found in surface soil derive from rock and precipitation. Urban areas contain more anthropogenic metals due to higher population density, traffic intensity, and proximity to industry. Their long-term deposition may reduce soil buffering capacity and cause soil and groundwater contamination [1]. Fruits accumulate soil metals during growth and processing. As metals are not biodegradable they may accumulate up the food chain. Although some are micronutrients, in high concentrations they are toxic [2]. The increased environmental heavy metal concentrations may lead to the increased human intake, causing serious illness [3-7]. Zinc, copper, iron, chromium and cobalt are essential and become toxic only in high concentrations, while lead and cadmium are highly toxic [8]. It is thus important to monitor metals in soil, fruit and fruit products. Many species of fruit used in nutrition and folk medicine grow in southeast Serbia.

Mulberry belongs to the genus *Morus* of the Moraceae family. Although it is extensively grown as food for silkworms in many countries, the mulberry fruit production is the main aim in Turkey, which is one of the most important mulberry fruit producers in the world [9,10]. Mulberry is found from temperate to subtropical regions of the northern hemisphere to the tropics of the southern hemisphere and it can grow in a wide range of climatic, topographical and soil conditions. It is widely spread throughout all regions from the tropics to the subarctic areas. Genus *Morus* is widespread in Asia, Europe, North and South America and Africa as well. Mulberry has a unique delicious fruit of the sour and refreshing taste. It has been used as a folk remedy to treat oral and dental diseases, diabetes, hypertension, arthritis and anemia [11]. The bright black and purple mulberry fruits, which have a very pleasant taste when eaten fresh, are also used in jams, juices, liquors, natural dyes, as well as in the cosmetics industry [12]. *Morus* species are deciduous

and in the period of low temperatures during the winter they are required to break dormancy. Mulberry fruits may be of white, red or black colour when they are ripe. Deep-coloured fruits are good sources of phenolics including flavonoids, anthocyanins and carotenoids [13-16], and mulberries are rich in phenolics [17].

Fruits and their extracts deserve special attention because of the important influence they have on human health. For the majority of the world population, fruits represent the primary source of the health care. Although the effectiveness of fruits is mainly associated with their constituents such as essential oils, vitamins, glycosides, etc., it was found that a prolonged intake can cause health problems due to the possible presence of heavy metals [3].

Mulberry fruit and leaves (*Morus alba* L. and *Morus nigra* L.) from rural southeast Serbia were analyzed (Table 1). Metals uptake from soil to leaves and fruit was determined. The health risk index estimates the risk due to the exposure.

Experimental

Reagents

All chemicals were of analytical grade. High purity deionized water (conductivity 0.05 $\mu\text{S cm}^{-1}$) was used in the solution preparation. Stock Cu, Mn, Zn, Fe, Pb, Ni and Cd solutions (1 g L⁻¹) were purchased from Merck (Darmstadt, Germany).

Sample preparation

Sampling points were selected by a standard procedure [18]. Points were at least 300 m from the main road, 100 m from the local road, and 5 m from the forest road to avoid pollution from vehicle exhaust. The samples were collected in southeast Serbia (locations in Table 3)

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during July 2014. Fruit and leaves were collected from the same plants. The soil sampling followed a zigzag principle at the depth of 0-30 cm.

Soil analysis

Air-dried soil samples were oven dried at 105 °C for two hours, then ground to pass through 2 mm sieves. Ten grams of the sample was suspended in deionized water in a 100 mL volumetric flask then filtered through Whatman No. 42 filter paper. The pH of the first 25 mL of the filtrate was measured. The remaining 75 mL was evaporated to dryness on a water bath, dissolved in 5 mL of 1:1 HNO₃:H₂O plus 5 mL of 1:1 HCl:H₂O, then diluted to 25 mL [18].

Fruit and leaves analysis

The fruit and leaves were washed with distilled water to remove dust and parasites, dried at 105 °C for 24 h, and homogenized [18]. The standard AOAC (2000) sample

preparation was followed [18]. The accurately weighed (1 g) sample in a silica crucible was ashed in a muffle furnace at 450 °C for 2 h and then 5 mL of 6 M HCl was added. Care was taken to ensure that all the ash came into contact with the acid. The sample was digested on a hot plate to obtain a clear solution. The residue was dissolved in 0.1 M HNO₃ and made up to 25 mL. Working standards were prepared by diluting stock solutions with 0.1 M nitric acid. The blank was deionized water.

Apparatus

Air-acetylene flame atomic absorption measurements were made using a Varian SpectrAA 20 spectrophotometer. Working conditions and limits of detection are shown in Table 1. Hanna Instruments pH meter was used to measure pH. Sigma pH 7,00 ± 0,01 and 4,00 ± 0,01 calibration buffers were used. Milli-Q apparatus prepared high purity deionized water.

Table 1. Working conditions and limits of detection

Metal	Calibration range (mg/l)	Limit of detection (LOD) (mg/l)	Wavelength (nm)	Slit (nm)	Acetylen flow (l/min)
Iron (Fe)	0,00-10,00	0,015	248,3	0,2	2,0
Copper (Cu)	0,00-1,00	0,007	324,8	1,0	1,8
Zink (Zn)	0,00-5,00	0,021	213,9	0,5	2,0
Lead (Pb)	0,00-1,00	0,002	217,0	1,0	1,6
Cadmium (Cd)	0,00-1,00	0,003	228,8	0,5	2,0
Manganese (Mn)	0,00-2,00	0,005	279,5	0,2	1,8
Nickel (Ni)	0,00-1,00	0,002	232,0	0,2	2,0

Data analysis

Calculation of oral intake of metals from soil through fruits

The daily intake of metals (DIM) from fruit was estimated following Jolly et al. [19].

$\text{DIM} = \text{daily fruit consumption} \times \text{mean fruit metal concentrations (mg day}^{-1}\text{, fresh weight)} \dots\dots\dots (1)$

The daily fruit consumption was assumed to be 300 g per person [20].

Calculation of the health risk index of metal contamination of fruits

Health risk was characterized by a Hazard Quotient (HQ) [21], the ratio between the exposure and the reference oral dose (RfD).

$\text{HQ} = \text{DIM} / \text{RfD} \dots\dots\dots (2)$

Where RfD is the oral reference dose for metal (mg day⁻¹) assuming 70 kg bodyweight. If HQ is less than 1 there will be no obvious risk. Although the HQ does not quantitatively estimate the probability of negative health effects, it provides an indication of the health risk [19].

Results and discussion

Metal concentration in soil, leaves and fruit

Results of Zn, Mn, Fe, Cu, Pb, Ni, Cu and Cd determinations in soil, leaves and fruit are shown in Table 2. The iron content was several times higher than that of other

metals, ranging from 18569,7 to 27987,7 ppm (mg kg⁻¹) in soil, 115,097 to 206,63 ppm in leaves, and 9,57 to 26,89 ppm in fruit. The maximum Fe set by FAO/WHO (1984) [22] in edible plants is 20 ppm. However, for medicinal plants WHO limits have not been established [23].

Zinc is important in protein synthesis enzymes, energy production, and in maintaining membranes. Zn concentrations ranged from 40,02–125,78 ppm in soil, 18,65–55,37 ppm in leaves, and 1,369–7,18 ppm in fruit. According to the literature data [24], soil Zn levels should not be greater than 200–300 ppm. For a majority of species, the symptoms of zinc deficiency appear with leaf concentrations of 20–30 ppm. However, all Zn levels we found were below those in literature. The FAO/WHO limit (1984) is 27,4 ppm Zn in edible plants. [22]

Mn varies from 108,92 to 162,04 ppm in soil, from 37,03 to 93,52 ppm in leaves and from 3,03 ppm (*Morus alba* L., “Mokra”) to 8,97 ppm (*Morus nigra*., “Pukovac”) in fruit. The FAO/WHO Mn limit in edible plants is 2 ppm. [22]

Copper ranges from 20,37 (*Morus alba* L., Aleksinac) to 40,0 (*Morus alba* L., Mokra) ppm in soil, 4,33–16,52 ppm in leaves, and 1,06–2,586 ppm in fruit. According to Allaway Cu in agricultural products should be between 4 and 15 ppm, and FAO/WHO (1984) recommends 3.00 ppm or less. [25, 22]

Table 2. Mean metal concentrations in soil, leaves and fruit

Location	Fruit	Sample	Fe mg/kg	Zn mg/kg	Cu mg/kg	Mn mg/kg	Ni mg/kg	Pb mg/kg	Cd mg/kg
Pukovac	Morus alba L.	Soil	20153,10±403	52,53±1,05	32,57±0,65	156,96±3,14	9,45±0,19	-	0,55±0,01
		Leaves	160,84±3,22	40,96±0,82	7,55±0,151	90,01±1,80	-	-	-
		Fruit	12,33±0,25	3,56±0,07	1,06±0,02	5,13±0,10	-	-	-
	Morus nigra L.	Soil	19895,20±397	71,12±1,42	28,65±0,53	130,25±2,61	10,36±0,21	-	0,63±0,01
		Leaves	143,87±2,88	42,37±0,85	5,69±0,11	81,59±1,63	-	-	-
		Fruit	10,36±0,21	3,56±0,07	1,52±0,03	8,96±0,18	-	-	-
Mokra	Morus alba L.	Soil	22136,50±442	81,69±1,64	40,06±0,80	125,67±2,51	6,35±0,13	4,67±0,09	0,87±0,01
		Leaves	183,82±3,68	22,72±0,45	9,47±0,19	84,71±1,69	-	-	-
		Fruit	15,82±0,32	7,18±0,14	1,90±0,04	3,03±0,06	-	-	-
	Morus nigra L.	Soil	19862,20±398	125,77±2,52	36,89±0,74	109,43±2,19	6,72±0,13	5,38±0,11	0,91±0,02
		Leaves	115,09±2,31	55,37±1,11	7,51±0,15	37,03±0,74	-	-	-
		Fruit	26,89±0,54	5,48±0,11	1,56±0,03	4,38±0,09	-	-	-
Svrljig	Morus alba L.	Soil	19985,30±399	45,78±0,92	26,21±0,52	162,04±3,25	10,25±0,20	1,00±0,02	0,39±0,01
		Leaves	120,56±2,42	26,85±0,54	6,52±0,13	93,52±1,87	-	-	0,20±0,01
		Fruit	9,57±0,19	1,37±0,03	1,14±0,02	8,55±0,17	-	-	0,12±0,01
	Morus nigra L.	Soil	18569,70±371	40,02±0,80	21,5±0,43	148,21±2,96	9,96±0,20	0,97±0,02	0,41±0,01
		Leaves	131,56±2,63	18,65±0,37	4,32±0,08	72,57±1,45	-	-	0,22±0,01
		Fruit	10,26±0,20	2,99±0,06	1,87±0,04	5,96±0,12	-	-	0,13±0,01
Aleksinac	Morus alba L.	Soil	26895,60±537	70,25±1,41	20,36±0,41	130,05±2,60	12,54±0,25	-	0,50±0,01
		Leaves	200,77±4,01	30,26±0,60	16,52±0,33	60,20±1,20	-	-	-
		Fruit	19,39±0,39	2,24±0,04	2,59±0,05	5,20±0,10	-	-	-
	Morus nigra L.	Soil	27987,70±559	56,37±1,13	22,05±0,44	108,92±2,18	11,22±0,22	-	0,52±0,01
		Leaves	206,63±4,13	28,99±0,58	15,23±0,30	44,53±0,89	-	-	-
		Fruit	23,00±0,46	2,69±0,05	1,96±0,04	4,96±0,10	-	-	-

Cadmium was 0,502–0,907 ppm in soil, 0,00–0,225 ppm in leaves and in fruit it was below the detection limit. Maobe et al. report FAO/WHO cadmium limits of 0.21 ppm in edible plants. [26] However, for medicinal herbs WHO, Canada, China and Thailand allow 0.3 ppm cadmium. Canadian cadmium limits in finished herbal products are 0.006 mg day⁻¹. [27]

Nickel was detected in soil (6,351–12,54 ppm). It was detected neither in leaves nor fruit. Maobe et al. [26] report FAO/WHO Pb limits in edible plants of 1,63 ppm.

Other authors have presented similar findings. The results are represented in Table 3.

Table 3. Contents of metal concentrations in soil, leaves and fruit from different countries

Location	Sample	Metal							References
		Fe	Zn	Cu	Mn	Pb	Cd	Ni	
Northwestern Romania	Soil			66,3 - 38,1		108–397	0,48-3,12		[25]
Mining region, Canada	Plant			2,64 -9,55		0,26-3,24	0,09-4,21		
Pakistan	Soil	447,01	1,65	115,25					[28]
	Leaves	906,81	29,53	65,78					
Mexico	Soil	245,99-324,76	17,81-93,19	4,98-71,89	111,04-7,62	2,08-16,57	0,15-1,99	3,90-23,53	[29]
	Plant	33,77-79,6	8,37-47,71	4,93-17,67	3,15-84,73	1,09-6,04	0,00-1,48	0,81-14,73	
West Algeria	Soil	3,56-74,228		0,588-35,808	15,214-9,844	0,562-85,656	0,013-5,036		[30]
	Plant	766,66-25359,6	31,13-849,74	69,87-213,63	Na-485,14	Nd-293,24			
	PUF	0,07-0,91	0,61-2,86	0,94-1,91	Nd-1,52	Nd-0,21			
Nigeria	Soil	4030-9390	21,15-731,08	4,32-15,32	79,03-244,94	7,99-39,14	1,89-10,18	7,99-39,14	[31]
	Plant	220-1690	432,15-918,89	38,14-44,97	56,52-73,72	5,42-28,47	2,24-6,15	5,42-28,47	
Dunhua, China	Soil	0,27-0,63	0,30-0,40	0,34-0,91		0,10-0,53	0,32-0,84	0,21-0,42	[32]
	Leaves	0,02-0,32	0,02-0,06	0,06-0,49		Nd-0,07	0,02-0,43	0,01-0,02	
Turkey	Soil					18,57-110,82	0,9-1,76		[33]
	Leaves					0,209	1,133		
Iraq	Soil		295,4-330,7	184,7-217,5	436,8-527,3	76,6-500,2	16,96-6,45	1,73-4,24	[34]
	Leaves		18,23,88	6,38-8,26	234-354,4	0,86-1,28	0,92-2,02	0,03-0,06	
	Plant		5,97-8,74	2,12-3,14	74,1-127,2	0,28-0,47	0,30-0,75	0,01-0,02	
Iraq	Soil	0.003-26%				29-183	105-208	11-41	[35]
	Leaves	56316 ppm				20-47	27-475	11-20	
	Plant	75-711				21-48	30-127	10-22	

Soil metals in northwestern Romania vary from 66,3 to 238,1 ppm (Cu), 108–397 ppm (Pb), and 0,48 to 3,12 ppm (Cd). Plant metal concentrations from this area are 2,64 to 19,55 ppm (Cu), 0,26 to 3,24 ppm (Pb) and 0,09 to 4,21 ppm (Cd) [25].

Soil metals uptake to leaves and fruit

Plant uptake of trace metals depends on soil pH, soil organics, and soil type, but the metals concentration in

the soil is the dominant factor [36]. The relationships among metals concentrations in soil, foliage, and fruit may be summarized by species-specific plant uptake factors (PUF) which measure the efficiency of metal accumulation [37]. They are defined by:

$PUF_{sf} = \text{fruit concentration} / \text{soil concentration}$

$PUF_{sl} = \text{leaf concentration} / \text{soil concentration}$

$PUF_{lf} = \text{fruit concentration} / \text{leaf concentration}$

These factors are shown in Table 4.

Table 4. PUF values

Location	Sample	PUF	Fe	Zn	Cu	Mn	Ni	Pb	Cd
Pukovac	Morus alba L.	Soil/Leaves	0,008	0,7797	0,2318	0,5734	-	-	-
		Soil/Fruit	$6,11 \cdot 10^{-4}$	0,0678	0,0324	0,0327	-	-	-
		Leaves/Fruit	0,076	0,087	0,1399	0,1399	-	-	-
	Morus nigra L.	Soil/Leaves	0,0072	0,5958	0,1988	0,6264	-	-	-
		Soil/Fruit	$5,21 \cdot 10^{-4}$	0,0500	0,0531	0,0688	-	-	-
		Leaves/Fruit	0,072	0,0839	0,2670	0,1099	-	-	-
Mokra	Morus alba L.	Soil/Leaves	0,0083	0,2781	0,2365	0,6740	-	-	-
		Soil/Fruit	$7,15 \cdot 10^{-4}$	0,0879	0,0475	0,0241	-	-	-
		Leaves/Fruit	0,0860	0,3160	0,2001	0,0356	-	-	-
	Morus nigra L.	Soil/Leaves	0,0058	0,4402	0,2037	0,3384	-	-	-
		Soil/Fruit	0,0013	0,0435	0,0423	0,0401	-	-	-
		Leaves/Fruit	0,0794	0,0989	0,2080	0,1184	-	-	-
Svrlijig	Morus alba L.	Soil/Leaves	0,0060	0,5865	0,2488	0,5772	-	-	0,5128
		Soil/Fruit	$4,79 \cdot 10^{-4}$	0,0300	0,0436	0,0528	-	-	0,3211
		Leaves/Fruit	0,0794	0,051	0,1751	0,0915	-	-	0,6262
	Morus nigra L.	Soil/Leaves	0,0071	0,4661	0,2010	0,4896	-	-	0,5420
		Soil/Fruit	$5,52 \cdot 10^{-4}$	0,0746	0,0869	0,0402	-	-	0,3289
		Leaves/Fruit	0,0779	0,1601	0,4323	0,0789	-	-	0,6067
Aleksinac	Morus alba L.	Soil/Leaves	0,0074	0,4308	0,8112	0,4629	-	-	-
		Soil/Fruit	$7,2 \cdot 10^{-4}$	0,0318	0,1269	0,0400	-	-	-
		Leaves/Fruit	0,0965	0,0740	0,1565	0,0864	-	-	-
	Morus nigra L.	Soil/Leaves	0,0074	0,5144	0,6903	0,4088	-	-	-
		Soil/Fruit	$8,2 \cdot 10^{-4}$	0,0478	0,8881	0,0455	-	-	-
		Leaves/Fruit	0,1113	0,0931	0,1286	0,1113	-	-	-

Cd, Cu and Zn are more efficiently concentrated in leaves, while Mn and Fe are less so. Cu, Fe and Zn accumulate more in fruit than in leaves, Mn and Cd much less so. Fe is deposited mostly in fruit.

Daily intake of metals from mixed fruit

Table 5. shows the approximate daily intake of metals

from mixed fruit. These were calculated from the average of the fruit metals contents (Table 2), assuming a daily fruit consumption of 300 g [20]. As fruit is mostly water, and the analyses are reported on a dry weight basis, these estimates of the metal intake tend to be high. The metals intake is thus within recommended limits of various agencies [38-41].

Table 5. Estimated daily intake of metal (DIM) and potential health hazards (HQ) from fruit

Metal	Average fruit concentration (mg/kg)	Daily intake (mg/kg)	R _d (mg/day)	HQ	References
Zn	36,335	10,9005	15,00	0,728	[19]
Mn	5,7725	1,7317	0,5-5,0	0,346	[19]
Fe	159,522	47,86	10,0-60,0	0,798	[19]
Pb	0	0	0,245	0	[42]
Ni	0	0	1,400	0	[43]
Cu	1,7001	0,5100	2,0-3,0	0,17	[19]
Cd	0,1302	0,0391	0,070	0,643	[43]

Potential health hazards (HQ)

The hazard quotients (HQ) represented in Table 5., for Zn, Mn, Fe, Pb, Ni, Cu and Cd were 0,728; 0,346; 0,798; 0; 0; 0,17 and 0,643 decreasing Fe>Zn>Cd>Mn>Cu>Pb=Ni. The HQ value for all the elements were below 1, suggesting that fruit consumption is safe.

Conclusion

Fe, Zn, Mn, Cu and Cd were determined in soil, leaves and fruit of mulberry (*Morus nigra* L. and *Morus alba* L.) from southeast Serbia. Pb and Ni were found in soil but not in leaves or fruit. The ratios among metals concentrations in soil, leaves and fruit were calculated. Strong correlations among the concentrations in soil, leaves and fruit were found.

The metal contents of *Morus nigra* L. and *Morus alba* L. pose no hazard to human health.

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Izvod

BIOAKUMULACIJA METALA U RAZLIČITIM VRSTAMA DUDA

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Atomsko absorpcionom spektroskopijom određena je koncentracija Zn, Mn, Fe, Ni, Cu i Cd u uzorcima zemljišta, lišća i plodovima dudu (*Morus alba* L. and *Morus nigra* L.) sa područja jugoistočne Srbije. Izračunata je translokacija metala iz zemljišta u lišće i plodove, procenjen dnevni unos metala i indeks zdravstvenog rizika. Takođe, utvrđena je i pozitivna korelacija između metala u zemljištu, lišću i plodovima.

Ključne reči: dud, metali, zemljište, lišće, plod