

FREE-LIVING RODENTS AS MONITORS OF ENVIRONMENTAL CONTAMINANTS AT A POLLUTED MINING DUMP AREA

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Abstract: Several toxicological studies have shown that some rodents, due to their feeding habits, easily accumulate high amounts of pollutants in relation to other mammal species. Their limited living space significantly reflects the contamination level of their biotope. The article presents the results of 142 rodent tissues biomonitoring from the area of mining dumps of various ages at the locality Banská Štiavnica (Central Slovak Neovolcanites). The investigation was realized in summer and in autumn. The contaminations of Fe, Mn, Cu, Zn, Pb, Cd and Bi were determined in liver, kidney and spleen tissues of three dominant rodents: *Apodemus flavicollis*, *Micotus arvalis* and *Clethrionomys glareolus*. There was discovered a close connection among heavy metal contents in the soils, food and organs of the small mammals in livers, spleen and kidneys. *Apodemus flavicollis* seems to be the most suitable species from the viewpoint of geotoxicity monitoring because of its number at all studied sites and because of notable differences between the pollution and control sites.

Keywords: dumps, tailings, heavy metals, toxic elements, rodents, liver, kidney, spleen

1. INTRODUCTION

Natural and anthropogenic activities are sources of heavy metals and other pollutants in the environment. The locality Štiavnické Bane - Lúky pod Tonádom is contaminated predominantly by mining and smelting activities. The land is considerably changed by historical exploitation of Au-Ag-polymetallic ores from the

14. century to 19. century. The phases of mining activities are well documented (Sombathy 1993). They finished in 1903. The following liquidation and reconstruction works lasted till 1910 and the part of them was also the forestation of the greatest dumps (Fig.1). Heavy metal residues in soil influence the vegetation and the fauna.

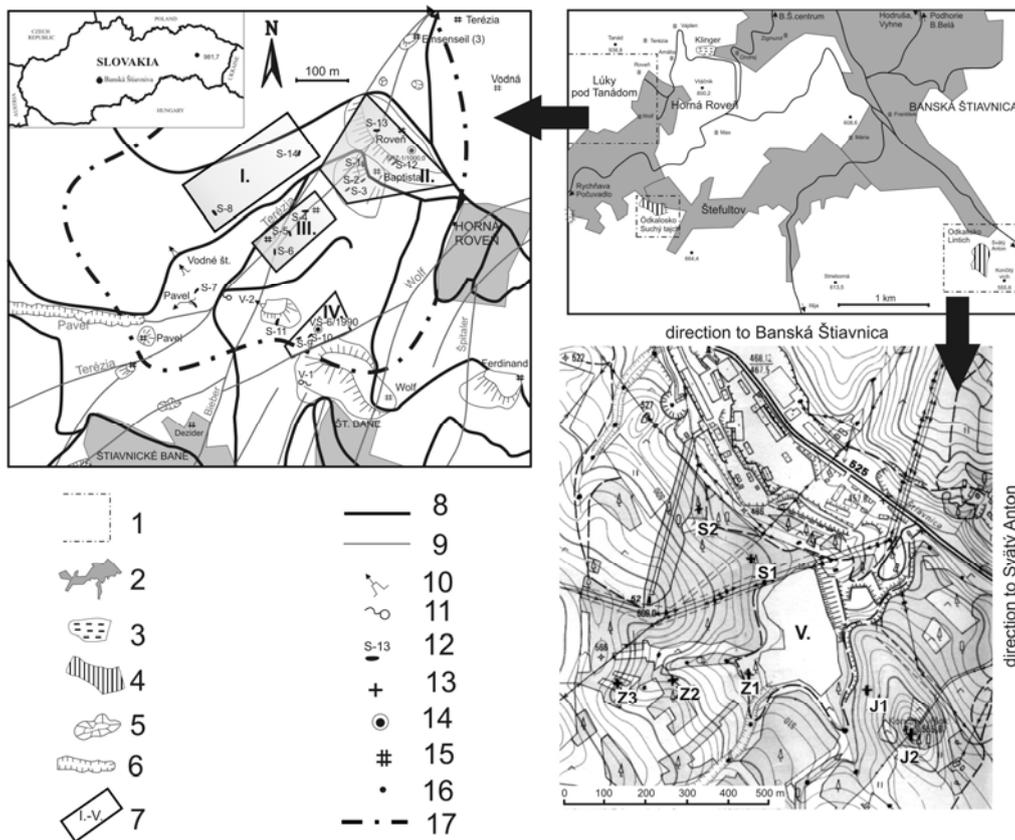


Fig. 1 - Scheme of investigated localities, sites and objects.

1 – investigated localities, 2 – village area, 3 – Klinger water reservoir, 4 – setting pit, 5 – dump, 6 – surface quarry, 7 – site (investigated area within locality), 8 – border of the microwatershed, 9 – route, 10 – ore vein, 11 – adit collar, 12 – spring, 13 – sonder, 14 – point of ecologically transported dust sampling, 15 – structural bore, 16 – shaft collar, 17 – borders of the watershed

During last several decades many investigations have been realized to reveal heavy metal contamination in free-living rodents. These field and laboratory studies have shown that high levels of heavy metals in the environment may appropriately be monitored by the assessment of their concentrations in target organs of rodents. The increased levels have usually been considered to relate to environmental pollution, and significant relationships were found between metal content in soil and body or tissues.

2. THE CHARACTERIZATION OF THE STUDIED SUBJECTS

The field research was performed in five areas (I – V) in 1999. At the reference area (site I) the soil is developed directly at the mechanically mounded pyroxenic andesite of the Tanadian complex. At the dump surface of the shaft Roveň (site II) the main component is the silicious-diorite porphyry from the mentioned shaft. At the surface of the dump is the layer of limnic sediment. The representative of old dumps from the 17. and 18. centuries is the dump Babčo (site III) created by various rocks, materials of gangues with the dominant abundance of timazite with the considerable proportion of vein-quartz and oxidized ore-filling. The oldest dumps from the 14. and 17. centuries (site III) have the various material compositions. The crystalline vein-quartz or material from argillitized zone dominate in some of them. In the drainage endorheic area of depression under the root of dump of the shaft Wolf (site IV) the cultural layer is thick 1 - 5 m and there can be differentiate 3 technogenous horizons. Each of them contains the cuts of the technical ceramics and the wood-coals. Additional was studied the settling pit Lintich (site V).

The soils developed on the heaps (Danáková and Vlčková, 1996) Typical soil on dumps ANm^b with good developed horizon Bv, contain the substratum mixture which consists of the vein-quartz with the addition of hydrothermal altered andesite and sulphide ore cuts. Bv horizon reaches the depth 25 – 30 cm and is humid, with the inexpressive polyedric structure and approximately 40% content of skeleton. Horizon C consists of mixture of quartz and illite (Križáni and Andráš 2005).

The studied vegetation at the heaps tends to the association *Arrhenatheretum elatioris* (Braun–Blaquet, 1991). The portion of synantrophic species does not change in later stages of development of vegetation but the portion of meadow species of the class *Molinio Arrhenatheretea* (Tüxen, 1937) increases. Out-washing of metals released from the dumps of mining-processing wastes in the form of colloids, ion solutions and organo-complexes causes their availability to vegetation (Šimko 1992; Šimko and Kačmár 1992). The change of plants specific spectrum occurs in consequence of soil-loading by heavy metals whereby the composition and the covering by vegetation at the dumps and soils not affecting by mining activity are markedly different. Dump-positions are inhabited mainly by competitive weak plant species. These are able to adapt to hindered conditions at the positions (Križáni et al. 2004)..

3. MATERIALS AND METHODS

The trapping of rodents was realized at the areas demarked near the probes excavated in the area of sites I - IV. The additional catching was realized at the settling pit Lintich (site V) in autumn.

Free-living rodents were trapped in summer and autumn 1999. The trapping was realized by bascule-trap with the wick and the mixture of edible oil and nuts bait was used. The trapping of rodents, their specific and sexual determination, internal organs taking, and also their preparing for analysis were realized at the Zoological Institute of the Slovak Academy of Sciences in Košice. For analysis of small mammals tissues were prepared cumulative charges, i.e. organs from the several specimens of concrete

species and genders were connected into samples with minimum 3g of tissue. These samples of internal tissues (liver, kidney and spleen) were after mineralization analyzed for content of Fe, Mn, Cu, Zn, Pb, Cd, and Bi in the laboratory of the Geological Institute of the Slovak Academy of Sciences in Banská Bystrica.

4. RESULTS AND DISCUSSION

The possibility of rodents utilization as the indicator of loading depends on their stressor sensibility and on extent of their reaction in time and area. The group of small mammals is the suitable modeling group for monitoring of environment toxicity (Talmage and Walton, 1991; Jančová 200; Ieradi et al. 2003). They have adequate length of the life which enable to manifest the influence of malignant agents. Their metabolism is intensive. This feature cause that the influence of the pollutants is identifiable even if its concentrations are very low. In connection with the quick diversification of generations it is possible follow the changes of the genetic material (Shore and Douben 1994).

The comparative study on value of pollutant concentrations in tissues of various body organs is often used method. The small mammals present very suitable group for monitoring from the view of their short-life (several months till year). Their living space is small (maximum 1 – 2 hectares), so they enable to monitor the particular site.

At all sites the dominant species are *Apodemus flavicollis* (53.9%, thereafter *AFLA*), *Microtus arvalis* (24.5%, thereafter *MARV*) and *Clethrionomys glareolus* (18.2%, thereafter *CGLA*). Species *Microtus (Pytymys) subterraneus* (*PSUB*) and the only representative of insectivorous animals *Crocidura suaveolens* have in populations the minor abundance (tab. 1).

Tab. 1 Species and numbers of little mammals from localities I – V

Species	Localities					n	%
	I	II	III	IV	V		
<i>Apodemus flavicollis</i>	23	11	26	17	-	77	53.9
<i>Clethrionomys glareolus</i>	22	1	1	2	-	26	18.2
<i>Microtus arvalis</i>	1	11	11	2	10	35	24.5
<i>Microtus (Pytymys) subterraneus</i>	2	-	1	-	-	3	2.1
<i>Crocidura suaveolens</i>	1	1	-	-	-	2	1.4
Total	48	24	40	21	10	143	100.1

Explanations:

I - reference area, II - dumps from 18. to 20. century, III - little dumps from 14. to 17 century, IV - outfallless water basin at the northern slope of the Wolf dump, V - setting pit Lintich

Determined spectrum and dominance of listed rodents are typical in the studied area (Štollmann and Dudich, 1988). Dump biotopes differ in dominant species of small mammals depending up their demand on vegetation cover. The plant-part predominates in food of studied species; the seeds create the significant part of food of *AFLA* and the vegetation parts of plants predominate in food of common vole and *CGLA*. It is known,

the reproducing organs of plants (seeds) show the considerable degree of resistance towards the contamination by heavy metals and other pollutants.

Tab. 2 Average contents of selected elements (mg.kg⁻¹) in free-living rodents tissues from summer sample set from sites I – IV

Element	Site	AFLA			MARV			CGLA		
		Liver	Kidney	Spleen	Liver	Kidney	Spleen	Liver	Kidney	Spleen
Fe	I	639,0	2,0	1,0				4,5	118,0	1,0
	II	53,0	146,0	1,0	1248,0	163,0	22,0			
	III	720,0	3028,0	1,0	1,0	1,0	8,0			
	IV	458,0	1,0	1,0				1837,0	240,0	952,0
Mn	I	9,5	1,0	0,1				13,0	0,1	11,0
	II	6,7	0,1	1,0	9,0	0,1	0,1			
	III	17,3	26,0	3,0	3,0	0,1	0,1			
	IV	11,3	1,0	1,0				47,0	0,1	0,1
Cu	I	5,9	1,0	0,1				12,0	4,0	0,1
	II	10,8	1,0	0,1	11,0	20,0			4,0	0,1
	III	9,4	21,3	0,1	4,0	2,0	0,1			
	IV	12,0	3,0	1,0				14,0	3,0	1371,0
Zn	I	25,5	2,0	21,0				34,0	24,0	30,0
	II	26,0	15,0	21,0	21,0	28,0	15,0			
	III	34,3	33,0	20,0	27,0	17,0	24,0			
	IV	35,0	1,0	19,0				24,0	29,0	61,0
Pb	I	15,0	0,1	0,1				4,0	7,0	3,0
	II	20,4	6,0	0,1	9,0	39,0	0,1			
	III	40,0	30,0	0,1	0,1	0,1	0,1			
	IV	5,4	0,1	0,1				26,0	87,0	122,0
Cd	I	1,8	1,0	2,0				1,5	1,0	0,1
	II	1,0	1,0	0,1	1,0	2,0	0,1			
	III	2,3	2,0	0,1	0,1	0,1	0,1			
	IV	1,0	0,1	0,1				1,0	2,0	5,0
Bi	I	0,1	0,1	0,1				0,1	0,1	0,1
	II	0,1	0,1	0,1	0,1	0,1	0,1			
	III	0,1	0,1	0,1	0,1	0,1	0,1			
	IV	0,1	0,1	0,1				0,1	0,1	0,1

The specific composition of plant cover underlies the dominance of different small mammal species inhabited the sites. Concentrations of heavy metals in tissues of studied species show considerable differences (tab. 2, 3). Accumulation trends of monitored metals in organ tissues of the same species differ according to season (summer – autumn) and body organs (liver, kidney, spleen).

Tab. 3 Average contents of selected elements (mg.kg⁻¹) in free-living rodents tissues from autumn sample set from sites I – V

Element	Site	AFLA			MARV			CGLA		
		Liver	Kidney	Spleen	Liver	Kidney	Spleen	Liver	Kidney	Spleen
Fe	I	302,0	80,0	559,0				334,0	118,0	241,0
	II	207,0	108,0	281,0	225,0	123,0	208,0			
	III	165,0	195,0	377,0	228,0	124,0	1,0			
	IV	203,0	101,0	672,0	297,0	1,0	454,0			
	V				282,	75,0	216,0			
Mn	I	7,3	2,6	4,0				6,0	4,0	0,1
	II	4,3	6,5	3,0	3,7	6,0	0,1			
	III	2,0	6,0	18,0	7,0	9,0	26,0			
	IV	4,4	5,0	21,0	12,0	0,1	0,1			
	V				3,4	6,0	115,0			
Cu	I	10,3	13,0	0,1				8,0	9,0	6,0
	II	8,2	18,0	0,1	1,4	25,0	0,1			
	III	9,0	0,1	0,1	1,4	10,0	75,0			
	IV	9,0	188,0	37,0	8,0	0,1	30,0			
	V				7,4	4,0	41,0			
Zn	I	271,0	305,0	477,0				281,0	284,0	275,0
	II	389,0	123,0	39,0	155,0	641,0	248,0			
	III	365,0	35,0	357,0	187,0	528,0	0,1			
	IV	266,0	309,0	570,0	750,0	575,0	0,1			
	V				177,0	12,0	42,0			
Pb	I	0,1	11,1	0,1				0,1	0,1	0,1
	II	0,1	0,1	0,1	0,1	3,0	0,1			
	III	0,1	0,1	0,1	0,1	40,0	89,0			
	IV	0,1	27,0	0,1	0,1	0,1	0,1			
	V				0,1	0,1	229,0			
Cd	I	3,0	1,1	1,0				3,0	1,5	1,0
	II	2,7	0,1	2,1	9,3	2,0	1,0			
	III	0,1	6,0	20,0	3,7	0,1	4,0			
	IV	7,7	0,1	2,0	8,0	6,0	36,0			
	V				4,3	1,0	8,0			
Bi	I	87,6	110,0	0,1				5,5	73,0	45,0
	II	51,0	119,0	15,0	0,1	164,0	35,0			
	III	50,0	18,0	177,0	9,1	203,0	44,0			
	IV	7,4	134,0	0,1	0,1	256,0	0,1			
	V				18,4	87,0	41,0			

Total 84 samples of livers, kidney and spleens were prepared for analysis from the population of above mentioned species. 34 of them were from the summer sampling and 50 of them from the autumn sampling. The average contents of selected

elements in summer set of tissues of species *AFLA*, *MARV* and *CGLA* from the sites I – IV are listed in tab. 2 and from the autumn set from the sites I – V in tab. 3.

Tab. 4 Cummulation coefficients ($K = xP : xT$) calculated from average contents of selected elements in soil (xP) from investigated localities (I - V) and average contents of the same elements in liver tissues of small mammals for summer and autumn sample sets

Element	Site	K = xP : xT					
		Summer			Autumn		
		<i>AFLA</i>	<i>MARV</i>	<i>CGLA</i>	<i>AFLA</i>	<i>MARV</i>	<i>CGLA</i>
Fe	I	63,8		9 067,0	135,0		122,0
	II	533	23,5		142,0	130,0	
	III	35,3	25 397,0		154,0	111,0	
	IV	91,4		22,8	206,0	141,0	
	V					112,0	
Mn	I	115,0		84,3	150,0		183,0
	II	520,0	387,0		310,0	942,0	
	III	127,0	730,0		1 095,0	313,0	
	IV	272,0		65,3	698,0	256,0	
	V					903,0	
Cu	I	3,1		1,5	1,8		2,3
	II	15,4	14,4		19,3	113,0	
	III	37,4	87,8		9,0	251,0	
	IV	3,8		3,2	5,0	5,6	
	V					62,4	
Zn	I	3,1		2,3	0,3		0,3
	II	15,1	18,7		1,0	2,5	
	III	21,4	27,2		2,0	3,9	
	IV	10,5		15,3	1,4	0,5	
	V					20,1	
Pb	I	5,7		21,3	853,0		853,0
	II	21,4	48,4		4 360,0	4 360,0	
	III	13,5	5 393,0		5 393,0	5 393,0	
	IV	122,0		2,3	6 580,0	6 580,0	
	V					16 640,0	
Cd	I	2,9		3,5	1,7		1,7
	II	4,0	4,0		1,5	0,4	
	III	2,7	61,0		61	1,7	
	IV	5,4		5,4	0,7	1,6	
	V					8,9	

The cummulation coefficients are suitable to compare the mobility of elements. They express using one number (without regard to absolute values of the element concentrations) the ratio of selected element in landscape components (content of the element in soil to its content in animal tissues – tab. 4, or ratio between selected

elements in animal tissues from summer and autumn set – tab. 5). The graphic representation in differences of tissues' contamination of livers, kidney and spleens of *AFLA* species at sites between the summer and autumn set of analyzed samples (Fig. 2) shows, that between the contamination degree of studied organs from the site I - IV is obvious similarity. Sites at younger (II) and older (III) dumps differ by proportional contamination of analyzed organs. The contents of individual elements at younger dumps from the summer and autumn set intersect and at the older dumps there is a differentiation between the summer and autumn set (III). It is similar to differentiation at the sites I and IV (Fig. 2).

Tab. 5 Cummulation coefficients ($K = S : A$) calculated from average contents of selected elements in tissues of *Apodemus flavicollis* in summer (S) and autumn (A) sample sets from localities I – IV

Tissue	Site	K = S : A						
		Fe	Mn	Cu	Zn	Pb	Cd	Bi
Liver	I	2,102	1,301	0,57	0,091	150,001	0,602	0,001
	II	0,261	1,562	1,26	0,072	204,002	0,371	0,002
	III	4,363	8,652	1,04	0,942	400,011	23,003	0,002
	IV	2,261	2,572	1,32	0,132	54,002	0,132	0,014
Kidney	I	0,032	0,381	0,08	0,007	0,009	0,911	0,001
	II	1,352	0,015	0,06	0,122	60,002	10,004	0,001
	III	15,531	4,332	210,03	0,941	300,011	0,302	0,006
	IV	0,011	0,203	0,02	0,003	0,004	1,011	0,001
Spleen	I	0,002	0,025	1,01	0,042	1,002	2,011	1,021
	II	0,004	0,302	1,03	0,541	1,021	0,052	0,007
	III	0,003	0,172	1,02	0,943	1,002	0,005	0,001
	IV	0,001	0,051	0,03	0,032	1,003	0,052	1,002

The evident insufficiency of bioaccumulation study is the fact, that the presence of chemical agents in tissues does not document the toxic effect. Opinions on effects of pollutants in higher concentrations on physiological or genetic changes are not united. The important role has the exposure time - the period while the animal is exposed to the activity of stressors. If the organs of short-living organisms are markedly contaminated, then it is very probable, that at animals with longer lifetime will the contamination of internal organs proportionally rise.

Cation Fe^{3+} predominates only in markedly acid environment (e.g. in acid mine drainage waters). In sediments of studied settling pits were determined hexa-hydrides of Fe and Mn crystallizing from the porous solutions at the end of capillaries. Mn in waters is present in various oxidation state, in soluble and insoluble form. In neutral until acid environment the simple hydrated cation $[Mn(H_2O)_6]^{2+}$ predominates from the soluble species (Pitter 1990).

In natural, mine and dump drainage waters Cu is in soluble form presented in forms of simple hydrated ion Cu^{2+} , carbonated complexes $[CuCO_3(aq)]^0$, $[Cu(CO_3)_2]_2-$ and hydro-complexes $[CuOH]$, $[Cu(OH)_2(aq)]^0$, $[Cu(OH)]^{3-}$ a $[Cu(OH)_4]^{2-}$. Cu is an essential element, which containing many metallo-enzymes and influencing the

heamatogenesis. It is the basic element by synthesis of hemoglobin and oxidative enzymes (Talmage and Walton, 1991; Sample et al. 1998).

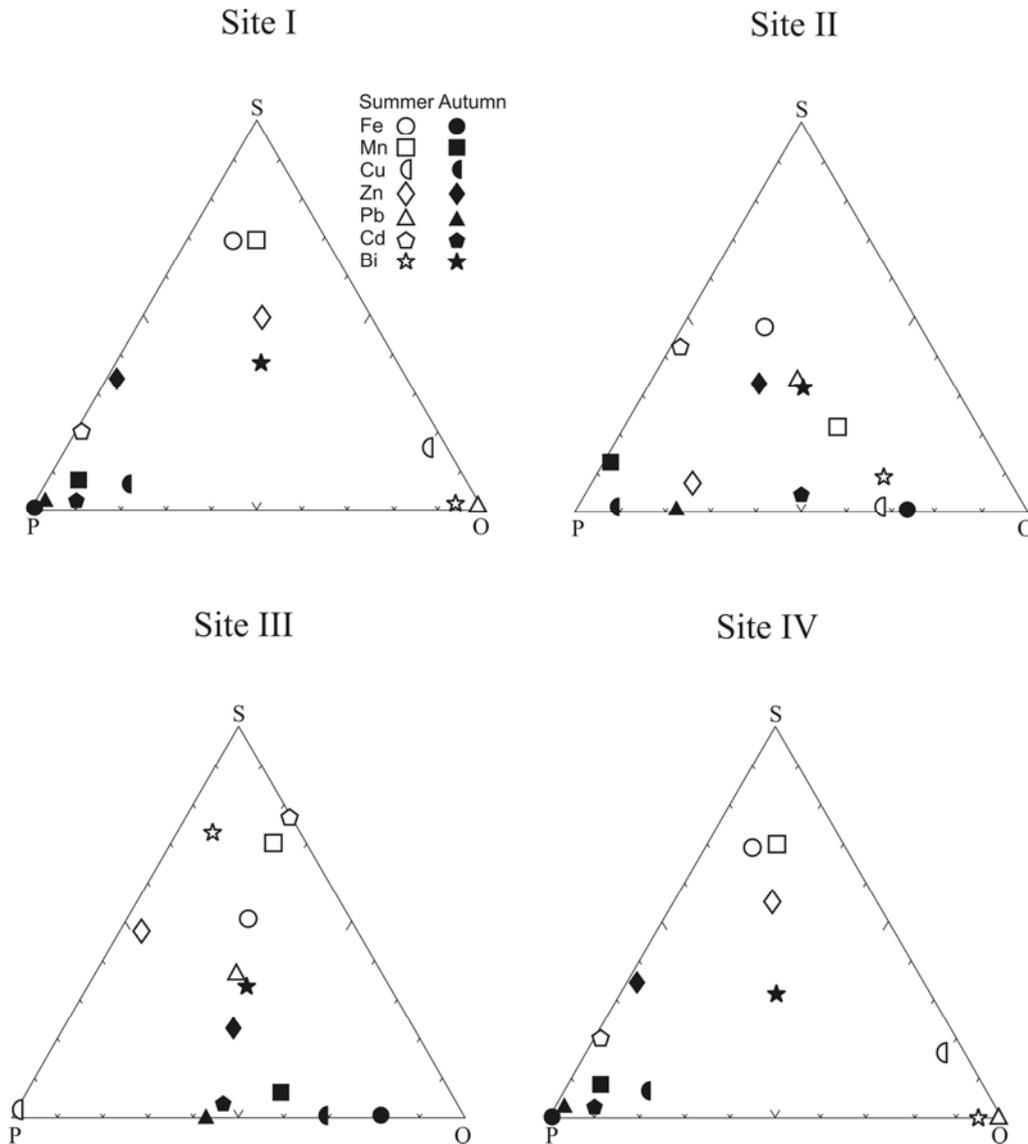


Fig. 2 – Triangular plots of rate concentrations of selected elements in tissues of livers (P), kidneys (O) and spleens (S) of *Apodemus flavicolis* species from sites I – IV.

The lack of Cu in organism causes the pathologic changes. It limits the transport of Fe ions and causing anemia although there is enough Fe in organism. The redundancy of Cu in organism is toxic, blocks membrane process. Cu overly secretes in urine and accumulates mainly in liver, kidney, bone marrow, cerebrum, cornea, and

in hair and can cause the degenerative changes. Intoxication can cause the damage of liver and kidney and also death (Melicherčík and Melicherčíková, 1997).

Zn^{2+} in waters with high concentration of sulphates can be presented as $[ZnSO_4(aq)]^0$. Long-time and excessive Zn intake is toxic. Besides the reduced re-sorption of phosphates there are presented the symptoms of anemia and other gastrointestinal symptoms (Pitter 1990; Uthe et al. 1979).

Pb^{2+} predominates among bio-available forms of Pb in hydrated compound $[PbCO_3(aq)]^0$. In alkaline area can be presented in higher concentrations also the complexes $[Pb(CO_3)_2]^{2-}$, $[Pb(OH)_2(aq)]^0$ a $[PbOH]^+$ (Melicherčík and Melicherčíková, 1997). Pb effects mainly on red-blood-pigment and blood corpuscles. It endangers the system nervous, musculature and blood vessels. The toxic effect it has also on digestive system, kidney and on ductless glands. Uthe et al. (1979) discovered that Pb is accumulating in liver and kidney of animals.

Cd and Bi belong to high toxic elements and have the tendency to accumulate in organisms where are binding in plasma to proteins (Virčíková and Pálffy, 1997). They accumulate mainly in kidney, spleen, genitals and liver. In contrast to Bi, which is geochemical related to Pb, Cd is related to Zn, but is more mobile than Zn mainly in acid environment. In solutions Cd is presented in forms of simple hydrated ion Cd^{2+} . So it becomes bio-available to plants. Cd contents in animal organisms decrease also in case of Ca deficit. It accumulates mainly in bones, liver and kidneys, at least in musculature and cerebrum (Kminiak, 1994; Pitter 1990). Higher contents of Cd were determined at predacious species from family *Insectivora*. This species was in our set represented only by 3 individuals. The differences of Cd contents in liver can be credit to differences in metabolism or in physiological anomalies of individuals (Kováčiková and Reichrtová, 1986). Talmage and Walton (1991) and Cherian and Vostal (1988) stated, the influence of contamination of main food components on overall Cd concentration in tissues of two species of rodents *Microtus agrestis* and *Apodemus sylvaticus*. *Microtus agrestis* consumes mainly herbs and grasses and *Apodemus sylvaticus* has the main component of food the fruges and seeds containing lower Cd concentrations as herbs and grasses. When the Cd concentration in liver reaches the threshold value it is secreted in urine connected to glutathione (Cherian and Vostal, 1988) but mainly to stools.

5. CONCLUSIONS

The herbivorous animals predominate in specific composition of small mammals inhabited the areas of dumps positions. Between herbivorous animals are presented the species those the main food component are the vegetation organs of plants and seeds. Higher contamination by heavy metals and toxic elements of analyzed tissues of internal organs was determined at animals consuming in preference vegetation organs of plants. Animals consuming mainly the seeds show lower contamination by heavy metals. The different contamination of internal tissues was shown at individual monitored elements from the summer and autumn sets of studied rodents within the individual sites and between the positions.

The dominant number position of *AFLA* compared to *CGLA* and *MARV* and the differences between the heavy metal contents in its tissues from polluted and control sites determine the former species a most suitable indicator of environmental pollution and genotoxic damage in the area. Similar conclusion was proposed by Abramsson-Zetterberg et al. (1997).

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