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Microtus guentheri (Danford & Alston) (Rodentia, Mammalia) : a bioindicator species for estimation of the influence of polymetal dust emissions

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ABSTRACT. Chemical pollution of ecosystems resulting from human activity is an ecological factor in the living world, affecting individuals, populations, communities and ecosystems as a whole. This laboratory investigation studied the toxic effects of polymetal ferosilicic dust on *Microtus guentheri* (Rodentia, Mammalia), measuring pathological changes in blood components (haemoglobin, erythrocyte, leucocyte and platelets number, erythrocyte sedimentation rate (ESR) and white cell count). Biochemical indices were established for levels of the following blood components : blood sugar, albumin, uric acid, triglycerides, cholesterol, total protein, creatinine, calcium and inorganic phosphorus. The results obtained indicate that *Microtus guentheri* can be used as a species for evaluation of the influence of polymetal dust emission.

KEY WORDS : *Microtus guentheri*, industrial polymetal dust, bioindication, biochemical and haematological indices

INTRODUCTION

Chemical pollution of ecosystems resulting from human activity and having an influence on individuals, populations, communities and the ecosystem as a whole is an ecological factor for wildlife. Detailed analysis of the entry of various such pollutants into the environment, their concentration in selected organs and their role in development and vitality of organisms is necessary for revealing the effects of chemical pollution.

Recently, dust pollution has increased, reaching its highest values in the Northern hemisphere. During the past 20 years the atmosphere has been polluted with 240,000 t of ferosilicic dust. In the atmosphere of Sofia, Bulgaria, the current amount of polymetal microsilicic dust emissions is 0.75 – 1.0 mg/ m^3 (FeSi-45%, Fe-3,11%, Pb–2, 03%, Mn-0,25%, Cd-0,57%, MgO-1,07%, SiO-7,65%, AlO-1,76%, KO-2,60%, NaO-0,85%) (EEABG, 2002). Iron, magnesium and manganese are microelements that are absolutely necessary for hemoglobin synthesis and enzyme activity, while cadmium, lead and aluminum are not nutritionally essential elements for animals. Exposure to acutely high, or chronically low levels may induce intracellular production of metallothionein, which has an important function as a store in zinc metabolism (CHAKRABORTY et al., 1987, FERNANDO et al., 1989). The high accumulation of cadmium can lead to food chain amplification (increases in concentration in animals at each step in the food chain), because metallothionein –bound cadmium has a long biological half-life in animals and because concentrations tend to increase with age (VERMEER & CASTILA, 1991).

The main aim of this study was to investigate the heavy metal accumulation and distribution in different organs, and the influence of polymetal microsilicic dust on some basic haematological and biochemical indices in the peripheral blood of the Guenther's vole, *Microtus guentheri* (Danford & Alston) (Rodentia, Mammalia) and to determine indices important for biological monitoring.

MATERIAL AND METHODS

Polymetal microsilicic dust emission is a waste product from iron-bearing alloy production of metallurgical works. The present laboratory eco-toxicological experiment assessed its effect on *Microtus guentheri* (Danford & Alston), which is a convenient subject for such investigations because of its high reproductive potential. Being herbivorous, it is also an important link in the trophic chain, and its stationary way of life determines the permanent influence of different pollutants on its food base.

The toxic effect of the polymetal dust was studied under experimental subchronic conditions. Eighty-five individuals were divided into one control (15 individuals) and two test groups (Group I and Group II with 35 individuals each). In order to best approximate natural conditions, the microsilicic dust was given through food in two concentrations : Group I at 5% and Group II at 10% of food quantity. Samples for analysis were taken at 30, 60 and 90 days for all groups.

The bioaccumulation and distribution of Cu, Pb, Zn, Cd, Mn and Fe in different organs and tissues were examined using an atomic absorption spectrometer (Perkin Elmer). The pathological changes caused by the toxicant to the haemoglobin content, erythrocyte, leucocyte and platelet numbers, erythrocyte sedimentation rate (ESR) and white cell count were measured using an automatic hematological counter (HC-333). The following biochemical indices were studied : blood sugar, albumin, uric acid, triglycerides, cholesterol, total protein, creatinine,

calcium and inorganic phosphorus levels using the biochemical analyzer Technicon RA-1000.

RESULTS AND DISCUSSION

The concentrations of the most toxic heavy metals are presented in Table 1. The elements considered were very unevenly distributed. It seems important to recognize the pattern of distribution because this may allow an estimate of the impact on organisms, as well as give an insight into possible detoxification mechanisms. The animal's organs differed from one another in respect to heavy metal concentration. The most sensitive organs were the kidneys, where the accumulated levels of lead and cadmium were the highest (29 mg/kg Pb and 32 mg/kg Cd for Group I on the 90th day and 40 mg/kg Pb and 125 mg/kg Cd for Group II on the 90th day), and the differences in cadmium and lead concentrations between experimental and control groups are statistically significant ($t=4.36$; $p < 0{,}0001$).

TABLE 1

Heavy metals concentrations in the whole body and in different organs of *M. guentheri* in mg/kg dry weight

		Control				30th Day				60th Day				90th Day			
	N	Fe	Mn	Pb	Cd	Fe	Mn	Pb	Cd	Fe	Mn	Pb	Cd	Fe	Mn	Pb	Cd
Whole body																	
X±	10	203.4	2.6	1.7	1.4												
Sd		48.1	0.5	0.5	0.4												
Group I																	
X±	10					217.2	2.4	1.9	3.8	257.8	1.5	4.0	2.8	281.3	1.4	3.2	2.1
Sd						40.6	1.6	0.6	1.8	76.1	0.6	1.5	1.9	24.8	1.1	1.3	0.5
Group II																	
X±	8					240.0	3.5	7.4	3.9	265.3	3.0	8.3	3.1	320.8	4.0	5.8	4.6
Sd						63.8	0.4	4.3	4.3	118.0	0.5	3.0	0.5	21.0	1.4	3.2	1.6
Kidneys																	
X±	10	479.7	3.9	7.5	7.6												
Sd		46.4	0.2	7.9	3.0												
Group I																	
X±	10					507.6	12.1	19.2	86.6	470.6	5.4	22.4	53.9	659.6	14.7	28.1	31.6
Sd						144.8	4.9	7.8	72.4	162.9	3.9	7.9	27.3	170.8	6.1	21.0	10.5
Group II																	
X±	10					439.6	10.1	28.7	107.2	389.7	12.1	44.2	152.9	683.1	12.3	40.3	125.1
Sd						97.5	2.9	15.6	45.0	43.1	2.5	18.1	54.4	213.7	5.7	16.1	20.8
Liver																	
X±	10	784.4	8.7	3.8	7.6												
Sd		31.9	2.4	1.0	2.6												
Group I																	
X±	10					1064.8	8.8	3.2	20.9	901.3	6.1	5.7	71.0	1103.2	7.2	2.8	20.1
Sd						70.9	2.3	1.9	19.6	49.8	2.7	1.9	19.3	343.2	5.6	1.6	9.7
Group II																	
X±	10					868.9	9.0	5.4	37.3	645.3	8.6	10.4	48.5	800.6	8.1	10.0	114.8
Sd						237.3	2.8	3.0	23.9	155.1	2.2	3.2	12.7	407.7	2.2	5.4	8.6

Cadmium and lead cause kidney toxicity in *M. guenthery.* The results concerning the accumulation of cadmium are very interesting. In the first variant of the experiment the bioaccumulation of this element was three times higher on the 30th day than on the 90th day, confirming data obtained by MILLS & DELGRANO (1972) and TOPASHKA-ANCHEVA et al. (1998). The high cadmium concentration on the 30th day may induce intracellular production of matallothionein, a low-molecular-weight protein rich in sulfur amino acids to which cadmium can be bound and, hence, rendered less toxic (SCHREIBER & BURGER, 2002). At the end of the experimental period a tendency towards compensation of the toxic effect occurred but it was not entirely effective.

From an ecological point of view, the data obtained for the total bioaccumulation of these heavy metals in the body of the Guenter's vole are very important because they show an average assessment of the organism's intoxication – a fact that could be used for prognoses in biological monitoring.

The changes in the average values of basic blood indices (Table 2) have an analogous character to that of the metal data. In both test groups there was a statistically significant reduction of the average values on the 30th day (with the exception of the average number of leucocytes), followed by a significant increase on the 60th day. At the end of the experimental period a tendency towards compensation of the changes occurred but, once more, it was not entirely effective.

The average values of albumin, creatinine, cholesterol and total protein varied similarly : at the beginning of the experiment there was an initial reduction followed by evidence of a compensatory mechanism. Group II exhibited a constant increase in blood sugar and uric acid, while triglycerides showed a decrease over the experimental time period to nearly that of the control value.

The results obtained show anemic effects during the experiment, which were surmounted. On the 60th day, a dissociation of erythrocyte number and hemoglobin concentration occurred as an expression of hypochromic ane-

TABLE 2

Hematological and biochemical values in exposed and control time

Indices	Referent values n = 90	30th day n = 15		60th day n = 15		90th day n = 15	
		Group I	Group II	Group I	Group II	Group I	Group II
Hemoglobin (g/l)	161.0±9.0	123.1±5.3	136.2±4.9	160.8±2.6	149.0±8.3	169.0±9.2	178.2±6.9
Erythrocytes (10^{12}/l)	4.9±0.3	3.7±0.9	4.3±1.1	9.5±0.3	9.0±0.2	5.1±0.6	5.3±0.4
Leukocytes (10^9/l)	4.1±0.3	3.9±0.8	6.4±0.7	9.6±1.4	14.4±1.3	3.8±0.9	8.6±0.6
ESR (mm/h)	1.2±0.3	-	-	0.8±1.9	1.5±2.2	0.8±0.9	0.8±1.3
Thrombocytes (10^9/l)	268.5±9.2	198.0±7.4	212.3±8.8	430.5±9.8	456.0±8.3	268.5±9.3	316.5±9.1
Blood sugar (mmol/l)	2.6±0.1	3.6±0.4	3.2±0.2	3.7±0.4	4.0±0.2	4.6±0.2	4.2±0.3
Albumin (g/l)	43.0±0.8	-	-	40.0±0.8	33.4±0.4	42.6±0.7	44.3±0.6
Uric acide (μmol/l)	86.1±0.3	-	-	80.1±0.4	108.2±0.3	105.8±0.4	194.2±0.6
Creatinine (μmol/l)	165.2±2.5	21.2±3.5	41.3±3.3	43.0±2.9	40.3±3.1	161.2±3.9	148.4±4.1
Cholesterol (mmol/l)	2.1±0.2	1.7±0.6	1.4±0.3	2.1±0.2	2.0±0.2	2.3±0.3	2.1±0.4
Triglycerides (mmol/l)	1.2±0.3	2.7±0.3	2.9±0.2	-	-	1.7±0.4	0.9±0.5
Total protein (g/l)	82.0±1.4	71.3±1.9	72.6±1.8	80.3±1.7	74.2±1.9	86.3±2.1	93.4±2.0
Calcium (mmol/l)	1.9±0.2	2.4±0.6	2.3±0.5	2.7±0.3	2.7±0.6	-	-
Inorganic Phosphorus (mmol/l)	2.5±0.3	2.3±0.7	2.2±0.9	1.7±0.8	2.1±0.6	-	-

mia. The acceleration of blood sedimentation rate on the 60th day supported this. Evidently toxic polymetal dust irritates marrow and stimulates erythropoiesis. Many young erythrocytes with reduced hemoglobin were pushed out to the periphery. On the 30th day the average number of leukocytes in Group II was twice as high as the control value. Consequently, this index is a very good marker for the toxic action of polymetal dust.

The changes of biochemical indices show that a hyperglycemic effect occurred during the whole experiment, and its expression depended to a certain extent on dose and time. The hyperuricaemic effect in Group II was the reason for reduced elimination of uric acid because of kidney damage resulting from exposure.

Physiological triglyceridemia in *M. guentheri* was reduced during the course of the experiment. Anabolic processes were affected leading to a reduction and stunting of growth. This is also confirmed by the suppressed synthesis of triglycerides. The increased amount of cholesterol shows an acute hepatotoxic effect on the 30th day. The opposite tendency in the changes of triglycerides and cholesterol is connected with lipoprotein exchange. Evidently the synthesis of lipoproteins in the liver is reduced.

The increased level of urea shows that a nephrotoxic effect is probably occurring with an impact at the tubular level. This is why, at the beginning of the experiment, urea concentrations increase but creatinine levels do not. The polyurea observed is due to hypoglycemia. Although Cd, Fe, Mn and Mg cause damage to the liver at very high concentrations, the critical organ is generally considered to be the kidney (Nyholm & Rueling, 2001). Nephropathy is indicated by proximal tubule cell necrosis, proteiuria, glucosuria, increased urinary cadmium and decreased cadmium content in kidneys (White at al., 1978).

The pathological changes observed in the tested blood indices, as a result of synergetic action of the metal captions or ferosilicic dust show that *M. guentheri* is a good bioindicator species for the evaluation of dust emissions.

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