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Age Differences in Bioaccumulation of Heavy Metals in Populations of the Black-Striped Field Mouse, *Apodemusagrarius*(Rodentia, Mammalia)

Blagojević, J.*, Jovanović, V., Stamenković, G., Jojić, V., Bugarski-Stanojević, V., Adnađević, T. and Vujošević, M.

University of Belgrade, Institute for Biological Research "SinišaStanković", 142 Despot Stefan Blvd., 11060 Belgrade, Serbia

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ABSTRACT: Bioaccumulation of heavy metals in the skulls of black-striped field mice (*Apodemusagrarius*) was compared for two localities in Serbia differing in the level of pollution. Eight heavy metals: Fe, Mn, Co, Cd, Zn, Ni, Pb and Cu, were quantitatively analyzed by atomic absorption spectrophotometry.Four of them (Mn, Cd, Fe and Ni) showed significantly higher concentrations at the polluted location than in the relatively unpolluted one. Concentrations of heavy metals in three age categories exhibited opposite patterns depending on pollution levels. At the unpolluted locality heavy metal concentrations were the highest in the youngest group and lower in older animals. In contrast, bone metal concentrations increased with age class at the more polluted site. At the same time, we found that young animals from the polluted location had a statistically significant lower relative body mass (RBM) than those from the unpolluted area($t = 7.155$, $p < 0.001$), which may have been caused by heavy metals or other factors associated with pollution. In general, we found that age is a critical factor for estimating the level of heavy metal pollution, so proper investigations should account for the age structure of the samples.

Key words: Heavy metal, Relative body mass (RBM), Age category, *Apodemusagrarius*

INTRODUCTION

Heavy metal (HM) pollution is a growing environmental problem, which requires constant attention (Nasrabadi *et al.,* 2010; Haruna *et al.,* 2011; Yu *et al.,* 2011; Kargar *et al.,* 2012; Divis *et al.,* 2012). As one of the major groups of pollutants they pose a threat to the environment because they are not degraded and can persist for a long time in the soil (AlimohammadKalhori *et al.,* 2012; Ashraf *et al.,* 2012; Okuku and Peter, 2012). Contamination with heavy metals poses a constant risk to human health (Mzoughi and Chouba, 2012; Ghaderi *et al.,* 2012; Mhadhbi *et al.,* 2012). Living organisms require varying amounts of essential heavy metals: iron, cobalt, copper, manganese, molybdenum and zinc but excessive levels can be harmful to the organism. Other heavy metals, xenobiotics, such as mercury, plutonium, cadmium and lead are toxic and their accumulation over time in the body of animals and humans may cause serious illness. When living organisms are exposed to metals, actively or passively, they can enter the organism at all stages of development (Serbaji *et al.,* 2012; Ogundiran *et al.,* 2012). Due to their wide distribution in both polluted and unpolluted areas, small mammals are suitable for studying the effects of pollution. Factors that may influence their level of exposure to pollutants are season, pollutant concentrations in the exposure area, physiology, body size, gender and age (Komarnicki, 2000; Beernaert *et al.,* 2007). The uptake and distribution of biologically essential metals (Fe, Mn, Cu, Zn, and Ni) is physiologically regulated, in contrast to non-essential elements. The specific tissues in which certain metals can be retained depend on the properties of the element, metabolic turnover and the state of the organism.

There are numerous sources of heavy metal pollution, including coal, natural gas, metal, paper and chlor-alkali industries. In addition, traffic is considered to be one of the largest sources of heavy metals. At least 90% of the metals in road runoff consist of copper, zinc and lead. Lead concentrations are directly dependent on gasoline quality. With the aim of controlling lead pollution, most countries are phasing out leaded fuel, and replacing it with an unleaded equivalent. Although lead, which can accumulate in bones continuously, does not damage them, it represents a permanent source for other organs (Rabinowitz, 1991). The main sources of cadmium emissions to the air are combustion in power plants,

*Corresponding author E-mail:jelena.blagojevic@ibiss.bg.ac.rs

industry and residential housing and other stationary locations, while nickel and cadmium are found in road runoff and exhaust fumes. The density of road nets in the world is positively correlated with the level of pollution even in unpopulated and relatively clean areas. The number of studies on bioaccumulation of metals and their toxic effects in small mammals is rising from year to year (Sheffield *et al.,* 2001; Berckmoes *et al.,* 2005; Świergosz-Kowalewska *et al.,* 2005; Scheirs *et al.,* 2006; Torres *et al.,* 2006; Beernaert *et al.,* 2007; Sánchez-Chardi and Nadal 2007; Sánchez-Chardi *et al.,* 2007; Schleich *et al.,* 2010). Small mammals accumulate larger amounts of heavy metals but that does not necessarily mean that those species are under the greatest risk of toxic effects from pollution. Species sensitivity is quite variable and numerous mechanisms to cope with toxins exist. Thus, wood mice in laboratory conditions were shown to prefer eating acorns from unpolluted sites over those from polluted ones (Beernaert *et al.,* 2008). However, the authors hypothesize that search time constrains animals in the field to forage selectively. Often, total metal concentrations in the soil at trapping locations are not an accurate predictor of their tissue concentrations in small mammals (Wijnhoven *et al.,* 2007), although in some cases multivariate analysis showed a significant correlation between metal levels in tissues and soils (Marcheselli, 2010). Shrew species accumulate more heavy metals than rodents due to their high metabolic rate and type of diet. Furthermore, from results for seven species of small mammals, Fritsch et al. (2010) concluded that age more than gender, species or trophic group influenced metallic trace element levels and their relationships with exposure to metals.

The black-striped field mouse, *Apodemusagrarius* (Pallas, 1771) is widespread in most of Europe and Asia.This species has a narrow home-range of about 2400m^2, but occasionally animals disperse depending on population abundance (Vukićević-Radić *et al.,* 2006). Its diet varies according to season and food availability and is mostly composed of green parts of plants, seeds, berries and insects, so populations of *Apodemusagrarius*can be found in all ecosystems from grassy fields to forests. All these characteristics qualify this species as a suitable biomarker.

The aims of this study were to analyse variation in accumulation of heavy metals (iron, manganese, cobalt, cadmium, zinc, nickel, lead and copper) in teeth and skull bones of *Apodemusagrarius* from two localities (Lešnica and Pančevo) in Serbia, differing in the level of pollution. Further we intended to establish if there is any correlation between bioaccumulation of heavy metals and relative body mass, as the measure of animal condition.

MATERIAL & METHODS

A total of 151 specimens of black-striped field mice, *Apodemusagrarius*, were collected using baited Longworth traps at two localities in Serbia. The first locality, Lešnica in west Serbia, is situated in a non-industrialized area without intensive pollution and with relatively low traffic density and is herein referred to as the unpolluted site. At this locality animals were sampled from 1994-1996 (54 male and 29 female). The area round the town of Pančevo is the second locality with two collection sites. Pančevo is one of the most polluted towns in Europe with heavy industry and traffic. The first site was located near the river Tamiš and was sampled in October 1992 (28 males and 20 females). The second one was near the large oil industrial installation "Petrohemija" where we collected 11 males and 9 females in November 2000. Sites one and two are approximately 7km apart.

Trapped animals were brought to the laboratory where their body mass (BM) was measured to the nearest 0.01 g and body length (BL) to the nearest 1 mm. Relative body mass (RBM) represents the residual index from linear regression of BW on BL (Jakob *et al.,* 1996).Positive values are associated with better animal condition and vice versa for negative ones. Groups or localities were compared using the parametric t-test (Statistica 6.0).Animals were sacrified and the skulls exposed to dermestid beetles, cleaned, dried and weighed with 0.1mg precision. Eye lenses were removed and prepared according the method of Nabaglo and Pachinger (1979) and then weighed to an accuracy of ± 0.1 mg using a Mettler (Germany) laboratory balance. Dry lens weight was used to estimate the age of the animals (Adamszewska-Andrzejewska, 1973). Age categories were: I – animals up to 4 months old, II – animals aged between 5 and 8 months and III – animals $\geq$ 9 months old.

Heavy metal concentrations were determined in 72 skulls from the two locations, Lešnica and Pančevo. Each location was represented by groups of males and females divided into the three age categories. Up to five skulls (without mandibles) were grouped per age and sex category, crushed in ceramic pots and digested in 10 ml of a 2:1 mixture of concentrated HNO_3 and $HClO_4$ (Merck, Germany). Digestion was completed by heating behind the protection shield on a hot plate until white fumes of perchloric acid were no longer noted and the solution became clear. After digestion the samples were filtered, transferred to volumetric flasks anddeionised water added to 50ml. Eight heavy metals (HM): Fe, Mn, Co, Cd, Zn, Ni, Pb and Cu, were quantitatively analyzed by atomic absorption spectrophotometry (Thermo Scientific Solaar S Series AA) and expressed in µg/g dry weight. As the heavy metal concentrations were not normally distributed, the nonparametric Mann-Whitney U-test was used for statistical comparisons. Significant differences were accepted at level of probability $p < 0.05$. Statistica 6.0 was used for all statistical analyses.

RESULTS & DISCUSSION

Bioaccumulation of heavy metals in living organisms can be affected by age and sex but their effects vary greatly between populations and species. Non-essential metals are undesirable in an organism and have toxic effects, while essential ones may have negative effects when they are deficient or in excess (Klaassen, 2001). The uptake and elimination kinetics of heavy metals are not constant during life, but alter during the growth of an animal.Heavy metal concentrations varied widely among the analyzed samples. The highest values were for Zn and the lowest for Cd (Table 1). Samples from the two sites at the Pančevo locality did not differ significantly in heavy metal concentrations, and further comparisons were made with pooled sites. Statistically significant differences in concentrations for Fe (3.891 ± 1.822 vs. 6.577 ± 2.958, U = 11.0, p = 0.027), Mn (0.848 ± 0.149 vs. 1.282 ± 0.148, U = 10.0, p = 0.020), Ni (18.228 ± 3.926 vs. 34.376 ± 5.168, U = 10.0, p = 0.020) and Cd (0.156 ± 0.034 vs. 0.245 ± 0.035, U = 10.0, p = 0.020) were found for Pančevo vs. Lešnica. In the whole sample, there was a significant ($p<0.05$) decrease in concentration of Fe, Mn, Co, Ni and Pb between age categories I and II and a significant increase in Ni concentration between age categories II and III. No gender dependent variation was detected for the concentration of any metal.

Table 1. Concentrations of heavy metals (µg/g) in the skulls of *Apodemusagrarius* from the localities, Lešnica and Pančevo (site 1- Pančevo1; site 2- Pančevo2); n - sample size; M -male, F - female

Locality	Sex	Age	n	Fe	Mn	Co	Cd	Zn	Ni	Pb	Cu
Lešnica	M	I	5	7,403	1,560	1,552	0,323	96,741	36,408	36,755	184,119
		II	5	3,139	0,701	0,748	0,128	53,783	12,291	17,460	42,420
		III	5	2,441	0,577	0,672	0,106	48,322	10,440	15,503	27,542
	F	I	5	4,250	0,910	0,875	0,141	60,108	21,179	26,297	38,037
		II	5	3,253	0,683	0,744	0,118	44,821	14,539	17,024	29,379
		III	5	2,863	0,659	0,747	0,118	55,509	14,514	16,358	23,476
Pančevo1	M	I	5	4,410	0,916	0,945	0,154	53,602	26,114	22,311	32,757
		II	5	4,012	0,760	0,841	0,135	51,183	17,007	18,414	27,121
		III	3	8,677	1,568	1,526	0,262	111,490	41,937	39,891	15,718
	F	I	5	5,172	1,090	1,014	0,177	69,210	24,363	27,633	62,365
		II	4	6,635	1,303	1,200	0,233	85,714	33,284	29,750	71,870
		III	2	10,832	1,952	1,676	0,367	116,902	58,451	41,283	125,485
Pančevo2	M	I	3	8,532	1,772	1,567	0,339	102,574	51,473	40,656	88,773
		II	5	3,189	0,718	0,713	0,138	47,650	14,989	15,158	16,828
		III	2	6,036	1,232	1,148	0,249	84,314	33,333	26,359	28,571
	F	II	5	3,263	0,737	0,742	0,146	46,385	14,873	15,793	16,995
		III	3	11,590	2,050	1,609	0,494	134,044	62,308	34,761	88,543
Total			72	5,629	1,129	1,078	0,213	74,256	28,677	25,965	54,118

Age-dependent variation showed different patterns at the polluted and unpolluted localities (Fig. 1). Concentrations of all studied heavy metals in the skulls from Lešnica were the highest in the group of young animals and decreased with age. Contrary to this, at Pančevo the highest concentrations of HM occurred in the group of oldest animals (from 9 months and older). Differences in HM concentrations between the localities were significant only for age category III. Mean values were 2 to 4 times higher at the polluted location, Pančevo, depending on the HM.

The body residual index (RI) is a measure of energy reserve which can be very important for longer survival, particularly of young animals. Specimens with positive RBM values are considered to be in good condition (Fig. 2). All age categories at Lešnica had positive RBM values, while for Pančevo, RBM was positive onlyin age category III. Significant differences between the localities were found in RBM (Lešnica: 0.816 ± 2.206; Pančevo: -0.996 ± 2.265; $t = 4.963$, $p < 0.001$). This was mostly due to the large difference in age category I ($t = 7.155$, $p < 0.001$). Differences in RBM for the other two age categories were not statistically significant (II: $t = 1.318$, $p = 0.193$; III: $t = 0.952$, $p = 0.348$). Effects of gender on RBM were not significant at either locality (Lešnica: $t = 1.101$, $df = 81$, $p = 0.274$; Pančevo: $t = -1.649$, $df = 66$, $p = 0.104$).

Kostial et al. (1978) showed that the early neonatal age is a critical period for metal accumulation and therefore for metal toxicity in rats. In natural populations of small mammals concentrations of heavy metals frequently decrease with age (Outridge and Scheuhammer, 1993; Lopes *et al.,* 2002; Scheirs *et al.,* 2006; Beernaert *et al.,* 2007; Sánchez-Chardi and Nadal, 2007), mostly in soft tissues, such as liver, kidneysand muscles. In general, this decrease is explained by high intake and incorporation of essential heavy metals during periods of intensive growth in young animals. Moreover, the higher metabolic rate of juveniles, implying a high uptake of food, may explain the increased amounts of xenobiotics, such as Cd and Pb, for polluted areas. Another explanation could be a decrease in intestinal absorption of certain metals in adults. Our results show that bioaccumulation levels did not differ significantly between young animals from polluted (Pančevo) and unpolluted (Lešnica) localities. At that age (neonates and juveniles) bioaccumulation is mainly affected by the high rate of metabolism, no matter how high the concentration of heavy metals is in the environment. Bioaccumulation may also be affected by the general fitness of young animals. Therefore, it is not surprising that bioaccumulation is more effective in an unpolluted locality due to the generally better condition of the animals.

Relative body mass showed that young animals from unpolluted Lešnica were in a significantly better condition than the equivalent group from the polluted locality. Regardless of heavy metal concentrations, bioaccumulation is limited by the rate of uptake. In the next stage of growth metabolic rate is slower and this

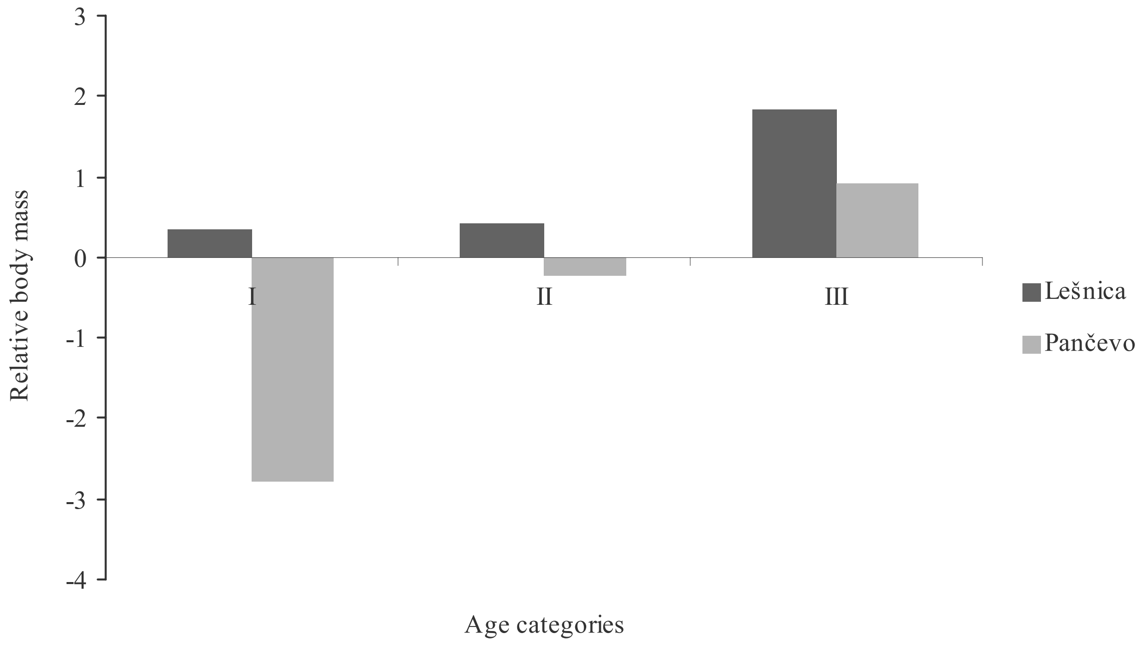

Fig. 1. Relative body mass (RBM) in three age categories of *Apodemusagrarius* at unpolluted (Lešnica) and polluted (Pančevo) localities

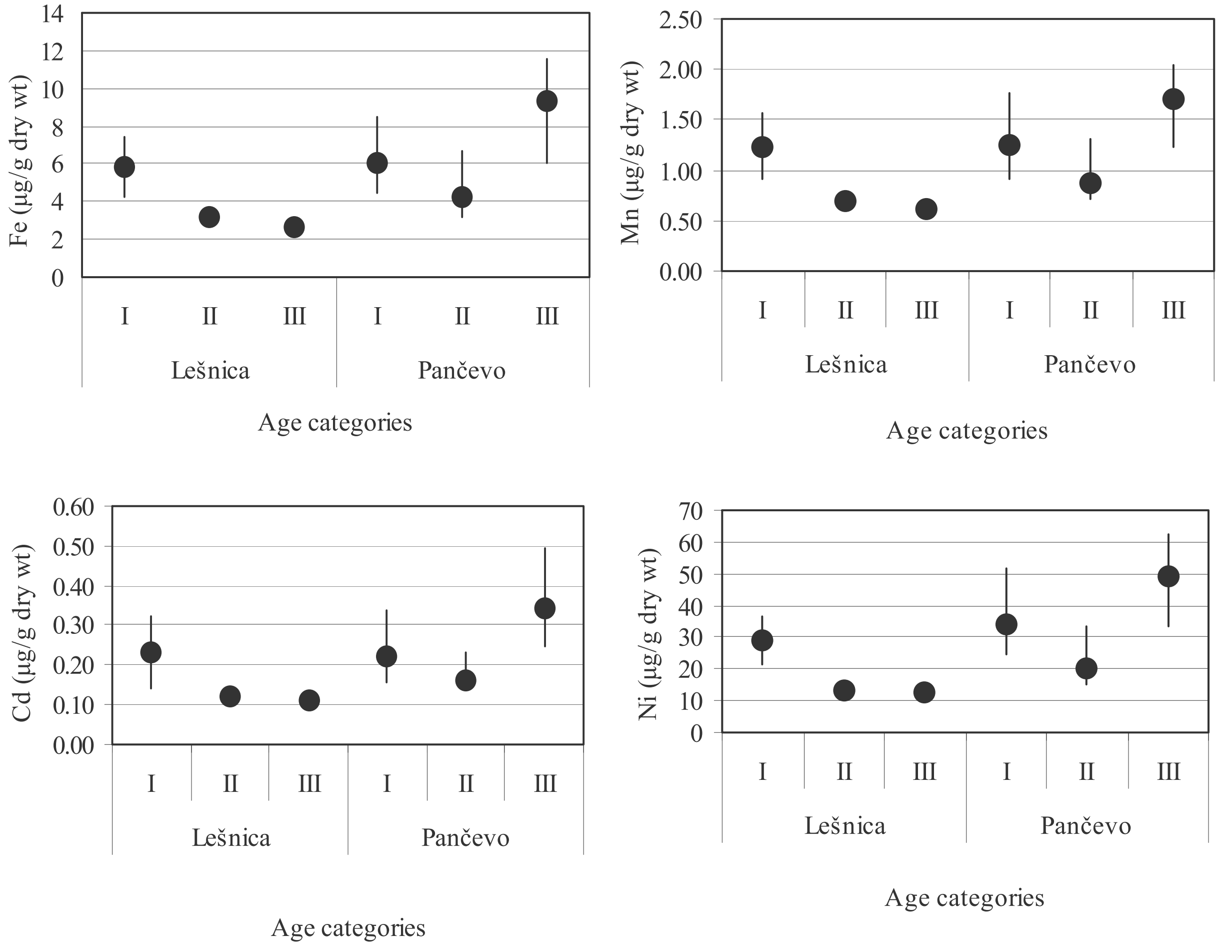

Fig. 2. Variation in concentrations of Fe, Mn, Cd and Ni among three age categories which differ significantly between unpolluted (Lešnica) and polluted (Pančevo) localities (circles – median values; lines – min-max values)

is clearly seen from the decrease of heavy metal concentrations. According to our results a difference between the polluted and unpolluted locality could be clearly seen only in old animals. We suppose that in unpolluted and moderately polluted areas bioaccumulation in young animals will be maximal but in older animals it will decrease as growth rate declines. Only in a highly polluted area like Pančevo, will bioaccumulation continually increase despite the decrease of metabolic rate. The same trend of accumulation was obtained for the striped dolphin, *Stenellacoeruleoalba* (Honda *et al.,* 1986), where Ni, Cd and Pb accumulated more efficiently during the suckling and juvenile period. The strong ability of small mammals to regulate some essential metals, such as Cu and Zn, homeostatically in their soft tissues (Hunter and Johnson, 1982; Alberici *et al.,* 1989; Damek-Poprawa and Sawicka-Kapusta, 2003) is also confirmed for bones by our findings. There was no accumulation in bones dependent on level of pollution.

Analysis of soft tissues could be an instant method for estimating pollution, while bone tissue could be used for total estimation of pollution. In the yellow necked mouse and bank vole, Martiniaková et al. (2010) showed that concentrations of Cd and Zn were higher in bones, while Cu and Fe accumulated mainly in the liver and kidneys. Bioaccumulation in bones followed prolonged exposure. A polluted environment may affect animal fitness and behavior (Homady *et al.,* 2002), or frequently provoke genotoxic effects (Scheirs *et al.,* 2006; Sánchez-Chardi et al. 2009). Additionally, decreased weights of body, testes, preputial glands, and seminal vesicles were also found in mice (Homady *et al.,* 2002) as an effect of heavy metal pollution. Comparisons between prehistoric and modern human teeth suggest that the impact of current environmental lead pollution is considerable (10–100 times higher), while that of cadmium pollution is much less (Grandjean and Jørgensen, 1990). We found no differences in bone accumulation of Pb at the studied localities, which can be explained by the wide presence of this heavy metal. Nickel is a carcinogen and overexposure to it can cause decreased body weight and may damage the heart and liver (Homady *et al.,* 2002).The effects of pollution in young animals mostly influence body condition which could be seen as a suitable parameter for measuring the effects of general pollution. Our results indicate that for accurate estimation of environmental pollution based on bioaccumulation of heavy metals, it is necessary to use only the oldest animals if the analysis does not include a separate study of age categories. Otherwise, comparison of samples originating from different seasons or different localities is not relevant, because samples could markedly differ in age structure, which might lead to wrong conclusions.

CONCLUSION

We found that bioaccumulation of heavy metals (HM) in bones is process whose efficiency depends both of the level of pollution and the individual age. Mechanisms of HM bioaccumulation are very similar in mammals whatever area they occupy, so the results could be extrapolated on man. Regardless of heavy metal concentrations, bioaccumulation is limited by the rate of uptake. At early age stages, due to increased metabolic rate, heavy metals are more rapidly accumulated. We established that in population settled in polluted area, young animals are in worst condition (measured by relative body mass - RBM) in comparison to those from unpolluted population($t = 7.155$, $p < 0.001$), so they accumulate HM at lower rate than expected. However, the process of accumulation continues during whole life, although older animals have lower rate of metabolism so accumulation of HM is directly dependent on the level of pollution. Concentrations of HM were 2 to 4 times higher at the polluted location.According to our results a difference between the polluted and unpolluted locality could be clearly seen only in old animals so this category should be the target of studies.

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REFERENCES

Adamszewska-Andrzejewska, K.A. (1973). Growth, variations and age criteria in *Apodemusagrarius* (Pallas, 1771).ActaTheriol.,**19**, 353-394.

Alberici, T.M., Dopper, W.E., Storm, G. L. and Yahner, R.H. (1989). Trace metals in soil, vegetation, and voles from mine land treated with sewage sludge. J. Environ. Qual., **18**, 115-120.

AlimohammadKalhori, A., Jafari, H. R., Yavari, A. R., Prohić, E. and AhmadzadehKokya, T. (2012). Evaluation of Anthropogenic Impacts on Soiland Regolith Materials Based on BCR Sequential Extraction Analysis. Int. J. Environ. Res., **6 (1)**, 185-194.

Ashraf, M. A., Maah, M. J. and Yusoff, I. (2012). Bioaccumulation of Heavy Metals in Fish Species Collected From Former Tin Mining Catchment. Int. J. Environ. Res., **6 (1)**, 209-218.

Beernaert, J., Scheirs, J., Leirs, H., Blust, R. and Verhagen, R. (2007).Non-destructive pollution exposure assessment by means of wood mice hair. Environ. Pollut.,**145 (2)**, 443–451.

Beernaert, J., Scheirs, J., Van Den Brande, G., Leirs, H., Blust, R., De Meulenaer, B., Van Camp, J. and Verhagen, R. (2008). Do wood mice (*Apodemussylvaticus* L.) use food selection as a means to reduce heavy metal intake? Environ. Pollut.,**151 (3)**, 599-607.

Berckmoes, V., Scheirs, J., Jordaens, K., Blust, R., Backeljau, T. and Verhagen, R. (2005). Effects of environmental pollution on microsatellite dna diversity in wood mouse (*Apodemussylvaticus*) populations. Environ. Toxicol. Chem., **24 (11)**, 2898-2907.

Damek-Poprawa, M. and Sawicka-Kapusta, K. (2003).Damage to the liver, kidney, and testis with reference to burden of heavy metals in yellow-necked mice from areas around steelworks and zinc smelters in Poland. Toxicology, **186 (1-2)**, 1-10.

Ghaderi, A.A., Abduli, M.A., Karbassi, A.R., Nasrabadi, T. andKhajeh, M. (2012).Evaluating the Effects of Fertilizers on Bioavailable Metallic Pollution of soils, Case study of Sistan farms, Iran.Int. J. Environ. Res., **6 (2)**, 565-570.

Grandjean, P.and Jørgensen, P.J. (1990).Retention of lead and cadmium in prehistoric and modern human teeth. Environ. Res., **53 (1)**, 6–15.

Homady, M., Helmi, H., Jiries, A., Mahasneh, A., Al-Nasir, F. and Khleifat, K. (2002).Survey of some heavy metals in sediments from vehicular service stations in Jordan and their effects on social aggression in prepubertal male mice.Environ. Res.,**8 (1)**, 43-49.

Honda, K., Fujise, Y. and Tatsukawa, R. (1986).Age-related accumulation of heavy metals in bone of the striped dolphin, *Stenellacoeruleoalba*. Mar. Environ. Res., **20**, 143-160.

Jakob, E.M., Marshall, S.D. and Uetz, G.W. (1996).Estimating fitness: a comparison of body condition indices.Oikos, **77 (1)**, 61–67.

Kargar, M., Khorasani, N. A., Karami, M., Rafiee, G. H. and Naseh, R. (2012).An Investigation on As, Cd, Mo and Cu Contents of Soils Surrounding the Meyduk Tailings Dam.Int. J. Environ. Res., **6 (1)**, 173-184.

Klaassen, C.D. (2001). Casarett and Doull's toxicology: The basic science of poisons. (New York: McGraw-Hill Medical Publishing Division).

Komarnicki, G.J.K. (2000). Tissue, sex and age specific accumulation of heavy metals (Zn, Cu, Pb, Cd) by populations of the mole (*Talpaeuropeae*L.) in a central urban area. Chemosphere, **41 (10)**, 1593-1602.

Kostial, K., Kello, D., Jugo, S., Rabar, I. and Maljković, T. (1978). Influence of age on metaletabolism and toxicity. Environ. Health Persp.,**25**, 81-86.

Lopes, P.A., Viegas-Crespo, A.M., Nunes, A.C., Pinheiro, T., Marques, C., Santos, M.C. and Mathias, M.L. (2002). Influence of age, sex, and sexual activity on trace element levels and antioxidant enzyme activities in field mice (*Apodemussylvaticus* and *Musspretus*). Biol. Trace. Elem. Res., **85 (3)**, 227–239.

Marcheselli, M., Sala, L. and Mauri, M. (2010).Bioaccumulation of PGEs and other traffic-related metals in populations of the small mammal *Apodemussylvaticus*. Chemosphere, **80 (11)**, 1247-1254.

Martiniaková, M., Omelika, R., Grosskopf, B. and Jančová, A. (2010).Yellow-necked mice (*Apodemusflavicollis*) and bank vole (*Myodesglareolus*) as zoomonitors of environmental contamination at a polluted area in Slovakia.Acta Vet. Scand., **52**, 58-62.

Mzoughi, N. and Chouba, L. (2012). Heavy Metals and PAH Assessment Based on Mussel Caging in the North Coast of Tunisia (Mediterranean Sea). Int. J. Environ. Res., **6 (1)**, 109-118.

Nabaglo, L. and Pachinger, K. (1979).Eye lens weight as an age indicator in yellow-necked mice.ActaTheriol.,**24**, 119-122.

Nasrabadi T., NabiBidhendi G. R., Karbassi A. R. and Mehrdadi N. (2010). Partitioning of metals in sediments of the Haraz River (Southern Caspian Sea basin), , Environmental Earth Sciences, **59**, 1111-1117.

Okuku, E.O. and Peter, H. K. (2012) Choose of Heavy Metals Pollution Biomonitors: A Critic of the Method that uses Sediments total Metals Concentration as the Benchmark. Int. J. Environ. Res., **6 (1)**, 313-322.

Outridge, P.M. and Scheuhammer, A.M. (1993). Bioaccumulation and toxicology of chromium: implications for wildlife. Rev. Environ. Contam.Toxicol.**130**, 31–77.

Ogundiran, M. B., Ogundele, D. T., Afolayan, P. G.and Osibanjo, O. (2012). Heavy Metals Levels in Forage Grasses, Leachate and Lactating Cows Reared around Lead Slag Dumpsites in Nigeria. Int. J. Environ. Res., **6 (3)**, 695-702.

Rabinowitz, B.M. (1991). Toxicokinetics of bone lead. Environ Health Perspect.,**91**, 33–37.

Sánchez-Chardi, A., Marques, C.C., Nadal, J. and Mathias, M.L. (2007).Metal bioaccumulation in the greater white-toothed shrew, *Crocidurarussula*, inhabiting an abandoned pyrite mine site. Chemosphere, **67 (1)**, 121–130.

Sánchez-Chardi, A. and Nadal, J. (2007). Bioaccumulation of metals and effects of a landfill in small mammals. Part I. The greater white-toothed shrew, *Crocidurarussula*. Chemosphere, **68 (4)**, 703–711.

Sánchez-Chardi, A., Oliveira Ribeiro, A. and Nadal, J. (2009). Metals in liver and kidneys and the effects of chronic exposure to pyrite mine pollution in the shrew *Crocidurarussula* inhabiting the protected wetland of Doñana. Chemosphere, **76 (3)**, 389-394.

Serbaji, M. M., Azri, C. and Medhioub, K. (2012).Anthropogenic Contributions to Heavy Metal Distributions in the Surface and Sub-surface Sediments of the Northern Coast of Sfax, Tunisia. Int. J. Environ. Res., **6 (3)**, 613-626.

Scheirs, J., De Coen, A., Covaci, A., Beernaert, J., Kayawe, M., Caturla, M., De Wolf, H., Baert, P., Van Oostveldt, P., Verhagen, R., Blust, R. and De Coen, W. (2006).Genotoxicity in wood mice (*Apodemussylvaticus*) along a pollution gradient: Exposure-, age-, and gender-related effects. Environ. Toxicol. Chem., **25 (8)**, 2154-2162.

Schleich, C.E., Beltrame, M.O.andAntenucci, C.D. (2010). Heavy metals accumulation in the subterranean rodent *Ctenomystalarum* (Rodentia: Ctenomyidae) from areas with different risk of contamination. Folia Zool., **59 (2)**, 108-114.

Sheffield, S.R., Sawicka-Kapusta, K., Cohen, J.B. and Rattner, B.A. (2001).Rodentia and Lagomorpha. (In: R.F. Shore, B.A. Rattner (Eds.), Ecotoxicology of Wild Mammals. pp. 215-314) Chichester: John Wiley and Sons.

Świergosz-Kowalewska, R., Gramatyka, M. and Reczyński, W. (2005).Metals distribution and interactions in tissues of shrews (*Sorex* spp.) from copper- and zinc-contamineted areas in Poland.J. Environ. Qual., **34**, 1519-1529.

Technique, Diviš, P., Machát, J., Szkandera, R. and Dočekalová, H. (2012).In situ Measurement of Bioavailable Metal Concentrations at the Downstream on the Morava River using Transplanted Aquatic mosses and DGT.Int. J. Environ. Res., **6 (1)**, 87-94.

Torres, J., Peig, J., Eira, C. and Borras, M. (2006). Cadmium and lead concentrations in *Skrjabinotaenialobata* (Cestoda: Catenotaeniidae) and in its host, *Apodemussylvaticus* (Rodentia: Muridae) in the urban dumping site of Garraf (Spain). Environ. Pollut.,**143 (1)**, 4–8.

Vukićević-Radić, O., Matić, R., Kataranovski, D. and Stamenković, S. (2006). Spatial organization and home range of *Apodemusflavicollis* and *A. agrarius* on Mt. Avala, Serbia. Acta Zool. Acad. Sci. H. **52 (1)**, 81–96.

Wijnhoven, S., Leuven, R.S., van der Velde, G., Jungheim, G., Koelemij, E.I., de Vries, F.T., Eijsackers, H.J. and Smits, A.J. (2007). Heavy-metal concentrations in small mammals from a diffusely polluted floodplain: importance of species- and location-specific characteristics. Arch. Environ. Contam.Toxicol.,**52 (4)**, 603–613.

Yu, Ch., Xu, Sh., Gang, M., Chen, G. and Zhou, L. (2011). Molybdenum pollution and speciation in Nver River sediments impacted with Mo mining activities in western Liaoning, northeast China. Int. J. Environ. Res., **5 (1)**, 205-212.