Journal of Elementology ISSN 1644-2296

Miska-Schramm A., Zakrzewska M., Hajduk J., Kruczek M. 2017. *How copper excess influences homeostasis of the bank vole (Myodes glareolus).* J. Elem., 22(2): 617-628. DOI: 10.5601/jelem.2016.21.4.1049

ORIGINAL PAPER

HOW COPPER EXCESS INFLUENCES HOMEOSTASIS OF THE BANK VOLE (*MYODES GLAREOLUS*)*

Agata Miska-Schramm, Marta Zakrzewska, Joanna Hajduk, Małgorzata Kruczek

Institute of Environmental Sciences
Jagiellonian University, Kraków, Poland

Abstract

Copper is a physiologically occurring trace element, the availability of which to wild small mammals is constantly increasing. This research deals with the questions of how copper in doses equivalent to those found in the natural environment from polluted areas (Cu I – 150 mg kg^{-1}, Cu II – 600 mg kg^{-1}) influences homeostasis of small rodents. The following were used as indicators: ratios of organs to body mass, blood parameters (haematocrit, haemoglobin), copper, iron and zinc concentrations in selected tissues and in excrements. The bank vole was used as a model species (n = 72). Haematocrit was assessed using a haematocrit reader, while haemoglobin was determined with a spectrophotometer. The content of Cu, Fe and Zn was analyzed using the flame method. Ratios of the liver and kidneys to body mass were significantly lower in animals exposed to 600 mg kg^{-1} Cu than in the control. Copper accumulated in the liver, kidneys, brain and excrements of the Cu II animals of both sexes. It also influenced the iron and zinc homeostasis by decreasing the iron concentration in the kidneys of both sexes, in the liver of females and in male testes. Additionally, it caused a decline in iron excretion in females. Compared to the control, the zinc concentration was significantly higher in the liver and brain of animals exposed to copper solutions as well as in the kidneys of copper treated males. Along with an increase in copper, the zinc concentration in faeces decreased significantly. In conclusion, copper pollution influences negatively the homeostasis of small mammals. Consequently it may interfere with trophic chains, which could affect adversely the degree of biodiversity.

Keywords: copper, iron, zinc, bank vole, homeostasis.

Agata Miska-Schramm, PhD, Institute of Environmental Sciences, Jagiellonian University, Gronostajowa 7, 30-387 Kraków, Poland, phone: (+48) 12 266 45 204; fax (+48) 12 664 69 12, e-mail: agata.miska.schramm@gmail.com

* This work was supported by the "Doctus – Małopolski fundusz stypendialny dla doktorantów" project co-funded by EU funds within the European Social Fund and by a grant from structural funds from the Faculty of Biology and Earth Sciences of the Jagiellonian University DS/MND/WBiNoZ/INoŚ/22/2013.

INTRODUCTION

The human impact on nature is continually increasing, adding to the contamination of the environment, one possible manifestation of which is the growing copper availability to wild animals. Decreasing numbers of small rodents have been observed in industrial regions (Kataev et al. 1994), which might be attributed to the harmful effects of contamination. Vertebrates living in the wild are commonly used in environmental studies as monitoring organisms that facilitate assessments of pollution in trophic chains (Zakrzewska et al. 2005, Babińska et al. 2008, Martiniakova et al. 2012). However, such studies do not allow researchers to monitor exact amounts of certain pollutants that are ingested by the model animal. Furthermore, it is impossible to observe how a certain level of a pollutant influences homeostasis of an organism, including the accumulation of this element in tissues, which might be linked with its availability for the higher level organisms in a trophic chain. Thus, the present research focused on the influence of copper concentrations corresponding to those found in the natural environments studied under laboratory conditions.

Copper and its alloys are used extensively in domestic and industrial applications (Stern 2010). Therefore, the element may easily pemeate into the natural environment and accumulate in plants and water, which makes it available to herbivores. This metal is an essential trace element for living organisms and is found in all organs, with the highest concentrations found in the liver and brain (Ozcelik, Uzun 2009). Copper is crucial for the functioning of many cellular enzymes (Tapiero et al. 2003), although is excess may be toxic to a living organism. It has been documented in rodents that excess copper interferes with reproductive abilities (Miska-Schramm et al. 2014), mainly due to its negative effect on the endocrine system (Kochman et al. 1997, Martin et al. 2003). Moreover, copper as an oxidative stress factor may affect adversely the liver function, which has been proven in rats (Zhang et al. 2000). Excess of copper in organisms may also interact with other essential elements, especially with iron and zinc.

Ceruloplasmin, the major copper-carrying protein in blood, or hephaestin, another copper-containing protein, both have a significant impact on the metabolism of iron (Arredondo, Nunez 2005). Limited iron mobilization caused by those proteins may decrease iron availability for new haemoglobin synthesis and result in anaemia (Reeves et al. 2005). On the other hand, zinc intake may induce the synthesis of intestinal metallothionein (a group of proteins binding metals and xenobiotics, important in detoxification), to which copper has higher affinity than to zinc and which will therefore bind in enterocytes (Arredondo, Nunez 2005). These characteristics suggest that copper deficiency may be reduced with high zinc intake. The following question arises: what happens to a zinc level provided the copper intake was raised? Taking into consideration the above information, the authors decided

to assess the accumulation of copper, iron and zinc in selected tissues and excrements of rodents exposed to copper concentrations equivalent to those found in the natural environment from polluted sites (TANG et al. 1999). Moreover, the influence of copper on blood parameters of the examined animals was explored. The bank vole (*Myodes glareolus*) was used as a model species. In its natural environment, this small rodent typically inhabits woodlands, but can also be found in parks, hedgerows and croplands (TORRE, ARRIZABALAGA 2008). It occurs in Europe and Asia and is considered to be a pest species in agriculture.

MATERIALS AND METHODS

Animals

The bank vole (*Myodes glareolus* Schreber, 1780) individuals originated from a laboratory colony of the Institute of Environmental Sciences, Jagiellonian University, Krakow. The original stock was obtained in 1976 from the Mammal Research Institute of the Polish Academy of Sciences (Białowieża) and has been maintained as an outbreed stock colony. The animals are housed in polyethylene cages (40 cm x 25 cm x 15 cm) under a 14 h photoperiod (7 am – 9 pm light, 9 pm – 7 am dark) at 21±1°C and 60% humidity. Wood shavings changed once a week served as bedding material. Standard pelleted chow for laboratory rodents (Labofeed H, Kcynia) and liquid in the form of deionized water or solutions of copper were available *ad libitum*.

For the study, four-week-old individuals were randomly divided into three experimental groups. The animals were treated with different metal solutions or given deionized water for 12 weeks. At the age of 16 weeks, individuals body mass, (g): females 17.0-29.0, males 19.6-33.6 were euthanized by cervical dislocation.

Experimental groups

C – control group given deionized water;

Cu I – (150 mg kg^{-1} dose): copper sulphate (II) 5 hydrate ($CuSO_4 \cdot 5H_2O$) AR purity grade (AVANTOR, Poland) at concentration of 150 mg Cu^{2+} l^{-1};

Cu II – (600 mg kg^{-1} dose): copper sulphate (II) 5 hydrate ($CuSO_4 \cdot 5H_2O$) AR purity grade (AVANTOR, Poland) at concentration of 600 mg Cu^{2+} l^{-1}.

Hematocrit and the level of hemoglobin

Blood samples were collected directly from the heart, right after cervical dislocation to two, one-end-closed capillaries. After 15 min of centrifugation

at 1000 rpm haematocrit (Ht; %) was assessed with a haematocrit reader. Haemoglobin (Hb) level was determinede on a spectrophotometer HemoCue AB (Allgelholm, Sweden).

Chemical analysis

Livers, kidneys, brains, intestines and (in males) testes were dissected from 12 individuals from each experimental group (together 36 males, 36 females), weighed and used for further chemical analyses. The accumulation of copper, iron and zinc was assessed in these tissues, using the spectrophotometric method after wet digestion in a 4:1 HNO_3:$HClO_4$ solution (Zakrzewska et al. 2005). The content of Cu, Fe and Zn was analyzed on a PerkinElmer Analyst 200 using the flame method. Simultaneously, blind reagent samples and standard samples were tested (NBS Bovine Liver 1577b, salvage: 97.2%-101%).

Statistical analysis

Means were compared using one-way analysis of variance (ANOVA) and the significance of differences was determined by the post hoc *Tukey* test. All results are presented as means ±SE with ranges and $p < 0.05$ is taken to indicate significance. All calculations were done using Statistica PL ver. 10.0.

Compliance with ethical standard

The experimental procedures for this study were approved by the Regional Committee on Animal Experimentation in Krakow (Protocol No. 36/2009), acting in compliance with the European Communities Council Directive of 24 Nov. 1986 (86/609/EEC).

RESULTS

Ratios of organs to body mass in the bank vole females and males treated with different copper doses are presented in Table 1. In both sexes, the liver to body mass ratio was significantly higher in animals treated with 600 mg kg^{-1} of Cu than in the control. The kidneys to body weight ratio was significantly higher in both sexes after administration of 150 mg kg^{-1} solution than in the control group. Moreover, both females and males from group Cu II had a significantly lower kidney to body mass ratio than those from group Cu I.

The results summarized in Table 2 show that copper did not affect blood parameters except for a decrease in haematocrit in males from group Cu II, which was was significantly lower than in both the control and Cu I males (Table 2).

As shown in Table 3, the highest copper concentration was found in ex-

Table 1

The ratio of the weight of the liver, kidneys and testes to the mass of bank vole females and males treated with different copper solutions

Mass ratio	Experimental group			$F_{(2.33)}$	p
	C	Cu I	Cu II		
Males					
Liver to body mass	4.24^{A} ± 0.15 3.74 – 5.36	4.71 ± 0.15 3.73 – 5.25	4.99^{A} ± 0.12 4.29 – 5.63	7.29	<0.01
Kidneys to body mass	1.28^{A} ± 0.03 1.1 – 1.46	1.62^{Aa} ± 0.05 1.39 – 1.98	1.43^{a} ± 0.05 1.25 – 1.71	14.01	<0.01
Testes to body mass	2.54 ± 0.08 2.11 – 2.96	2.93 ± 0.14 2.26 – 3.59	2.83 ± 0.15 2.08 – 3.64	2.59	NS
Females					
Liver to body mass	4.32^{a} ± 0.2 3.45 – 5.29	5.13 ± 0.24 3.88 – 6.16	5.39^{a} ± 0.36 3.86 – 7.29	4.18	<0.05
Kidneys to body mass	1.22^{A} ± 0.03 1.08 – 1.39	1.44^{Aa} ± 0.05 1.21 – 1.64	1.18^{a} ± 0.05 0.89 – 1.43	9.06	<0.01

Means assigned the same superscripts differ significantly; $a - p < 0.05$, $A - p < 0.01$; (means ±SE, min. and max. values).

Table 2

Haematocrit and haemoglobin concentrations in blood of bank vole females and males treated with different copper solutions and deionized water

	Experimental group			$F_{(2.33)}$	p
	C	Cu I	Cu II		
Males					
Ht (%)	42^{a} ± 1 35 - 50	41^{b} ± 1 30 - 42	36^{ab} ± 1 30 - 42	5.12	<0.05
Hb (g/L)	135 ± 4 115 - 153	120 ± 4 101 - 152	127 ± 7 88 - 158	2.23	NS
Females					
Ht (%)	41 ± 2 30 - 49	38 ± 1 28 - 46	37 ± 2 27 - 43	1.96	NS
Hb (g/L)	123 ± 3 102 - 136	121 ± 6 78 - 166	114 ± 6 80 - 141	0.76	NS

crements and livers of both males and females from group Cu II. The copper concentration in tissues sampled from both sexes was also significantly higher in Cu II than in control and Cu I groups (Table 3). In excrements, copper concentration was higher in Cu I animals than in the control. The kidneys of Cu II females and males accumulated significantly more copper than both Cu I and control animals. The highest copper accumulation in brains was

Table 3

Copper concentration (μg g^{-1} d.w.) in the liver, kidneys, brain, testes and excrements of bank vole females and males treated with different copper solutions and deionized water

	Cu concentration (μg g^{-1} d.w.)			$F_{(2.33)}$	p
	C	Cu I	Cu II		
Males					
Liver	$14.9^{A} \pm 0.7$ 11.9 – 18.2	$56.12^{a} \pm 8.7$ 23.4 – 102.6	$825.8^{Aa} \pm 175.3$ 107.9 – 1886.6	20.31	<0.01
Kidneys	$14.7^{A} \pm 1.03$ 10.4 – 22.1	$27.1^{B} \pm 3.3$ 13.1 – 54.8	$85.9^{AB} \pm 15.8$ 24.7 – 200.4	16.53	<0.01
Brain	$13.1^{a} \pm 0.3$ 12.4 – 13.9	16.8 ± 1.1 10.7 – 21.4	$20.4^{a} \pm 2.1$ 9.2 – 38	3.6	<0.05
Excrement	$64.4^{Aa} \pm 6.2$ 34.6 – 101.4	$922.5^{Ba} \pm 71.5$ 531.3 – 1390	$2214.1^{AB} \pm 384.1$ 383.1 – 4252.5	22.97	<0.01
Testes	11.2 ± 0.8 8.1 – 17.9	10.8 ± 0.4 9.4 – 14.2	12.5 ± 0.4 9.8 – 15	2.41	NS
Females					
Liver	$16.3^{A} \pm 1.1$ 10.3 – 22.1	$121^{B} \pm 26.8$ 27.3 – 341.3	$1638.5^{AB} \pm 237.2$ 553.5 – 2893.6	43.39	<0.01
Kidneys	$24.6^{A} \pm 2.5$ 14.1 – 43.4	$24.3^{B} \pm 3.3$ 9.5 – 46.1	$73.1^{AB} \pm 11.3$ 34.4 – 139.7	17.53	<0.01
Brain	$13.1^{Aa} \pm 0.3$ 11.9 – 14.3	$15.6^{Ba} \pm 0.5$ 14 – 19.2	$18.2^{AB} \pm 0.6$ 15.9 – 22.4	21.3	<0.01
Excrement	$72^{AB} \pm 4$ 54- 101	$1134^{AC} \pm 73$ 8312 – 5009	$3722^{BC} \pm 291$ 2095 – 5009	117.2	<0.01

Means assigned the same superscripts differ significantly; a – $p < 0.05$, A,B – $p < 0.01$; (means ±SE, min. and max. values).

observed in animals from Cu II groups. In females, copper accumulation in the brain was significantly higher in Cu II than in the other experimental groups (Table 3); in males, however, the copper content was significantly higher in Cu II groups than in the control. In males treated with 600 mg kg^{-1} Cu, the iron concentration in kidneys significantly decreased when compared to the control (Table 4). There was also less iron accumulated in testes of Cu I males compared to the control group. When compared to control females, copper causes a decrease in the iron concentration in the liver, kidneys and excrements of Cu II females. There was also significantly less iron in livers of Cu II females than in Cu I. Iron concentration in kidneys and in excrements was significantly higher in control females than in Cu I groups.

Zinc concentrations in male and female tissues are contained in Table 5. Compared to the control, significantly higher zinc concentrations were observed in the liver, kidneys and brain of Cu II males. Zinc accumulation was also higher in the livers of Cu I males than in the control. On the other hand, zinc concentration in the kidneys and brains of control males was lower than in Cu I ones. Significantly smaller zinc concentration was observed

Table 4
Iron concentration (μg g^{-1} d.m.) in the liver, kidneys, brain, testes and excrements of bank vole females and males treated with different copper solutions and deionized water.

	Fe concentration (μg g^{-1} d.w.)			$F_{(2.33)}$	p
	C	Cu I	Cu II		
Males					
Liver	2452 ± 441 1102 – 6639	2979 ± 361 1424 – 5139	2365 ± 225 1392 – 3993	0.88	NS
Kidneys	422^{a} ± 22 316 – 553	341 ± 22 255 – 466	323^{a} ± 28 247 – 594	4.73	<0.05
Brain	155 ± 6 143 – 173	134 ± 9 92 – 192	140 ± 5 112 – 163	1.42	NS
Excrement	870 ± 37 661 – 1075	835 ± 35 576 – 974	998 ± 72 597 – 1447	2.86	NS
Testes	152^{AB} ± 8 85 – 192	115^{A} ± 6 80 – 158	111^{B} ± 8 90 – 176	9.46	<0.01
Females					
Liver	2015^{a} ± 237 988 – 3542	2174^{B} ± 177 564 – 2737	1330^{aB} ± 130 704 – 2298	5.78	<0.01
Kidneys	528^{ab} ± 29 359 – 667	409^{a} ± 29 226 – 550	422^{b} ± 25 215 – 540	5.62	<0.05
Brain	141 ± 11 110 – 183	116 ± 8 61 – 164	121 ± 6 78 – 145	2.01	NS
Excrement	459^{AB} ± 26 327 - 591	227^{A} ± 10 217 – 331	244^{B} ± 13 154 – 298	43.22	<0.01

Means assigned the same superscripts differ significantly; $a - p < 0.05$, $A,B - p < 0.01$; (means ±SE, min. and max. values).

in excrements of Cu I males than in the control. Relative to the control females, the highest zinc concentration in the liver and brain tissues was observed in group Cu II. The Cu I females had more zinc in the liver and brain than the control females. Copper decreased the zinc concentration in faeces of females; Cu I and Cu II females had less zinc in excrements than did the control ones (Table 5).

DISCUSSION

Differences in the mass of organs between the treatment groups are often accompanied by differences in body mass between the examined groups, making any interpretation of organ weight differences more difficult (Bailey et al. 2004, Michael et al. 2007). Therefore, we have evaluated the organ and body mass ratios to detect copper toxicity towards the target organ. The weights of livers, kidneys and testes were taken into consideration. The liver and, in somecases, the kidney are primary targets of repea-

Table 5

Zinc concentration (μg g^{-1} d.m.) in the liver, kidneys, brain, testes and excrements of bank vole females and males treated with different copper solutions and deionized water

	Zn concentration (μg g^{-1} d.w.)			$F_{(2.33)}$	p
	C	Cu I	Cu II		
Males					
Liver	96^{A} ± 3 77 – 115	110^{b} ± 5 84 – 154	129^{Ab} ± 5 103 – 166	13.74	<0.01
Kidneys	74^{ab} ± 4 44 – 92	88^{a} ± 4 67 – 108	87^{b} ± 4 75 – 120	4.27	<0.05
Brain	47^{AB} ± 1 45 – 49	96^{A} ± 7 56 – 162	95^{B} ± 9 58 – 162	6.51	<0.01
Excrement	391^{A} ± 26 255 – 261	299^{A} ± 14 241 – 418	357 ± 20 217 – 487	5.09	<0.01
Testes	138 ± 4 122 – 178	148 ± 2 133 – 156	121 ± 23 0 – 250	1.04	NS
Females					
Liver	106^{AB} ± 4 85 – 141	142^{A} ±4 110 – 157	154^{B} ± 6 124 – 182	28.90	<0.01
Kidneys	87 ± 4 68 – 113	87 ± 4 50 – 107	89 ± 9 32 – 134	0.04	NS
Brain	49^{AB} ± 1 47 – 51	74^{A} ± 1 65 – 82	69^{B} ± 2 53 – 80	42.4	<0.01
Excrement	459^{AB} ± 26 327 - 591	277^{A} ± 10 216 – 331	243^{B} ±13 154 – 298	43.22	<0.01

Means assigned the same superscripts differ significantly; $a - p < 0.05$, $A,B - p < 0.01$; (means ±SE, min. and max. values).

ted-dose toxicity (Aburto et al. 2001). Their cells can store significant amounts of metal ions without any damage to the organism (Linder 2001). In extreme cases, an excessive intake of elements leads to cell and organ damage and disruption of their functions (Nikolov et al. 2010), which may cause changes in the ratios of these organs to body mass. Our research has shown that copper caused a decrease in the liver and kidney to body mass ratios in both sexes. This suggests that those are the organs most highly susceptible to excessive amounts of copper in the organism. No such difference in the testes to body mass ratio between the experimental groups was detected. However, Chattopadyay and collaborators (2005) as well as Miska-Schramm with colleagues (2014) indicated direct harmful effects of copper on functions of rodents' testes, which, as emerges from the present results, may go undetected by the testes to body mass ratio parameter. At the same time, no differences in the copper accumulation in testes between the experimental groups were found, which is consistent with other research on wild rodents (Jančová et al. 2006). However,

copper accumulated in the liver, kidneys and brain, especially in 600 mg kg^{-1} Cu treated animals of both sexes, and was also found in large quantities in excrements.

Sanchez-Chardi and Nadal (2007) found that the liver accumulated much more copper, zinc and iron than kidneys, which our research has confirmed only in animals exposed to 600 mg kg^{-1} Cu. Compared to the control, there was *circa* ten times as much copper in livers as in kidneys in 600 mg kg^{-1} Cu treated males and about twenty-fold more copper in livers than in kidneys in 600 mg kg^{-1} Cu treated females. Since the copper concentration in the liver and kidneys did not increase in 150 mg kg^{-1} Cu treated bank voles nor in 50 mg kg^{-1} Cu treated laboratory mice (Allen et al. 2006), it might be suggested that copper in lower concentrations can be effectively regulated by an organism. Indeed, copper in significantly higher concentrations than in the control was found in excrements of copper treated males and females. The same was reported for goats (Solaiman et al. 2006). Moreover, copper excretion in females is much more effective than in males, indicating that females possess better homeostasis regulation than males. The main role in homeostatic regulation is played by metallothioneins, which protect an organism against metals and excessive quantities of physiologically occurring elements (Suzuki et al. 2002). Testes, being a male's reproductive organ, also seem to be protected from the excess of copper since no copper accumulation in has been found in testes of copper treated males. This stands in contrast to the brains, where copper accumulated in both sexes. A similar effect of copper excess in brains was observed in pigs and rats (Ozcelik, Uzun 2009). Excessively high copper levels may have caused degenerative changes in the brain (Linder 2001) and affected the whole organism. Copper induced degenerative changes with the appearance of concentric spaces around the nerve fiber bundles in rats' brains (Tariq Zaidi et al. 2002).

Copper excess also caused disorders in the zinc and iron homeostasis in bank voles. A significant increase in the zinc concentration in livers and brains of both sexes and in kidneys of males was determined in copper treated animals. On the other hand, at higher copper availability, significantly less zinc was excreted. The impact of copper on zinc homeostasis was also observed in pigs and in *tx* mice (Gipp et al. 1973, Allen et al. 2006). In out study, as the amount of copper increase, a decline in the iron concentration in kidneys of bank vole females and males, in testes, and in livers and excrements of females was observed. Like zinc, this element also seems to be synergic with copper, which has already been confirmed in a study on pigs, where individuals exposed to 250 mg kg^{-1} of copper had significant less iron accumulated in the liver than those administrated 2 or 10 mg kg^{-1} Cu (Gipp et al. 1973). On the other hand, Bureau et al. (2003) found that with declining copper concentrations, the iron concentration increases in rats, while Prohaska (1983) observed that the iron level in mice remains unchanged at decreasing copper concentrations.

Copper did not influence the blood parameters of bank voles, except for a drop in the haematocrit level in males treated with 600 mg kg^{-1} Cu. Low haematological levels in 600 mg kg^{-1} Cu treated males were probably caused by some deficiency of ceruloplasmin and cytochrome c oxidase, which are copper-dependent enzymes required for iron metabolism (Ramírez-Cárdenas et al. 2005). The absence of changes in haematocrit levels in females may be explained by the suggested increased effectiveness of the homeostatic regulation of copper in this sex.

The results presented above illustrate how the constantly growing copper contamination of the environment influences homeostasis of small mammals. Copper pollution, through harmful effects on organisms of rodents, may play a role in the disruption of trophic chains and subsequently affect biodiversity.

CONCLUSION

Pollution of the natural environment may lead to irreversible changes in biodiversity, especially in developed countries. The results of our work show that pollution due to excess copper alters homeostasis of the bank vole. The documented changes may ultimately reduce wild populations of small rodents. Therefore, our results should contribute to a debate on environmental contamination and its consequences for living organisms.

REFERENCES

Aburto E.M., Cribb A., Fuentealba I.C., Ikede B.O., Kibenge F.S.B., Markham F. 2001. *The failure of selenium supplementation to prevent copper-induced liver damage in Fischer 344 rats.* Can J. Vet. Res., 65: 104-110.

Allen K.J., Buck N.E., Cheah D.M., Gazeas S., Bhathal P., Mercer J.F. 2006. *Chronological changes in tissue copper, zinc and iron in the toxic milk mouse and effects of copper loading.* Biometals, 19: 555-564. DOI: 10.1007/s10534-005-5918-5

Arredondo M., Nunez M.T. 2005. *Iron and copper metabolism.* Mol. Aspects Med., 26: 313-344.

Babińska I., Szarek J., Binkowski Ł., Skibniewska K., Wojtacka J., Markiewicz E., Felsmann M.Z., Zakrzewska M., Gesek M., Dublan K. 2008. *Grey herons (Ardea cinera* L.*) as a tool for monitoring the environment for metal concentrations in the vicinity of pesticide tomb in the Iławskie Lake District*. Fresenius Environ. Bull., 17: 98-102.

Bailey S.A., Zidell R.H., Perry R.W. 2004. *Relationships between organ weight and body/brain weight in the rat: What is the best analytical endpoint?* Toxicol. Pathol., 32: 448-466. DOI: 10.1080/01926230490465874

Bureau I., Gueux E., Mazur A., Rock E., Roussel A.M., Rayssiguier Y. 2003. *Female rats are protected against oxidative stress during copper deficiency.* J. Am. Coll. Nutr., 22: 239-246. DOI: 10.1080/07315724.2003.10719299

Chattopadhyay A., Sarkar M., Biswas N.M. 2005. *Dose-dependent effect of copper chloride on male reproductive function in immature rats.* KUMJ, 3: 392-400.

Gipp W.F., Pond W.G., Tasker J., Van Campen D., Krook L., Visek W.J. 1973. *Influence of level*

of dietary copper on weight gain. Hematology and liver copper and iron storage of young pigs. J. Nutr., 103: 713-719.

Jančová A., Massányi P., Naď P., Koréneková A., Skalická M., Drábenková J., Baláž J. 2006. *Accumulation of heavy metals in selected organs of yellow necked mouse*. Ekologia (Bratislava), 25(1): 19-26.

Kataev G.D., Suomela J., Palokangas P. 1994. *Densities of microtine rodents along a pollution gradient from a copper-nickel smelter*. Oecologia, 97: 491-498. DOI: 10.1007/BF00325887.

Kochman K., Gajewska A., Kochman H., Kozlowski H., Masiukiewicz E., Rzeszotarska B. 1997. *Binding of Cu2+, Zn2+, and Ni2+-GnRH complexes with the rat pituitary receptor*. J. Inorg. Biochem., 65: 277-279.

Linder M.C. 2001. *Copper and genomic stability in mammals*. Mutat. Res. Fund. Mol. Mech. Mut., 475: 141-152. DOI: 10.1016/S0027-5107(01)00076-8

Martin M.B., Reiter R., Pham T., Avellanet Y.R., Camara J., Lahm M., Pentecost E., Pratap K., Gilmore B.A., Divekar S., Dagata R.S., Bull J.L., Stoica A. 2003. *Estrogen-like activity of metals in MCF-7 breast cancer cells*. Endocrinology, 144: 2425-2436. DOI: 10.1210/en.2002-221054

Martiniakova M., Omelka R., Jancova A., Formicki G., Stawarz R., Bauerova M. 2012. *Accumulation of risk elements in kidney, liver, testis, uterus and bone of free-living wild rodents from a polluted area in Slovakia*. J. Environ. Sci. Health (A), 47: 1202-1206. DOI: 10.1080/10934529.2012.672062

Michael B., Yano B., Sellers R.S., Perry R., Morton D., Roome N., Johnson J.K., Schafer K., Pitsch S. 2007. *Evaluation of organ weights for rodent and non-rodent toxicity studies: a review of regulatory guidelines and a survey of current practices*. Toxicol. Pathol., 35: 742-750. DOI: 10.1080/01926230701595292

Miska-Schramm A., Kruczek M., Kapusta J. 2014. *Effect of copper exposure on reproductive ability in the bank vole (Myodes glareolus)*. Ecotoxicology, 23: 1546-1554. DOI: 10.1007/s10646--014-1295-6

Nikolov I.G., Joki N., Vicca S., Patey N., Auchère D., Benchitrit J., Flinois J.P., Ziol M., Beaune P., Drüeke T.B., Lacour B. 2010. *Tissue accumulation of lanthanum as compared to aluminum in rats with chronic renal failure - Possible harmful effects after long-term exposure*. Nephron. Exp. Nephrol., 115: e112-e121. DOI: 10.1159/000313492

Ozcelik D., Uzun H. 2009. *Copper intoxication; antioxidant defenses and oxidative damage in rat brain*. Biol. Trace Elem. Res., 127: 45-52. DOI: 10.1007/s12011-008-8219-3

Prohaska J.R. 1983. *Changes in tissue growth, concentrations of copper, iron, cytochrome oxidase and superoxide dismutase subsequent to dietary or genetic copper deficiency in mice*. J. Nutr., 113: 2048-2058.

Ramírez-Cárdenas L., Costa N.M.B., Reis F.P. 2005. *Copper-iron metabolism interaction in rats*. Nutr. Res., 25: 79-92. DOI: http://dx.doi.org/10.1016/j.nutres.2004.07.003

Reeves P.G., Demars L.C., Johnson W.T., Lukaski H.C. 2005. *Dietary copper deficiency reduces iron absorption and duodenal enterocyte hephaestin protein in male and female rats*. J. Nutr., 135: 92-98.

Sanchez-Chardi A., Nadal J. 2007. *Bioaccumulation of metals and effects of landfill pollution in small mammals*. Part I. *The greater white-toothed shrew, Crocidura russula*. Chemosphere, 68: 703-711. DOI: 10.1016/j.chemosphere.2007.01.042

Solaiman S.G., Shoemaker C.E., D'Andrea G.H. 2006. *The effect of high dietary Cu on health, growth performance, and Cu status in young goats*. Small Ruminant Res., 66: 85-91. DOI: http://dx.doi.org/10.1016/j.smallrumres.2005.07.024

Stern B.R. 2010. *Essentiality and toxicity in copper health risk assessment: overview, update and regulatory considerations*. J. Toxicol. Environ. Health (A), 73: 114-127. DOI: 10.1080/15287390903337100

Suzuki K.T., Someya A., Komada Y., Ogra Y. 2002. *Roles of metallothionein in copper homeostasis: responses to Cu-deficient diets in mice*. J. Inorg. Biochem., 88: 173-182.

Tang S., Wilke B.M., Huang C. 1999. *The uptake of copper by plants dominantly growing on copper mining spoils along the Yangtze River, the People's Republic of China*. Plant Soil, 209: 225-232.

Tapiero H., Townsend D.M., Tew K.D. 2003. *Trace elements in human physiology and pathology*. Copper Biomed Pharmacother, 57: 386-398.

Tariq Zaidi M., Faruqi N.A., Abidi S.H.M. 2002. *Effects of copper chloride toxicity on corpus striatum of rat brain - a histological study*. J. Anat. Soc. India, 51: 55-56.

Torre I., Arrizabalaga A. 2008. *Habitat preferences of the bank vole Myodes glareolus in a Mediterranean mountain range*. Acta Theriol., 53: 241-250. DOI: 10.1007/BF03193120

Zakrzewska M., Sawicka-Kapusta K., Aleksandrowicz P., Stochmal M., Szarek J., Grzybowski M., Skibniewska K.A., Guziur J., Zmysłowska I. 2005. *Heavy metal concentrations in tissues of bank voles (Clethrionomys glareolus) from areas around a pesticide tomb*. J. Elem., 10(3): 851-856. (in Polish)

Zhang S.S., Noordin M.M., Rahman S.O., Haron J. 2000. *Effects of copper overload on hepatic lipid peroxidation and antioxidant defense in rats*. Vet. Hum. Toxicol., 42: 261-264.