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# ACCUMULATION OF SELECTED HEAVY METALS IN THE FEMORA OF SMALL TERRESTRIAL MAMMALS

## KUMULACJA WYBRANYCH METALI CIĘŻKICH W KOŚCI UDOWEJ DROBNYCH SSAKÓW LĄDOWYCH

**Abstract:** The accumulation of lead, cadmium, iron, nickel, copper and zinc in the femora of yellow-necked mouse (*Apodemus flavicollis*) and bank vole (*Clethrionomys glareolus*) living near the site of coal power station Novaky (Slovakia) was investigated. The content of heavy metals in the bones was assessed by atomic absorption spectrophotometry method. Altogether 20 femora of adult individuals were analysed. Higher concentrations of Cd, Ni, Cu and Zn were detected in the bones of bank vole. Significant differences were observed for the concentrations of Cd, Ni and Zn ($p < 0.05$). On the contrary, higher concentrations of Pb and Fe were found in the femora of yellow necked mouse. However, the differences were not significant. Our results indicate that *Clethrionomys glareolus* may be considered as more bone loaded zoomonitor in comparison with *Apodemus flavicollis*.

**Keywords:** heavy metals, bone, yellow necked mouse, bank vole, environment

The dynamic development of industry and motorization, as well as the continuing over-intensive use of various compounds in agriculture, cause levels of toxic heavy metals in the environment to constantly be on the increase [1]. Among the investigated elements, copper, zinc, and manganese play an important role in metabolism as components or activators of enzymes and their tissue concentrations are effectively controlled over a wide range of metal intake. Other elements, called xenobiotics such as

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cadmium and lead, are non-essential and their concentrations are physiologically more poorly regulated [2, 3].

Small terrestrial mammals have often been used as bioindicators of pollution, as residues being determined in either the carcass or in specific organs. In addition, they are small, easy to catch, have a territory of limited range, fairly short life span and they are closely adjusted to their environment [4, 5]. The yellow-necked mouse (*Apodemus flavicollis*, *Rodentia*) is slightly larger and more brightly coloured than the wood mouse (*Apodemus sylvaticus*, *Rodentia*). It is found mostly in mountainous areas of southern Europe, but extends north into parts of Scandinavia and Britain. The bank vole (*Clethrionomys glareolus*, *Rodentia*) is a small microtiny rodent that is common throughout Europe. There are only few studies focused on distribution of heavy metals in selected organs of small terrestrial mammals. They are mainly concentrated on accumulation of selected toxic elements in kidney, liver, testis, uterus and teeth [6–10]. The distribution of heavy metals in their bones is only rarely found in the literature [10, 11].

Bones have some advantages over soft tissues (such as liver and kidney) as markers of exposure to environmental pollution. It is generally known that toxic elements become incorporated into the mineral phase of bone tissue which is subject to little turnover. Anyway, an accurate historical record of exposure to various elements is retained in the bone.

In the present study, the concentrations of selected heavy metals in the femora of *Apodemus flavicollis* and *Clethrionomys glareolus* from polluted region Novaky (Slovakia) were analysed.

## Material and methods

The individuals of yellow-necked mouse (*Apodemus flavicollis*) and bank vole (*Clethrionomys glareolus*) were obtained by means of the standard teriological methods and procedures from wood ecosystems [12] in the surrounding of the power station Novaky (Prievidza district, Slovakia). All animals used in the experiment were adult, in good physical condition, without pathological-anatomical changes. Our research focused on 20 femora taken from the adult mammals (12 from *Apodemus flavicollis* and 8 from *Clethrionomys glareolus*). The concentrations of selected heavy metals (Pb, Cd, Ni, Fe, Cu and Zn) were determined with the method of atomic absorption spectrophotometry (Perkin Elmer 4100 ZL) in a graphite furnace. The tissue samples were kept at –18 $^{o}$C until analysis. In the laboratory the samples were dried at 105 $^{o}$C until dry mass was obtained. Then, the bones were weighed (minimum 2 g) and digested in concentrated nitric(V) acid at 90 $^{o}$C for 10 hours. Before the analysis, the samples were diluted to 25 cm$^3$ with distilled water. All metal concentrations were expressed on a dry weight basis in mg $\cdot$ kg$^{-1}$. All samples were measured on the same day. From the final data, basic statistical characteristics were calculated (mean, standard deviation, minimum, maximum, median). Since the distribution of observed levels of heavy metals was normal according to Shapiro-Wilk test, the parametric T-test were used for group comparisions with Statistica 7.0 program.

## Results and discussion

Concentrations of selected heavy metals (Pb, Cd, Fe, Ni, Cu and Zn) in the femora of *Apodemus flavicollis* and *Clethrionomys glareolus* are listed in Table 1. Higher concentrations of Cd, Ni, Cu and Zn were detected in the bones of bank vole. Significant differences were observed for the concentrations of Cd, Ni and Zn ($p < 0.05$). On the other hand, higher concentrations of Pb and Fe were found in the femora of yellow necked mouse. However, the differences were not significant.

Table 1

The concentrations of selected heavy metals in the femora of small terrestrial mammals

| Investigated species | Symbol | Pb | Cd | Ni | Fe | Cu | Zn |
|---|---|---|---|---|---|---|---|
| | | [mg · kg$^{-1}$] | | | | | |
| *Apodemus flavicollis* | x | 20.18 | 2.53 | 7.95 | 156.61 | 3.60 | 126.88 |
| | sd | 3.87 | 0.77 | 1.94 | 31.64 | 0.47 | 10.35 |
| | min. | 15.28 | 1.93 | 6.29 | 115.98 | 2.89 | 110.96 |
| | max | 26.68 | 3.95 | 11.34 | 204.45 | 4.27 | 141.35 |
| | med. | 18.84 | 2.76 | 7.07 | 168.57 | 3.81 | 129.14 |
| *Cleithrionomys glareolus* | x | 20.13 | 4.61* | 9.82* | 138.98 | 3.78 | 176.49* |
| | sd | 9.51 | 1.13 | 1.89 | 10.15 | 0.74 | 11.20 |
| | min. | 14.08 | 3.71 | 7.93 | 128.19 | 3.20 | 164.21 |
| | max | 31.09 | 5.88 | 11.69 | 140.42 | 4.61 | 186.14 |
| | med. | 19.84 | 3.76 | 8.17 | 138.17 | 3.81 | 174.14 |

x – mean, sd – standard deviation, min – minimum, max – maximum, med – median, (*) – $p < 0.05$.

In general, there is a significant relationship between the amount of risk elements in soil, water, also in food and in the organs of mammals, first of all in liver and kidneys [8]. However, lead accumulates mainly in bone [13] and cadmium causes damage primarily to kidney, bone and lungs [14]. Cadmium alters the calcium metabolism in the bone, which leads to osteomalacia [15]. In the study by Milton et al [10] who determined lead, zinc and cadmium concentrations in a range of tissues from wild populations of bank voles trapped on an abandoned metalliferous mine site in United Kingdom, the hierarchy of Pb concentrations was bone > kidney > liver > muscle. The hierarchy of Zn concentration was bone > liver > kidney > muscle and the hierarchy of Cd in tissues was kidney > bone > liver > muscle.

It is generally known that a coal power station Novaky has a negative effect on environmental (mainly soil) pollution resulting from mine work and/or from road traffic. According to Iearadi et al [3], Roberts and Johnson [16] one of the most important sources of environmental contamination with toxic elements is the coal industry. The dust emitted contains zinc, copper, lead and cadmium, and this contamination may increase the content of the elements in the tissues of mammals inhabiting polluted areas.

We observed higher concentrations of Cd and Fe in the femora of *Apodemus flavicollis* from the area of Novaky in comparison with the data published by Damek-Poprawa and Sawicka-Kapusta [11] for the rodents caught in polluted region Bukowno (Poland). On the other hand, Pb and Zn concentrations were higher in their study. In comparison with our previous study [17], higher concentrations of Cd, Ni, Fe, Cu, and Zn were detected in the bones of yellow-necked mice from the area of Kolinany (relatively polluted region in Nitra district which is located approximately 100 km far from the town Novaky). The animals living near the site of the coal power station Novaky disposed only higher concentration of Pb. One possible reason for this phenomenon might be intensive agricultural production and the use of chemicals that is characteristic for the whole region of Nitra. There is also a possibility of fallout of dust transported in the air from big industrial regions such as Bratislava, Vienna, Budapest, or factories nearby the Nitra region. This hypothesis may also be confirmed by studies indicating the possibility of the long range transportation of toxic elements [18]. The concentration of Pb in the femora of *Clethrionomys glareolus* was lower in comparison with the one from the study by Milton et al [10]. On the contrary, Zn and Cd concentrations in the bone were higher in our study.

According to Pokarzhevskij [19] the concentration in animal organism (also in bones) of a given element is practically directly proportional to its contents in the food. The *Apodemus* food could be determined like the "purest" in comparison with all other zoomonitors. This mouse feeds mainly on seeds and fruits. The green parts of the plants which are more dusted are present in lower degree in its feeding. Really, heavy metal concentration in liver of *Apodemus* is in the last place. However, the body heavy metal loading is relatively high [6]. It may be due to lower excretion by the kidneys. It is possible that this factor results in the greater sensitivity of *Apodemus* to heavy metal pollution. On the contrary, the voles are characterized by good excretion and therefore lower contaminants remaining in the organism. But the voles feed mainly on the green parts of the plants. There are polluted in the greatest degree like more accessible to the precipitations, atmosphere dust, etc. Also, seeds and roots are present in the food of bank vole.

It is interesting to note the observations of Sawicka-Kapusta et al [20] who have investigated heavy metal content in rodents populating industrial polluted forests in southern Poland. The authors have recorded that Cd, Pb, Cu and Zn concentrations in *Apodemus flavicollis* are significantly lower than those in *Clethrionomys glareolus*. The same correlations have been established in the study by Metcheva et al [6] who studied heavy metal contentration in the liver and body of small terrestrial mammals living in different Bulgarian regions. These results are in agreement with the data obtained for Central Europe [21]. In our study, higher concentrations of Cd, Ni, Cu and Zn have been detected in the bones of bank vole as compared with yellow-necked mouse. Therefore, we presume that *Clethrionomys glareolus* may also be considered as more bone loaded zoomonitor in comparison with *Apodemus flavicollis*.

In conclusion, our contribution is a pilot study about accumulation of selected heavy metals in the bone of small terrestrial mammals living near the site of the coal power station Novaky. Further research in this direction will need to extend the number of

analysed skeletal elements and to verify the results that were obtained from our skeletal samples.

## Conclusions

Accumulation of selected heavy metals in the femora of small terrestrial mammals (*Apodemus flavicollis* and *Clethrionomys glareolus*) living in polluted region in Slovakia was studied. We observed higher concentrations of Cd, Ni, Cu and Zn in the bones of *Clethrionomys glareolus*. Significant differences were observed for the concentrations of Cd, Ni and Zn ($p < 0.05$). On the contrary, higher concentrations of Pb and Fe were detected in the femora of *Apodemus flavicollis*. However, the differences were not significant. According to our results bank vole may be considered as more bone loaded zoomonitor in comparison with yellow-necked mouse.

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## KUMULACJA WYBRANYCH METALI CIĘŻKICH W KOŚCI UDOWEJ DROBNYCH SSAKÓW LĄDOWYCH

**Abstrakt:** Zbadano kumulację ołowiu, kadmu, żelaza, niklu, miedzi i cynku w kości udowej myszy leśnej (*Apodemus flavicollis*) i nornicy rudej (*Clethrionomys glareolus*) zasiedlających tereny w pobliżu elektrowni Novaky na Słowacji. Zawartość metali ciężkich w kościach zmierzono metodą spektrofotometrii atomowej. Przebadano 20 kości udowych podchodzących od dorosłych osobników. Większe zawartości Cd, Ni, Cu i Zn stwierdzono w kościach nornicy rudej niż w kościach myszy leśnej. Istotne statystycznie różnice między badanymi ssakami dotyczyły zawartości Cd, Ni i Zn ($p < 0.05$). Z drugiej strony kości myszy leśnej zawierały więcej Pb i Fe niż kości nornicy rudej. Różnice te nie były jednak istotne statystycznie. Uzyskane wyniki wskazują, że kości nornicy rudej kumulują więcej metali ciężkich niż kości myszy leśnej, co może mieć znaczenie dla przyszłych badań monitoringowych.

**Słowa kluczowe:** metale ciężkie, kości, mysz leśna, nornica ruda, środowisko