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The Effect of Contamination of the Kraków Region on Heavy Metals Content in the Organs of Bank Voles (*Clethrionomys glareolus*, Schreber, 1780)

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Abstract

Tests were carried out on the levels of cadmium, copper, iron and zinc in selected organs (liver, kidneys and spleen) of bank voles (*Clethrionomys glareolus*, Schreber, 1780) caught in autumn 1998 and 1999 in a number of forests situated at varying distances east and south of Kraków. All the results were compared with the levels of these metals present in voles caught from a control area of Borecka Forest. Cadmium content was determined with a graphite tray, using a Perkin Elmer AAnalyst 800 atomic absorption spectrophotometer, after wet digestion of samples in a mixture of inorganic acids (HNO_3 : $HClO_4$). The concentrations of physiological elements (Cu, Fe, and Zn) were analyzed using the flame method of an IL-251 atomic absorption spectrophotometer. The levels of heavy metals present depended on the organ being tested and the area in which the rodents were caught. The relationships between the content of these elements and the age and sex of the animals were also observed.

Keywords: pollution, heavy metals, cadmium, bank vole, bioindicator, the Kraków region

Introduction

The dynamic development of industry and motorization, as well as the continuing over-intensive use of various chemical compounds in agriculture, cause levels of toxic heavy metals in the environment to constantly be on the increase. Cadmium is on the list of the most dangerous metals, causing acute and chronic environmental contamination [1]. Copper, iron and zinc are among the physiological elements essential for the correct functioning of living organisms. The high concentrations of these metals can also be harmful to organisms, but it is more often their deficiency that causes interactions between them and toxic metals. Increased content of heavy metals can disrupt the absorption of physiological elements and as a consequence the deficiencies of these latter can intensify the toxicity of harmful metals [2,3].

Both natural and anthropogenic factors have a significant influence on pollution levels in the urban-industrial area of Kraków. The city, located in the Vistula valley, is characterized by poor air circulation, with weak winds blowing mainly among the west-east axis. Worse, it is along this axis that the main sources of atmospheric pollution are situated: the industrial region of Silesia begins 30 km to the west of Kraków, 15 km away to the south-west is the Elektrownia "Skawina" S.A. power plant, and at a distance of 125 km the heavy industry region of Morawska Ostrawa. Little more than 10 km east of the centre of

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Kraków are the Huta im. T. Sendzimira steelworks and the Elektrociepłownia "Kraków" S.A. power plant. At a distance of about 70 km to the east, the Małopolska Upland ends in the highly industrialised region of Tarnów. Another serious problem in the whole province, especially in Kraków itself, is motor transport. Another factor of some importance is low emission, which is difficult to estimate [4,5].

In order to be in a position to detect the earliest risks, it is necessary to estimate the extent to which living organisms are exposed to them [6,7]. An advantage of biological methods is the fact that living organisms, in addition to fulfilling monitoring functions, record the degree of changes in their surroundings and react directly to environmental pollution. Animals living in different ecosystems are bioindicators of their environments. Food and water are the main sources of exposure to the action of various toxic compounds, especially heavy metals.

Bank voles (*Clethrionomys glareolus*) satisfy all the conditions of a good bioindicator: they are small, easy to catch, inhabit a small area, and have a short lifespan [8,9,10].

The aim of this study was to assess the pollution of a number of forest areas situated at varying distances from Kraków, on the basis of the heavy metals content in the organs of bank voles (*Clethrionomys glareolus*).

Material and Methods

Study Areas

The bank vole (*Clethrionomys glareolus,* Schreber, 1780) was chosen for this study. The animals used in this investigation were caught in the autumn of 1998 and 1999 from seven woodland areas. The study areas were divided into two zones – southern and eastern, taking into consideration the distance from the centre of Kraków, the similarity of the habitats, predominant wind direction and proximity of significant sources of contaminants emission.

The woodland areas, which were qualified under the southern zone (Rajsko, Mogilany, Kornatka and Węglówka) are under the influence of the urban conurbation and motor contaminants from the Kraków-Zakopane clearway. The points of study situated east of Kraków (Staniątki, Koło, Ispina) were under the influence of dusts and gas contaminants emitted from the GOP (Upper Silesian Industrial Region) industrial area as well as from Kraków industry, chiefly the Huta im. T. Sendzimira steelworks [4,5]. As a control region, the Borecka Forest, located in Pojezierze Mazurskie (the Mazurian Lake District) and counted among Poland's cleanest areas, was selected [11].

Chemical Analysis

After being caught, the animals were weighed, and the livers, kidneys and spleens were removed and used to determine the content of cadmium and physiological metals (copper, iron and zinc) using the spectrophotometric method, after wet digestion in a 4:1 HNO_3 : $HClO_4$ solution [12]. The cadmium concentrations of the organs were determined in a Perkin Elmer AAnalyst 800 AAS with a graphite tray, at a wavelength of 228.8 nm, while the contents of physiological elements (Cu, Fe, Zn) were analyzed by the flame method, with an IL-251 AAS at the following wavelengths: Cu – 324.7 nm, Fe – 248.3 nm, Zn – 213.9 nm.

On the basis of the lens mass, the age of the bank voles caught was estimated; the animals were divided into three age ranges:

I - animals up to the third month of life,
II - 4-6-month-old voles, and
III - 7-10-month-old animals.

The bank voles caught in 1998 and 1999 were divided into 2 groups by sex: males (M) and females (F).

Statistical Analysis

As there were no significant differences in the content of the metals between the two years in which research was carried out, all the results received were expressed as arithmetical means. 96 rodents were tested. For the statistical analysis of the results obtained, an analysis of variance and Tukey's test (*a posteriori*) were used, or in the case of heterogeneous data, the nonparametric alternative to straight classification, i.e. the Kruskal–Wallis test [13,14].

All the calculations were carried out using the computer programme Statistica 5.0. for Windows.

Results

The values of the average concentrations of selected heavy metals in the livers, kidneys and spleens of the bank voles caught in areas situated at the various different distances from Kraków were compared with each other and with the results gained in the control area (Tab. 1).

The cadmium content differed depending on the organ and varied from 0.23 μg g^{-1} in the spleen to 7.76 μg g^{-1} in the kidneys. The average concentration of Cd in the livers of animals caught in the Kraków area ranged from 0.71 μg g^{-1} in Staniątki to 2.38 μg g^{-1} in Rajsko, while the equivalent figure from the Borecka Forest was - 0.50 μg g^{-1}. Statistically higher concentrations of this metal than in the control area were affirmed in all the areas under study with the exception of Staniątki. The highest cadmium quantities in the kidneys of *Clethrionomys glareolus* were recorded in Rajsko (7.76 μg g^{-1}) and the lowest in Staniątki (3.15 μg g^{-1}). In Borecka Forest the content of cadmium was 2.99 μg g^{-1} on average. No statistically significant differences between the areas analyzed were affirmed. In the spleens of bank voles from woodland areas near Kraków the levels of cadmium ranged from 0.93 μg g^{-1} in Kornatka to 2.98 μg g^{-1} in Mogilany. All the results were significantly higher than the result recorded in the control area (0.23 μg g^{-1}).

Analysis of the copper concentrations in the investigated samples showed that the lowest quantities of this element were recorded for the spleens (8.4 μg g^{-1})

Table 1. The average concentrations of Cd, Cu, Fe and Zn (μg g^{-1} d.m.) in the selected organs of bank vole from the areas situated at varying distances from Kraków.

Organ	Area	N	Cadmium $X_{śr.} \pm SE$	Copper $X_{śr.} \pm SE$	Iron $X_{śr.} \pm SE$	Zinc $X_{śr.} \pm SE$
Liver	Rajsko	1	2.38	22.2	636	90
	Mogilany	6	1.57 ± 0.288 bc	24.4 ± 1.15 bc	602 ± 24.2 a	94 ± 5.0 a
	Kornatka	16	1.48 ± 0.228 bc	21.1 ± 1.00 bc	542 ± 29.8 a	86 ± 3.3 a
	Węglówka	18	1.78 ± 0.212 b	21.6 ± 0.76 bc	614 ± 29.7 a	89 ± 2.2 a
	Staniątki	11	0.71 ± 0.124 ac	18.5 ± 0.87 ac	571 ± 20.8 a	92 ± 3.4 a
	Koło	10	1.75 ± 0.260 b	19.5 ± 1.25 ac	637 ± 29.9 a	89 ± 6.2 a
	Ispina	14	1.10 ± 0.209 bc	21.9 ± 0.96 bc	638 ± 33.6 a	90 ± 2.7 a
	Borecka Forest	10	0.50 ± 0.125 a	15.6 ± 1.07 a	505 ± 33.6 a	83 ± 4.5 a
Kidneys	Rajsko	1	7.76	18.3	426	79
	Mogilany	6	5.69 ± 1.280 a	21.9 ± 1.59 bc	496 ± 35.3 a	97 ± 4.4 b
	Kornatka	16	5.91 ± 1.021 a	21.6 ± 0.72 bc	384 ± 25.4 ab	77 ± 1.8 d
	Węglówka	18	5.22 ± 1.009 a	19.8 ± 0.50 bc	321 ± 13.2 b	83 ± 1.3 bd
	Staniątki	11	3.15 ± 0.586 a	16.2 ± 0.56 a	389 ± 20.1 ab	95 ± 3.6 b
	Koło	10	5.65 ± 1.244 a	22.2 ± 1.13 bc	423 ± 19.4 a	92 ± 4.2 b
	Ispina	14	4.20 ± 1.025 a	20.6 ± 1.03 bc	455 ± 19.0 a	91 ± 1.8 b
	Borecka Forest	10	2.99 ± 0.573 a	14.3 ± 0.42 a	334 ± 13.3 b	54 ± 2.2 a
Spleen	Rajsko	-	-	-	-	-
	Mogilany	6	2.98 ± 0.881 bc	18.8 ± 1.45 b	507 ± 16.0 a	106 ± 2.8 ac
	Kornatka	10	0.93 ± 0.246 b	14.7 ± 0.56 ab	288 ± 8.0 b	72 ± 3.0 d
	Węglówka	16	1.99 ± 0.607 bc	13.8 ± 0.46 ab	365 ± 17.4 b	92 ± 3.0 bc
	Staniątki	11	1.36 ± 0.164 b	17.4 ± 1.70 b	356 ± 10.2 b	82 ± 2.2 bd
	Koło	9	1.65 ± 0.299 b	11.2 ± 1.05 ac	306 ± 7.2 b	80 ± 3.1 bd
	Ispina	13	1.83 ± 0.289 b	16.2 ± 0.60 b	638 ± 10.4 a	87 ± 1.8 bc
	Borecka Forest	10	0.23 ± 0.077 a	8.4 ± 0.39 c	526 ± 7.8 a	113 ± 7.4 a

N – the number of animals; a, b, c, d – the same letters mean no statistically significant differences between the areas within the given organs; (P > 0,05).

and the highest for the livers (24.4 μg g^{-1}) (Tab.1). In the livers of *Clethrionomys glareolus* the lowest copper contents were obtained in Staniątki (18.5 μg g^{-1}) and the highest in Mogilany (24.4 μg g^{-1}). The values recorded for the Kraków area did not differ greatly from each other, but they were significantly higher (except for the copper concentrations in Staniątki and Koło) than the average value in Borecka Forest (15.6 μg g^{-1}). The levels of copper in the kidneys of animals from the areas near Kraków ranged from 16.2 μg g^{-1} in Staniątki to 22.2 μg g^{-1} in Koło. The copper concentrations in Staniątki and Borecka Forest (14.3 μg g^{-1}) were significally lower than the values recorded elsewhere. In the spleens of bank voles from the urban area, by far the lowest quantities were recorded for animals in Koło (11.2 μg g^{-1}), and only this value was not significantly different from the results gained in the control area (8.4 μg g^{-1}).

The iron content in the rodents' organs ranged from 288 μg g^{-1} (spleen) to 638 μg g^{-1} (liver, spleen) (Tab.1). The minimum concentrations of Fe in the livers of bank voles caught near Kraków amounted to 542 μg g^{-1} in Kornatka, while the maximum value was 638 μg g^{-1} in Ispina. The quantities recorded for the woodland areas around Kraków differed neither from each other nor from those recorded in the control area (505 μg g^{-1}). The highest level of iron was found in the kidneys of

Table 2. The accumulation of heavy metals (μg g^{-1} d.m.) in the liver, kidneys and spleen of bank voles according to age.

Organ	Age class (months)	N	Cadmium	Copper	Iron	Zinc
Liver	1 – 3	49	1.02 a	20.5 a	546 a	89 a
	4 – 6	29	1.44 b	20.2 a	636 b	90 a
	7 – 10	18	1.29 b	18.0 a	572 ab	85 a
Kidneys	1 – 3	49	4.06 a	18.9 a	387 a	78 a
	4 – 6	29	3.94 a	18.8 a	384 a	81 a
	7 – 10	18	6.27 b	17.6 a	362 a	76 a
Spleen	1 – 3	45	1.38 a	13.5 a	434 a	92 ab
	4 – 6	26	1.11 b	14.4 a	478 a	89 a
	7 – 10	16	1.38 a	13.6 a	461 a	107 b

N – the number of animals; a,b – the same letters mean no statistically significant differences between the age classes within the given organs; (P > 0,05).

animals caught in Mogilany (496 μg g^{-1}), and the lowest in Węglówka (321 μg g^{-1}). The concentration of Fe in Borecka Forest (334 μg g^{-1}) was significantly lower than the values from Ispina, Koło and Mogilany. The iron content in the spleens ranged from 288 μg g^{-1} in Kornatka to 638 μg g^{-1} in Ispina. The value recorded for Borecka Forest (526 μg g^{-1}) did not differ significantly from the results obtained in the samples from Mogilany and Ispina.

The levels of zinc were even for all the organs (Tab. 1). In the livers of *Clethrionomys glareolus* the average concentrations of this metal ranged from 86 μg g^{-1} in Kornatka to 94 μg g^{-1} in Mogilany. The zinc content in Borecka Forest was 83 μg g^{-1}. No statistically significant differences between the different points analyzed within the Kraków area or in comparison with the control area were noted. The average concentrations of zinc in the kidneys of bank voles ranged from 77 μg g^{-1} in Kornatka

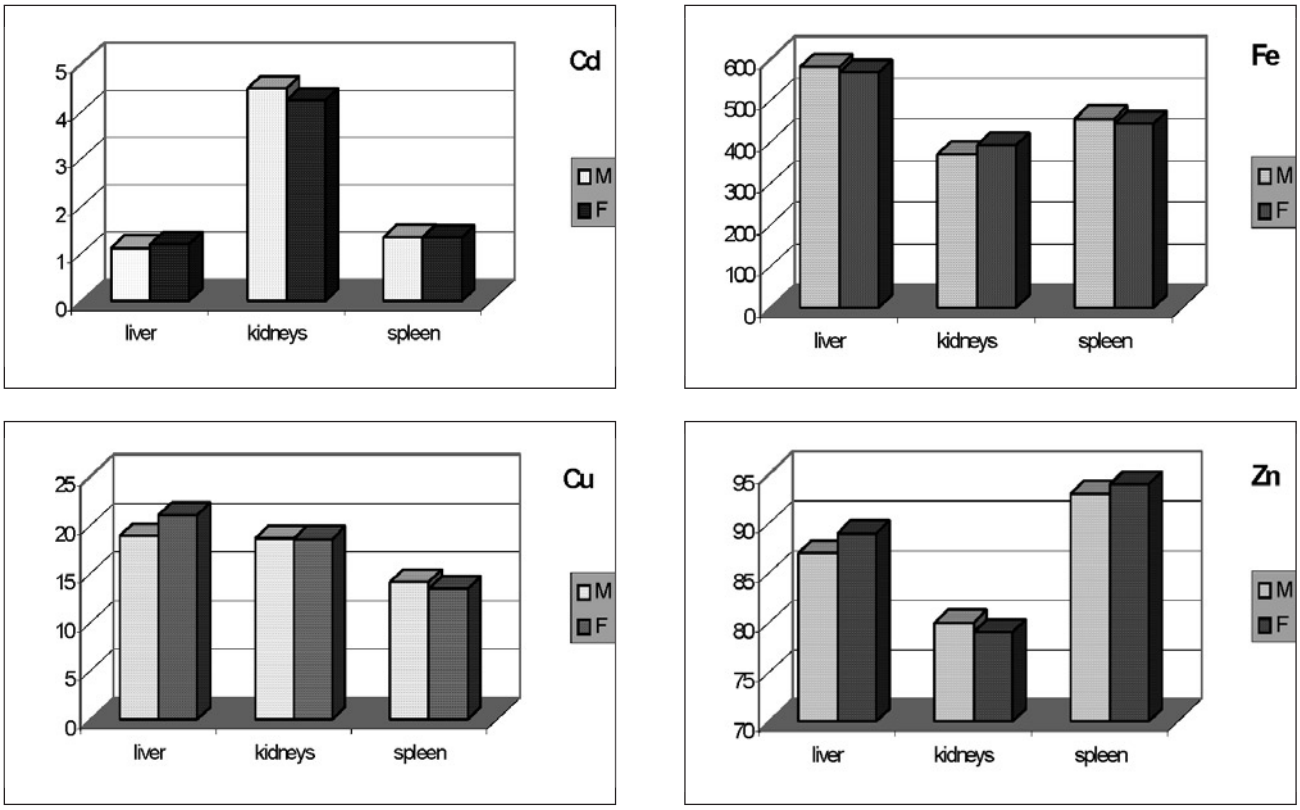

Fig.1. The accumulation of selected heavy metals (μg g^{-1}d.m.) in the liver, kidneys and spleen of bank voles according to sex.

to 97 μg g^{-1} in Mogilany. The content of this element in the kidneys of animals from the areas around Kraków in comparison with samples from the control area (54 μg g^{-1}) was not significantly higher. The highest Zn content in the spleens of bank voles from the forests around Kraków was 106 μg g^{-1} in Mogilany, and the lowest 72 μg g^{-1} in Kornatka. All the recorded values, with the exception of the zinc level in the organs of the rodents from Mogilany, were significantly lower than the level obtained in Borecka Forest (113 μg g^{-1}) (Tab. 1).

The relationships between the heavy metal content and the age (Tab. 2) and sex of the animals (Fig. 1) were observed.

Statistical analysis showed significant differences between the level of cadmium in organs depending on vole age (Tab. 2). In the livers of the youngest animals (to the third month of their life) cadmium content (1.02 μg g^{-1}) was significantly lower than in older animals; a similar tendency was observed in the case of the content of this element in the kidneys. Both in the livers and in the kidneys of bank voles, a decreasing tendency of copper quantities was observed as the animals' age increased. The average iron concentrations in the livers ranged from 546 μg g^{-1} in the youngest voles to 636 μg g^{-1} in older animals (those from the second age range). In the kidneys the level of Fe decreased as the animals grew older. The content of zinc in the animals analyzed did not differ significantly between the individual age classes (Tab. 2).

A lack of significant differences in the concentrations of metals between sexes within investigated organs was noted (Fig. 1). The lowest cadmium concentrations were recorded for the livers of bank voles – 1.12 μg g^{-1} in males and 1.20 μg g^{-1} in females. In the case of the kidneys and spleens the content of this metal was somewhat higher in males than in females, in contrast with the copper levels in the livers – 18.9 μg g^{-1} in males and 21.1 μg g^{-1} in females. The concentrations of this metal in the kidneys and spleens were almost equal in both sexes. The iron levels in the livers and spleens of males were higher than in females. However, the concentrations of Fe in the kidneys were higher in females (391 μg g^{-1}) than in males (369 μg g^{-1}). The average zinc concentrations were identical in both sexes and very evenly weighted between the organs (Fig. 1).

Discussion

An Estimation of the Pollution of Woodland Areas on the Basis of the Heavy Metal Content of the Organs of Bank Voles

The various cadmium concentrations in the livers of bank voles caught in the forest areas situated south of Kraków were almost the same high level, regardless of distance from the urban area. They were somewhat higher than noted in the livers of bank voles from the areas situated east of the city, possibly because of the extremely high proportion of wet cadmium deposition in these areas. Thus the concentrations noted in the livers and kidneys of animals from Koło and Ispina did not provide unambiguous evidence of the influence of the HTS steelworks, which is possibly due to the fact that the molecules of this metal are very small. This causes it to act like a gaseous substance and it is transmitted far away from the source of emission. Only in the spleens of animals caught in these areas in 1999 were significantly increased concentrations of cadmium found in comparison with samples from the southern zone (Tab. 1).

According to Turzański [4], not only the most polluted western part of the Kraków area but also the southern part reveals an increased concentration of contaminants. This is caused by the northwesterly direction of the wind that is predominant in this area from May to September and is responsible for carrying contaminants from outside the area of the former Kraków Province, mainly from Silesia. All the results obtained concerning the content of cadmium in the livers of *Clethrionomys glareolus* showed that the Kraków area is exposed to a significantly higher quantity of cadmium than Borecka Forest, situated in the cleanest region of our country (in the northeast). Research conducted by monitoring stations affirms that the total fall of cadmium in Borecka Forest was lower than the minimum concentration of this metal recorded for the areas studied [4, 11].

In analyzed organs the content of copper, like that of cadmium, showed a higher accumulation in the areas situated south of Kraków than in regions located in the eastern part of the former Kraków Province (Tab. 1); it may supposed that the source of the emission of copper is not only local, but also from further away [15]. Copper is deposited in both dry and wet forms. According to Turzański and Godzik's [15] study, the proportion of the total amount of Cu deposited is made up of dry deposition much higher not far from the centre of Kraków (hence a higher concentration of Cu in Mogilany, located 14 km from Kraków, than in Węglówka, which is 34.5 km away). Analysis of the copper content in the organs of bank voles showed that in all locations (except in Staniątki and Koło in 1999) the concentrations of this metal in the livers and kidneys were significantly higher than in Borecka Forest (Tab. 1). In comparison with the dry deposition of copper in the vicinity of Kraków, the deposition of this element was significantly lower in Borecka Forest [4,11]. In all the woodland areas studied in the vicinity of Kraków, similar concentrations of iron in the livers of bank voles were found; neither did the results differ from the content of this metal found in Borecka Forest (Tab.1). The results of ecological monitoring showed that there is a bigger load of dust containing iron compounds near HTS and in the eastern part of the province. Ispina is nearest to the Huta im. T. Sendzimira steelworks, east of the factory and the urban area, which in connection with the predominance of winds blowing from west to east places this area in a position of extremely high exposure to dust fall containing Fe. As the level of iron in the organs of bank voles from Ispina ar-

eas was not significantly different in comparison with the other areas, it is to be supposed that the concentrations of iron in the environment were not very high despite the neighbourhood of the Huta im. T. Sendzimira steelworks. The results obtained from the research into bank voles confirmed data from ecological monitoring, which also indicated a low deposition of Fe with dust on the southern limits of the province [4]. The concentrations of zinc in bank voles in the studied areas were almost equal, only in the spleens were significantly lower concentrations of this metal found in comparison with the results from Borecka Forest (Tab. 1). Taking into consideration the fact that most zinc in the province was deposited with dry fall, it is probable that the main danger connected with emission of this element is posed by local sources. Data from monitoring showed that the lowest fall of this metal was in the southern part of the province, whereas its highest value was in the direct neighbourhood of the Huta im. T. Sendzimira steelworks [4].

Environmental pollution with physiological elements (e.g. Cu, Fe, Zn,) in contrast to toxic elements (e.g. Cd) had minimal influence on their content in the organs, which depended not only on food supply, but on physiological processes in the individual specimen. Animals are capable of homeostatic regulation of the level of metals responsible for the correct functioning of these processes. It is therefore important to remember that it is difficult to draw conclusions about the accumulation of copper, iron and zinc in the web chain on the basis of differences in their concentrations.

The Accumulation of Heavy Metals in the Organs of Bank Voles According to Age and Sex

Toxic metals have a tendency to accumulate in animals' bodies with age [16]. Cadmium is one example of this because its content, especially in the livers and kidneys, was considerably higher in older animals than in younger ones [3,17]. Statistical analysis of the results obtained in this study also showed that cadmium concentrations in the livers of the youngest bank voles were significantly lower than the values in older animals. In the case of the kidneys there was a significant difference between individuals from the two lower age groups and the oldest animals (Tab. 2). Similar results were obtained by Hunter [17] in his investigations carried out on *Apodemus sylvaticus, Microtus agrestis* and *Sorex araneus* and by Milton amd Johnson [18]. In the livers and kidneys the highest copper content was found in the youngest animals, just as in the case of the average iron concentrations in the livers. However, in the kidneys the concentrations of Fe decreased with the age of bank voles. Zinc content in the organs appeared to be constant and independent of their age. This reflects the evidence of non-accumulation and homeostatically regulated consumption of physiological elements (Tab. 2).

One of the aims of this study was to estimate the influence of the sex of the animal on the accumulation of heavy metals, especially cadmium. Owing to the fact that males and females have different food preferences in different seasons, it would appear that the content of heavy metals in the bodies of bank voles depends on their sex. It has been confirmed that in the spring and summer females eat more animal food than males [19]. On the basis of considerations from the previous chapter regarding the interdependence of heavy metal accumulation on diet composition, it could be propounded that the higher cadmium accumulation in the females' bodies is due to their larger consumption of invertebrates, which are more contaminated with this element than plants. The results obtained in this study confirmed that the content of cadmium is higher in females, but this concerned only the livers (Fig. 1). In the case of the kidneys the situation was the opposite – larger quantities of this element were accumulated in males. It should be emphasized that the differences between the sexes were not statistically significant. Significantly higher contents of physiological elements in the females' organs were not noted (Fig. 1).

Conclusions

The highest cadmium concentrations were found in the livers of animals from Rajsko and Węglówka, and the lowest in bank voles from Staniątki, which means that the areas situated along the southern zone are somewhat more contaminated with cadmium than those east of Kraków. The Kraków region is much more loaded with this toxic metal than Borecka Forest.

The analysis of the copper content also showed higher accumulation in the areas in the southern zone in comparison with the points of study located in the eastern part of the province.

However, the highest concentrations of iron and zinc were found in the organs of animals examined from the vicinity of the Huta im. T. Sendzimira steelworks.

Relationships between the accumulation of heavy metals in the tissues of bank voles and their sex and age were observed. The cadmium concentrations increased with the age of the animals, as did the copper content in the livers and kidneys and iron in the livers. In the kidneys the level of Fe decreased in the higher age classes of bank voles. Zinc levels appeared to be constant, independent of age.

The content of heavy metals in the bank voles' organs in general conformed with the level of contamination in the Kraków region, which confirmed the legitimacy of the selection of *Clethrionomys glareolus* as a good bioindicator with a useful role to play in the estimation of pollution of woodland areas and in making possible the prediction of changes at the level of higher links of the trophic chain.

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