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Chapter 9

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Bioindication of the Environment Contamination by Heavy Metals

Bioindicating of Heavy Metal Contamination of the Environment

Technical monitoring provides information about the quantity of multifarious chemical elements as well as chemical compounds present in the natural environment. These data are indispensable for the evaluation of air, water and soil pollution, although they do not provide information about the influence of toxic chemical compounds on living organisms. Biological monitoring completes these studies and supplies information about the integrated effect of all environmental factors on living organisms. Bioindication consists in the assessment of the value of the natural environmental by means of biological tests which, in turn, enable evaluating the level of many substances introduced to ecosystems from industrial, agricultural and communication sources. Living organisms employed in biomonitoring record the extent of intensification of changes in their habitat and show a direct response to environmental contamination. In order to be reliable, a bioindicator should be common in the analyzed area, tolerate some level of contamination as well as display detectable responses to changes in the contamination degree – changes in the appearance, structure or chemical composition. Thus, lichen, as well as organisms of plant and animal origin, may serve as bioindicators.

Grodziński and Yorks (1981) and Ten Houten (1983) classified bioindicators into three main categories: **species scales** (species composition of organisms changes depending on contaminant concentration), **real indicators** (organisms that show a varied degree of damage depending on the level of contamination), and **accumulators** (organisms accumulating contaminants in their tissues which can be quantified). On the other hand, Martin and Coughtrey (1982) postulated the following classification of bioindicators: **indicators** (species that show a strong response to increasing contamination and dye in the contaminated environment) and **monitors** (organisms that enable monitoring changes in the degree of environment degradation).

Animal bioindicators may be representatives of the invertebrates as well as of the vertebrates. Amongst the invertebrates, common use has been made of earthworms and anthropoda that were applied as indicators of soil and litter contamination with heavy metals. Nowadays, earthworms, snails and a few more species of anthropoda, springtails in particular, are employed in biomonitoring studies as well.

In biomonitoring studies, the vertebrata have been used for approximately 30 years. They include small rodents and birds. Deer antlers and magpie feathers have been found to be very good indicators of environment contamination with heavy metals.

Small mammals are a reliable bioindicator as they have a small body size, can be caught easily, occupy a restricted territory, and what is more, are characterized by a relatively short lifespan. According to these criteria, potential good bioindicators are three rodent species: wood mouse (*Apodemus sylvaticus*), bank vole (*Myodes glareolus*) and field vole (*Microtus agrestis*). These animals are widely used for the determination of the level of environment contamination based on the concentration of heavy metals in different organs, tissues or in whole bodies. For biomonitoring studies, mainly the detoxifying organs (kidneys and liver, but also bones, fur, muscles and brain) are employed due to their high capability for accumulating heavy metals.

Small mammals were used in studies assessing the heavy metal content in the environment adjacent to iron works, mines and post-mine waste-tips. The carnivorous mammals which are placed in a higher position of the trophic chain are frequently employed in biomonitoring studies as well. This enables an additional risk evaluation of the higher links in the trophic chain, which is highly significant from an ecological point of view. Shrews have been applied in the assessment of the degree of contamination with trace elements of grassy ecosystems located in the proximity of post-mine waste-tips in which metal wastes had been stored. In the 1970s, the content of lead was analyzed in tissues of carnivorous mammals living close to roads. Currently, studies using small mammals as bioindicators are undertaken as well. These studies are conducted in the area of post-mine waste-tips, in the vicinity of abandoned mines, on waste dumping sites or close to power stations. Rodents (*i.e. Myodes glareolus* and *Apodemus sylvaticus*) as well as shrews (*i.e. Sorex sp*. or *Crocidura russula*) are used for such studies. In Poland, small mammals are employed in biomonitoring studies as well. Rodents have been used for the assessment of the contamination degree of different Polish forest terrains as well as in environments close to zinc and steel mines. On the basis of heavy metal contamination in the voles and common shrews, a comparison of the environmental contamination of Polish National Parks has been conducted. Rodents have also been used in the assessment of the influence of a pesticide dump on animals.

The aim of the study was to assess the level of selected heavy metals in tissues of bank voles (*Myodes glareolus*) caught in the area affected by a pesticide dump at different times of its existence and to successively evaluate if, and to what extent, the liquidation of the pesticide dump affected the status of the adjacent environment.

Bank vole (*Myodes glareolus*) as a bioindicator of heavy metal contamination of the environment near a pesticide dump

The study was performed in the proximity of a pesticide dump located in Warlity Wielkie (ca. 6 km from Ostróda, Poland) at three different experimental periods (more information about the study area in Chapter 5). The first period (2002-2004) covered the years before the closure of the dump, the second (2005) – the spring and autumn after closing down, and the final (2006) – two years after closing down the pesticide dump. The studies were conducted in three zones, in the east-west transect, located ca. 50 m (zone A) and 500 m (zone B) away from the pesticide dump and in the control zone (zone K) situated in an area not influenced by the pesticide dump. In 2002-2005, the control area was located approximately 4 km south-east of the study area. In 2006, the new control area was established, south-west of the liquidated dump. The voles were caught with the trapline method. In 2002-2006, the catch was performed in October and in 2005 an additional one was performed in May.

Samples (liver, kidneys, thigh bones and faeces) were mineralized after drying in a mixture of spectrally clean, concentrated acids HNO_3+$HClO_4$ (volume ratio 4:1). AAS Perkin Elmer AAnalyst 800 with graphite cuvette was applied for cadmium and lead determination. Zinc, copper and iron were analyzed with the flame method using an IL 251 spectrophotometer. Reference material (Bovine Liver 1577 b) was evaluated as well. Recovery was at a level of 90-105%.

In order to demonstrate statistically significant differences, a two-way analysis of variance was applied, followed by an *a posteriori* Tukey's test. In the case of heterogeneous data, use was made of a non-parametric Kruskal-Wallis test.

The concentration of heavy metals in tissues and faeces of bank vole caught in the autumn at different experimental periods

The highest concentrations of **cadmium** were found in all analyzed samples originating from animals trapped in October 2005, *i.e.* nearly one year after the closure of the pesticide dump. Despite the fact that the values obtained were much higher than those obtained in 2002-2004 and 2006, in most cases no statistically significant differences were noted (Table 1).

In bank voles' kidneys, the concentration of cadmium was within the range from 0.85 $mg \cdot kg^{-1}$ (control sample, 2002-2004) to 4.13 $mg \cdot kg^{-1}$ (zone A, 2005). The cadmium concentration in liver was between 0.17 $mg \cdot kg^{-1}$ (zone B, 2002-2004) to 2.20 $mg \cdot kg^{-1}$ (zone A, 2005). However, although in the period before closing down the pesticide dump the conducted survey indicated differences in cadmium concentration in kidneys and liver of the bank voles, the statistical analysis of data from the further periods did not show any differences, which suggests an increase in the cadmium content of bank vole tissues after closure (Table 1).

Table 1

Cadmium concentrations (mg·kg^{-1} d.w.) in tissues and faeces of *Myodes glareolus*

Tissue	Year	Zone A	Zone B	Control area
Kidneys	2002-2004	**2.44** [1,a] ± 0.43	**1.32** [1,a,b] ± 0.21	**0.85** [1,b] ± 0.22
		N = 40	N = 31	N = 19
	2005	**4.13** [1] ± 1.01	**2.30** [1] ± 0.79	**1.63** [1] ± 0.27
		N = 8	N = 3	N = 9
	2006	**1.70** [1] ± 0.43	**1.79** [1] ± 0.55	**1.22** [1] ± 0.24
		N = 6	N = 6	N = 8
Liver	2002-2004	**0.28** [1,a] ± 0.04	**0.17** [1,b] ± 0.03	**0.26** [1,a,b] ± 0.10
		N = 40	N = 31	N = 19
	2005	**2.20** [2] ± 0.70	**0.45** [1] ± 0.18	**0.65** [1] ± 0.22
		N = 8	N = 3	N = 9
	2006	**0.19** [1] ± 0.02	**0.27** [1] ± 0.09	**0.19** [1] ± 0.03
		N = 6	N = 6	N = 8
Faeces	2002-2004	n.d.	n.d.	n.d.
	2005	**4.30** [1] ± 1.25	**0.46** [1] ± 0.04	**2.19** [1] ± 0.49
		N = 8	N = 3	N = 9
	2006	**0.30** [2,a] ± 0.04	**1.05** [1,b] ± 0.35	**0.62** [2,a,b] ± 0.17
		N = 6	N = 6	N = 8

n.d. – no data
[1,2] – different numbers indicate statistical differences in levels of the metal between years ($p < 0,05$)
[a,b] – different letters indicate statistical differences in levels of the metal between zones ($p < 0,05$)

The mean cadmium concentration in liver of the bank voles from zone A, caught in 2005 in Warlity (Poland) was only slightly lower when compared to the mean values obtained for livers of animals collected from the Starczynowska Desert (Poland) – area influenced by the Mining and Metallurgic Plant ”Bolesław” in Bukowno near Olkusz (Poland). In turn, the content of cadmium in kidneys of the bank voles from the contaminated area was considerably higher than in those of the animals originating from Warlity. The concentrations of cadmium excreted with faeces were within the lower range of values obtained for the bank voles from Olkusz Forests.

Concentrations of cadmium in kidneys and livers of animals trapped in 2002-2004 were at a level similar to that those noted in soft tissues of bank voles collected from areas used as a control for this type of studies. Klimkówka, located in Podkarpacki Province (Poland), is recognized as such an uncontaminated area. Animals caught in the autumn of 2006 in this area were found to accumulate cadmium at an average level of 0.87 mg·kg^{-1} in kidneys, 0.41 mg·kg^{-1} in liver, and 2.04 mg·kg^{-1} in faeces.

The highest levels of **lead**, especially in zone A, were found in tissues and faeces collected from bank voles caught in 2005. Nevertheless, as in the case of cadmium, the results obtained were not statistically significantly different between the analytical periods (Table 2). Both in kidneys (3.12 $mg \cdot kg^{-1}$) as well as in liver (3.19 $mg \cdot kg^{-1}$) of voles collected in zone A in 2005, the concentrations of lead were significantly higher in comparison to those noted in tissues of voles caught in zone B (Table 2). The concentration of lead in kidneys of bank voles caught in the other analytical periods were within the range of 0.18 to 1.80 $mg \cdot kg^{-1}$ whereas in livers were at a level of 0.05 to 0.35 $mg \cdot kg^{-1}$. These values were comparable to those obtained for tissues of animals originating from Klimkówka (0.72 $mg \cdot kg^{-1}$ for kidneys and 0.30 $mg \cdot kg^{-1}$ for liver on average). The bank voles caught in the contaminated area (Starczynowska Desert) accumulated lead at an average level of 15.17 $mg \cdot kg^{-1}$ in kidneys and 3.51 $mg \cdot kg^{-1}$ in liver.

In the case of both cadmium as well as lead, their excretion with faeces increased considerably in the voles caught in 2005 in zone A and also in the control zone. A similar situation was noted in 2006 in zone B (Table 1 and 2).

Table 2

Lead concentrations ($mg \cdot kg^{-1}$ d.w.) in tissues and faeces of *Myodes glareolus*

Tissue	Year	Zone A	Zone B	Control area
Kidneys	2002-2004	**1.03** [1] ± 0.20	**0.71** [1] ± 0.16	**1.80** [1,2] ± 0.88
		N = 40	N = 31	N = 19
	2005	**3.12** [1,a] ± 0.62	**0.07** [1,b] ± 0.03	**1.47** [1,a,b] ± 0.39
		N = 8	N = 3	N = 9
	2006	**0.45** [1] ± 0.10	**0.18** [1] ± 0.08	**1.16** [2] ± 0.05
		N = 6	N = 6	N = 8
Liver	2002-2004	**0.20** [1] ± 1.05	**0.22** [1] ± 0.05	**0.35** [1,2] ± 0.16
		N = 40	N = 31	N = 19
	2005	**3.19** [1,a] ± 1.04	**0.02** [1,b] ± 0.01	**0.70** [1,a,b] ± 0.24
		N = 8	N = 3	N = 9
	2006	**0.10** [1] ± 0.02	**0.05** [1] ± 0.02	**0.05** [2] ± 0.01
		N = 6	N = 6	N = 8
Bones	2002-2004	**1.27** [1] ± 0.26	**0.49** [1] ± 0.06	**1.36** [1] ± 0.41
		N = 40	N = 31	N = 19
	2005	**2.12** [1] ± 0.70	**0.04** [2] ± 0.02	**1.38** [1] ± 0.45
		N = 8	N = 3	N = 9
	2006	**0.77** [1] ± 0.16	**0.15** [2] ± 0.05	**0.84** [1] ± 0.21
		N = 6	N = 6	8
Faeces	2002-2004	n.d.	n.d.	n.d.
	2005	**6.63** [1] **± 1.73**	**2.09** [1] **± 0.90**	**2.15** [1] **± 0.59**
		N = 8	N = 3	N = 9
	2006	**3.89** [1] **± 1.30**	**6.31** [1] **± 2.04**	**2.69** [1] **± 0.56**
		N = 6	N = 6	N = 8

[1,2,a,b] – for description see Table 1

Lead is an element whose high accumulation in animal bones provides information about the high contamination of the environment with that metal. Its mean concentrations found in bones of the bank voles caught in Warlity (Table 2) were much lower than most of the values determined in bones of bank voles caught in areas influenced by the Mine and Metallurgic Plant ”Bolesław” (23.26 mg·kg^{-1}). This indicates that lead contamination in the area close to the dump was low in the year it had was dug out and liquidated. The concentrations of lead in bones of animals collected from the uncontaminated area of Klimkówka (1.06 mg·kg^{-1}) were comparable to those obtained in bones of voles from Warlity (Table 2).

The concentrations of **zinc** in kidneys, liver and bones were at a similar level, irrespective of the analytical period or of the zone in which the voles were caught (Table 3).

Table 3

Zinc concentrations (mg·kg^{-1} d.w.) in tissues and faeces of *Myodes glareolus*

Tissue	Year	Zone A	Zone B	Control area
Kidneys	2002-2004	**101** [1,a] ± 3	**90** [1,b] ± 3	**91** [1,b] ± 11
		N = 40	N = 31	N = 19
	2005	**77** [2] ± 4	**90** [1] ± 1	**78** [1] ± 6
		N = 8	N = 3	N = 9
	2006	**108** [1] ± 5	**93** [1] ± 5	**98** [1] ± 5
		N = 6	N = 6	N = 8
Liver	2002-2004	**98** [1] ± 2	**96** [1] ± 3	**88** [1] ± 5
		N = 40	N = 31	N = 19
	2005	**95** [1] ± 4	**104** [1] ± 6	**87** [1] ± 4
		N = 8	N = 3	N = 9
	2006	**107** [1] ± 6	**104** [1] ± 1	**102** [1] ± 3
		N = 6	N = 6	N = 8
Bones	2002-2004	**166** [1] ± 3	**151** [1] ± 6	**160** [1] ± 4
		N = 40	N = 31	N = 19
	2005	**127** [1] ± 6	**173** [1] ± 3	**153** [1] ± 9
		N = 8	N = 3	N = 9
	2006	**146** [1] ± 4	**169** [1] ± 6	**171** [1] ± 8
		N = 6	N = 6	N = 8
Faeces	2002-2004	n.d.	n.d.	n.d.
	2005	**103** [1,a] ± 8	**346** [1,b] ± 41	**131** [1,a,b] ± 10
		N = 8	N = 3	N = 9
	2006	**1377** [2] ± 534	**2163** [1] ± 762	**1088** [2] ± 252
		N = 6	N = 6	N = 8

[1,2,a,b] – for description see Table 1

Only in kidneys of voles trapped in zone A in 2002-2004 was the concentration of this element significantly higher in comparison to the other zones. In the mentioned zone in 2005, the content of zinc in kidneys was significantly lower compared to the other periods of analysis (Table 3). In contrast, significant differences were noted in the case of faeces. Samples collected in 2006 were characterized by significantly higher contents of zinc when compared to the samples collected a year before. This significant increase in zinc content in faeces is likely to indicate environment contamination with zinc originating from the dump liquidated a year earlier or was linked with the closing down works. Due to the fact that zinc content in organisms is maintained through the homeostasis mechanism, elevated concentrations of this element were not found in tissues of the bank voles (Table 3).

Table 4

Copper concentrations (mg·kg^{-1} d.w.) in tissues and faeces of *Myodes glareolus*

Tissue	Year	Zone A	Zone B	Control area
Kidneys	2002-2004	**24.4** ^{1,a} ± 1.8	**20.6** ^{1,a,b} ± 1.2	**18.7** ^{1,b} ± 1.3
		N = 40	N = 31	N = 19
	2005	**15.7** ^{2} ± 0.9	**19.6** ^{1} ± 0.8	**15.7** ^{1} ± 1.1
		N = 8	N = 3	N = 9
	2006	**19.9** ^{1,2,a} ± 1.2	**17.1** ^{1,a,b} ± 1.0	**13.4** ^{1,b} ± 1.5
		N = 6	N = 6	N = 8
Liver	2002-2004	**19.3** ^{1,a} ± 0.6	**19.5** ^{1,a} ± 0.7	**16.3** ^{1,b} ± 0.8
		N = 40	N = 31	N = 19
	2005	**16.8** ^{1,a,b} ± 0.5	**18.9** ^{1,a} ± 0.1	**15.2** ^{1,b} ± 0.4
		N = 8	N = 3	N = 9
	2006	**18.7** ^{1} ± 1.4	**18.4** ^{1} ± 0.6	**17.3** ^{1} ± 1.2
		N = 6	N = 6	N = 8
Bones	2002-2004	**10.2** ^{1,a} ± 1.1	**7.2** ^{1,b} ± 1.1	**7.5** ^{1,a,b} ± 0.5
		N = 40	N = 31	N = 19
	2005	**5.6** ^{2} ± 0.5	**6.3** ^{1} ± 0.4	**5.7** ^{1,2} ± 0.5
		N = 8	N = 3	N = 9
	2006	**6.0** ^{1,2} ± 1.2	**7.6** ^{1} ± 1.8	**3.1** ^{2} ± 1.0
		N = 6	N = 6	N = 8
Faeces	2002-2004	n.d.	n.d.	n.d.
	2005	**14.1** ^{1} ± 2.5	**15.4** ^{1} ± 2.8	**21.5** ^{1} ± 2.1
		N = 8	N = 3	N = 9
	2006	**17.6** ^{1} ± 3.7	**27.5** ^{1} ± 5.4	**22.7** ^{1} ± 4.8
		N = 6	N = 6	N = 8

^{1,2,a,b} – for description see Table 1

The concentrations of zinc in tissues of the bank voles originating from Warlity were at a similar level to those reported for animals from Klimkówka and Starczynowska Desert. The only exception was the zinc concentration in livers of animals originating from the Olkusz Forests, being approximately 3-fold higher than that reported for livers of voles originating from Warlity.

The content of **copper** in kidneys of bank voles caught in the area affected by the pesticide dump was significantly higher in zone A than in the control zone, both in 2002-2004 (24.4 mg·kg^{-1}) and in 2006 (19.9 mg·kg^{-1}). In 2005, the determined concentration of copper reaching 15.7 mg·kg^{-1} was significantly lower than the values obtained in previous years. A similar dependency was also noted in the bones. Simultaneously, levels of this element noted in soft tissues of the voles originating from zone A were significantly higher when compared to the control samples (Table 4). This may suggest a low level of environmental contamination with copper as a result of the longstanding activity of the dump.

Iron is an essential element for the correct functioning of living organisms. Its concentration in animal bodies, irrespective of their habitat, is usually in a broad range. All results obtained in this study (Table 5) are within the range of the values determined in tissues of bank voles caught from the uncontaminated environment (Klimkówka).

Table 5

Iron concentrations (mg·kg^{-1} d.w.) in tissues and faeces of *Myodes glareolus*

Tissue	Year	Zone A	Zone B	Control area
Kidneys	2002-2004	**373 [1] ± 14**	**375 [1] ± 28**	**314 [1] ± 20**
		N = 40	N = 31	N = 19
	2005	**309 [1] ± 33**	**416 [1] ± 75**	**258 [1] ± 31**
		N = 8	N = 3	N = 9
	2006	**420 [1] ± 17**	**388 [1] ± 47**	**379 [1] ± 21**
		N = 6	N = 6	N = 8
Liver	2002-2004	**675 [1,a] ± 51**	**694 [1,a] ± 68**	**401 [1,b] ± 34**
		N = 40	N = 31	N = 19
	2005	**482 [1] ± 55**	**668 [1] ± 126**	**332 [1] ± 27**
		N = 8	N = 3	N = 9
	2006	**793 [1] ± 109**	**877 [1] ± 142**	**501 [1] ± 55**
		N = 6	N = 6	N = 8
Bones	2002-2004	**131 [1] ± 5**	**135 [1] ± 8**	**147 [1] ± 6**
		N = 40	N = 31	N = 19
	2005	**96 [2,a] ± 7**	**107 [1,2,b] ± 9**	**98 [2,a,b] ± 4**
		N = 8	N = 3	N = 9
	2006	**72 [2] ± 18**	**77 [2] ± 13**	**96 [2] ± 13**
		N = 6	N = 6	N = 8
Faeces	2002-2004	n.d.	n.d.	n.d.
	2005	**411 [1] ± 84**	**513 [1] ± 8**	**317 [1] ± 75**
		N = 8	N = 3	N = 9
	2006	**791 [1] ± 394**	**934 [1] ± 281**	**662 [1] ± 235**
		N = 6	N = 6	N = 8

[1,2,a,b] – for description see Table 1

The concentration of metals in tissues and faeces of bank voles caught in 2005

The highest **cadmium** concentration was found in kidneys of the voles caught in May 2005, in zones located close to the closed dump. Results obtained were at a similar level, i.e. 4.47 mg·kg^{-1} in kidneys of voles from zone A and 4.99 mg·kg^{-1} in those from zone B. In kidneys of rodents caught in October 2005 in zone A, the mean concentration of cadmium (4.13 mg·kg^{-1}) was similar, whereas that noted in kidneys of the animals from zone B – 2 times lower (2.30 mg·kg^{-1}) than in the samples collected in May (Figure 1). The mean values were not statistically significantly different. This is likely to be due to a very small number of the voles caught in zone B in May as well as in October and in control zone in May.

Lower concentrations of cadmium were noted in livers when compared to kidneys of the bank voles. Moreover, a substantially higher concentration of this element was noted in the voles collected from zone A in October and from zone B in May. The analysis of faeces showed a similar tendency in the concentration of Cd as that found in livers (Figure 1).

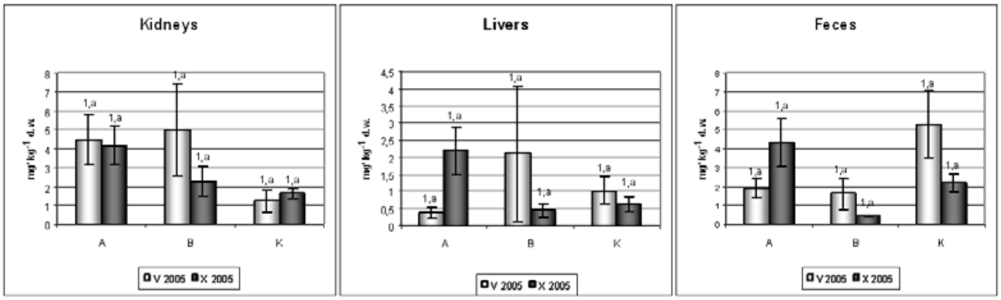

Figure 1. Cadmium concentration (mg·kg^{-1}d.w.) in tissues and faeces of bank voles (*Myodes glareolus*) captured in particular zones after pesticides dump closure.
1,2 – different numbers indicates statistically important differences in metal concentration in animal tissues between investigated months (p< 0,05)
a,b – different letters indicates statistically important differences in metal concentration in animal tissues between zones for the selected month (p< 0,05)

Kidneys of the bank voles caught in zone III in May were additionally characterized by a high concentration of **lead** (22.74 mg·kg^{-1}). This value was significantly higher in comparison to the lead content of kidneys of the rodents caught in October. Simultaneously, it was similar to the value found in 2005 in kidneys of *Myodes glareolus* originating from the contaminated area of the Olkusz Forests (30.02 mg·kg^{-1}) and to the maximal value (23.15 mg·kg^{-1}) found in the voles caught from the same area, but in 2006. A similar tendency of the higher accumulation of lead was observed in all analyzed tissues from this zone, however, statistically significant differences between the values were found for bone samples (Figure 2).

The voles caught from zone A in May accumulated lower contents of lead in tissues than those caught in zone B. In turn, the highest concentration of this element was noted in the samples collected in October from zone A (Figure 2). On the basis of the results obtained, it can be concluded that the area in the dump's proximity was contaminated with iron during its liquidation, thus, distinctly higher contents of lead were noted in the tissues and faeces of the voles caught in May, 2005.

The increased concentrations of cadmium and lead in tissues of the bank voles caught in zone B in May suggest that the rodents were especially exposed to the toxic effects of these two metals in the first months after the dump's closure due to the fact that their concentrations in the environment were higher. This was also confirmed by high concentrations of Cd and Pb in faeces (Figure 1, 2). In addition, in the winter and spring time, the voles ingest higher quantities of food of animal origin (the invertebrata), including insects and earthworms, which might accumulate significant amounts of heavy metals in their bodies, which – in turn – results in an elevated content of these elements in tissues of rodents. The content of lead in faeces, especially in the samples collected in May, confirmed its effective excretion from the gastrointestinal tract.

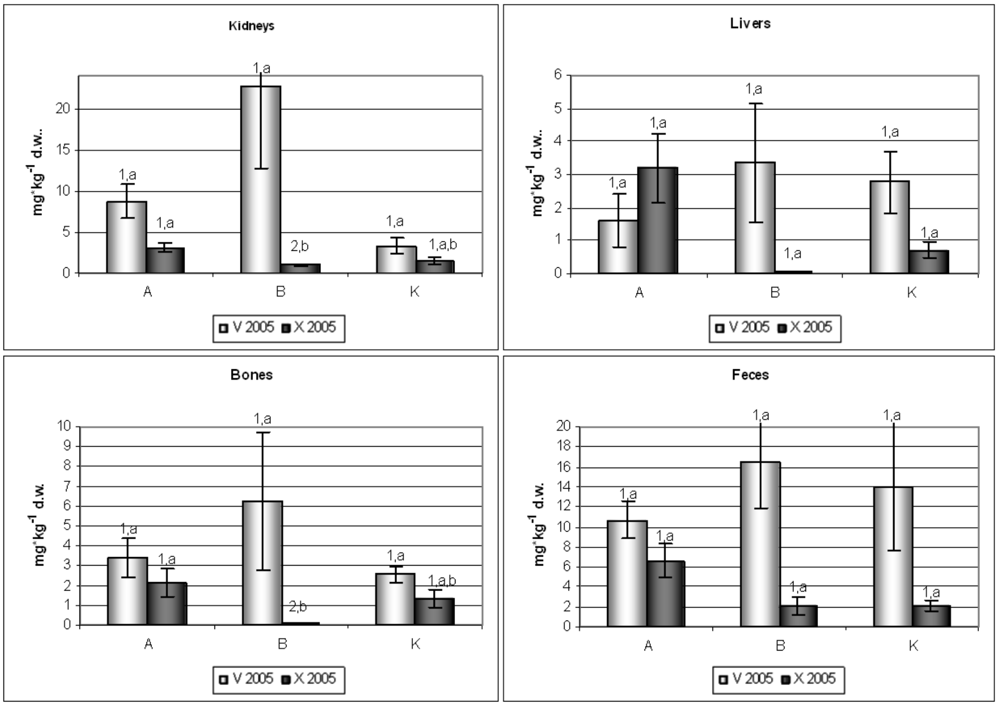

Figure 2. Lead concentration (mg·kg^{-1}d.w.) in tissues and faeces of bank voles (*Myodes glareolus*) captured in particular zones after pesticides dump closure.
1,2,a,b – for description see Fig. 1

In the case of **zinc**, in most of the samples of the voles caught in zones A and B, the content of this element was higher when the rodents were trapped in October (Table 6). Its contents in kidneys, bones and in feaces were found to be statistically significant different. Higher contents of this microelement were determined in tissues of the voles caught in May in the control zone in comparison to those caught in zone A and B. In October, the highest level of zinc was noted in the samples collected from zone B.

Table 6

Mean concentrations (± SE) of zinc (mg·kg^{-1}d.w.) in bank vole tissues (*Myodes glareolus*) captured in particular zones after dump closure

Tissue	Date	Zone A	Zone B	Control area
Kidneys	May 2005	**66** ^{1,a,b} ± 7	**51** ^{1,a} ± 8	**90** ^{1,b} ± 8
		N = 8	N = 3	N = 3
	October 2005	**77** ^{1,a} ± 4	**90** ^{2,a} ± 1	**78** ^{1,a} ± 6
		N = 8	N = 3	N = 9
Livers	May 2005	**78** ^{1,a} ± 6	**80** ^{1,a} ± 6	**95** ^{1,a} ± 5
		N = 8	N = 3	N = 3
	October 2005	**95** ^{1,a} ± 4	**104** ^{1,a} ± 6	**87** ^{1,a} ± 4
		N = 8	N = 3	N = 9
Bones	May 2005	**127** ^{1,a} ± 5	**112** ^{1,a} ± 18	**149** ^{1,a} ± 4
		N = 8	N = 3	N = 3
	October 2005	**127** ^{1,a} ± 6	**173** ^{2,b} ± 3	**153** ^{1,a,b} ± 9
		N = 8	N = 3	N = 9
Faeces	May 2005	**308** ^{1,a} ± 82	**73** ^{1,b} ± 5	**137** ^{1,a,b} ± 10
		N = 8	N = 3	N = 3
	October 2005	**103** ^{2,a} ± 8	**346** ^{2,b} ± 41	**131** ^{1,a,b} ± 10
		N = 8	N = 3	N = 9

^{1,2,a,b} – for description see Fig. 1

Taking into account the concentration of cadmium and lead in the analyzed samples taken from May and October, a constant dependency may additionally be observed between contents of these toxic elements and zinc, *i.e.* the more cadmium and lead that was accumulated in tissues, the lesser was the accumulation of zinc. This observation was especially tangible in zone B (Figure 1, 2; Table 6).

Similar to zinc content, higher concentrations of **copper** were found in organs of the bank voles caught in zone A and B in October. In kidneys of the voles trapped in zone A, as well as in livers of the animals caught in zone B, the differences observed were statistically significantly different (Table 7). Significantly higher concentrations of copper were determined in kidneys and faeces of control animals caught in May as compared to the rodents originating from zone A and B.

In analyzing the mean values of **iron** content in particular tissues of bank voles, caught in different months, the same trend may be observed. In zone A, higher levels of iron were found in tissues of animals caught in May, whereas in zone B – conversely – in tissues of voles caught in October (Table 8).

Table 7

Mean concentrations (± SE) of copper (mg·kg^{-1}d.w.) in bank vole tissues (*Myodes glareolus*) captured in particular zones after dump closure.

Tissue	Date	Zone A	Zone B	Control area
Kidneys	May 2005	**11.5** [1,a] ± 1.0	**14.6** [1,a,b] ± 0.5	**20.4** [1,b] ± 2.8
		N = 8	N = 3	N = 3
	October 2005	**15.7** [2,a] ± 0.6	**19.6** [1,a] ± 0.8	**15.7** [1,a] ± 1.1
		N = 8	N = 3	N = 9
Livers	May 2005	**16.3** [1,a] ± 1.5	**12.0** [1,a] ± 1.4	**15.7** [1,a] ± 0.3
		N = 8	N = 3	N = 3
	October 2005	**16.8** [1,a] ± 0.5	**18.9** [2,a] ± 0.1	**15.2** [1,a] ± 0.4
		N = 8	N = 3	N = 9
Bones	May 2005	**3.8** [1,a] ± 0.5	**3.2** [1,a] ± 1.3	**6.3** [1,a] ± 1.4
		N = 8	N = 3	N = 3
	October 2005	**5.6** [1,a] ± 0.5	**6.3** [1,a] ± 0.4	**5.7** [1,a] ± 0.5
		N = 8	N = 3	N = 9
Faeces	May 2005	**17.3** [1,a,b] ± 1.9	**5.2** [1,a] ± 1.6	**24.0** [1,b] ± 1.6
		N = 8	N = 3	N = 3
	October 2005	**14.1** [1,a] ± 2.5	**15.4** [1,a] ± 2.8	**21.5** [1,a] ± 2.1
		N = 8	N = 3	N = 9

[1,2,a,b] – for description see Fig. 1

It suggests that the contamination caused by the closure of the pesticide dump was spreading out to reach zone B even by the autumn. In livers of voles caught in the control zone, the levels of this element were found to be lower in comparison to those noted for animals from zone A and B. Due to the fact that iron is an element whose quantity in the body is regulated homeostatically, its surplus is excreted by faeces. Thus, its increased concentration was observed in faeces of voles collected in zone A in May, and in zone B in October.

The results obtained in this study indicate the influence of the pesticide dump on xenobiotic accumulation in tissues of *Myodes glareolus* inhabiting the zones in the closest vicinity to the dump. The exposure of the voles to metals decreased once the dump had been liquidated. This was confirmed by lower concentrations of these elements in the analyzed samples. It may be speculated that the existence of the pesticide dump posed a risk to the environment due to the penetration of accumulated toxic compounds into the soil. They were transmitted with a water run-off at considerable distances, which led to higher concentrations of the metals also in tissues of the voles inhabiting zone B. Additionally, during the closure of the dump in the autumn 2004, some quantities of metals were introduced to the environment but it did not have any permanent effect on its contamination.

Table 8

Mean concentrations (± SE) of iron (mg·kg^{-1}d.w.) in bank vole tissues (*Myodes glareolus*) captured in particular zones after dump closure.

Tissue	Date	Zone A	Zone B	Control area
Kidneys	May 2005	**494** [1,a] ± 89	**305** [1,a] ± 20	**360** [1,a] ± 88
		N = 8	N = 3	N = 3
	October 2005	**309** [1,a] ± 33	**416** [1,a] ± 75	**258** [1,a] ± 31
		N = 8	N = 3	N = 9
Livers	May 2005	**676** [1,a] ± 37	**338** [1,b] ± 26	**322** [1,b] ± 26
		N = 8	N = 3	N = 3
	October 2005	**482** [2,a,b] ± 55	**668** [2,a] ± 126	**332** [1,b] ± 27
		N = 8	N = 3	N = 9
Bones	May 2005	**161** [1,a] ± 39	**103** [1,a] ± 8	**107** [1,a] ± 6
		N = 8	N = 3	N = 3
	October 2005	**96** [1,a] ± 7	**107** [1,a] ± 9	**98** [1,a] ± 4
		N = 8	N = 3	N = 9
Faeces	May 2005	**739** [1,a] ± 152	**244** [1,a] ± 6	**266** [1,a] ± 77
		N = 8	N = 3	N = 3
	October 2005	**411** [1,a] ± 84	**513** [1,a] ± 8	**317** [1,a] ± 75
		N = 8	N = 3	N = 9

[1,2,a,b] – for description see Fig. 1

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