Contemporary Problems of Management and Environmental Protection
Vol. 5, 2010. Influence of the Pesticide Dump on the Environment: 91-106

Chapter 8

Katarzyna Sawicka-Kapusta, Paulina Baran, Marta Zakrzewska,
Józef Szarek, Joanna Hajduk, Justyna Porębska, Krystyna A. Skibniewska,
Janusz Guziur, Mirosław Grzybowski, Izabella Babińska

Effect of a Pesticide Dump on the Level of Heavy Metals in Tissues of Mice

Problem of pesticide dumps vs. heavy metals in the environment

The dumping sites of overdue pesticides currently constitute not only one of the most significant sources of environment pollution with chemicals acknowledged as persistent organic pollutants (POP) covered by the provisions of the Stockholm Convention, but also may be a source of environment contamination with heavy metals. Heavy metals contain both physiological elements (such microelements as: zinc, copper, iron) and elements indispensable for living organisms (xenobiotics, *e.g.* cadmium, lead, mercury or arsenic). Their common characteristic is that they are not subject to biodegradation, thus persist in the environment for long periods and are accumulated in trophic chains. Despite the introduction of a number of environmental protection standards, severe anthropogenic sources of biosphere contamination with heavy metals still exist, with industry, power engineering, roads and waste dumping sites being of particular concern.

Currently, as a result of a ban imposed in many countries on the use of chemical substances especially detrimental to the environment, pesticides or artificial fertilizers are no longer such a significant source of environment contamination with heavy metals. However, when extensively applied in previous decades they were major carriers of heavy metals. They contained considerable quantities of metal compounds, including active forms of arsenic, copper, mercury, zinc and lead. Currently, there are pesticide dumps containing vast quantities of out-of-use pesticides that constitute an additional source of environmental contamination with heavy metals.

The effect of heavy metals on animals

Heavy metals of various origin exert a significant effect on living organisms. The susceptibility of an animal to toxic effect of metals is determined by its species, genotype, physiological status and age. In turn, the toxicity of heavy metals depends on the extent of contamination, but also on the chemical form of an element, route of penetration to an organism, degree of solubility in body fluids as well as on the biochemical reactions in which they participate in metabolic processes. Deficit, excess or harmfulness of some trace elements may be a secondary phenomenon elicited by interactions proceeding between them in the body.

Cadmium. It does not play any biochemical nor physiological function in animal organisms. Even in small concentrations, it is highly toxic to an organism, to which it penetrates relatively easily, displaying a considerable tendency for accumulation. Cadmium is absorbed mainly through the respiratory system – on average 25% of its dose is absorbed in the pulmonary alveoli. Less effective is its absorption from the digestive tract, which accounts for 5%, on average. Irrespective of the route it penetrates into the body, cadmium is transported throughout the organism with blood in the form of complexes with low-molecular proteins, and then deposited in organs, kidneys and the liver, in particular. In kidneys, it displays peculiarly long biological half-life, *i.e.* up to 10-30 years in humans and 700 days in mice and rats, depending on the form of uptake and individual traits of an organism. Therefore, the concentration of cadmium in organs increases with age and reflects chronic exposure, whereas its presence in blood points to both short-term exposure and chronic intoxication. Critical organs to intoxication with cadmium are the kidneys, liver and lungs. Kidney damage and dysfunction are claimed to be typical symptoms of cadmium toxicity. Chronic exposure also results in damage to the liver, calcium metabolism disorders, disorders of reproductive functions (*e.g.* damage to the testicles) and neoplasmic lesions. It also suppresses the effectiveness of the immune system of a body by inducing apoptosis of healthy leucocytes. Furthermore, cadmium is one of the strongest carcinogens and teratogens amongst the heavy metals.

Lead. Like cadmium, it does not serve any physiological nor biochemical function in the body and displays toxicity even in low concentrations. It causes severe biochemical and neurophysiological disorders, histopathological changes and is teratogenic, thus posing a threat to both animals and humans. In the case of wild animals, lead is absorbed mainly through the digestive tract with contaminated food. In adult animals, ca. 2-16% of the dose is absorbed along this route, whilst young animals are exposed to higher absorption, reaching 45-50%. The absorption of lead is also reported to increase to 60% in the case of calcium and phosphorus deficiencies. Low concentrations are absorbed more readily than high ones. The absorption of lead through respiratory airways, depending on the size of molecules of suspended dust and efficiency of the lung cleansing mechanism, accounts for ca. 30%. In the body, lead is transported with blood. It first reaches soft tissues, including the liver and kidneys, and then gonads, bone marrow and the central nervous system, thus overcoming the blood-brain barrier. A more stable fraction of lead accumulated in an organism is the osseous system, which contains 90% of the total content of this element. The concentration of lead in tissues is usually correlated with its level in the environment and with the time span of exposure, which is linked with its relatively long biological half-life. The lead content of osseous tissue depicts chronic exposure of a body, whereas its presence in blood and soft tissues points to a relatively recent exposure. In the case of lead, critical organs include the kidneys, liver and bone marrow and its toxic effects largely affect the four following systems: nervous, cardiovascular, gastrointestinal and excretory.

Copper. Copper is an element indispensable for the functioning of a living organism, in spite of the fact that being a heavy metal it also displays toxic properties. It occurs in all animal tissues, in some of them even showing a tendency for accumulation. The highest concentration of copper is reported in liver, which is a reservoir of this element and regulates its content in the body through the control of its secretion to bile ducts. Copper also occurs in relatively high concentrations in the brain and muscles, yet this pool cannot be activated in the case of a copper deficiency. One of its key roles in the body is as an electron carrier in redox processes. It additionally serves a significant function in incorporating iron to hemoglobin; one of the symptoms of copper deficiency is a low content of hemoglobin and anemia. Despite a number of significant functions in the body, excess quantities of copper pose serious health risks. In the case of impairment of a copper excretion mechanism or its excess in the environment of an animals, free ions may be subject to accumulation and may generate free hydroxyl radicals, thus causing damage to cell structures. At a level of an organism, an excess of copper triggers a variety of metabolic changes resulting in damage to the liver, kidneys or brain tissue.

Zinc. It is indispensable for an animal organism and naturally occurs therein. It is one of the elements occurring in the body in the highest concentrations, and 85% of its content is absorbed in muscle tissue and bones. In the body, zinc appears exclusively intracellularly – in cell nucleus, organelles, cytoplasm and in specialized alveoli. In contrast to other heavy metals, it displays lower toxicity and a more frequent unbeneficial symptom in the body is its deficiency rather than excess. The level of zinc absorption from the environment is regulated homeostatically. In the mammals, the absorption proceeds mainly through the digestive system in the small intestine. Zinc takes part in ca. 300 enzymatic reactions, in which it plays catalytic and structure-forming roles. In addition, it is co-factor to over 200 enzymes. Furthermore, zinc serves the function of an antioxidant, which may be linked with its capability to induce the production of metallothioneins, which protect the cellular membrane against free radicals. Zinc exhibits a relatively low toxicity to organisms, though its excess is implicated in both acute and chronic intoxications. The most known toxic effect of zinc is its antagonism against copper and iron, which leads to their secondary deficiencies in the body and resultant growth deceleration and anemia.

Iron. From the biological perspective, the key characteristic of iron is its readiness for receiving and giving back electrons, owing to which it may participate in redox reactions. In organisms of mammals, irons occurs in all tissues, but its highest quantities are accumulated in the liver and spleen, constituting a pool to be activated upon body demand. 60-70% of the total iron content of the body occurs in the form bound with haeme – in haemoglobin and myoglobin. Owing to a lack of any physiological mechanisms of iron excess excretion, the key process regulating its level in the body is controlled intestinal absorption, that proceeds mainly in enterocytes of the duodenum and small intestine. The capability of receiving and giving back electrons as well as transiting into various states of oxidation is also linked with the detrimental effect of iron. Under aerobic conditions, it is capable of generating free radicals, which induces oxidative stress and may lead to tissue damage. In those processes, damage may also be caused to DNA, which is included in the carcinogenic effects of iron.

Effect of a pesticide dump on the level of heavy metals in tissues of yellow-necked mice (*Apodemus flavicollis*)

In the province of Warmia and Mazury, 17 pesticide dumps have been located to date, and the total quantity of pesticides deposited in those dumps was estimated in 2004 to reach 983 Mg. With the end of XX century the government of the province elaborated a “Socio-economic development strategy for the Warmia and Mazury Province”, which contained a plan to remove the pesticide dumps. First, closing procedures were applied to the sites posing the most severe threat, including, among others, the unfortunately-located (on a hill and in permeable soil) pesticide dump in Warlity Wielkie (more details in Chapter 5). The closing down work was conducted from 18.10.2004 to 05.11.2004.

The study has been performed to determine the concentrations of selected heavy metals in tissues of yellow-necked mice (*Apodemus flavicollis*) caught in areas adjacent to pesticide dumps and to verify, on that basis, whether the operation and closing down of that site have elicited any changes in the environment.

In the year 2002, zones were established that were located in the area between the pesticide dump and the coast line of Lake Szeląg Wielki, in the east-west transect, *i.e.:* zone A – in the closest vicinity to the pesticide dump, followed by zone B and control area (K). The division followed a successive decrease in contamination level along with an increasing distance from the spot source.

Each zone was encompassing forest and meadow ecosystems, inhabited by, among others, such rodents as the yellow-necked mouse (*Apodemus flavicollis*).

Zone A, located in the closest vicinity of the pesticide dump, covered a hillside overgrown with grass where the pesticide dump was located, with a south-eastern exposure and slightly water-logged foothill and meadow vegetation. This zone also encompassed afforested area located on the other side of the road, with a predominating share of pine and a high contribution of lime and oak. The hilltop the pesticide was located on is currently covered with sandy soil owing to the relatively recent closing-down work.

Zone B, covered area located ca. 500 m away of the pesticide dump. Its afforested part constituted a narrow belt of leafy forest with such species as: lime, oak, maple tree, hornbeam, poplar, and wild lilac, spreading along the shore of Lake Szeląg Wielki.

Control area (K) in the years 2002-2005 covered a dyke located 4 km south-east of the pesticide dump. This was a grassy area with an admixture of aquatic flora (reed-mace). It covered a narrow belt of plantings in the form of an alder marshy meadow, being a typical habitat of yellow-necked mice (A*podemus flavicollis*). However, owing to the high concentration of lead found in organs of animals originating from the control zone in 2005, in subsequent years the control area was established in a different site. It was located ca. 3 km south-west of the pesticide dump. That area covered a narrow fragment of a mixed forest on both sides of a forest road. The planting was predominated by pine and spruce and a small contribution of oak and hornbeam.

One of the methods used for the evaluation of environmental contamination with heavy metals is biomonitoring, which uses small mammals, *e.g.* rodents as bioindicators. They are selected as an analytical object due to their low position in the trophic chain, high reproducibility and short lifespan, as well as owing to their high population number and determined, known individual territory. An additional advantage is the high rate of their metabolism linked with their small body size. For this reason, it may be speculated that exposing these animals to contamination is more intensive than in large animals with a low metabolic rate.

The object of the study were yellow-necked mice (*Apodemus flavicollis*). The range of occurrence of this species covers all of Poland. They occur both in highlands and lowlands, and are especially found in old forests with meager underbrush – both leafy and mixed, in parks and compact scrubs. Yellow-necked mice feed mainly on seeds and green parts of plants as well as animal feed – insects, snails and annelida. Depending on the season, the analysis of stomach contents of animals of this species demonstrates that from 15 to 37% of their digesta are of plant origin. The mean individual lifespan of the yellow-necked mouse reaches 2.9-3.6 months.

Collection of the rodents was conducted annually in the autumn, in the years 2002-2007, with the trapline method. Concentrations of heavy metals (Cd, Pb, Cu, Zn, Fe) were determined in livers, kidneys and thigh bones of the collected animals using atomic absorption spectrometry after mineralization in a mixture of nitric(V) and perchloric acids, at a ratio of 4:1. In addition, in the years 2005-2007 concentrations of metals were also assayed in faeces of the animals examined. Bovine Liver 1577 b was used as a reference material. The recovery accounted for 90-105%. All values obtained were converted into $mg \cdot kg^{-1}$ dry weight (d.w.).

All calculations were made with Statistica 7.1 software. The obtained results were determined for normality of distribution and subjected to Laven's test to examine the variance homogeneity of the variables. In order to demonstrate statistically significant differences between organs in various zones, use was made of the Kruskal-Wallis test. A comparison of results obtained in different years was conducted with a two-way analysis of variance, followed by a parametric Tukey's test or non-parametric Kruskal-Wallis test, when conditions of the parametric test were not fulfilled.

Contents of cadmium and lead in tissues of yellow-necked mice

A comparative analysis of the accumulation of **cadmium** in organs of yellow-necked mice caught in particular experimental years demonstrated that the animals were the most exposed to that element in 2005, *i.e.* nearly a year after closing down procedures. In livers of yellow-necked mice caught in this period in zone A, the concentration of cadmium accounted to 1.38 mg·kg^{-1} and was statistically significantly higher than in the years 2002-2004. This value was also distinctly higher than the results obtained in the same zone in the subsequent years of the study: 0.11 mg·kg^{-1} in 2006 and 0.09 mg·kg^{-1} in 2007, although the statistical analysis did not demonstrate any differences. In the case of cadmium concentration in livers of mice originating from zone B, the highest value was also reported in the year 2005, which was a significantly higher result than the value of 0.05mg·kg^{-1} recorded a year later.

Similarly, in kidneys of yellow-necked mice the concentration of cadmium in 2005 was tangibly distinguished. It ranged from 2.38 mg·kg^{-1} in zone A (it was also the highest result obtained for cadmium) to 0.74 mg·kg^{-1} in the control zone. Even the lowest concentration of cadmium reported in that year was higher than the results determined both before and after closing down the pesticide dump (Table 1).

Table 1

Cadmium concentration (mg·kg^{-1} d.w.) in tissues of yellow-necked mice (*Apodemus flavicollis*)

	Zone		2002-2004	2005	2006	2007
LIVER	A	X ± SE	0.12^{1} ± 0.02	1.38^{2} ± 0.54	$0.11^{1,2,A}$ ± 0.01	$0.09^{1,2,AB}$ ± 0.02
		N	32	6	6	10
	B	X ± SE	$0.12^{1,2}$ ± 0.02	0.38^{1} ± 0.18	$0.05^{2,B}$ ± 0.01	$0.13^{1,2,A}$ ± 0.03
		N	31	5	7	10
	K	X ± SE	0.14 ± 0.03	0.47 ± 0.18	0.06^{AB} ± 0.02	0.05^{B} ± 0.01
		N	26	10	6	15
KIDNEYS	A	X ± SE	0.62 ± 0.17	2.38 ± 1.37	0.44 ± 0.06	0.36 ± 0.07
		N	32	6	6	10
	B	X ± SE	0.42 ± 0.05	1.03 ± 0.49	0.17 ± 0.07	0.63 ± 0.16
		N	32	5	7	10
	K	X ± SE	0.72 ± 0.20	0.74 ± 0.29	0.23 ± 0.06	0.25 ± 0.05
		N	28	10	6	15

^{1,2} – different numbers indicate statistically important differences in metal concentration between investigated years in particular zone (p<0,05)
^{A,B} – different letters indicate statistically important differences in metal concentration between zones in particular year (p<0,05)

Both in kidneys and livers of the mice, the statistical analysis did not demonstrate any significant differences between mean concentrations of cadmium reported in the years 2002-2004 and 2006-2007. In kidneys of yellow-neck mice, those concentrations were observed to range from 0.17 mg·kg^{-1} in zone B in the year 2007 to 0.72 mg·kg^{-1} in the control zone in the years 2002-2004. In livers, concentrations of cadmium were lower and ranged from 0.05 mg·kg^{-1} (zone B in 2006 and zone K in 2007) to 0.14 mg·kg^{-1} (zone K in the years 2002-2004). These concentrations may be found low and comparable with results obtained in analyses conducted in ecologically-clean areas, *i.e.*: 0.55 $mg \cdot kg^{-1}$ in kidneys and 0.16 $mg \cdot kg^{-1}$ in liver of yellow-necked mice originating from the area of Borecka Primeval Forest, one of the cleanest areas of Poland. Also, organs of yellow-necked mice caught in the vicinity of Klimówka – an area acknowledged as clear and control for monitoring studies – contained similar concentrations of that element (kidneys – 0.26 $mg \cdot kg^{-1}$, livers – 0.16 $mg \cdot kg^{-1}$ on average).

The concentrations of cadmium determined in tissues of yellow-necked mice in 2005 considerably exceeded results reported for that metal on non-contaminated areas, whereas in the case of organs of animals from zone A they were higher than those recorded in tissues of yellow-necked mice and the activity areas of steelworks in Cracow and Warsaw (respectively: 1.16 $mg \cdot kg^{-1}$ and 1.19 $mg \cdot kg^{-1}$ in kidneys). This suggests that some quantities of cadmium might have penetrated into the environment during the closing-down work.

The highest concentrations of **lead** were accumulated by tissues of yellow-necked mice caught in October of 2005, whereas the lowest ones – by those of animals caught in October 2006. The statistical analysis confirmed this observation in most cases. In thigh bones, which reflect chronic exposure of animals to that heavy metal, the highest concentrations of lead were found in 2005 in yellow-necked mice originating from zone A (3.70 $mg \cdot kg^{-1}$). That result was statistically significantly higher than those obtained in that zone in the subsequent years of the study – 0.23 $mg \cdot kg^{-1}$ in 2006 and 0.35 $mg \cdot kg^{-1}$ in 2007. In contrast, the lowest content of cadmium was accumulated in thigh bones of mice caught in zone B in the year 2006 – 0.07 $mg \cdot kg^{-1}$. Except for bones of the yellow-necked mice caught in zone B in 2005, results determined in years preceding the closing of the pesticide dump and in 2005 are statistically significantly higher than those obtained in the subsequent years, *i.e.* 2006 and 2007. This is likely to suggest that the existence of the pesticide dump and its liquidation had some effects on long-term exposure of yellow-necked mice to lead (Table 2).

As in the case of cadmium, livers of yellow-necked mice caught in 2005 were characterized by high concentrations of lead. In zone A, this concentration (3.43 $mg \cdot kg^{-1}$) was significantly higher as compared to results noted in the successive years (0.04 $mg \cdot kg^{-1}$ in 2006 and 0.06 $mg \cdot kg^{-1}$ in 2007), whereas in zone B – as compared to very low concentrations of cadmium determined in the year 2006. In none of the zones, however, were differences noted in the concentration of lead in livers of animals caught before the closing of the pesticide dump and in the subsequent year (2005), although the results of 2005 were considerably higher. The contents of lead in kidneys of yellow-necked mice caught in zones A and B confirmed the tendency observed in the other tissues, namely, that the results obtained in 2002-2004 (zone A: 1.36 $mg \cdot kg^{-1}$) are not statistically significantly different from those recorded in 2005 (zone A: 10.29 $mg \cdot kg^{-1}$), whereas concentrations of lead in both these experimental periods are significantly higher than mean contents of that metal in kidneys of mice examined in 2006 and 2007 (zone A – 0.22 $mg \cdot kg^{-1}$ and 0.21 $mg \cdot kg^{-1}$, respectively), whilst those do not differ between each other.

Table 2

Lead concentration (mg·kg^{-1} d.w.) in tissues of yellow-necked mice (*Apodemus flavicollis*)

	Zone		2002-2004	2005	2006	2007
LIVER	A	X ± SE	$0.20^{1,2} \pm 0.02$	$3.43^{1} \pm 1.32$	$0.04^{2,3,A} \pm 0.02$	$0.06^{3} \pm 0.03$
		N	33	6	6	10
	B	X ± SE	$0.24^{1} \pm 0.04$	$1.84^{1} \pm 1.18$	$0.00^{2,B} \pm 0.00$	$0.10^{1,2} \pm 0.04$
		N	31	5	7	10
	K	X ± SE	$0.18^{1} \pm 0.02$	$0.99^{1,3} \pm 0.55$	$0.01^{2,AB} \pm 0.00$	$0.08^{2,3} \pm 0.03$
		N	26	6	6	15
KIDNEYS	A	X ± SE	$1.36^{1} \pm 0.22$	$10.29^{1} \pm 4.14$	$0.22^{2,A} \pm 0.05$	$0.21^{2} \pm 0.05$
		N	32	6	6	10
	B	X ± SE	$2.07^{1} \pm 0.54$	$2.22^{1} \pm 0.93$	$0.07^{2,B} \pm 0.02$	$0.65^{1,2} \pm 0.23$
		N	32	5	7	10
	K	X ± SE	$1.58^{1} \pm 0.37$	$0.54^{2} \pm 0.39$	$0.13^{2,AB} \pm 0.04$	$0.58^{2} \pm 0.21$
		N	28	7	6	15
THIGH BONES	A	X ± SE	$1.04^{1} \pm 0.17$	$3.70^{1} \pm 1.59$	$0.23^{2} \pm 0.07$	$0.35^{2} \pm 0.11$
		N	33	6	6	10
	B	X ± SE	$0.71^{1} \pm 0.09$	$0.66^{1,2} \pm 0.43$	$0.07^{2} \pm 0.02$	$0.10^{2} \pm 0.04$
		N	32	5	7	10
	K	X ± SE	$0.77^{1} \pm 0.10$	$1.71^{1} \pm 0.88$	$0.11^{2} \pm 0.03$	$0.29^{2} \pm 0.07$
		N	27	7	6	15

[1,2,A,B] – for description see Table 1

The concentrations of lead determined in thigh bones, livers and kidneys in 2005, especially in zone A, exceed not only the concentrations occurring in analogous tissues of mice caught in the area of the Borecka Primeval Forest (0.22 mg·kg^{-1} in bones, on average), but also those noted in tissues of mice from the areas of steelworks in Warsaw (0.82 mg·kg^{-1}) and Cracow (0.87 mg·kg^{-1}). In the case of kidneys, the mean concentration of Pb in zone A (10.29 mg·kg^{-1}) was almost fivefold higher than the mean content of this metal recorded in yellow-necked mice originating from zinc steelworks in Miasteczko Śląskie (2.51 mg·kg^{-1}) and twofold higher than the lead concentration in tissues of yellow-necked mice caught in the Starczynowska Desert, ca. 5 km away from the "Bolesław" Mining and Metallurgic Plant in Bukowno (5.04 mg·kg^{-1}). This value, however, was definitely lower than the mean concentrations determined in kidneys of mice originating from the close vicinity of this steelworks (91.34 mg·kg^{-1}).

They above-presented results may indicate that the existing pesticide dump affected the presence of lead in the adjacent environment, and that the liquidation works contributed to the release of high quantities of this metal. Also noteworthy are the relatively higher results obtained in 2002-2004 and 2005 in the samples originating from the control area – in most cases they are significantly higher than those determined in control samples in the subsequent years of the study. Such results are likely to be due to the effect of local contamination of the control area with lead, which was avoided in successive years by establishing new control areas. The existence of a local source of lead contamination in that area has also been suggested elsewhere.

Contents of selected physiological elements in tissues of yellow-necked mice

The highest contents of **copper** were found in livers of the animals assayed – 16.01 mg·kg^{-1} on average. In all experimental years and zones, concentrations of copper in this organ were equal and did not show significant differences. They were, additionally, comparable with the results obtained for the samples collected from the uncontaminated area of Klimkówka. The lowest concentrations of this physiological element were reported in kidneys of yellow-necked mice caught in 2005 and accounted on average for 13 mg·kg^{-1}, whereas in the other years the respective values ranged from 14.7 mg·kg^{-1} (zone K, 2006) to 18.2 mg·kg^{-1} (zone K, 2002-2004) (Table 3). The diminished level of copper in kidneys of the animals caught in 2005 might have been due to the relatively high concentrations of cadmium occurring in that organ, for cadmium is a copper antagonist and is capable of inducing its secondary deficiency.

Table 3

Copper concentration (mg·kg^{-1}d.w.) in tissues of yellow-necked mice (*Apodemus flavicollis*)

	Zone		2002-2004	2005	2006	2007
LIVER	A	X ± SE	17.0 ± 0.63	16.4 ± 1.7	16.7 ± 1.0	15.8 ± 1.0
		N	33	6	6	10
	B	X ± SE	16.8 ± 0.51	15.1 ± 0.9	17.8 ± 2.0	17.4 ± 0.7
		N	31	5	7	10
	K	X ± SE	15.2 ± 0.5	13.9 ± 0.6	15.3 ± 0.6	14.6 ± 0.8
		N	26	12	6	15
KIDNEYS	A	X ± SE	17.0 ± 0.8	12.8 ± 1.7	16.7 ± 1.2	15.0 ± 0.3
		N	32	6	6	10
	B	X ± SE	16.8 ± 0.61	13.9 ± 1.2	15.4 ± 0.7	15.3 ± 0.9
		N	32	5	7	10
	K	X ± SE	18.2[1] ± 0.94	12.2[2] ± 0.9	14.7[1,2] ± 0.7	15.3[1,2] ± 0.6
		N	28	12	6	15
THIGH BONES	A	X ± SE	6.3 ± 0.51	4.0 ± 0.5	8.1 ± 2.2	6.5 ± 0.7
		N	33	6	6	10
	B	X ± SE	6.3[1] ± 0.45	4.7[1,2] ± 0.3	4.0[2] ± 0.2	4.1[1,2] ± 0.2
		N	32	5	7	10
	K	X ± SE	6.5 ± 0.50	4.8 ± 0.4	5.3 ± 1.1	4.5 ± 0.9
		N	27	12	6	15

[1,2] – for description see Table 1

The highest quantities of **zinc** in the body are accumulated in liver and kidneys as well as in the osseous tissue. Livers of yellow-necked mice caught in the 2006 in zones A and B were, indeed, characterized by an elevated concentration of that microelement, which may point to a recent exposure (zinc accumulated in liver constitutes a mobile pool, its biological half-life in the body reaches 16-28 days). In thigh bones, the concentration of zinc ranged from 109 mg·kg^{-1} (zone B, 2007) to 142 mg·kg^{-1} (control zone, 2002-2004), and those values matched the physiological level, which in mammals fluctuates between 75 and 170 mg·kg^{-1}. Relatively low concentrations of zinc were, in turn, observed in kidneys (Table 4), yet they corresponded to physiological levels and were comparable to results of similar studies conducted on the same species of rodents.

An analysis of **iron** content of yellow-necked mice tissues found the relatively lowest concentration of this element in tissues of mice caught in 2006. In liver, these values ranged from 275 mg·kg^{-1} in the control zone to 416 mg·kg^{-1} in zone B (Table 5), whilst analogous research carried out on the area of the Woliński National Park showed that livers of yellow-necked mice contained iron at a level of 542-585 mg·kg^{-1}. The low content of this element in the animals caught in 2005 is likely to indicate their poor physiological state. This might have been due to diminished availability of iron in the ingested food, disorders in its absorption or interactions with other elements.

Table 4

Zinc concentration (mg·kg^{-1} d.w.) in tissues of yellow-necked mice (*Apodemus flavicollis*)

	Zone		2002-2004	2005	2006	2007
LIVER	A	X ± SE	100^{AB} ± 5	84^{AB} ± 14	122 ± 6	105^{AB} ± 16
		N	33	6	6	10
	B	X ± SE	109^{A} ± 5	116^{A} ± 77	122 ± 12	121^{A} ± 10
		N	31	5	7	10
	K	X ± SE	$91^{1,B}$ ± 4	$75^{2,B}$ ± 2	99^{1} ± 4	$80^{1,2,B}$ ± 2
		N	25	12	6	15
KIDNEYS	A	X ± SE	72 ± 3	81^{AB} ± 5	76 ± 5	77 ± 12
		N	32	6	6	10
	B	X ± SE	$77^{1,2}$ ± 2	$84^{1,A}$ ± 5	66^{2} ± 2	$74^{1,2}$ ± 6
		N	32	5	7	10
	K	X ± SE	77^{1} ± 4	$63^{1,2,B}$ ± 5	$67^{1,2}$ ± 1	62^{2} ± 4
		N	28	12	6	15
THIGH BONES	A	X ± SE	115^{A} ± 4	121 ± 4	123^{AB} ± 1	115 ± 18
		N	33	6	6	10
	B	X ± SE	$140^{1,B}$ ± 9	$118^{1,2}$ ± 7	$115^{2,A}$ ± 4	$109^{1,2}$ ± 15
		N	32	5	7	10
	K	X ± SE	$142^{1,B}$ ± 5	111^{2} ± 4	$131^{1,2,B}$ ± 4	119^{2} ± 5
		N	27	12	6	15

^{1,2,A,B} – for description see Table 1

Table 5

Iron concentration (mg·kg^{-1} d.w.) in tissues of yellow-necked mice (*Apodemus flavicollis*)

	Zone		2002-2004	2005	2006	2007
LIVER	A	X ± SE	522 ± 26	561 ± 92	364 ± 76	522 ± 80
		N	33	6	6	10
	B	X ± SE	538 ± 35	560 ± 44	416 ± 139	587 ± 83
		N	31	5	7	10
	K	X ± SE	470 ± 33	359 ± 34	275 ± 27	371 ± 30
		N	26	12	6	15
KIDNEYS	A	X ± SE	338 ± 14	305 ± 29	288 ± 17	356 ± 24
		N	32	6	6	10
	B	X ± SE	320 ± 11	351 ± 37	258 ± 23	346 ± 19
		N	32	5	7	10
	K	X ± SE	328[1] ± 9	237[2] ± 23	272[1,2] ± 21	293[1,2] ± 12
		N	28	12	6	15
THIGH BONES	A	X ± SE	171[1] ± 21	129[1,2] ± 26	82[2] ± 16	92[2] ± 6
		N	31	6	6	10
	B	X ± SE	122[1] ± 6	96[1,2] ± 16	81[2] ± 10	104[1,2] ± 14
		N	30	5	7	10
	K	X ± SE	122[1] ± 6	90[2] ± 6	59[2] ± 9	98[1,2] ± 8
		N	26	12	6	15

[1,2,A,B] – for description see Table 1

Concentration of heavy metals in faeces of animals examined in the years 2005-2007

The highest concentrations of **cadmium** were determined in faeces of yellow-necked mice caught in 2005 in all zones compared. In zone A, the mean concentration of this metal accounted for 1.70 mg·kg^{-1} and was significantly different than the values recorded in the subsequent years of the study (0.47 mg·kg^{-1} and 0.48 mg·kg^{-1}). A similar tendency was observed in zone B (Figure 1). Levels of cadmium in faeces of the animals from the control zone did not display any differences. Results obtained for faeces seem to confirm the conclusions, reached in the analysis of cadmium levels in tissues of animals, on the impact of closing down work in the presence of this element in the environment and food of the animals.

A very high concentration of **lead** was determined in faeces of yellow-necked mice caught from zone A in 2006 (14.79 mg·kg^{-1}) and from zone B in 2007 (12.87 mg·kg^{-1}) (Figure 1). Such a result might indicate that the environmental contamination with lead which had occurred as a result of digging up the pesticide dump and was detected in animal tissues till 2005, has spread out in subsequent years. Nevertheless, as the high concentrations of lead determined in faeces of yellow-necked mice in the years 2006-2007 are not reflected in tissues, it may be concluded that lead was effectively excreted by the animals.

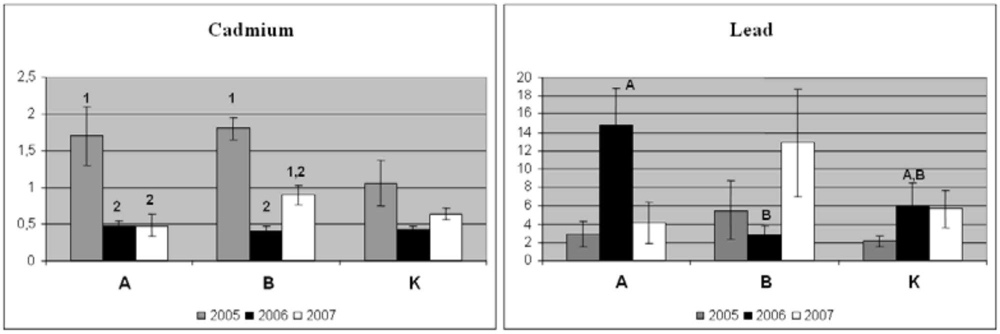

Figure 1. Cadmium and lead concentrations (mg·kg^{-1} d.w.) in feaces of yellow-necked mice (*Apodemus flavicollis*) captured in 2005-2007
[1,2,A,B] – for description see Table 1

The analysis of **copper** level in faeces of the animals examined found that with each year the animals were excreting higher quantities of this element. It points to the existence of some source of contamination with that metal, most likely independent of the pesticide dump, because a year after its closing down, the concentrations of copper were reported to be the lowest (Figure 2).

Attention is drawn to the very high concentrations of **zinc** demonstrated in the analysis of faeces of yellow-necked mice caught in Warlity Wielkie. In 2005, the highest mean concentration of zinc was noted in zone B – 3345 mg·kg^{-1}, in the year 2006 – in zone A (2537 mg·kg^{-1}), whereas in 2007 – in zone B (4480 mg·kg^{-1}, this was simultaneously the highest result). Owing to the fact that samples of faeces of the animals caught were not analyzed in the previous years, it is impossible to determine what quantities of zinc were excreted in the period preceding the closing of the pesticide dump. The results obtained do not depict any explicit trend (Figure 3).

According to literature data, ca. 70% of the dose of this element absorbed with food are excreted with faeces. This indicates that food ingested by the animals contained even higher concentrations of zinc. These are doses that considerably exceed the zinc levels occurring, for instance, in cereal grains from unpolluted areas – ca. 46 mg·kg^{-1}, or even in cereal grains originating from industrial areas or soils fertilized with municipal wastes (87 mg·kg^{-1} on average). Thus, an explanation of this phenomenon ought to be the presence of an adjacent strong source of rodent food contaminated with zinc.

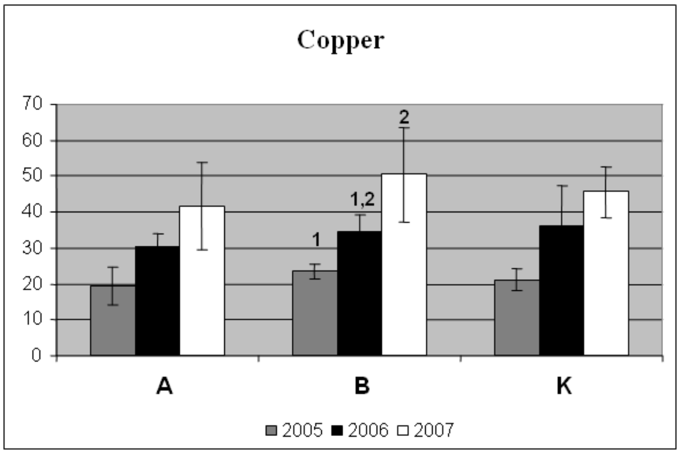

Figure 2. Copper concentration (mg·kg^{-1} d.w.) in feaces of yellow-necked mice (*Apodemus flavicollis*) captured in 2005-2007
1,2 – for description see Table 1

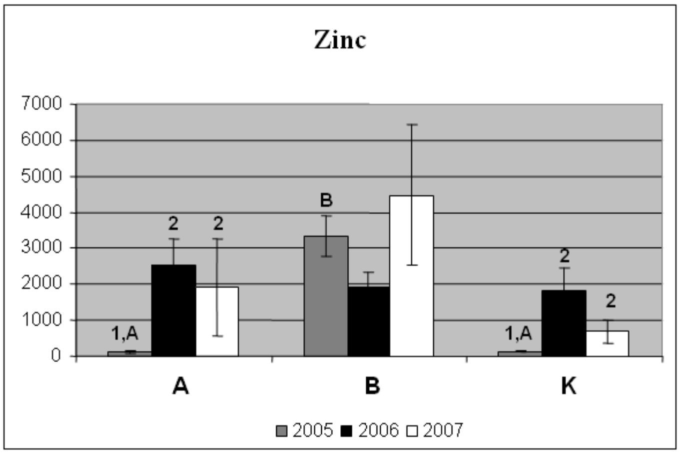

Figure 3. Zinc concentration (mg·kg^{-1} d.w.) in feaces of yellow-necked mice (*Apodemus flavicollis*)
1,2,A,B – for description see Table 1

Worthy of attention is the fact that high quantities of seeds dressed with pesticides were dug out while liquidating the pesticide dump in Warlity Wielkie (oral information). It seems likely that the grain could have been dressed with zinc phosphide. That pesticide exhibits strong toxic effects once ingested and is applied for eradicating rodents, the arvicolines and murids in particular. It is insoluble in water and alcohol, whereas in the presence of acids and bases it is subject to degradation into zinc oxides or salts, and a strongly-toxic hydrogen phosphide, releasing a typical odor resembling that of garlic. That rodenticide has been applied worldwide for a number of years, including Poland, as an alternative to strychnine – owing to its relatively slow rate of action and a lack of accumulation in tissues of rodents, which diminishes the likelihood of its bioaccumulation in the food chain. It is referred in literature as one of the sources of rodents' exposure to elevated concentrations of zinc.

Interesting conclusions may be reached when comparing the contents of zinc in faeces of yellow-necked mice caught in 2005 and 2006. In 2005, its content in faeces of mice originating from zone A was relatively low (120 $mg\cdot kg^{-1}$), whereas in zone B it reached the value of 3345 $mg\cdot kg^{-1}$, which exceeds substantially concentrations of that metal reported in 2006. A year after liquidating the pesticide dump, concentrations of this toxic compound in the environment must still have been very high, which is seen in the example of animals originating from zone B. Most likely, in zone A (located in the closest proximity to the pesticide dump) the contamination was so high that the animals having contact with the dressed grain – and thus absorbing high doses of the pesticide – did not survive. In the successive years, the contamination might have spread out to the other zones (Figure 3).

Faeces are the main excretion route of **iron** not absorbed by the organism. Samples of faeces collected from yellow-necked mice in 2006 and 2007 were characterized by a high concentration of iron, which points to its presence in feed. In the case of the animals caught in 2006, in tissues of which the content of this element was observed to decrease, its high level in faeces indicates suppressed intestinal absorption (Figure 4).

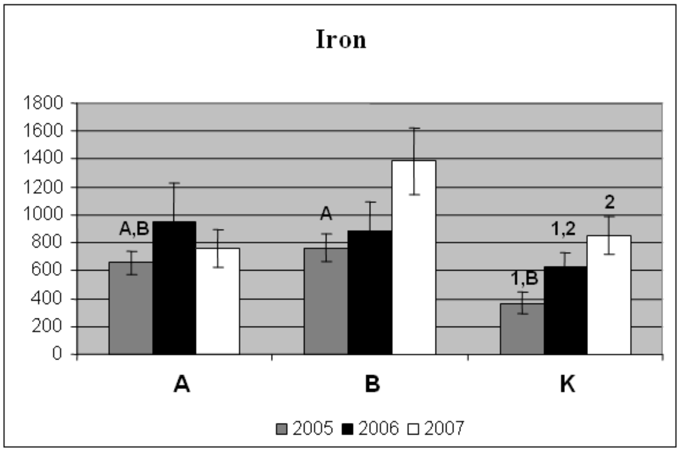

Figure 4. Iron concentration ($mg\cdot kg^{-1}$ d.w.) in feaces of yellow-necked mice (*Apodemus flavicollis*) captured in 2005-2007
1,2,A,B – for description see Table 1

Iron is a metal whose availability to an animal body is negatively affected by the presence of such metals as cadmium, lead or zinc. In the case of the animals discussed, the high concentrations of iron in faeces are partly consistent with high quantities of excreted lead and zinc (2006 – zone A, 2007 – zone B) (Figure 1, 3, 4). This suggests that lead and zinc contamination of the animals' habitat exerts a negative effect on their iron absorption.

It can be concluded that digging out the pesticide dump evoked a rapid, though short-term, increase in the concentrations of cadmium and lead in tissues of yellow-necked mice caught in 2005, *i.e.* immediately after dump's liquidation. The concentrations of heavy metals determined in some samples originating from the years 2006-2007 were statistically lower compared to the results obtained when the pesticide dump did exist. This indicates that the presence of the pesticide dump posed a risk to the surrounding environment, since – presumably – some part of detrimental substances penetrated outside.

Acknowledgements

The experimental part of the study was financed from the Polish research found as a research projects.

Literature

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

Il'yasova D., Schwarz G. G. 2005. Review. Cadmium and renal cancer. Toxicol. Appl. Pharmacol., 207: 179-186.

Merian E., Anke M., Inhat M., Stoeppler M. 2004. Elements and their Compounds in the Environment. Occurrence, Analysis and Biological Relevance. 2nd, Completely Revised and Enlarged Edition. Vol. 2 Metals and Their Compounds. Wiley–VCH Verlag GmbH & Co. KGaA, Weinheim.

Nicholson F. A., Smith S. R., Alloway B. J., Carlton-Smith C., Chambers B. J. 2003. An inventory of heavy metals inputs to agricultural soils in England and Wales. Sci. Total Environ., 311: 205-219.

Nordberg G. F., Fowler B. A., Nordberg M., Friberg L. 2007. Handbook on the toxicology of metals. 3rd edition. Elsevier Science Publishers B. V. Amsterdam, NY, Oxford.

Papanikolaou G., Pantopoulos K. 2005. Review. Iron metabolism and toxicity. Toxicol. Appl. Pharmacol., 202: 199-211.

Pereira R., Pereira M. L., Ribeiro R., Gonçalves F. 2006. Tissues and hair residues and histopathology in wild rats (*Rattus rattus* L.) and Algerian mice (*Mus spretus* Lataste) from an abandoned mine area (Southeast Portugal). Environ. Pollut., 139: 561-575.

Piao F. Yokoyama K., Ma N., Yamauchi T. 2003. Subacute toxic effects of zinc on various tissues and organs of rats. Toxicol. Lett., 145: 28-35.

Sheffield S.R., Sawicka-Kapusta K., Cohen J.B., Rattner B.A. 2001. Rodentia and Lagomorpha. In.: Ecotoxicology of Wild Mammals, Shore R. and Rattner B. (eds.). Ecological and Toxicology Series, John Wiley and Sons, London.

Talmage, S.S., Walton, B.T. 1991. Small mammals as monitors of environmental contaminants. Rev. Environ. Contam. Toxicol. 119: 47-145.

Paulina Baran[1], Katarzyna Sawicka-Kapusta[1], Marta Zakrzewska[1], Józef Szarek[2], Joanna Hajduk[1], Justyna Porębska[1], Krystyna A. Skibniewska[3], Janusz Guziur[4], Mirosław Grzybowski[5], Izabella Babińska[2]

[1]Jagiellonian University in Krakow
Faculty of Biology & Earth Sciences
Institute of Environmental Sciences
Gronostajowa 7, 30-387 Kraków, Poland
e-mail: katarzyna.sawicka-kapusta@uj.edu.pl
marta.zakrzewska@uj.edu.pl

[2]University of Warmia & Mazury in Olsztyn
Faculty of Veterinary Medicine
Chair of Pathophysiology, Forensic Veterinary Medicine & Administration
Oczapowskiego 13, 10 – 719 Olsztyn, Poland
E-mail: szarek@uwm.edu.pl
izabella.babinska@uwm.edu.pl

[3]University of Warmia & Mazury in Olsztyn
Faculty of Technical Science
Chair of Safety Foundations
Okrzei 1A, 10-266 Olsztyn, Poland
e-mail: kas@uwm.edu.pl

[4]University of Warmia & Mazury in Olsztyn
Faculty of Environmental Protection & Fisheries
Department of Fish Biology and Pisciculture
Oczapowskiego 5, 10-719 Olsztyn, Poland
e-mail: jguziur@uwm.edu.pl

[5]University of Warmia & Mazury in Olsztyn
Faculty of Environmental Protection & Fisheries
Department of Applied Ecology
Oczapowskiego 5, 10-719 Olsztyn, Poland
e-mail: grzybomi@uwm.edu.pl