COPPER, ZINC, CADMIUM AND LEAD LEVELS IN FAT BODIES OF ADULT FEMALE RANA ESCULENTA L. DURING HIBERNATION

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ABSTRACT

The total trace element content in fat bodies were determined. The concentration of Zn was determined with a flame atomic absorption spectrophotometer (Cole-Parmer, model 200A). Cu, Pb and Cd were measured after methyl isobutyl ketone-diethyl dithiocarbamate treatment by flame atomic absorption spectrometry. The relationships between the metals in fat bodies show interaction between the investigated elements: synergism and antagonism. This study demonstrated that there are significant relationships between trace elements in fat bodies of *Rana esculenta*. The method of investigation allowed regressions for pairs of metals to be calculated.

INTRODUCTION

The effects of metals on wild animals are often assessed by analyzing residues in body organs. Over the last few years, cadmium (Cd) has been one of the main components of environmental pollution (Alloway, 1990). Low levels of cadmium have little effect on animals, but if it is concentrated over time it becomes toxic above a critical concentration. This metal tends to accumulate in tissues, but it can move out of tissue and the body by means of different systems. Numerous studies have been conducted on the effect of cadmium on liver metabolism of rats (Liu, Liun, 1990; Bell et al., 1991). Cadmium changes the metabolism through biochemical changes and morphological changes.

Among the heavy metals of industrial and toxicological interest, lead (Pb) has probably the widest distribution in the human environment (Davis, 1990). When released into the environment it persist longer than most other pollutants. Lead compounds tend to accumulate in soils and sediments. Because of its low solubility and relative freedom from microbial degradation, it will remain accessible to the food chain and the human metabolism far into the future (Davis, 1990). Lead toxicity causes haematological, gastrointestinal and neurological dysfunction in animals. Prolonged exposure may also cause chronic nephropathy and reproductive impairment.

Copper and zinc are physiological elements. Zinc and copper are essential nutrients in humans and animals, necessary for the functioning of a large number of metalloenzymes. These enzymes include alcohol dehydrogenase, alkaline phosphatase, superoxide dismutase and many others.

Zinc is required for normal nucleic acid, protein and membrane metabolism, as well as cell growth and division. Certain levels of zinc and copper intake and storage by animals are recommended. Cd and Zn as trace amounts are required for most biological systems to survive (Dulka, 1976). An accumulation or deficiency of certain essential metals is known to cause disturbances in different animal systems.

Frogs are more vulnerable than other vertebrates to environmental contaminants because the frog egg is not protected by a semi-impervious shell and their skins are water-permeable (Duellman, Trueb, 1986). *Rana esculenta* is one of the more often then not species which is of great importance in culinary. This frog is caught in their natural environment or raising in ponds and is exported to countries where is delicacy.

Amphibians can be used as biomonitors because they show a low rate of migration. Migration might mask the negative effects of environmental pollution (Wren, 1986). Their long life expectancy makes it possible to estimate possible long-term effects, e.g., the accumulation of heavy metals.

Fat bodies associated with the gonads are characteristic of all amphibians. In anurans they are aggregated at the anterior end of the gonads. Fat bodies are in the form of many fingerlike projections and are larger in males than in females. They are suspended from the body wall by a dorsal mesentery which is fused with the gonadal mesentery (Noble, 1931b). Fat bodies are composed of typical adipose tissue consisting of large cells, each with an oil vacuole. They are a source of nutrients for the gonads. The bodies are largest just before hibernation and smallest just after breeding (Krawczyk, 1974).

The aims of this study were to investigate the levels of copper, zinc, cadmium and lead in fat bodies of adult female *Rana esculenta L.* during hibernation, and to determine their distribution and the correlation between elements.

MATERIAL AND METHODS

Rana esculenta is an aquatic frog widely distributed in Poland. Ten females of adult *Rana esculenta L.* were selected for analysis of metal residues in their fat bodies. The specimens were caught in their natural habitat near Cracow (Węgrzce Wielkie 50°04`N, 20°08`E, 220 m a. s. l.) during hibernation (3rd decade of December).

The specimens selected for metal analysis were dissected and the fat bodies were weighed separately. For analysis of Zn, Cu, Pb and Cd, fat bodies were dried for two days at 60°C and then at 105°C until constant mass. Dry mass was reduced to ash at 450°C. The samples were digested to a transparent solution with a mixture of nitric, perchloric and sulfuric acids in a Kjeldahl flask (100 ml volume). The resulting solutions were diluted to a known volume with deionized water and transfered to acid-washed test tubes with Teflon screw-caps. The concentration of Zn was determined with a flame atomic absorption spectrophotometer (Cole-Parmer, model 200A). Cu, Pb and Cd were measured after methyl isobutyl ketone-diethyl dithiocarbamate treatment by flame atomic absorption spectrometry (Honda et al., 1982). Correction for background interference was made by running the appropriate blanks. The accurancy of this method was determined by spiking fat bodies of frogs with known amounts of Zn, Cu, Pb and Cd, with recoveries of 90.0 to 99.9%. The results were calculated in micrograms per gram dry mass (µg/g d.m.)

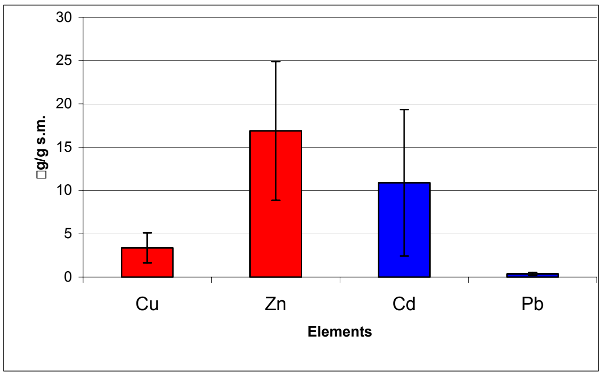
RESULTS

In order to establish a correlation between concentration of trace metals in fat bodies a graphical format and data tables has been chosen. Metals have been grouped together, proportions and correlation ratio [R] and variance [F] were counted. Relationships between metals were plotted in figures.

Tab. 1. Copper, zinc, cadmium and lead concentration in fat bodies of adult female frog *Rana esculenta L.* during hibernation (µg/g d.m.).

Number of individual	Cu	Zn	Cd	Pb
1	4.79	16.46	8.41	0.05
2	6.95	37.39	33.95	0.45
3	4.99	18.95	11.94	0.35
4	2.28	13.09	6.20	0.58
5	3.80	17.09	11.10	0.45
6	1.99	15.93	9.73	0.35
7	2.21	18.47	9.11	0.35
8	2.93	13.04	8.32	0.45
9	2.10	9.92	6.60	0.06
10	1.66	8.46	3.57	0.45
Mean	3.37	16.88	10.89	0.35
Standard deviation	±1.73	±8.01	±8.46	±0.17
Median	2.61	16.20	8.76	0.40

Fig. 1. Copper, zinc, cadmium and lead concentration in fat bodies of adult female *Rana esculenta L.* during hibernation.



Tab. 2. Proportions of investigated elements in selected individuals, correlation ratio [R], variance [F] and Student-Gosset test for investigated elements in fat bodies of adult female *Rana esculenta L.* during hibernation.

Number of individual	**Cu/Zn**	**Cu/Cd**	**Cu/Pb**	**Cu/Ni**	**Zn/Cd**	**Zn/Pb**	**Zn/Ni**	**Cd/Pb**	**Cd/Ni**	Ni/Pb
1	0.3	0.6	96	0.5	2	329	1.7	168	0.9	200
2	0.2	0.2	15	0.5	1.1	83	2.7	75	2.4	31
3	0.3	0.4	14	0.2	1.6	54	0.8	34	0.5	66
4	0.2	0.4	4	0.2	2	23	1.2	11	0.5	19
5	0.2	0.3	8	0.2	1.5	38	0.7	25	0.5	51
6	0.1	0.2	6	0.2	1.6	46	1.2	28	0.7	37
7	0.1	0.2	6	0.2	2	53	1.9	26	0.9	28
8	0.2	0.4	6	0.2	1.6	29	1	18	0.7	28
9	0.2	0.3	35	0.5	1.5	165	2.5	110	1.7	67
10	0.2	0.5	3	0.6	2.4	19	2.9	8	1.2	6
R	0.843	0.827	-0.046	0.524	0.974	0.170	0.406	0.171	0.336	0.304
F	19.57	17.33	0.17	3.02	145.9	0.24	1.58	0.24	1.02	0.81
T	6.45*	3.35*	5.47*	4.71*	9.75*	6.55*	1.74	3.95*	0.54	5.66*

Relationships between metals in fat bodies.

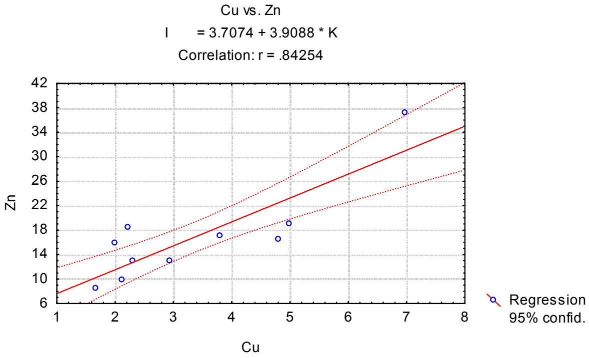


Fig. 2. Correlation between copper and zinc in fat bodies of adult female *Rana esculenta L.* during hibernation.

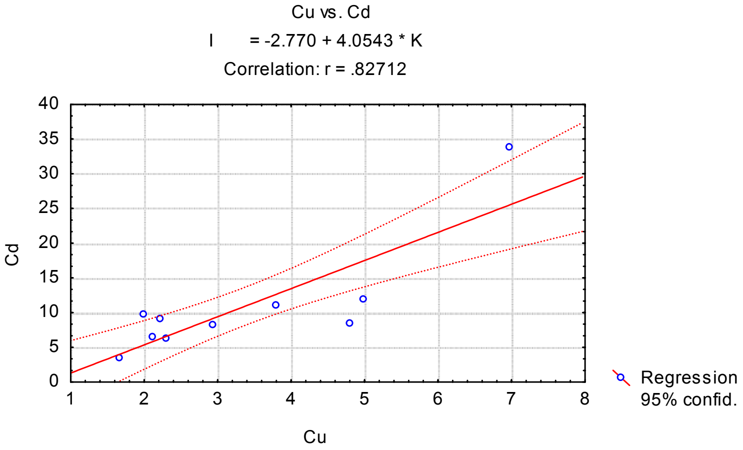


Fig. 3. Correlation between copper and cadmium in fat bodies of adult female *Rana esculenta L.* during hibernation.

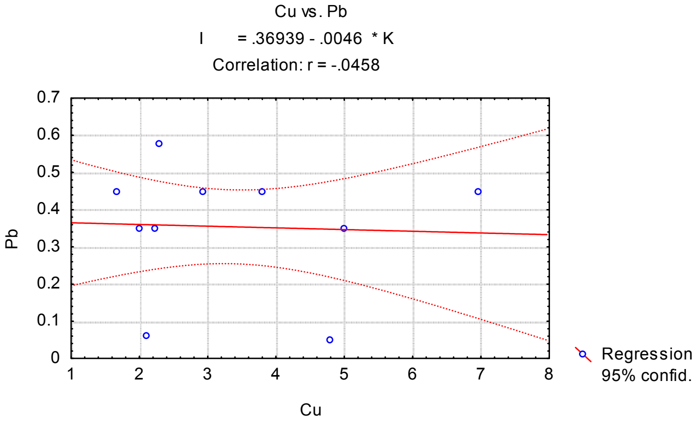


Fig. 4. Correlation between copper and lead in fat bodies of adult female *Rana esculenta L.* during hibernation.

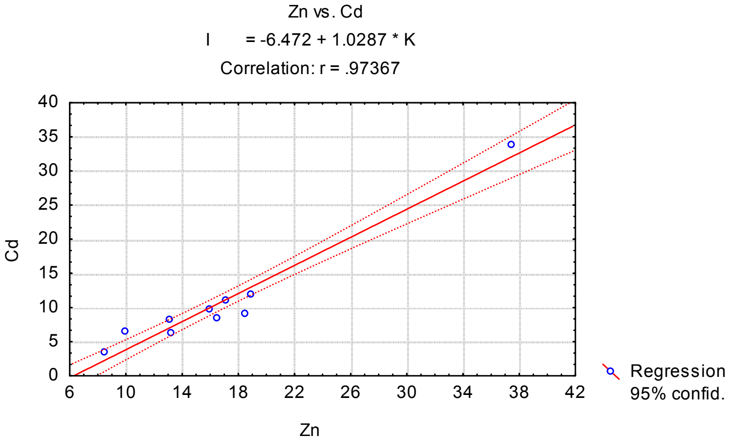


Fig. 5. Correlation between zinc and cadmium in fat bodies of adult female *Rana esculenta L.* during hibernation.

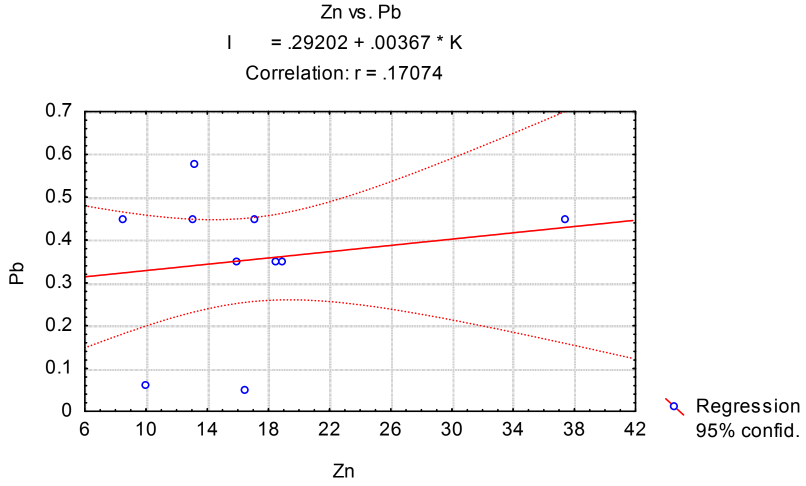


Fig. 6. Correlation between zinc and lead in fat bodies of adult female *Rana esculenta L.* during hibernation.

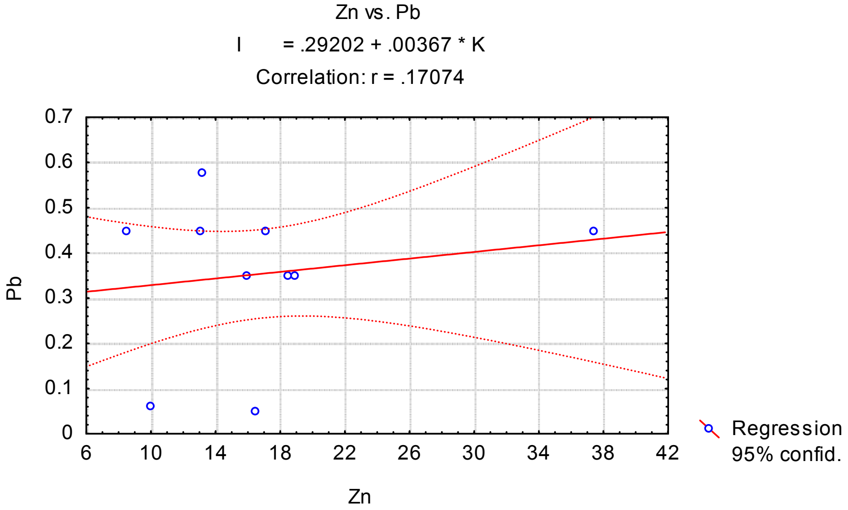


Fig. 7. Correlation between cadmium and lead in fat bodies of adult female *Rana esculenta L.* during hibernation.

DISCUSSION

During the hibernation period the lipid content of fat bodies gradually lowers until the breeding period (3^{rd} decade of May). Their water content varies in the opposite direction. In the 3^{rd} decade of December, low fat level and high water content are characteristic (Krawczyk, 1974).

High levels of zinc are probably associated with its biological role. It is the result of the accumulation process, which is an effect of fat-body hydration, increasing during the investigated period. Fat bodies can accumulate zinc. This element is basic for egg production during the mating period. Zinc is an essential trace element; it is homeostatically regulated in vertebrates and there is no interaction between tissue content and environmental load (Johnson et al., 1978; Andrews et al., 1989b). The zinc distribution in the vertebrate body is not localized to particular soft tissue organs.

The level of copper was lower than that of zinc. Many authors have shown that the liver is the main organ for copper accumulation. Cu is absorbed mainly in the stomach (Dodds-Smith et al., 1992b), and then transported to the liver where is stored. Frogs do not eat during the winter, so the copper had to be stored just before, during the active period. Some copper is stored in fat bodies also, but competition with zinc lowers its level. Copper is also essential for skin pigmentation (Voitkevich, 1966).

Cadmium is a nonessential trace element. It has no essential biological function in animals, and is not subject to physiological retention control. It has a high potential for food chain transfer (Andrews et al., 1984; Hunter et al., 1987b). The main target for cadmium is the kidneys, followed by the liver. (Dodds-Smith et al., 1992b; Shore, Douben 1994a). The very high concentration of Cd in fat bodies of the frog shows that in amphibians this tissue is critical for this metal, as is the kidney.

Lead content in the fat bodies was lower. Most of the Pb intake via ingestion (90%) is transferred to the bones, which are the main target organs for Pb accumulation in vertebrates. (Sharma, Shupe, 1977; Stawarz 1998). The level of Pb is usually much higher than that of Cd in most tissues and organs (Stawarz, 1998). The fat bodies are an exception to this pattern.

The relationships between the metals in fat bodies show interaction between the investigated elements: synergism and antagonism. The method of investigation allowed regressions for pairs of metals to be calculated. Levels of elements were measured in each specimen separately.

Increased zinc level was associated with increased copper in fat bodies. The correlation coefficient is 0.843 (fig. 3). A similar interaction is clear for copper and cadmium (r=0.827; fig. 4), and for zinc and cadmium (r=0.974; fig. 7). Cd can be a catalyst of the same enzymes as Zn and Cu. Thus this element is stored in fat bodies, but its possible future role is not clear. In humans the Cd/Zn proportions in kidneys is 2:3, in another tissues 1:7 (Gaździk, 1984). In fat bodies of the frog this proportion is similar to that in human kidneys: 1:2 (r=0.9). In human kidneys, where accumulation of cadmium is rather low, increased cadmium level is associated with increased zinc. The same finding applied to fat bodies of the frog.

There is no relationship between copper and lead. This is probably an effect of antagonism between them. Lead and copper competition in melanophores of melanocytes and their role in producing melanins makes the level of lead lower than copper, because there is no reason to store both these elements in the fat bodies. Their level is much higher in liver (copper) and skin (lead) (no reported studies on *Rana esculenta L.* and *Rana temporaria L.*).

Zinc, like cadmium and copper, may be bound to metallothioneins for detoxification and homeostasis. Metallothioneins – metal-binding proteins (MT) – play an important role in metal metabolism in animals. These low-molecular-weight molecules have a unique amino acid composition, with high content of cysteine residues, giving them a very high affinity for heavy metals such as zinc, copper and cadmium (Kagi et al., 1979). MT have been isolated from animals and human liver (Clarkson et al., 1985), and also from the small intestine where they act as regulators of trace element absorption and excretion (Cousins, 1979).

Elements are redistributed among tissues as a MT-metal complexes. Their migration to fat bodies is probably an effect of transport with water. In these organs where cadmium and zinc competition is high in enzyme-active centers (kidney, liver), the presence of cadmium lowers the zinc level (Ashby et al., 1980;). Cadmium does not change zinc content in the pancreas and heart. This is an effect of the low level of MT in these organs. Zinc protects against the toxic action of cadmium in many tissues.

There is a strong relationship between cadmium and copper levels (r=0.827; fig. 4). Generally cadmium ions reduce copper uptake in the small intestine of mammals and birds. This phenomenon is an effect of competition for binding places in MT, which is produced in the liver and intestine. Cadmium and copper ions are often the catalyst of the same enzymes. (Campbell, Mills, 1974, Mazur et al., 1960). The data about the relationships between cadmium and copper are contradictory. Some of them suggest that increasing the cadmium level causes an increase of the copper level. This is the situation we have detected in fat bodies of the frog. Other authors have suggested that this relationship is exactly inverse. It probably depends on their location in body structures, tissues and organs (Ashby et al., 1980, Banis et al., 1969). Laboratory-accumulated Cd did not correlate with the concentration of any other metal, but it inhibited redistribution of copper and stimulated production of ceruloplasmin (Klaassen, 1978).

The present study demonstrated that there are significant relationships between trace elements in fat bodies of *Rana esculenta*. The correlations between the concentrations of each of the various metals located in the fat bodies are often significant. Where significant correlations between metal concentrations were found, the slope of the regression line of the first metal on the second was always positive, indicating that a high concentration of one metal was accompanied by a high concentration of the second metal.

BIBLIOGRAPHY

Alloway, B. J. (1990). Heavy metals in soils. *Blackie, Glasgow, London, John Wiley and Sons. New York*, pp 100–124.

Andrews, G. K, Adamson, E. D., and Gedamu L. (1984). The ontogeny of expression of murine metallothionein: comparison with the alpha-fetoprotein gene. *Developmental Biology.* **103**, 294–303.

Ashby, S. L., King, L. J., Parke, D. V. (1980). Effect of acute administration of cadmium on the disposition of copper, zinc, and iron in the rat. *Environmental Research.* **21**, 177–85.

Banis, R. J., Pond, W. G., Walker, E. F., and O Connor, J.R., (1969). Dietary cadmium, iron, and zinc interactions in the growing rat. *Proceedings of the Society for Experim. Biology and Medicine*. **130** 802–6.

Bell, R. R., Nonavinakere, V. K. , Soliman, M. R., and Early, J. L., (1991). Effect of in vitro treatment of rat hepatocytes with selenium, and/or cadmium on cell viability, glucose output, and cellular glutathione. *Toxicology* **69** 111–9.

Campbell, J. K., Mills, C. F., Effects of dietary cadmium and zinc on rats maintained on diets low in copper. (1974). *Proceedings of the Nutrition Society*. **33** 15A–16A.

Clarkson, J. P., Elmes, M. E., Jasani, B., and Webb, M., (1985). Histological demonstration of immunoreactive zinc metallothionein in liver and ileum of rat and man. *Histochemistry Journal.* **17** 343–52.

Cousins, R. J., (1979). Regulatory aspects of zinc metabolism in liver and intestine. *Nutrition Revievs.* **37** 97–103.

Davis, W. F., (1990). Case study of lead in drinking water: protocol, methods, and investigative techniques. *American Industrial Hygiene Association Journal.* **51,** 620–4.

Dodds-Smith, M. E., Johnson, M. S., and Thompson, D. J., (1992). Trace metal accumulation by the shrew Sorex araneus. II. Tissue distribution in kidney and liver. *Ecotoxicology and Environmental Safety.* **24**, 118–30.

Dulka, J. J., Risby, T. H., (1976). Ultratrace metals in some environmental and biological systems. *Analytical Chemistry.* **48**, 640A–653A.

Duellman, W. E., Trueb, L., (1986). Biology of Amphibians. *McGraw–Hill Book Company, New York.*

Grzybek, H., Gaździk, M., and Panz, B., (1984). Interakcje kadmu i innych metali w organizmie człowieka i zwierząt. *Polski Tygodnik Lekarski*. XXXIX **12**.

Hunter, B. A., Hunter, L. M., Johnson, M. S., and Thompson, D. J., (1987). Dynamics of metal accumulation in the grasshopper Chorthippus brunneus in contaminated grasslands. *Archives of Environmental Contamination and Toxicology.* **16**, 711–6.

Johnson, P. E., Evans G. W., (1978). Relative zinc availability in human breast milk, infant formulas, and cow's milk. *American Journal of Clinical Nutrition* **31**, 416–21.

Kagi, J. H., Kojima, Y., Kissling, M. M., and Lerch, K., (1979). Metallothionein: an exceptional metal thiolate protein. *Ciba Foundation Symposium.* **72**, 223–37.

Krawczyk, S., (1974). Annual cycle of changes in lipid and water content of some organs of the edible frog (*Rana esculenta L.*). *Folia Biologica* (Krakow). **22**, 309–26.

Liu, R. M., Liun, Y. G., (1990). Effects of cadmium on the energy metabolism of isolated hepatocytes: its relationship with the nonviability of isolated hepatocytes caused by cadmium., *Biomedical Environmental Science.* **3**, 251–61.

Sharma, R. P., Shupe, J. L., (1977). Lead, cadmium, and arsenic residues in animal tissues in relation to those in their surrounding habitat. *The Science of The Total Environment.* **7**, 53–62.

Shore, R. F., Douben, P. E., (1994). The ecotoxicological significance of cadmium intake and residues in terrestrial small mammals. *Ecotoxicology and Environmental Safety.* **29**, 101–12.

Stawarz, R., (1998). Analiza zawartości niektórych pierwiastków u żaby trawnej *Rana temporaria L.* w okresie odrętwienia zimowego i pory godowej. *Praca doktorska.* Kraków.

Voitkevich, A. A., (1966). Melanocytes and the hormonology of pigment reactions. *Uspekhi Sovremennoj Biologi.* **61**, 471–90.

Wren, C. D., (1986). A review of metal accumulation and toxicity in wild mammals. I. Mercury. *Environmental Research*. **40**, 210–44.