Arch. Pol. Fish.	Archives of Polish Fisheries	Vol. 16	Fasc. 2	155-166	2008

STUDIES ON THE BIOAVAILABILITY OF HEAVY METALS (Cd, Pb, Cu, Zn) FROM BOTTOM SEDIMENTS TO GUPPIES, *POECILIA RETICULATA* PETERS

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ABSTRACT. The experiment was carried out to determine the potential of heavy metals (Cd, Pb, Cu and Zn) passing from bottom sediments to fish and to identify the correlations between concentrations of these metals in bottom sediments and those in fish. The study material consisted of guppies *Poecilia reticulata* Peters and bottom sediments with known concentrations of the determined metals in particular fractions. As result of the 60-day exposure of the fish to environments with bottom sediments containing various heavy metals loads, an increasing trend was observable for cadmium and lead concentrations in guppies. Comparative analysis showed that the degree of bottom sediment heavy metal contamination influenced concentrations of these in the guppies. It was found that even small changes in environmental conditions affected the release of metals from bottom sediments, which resulted in notable differences in the concentrations of the examined metals in the fish.

Key words: HEAVY METALS, GUPPIES, FISH, BOTTOM SEDIMENTS, BIOAVAILABILITY

INTRODUCTION

The continuous, aggressive inflow of heavy metals into aquatic ecosystems is a major environmental problem (Rayms-Keller et al. 1998). Metal bioavailability resulting from, among other factors, changeable environmental conditions, as well as the ability of heavy metals to pass from one carrier to another, contribute to difficulties in determining the risk of metal contamination in organisms living in an examined area.

A portion of the heavy metals in aquatic environments is bound to the bottom sediments, so the harmful effects of them is "dormant". The bioavailability of heavy metals to organisms is determined by their occurrence in soluble fractions. It is known that the desorption of metals is possible at the moment environmental physical, chemical, and bio-

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chemical conditions change. Furthermore, attention must be paid to the fact that metals of anthropogenic origin are usually more mobile than those of lithogenic origin (Chlopecka et al. 1996), which means there is an increased risk of contamination to living organisms.

When estimating the potential bioavailability of pollutants deposited in bottom sediments, physical, chemical, and biochemical processes occurring in the environment must be taken into account. A number of studies have discussed in detail the factors influencing the rate of heavy metals sorption and desorption from bottom sediments (Bourg 1988, Berg et al. 2001, Singh et al. 2001, Impellitteri et al. 2002, Gao et al. 2003, Li et al. 2005) and metal bioavailability (Luoma 1983, Blais et al. 1992, McKinney and Rogers 1992). The chemical monitoring of abiotic elements in ecosystems does not fully reflect the toxic influence of xenobiotics contained in bottom sediments since this is characteristic for particular environments. More precise data on this topic are provided by bio-monitoring, as it takes into account differences in the bioavailability of elements in different ecosystems (Borgmann 2000).

The aim of the present study was to examine the potential of some metals (Cd, Pb, Cu and Zn) contained in bottom sediments to accumulate in guppies, *Poecilia reticulata* Peters, a fish frequently used in toxicology studies. The effect of external factors on the studied parameters was eliminated by ensuring stable, optimum experimental conditions.

MATERIALS AND METHODS

The simulation experiment carried out under laboratory conditions was aimed at examining the possibilities of heavy metals passing from bottom sediments to the water and then to fish. In the experiment, eight bottom sediment batches from the Oder River with known and varied metal concentrations were used along with guppies as bio-indicators. Measurements of water reaction (pH) were taken with a Eutech Cybernetics pH Scan BNC pH-meter to the nearest ± 0.1 pH.

The guppies used in the study were bought in an aquarist shop. After delivery to the laboratory, the fish were stocked into an aerated tank filled with dechlorinated tap water for a 14-day acclimation period. The laboratory conditions were stable throughout the experiment. The study involved a total of 108 guppies weighing 24.2±1.24 g and measuring 43±5.5 mm. During both the acclimation period and the experiment, the fish were fed feed for aquarium fish.

The study involved nine groups of guppies of twelve individuals each. The control group (C) was comprised of fish placed in a tank without any sediment, while the other eight groups were comprised of fish placed in water with the addition of bottom sediments (groups S1 to S8 depending on the sediment used; Table 1). The sediments were collected at eight sites on the Oder River (Table 2). The experiment was carried out in eight plastic aquaria, into which 200 g of selected bottom sediments were placed, while in the ninth aquarium (the control group) there was no bottom sediment. The aquaria were filled with 6 dm^3 of tap water. The aquaria were aerated and the temperature was maintained at a stable 24°C for a week prior to stocking the fish. Thereafter, 12 guppies were placed in each aquarium. During the experiment, a stable water temperature of 24°C was maintained. The duration of fish exposure to heavy metals contained in the bottom sediments was 60 days. Water reaction (pH) measurements were taken on the third, thirtieth, and sixtieth days of the experiment.

TABLE 1

Microwave mineralization protocol applied to fish tissues in an MDS 2000 oven (application for 12 containers)

	Level				
Parameter	I	II	III	IV	V
Power (%)	100	100	100	100	100
Pressure (psi)	20	40	85	135	175
Time (min)	10	10	10	10	5

The whole guppies were analyzed. Samples were placed in Teflon® containers and supplemented with 3 cm^3 of HNO_3. Mineralization was conducted with a CEM MDS 2000 microwave system. Containers were twisted and samples mineralized in a microwave oven according to the protocol presented in Table 1. Afterwards, the samples were transferred quantitatively into polyethylene bottles and filled with deionized water to a mass of 40 g. The samples were then analyzed for Cd, Pb, Cu, and Zn contents.

Lead and cadmium were determined with the flameless atomic absorption spectrometry method with graphite furnace atomization (GF-AAS) with a Perkin Elmer ZL 4110 apparatus, whereas zinc and copper were determined with the atomic emission spectrometry method in inductively coupled plasma (ICP-AES) in a Jobin Yvon JY-24 apparatus. Determinations were made in three parallel replicates.

The results were analyzed statistically with Statistica 6.0 software according to single- and multi-factor analysis of variance (ANOVA, post hoc Dunnett test) at significance levels of P = 0.05 and P = 0.01. The results were checked for similarity to the normal distribution with the Shapiro-Wilk test. As the results were approximated well by the normal distribution, the parametric Dunnett test was applied. Statistical analysis was based on results obtained from all twelve individuals from each group (N = 12).

TABLE 2

Mean concentrations of heavy metals (μg g^{-1} d.m.) bound to bottom sediments in different chemical fractions (n = 12)

Sediment samples	S1	S2	S3	S4	S5	S6	S7	S8
Cd								
Exchangeable fraction	0.13	1.84	0.18	1.88	1.71	2.04	0.77	0.30
Carbonate fraction	0.01	0.01	N.D.*	0.02	0.02	0.02	0.01	0.01
Easily reducible	4.45	9.29	3.94	4.32	2.94	0.67	0.12	1.18
Total concentration	8.24	27.98	8.59	18.85	13.45	6.38	1.86	3.09
Pb								
Exchangeable fraction	0.4	1.1	0.6	1.3	0.8	0.3	0.2	0.2
Carbonate fraction	0.8	1.6	1.7	2.0	0.1	0.2	N.D.	N.D.
Easily reducible	1.4	4.1	3.1	4.7	1.9	3.9	8.0	6.7
Total concentration	128.5	306.4	200.9	213.6	204.0	154.5	80.1	119.2
Zn								
Exchangeable fraction	11.5	118.9	19.9	111.5	76.9	97.2	51.9	10.8
Carbonate fraction	169.0	444.4	242.6	350.2	301.2	191.2	74.3	67.6
Easily reducible	326.7	505.0	471.2	400.0	447.9	255.5	61.9	200.8
Total concentration	954.9	1890.0	1478.8	1482.2	1079.4	1034.9	512.8	513.2
Cu								
Exchangeable fraction	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Carbonate fraction	0.2	1.1	0.2	1.1	1.8	1.8	1.8	0.4
Easily reducible	N.D	N.D.	N.D.	N.D.	N.D	N.D	N.D.	N.D.
Total concentration	107.3	295.8	128.6	197.1	128.9	99.7	54.1	59.2

**N.D. – not detectable, d.m. – dry mass, n –number in one group. Sediment sample collection sites: S1 – West Oder – Mescherin, S2 – East Oder – Gryfino, S3 – West Oder – Krainka, S4 – East Oder – Lake Dąbie, S5 – Roztoka Odrzańska, S6 – Szczecin Lagoon, S7 – Lake Wrzosowskie, S8 – Pomeranian Gulf*

RESULTS

For comparative assessment, data on the concentrations of the analyzed heavy metals (Cd, Pb, Zn, Cu) in bottom sediments (exchangeable, carbonate, easily reducible forms, and total concentration) were used (Table 2). The highest concentration of heavy metals content was found in S2. The pH of water fluctuated over the course of the experiment from slightly acidic (pH = 6.4-6.9) to alkaline (pH = 7.4-7.9) (Table 3). The mean metal concentrations determined in the guppies were compared with those in the bottom sediments.

TABLE 3

Changes in water pH values during the experiment

Section	pH		
	day 3	day 30	day 60
C	5.6	6.1	7.6
S1	6.9	7.6	7.1
S2	6.7	7.4	7.3
S3	6.8	7.6	7.3
S4	6.6	7.4	7.4
S5	6.8	7.3	7.6
S6	6.7	7.8	7.5
S7	6.7	7.9	7.3
S8	6.4	7.4	7.3

As a result of 60 days of exposure to bottom sediments with various heavy metal contamination levels a positive trend was noted for cadmium and lead concentrations in the guppies (Fig. 1, 2). Heavy metal concentrations in fish were varied. It was found that in the control group the concentrations of the analyzed elements in the guppies were markedly lower than in the sediment-exposed groups (Table 4). These differences, however, were statistically non-significant in most cases.

The statistical comparison of mean cadmium concentrations between respective groups of guppies showed that the concentration of this element in the control group differed significantly from that in the guppies from groups S5 ($P < 0.01$) and S7 ($P < 0.05$). The fish from groups S5 and S7 also had the largest amounts of this element (Table 4). The lead concentrations in the guppies from groups S3 and S6 differed significantly ($P < 0.01$) from those in the control group. The fish in the two sedi-

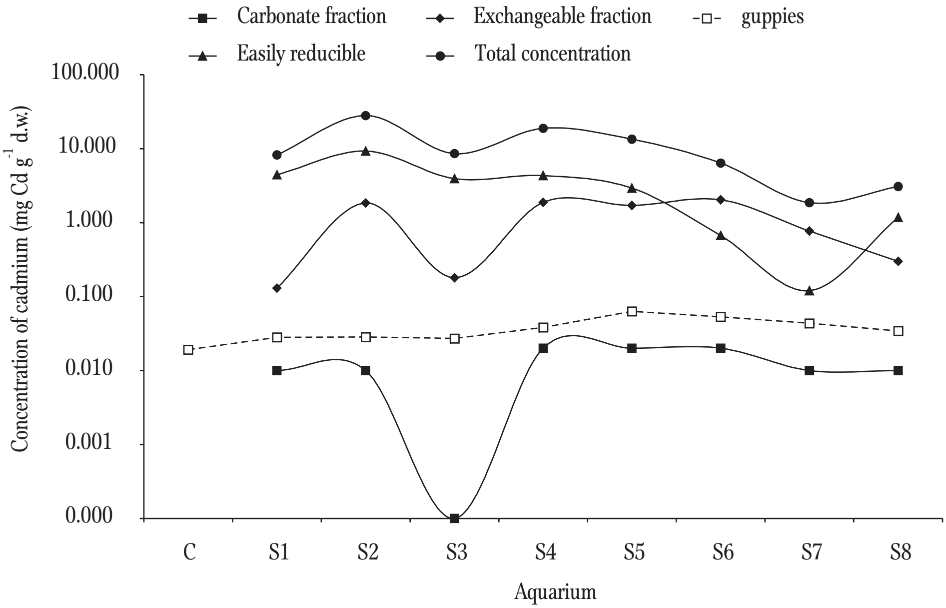

Fig. 1. Log-log plot for mean Cd concentrations (μg g^{-1} d.w.) in guppies and exchangeable, carbonate, easily reducible fractions and total concentration in the bottom sediments.

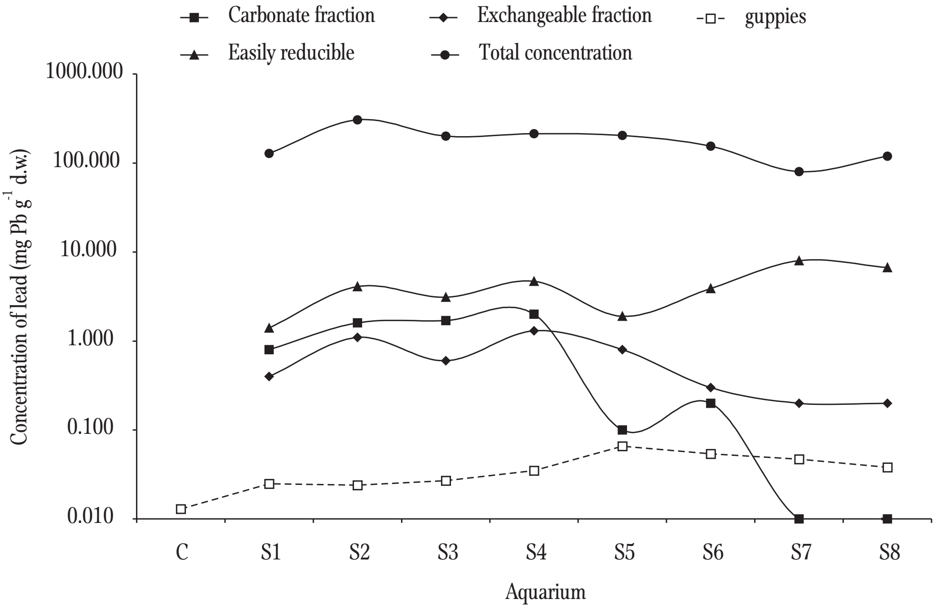

Fig. 2. Log-log plot for mean Pb concentrations (μg g^{-1} d.w.) in guppies and exchangeable, carbonate, easily reducible fractions and total concentrations in bottom sediments.

TABLE 4

Mean concentrations of heavy metals (μg g^{-1} d.m.) in guppies (N=12) exposed to selected bottom sediments from the control (C) and experimental groups (S1-S8)

Sample	Element			
	Cd (±SD)	Pb (±SD)	Zn (±SD)	Cu (±SD)
C	0.014±0.007	0.03±0.02	49.0±14.7	1.04±0.32
S1	0.046±0.012	0.35±0.39	55.5±25.4	0.26±0.02*
S2	0.075±0.047	0.43±0.27	47.8±13.0	0.43±0.43*
S3	0.054±0.040	0.71±0.33*	31.6±4.8	1.21±0.14
S4	0.066±0.056	0.33±0.20	44.1±19.4	1.88±1.81*
S5	0.139±0.238*	0.39±0.46	82.7±24.7*	1.19±0.15
S6	0.035±0.023	1.06±0.96*	60.7±19.5	1.14±0.26
S7	0.117±0.080*	0.14±0.10	66.9±24.9	0.32±0.10*
S8	0.025±0.010	0.35±0.19	41.7±12.5	1.07±0.44

**Significant difference between control and experimental groups*

ment-exposed groups also had the largest concentrations of this metal. Copper concentrations in the fish from groups S3, S5, S6, and S8 were similar to those found in the fish from the control group. The copper concentrations in the fish from the other groups (S1, S2, S4, S7) differed significantly ($P < 0.05$) from concentrations of this metal in the control group guppies (Table 4). The mean zinc concentration in the guppies from group S5 was significantly higher ($P < 0.01$) than that in the control group. The zinc concentrations in the guppies from groups S2, S4, and S8 fluctuated around the mean zinc concentration in the control group guppies. This concentration was higher in the other guppy groups, except in S3 (lower concentration).

There was no significant relationship between the metal concentrations in bottom sediments and those in the guppies exposed to the sediments (Pearson's correlation, $P > 0.05$). Correlation coefficients were low, even though, in case of cadmium (Fig. 1) and lead (Fig. 2), a positive trend was noted between their concentrations in bottom sediments and in the bodies of guppies.

DISCUSSION

The basic indicator of the toxicity of a substance is its bioavailability (Johansson 2002). On the other hand, bioavailability is a complex function of many factors that include the total concentration and speciation of metals, mineralogy, pH, redox potential, temperature, organic matter content, and water flow rate (Luoma 1989, Prosi 1989). The degree of association is also important, as it influences the pollutants routes of movement and their carriers, and physicochemical conditions such as pH and temperature can change this significantly (Johansson 2002).

The current experiment showed that even small environmental changes induced the release of some metals from the sediments to the water column and the accumulation of them in fish. This was apparent in the cases of cadmium and lead, as the concentrations of these two elements in the sediment-exposed guppies were higher than those in fish from the control group. It should be noted that the bottom sediments were placed in water that differed in chemical content and properties from water in the natural environment and that an “individual” state of equilibrium was established between the solid and fluid phases for each system.

More importantly, in this project there were three variables that could have influenced guppy metal accumulation, all of which varied between the various treatments: pH, metal concentration, and the relative proportions of the metal species in each sediment used (i.e., the easily reducible fraction of the each metal compared to total metal varied among the sediments). Therefore, a linear model was developed and expressed as the following equation:

$$[\text{element}]_{\text{fish}} = \text{pH} + [\text{element}]_{\text{sediment total}} + [\text{element}]_{\text{sediment easily reducible}} + [\text{element}]_{\text{sediment carbonate fraction}} + [\text{element}]_{\text{sediment exchangeable fraction}}$$

The kinetics of heavy metal release into a solution differs and depends on pH changes and water composition (Zhou and Kot 1995, Kaoser 2003, Maes et al. 2003). For example, the ability of metal to pass into a solution decreases in the following sequence: Cd > Cu = Zn > Pb under the influence of ammonium acetate extraction (pH = 7).

The water pH values during the experiment changed considerably: at the beginning of experiment they ranged from 6.4 to 6.9 (depending on the aquarium), then they increased over thirty days and ranged from 7.3 to 7.9, followed by an insignificant decrease (7.1 ÷ 7.6) by the end of experiment (Table 3). Considering the fact that bottom sediments from the Oder River used in the experiment were characterized by weak and medium buffer properties (Helios-Rybicka et al. 1995), it should be kept in mind that even insignificant changes in environmental pH could have induced the release of considerable amounts of metals from the solid phase (increased bioavailability) or could have contributed to their passage to the bottom sediment (decreased bioavailability).

The observed changes in aquarium water pH might have been caused by redox processes occurring in the bottom sediments and the presence of microorganisms in the water (Blais et al. 1992), as well as by metabolic products excreted by the fish. Redox processes in a solution are an important parameter of heavy metals speciation, because they change their toxicity, adsorption ability and way of transportation drastically (Matagi et al. 1998).

Helios-Rybicka and Kyzioł (1991) stated that over 50% of the cadmium adsorbed is bound on cation-exchangeable positions, i.e., in mobile forms. According to Suzuki et al. (1979), the adsorption of cadmium takes place mainly in organic materials and it accumulates very easily in aquatic organisms. The process of cadmium dissolution from bottom sediments depends on its form, among other factors. In the case of the bottom sediments used in the current experiment, the largest amounts of this element occurred in exchangeable and easily reducible fractions, i.e., in the fractions that are potentially the most easily mobilized under the influence of environmental changes. The exchangeable and carbonate cadmium fractions are very sensitive to pH changes, and their release into solutions occurs at pH = 7 (Dojlido and Best 1993, Zhou and Kot 1995, Maes et al. 2003).

At the prevailing pH during the experiment, lead forms were also involved in transformations. The soluble forms of lead could precipitate gradually together with the increase of water pH during the experiment, while they could precipitate almost totally in the form of hydroxides at pH = 7 (Helios-Rybicka and Kyzioł 1991). It is conceivable that concentration of the available forms changed continuously under the influence of pH changes, which could affect the concentration of this metal in fish. This may explain the lack of significant correlations between lead concentrations in fish and bottom sediments in this study. Bearing the above in mind, it could be concluded that water pH in the present experiment was one of main factors determining metal sorption and desorption from bottom sediments. Concentrations of cadmium and lead in the fish seemed to be influenced by the metals concentrations in the bottom sediments (Fig. 1, 2), although the correlations detected were statistically non-significant.

Despite significant differences between copper concentrations in the guppies from aquaria S1, S2, S4, and S7 and those in the guppies from the control group (Table 4), no correlations were found between copper concentrations in bottom sediments and those in fish. This is not surprising, as copper concentration in carbonate fraction was vestigial, while in exchangeable and easily reducible fractions it was below the method detection level.

The degree aquatic organisms are exposed to contamination with heavy metals depends on the concentration of water-soluble forms, as metals in this form can be easily absorbed and used by organisms (Campbell 1995, Pizarro et al. 2003). In this case, Cu and Zn concentrations in mobile fractions were small. Moreover, both Cu and Zn are indispensable elements and animals have evolutionary considerably better developed mechanisms of their absorption and maintaining their concentration at necessary physiological level than those occurring in case of toxic metals such as cadmium and Pb.

The way in which the metals were bound to the bottom sediments contributed to some insignificant differences in metal concentrations between the guppies from the sediment-exposed and control groups. As lead, zinc, and copper were mostly in not readily available forms, their passage to the water and their accumulation in the fish were impeded. The rate of desorption could have been small, as the changes that occurred were not induced artificially (the limited influence of external factors), but spontaneously followed a natural course.

CONCLUSIONS

The main factor influencing processes of metal release from the bottom sediments during the experiment was pH change wile the temperature remained stable.

A distinct increase of cadmium and lead concentrations in fish bodies occurred in all sediment-exposed groups.

Zinc concentration in exchangeable fractions was small (1.2 to 10% of the total zinc content in the bottom sediments). In general, no significant increase of zinc concentration in the bodies of the sediment-exposed guppies was determined.

Altogether, the results of this study showed that even small spontaneous changes in environmental conditions can result in notable increases of heavy metal bioavailability to aquatic organisms.

REFERENCES

Berg M., Arnold C.G., Muller S.R., Muhlemann J., Schwarzenbach R.P. 2001 – Sorption and desorption behavior of organotin compounds in sediment-pore water systems – Environ. Sci. Technol. 35: 3151-3157.

Blais J.F., Tyagi R.D., Auclair J.C., Lavoie M.C. 1992 – Indicator bacteria reduction in sewage sludge by a metal bioleaching process – Water Res. 26: 487-495.

Borgmann U. 2000 – Methods for assessing the toxicological significance of metals in aquatic ecosystems: bio-accumulation-toxicity relationships, water concentrations and sediment spiking approaches – Aquat. Ecosyst. Health. Menag. 3: 277-289.

Bourg A.C.M. 1988 – Metal in aquatic and terrestrial systems: Sorption, speciation, and mobilization – In: Chemistry and biology of solid waste (Eds.) W. Salomons, U. Forstner, Springer-Verlag, Berlin: 3-32.

Campbell P.G.C. 1995 – Interactions between trace metals and aquatic organisms: a critique of the free-ion activity model – In: Metal speciation and bioavailability in aquatic system (Eds.) A. Tessier, D. Turner, John Wiley and Sons: 45-402.

Chlopecka A., Bacon J.R., Wilson M.J. 1996 – Forms of cadmium, lead and zinc in contaminated soils from South West Poland – J. Environ. Qual. 25: 69-79.

Dojlido J., Best G.A. 1993 – Chemistry of water and water pollution – Ellis Harwood Ltd, West Sussex.

Gao Y., Kan A.T., Tomson M.B. 2003 – Critical evaluation of desorption phenomena of heavy metals from natural sediments – Environ. Sci. Technol. 37: 5566-5573.

Helios-Rybicka E., Kukułka L., Wardas M. 1995 – Heavy metals in bottom sediments as an indicator of the contamination of the Oder River environment – In: Issues of sewage treatment and water protection in the Oder River catchment, Polanica Zdrój: 241-252 (in Polish).

Helios-Rybicka E., Kyzioł J. 1991 – The role of clays and clay minerals in binding heavy metals in aquatic environments – Zesz. Nauk. AGH, Sozologia i Sozotechnika 31: 45-67 (in Polish).

Impellitteri C.A., Lu Y., Saxe J.K., Allen H.E., Peijnenburg W.J. 2002 – Correlation of the partitioning of dissolved organic matter fractions with the desorption of Cd, Cu, Ni, Pb and Zn from 18 Dutch soils – Environ. Int. 28: 401-410.

Johansson H. 2002 – On distribution coefficients in aquatic systems – Acta Univ. Ups. Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology 687: p. 20.

Kaoser S. 2003 – Concept of copper mobility and compatibility with lead and cadmium in landfill liners – PhD Thesis, Department of Bioresource Engineering, McGill University Montreal, Quebec, Canada, 211 p.

Li Y., Wang X.L., Wang Y., Dong D.M., Zhang H.P., Li Q.S., Li X.C. 2005 – Comparison of Pb and Cd adsorption to the surface coatings and surficial sediments collected in Xianghai Wetland – J. Environ. Sci. (China) 17: 126-129.

Luoma S.N. 1983 – Bioavailability of trace metals to aquatic organisms – A review – Sci. Total Environ. 28: 1-22.

Luoma S.N. 1989 – Can we determine the biological availability of sediment-bound trace elements? – Hydrobiology 176/177: 379-396.

Maes A., Vanthuyne M., Cauwenberg P., Engels B. 2003 – Metal partitioning in a sulfidic canal sediment: metal solubility as a function of pH combined with EDTA extraction in anoxic conditions – Sci. Total Environ. 312: 181-193.

Matagi S.V., Swai D., Mugabe R. 1998 – A review of heavy metal removal mechanisms in wetlands – Afr. J. Trop. Hydrobiol. Fish. 8: 23-35.

McKinney J., Rogers R. 1992 – Metal bioavailability – Environ. Sci. Technol. 26: 1298-1299.

Pizarro J., Rubio M.A., Castillo X. 2003 – Study of chemical speciation in sediments: An approach to vertical metals distribution in rapel reservoir (Chile) – J. Chil. Chem. Soc. 48: 45-50.
Prosi F. 1989 – Factors controlling biological availability and toxic effects of lead in aquatic organisms – Sci. Total Environ. 79: 157-169.
Rayms-Keller A., Olson K.E., McGaw M., Oray C., Carlson J.O., Beaty B.J. 1998 – Effect of heavy metals on *Aedes aegypti* (Diptera: Culicidea) larvae – Ecotoxicol. Environ. Saf. 39: 41-47.
Singh S.P., Ma L.Q., Harris W.G. 2001 – Heavy metal interactions with phosphatic clay. Sorption and desorption behavior – J. Environ. Qual. 30: 1961-1968.
Suzuki M., Yamada T., Miyazahi T., Kawazoe K. 1979 – Sorption accumulation of cadmium in the sediment of the Tama river – Water Res. 13: 57-65.
Zhou X.D., Kot S.C. 1995 – Heavy metal ion adsorption on sediments of the Weiho and Hanjiang Rivers, China – J. Environ. Hydrol. 3: 1-5.

Received – 24 September 2007

Accepted – 08 January 2008

STRESZCZENIE

BADANIA BIODOSTĘPNOŚCI METALI CIĘŻKICH (Cd, Pb, Cu, Zn) ZAWARTYCH W OSADACH DLA GUPIKÓW, *POECILIA RETICULATA* PETERS

Wykonano doświadczenie, mające na celu zbadanie możliwości przechodzenia metali ciężkich (Cd, Pb, Zn i Cu) z osadów do ryb oraz zbadanie zależności między stężeniami tych metali w osadach a ich stężeniami w rybach. Materiał badawczy stanowiły gupiki, *Poecilia reticulata* Peters oraz osady denne o znanych stężeniach oznaczanych metali w poszczególnych formach. W wyniku 60-dniowej ekspozycji ryb na działanie środowiska z osadami o różnym stopniu zanieczyszczenia metalami ciężkimi zauważono tendencję wzrostową stężeń kadmu i ołowiu w gupikach. Analiza porównawcza wykazała, że stopień zanieczyszczenia osadów metalami ciężkimi miał wpływ na ich stężenia w gupikach. Stwierdzono, że nawet niewielkie zmiany warunków środowiska mają wpływ na uwalnianie metali z osadów, co powoduje zauważalne różnice w stężeniach badanych metali w rybach.