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CHANGES OF CADMIUM CONTENT IN VARIOUS ORGANS OF COMMON CARP (*CYPRINUS CARPIO* L.) DURING THE FAST GROWTH PERIOD FOLLOWING INITIAL REARING IN CONTAMINATED WATER

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ABSTRACT. In this paper, the dynamics of cadmium elimination were assessed in various organs of freshwater common carp, *Cyprinus carpio* L. The fish used in the study were undergoing a period of intensive growth between the third and sixth months of life. The aim of this study was to assess comparatively cadmium elimination dynamics by various organs of common carp after transferring them to a laboratory environment, taking into account their growth rate and accompanying seasonal changes. The study focused on individuals of common carp obtained from an experimental station where they had been kept in post-cooling water. The study was divided into four stages during which various fish organs and tissues (liver, kidneys, skin, gills, alimentary tract, muscles) were examined and subjected to assays for cadmium content at pre-determined periods of time. Average cadmium contents in the examined carp organs ranged from 0.004 to 0.053 μg g^{-1} wet weight. The highest values of Cd content were recorded in the liver, the mid posterior section of the alimentary tract, and the gills, while the lowest value was noted in the muscles.

Key words: COMMON CARP (*CYPRINUS CARPIO*), CADMIUM, ELIMINATION

INTRODUCTION

Fish are at the top of the food chain in freshwater ecosystems. The contamination of water reservoirs can lead to the accumulation of toxic compounds in living organisms – both plants and animals. Being at the top of the trophic pyramid in freshwater ecosystems, fish can accumulate high amounts of toxic compounds in their bodies. Post-cooling water is often used for fish breeding and farming. For many years this type of aquaculture had been conducted in a channel receiving post-cooling water from the Dolna Odra Power Plant (northwest Poland). An investigation performed by the Inspec-

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torate for Environmental Protection confirmed that both post-cooling water from the Dolna Odra facility and human activity can contribute significantly to the pollution of the natural environment. In fact, physical disruptions such as drainage are also significant (hydropower). The power plant discharge waters and the adjacent waters of the Oder River contain trace amounts of toxic compounds, including cadmium (Anonymous 2004). However, the concentrations determined did not exceed permissible limits (Anonymous 1991, Anonymous 2002a) and were lower than the maximum limits for class III water quality standards (category A3) (Anonymous 2002b, Anonymous 2004).

The aim of this study was to assess comparatively the dynamics of cadmium distribution and the elimination by various organs of common carp, *Cyprinus carpio* L., individuals reared initially for three months in post-cooling water from the Dolna Odra Power Plant. Additionally, the fish growth rates and spring-summer seasonal changes were measured and correlated to cadmium concentrations.

MATERIALS AND METHODS

The study examined individuals of common carp obtained from the experimental station of the Agricultural University of Szczecin (Poland), where they had been kept in post-cooling water from the Dolna Odra Power Plant. The post-cooling water is collected in a channel and discharged into the Oder River. The channel provides good conditions for year-round fish cultivation due to the near constant water temperature that ranges from 20 to 22°C. At the beginning of the study the fish weighed 46.2 ± 5.1 g and were 8.4 ± 2.5 cm in body length (BL). After the four-month study, fish weight had increased to 125.3 ± 13.4 g at an increased body length of 19.1 ± 2.1 cm. A total of 120 individuals measuring 10.9-20.2 cm and weighing 51.3-138.7 g were examined. At the beginning and end of the study, the carp were three and six months old, respectively. After one month 30 fish were sacrificed. At the conclusion of each subsequent month of the study an additional 30 fish were sacrificed. After the fish were delivered to the laboratory, 20 individuals were stocked into each of six 120-l aerated tanks filled with tap water. Forty-eight hours prior to stocking the fish, the tanks were filled and aerated in order to dechlorinate the water and raise its temperature to the ambient level. The carp were fed with Aller Aqua (Poland) pelleted feed mix that contained 37% protein and

12% fat; this was the same feed applied in the culture at the experimental station. The daily food ration was 3.4 ± 0.2 g fish^{-1}.

Throughout the study, the tank water temperature range was 21 ± 2°C; the dissolved oxygen content, pH, and water hardness were 8.0-9.0 mg l^{-1}, 7.0-7.5, and 10.71 mval l^{-1}, respectively. The tank water was changed every second day to remove toxic compounds excreted by the fish. The water was not subjected to chemical assays of cadmium content due to its frequent rotation and significant contamination by food residues and fish excrement. The cadmium content in the tank water in the laboratory was 0.003 ± 0.002 μg ml^{-1}.

Chemical assays were performed on samples from the gills, anterior and mid posterior sections of the alimentary tract, liver, kidneys, skin, and muscles dissected from each fish from each of the four samplings. The samples were collected in four stages – at the beginning of the study and then at one-month intervals. The tissues were frozen and kept at a temperature of -20°C until analysis. Prior to the assay, 1 g tissue samples (weighing 0.001 g) were mineralized wet in 3 ml concentrated HNO_3 in a CEM MDS 2000 microwave oven. The solution obtained was transferred quantitatively to polyethylene bottles and brought to 30 g with deionized water. These samples were then assayed for cadmium content.

Cadmium was determined with flameless graphite furnace atomic absorption spectrometry (GF-AAS) in a ZL 4110 Perkin Elmer spectrometer. The cadmium content in the tissue was calculated from a relevant calibration curve after correcting the data with blind sample results. Cadmium contents are presented in μg g^{-1} wet weight (w.w.)

The results were analyzed statistically with Statistica 6.0 software with one- and multi-way analysis of variance (ANOVA, Scheffé's test) at a significance level of P = 0.05.

RESULTS

The mean cadmium contents in the examined organs ranged from 0.004 to 0.053 μg g^{-1} w.w. The highest values of cadmium content were noted in the liver (0.053 μg g^{-1} w.w.), the mid posterior section of the alimentary tract (0.040 μg g^{-1} w.w.), and the gills (0.039 μg g^{-1} w.w.), while the lowest value was noted in the muscles (0.004 μg g^{-1} w.w.) (Table 1).

TABLE 1

Cadmium contents in the organs of the examined fish

Organ	Cd content (μg g^{-1} w.w.)*				Statistical significance of differences, P < 0.05*
	mean	± SD	min.	max.	Within groups = changes over time
Liver	0.053	0.007	0.041	0.067	ns
Alimentary tract	0.030	0.004	0.026	0.035	ns
Anterior section of alimentary tract	0.019	0.003	0.014	0.031	ns
Mid posterior section of the alimentary tract	0.040	0.009	0.026	0.065	ns
Gills	0.039	0.006	0.025	0.053	ns
Skin	0.022	0.003	0.018	0.028	ns
Muscles	0.004	0.001	0.002	0.006	ns
Kidneys	0.031	0.006	0.021	0.046	ns

**see text for explanation of group symbols; SD – standard deviation; sample size: N=120; ns – changes over time non-significant, w.w. – wet weight*

In the liver, the cadmium content increased slightly after the fish had been held in non-polluted tank water for one month. After this, the toxic compounds began to be eliminated from the liver. At the end of the study, the cadmium content in the liver was lower by an average of 20% compared to the highest measured value. Conversely, cadmium elimination from the length of the alimentary tract began at the beginning of the study and continued throughout the four-months of its duration. The mean cadmium content in the alimentary tract decreased by 26% compared to the value noted at the beginning of the study (Fig. 1).

The cadmium content in the anterior section of the alimentary tract fluctuated slightly by an average of 13%. Over the course of the four-month study, cadmium was eliminated from the mid posterior section of the alimentary tract by an average of 32% compared to the initial value. In both the anterior and mid posterior sections of the alimentary tract, the elimination of cadmium started as soon as the fish were transferred to the laboratory environment (Fig. 2).

The cadmium content in the gills decreased by an average of 24.5% over the four months of laboratory tank culture. Conversely, cadmium was eliminated from the skin

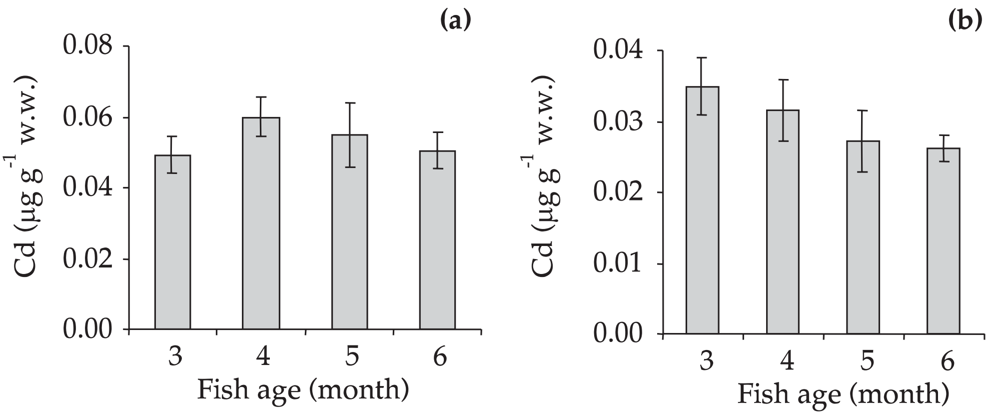

Fig. 1. Changes of cadmium contents in the liver (a) and alimentary tract (b) throughout the intensive carp growth period between months three and six of life (mean ± SD).

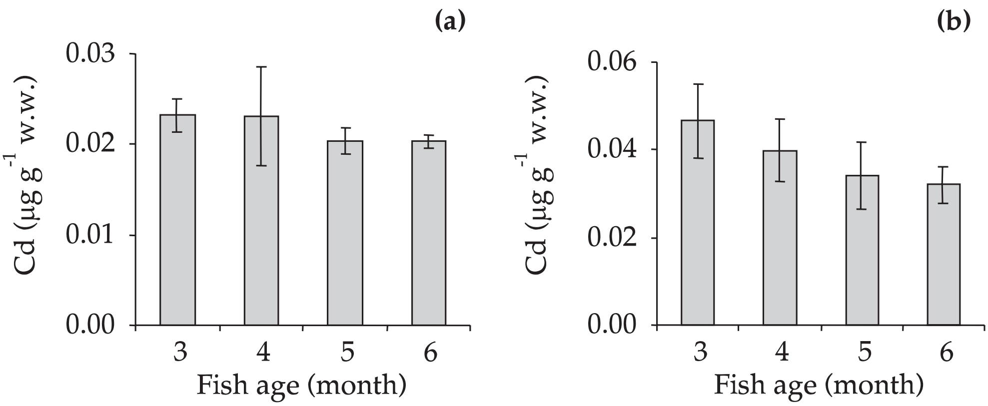

Fig. 2. Changes of cadmium contents in the anterior (a) and mid posterior sections of the alimentary tract (b) throughout the period of intense carp growth between months three and six of life (mean ± SD).

very slowly – only by an average of 4.5% of the initial value over the course of the four-month study (Fig. 3).

Correlation coefficients and linear regression equations were calculated for changes of cadmium contents in all the examined organs (Table 2). The highest determination coefficient R^2 values were noted for relationships between study duration and cadmium content throughout the length of the alimentary tract (0.953), which included

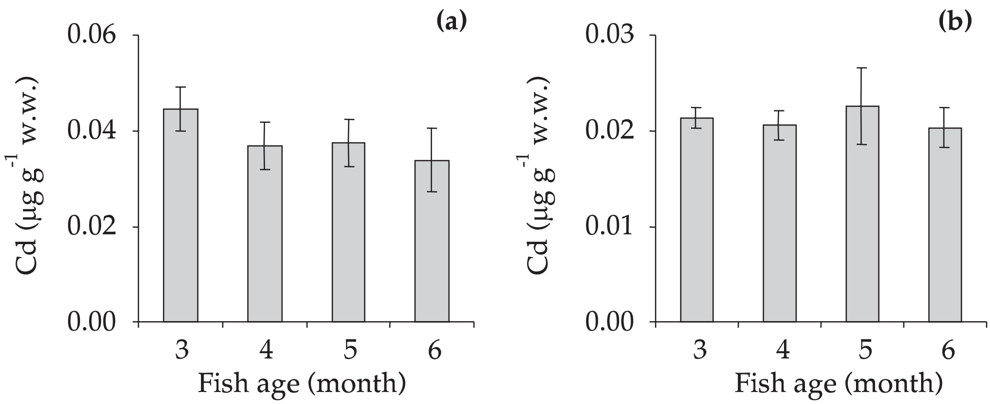

Fig. 3. Changes of cadmium contents in the gills (a) and skin (b) throughout the period of intense carp growth between months three and six of life (mean ± SD).

both the anterior (0.817) and mid posterior (0.953) sections. Neither for the liver nor for the skin were statistically significant relationships noted between the duration of the study and cadmium content.

TABLE 2

Linear regression equations and determination coefficients for the studied interactions: cadmium in organs (y) over time (x)

Organs	Linear regression equations	Determination coefficient R^2
Liver	y = -0.0001x + 0.0538	R^2 = 0.001
Alimentary tract	y = -0.00031x + 0.0037	R^2 = 0.953*
Anterior part of alimentary tract	y = -0.0011x + 0.0246	R^2 = 0.817*
Mid posterior section of the alimentary tract	y = -0.005x + 0.0506	R^2 = 0.953*
Gills	y = -0.0031x + 0.0461	R^2 = 0.795*
Skin	y = -0.0001x + 0.0215	R^2 = 0.016
Muscles	y = -0.0005x + 0.0045	R^2 = 0.598*
Kidneys	y = 0.0011x + 0.0274	R^2 = 0.633*

** – statistically significant determination coefficients, P < 0.05, N=120*

Cadmium content in the muscles decreased by an average of 25% during the four-month period after the fish had been transferred to the laboratory tanks. On the other hand, cadmium content in the kidneys increased over the same period by an average of 10% compared to the initial value observed at the beginning of the study (Fig. 4).

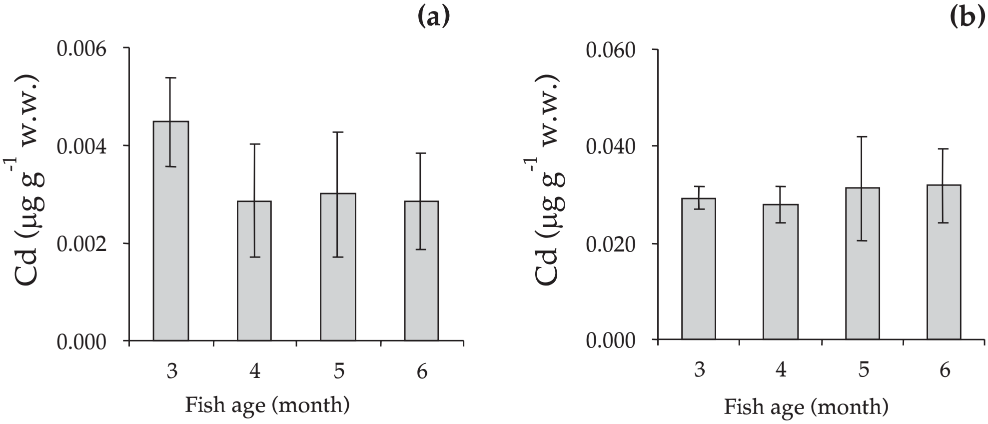

Fig. 4. Changes of cadmium contents in the muscles (a) and kidneys (b) over the period of intensive carp growth between months three and six of life (mean ± SD).

DISCUSSION

Aquatic environments are degraded mainly through intensive industrial development and the application of chemical agents in agriculture (Singh et al. 2000). Industrial power plants produce significant amounts of post-cooling water, which are discharged into natural waters thus raising temperatures and altering water chemical composition. Year-round fish cultivation is possible in the post-cooling waters from the Dolna Odra Power Plant thanks to the near constant temperature conditions; however, the fish may be exposed to toxic compounds. The requirements for freshwater quality for fish culture are put forward in the Decree of the Minister of Environment of 4 October 2002 (Anonymous 2002a), which incorporates into Polish legislation the Council Directive No 79/659/EEC regarding the protection and improvement of freshwaters in order to maintain fish life (Anonymous 2002b). Monitoring data from the western Pomeranian Province from 2002-2003 indicate that the concentrations of total phosphorus and nitrogen in nitrite forms in waters inhabited by fish were usually slightly above the permissible levels and that river waters were poor in oxygen (Anonymous 2004). However, the concentrations of heavy metals were within permissible limits, one of which, namely cadmium, is the subject of the current paper. Although this element is toxic to fish, it is not very toxic to humans as it is absorbed in less than 5%. In most countries, including Poland, the permissible cadmium concentration in potable water is 5 μg l^{-1}, while in sewage discharged into surface waters it is 100 μg l^{-1} (Anonymous 1991). The permissible concentration of cadmium in natural waters of quality class I is 5 μg l^{-1} (Anonymous 1991).

In the current study the dynamics of absorption, distribution, and, especially, the elimination of cadmium from carp as well as the processes occurring during fish recovery were followed. Both environmental and internal factors affect these processes. The accumulation of metals, including cadmium, in fish inhabiting contaminated water depends on the concentration of these metals in the water and food, physiochemical factors, and the duration of exposure. The toxic effect of metals depends on the site of their deposition in the body. Various metals show different degrees of affinity to various organs and are accumulated in them in different amounts (Jezierska and Witeska 2001). The highest cadmium accumulation was observed in the liver (0.053 μg g^{-1} w.w.) and gills (0.039 μg g^{-1} w.w.). The highest concentration of cadmium in whole carp was noted by Szakolczai et al. (1992). One month following the transfer of fish to laboratory basins with uncontaminated water the process of the elimination of cadmium from the liver was observed (Fig. 1a). High concentrations of cadmium in the kidneys and liver are related to detoxification processes that occur in these organs.

The elimination process was also observed in the alimentary tract (Fig. 1b) both in the anterior (Fig. 2a) and mid posterior sections (Fig. 2b). Between months four and six, cadmium was distributed in the anterior section of the alimentary tract, while as of month four, the metal began to be eliminated. Cadmium was transported from the anterior section of the alimentary tract to the liver. This process continued until the end of the study. The slow depuration of cadmium from the gills was observed throughout the study (Fig. 3a).

Carpene et al. (1994) observed maximum cadmium concentration in the gills (0.37 μg g^{-1} dry weight (d.w.)) and kidneys (1.21 μg g^{-1} d.w.). The level of cadmium in the skin was constant for the four months of the current study (Fig. 3b). This confirms statistically significant differences between the cadmium contents in the studied tissues (Table 2). One month after the fish were transferred to basins with clean water a reduction in cadmium levels was observed in the muscles (Fig. 4a). This was confirmed by statistical calculations; negative linear regression equations were obtained for all the studied organs with the exception of the kidneys (Table 2). Maximum cadmium concentrations in the muscles of common carp from natural water bodies were observed by Markiewicz et al. (1998) (0.004 μg g^{-3} w.w.) and Szulkowska-Wojaczek et al. (1992) (0.72 μg g^{-3} w.w.).

The duration of exposure plays a key role in the metal deposition pattern. The time of exposure affects the final metal accumulation in the tissues, but it is difficult to assess under natural conditions. The accumulation of metals in the organs of fish is a function of uptake and depuration rates, and metal concentrations in various organs may change during exposure, according to various patterns. Time affects not only the whole body metal concentration but also its distribution in various organs (Jezierska and Witeska 2001). At the beginning of the current study elevated cadmium levels were noted in all the examined organs and tissues. Then cadmium levels decreased as a result of transferring the fish to laboratory tanks. This process is known as recovery (Jezierska and Witeska 2001). Fish growth and recovery after exposure were the main factors determining cadmium levels in the present study.

Heavy metals, including cadmium, are natural elements that are always present in nature and participate in the circulation of matter. Human activity has intensified the introduction of heavy metals into the processes of matter exchange (Lee et al. 2000, O'Day et al. 2000). The increase of heavy metal concentrations in aquatic environments influences cadmium uptake in fish. Metals can penetrate the fish through the alimentary tract, the skin, and gills where they are absorbed directly from the water or food (Protasowicki 1991, Protasowicki and Chodyniecki 1992, Sreedevi et al. 1992). Many factors influence the amounts of metals retained. Some of them include fish species, age, body weight and length, sex, season of the year, and fishing ground (Liang et. al. 1999, Liang and Wong 2000, Cain et al. 2004). An important factor is bioavailability from food and water. Trace amounts of cadmium determined in the three-month-old carp may have resulted from the contamination of post-cooling water from the Dolna Odra Power Plant by atmospheric deposition, sewage disposal, and/or drain water discharge. This is confirmed by the fact that the highest cadmium concentrations were noted in the liver, alimentary tract, and gills, while the lowest was noted in the muscles. Protasowicki (1991) and Markiewicz et al. (1998) drew similar conclusions in their investigations of cadmium accumulation. They noted that most of the cadmium accumulated in the organs responsible for absorption and elimination (gills, alimentary tract, liver, and kidneys), which influenced substantially the life processes of fish.

The slight decrease of cadmium concentration in the organs of carp observed over the course of the four months of the study was the result of transferring the fish to clean laboratory tanks filled with tap water containing slight amounts of toxic compounds present in the external environment (see Materials and Methods section).

The second factor was the intense growth of the carp and the accompanying elimination of cadmium to the water. The study was performed in the second quarter of the year, which could have contributed to the fact that high correlation coefficients were obtained. If fish double in size, the cadmium content will still be absorbed; however, some organs (liver, gills, kidneys) will eliminate it. The higher ambient temperature and increasing daylight period probably caused increased fish activity during the day. Due to this, their metabolic rates increased and toxic compounds were eliminated more rapidly from the fish. This conclusion is confirmed by the fact that cadmium content decreased in carp organs by an average of 4.5 to 32%. The most effective elimination of cadmium occurred in the mid posterior section of the alimentary tract (32%), the gills (24.5%), and the muscles (25%). The least effective elimination was noted in the skin (4.5%). De Boeck et al. (1997) came to similar conclusions in their study of the influence of photoperiod on carp physiology and iron accumulation. The ability to eliminate cadmium rapidly by young fish during the period of intense growth is very important considering their value as a consumer product.

CONCLUSIONS

1. The average cadmium contents in the examined carp organs ranged from 0.004 to 0.053 µg g^{-1} w.w.
2. The highest cadmium concentrations were found in the liver and the mid posterior section of the alimentary tract, while the lowest was noted in the muscles.
3. The cadmium contents in the organs of the studied carp fluctuated significantly with the exception of the values for the liver and skin throughout the study that was conducted between months three and six of life during the intense growth period.

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STRESZCZENIE

ZMIANY ZAWARTOŚCI KADMU PODCZAS SZYBKIEGO WZROSTU W POSZCZEGÓLNYCH NARZĄDACH KARPIA (*CYPRINUS CARPIO* L.), W TRAKCIE HODOWLI PO PRZENIESIENIU Z ZANIECZYSZCZANEJ DO CZYSTEJ WODY

W pracy przedstawiono dynamikę eliminacji kadmu z wybranych narządów ryb słodkowodnych na przykładzie karpia, *Cyprinus carpio* L. Ryby wzięte do badań znajdowały się w okresie intensywnego wzrostu między 3 a 6 miesiącem życia. Badania obejmowały prześledzenie zmian poziomu kadmu u karpi po ich przeniesieniu do czystego środowiska laboratoryjnego. Karpie przed rozpoczęciem badań przebywały w zrzutowych wodach pochłodniczych. Eksperyment został podzielony na 4 etapy czasowe, podczas których pobierano od ryb do badań tkanki i narządy (wątrobę, nerki, skórę, skrzela, przewód pokarmowy i mięśnie). Średnia zawartość kadmu w badanych narządach karpi kształtowała się w przedziale od 0,004 do 0,053 μg g^{-1} mokrej masy (tab. 1). Najwyższą zawartość Cd oznaczono w wątrobie (rys. 1a), środkowo-końcowej części przewodu pokarmowego (rys. 2b) i w skrzelach (rys. 3a), a najniższą w mięśniach (rys. 4a).