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ORIGINAL PAPER

CADMIUM ACCUMULATION IN THE SCALES OF PRUSSIAN CARP (*CARASSIUS GIBELIO* BLOCH, 1782) FOLLOWING EXPOSURE TO CADMIUM IN WATER*

Ewa Łuszczek-Trojnar, Damian Ryndak, Ewa Drąg-Kozak

**Department of Animal Nutrition and Biotechnology, and Fisheries
University of Agriculture in Krakow, Poland**

Abstract

The aim of the study was to investigate the accumulation of cadmium in the scales of Prussian carp (*Carassius gibelio* B.) as an indicator tissue of water contamination by cadmium. The study was carried out on 70 mature Prussian carp, which were placed in three 300L tanks. The fish were exposed to cadmium dissolved in water at a concentration of 0.0, 0.4 or 4.0 mg L^{-1} for a period of 84 days. After 42 and 84 days of exposure, 10 fish were randomly sampled from each tank. The fish were decapitated and all their scales were collected. The scales were rinsed and each was then carefully sectioned into an inner part and an outer part, removing the outermost growth ring, which most likely included scale increment from the exposure period. Cadmium concentration in the samples was then measured using the atomic absorption method. The analysis of the results showed that the concentration of cadmium in the scales increased with the duration of exposure and was significantly higher in the outer growth rings. The accumulation of cadmium in this part of the scales was correlated with the dose of cadmium and the duration of exposure. The results obtained confirmed the hypothesis that the concentration of cadmium in the outermost growth rings of fish scales reflects the most recent status of environmental contamination by this metal. Scales, which – unlike other tissues such as the liver and muscle – can be sampled non-lethally, seem to be an excellent bioindicator of environmental contamination by heavy metals.

Keywords: cadmium, fish scales, Prussian carp, bioindicator

Ewa Łuszczek-Trojnar, prof. URK, Department of Animal Nutrition and Biotechnology, and Fisheries, Agriculture University in Krakow, Spiczakowa 6, 30-198 Krakow, Poland, phone: 0048 12 637 76 51; fax: 48 12 638 59 79, e-mail: ewa.trojnar@urk.edu.pl
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INTRODUCTION

Fish are considered to be excellent indicator organisms. They are used in the biomonitoring of surface waters for the ichthyological assessment of watercourses. For this purpose, they are sampled mainly by electrofishing, identified to the species level, weighed, measured and then released back to water (EFI 2009, Prus et al. 2016). The Water Framework Directive (2000) allows for the lethal sampling of fish to measure the concentrations of priority substances in entire fish samples or in individual fish tissues or organs, such as the liver and kidneys (Guidance Document No. 32). In order to protect fish life and ensure that fish are not killed unnecessarily, the use of indicator tissues that can be sampled non-lethally should be considered. Fish scales are exactly such a tissue. They grow in the dermis throughout the entire life of fish and can be sampled non-lethally.

For many decades, ichthyologists have used fish scales as a tool for non-lethal age determination in fish (Hile 1936). Furthermore, the analysis of scale malformations and deformities can demonstrate the negative effects of xenobiotics present in water (Sultana et al. 2017). In one study, archived fish-scale samples, collected over the four-decade history of the Římov Reservoir in the Czech Republic, were successfully used to determine long-term trends in CO_2 concentrations, which shows that fish scales can serve as an excellent indicator tissue for long-term monitoring of biochemical parameters in the aquatic environment (Vašek et al. 2021). Fish scales are a tissue which allows for the measurement of the concentrations of particular substances and determination of the age of fish without any negative consequences for the animals (Zydlewski 2010). Each scale collected for analysis is replaced by a new scale to ensure complete protection of the fish's body. An idea worth considering would be to develop a model for a non-lethal collection of scales during the monitoring of fish to estimate the level of environmental pollution with heavy metals. There are studies which confirm that the levels of certain heavy metals in scales are correlated with the pollutant concentration in whole fish (Łuszczek-Trojnar et al. 2013, 2015, Łuszczek-Trojnar, Nowacki 2021). As the scale grows, new sclerites are formed out of calcium, phosphorus and other elements which are taken up by the fish and present in its blood (Saueri, Watabe 1989, Wells et al. 2000, Ramsay et al. 2011), including heavy metals that are found in the fish's body. Łuszczek-Trojnar, Nowacki (2021) analysed three consecutive annuli on scales collected from carp kept in a pond farm. They found that the more recent the annulus, the higher the levels of metals it contains. The body weight of fish increases with age, which would explain their increasing feed intake and the increasing uptake of heavy metals. While some of the metals are eliminated, those that accumulate in hard tissues (bones and scales) remain there for many months or even years (Łuszczek-Trojnar et al. 2013). It may happen that the soft tissues of an individual fish contain no heavy metals, but its scales and bone tissue show increased heavy metal concentrations, indicating the presence of environmental contamination at a much earlier point in time – several months or years earlier. In light of the current knowledge, it would be very interesting to learn whether the identified environmental contamination with a given heavy metal that is present during a particular period of an individual fish's life will be reflected in the scale increment corresponding with the period of exposure to this metal.

Therefore, the aim of the study was to investigate the concentration of cadmium in appropriately sectioned scales of fish exposed to cadmium in water for a period of 84 days. The fish species used for the study was Prussian carp. The species was chosen due to its size (it is large enough for easy collection and sectioning of scales and small enough to be kept in tanks) and resilience to unfavourable environmental conditions. These features make it an excellent experimental model in toxicological studies on cyprinids.

MATERIAL AND METHODS

The experiments were performed in accordance with the research protocols approved by the Local Animal Ethics Committee in Krakow, Poland (No. 144/2019). The study was performed on 70 Prussian carp (*Carassius gibelio* B.) aged 3 years with a mean body weight of 205 g and mean body length of 23 cm. The fish were sourced from the Fisheries Experimental Station of the University of Agriculture in Krakow, which is where the experiment was conducted. After the wintering period, the fish were caught during the spring fishing period and transported to tanks. After a 30-day acclimation period, 10 fish were randomly sampled, and their scales were collected for cadmium concentration analysis. The fish formed the basal group. The remaining fish were divided into three groups: group Cd1, group Cd2 and control group C, each comprising 20 fish (two repetitions). The groups were placed in separate 300L tanks and kept under a 14:10 light-dark cycle. The water in all the tanks was changed every 2 days to maintain a constant level of contamination of fish in the experimental groups. The physicochemical parameters of water were as follows: temperature – 18°C, pH – 7.6, dissolved oxygen concentration – 9.5 mg L^{-1}, water hardness – 186 mg $CaCO_3$ L^{-1}. In addition, the water was aerated. During the acclimation period and the experiment, the fish were fed commercial complete feed for carp. The feed contained cereal seeds, fish meal, oil, vitamins and supplements. It comprised 12% crude fat, 37% protein and 32.5% carbohydrate. During the experiment, the fish were fed a ration of approximately 3% of body weight daily. The fish in the control group were kept in water without the addition of cadmium, fish in the Cd1 group – in water with a cadmium concentration of 0.4 mg L^{-1} and fish in the Cd2 group – in water with a cadmium concentration of 4.0 mg L^{-1}. The stock solutions were prepared using cadmium chloride. The cadmium concentrations used in the study were chosen based on the literature data, stating that cadmium concentrations in polluted waters can range between 1 μg L^{-1} and over 16.1 mg L^{-1} (Tilton et al. 2003, Peng et al. 2009).

During the period of exposure, scale samples were collected twice – after 42 and after 84 days. For this purpose, 10 fish were randomly sampled from each group. The fish were decapitated and all scales from each fish were then collected for analysis. The scales sampled were stored at a temp. of -20°C until concentration analysis. Once the scales were thawed and rinsed with deionised water, each scale was carefully sectioned into two parts, removing the outer, most recent growth increment with a width of approximately 1 mm, where the mean scale diameter was 10 mm. The cutting line was parallel to the edges of annual growth rings on the scales. To ensure a precise cut, the scales were sectioned manually using specialist tools such as serrated tweezers, which were used to hold the scales, and curved scissors. The prepared scales from each fish were divided into: subgroups Cd1-in and Cd2-in – which comprised the inner parts of scales, including growth rings, collected from fish exposed to 0.4 mg Cd L^{-1} and 4.0 mg Cd L^{-1}, respectively; and subgroups Cd1-out and Cd2-out – which comprised the outer parts of scales, including the last growth ring, which most probably included the increment formed during the exposure to 0.4 mg Cd L^{-1} and 4.0 mg Cd L^{-1}, respectively. Scales collected from the control fish after 42 and 84 days of the experiment were sectioned in accordance with the procedure described above, whereas scales collected prior to exposure were left uncut to compare the concentration of cadmium in particular parts of scales to its concentration in whole scales collected before the period of exposure (day 0).

The scale samples, divided into groups and subgroups, were subjected to mineralisation and Cd concentration analysis using the atomic absorption method, in accordance with the methodology described by Łuszczek-Trojnar and Nowacki (2021). The results of the cadmium concentration analysis were expressed as mg/kg of wet tissue weight.

Based on the results obtained, bioconcentration factors were calculated in accordance with the following formula:

BCF – Cd concentration in fish/Cd concentration in water.

The results were analysed statistically using one-way analysis of variance. Student's *t*-test was used to determine the significance of differences in cadmium concentration in the scale fragments analysed. Differences in cadmium concentration between particular parts of scales in the same individual and differences between the experimental groups and the control were analysed. In addition, Pearson's correlation analysis was used to assess the correlation between Cd concentration in scales and the exposure concentration of the metal and the duration of exposure. The data were analysed using Statistica 12.

RESULTS

The mean cadmium concentration in the scales collected from randomly caught fish before exposure to cadmium (day 0) was 0.021 mg kg^{-1}. During the exposure period, a dose- and time-dependent increase in Cd concentration in both the inner and outer parts of scales was observed. After 42 days of exposure, cadmium concentration in the inner parts of the scales of fish exposed to 0.4 mg Cd L^{-1} was 0.122±0.010 mg Cd kg^{-1}, whereas the concentration of the metal in the outer parts of the scales was more than twice as high, i.e. 0.257±0.018 mg kg^{-1}. The fish exposed to the higher concentration of cadmium (4.0 mg Cd L^{-1}) also accumulated cadmium in their scales. However, no statistically significant difference in Cd concentration was observed between the outer and inner parts of scales collected from those fish. After 84 days of exposure, an increase in cadmium concentration was observed in each of the experimental groups studied. The highest concentration of cadmium (3.964±0.290 mg kg^{-1}) was observed in the outer parts of scales collected from fish that were exposed to cadmium at a concentration of 4.0 mg L^{-1} for a period of 84 days. The concentration was more than twice as high as the concentration of cadmium in the inner parts of the scales, and was statistically significantly different from the Cd concentration found in the scales of other fish groups and at different sampling times (Table 1).

After 42 days of exposure, it was found that cadmium concentration in the scales of fish in group Cd1, which were exposed to cadmium at a concentration of 0.4 mg L^{-1}, increased to a level that was statistically significantly higher compared with the concentration found in the control group. Moreover, a significant difference in Cd concentration between the outer (0.257±0.018 mg kg^{-1}) and inner parts (0.122±0.010 mg kg^{-1}) of scales collected from those fish was observed after the shorter period of exposure.

Table 1

Comparison of Cd concentrations (±SE) in the fragments of scales collected from fish exposed to Cd at a concentration of 0.4 mg Cd L^{-1} (group Cd1) or 4.0 mg Cd L^{-1} (group Cd2).

Duration of exposure [days]	C-in	C-out	Cd1-in 0.4 mg Cd L^{-1}	Cd1-out 0.4 mg Cd L^{-1}	Cd2-in 4.0 mg Cd L^{-1}	Cd2-out 4.0 mg Cd L^{-1}
0	0.021±0.001 *A*					
42	0.049±0.001*Ba*	0.069±0.004*Ba*	0.122±0.010*Bb*	0.257±0.018*Bc*	0.959±0.197*Bd*	1.356±0.107*Bd*
84	0.062±0.005*Ba*	0.161±0.031*Cb*	0.311±0.071*Cb*	0.949±0.280*Cc*	1.581±0.153*Bc*	3.964±0.290*Cd*

C-in, Cd1-in and Cd2-in – inner part of scales, C-out, Cd1-out and Cd2-out – outermost growth ring of scales. Small letters *abcd* denote statistically significant (p<0.05) differences in means between the groups (in rows), whereas capital letters *ABC* indicate significant differences in means between exposure times within particular groups (in columns).

Measurements performed after 84 days of exposure showed that Cd concentration in the inner parts of scales collected from fish in that group increased to 0.311±0.071mg kg^{-1} and was almost three-fold higher compared with the previous measurement, whereas Cd concentration in the outer parts of the scales was 0.95 mg kg^{-1} and was also over three-fold higher compared with the previous measurement.

In group Cd2, i.e. fish exposed to 4.0 mg Cd L^{-1}, measurements performed after 42 days of exposure showed that Cd concentration in the inner parts of scales increased to 0.959±0.197 mg kg^{-1}, whereas Cd concentration in the outer parts of scales increased to 1.36 mg kg^{-1}. The difference in Cd concentration between the inner and outer parts of scales was not statistically significant. During further exposure, Cd concentration in the inner parts of scales did not increase statistically significantly (1.581±0.153 mg kg^{-1}), whereas Cd concentration in the outer parts of scales increased three-fold and the increase was highly statistically significant (3.964±0.290 mg kg^{-1}) – Table 1.

After 42 days of exposure, bioconcentration factor values for cadmium in the scales analysed were higher in fish exposed to the lower concentration of the metal in water, both in the inner and outer parts of the scales. However, after 84 days of exposure, no statistically significant differences in BCF values were observed between Cd1-in and Cd2-in and between Cd1-out and Cd2-out (Table 2).

The analysis of changes in BCF values over the exposure period showed a significant increase in the BCF both in the group of fish exposed to 0.4 mg Cd L^{-1} and that exposed to 4.0 mg Cd L^{-1}. However, the significance level for the increase in group Cd1 was $p<0.05$, whereas in subgroups Cd2-in and Cd2-out it was $p<0.01$ and $p<0.001$, respectively (Table 2).

The Pearson's correlation analysis showed that Cd concentration in all the parts of scales analysed was statistically significantly correlated with the dose of the metal and the duration of exposure (Table 3).

DISCUSSION

This paper presents the results of the first-ever study on the accumulation of cadmium in different parts of fish scales during exposure to cadmium in water.

As the scale grows, its diameter increases as a result of sclerite formation under its entire surface, where it adheres to the dermis. The outermost region (ring) is entirely new and contains microelements incorporated over the most recent period in the fish's life – and this is where the highest increase in the concentration of microelements to which the fish is currently exposed is expected. However, the inner part of the scale may also contain

Table 2

Comparison of the bioconcentration factor values of Cd (BCF) (±SE) in the inner and outer parts of the scales of Prussian carp during exposure to 0.4 mg L^{-1} or 4.0 mg L^{-1} of cadmium in water

Duration of exposure (days)	Cd1-in	Cd1-out	Cd2-in	Cd2-out
42	0.31±0.026 *a*	0.51±0.081 *b*	0.19±0.047 *c*	0.27±0.043 *ac*
84	0.67±0.17 *ac*	2.03±0.639 *b*	0.4±0.038 *a*	0.99±0.072 *bc*
Significance level	p<0.05	p<0.05	p<0.01	p<0.001

Letters *abc* denote statistically significant (p<0.05) differences in means between the groups (in rows); in the last row, the level of significance of differences in BCF values between the 42nd and 84th day of exposure is provided (in columns).

Table 3

Comparison of Pearson's correlation coefficients R (including the level of significance p) confirming the correlation between Cd concentration and the dose and duration of exposure

Specification	Cd1-in	Cd2-in	Cd1-out	Cd2-out
R (exposure concentration)	0.75		0.87	
Significance level	p<0.0001		p<0.0001	
R (time)	0.67	0.82	0.50	0.92
Significance level	p<0.01	p<0.0001	p<0.01	p<0.0001

new microelements that accumulate in the bottom layer during scale growth. Therefore, the concentration of Cd in this part of the scale was also expected to increase, but to a lesser extent compared with the outer part.

The analysis of the results obtained showed that the concentration of cadmium in the scales of Prussian carp exposed to Cd at a concentration of 0.4 mg L^{-1} for a period of 42 days increased statistically significantly both in the inner parts of scales, where a six-fold increase in Cd concentration was observed, and in the outer parts of scales, where a 13-fold increase was observed. In turn, in fish exposed to 4.0 mg Cd L^{-1}, a 48-fold increase in Cd concentration was observed in the inner parts of scales and a 68-fold increase in the concentration of the metal was found in the outer parts of scales, which confirms the sensitivity of scale tissue to the presence of cadmium in water. The results of the present study confirm that fish scales can accumulate cadmium and can therefore be used as a bioindicator tissue. In another study, which investigated the effect of chronic exposure of sea bass (*Dicentrarchus labrax)* to 0.5 µg Cd L^{-1} (for 8 consecutive days, 4 hours per day), a significant increase in the concentration of cadmium (from 0.052 to 0.147 µg g^{-1}) was observed in the scales after three days of exposure.

Moreover, the concentration of cadmium in the scales of the exposed fish remained significantly higher compared with control fish until day 21 of exposure. In contrast, the exposure to cadmium did not have a significant impact on the concentration of the metal in the gills of the fish studied (Faucher et al. 2008). A study by Rashed (2001) on the concentration of various elements in the tissues of Nile tilapia (*Tilapia nilotica*) found that the level of accumulation of the elements differed between tissues. In that study, the highest concentrations of zinc, copper and cobalt were found in the liver, the highest levels of iron and manganese – in the tissues of the digestive tract, and the highest levels of Cr, Ni and Sr – in the scales of the fish. The gills of Nile tilapia showed the lowest levels of the elements analysed, even though they serve as the first barrier between the environment and the fish.

The analysis of the present findings showed that while the concentration of cadmium in the scales of Prussian carp exposed to the higher concentration of Cd was higher, significantly lower BCF values, both for the inner and outer parts of scales, were found in those fish after 42 days of exposure (Table 2). The results indicate that fish accumulate higher proportions of cadmium when exposed to lower concentrations of this metal. It is very important to ensure that low levels of heavy metals in the environment, those that slightly exceed the permitted thresholds, are not disregarded during the monitoring of the chemical status of open waters in the belief that fish can handle them. The findings from the present study indicate that chronic exposure to heavy metals also results in their accumulation. This is confirmed even more strongly by the increase in Cd concentration in the scales of control fish, which were kept in mains-water without the addition of cadmium. The increase in Cd concentration in the scales collected from those fish was probably due to the trace levels of Cd present in the standard feed given to the fish. A study by Łuszczek-Trojnar and Nowacki (2021) confirms that the concentration of heavy metals in the scales of cyprinids increases with time, even in those fish that are not experimentally exposed to heavy metals.

In the present study, a further increase in Cd concentration was observed after 84 days of exposure in group Cd1 and group Cd2. It was only in the case of the inner parts of the scales of fish in the control group and group Cd2 that the increase in Cd concentration was not statistically significant (Table 1). The results obtained indicate that the accumulation of cadmium in fish scales is correlated with both Cd dose and the duration of exposure. This is also confirmed by the highly statistically significant correlation coefficients between the concentration of cadmium in scales and the exposure concentration of the metal, which were 0.75 and 0.87 (p<0.0001), respectively, for the inner and outer parts of scales, and the highly statistically significant correlation coefficients between Cd concentration in scales and the duration of exposure, which were 0.67 and 0.82 (p<0.0001), respectively, for the inner parts of scales collected from fish in groups Cd1 and Cd2, and 0.5 and 0.92 (p<0.0001), respectively, for the outer parts of scales collected from fish in groups Cd1 and Cd2 (Table 3).

The bioconcentration factor increased with the duration of exposure to cadmium. The increase was statistically significant in each of the groups studied. However, differences in the BCF values between day 42 and 84 of exposure were more statistically significant for fish in group Cd2 (p<0.0001). Differences in the BCF values between the Cd1-in and Cd2-in subgroups and between the Cd1-out and Cd2-out subgroups ceased to be statistically significant, which indicates that the longer the period of exposure, the more cadmium accumulated in fish from group Cd2.

It seems that metal concentration in the outer parts of scales reflects the recent status of environmental pollution, but is also subject to dynamic changes where the contamination continues. The analysis of two consecutive 42-day periods of exposure of Prussian carp to different concentrations of cadmium in water showed that the increase in the concentration of the metal in the scales analysed was not proportional to either the dose of the metal or the duration of exposure. Despite the same dose and a further 42-day period of exposure, the increase in the concentration of cadmium was lower than at baseline. A study by Łuszczek-Trojnar et al. (2013), in which fish were exposed to different concentrations of lead in feed, found that the accumulation of the metal in scales was highest at the beginning of the period of exposure and that the levels of the metal in particular tissues stabilised in the subsequent months of chronic exposure, depending on the concentration of the metal in the environment. The significant decrease in the level of cadmium accumulation observed in the present study after 84 days of exposure indicates that the concentration of the metal in the fish studied was approaching a plateau and that a steady, yet statistically insignificant increase in cadmium levels in scales would have been observed if the period of exposure had been extended.

Studies by other authors confirm the usefulness of fish scales as a bioindicator of environmental contamination by heavy metals (Rauf et al. 2009, Valova et al. 2012, Zubcov et al. 2012). Łuszczek-Trojnar and Nowacki (2021) investigated the concentration of selected heavy metals in particular annuli of the scales of carp from two pond farms in southern Poland. They found that the concentration of the metals differed between particular parts of the scales, increasing towards the outer growth rings. Understanding the concentrations of heavy metals in particular annuli of the scales of fish from unpolluted environments should be part of basic knowledge, necessary to determine reference values which could be used in the future as a benchmark for metal concentration thresholds in the scales of fish living in the natural environment. The concentrations of cadmium found by Łuszczek-Trojnar and Nowacki (2021) in the scale annuli of carp formed in the first, second and third year of life were 0.2, 0.25 and 0.48 mg kg^{-1}, respectively. The difference in cadmium concentration between the annuli formed in the first and third year of life was statistically significant.

Cadmium accumulates in scales to a much lower degree compared with other recognised indicator tissues, such as the liver or kidney. Studies by Drąg-Kozak et al. (2018, 2019) found that the concentration of cadmium in the kidneys of Prussian carp exposed to 0.4 mg Cd L^{-1} for a period of 7 weeks was 16.1 ±1.25 mg Cd kg^{-1} and increased to 23±1.41 mg kg^{-1} after the exposure period was extended to 13 weeks. In contrast, in fish exposed to 4.0 mg Cd kg^{-1}, the concentration of cadmium did not increase from the level of 112.5±3.92 mg Cd kg^{-1} recorded after 7 weeks of exposure when the exposure period was further extended to 13 weeks. The kidney is a recognised bioindicator tissue of cadmium accumulation as it is highly sensitive to the presence of the metal in the fish body. This is confirmed by the nearly 300-fold higher concentration of Cd found in the kidneys of fish exposed to cadmium at a concentration of 4.0 mg L^{-1} compared with control fish. However, kidney tissue does not grow with time and no further increase in the accumulation of cadmium in the tissue is observed once it has become saturated with Cd, despite continued exposure to the metal. Similarly, it was found that the concentration of Cd in the blood of fish exposed to 4.0 mg Cd L^{-1} for a period of 13 weeks was 1.13±0.08 mg kg^{-1} and did not differ significantly from the blood concentration of the metal recorded after 7 weeks of exposure – 1.23 mg Cd kg^{-1} (Drąg-Kozak et al. 2019). In turn, in the present study, the concentration of Cd in scales increased continuously until day 84 of exposure (Table 1).

CONCLUSIONS

The results of the present study on Prussian carp (*Carassius gibelio* B.) showed that Cd can accumulate in fish scales and that its concentration in scales is correlated with the exposure dose of the metal and the duration of exposure, which might be an arguement in favour of using fish scales as a bioindicator of cadmium contamination in aquatic ecosystems. Fish scales seem to be an excellent indicator tissue as they reflect very well the status of environmental pollution and are easy to sample non-lethally. Scales can be collected for analysis during the standard fish sampling procedure to assess the ecological status of waters under the National Surface Water Quality Monitoring programme. Each scale collected for analysis is replaced by a new scale, which prevents potential bacterial, viral and parasitic infections that could be caused through the contact of the skin with water. The protective barrier of the tegument remains intact and the fish whose scales have been collected recovers quickly and returns to feeding.

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