www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 14: 939-946 (2014) DOI: 10.4194/1303-2712-v14_4_13

Bioaccumulation and purification of cadmium in *Tubifex tubifex*

Łuszczek-Trojnar E.[1], Sroka K.[1], Klaczak A.[1], Nowak M.[1], Popek W.[1]

[1] University of Agriculture in Krakow, Department of Ichthyobiology and Fisheries, Spiczakowa, 6, 30-199 Kraków-Mydlniki.

* Corresponding Author: Tel.: +48.12 6375176; Fax: +48.12 6385979;
E-mail: e.trojnar@ur.krakow.pl

Received 5 August 2014
Accepted 23 December 2014

Abstract

This study compared accumulation of cadmium (Cd) in Tubifex worms (*Tubifex tubifex*) during exposure to different doses of the metal (0.9 and 2.5 mg/kg) in bottom sediments and in water (0.9 and 2.5 mg/l). Elimination of Cd from these invertebrates by prior exposure was also examined. Cd concentration in water, sediments and worms was analysed by Atomic Absorption Spectroscopy (AAS) method.

The results showed higher sensitivity of Tubifex worms to Cd concentration in water (Concentration factors from 16 to 60) than bottom sediments (CF from 0.44 to 0.77). Cd bioaccumulation in worms was positively correlated with its dose and exposure duration (0.88 to 0.94). The highest level of Cd in worms (150 mg/kg) was observed after two days of exposure in water to 2.5 mg Cd/L. The results of purification that was studied the first time after the previous exposure to Cd showed that *Tubifex tubifex* rapidly reduced Cd concentration (from 28.5 to 0.13 mg/kg) in 3 days after exposure cessation.

The results indicate that it is possible to purify Tubifex worms taken from a population existing in contaminated environment to obtain safe food for aquarium fish. Additionally, Tubifex worms can be considered as an ideal cumulative bioindicator of Cd in the water environment.

Keywords: *Tubifex tubifex*, cadmium, bioaccumulation, purification.

Introduction

The *Tubifex tubifex* is a widely distributed freshwater oligochaete, which lives in the water/sediment interface. The anterior part burrows in the sediment while it's posterior undulates in the overlying water. All aquatic invertebrates accumulate trace metals in their tissues, whether or not these metals are essential to metabolism (Eisler, 1981). Oligochaete living in contaminated environment can be a potential source of toxic pollutants for higher organisms in a trophic chain. Cd is one of the most deleterious contaminations present in the environment. As a heavy metal it does not undergo biodegradation and its level in the environment increases. Negative effects of Cd in surface water organisms were observed at concentrations as low as 0.5 µg/L (Hall *et al.*, 1988). The most anthropogenic sources of metals are industrial, petroleum contamination and sewage disposal. Lake sediments are normally the final pathway of both natural and anthropogenic components produced or derived into the environment. Sediment quality is a good indicator of pollution in water column, where it tends to concentrate the heavy metals and other organic pollutants. In some cases, sediments may hold over 99% of the total amount of heavy metals present in an ecosystem (Renfiro, 1983). Many metals associate readily with particulates and become adsorbed or co-precipitated with carbonates, oxyhydroxides, sulphides and clay minerals. Consequently, sediments accumulate contaminants and may act as long-term stores for metals in the environment (Spencer and McLoyed, 2002). Polish Environmental Quality Standards (EQS) for the priority substances in surface waters permits maximum Cd concentration of 1.5 µg/L, but average annual values cannot exceed 0.2 µg/L (Journal of Laws, 2011). According to Polish law, permitted maximal Cd level in bottom sediments cannot exceed 7.5 mg/kg dry weight (Journal of Laws, 2002). Cd undergo easy bioaccumulation in invertebrate organisms such as Tubifex worms (Bouche *et al.*, 2000; Kaonga *et al.*, 2010) which may pose a risk to organisms high up in the food chain, especially non-predatory fish. Tubifex are tolerant to pollution, as such dominate in contaminated water environments. In sublethal Cd concentrations they demonstrate immune mechanisms (Arendarczyk *et*

al., 2014). Tubifex are often sold as food for aquarium fish. The question is if Tubifex worms are taken from polluted environment can they be toxic to aquarium fish?

The aim of this work was to compare Cd bioaccumulation in Tubifex worms through exposure: in sediments and water. Additionally the study aimed at establishing if Cd bioaccumulation is a reversible process and after exposure cessation.

Materials and Methods

The studies were conducted in the Department of Ichthyobiology and Fisheries of University of Agriculture in Kraków, Poland. Tubifex worms of 1800 g were purchased in the aquarium stock market.

Sediment Exposures (14 days)

Nine kilograms of sediments were collected from a Cd-free carp pond located in the area of Experimental Station of the Department of Ichthyobiology and Fisheries, which is supplied with water from the Rudawa River (the Upper Vistula drainage, SE Poland). Three sets of experimental sediments were prepared in plastic containers. Sediments were spiked with the appropriate aliquot of Cd and shacked. Tap water was used in sediment exposure experiments. The first one was contaminated with cadmium chloride $CdCl_2 \cdot 2.5\ H_2O$ (produced by ChemPur in Piekary Śląskie) to achieve a concentration 0.9 mg Cd/kg (Cd1 group), the second one was contaminated with 2.5 mg Cd/kg (Cd2 group) and the third was not contaminated and acted as control set. Before exposure *T. tubifex* were placed in tap water for 3 days and then divided into 3 groups (each 300 grams weight), which were located in previously prepared sediments sets. The density of worms in sediments was 100 g per 1000 g. Water above the sediments, of temperature 17°C, was circulating through the pump and aerated. Dissolved oxygen was kept at 60% saturation (Figure 1). Experiment was conducted in duplicate. The worms were not additionally fed during the experiment to avoid any interaction between contaminant and food.

Water-Only Exposures (6 days)

Water-only experiment was conducted under static conditions for 6 days. The exposure consisted of an unspiked control and two spiked concentrations. Tubifex were divided into 3 groups (each of 300 g weight), which were located in previously prepared containers in tap water contaminated with Cd at the level 0.9 mg/L or 2.5 mg/L and uncontaminated (groups: Cd3, Cd4 and control-in-water, respectively). These sets did not contain any sediments. Water quality characteristics were: conductivity 270 to 350 µS/cm; pH 7.5 to 8.5; TDS 120 to 140 mg/L; alkalinity 75 to 100 mg/L. Water was circulating through a pomp and additionally was aerated in the aim of providing comfortable conditions (Figure 2). After 48 hours of the exposure, worms of group Cd3 were divided into two subgroups, of which one was still under Cd exposure and the second purified in clear tap water (Cd3-dx). Tubifex of group Cd4 were not purified because of 100% mortality occurring after 48 hours of exposure.

Survival Rate

After 2, 7, 10, 12 and 14 days of exposure survival of Tubifex from sediments groups (Cd1, C2 and control-in-sediment) was determined.

After 24 and 48 hours and later after 3 and 6 days of exposure in water-only worms' groups survivability was determined.

Sample of 10 g Tubifex was collected from each group, and alive and dead worms were counted using stereomicroscope under the magnification 6.3 x (PZO-Warszawa). Worms were considered dead when no response to physical stimulation was

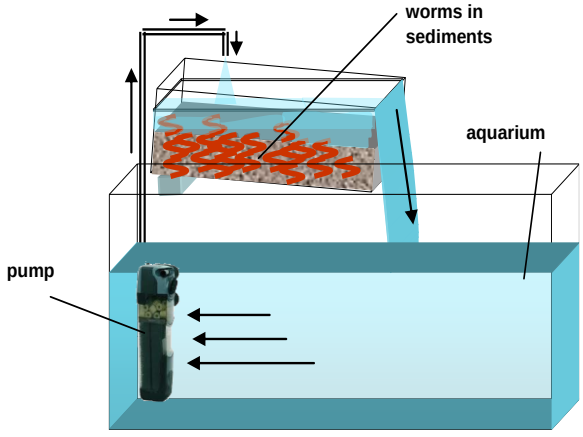

Figure 1. Schedule of a closed circuit of water used in the sediment-exposure. The arrows show the direction of water flow. The container with the sediments is in slight inclination of 8% in order to have drop in water levels.

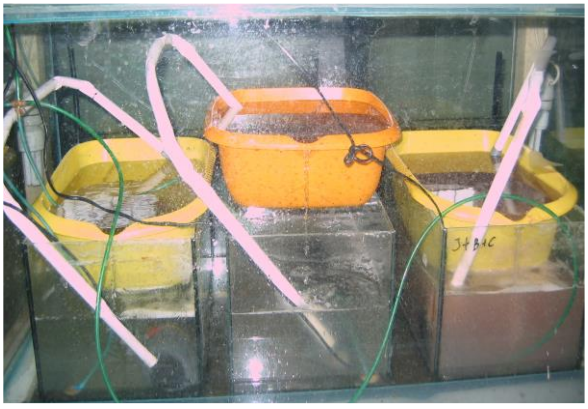

Figure 2. Three plastic container sets of worms in only-water exposure. The container with the worms is in slight inclination of 8% in order to have drop in water levels. Water is circulating through the pump located in the aquarium under the container.

observed. During the study of survivability worms were observed to monitor any visible toxic effects.

Bioaccumulation

Samples of water above sediments and samples of worms were collected after the seventh day of exposure with the aim of analysing Cd concentration in groups Cd1, Cd2 and sediments control. After 24 and 48 hours, and later after 3 and 6 days of exposure only-in-water samples of worms from groups Cd3, Cd4 and control-in-water were collected with the aim of Cd concentration analysis. Always 5 samples of worms weighing 5 g were taken from each group.

All collected samples of water, sediments and worms were kept at -20°C before Cd analysis. Prior to the analysis samples were mineralized. Samples of worms (5 g) were placed individually in glass cylinders and mineralized in the presence of 10 mL HNO_3 and $HClO_4$ (3:1) at room temperature. After 20 hours samples were heated for 5-6 hours up to a maximum temperature of 180°C using mineralizer Velp 20/26.

Samples of sediments (20 g) were filled with 10 mL HNO_3, and crushed with ceramic mortar for 5 minutes with the aim of extraction of labile Cd forms, which could be accumulated by living invertebrates. Next each sample was filtered and diluted with deionized water to 25 ml. From the diluted extract subsamples of 5 mL were taken and placed individually in glass cylinders in the presence of 10 mL HNO_3 and $HClO_4$ (3:1) at room temperature. After 20 hours samples were heated for 5-6 hours up to a maximum temperature of 180°C as in case of worms.

Water samples (5 ml) were mineralized in the presence of 10 mL of HNO_3 at room temperature for 20 hours and 5 hours at 180°C.

All samples after the mineralization process were diluted to 10 mL with deionized water and kept at 4^0C before Cd concentration analysis.

Atomic absorption spectrometry was used to determine Cd concentration in all samples (spectrometer - Unicam 929) (Agemian *et al.,* 1980). The concentrations were read from the standard curve generated using the standards prepared based on atomic absorption standards made at the Office of Weights and Measures in Warsaw. Each sample was assayed in duplicate. Calibration was repeated after every ten samples. The results were presented in milligrams of Cd per kilogram wet weight (w.w.) of worms or sediments, and milligrams per liter of water. Detection limit for Cd was 0.001 mg/kg. Bioaccumulation coefficients were calculated as the relation of Cd concentration in worms to Cd concentration in sediments (sediment exposure) or in water (only-water exposure).

The results were subjected to Kruskal-Wallis test, and significant differences were determined between the groups using Man Whitney' test. Spearman's correlation coefficients were calculated to examine the relationship between Cd concentration in worms and kind, dose and time of exposure.

Results

Sediment Exposures

Cd concentration analysis in bottom sediments showed that in group Cd1 was 0.9 mg/kg, and in group Cd2 2.5 mg/kg, while sediments of control group contained 0.2 mg Cd/kg (Figure 3). After 7 days of exposure statistically significant differences were observed in Cd concentration in worms

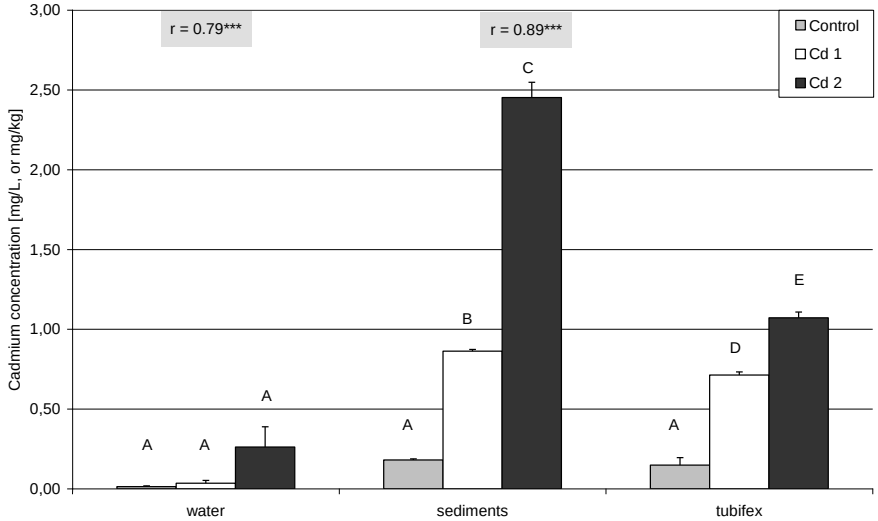

Figure 3. Comparison of Cd concentration (±SE) in samples of water above the sediments, sediments and worms after 7 days of exposure in sediments to 0.9 and 2.5 mg Cd/kg. Different letters indicate statistical significance of mean values ($P<0.01$). Spearman's correlation coefficients r indicating the dependence of Cd accumulation in worms on metal concentration in water above sediments, and in sediments are presented above bars respectively. *** means statistical significance at $P<0.0001$.

dependent on the dose of exposure (Figure 3). Cd level in worms was significantly correlated to its concentration in sediments ($r = 0.89$ $P<0.0001$) and in water above the sediments ($r = 0.79$ $P<0.0001$) (Figure 3).

Survivability of worms after 2 days was 99% in group Cd2, after 7 days 88% and 82% in groups Cd1 and Cd2, while after 14 days of exposure decreased to 0% in both exposed groups Cd1 and Cd2 (Table 1). Concentration factors for both kinds of exposure (in sediments and in water) are presented in Table 2.

Exposure in Water-Only

Survivability of worms in subsequent days of exposure to Cd in water-only is presented in Table 3. Statistically significant differences in Cd concentration in Tubifex in dependence on metal dose exposure have been shown. The highest Cd level (150 mg/kg) was noted in worms of group Cd4 after 48 hours of exposure to 2.5 mg Cd/L, with 100% mortality rate observed the following day (Figure 4, Table 3).

The highest Cd concentration in worms of group Cd3 was noted after 3 days of exposure to 0.9 mg (43.1 mg/kg), just prior to 100% mortality of this group worms observed on the subsequent day. After 24 hours of exposure in water-only Cd level in worms was significantly higher than in the control, and in the group Cd4 was significantly higher than in Cd3 group. In worms of Cd3-dx group statistically significant decrease of Cd level was noted after 4 days of purification (Figure 4).

There was strong correlation between Cd level in worms and in water during successive days of exposure. Also in the group of worms under purification (Cd3-dx) after the first day the dependence of Cd concentration in worms was significant ($r = 0.88$), while after 4 days was already insignificant ($r = 0.45$) (Table 3).

During the survivability examination, the autotomy of the caudal region of the worms was observed in 12% of Tubifex of groups Cd4 after 24 hours of exposure and in 10% of worms in group Cd3 after 48 hours of exposure to Cd respectively (Table 4, Figure 5). Additionally, the increase in mucus production by the worms exposed to Cd was observed.

Discussion

Survivability is a suitable parameter to indicate the stress status of organisms (Biagianti-Risbourg and Bastide, 1995). The increase in mortality rates

Table 1. Survivability of Tubifex during 14 days of exposure in sediments to 0,9 and 2,5 mg Cd/kg

Days	Control (%)	Cd1 (%)	Cd2 (%)
2	100	100	99
7	84	88	82
10	83	60	30
12	81	35	0
14	80	0	0

Table 2. Comparison of concentration factors depending on kind of exposure, Cd dose, and exposure duration

	Control	Cd1/Cd3	Cd2/Cd4
After 7 days of exposure in sediments	0.50	0.77	0.44
After 24 hours of exposure in water-only	1	23.3	16
After 48 hours of exposure in water-only	1	47	60
After 3 days of exposure in water-only	1	47.8	-

Table 3. Comparison of *T. tubifex* survivability and autotomy of caudal region during exposure to 0.9 and 2.5 mg Cd/L in water-only, and during purification (Cd 3dx-group)

Exposure [days]	Purification [days]	Groups							
		Control (0.1 mg Cd/L)		Cd 3 (0.9 mg/L)		Cd 4 (2.5 mg/L)		Cd 3dx (0.1 mg/L)	
		survival	autotomy	survival	autotomy	survival	autotomy	survival	autotomy
		%		%		%		%	
1	-	100	0	100	0	40	12	-	
2	-	96	0	30	10	0	-	-	
3	1	90	0	20	20	-		50	0
6	4	85	0	0	-	-		10	0

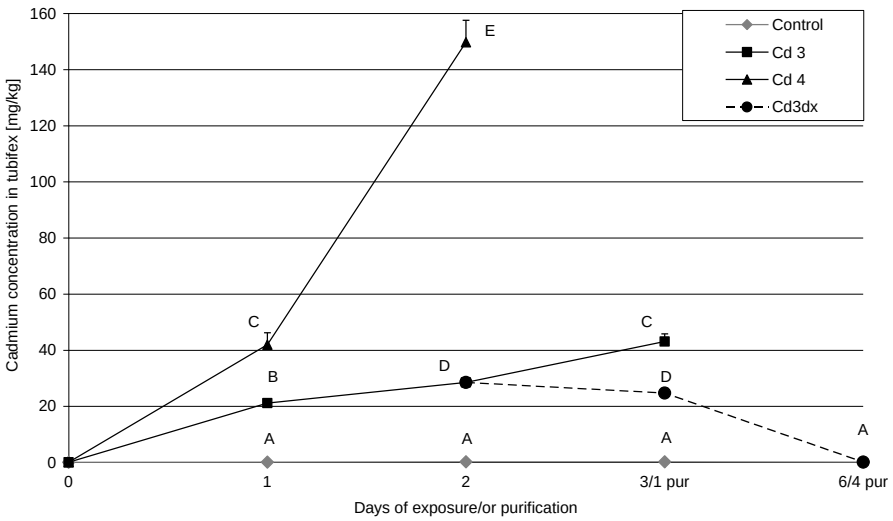

Figure 4. Comparison of Cd concentration changes in worms during exposure to this metal in water-only (groups Cd3 and Cd4) and during purification (group Cd3-dx). Different letters indicate statistical significance of mean values (P<0.01).

Table 4. Comparison of Spearman's correlation coefficients r indicating the dependence of Cd accumulation in worms and metal concentration in water in successive days of the experiment

Time	Control/Cd3/Cd4	Control/Cd3-dx
24 hours exposure	0.94***	-
48 hours exposure	0.88***	-
3 days of exposure or 1 day of purification	0.87***	0.88***
4 days of purification	-	0.45

** P<0.01
***P<0.001

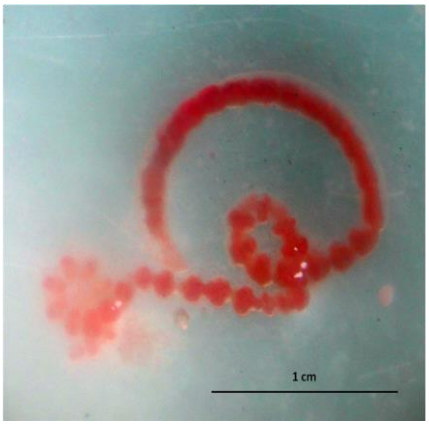

Figure 5. Tubifex worm with autotomy of the caudal region.

indicates the transition to the final stage of stress (exhaustion). In the present study the same dose of Cd in sediments and in water-only exposure caused differences in the survival of worms. While 82% of worms exposed to 2.5 mg Cd/kg in sediments survived more than 7 days (Table 1), the same Cd concentration in water-only exposure caused the mortality of 60% already after 24 hours (Table 3). This was similar in group Cd1 and Cd3, where Tubifex were exposed to 0.9 mg Cd per kilogram of sediments (Cd1 group) or per liter of water (Cd3 group). After 7 days of exposure to Cd in sediments survivability was at the level of 88%, while during the exposure in water-only after 3 days only 20% of worms survived, and on the next day 100% mortality was noted (Table 1, and 3). Bouche *et al.* (2000), studying the acute toxicity of Cd in *Tubifex tubifex*, noted complete mortality after 3 days of exposure to 0.1 mg Cd /L. These authors determined the value of lethal toxicity LC_{50} as 0.064 mg Cd/L after 48 hours. Brkovic-Popovic and Popovic (1977) indicated even lower dose of Cd as LC_{50} after 48 h of 0.003 mg/L. Also other authors presented higher Cd concentrations as LC_{50} for Tubifex, from 0.4 to 47.5 mg/L (Chapman

et al., 1982; Khangarot, 1991; Fargasova, 1994; Reynoldson *et al.*, 1996, Milani *et al.*, 2003), what shows that the little difference in test conditions may significantly influence the results. Several authors found that worms with a long-term history of exposure to a metal were more tolerant when they were further exposed (Bryan and Hummerstone, 1971; Pesch and Hoffman, 1982; Reinecke *et al.*, 1999). Cd toxicity for Tubifex depends on environmental parameters (water or sediments environment, pH, O_2 saturation, water hardness). This is probably why the results of worms survivability in the present study were so different between the sediment exposure and in water-only exposure conditions (Table 1, and 3).

Invertebrates, particularly annelids, are known to have a great ability to accumulate metals (Dallinger, 1994). Results of the present study indicate that Cd bioaccumulation process in worms is dependent on its dose, on exposure duration and on the environment (sediments or water-only conditions). In sediments the highest Cd concentrations (1.1 ± 0.002 mg/kg) were observed in worms after 7 days of exposure to 2.5 mg Cd/kg, and were significantly higher than in control (0.1 ± 0.001 mg/kg) and higher than in group Cd1 (0.7 ± 0.003 mg/kg) exposed to lower Cd concentration 0.9 mg/kg (Figure 3). The dependence of Cd accumulation in worms on the sediment contamination was confirmed by statistically significant Spearman's coefficients r = 0.89 ($P<0.0001$) (Figure 3). Concentration factors during the sediment exposure were: 0.5; 0.77 and 0.44 respectively for control, Cd1, and Cd2 groups (Table 2). The water-only exposure to the same Cd doses effected an increasing Cd concentration in worms of groups Cd3 and Cd4 respectively: 21 and 42 mg Cd/kg after 24 hours, 28.5 and 150 mg Cd/kg after 48 hours and 43.1 mg Cd/kg after 3 days of exposure in group Cd3 (Fig.4). Concentration factors observed in water-only exposure were much higher than during sediment exposure, and ranged from 23 to 47.8 in group Cd3 and from 16.8 to 60 in group Cd4 (Table 2). Bouche *et al.* (2000) exposed Tubifex to 0.005 mg Cd/L and noted concentration factors at the level of 15 040, and in case of exposure to 0.01 mg/L values of concentration factors amounted to 18 430, but these authors in tests used distilled water, which significantly influenced the observed results of accumulation. Distilled water is free of ions of calcium or other elements, which are present in tap water, and during exposure may compete with Cd. Results of the present study indicate that Cd uptake is rapid and efficient. Back (1990) studying the accumulation of Cd, Zn and Pb in annelids showed that just Cd is absorbed in the highest concentrations in comparison to other metals. In addition to passive diffusion, some metals may enter by active transport, and for Cd, through calcium ion pumps (Depledge *et al.* 1994). Cd uptake would first be a passive phenomenon; then, active transport would take place when equilibrium was reached (Dhainaut-Courtois *et al.*, 1991).

Accumulation of Cd in worms was studied several times and in spite of contrary results it is known that Cd bioaccumulation depends on the dose, and duration of exposure, which is also confirmed in the results of the present work. Much more interesting was studying the depuration of this metal from the organism of Tubifex previously exposed to Cd in water-only. In this experiment after the second day of exposure, worms of the group Cd3 exposed to 0.9 mg Cd/L were divided into two subgroups, of which one was placed in uncontaminated tap water and started the purification. After one day, the decrease of Cd concentration was observed in purified worms, and full purification after 4 days in control conditions occurred (Figure 4). No autotomy was noted in this group, in spite of 50% of mortality after one day and 90% after 4 days (Table 3).

Observations of the worms revealed that Cd induced morphological alternations. One of which is increased mucus secretion in worms. Mucus secretion is considered to be an adaptive response related to the physiological resistance phase. When emitted at the surface of the body mucus containing metal represents a detoxification mechanism (Dhainaut-Courtois *et al.*, 1988). However mucus produced in large amounts may induce physiological disturbances in gas exchange (Whitley, 1967).

Another sublethal effect induced by Cd was the loss of the caudal region of the worms. The animal becomes constricted, isolating the posterior part from the rest of the body. Constriction occurred at the beginning of the posterior region (Figure 5). In the first step of degenerating each metamere formed a sphere, leading to a beaded appearance. In the second step the tail was lost, when degenerating part separated from the body. The percentages of worms undergoing this process of autotomy increased proportionally with the duration of the exposure. After 24 hours of exposure in water only, worms of group Cd3 showed no symptoms of autotomy except at 48 hours when 10% of them did. This phenomenon was also observed by Bouche *et al.* (2000), under conditions of exposure to 0.1 Cd mg/L. But these authors noted additionally the third step of the autotomy – the closing and healing of the wound. In this case autotomy may be thought as a way of eliminating part of accumulated metals from organism, especially that the highest concentration of heavy metals is determined just in posterior segments (Back, 1990). Other authors also mention fragmentation of *Tubifex tubifex* or *Lumbriculus variegates* as the effect of exposure to various heavy metals (Brkovic-Popovic and Popovic, 1977; Bailey and Liu, 1980; Khangarot, 1991; Lucan-Buche *et al.*, 1999). Meller *et al.* (1998) and Bouche *et al.* (2000) suggest that autotomy could constitute a valid endpoint of sublethal toxicity, since this phenomenon is easily monitored and is time and concentration

dependent.

Bioaccumulation of Cd in worms represents a major risk. It is commonly known that heavy metals are not biodegradable and accumulating in living organisms are easily biomagnificated and transferred to higher organisms in food chains. Ali and Fishar (2005) studying heavy metals in various elements of Qarun Lake in Egypt indicated that Cd concentration in water was as low as 0.22 µg/L, in bottom sediments little higher ranging from 0.94 to 1.80 mg/kg, and on benthic invertebrates from 0.22 to 0.25 mg/kg while at the higher level of food chain in fish feeding benthos Cd concentrations ranged from 1.16 to 1.87 mg/kg. These results show that the level of contamination increases in the subsequent links of food chain, this is why it is important to know the level of contamination in the whole food chain. The results obtained in the present work indicated that worms kept in the sediments showed higher survival and lower Cd bioaccumulation than worms exposed to Cd in water-only, although the same Cd concentration level in both kinds of exposure. Various ions present in sediments more abundant than in tap water, competing with Cd probably protected the worms and observed accumulation was not as rapid and efficient as in case of water-only exposure. Repeatedly aquarists are not sure if the natural food for fish – worms, that they buy is safe for fish, because it may come from contaminated environment. The results of the present work indicate that high Cd concentrations noted in worms after the exposure decreased during short and effective purification. This means that it is possible to purify worms from contaminated environments and use them as food for fish.

Acknowledgements

The research was financed from DS - Department of Ichthyobiology and Fisheries of the University of Agriculture -3202/KIiR.

References

Agemian, H., Sturtevant, D.P., Austen, K.D. 1980. Simultaneous acid extraction of six trace metals from fish tissue by hot-block digestion and determination by atomic-absorption spectrometry. Analyst, 105(1247): 125–130. doi: 10.1039/AN9800500125

Ali MHH, Fishar MRA. 2005. Accumulation of trace metals in some benthic invertebrate and fish species relevant to their concentration in water and sediment of Lake Qarun, Egypt. Egyptian Journal of Aquatic Research, 31(1): 289-301. http://hdl.handle.net/1834/1215

Arendarczyk A., Jakubowska A., Zgórska A., Grabińska-Sota E. 2014. Toxic effects of cadmium-spiked sediments in *Tubifex tubifex*: enzyme biomarkers measurements. Desalination and Water Treatment, 52(19-21): 3798-3803. doi: 10.1080/19443994.2014.884793

Back H. 1990. Epidermal uptake of Pb, Cd and Zn in tubificid worms. Oecologia, 85: 226-232. doi: 10.1007/BF00319405

Bailey H.C., Liu D.H.W. 1980. *Lumbriculus variegates*, a benthic oligochaete, as a bioassay organism. In J.C. Eaton, P.R. Parrish and A.C. Hendricks (Eds), Aquatic Toxicology ASTM STP 707, Philadelphia, 205-215.

Biagianti-Risbourg S., Bastide J. 1995. Hepatic perturbations induced by a herbicide (atrazine) in juvenile grey mullet *Liza ramada* (Mugilidae, Teleostei): An ultrastructural study. Aquatic Toxicology, 31: 217-229. doi: 10.1016/0166-445X(94)00065-X

Bouche ML., Habets F., Biagianti-Risbourg S., Vernet G. 2000. Toxic effects and bioaccumulation of cadmium in the aquatic Oligochaete *Tubifex tubifex*. Ecotoxicology and Environmental Safety, 46: 246-251. doi: 10.1006/eesa.2000.1919

Brkovic-Popovic I., Popovic M. 1977. Effects of heavy metals on survival and respiration rate of tubificid worms. Part I: Effects on survival. Environmental Pollution 13: 65-72. doi: 10.1016/0013-9327(77)90093-3

Bryan G.W., Hummerstone L.G. 1971. Adaptation of the polychaete *Nereis diversicolor* to estuarine sediments containing high concentrations of heavy metals. I. General observations and adaptation to copper. Association of the United Kingom. Journal of the Marine Biological Association of the United Kingdom, 51: 845-863. doi: 10.1017/S0025315400018014

Chapman P. M., Farrell M. A., Brinkhurst R. O. 1982. Relative tolerances of selected aquatic oligochaetes to individual pollutants and environmental factors. Aquatic Toxicology 2: 47-67. doi: 10.1016/0166-445X(82)90005-4

Dallinger R. 1994. Invertebrate organisms as biological indicators of heavy metal pollution. Applied Biochemistry and Biotechnology, 48: 27-31. doi: 10.1007/BF02825356

Depledge M.H., Weeks J.M., Bjerregaard P. 1994. Heavy metals. In P. Calow (Ed) Handbook of Ecotoxicology. Second edition, Blackwell Scientific, Oxford, 79-105.

Dhainaut-Curtois N., Arrouijal F. Z., Demuynek S. 1988. Effets biologiques de trois metaux lourds (chrome, nickel et plomb) sur *Nereis diversicolor* (Annelide polychete). Oceanis, 14: 423-433.

Dhainaut-Curtois N., Demuynek S., Salzet-Reveillon B. 1991. Mecanismes de detoxication chez les poissons et invertebres marins. Oceanis, 17, 403-419.

Eisler R. 1981. Trace metal concentrations in marine organisms. Pergamon Press, Oxford.

Fargasova A. 1994. Toxicity of metals on *Daphnia* and *Tubifex tubifex*. Ecotoxicology and Environmental Safety, 27: 210-213. doi: 10.1006/eesa.1994.1017

Hall L.W.Jr., Scott M.C., Killen W.D. 1988. Ecological risk assessment of copper and cadmium in surface waters of Chsapeake Bay watershed. Environmental Toxicology and Chemistry, 17: 1172-1189. doi: 10.1002/etc.5620170626

Journal of Laws 2002. Sorts and concentrations of substances which cause that the spoil is contaminated. Regulation of the Minister of the Environment No 55 item 498 (accessed 16 April 2002).

Journal of Laws 2011. Regulation of the Minister of Environment on the classification of the status of surface waters and environmental quality standards for priority substances. No 257 item. 1545, (accesed 9 November 2011)

Kaonga C.C., Kumwenda J., Mapoma H.T. 2010. Accumulation of lead, cadmium, manganese, copper and zinc by sludge worms: *Tubifex tubifex* in sewage sludge. International Journal of Environmental Science & Technology, 7(1): 119-126. doi: 10.1007/BF03326123

Khangarot B. S. 1991. Toxicity of metals to a freshwater tubificid worm, *Tubifex tubifex* (Muller). Bulletin of Environmental Contamination and Toxicology, 46: 906-912. doi: 10.1007/BF01689737

Lucan - Bouche M., Biagianti-Risbourg S., Arsac F., Vernet G. 1999. An original decontamination process developed by the aquatic oligochaete *Tubifex tubifex* exposed to copper and lead. Aquatic Toxicology, 45: 9-17. doi: 10.1016/S0166-445X(98)00091-5

Meller M., Egeler P., Römbke J., Schallnass H., Nagel R., Streit B. 1998. Short-term toxicity of lindane, hexachlorobenzene, and copper sulfate to tubificid sludgeworms (Oligochaeta) in artificial media. Ecotoxicology and Environmental Safety, 39: 10–20. doi: 10.1006/eesa.1997.1603

Milani D., Reynoldsom TB, Bormann U., Kolasa J. 2003. The relative sensitivity of four benthic invertebrates to metals in spiker-sediment exposures and application to contaminated field sediment. Environmental Toxicology and Chemistry, 22: 845-854. doi: 10.1002/etc.5620220424

Pesh C. E., Hoffman G. 1982. Adaptation of the polychaete *Neanthes arenaceodentata* to copper. Marine Environmental Research, 6: 307-317. doi: 10.1016/0141-1136(82)90044-7

Reinecke S.A., Prinsloo M.W., Reinecke A.J. 1999. Resistance of *Eisenia fetida* (Oligochaeta) to cadmium after a long-term exposure. Ecotoxicology and Environmental Safety, 42: 75-80. doi: 10.1006/eesa.1998.1731

Renfro W.C. 1983. Transfer of 65Zn from Sediments by Marine Polycheate Worm. Marine Biology, 21: 305-316. doi:10.1007/BF00381087

Reynoldson T.B., Rodriguez P., Madrid M.M. 1996. A comparison of reproduction, growth, and acute toxicity in two populations of *Tubifex* (Muller, 1774) from the North American Great Lakes and Northern Spain. Hydrobiologia, 334: 199-206. doi: 10.1007/BF00017370

Spencer K.L., MacLeod C.L. 2002. Distribution and Partitioning of Heavy Metals in Estuarine Sediment Cores and Implications for the Use of Sediment Quality Standards. Hydrology and Earth System Sciences, 6(6): 989-998. doi: 6/989/2002/hess-6-989-2002

Whitley L. S. 1967. The resistance of tubificid worms to three common pollutants. *Hydrobiologia* 32: 193-205. doi: 10.1007/BF00179550