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Toxic effects of cadmium-spiked sediments in *Tubifex tubifex*: enzyme biomarkers measurements

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ABSTRACT

Effects of cadmium-spiked sediment exposure (0.001, 0.01, and 0.1 mg·kg^{-1} dm.) on the oligochaete *Tubifex tubifex* were evaluated. $CdCl_2$ which is easily soluble in water was used as a cadmium source. Catalase (CAT) and gluthatione S-transferase (GST) enzymes belonging to the antioxidant system which protects organisms from harmful impact caused by reactive oxygen species and carboxylesterase (CarE) which takes part in the phase I of detoxification were analyzed. Enzyme activity was measured in tissues of *T. tubifex* after 4, 24, 72, and 168 h of exposition. Cadmium, generally, caused an increase in *T. tubifex* CarE activity by comparing the control sediment prepared in accordance with the OECD 233 guideline. CAT activity demonstrates a decrease in a dose-responsive manner after 4-h exposure and an increase after 24-h exposure. No significant changes in GST activity under sublethal concentrations of cadmium were reported.

Keywords: Cadmium; Oxidative stress; Oligochaete *Tubifex tubifex*; Catalase; Gluthatione S-transferase; Carboxylesterase

Cadmium is present in the water environment mostly due to the industrial waste. The main sources causing the pollution are galvanization waste, steel industry, dyes production, phosphonium feritilizers, and carbon combustion [1]. Cadmium causes numerous toxic effects in organisms belonging to many trophic levels. The toxicity of cadmium to aquatic species was proved through many studies, focusing on organisms ranging from unicellular to vertebrates [2–7]. Toxic effects of cadmium are caused by its multidirectional bioactivity. It is proved that cadmium acts as an endocrine disruptor [8] and causes oxidative stress by inducing the formation of reactive oxygen species [9].

Due to their bioaccumulating capacities, sediments can be a source of contaminants in the aquatic environment. Therefore, benthic species are of a great interest in the environmental toxicity studies as they

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represent organisms that have a direct contact with the bioaccumulated contaminants. Many studies all around the world concerned the cadmium content in the river sediments. The level of cadmium may differ significantly depending on the river under investigation. The examples of cadmium content studies in different rivers are shown in Table 1.

Tubifex tubifex is a worm widely distributed in the aquatic sediments. It lives in the water–sediment interface, hence it experiences the contact with contaminants cutaneously or by ingestion in sediment as well as water pore and water column. *T. tubifex* is a standard model organism for ecotoxicity testing because of its intermediate position in the trophic network and the ease of breeding in the laboratory conditions [15].

There are many studies reporting multidirectional approaches undertaken to investigate the impact of cadmium to *T. tubifex* [16–20]. The toxicity of cadmium to this sediment worm is known to be high and temperature dependent. This heavy metal is easily absorbed by the organism and it causes nuclear growth. The present study gives a new perspective on cadmium activity in *T. tubifex*. Its aim was to evaluate the enzymatic response of catalase (CAT), gluthatione S-transferase (GST), and carboxylesterase (CarE) of *T. tubifex* to sublethal concentrations of cadmium. CAT and GST are enzymes belonging to the antioxidant system which protects organisms from harmful effects caused by reactive oxygen species. Its role is to remove free radicals and undesirable products of their activity as well as to repair the damage caused by oxidative stress, such as DNA alterations, strand breaks, or mutations. CAT catalyzes the reaction of hydrogen superoxide disproportion. GST takes part in removing the oxidized glutathione formed in the hydrogen superoxide reduction by glutathione [21]. CarE is an enzyme which takes part in the phase I of detoxification. If a toxicant appears it is hydrolyzed by CarE into the intermediate product which is subsequently conjugated with glutathione, glycine, glucuronic acid, or sulphides and removed from cell [22]. Thus, changes in the activity level of those enzymes are good biological indicators of whether and with what strength the tested substance acts like a toxicant and oxidative stress inducer. The present study verifies the utility of measuring CAT, GST, and CarE levels in the material taken from *T. tubifex* in order to define the toxic impact of cadmium.

1. Materials and methods

1.1. Estimation of LC_{50} value

To select the sublethal concentrations of cadmium for *T. tubifex* and further enzymatic analysis, the mortality test was conducted. The 96 h LC_{50} value for *T. tubifex* exposed to cadmium-spiked sediments was estimated using the probit method [23]. $CdCl_2$ which is easily soluble in water was used as a cadmium source. The exposition of *T. tubifex* was conducted for the cadmium concentrations 0.1, 0.5, 1.25, 2.5, and 5 mg kg^{-1} ds, at the same conditions like the one described below.

1.2. Culture conditions and exposition of T. tubifex

All the organisms used in tests were in the same life stage, had a similar body length, and were cultured at the conditions described below for the cadmium exposition. New cocoons were transferred from the sediment into new containers twice a week as well as replacing supernatant and washing the sediments. The artificial sediment and the supernatant were prepared in accordance with the OECD 233 guideline [24]. Cultured organisms were fed with Spirulina twice a week.

T. tubifex individuals were exposed to cadmium-(Sigma–Aldrich, cadmium chloride) spiked sediment samples (concentrations: 0, 0.001, 0.01, and 0.1 mg kg^{-1}) in a plastic container of 10 cm diameter and 1 cm layer of sediment with slight aeration. The sediment water: volume ratio was 1:4. Tested organisms were fed once in the beginning of the test with *Spirulina* [25]. Those

Table 1
Cadmium content in river sediments investigated in five independent studies

River	Cd [mg/kg]	Reference
Phraya river, Thailand	208,000–493,000	[10]
Odra, Vistula and their tributaries, Poland	0.11–1,490	[11]
Nemunas, Lithuania	0.14–0.35	[12]
Danube, Serbia and Montenegro	2.12–4.03	[13]
Orogodo, Nigeria	16.65–77.03	[14]

tests were performed under conditions of 12:12 light: dark photoperiod for 7 d, 21 ± 1℃ [26].

1.3. Enzymatic assays

Enzymatic assays for each experimental group were applied in six replicates. Enzyme activity was measured in tissues of *T. tubifex* after 4, 24, 72, and 168 h of exposition.

1.3.1. Sample preparation and protein concentration measurements

Animals (six per sample) were anaesthetized on ice and subsequently homogenized in a mechanical PRO 200 homogenizer at 4℃ in 3 ml of 0.05 M Tris–HCl, pH 7.4. The homogenates were centrifuged for 20 min at 12,000 *g*. After decanting, the supernatants were used for assays. Protein contents were measured according to Bradford [27] using bovine serum albumin as the reference standard.

1.3.2. Enzyme activity

Carboxylesterases (EC 3.1.1.1) were measured spectrophotometrically (Marcel Media Eko) in the submitochondrial fraction [28], with *p*-nitrophenyl acetate (*p*-NPA) as the substrate. Changes in the substrate hydrolysis product (*p*-nitrophenol) concentration were measured at 400 nm. Results were expressed in μmol *p*-NP min^{-1} mg^{-1} protein.

Gluthatione *S*-transferase (E.C. 2.5.1.18) activity was determined in the post-mitochondrial fraction, as the rate of glutathione conjugation with 1-chloro-2,4 dinitrobenzene (CDNB, 340 nm) [29,30]. The product of glutathione-CDNB conjugation constitutes S-(-1-chloro-2,4-dinitrophenyl) glutathione. Results were expressed in μm of glutathione-CDNB conjugate•min^{-1} mg^{-1}protein.

Catalase (EC 1.11.1.6) activity was measured by the assay based on formation of hydrogen peroxide stable complex with ammonium molybdate [31]. The concentration of the yellow H_2O_2 and ammonium molybdate complex was measured at 405 nm. The results were expressed in mM H_2O_2•min^{-1} mg^{-1}protein.

1.4. Statistical analysis of the results

The results of enzymatic assays were analyzed statistically using Shapiro–Wilk method for normality testing. Subsequently the nonparametric Wilcoxon test was performed using Statistica® (version 10). Differences between groups were taken statistically significant for $p < 0.05$.

2. Results and discussion

The 96 h—LC_{50} was estimated to be 0.45 mg kg^{-1} ds. Current study indicates the linear relation between *T. tubifex* mortality and cadmium concentration in spiked sediments—R^2 = 0.943 (Fig. 1).

Generally, cadmium caused an increase in CarE activity (Fig. 2) by comparing the control sediment but it differed statistically for 0.001 after 168 h, 0.01 mg kg^{-1} after 4 h, and 0.1 mg kg^{-1} after 4 and 168 h of exposure time. Stimulation of CarE activity by cadmium, especially for the highest tested concentration and 168 h of exposure, can result from an indirect influence on CarE gene expression, like to pesticides and allelochemicals [32,33]. Perhaps, cadmium also stimulates the production of metabolites that are CarE substrates as in *Poecilus cupreus* tissues [34]. What is more, CarE activity was statistically lower for the highest tested cadmium concentration and the shortest time of *T. tubifex* exposure. Time-responding manner could be observed for that concentration.

Cadmium exposure levels used in the present study had no significant effect on the GST activities measured in the *T. tubifex* tissues (Fig. 3). Chandrasekera et al. [35] also stated that in low levels of cadmium (0.001–0.01 mg L^{-1}) there was no effect on the hepatic GST activities in *Oreochromis nilotivus* fish but at the same time there was stimulation of metallothionein synthesis in concentrations above 0.005 mg L^{-1}. For *Daphnia magna*, no significant changes in GST activity under sublethal doses of cadmium were reported either [36]. What is more, it is generally believed that pollutants like fungicide and pesticide could decrease GST activities in organisms like *T. tubifex* and *Chironomus xantus* [37,38]. However, in the case of GST, glutathione seems to protect cells from harmful effects caused by reactive oxygen species induced by metals [39]; thus, some increase of GST activity in cadmium-stressed organisms was also

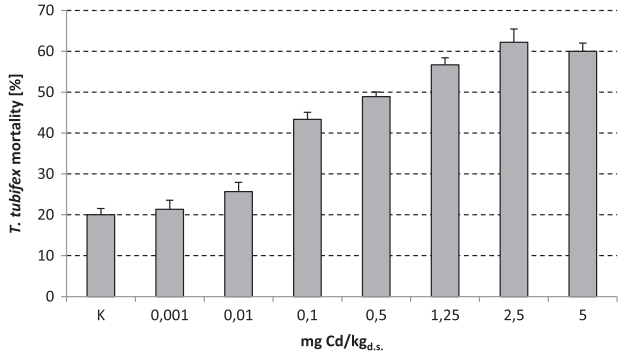

Fig. 1. Cadmium toxicity in sediments towards *T. tubifex*.

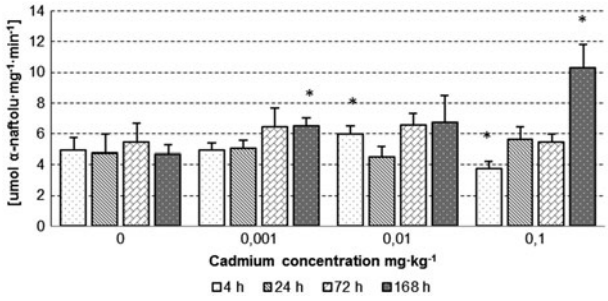

Fig. 2. CarE activity (against pNPA) in *T. tubifex* vs. cadmium concentrations in sediments. "∗" indicates significant differences between control and treatment groups (U Mann–Whitney test).

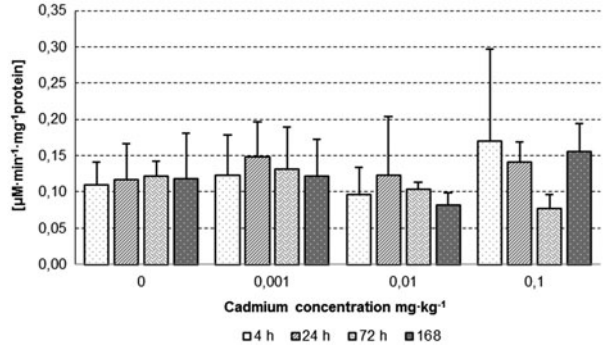

Fig. 3. GST activity (against CDNB) in *T. tubifex* vs. cadmium concentrations in sediments. "∗" indicates significant differences between control and treatment groups (U Mann–Whitney test).

proved. Such results were obtained in bivalves, *Macoma balthica*, *Perna perna*, and *Mytilus galloprovincialis* [40].

Data obtained for CAT activity unexpectedly indicated that in the control group CAT activity changed with exposure time (Fig. 4), which might have been caused by natural enzymatic activity fluctuation [37,41,42]. It was also noted that CAT activity demonstrates a decrease in a dose-responsive manner after 4-h exposure and an increase after 24-h exposure. Components of the antioxidant system, like CAT, can be targets for radicals formed under oxidative stress [43]. Sandrini et al. [42] suggested that the reduction of CAT activity could be a consequence of oxidative damage of the enzyme molecule. The highest CAT activity was recorded after 168 h of *T. tubifex* exposition to cadmium-spiked sediments but there was no dose–response dependence for that time of exposure (Fig. 4). An increase in CAT activity especially in the highest cadmium concentration could be explained by binding of cadmium to specific soluble

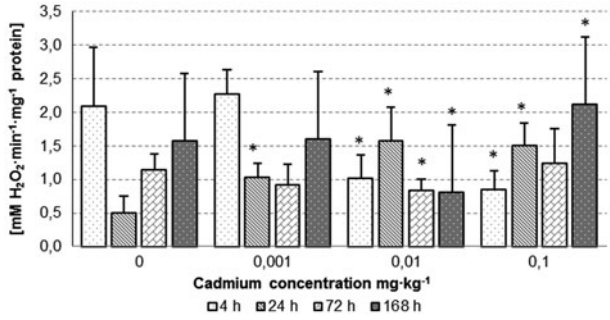

Fig. 4. CAT activity in *T. tubifex* vs. cadmium concentrations in sediments. "∗" indicates significant differences between control and treatment groups (U Mann–Whitney test).

ligands or cadmium storage in insoluble granules [44] so that oxidative damage to enzyme molecule could be limited.

In conclusion, research findings demonstrate that sublethal concentrations in sediments of cadmium interfere with CarE and CAT activities measured in *T. tuibifex* tissues, so that they could be used as cadmium exposure biomarkers. The obtained results confirm that when studying enzymes under metal stress one should also consider the long-term time exposures.

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