Application of selected detoxifying and antioxidative enzymes of the *Tubifex tubifex* (Oligochaeta) as potential indicators of river sediment contamination

Anna Arendarczyk*, Elżbieta Grabińska-Sota

*Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Pszczyna Branch, ul. Doświadczalna 27, 43-200 Pszczyna, Poland, Tel. +0048-32-210-30-81; email: anna.arendarczyk@ipo.lukasiewicz.gov.pl (A. Arendarczyk)

Environmental Biotechnology Department, Faculty of Power and Environmental Engineering, The Silesian University of Technology, ul. Akademicka 2, 44-100 Gliwice, Poland, Tel. +0048-32-237-29-15; email: elzbieta.grabinska-sota@polsl.pl (E. Grabińska-Sota)

Received 22 September 2019; Accepted 16 April 2020

**Abstract**

Tissues of the oligochaete *Tubifex tubifex* were assayed for selected enzymatic biomarkers: superoxide dismutase (SOD), catalase (CAT), carboxylesterase (CarE), and glutathione-S-transferase (GST) in order to evaluate the possibility of applying those biomarkers in the process of evaluating the contamination of river sediments sampled from four rivers in Upper Silesia: Bajerka, Odra, Jamna, and Gostynia. The enzymatic activity in tissues of the sludge worm was measured after exposure periods of 4, 24, 72, and 168 h. The obtained data led to the conclusion that CarE activity reflects the level of contamination of the environmental sediment samples, not only with regard to organic compounds such as PAHs, but also heavy metals. In addition to the increase in CarE activity, a significant decrease in GST and CAT was noted for the sediment containing unacceptable PAH concentrations. Moreover, a strong response in the form of an increase in GST activity was observed after exposure to sediment contaminated with heavy metals. Thus, the analyzed enzymatic activities of *T. tubifex* could be considered a potential biomarker of sediment contamination.

**Keywords:** River sediments; Oxidative stress; Oligochaete *Tubifex tubifex*; catalase; Glutathione-S-transferase; Carboxylesterase; Superoxide dismutase

1. Introduction

Surface water sediments are formed as a result of the sedimentation of allochthonous and autochthonous materials. Allochthonous materials, such as sand, silt, and gravel, are formed at the bottom and on the banks of water bodies in the course of their degradation. The allochthonous material also includes mineral and organic suspensions which flow to reservoirs along with surface runoff, tributary water, and wastewater [1–4]. Each type of sediment contains individual fauna and flora. The prevalence of organisms and their metabolic activity is much greater in the surface layer of sediment than in water itself. The deeper the sediment layer, the fewer the organisms [5,6].

Apart from the components vital to the proper functioning of aquatic ecosystems, river sediments also accumulate relatively high quantities of pollutants. These are deposits of significant amounts of heavy metals, polycyclic aromatic hydrocarbons (PAHs), pharmaceutics, or pesticides [7–9]. In unfavorable hydrological conditions, pollutants contained in solid fraction may be re-released into the water, causing its secondary pollution [4,10]. This contamination can, in turn, cause almost the entire elimination of invertebrates inhabiting the bottom of a reservoir and replace...
species sensitive to pollution with species with a higher tolerance. Changes to the species composition may affect processes occurring in the ecosystem, such as energy flow, as well as its productivity [6]. The issue of managing polluted sediments also constitutes a serious problem, as sediment management generates great costs related to its extraction, treatment, and dumping [11]. The available data confirms that sediments in Polish rivers contain a wide array of pollutants, including metals and metalloids, PAHs, polychlorinated biphenyls, and organochlorine pesticides [12].

For the purposes of surface water monitoring, the evaluation of river sediment quality in the context of its pollution with heavy metals or harmful organic compounds is done based on geochemical criteria [13]. The sediment is deemed as polluted even if the admissible content for only one element is exceeded [2]. In order to evaluate adverse effects of trace elements, PAHs, polychlorinated biphenyls, and organochlorine pesticides contained in sediments in relation to aquatic organisms, the State Environmental Monitoring uses only threshold pollution content values, that is, PEL values (probable effects level; the maximum concentration is high [31,32]. Tests conducted on organisms inhabiting reservoir and watercourse beds, including larvae from the Chironomus genus [18,25–27], the Polychaeta genus [27], as well as clams from the Ruditapes genus [27]. The oligochaetes Tubifex tubifex are one of the aquatic organisms that are deemed to be suitable for the analysis of the quality and pollution of river sediments [28–30]. Sludge worms ingest a great amount of sediments, thus feeding on the dead organic matter contained inside. The pollutants that occur in sediments are carried to the worm’s digestive system and cause a toxic effect if their concentration is high [31,32]. Tests conducted on organisms collected from and/or exposed to pollutants in natural ecosystems may lead to false interpretation of the obtained results. Aquatic ecosystems such as areas around harbors or waterways are often subject to changes with regard to the level of pollution due to such factors as periodical dredging, which can additionally inhibit the growth of a permanent population in a given area. Moreover, the organisms collected from the areas which are subject to constant and intensive environmental pollution may develop permanent adaptive mechanisms [33]. The use of laboratory-grown populations exposed both in situ and ex situ allows us to minimize the likelihood of misinterpreting the results [27,34].

The aim of the analysis was to evaluate the ecotoxicity of sediments from selected rivers in the Upper Silesia region and to verify the application of enzymatic biomarkers such as the activity of CarE, CAT, GST, and SOD in the tissues of the sludge worm Tubifex tubifex after a certain time of exposure.

2. Materials and methods

2.1. Localization and collection of environmental samples from river sediments

The river sediment samples were collected from four rivers in the Upper Silesia region: Bajerka (Chybie), Odra (Racibórz), Jamna (Ruda Śląska), Gostynia (Bieruń). According to tests carried out by the Voivodship Inspectorate for Environmental Protection, each of these rivers had been characterized by high pollution levels, mostly caused by anthropogenic factors [35]. The sediment samples were collected three times from each of the rivers, at a distance of up to 5 m along the riverbank, and at the depth of up to approximately 5 cm of sediment. The samples were collected with the use of an Ekman grab and a spade.

Bajerka is a right-bank tributary of the Vistula River which naturally flows into Goczałkowice Reservoir. The headwaters of the river flow through a scattered residential area and cropland. The middle and lower courses of Bajerka flow through a woodland area. Odra flows through Racibórz, whose key economy sectors are agriculture and industry, mainly chemical, machine, and agri-food. Municipal and industrial waste run-off constitutes the main source of water pollution in Odra [12]. Jamna is a left-bank tributary of Kłodnica, which belongs to Odra’s drainage basin. Kłodnica’s headwaters flow through industrial areas of the Upper Silesia Industrial Region. The main source of pollution is saline water drained from mines. Gostynia is a tributary of Mala Wisla. The quality of water in Gostynia is significantly affected by municipal waste drained from cities as well as water drained from black carbon mines [35].

2.2. Culture conditions and exposure of T. tubifex

All of the organisms used in the tests were in the same life stage, had a similar body length and were cultured in the conditions described below for river sediment exposure. Twice a week, new cocoons were transferred from the sediment into new containers, the supernatant was replaced and the sediments were washed. The artificial sediment with/without water was fed with Spirulina twice a week. Tubifex individuals were exposed to river sediment in a plastic container measuring 10 cm in diameter containing a 1 cm layer of sediment with slight aeration. Each container contained 30 individuals. The sediment’s water – volume ratio was 1 : 4. The tested organisms were fed once at the beginning of the test with Spirulina [37]. Tests were performed under the following conditions: 12:12
light:dark photoperiod for 7 d, at 21°C ± 1°C. The control sample was prepared in the same way with the use of reference sediment [36].

2.3. Testing evaluation of the mortality of T. tubifex exposed to river sediment

In order to determine the toxicity level of the analyzed river sediments and to determine the length of the experiments in which the sublethal effects were to be measured, the mortality of the sludge worm T. tubifex was evaluated. The evaluation of the mortality of T. tubifex was conducted for 168 h. The individuals were exposed in conditions analogous to the tests during which the tissues were collected for enzymatic assays. Each testing container contained 30 individuals. The test was performed in five replicates.

2.4. Enzymatic assays

The enzymatic assays were conducted in six replicates for each experimental group. Enzyme activity was measured in tissues of T. tubifex after 4, 24, 72, and 168 h of exposure.

2.5. Sample preparation and protein concentration measurements

The animals (six per replicate) were anaesthetized on ice and subsequently homogenized in a mechanical PRO 200 homogenizer at 4°C in 3 mL of 0.05 M Tris–HCl, pH 7.4. The homogenates were centrifuged for 20 min at 12,000 g, 4°C. After decanting, the supernatant was used for assays. The supernatant was batched and frozen at −40°C until enzymatic assays were completed. Protein contents were measured according to Bradford [39] using bovine serum albumin as the reference standard.

2.6. Enzyme activity

The activity of CAT, which catalyses the decomposition of hydrogen peroxide into water and oxygen, was assayed in a cytoplasmic fraction with the use of the Goth method [40]. The assumed activity unit was the amount of the enzyme that decomposes 1 mmol of hydrogen peroxide in 1 min. The obtained results were expressed in mM H₂O₂·mg protein⁻¹·min⁻¹.

The activity of CarE was measured according to the van Asperen method [41], with the use of α-naphthyl acetate (α-NA) as a substrate. The enzyme’s specific activity was expressed in μmol of α-naphthyl mg protein⁻¹·min⁻¹.

GST was assayed using a procedure according to Habig et al. [42], which was modified by Yu [43], with 1-chloro-2,4-dinitrobenzene (CDNB) used as a substrate. The specific activity was expressed in μmol of glutathione conjugates of CDNB (μmol CDNB mg protein⁻¹·min⁻¹).

The activity of SOD was assayed in a cytoplasmic fraction with the use of the adenine method according to Mistra and Fridovich [44]. The activity was expressed in arbitrary units of U mg protein⁻¹·min⁻¹. The arbitrary unit (U) was defined as the amount of enzyme which causes 50% inhibition of the rate of adrenaline autoxidation in the course of 1 min in comparison with a blind sample.

2.7. Analysis of selected physical and chemical parameters of the analyzed samples of river sediments

The assay of selected physical and chemical parameters of river samples such as: pH value, content of organic matter, organic carbon, humus, and hygroscopic water, was conducted based on the methodology included in Polish norms [45–48].

The assay of PAHs was conducted using extraction with dichloromethane and hexane, followed by an assay with the use of gas chromatography–mass spectrometry, using an internal standard and a standard addition method, United States Environmental Protection Agency 8270. The content of the selected heavy metals Cd, Pb, Cu, and Cr was assayed with the use of atomic absorption spectroscopy with electrothermal atomization in Solecrt-AA 880 Zeeman (Varian) apparatus.

2.8. Statistical analysis of the results

Statistical data analysis were conducted using Statistica® (version 10). Data normality was evaluated using Shapiro–Wilk normality test. Therefore, data were run with Tukey’s test to elucidate significant differences between sample means, p < 0.05, α = 0.05.

3. Results

3.1. Evaluation of physical and chemical parameters of river sediments

River sediments were subject to physical and chemical analysis immediately after they arrived at the laboratory. The basic parameters are listed in Table 1. The pH value of the analyzed sediments was between 6.5 for Bajerka and 7.4 for Jamna. The highest contents of organic matter and organic carbon were observed in Bajerka sediment and the lowest – in Jamna and Gostynia sediments. The sediment collected from Jamna was characterized by the highest percentage of dry matter.

Table 2 shows the average concentration of selected metals in individual samples of river sediment. The highest concentration of cadmium was observed in the sediment from Bajerka. It was three times higher than the concentration of cadmium in the sediment from Jamna. The former also contained the highest concentration of lead. The range of copper concentration was similar for all of the analyzed sediments, and the concentration of chrome in Bajerka was over 13 times higher than the one in Jamna. With regard to geochemical criteria, the analyzed sediments were classified as not polluted. The obtained values for the analyzed metals reflected the so-called geochemical background [13,49].

The selected PAHs in the collected sediments were also analyzed (Table 3). Out of all the analyzed sediments, only the sample collected from Odra showed a PAH concentration exceeding the minimum detection limits of the applied analyser. Substances with the highest concentration in this sample were fluoroanthene and pyrene, at 6.133 and 4.553 mg kg⁻¹·m, respectively. The total average content of the assayed PAHs in the sediment sample from Odra was 29.33 mg kg⁻¹·m. After comparing the results presented...
in Table 2 with PEC values [11], the sediment from Odra was classified as polluted.

Based on tests conducted by the Chief Inspectorate of Environmental Protection in 2011, before the sediments for analysis were collected, the sediments from Gostynia and Odra were classified as polluted due to increased concentrations of heavy metals, PAHs, organochlorine pesticides, and polychlorinated biphenyls. The sediments from Jamna and Bajerka are not monitored by the Inspectorate [49].

3.2. Testing the mortality of T. tubifex exposed to river sediment

In order to determine the toxicity of the analyzed river sediments, the mortality of T. tubifex was estimated prior to enzymatic analyses during a 7 d toxicity test (Fig. 1). The duration of the test was analogous to the maximum exposure duration of organisms destined for conducting enzymatic assays. Results obtained for T. tubifex are presented in Fig. 1. The highest mortality rate of sludge worms was
determined in the sediment from Gostynia and the lowest – in the sediment from Bajerka. What is more, Bajerka was characterized with the lowest pH value and the highest organic matter content among the tested locations (Table 1). The mortality observed in Gostynia, Jamna, and Odra was significantly higher than the mortality determined in the control sample (Student’s t-test, p < 0.05), however none of them exceeded 22%.

3.3. Analysis of the activity of detoxifying and antioxidative enzymes in tissues of T. tubifex exposed to river sediments

Fig. 2 shows the average activity of CarE in tissues of T. tubifex exposed to environmental samples of river sediments. CarE activity mainly showed an upward trend both in terms of the duration of the experiment as well as in comparison to control samples at a given sampling time. CarE activity increased significantly with the duration of exposure to environmental samples of river sediments collected from Bajerka and Odra. A statistically significant increase in CarE activity for Bajerka was observed after 72 and 168 h of exposure and for Odra after 168 h of exposure. CarE activity in T. tubifex tissues exposed to sediments from Jamna and Gostynia was not dependent on the time of exposure. The highest activity after 7 d exposure was observed in organisms exposed to sediments from Odra and it was 2.5 times higher than the activity after 4 h of exposure and 2.2 times higher than the activity in the control group after 7 d exposure (Fig. 2). Moreover, it should be noted that after 72 h of exposure, CarE activity was statistically higher for all of the tested locations in comparison to the control group. A significant increase in comparison to the control samples was also observed for Bajerka and Jamna after a 4 h exposure time and for Bajerka, Odra, and Gostynia after 7 d of exposure. CarE activity in the tissues of T. tubifex exposed to sediments from Jamna and Gostynia did not change significantly with the duration of the experiment; differences also weren’t observed in comparison to the control groups after 4, 72, and 168 h of exposure (Fig. 3). Activity of CarE decreased significantly starting from 24 h of exposure to sediment from Odra. After exposure to these samples, activity of CarE was 3.5 times lower after 7 d exposure than after 4 h. Moreover, differences in CarE activity after exposure to Odra sediment in comparison to the control group at 24, 72, and 168 h were observed. A different pattern of CarE activity emerged during exposure to sediment from Jamna River. CarE activity decreased significantly at the beginning of the experiment; after 4 h it was almost seven times lower than for the control group. A sudden increase in CarE activity was recorded after 168 h of exposure to Jamna sediment but it did not differ in comparison to the control group. There was a slight increase in CarE activity in the course of the experiment after exposure to Gostynia sediment but the mean CarE activity was statistically lower than the mean for the control groups only after 4 and 24 h of exposure (Fig. 3).

Fig. 4 shows the average activity of GST in tissues of T. tubifex exposed to environmental samples of river sediments; *denotes statistically significant differences between the average activity of CarE in T. tubifex tissues in the control sample and the activity of the analyzed sediments in each tested location at a given exposure time, p > 0.05; the letters indicate significant differences in the mean activity of enzymes after different times of exposure at the same location, p > 0.05.
sediment. GST activity of *T. tubifex* increased randomly after 168 h of exposure to the sediment from Bajerka. This activity change differs significantly from the control group as well as from the mean GST activity at the remaining exposure times. The exposure of *T. tubifex* to sediment from Odra did not cause any change in GST activity during the experiment. It was only observed that the GST activity was slightly lower after 168 h in comparison to the control group (Fig. 4). After exposure to sediment from Jamna, GST activity was lower than in the control group but these changes were significant only after 4 and 24 h. It should be noted that there was an upward trend in GST activity after exposure to Jamna sediment during the experiment, with GST activity differing statistically between 4 and 168 h of exposure. After exposure to sediment from Gostynia, a fluctuation of GST activity was observed in the course of the experiment, with the activity significantly lower after 4 and 72 h.

Fig. 4 shows the average activity of SOD in tissues of *T. tubifex* exposed to environmental samples of river sediment. After the exposure to Odra sediment, SOD activity was significantly lower than that of the control group at each sampling time, but there was no time-dependent SOD activity in this case. After exposure to Jamna, SOD activity increased in the course of the experiment, but after 4 and 24 h of exposure it was statistically lower and after 168 h – statistically higher than in the control groups. SOD activity after the exposure to Gostynia sediment was time-dependent. After the first two exposure times it was statistically higher and then the activity significantly decreased. The changes were also observed when compared to the control groups at each sampling time (Fig. 5).

4. Discussion

Xenobiotics cause changes in aquatic organisms on different levels of their biological organization: the higher the structural level, the higher the likelihood of significant or permanent changes [27,50]. However, organisms have developed biochemical defence mechanisms, which are activated in the very first stages of exposure to a xenobiotic. The responses on basic cellular levels are considered early warning tools when it comes to exposure to environmental stress, as well as biomarkers of potential changes to the ecosystem [51].

The above-mentioned mechanisms include CarE activity responsible for detoxicating organic compounds [21,22]. CarEs hydrolyse many esters of an endo- and exogenous origin, both aromatic and aliphatic, and are used in the amide range. They are important enzymes in the process of hydrolytic detoxification of organophosphorus insecticides and play an important protective role through binding and phosphorylation of insecticides [21,22]. They have also been shown to contribute to the metabolic degradation of pyrethroid insecticides in many insect species [17,20,52]. CarE activity increased along with the duration of exposure of *T. tubifex* to environmental samples of river sediments (Fig. 2). The highest activity after 7 days exposure was observed in the sediments from Odra, Bajerka, and Gostynia and the values were statistically different from the control sediment. Moreover, it should be noted that after 72 h of exposure the CarE activity was statistically higher for all of the tested locations. Based on a chemical analysis it was concluded that Odra was polluted with high concentrations of PAHs (Table 3) and Bajerka – due to its location – was exposed to organic pollutants flowing from a near-by cropland [35]. However, the contents of heavy metals (geochemical criteria) and PAHs (PEC values) were not exceeded in Bajerka (Tables 2 and 3). The literature has confirmed a direct correlation between CarE activity in tissues of invertebrates and the concentration of organophosphorus compounds and carbamates. In many cases CarE exhibits significantly higher sensitivity than the commonly used biomarker which is acetylcholinesterase (AChE) activity [53,54]. What is more, the sediment from Bajerka was characterized by the highest concentrations of metals, including cadmium, lead, and chrome (Fig. 2), as well as the lowest pH value and the highest percentage of organic matter (Table 1), which

---

**Fig. 4. Average activity of glutathione-S-transferase in tissues of *T. tubifex* exposed to environmental samples of river sediment; *denotes statistically significant differences between the average activity of GST in *T. tubifex* tissues in the control sample and the activity of the analyzed sediments in each tested location at selected exposure time, p < 0.05; the letters indicate significant differences in the mean activity of enzymes after different times of exposure at the same location, p > 0.05.

**Fig. 5. Average activity of superoxide dismutase in tissues of *T. tubifex* exposed to environmental samples of river sediment; *denotes statistically significant differences between the average activity of SOD in *T. tubifex* tissues in the control sample and the activity of the analyzed sediments in each tested location at selected exposure time, p > 0.05; the letters indicate significant differences in the mean activity of enzymes after different times of exposure at the same location, p > 0.05.
could have affected the level of metal bioavailability [55]. So far it has been observed that CarE activity increases when \( T.\ tubifex \) is exposed to sediments polluted by cadmium in a concentration range of 1 to 100 \( \mu \text{g} \cdot \text{kg}^{-1} \cdot \text{s} \) of sediment [31], which constitutes the range of the geochemical background [13]. Moreover, it is also known that cadmium leads to the production of substances that are substrates of CarE in tissues of \( Poecilus\ cupreus \) [56].

GSTs is a group of enzymes which perform a major function both in detoxication and in prevention of lipid peroxidation. GST conjugates reduced glutathione (GSH) with electrophilic lipophilic compounds such as xenobiotics, including carcinogens, mutagens, and drugs [16,57], increasing the solubility of the developed products [17,42,58]. Pollutants, such as petrochemicals, PAHs, and their halogenated derivatives, aromatic amines, aflatoxins, or antioxidants [59,60], induce the activity of this enzyme group. The influence of metals on the mentioned enzyme group has also been observed, however authors describe various mechanisms – from activity induction [61] to its inhibition [62,63]. Exposure of \( T.\ tubifex \) to Odra sediment with PAH concentrations exceeding PEC values resulted in a significant decrease in GST activity after 24 and 168 h in comparison to the control groups (Fig. 4), but the activity of these samples did not differ depending on the time of exposure. The greatest increase in GST activity was observed after 168 h of exposure to sediment from Bajerka (Fig. 4). A similar tendency was observed during tests involving \( Chironomus\ riparius \), which was exposed to cadmium and nonylphenol. Along with an increase in the concentration of the analyzed substances, the mRNA expression of genes coding GST increased [64]. Therefore, it can be assumed that the increase in the activity of GST with the simultaneous increase in the CarE activity may indicate that other highly contaminating organic substances are present in the sediment from Bajerka. The sediment from Bajerka was characterized by the lowest pH value (Table 1), as well as the highest concentration of metals out of all the analyzed sediments (Table 2). The pH of the environment has a very significant effect on the solubility of heavy metals. The higher the pH, the more metals turn into a form that is poorly soluble, thus becoming less available to living organisms. Lower pH values, even around 6.0, promote better solubility of metals, thus increasing their toxicity. Swiderska-Bróz [55] has presented the dependency of heavy metal solubility on pH. Another factor that may have a decided effect on the content of heavy metals in water and sediment in Bajerka is its high content of organic carbon (Table 1). Tests conducted by Działoszyńska-Wawrzkiewicz [11] confirmed that organic carbon may become a heavy metal adsorber. Bajerka River is a direct and natural tributary of Goczałkowice Reservoir, which constitutes the main reservoir of the voivodeship. A high content of heavy metals and its visible toxic effect would have an adverse outcome.

CAT and SOD are another group of enzymes that are responsible for cellular antioxidative mechanisms. CAT is a common enzyme found in nearly all living organisms exposed to oxygen. It catalyses the decomposition of hydrogen peroxide to water molecules and oxygen [19,65]. SOD is an enzyme that alternately catalyses the dismutation of the superoxide radical into either molecular oxygen or hydrogen peroxide [19]. In the case of CAT, attention should be paid to the activity of enzymes in the tissues of \( T.\ tubifex \) exposed to sediments from Odra and Jamna (Fig. 3), where different patterns of activity were observed: a sudden decrease in CAT activity in comparison to the control group after 4, 24, and 72 h, and then an increase in the level of the control group at the end of the experiment after exposure to Jamna sediment. A different situation concerning CAT activity was observed by Mosleh et al. [66,67], who noted an increase in CAT activity in tissues of \( T.\ tubifex \) exposed to aquatic solutions of organic matter such as chitosan, a derivative of chitin used as a biopesticide, and a fungicide called fenhexamid. However, there was a heavy decrease in CAT activity after the exposure to Odra sediment containing high concentrations of PAH after 24, 72, and 168 h of exposure (Fig. 3). It is worth noticing that a situation similar to the CAT activity was observed for SOD in tissues exposed to the sediment from Jamna River (Fig. 5). When it comes to the content of heavy metals in river sediments, the lowest pollution was observed in Jamna (Table 2). Until 2006, Jamna was one of the most polluted tributaries of Kłodnica River. Jamna was the main receiver of municipal waste from the town of Mikołów, resulting in decreased quality of waters and significant cumulation of pollutants in the river’s sediments [35]. The results of an analysis of river sediments from Kłodnica basin carried out by Działoszyńska-Wawrzkiewicz [11] confirmed a low level of heavy metal pollution of Jamna. In the case of Jamna sediment, the key issue seems to be the amount of organic matter, which may be reflected in the graph determining the mortality of \( T.\ tubifex\) or/and its enzyme activity. Activity of SOD in \( T.\ tubifex\) exposed to Odra River was only decreased in comparison to the control groups at each sampling time, and no time-responsive manner occurred (Fig. 5). Perhaps the decrease in SOD and CAT activities is a result of a greater participation of glutathione peroxidase (GSH-Px) in \( H_{2}O_{2} \) neutralization [19], however the activity of that enzyme was not analyzed during the test described herein. The lowered SOD activity compared to the control sample may also have been induced by an increased involvement of non-enzymatic reactions in the production of hydrogen peroxide [68]. The decrease in CAT and SOD activity could also have resulted from the harmful influence of sediment contaminants on the antioxidative system.

5. Conclusions

A comprehensive assay of detoxifying and antioxidative enzymes in \( T.\ tubifex \) confirmed the possibility of their application in the evaluation of the toxicity of environmental river sediment samples. Based on the obtained data, it was concluded that the sediments which do not exhibit notable lethal effects cause significant physiological changes to the tissues of aquatic invertebrates. In particular, CarE activity reflects the level of contamination of the environmental sediment samples, not only with regard to organic compounds such as PAHs, but also heavy metals. In addition to the increase in CarE activity, a significant decrease in GST and CAT was observed for sediment containing unacceptable PAH concentrations. Also a strong response in the form of an increase in GST activity after exposure to
sediment contaminated with heavy metals was observed. The activity of the analyzed enzymes largely corresponded with the level of their contamination as well as with the length of exposure. There seems to be a need for new methods of ecotoxicological evaluation of river sediments due to a cumulation of a wide array of pollutants as well as significant fluctuations in their concentrations, which are largely dependent on physical and chemical factors.

References


