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Research article

Ecotoxicological implications of leachates from concrete demolition debris on oligochaetes: survival and oxidative stress status

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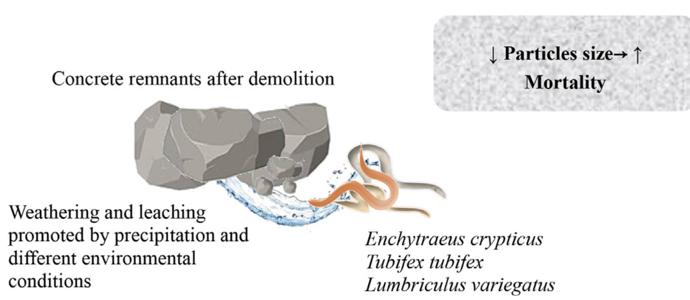
HIGHLIGHTS

- Leachate from smaller concrete particles was the most toxic.
- *Enchytraeus crypticus* was the most resilient to concrete leachate.
- Sediment decreased the adverse effects of the concrete leachate.

GRAPHICAL ABSTRACT



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ABSTRACT

Urbanization and population growth demand the construction of structures to facilitate the need for space, and old infrastructures must make space for new ones leading to demolition and concrete debris. In addition to demolition, aging and weather are factors leading to concrete deterioration and, thus, a new challenge as an environmental pollutant. Studies on how concrete debris and leachate affect biota in the environment are limited. The present study aimed to understand the effects of leachate from various sizes of concrete debris on the three oligochaete species *Enchytraeus crypticus*, *Tubifex*, and *Lumbriculus variegatus*. Acute toxicity testing was carried out to determine the adverse effects over time. The oligochaetes' survival was monitored as well as the activity of the biotransformation enzyme glutathione S-transferase and the antioxidative enzyme catalase as indicators of the oxidative stress status. Leachate from the smallest concrete particle size (<1 mm) was found to be the most toxic as it caused, on average, 6-fold increased oligochaete mortality compared to the larger pieces (2–5 cm) after 96 h of exposure, potentially due to the larger surface area facilitating the release of toxicants. Substrate buffered the toxic effect of the leachate with 42 ± 12% fewer mortalities and reduced adverse effects on the enzymes. Of the three oligochaetes, *E. crypticus* was the most resilient to the concrete leachate. The study is the first to investigate the effects of concrete leachate on oligochaetes.

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1. Introduction

With the prediction that 80% of the world's population is expected to live in cities by 2050, there is an urgent need to provide sufficient living spaces and facilities to accommodate increasing urbanization (Kiss et al., 2015). The rapid growth and development of cities are changing the world's surfaces to less permeable, which is associated with several environmental problems, as reviewed by Shuster et al. (2005) and Strohbach et al. (2019). The construction of new and larger structures to facilitate a growing population demands space, and old buildings and bridges must make space for new ones leading to demolition. The waste materials resulting from the destruction of concrete features are becoming a significant challenge as environmental pollutants (Chen et al., 2021). Apart from demolishing, aging and weathering cause concrete to crumble with time, and the concrete waste and debris are either transported to landfills, recycled, or left in the environment.

Millions of tons of construction waste are being produced worldwide, especially in megacities (Duan and Li, 2016; Wu et al., 2016). The construction and demolition industry is responsible for one-third of the total waste in the EU (Eurostat, 2019). Ecotoxicological risk assessment is necessary to evaluate the immediate environmental impact and develop methods to safely discard or reuse these materials (Schlanbusch et al., 2016). The constantly increasing quantity of waste is not only an environmental issue but also a possible hazard for humans via contact with particulate material associated with demolition (Cui et al., 2019). Studies have proven the danger of exposure to particulate materials and their impacts on human health (Alemayehu et al., 2020). However, the data on the ecotoxicity caused by the particles and derived leachates from construction products and demolition material on biota are still limited (Kobetičová and Černý, 2017).

Irrespective of whether concrete debris is processed by contractors as concrete and demolition waste, recycled, or left to decay onsite, one of the problems associated with construction products is the hazard of pollutant percolation. Concrete is very porous, and once water infiltrates the structure, it will dissolve hydration products within the concrete, especially calcium. Leaching of a complex mixture of substances from the fine particles of concrete can occur due to physical and chemical processes (e.g., mechanical abrasion, rain, snow, ice, wind, aging, temperature), potentially increasing the risk of contamination of soil, water sources, and air (Togerö, 2006; Siddique et al., 2012). The chemistry of concrete leaching is comprehensively described by Ekström (2001). In specific scenarios, like during demolition and construction, the available surface area for promoting leaching increases (Roussat et al., 2008), directly influencing and accelerating effects on the biota in contact with them (Cabrera et al., 2019). It is pertinent to comprehend the behavior of the particulate material from construction products and their leachates to clarify the interaction of these substances with the environment and develop safe methodologies for the reuse of building materials and the possible contamination due to these particles. Concrete is produced from natural aggregates, water, and cement; however, other materials, such as fly ash and recycled concrete aggregates, may be added to increase robustness and durability. Many studies have assessed the chemical composition of leachates from concrete (as reviewed by Kurda et al. (2018)) and identified heavy metals, certain trace elements, and sulfates as the most concerning regarding their potential environmental impacts.

Invertebrates, especially oligochaetes, are ecologically essential bioindicators of the toxicity and influence of chemicals in aquatic as well as terrestrial environments (Römbke et al., 2013). These organisms are abundant and widespread in many soils and are represented globally (Scanes, 2018). They can detect early stages of contamination and modification in the environment through many receptors (Didden, 2003). However, the species selected for the ecotoxicity assays are not only based on their environmental prevalence but also their sensibility. Different species might react uniquely when exposed to the same substance and concentration. Instead of focusing on only one organism, more reliable information can be obtained using multiple bioindicators for exposure scenarios, and consequently, a more critical evaluation of the impact of the substance can be performed.

The environmental impact of construction materials is typically based on Life Cycle Assessments (LCA), where the toxicity is related to the raw materials (Kobetičová and Černý, 2017); however, ecotoxicological bioassays with actual samples are required to understand the potential environmental risks. Therefore, the present study aimed to investigate the toxicity of the leachates from the concrete waste collected from an actual demolition site. The impact of particle size was considered in the toxicological investigation. To achieve the aim of assessing the toxicity of concrete leachate, the objectives were to a) assess oligochaete mortality with exposure to leachate from various sizes of concrete particles and 2) measure associated oxidative stress, which was assessed by measuring the enzyme activity of the antioxidative stress enzymes catalase and the biotransformation enzyme glutathione S-transferase. Tests performed in this study were intended to fill the knowledge gaps regarding the exposure of oligochaetes to leachates from demolition site concrete particles. This research used three different species of oligochaetes (*Enchytraeus crypticus*, *Tubifex*, and *Lumbriculus variegatus*) representing both terrestrial and aquatic environments to compare the ecotoxicity based on their particular sensitivities. *E. crypticus* (Castro-Ferreira et al., 2012), *T. tubifex*, and *L. variegatus* (Chapman et al., 1999; Chapman, 2001) are commonly used model species in ecotoxicology to assess the harmful effects of pollutants.

2. Material & methods

2.1. Oligochaete cultures

Tubifex, which typically inhabits the sediments of lakes, was cultured in the Joint Laboratory of Applied Ecotoxicology at the University of Helsinki for 12 months prior to experimentation. The aquariums (40 cm × 25 cm × 30 cm, 30 L) housing the worms were filled with 2 cm of ultrapure quartz sand and water reconstituted according to ISO 7346–3:1996 (ISO 1996). The worms were fed once a day with TetraMin® (Tetra, Blacksburg, VA, USA) added to the water surface of the culture tanks. The worms were cultured under a light intensity of 18 μmol photons/m^{2}s and a light/dark cycle of 14:10. The ambient temperature was kept constant at 22 °C ± 1 °C. One-third of the water in the tank was replaced every three days.

Lumbriculus variegatus is a sediment-dwelling oligochaete native to Europe and North America in lake and river sediments. *L. variegatus* has been widely used and recommended as a standard bioindicator organism for water and sediment quality (U.S. EPA 600/R-99/064, 2000). Culture conditions were described by Phipps et al. (1993) and Brunson et al. (1998), which are similar conditions to those used to cultivate *T. tubifex*, except for the addition of paper towel cuttings as a substrate. Worms were fed with ground TetraMin® (Tetra, Blacksburg, VA, USA).

Enchytraeus crypticus, a semi-aquatic, terrestrial species, was kept as a permanent culture under conditions outlined by Kobeticova et al. (2010) and Castro-Ferreira et al. (2012). In brief, the culture of *E. crypticus* was maintained in a commercially available soil substrate (pH 5.85 ± 0.04; 60% water holding capacity) purchased from MyWoody (Germany) in the dark. The ambient temperature was 15 ± 2 °C. The cultures were fed with oatmeal once a week by mixing it into the soil substrate. Adults with a well-developed clitellum were used for the tests.

2.2. Solid concrete and leachates

Concrete blocks (5 cm–15 cm) were randomly collected from the demolition site of an old concrete car bridge near Lahti (Finland). The concrete blocks were manually pulverized into concrete particles, which were separated by size using stainless steel sieves (diameter of 200 mm) with woven wire meshes (sieves' mesh sizes ranging from 125 mm to 20 μm) (Retsch, GmbH, Haan, Germany) according to ISO 3310–1:2016 (ISO, 2016) and ASTM E11-20 (2020). Three size fractions were selected for the exposures, namely concrete powder with particles smaller than 1 mm, 0.5 cm–1.0 cm fragments, and 2 cm–5 cm concrete pieces.

Leaching was performed according to the Swedish standard SS-EN 12457–4 (SIS, 2003), with some modifications. The leaching time was increased from 24 h to 72 h, and a temperature of 50 °C was used to induce increased leaching. The material (200 g of the different particle sizes of concrete material) was mixed with artificial water (ISO, 1996) to reach a liquid to solid ratio (L/S) of 10:1. The complete leaching process was done in the dark in round bottom flasks on a rotary mixer (Hei-VAP Valve, Heidolph-Instruments, Schwabach, Germany) at 21 rpm. The liquid and solids were separated by vacuum filtration on Whatman borosilicate glass microfiber filters grade GF/F with particle retention down to 0.7 μm. This filter is recommended in the EPA method 1311 (EPA, 1992) for toxicity characteristic leaching procedure. The leachate was immediately used for the exposures and not stored.

2.3. Experimental set-up

First, *E. crypticus*, *T. tubifex,* and *L. variegatus* were exposed to the leachates prepared from the various sizes of concrete particles (2 cm–5 cm pieces, 0.5 cm–1 cm fragments, and powder with particles smaller than 1 mm) undiluted as well as at various dilutions (1000x, 500x, 100x, 50x, 10x, 5x, and 1x) in water for 96 h during the first exposure regime. Artificial water (ISO, 1996) was used to dilute the concrete particle fractions.

In the second exposure regime, the worms were exposed to the concrete powder (<1 mm) leachate in a substrate representative of their common habitat (soil/sediment) and water for 96 h. *E. crypticus* was exposed to the leachate in soil (6 g of soil from MyWoody, Germany; pH 5.85 ± 0.04; 60% water holding capacity), and *T. tubifex* and *L. variegatus* were exposed in pre-washed sediment (6 g) collected from lake Vesijärvi (Lahti, Finland). Leachate was added (15 mL) to the substrates, and after allowing time for the sedimentation of particles (24 h), the oligochaetes were added.

Time-dependency of survival was monitored in water with two dilutions of 1000x and non-diluted leachates after 0, 0.5, 1, 2, 6, 24, 48, 72, and 96 h. As before, the oligochaete survival over time assessment was repeated in a substrate-containing system with exposure to leachate from the <1 mm particles (undiluted and 1000x).

Each exposure treatment and control for all experiments were performed in quintuplicate, each containing ten oligochaetes per treatment, respectively. Borosilicate crystallization beakers (90 mm × 50 mm) were used for all experiments. The exposures were maintained at a temperature of 22 °C ± 0.5 °C with no feeding of the oligochaetes during the 96 h exposure period. Survival of the oligochaetes was assessed by manually counting the living worms in the treatments and compared to untreated controls after 96 h.

2.4. Enzymatic measurements

Live worms were collected at the end of the exposures, frozen in liquid nitrogen, and stored at -80 °C until their antioxidative stress enzymes, catalase and glutathione S-transferase were measured.

The S9 fraction containing the antioxidative enzymes was extracted from the frozen worms, according to Scopetani et al. (2020). Worms were homogenized using 0.1 M potassium phosphate buffer pH 6.5 containing 2.17 M glycerol, 1 mM ethylene-diamine-tetra acetic acid (EDTA), and 1.4 mM dithioerythritol (DTE). Cell debris was removed by centrifugation (10 min at 13,000 × g), and the supernatant was used to assess enzyme activity. The protein content of the samples was determined according to Bradford (1976), which was used to normalize the enzyme activities. Soluble (cytosolic) glutathione S-transferase (E.C. 2.5.1.18) enzyme activity was determined using the standard model substrate 1-chloro-2,4-dinitrobenzene (CDNB) according to Habig et al. (1974). Catalase (E.C. 1.11.1.6) activity was measured according to Claiborne (1985), using hydrogen peroxide as substrate. The kinetics were followed at 240 nm for 5 min.

Enzyme activities are calculated in μkat/mg protein (catalase) and nkat/mg protein (glutathione S-transferase), i.e., using the standard SI unit katal, the conversion rate of one mol substrate per second.

2.5. Statistical analysis

IBM® SPSS® Statistics 28.0.0.0 (190) (2021) was used to perform descriptive analyses on all data sets. The data satisfied the requirements of sphericity and homogeneity and were thus analyzed using a factorial repeated-measures analysis of variance (ANOVA). Multiple comparisons were made using the Bonferroni test, observing an alpha value of 0.05. For those data sets not meeting the requirements (enzyme analysis), the non-parametric Kruskal-Wallis test with pairwise comparisons was used, observing an alpha value of 0.05 (Sokal and Rohlf, 1997).

3. Results and discussion

3.1. The survival responses of the three oligochaetes

In the first experimental set-up with exposures executed in water to exclude the possible effects of substrate binding, the three oligochaetes were exposed to different dilutions of the concrete leachates produced from the three particle size fractions for 96 h (Figure 1A-C).

The survival of *E. crypticus* (Figure 1A) linearly increased with dilution of the leachate from the largest particle size (2 cm–5 cm: R^2 = 0.975); however, for the leachate from the smaller fragments (0.5 cm–1 cm) and powder (<1 mm), the survival with dilution increased logarithmically (0.5–1 cm: R^2 = 0.986, <1 mm: R^2 = 0.987). *E. crypticus* survival (Figure 1A) significantly depended on the size of the concrete particle the leachate was produced from (Wilks'Lambda = 0.006 F (8,5) = 108.7, $p < 0.001$, partial eta^2 = 0.994) and the interaction between the dilution and the leachate from the three particle sizes was also significant (Wilks'Lambda = 0.010 F (16,10) = 5.568, $p = 0.005$ and partial eta^2 = 0.899). Leachate from the larger particles (2 cm–5 cm) resulted in significantly lower mortalities compared to the leachate from the 0.5 cm–1 cm fragments ($p < 0.001$), and exposure to this leachate caused significantly less mortality compared to the leachate from the concrete powder (<1 mm: $p < 0.001$).

T. tubifex (Figure 1B) survival polynomially increased with dilution of leachate from the larger particles (2–5 cm: R^2 = 0.977), linearly with dilution of leachate from fragment (0.5 cm–1 cm: R^2 = 0.977), and logarithmically for leachate from powder (<1 mm: R^2 = 0.994). For *T. tubifex*, there was also a significant effect of the leachate from each particle size on survival (Wilks'Lambda = 0.001 F (8,5) = 461.3, $p <$ 0.001, partial eta^2 = 0.999) as well as interaction between size and dilution on survival (Wilks'Lambda = 0.018 F (10,16) = 4.098, p = 0.014, partial eta^2 = 0.868). As with *E. crypticus*, exposure to the leachate from the larger particles (2 cm–5 cm) was associated with a larger number of survivors than the two smaller sizes (0.5 cm–1 cm and <1 mm) ($p < 0.001$).

L. variegatus survival increased polynomially with both the larger particles and fragments (2 cm–5 cm: R^2 = 0.994; 0.5 1 cm: R^2 = 0.998) and linearly decreased with the leachate from powder (R^2 = 0.989). The particle size from which the leachate was produced (Wilks'lambda = 0.002 F (8,5) = 338.2, $p < 0.001$, partial eta^2 = 0.998) significantly influenced the survival of *L. variegatus* but not the interaction between size and dilution (Wilks'lambda = 0.038 F (16,10) = 2.575 $p = 0.066$, partial eta^2 = 0.805).

The concrete particle size from which the leachate was derived played a significant role in the toxic effects on the oligochaetes, where a smaller particle size correlated with increased toxicity. Likely, the larger surface area, and thus providing a larger reactive surface, associated with a smaller particle size facilitated a more significant concentration of toxicants to be released from the concrete particles (Hillier et al., 1999).

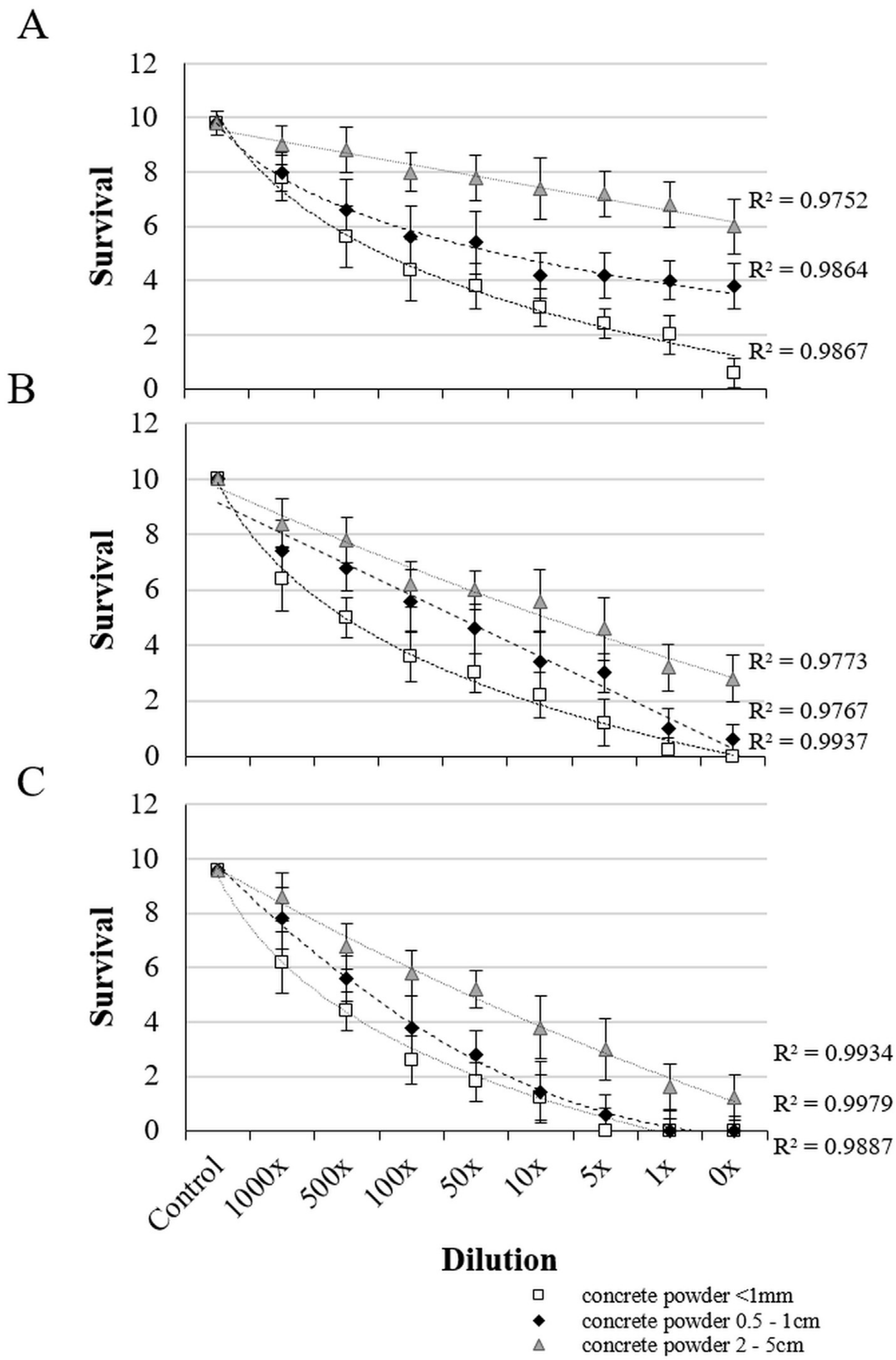

Figure 1. Comparison of the survival of the three oligochaetes A) *Enchytraeus crypticus*, B) *Tubifex,* and C) *Lumbriculus variegatus* exposed to dilutions of leachates from various sizes of concrete fragments in water for 96 h. Data points represent mean live worms ±standard deviation (n = 5). R^2 value denotes trendline fit for linear, logarithmic, exponential survival trend with dilution of the leachate.

T. tubifex and *L. variegatus* were more sensitive to the adverse effects of the leachates and dilutions compared to *E. crypticus,* indicated by significantly fewer survivors per dilution ($p < 0.05$). For *T. tubifex* and *L. variegatus,* 53% and 80% fewer survivors were observed than *E. crypticus* when exposed to the undiluted leachate from 2 cm–5 cm large particles; 84% and 100% for 0.5 cm–1 cm fragments, and 100% less for both with leachate from <1 mm powder. Mocová et al. (2019) also remarked on the different sensitivities of organisms to concrete leachate, where, e.g., leachate from a specific concrete sample was lethal to *Daphnia magna* but not *Desmodesmus subspicatus* and *L. minor*.

The pH of the undiluted leachate was 11.0 ± 0.4 and decreased to 7.4 ± 1.0 with 1000x dilution. Concrete leachates are typically highly alkaline, which could lead to mortality (Mocová et al., 2019). Mocová et al. (2019) demonstrated that pH adjustment reduced the ecotoxic effect of concrete leachates on *D. subspicatus*, *Lemna minor*, and *D. magna*. However, *T. tubifex* and *L. variegatus* are more tolerant to alkaline pH (Chapman et al., 1982; Berezina, 2001) than *E. crypticus* (Kuperman et al., 2006). Therefore, considering the lower survivorship in *T. tubifex* and *L. variegatus* compared to *E. crypticus*, the alkaline pH may not be the factor responsible for mortality.

Heavy metals are commonly reported to leach from concrete (Hartwich and Vollpracht, 2017) which may be toxic to the oligochaetes. However, *T. tubifex* (Lucan-Bouché et al., 1999; Bouché et al., 2000) and *L. variegatus* (Xie et al., 2008; Chapman et al., 1999) have been found to be resilient to heavy metals such as copper, lead and cadmium. Whereas copper, cadmium (Cedergreen et al., 2013), and lead (Luo et al., 2014) have been shown to be toxic to *E. crypticus,* which had the highest tolerance to the leachate in the present study. It indicates that other toxic compounds in the leachate may be responsible for the mortality trends observed. Nevertheless, Chapman et al. (1999) reported that *L. variegatus* is more sensitive to metal toxicity than *T. tubifex*. In the present study, fewer *L. variegatus* worms survived when exposed to the leachate and its diluents than *T. tubifex,* indicating higher susceptibility to toxicants.

Figure 2 illustrates the difference in survival of the oligochaetes in the absence (Figure 2A) and presence (Figure 2B) of a substrate. Significantly fewer organisms from all three species survived in the absence of a substrate ($p < 0.05$). In the absence of sediment a retarded (polynomial) increase in survivorship with increased dilution (2 cm–5 cm: $R^2 = 0.979$; 0.5 cm–1 cm: $R^2 = 0.989$; <1 mm: $R^2 = 0.075$) was observed. In aqueous solutions, the contaminants are freely dissolved and result in direct contact exposure. However, sediments may buffer the effects through adsorption or dilution (Ter Laak et al., 2007; Pflugmacher et al., 2020). Irrespective of the presence or absence of sediment, *E. crypticus* was less affected by the leachate than the other two species.

Figure 3A-C depicts the survival of the three oligochaetes with exposure to the leachates from the three concrete sizes over 96 h. Within the first 30 min of exposure, *E. crypticus* and *L. variegatus* survivorship were adversely affected in the presence of the leachate from the concrete powder (<1 mm: $p > 0.05$). Neither oligochaete species survived after 48 h of exposure to the leachate from the powder. None of the *T. tubifex* worms survived after 72 h of exposure. *E. crypticus* was the most tolerant of the three worms, and *L. variegatus* was the least tolerant over time with exposure to the leachates.

As observed with exposure to the various dilutions in the presence of a substrate (Figure 2B), the same buffering effect is observed in the presence of a substrate over time (Figure 4B). *T. Tubifex* survival decreased polynomially with time when exposed to the leachate from <1 mm concrete powder in water ($R^2 = 0.958$), whereas the survivorship of *L. variegatus* ($R^2 = 0.968$) and *E. crypticus* ($R^2 = 0.981$) decrease exponentially. None of the organisms survived after 96 h of exposure to the leachate in water (Figure 4A). However, in sediment (Figure 4B), 52% *E. crypticus*, 30% *L. variegatus*, and 26% *T. tubifex* survived after 96 h of exposure to the undiluted leachate from <1 mm concrete powder.

3.2. Antioxidative response

The activities of the biotransformation enzyme GST were unaffected when *E. crypticus* was exposed to leachate from 2 cm–5 cm concrete pieces (Figure 5A); however, with exposure to the 0.5 cm–1 cm fragment (Figure 5B) and <1 mm powder (Figure 5C) derived leachate, around a 100 times dilution caused a significant increase ($p < 0.05$). With exposure to the powder-derived leachate, the GST activity was 83.5%

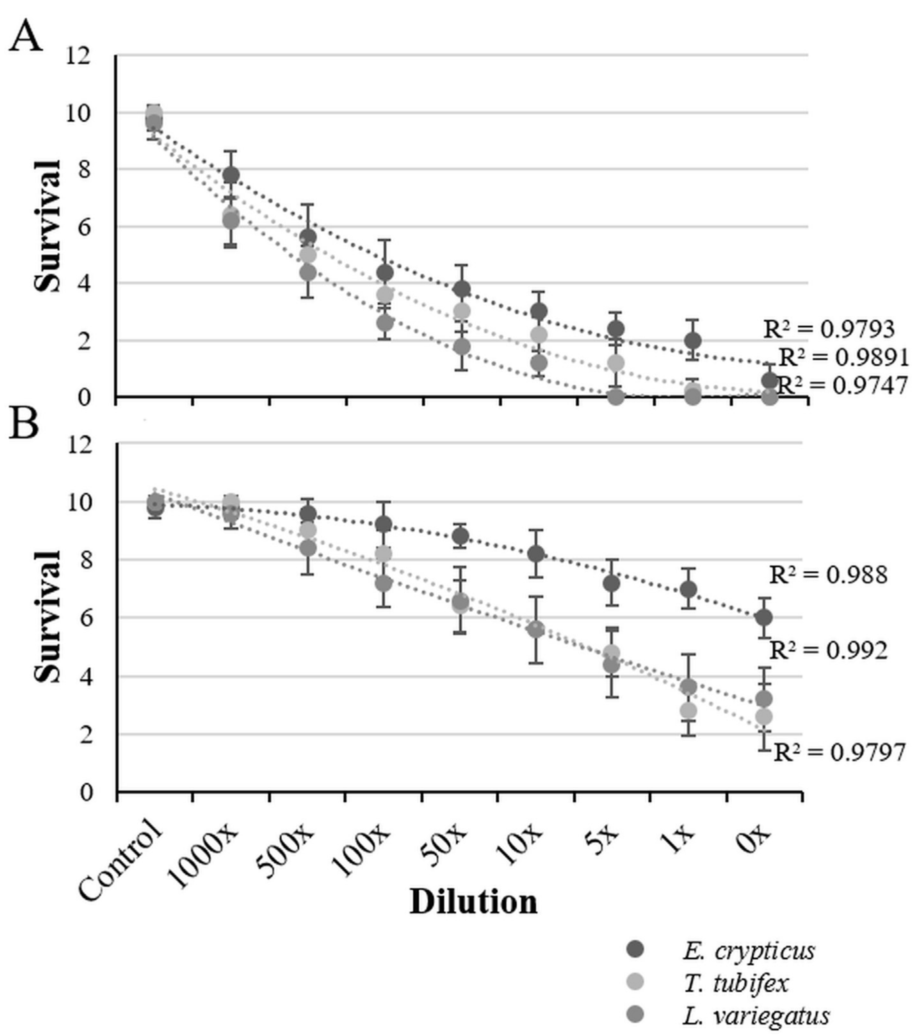

Figure 2. Comparison of the survival of the three oligochaetes (*Enchytraeus crypticus*, *Tubifex*, and *Lumbriculus variegatus*) exposed to leachates from concrete powder (<1 mm particles) in A) water versus B) soil for 96 h. Data points represent mean live worms ±standard deviation (n = 5). R^2 value denotes trendline fit for linear, logarithmic, exponential survival trend with dilution of the leachate.

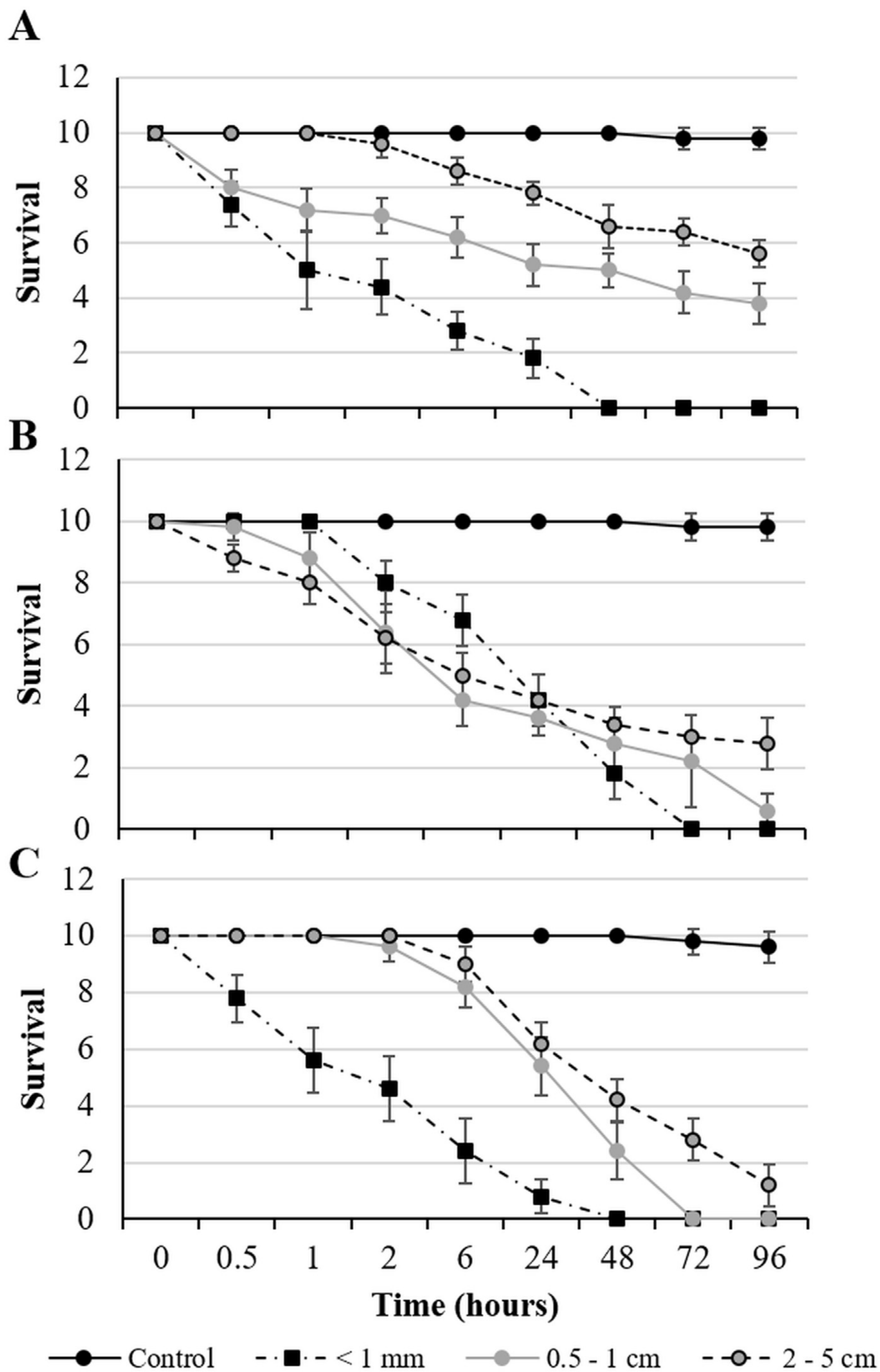

Figure 3. Exposure of the three oligochaetes (A) *Enchytraeus crypticus* (B) *Tubifex* (C), and *Lumbriculus variegatus* to the undiluted leachate from each of the three concrete size fractions throughout 96 h in water devoid of a substrate. Data points represent mean live worms ±standard deviation (n = 5).

decreased compared to the control ($p = 0.020$). The GST activity of *T. tubifex* (Figure 5D) and *L. varietagus* (Figure 5G) increased with exposure to the 5-fold and 100-fold dilutions of the leachate from the pieces (2 cm–5 cm), respectively ($p < 0.05$). For both organisms, inhibition of GST was observed with lower dilutions and undiluted leachate from the fragments (0.5 cm–1 cm; Figure 5E and H) and the powder (<1 mm; Figure 5 F and I). Markad et al. (2012) studied the adverse effects of fly ash, which is used in the production of concrete, on the earthworm *Dichogaster curgensis.* They reported that the GST activity, as well as those of other antioxidative enzymes such as superoxide dismutase (SOD) and glutathione peroxidase, increased in a dose-response manner with increasing concentrations of fly ash. In a study investigating the adverse effects of copper on *Enchytraeus albidus*, the authors found that the activities of the antioxidative enzymes tested, including CAT and GST increased with increasing amounts of copper; however, they remarked that the adverse effects were transient (Howcroft et al., 2009). Thus, no adverse effect observed with exposure to leachate from concrete pieces may be due to the oxidative stress balance already being rectified within 96 h.

E. crypticus's antioxidative enzyme, CAT, was elevated with lower dilutions of the leachates and the undiluted leachate (Figure 6A-C). However, the activity of CAT in *T. tubifex* (Figure 6D-F) and *L. variegatus* (Figure 6G-I) followed the same trend as GST, i.e., elevated activity with 100 to 50-times dilutions of the leachate followed by an inhibition of the

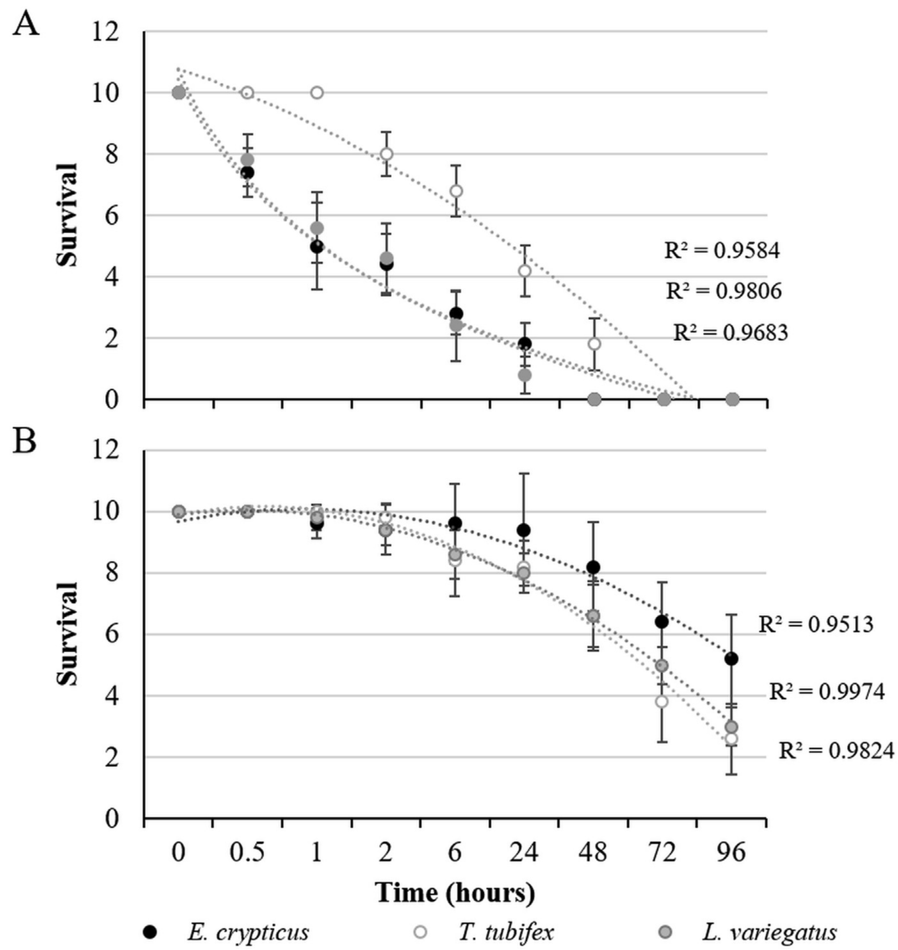

Figure 4. Exposure of the three oligochaetes [*Enchytraeus crypticus*, *Tubifex, and Lumbriculus variegatus*] to the undiluted leachate from the powdered concrete fraction (<1 mm) over 96 h in (A) water versus (B) substrate. Data points represent mean live worms ±standard deviation (n = 5). R^2 value denotes trendline fit for linear, logarithmic, and exponential survival trends with time.

activity with lower dilutions and undiluted leachate. The CAT activity in *L. variegatus* was most sensitive to exposure, with complete inhibition already observed at 5-times dilutions of the leachate ($p < 0.05$). Markad et al. (2012) reported that in the earthworm *D. curgensis,* the response in CAT activity did not follow an apparent trend when exposed to increasing fly ash concentrations. The authors remarked that, in accordance with other studies, this result verified that CAT plays a minor role in ROS breakdown in earthworms compared to other antioxidative enzymes. Nevertheless, in the current study, CAT activity stimulation with decreasing concentrations of the cement leachate suggests that the enzyme is actively involved in establishing oxidative stress homeostasis in *E. crypticus but not T. tubifex and L. variegatus*.

When considering the survival data, *E. crypticus* had the highest survival rates. This may be due to the increased activities of the anti-oxidative stress enzyme CAT combatting increased reactive oxygen species associated with leachate exposure and the biotransformation of xenobiotics due to GST conjugation. Kono and Fridovich (1982) demonstrated that when the superoxide anion concentration exceeds the dismutation capacity of SOD, they inhibit CAT. This explains the inhibition of CAT in *T. tubifex* and *L. variegatus* exposed to undiluted and low dilutions of the leachate. Similarly, GST activity was inhibited with low dilutions and undiluted leachate exposures, which was likely due to various metals (Dobritzsch et al., 2020) in the leachate (Markad et al., 2012). When ROS homeostasis is not maintained within the cell, various cellular damages may occur (as reviewed by Jakubczyk et al. (2020)) including lipid peroxidation as well as protein and DNA damage, leading to death. Thus, in the present study, as oxidative stress could not be overcome due to inhibited enzymes at lower dilutions and undiluted leachate exposure, the corresponding cellular damage likely resulted in decreased survivorship.

Comparing the GST response of *E. crypticus* with exposure to the leachate in water (Figure 5C) versus sediment (Figure 7A), it can be noted that the activity was not inhibited in the presence of sediment. Similarly, the GST activity of *L. variegatus* was not decreased compared to the control (Figure 7C). However, the GST activity of *T. tubifex* (Figure 7B) was inhibited with the 1-times dilution and undiluted leachate.

In the presence of a substrate (Figure 7D), the CAT activity increased by 53.3% compared to the CAT activity of *E. crypticus* exposed to the leachate in water (Figure 6C). Unequivocally, the CAT activities of *T. tubifex* (Figure 7E) *and L. variegatus* (Figure 7F) with exposure to the lower dilutions and undiluted leachate were inhibited to a lesser degree in the presence of sediment. With the increased antioxidative activity in *E. crypticus* and the decrease in *T. tubifex* and *L. variegatus*, antioxidative defense and biotransformation of the toxic xenobiotics in the leachate becomes applicable and appropriate; thus, supporting the finding of the adverse effects of the oligochaetes exposed to the leachate in the presence of sediment compared to those exposed only in water.

Studies investigating how concrete and their leachates affect biota are limited, especially regarding oligochaetes. However, Peng et al. (2022) demonstrated that decreased survival in *Daphnia magna* exposed to fly ash and coal gangue leachate was associated with heavy metals, specifically Cr and Ni. Peng et al. (2022) reported that the oxidative status of

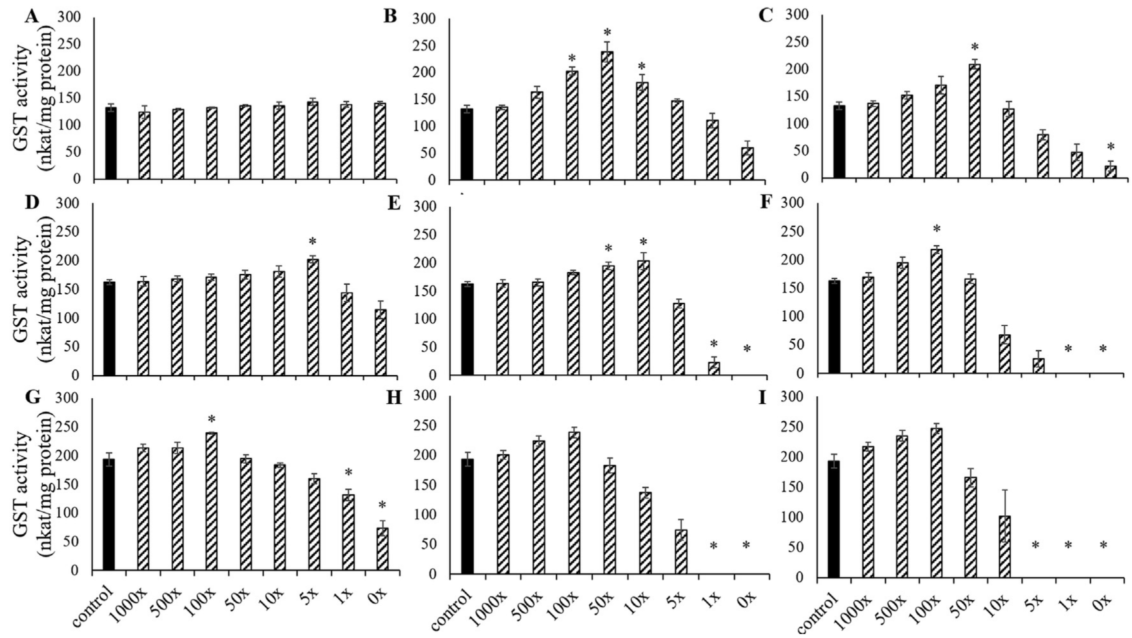

Figure 5. GST activity of (A–C) *Enchytraeus crypticus* (D–F), *Tubifex*, and (G–I) *Lumbriculus variegatus* exposed to leachate from concrete pieces of 2 cm–5 cm (A, D, G), concrete fragments between 0.5 cm and 1 cm (B, E, H), and concrete powder <1 mm (C, F, I) in water. Data presents average enzyme activity (nkat/mg protein) ± standard deviation (n = 5). Asterisks (*) indicate statistical significance compared to the control ($p < 0.05$).

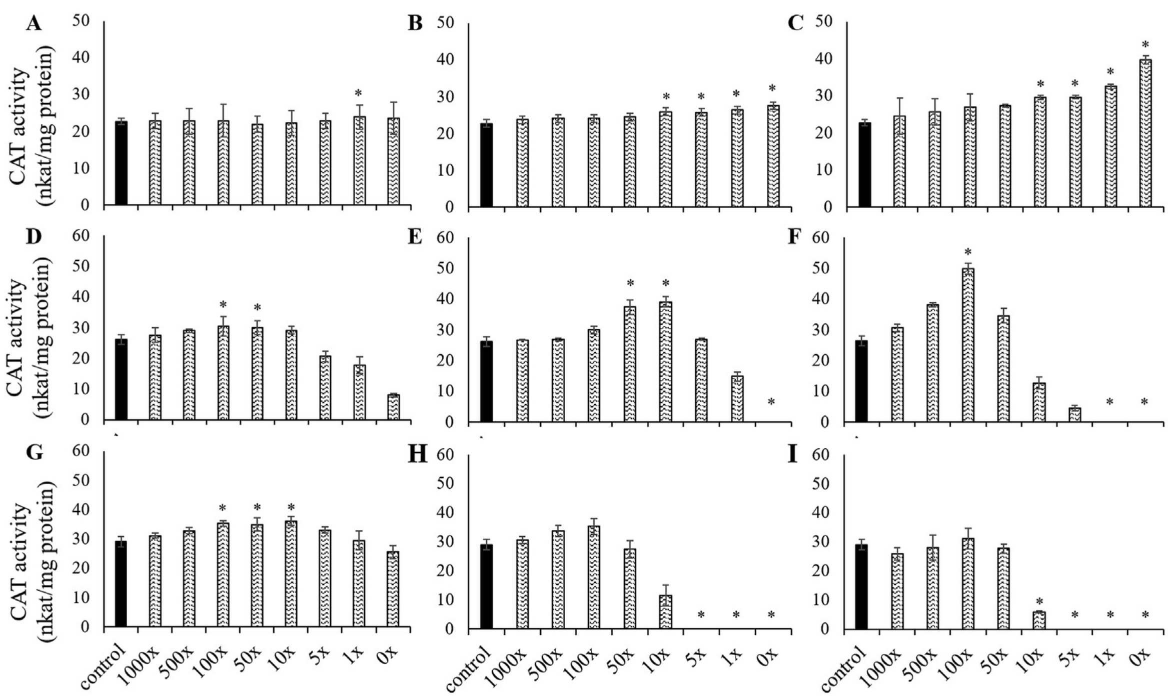

Figure 6. CAT activity of (A–C) *Enchytraeus crypticus* (D–F), *Tubifex*, and (G–I) *Lumbriculus variegatus* exposed to leachate from concrete pieces of 2 cm–5 cm (A, D, G), concrete fragments between 0.5 cm and 1 cm (B, E, H), and concrete powder <1 mm (C, F, I) in water. Data presents average enzyme activity (nkat/mg protein) ± standard deviation (n = 5). Asterisks (*) indicate statistical significance compared to the control ($p < 0.05$).

the daphnids was affected. Maity et al. (2009) investigated the metallothionein response in the earthworm *Lampito mauritii* exposed to fly ash. Metallothioneins are metal-binding proteins that play an essential role in oxidative stress as radical scavengers. Fly ash was found not to affect the earthworms' survival. However, the metallothionein concentration was significantly elevated. The authors concluded that the elevated metallothionein concentration protected the worms from increased oxidative stress caused by fly ash exposure which may be caused by associated heavy metals (such as, Cd, Bi, Hg) and organic compounds (such as polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs)) Thus, the adverse effects observed in the present study may be attributed to the concentrations and types of heavy metals and organic compounds in the leachates and should be investigated in the future. Additionally, it would be beneficial to conduct an impact assessment of leachate pollution (Sharma et al., 2020) on demolition sites. In the future, a complete analysis of the adverse effects of heavy metals is required as well as an onsite impact assessment of the leachates to overcome the limitations of this study.

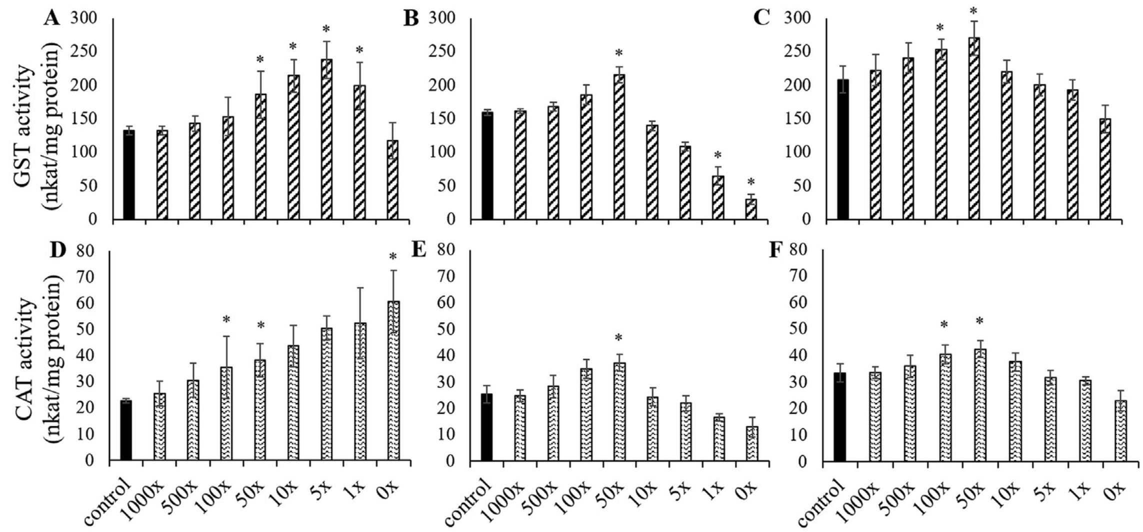

Figure 7. GST activity of (A) *Enchytraeus crypticus* (B), *Tubifex*, and (C) *Lumbriculus variegatus*, as well as CAT activity of (D) *Enchytraeus crypticus* (E), *Tubifex*, and (F) *Lumbriculus variegatus* exposed to leachate from concrete powder (<1 mm) in sediment. Data presents average enzyme activity (nkat/mg protein) ± standard deviation (n = 5). Asterisks (*) indicate statistical significance compared to the control ($p < 0.05$).

4. Conclusion

Concrete leachate affected the survival of the three oligochaetes tested. Overall, *E. crypticus* was the most tolerant of the concrete's toxic properties. Activities of the biotransformation enzyme, GST, and antioxidative enzyme, CAT, were elevated at mid-dilutions of the leachate. However, these enzymes were inhibited when the oligochaetes were exposed to leachates from the concrete. Leachate from smaller concrete particles proved to be more toxic, potentially as a larger surface area resulted in the release of a higher concentration of toxicants. Substrate significantly diminished the toxicity of the leachate and allowed the antioxidative stress system to continue functioning, potentially resulting in a higher number of survivors seen in the substrate-containing exposure system. International policies and regulations for improving the management of concrete and demolition waste are currently lacking. As an EU Member State, Finland has committed to utilizing 70% of its building and demolition waste by 2020. Even so, utilization remains presently below 60%. Nevertheless, the data generated in this study illustrate the importance of adequate handling and recycling of concrete and demolition waste in minimizing adverse effects on the environment, especially oligochaetes, and in so doing, preserving biodiversity.

Declarations

Author contribution statement

Maranda Esterhuizen, Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Marya Anne von Wolff: Performed the experiments.

Young Jun Kim: Contributed reagents, materials, analysis tools or data.

Stephan Pflugmacher: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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