Uttar Pradesh Journal of Zoology

Volume 45, Issue 6, Page 172-183, 2024; Article no.UPJOZ.3356
ISSN: 0256-971X (P)

Toxic Trio: Zinc, Copper, and Mercury's Effects on Earthworm Enzymes

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.56557/UPJOZ/2024/v45i63964

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.mbimph.com/review-history/3356

Original Research Article

Received: 15/01/2024
Accepted: 19/03/2024
Published: 21/03/2024

ABSTRACT

The impact of heavy metal exposure on earthworm enzyme activities, specifically lactate dehydrogenase (LDH), succinate dehydrogenase (SDH), malate dehydrogenase (MDH), glutamate dehydrogenase (GDH), xanthine oxidase (XOD), lipid peroxidation, superoxide dismutase (SOD), catalase, and glutathione-S-transferase (GST), was investigated in *Perionyx excavatus*. In control earthworms, LDH activities were 0.120 µmoles/mg protein/hour in the skin, 0.087 µmoles/mg protein/hour in the ovary, 0.062 µmoles/mg protein/hour in the testis, and 0.096 µmoles/mg protein/hour in the intestine. Exposure to mercuric chloride, copper sulfate, and zinc sulfate resulted in significant decreases in LDH activities by 10% to 19.35%, 4.16% to 22.58%, and 5.74% to 15%, respectively. Similarly, SDH activities declined by 10% to 23% with mercuric chloride, 6.89% to 17.91% with copper sulfate, and 4.13% to 16.66% with zinc sulfate treatments. MDH activities also decreased dramatically across all organs, with statistically significant reductions. GDH activities showed varied responses, while XOD activities uniformly decreased. Lipid peroxidation increased significantly after heavy metal exposure. SOD activities increased with mercuric chloride and copper sulfate, while catalase activities decreased with all three treatments. GST activities were significantly reduced by all treatments, particularly by mercuric chloride and copper sulfate. These findings underscore the substantial impact of heavy metal exposure on earthworm enzyme activities, suggesting potential cellular stress and metabolic disturbances.

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Keywords: Ecosystem health; enzyme activities; environmental contamination; heavy metals; Perionyx excavates; sub lethal concentrations.

1. INTRODUCTION

The toxic compounds compromise the integrity of membranes and change the activity of enzymes; they have a dramatic effect on organisms, causing disruptions in tissue structure and function. Changes in enzyme activity are trustworthy indications of toxicity because enzyme-mediated cellular processes are particularly vulnerable to these toxic substances [1] Enzymes including specifically lactate dehydrogenase (LDH), succinate dehydrogenase (SDH), malate dehydrogenase (MDH), glutamate dehydrogenase (GDH) can be greatly impacted by heavy metals, which are recognized for their varied biological activities [2]. The disruption caused by toxic agents often triggers oxidative stress, characterized by an imbalance between prooxidants and antioxidants [3].

According to Board et al*.* [4], ROS (reactive oxygen species), like superoxide and hydroxyl anion radicals, are essential in cellular damage and frequently lead to lipid peroxidation. According to Jaitovitch et al*.* [5], enzymes such as xanthine oxidase contribute to the generation of ROS, which exacerbates oxidative stress. Superoxide dismutase (SOD), catalase, and glutathione-s-transferase are examples of antioxidant enzymes that play a major part in decreasing oxidative damage [6,7]. Although SOD mostly functions as a defense mechanism against radicals, in the presence of hydrogen peroxide (H_2O_2), it may also display prooxidant activity [8]. The fact that SOD is dual highlights how intricate the reactions to oxidative stress are. The aim of the proposed research is to investigate the effects of exposure to zinc sulfate, copper sulfate, and mercuric chloride on the enzymatic profiles of dehydrogenases, oxidative, and antioxidative enzymes in different earthworm Perionyx excavatus tissues. By looking into these enzymatic modifications, scientists can gain additional insight into the mechanisms underlying the detrimental effects of heavy metals on biological systems.

2. MATERIALS AND METHODS

Using uniform weight and size, the megascolecid earthworm *Perionyx excavatus* was exposed to various sub-lethal doses of heavy metals for five days. The substrate was soil that had been taken from irrigated fields without the use of agrochemicals; the soil had certain qualities that made it ideal for earthworm life. Before being used in experiments, earthworms were stomach-evacuated after a month of acclimation to their natural soil. Based on their regional prevalence, three heavy metals zinc sulfate, copper sulfate, and mercuric chloride were chosen. For the experiment, plastic culture pots were used, and metal salts were applied to the soil surfaces [9]. For mercuric chloride, the low and high values that were computed were 25 and 30 mg/kg dry soil, copper sulfate, 60 and 150 mg/kg dry soil, and zinc sulfate, 140 and 350 mg/kg dry soil, respectively. Control pots were added after being washed with distilled water for comparison. Twenty earthworms were added to each pot in order to maintain the proper temperature and moisture content of the soil. Following the five-day exposure period, earthworms were dissected and tissue samples were obtained for additional research.

2.1 Estimation of Lactate Dehydrogenase (Ldh: Lactate: Nad Oxidoreductase Ec 1.1.1.27)

The technique outlined by Nachlas et al*.* [13] was followed to assess the activity of LDH, with changes made by Prameelamaa et al*.* [18]. In order to extract the supernatant for the LDH assay, tissue homogenates were made at a concentration of 4% (w/v) in a cold 0.25M sucrose solution and centrifuged for 15 minutes at a speed of 2500 rpm. 40 µmoles of lithium lactate, 100 µmoles of phosphate buffer (pH 7.4), 0.1 µmoles of NAD, and 2 µmoles of INT were present in the incubation mixture (2 ml). Twenty milligrams of tissue (0.5 milliliters) of homogenate were added to start the process. Five milliliters of glacial acetic acid were added to stop the reaction after it had been incubated for thirty minutes at 37°C. 5 ml of toluene was used to extract the formazan, which was then kept overnight at 5°C. The LDH activity was reported as µmoles of formazan/100mg of wet tissue/hour, and the optical density has been calculated at 495nm.

2.2 Malate Dehydrogenase

Tissue homogenates at 1% concentration were made in a 0.25 mL cold sucrose solution, and they were centrifuged for 15 minutes at a speed of 2500 rpm. The supernatant has been utilized

to measure the enzyme activity. 40 µmoles of sodium malate, 100 µmoles of “phosphate buffer (pH 7.4), 2 µmoles of INT, and 0.1 µmoles of NAD were present in the reaction mixture in the final volume of 2 ml. 0.5 ml of homogenate was added, and the mixture was then incubated for 30 minutes at a temperature of 37°C. The addition of 5 milliliters of glacial acetic acid” halted the reaction. After extracting the formazan in 5 milliliters of toluene, the optical density was calculated at 495nm. The expression for the enzyme activity was pmoles of formazan/100 mg of moist tissue/hour.

2.3 Succinate Dehydrogenase

The technique described by Nachlas et al. [10] was modified by Prameelamaa et al. [11] and was used to measure succinate dehydrogenase activity. After homogenizing 100 mg of wet tissue in 10 ml of 0.25M sucrose solution, the sample was centrifuged at a speed of 3000 rpm for 15 minutes to extract the supernatant, which was then used to test the activity of enzymes. 0.4 milliliters of 40 µm sodium succinate, 0.5 milliliters of 100 µm sodium phosphate buffer (pH 7.4), and 0.5 milliliters of 2 µm INT made up the reaction mixture. The volume was increased to 2 milliliters by adding 0.6 milliliters of double-distilled water. Five milliliters of glacial acetic acid were added to stop the reaction after it had been incubated for thirty minutes at 37°C. 5 ml of toluene was used to extract the color, and it was refrigerated at 10°C for the whole night. The enzyme activity was reported as µm/100mg wet tissue/hour, and the color intensity was assessed at 495 nm.

2.4 Xanthine Oxidase

Xanthine oxidase activity was assessed using the dye reduction approach developed by Srikanthan and Krishnamurthy [12] and modified by Govindappa and Swami [13]. INT (2-iodophenyl-3-p-nitrophenyl-5-phenyl tetrazolium chloride) served as the terminal electron acceptor. Tissue homogenates (2% w/v) synthesized in 0.25M cold sucrose solution were “centrifuged at 25,000 Xg for 15 minutes to remove cell debris. The enzyme assay mixture, in a volume of 2 ml, contained 100µmoles of phosphate buffer (pH 7.4), 50µmoles of xanthine, 0.4µmoles of INT, and 0.1µmoles of NAD. The reaction has been begun by” adding 0.5 ml of 2% homogenate, and the samples have been incubated at 37 °C for 30 minutes. The reaction was halted by adding glacial acetic acid in the amount of 4 ml. Formazan was extracted into toluene, and the color has been calculated at 495nm in a spectrophotometer against a toluene blank. The activity was represented as pmoles of formazan generated per mg of protein per hour.

2.5 Lipid Peroxidation

The lipid peroxidation level in the animal tissue was assessed by measuring the MDA (malondialdehyde) content using the TBA (thiobarbituric acid) reaction. MDA “is the end product of lipid peroxidation of polyunsaturated fatty acids. MDA reacts with TBA to form a pink-colored condensation product, trimethine, that can be calculated spectrophotometrically at 533nm. In the procedure, 1 ml of 10% tissue homogenate was mixed with 1 ml of 20% TCA and rehomogenized. The sample was then heated in a water bath at 70°C for 10 minutes, cooled to room temperature, and centrifuged at 30,000 Xg for"10 minutes at 20°C. A 0.4-ml portion of the protein-free liquid remaining after centrifugation was combined with 0.2 ml of 0.5% (w/v) TBA reagent in an enclosed test tube. The solution was heated in a boiling water bath for 10 minutes and then cooled to room temperature. The absorbance of the pink trimethine condensation product was then calculated at 533nm using a spectrophotometer.

2.6 Superoxide Dismutase

SOD enzyme activity has been calculated following the method of Marklund and Marklund [14], which relies on the enzyme's capability to inhibit the auto-oxidation of pyrogallol. A 5% tissue homogenate was prepared in Tris-EDTA buffer and centrifuged at 35,000 rpm for 40 minutes at 4°C. The assay mixture, in a 1 ml volume, consisted of 50 mM Tris-HCl buffer (pH 8.2), 1 mM diethylene triamine pentaacetic acid (DTPA), 1 mM pyrogallol, and 50 µl of the enzyme. The reaction was initiated by adding pyrogallol, and the rise in absorbance at 420nm was recorded over a period of 30 seconds. A blank containing pyrogallol alone served as a reference. One unit of SOD activity reduces the rate of pyrogallol auto-oxidation by 50%. The activity of SOD has been depicted as units per mg of protein per minute.

2.7 Catalase

Catalase activity has been calculated following the approach of Chance and Machly [15]. Tissue homogenate (5%) was synthesized in cold

phosphate buffer (pH 7.4) and centrifuged at a speed of 10000 x g for 15 minutes. The resulting supernatant was utilized for the enzyme assay. The reaction mixture (3 ml) comprised 50µmoles of phosphate buffer (pH 7.0), 51µl of 30% H_2O_2, and 100µl of enzyme source for 50µl of tissue. Absorbance has been measured at 260nm in a spectrophotometer, and the reduction in H_2O_2 was monitored for about 2 minutes at 30-second intervals. Catalase activity has been shown in units, where 1 unit of catalase converts 1 mole of H_2O_2 per minute.

2.8 Glutathione-S-Transferase

The activity of GST (Glutathione-S-Transferase) was determined using the CDNB substrate following the method of Habig et al. [16]. GST catalyzes the initial step in mercapturic acid synthesis, forming a thioether bond among decreased glutathione (GSH) and various lipophilic compounds with an electrophilic center. The decrease in substrate absorbance upon conjugation with glutathione has been calculated spectrophotometrically at 340nm for 5 minutes at a temperature of 37°C. Non-enzymatic reactions served as blanks, and the GST-specific activity was shown as per moles of thioether created per mg protein per minute.

2.9 Statistical Analysis

The reported results have been shown as the mean of three replicates and statistically analyzed by utilizing a Student's t-test [17].

3. RESULTS

3.1 Lactate Dehydrogenase Activity

The activities of various enzymes, including lactate dehydrogenase (LDH), were analyzed in different organs of the earthworm *Perionyx excavatus* treated with sublethal concentrations of mercuric chloride, copper sulfate, and zinc sulfate. In control earthworms, LDH activities have been observed to be 0.120µmoles of formazan liberated/mg protein/hour in the skin, 0.087µmoles/mg protein/hour in the ovary, 0.062µmoles/mg protein/hr in the testis, and 0.096µmoles/mg protein/hr in the intestine. Upon treatment with mercuric chloride, LDH activities decreased by 10% to 19.35% in different organs, with statistical significance ($P<0.05$). Copper sulfate treatment led to decreases of 4.16% to 22.58% in LDH activities, which were also statistically significant. Zinc sulfate treatment resulted in decreases of 5.74% to 15% in LDH activities, which is also statistically significant. These results imply that this heavy metal exposure can have a substantial impact on earthworm LDH activity, suggesting possible cellular stress and metabolic changes.

3.2 Succinate Dehydrogenase (Sdh) Activities

Succinate dehydrogenase (SDH) activity in the skin, ovary, testis, and gut of control earthworms was measured to be 0.145 µmoles/mg protein/hour, 0.067 µmoles/mg protein/hour, 0.072 µmoles/mg protein/hour, and 0.135 µmoles/mg protein/hour, respectively. SDH activities dropped by 10% to 23% in various organs after mercuric chloride therapy, with statistical significance ($P<0.05$). Treatment with copper sulfate resulted in statistically significant reductions in SDH activity ranging from 6.89% to 17.91%. Treatment with zinc sulfate reduced SDH activities by 4.13% to 16.66%, and these reductions were statistically significant. These results imply that heavy metal exposure can have a substantial impact on earthworm SDH activity, suggesting possible disturbances in oxidative processes and metabolic activities.

3.3 Malate Dehydrogenase (Mdh) Activities

Malate dehydrogenase (MDH) activities were measured in the skin, ovary, testis, and intestine of the *Perionyx excavatus* control group. The skin showed 0.150 pmoles of formazan liberated/mg protein/hr, the ovary 0.048 µmoles, the testis 0.038 µmoles, and the intestine 0.053 µmoles of formazan liberated/mg protein/hr. Malate dehydrogenase (MDH) activities declined dramatically in all organs after exposure to mercuric chloride (25 and 30 mg/kg soil): in the skin, they were reduced by 8% and 14%, in the ovary, by 27% and 33%, in the testis, by 20% and 33%, and in the intestine, by 9.43% and 20.75%. $P<0.05$ indicated that these decreases were statistically significant. Similar reductions in Malate dehydrogenase (MDH) activities were seen after treatment with copper sulfate (60 and 150 mg/kg soil): by 7.54% and 10% in the epidermis, 7% and 27% in the ovary, 18% and 34% in the testis, and 15.09% and 26.41% in the gut. Additionally, these alterations were statistically significant ($P<0.05$). Malate dehydrogenase (MDH) activities, on the other hand, were reduced following zinc sulfate treatment (60 and 150 mg/kg soil), but to a lesser

degree: by 2.66% and 6.66% in the epidermis, 8.33% and 5.26% in the ovary, 23.68% and 31.57% in the testis, and 7.54% and 15.09% in the gut. P<0.05 indicated that these decreases were statistically significant. All three therapies had a considerable impact on Malate dehydrogenase (MDH) activities overall, with varying patterns seen in different organs.

3.4 Glutamate Dehydrogenase (GDH) Activities

Glutamate dehydrogenase (GDH) activities were measured in the skin, gut, and nephridia of the control group of Perionyx excavatus. The results showed that the skin had 0.159 pmoles of formazan liberated/mg protein/hour, the intestine had 0.162 µmoles, and the nephridia had 0.174 µmoles of formazan liberated/mg protein/hour. The epidermis and intestine showed a minor increase in glutamate dehydrogenase activities after being treated with mercuric chloride (25 and 30 mg/kg soil), but the nephridia showed a decrease in these activities. In particular, the activities in the skin dropped by 8.80% and 11.94%, in the intestine by 4% and 10%, and in the nephridia by 12% and 16%. A statistically significant difference has been found (P<0.05). In a similar vein, Glutamate dehydrogenase (GDH) activities were reduced by 7.54% and 10% in the skin, 6.17% and 12.96% in the gut, and 8% and 14% in the nephridia after exposure to copper sulfate (60 and 150 mg/kg soil). Additionally, these alterations were statistically significant (P<0.05). Glutamate dehydrogenase (GDH) activities were reduced by zinc sulfate treatment (60 and 150 mg/kg soil) by 5.66% and 6.91% in the epidermis, 3.70% and 12.34% in the intestine, and 7% and 9% in the nephridia. P<0.05 indicated that these decreases were statistically significant. All three treatments had a considerable impact on Glutamate dehydrogenase (GDH) activities overall, with varying patterns seen in different organs.

3.5 Xanthine Oxidase (Xod) Activities

Xanthine oxidase (Xod) activity in the epidermis, gut, and nephridia of *Perionyx excavatus* was 0.166 µmoles/mg protein/hour, 0.130 µmoles/mg protein/hour, and 0.122 pmoles/mg protein/hour, respectively, in the control group. Xanthine oxidase (Xod) activities considerably decreased in all organs upon treatment with mercuric chloride (25 and 30 mg/kg soil): in the epidermis, they decreased by 8.43% and 32.53%, in the intestine, by 6.15% and 9.23%, and in the nephridia, by 21.31% and 25.40%. In a similar vein, xanthine oxidase (Xod) activities were reduced by 6.15% and 9.23% in the epidermis, 3.84% and 6.15% in the intestine, and 18% and 19% in the nephridia after exposure to copper sulfate (60 and 150 mg/kg soil). Reductions were also brought about by zinc sulfate treatment (60 and 150 mg/kg soil): these occurred in the skin by 2.40% and 7.22%, in the intestine by 1.53% and 6.15%, and in the nephridia by 14.75% and 19.67%. P<0.05 indicated that these decreases were statistically significant.

3.6 Lipid Peroxidation

The lipid peroxidation activities in the skin, gut, and nephridia of the control *Perionyx excavatus* group were assessed as follows: 0.230 µmoles of MDA/gm wet weight of tissue, 0.280 µmoles of MDA/gm wet weight of tissue, and 0.165 µmoles of MDA/gm wet weight of tissue. Lipid peroxidation activities rose considerably in all organs after exposure to mercuric chloride (25 and 30 mg/kg soil): in the skin, they increased by 4.78% and 8.26%, in the intestine, by 3.21% and 4.28%, and in the nephridia, by 2.42% and 4.24%. Similar increases in lipid peroxidation activities were seen after treatment with copper sulfate (60 and 150 mg/kg soil): 3.47% and 6.52% in the epidermis, 1.78% and 3.21% in the intestine, and 1.2% and 3.03% in the nephridia. Lipid peroxidation activities increased in response to zinc sulfate treatment (60 and 150 mg/kg soil) by 2.17% and 4.78% in the epidermis, 1.78% and 2.5% in the intestine, and 1.78% and 2.5% in the nephridia. P<0.05 indicated that these increases were statistically significant.

3.7 Superoxide Dismutase (Sod)

Superoxide dismutase (Sod) activities were measured in the skin (0.310 units/mg protein), gut (0.289 units/mg protein), and nephridia (0.319 units/mg protein) of the Perionyx control group. Superoxide dismutase (Sod) activities increased dramatically in all organs after treatment with mercuric chloride (25 and 30 mg/kg soil): by 6.45% and 8% in the epidermis, 3.80% and 4.84% in the intestine, and 2.50% and 3.76% in the nephridia. Superoxide dismutase (Sod) activities were also elevated in response to exposure to copper sulfate (60 and 150 mg/kg soil): by 3.54% and 7.41% in the epidermis, 3.11% and 3.80% in the intestine, and 1.25% and 2.19% in the nephridia. On the other hand, Superoxide dismutase (Sod) activities

increased slightly after being treated with zinc sulfate (60 and 150 mg/kg soil), but these increases were not statistically significant. Overall, treatments with mercuric chloride and copper sulfate significantly increased superoxide dismutase (Sod) activity; zinc sulfate had no statistically significant effect.

3.8 Catalase Activities

Catalase activity was evaluated in the skin (0.65 units/mg protein), gut (0.40 units/mg protein), and nephridia (0.72 units/mg protein) of the Perionyx control group. Catalase activities considerably decreased in all organs after exposure to mercuric chloride (25 and 30 mg/kg soil): in the skin, they decreased by 38.46% and 56.92%, in the intestine, by 37.5% and 45%, and in the nephridia, by 13.88% and 33.33%. Likewise, catalase activities were reduced by 21% and 24% in the skin, 22.5% and 30% in the intestine, and 8.33% and 16.66% in the nephridia after treatment with copper sulfate (60 and 150 mg/kg soil). Conversely, somewhat smaller reductions in catalase activity were brought about by zinc sulfate treatment (60 and 150 mg/kg soil): 7.69% and 20% in the skin, 12.5% and 25% in the colon, and 4.16% and 12.5% in the nephridia. $P<0.05$ indicated that these decreases were statistically significant. All three treatments had a considerable impact on catalase activity overall, with mercuric chloride having the most noticeable effects.

3.9 Glutathione-S-Transferase (GST)

Glutathione-S-Transferase GST activities were examined in the skin, gut, and nephridia of the *Perionyx excavatus* control group. The results showed that the skin had 0.38µmoles of formazan liberated/mg protein/hour, the intestine had 0.30µmoles, and the nephridia had 0.25µmoles. Glutathione-S-transferase (GST) activities considerably decreased in all organs upon exposure to mercuric chloride (25 and 30 mg/kg soil): in the skin, they decreased by 44.73% and 52.63%, in the intestine, by 36.33% and 53.33%, and in the nephridia, by 48% and 56%. Likewise, Glutathione-S-Transferase GST activities were reduced by treatment with copper sulfate (60 and 150 mg/kg soil) by 34.21% and 39.47% in the epidermis, 23.33% and 33.33% in the intestine, and 8.33% and 16.66% in the nephridia. On the other hand, glutathione-S-transferase activities were likewise reduced by zinc sulfate treatment (60 and 150 mg/kg soil): by 16.67% and 25% in the skin, 12.5% and 22.2% in the intestine, and 0% and 12.5% in the nephridia. $P<0.01$ indicated that these decreases were statistically significant. All three treatments had a considerable impact on Glutathione-S-Transferase GST rase activities overall, with mercuric chloride and copper sulfate treatments having the strongest effects.

4. DISCUSSION

Metal intoxication induces a complex array of physiological responses in animals, which often manifest as alterations in various biochemical pathways. Our study delved into the impact of heavy metal exposure on key enzymes involved in energy metabolism and oxidative stress response. At first, there was a decrease in LDH activity, particularly in the testes of worms exposed to mercuric chloride at lower sub lethal concentrations. LDH is crucial for energy production as it catalyzes the conversion of lactate to pyruvate, and its reduced activity suggests a disruption in energy metabolism [18,9].

The study aligns with previous research, demonstrating the physiological stress induced by metal intoxication. Investigations by Sharma & Dubey [19], have extensively documented non-specific biochemical responses in animals subjected to toxicity stress, shedding light on the profound impact on metabolic pathways. For instance, the reduction in lactate dehydrogenase (LDH) activity observed in our study resonates with similar decreases reported in response to cadmium exposure by Srikanthan & Krishnamurthy [12] and heavy metal exposure by Vaidya, [20] indicating a consistent pattern across different heavy metals.

Similarly, succinate dehydrogenase (SDH), a pivotal enzyme in the tricarboxylic acid (TCA) cycle, exhibited a notable decrease in activity, especially in the testis of worms exposed to mercuric chloride. This decline in SDH activity indicates a compromised Krebs cycle, potentially due to hepatocyte degeneration caused by heavy metal toxicity [9]. Similarly, research findings regarding the decline in Succinate dehydrogenase (SDH) and malate dehydrogenase (MDH) activities echo those of previous research by Ikediabi & Michael [21]. These studies provide further evidence of the disruption of energy production pathways induced by metal toxicities, underscoring the detrimental effects on cellular metabolism [22,23,24,25].

Table 1. % variation in the activity of the enzyme lactate dehydrogenase in the various Perionyx excavatus earthworm tissues following five years of exposure to heavy metal pesticide

Tissue	Control	Mercuric chloride		Copper sulphate		Zinc sulphate	
		Lower sublethal 25mg/kg soil	Highsublethal 30 mg/kg soil	Lowsublethal 60mg/kg soil	High sublethal 150mg/kg soil	Low sublethal 140mg/kg soil	High sublethal 350mg/kg soil
Skin	0.12±0.21	-10%	-14.16%	-4.16%	-9.16%	-11.66%	-15%
Ovary	0.087±0.6	-8.40%	-10.34%	-4.59%	-14.94%	-5.745%	-8.04%
Testis	0.062±0.2	-11.29%	-19.35%	-17.74%	-22.58%	-11.29%	-14.51%
Intestine	0.096±0.5	-9.16%%	-16.66%	-10.41%	-12.50%	-6.25%	-11.45%

Table 2. % change in the activity of the enzyme succinate dehydrogenase in the various Perionyx excavatus earthworm tissues following a five-day exposure to a heavy metal pesticide

Tissue	Control	Mercuric chloride		Copper sulphate		Zinc sulphate	
		Lowsublethal 25mg/kg soil	High sublethal 30 mg/kg soil	Lowsublethal 60mg/kg soil	High sublethal 150mg/kg soil	Low sublethal 140mg/kg soil	High sublethal 350mg/kg soil
Skin	0.145 ± 0.25	-10%	-13.79%	-6.89%	-11.09%	-4.13%	-10.34%
Ovary	0.067 ± 0.12	-11%	-22%	-10.44%	-17.91%	-7.46%	-10.44%
Testis	0.072 ± 0.2	-16%	-23%	-12.50%	-13.88%	-8.33%	-16.66%
Intestine	0.135 ± 0.2	-8.14%	-10.97%	-5.18%	-7.40%	-6.25%	-11.45%

Table 3. % change in the activity of the enzyme Malate Dehydrogenase in the various Perionyx excavatus earthworm tissues following a five-day exposure to a heavy metal pesticide

Tissue	Control	Mercuric chloride		Copper sulphate		Zinc sulphate	
		Low sublethal 25mg/kg soil	High sublethal 30 mg/kg soil	Lower sublethal 60mg/kg soil	High sublethal 150mg/kg soil	Low sublethal 140mg/kg soil	Highsublethal 350mg/kg soil
Skin	0.150 ± 0.25	-8%	-14%	-7.54%	-10%	-3%	-7%
Ovary	0.048 ± 0.17	-27%	-33%	-7%	-27%	-9%	-5%
Testis	0.038 ± 0.25	-20%	-33%	-18%	-34%	-23%	-32%
Intestine	0.053 ± 0.23	-9%	-20.75%	-15%	-27%	-8%	-15%

Table 4. % change in Glutamate Dehydrogenase enzyme activities in the different tissues of earthworm *Perionyx excavatus* after exposure to heavy metal pesticide for 5 days

Tissue	Control	Mercuric chloride		Copper sulphate		Zinc sulphate	
		Lowsublethal 25mg/kg soil	High sublethal 30mg/kg soil	Low sublethal 60mg/kg soil	High sublethal 150mg/kg soil	Low sublethal 140mg/kg soil	High sublethal 350mg/kg soil
Skin	0.159 ± 0.25	+9%	+12%	+8%	+10%	+6%	+7%
intestine	0.162 ± 0.23	+4%	+10%	+6%	+13%	+4%	+13%
Nephridia	0.174 ± 0.2	+12%	+16%	+8%	+14%	+7%	+9%

Table 5. % change in the activity of the Xanthine Oxidase enzyme in the various Perionyx excavatus earthworm tissues following a five-day exposure to a heavy metal pesticide

Tissue	Control	Mercuric chloride		Copper sulphate		Zinc sulphate	
		Low sublethal 25mg/kg soil	High sublethal 30 mg/kg soil	Low sublethal 60mg/kg soil	High sublethal 150mg/kg soil	Low sublethal 140mg/kg soil	High sublethal 350mg/kg soil
Skin	0.166 ± 0.15	-8%	-32%	-6%	-9%	-2%	-7%
Intestine	0.130 ± 0.28	-6%	-9%	-4%	-6%	-2%	-6%
Nephridia	0.122 ± 0.21	-21%	-25%	-18%	-19%	-14%	-20%

Table 6. % change in the activity of the lipid peroxidation enzyme in the various Perionyx excavatus earthworm tissues following a five-day exposure to a heavy metal pesticide

Tissue	Control	Mercuric chloride		Copper sulphate		Zinc sulphate	
		Low sublethal 25mg/kg soil	High sublethal 30 mg/kg soil	Low sublethal 60mg/kg soil	High sublethal 150mg/kg soil	Low sublethal 140mg/kg soil	High sublethal 350mg/kg soil
Skin	0.230 ± 0.31	+4%	+8.26%	+3.47%	+6.52%	+2.17%	+5%
intestine	0.280 ± 0.28	+3.21%	+4.28%	+1.78%	+3.21%	+2%	+3%
Nephridia	0.165 ± 0.39	+2.42%	+4.24%	+1.20%	+3.30%	+2%	+3%

Table 7. % change in Superoxide Dismutase enzyme activity in the various tissues of the earthworm Perionyx excavates

Tissue	Control	Mercuric chloride		Copper sulphate		Zinc sulphate	
		Low sublethal 25mg/kg soil	High sublethal 30 mg/kg soil	Low sublethal 60mg/kg soil	High sublethal 150mg/kg soil	Low sublethal 140mg/kg soil	High sublethal 350mg/kg soil
Skin	0.310 ± 0.19	+6.45%	+8%	+3%	+7%	+2%	+5%
intestine	0.289 ± 0.22	+4%	+5%	+3%	+4%	+3%	+4%
Nephridia	0.319 ± 0.23	+3%	+4%	+1%	+2%	+1%	+2%

Table 8. Percentage change in the activity of the catalase enzyme in the various tissues of the earthworm Perionyx excavates

Tissue	Control	Mercuric chloride		Copper sulphate		Zinc sulphate	
		Low sublethal 25mg/kg soil	High sublethal 30 mg/kg soil	Low sublethal 60mg/kg soil	High sublethal 150mg/kg soil	Low sublethal 140mg/kg soil	High sublethal 350mg/kg soil
Skin	0.65± 0.2	-38%	-56%	-21%	-24%	-44%	-52%
intestine	0.4± 0.23	-37%	-45%	-22%	-30%	-36%	-53%
Nephridia	0.72± 0.51	-13%	-33%	-8%	-17%	-46%	-56%

Table 9. % change in the activities of the Glutathione-s-transferase enzyme in the various tissues of the earthworm Perionyx excavates

Tissue	Control	Mercuric chloride		Copper sulphate		Zinc sulphate	
		Low sublethal 25mg/kg soil	High sublethal 30 mg/kg soil	Low sublethal 60mg/kg soil	High sublethal 150mg/kg soil	Low sublethal 140mg/kg soil	High sublethal 350mg/kg soil
Skin	0.38±0.2	-44%	-52%	-34%	-40%	-21%	-28%
Intestine	0.3±0.29	-36%	-53%	-23%	-33%	-17%	-26%
Nephridia	0.25±0.6	-48%	-56%	-8%	-17%	-12%	-26%

The decrease in Malate dehydrogenase (MDH) activity observed in our study reflects the response observed for SDH, further suggesting impaired Krebs cycle function. This impairment could arise from limitations in substrate flow or mitochondrial dysfunction induced by heavy metal exposure [26] Furthermore, there was a noticeable drop in the activity of GST, which is essential for detoxification and protection against oxidative stress. This was especially noticeable in the nephridia of worms exposed to mercuric chloride. This decrease is consistent with other research showing a reduced ability to respond to heavy metal toxicity by detoxifying and defending against free radicals [27,2,19].

The decline in superoxide dismutase (SOD) activity in heavy metal-treated earthworms suggests disruptions in free radical metabolism, potentially stemming from increased free radical production due to heavy metal exposure. Additionally, decreased catalase activity across all tissues may be attributed to reduced availability of hydrogen peroxide (H_2O_2) resulting from decreased SOD activity. Furthermore, decreased GST activity indicates the accumulation of organic hydroperoxides, exacerbating oxidative stress [28]. Moreover, research highlights the impact of heavy metal toxicity on antioxidant defense mechanisms. The observed decreases in catalase and glutathione-S-transferase (GST) activities are consistent with findings by Wang et al. [29] Yim et al. [8] and Yadav et al. [27] indicating compromised cellular defense against oxidative stress, a common consequence of heavy metal exposure.

Furthermore, the investigation sheds light on the association between heavy metal exposure and free radical proliferation, as evidenced by the increased xanthine oxidase activity and lipid peroxidation. This aligns with the broader understanding of the link between free radical metabolism and heavy metal stress, as documented by Ikediabi [21] and other recent studies [30].

5. CONCLUSION

In conclusion, our research underscores the intricate interplay among exposure to heavy metals, alterations in energy metabolism, and the mechanisms governing oxidative stress response. These findings contribute significantly to our understanding of the physiological repercussions of heavy metal overdose. Moving forward, it is imperative to delve deeper into this domain to mitigate the detrimental impacts on both the environment and public health. Future research should focus on elucidating the underlying mechanisms driving these interactions and exploring potential therapeutic interventions to counteract heavy metal toxicity effectively. By continuing to investigate this area, we can pave the way for more targeted strategies aimed at safeguarding human health and preserving ecological balance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://prh.mbimph.com/review-history/3356