Evaluating Polyaromatic Hydrocarbons (PAHs) Toxicity in Terrestrial Ecosystems Using Enzymatic Biomarkers in Achatina Fulica

Perfect Donga*

Applied Biology and Biochemistry, National University of Science and Technology, Bulawayo, Zimbabwe

***Corresponding author**

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ABSTRACT

Polyaromatic hydrocarbons (PAHs) have been linked to various human activities such as cooking, domestic heating, and the use of fuel for operating automobiles, resulting in common background levels of PAHs in soils. Although much has been revealed about the effects of PAHs on aquatic organisms, comparatively little is known about their impact on terrestrial organisms. This study investigates response of the model antioxidant, phase II and carbohydrate enzymes to stress induced by benzo(a)pyrene + pyrene and PAH associated pollution using the land snail *Achatina fulica*. The snails were exposed in triplicates to soil spiked with benzo(a)pyrene+pyrene and naturally polluted soil for a maximum of 21 days, with sampling conducted on days 1, 11, and 21 of exposure. Specific activities were significantly elevated with increase in exposure as revealed by RM Two-way ANOVA at 95% confidence level with the Giesser-Greenhouse correlation for superoxide dismutase ($P<0.0001$), catalase ($P<0.0001$), glutathione-S-transferase ($P<0.0001$), and lactate dehydrogenase [time of exposure ($P < 0.0111$) and between samples ($P = 0.0001$)]. In contrast, succinate dehydrogenase activity [time of exposure ($P < 0.0017$) and between samples ($P = 0.0027$)] was notably suppressed during the same time points. These results demonstrate significant stress induction by PAH-associated pollutants. Thus, enzyme activity in *A. fulica* proves to be a promising and practical biomarker for monitoring terrestrial pollution by polycyclic aromatic hydrocarbon.

Keywords: Polyaromatic hydrocarbons, *Achatina fulica*, biomarkers, bio-monitoring, benzo(a)pyrene, pyrene

INTRODUCTION

Polyaromatic hydrocarbons (PAHs) are a class of persistent organic pollutants widely distributed in the environment, primarily originating from anthropogenic activities such as cooking, domestic heating, and fossil fuel combustion (Patel et al., 2020). These compounds frequently accumulate in terrestrial ecosystems, particularly in soils, where they pose potential risks to resident organisms. While the toxicological effects of PAHs have been extensively studied in aquatic biota (Basopo et al., 2014), the impact on terrestrial organisms remains comparatively understudied. Land snails, such as *Achatina fulica,* are valuable bioindicators due to their widespread distribution, ecological relevance, and sensitivity to environmental contaminants (Ndebele, 2023). In addition, land snails are closely associated with soil when referring to their hibernation and mechanism of movement that is soil based, they become suitable environmental indicators to PAHs pollution.

Over the past few decades, increasing industrial activities have posed significant challenges to environmental monitoring due to the widespread release of anthropogenically produced toxic substances, many of which are by-products of manufacturing processes (Juhasz & Naidu, 2000; Agency for Toxic Substances and Disease Registry, 2012). These contaminants have been detected in various environmental media including food, water, soil, and airborne particulates. They enter organisms primarily through ingestion, inhalation, or dermal

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absorption, often via association with organic and inorganic particles in the environment. In many cases, these substances are transferred through the food chain, resulting in bioaccumulation, particularly at higher trophic levels. Therefore, it is very common to detect background levels of PAHs in soils, this being performed by environmental protection agencies (Carter, 2018).

Over the past few decades, the antioxidative defence enzymes have been proposed as biomarkers of contaminant mediated oxidative stress in a variety of organisms *Oreochromis mossambicus* (Srivastava et al., 1999), *Helisoma duryi* (Basopo et al., 2014), *Mullus barbatus* (Maehly & Chance, 1955), *Clarias gariepinus* (Osuagwu et al., 2023) as well as *Achatina fulica* (Ndebele, 2023). In these and other related studies it has been indicated that antioxidative defence system plays a crucial role in maintaining cell homeostasis; it may be induced after exposure to pollutants, this response reflecting an adaptation of the species to their environment (Ighodaro & Akinloye, 2018). This phenomenon may be utilised in experimental exercise as stress indicators and biological makers in animals exposed to environmental contaminants. One of the key functions of biomarkers is to provide early warning signals of significant biological effects (Vidal-Linan et al., 2010) and it is generally believed that sub-organismic (molecular, biochemical and physiological) responses precede those that occur at higher levels of biological organization such as population, community or ecosystem (Lam, 2009).

In recent years, such toxicants have garnered significant scientific attention not only due to their acute and chronic toxicity but also because of their environmental persistence and potential for long-term accumulation in ecosystems (Poulain et al., 2014). Among these, organic pollutants especially those derived from petroleum and fuel industries are of particular concern.

Land snails are ecologically significant invertebrates and have been widely recognized as sensitive bioindicators of terrestrial environmental pollution. Among them, *Achatina fulica* is particularly suitable for ecotoxicological studies due to its broad geographical distribution, ease of collection, and soil-surface-based locomotion, which increases its contact with contaminated substrates such as PAH-laden soils. Their close association with the soil environment makes them especially vulnerable to xenobiotic exposure, including polycyclic aromatic hydrocarbons (PAHs), which are known to persist and accumulate in terrestrial ecosystems. Beyond their ecological role, land snails contribute to food webs by serving as prey for birds, amphibians, mammals, and arthropods. They are also consumed by humans in some regions and have commercial applications in cosmetics and jewellery, while playing a functional role in nutrient cycling through the decomposition of organic matter (Barker, 2001).

In this study, *A. fulica* was selected as a model organism to evaluate the effects of PAHs on terrestrial invertebrates. The research aims to elucidate the biochemical responses of key enzymes involved in antioxidant defense (superoxide dismutase, catalase), phase II detoxification (glutathione-S-transferase), and energy metabolism (lactate dehydrogenase and succinate dehydrogenase) following exposure to both artificially spiked and naturally contaminated soils. By assessing enzyme activity over a 21-day period, the study investigates the potential of *A. fulica* as a reliable biomonitoring for terrestrial PAH pollution.

Polycyclic aromatic hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) are a class of organic compounds composed of two or more fused aromatic rings (Lee, 2010). They are primarily generated through anthropogenic activities, particularly the incomplete combustion of organic materials such as fossil fuels, wood, and biomass. As a result, PAHs are ubiquitous environmental contaminants found in air, water, and soil matrices (Groova et al., 2005; Wretling et al., 2010). Many PAHs are known to possess toxic, mutagenic, and carcinogenic properties, making their presence in the environment and food chain a significant public health concern.

Due to their harmful effects, various regulatory agencies worldwide have established maximum allowable concentrations of specific PAHs in drinking water, food additives, cosmetics, occupational environments, and industrial emissions (Table 1). Importantly, exposure to PAHs in real-world scenarios rarely involves isolated compounds; rather, humans and animals are typically exposed to complex mixtures of PAHs, which may exert synergistic or cumulative toxic effects (Igwe & Ukaogo, 2015).

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Table 1. PAHs recommended limits by Agency of Toxic substances and disease registry

Medium	**Interval limits of**		
	Total PAHs	Carcinogenic PAHs	Benzo(a)pyrene
Food	1.6 to <16.0 µg/day	--	0.16 to <1.6 µg/day
Drinking water	0.01 to <0.2 µg/L	<0.002 µg/L	<0.0006 µg/L
Air	<0.01 µg/m^3	<0.002 µg/m^3	<0.0005 µg/m^3

Source: Agency for Toxic Substances and Disease Registry as of May 2023

One has to know that PAHs may be divided into two groups, low-molecular-weight PAHs, containing three or fewer aromatic rings and high-molecular-weight PAHs, containing more than three aromatic rings (Patel et al., 2020). The typical examples of PAHS are indicated in Fig 1 using the structure of bezo(a)pyrene and pyrene.

Fig 1. Structure of pyrene on the left and benzo(a)pyrene on the right (Patel et al., 2020).

Metabolism of Reactive Exogenous Matter

Animals are frequently exposed to polycyclic aromatic hydrocarbons (PAHs) through various environmental routes, including ingestion, inhalation, and dermal absorption. Under normal physiological conditions, organisms maintain a dynamic balance between the generation and neutralization of reactive oxygen species (ROS). However, exposure to xenobiotic compounds such as PAHs disrupts this equilibrium, leading to excessive production of ROS including superoxide anion radicals ($O_2\cdot$), hydrogen peroxide (H_2O_2), hydroxyl radicals (·OH), and peroxyl radicals (ROO·) that exceeds the organism's antioxidative capacity (Halliwell & Gutteridge, 2007).

To mitigate ROS-induced cellular damage, organisms possess a sophisticated antioxidant defence system (ADS), comprising both enzymatic and non-enzymatic components (Birben et al., 2012). The enzymatic defence includes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) (Halliwell & Gutteridge, 2007). SOD catalyses the dismutation of superoxide radicals into hydrogen peroxide, which is subsequently detoxified by CAT into water and molecular oxygen, or by GSH-Px, which reduces hydrogen peroxide and organic peroxides to water and alcohols, respectively, using glutathione (GSH) as a reducing agent (Ighodaro & Akinloye, 2018).

Glutathione reductase (GR) plays a vital role in regenerating GSH from its oxidized form (GSSG), ensuring a continuous supply of GSH for ROS neutralization. Additionally, glutathione-S-transferase (GST) contributes to the detoxification process by conjugating xenobiotic compounds with GSH, facilitating their solubilization and subsequent excretion from the body (Čolak & Žorić, 2019). Collectively, these enzymes function as an integrated system to protect cellular structures from oxidative damage induced by reactive exogenous substances like PAHs.

MATERIALS AND METHODS

Snails and soil sampling

Achatina fulica of about (20 ± 6.8) g were collected for three consecutive days making a total of 150 ± 5 live snails from selected, minimally polluted terrestrial biota at National University grounds in Bulawayo. They were

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acclimatised in native cleaned soil which was also used as control soil during exposures and fed on lettuce for 21 days prior to experimental exposures.

The polluted soil was collected 5 meters downgradient from incineration area at Ngozi Mine landfill in Bulawayo within an area on $5m^2$ at a depth of 5 to 20 cm of the soil profile. Ngozi mine is located 15 km North-west of Bulawayo CBD and 0.8 km from surrounding settlement areas.

Exposures

The snails were separately exposed in triplicates to control, spiked and polluted soil. The spiked soil was prepared by carefully mixing 1 mg pyrene and 1 mg benzo(a)pyrene with 1 kg of control soil. Control soil sample was simply native cleaned soil and polluted soil was sampled and used directly as is. Each tank contained 1 kg of soil was allocated for seven snails; therefore, each treatment had 36 snails, making a total of 108 snails for all three treatments. The exposure period was 21 days and sampling done at day 1, 11 and 21. Each time the snails were quickly euthanized after sampling, homogenised and post mitochondrial fractions prepared.

Preparation of post mitochondrial fractions

The homogenates were prepared using a modified method of Loggini et al. (1999). Samples were euthanized on ice and homogenized in separate batches using a pestle and mortar in 0.1 M potassium phosphate buffer (pH 7.0) at 4 ± 2 °C, maintaining a tissue-to-buffer ratio of 1 g:5 ml. The resulting homogenate was centrifuged at 10,000 × g for 15 minutes, after which the supernatant was carefully collected and stored at −70 °C. Total protein content was quantified, and the samples were subsequently diluted with phosphate buffer to a final protein concentration of 1 mg/ml. These preparations were then used to assess enzyme-specific activity.

Practical assays

Each and every standard and sample were done in triplicates. The molar absorptivity's (M^{-1} cm^{-1}) were calculated for each practical assay using Beer lambert law equation that states that absorbance is equal to a product of cuvette length, molar absorptivity of the solution and concentration; length of the cuvette was always 1cm.

Protein assay

Protein content was estimated by the method of Lowry et al., (1951).

The test tubes containing standard bovine serum albumin (BSA) of seven different concentrations [0, 0.05, 0.1, 0.15, 0.2, 0.25 and 0.3 mg/ml] were prepared, with each tube having a total volume of 360 µl. For determining protein content in homogenates, the standard BSA was replaced by 360 µl of post mitochondrial fraction of each sample. Into each tube 2400 µl of alkaline copper reagent was added and tubes were incubated at room temperature for 10 minutes. To each test tube 240 µl of 1N Folin Ciocalteau phenol reagent was added, mixed and then incubated at room temperature for 30 minutes. The absorbance of each tube was measured at 750 nm. The standard curve was drawn using concentration of standard BSA against absorbance, from which the protein concentration of samples was calculated. The samples were then diluted each against its protein content so that the final concentration of protein becomes 1 mg/ml.

Superoxide dismutase activity

Superoxide dismutase activity was estimated by the method of Sun et al., (1988).

The test tubes containing standard Superoxide dismutase of ten different concentrations [0, 15, 30, 45, 75, 105, 135, 165, 210 and 270 ng/tube] were prepared, with each tube having a total volume of 0.5 ml. For post mitochondrial fraction samples; standard SOD was replaced by 0.5 ml of sample. Into each tube 2.45 ml of Superoxide dismutase assay reagent (SODAR) prepared by mixing 0.3 mM Xanthine solution, 0.6 mM EDTA solution, 150 µM Nitroblue Tetrazolium, 400 mM sodium carbonate buffer (pH 10.2) and 1 g/L Bovine serum, in the ratio 4:2:2:1.2:0.6 respectively by volume was added. The tubes were placed in a water bath at 25ºC

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followed by addition of 50 µl xanthine oxidase to each tube at 30 second intervals. The tubes were incubated for 20 minutes and then the reaction was terminated by adding of 1 ml of 0.8 mM $CuCl_2$ to each tube at 30 second intervals. The absorbance of each tube was read on a spectrophotometer at 560 nm. The enzyme activity was estimated by following SOD inhibition of 50% formazan formation, activity of samples calculated from standard curve using linearized rate against enzyme activity.

Catalase activity

The catalase activity was estimated by the method of Claireborne, (1985).

A dilution of 30% hydrogen peroxide using 50mM potassium phosphate buffer of pH 7 to make a concentration of 19 mM H_2O_2 with absorbance of approximately 0.83 was done in a 500 ml beaker. This was followed by addition of 2.9 ml of this solution into the cuvette followed by pipetting 0.1 ml post mitochondrial fraction sample into the cuvette. The decrease of absorbance per second at 240 nm in a spectrophotometer thermostated at 25°C was read for a total of 30 seconds for each sample. The blank solution was prepared by adding 3 ml of 50 mM potassium phosphate buffer pH 7 into the cuvette. Catalase activity was determined by the decrease in absorbance that was directly proportional to decomposition of H_2O_2 with time.

Glutathione-S-transferase activity

The Glutathione-S transferase activity was estimated by the method of Habig *et., al.* (1974).

Cytosolic Glutathione-S-transferase activity was measured using 1-Chloro-2,4-Dinitrobenzene (CDNB) as substrate. Briefly, the reaction mixture contained 2.64 ml of 0.1 M Potassium phosphate buffer of pH 6.5, 0.12 ml of 25 mM reduced Glutathione, 0.12 ml of post mitochondrial fraction and 0.12 ml of 25 mM 1-chloro-2,4-dinitrobenzene being added last into the cuvette. The change in absorbance was determined at 30 seconds interval for five minutes at room temperature on a spectrometer at 340 nm. The blank solution was prepared by adding 0.12 ml of 0.1 M Potassium phosphate buffer of pH 6.5 in place of post mitochondrial fraction into the cuvette. The specific activity of glutathione peroxidase was estimated by calculating the difference in absorbance values during the oxidation of NADPH.

Succinate dehydrogenase activity

The Succinate dehydrogenase activity was estimated by the method of Ackrell et al*.,* (1978).

Succinate dehydrogenase activity was measured using succinate obtained from dissociation of sodium succinate as substrate. The reaction mixture consisted of 1.5 ml of 50 mM potassium phosphate buffer pH 7.4 mixed with 0.5 ml of 0.01% 2.6-DCPIP and 0.25 ml of post mitochondrial fraction. To the reaction mixture 0.5 ml of 0.1M sodium succinate was added with subsequent addition of 2 drops of paraffin oil to provide anaerobic medium. The blank solution was prepared by adding 3 ml of 50 mM potassium phosphate buffer pH 7.4. The change in absorbance was determined at 30 seconds interval for five minutes at room temperature on a spectrometer at 600 nm. Succinate dehydrogenase and its coenzyme flavin adenine dinucleotide (FAD), represented as the complex E-FAD, oxidize the metabolite succinate to fumarate. Since the reaction liberates hydrogen atoms that are quickly taken up by FAD, the rate of enzyme was estimated by following the reduction of an artificial electron acceptor DCPIP by the E-FADH2.

Lactate dehydrogenase activity

The Lactate dehydrogenase activity was estimated by the method of King, (1959).

Cytosolic Lactate dehydrogenase activity was measured using lactate obtained from dissociation of sodium lactate as substrate. Briefly, the reaction mixture contained 1.25 ml of 0.1 M Sorensen glycine buffer pH 10 followed by addition of 0.05 ml of 10 mg/ml oxidised NAD. To the reaction mixture 125 µl of post mitochondrial fraction was added followed by 2.5 ml of 0.2 M buffered sodium lactate substrate of pH 10. The change in absorbance was determined at 30 seconds interval for five minutes at room temperature on a spectrometer at 340

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nm. The blank solution was prepared by adding 3 ml of 0.2 M buffered sodium lactate substrate of pH 10, into the cuvette. Lactate is converted to pyruvate by Lactate dehydrogenase enzyme with use of oxidised NAD as electron acceptor under alkaline conditions of significantly high pH. The specific activity of Lactate dehydrogenase was estimated by calculating the difference in absorbance values during the reduction of NAD.

Statistics

The data was analysed using Graphpad Prism version 10.5.0 and are expressed as mean ± standard deviation. Significant differences between groups and sampling period were determined using Repeated Measures (RM) Two-way ANOVA with the Giesser-Greenhouse correlation, followed by the Multiple non parametric t-test using a Two stage linear step-up procedure of Benjamin, Krieger and Yekutieli for comparing differences between means of all treatments with control. Only values $p < 0.05$ was accepted as significant for indicating statistical differences.

RESULTS

Superoxide dismutase

There were no significant differences between snail samples after 1 day of exposure. Superoxide dismutase specific activity showed an increase after 11 and 21 days on snails exposed to spiked and polluted soil compared to the control as shown in Fig 2. RM Two-way ANOVA reveals an overall significant increase of superoxide dismutase specific activity in snails from both treatment ($P<0.0001$) from day 1 to day 21. A significant difference was observed on day 11 for spiked ($P<0.0027$) and polluted ($P<0.0030$), and day 21 for spiked ($P<0.0056$) and polluted (0.0064) against the control as shown in Appendix 1B.

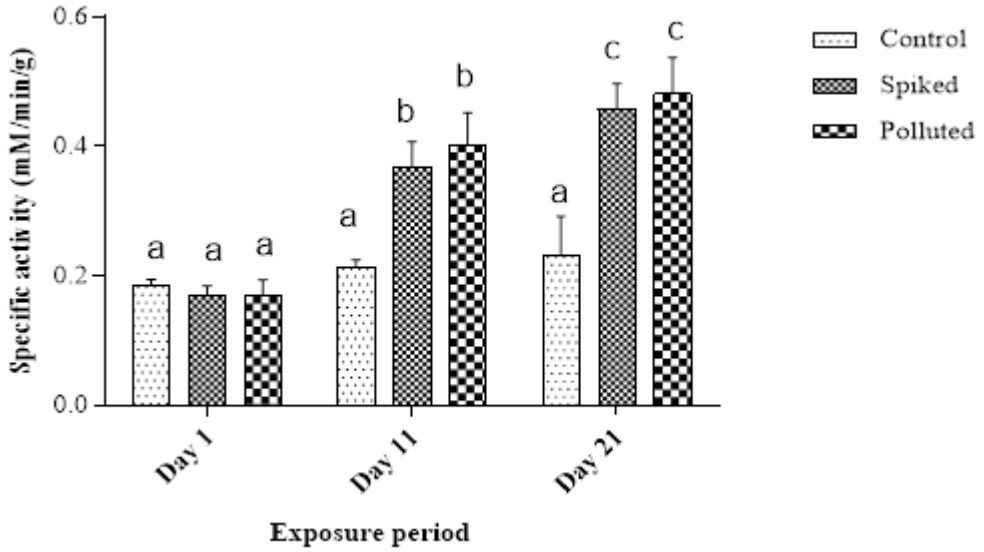

Fig 2: Specific activity of superoxide dismutase in the treatment samples after 1, 11 and 21 days. Superoxide dismutase specific activity expressed in mM/min/mg protein for data expressed in means ± standard deviation. Means indicated by the same letters above columns do not differ significantly ($P < 0.05$).

Catalase

There were no significant differences between the enzyme activity of snails after 1 day of exposure. Catalase specific activity showed an increase after 11 and 21 days on snails exposed to spiked and polluted soil compared to the control as shown in Fig 3. RM Two-way ANOVA reveals an overall significant increase of Catalase specific activity in snails from both treatment ($P<0.0001$) from day 1 to day 21. A significant difference was observed on day 11 for spiked ($P<0.00055$) and polluted ($P<0.00007$), and day 21 for spiked ($P<0.00053$) and polluted (0.00040) against the control as shown in Appendix IIB.

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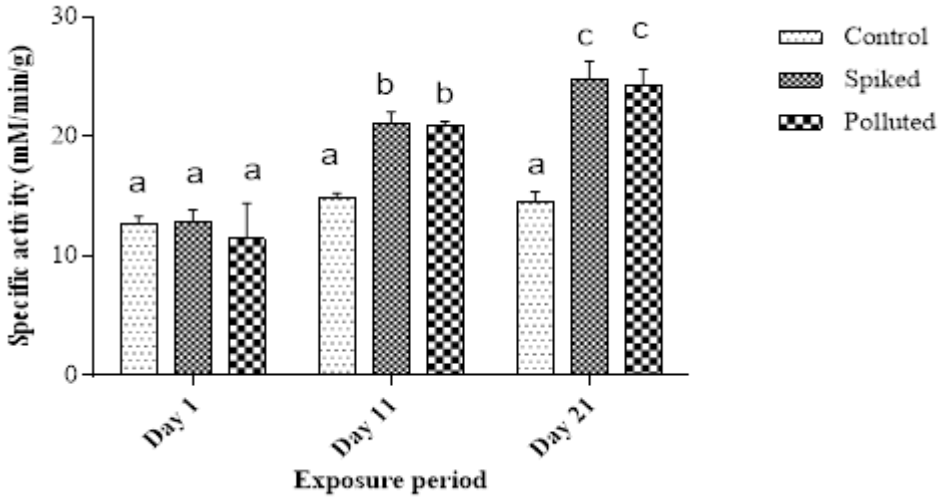

Fig 3: Specific activity of catalase in the treatment samples after 1, 11 and 21 days. Specific activity expressed in mM/min/mg protein for data expressed in means ± standard deviation. Means indicated by the same letters above columns do not differ significantly ($P < 0.05$).

Glutathione-S-transferase

Glutathione-S-transferase specific activity showed an increase after 11 and 21 days on snails exposed to spiked and polluted soil compared to the control as shown in Fig 4. RM Two-way ANOVA reveals an overall significant increase of Glutathione-S-transferase specific activity in snails from both treatment ($P<0.0001$) from day 1 to day 21. A significant difference was observed on day 11 for spiked ($P<0.00059$) and polluted ($P<0.00109$), and day 21 for spiked ($P<0.00913$) and polluted (0.00057) against the control as shown in Appendix IIIB.

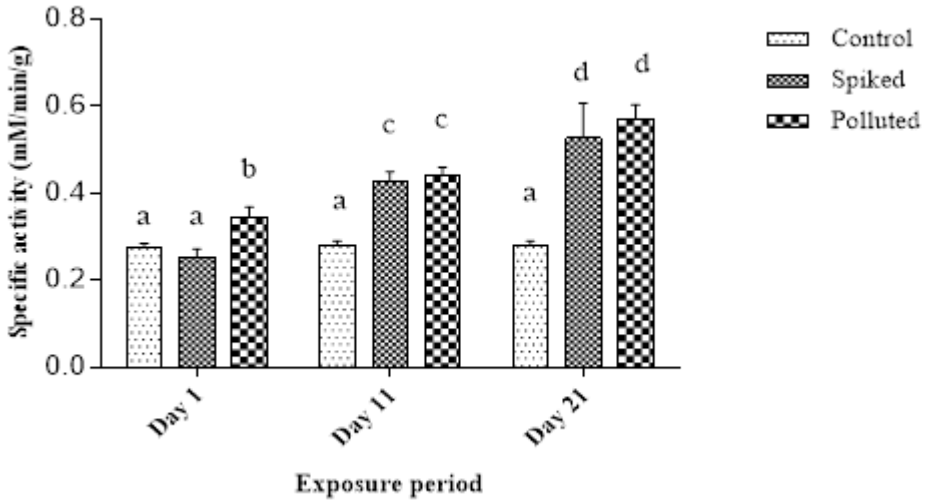

Fig 4: Specific activity of glutathione-S transferase in the treatment samples after 1, 11 and 21 days. Specific activity expressed in mM/min/mg protein for data expressed in means ± standard deviation. Means indicated by the same letters above columns do not differ significantly ($P < 0.05$).

Succinate dehydrogenase

Succinate dehydrogenase specific activity showed a decrease after 11 and 21 days on snails exposed to spiked and polluted soil compared to the control as shown in Fig 5. RM Two-way ANOVA revealed an overall significant decrease between time of exposure ($P < 0.0017$) and between samples ($P = 0.0027$) from day 1 to day 21. A significant difference was observed on day 11 for spiked ($P<0.03884$) and polluted ($P<0.00442$), and day 21 for spiked ($P<0.04107$) but not for polluted (0.0505) against the control as shown in Appendix IVB.

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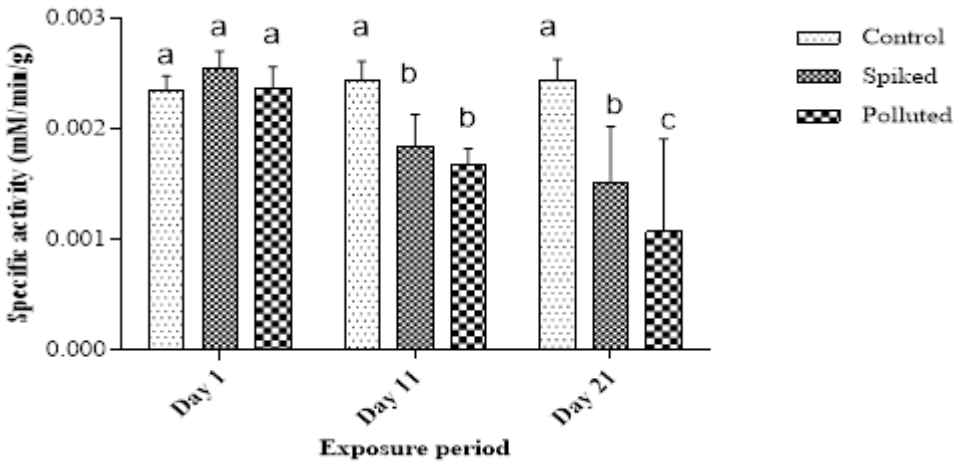

Fig 5: Specific activity of succinate dehydrogenase in the treatment samples after 1, 11 and 21 days. Specific activity expressed in mM/min/mg protein for data expressed in means ± standard deviation. Means indicated by the same letters above columns do not differ significantly ($P < 0.05$).

Lactate dehydrogenase

There were no significant differences in enzyme activity for snail samples after 1 day of exposure. Lactate dehydrogenase specific activity showed an increase after 11 and 21 days on snails exposed to spiked and polluted soil compared to the control as shown in Fig 6. RM Two-way ANOVA revealed an overall significant decrease between time of exposure ($P < 0.0111$) and between samples ($P = 0.0001$) from day 1 to day 21. A significant difference was observed on day 11 for spiked ($P<0.00009$) and polluted ($P<0.00484$), and day 21 for spiked ($P<0.00735$) and polluted (0.00588) against the control as shown in Appendix VB.

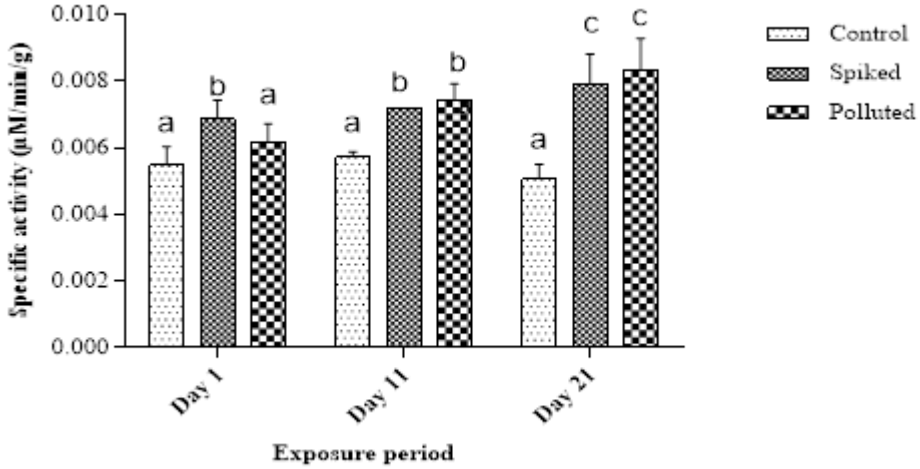

Fig 6 Specific activity of lactate dehydrogenase the treatment samples after 1, 11 and 21 days. Specific activity expressed in mM/min/mg protein for data expressed in means ± standard deviation. Means indicated by the same letters above columns do not differ significantly ($P < 0.05$).

DISCUSSION

Given the wide array of chemical compounds present in terrestrial environments, it is essential to investigate their interactions with biochemical parameters. This study contributes to a deeper understanding of enzymatic responses to chemical intoxication in the land snail *Achatina fulica* exposed to a complex mixture of pollutants. These pollutants included both field-collected soil from Ngozi mine Bulawayo suspected to contain elevated levels of polycyclic aromatic hydrocarbons (PAHs) since it is obtained from an incineration site and an artificial mixture of two well-characterized PAHs thus benzo(a)pyrene and pyrene.

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Superoxide dismutase (SOD) activity was significantly induced in snails following 21 days of exposure to both treatments, with increases recorded in the spiked group (from 0.168633 to 0.457767 µmol/min/g) and the polluted soil group (from 0.170000 to 0.480567 µmol/min/g). As a key first-line antioxidant defence enzyme, SOD showed significant activation by day 11 in both groups ($P < 0.05$; Fig. 2), indicating a rapid physiological response to oxidative stress induced by the pollutants. Notably, the highest specific SOD activity was consistently observed in the group exposed to the naturally polluted soil across the entire experimental period.

This heightened enzymatic activity may be attributed to the presence of a complex mixture of toxicants, necessitating a robust and coordinated antioxidant response to maintain cellular homeostasis. The observed trend aligns with previous findings suggesting that the activity of antioxidant enzymes can either increase or decrease under chemical stress, depending on the nature, intensity, and duration of exposure, as well as the inherent sensitivity of the exposed organism (Forrester et al., 2018). Previous studies support these findings. *Helisoma duryi*, an aquatic snail species, exhibited increased SOD activity upon exposure to pollutants known to induce oxidative stress, such as polycyclic aromatic hydrocarbons (PAHs), accompanied by a concurrent elevation in catalase activity (Basopo et al., 2014). SOD plays a critical role in cellular detoxification by providing cytoprotection against oxidative damage. It achieves this by catalysing the dismutation of superoxide radicals primarily generated in peroxisomes and mitochondria into hydrogen peroxide, thereby mitigating the harmful effects of reactive oxygen species (ROS) (Wang et al., 2018).

Research suggests that the activation of superoxide dismutase (SOD) can act synergistically to stimulate the activities of other key antioxidant enzymes, such as catalase (CAT) and glutathione peroxidase (GPx), all of which constitute the first line of cellular defense against oxidative stress (Ighodaro & Akinloye, 2018; Vives-Bauza et al., 2007). This pattern was confirmed in the present study, as a significant increase in catalase activity was observed in snails exposed to both spiked and polluted soil treatments.

Throughout the entire exposure period, there was a statistically significant elevation ($P < 0.0001$) in the specific activities of catalase and glutathione-S-transferase (GST). The highest mean specific activities for both enzymes were recorded in snails exposed to the polluted soil treatment catalase in Fig. 3 and GST in Fig. 4. These findings are consistent with previous studies in which catalase activity was upregulated in response to PAH exposure, as observed in the red mullet (*Mullus barbatus*) and the African sharp-tooth catfish (*Clarias gariepinus*) (Osuagwu et al., 2023; Maehly & Chance, 1955).

Catalase typically plays a relatively minor role in hydrogen peroxide decomposition under normal physiological conditions, particularly when the rate of hydrogen peroxide generation is low (Jones et al., 2002). However, during oxidative stress, when hydrogen peroxide levels are elevated, catalase becomes a critical component of the cellular stress-response mechanism (Borkovic et al., 2005). This is evident in the current study, where catalase specific activity increased from 12.82 to 24.78 µmol/min/g in the spiked treatment and from 11.46 to 12.87 µmol/min/g in the polluted soil treatment.

A parallel trend was observed for glutathione-S-transferase (GST), whose activity rose from 0.2550 to 0.5268 µmol/min/g in the spiked group and from 0.3448 to 0.5715 µmol/min/g in the polluted group. GST is a multifunctional detoxification enzyme that facilitates the conjugation of reactive epoxides such as those formed from PAHs with glutathione. This conjugation enhances the water solubility of toxic intermediates, thereby promoting their excretion and reducing intracellular damage (Camus et al., 2002).

These findings are supported by the work of Mathews (2003), who reported increased GST activity in Mozambique tilapia, *Oreochromis mossambicus* following a three-week exposure to 1.5 ppm and 3.0 ppm of water-accommodated fractions of crude oil. However, the response of GST can be compound-specific; for instance, certain metabolites of benzo(a)pyrene have been shown to inhibit specific GST isoenzymes. Such inhibition suggests that some conjugation products may interfere with enzyme function, ultimately reducing the efficacy of detoxification pathways (Allocati et al., 2018).

Succinate dehydrogenase (SDH) activity was markedly altered after 21 days of exposure to contaminated substrates (Fig. 5). In the spiked-soil treatment, SDH activity fell from 0.002542 to 0.001516 µmol min^{-1} g^{-1}, whereas in the field-polluted soil it increased from 0.001072 to 0.002370 µmol min^{-1} g^{-1}. Lactate dehydrogenase

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(LDH) displayed the reverse trend, showing a slight rise in the spiked group ($0.006860 \rightarrow 0.007929$ µmol min^{-1} g^{-1}) but a pronounced decline in the polluted-soil group ($0.006188 \rightarrow 0.002139$ µmol min^{-1} g^{-1}).

Because SDH is a key enzyme of the tricarboxylic acid cycle and the mitochondrial electron-transport chain, its inhibition indicates a shift away from aerobic carbohydrate oxidation, leading to diminished ATP generation. In the intact pathway, reducing equivalents from SDH-bound $FADH_2$ are transferred sequentially through the respiratory chain, ultimately driving oxidative phosphorylation. Suppressed SDH turnover therefore lowers the cellular pools of NADH and $FADH_2$, curtails oxidative phosphorylation, and reduces overall energy output (Bouillaud, 2023). Similar SDH suppression has been reported in American oysters chronically exposed to crude-oil contaminants in mesocosm studies (Janice et al., 1979).

In general, exposure to stress-inducing substances has been shown to disrupt the respiratory metabolism of snails. Consequently, any perturbation in intermediary metabolism resulting from PAH-induced stress is likely to influence the activities of key energy-metabolizing enzymes, such as lactate dehydrogenase (LDH) and succinate dehydrogenase (SDH) (Kalpan et al., 2009). LDH, a critical glycolytic enzyme ubiquitous in most tissues, is actively involved in carbohydrate metabolism and has been widely used as a biomarker of chemical stress exposure.

In the present study, snails exposed to benzo(a)pyrene + pyrene and naturally polluted soil exhibited a statistically significant difference in enzyme activity compared to the control group after both 11 and 21 days of exposure ($P < 0.05$; Fig. 2–6). Activation of energy metabolism enzymes occurred as early as the first day, suggesting a rapid shift toward anaerobic energy production. However, no significant increase ($P > 0.05$) in enzyme activity was observed between days 11 and 21, indicating stabilization of the metabolic response over time.

The activation of LDH in snails exposed to polluted soil was significantly elevated ($P < 0.05$) at day 21 relative to the control, suggesting a delayed but measurable metabolic adjustment. This delay likely reflects a sustained reliance on aerobic energy metabolism during the early exposure period (Fig. 5). The initial elevation of LDH activity between day 11 and day 21 (Fig. 6) can be interpreted as a compensatory mechanism to fulfil energy demands when oxidative phosphorylation is impaired. This observation underscores the role of LDH in maintaining energy homeostasis through enhanced anaerobic glycolysis under conditions of mitochondrial dysfunction.

Functionally, LDH serves as a key regulatory enzyme linking glycolysis and the Krebs cycle. Under anaerobic conditions, it catalyses the conversion of pyruvate to lactate, thereby facilitating continued ATP production in the absence of sufficient oxygen or impaired oxidative pathways (Farhana & Lappin, 2023). A metabolic shift characterised by elevated lactate-dehydrogenase (LDH) activity and concurrent suppression of succinate-dehydrogenase (SDH) activity in the present results is therefore interpreted as evidence of a comparable transition from aerobic to anaerobic respiration in *Achatina fulica*.

In *A. fulica*, exposure to xenobiotics provokes broad homeostatic adjustments in metabolic enzymes. Biotransformation of these foreign compounds accelerates the generation of reactive oxygen species (ROS); if not rapidly scavenged, ROS react with membranes, proteins and nucleic acids, inflicting cellular damage. The snail's antioxidant defence network including SOD, catalase and glutathione-S-transferase is thus highly inducible and provides a sensitive biomarker suite for detecting contaminants such as benzo(a)pyrene, pyrene and complex polluted soils.

CONCLUSION

Overall, our data indicate that *A. fulica* is an effective sentinel for terrestrial pollution. Suppression of SDH, together with the induction of antioxidant and phase-II detoxification enzymes, forms a consistent, quantifiable response irrespective of whether the stressor is a single PAH or a heterogeneous mixture. These enzyme endpoints therefore have strong potential as early-warning biomarkers for assessing the ecological health of terrestrial ecosystems found at around the polluted sampling site.

Ethical Considerations

The research presented here was closely monitored to adhere to good laboratory practise by Department of Environmental Science and Health Ecotoxicology Laboratory, National University of Science and Technology (NUST), Zimbabwe. Animal euthanasia was performed following humane methods approved by the institution and consistent with American Veterinary Medical Association guidelines for the Euthanasia of Animals, 2020.

Conflict of Interest

The author declares that there are no competing interests or conflicts of interest related to this manuscript.

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