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RESEARCH ARTICLE

Laevicaulis stuhlmanni slugs as accumulation bio-indicators of lead metal pollution: immunotoxic, physiological, and histopathological alterations

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Abstract

Trace metal pollution of soils is a widespread consequence of anthropogenic activity. Land slugs can be used as bio-indicators of the metals' pollution in the soil, so the present study aimed to determine the metal in the soil and *Laevicaulis stuhlmanni* land slug tissues by studying its effects on different physiological parameters. Slugs and soil samples were collected from fields in Abu-Rawash, Giza, Egypt. Slugs were identified, and the metals were determined in slug tissues and soil samples. On the other hand, slugs were reared in the laboratory and the new generation was fed on lettuce dipped in 0.027 µg/ml lead (Pb) for 10 days. The results revealed that the soil and slug tissues contained copper, manganese, lead, and zinc; the lead metal bioaccumulation factor was the highest. Also, the results showed that the hemocytes' count, testosterone, and estradiol hormones were significantly decreased. At the same time, the phagocytic index was increased considerably, and some morphological alterations in the granulocytes and hyalinocytes were observed after treatment with 0.027 µg/ml lead compared to untreated slugs. On the other hand, all the oxidative stress parameters were significantly increased in the treated slugs compared with the control. Concerning the histopathological studies, lead caused a rupture, vacuolation, or degeneration in the digestive cells of treated slugs. Finally, it can be concluded that the land slugs were sensitive to lead which was reflected by endocrine disruption, immunotoxicity, and increased oxidative stress parameters with histopathological damages. Hence, *Laevicaulis stuhlmanni* can be used as a metal accumulation bio-indicator to reflect the metal pollution in the soil.

Keywords Land slugs · Metals · Oxidative enzyme · Hormones · Histopathology

Introduction

One of the world's most serious issues is the presence of environmental metal contamination that could deteriorate human health (Salih et al. 2021; Rašković et al. 2022). Both anthropogenic and geogenic factors are responsible for the presence of the metals in the soil (Ugbaja et al. 2020; Briffa et al. 2020). In relation to living organisms, there are two categories of trace metals: Metals that are not required because they are not known to play a physiological role in living things, such as Cd, Hg, and Pb (Kibria et al. 2016). Conversely, essential metals (such as Co, Cu, Fe, Mn, Mo, and Zn) are important for a wide range of physiological and biochemical activities (Dhiman and Pant 2021) since they function as co-factors, acceptors, donors of electrons in redox reactions, and promoters of allostery (Dar et al. 2019).

The continuous anthropogenic release of trace metals into the soil creates ecological risk because most animals are frequently unable to control internal concentrations of certain metals (Morais et al. 2022). When trace metal concentrations rise above a threshold, organisms either accumulate these metals or cannot utilize detoxifying systems, which causes a negative impact on the physiological homeostasis of some terrestrial hosts, such as land snails (El Mageed et al. 2023). The propensity of a specific trace metal to bioaccumulate is determined by the metal's biochemistry and the invertebrate's ability to regulate its concentrations, not by whether the element is essential or not (Dar et al. 2019).

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Lead (Pb^{2+}) metal might cause cognitive and behavioral impairments in adults and children at high exposure levels. It can cause diverse neurological effects, alter presynaptic neurotransmission, and have different targets and mechanisms of action in the brain (Sultana et al. 2015). One of the main routes of lead input in the ecosystem is traffic, when gasoline additives burn in cars, lead contamination results, which is then carried to the land by precipitation from the atmosphere (Neal and Guilarte 2013). Because the chemicals enter snails through their diet of flora, their buildup may impact their ability to survive, reproduce, and perform physiological tasks (Briffa et al. 2020).

To monitor environmental changes and their impact on human society, natural biomonitors are employed in the evaluation of the state of the environment (Singh and Gupta 2012). A collection of species known as "bioindicators" represents the environment's natural status, including biotic and abiotic alterations to the habitat (McGeogh 1998). Mollusks are frequently employed as bioindicators and biosensors of metal toxicity because they rapidly accumulate harmful contaminants (Allah et al. 1997; Amusan et al. 2002; Ugbaja et al. 2020). Mollusks' wide geographic spread and sedentary, sessile habit make them a promising biomonitor for metal contamination in ecosystems. Because they are filter-feeders, several pollutants bioaccumulate in their tissues at a significantly faster rate (Elder and Collins 1991). As a result, the metal body burden in mollusks may be indicative of the quality of the surrounding environment, similar to the amounts of metals in water and sediment (Singh and Gupta 2012). Freshwater mollusks were considered accumulation indicators for monitoring metal pollution (Zadory 1984). Many biological functions necessary for the growth and maintenance of molluscan populations, including feeding, growth, reproduction, general physiological functions, and maturity, are impacted by the accumulation of the metals (Adriano 2001; Singh and Uma 2009; Taslima et al. 2022).

Land mollusks, such as slugs, are one of the many biological organisms used as bio-indicators for describing soil quality (Parmar et al. 2016). Slugs possess many characteristics that qualify them as bioindicators: their ability to store the metal in their tissues (Marigómez et al. 1998), wide distribution, easy collecting (Cofone et al. 2020; Baroudi et al. 2020), and hence, they are used in ecotoxicological biomonitoring research works. The slug *Laevicaulis stuhlmanni* is a common species of terrestrial slug in Egypt (Ali et al. 2022). It might be helpful as an indicator of the level of metals in the soil. The slugs potentially represent the time and space-integrated determination of metal pollution in soil that the slugs uptake from plants living in the sampled area (Popham and D'Auria 1980). The digestive gland of terrestrial gastropods is a target organ for metal accumulation and storage in its tissues (Dallinger et al. 1993), leading to many histopathological alterations like rapture, degeneration, and vacuolation of the digestive and secretory cells and might even lead to the death of these slugs (Abdel-Tawab et al. 2022).

Therefore, the present study aims to determine the lead metal concentration in tissues of *Laevicaulis stuhlmanni* slugs and then study its bioaccumulation on the immunotoxic, histopathological, endocrine disruption, and oxidative stress alteration in slugs' tissues.

Materials and methods

Experimental slugs and soil samples

The slugs and the soil surrounding them were collected in the late evening from an agriculture field in Abu-Rawash, Giza Governorate, Egypt (latitude, 30° 01′ 33.00″ N longitude, 31° 04′ 18.00″ E). Within 20 cm of the soil's depth, soil samples were taken. Three to five soil samples were collected, each of 0.5 kg, were collected from the surface around slug locations (Shi et al. 2019; Salih et al. 2021). The collected samples were transferred to the Medical Malacology Department, Theodor Bilharz Research Institute (TBRI), Giza, Egypt. The animals were placed in small glass boxes containing moist soil (1:1 mixture of clay and sand, 10 cm high)**.** Each box was provided with fresh green lettuce leaves and was covered with a muslin cloth secured with a rubber band, to prevent slugs from escaping, and it was maintained under 20 °C ± 2 °C in the laboratory for 2 weeks for acclimatization.

Slug morphological parameters

One group of slugs (10 slugs) was preserved in 85% ethanol, and some morphological parameters were measured by using an electronic caliper like, total body length (mm), total body width (mm), and weight of the body (g) (Ali et al. 2022).

Metal determination in slug tissues and soil samples

i. Metal determination in slug tissues

Fifty slugs were divided into five replicates (one gram each) and dried at 50 °C, then ground and wet-digested in 1 ml of HNO_3 conc., (70%) for 2 h at 70 °C, and then diluted with 5 ml of deionized water for analyzing metals (Abdel Kader et al. 2016).

ii. Metal determination in soil sample

The soil samples were divided into three replicates and dried in an oven at 120 °C for 4 h. The samples were then passed through a 0.5-mm sieve. Soil samples were dispersed with nitric acid (1 g/10 ml HNO_3) and filtered. The leachate samples were diluted to 100 ml in a volumetric flask with double–distilled water

(Mohammadein et al. 2013; Shaaban et al. 2017). A blank determination was carried out for the calibration of the instrument (Kacholi and Sahu 2018).

The metals such as Cu, Mn, Pb, and Zn were determined in the whole slug tissues, and the collected soil sample, using automatic absorption spectrophotometry (AAS) (GBC AVANTA 3000, Australia) in Environmental Research Laboratory; Theodor Bilharz Research Institute (TBRI) according to Abdel Kader et al. (2016). The instrument software automatically changed each element's current, wavelength, and slit bandwidth. Standards: Metals stock standard solutions were obtained from Merck, Darmstadt, Germany (Merck's ampoules; 1000 mg).

The bioaccumulation factor (BAF) was calculated according to (Gobas and Morrison 2000; Mohammadein et al. 2013) using the following equation:

$$\mathrm{BAF} = \frac{\text{Metal concentration in snail tissues } (\mu \text{ tissue/g dry weight})}{\text{Metal concentration in soil jr } (\mu g/\text{g dry soil})}$$

Slug rearing

The third group of slugs was kept in glass cages containing sterilized soil (sandy, clay, and peat-mous soil, 1:1:1), moistened with water three times a week, and held at 20 ± 2 °C with 80 ± 5.0 R.H.% (relative humidity %). The boxes were checked daily, searching for clutches of eggs. Newly deposited clutches were removed with soil, placed in another box, and observed daily until hatching to be used in the next experiments (Mohammad et al. 2021).

Treatment of slugs produced in the laboratory with Lead (Pb)

Sixty new slugs produced in the laboratory were divided into six groups (ten slugs each), three groups for treatment and another for control. Animals were put in plastic containers and covered with perforated cloth for ventilation. A stock solution (1000 mg/l) of lead nitrates {Pb $(NO_3)^2$}, Fisher Scientific, Fair Lawn, NJ, USA, and 0.027 µg/g was prepared. Disks of fresh lettuce leaves were dipped for 20 s, in the prepared concentration of Pb. Three groups of slugs were fed on treated lettuce for 10 days, and control groups were fed on untreated lettuce disks. Dead animals were counted daily and removed (Mohammad et al. 2021).

Immunotoxic studies

Total count, morphological alterations of hemocytes and phagocytic index measurement The hemolymph of treated and untreated slugs from each group was collected according to Nduku and Harrison (1980) and divided into two groups: one group to measure the phagocytic index, and another to measure some reproductive system hormones, *i.e.* testosterone and estradiol hormones.

Total hemocytes count was done by a Bürker- Turk hemocytometer according to Der Knaap et al. (1981). To display the various hemocyte morphologies, hemolymph smears were performed as monolayers (Ibrahim et al. 2018). A 100 µl hemocyte suspension was taken from each specimen, spread onto glass slides, and incubated for 1 h at 37 °C in a humid environment with activated charcoal particles to promote cell adhesion. The following formula was used to determine the phagocytic index (Guria 2018; Ibrahim and Hussein 2022):

$$\text{Phagocytic index} = \frac{\text{Number of cells with phagocytosed charcoal particle}}{\text{Total number of cells counted}} \times 100$$

Biochemical studies

Sample preparation One gram of the slugs' soft tissues (digestive and hermaphrodite glands) of the control and exposed groups was homogenized in 10 ml of phosphate buffer by a glass dounce homogenizer and a part of them then centrifuged under cooling at 3000 rpm/10 min for determining total antioxidant capacity and SOD activity. Another part was centrifuged under cooling at 4000 rpm/15 min to determine MDA. After that, the supernatant was used to determine the enzymes (Morad et al. 2022).

Testosterone and estradiol hormone determination Testosterone and estradiol hormone concentrations were assayed according to the manufacturer instructions of T EIA kit (Enzo Life Science, Michigan, USA, ADI-900–065) and E EIA kit (Cayman Chemical Company, Michigan, USA, item no. 582251) (Ibrahim et al. 2023a). Using fresh disposable tips, 25 µl of each standard, control, and sample (exposed) extracts were dispensed in duplicate into the corresponding wells on a microplate coated with T or E monoclonal antibody. Subsequently, 200 µl of the enzyme conjugate was added to each microplate well, mixed well, and incubated

for 60 min at room temperature. The microplates were then treated, and the absorbance was determined (Ibrahim et al. 2023b).

Superoxide dismutase (SOD), malondialdehyde (MDA), and total antioxidant capacity determination SOD was determined according to Damerval (Damerval et al. 1986) by using Biodiagnostic kits. Malondialdehyde (Lipid peroxidase, MDA) was done according to (Ohkawa et al. 1979), catalase (CAT), and total antioxidant capacity (TAC) were measured by (Cat. No. TA 2513) (Koracevic et al. 2001).

Histological studies

The digestive gland was separated from exposed and unexposed slugs, then it was fixed in 10% formalin for 12 h, processed in ascending series of ethanol concentrations (80%, 90%, 100%) for 3 h in each concentration, cleared in 1:1 solution of xylene and absolute alcohol for 30 min, 3 times, embedded in equal volumes of xylene and paraffin (1:1), then left in the incubator at 55 °C, for 45 min. Tissues were blocked in equal volumes of paraffin and barablast (1:1) for 30 min. Blocks were then sectioned at 5 µm into ribbons on cold water using a microtome, and then sections were placed on slides, de-waxified in xylene, and stained with hematoxylin and eosin (Bancroft et al. 1996). Slides were finally covered by using DPX and glass slips and examined under a light microscope (Morad et al. 2023).

Statistical analysis

The half-lethal and lethal concentration values were defined by probit analysis (Finney 1971). Analysis of data was carried out by one-way analysis of variance (ANOVA) followed by Duncan's test to assess the significance of differences among control and exposed groups. The obtained data were analyzed using the statistical program SPSS version 20 (SPSS, Inc., Chicago, IL) for Windows. Significance at $p < 0.05$ and values were expressed as mean ± S.D (Murray 1981).

Results

The external morphological description of the *Laevicaulis* stuhlmanni slug

Laevicaulis stuhlmanni (family Veronicellidae) has a dark to light brown color dorso-ventrally flattened body with a light longitudinal color band in the center (Fig. 1). The hyponata are uniformly light brown. The head has two pairs of tentacles that are hidden under the notum, the first (lower) shorter chemotactic pair and a longer (upper) pair of ocular tentacles. The male genital pore is located interiorly on the body surface below the mouth and the female genital pore is in the left hyponotum in the posterior portion of the body. Adults are on average 5.1 ± 1.2 cm long and 2.05 ± 0.1 cm wide. The weight of an adult averages around 3.35 ± 1.01 g (Table 1).

Metal concentrations in *L. stuhlmanni* slugs' tissue and soil samples are shown in Table 2. The results showed that

Table 1 Morphometric parameters of the sample ($N = 16$)

Morphometric parameter	Minimum	Maximum	Mean ± SD
Length (cm)	4.1	6.2	5.1 ± 1.2
Width (cm)	1.8	2.2	2.05 ± 0.1
Foot length (cm)	2.6	4.8	3.7 ± 1.3
Live weight (g)	2.5	4.3	3.35 ± 1.01

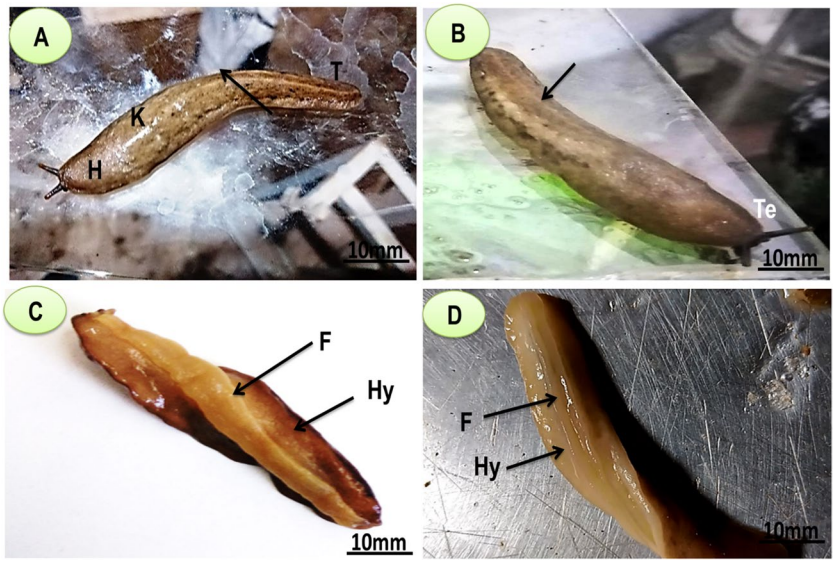

Fig. 1 Morphological features of garden slug: **A** dorsal view; **C**, **D** ventral view. (F) foot; (H) head region; (Hy) hyponotum; (K) keel; (T) tail region; (Te) tentacle. The black arrow: a light longitudinal color band

Table 2 Concentration and Bioaccumulation of metals in *L. stulhmanni'* soft tissue and soil samples

Metal	Conc. in soft tissue of slug (µg/g dry weight)	Conc. in soil (µg/g dry weight)	Bioaccumulation factor
Copper (Cu)	4.609	2.084	2.215
Manganese (Mn)	0.25	0.097	2.577
Lead (Pb)	0.130	0.027	4.814
Zinc (Zn)	0.181	0.101	1.79

Conc. concentration

the tissue of the slug, and soil samples contained copper, manganese, lead, and zinc. Moreover, the results revealed that the bioaccumulation factor of Pb metal was the highest one (Pb > Mn > Cu > Zn) for tissue.

Laboratory experiment

Examining the slug hemocyte monolayers revealed that the typical control slug included two distinct cell morphological types (Fig. 2): the larger (type 2), which divided into granulocytes and hyalinocytes, and the smaller (type 1), which were more numerous. When slugs' hemocytes were exposed to 0.027 µg/g of lead, the granulocytes and hyalinocytes displayed some morphological changes, while the granulocytes had a large number of granules and vacuoles. The cell's outer membrane had pseudopodia and protrusions. The nuclei of hyalinocytes shrunk, and some of them developed pseudopodia with uneven outer membranes (Fig. 2D–F).

According to the current findings, the slugs exposed to 0.027 µg/g of lead for 10 days presented a mean total number of hemocytes that was a substantially mean lower ($p < 0.05$) than the control group, while their phagocytic index was significantly higher (Fig. 3).

Testosterone significantly decreases its concentration decreasing from 20 nmol/l levels to 12 nmol/l levels after exposure to lead and in the case of the estradiol significantly decreased ($p < 0.05$) than in the control group from 85 pg/ml level to 35 pg/ml (Fig. 4).

The current results showed that following exposure to 0.027 µg/g of Pb metal, the MDA, SOD, CAT, and TAC contents µg/g increased significantly ($p < 0.05$) in comparison to the control group (Fig. 5).

In control slugs, the digestive gland was made up of digestive tubules coated with secretory and digestive cells that rested on a basement membrane enclosing the lumen (Fig. 6A). The current findings demonstrated that slugs exposed to 0.027 µg/g of lead experienced changes in their histological architecture, including numerous ruptured, vacuolated, or degraded digestive cells. Furthermore, the secretory cells had broken or deteriorated. In addition, there were more vacuoles and more lumen inside the tubules (Fig. 6B).

Discussion

The present results showed that *L. stuhlmanni* slugs have a dorso-ventrally flattened dark to light brown color in the center. Its hyponata are uniform with a light brown color and it has two pairs of tentacles. The male genital pore is located anteriorly on the body surface below the mouth, and the female genital pore is in the left hyponotum in the posterior portion of the body. These descriptions were similar to (Ali et al. 2022) who reported that these slugs have a 44 mm length (range, 36–52 mm), mean width 16 mm and a dorsal-ventrally flattened body with a light longitudinal color band running down the center; in the back, there are rows of dark spots. Two pairs of tentacles, which are hidden under the notum, were found in the head region. Also, (Das and Parida 2015) emphasized the morphometric description of a tropical leather leaf slug, *Laevicaulis alte* depending on three sets of morphometric variables, which were live weight and length, and length-circumference. Ali (2017) confirmed the

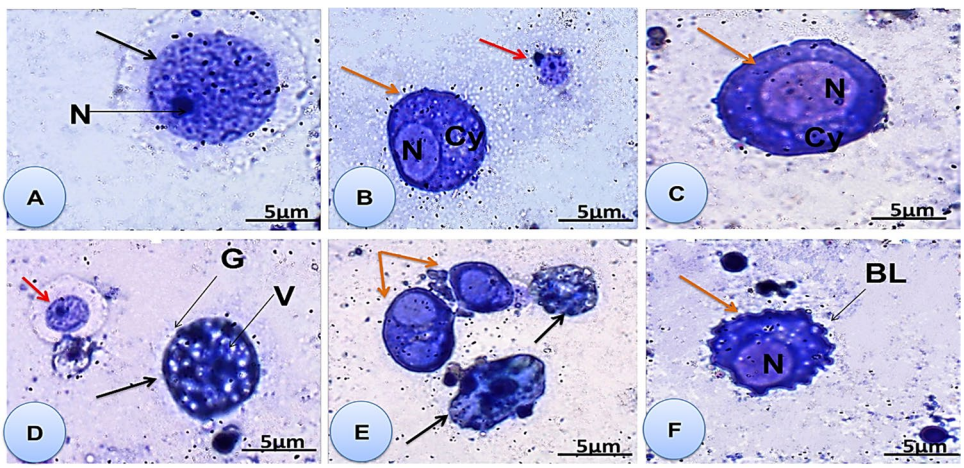

Fig. 2 Photomicrographs show normal slug hemocytes (**A**, **B,** and **C**) (×40). **D**, **E,** and **F**: hemocytes of slugs that exposed to 0.027 µg/g of Pb. Cy, cytoplasm; BL, blebbing; G, granules; N, nucleus; V, vacuole; small granulocytes, red arrow; type 2 (granulocytes), black arrow; type 2 (hyalinocytes), orange arrow

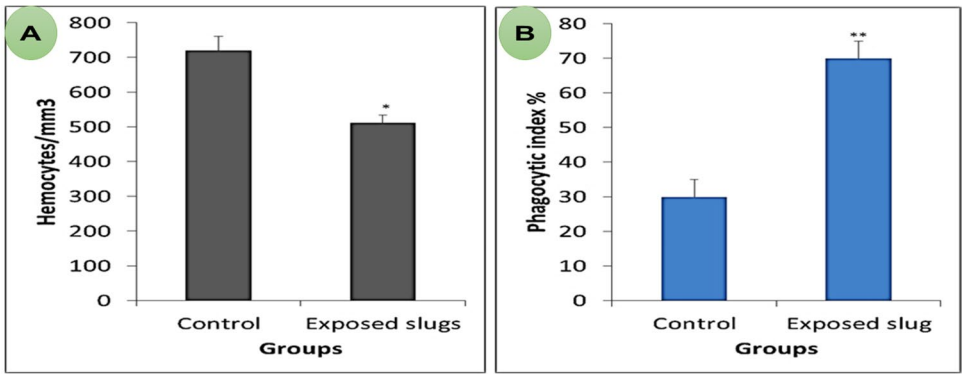

Fig. 3 Toxic effect of Pb metal on total number of hemocytes/mm.^{3} (**A**), and phagocytic index (**B**) of *L. stulhmanni* slugs. $N = 10$; data are expressed as mean ± SD.*Significantly different at $P < 0.05$. **Significantly different at $P < 0.01$

presence of the veronicellid slug *L. stuhlmanni aegypti* Ali & Robinson as a subspecies found in Egypt and it is widely distributed in the tropical and subtropical regions.

Metal pollution caused dangerous and dramatic consequences for organisms (Ali et al. 2019; Salih et al. 2021). Gastropods are largely used as bioindicators for metal accumulation (Allah et al. 1997; Ibrahim and Sayed 2019; Ugbaja et al. 2020; Dhiman and Pant 2021). Because they are filter-feeders, several pollutants bioaccumulate in their tissues at a significantly faster rate (Elder and Collins 1991). Freshwater mollusks were thought to be markers of metal pollution accumulation (Zadory 1984). The present study indicated that there are at least four metals accumulated in the tissue of slug and soil samples and they are: Cu, Mn, Zn, and Pb. Also, results indicated that the bioaccumulation of Pb in *L. stuhlmanni* slugs' tissue and in the soil, was the highest one compared with the other studied metals. Similarly, (Akhrasy and El-Sayd 2020) stated that the land snails could be used as perfect metal pollution bioindicators in the case of Mo (molybdenum), Pb (lead), Cd (Cadmium), Cu (copper), and Zn (zinc). They concluded that the highest metal concentration was Mo with *Monacha cartusiana* (Müller) while the lowest concentration was Zn in soil and snail samples from Sharqia and Qalyubiya Governorates, and they attributed this variation in the metal concentrations to anthropogenic activities and the metal traffic load (El Mageed et al. 2023). Ugokwe et al. (2020) stated that *Archachatina papyracea* snails could transfer and accumulate two non-essential metals (cadmium and lead) in the food chain and hence could harm humans due to their consumption. Snails can be used as quantitative indicators of metal (and Pb) accumulation in their soft tissues (Berger and Dallinger 1993), especially in the hepatopancreas because Pb is less toxic (5–6) times for invertebrates compared to Cd and Zn (Nowakowska 2014). Nica et al (2012) stated that Cu, Zn, Cd, and Pb caused serious problems to environmental health because they bioaccumulated in the terrestrial ecosystems.

Hemocytes are the first line of defense against different environmental toxins (Morad et al. 2023). They perform the main immunological functions like encapsulation, phagocytosis, and cytotoxicity after exposure to pathogens (Penagos-Tabares et al. 2018; Mansour and Ibrahim 2023). The main immune effector hemocytes are granulocytes that exhibit strong phagocytic efficiency and generate of high levels of nitric oxide and superoxide anion (Ibrahim et al.

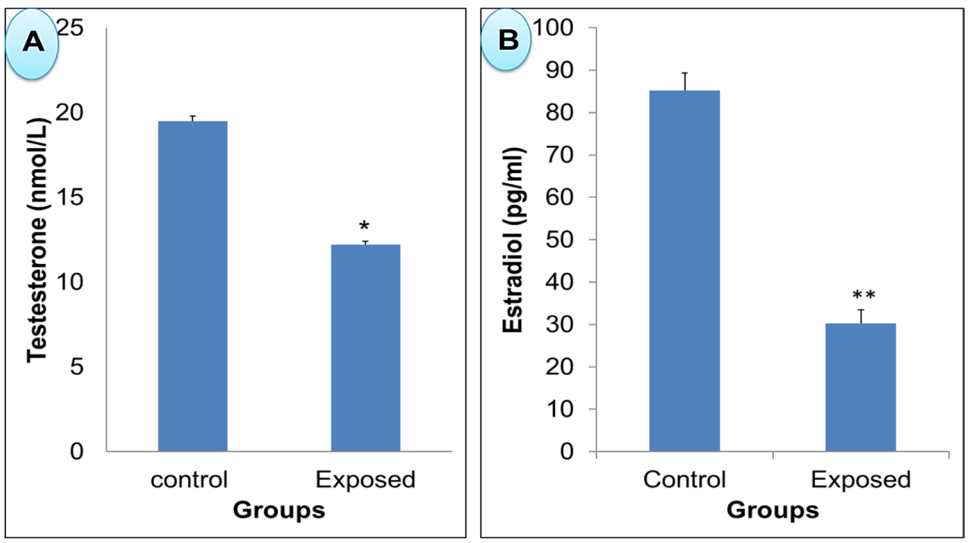

Fig. 4 Effect of 0.027 µg/g of Pb metal on testosterone (**A**) and estradiol (**B**) levels of the slugs after 24 h of exposure. $N = 10$; data are expressed as mean ± SD.*Significantly different at $P < 0.05$. **Significantly different at $P < 0.01$

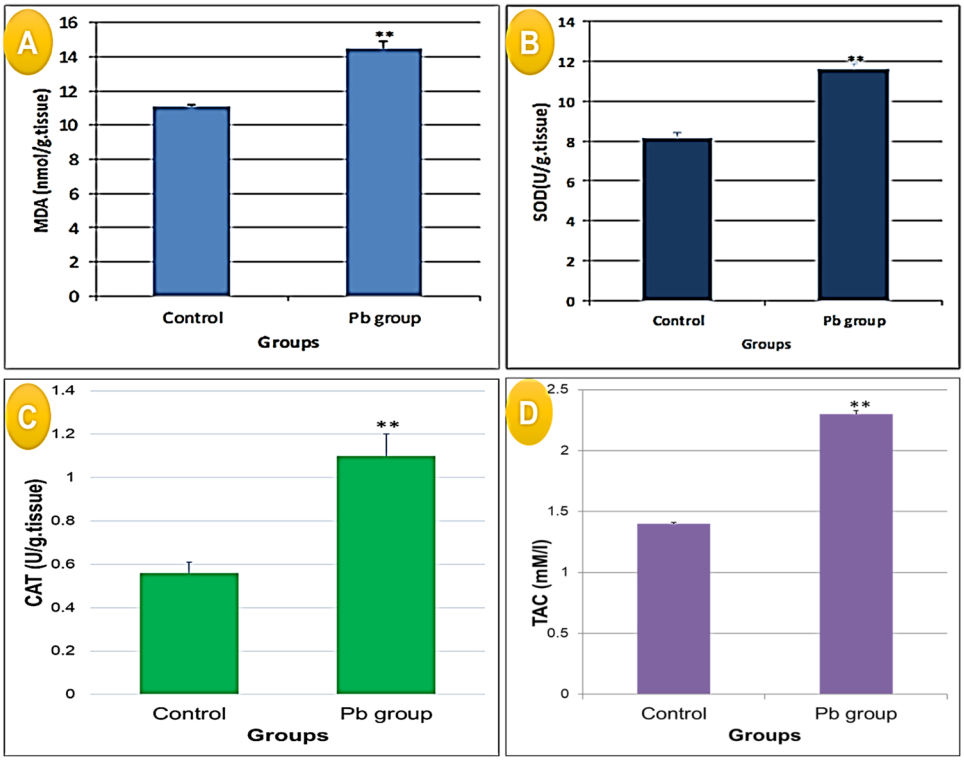

Fig. 5 Effect of 0.027 µg/g of Pb metal on malonaldehyde (MDA) (**A**), superoxide dismutase (SOD) (**B**), catalase (CAT) (**C**), and total antioxidant capacity (TAC) (**D**)levels of the slugs after 24 h of exposure. $N = 10$; data are expressed as mean ± SD.*Significantly different at $P < 0.05$. **Significantly different at $P < 0.01$

2022). The present results showed that slugs exposed to 0.027 µg/g had a significant reduction in the mean total number of hemocytes; while the phagocytic index was significantly higher than the control slug group. The decrease in total blood count could be due to the hemocytes' participation in the healing and repairing the damaged hermaphrodite and digestive glands after exposure to Pb (Esmaeil 2009; Ibrahim et al. 2018; Ibrahim and Abdel-Tawab 2020). However, the increase in the phagocytic index relied on the type and the nature of the toxins (Ibrahim and Sayed 2020). Phagocytosis is a broad immunological reaction to foreign substances and is one of the many functions of hemocytes. Hemocyte activity for an immune response involves invaginating the cell membrane, forming pseudopods, and ingesting foreign particles into an endocytic vacuole (Ibrahim et al. 2023b).

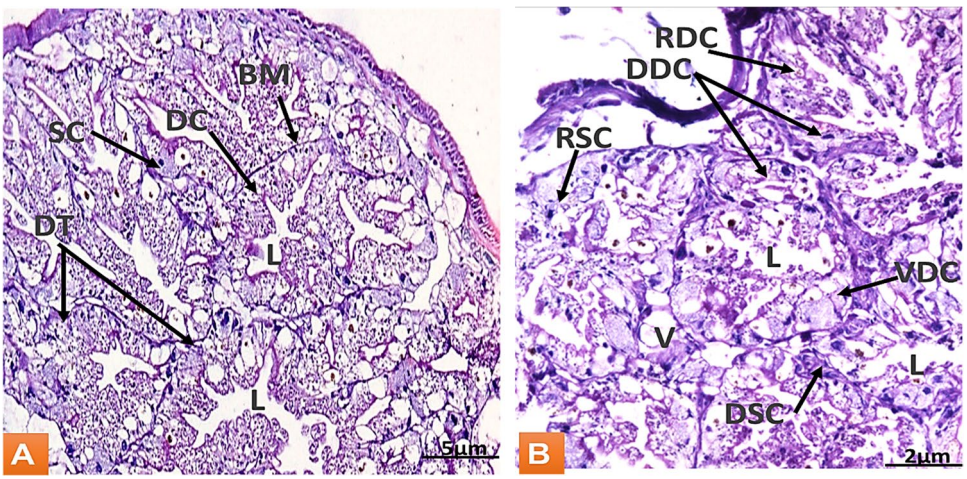

Fig. 6 **A** The digestive gland of untreated slugs (hematoxylin and eosin stain). Each digestive tubule (DT) is lined with digestive cells (DC) and secretory cells (SC) resting on a basement membrane (BM) and are arranged around a narrow lumen (L). **B** Sections in slug tissues under experiment show alterations in the histological architecture where many digestive cells were ruptured (RDC), vacuolated (VDC) or degenerated digestive cells (DDC). Also, the secretory cells were ruptured (RSC) or degenerated (DSC). Besides, vacuoles (V) and the lumen (L) inside tubules were increased

Also, this concentration of Pb resulted in some morphological alterations in the granulocytes and hyalinocytes. Granulocytes had numerous granules and vacuoles. The outer cell membrane was irregular with protrusions and blebbing. Hyalinocytes nuclei shrank and some had irregular outer membranes with blebs. Similarly, the hemocytes cytomorphology of *Lamellidens marginalis* was altered after exposure to lead (Pb) which led to the formation of membrane blebs, cytoplasm vacuolization, plasma membrane rupture, and degeneration of the nuclei of the hemocytes (Guria 2018). Blebbing is a toxicological phenomenon observed in single cells also in other mollusks under the influence of metals like Cd and Cu due to the disruption of energy metabolism accompanied by the impairment of the cellular actin filament skeleton (Manzl et al. 2004). The pseudopodia formation of the granulocyte is used to engulf the toxins or the pathogens after exposure (Ibrahim et al. 2022). (Penagos-Tabares et al. 2018) concluded that Adult slug species (*Arion lusitanicus*, *Limax maximus*) and giant snails (*Achatina fulica*) Giemsa-stained haemolymph smears revealed the presence of high numbers of intact hemocytes of two types; type I (small) haemocytes were more abundant than type II (large) hemocytes and reported that similarities exist between the innate immune system of vertebrates and invertebrates and these results were in good accordance with the present findings.

Exposure to metals causes deleterious health effects in all body organs (Abdel-Tawab et al. 2022). It disturbed steroidogenesis, hormonal regulation, and gametogenesis (Verma et al. 2018; Ibrahim et al. 2023a). The present results showed that both levels of testosterone and estradiol were significantly decreased ($p < 0.05$) after exposure to 0.027 µg/g compared with the control group. Hence, the metal pollution could disrupt the endocrine system (Luo et al. 2020). It was found that there was an association between metals and sex hormones in males (Kresovich et al. 2015).

Lead is a toxicant found in the environment that can cause oxidative stress (OS) through the production of reactive oxygen species (ROS), which has been identified as a key mechanism underlying lead toxicity. When the production of ROS surpasses the capacity of the antioxidant system to protect cells from oxidized molecules, OS arises leading to damage to the tissues and cells (Almeida Lopes et al. 2016). The alterations in MDA, SOD/CAT, and TAC levels could be used as biomarkers of metal pollution (Ibrahim et al. 2023a). The present results showed that exposure of slugs to 0.027 µg/g of Pb exhibited a significant increase in MDA, SOD, CAT, and TAC contents compared with the control group. (Atailia et al. 2016) studied the effect of several metals' exposure to the terrestrial land snail *Helix aspersa* and accordingly reported that CAT activity and MDA content were significantly higher in snails exposed to high concentrations of metal dust. Metal toxicity is linked to reactive oxygen production (ROS) in biological systems (Bakr et al. 2022). The defensive responses of the free radical scavenging system against ROS attacks are most important to decrease the damages that occurred (Nowakowska 2014).

Malonaldehyde is a product of lipid peroxidation and could be identified as an indicator of oxidative stress (Cordiano et al. 2023). Therefore, the high increase of MDA content might be due to the effect of Pb metal (Ibrahim and Sayed 2021). The enzymatic antioxidant system SOD/CAT is the first defense line against ROS (Ibrahim and Sayed 2019). The metals could result in oxidative stress in mollusks (Hussein et al. 2023). Bakr et al. (2022) reported that the land slugs (*Lehmannia nyctelia*) had significant increases in total lipid, lipid peroxidation (LPO), and DNA fragmentation after exposure to Ag NPs and AgNO3 for 15 days.

The present results showed that slugs exposed to 0.027 µg/g had many ruptured, vacuolated, or degenerated digestive or secretory cells. Besides, an increased number of vacuoles were present and the lumen inside tubules was increased. Atailia et al. (2016) reported an increase in the MDA level of the land snail *Helix aspersa* after exposure to several metals and linked these results with the great damage that occurs in the digestive gland of this snail (Abd El Mageed et al. 2023). Bakr et al. (2022) stated that silver nitrate $AgNO_3$ has harmful effects on the land slugs (*Lehmannia nyctelia*) after exposure for 15 days. It caused histopathological damage in the digestive glands, which can be considered the main cause of the death of these slugs. These histopathological damages might be due to the direct toxic effects of Pb on the edible organs of slugs (Abdel-Tawab et al. 2022).

Conclusion

Therefore, the non-essential metal lead (Pb) can accumulate in the slugs' tissues leading to disturbances in its physiological functions. Hence, *L. stuhlmanni* slug could be used as a sensitive bioindicator of metal pollution and reflect the effect of its pollution in soil.

Author contribution All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Amina M. Ibrahim and Soha A. Mobarak. The first draft of the manuscript was written by Amina M. Ibrahim and Soha A. Mobarak. Both authors commented on previous versions of the manuscript and approved the final one.

Data availability Data are available on request from the authors.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

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