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Impact of the Heavy Metals on Some Species of Freshwater Snails During Two Seasons on Al-Shaeir Island, Qalubia, Egypt

Hebat-Allah A. Dokmak[1, #*], Olfat A. Hammam[2], Khaled M. Zayed[1, #*]

[1]Medical Malacology Department, Theodor Bilharz Research Institute, Giza12411, Egypt
[2]Pathology Department, Theodor Bilharz Research Institute, Giza 12411, Egypt
[#]Same contribution as the first authors: Khaled M. Zayed, Hebat-Allah A. Dokmak
[*]**Corresponding Author: k.zayed@tbri.gov.eg**

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ABSTRACT

This study highlights significant heavy metal pollution on Al-Shaeir Island, Qalubia, Egypt, during 2022 and 2023 with levels exceeding international standards. The bioaccumulation of Fe, Mn, Pb, and Cd in five freshwater snail species (*Cleopatra ferruginea*, *Helisoma duryi*, *Lanistes carinatus*, *Physa acuta*, and *Theodoxus niloticus*) underscores the potential ecological risks. Selected for their bioindicator potential, these species provide crucial insights into heavy metal impacts on freshwater ecosystems, emphasizing the need for further monitoring and remediation efforts. Water analysis revealed metal concentrations exceeding FAO and USEPA standards, indicating significant pollution. Bioaccumulation factor (BAF) analysis showed species-specific variations, with *Physa acuta* exhibiting the highest accumulation levels. Elevated nitric oxide (NO) levels in all tested species suggest a physiological response to metal exposure. Increased ALT and AST enzyme activities correlate with higher BAFs, indicating potential liver toxicity. Histological examination revealed excessive lipofuscin accumulation in digestive glands, suggesting oxidative stress and cellular damage. Additionally, comet assay results confirmed significant DNA damage, highlighting the genotoxic effects of heavy metal contamination. These findings emphasize the role of freshwater snails as bioindicators and highlight the importance of environmental monitoring in polluted aquatic ecosystems to support sustainable development.

INTRODUCTION

Heavy metals pose a significant hazard because of their potential to bioaccumulate in aquatic ecosystems and organisms, persisting due to their resistance to biodegradation and long biological half-lives. Human activities are the primary sources of heavy metal contamination in water bodies **(El-Sayed *et al*., 2015)**.

Aquatic organisms accumulate heavy metals from both food and water sources. Factors such as dissolved oxygen levels, metal interactions, physicochemical conditions, and the presence of dissolved metals influence metal accumulation. In addition,

geographical variations, seasonal fluctuations, food availability, sediment attributes, and internal factors such as organism growth patterns impact accumulation **(Çougun *et al.*, 2006)**.

Snails, especially molluscs, are valuable for monitoring aquatic contamination due to their abundance and ease of collection across various habitats. Their limited mobility and larger size make them advantageous for biomonitoring, as they effectively accumulate pollutants such as heavy metals **(Lau *et al.*, 1998)**. **Vukašinović-Pešić *et al.* (2017)** emphasized the significant impact of heavy metals on freshwater snail tissues, highlighting the efficacy of species such as *V. mamillatus*, which is an efficient biomonitor tool for assessing iron (Fe), lead (Pb), and cadmium (Cd) levels in aquatic environments.

The toxicity, bioaccumulation potential, and persistence of heavy metals have raised significant concerns about their impact on aquatic ecosystem health **(Wang *et al.*, 2015; Che *et al.*, 2020)**. Even minimal quantities of heavy metals can be toxic to organisms, according to **Mehmood *et al.* (2019)**. Lead, mercury, and cadmium are particularly harmful due to their limited utilization by plants and animals, as noted by **Singh *et al.* (2018)**. **Pyatt *et al.* (2002)** reported adverse effects on the behavior of *Lymnaea stagnalis* in lead-contaminated water, with significant lead bioaccumulation in the stomach and buccal mass tissues of the snail.

Heavy metals induce the production of reactive oxygen species (ROS) in aquatic organisms, causing significant stress due to poor degradability and bioaccumulation tendencies **(Company *et al.*, 2004; Nunes, 2011; Bianco *et al.*, 2013)**. Molluscs, known for their resilience to toxic substances such as metals, serve as effective biomonitors **(Salànki *et al.*, 2003; Giarratan *et al.*, 2010; Mahajan, 2015; Rehman *et al.*, 2016)**. These metals can influence oxidative stress parameters in the hepatopancreas, such as protein carbonylation, malondialdehyde (MDA), and total protein levels.

In contrast, a control group of snails presented a significant decrease in liver enzyme levels despite slightly greater metal accumulation. As highlighted by **Zhang *et al.* (2019)**, the liver acts as the primary organ for detoxification in animals, playing crucial roles in both pro- and anti-inflammatory responses during toxin detoxification processes.

Advocate histological examinations are crucial tools for assessing the lasting impacts of water pollution and quantifying contamination levels **(Mohamed & Sabae 2015; Ibemenuga *et al.*, 2019)**. Alkaline single-cell gel electrophoresis (SCGE), also known as the "comet" assay, was pioneered by **Singh *et al.* (1988)**. It was buildt upon the initial 'neutral' version developed by **Östling and Johanson (1984)**. This cost-effective technique is pivotal in enhancing our understanding of pollutant-induced genotoxicity and its risks to various organisms.

This study evaluated the bioaccumulation of heavy metals in five freshwater snail species (*Cleopatra ferruginea, Helisoma duryi*, *Lanistes carinatus*, *Physa acuta,* and

Theodoxus niloticus) during the 2022 and 2023 seasons in the waters of Al-Shaeir Island, Qalubia, Egypt. It investigated bioaccumulation factors in snail tissues and their impact on nitric oxide (NO) levels, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) activities, along with histological changes in the digestive gland and DNA damage assessed through comet assay analysis.

MATERIALS AND METHODS

1. Physicochemical parameters

The physicochemical parameters at the water site on Al-Shaeir Island, Qalubia, Egypt, were measured using specialized instruments: a portable pH meter (Hanna Instruments HI 9024) for temperature and pH, a portable conductivity meter (Hanna Instruments HI 9635) for electrical conductivity (EC) and total dissolved solids (TDS), and a portable DO meter (Hanna Instruments HI 8543) for dissolved oxygen (DO) levels **(Jannat *et al*., 2019)**. Measurements were conducted at noon, 20cm below the water's surface.

1.1. Water samples

The water samples, which were collected following the **WHO (2011)** guidelines 50cm below the water surface and stored in dark polythene bottles, were 500ml/ each. To prevent heavy metal adhesion, 5ml of nitric acid was added to each sample after collection. In the laboratory, the samples were filtered through 50ml of Whatman No. 42 filter paper and a 0.45µm pore size syringe filter. The filtered samples were refrigerated until analysis. Heavy metal analysis (Fe, Cd, Mn, Pb, and Cd) was conducted via an atomic absorption spectrophotometer (GBC AVENTA) **(Abdel Kader *et al*., 2016)**.

2. Collection of freshwater snail species

Freshwater snail species, including *Cleopatra ferruginea, Helisoma duryi*, *Lanistes carinatus*, *Physa acuta*, and *Theodoxus niloticus*, were collected using a standard 300mm dip-net sieve in the study location (Fig. 1). The samples were placed in plastic cases with site data recorded and transferred to the lab for cleaning, sorting, and examination for natural parasite infections. Healthy, uninfected snails were kept in plastic aquaria (10 snails/L of field water) under controlled conditions at a constant temperature of 24± 1°C. Taxonomic classification followed **Hynes (1984)**, with identification confirmed by the Medical Malacology Department at Theodor Bilharz Research Institute, Imbaba, Giza, Egypt. For the control study, 200 uniformly sized snails from each species were collected from the site and maintained in a controlled environment, free from site pollution.

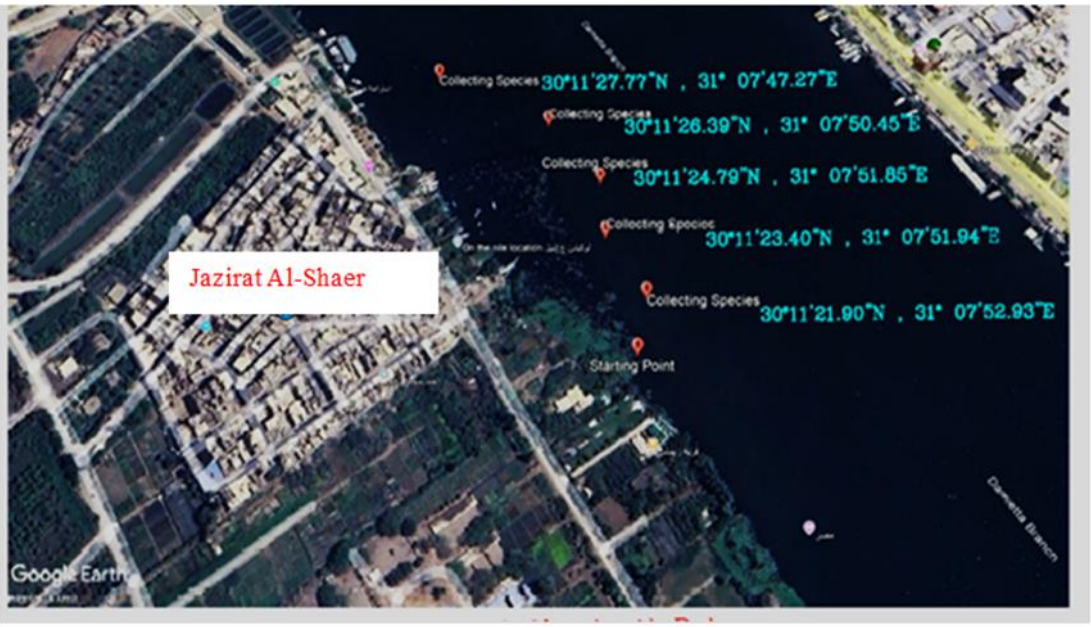


Fig. 1. Studying area of Al-Shaeir Island, Qalubia, Egypt, coordinate system (WGS 840)

3. Bioaccumulation of heavy metals

Snail samples from both the control group and field snails had their shells removed, and the tissues were dried at 80°C before being weighed. Each tissue sample was then digested with 1ml of concentrated nitric acid (HNO3) at 70°C for 2 hours, followed by dilution with 5ml of ultrapure deionized water for heavy metal analysis **(Federici *et al.*, 2007)**. The concentrations of heavy metals, including Fe, Mn, Pb, and Cd, in the digested tissue samples were measured using an atomic absorption spectrophotometer (GBC AVANTA™). The bioaccumulation factor (BAF) was calculated using the formula: BAF = concentration of the metal in snail tissues (mg/g or µg/g dry weight) / concentration of the metal in water (mg/L). According to **Ávila *et al.* (2017)**, BAF values below 1 indicate no contamination, values between 1 and 10 suggest tolerance, and values above 10 imply hyperaccumulation.

4. Biochemical analysis

The tissues were weighed and homogenized via a UP 200H ultrasonic processor with 1g of tissue in 2ml of dechlorinated water. The suspension was subsequently centrifuged at 25°C for 45 minutes at 4000rpm. The fresh supernatant was used for biochemical tests, and the pellet was discarded following the protocol of **El-Khayat and Abu-Zikri (2004)**.

4.1. Nitric oxide (NO) measurement

The concentration of nitric oxide (NO) in the tissue homogenate of freshwater snails was measured via a colorimetric NO kit (Biodiagnostic Company, Dokki, Giza, Egypt; Cat. No. GR 2511) following the method of **Montgomery and Dymock (1962)**.

4.2. Enzyme activities

In this study, the enzyme activities of ALT and AST in freshwater snail homogenate tissues were assessed. Three replicates were prepared for each species, with ten snails per liter. The soft tissues were separated from the shells, and aminotransferase activities for aspartate (AST) and alanine (ALT) were determined following the methods of **White *et al*. (1970)**. The chemicals for these assays were obtained from QCA Ltd., Spain.

5. Histological investigationof the digestive glands of freshwater snail species

A study investigated the impact of heavy metals on freshwater snail species through histological examination of the digestive gland. The digestive glands were removed, preserved in Bouin's solution for 24 hours, and then dehydrated in a series of ethanol concentrations. After being cleaned in xylene, the tissues were embedded in melted wax and sectioned at 5µm using a rotary microtome. The sections were stained with Delafield's hematoxylin and eosin, mounted with DPX, and examined via a Carl Zeiss microscope **(El-Nahas & El-Deeb, 2007)**.

6. Assessment of genotoxicity via the comet assay

A study on DNA damage was conducted in both the control group and bioaccumulated snails from the study site, drawing insights from the works of **Singh *et al*. (1988)** and **Grazeffe *et al*. (2008)**.

7. Statistical analysis

For the statistical analysis, the standard deviation (SD) or mean ± SD was used to represent the biochemical parameters. The procedure outlined by **Sokal and Rohlf (1995)** was followed, utilizing the Student's t-test to determine significant differences between the control and field snail groups. A 95% confidence level was applied as the threshold for statistical significance, with $P< 0.05$ indicating significance and $P< 0.001$ indicating high significance.

RESULTS

Physicochemical parameters of the examined water samples

In Table (1), the mean levels of temperature, pH, dissolved oxygen, electrical conductivity (EC), and total dissolved solids (TDS) in the water from Al-Shaeir Island, Qalubia, Egypt, during the 2022 and 2023 seasons are presented, alongside the permissible heavy metal levels set by the FAO and USEPA.

The recorded environmental parameters are as follows: temperature 30.56 ± 0.053°C, pH 6.36 ± 0.053, dissolved oxygen 9.89 ± 0mg/ L, electrical conductivity (EC) 847.44 ± 0.90µS, and total dissolved solids (TDS) 239.2 ± 0.16mg/ L.

The concentrations of heavy metals detected include iron (Fe) at 12.53 ± 0.27 mg/L, manganese (Mn) at 13.72 ± 0.26 mg/L, lead (Pb) at 11.7 ± 1.78 mg/L, and cadmium (Cd) at 12.86 ± 1.62 µg/g.

These values are compared against the allowable limits established by the Food and Agriculture Organization (FAO) and the United States Environmental Protection Agency (USEPA), as presented in Table (1).

Table 1. Physico-chemical parameters of water samples collected from Al-Shaeir Island, Qalubia, Egypt, during the 2022 and 2023 seasons

Physical parameters of examined water site		**Allowable levels, as outlined by the FAO and USEPA**
Parameter	**Concentration**	
Temperature (◦C)	30.56 ±0.053	25 ◦C
pH	6.36±0.053	6.5–8.4
D.O (mg/l)	9.89 ± 0	≥5 mg/l
TDS (mg /l)	239.2 ±0.16	< 450
Electric Conductivity EC (µmhos/cm)	847.44 ±0.90	<3 m mhos/cm
(Fe) (mg /l)	12.53 ±0.27	0.5–50 (mg /l)
(Mn) (mg/l)	13.72 ±0.26	0.5 (mg /l)
(Pb) (mg/ l)	11.7 ±1.78	0.05 (mg/ l)
(Cd) (µg/g)	12.86 ±1.62	0.00025 (µg/g)

Bioaccumulation of the heavy metals iron (Fe), manganese (Mn), lead (Pb), and cadmium (Cd) in the dried tissues of selected snail species

The data presented in Table (2) and Fig. (2A) reveal varying concentrations (mg/g) of Fe in the dried tissues of the freshwater snail species under study. *Theodoxus niloticus* presented the highest concentration at 492.30 ± 0.14mg/ g, significantly exceeding the control group's Fe level of 154.52 ± 0.01mg/ g ($P< 0.001$). Similarly, *Physa acuta* had a concentration of 480.42 ± 0.01mg/ g, whereas the control group had a value of 413.73 ± 10.5mg/ g. Additionally, *Helisoma duryi*, *Lanistes carinatus*, and *Cleopatra ferruginea* presented distinct Fe levels, with *Helisoma duryi* at 386.71 ± 0.01mg/ g, *Lanistes carinatus* at 380.82 ± 0.05mg/ g, and *Cleopatra ferruginea* at 108.31 ± 0.09mg/ g. These values were significantly greater than those of the control group ($P< 0.001$). On the other hand, Table (2) and Fig. (2B) depict the accumulation of the heavy metal Mn in dried tissues, with a focus on various species. *Physa acuta* had a significantly greater concentration (410.36 ± 0.01mg/ g) than did the control group (156.40 ± 0.46mg/ g) ($P< 0.001$). Compared with the control, the concentration of *Cleopatra ferruginea* increased markedly to 404.49 ± 0.005mg/ g, with a value of 268.3087 ± 0.324mg/ g. *Helisoma duryi* was 369.75 ± 0.02mg/ g, whereas it was 297.5937 ± 0.889mg/ g in the control group. *Lanistes carinatus* compared with that of the control, the content of the former significantly increased to 115.6737 ± 0.106mg/ g, whereas that of the former was 91.035 ± 0.0096mg/ g, while *Theodoxus niloticus* was 144.641 ± 0.265mg/ g, Compared to 97.765 ± 0.103mg/ g in the control group. These results highlight significant variations in Mn concentrations in the tissues of these snail species compared with their respective control groups ($P< 0.001$).

The dried tissues of freshwater snails collected from Al-Shaeir Island, Qalubia, Egypt, in this study presented notable differences in lead (Pb) accumulation (µg/g) compared with those of the control groups, as detailed in Table (1) and Fig. (2C). *Helisoma duryi* presented a significant increase in lead accumulation of 449.17 ± 0.05µg/ g, which surpassed that of the control group (254.44 ± 0.02µg/ g; $P< 0.001$). *Physa acuta* presented lead accumulation of 343.12 ± 0.01µg/ g in dried tissues, which contrasted with that of the control group (92.21 ± 0.009µg/ g; $P< 0.001$). The lead accumulation of *Cleopatra ferruginea* was 259.73 ± 0.005µg/ g, whereas that of the control *Cleopatra ferruginea* species was 182.40 ± 0.005µg/ g. *Theodoxus niloticus* and *Lanistes carinatus* presented lead accumulations of 212.3 ± 0.092µg/ g and 208.1333 ± 0.0282µg/ g, respectively, compared with 87.006 ± 0.002µg/ g and 144.24 ± 0.03µg/ g in their respective control groups ($P< 0.001$).

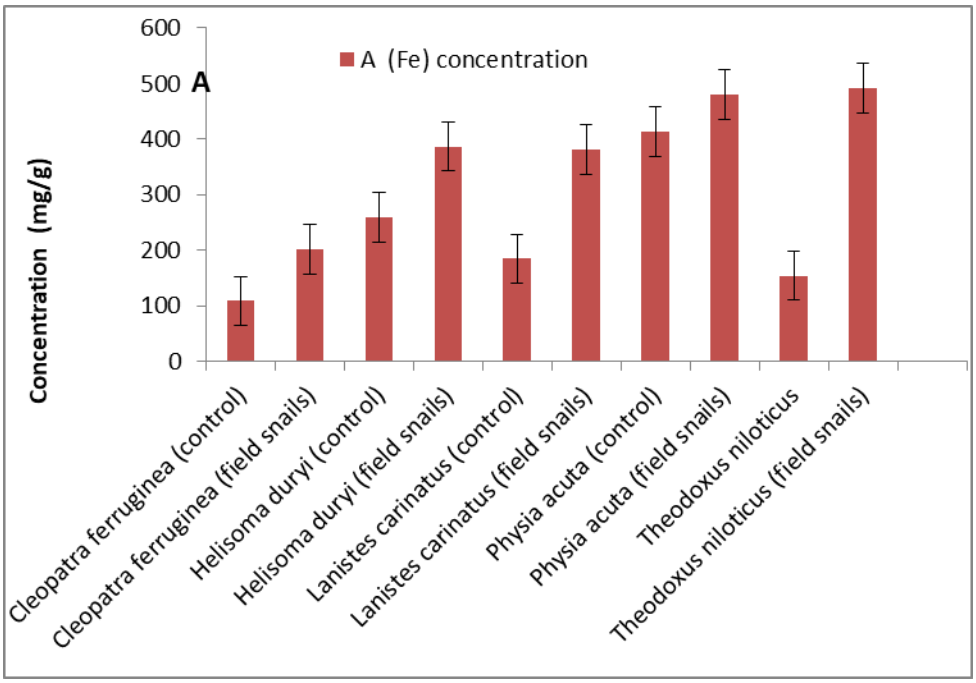
One of the heavy metals considered to be the most harmful to aquatic organisms is cadmium (Cd). Table (1) and Fig. (2D) present the accumulation of cadmium in the dried tissues of freshwater snail species collected in the present study. *Physa acuta* recorded the highest significant value, 500.3 ± 0.16µg/ g, compared with 346.6 ± 0.16µg/ g in the control group. *Cleopatra ferruginea* living in polluted water presented a highly significant value of 387.02 ± 0.0002µg/ g compared with 299.008 ± 0.0002µg/ g in the control group ($P< 0.001$).

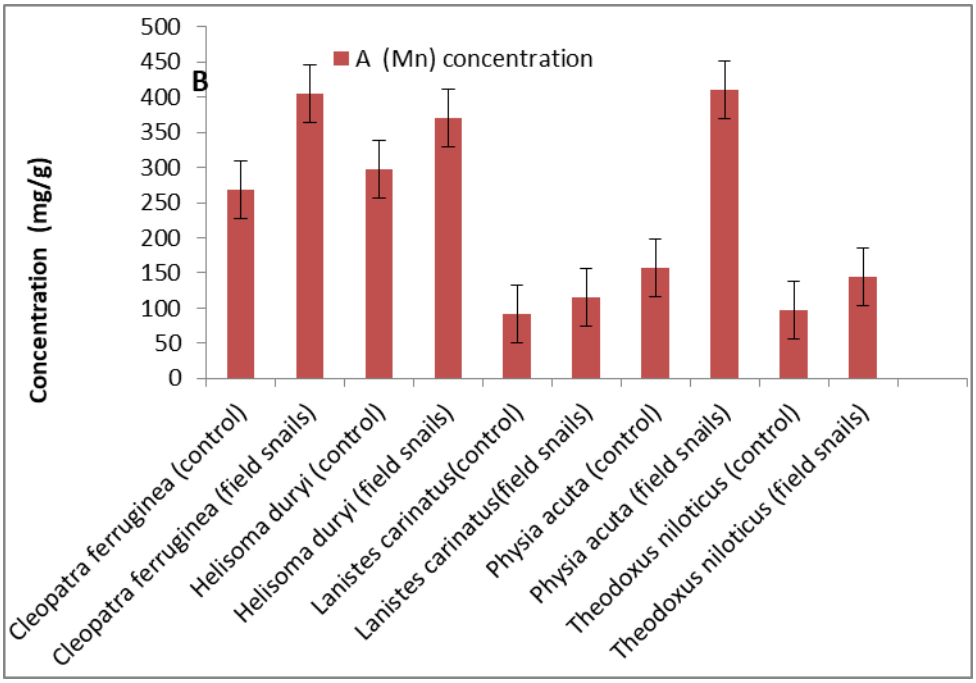
Theodoxus niloticus collected from the same polluted site presented a highly significant value of 335.6 ± 0.1µg/ g compared with 243.2 ± 0.1µg/ g in the control group, where the water was changed weekly. *Helisoma duryi* and *Lanistes carinatus* showed cadmium accumulation in their dried tissues, with values of 305.4133 ± 0.0141µg/ g compared with 190.4333 ± 0.0533µg/ g and 129.0133 ± 0.0213µg/ g compared with 83.0033 ± 0.00533µg/ g in the control group ($P< 0.001$).

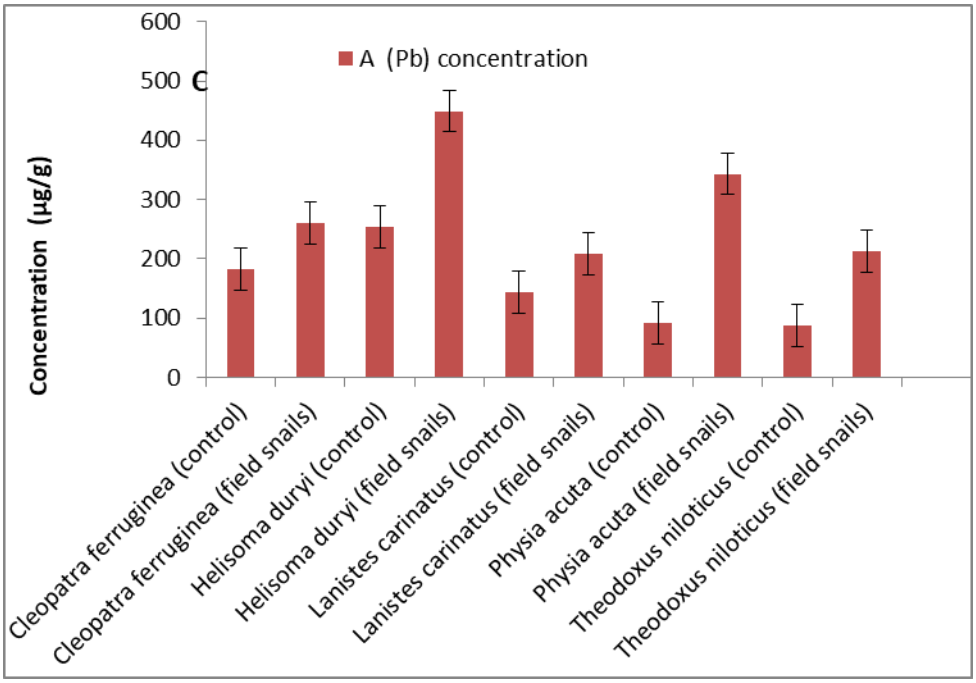
Table 2. Bioaccumulation of iron (Fe), manganese (Mn), lead (Pb), and cadmium (Cd) in dried tissues of freshwater snail species from Al-Shaeir Island, Qalubia, Egypt, during the 2022 and 2023 seasons

Species of fresh water snails	Fe (mg/g)	Mn (mg/g)	Pb (mg/g)	Cd (µg/g)
***Cleopatra ferruginea* (control)**	108.31 ±0.09	268.30 ±0.003	182.40 ±0.005	299.008 ±0.02
***Cleopatra ferruginea* (field snails)**	202.35 ±0.05	404.49 ±0.005	259.73 ±0.005	387.02±0.02
***Helisoma duryi* (control)**	259.34 ±0.005	297.5937 ±0.889	254.44 ±0.02	190.43 ±0.05
***Helisoma duryi* (field snails)**	386.71 ±0.01	369.75 ±0.02	449.17 ±0.05	305.41±0.01
***Lanistes carinatus* (control)**	184.81 ±0.004	91.035 ±0.0096	144.24 ±0.03	83.003 ±0.005
***Lanistes carinatus* (field snails)**	380.82 ±0.05	115.67 ±0.106	208.13 ±0.02	129.01 ±0.02

Physia acuta (control)	413.73 ±10.5	156.403 ±0.467	92.21 ±0.009	346.6 ±0.16
***Physia acuta* (field snails)**	480.42 ±0.01	410.36 ±0.01	343.12 ±0.01	500.3 ±0.16
Theodoxus niloticus (control)	154.52 ±0.01	97.765 ±0.1036	87.006 ±0.002	243.2 ±0.16
***Theodoxus niloticus* (field snails l)**	492.30 ±0.14	144.641 ±0.265	212.3 ±0.09	335.6 ±0.16







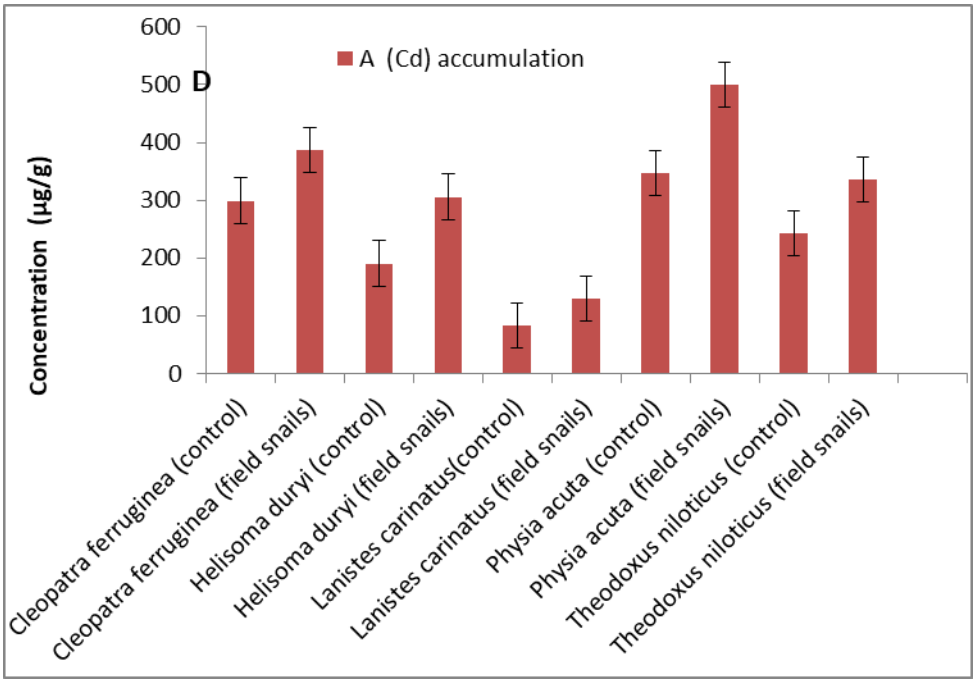


Fig. 2. Bioaccumulation of heavy metals in dried tissues of freshwater snail species: (**A)** Iron (Fe), **(B)** Manganese (Mn), **(C)** Lead (Pb), and **(D)** Cadmium (Cd)

The results in Table (3) and Figs. (3A, B, C and D) show high significant variations in BAF among freshwater snail species. The BAF in response to Fe (Fig. 3A) was as follows: 39.27 ± 0.01 for *Theodoxus niloticus* and 12.32 ± 0.01 for the control, *Physa*

acuta at 38.33 ± 0.01, compared with the control at 33.04 ±0.03; *Helisoma duryi* at 30.66 ± 0.1, compared with the control at 20.64 ± 0.04; and *Lanistes carinatus* with a BAF of 30.33 ± 0.04, compared with the control at 14.73 ± 0.01. *Cleopatra ferruginea* also presented notable results, with a BAF of 16.15 ± 0.02, whereas that of the control was 8.65 ± 0.01 ($P< 0.001$).

The bioaccumulation factors (BAFs) for manganese (Mn) (Fig. 3B) in the five tested freshwater snails were recorded. The *Physa acuta* group presented the highest BAF at 29.88 ± 0.01, whereas the BAF of the control group was 11.37 ± 0.01. This was followed by *Cleopatra ferruginea*, whose BAF was 29.47 ± 0.01, whereas it was 19.54 ± 0.01 in the control group. *Helisoma duryi* had a BAF of 26.92 ± 0.01, whereas the BAF of *Lanistes carinatus* was 21.63 ± 0.04 in the control group. It had a BAF of 8.42 ± 0.005, whereas it was 6.62 ± 0.005 in the control group ($P< 0.001$).

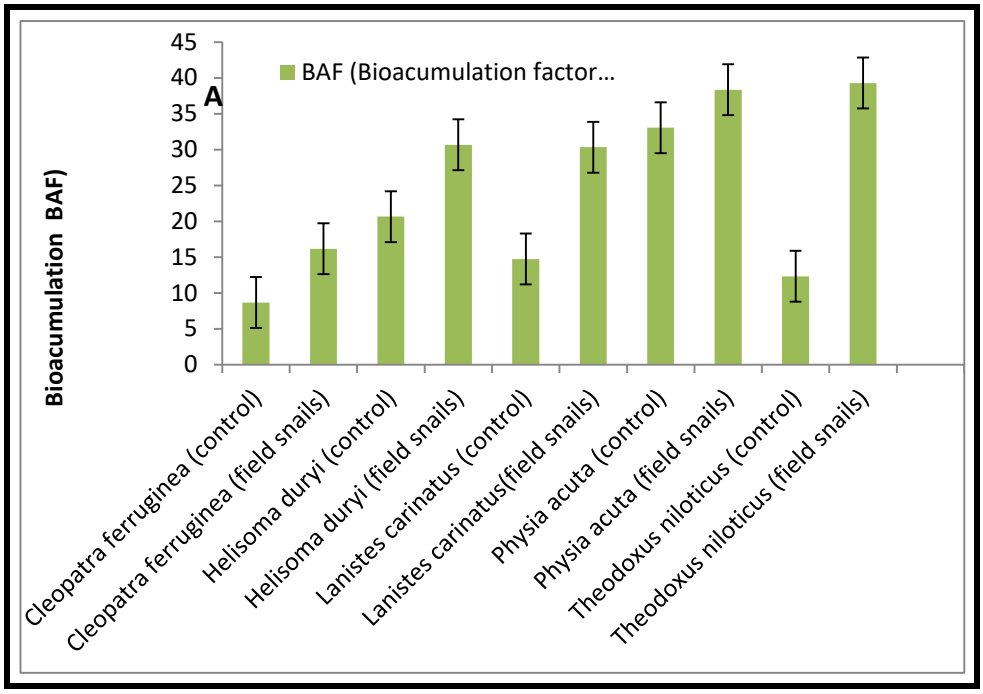
The factors associated with the bioaccumulation of lead (Pb, Fig. 3C) in the freshwater snails were also recorded. *Helisoma duryi* presented the highest bioaccumulation factor at 38.85 ± 0.03, whereas it was 20.94 ± 0.03 in the control group, followed by *Physa acuta* at 29.31 ± 0.01, compared with 7.87 ± 0.005 in the control group, and *Cleopatra ferruginea* at 22.17 ± 0.01, compared with 15.57 ± 0.009 in the control group. In contrast, *Lanistes carinatus* and *Theodoxus niloticus* presented BAFs of 17.76 ± 0.01 and 18.15 ± 0.02, respectively, whereas the corresponding control groups presented BAFs of 12.33 ± 0.02 and 7.44 ± 0.01.

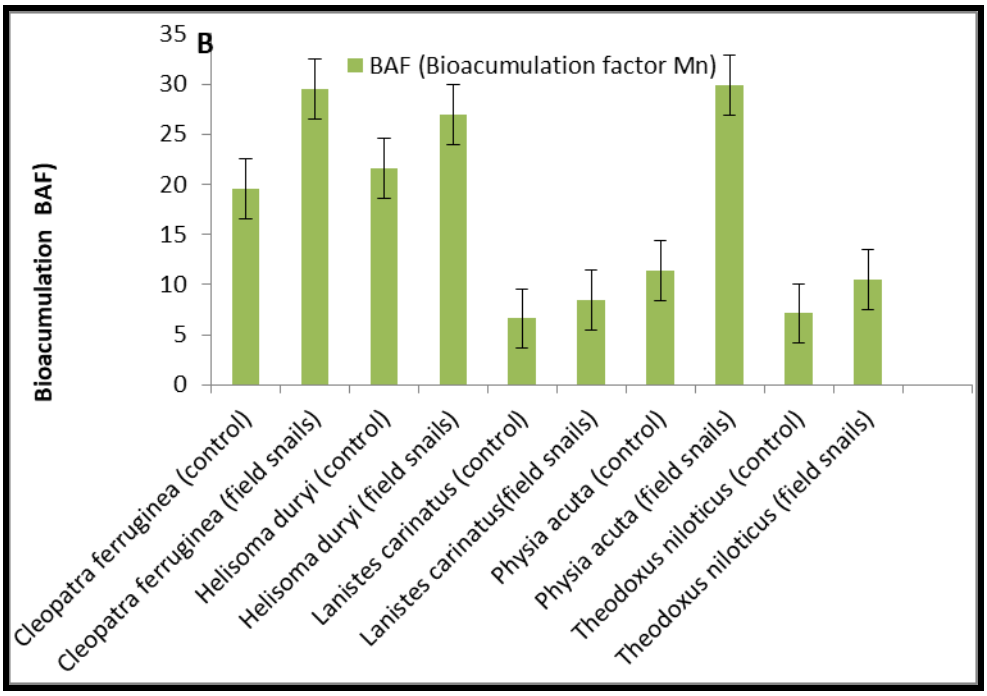
The bioaccumulation factors for cadmium (Cd, Fig. 3D), which represent the ratio of the cadmium concentration in freshwater snails or biota to the concentration in water, are presented in Table (3). *Physa acuta* reported the highest BAF (38.76 ± 0.2), followed by *Cleopatra ferruginea* (30.06 ± 0.005), *Theodoxus niloticus* (26.05 ± 0.03), *Helisoma duryi* (23.72 ± 0.01), and *Lanistes carinatus* (10.03 ± 0.009), compared with those of the control groups for each tested species, where the water was changed weekly ($P< 0.001$).

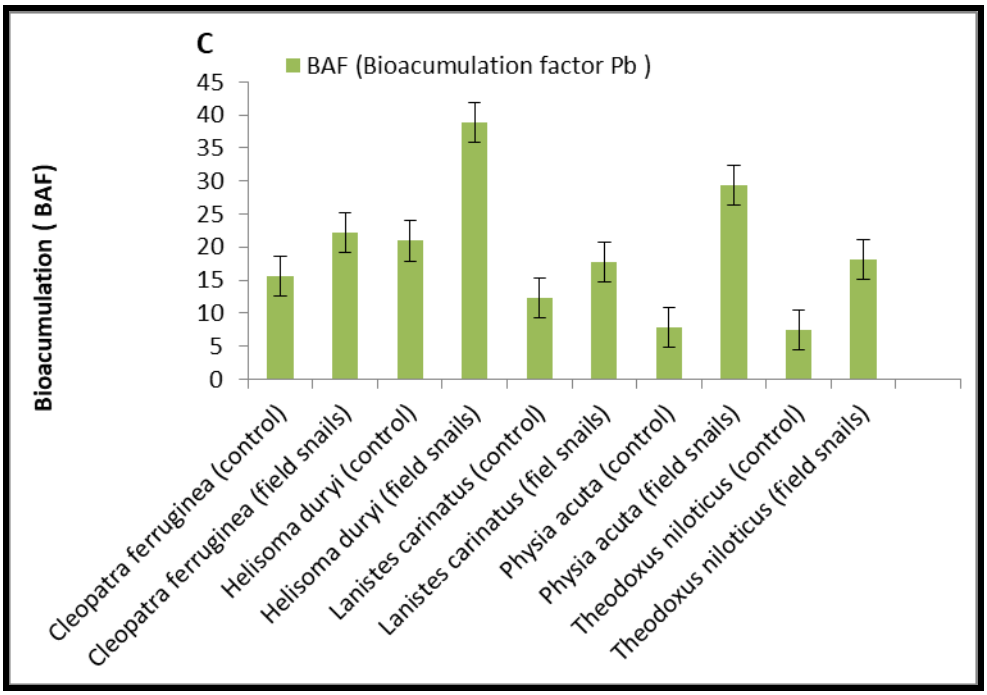
Table 3. Bioaccumulation factor of heavy metals (Iron (Fe), manganese (Mn), lead (Pb), and cadmium (Cd)) in dried tissues of freshwater snails from Al-Shaeir Island, Qalubia, Egypt, during the 2022 and 2023 seasons

Species of fresh water snails	(BAF) Fe	(BAF) Mn	(BAF) Pb	(BAF) Cd
Cleopatra erruginea (control)	8.65 ±0.01	19.54 ±0.01	15.57 ±0.009	23.23 ±0.01
Cleopatra ferruginea (field snails)	16.15 ±0.02	29.47 ±0.01	22.17 ±0.01	30.06 ±0.005
Helisoma duryi (control)	20.64 ±0.04	21.63 ±0.04	20.94 ±0.03	14.82 ±0.01
Helisoma duryi (field snailsl)	30.66 ±0.1	29.47 ±0.01	38.85 ±0.03	23.72 ±0.01
Lanistes carinatus (control)	14.73 ±0.01	6.62 ±0.005	12.33 ±0.02	6.44 ±0.005

Lanistes carinatus (field snails)	30.33 ±0.04	8.42 ±0.005	17.76 ±0.01	10.03 ±0.009
Physia acuta (control)	33.04 ±0.03	11.37 ±0.01	7.87 ±0.005	26.92 ±0.02
Physia acuta (field snails)	38.33 ±0.01	29.88 ±0.01	29.31 ±0.01	38.76 ±0.2
Theodoxus niloticus (control)	12.32 ±0.01	7.12 ±0.01	7.44 ±0.01	18.92 ±0.01
Theodoxus niloticus (field snails)	39.27 ±0.01	10.53 ±0.01	18.15 ±0.02	26.05 ±0.03




B
BAF (Bioacumulation factor Mn)
Bioacumulation BAF)
35
30
25
20
15
10
5
0
Cleopatra ferruginea (control)
Cleopatra ferruginea (field snails)
Helisoma duryi (control)
Helisoma duryi (field snails)
Lanistes carinatus (control)
Lanistes carinatus(field snails)
Physia acuta (control)
Physia acuta (field snails)
Theodoxus niloticus (control)
Theodoxus niloticus (field snails)


C
BAF (Bioacumulation factor Pb)
Bioacumulation (BAF)
45
40
35
30
25
20
15
10
5
0
Cleopatra ferruginea (control)
Cleopatra ferruginea (field snails)
Helisoma duryi (control)
Helisoma duryi (field snails)
Lanistes carinatus (control)
Lanistes carinatus (fiel snails)
Physia acuta (control)
Physia acuta (field snails)
Theodoxus niloticus (control)
Theodoxus niloticus (field snails)

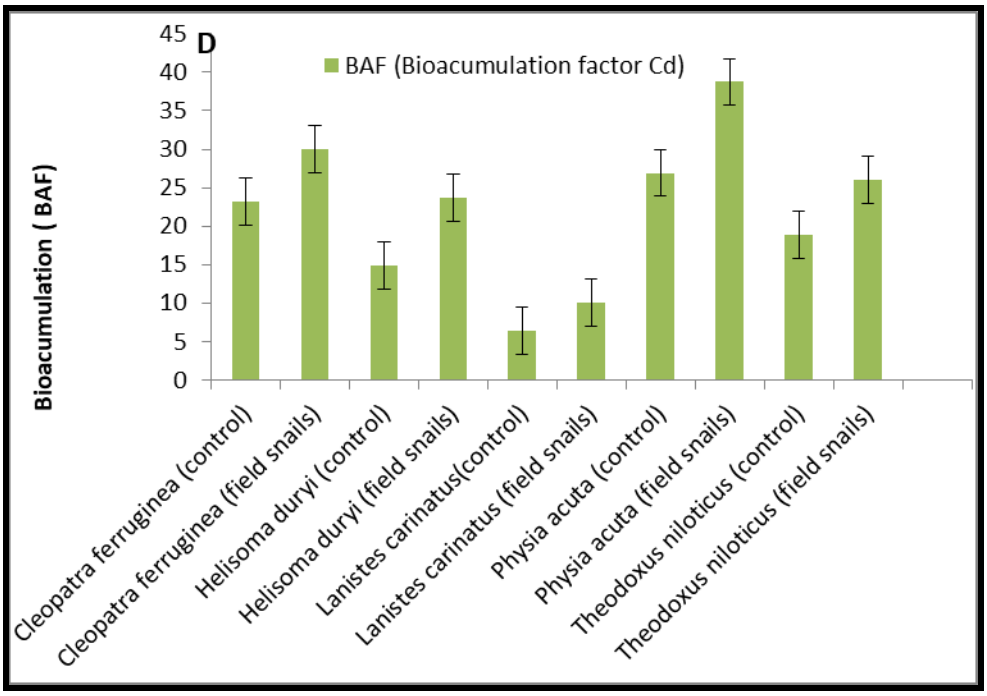


Fig. 3. Bioaccumulation factor (BAF) in freshwater snail species for **(A)** Iron (Fe), **(B)** Manganese (Mn), **(C)** Lead (Pb), and **(D)** Cadmium (Cd)

Impact of heavy metals on nitrogen oxide (NO) levels in the homogenized tissues of fresh water snails

The data presented in Table (4) and Fig. (4A) indicate a significant difference in nitric oxide (NO) levels in freshwater snails subjected to bioaccumulation of heavy metals (Fe, Mn, Pb, and Cd) in the homogenized tissues compared with those in the control group for each species ($P > 0.001$). In *Theodoxus niloticus*, a highly significant increase was observed between samples collected from the test site and those from the control group, with levels recorded at 12.2 ± 0.16nmol/ L compared with 8.2 ± 0.16nmol/ L in the control group. *Lanistes carinatus* and *Physa acuta* presented levels of 7.8 ± 0.05nmol/ L compared with 3.53 ± 0.05nmol/ L and 7.46 ± 0.10nmol/ L compared with 3.56 ± 0.05nmol/ L in the control. *Helisoma duryi* presented NO levels of 6.8 ± 0.05nmol/ L compared with 6.16 ± 0.10nmol/ L in the control *Helisoma duryi* group. Additionally, the concentration of *Cleopatra ferruginea* collected from the test site was 3.73 ± 0.05nmol/ L, whereas the concentration of *Cleopatra ferruginea* collected from the control site was 2.7 ± 0.26nmol/ L.

Table 4. Impact of heavy metals bioaccumulation on nitrogen oxide (NO) (Nmol/l) in homogenized tissues of freshwater snail species from Al-Shaeir Island, Qalubia, Egypt, during the 2022 and 2023 seasons

Freshwater snail species	**(NO) nmol/l**
Cleopatra ferruginea (control)	**2.7±0.26**
Cleopatra ferruginea (field snails)	**3.73 ±0.05**
Helisoma duryi (control)	**6.8±0.05**
Hilesoma duryi (field snails)	**7.8±0.05**
Lanistes carinatus (control)	**3.53±0.05**
Lanistes carinatus (field snails)	**6.16±0.10**
Physia acutan (control)	**3.56±0.05**
Physia acuta (field snails)	**7.46±0.10**
Theodoxus niloticus (control)	**8.2±0.16**
Theodoxus niloticus (field snails)	**12.2±0.16**

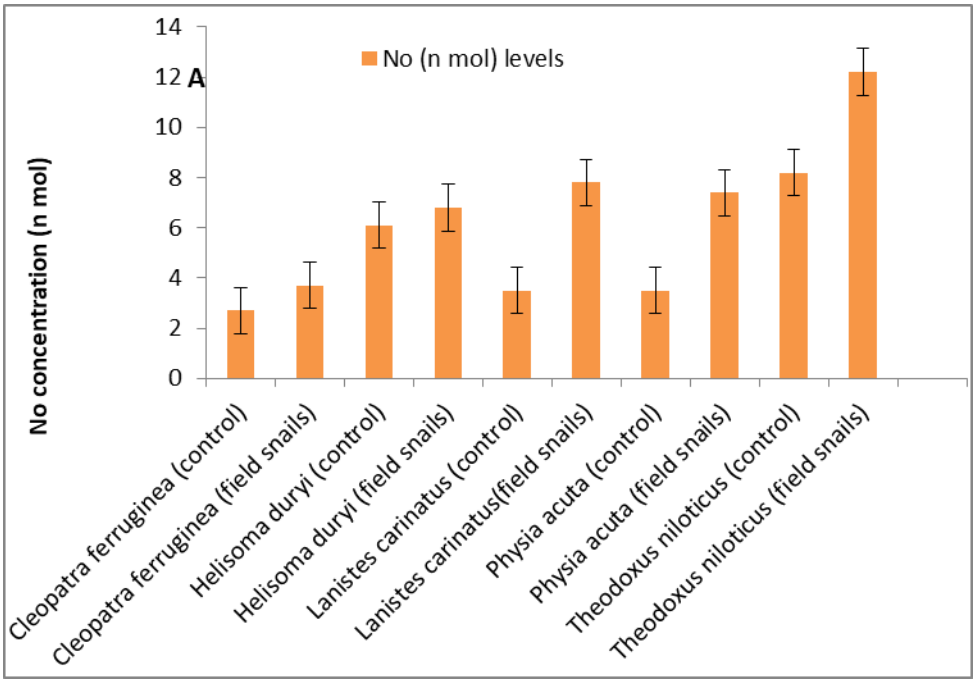


Fig. 4. Nitrogen oxide (NO) levels in bioaccumulated tissues with tested heavy metals in freshwater snail species

Impact of heavy metal bioaccumulation on ALT and AST enzyme activities in freshwater snails from Al-Shaeir Island, Qalubia, Egypt, during the 2022 and 2023 seasons

Table (5) and Fig. (5A) clearly demonstrate that snails with elevated bioaccumulation factors (BAFs) presented significantly greater ALT and AST enzymes activities ($P<$ 0.001) than did the control snails because of the increased heavy metal content in their

homogenized tissues. Specifically, in the homogenized tissues of the *Theodoxus niloticus* snail species, the recorded values for these enzymes were 148.33 ± 0.5 and 136.6667 ± 0.533U/ g tissue for the ALT and AST enzymes, respectively, whereas the values were 102 ± 1.6 and 98.66 ± 0.5U/ g tissue in the control group. In the case of *Lanistes carinatus*, the enzyme activities of the ALT and AST enzymes were 93.33 ± 5.3 and 87.33 ± 0.5U/ g tissue, respectively, whereas those of the control group were 41.33 ± 0.5 and 37.66 ± 0.5, respectively. For *Physa acuta*, the ALT and AST enzyme activities were 88.3333 ± 0.533 and 76.33 ± 0.5U/ g tissue, respectively, whereas in the control group, the enzyme activities were 35.33 ± 0.533 and 30.33 ± 0.5U/ g tissue, respectively. *Helisoma duryi* exhibited ALT and AST enzyme activities of 73.33 ± 0.5U/ g and 70.66 ± 1.06U/ g, respectively, compared with 64.33 ± 0.5 and 62.33 ± 0.5, respectively, in the control group. For *Cleopatra ferruginea*, the data in Table (5) indicate that the ALT and AST enzyme activities were 25.66 ± 0.5 and 28.66 ± 0.5U/ g tissue, respectively, whereas they were 24.33 ± 0.5 and 22.61 ± 0.5U/ g tissue, respectively, in the control group.

Table 5. Impact of heavy metal bioaccumulation on enzyme activities (ALT and AST) (U/g tissue) in homogenized tissues of freshwater snail species from Al-Shaeir Island, Qalubia, Egypt, during the 2022 and 2023 seasons

Freshwater snail tissues	ALT (U/g tissues)	AST (U/g tissues)
***Cleopatra ferruginea* (control)**	24.33 ±0.5	22.61 ±0.5
***Cleopatra ferruginea* (field snails)**	25.66 ±0.5	28.6667 ±0.5
***Helisoma duryi* (control)**	64.33 ±0.5	62.33 ±0.5
***Hilesoma duryi* (field snails)**	73.33 ±0.5	70.66 ±1.05
***Lanistes carinatus* (control)**	41.33 ±0.5	37.66 ±0.5
***Lanistes carinatus* (field snails)**	93.33 ±5.3	87.33 ±0.5
***Physia acuta* (control)**	35.33 ±0.5	30.33 ±0.5
***Physia acuta* (field snails)**	88.3333 ±0.5	76.3333 ±0.533
***Theodoxus niloticus* (control)**	102 ±1.6	98.66 ±0.5
***Theodoxus niloticus* (field snails)**	148.33 ±0.5	136.66 ±0.5

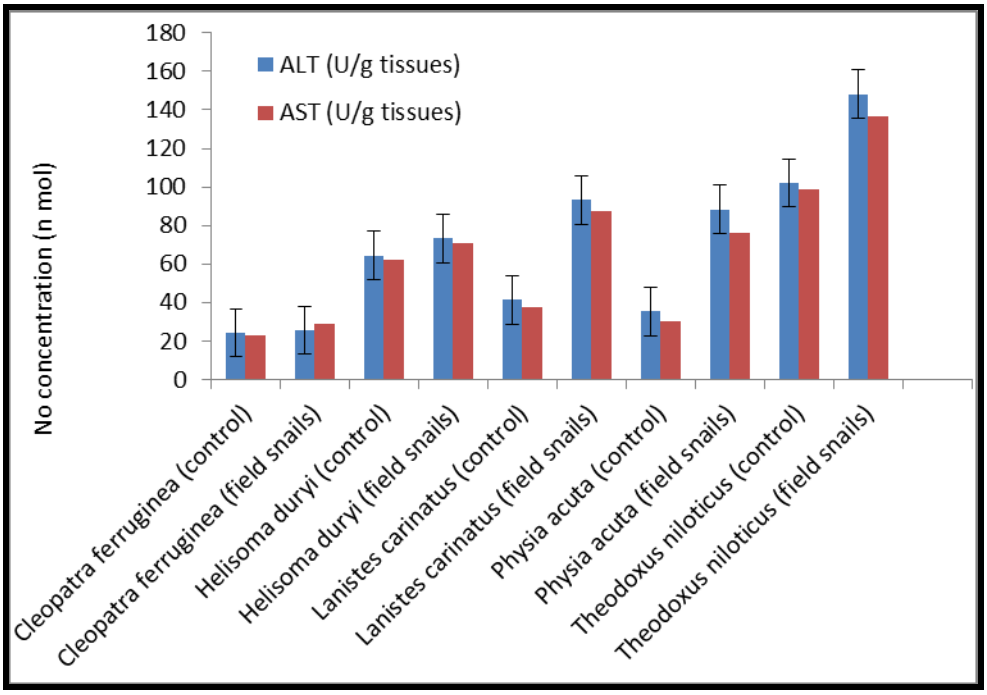


Fig. 5. Enzyme activity levels of ALT and AST levels in bioaccumulated tissues with tested heavy metals in freshwater snail species

Histological examination of digestive gland in freshwater snails: *Cleopatra* sp., *Helisoma* sp., *Lanistes* sp., *Physa* sp., and *Theodoxus sp*.

Histological examination of the digestive gland from each of the freshwater snails *Cleopatra ferruginea*, *Helisoma duryi*, *Lanistes carinatus*, *Physa acuta*, and *Theodoxus niloticus* was performed on both the collected field snail species and the control group. Under controlled conditions, the histology of the digestive gland revealed columnar epithelial cells and secretory cells arranged on basement membranes along each tubule. The lumens within the digestive tubules appeared constricted, with a sparse layer of connective tissue filling the interlobular spaces (illustrated in Figs. 6, 7, and 8). A noticeable excessive accumulation of lipofuscin within the cells was observed compared to the control group. This surplus lipofuscin has the potential to disrupt cellular functions, impede metabolic processes, and act as an indicator of oxidative stress and cellular damage in each snail species (B, D, F, and H in the respective species of *Cleopatra ferruginea*, *Helisoma duryi*, *Lanistes carinatus*, *Physa acuta*, and *Theodoxus niloticus*).

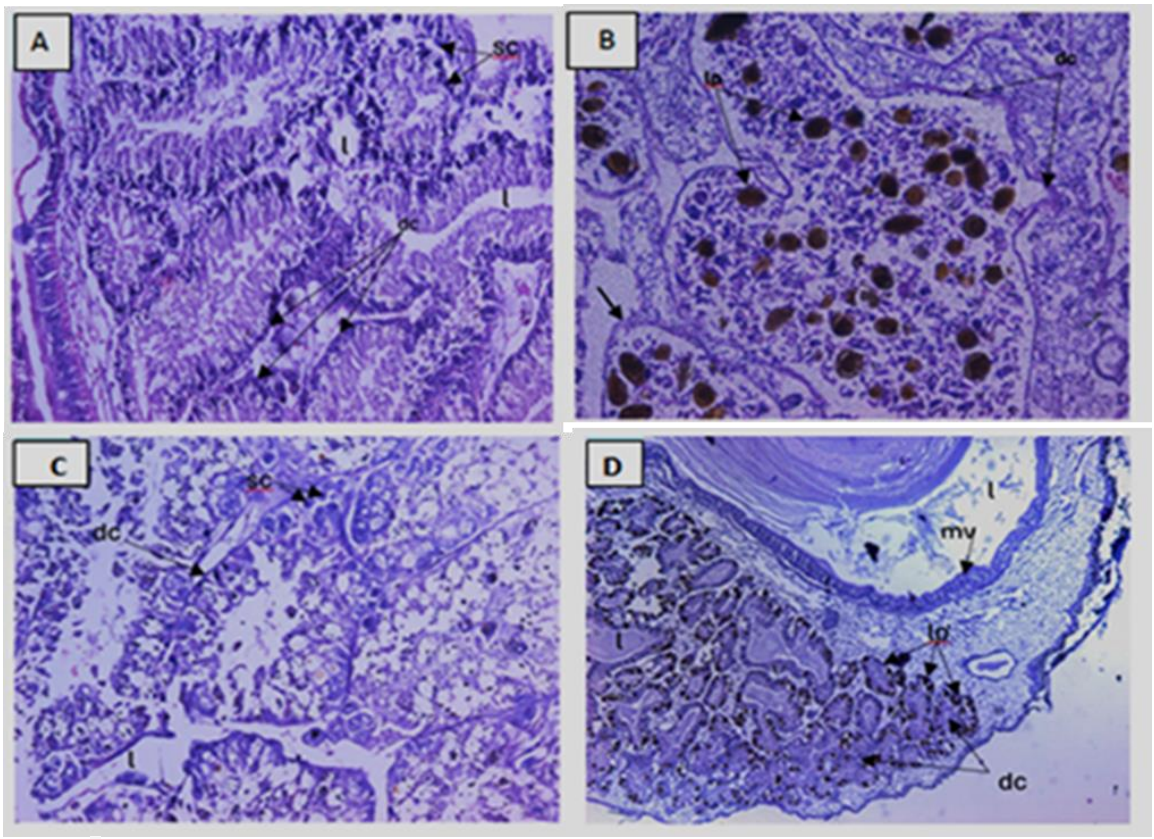


Fig. 6. (A) Light micrograph digestive tissue in *Cleopatra ferruginea*, (control*)* showing normal digestive cells (dc), secretory cells (sc), and the lumen (l). (H&E x100, x200, x400). **(B)** Light micrograph of digestive tissue of *Cleopatra ferruginea* (collected snail), showing digestive cells (dc) with a marked accumulation of lipofuscin (lp). (H&E x100, x200, x400). **(C)** Control image of *Helisoma duryi*, showing digestive cells (dc), secretory cells (sc), and the lumen (l) (H&E x100, x200, x400). **(D)** Light micrograph of digestive tissue in *Helisoma duryi* (collected snail), showing digestive cells (dc) with a marked accumulation of lipofuscin (lp) and microvilli (mv)

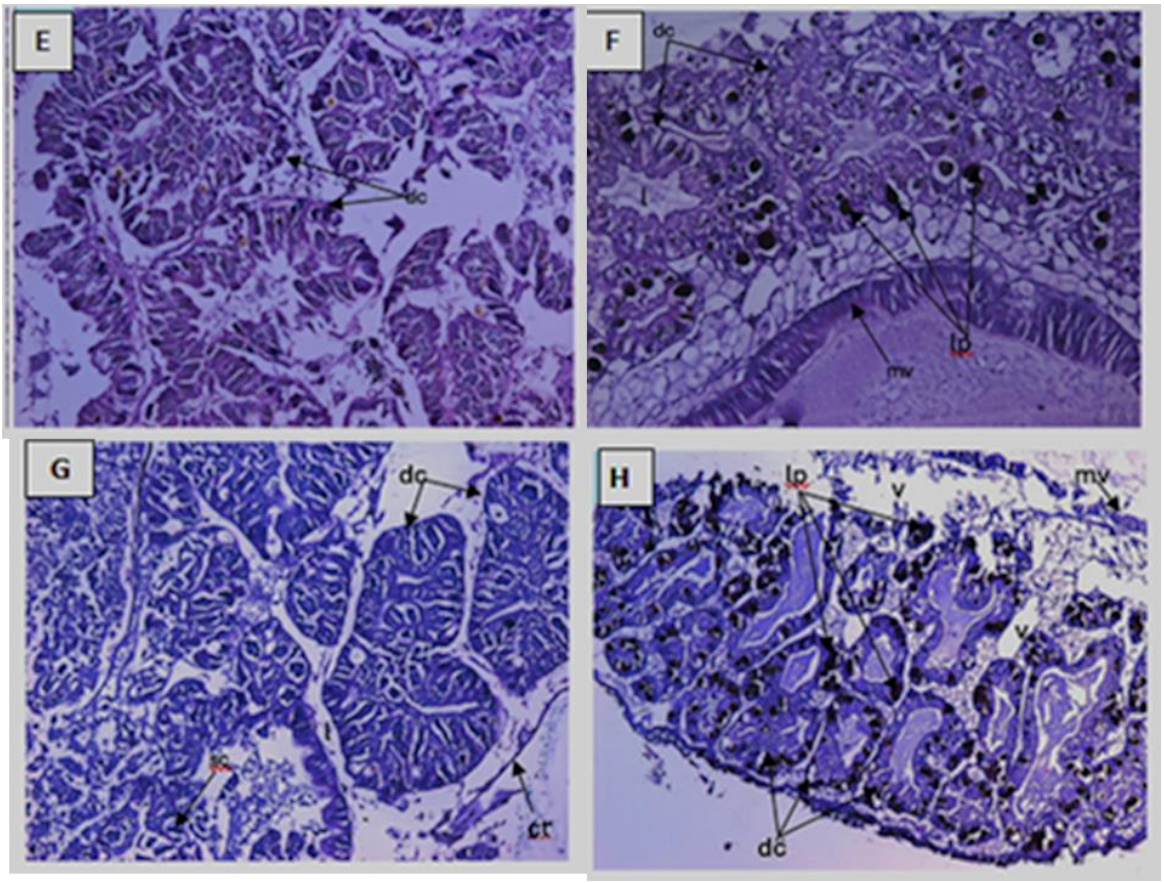


Fig. 7. (E) Light micrograph of digestive tissue in *Lanistes carinatus* (control), showing normal digestive cells (dc), secretory cells (sc), and the lumen (l). **(F)** Light micrograph of digestive tissue in *Lanistes nyassanus* (collected snail) showing digestive cells (dc) with marked dark lipofuscin (lp) and microvilli (mv) of digestive tissue. (H&E x100, x200, x400). **(G)** *Physia acuta* (control) showing normal digestive cells (dc), secretory cells (red arrow), the lumen (l) and connective tissue (ct) (H&E x100, x200, x400). **(H)** Light micrograph of digestive tissue in *Physia acuta* (collected snail) showing digestive cells (dc) with marked accumulation of lipofuscin (lp)

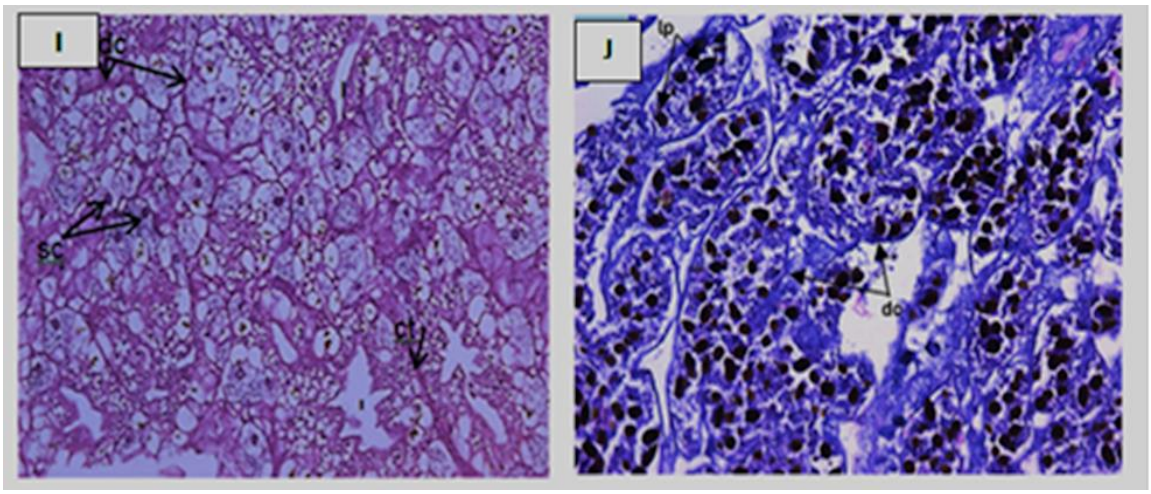


Fig. 8. (I) Light micrograph of digestive tissue in *Theodoxus niloticus* (control) showing normal digestive cells (dc) and the lumen (H&E x100, x200, x400). **(J)** Light micrograph of digestive tissue in *Theodoxus niloticus* (collected snail) showing digestive cells (dc) with a marked accumulation of lipofuscin (lp)

Effects of Heavy Metal Bioaccumulation on Comet Assay Parameters in Freshwater Snail Species: *Cleopatra* sp., *Helisoma* sp., *Lanistes* sp., *Physa* sp., and *Theodoxus* sp.

The comet assay results for various freshwater snail species underscore the genotoxic effects of heavy metal bioaccumulation, as recorded in Table (6) and Figs. (9, 10, and 11). In *Cleopatra ferruginea*, there was a significant increase in DNA damage, increasing from 11.76% ± 0.2% in the control group to 16.76% ± 0.2% in the bioaccumulation group ($P< 0.001$). Furthermore, the bioaccumulated snails presented a slightly longer tail length (10.18µm ± 1.1) than the control snails (9.76µm ± 0.9). Conversely, the percentage of DNA in the tail was notably lower in the bioaccumulation group, at 8.32% ± 1.1%, than in the control group, at 9.67% ± 2.1%. The tail moment parameter showed a minimal but statistically significant difference, measuring 0.81 in the bioaccumulation group compared with 0.84 in the control group.

In *Helisoma duryi*, the impact of bioaccumulation included significant increase in DNA damage from 10.76% ± 0.2 in the control group to 17.93% ± 0.3 in the bioaccumulated group, along with a notable significant increase in tail length (9.65µm ±0.1 in the control group compared with 11.4µm±1.4 in the bioaccumulated group). Furthermore, the percentage of DNA in the tail significantly decreased from 8.89% ±2.4% in the control group to 4.90% ±0.5% in the bioaccumulated group, whereas the tail moment decreased from 0.91±0.1% in the control group to 0.58 ±0.1% in the bioaccumulated group, all of which demonstrated highly significant genotoxic effects due to heavy metal bioaccumulation ($P< 0.001$).

In the case of *Lanistes carinatus*, bioaccumulation significantly increased DNA damage, with levels increasing from 14.33% ± 0.2% in the control group to 25.56% ± 0.4% in the bioaccumulated group. Although a slightly shorter tail length was observed in the bioaccumulated snails (7.62µm ±1.4 compared with 8.06µm ±0.8 in the control

group), there was a greater percentage of DNA in the tail (12.18% compared with 11.29% in the control group) and a notable increase in the tail moment (1.04% compared with 0.82% in the control group), all indicating the pronounced genotoxic impacts experienced under heavy metal bioaccumulation conditions ($P< 0.001$).

In *Physa acuta*, the impact of bioaccumulation significantly increased, resulting in a substantial increase in DNA damage from 11.36% ± 0.3% in the control group to 24.5% ± 0.4% in the bioaccumulated group. Additionally, there was a marginal increase in tail length (9.51µm ±1.2 compared with 9.55µm ±0.5), a notable increase in the percentage of DNA in the tail (6.16% compared with 11.92%), and a greater tail moment (0.55% compared with 0.5%). 0.83). These findings highlight the pronounced genotoxic effects of heavy metal bioaccumulation on *Physa acuta*.

With respect to *Theodoxus niloticus*, bioaccumulation significantly increased DNA damage, with levels increasing from 12.23% ± 0.2% in the control group to 20.13% ± 0.9% in the bioaccumulated group. Furthermore, there was an increase in tail length (6.8µm in contrast to 7.6µm), a decrease in DNA percentage in the tail (15.79% in the control group compared with 14.00% in the bioaccumulated group), whereas the tail moment remained similar (1.18% in the control group and 1.12% in the bioaccumulation group). These results indicated species-specific reactions to metal exposure, emphasizing the comet assay's sensitivity in assessing the effects of environmental stress on aquatic life.

Table 6. Impact of heavy metal bioaccumulation on comet assay parameters in freshwater snail species from Al-Shaeir Island, Qalubia, Egypt, during the 2022 and 2023 seasons

Freshwater snail	Percentage of damage	Tail length (µm)	DNA % in tail	Tail moment
Cleopatra ferruginea (control)	11.76 ±0.2	9.76±0.9	9.67 ±2.1	0.84 ±0.09
Cleopatra ferruginea (field snails)	16.76 ±0.2	10.18 ±1.1	8.32 ±1.1	0.81 ±0.09
Helisoma duryi (control)	10.76±0.2	9.65 ±0.1	8.89 ±2.4	0.91 ±0.1
Hilesoma duryi (field snails)	17.93 ±0.3	11.4 ±1.4	4.90 ±0.5	0.58 ±0.1
Lanistes carinatus (control)	14.33 ±0.2	8.06 ±0.8	11.29 ±2.3	0.82 ±0.06
Lanistes carinatus (field snails)	25.56 ±0.4	7.62 ±1.4	12.18 ±0.6	1.04 ±0.1
Physia acuta (control)	11.36 ±0.3	9.51 ±1.2	6.16 ±0.2	0.55 ±0.03
Physia acuta (field snails)	24.5 ±0.4	9.55 ±0.5	11.92 ±1.3	0.83 ±0.1
Theodoxus niloticus (control)	12.23 ±0.2	6.8 ±0.06	15.79 ±0.7	1.18 ±0.1
Theodoxus niloticus (field snails)	20.13 ±0.9	7.6 ±1.1	14.00 ±2.4	1.12 ±0.1

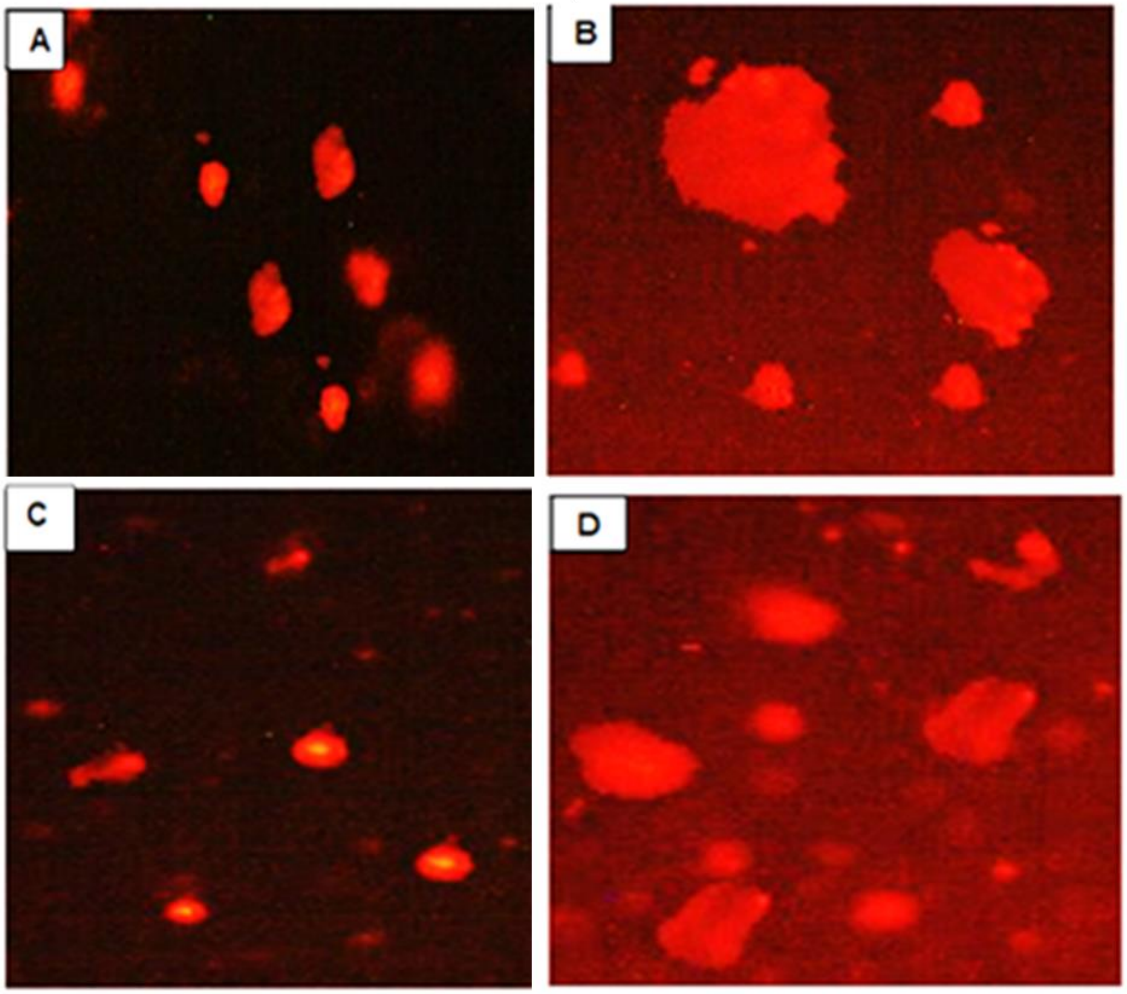


Fig. 9. (A, B, C, and D) The impact of heavy metal bioaccumulation on comet assay parameters in freshwater snail species. Panel **(A)** shows *Cleopatra ferruginea* under control conditions, while **(B)** shows *Cleopatra ferruginea* of field snails. Panel **(C)** depicts *Helisoma duryi* under control conditions, and **(D)** shows *Helisoma duryi* of field snails

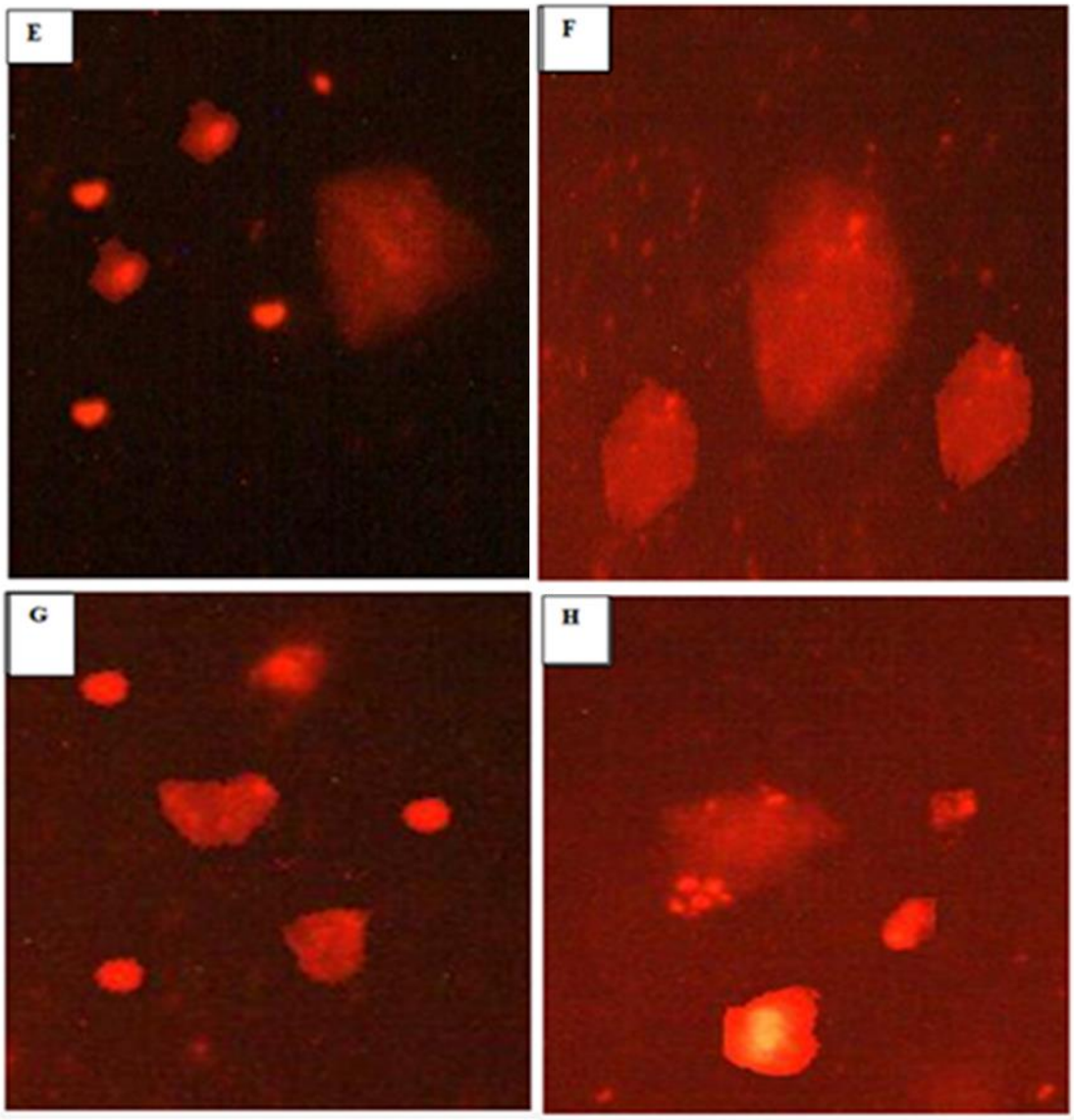


Fig. 10. (E, F, G, and H) demonstrates the impact of heavy metal bioaccumulation on comet assay parameters in freshwater snail species. Panel **(E)** shows *Lanistes nyassanus* under control conditions, while **(F)** shows *Lanistes nyassanus* of field snails. Panel **(G)** depicts *Physia acuta* under control conditions, and **(H)** shows *Helisoma duryi* of field snails

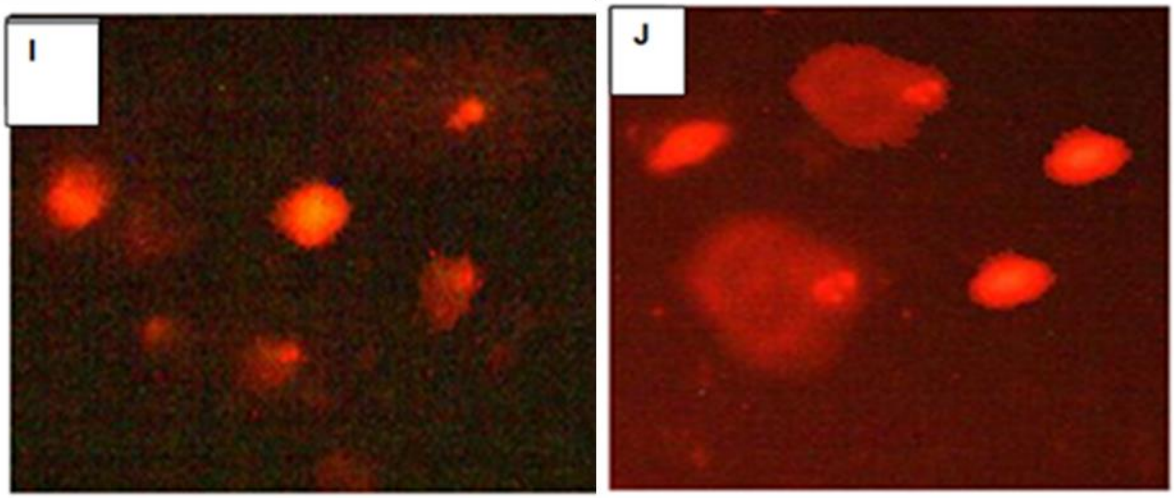


Fig. 11. (I and J) demonstrate the impact of heavy metal bioaccumulation on comet assay parameters in freshwater snail species. Panel **(I)** shows *Theodoxus niloticus* under control conditions, while **(J)** shows *Theodoxus niloticus* of field snails

DISCUSSION

The elevated heavy metal concentrations observed at the surveyed site are linked to the accumulation of these metals in the selected snail species. This suggests that anthropogenic activities, such as pollution, are likely contributing to the poor water quality, as indicated by low dissolved oxygen (DO) levels **(Huange *et al.*, 2017)**. High total dissolved solids (TDS) further support the idea of human impact, pointing to environmental stress in the aquatic ecosystem. Both geological and human influences are reflected in the river water and sediment characteristics, providing valuable insights into the health of the ecosystem **(Yuan *et al.*, 2014)**. Key environmental factors, such as pH, salinity, DO, suspended solids, and sediment size, play a significant role in metal behavior in the water **(Atkinson *et al.*, 2007; Butler, 2009)**. Although the effect of temperature on metal transport remains debated, some studies suggest it has an inconsistent impact **(Aston *et al.*, 2010; Zhang *et al.*, 2013)**. Overall, the accumulation of heavy metals in the environment is harmful to aquatic organisms, including oxygen levels, plankton, fish, and benthic organisms, all of which are essential for maintaining the health and balance of the ecosystem **(Sharma *et al.*, 2024)**. Therefore, the site appears to be under significant anthropogenic stress, which is negatively affecting its ecological integrity.

The release of heavy metals from sediments, caused by both natural processes and human activities, impacts environmental quality and poses risks to ecosystems and human health **(Huang *et al.*, 2017)**. While naturally occurring, human activities have significantly increased their concentrations, classifying them as contaminants **(Zhang *et al.*, 2017; El-Kady *et al.*, 2018)**. Trace heavy metals are particularly concerning due to their persistent toxicity and associated risks to ecosystems **(Payán *et al.*, 2012)**.

Dissolved metals are more bioavailable and pose greater risks than particulate forms **(Atkinson *et al.*, 2007)**, impacting surrounding water and accumulating in living organisms **(Zhang *et al.*, 2009; Pokorny *et al.*, 2015)**.

The current study documented the diverse abilities of freshwater snail species, which are commonly found on Barley Island, Egypt, to accumulate metals such as Fe, Mn, Pb, and Cd within their tissues. Molluscs are known to absorb essential metals, as noted by **El-Moselhy and Yassien (2005)**. These findings highlight the bioavailability of these metals in the environment and the significant assimilation capacities of molluscs for Fe, Mn, Pb, and Cd, reflecting their role in metal uptake.

Duquesne *et al.* (2000) demonstrated that mollusk species preferentially accumulate specific components. Furthermore, **Krupnova *et al.* (2017)** reported elevated concentrations of Fe in the shells of *Contectiana listeri*, *Lymnaea stagnalis*, and *Bithynia tentaculata*, contributing to their dark coloration. Variations in Mn levels in dehydrated tissues may be associated with differences in shell thickness. **Ramadan and Shata (1993)** reported iron concentrations ranging from 100--309 parts per million in the shells of *Anadara diluvi* in the Mediterranean Sea. **King *et al.* (2004)** attributed the bioaccumulation of heavy metals in filter- and deposit-feeding benthos to their feeding behaviors, their exposure to metals in pore water and suspended particulate matter, and the ingestion of sediments.

The bioaccumulation of metals in aquatic gastropods varies due to factors such as species, metal type, and location, as noted by **Yap and Cheng (2013)**. Individual characteristics such as age, nutrition, and growth rate also influence metal accumulation **(Mance, 1990; Tan Chi Yen & Su'Ut, 1998)**. **Abdel Kader *et al.* (2016)** reported positive correlations between heavy metal concentrations (Cd, Cu, Fe, Pb, Zn) and snail species in water canals, indicating that higher metal concentrations are associated with more species. Snail feeding behavior, such as the detritus-feeding habit of *Melanoides tuberculata*, plays a key role in metal uptake. Species differences, such as greater Fe and Mn contents in *Melanopsis praemorsa* and greater Cd and Co contents in *Theodoxus jordani*, are linked to physiological factors **(Swaileh *et al.*, 1994)**. Significant variations in bioaccumulation factor (BCF) levels, which are influenced by metal type and snail species, have been reported **(Abdel Kader *et al.*, 2016; Ibrahim *et al.*, 2022)**. The ability of molluscs to accumulate metals highlights their potential as effective biomonitors of environmental contamination **(Salànki *et al.*, 2003; Giarratan *et al.*, 2010)**.

Controlled snail species show reduced heavy metal bioaccumulation and lower bioaccumulation factors (BAFs), likely due to defense mechanisms against reactive oxygen species (ROS). Nonenzymatic antioxidants, such as metallothioneins, glutathione, and vitamin E, help combat oxidative stress **(Halliwell & Gutteridge, 2007)**. Antioxidant enzymes such as glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD) play key roles in neutralizing harmful radicals across various cellular compartments **(Wiegand *et al.*, 2001; Wong *et al.*, 2002; Tilton *et al.*, 2008)**.

Elevated nitric oxide (NO) levels in snails with heavy metal bioaccumulation indicate a protective response to oxidative damage. The accumulation of essential metals such as zinc, copper, and nickel in tissues without proper metabolism poses risks to aquatic life, threatening biodiversity in aquatic ecosystems **(Adams & Rowland, 2003; Luoma & Rainbow, 2008; El-Taher *et al*., 2018)**. Research has demonstrated that exposure to derivatives of pyridyl phenylurea can increase nitric oxide levels in snails, as observed by **Wang *et al*. (2018)** in *B. straminea* snails. Similarly, freshwater clams (*Corbicula fluminea*) exposed to copper presented increased activities of SOD, GST, and glutathione reductase **(Netpae *et al*., 2012)**. Heavy metals such as copper are known to stimulate ROS production in aquatic organisms, representing a significant stressor due to their poor degradability and bioaccumulation tendencies in aquatic food webs **(Company *et al*., 2004; Nunes, 2011; Bianco *et al*., 2013)**. Furthermore, antioxidant enzymes such as SOD, CAT, and GST are activated in *Physa acuta* when exposed to the insecticide amectin, with enzyme activity returning to normal levels during the recovery phase **(Ma *et al*., 2014)**.

The bioaccumulation of heavy metals in the digestive gland histological segment can induce cellular damage and oxidative stress, triggering an inflammatory response and potentially increasing the levels of liver enzymes such as AST and ALT, which are indicative of hepatocellular damage **(Todd, 1964; Health, 2018)**. Reports of histopathological findings align with marked hepatocyte degeneration and necrosis, which is supported by prior studies **(Kristaffersson & Okari, 1974)**. **Bici *et al*. (2023)** noted significant bioaccumulation of Pb, Zn, and Ni in snail shells (*Helix pomatia* L.), leading to elevated oxidative stress markers in the hepatopancreas. In contrast, the control snail group presented decreased liver enzyme levels alongside slightly increased metal accumulation. The liver, which is crucial for detoxification, displays pro- and anti-inflammatory responses essential for toxin detoxification processes **(Zhang *et al*., 2017)**.

The pathological accumulation of lipofuscin in the digestive glands of bioaccumulated snail species is associated with heavy metal deposition, leading to cellular aging and dysfunction **(Porta, 2002; Singh Kushwaha *et al*., 2019)**. In *L. carinatus*, fertilizer-induced lipid peroxidation and lipofuscin pigment accumulation accelerate digestive gland aging **(Sheir, 2015). Hödl *et al*. (2010)** reported that cadmium-induced oxidative stress can cause irreversible macromolecular breakdown, resulting in lipofuscin buildup in snail tissues. When a critical cadmium threshold is reached, saturated metallothionein proteins induce cellular toxicity. Controlled conditions reduce lipofuscin accumulation in snail digestive glands, which is crucial for maintaining function and delaying age-related pathologies **(Jolly *et al*., 2002; Terman & Brunk, 2004)**.

Animals residing in proximity to industrial zones have shown histological alterations in vital organs, notably the liver, as documented by **Kar *et al*. (2015)**. **Mohamadein and Desouky (2002)** stated that the marine clam *Venerupisa* urea

exhibited morphological and histological abnormalities in its siphons and gills following exposure to heavy metals. The digestive system has been a focal point in various research endeavors, such as investigations on *Lymnaea stagnalis*, a freshwater snail, conducted by **Lance *et al*. (2010)**, and studies on *Helicella vestalis*, a land snail, carried out by **Sharaf *et al*. (2015)**.

The comet assay, which measures DNA damage through indicators such as tail length, %DNA in the tail, and the tail moment, presents unique challenges in study design and data interpretation **(Hagger *et al*., 2006; Chapman *et al*., 2013; Yoshiyama, 2019)**. In the present study, in freshwater snails, including species such as *Cleopatra ferruginea* and *Helisoma duryi*, a decrease in DNA damage was observed across the control groups. The decrease in DNA damage observed may be due to defense mechanisms that prevent mutation spread, such as cell cycle arrest and programmed cell death. Repair pathways such as base excision repair (BER) and nucleotide excision repair (NER) also help rectify DNA damage **(Martins & Costa, 2015)**. However, exposure to toxins can impair these repair systems (**Mukherjee *et al*., 2008; Costa *et al*., 2010)**.

Environmental pollutants such as Cd and Cu can interfere with DNA repair enzymes, such as formamidopyrimidine DNA glycosylase (FPG), by competing with zinc in Zn-finger domain enzymes. Factors such as dehydration, high temperatures, UV radiation, infections, and toxins can cause DNA damage, including strand breaks and base alterations. DNA damage can disrupt transcription and replication, leading to chromosomal abnormalities, mutations, or cell death **(Yoshiyama *et al*., 2019)**.

However, in a month-long study by **Baussant *et al*. (2009)**, marine clams and mussels were exposed to dispersed crude oil at different concentrations. This study revealed no clear correlation between the comet assay results and biomarkers related to oxidative stress or PAH metabolism, such as glutathione S-transferase (GST) activity, catalase activity, and total oxidative scavenging capacity (TOSC). **Frenzilli *et al*. (2001)** reported a positive correlation between DNA strand breakage, total oxidative scavenging capacity (TOSC), and hydroxyl radicals in mussels from a contaminated lagoon. Unlike studies focused on contamination and bioaccumulation, this research emphasizes the complex relationships among DNA damage, oxidative stress markers, and pollutant exposure, underscoring the need for further investigations into these interactions.

CONCLUSION

In conclusion, these findings underscore the detrimental impact of heavy metal bioaccumulation on freshwater snails in the studied area, which manifests as elevated stress markers, histopathological anomalies, and genetic damage. These results emphasize the urgent need for environmental remediation efforts to mitigate the adverse effects of anthropogenic activities on aquatic ecosystems and their inhabitants.

REFERENCES

Abdel Kader A.; Ismail N.M.M. and Abdel-motleb A. (2016). Bioaccumulation of heavy metals in freshwater snails in relation to lining of water courses in Egypt. J Biosci Appl Res., 2: 561–573.

Adams, W.J. and Rowland, C.D. (2003). Aquatic toxicology test methods. In: Hoffman, D. J., Rattner, B.A., Burton, G.A., Cairns, J. (Eds.), Handbook of Ecotoxicology. CRC Press, LLC, New York, pp. 19–38

Aston, J.E.; Apel, W.A.; Lee, B.D. and Peyton, B.M. (2010). Effects of cell condition, pH, and temperature on lead, zinc, and copper sorption to Acidithiobacillus caldus strain BC13, Journal of Hazardous Materials, 184(1–3), pp. 34–41.

Atkinson, C.A.; Jolley, D.F. and Simpson, S.L. (2007). Effect of overlying water pH, dissolved oxygen, salinity and sediment disturbances on metal release and sequestration from metal contaminated marine sediments, Chemosphere, 69(9), pp. 1428– 1437.

Ávila, L.V.; Leal Filho, W. and Brandli, L. (2017). Barriers to innovation and sustainability at universities around the world. J Clean Prod 164:1268–1278

Baussant, T.; Bechmann, R. K. and Taban, I. C. (2009). Enzymatic and cellular responses in relation to body burden of PAHs in bivalve molluscs: a case study with chronic levels of North Sea and Barents Sea dispersed oil. Mar. Pollut. Bull., 58: 1796–1807

Bianco, K.; Yusseppone, M.S.; Otero, S.; Luquet, C.; Rios de Molina, M.C. and Kristoff, G. (2013). Cholinesterase and Neurotoxicity as Highly Sensitive Biomarkers for an Arganophosphate Insecticide in a Freshwater Gastropod (Chilina gibbosa) with Low Sensitivity Carboxylesterases. Aquatic Toxicology, 26-35, 144-145.

Bici, M.; Mazreku, I.; Hyseni, B.; Halili, J.; Krasniqi, Y. and Bislimi, K. (2023). Effect of lead, nickel, and zinc pollution in some parameters of oxidative stress in hepatopancreas of snail Helix pomatia L. In power plant of obiliq. Journal of Ecological Engineering, 24(9).

Butler, B.A. (2009). Effect of pH, ionic strength, dissolved organic carbon, time, and particle size on metals release from mine drainage impacted streambed sediments, Water Research, 43(5), pp. 1392–1402.

Chapman, P. M.; Wang, F. and Caeiro, S. S. (2013). Assessing and managing sediment contamination in transitional waters. Environ. Int., 55: 71–91.

Che, F.; Chen, J.; Zhang, B.; Jiang, X. and Wang, S. (2020). Distribution, risk and bioavailability of metals in sediments of Lake Yamdrok Basin on the Tibetan Plateau, China. J. Environ. Sci., 97: 169–179.

Company, R.; Serafim, A.; Bebianno, M.J.; Cosson, R.; Shillito, B. and Fiala-Médioni, A. (2004) Effect of Cadmium, Copper and Mercury on Antioxidant

Enzyme Activities and Lipid Peroxidation in the Gills of the Hydrothermal Vent Mussel Bathymodiolus azoricus. Marine Environmental Research, 58: 377-381.

Costa, P. M.; Chicano-Gálvez, E.; López Barea, J.; DelValls, T. A. and Costa, M. H. (2010). Alterations to proteome and tissue recovery responses in fish liver caused by a short-term combination treatment with cadmium and benzo[a]pyrene. Environ. Pollut., 158: 3338–3346

Çougun, H.; Yüzerero_glu, T.; Firat, Ö.; Gök, G. and Kargin, F. (2006). Metal concentrations in fish species from the northeast Mediterranean Sea. Environ. Monit. Assess. 121, 431e438.

Duquesne, M.; Riddle, R. and Schulz, M. Liess (2000). Effect of contaminants in the Antarctic environment potential of the gammarid amphipod crustacean *Paramorea walkeri* as a biological indicator for Antarctic ecosystems based on toxicity and bioaccumulation of copper and cadmium.Aquat.Toxicol., 49: 131-143

EL-KADY, Ahmed A.; ABDEL-WAHHAB and Mosaad A. (2018). Occurrence of trace metals in foodstuffs and their health impact. Trends in food science & technology, 75: 36-45.

El-khayat, H.M.M. and Abu-Zikri, M. (2004). Biochemical situation in Biomphalaria alexandrina infected with Schistosoma mansoni during twelve weeks post infection. J. Egypt. Ger. Soc. Zool., 43 (A): 57-75.

El-Moselhy and Yassien (2005). Accumulation patterns of heavy metals in two venus clams, *Paphia undulata* (Born, 1780) and *Gafrarium pectinatum* (Linnaeus, 1758), from Lake Timsah Suez Canal, Egypt.

El-Nahas, H.A.; El-Sayed, M.M.; El-Deeb,F.A. and El- Wakil, E.A. (2003). Laboratory and semifiled assessment of three egyption plants as molluskicidal agenta. Egypt Journal of Applied science. 18 (9) 102-120.

El-Sayed, S.; Moussa, E. and El-Sabagh, M. (2015). Evaluation of heavy metal content in Qaroun Lake, El-Fayoum, Egypt. Part I: bottom sediments. J. Radiat. Res. Appl. Sci. 8, 276e285.

El-Taher A.; Zakaly H.M.H. and Elsaman R. (2018). Environmental implications and spatial distribution of natural radionuclides and heavy metals in sediments from four harbors in the Egyptian Red Sea coast.Applied Radiation and Isotopes; 131: 13-22.

Federici G.; Shaw B.J and Handy R.D. (2007). Toxicity of titanium dioxide nanoparticles to rainbow trout Oncorhynchus mykiss): Gill injury, oxidative stress, and other physiological (effects. Aquat Toxicol 84:415–430.

Frenzilli, G.; Nigro, M.; Scarcelli, V.; Gorbi, S. and Regoli, F. (2001). DNA integrity and total oxyradical scavenging capacity in the Mediterranean mussel, Mytilus galloprovincialis: a field study in a highly eutrophicated coastal lagoon.Aquat.Toxicol., 53: 19–32

Giarratan, O. E.; Duarte, C. A. and Amin, O. A. (2010). Biomarkers and heavy metal bioacumulation in mussels transplanted to coastal waters of the Beagle Chanel. Ecotoxicol.Environm.Saf., 73: 270–279.

Grazeffe V.S.; De Freitas, T.L. and De Sa P.A. (2008). Establishment of the comet assay in the freshwater snail Biomphalaria glabrata(Say, 1818). Mutat Res Toxicol Environ Mutagen, 654:58–63

Hagger, J. A.; Jones, M. B.; Leonard, D. R.; Owen, R. and Galloway, T. S. (2006). Biomarkers and integrated environmental risk assessment: are there more questions than answers? Integr. Environ. Assess. Manag., 2: 312–329.

Halliwell, B., and Gutteridge, J.M.C. (2007). Free radicals in biology and medicine, Fourth ed. Oxford University Press, New York.

Health. A. G. (2018). Water pollution and fish physiology. CRC press Inc. Boca. Raton Florida. U.S.A. https://doi.org/10.1201/9780203718896

Hödl E.; Felder E.; Chabicovsky M. and Dallinger R. (2010). Cadmium stress stimulates tissue turnover in Helix pomatia: increasing cell proliferation from metal tolerance to exhaustion in molluskan midgut gland. Cell Tissue Res. 341:159–171.

Huang, Y.; Zhang, D.; Xu, Z. Yuan,; S. Li, Y. and Wang, L. (2017). Effect of overlying water pH, dissolved oxygen and temperature on heavy metal release from river sediments under laboratory conditions.Archives of Environmental Protection. 43 (2): 28–36

Hynes, H.B.N. (1984). The relationships between the taxonomy and ecology of aquatic insects. In The Ecology of Aquatic Insects. Praeger, New York, pp. 9–23.

Ibrahim, A.M. Abdel-Haleem, A. and Taha, R.G. (2022). Biomphalaria alexandrina snail as a bioindicator of pollution with Manganese metal and its effect on physiological, immunological, histopathological parameters and larvicidal potenciesEnviron Sci Pollut Res Int. 30(48): 105967–105976

Jannat, N.; Mottalib, M.A. and Alam, M.N. (2019). Assessment of physico-chemical propertiesof surface water of Mokeshbeel, Gazipur. Bangladesh. J. Environ. Sci. 2(3): 1–6.

Jolly R.D.; Palmer D.N. and Dalefield R.R. (2002). The analytical approach to the nature of lipofuscin (age pigment) Arch. Gerontol.Geriatr. 34: 205–217.

Kar, I.; Mukhopadhayay, S.K.; Patra, A.K. and Pradhan, S. (2015). Metal Concentrations and Histopathological Changes in Goats (Capra hircus) Reared Near an Industrial Area of West Bengal, India. Arch Environ Contam Toxicol, 69: 32–43.

King, C.K.; Dowse, M.C.; Simpson, S.L. and Jolley, D.F. (2004). An assessment of five Australian polychaetes and bivalves for use in whole-sedimenttoxicity tests: toxicity and accumulation of copper and zinc from water and sedimentArch. Environ. Contam.Toxicol., 47: 314-323

Kristaffersson R. and Okari P. (1974). Effect of sublethal concentration of phenol on plasma enzyme activities in pike in brackish water. Ann. Zoo). FennieL 77: 220-223.

Krupnova, T.G.; Mashkovaa, I.V.; Kostryukova, A.M.; Schelkanova, E.E. and Gavrilkina S.V. (2017). Gastropods as potential biomonitors of contamination caused by heavy metals in South Ural lakes, Russia. Ecological Indicators, 95: 1001-1007.

Lance, E.; Josso, C.; Dietrich, D.; Ernst, B.; Paty, C.; Senger, F.; Bormans, M. and Gerard, C. (2010). Histopathology and microcystin distribution in Lymnaea stagnalis (Gastropoda) following toxic cyanobacterial or dissolved microcystin-LR exposure. Aquat Toxicol, 98: 211–220.

Lau, S.; Mohamed, M.; Tan Chi Yen, A. and Su'Ut, S. (1998). Accumulation of heavy metals in freshwater molluscs.*Science* of the *Total Environment*, 214, 113-121.

Luoma, S. N. and Rainbow, P. S. (2008). Metal contamination in aquatic environments: science and lateral management. Cambridge: Cambridge university press. (Vol. 126)

Ma, J.; Zhou, C.; Li, Y. and Li, X. (2014). Biochemical Responses to the Toxicity of the Biocide Abamectin on the Freshwater Snail *Physa acuta*. Ecotoxicology and Environmental Safety, 101, 31-33.

Mahajan, P.R. (2015). Lead bioaccumulation in whole body tissues of freshwater gastropod snail, Bellamya bengalensis after chronic lead intoxication, Ciotech Journal of Bio-Protocols, 4(2): 15-18.

Mance, G. (1990). Pollution Threat of Heavy Metal in Aquatic Environments. New York, USA, Elsevier Applied Science., 372 pp.

Martins, M.; Pedro M. and Costa, P.M. (2015). The comet assay in Environmental Risk Assessment of marine pollutants: applications, assets and handicaps of surveying genotoxicity in nonmodel organisms. Mutagenesis, 30, 89–106.

Mehmood, M. A.; Qadri, H.; Bhat, R. A.; Rashid, A.; Ganie, S. A.; Dar, G. H. and Shafiq-ur-Rehman. (2019). Heavy metal contamination in two commercial fish species of a trans-Himalayan freshwater ecosystem.Environmental Monitoring and Assessment Environment, 191: 104.

Mohamadein, A., and Desouky, M.M. (2002). Effect of cadmium as a water pollution on the filtration rate and the histology of the siphon of the marine clam Venerupisa urea. Eypt J Aquat Biol Fish, 6: 125–139.

Montgomery HAC and Dymock J.F. (1962).The rapid determination of nitrate in fresh and saline waters.Analyst. https://doi. org/10. 1039/an962 87003 74

Mukherjee, J. J.; Gupta, S. K. and Kumar, S. (2008). Inhibition of benzopyrene diol epoxide-induced apoptosis by cadmium(II) is AP-1-independent: role of extracelluler signal related kinase. Chem. Biol. Interact., 172: 72–80.

Netpae, T.; Phalaraksh, C., and Wongkham, W. (2012). Antioxidant Enzyme Activities and DNA Damage as Biomarker of Copper Effect on Corbicula fluminea. Electronic Journal of Biology, 8: 19-23.

Nunes, B. (2011). The Use of Cholinesterases in Ecotoxicology. Reviews of Environmental Contamination and Toxicology, 212, 29-59.

Östling, O. and Johanson, K. J. (1984). Microelectrophoretic study of radiation-induced DNA damages in individual mammalian cells. Biochem.Biophys. Res. Commun., 123: 291–298.

Payán, M.C.; Galan, B.; Coz, A.; Vandecasteele, C. and Viguri, J.R. (2012). Evaluation through column leaching tests of metal release from contaminated estuarine sediment subject to CO2 leakages from Carbon Capture and Storage sites, Environmental Pollution, 171(0): 174–184.

Pokorny, P.; Pokorny, J.; Dobicki, W.; Senze, M. and Kowalska, G.M. (2015). Bioaccumulations of heavy metals in submerged macrophytes in the mountain river Biala Ladecka (Poland, Sudety Mts.), Archives of Environmental Protection, 41(4): 81–90.

Porta E.A. (2002). Pigments in aging: an overview. *Ann. N. Y. Acad. Sci.* 959:57–65. doi: 10.1111/j.1749-6632..tb02083.x.

Pyatt, A.J; Pyatt ,F.B. and Pentreath, V.W (2002). Lead toxicity, locomotion and feeding in the freshwater snail, Lymnaea stagnalis (L.) Invertebrate Neuroscience 4(3):135-40

Ramadan, S.E. and Shata A., A. (1993). Biogeochemical studies on the mollusk bivalve Anadara diluvii (Lamarck, 1805) (Pteriomorpha Arcidae)Bul. Na Inst. Ocn.& Fish, (19): 145-157

Rehman, H. Ur.; Ullah Khan, R. and Sajed, M. (2016). Estimation of Heavy Metal of Molluska Shell, Water and Soil Collected from Darmalak Dam, Tehsil Lachi District Kohat. *World Journal of Zoology*, 11 (1): 01-05.

Salánki, J.; Farkas, A.; Kamardina, T. and Rozsa, K.S. (2003): Molluscs in biological monitoring of water quality Toxicol. Lett., 140-141 (11): 403-410.

Sharaf, H.M.; Salama, M.A. and Abd El-Atti, M.S. (2015). Biochemical and Histological Alterations in the Digestive Gland of the Land Snail Helicella vestalis (Locard, 1882) Exposed to Methiocarb and Chlorpyrifos in the Laboratory. Internat J Sci Res, 4: 334–343.

Sharma, M., Kant, R., Sharma, A. K., & Sharma, A. K. (2024). Exploring the impact of heavy metals toxicity in the aquatic ecosystem. International Journal of Energy and Water Resources, 1-14.

Sheir, S. K. (2015). The role of Caselio (plant fertilizer) exposure on digestive gland histology and heavy metals accumulation in the freshwater snail, *Lanistes carinatus*. Journal of Bioscience and Applied Research, 1 (5): 223-233.

Singh N.P.; McCoy M.T.; Tice R.R. and Schneider E.L. (1988). A simple technique for quantitation of low levels of DNA damage individual cells. Exp Cell Res., 175:184–191

Singh Kushwaha S.; Patro N. and Kumar Patro I. (2019). A sequential study of age-related lipofuscin accumulation in hippocampus and striate cortex of rats. *Ann. Neurosci.,* 25:223–233.

Singh, D. V.; Bhat, J. I. A.; Bhat, R. A.; Dervash, M. A., and Ganei, S. A. (2018). Vehicular stress a cause for heavy metal accumulation and change in physico-chemical characteristics of road side soils in Pahalgam. Environmental Monitoring and Assessment, 190(6): 353.

Swaileh, K.M.; Mesmar, M.N. and Ismail , N.S. (1994). Heavy metal concentrations in common freshwater snails of Azraq Oasis, Jordan.*Hydrobiologia* 286: 167-173.

Terman A., and Brunk U.T. (2004). Lipofuscin. Int. J. Biochem. Cell Biol. 36:1400–1404. doi: 10.1016/j.biocel.2003.08.009.

Tilton, F.; La Du, J.K. and Tanguay, R.L. (2008). Sulfhydryl systems are a critical factor in the zebrafish developmental toxicity of the dithiocarbamate sodium metam (NaM). Aquatic Toxicology 90: 121–127.

Todd. A.S. (1964). Localization of fibrinolytic activity in tissues. Brit. Med. Bull. 20: 212-221.

Vukašinović-Pešić, V.; Blagojević, N.; Vukanović, S.; Savić, A. and Pe š ić,V. (2017). Heavy Metal Concentrations in Different Tissues of the Snail *Viviparus mamillatus* (Küster, 1852) from Lacustrine and Riverine Environments in Montenegro. Turkish Journal of Fisheries and Aquatic Sciences 17: 557-563.

Wang, W.; Mao, Q.; Yao, J.; Yang, W.; Zhang, Q.; Lu, W. and Duan, L. (2018). Discovery of the pyridylphenylureas as novel molluscicides against the invasive snail Biomphalaria straminea, intermediate host of Schistosoma mansoni. Parasites & Vectors, 11: 1-8.

White, B.A.; Erickson, M.M. and Stevens, S.C. (1970). Determination of GOT and GPT enzymes; in Chemistry for Medical Technologists, pp. 293-296.

WHO, (2011). The use of ddt in malaria vector control: who position statement (no. who/htm/gmp/2011). World health organization.

Wiegand, C.; Krause, E.; Steinberg, C. and Pflugmacher, S. (2001). Toxicokinetics of atrazine in embryos of the zebrafish(*Danio rerio*). Ecotoxicology and Environmental Safety 49: 199–205.

Wong, C.M.; Zhou, Y.; Ng, R.W.; Kung, H.F. and Jin, D.Y. (2002). Cooperation of yeast peroxiredoxins Tsa1p and Tsa2p in the cellular defense against oxidative and nitrosative stress. Journal of Biological Chemistry 277: 5385–5394.

Yap, C. K. and Cheng, W.H. (2013). Distributions of heavy metal concentrations in different tissues of the mangrove snail *Nerita lineata*. *Sains Malaysiana*, 42: 597-60.

Yoshiyama, K.O. (2019). Mechanisms of genome maintenance in plants: From DNA repair to telomere maintenance. Frontiers in Plant Science, 10(653): 5-10.

Yuan, X.; Zhang, L.; Li, J.; Wang, C. and Ji, J. (2014). Sediment properties and heavy metal pollution assessment in the river, estuary and lake environments of a fl uvial plain, China, *CATENA*, 119(0): 52–60.

Zhang, D.; Lee, D.J. and Pan, X. (2013). Desorption of Hg(II) and Sb(V) on extracellular polymeric substances: Effects of pH, EDTA, Ca(II) and temperature shocks, Bioresource Technology, 128(0): 711–715.

Zhang, H.; Huang, B.; Dong, L.; Hu, W.; Akhtar, M.S.; Qu, M. (2017). Accumulation, sources and health risks of trace metals in elevated geochemical background soils used for greenhouse vegetable production in southwestern China. Ecotoxicol. Environ. Saf., 137: 233–239.

Zhang, M.; Cui, L.; Sheng, L. and Wang, Y. (2009). Distribution and enrichment of heavy metals among sediments, water body and plants in Hengshuihu Wetland of Northern China, Ecological Engineering, 35(4): 563–569.