https://doi.org/10.33472/AFJBS.6.13.2024.1304-1317

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Toxicological Effect of Cadmium and Zinc Oxide Nanoparticle Mixtures on the Land Snails, Helix Aspersa

Gouasmia Yassine[1*], Atailia Amira[2], Tadjine Aicha[3], Djeddi Khaled[4], Cherb Nora[5], Haddad Rafika[6], Boufendi Houda[7], Temime Asma[8], Rabah Siham[9]

[1*,3]Department of Biology, Faculty of Life and Natural Sciences, Research Laboratory of Functional and Evolutionary Ecology, Chadli Bendjedid University, El Tarf, BP 73, 36000, Algeria
[5,6,7,8]Biotechnology Research Center - C.R.Bt Constantine ALGERIA
[2]Department of Biology, Faculty of Science, University of Badji Mokhtar, BP 12, 23000, Annaba, Algeria
[4]Institute of Agriculture and Veterinary Sciences, Laboratory of Life Sciences and Techniques, Souk-Ahras University, Algeria
[9]Ministry of the Interior and Local Authorities, Birelater, Tebessa 12000, Algeria
To whom should be addressed. * Dr. GOUASMIA Yassine,
Email. y.gouasmia12@gmail.com

Article Info

Volume 6, Issue 13, July 2024

Received: 02 June 2024

Accepted: 30 June 2024

Published: 24 July 2024

doi: 10.33472/AFJBS.6.13.2024.1304-1317

ABSTRACT:

This study investigated the toxicological effect of cadmium and zinc oxide nanoparticles (ZnONPs) in land snails (*Helix aspersa*). Snails were exposed for 28 days to cadmium combined with ZnONPs at increasing concentrations (250, 750, 2250, 6750, and 20250mg/kg dry weight of soil "dwt"). Exposure to Cd and ZnONPs mixtures resulted in slight decrease inbody weight, and weights of vital organs (hepatopancreas, kidney, and smooth muscle). Oxidative stress effect was evidenced by significant increase in malondialdehyde (MDA, a marker of oxidative stress) content, and decrease in glutathione (GSH, an antioxidant) content, and the enzymatic activity of catalase (CAT), glutathione S-transferase (GST) and the neurotransmitter enzyme acetylcholinesterase (AChE) in hepatopancreas and kidney tissues. Histological analysis revealed gradual histological alterations to the kidney and hepatopancreas in Cd/ZnONPs mixture treatments. These findings suggest that and Cd and ZnONPs mixtures caused a toxicity on the antioxidant defense system and physiology of *Helix aspersa.*

Keywords: Zinc oxide nanoparticles (ZnONPs), Cadmium (Cd), GSH, CAT, AChE, kidney, Hepatopancreas, *Helix aspersa*

1. Introduction

Land snails have emerged as a valuable tool for assessing heavy metal contamination in terrestrial ecosystems due to their unique biological characteristics. Their sedentary lifestyle confines them to a specific area, reflecting the local soil composition and potential contaminant sources (Nica et al., 2013). As detritivores or herbivores, they accumulate heavy metals from ingested soil and plant material through a process known as bioaccumulation (Thanh-Nho et al., 2019). This bioaccumulation allows researchers to analyze the metal concentrations within snail tissues, providing a direct measure of the environmental burden. Additionally, land snails exhibit a high sensitivity to a range of heavy metals, and the specific metal profile within their tissues can offer insights into the types of pollutants present (Nica et al., 2013; Thanh-Nho et al., 2019).

Cadmium (Cd) is highly toxic and omnipresent aquatic environmental metal (Gnatyshyna et al. 2023; Xu et al. 2017), due to the increased agricultural and industrial human activities (L.-M. Cai et al., 2019). Cd is an accumulative metal in aquatic organisms, inducing generation reactive oxygen species (ROS) resulting in an imbalance of antioxidants (Gnatyshyna et al., 2023; Wu et al., 2023). In addition, biochemical, organ weights and growth are the main fundamental parameters in aquatic organisms for evaluating toxicity(Cai et al., 2020).

Nanoparticles (NPs), with their unique size-dependent properties, have revolutionized various fields. Their applications range from targeted drug delivery in medicine (Ayub and Wettig, 2022)to lightweight and strong materials in engineering(Manocha et al., 2006). NPs can enter ecosystems unintentionally through industrial processes or wastewater treatment plants(Zahra et al., 2020). In the cell, the small sized of NPs can interact with cell components, and consequently induce the production of reactive oxygen species (ROS), leading to oxidative stress and cell damage(Wang et al. 2015). NPs can interact with metals in mixtures to induce whether high or/and less toxic effects on target or non-target organisms. However, research is ongoing in areas like using NPs for environmental remediation or developing NPs for disease diagnosis and treatment with minimal side effects(Palit, 2018). Also, zinc oxide nanoparticles (ZnONPs) and offer a unique advantage due to their high surface area and reactivity(Akhtar et al., 2021).Recent research suggests a potential benefit for mitigating the toxicity of other heavy metals in aquatic environments(Kumar et al., 2022). As the impact of ZnONPs on Cd toxicity remains unclear, this study aimed to elucidate the optimal ZnONPs concentrations for alleviating or/and increasing Cd-mediated oxidative stress and physiological impairments in this ecologically relevant invertebrate.

2. Materials and Methods

Chemicals

Zinc oxide nanoparticles (ZnONPs) with the following information: 10–50 nm average size, +99% purity, 5.606 g/cm^3 density, and approximately 20–60 m2 /g surface area were purchased from Sigma-Aldrich Chemical Company (Dorset, UK) (**Figure 1**), cadmium chloride (CdCl2), DMN, thiobarbituric acid(TBA), and 5′-5′-dithiobis-2-nitrobenzoic (DTNB)were also supplied by Sigma-Aldrich Chemical Company (Dorset, UK). All other reagents of analytical grade were procured from local suppliers.

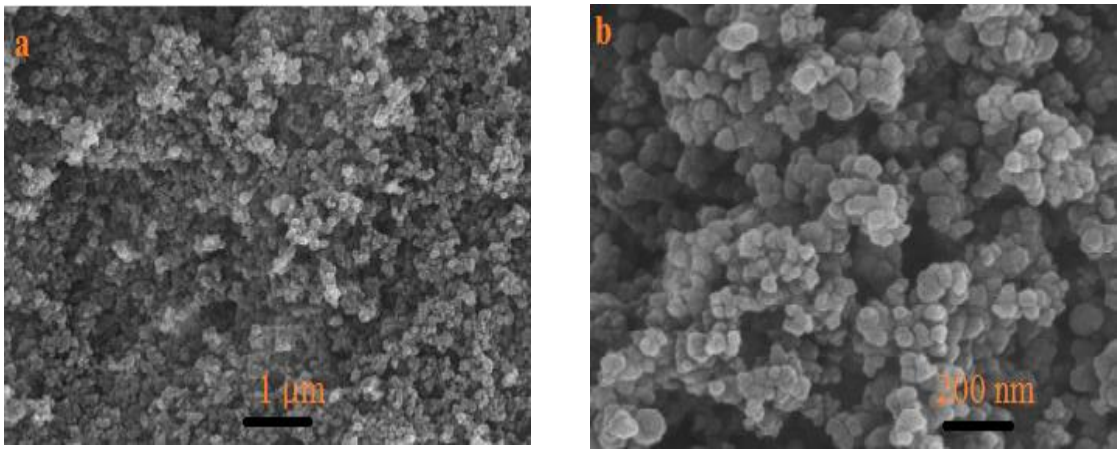


Figure 1. SEM images of ZnO nanoparticles. **a.**ZnONPs in 1µm scale, and **b.**ZnONPs in 200 nm scale.

Animals

Land snails, *Helix aspersa*, obtained from a snail farm in Bouchegouf region of Guelma city (northeastern Algeria) were acclimatized under standard laboratory conditions (20±2◦C, 16h/8h light/dark, and 80–85% humidity) as previously described (Gomot-De Vaufleury, 2000). Animals were placed into transparent perforated plastic boxes (25x15x15 cm, 5625 cm^3) supported with a perforated lid to ensure good ventilation, and were had ad libitum acess to food (wheat flour and lettuce leaves).

Experimental procedures

After 2 months of acclimatization period, five replicates of 25 snail individuals for each experimental group of control and five treated groups received respectively (mg/kg dry soil weight "dwt"), 250mg Zinc oxide nanoparticles (ZnONPs)/kg dwt + 1500mg $CdCl_2$/kg dw, 750mg ZnONPs/kg dwt + 1500mg $CdCl_2$/kg dwt, 2250mg ZnONPss/kg dwt + 1500mg $CdCl_2$/kg dwt, 6750mg ZnONPs/kg dwt + 1500mg $CdCl_2$/kg dwt, and 20250mg ZnONPs/kg dwt + 1500mg $CdCl_2$/kg dwt for 28 consecutive days. Of note, body weight of control and treated animals were measured every week throughout the study period. After that, the snails were sacrificed by freezing (−80 C), and then the hepatopancreas, and kidney were removed and fixed for histological study and antioxidants evaluations.

Determination of oxidative stress markers

The frozen tissue of kidney and hepatopancreas samples were thawed in crushed ice and homogenized using a manual homogenizer with an ice-cold Tris-HCl buffer. The homogenate was then centrifuged at 10,000g for 10 minutes at 4°C to separate the supernatant containing the proteins. Aliquots of the supernatant were used to measure the total protein content using the Bradford dye-binding assay, with bovine serum albumin as the standard as previously described (Bradford, 1976), and the major oxidative markers. The GSH content was determined in tissue homogenates using using Elman's reagent (DTNB) as previously reported (Weckbecker and Cory, 1988). In brief, a reaction mixture for each sample containing 500 µL of the supernatant , 1.0 ml of Tris-EDTA buffer (pre-made, 0.02 M, pH 9.6) and 25 µL of Ellman's reagent (DTNB, pre-made, 0.01 M) was prepared, and then incubate at room temperature for 5 minutes. The fluorescence of each sample was measured at a wavelength of 412 nm, and the resulting fluorescence intensity can then be compared to a standard curve generated using known concentrations of pure GSH. The GSH content was expressed in micromoles of glutathione formed per milligram of protein (µmol GSH/mg

protein).GST activity was determined as described elsewhere (Habig et al., 1974), based on the measurement of the enzyme's ability to conjugate glutathione (GSH) to a substrate, 1-chloro-2,4-dinitrobenzene (CDNB), resulting in a product that absorbs light at 340 nm. The rate of increase in absorbance at 340 nm is directly proportional to GST activity. GST activity was expressed in µmol /mn /mg protein. Catalase (CAT) activity was determined based on the disappearance of hydrogen peroxide (H2O2) at a wavelength of 240 nm(Beers and Sizer, 1952), following the presence of a reaction mixture containing phosphate buffer (pH 7.0), H2O2, and a small amount of tissue homogenates. A single unit of CAT activity is defined as the quantity of enzyme that can convert 1 µmol of H2O2 to another molecule per minute. The specific activity of CAT is reported in units that reflect the amount of enzyme activity per milligram of protein in the sample. The level of malondialdehyde (MDA), a marker of lipid peroxidation, in tissue homogenates was measured as previously described (Pothiwong et al., 2007), using the thiobarbituricacid reactive substances (TBARS) assay. This involves mixing the homogenate with a solution containing acetic acid and a detergent to break down cellular components and release MDA. After adding thiobarbituric acid (TBA), the mixture was incubated at high temperatures to trigger a reaction between MDA and TBA, forming a colored product. Following cooling and centrifugation, the intensity of this red color is spectrophotometrically measured 532 nm. The amount of MDA in samples was provided in µM/mg protein. At the end of the fourth week of exposure, the snails were weighed, and then, 5 snails were randomly chosen from each treated group. Snails were killed by decapitation, and the head of each animal was excised quickly, for the determination of acetylcholinesterase (AChE) activity. Moreover, the enzymatic activity of acetylcholinesterase (AChE) was determined according to previously described protocol (Dingova et al., 2014), which somehow is close to Ellman's method, involving the preparation of a reaction mixture with homogenate supernatant, a buffer solution, and a substrate like acetylthiocholine (ATC). Following incubation, the reaction is halted, and the remaining unreacted ATC is measured using a colorimetric technique. AChE breaks down ATC, and the difference between the initial ATC concentration and the remaining amount reflects the enzyme's activity. This difference is converted into a color intensity, which can be measured by a spectrophotometer at 412nm. Finally, the AChE activity is calculated and typically expressed as micro-molar per milligram of protein in the homogenate.

Histopathological evaluation of *H. aspersa* tissues

The histopathology of *H. aspersa* was studied according to the routine histological technique (Houlot, 1984).Tissue samples from hepatopancreas and kidney of control and treated snails were preserved with formalin, dehydrated with alcohol, cleared with a solvent, and then embedded in wax. Thin slices (2-5 microns) were cut, placed on slides, and stained with dyes to highlight cellular structures. These slides were examined under a powerful microscope (Leica DM 1000 LED) for abnormalities, which were documented and photographed in high definition.

Statistical analysis

Data were provided as mean ± SE, and statistically analyzed using PrismPad software. Multiple comparisons between groups were tested by one-way ANOVA coupled with the Tukey's test. $P < 0.05$, $P< 0.01$, and $P < 0.001$ were considered significant for all analyses.

3. Results and discussion

Effect on body weight and hepatopancreas and kidney weights of *H. aspersa*

Table 1 shows a non-significant decrease in body weight, and a non-significant in kidney weight in all Cd/mixtures treated animals compared with controls. In addition, smooth tissue weight decreased significantly (P<0.001) in Cd/ZnONPs mixtures, and similarly hepatopancreas weight decreased non-significantly in M1 and M2, but increased in M3, M4 and M5 compared with control animals.

Table 1. Changes in weights of kidney, hepatoponcreas and smooth tissues of control land snails and those exposed to various mixtures (M1), containing each 1500mg $CdCl_2$/kg dry soil weight "dwt"), and increased concentrations of ZnONPs; M1 (Cd/250mg ZnONPs/kg dwt), M2 (Cd/750mg ZnONPs/kg dwt), M3 (2250mg ZnONPs/kg dwt), M4 (6750mg ZnONPs/kg dwt), and M5 (20250mg ZnONPs/kg dwt) for 28 consecutive days.

Parameters	**Body weight**	**Organ weights (g)**		
Groups		Hepatopancreas	Kidney	Smooth tissue
Control	l10.24±1.7	90.94± 0.06	0.55±0.06	6.83±0.78
M1($CdCl_2$/ZnONPs)	9.13± 0.82ns	0.93±0.08ns	0.43 ± 0.04ns	5.07±0.19***
M2($CdCl_2$/ZnONPs)	9.83±0.97ns	0.73±0.04ns	0.41±0.03ns	5.54±0.22***
M3($CdCl_2$/ZnONPs)	9.81 ±0.06 ns	0.82±0.06ns	0.46±0.03ns	5.59±0.28***
M4($CdCl_2$/ZnONPs)	9.60±0.67 ns	0.74±0.04ns	0.42±0.02ns	5.2±0.26***
M5($CdCl_2$/Zn0NPs)	9.89 ± 0.92ns	0.92±0.07ns	0.49±0.02ns	5.47±0.22***

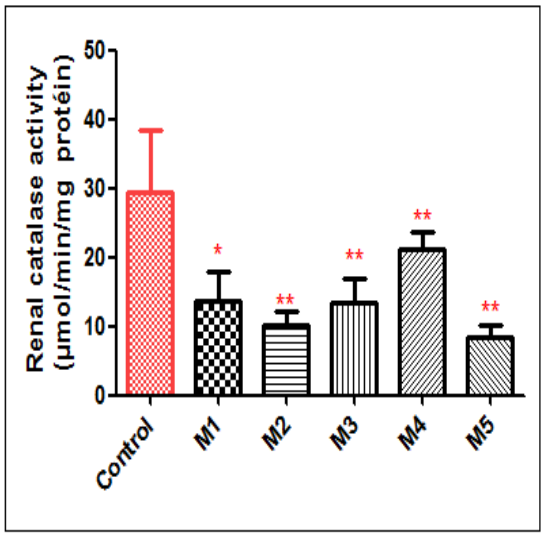
Each value is displayed as mean ± SEM (n = 6).
Values with superscripts are statistically different *p* value
**p*< 0.05, ****p*< 0.01, ***p*< 0.01 and ns: no significant versus control group.

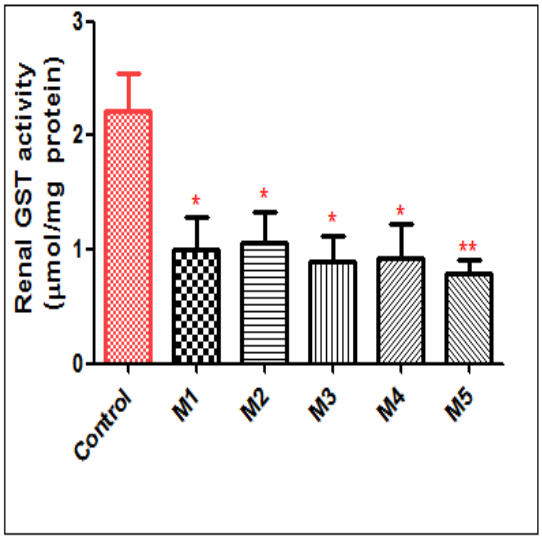
Oxidative stress markers

As shown in **Figure 1**, the enzymatic activity of catalase significantly decreased in the hepatopancreas of land snails treated with Cd/ZnONPs mixtures (M) M1, M2 (p<0.05), M3, M4 and M5 (p<0.001) and combined treatments (Cd/ZnONPs), and similarly in kidney tissue in M1(p<0.05), and M2, M3, M4 and M5 (p<0.01) compared with control group. Further, a marked decrease in the glutathione S-transferase (GST) activity in hepatopancrea was noticed in snails exposed to M3 (p<0.05), M4(p<0.01) and M5 (p<0.001), but not significant in M1 and M2. Also, the GST activity in kidney tissue deceased significantly in M1, M2, M3, M4 (p<0.05), and M5 (p<0.01) as compared to control animals.

In **Figure 2**, we noticed asignificant decreased in hepatic GSH content in (p<0.05) M3, (p<0.01) M2, and (p<0.001) M3, but not significant in M1 and M2 treatments. Similarly, the renal GSH content decreased significantly in M2and M3 treatments (p<0.05), M4 and M5 (p<0.001), but not significantly in M1 treated animals compared with controls. However, the malondialdehyde (MDA)content increased significantly in heaptopancreas of snails of M4(p<0.01), and hepatopancreas and kidney of snails of M5 (p<0.001) treatments, but not

significantly in hapatopancreas of snails of M1 and M2, and kidney of snails of M1, M2, M3 and M4 treatments compared to the control group.





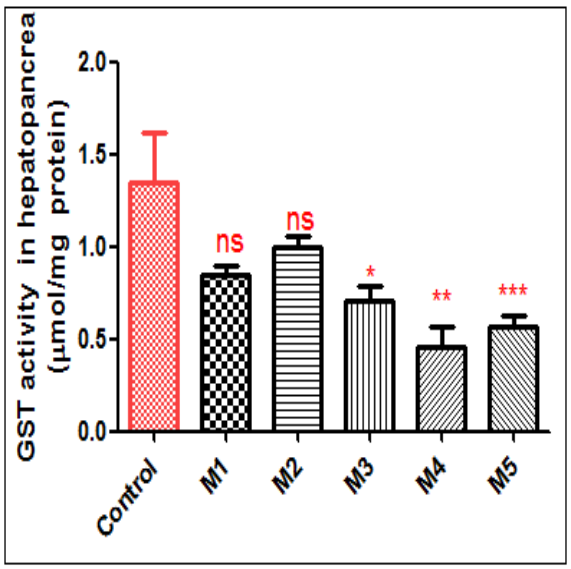
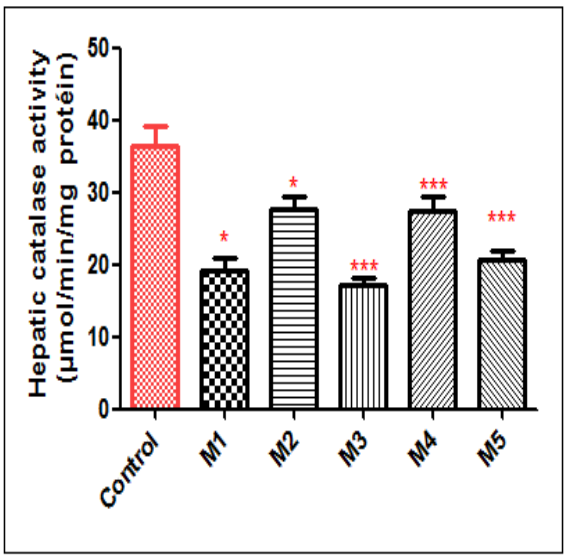
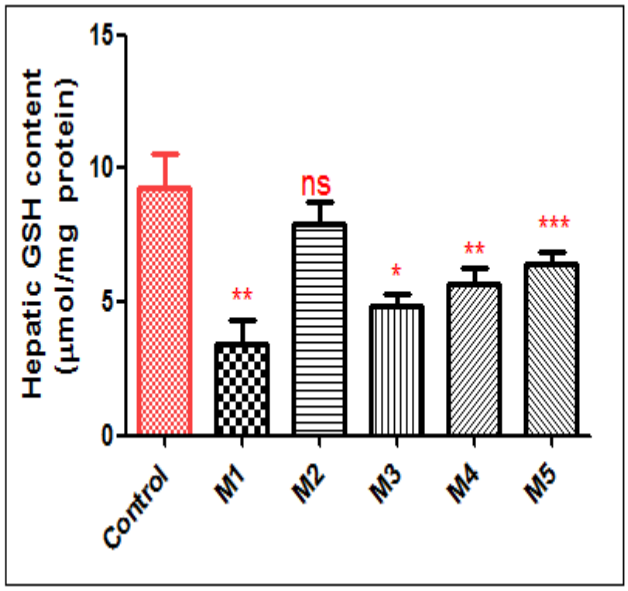


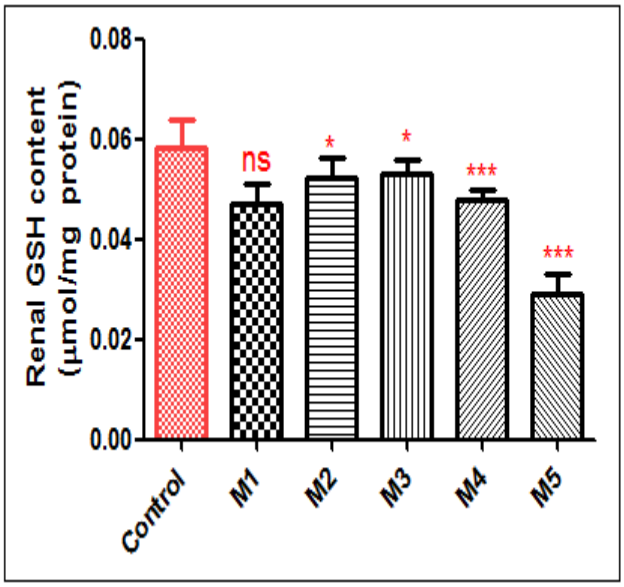
Figure 1. Changes in the enzymatic activity of catalase and glutathione S- transferase (GST) in hepatopancreas and kidney of control land snails and those exposed to various mixtures (M1), containing each 1500mg $CdCl_2$/kg dry soil weight "dwt"), and increased concentrations

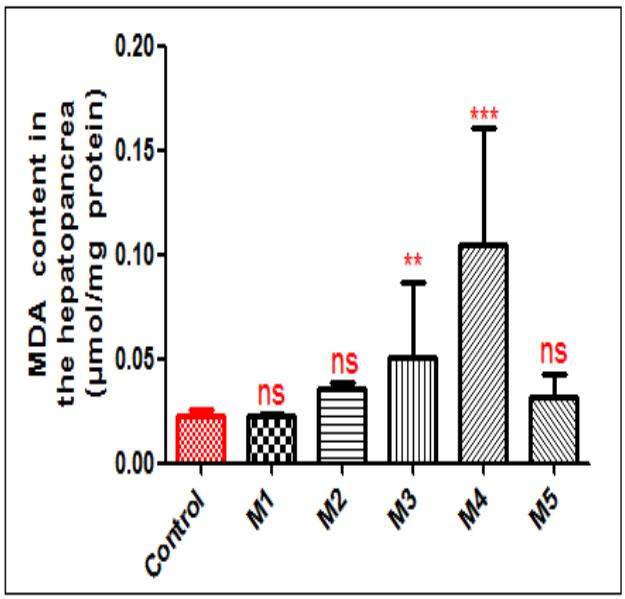


of ZnONPs; M1 (Cd/250mg ZnONPs/kg dwt), M2 (Cd/750mg ZnONPs/kg dwt), M3 (2250mgZnONPs/kg dwt), M4 (6750mg ZnONPs/kg dwt), and M5 (20250mg ZnONPs/kg dwt) for 28 consecutive days.

*p< 0.05, ***p< 0.01 and **p< 0.01 versus control group. ns: not significant versus control group.




Renal GSH content (µmol/mg protein)
0.08
0.06
0.04
0.02
0.00
ns
*
*
***
***
Control
M1
M2
M3
M4
M5


MDA content in the hepatopancrea (µmol/mg protein)
0.20
0.15
0.10
0.05
0.00
ns
ns
**
***
ns
Control
M1
M2
M3
M4
M5

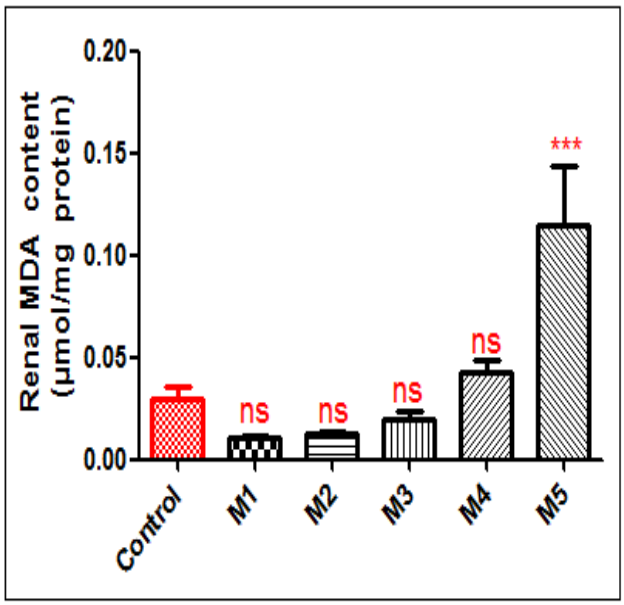

Renal MDA content (µmol/mg protein)
0.20
0.15
0.10
0.05
0.00
ns
ns
ns
ns
***
Control
M1
M2
M3
M4
M5

Figure 2. Changes in the GSH and MDA levels in hepatopancreas and kidney of control land snails and those exposed to various mixtures (M1), containing each 1500mg $CdCl_2$/kg dry soil weight "dwt"), and increased concentrations of ZnONPs; M1 (Cd/250mg ZnONPs/kg dwt), M2 (Cd/750mg ZnONPs/kg dwt), M3 (2250mg ZnONPs/kg dwt), M4 (6750mg ZnONPs/kg dwt), and M5 (20250mg ZnONPs/kg dwt) for 28 consecutive days.
*p< 0.05, ***p< 0.01 and **p< 0.01 versus control group. ns: not significant versus control group.

Effect on acetylcholine esterase activity (Ach E)
The enzymatic activity of Ach E decreased significantly and in M1($p<0.01$), M2, M3, M4 and M5($p<0.001$) treatments compared with control snails (**Figure 3**).

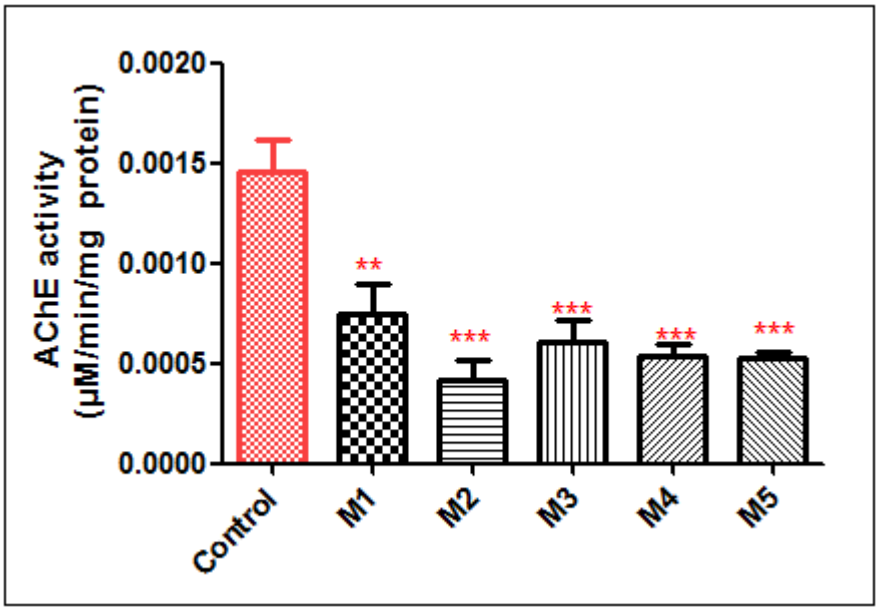


Figure 3. Changes in the AChE activity in control land snails and those and those exposed to various mixtures (M1), containing each 1500mg $CdCl_2$/kg dry soil weight "dwt"), and increased concentrations of ZnONPs; M1 (Cd/250mg ZnONPs/kg dwt), M2 (Cd/750mg ZnONPs/kg dwt), M3 (2250mg ZnONPs/kg dwt), M4 (6750mg ZnONPs/kg dwt), and M5 (20250mg ZnONPs/kg dwt) for 28 consecutive days.
*p< 0.05, ***p< 0.01 and **p< 0.01 versus control group. ns: not significant versus control group.

Hepatopancreas and kidney histopathological results
Figure 4 shows the different anomalies caused in hepatopancreas of snails compared to their control. The hepatopancreas control group, (**Fig. 4A**) reveals a healthy histology, formed by several tubules composed of secretory cells, absorbent cells and basal cells separated by fibrous connective tissue. The sections of the group treated with Cd/ZnONPs revealed serious anomalies evidencedby atrophy, degeneration and fat vaculationin addition to necrotic change of tubule cells. However, the severity of histological alterations decreased in M5: **Fig. 4B**, M4: **Fig. 4C**, M3: **Fig. 4D**, M2: **Fig. 4E, and** M1:**Fig. 4F**.
As shown in **Figure 5**, the kidney sections from control snails revealed a normal structure of hemolymphatic lacunae, and prismatic epithelium composed of homogeneous known asnephrocytes (**Fig. 5A**). After exposure to the Cd/ZnONPs mixtures, the kidney developed cellular hyperplasia with a vascular conjunction, and necrotic cells. While, these histological

alterations in the kidney are significantly reduced in M5: **Fig. 5B**, M4: **Fig. 5C**, M3: **Fig. 5D**, and M4: **Fig. 5E,** M5: **Fig. 5F treatments**.

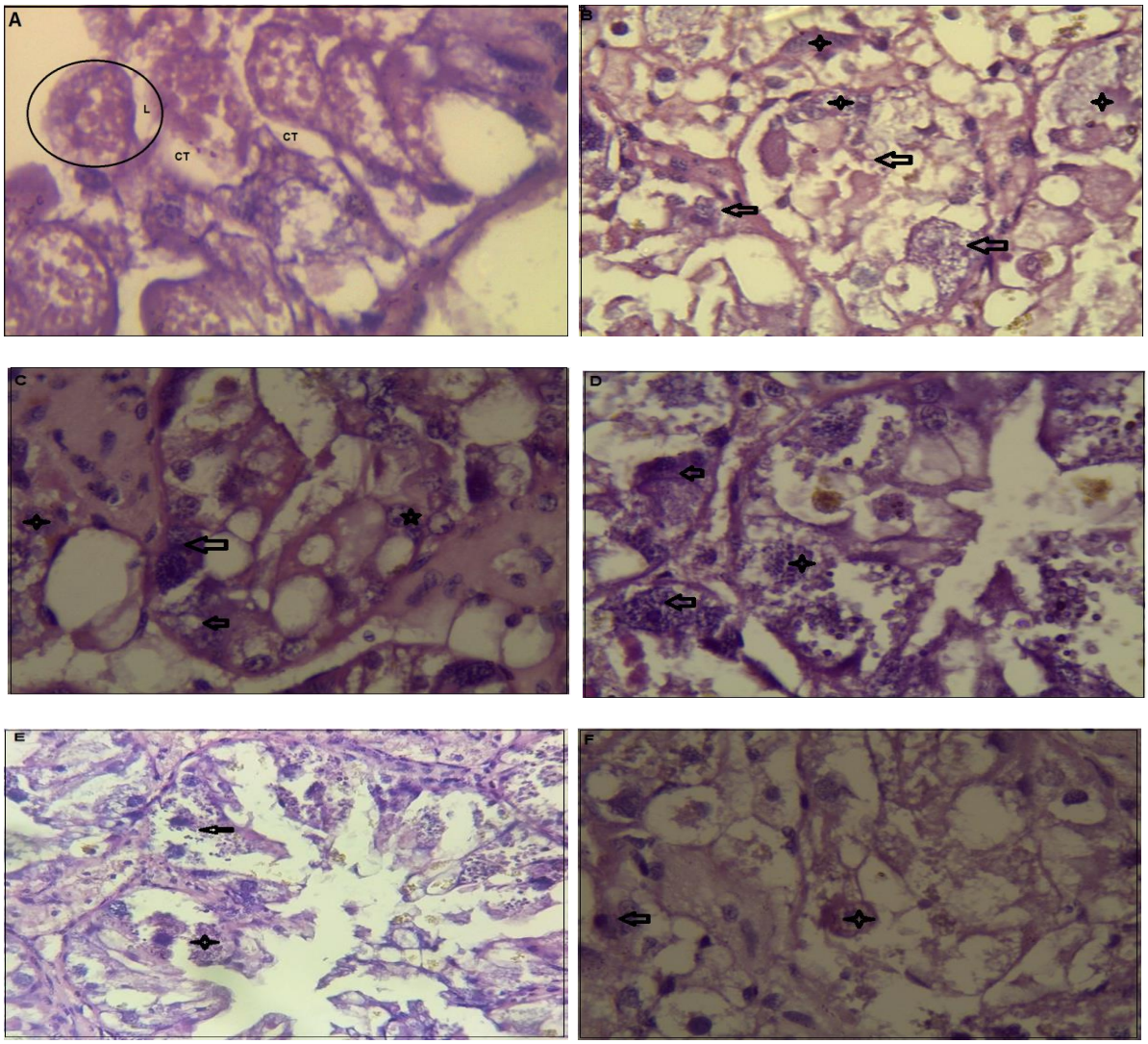
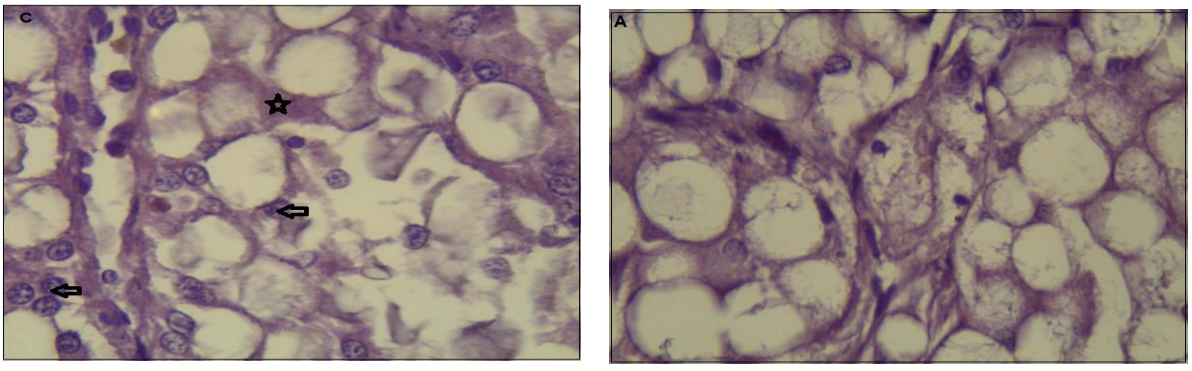


Figure 4. Histological alterations in the hepatopancreas (x 40) of control snails (**A**), and snails exposed to M5 (**B**) (1500mg Cd + 20250mg ZnONPs/kg dry soil weight "dwt"), M4 (**C**) (1500mg Cd + 6750mg ZnONPs/kg dwt), M3 (**D**) (1500mg Cd + 2250mg ZnONPs/kgdwt), M2 (1500mg Cd + 750mg ZnONPs/kg dwt) (**E**) and M1 (1500mg Cd + 250mg ZnONPs/kg dwt) (**F**)for 28 consecutive days.**CT**: Connective tissue, **Cercle with L**: Lobule, **Stars**: Atrophy, degeneration and fat vaculation, **Arrows**: necrotic change of cells of tubules.



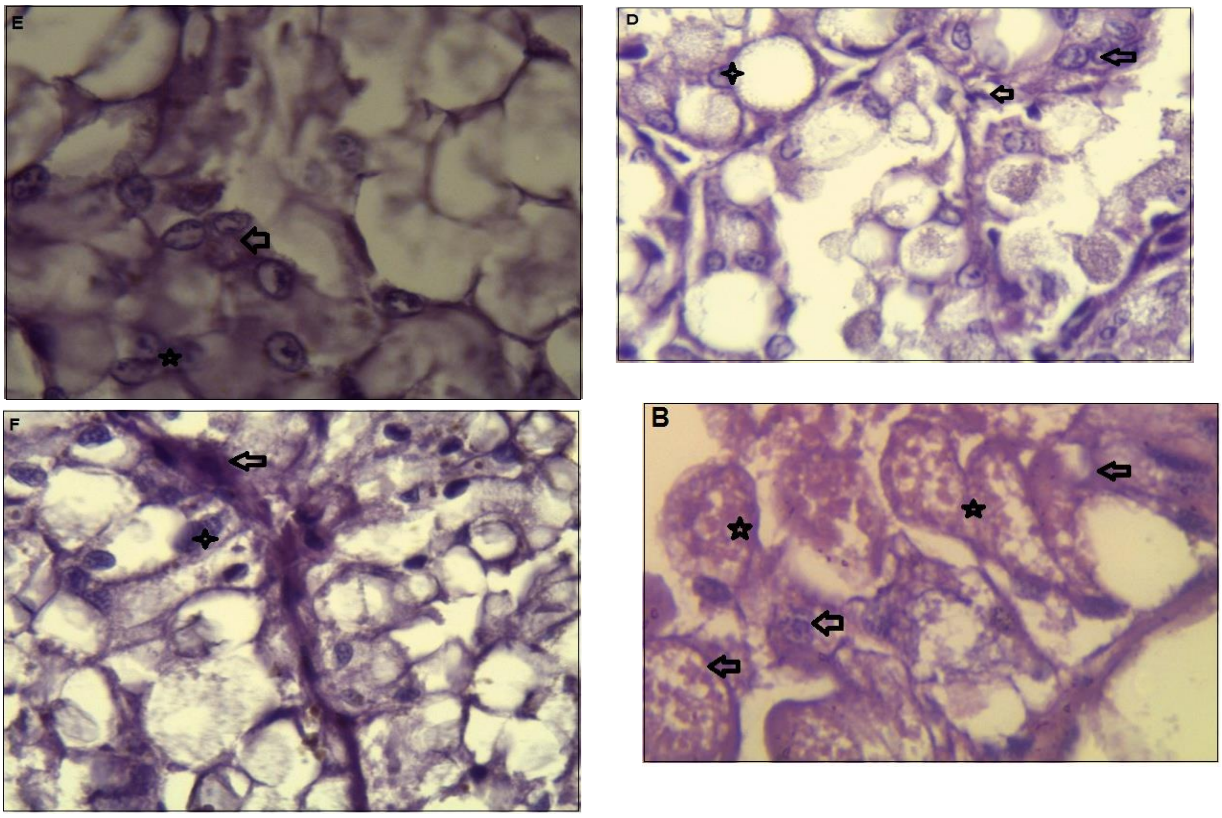


Figure 5. Histological alterations in the kidney of control snails and snails exposed to M5 (**B**) (1500mg Cd + 20250mg ZnONPs/kg dry soil weight "dwt"), M4 (**C**) (1500mg Cd + 6750mg ZnONPs/kg dwt), M3 (**D**) (1500mg Cd + 2250mg ZnONPs/kgdwt), M2 (1500mg Cd + 750mg ZnONPs/kg dwt) (**E**) and M1 (1500mg Cd + 250mg ZnONPs/kg dwt) (**F**)for 28 consecutive days. **Arrow**: necrosis, **Star:** Cellular Hyperplasia. (x 40)

4. Discussion

Heavy metals, in particular, cadmium are particularly harmful to snails due to their impact on vital organs, including the hepatopancreas where metals can accumulate, and the kidneys, interfering with their role in filtering waste and maintaining water balance (Huang et al., 2018; Qiu et al., 2021). Also, ZnONPs were reported to be toxic at high concentrations for non target organisms, and thus their combination with cadmium might have considerable toxicities on human and animal health (Abdel-Azeem and Osman, 2021; Ali et al. 2012). In this regard, ZnONPs+ Cd mixtures decreased body weight of animals but not significantly as compared with controls. This result suggests the less toxicity of these mixtures on animals' growth. The obtained results revealed decreased heaptopancreas, kidney and smooth tissue in mixture treatments compared to the control group. This result showing reduced weight of the soft tissues following the oral exposure of snails to metals has been previously reported in some previous studies (Helmy et al., 2022; Korni et al. 2022). The decreased organ weights in cadmium and/or ZnONPs exposed snails can be explained their involvement in the process of detoxification and excretion of toxicants (Yap et al., 2023). In addition, cadmium and /or ZnONPs can interfere with calcium uptake and utilization by the body. This can lead to smooth muscle dysfunction and hypertrophy as the muscles try to compensate for the altered calcium levels(Pinkina et al., 2022). Moreover, Cd/ZnONPs mixtures caused increased MDA content in hepatopancreas and kidney, which somehow proves the induction of ROS

generation responsible for lipid peroxidation and cell damage (De Silva et al., 2023). As a result, metal mixture treatments decreased the enzymatic activity of catalase, GST, and the content of GSH in the heaptopancreas and kidney. This finding was reported in some previous studies (Jiang et al., 2018; Ju et al., 2024), suggesting the increased production of reactive oxygen species, as well as the inhibiting effect of cadmium on the antioxidant enzymes to neutralize free radicals and detoxify harmful compounds. In addition, cadmium exposure can deplete GSH stores by increasing its utilization for detoxification. Additionally, cadmium and ZnONPs might inhibit the enzymes involved in GSH synthesis, leading to a decrease in its overall content. On top of that, results revealed a significant decrease in the enzymatic activity of acetylcholinesterase (AChE) in cadmium and ZnONPs treated snails. The effect of cadmium + ZnONPson Ach E may be associated with their direct bind to the active site of AChE, hindering its ability to break down the neurotransmitter acetylcholine (ACh). This leads to accumulation of ACh at nerve synapses, disrupting normal nerve impulse transmission. Also, cadmium and ZnONPs exposure promotes free radical generation, leading to oxidative stress. These free radicals can damage AChE molecules, further reducing their activity (Arruebarrena et al., 2023).Furthermore, the altered biochemical parameters were significantly supported by the histological observations of liver and kidney tissues. In accordance with these findings, previous studies conducted on cadmium intoxicated snails revealed marked tissue alterations characterized by tubular necrosis and hypertrophy(Manzl et al., 2004), and similarly, ZnONPs were found to cause histological abnormalities evidenced by cell necrosis and congestion (Ali et al., 2012).

5. Conclusion

The study demonstrates that ZnONPs combined with cadmium caused toxicity in land snails evidenced by marked changes in hepatopancreas and kidney weights, but no significant effect on body weight, and marked alterations in the biochemical and antioxidant parameters, in addition to serious histological damages.

Acknowledgements.
The authors would like to express their thanks to the Ministry of Higher Education and Scientific Research, the General Directorate of Scientific Research and Technological Development (DGRSDT) as well as for the financing of this work. We also thank Mr. AZIOUN Amar, Director of the Constantine Biotechnology Research Center, for his help.
Conflict of interest: The authors declare no competing interests.

6. References

1. Abdel-Azeem, H. H. and Osman, G. Y. (2021). Oxidative stress and histopathological effect of zinc oxide nanoparticles on the garden snail Helix aspersa. Environ Sci Poll. Res, 28, 9913–9920.
2. Akhtar, N., Khan, S., Rehman, S. U., Rehman, Z. U., Mashwani, Z. U. R., Rha, E. S. and Jamil, M. (2021). Zinc oxide nanoparticles enhance the tolerance and remediation potential of Bacillus spp. against heavy metal stress. Adsorp Sci Tech, 2021, 1774528.
3. Ali, D., Alarifi, S., Kumar, S., Ahamed, M. and Siddiqui, M. A. (2012). Oxidative stress and genotoxic effect of zinc oxide nanoparticles in freshwater snail Lymnaea luteola L. Aquat toxicol, 124, 83–90.
4. Arruebarrena, M. A., Hawe, C. T., Lee, Y. M., and Branco, R. C. (2023). Mechanisms of cadmium neurotoxicity. International J Mol Sci, 24(23), 16558.
5. Ayub, A., & Wettig, S. (2022). An overview of nanotechnologies for drug delivery to the brain. Pharm, 14(2), 224.

6. Beers, R. F., & Sizer, I. W. (1952). A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J Biol chem, 195(1), 133–140.
7. Bradford M. (1976). A rapid and sensitive method for the quantities of microgram quantities of protein utilizing the principle of protein binding. Anal Biochem, 72, 248–254. https://doi.org/10.1016/j.cj.2017.04.003
8. Cai, L.-M., Wang, Q.-S., Wen, H.-H., Luo, J. and Wang, S. (2019). Heavy metals in agricultural soils from a typical township in Guangdong Province, China: Occurrences and spatial distribution. Ecotoxicol environ saf, 168, 184–191.
9. Cai, Y., Yin, Y., Li, Y., Guan, L., Zhang, P., Qin, Y., et al. (2020). Cadmium exposure affects growth performance, energy metabolism, and neuropeptide expression in Carassius auratus gibelio. Fish physiol biochem, 46, 187–197.
10. De Silva, N. A. L., Marsden, I. D., Gaw, S. and Glover, C. N. (2023). Physiological and biochemical responses of the estuarine pulmonate mud snail, Amphibola crenata, sub-chronically exposed to waterborne cadmium. Aquat Toxicol, 256, 106418.
11. Dingova, D., Leroy, J., Check, A., Garaj, V., Krejci, E. and Hrabovska, A. (2014). Optimal detection of cholinesterase activity in biological samples: Modifications to the standard Ellman's assay. Anal biochem, 462, 67–75.
12. Gnatyshyna, L., Khoma, V., Martinyuk, V., Matskiv, T., Pedrini-Martha, V., Niederwanger, M., et al. (2023). Sublethal cadmium exposure in the freshwater snail Lymnaea stagnalis meets a deficient, poorly responsive metallothionein system while evoking oxidative and cellular stress. Comparative Biochem Physiolt C: Toxicol Pharmacol, 263, 109490.
13. Gomot-De Vaufleury, A. (2000). Standardized growth toxicity testing (Cu, Zn, Pb, and Pentachlorophenol) with Helix aspersa. Ecotoxicol Environ Saf, 46(1), 41–50.
14. Habig, W. H., Pabst, M. J. and Jakoby, W. B. (1974). Glutathione S-transferases the first enzymatic step in mercapturic acid formation. J Biol Chem, 249(22), 7130–7139.
15. Helmy, E. T., Ali, M. A., Ayyad, M. A., Mohamedbakr, H. G., Varma, R. S. and Pan, J. H. (2022). Molluscicidal and biochemical effects of green-synthesized F-doped ZnO nanoparticles against land snail Monacha cartusiana under laboratory and field conditions. Environ Poll, 308, 119691.
16. Houlot, R. (1984). Techniques d'histopathologie et de cytopathologie. Ed Maloine, 19, 225–227.
17. Huang, F., Peng, L., Zhang, J., Lin, W. and Chen, S. (2018). Cadmium bioaccumulation and antioxidant enzyme activity in hepatopancreas, kidney, and stomach of invasive apple snail Pomacea canaliculata. Environ Sci Poll Res, 25, 18682–18692.
18. Jiang, J., Pi, J. and Cai, J. (2018). The advancing of zinc oxide nanoparticles for biomedical applications. Bioinor Chem Applicat, 2018(1), 1062562.
19. Ju, Z., Ndandala, C. B., Lei, Y., Shija, V. M., Luo, J., Wang, P., et al. (2024). Cadmium-induced oxidative stress, histopathology, and transcriptomic changes in the hepatopancreas of Fenneropenaeus merguiensis. Aquacul Rep, 36, 102061.
20. Korni, F. M. M., Moawad, U. K., Mohammed, A. N. and Edrees, A. (2022). Prevention of Citrobacter freundii (MW279218) infection in Nile tilapia, Oreochromis niloticus using zinc oxide nanoparticles. Korean Pathol J, 35(1).
21. Kumar, N., Kumar, S., Singh, A. K., Gite, A., Patole, P. B. and Thorat, S. T. (2022). Exploring mitigating role of zinc nanoparticles on arsenic, ammonia and temperature stress using molecular signature in fish. J Trace Elem Med Biol, 74, 127076.
22. Manocha, L. M., Valand, J., Patel, N., Warrier, A. and Manocha, S. (2006). Nanocomposites for structural applications.
23. Manzl, C., Krumschnabel, G., Schwarzbaum, P. J. and Dallinger, R. (2004). Acute

toxicity of cadmium and copper in hepatopancreas cells from the Roman snail (Helix pomatia). Comp Biochem Physiol C: Toxicol Pharmacol, 138(1), 45–52.
24. Nica, D. V, Bordean, D.-M., Borozan, A. B., Gergen, I., Bura, M. and Banatean-Dunea, I. (2013). Use of land snails (Pulmonata) for monitoring copper pollution in terrestrial ecosystems. Rev Environ Contam Toxicol, 95–137.
25. Palit, S. (2018). Recent advances in the application of engineered nanomaterials in the environment industry—a critical overview and a vision for the future. Handbook of nanomaterials for industrial applications, 883–893.
26. Pinkina, T., Zymaroieva, A. and Fedoniuk, T. (2022). Cadmium impact on the growth and survival rate of great pond snail (Lymnaea stagnalis) in the chronic experiment. Biologia, 77(3), 749–756.
27. Pothiwong, W., Laorpaksa, A., Pirarat, N., Sirisawadi, S., Intarapanya, J. and Jianmongkol, S. (2007). Autoxidation of brain homogenates from various animals as measured by thiobarbituric acid assay. J Pharmacol Toxicol Meth, 56(3), 336–338.
28. Qiu, H., Liu, W., Yan, Y., Long, J. and Xie, X. (2021). Effects of waterborne cadmium exposure on Spinibarbus sinensis hepatopancreas and kidney: Mitochondrial cadmium accumulation and respiratory metabolism. Comp Biochem Physiol C: Toxicol Pharmacol, 248, 109115.
29. Thanh-Nho, N., Marchand, C., Strady, E., Huu-Phat, N. and Nhu-Trang, T.-T. (2019). Bioaccumulation of some trace elements in tropical mangrove plants and snails (Can Gio, Vietnam). Environ Poll, 248, 635–645.
30. Wang, S., Liu, H., Zhang, Y. and Xin, H. (2015). The effect of CuO NPs on reactive oxygen species and cell cycle gene expression in roots of rice. Environ Toxicol Chem, 34(3), 554–561.
31. Weckbecker, G. and Cory, J. G. (1988). Ribonucleotide reductase activity and growth of glutathione-depleted mouse leukemia L1210 cells in vitro. Cancer Lett, 40(3), 257–264.
32. Wu, Y. Y., Tian, W. F., Cheng, C. X., Yang, L., Ye, Q. Q., Li, W. H. and Jiang, J. Y. (2023). Effects of cadmium exposure on metabolism, antioxidant defense, immune function, and the hepatopancreas transcriptome of Cipangopaludina cathayensis. Ecotoxicol Environ Saf, 264, 115416.
33. Xu, Y., Wu, Y., Han, J. and Li, P. (2017). The current status of heavy metal in lake sediments from China: Pollution and ecological risk assessment. Ecol Evol, 7(14), 5454–5466.
34. Yap, C. K., Pang, B. H., Cheng, W. H., Kumar, K., Avtar, R., Okamura, H., et al. (2023). Heavy metal exposures on freshwater snail Pomacea insularum: Understanding its biomonitoring potentials. Appl Sci, 13(2), 1042.
35. Zahra, Z., Habib, Z., Chung, S. and Badshah, M. A. (2020). Exposure route of TiO2 NPs from industrial applications to wastewater treatment and their impacts on the agro-environment. Nanomaterials, 10(8), 1469.