Evaluation of iron in *Cyclophorus volvulus* at Mopa Airport site and trace metal toxicity in Aquatic snail *Littorina scutulata*

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Evaluation of iron in *Cyclophorus volvulus* at Mopa airport site and trace metal toxicity in Aquatic snail *Littorina scutulata*

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COMPLETION CERTIFICATE

This is to certify that the dissertation "Evaluation of trace metals in Cyclophorus volvulus at Mopa airport site and trace metal toxicity in Aquatic snail Littorina scutulata" is a bonafide work carried out by Ms. Rashmi Prakash Anurlekar under my supervision/mentorship in partial fulfilment of the requirements for the award of the degree of Master of Science in Zoology in the Zoology Discipline at the School of Biological Sciences and Biotechnology, Goa University.

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DECLARATION BY STUDENT

I hereby declare that the data presented in this Dissertation entitled, "Evaluation of trace

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Zoology Discipline at the School of Biological Sciences and Biotechnology, Goa University

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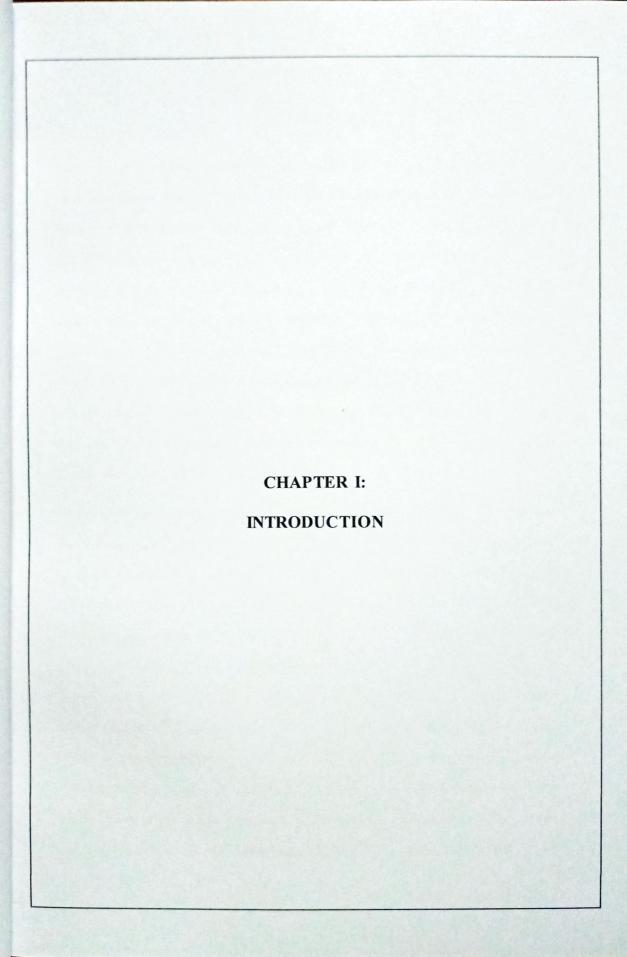
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1. INTRODUCTION

Trace elements are natural parts of the earth layer and cannot be degraded, the regular release of these elements threatens both the environment and human health due to their toxicity through the food chain and other human exposure routes. One of the main sources of soil pollution are human activities, such as the burning of fossil fuels, smelting and the use or production of metal compounds, which are responsible for most of the trace elements. Heavy metal pollution in the environment is increasingly problematic and causes widespread concern due to its negative consequences. Due to the rapid development of agriculture and metal industry, as well as improper waste disposal, these inorganic pollutants enter the water, soil and atmosphere. Heavy metals can be released into the atmosphere by both natural and anthropogenic factors. The main sources of emissions are man-made, especially mining. (Hutton and Symon, 1989; Battarbee et al. 1988)

These metals are washed out and in disturbed areas, travel downstream in waterways, contaminating the water and surrounding land. Metals move through rivers and streams either as dissolved species in the water or as solids in suspended sediments. They can then be stored in river bottom sediments or seep into underground sources, especially wells; and the extent of contamination depends on the proximity of the water body to the construction site. Heavy metals occur in nature in the aquatic environment mostly in very small concentrations. Increased concentrations of these pollutants are caused by various human activities such as mining, offshore oil exploration, industrial discharges such as pesticides, fertilizers, medical waste, and agricultural runoff. The toxicity of a metal depends on its nature and concentration in the environment. Some metals, in certain concentrations, are essential for the survival of aquatic organisms. Metals can also interact with suspended particles in water and this can be one of the mechanisms of their concentration and distribution patterns. These metals are later

incorporated into water sediments, where concentrations typically exceed those in the water column many times over. These high concentrations can have several consequences for benthic organisms. Release of heavy metals into a river or any marine environment can cause drastic changes in marine species diversity and marine ecosystem due to their toxicity and cumulative behaviour (Bat et al., 2009). Marine organisms such as fish can accumulate heavy metals at concentrations many times higher than in water or sediments (Bat et al. 2009; Boran and Altinok, 2010).

The toxicity of heavy metals is related to their ability to inhibit enzymatic processes and their low mobility in the body due to their small size and unsaturated chemical bonds, which promotes their accumulation and the resulting change in the metabolism of organisms. The toxicity of this heavy metal in aquatic organisms and its balance depends on several limnological factors that determine its concentration in the environment and its availability to aquatic organisms, such as pH, alkalinity, hardness, organic matter, total solids and sedimentation.

Toxicity occurs when the rate of intake of a metal from all sources exceeds the rate of detoxification and excretion of that metal. The biodynamic model of trace metal bioaccumulation allows to predict and explain very different accumulated concentrations of trace metals in organisms by combining geochemical analyses of environmental metal concentrations with measurements of the basic physiological parameters of the species in the considered area.

1.1 Status of Metal Pollution in Goa

Goa has been a mining hub for so many years that this man-made activity has caused enough destruction and metal pollution in the state in previous years. Promoting the development of the country, the state and the central government have come up with a new international

airport project in North Goa. The new airport has created problems for local residents and the surrounding flora and fauna. Conversion of 2,132 hectares of fertile plains into an airport has cause massive disruption to the surrounding biodiversity.

Water and sediment pollution along the coast of Goa due to organic and inorganic pollutants is a major problem. According to the Indian Bureau of Mines, Ministry of Mines (Government of India), Goa accounts for 18% of India's iron ore and 3% of its manganese ore (IBM, 2014). According to Dessai and Nayak (2008), surface sediments of the Zuari estuary are mostly contaminated by manganese, followed by cobalt and the least by chromium, zinc, copper and iron. They further report that the main source of these metals is mining and related activities such as ore transportation via offshore platforms, ore loading and barge construction.

The previous studies suggest that metal pollution has become a concern in Goa. Agadi et al. in 1978 found that marine algae collected from five localities of Goa had considerable variations in their concentration of Co, Cu, Fe, Mn, Ni, Pb, and Zn, suggesting that different seaweeds could be used as indicators of metal pollution. Kerkar et al. in 2011 analysed that the sediments of the Divar mangrove swamp in the Mandovi estuary have a higher Pollution Load Index (PLI) than the Tuvem mangrove swamp along the Chapora River, indicating greater anthropogenic inputs into the ecosystem. Singh et al. (2016) study reveals that most of the groundwater samples during the monsoon and post-monsoon seasons were found less polluted with respect to heavy metals concentration. The concentrations of Pb, Cu, Mn, Zn, Fe, Cd and Cr were found within limit except for Fe content during the monsoon season in few locations which is above desirable limit recommended for drinking water by the Bureau of Indian Standard (BIS 2003).

The metal distribution in the Mandovi estuary revealed preferential input through open-cast iron-ore mining, industrial, fishing and agricultural activities. The heavy riverine runoff associated with high rainfall influenced the distribution of Mn, Zn and Pb during monsoon season. In addition, sediment grain size and associated organic matter governed metal distribution in surface sediments. The role of grain size and organic matter along with Fe-Mn oxides in the distribution of metals was construed through correlation and factor analysis. Geo-accumulation index, contamination factor and potential contamination index indicated contamination of surficial sediments of the Mandovi estuary with Cr and Pb. (Goankar et al., 2021)

In mining areas of Goa, Yellishetty et al. (2009) reported minerals such as pyrrhotite, platinum-bearing marcasite, magnemite and gold bearing arsenopyrite in the waste rocks; all are capable of producing acid mine drainage and are also capable of releasing metals.

The major natural sources of metals include weathering of river basin rocks and wind transport (Bianchi 2007; Nasnodkar and Nayak 2019), whereas the discharge of metals through anthropogenic sources generally occurs via ore mining, industries, agricultural, domestic and municipal activities (Prego et al., 2008; Nayak and Noronha D'Mello 2018; Gaonkar et al., 2021).

The trace metal concentration in water and sediment of Mandovi was higher than that of Chapora. The concentration in the tissues was in the range of 1205.2-2506.7 ppm for Paphia malabarica, 1906.2-2802.6 ppm for Perna viridis and 778.7-1607.5 ppm for Saccostrea cucullate in Mandovi estuary. The values for Chapora were 199.4-625.8 ppm for P. malabarica, 812.6 – 1220.2 for P. viridis and 392.5-418.6 ppm for S. cucullate. The anthropogenic input of metal in Mandovi estuary appears to be mainly responsible for the high accumulation of trace metals.

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1.3 Snail as bioindicator of metal pollution

The use of animals as bioindicators is extremely important, especially in the evaluation of environmental effects caused by human activities, such as sewage and water discharges. Bioindicators are species used as primary indicators of environmental pollution defined by the response of an organism to an environmental pollutant that can be measured in a single biological matrix indicating a deviation from its homeostasis. Organisms such as aquatic plants, algae, crayfish, molluses, fish, mammals, birds can be considered as bioindicators. (Sanches Filho et al., 2017)

An ideal bioindicator should live in a healthy environment and survive by resisting the pollutants to which it is exposed. It is also important to consider the abundance of this species in the environment and its adaptability to laboratory experiments.

To be a robust control species for environmental monitoring, the following criteria must be met: wide range, known biology, immobility, early warning capacity, key ecosystem function, homogeneous response to pollutants, and presence of detectable toxic effects to the level of pollution. (Hilty and Merenlender, 2000; Goodsell et al., 2009)

There is a growing need for appropriate and reliable bioindicator methods to assess human impacts on aquatic ecosystems and for restoration and remedial measures. The growing need for methods to assess the actual state of ecosystems and monitor their rate of change has encouraged research into how communities of organisms can be used as indicator systems of human-induced environmental change or as a basis for conservation priority or restoration criteria tools (Rosenberg and Resh, 1993). Many exotic species are effectively used in toxicity tests (Bertoletti et al., 2009). However, the use of non-commercial and easily caught-bred local species as bioindicators is important from the point of view of environmental protection, mainly because the introduction of foreign species into the natural environment is

undesirable, because they damage the local water community reproduction and competition for resources.

To assess the effect of contaminants in the matrix, species representative of the environment must be studied. In fact, a biomonitor can be identified as an organism or a group of organisms that is important for the qualitative and quantitative determination of environmental pollution (Fränzle, 2006). Many studies have shown that molluscs, including land snails, are bioindicators of pollution, including metals, pesticides, PAHs, PCBs and PBDEs (Beeby and Richmond, 2002; Fu et al., 2011; De Vaufleury and Pihan, 2000; Silva et al., 2019). Such species play an important role in soil, air, and vegetation interactions in the environment and contain many sources of contamination (soil, atmosphere, plants) through the gastrointestinal tract, respiratory tract, and skin. Several terrestrial invertebrates, have been used as bioindicators of metal contamination to assess metal contamination in highway environments (Berger and Dallinger 1993; Newman et al., 1994). Terrestrial invertebrates are often used to monitor air and soil pollution (Dallinger 1994). This is because they have the ability to accumulate organic and inorganic chemicals of different origins and to react to them both at the organismic and cellular level (Snyman et al., 2000; Ragoli et al., 2005). Several snail species were observed, for example Papillifera papillaris (Emilia et al., 2016), Indothais gradata (Proum et al., 2016), Pomacea canaliculata (Ramli et al., 2019; Dummee et al., 2012), Cantareus apertus (Meiki et al., 2016), Helix aspersa (Viard et al., 2004; Abdel-Halim et al., 2013), Eobania vermiculata (El-Shenawy et al., 2012; Itziou and Dimitriadis Itziou, 2011), Cepaea nemoralis (Boshoff et al., 2015), Bellamya aeruginosa (Yin et al., 2014), Theodoxus niloticus (Abdel Gawad, 2018) and Achatina fulica (Cho et al., 2019). Such snails live in most environments and are often found at the edges of agricultural and forest areas. Invertebrate species accumulate pollutants as a result of absorptionassimilation, storage, transfer and excretion processes (Regoli et al., 2006). Once pollutants are absorbed, land snails have a regulatory strategy influenced by calcium metabolism (Notten et al., 2005) and the need to avoid excessive water loss (Dallinger et al., 2001). There are actually two main parts to consider: the leg and the internal organs. Guts refer to the organs in the shell and include the kidney, hepatopancreas, heart and part of the reproductive system that extends to the leg. The leg basically consists of the front part of the digestive system and the nervous system. It should also be noted that snails are involved in several food chains: carabids, amphibians, mammals, birds, other arthropods and humans. Thus, heavy metal contamination of snails can be transferred to consumers, leading to the risk of secondary poisoning. In contrast, several species have shown the ability to resist and accumulate pollutants (Notten et al., 2005; Radwan et al., 2010; Scheifler et al., 2002). Therefore, in the last two decades, many researchers around the world have focused on the bioaccumulation of trace elements in snail species. Fluctuations in the content of trace elements in snails depend on the feeding system of the snails, agricultural activities and metal bioavailability in the natural environment. Snails are distinguished from other mollusc groups because they can accumulate trace elements in higher concentrations. Thus, snails can provide an opportunity to assess residual pollutants by acting as bioindicators.

Due to their wide distribution, snails can be used to assess the bioavailability of soil pollutants by measuring soil-snail or soil-plant-snail translocation, as well as the bioavailability of environmental pollutants by measuring their concentration in caged snails. Snails can reflect atmospheric quality by accumulating some of the toxic pollutants found in their habitat in their spiracles or visceral pulp and shell. Their mucus protects them against external threats and bacterial and fungal infections (De Vaufleury and Gimbert, 2009). Land snails are sensitive to pollution and soil acidification. Therefore, snails have been proposed as useful animals for bioindication and biomonitoring of heavy metals in terrestrial environments, as they can accumulate metals in their tissues. In the past, snails were used to

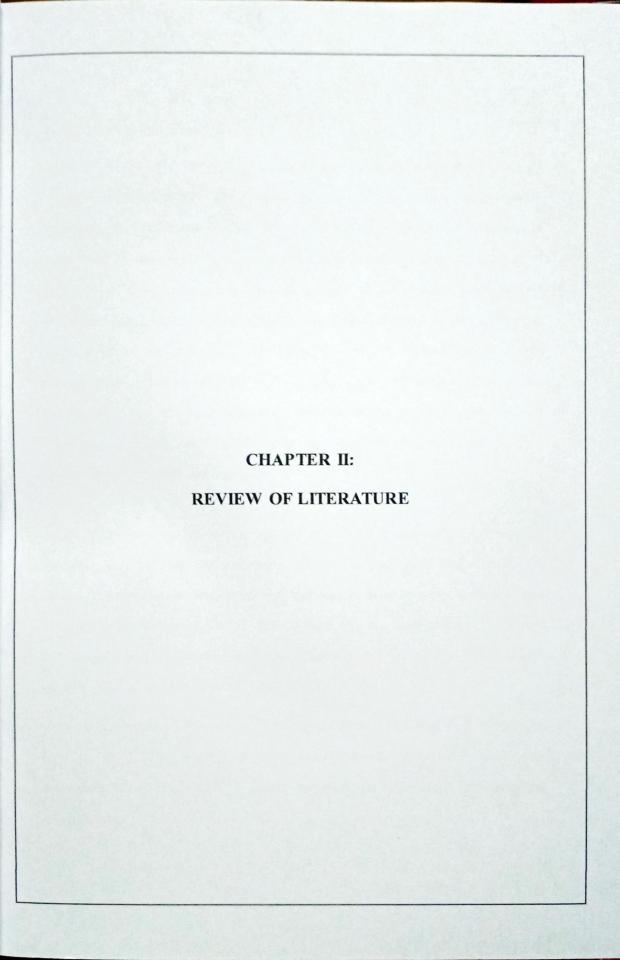
evaluate heavy metal remediation techniques in waste management (Pihan and de Vaufleury 2000). In fact, snails are a suitable tool for diagnosing heavy metal-contaminated sites and can concentrate large amounts of metals in their soft tissues without significant metabolic disturbances (Swaileh et al., 2001; Notten et al., 2005; Yap and Cheng 2013; Mahmoud)

The evaluation of the accumulation of pollutants, depends on the evaluation of internal concentrations after a certain period of exposure and provides an opportunity to evaluate the accumulation capacity of snails, their bioavailability and the force of transfer of pollutants from the environment (Gimbert et al., 2006). Snails can be easily collected, grown, identified and found almost anywhere. In addition, snails are exposed to a variety of pollutants that they consume through the skin, gastrointestinal tract, and respiratory tract from water, plants, and contact with soil (De Vaufleury and Pihan, 2000).

For example, gastropod molluscs were shown to have a high capacity to accumulate heavy metals, especially Cu, Zn, Cd and Pb, as shown by Viard et al., (2004). In fact, it was found that approximately 68% of consumed cadmium (Cd), 90% of copper (Cu), 43% of lead (Pb), and 60% of zinc (Zn) accumulated in *Helix aspera* snail soft tissues, shells and faeces were analyzed by flame atomic absorption spectrometry after mineralization and extraction with concentrated HNO₃. Snails appear to be a more important transport route in the food chain for Cu and Cd than for Zn and Pb, and they are unable to accumulate large amounts of metals in their shells. The resistance of snails to the effects of metal accumulation can contaminate their predators (Laskowski and Hopkin, 1996). Some studies have shown that high doses and duration of exposure to metals lead to more severe consequences, such as kidney failure caused by a combination of inorganic arsenic and cadmium, compared to low doses and individual exposure to different elements (Wang and Fowler, 2008; Nordberg et al., 2005).

1.4. Objectives

- To evaluate the accumulation of iron in Cyclophorous volvulus near Mopa Airport construction site.
- To identify the concentration of iron present in the sediments and water bodies near the airport construction site.
- To analyze the toxic effects of trace metals in Littorina scutulata under laboratory exposure conditions.



REVIEW OF LITERATURE

The studies carried out on heavy metal in previous years collectively suggest that heavy metal pollution is a global problem caused mainly by human activities such as industrialization, urbanization, and agricultural activities. Su et al., (2014) compared and analysed soil contamination of heavy metals in various countries, while Tchounwou et al., (2012) provided an analysis of heavy metal toxicity and their potential effects on human health and the environment. Hadia-e-Fatima (2018) highlighted the hazardous impact of heavy metals on human health and the environment, while Huang et al., (2013) analysed the heavy metal pollution status in surface sediments of Swan Lake lagoon and Rongcheng Bay in the northern Yellow Sea. The studies suggest that heavy metals such as mercury, cadmium, lead, chromium, and arsenic are of public health significance and can cause multiple organ damage, genotoxicity, and carcinogenicity.

Sultana (2020) notes that, the level of heavy metals in water is significantly higher than their respective local guidelines and international standards in many parts of the world. The continuous industrialization, urbanization and unplanned dumping of waste in water bodies are considered to be main factors of increasing extent of heavy metals in water. The process of pollution by trace elements of water resources has been observed in several regions (Sun et al., 2011; Hu et al., 2013; Subramanian et al., 2015).

Heavy Metal Pollution Assessment in Surface Water Bodies carried out in Tamil Nadu by Khan et al., in 2005, states that soil contaminated with heavy metals higher than recommended values mentioned in quality guidelines are not suitable for agricultural purposes.

According to Dessai and Nayak (2008), surface sediments of the Zuari estuary are mostly contaminated by manganese, followed by cobalt and the least by chromium, zinc, copper and iron. They further report that the main source of these metals is mining and related activities such as ore transportation via offshore platforms, ore loading and barge construction.

According to Baroudi, et al., (2020), including the pollution signal across a period of time or an area has been done by using snails as sensitive environmental pollution indicators. These species are also used as sentinels because they have a weak toxic reaction or minimal control over the levels in their tissues. Studying the impact of metals and other pollutants on organism physiology contributes to the development of many toxic studies which can be used as an environmental evaluation tool. For all of these reasons, it is strongly advised to employ snails as a monitoring matrix for a variety of environmental toxins that may be present in the environment.

In another study Filho et al., (2017), concluded that the lead nitrate II was the most toxic salt for the *Phalloceros caudimaculatus* species, followed by cadmium nitrate, zinc sulfate and potassium dichromate respectively. Despite the bioconcentration, *P. caudimaculatus* is an efficient bio-indicator of Cd, Cr, Pb and Zn.

In order to determine whether the land snail *Helix aspersa* may be used as a biomarker for terrestrial ecosystems, Sidiropoulou et al., (2018) examined the biological reactions brought on by iron oxide nanoparticles (IONPs) and iron oxide NPs embedded with zeolite (IONPZ) in relation to oxidative stress. In comparison to untreated animals, both effectors caused oxidative stress in the hemocytes of snails. The findings indicated that the factors evaluated in conjunction with one another would make for potentially trustworthy biomarkers for the assessment of NPs toxicity. Jordaens et al., (2006) studied the sensitivity of Land snails found

in terrestrial environments to pollutants and soil acidification. These are available for collection in various environments including urban areas.

Vaufleury et al., (2008) in his study 'Methods for toxicity assessment of contaminated soil by oral or dermal uptake in land snails: Metal bioavailability and bioaccumulation' concluded that, the metal burden per snail enables estimation of the risk of secondary poisoning encountered by predators, this study also provides data on the bioavailability of metals in soils and on the relationships between bioaccumulation and sublethal effects.

Nica et al., (2012) assessed the heavy metal transfer in a soil-plant-snail food chain and concluded that snails can tolerate many contaminants, including heavy metals, through bioaccumulation. Consequently, land snails have been recommended as premeditating animals for bioindication and biomonitoring heavy metals under terrestrial environments, as these can accumulate metals in their tissues.

In the various tissues of *Chicoreus capucinus*, Berandah et al., (2010) investigated the bioaccumulation and distribution of heavy metals (Cd, Cu, Fe, Ni, Pb, and Zn). Since they can accumulate larger amounts of trace elements, snails can be identified from other kinds of molluscs. As a result, snails can serve as bioindicators and offer a chance to assess trace contaminants. Due to this, many scientists have been concentrating on the bioaccumulation of trace metals via snail species over the past 20 years. The differences in trace element content in snails are influenced by the snail's nutrient system, farming practices, and the bioavailability of metals in the environment.

Massadeh et al., (2016) carried out analysis of Zn, Cd, As, Cu, Pb, and Fe in snails as bioindicators and soil samples near traffic road. They concluded that snails are suitable species for biomonitoring of heavy metal pollution. In particular, H. pelasga accumulates high amounts of Pb and Cu, and E. vermiculata accumulates high amounts of As and Cd.

Also, the results showed that there was a positive correlation between metal concentration for Pb and Fe in snail samples and soil sample.

Another study on tissues of land snails *E. vermiculata*, either taken from polluted locations or treated with heavy metals in the lab, Itziou et al., (2011) conducted a thorough investigation of a suite of biomarkers. The overall findings demonstrate this land snail's value as a bioindicator organism and lend support to the adoption of this ecotoxicologic approach to evaluate the biological effects of pollutants. Neutral lipids were successfully used as a biomarker for the snail *E. vermiculata* in both the laboratory and the field. The results revealed that both acute laboratory exposure of the snails and chronic exposure to polluted conditions in the field resulted in persistent responses on each biomarker as well as statistically significant correlations among the biomarkers used. Jordaens et al., (2006) studied the sensitivity of Land snails found in terrestrial environments to pollutants and soil acidification. These are available for collection in various environments including urban areas.

Fu et al., (2011) conducted a study on apple snail and concluded that apple snails could reflect the contamination status of PCBs and PBDEs in their habitat, confirming that apple snail is a reliable bioindicator for these two POPs. Furthermore, apple snail might be used as a novel bioindicator at a larger scale for monitoring POPs contamination because they are not only distributed in this e-waste dismantling region but also in many regions around the world. In 2015, Azam et al., looked at the danger that increasing Cu, Cr, Cd, Zn, and Ni metal concentrations pose to both human and insect life in Gujarat's industrial areas. By aggressively enforcing pollution control laws and mandating safe disposal of industrial effluents in industrial zones, the study thus promotes the necessity for appropriate actions to be done to minimise increasing environmental contamination.

Srivastava and Singh (2020) reviewed that Gastropod response to pollutants indicated by the decrease in the abundance, size change and can affect the community structure. Environmental factors, body size, weight, and gender influence in the accumulation of heavy metals. Heavy metals can affect hardness, thickness, volume, and color of the shell.

The soft body of gastropods is divided into four main parts, according to Samsi et al. (2017) in their review: the head, which is typically conspicuous in the anterior of the shell; legs, which are stomach muscular organs with the essentially flat part that is used to crawl or burrow; visceral mass, which fills the back of the spire shell and contains important organ systems; and coat, an integument. Yamada et al., (2007) found out that the viscera of the alimentary tract of *Turbo marmoratus* collected from the coral reef of Ryukyu Island, accumulated some metals, especially Zn and Fe.

According to Salánki et al. (2003), the two main advantages that snails and bivalves have over most other freshwater organisms for biomonitoring research are their large size and constrained mobility. They are also common in a variety of freshwater environments, are comparatively simple to collect, and can be identified. Freshwater snails and bivalves have been employed frequently as bioindicators of both organic and inorganic pollutants, according to a study by Elder and Collins (1991).

According to Stankovic et al. (2014), the snails' tiny size, restricted movement, and place in the food chain make them superior ecological indicators. The majority of snail species serve a presiding role in fresh waters by feeding fish and improving water quality by devouring enormous amounts of debris and algae.

Walters (2018), studied that molluscs are in direct contact with the ambient medium, such as water or soil, because they lack an exoskeleton, which is present in arthropods. Chemicals can therefore be absorbed through the skin, including the respiratory system in aquatic

species, not only through the diet but also from ambient water or soil, leading to a significant accumulation potential for pollutants.

According to Amadi et al. (2020), studies on cross sections of snails taken from polluted sites can reveal the effects of prolonged exposure of the contaminants on snails' health. The majority of pollutants dispersed in their environment, such as pesticides and heavy metals, are toxic to snails. Therefore, studying the cellular biochemical characteristics of snails in the field provides an opportunity to gauge how sensitive they are to environmental stress.

In addition to environmental factors including water flow, water availability, pH, sediment, and salinity, Gundacker (2020) noted that body size, weight, and gender also have a significant impact in the bioaccumulation of heavy metals by gastropods.

Jaishankar et al. (2014) reported that bioaccumulation and acute and chronic toxicity are strongly dependent on metal speciation. This is because toxicity and bioaccumulation are not congruent with each other due to this metal speciation effect.

According to Atobatele and Olutona (2015), heavy metal builds up in the bodies of animals with soft parts as well as in gastropods' shells. Additionally, they noted that the distribution of heavy metals in the body tissues of gastropods is determined by physiological tissue and metal bonding, storage, and detoxification techniques. The large heavy metal content in each of the soft tissues and shells of gastropods were also found to be varied.

Evidenced in some studies and as stressed by Luoma (1983) Several methods of extraction and a few research using aquatic sediments have revealed relationships between metal bioavailability (Diks and Allen, 1983). However, a large number of studies investigated the metal bioaccumulation from waterborne and dietary routes separately, but paid a little attention to the interaction between the two uptake routes (Wang and Rainbow, 2008).

From reports of other metals tested on *H. aspersa* in the literature (Pb, Cu, Zn, and Cd), only Cd has been reported to show negative effects at lesser concentrations (Laskowski & Hopkin 1996).

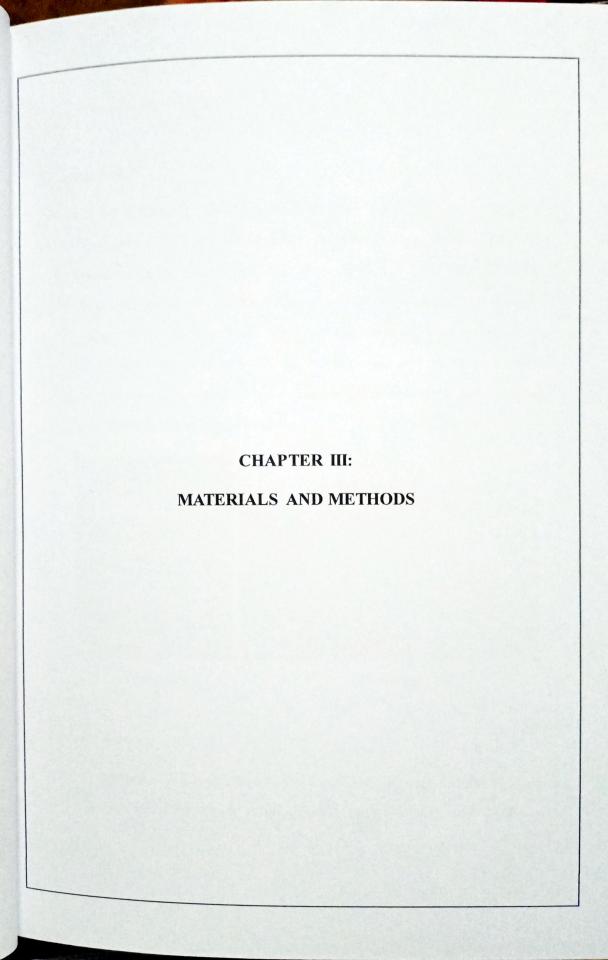
In view of this importance, many studies have been conducted on histopathological changes in gastropods caused by acute and chronic toxicity of heavy metals (Otitoloju et al., 2009; Sawasdee et al., 2011; Dummee et al., 2012; Hamlet et al., 2012; Bighiu et al., 2017). The snails were exposed to sublethal concentrations of Cu and Pb in the form of CuSO₄ (2.35mM) and Pb (NO₃)₂ (5.79mM). Exposure to sublethal copper concentrations resulted in a five-fold and three-fold accumulation of the metal in the hepatopancreas and muscular foot of snails exposed to the test metals compared to the control animal.

Since they are intended for consumption of humans and are readily eaten by animals, terrestrial snails can also be valuable tool in risk assessment (Madejón et al., 2013). Trace element concentrations in the snail bodies are still of concern for human consumption.

The freshwater snail Lymnaea acuminata was used as the test organism, and Khangarot et al., (1982) found that mixtures of compounds containing Zn₂, Cu₂, and Ni₂ produced significantly higher mortality counts in the test organisms than did the individual metal components.

The reproductive organs of molluscs are also known to be targets for metal toxicity, affecting the structure and numbers of gametes particularly (Russell et al., 1981).

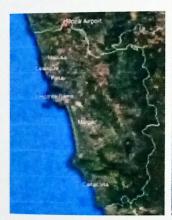
Pihan (2000) used snails for passive and active biomonitoring of an industrial waste dump, finding them useful for evaluating contamination and remediation. However, Cowie (2001) cautions against using snails as biocontrol agents due to their potential nontarget effects, indicating that their use as bioindicators may also have unintended consequences. Overall, while snails may be useful as bioindicators for pollutants and heavy metals, further research is needed to determine their effectiveness as bioindicators in Goa specifically.



3. MATERIALS AND METHODS

3.1 Study Area

The study area Nanachepani, Varconda — Nagzar (15°44'11.50"N 73°50'52.65"E) is located in the Northern part of Goa, and is situated approximately 500mtrs away, on the outskirts of the Goa International Airport, Mopa Pernem. This area was a home to diverse range of floral and faunal species. Apparently, after the onset of airport construction, in August 2016 an area of 2312 acres, mainly the agricultural plateau suffered destruction due to which the sedimentation rate in the water bodies in and around the area elevated due to excessive surface runoff. Hence a collapse of metal contaminates paved way in the surrounding ecosystem tailings its way to hazardous pollution.





(a) Mopa airport site

(b) Study area

3.2 Study Animal

 Cyclophorous volvulus (Muller, 1774) is a species of operculated land snail belonging to the family Cyclophoridae. Usually found in Monsoons from mid-June to early October in marshy areas, around leaf litter and on riverside. Feeds extensively on leaves, algae and other small creatures.

Classification

Phylum - Mollusca

Class - Gastropoda

Order – Architaenio glossa

Family - Cyclophoridae

Genus - Cyclophorus

Species - Cyclophorus volvulus



(a) specimen of Cyclophorous volvulus

2. Littorina scutulata (Gould, 1849) is a species of sea snail, a marine gastropod mollusc in the family Littorinidae. Commonly also known as the winkles or periwinkle snails. They live on rocks on the shore, with migration up and down the rocks with the tide. They crawl out of the tidepools at night. It mainly feeds on microalgae, lichens etc. Littorina scutulata are found on a rocky seashore throughout the year.

Classification

Phylum - Mollusca

Class - Gastropoda

Order – Architaenio glossa

Family - Littorinidae



(b) specimen of Littorina scutulata

Genus - Littorina

Species - Littorina scutulata

3.3 Sampling methods

3.3.1 Biota sampling

During each sampling, around 30 study organisms (*C. vulvulus*) were procured from the study area. A total of three samplings were done, each after every fifteen days, starting from 15 August 2022, for the field studies. Due care was taken that the organisms were safe and unharmed in any way during transportation.

3.3.2 Water sampling

At the time of each biota sampling, 1 L of water was collected in clean amber coloured glass bottles (500 ml X 2) from the same study site for metal analysis. Care was taken that no sediments were disturbed while collecting the water so as to avoid extra debris in the water sample.

3.3.3 Sediment Sampling

Sediment samples (~ 500 g) were collected from the study site and stored in clean labelled polythene bags. These samples were stored at 4°C and carried to the laboratory for further analysis.

3.4 Quality assurance

To prevent contamination, all glassware was thoroughly cleaned, sterilized, and placed in a hot air oven. Experiments were conducted in a clean and sterile environment while wearing clean lab coats. To avoid contamination, all samples were constantly covered with aluminium foil. All the chemicals used were of analytical grade from Hi-Media and SD fine.

3.5 Iron analysis

3.5.1. Water sample analysis (Berth and Magnusson, 1981).

Apparatus required:

- i. Flask
- ii. Measuring cylinders
- iii. Beaker
- iv. Separating funnel
- v. Pipettes
- vi. Centrifuge tube
- vii. Flame Atomic Absorption spectroscopy

Chemicals required:

- i. 70% HNO₃
- ii. 1% Ammonium pyrolidine dithiocarbamate (APDC)
- iii. Methyl isobutyl ketone (MIBK)
- iv. 6N HNO₃
- v. Distilled water

Preparation of Chemicals:

70% HNO3

0.7mL of concentrated HNO3 was added to 0.3mL of distilled water.

1% Ammonium pyrolidine dithiocarbamate (APDC)

0.1mg of APDC powder was dissolved in 10mL of distilled water.

6N HNO3

19.5mL of concentrated Nitric acid was added to 35.5mL distilled water.

Procedure:

One litre of water was acidified with 1mL of 70% HNO₃. One mL of 1% ammonium pyrolidine dithiocarbamate (APDC) and 10mL of methyl isobutyl ketone (MIBK) were added to the acidified water in a separating funnel and shaken for 5 minutes. This mixture was allowed to stand for about 15-20 minutes to allow phase separation. The organic layer obtained was transferred to another separating funnel and back-extracted with 50 ml of 6N HNO₃ in another separating funnel. The lower aqueous phase was collected in a centrifuge tube and analyzed for the presence of iron using a flame atomic absorption spectroscopy (Shimadzu AA-6300). The concentration of metals was expressed as mg/L of sample. (Berth, Magnusson, 1981).

3.5.2 Sediment sample analysis (Massadeh et al., 2016)

Apparatus required:

- i. Flask
- ii. Measuring cylinders
- iii. Beaker
- iv. Hot plate

- v. Pipettes
- vi. Centrifuge tube
- vii. Hot air oven
- viii. Whatman's Filter paper (No. 1)
 - ix. Flame Atomic Absorption spectroscopy

Chemicals required:

- i. 50% HNO₃
- ii. Distilled water

Preparation of Chemicals:

50% HNO₃

5mL of concentrated HNO3 was added to 5mL of distilled water.

Procedure:

1-2 gm of oven-dried sediment was sieved and were digested with 10 ml of 50% HNO₃ and heated on a hot plate at 85°C for 2 hours. The digestate was then diluted to a final volume of 25 mL with distilled water and filtered using a Whatman's filter paper (No. 1) and analyzed for the presence of iron using a flame atomic absorption spectroscopy (Shimadzu AA-6300). The concentration of metals was expressed as mg/g of dried sediment.

3.5.3 Tissue sample analysis (Newman et al., 1994).

Apparatus required:

- i. Flask
- ii. Measuring cylinders

- iii. Beaker
- iv. Hot plate
- v Pipettes
- vi. Centrifuge tube
- vii. Hot air oven
- viii. Whatman's Filter paper (No. 1)
- ix. Flame Atomic Absorption spectroscopy

Chemicals required:

- i 50%. HNO₃
- ii. Distilled water

Preparation of Chemicals:

50% HNO₃

5mL of concentrated HNO3 was added to 5mL of distilled water.

Procedure:

Whole tissue samples (~ 2 g in triplicates) were oven dried at 80°C for 18 hours and were digested with 10 ml of 50% nitric acid, in crucible and heated at 85°C for 1 hour. The solution was then allowed to evaporate to 5 ml in a Teflon beaker using a hot plate and then cooled and diluted up to 50 ml using deionized water. Concentrations of iron (Fe) were determined using a Flame Atomic Absorption Spectrophotometry (Shimadzu AA-6300). Results were expressed as mg/g of wet tissue (Newman et al., 1994).

3.6 Toxicological tests:

3.6.1 Test for condition index (Hassan et al., 2020)

<u>Principle</u>: The condition factor or index is an evaluation of the general wellbeing of the gastropods. This is on the premise that heavier individuals of a given length are in healthier condition than less weightier individuals. Condition index shows the inter-relation of weight and length of the snail and its combined effect on the health and growth status.

Apparatus required:

- i. Electronic digital weighing balance
- ii. Measuring scale

Procedure:

Snails were cleaned, de-shelled and the soft tissue was carefully separated from the hard shells. Both the soft tissue and the shells were dried in an oven at 60°C overnight to determine their dry weights, Condition index (CI) was then calculated as follows:

Condition index =
$$\frac{\text{Dry soft tissue weight}}{\text{Dry shell weight}} \times 100$$

3.6.2 Protein Estimation (Lowry et al., 1951)

Principle: Copper from alkaline copper reagent reacts with protein to form a protein complex. Amino acids from the complex reacts with tugstenic acid from Folin CioCalteu reagent, to give blue colour. The intensity of blue colour is directly proportional to the amount of tyrosine and tryptophan present and can be measured at 750 nm.

Apparatus required:

3.6.1 Test for condition index (Hassan et al., 2020)

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- Electronic digital weighing balance
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Amino acids from the complex reacts with tugstenic acid from Folin CioCalteu reagent, to give blue colour. The intensity of blue colour is directly proportional to the amount of tyrosine and tryptophan present and can be measured at 750 nm.

Apparatus required:

- i. Test tubes
- ii. Test tube stand
- iii. Micropipettes
- iv. Spectroscopy

Chemicals required:

- i. Lowry C
- ii. Bovine Serum Albumin (BSA)
- iii. Folin's reagent
- iv. Distilled water

Preparation of chemicals

Lowry C

- Solution A (100 ml): 0.57g NaOH and 2.86 g Na₂CO₃ were dissolved in 100ml distilled water.
- Solution B (50ml): $0.7g\ CuSO_4.5(H_2O)$ was dissolved in 50 ml of distilled water.
- Solution C (50ml): 1.4g sodium tartrate was dissolved in 50 ml of distilled water.
- · Solution A, B, and C were mixed in a ratio of 100:1:1 respectively to prepare Lowry C.

BSA -- 0.01g of BSA was dissolved in 100ml of distilled water.

Folin's reagent -- 5ml of Folin's CioCalteu reagent was mixed with 6ml of distilled water in a test tube and wrapped in aluminium foil to avoid degradation by light.

Extraction:

Snail was dissected and the soft muscle tissue was taken out. I gm of the muscle tissues was weighed and homogenized with cold phosphate-buffered saline and centrifuged at 4000 rpm for 20 mins. The supernatant obtained was used for protein estimation.

Procedure:

2.5 ml of tissue extract was taken in a test tube. To this, 3.5 ml of the Lowry's reagent was added and incubated at room temperature for 20 minutes in dark. 0.5 ml Folin CioCalteu reagent was added and the mixture was further incubated for 10 minutes. The intensity of blue coloured complex was measured against suitable blank at 750 nm. Quantification of protein content of the same was done with the help of standard curve of Bovine serum albumin (BSA).

3.6.3 Carbohydrate Estimation (Anthrone Method)

Principle: Carbohydrates are dehydrated by conc. H₂SO₄ to form furfural. Active form of the reagent is anthranol, the enol tautomer of anthrone, which reacts by condensing with the carbohydrate furfural derivative to give a green colour in dilute and a blue colour in concentrated solutions, which is determined colorimetrically. The blue-green solution shows absorption maximum at 620nm.

Apparatus required:

- i. Test tubes
- ii. Test tube stand
- iii. Micropipettes
- iv. Spectroscopy
- v. Vortex mixer

vi Hot Water Bath

Chemicals required:

- : Anthrone powder
- ii. Conc, H₂SO₄
- iii. Distilled water

Preparation of chemicals:

Anthrone reagent:

0.2g of Anthrone powder was dissolved in 100ml of concentrated H₂SO₄.

Procedure:

2ml of tissue extract was taken in a test tube. To this, 2ml of the Anthrone reagent was added and incubated at 90 °C for 10 minutes in a boiling water bath. The intensity of green coloured complex was measured against suitable blank at 620 nm. Quantification of carbohydrate content of the same was done with the help of standard curve of glucose.

3.6.4. Micronucleus Test (Barsiene et al., 2006)

Principle: A micronucleus (MN) test is a test used in toxicological screening for potential genotoxic compounds. The assay is now recognized as one of the most successful and reliable assays for genotoxic carcinogens. This test is based on the formation of number of MN in treated cells. (Emam et al., 2014)

Apparatus:

- i Slides
- ii. Dropper

- iii. Beakers
- iv. Coplin jars
- Olympus BX53 trinocular research microscope

Chemicals required:

- 1. Acetic-methanol (1:3)
- 2. 5% Giemsa stain
- 3. Phosphate Buffer
- 4. Immersion oil

Preparation of chemicals:

Acetic-methanol

10 mL of acetic acid was added to 30mL of methanol.

5% Giemsa stain

5 mL of Giemsa stain stock was added to 95 mL phosphate buffer.

Phosphate Buffer

6.8 g of Potassium dihydrogen phosphate(KH_2PO_4) and 8.88 g of disodium monohydrogen phosphate (Na_2HPO_4) was dissolved in 200mL of distilled water. The pH was adjusted to 7.4 by the addition of HCl.

Procedure:

The Micronucleus (MN) test was performed following the protocol outlined by Barsiene et al., A portion of the gill tissue was placed in a drop of methanol acetic acid mixture (3:1) on a clean glass slide. This tissue was then gently nipped with tweezers for a few minutes and the resulting cell suspension was then smeared and air-dried. The slide was stained with 5%

3.7.2 Exposure set-up:

A) Toxicant: Ferric oxide

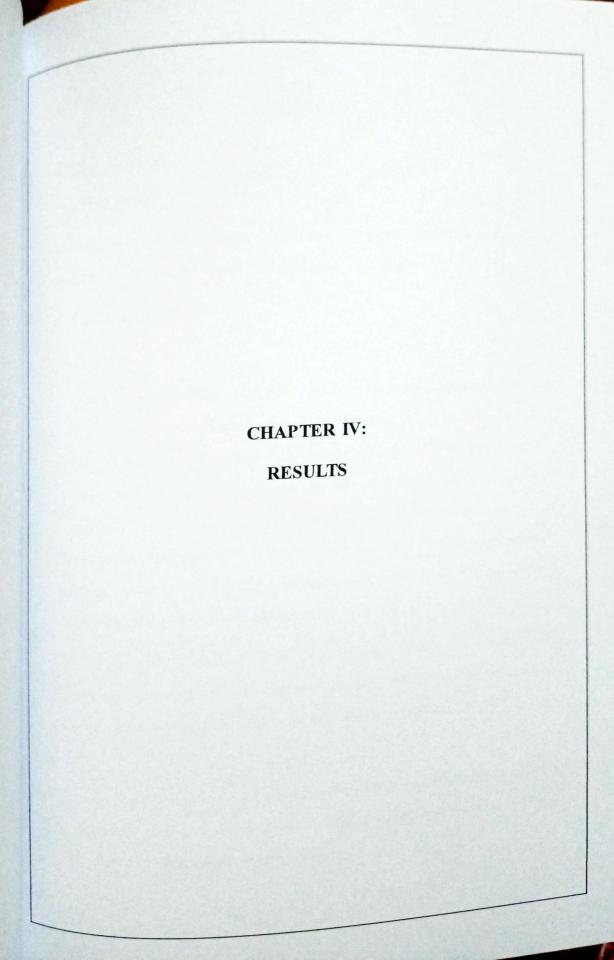
Acute toxicity tests were performed in triplicate in static environment without water change for 96 hr. For these tests, the snails were separated into batches of 15 individuals per tank, given three treatments with different concentrations (0.1µg/ml, 0.5ug/ml, 1.0ug/ml), and a control treatment. A total of 60 individuals were used per toxicant.

B) Toxicant: Manganese Sulphate

Acute toxicity tests were performed in triplicate in static environment without water change for 96 hr. For these tests, the snails were separated into batches of 15 individuals per tank, given three treatments with different concentrations (0.5µg/ml, 1.5ug/ml, 2.5ug/ml), and a control treatment. A total of 60 individuals were used per toxicant.

3.7.3 Statistical analysis:

Data sets were presented as mean values with standard deviations. To detect significant variation in toxicological parameters and trace metals, one-way ANOVA was used. Correlation analysis was also performed to investigate the association between toxicological indicators. Data was considered significant at p < 0.05, p < 0.01, p < 0.001. To compare the statistically significant difference between two groups, an unpaired t-test was used. To compare statistically significant difference between all possible pairs of means, a single step multiple comparison Tukey's range test was used.



4. RESULTS

4.1 FIELD STUDIES

Trace Metal analysis

The concentration of iron in water, sediment and biota sample collected from the site was found to be 19.59mg/L, 8.5260mg/L and 6.8557mg/L respectively.

4.2 EXPOSURE RESULTS

Protein analysis

The results for protein estimation of snails exposed to iron and manganese are shown in graph 4.1.1 and 4.1.2 respectively. For iron, the protein concentration was highest in experimental group 3 having 1mg/L of ferric oxide, whereas the lowest protein concentration was observed in the control group. It was seen that with increase in the concentration of toxicant, there was an increase in the protein content. On the other hand, protein concentration of snails exposed to manganese sulphate showed highest protein content in experimental group 1 i.e., 0.5mg/L of manganese, whereas experimental group 3 with 2.5mg/L of manganese concentration had the lowest protein content.

Using one-way ANOVA, for iron exposed snails, significant variation was observed in the protein content across different concentrations (F=12.22, p=0.0023). Also snails exposed to manganese showed a significant variation across different concentration (F=7.616, p=0.0099).

The results for unpaired t-test for iron exposed snails, showed a significance between control and experimental group 1, 2 and 3. Tukey's multiple comparison also showed a significant variation between control and each experimental group.

For manganese exposed snails, the unpaired t-test results showed significance between control and experimental 1 group, whereas Tukey's multiple comparison test showed significance between control and experimental 1, and also between experimental 1 and experimental 3.

Carbohydrate analysis

The results for carbohydrate estimation of snails exposed to iron and manganese are shown in graph 4.2.1 and 4.2.2 respectively. For iron, the carbohydrate concentration was highest in control group, whereas the lowest carbohydrate concentration was observed in experimental group 2 having 1.5mg/L of ferric oxide concentration. It was seen that with increase in the concentration of toxicant, there was a decrease in the carbohydrate content, except for experimental group 3. On the other hand, carbohydrate concentration of snails exposed to manganese sulphate showed highest carbohydrate content in control group, whereas experimental group 3 with 2.5mg/L of manganese concentration had the lowest carbohydrate content.

Using one-way ANOVA, for iron exposed snails, significant variation was observed in the carbohydrate content across different concentrations (F = 11.69, p = 0.0027), likewise snails exposed to manganese also showed significant variation in carbohydrate content across different concentrations (F = 7.502, p = 0.0103).

For iron exposed snails, the unpaired t-test results showed significance between control and experimental 1 and 2 group, whereas Tukey's multiple comparison test showed significance between control and experimental 1, control and experimental 2 and also between experimental 2 and experimental 3.

The results for unpaired t-test, for manganese exposed snails showed a significance between control and experimental group 3. On the other hand, Tukey's multiple comparison showed a significance between control and experimental 3, and also between experimental 2 and experimental 3.

Condition index

The results for condition index for iron and manganese exposed samples are depicted in graph 4.3.1 and 4.3.2 respectively. The graph for iron exposure, indicates that the condition index was high in the control group of snails, whereas the lowest was observed in experimental group 2. Similar results were observed for manganese exposure snails with control group having the highest condition index and experimental group 3 with the lowest condition index.

One-way ANOVA analysis showed a significant variation between the condition index of snails exposed to both iron and manganese. (Fe; F = 10.62, p = 0.0037, Mn; F = 20.24, p = 0.0004)

The results for unpaired t-test, for iron exposed snails showed significance between control and experimental group 2 and control and experimental group 3. On the other hand, Tukey's multiple comparison showed significance between control and experimental 1, and also between experimental 2 and experimental 3.

For manganese exposed snails, the unpaired t-test results showed significance between control and experimental group 1, 2 and 3, whereas Tukey's multiple comparison test showed significant variation between control and experimental 1, control and experimental 2, control and experimental 3 and also between experimental 1 and experimental 3.

Micronucleus test

The results for micronucleus test of snails exposed to iron and manganese are shown in graph 4.4.1 and 4.4.2 respectively. In the category of iron exposed samples, it indicates that highest number of micronuclei were recorded in experimental group 2 and lowest number of micronuclei were recorded in control group. Similar results were observed in snails exposed to manganese, highest number of micronuclei were recorded in experimental group 2 and lowest number of micronuclei were recorded in control group.

Using one-way ANOVA, for iron exposed snails, significant variation was observed in the micronuclei across different concentrations (F = 7.917, p = 0.0089), likewise snails exposed to manganese also showed significant variation in micronuclei across different concentrations (F = 31.44, p = < 0.0001).

For iron exposed snails, the unpaired t-test results showed significance between control and experimental group 1, 2 and 3, whereas Tukey's multiple comparison test showed significant variation between control and experimental 1, control and experimental 2, control and experimental 3 and also between experimental 1 and experimental 3.

The results for unpaired t-test, for snails exposed to manganese, showed significance between control and experimental group 1, 2 and 3. On the other hand, Tukey's multiple comparison showed significance between control and experimental 1, control and experimental 2, control and experimental 3, experimental 1 and experimental 2 and experimental 1 and experimental 3.

Histological analysis

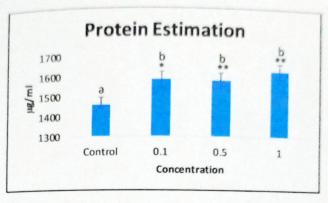
The resulting slides of histological analysis of snails exposed to iron and manganese are shown in plate i and ii respectively. It indicates that, the foot tissue in snails exposed to different concentration of ferric oxide, had highly ruptured and vacuolated muscular tissue, with thin damaged epithelial covering, whereas, muscular tissue and epithelial covering in that of control set was intact and less vacuolated. Similar results were observed in snails exposed to manganese sulphate.

Pearson's correlation

For iron exposed snails, Pearson's correlation analysis showed a negative correlation between carbohydrate vs micronucleus test (p= 0.0065), and micronucleus test vs condition index (p= 0.0215). (Table 4.1)

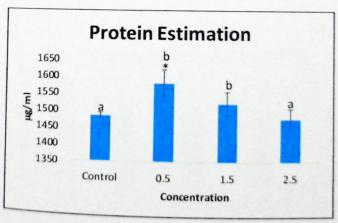
Pearson's correlation analysis, for manganese exposed snails, showed a positive correlation between carbohydrate vs protein (p=0.026), and carbohydrate vs condition index (p=0.0209), and negative correlation was observed between micronucleus test vs condition index (p=0.0002). (Table 4.2)

Graph 4.1.1. Protein estimation of Snails exposed to different concentrations of iron axide.



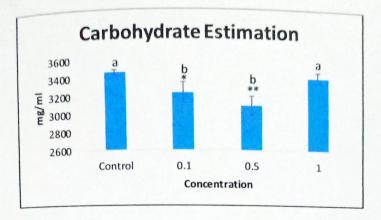
Different letters indicate significant difference between groups; same letters indicate nonsignificance between groups as per Tukey's analysis

Graph 4.1.2. Protein estimation of Snails exposed to different concentrations of manganese sulphate.



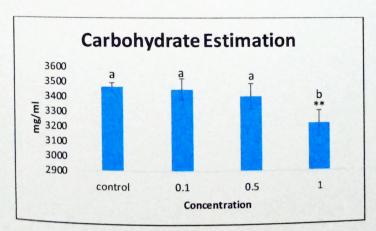
Different letters indicate significant difference between groups, same letters indicate nonsignificance between groups as per Tukey's analysis

Graph 4.2.1. Carbohydrate estimation of Snails exposed to different concentrations of iron oxide.



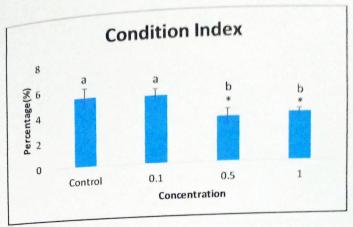
Different letters indicate significant difference between groups, same letters indicate nonsignificance between groups as per Tukey's analysis

Graph 4.2.2. Carbohydrate estimation of Snails exposed to different concentrations of manganese sulphate.



Different letters indicate significant difference between groups, same letters indicate nonsignificance between groups as per Tukey's analysis

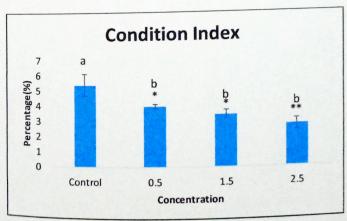
Graph 4.3.1. Condition Index of Snails exposed to different concentrations of iron oxide.



Different letters indicate significant difference between groups, same letters indicate nonsignificance between groups as per Tukey's analysis

p < 0.05*

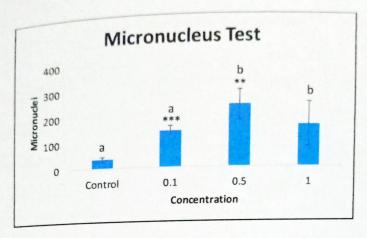
Graph 4.3.2. Condition Index of Snails exposed to different concentrations of manganese sulphate.



Different letters indicate significant difference between groups, same letters indicate nonsignificance between groups as per Tukey's analysis

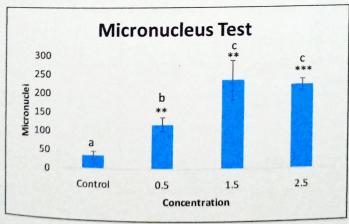
p<0.05*, p<0.01**

Graph 4.4.1. Micronucleus test of Snails exposed to different concentrations of iron oxide.



Different letters indicate significant difference between groups, same letters indicate nonsignificance between groups as per Tukey's analysis

Graph 4.4.2. Micronucleus test of Snails exposed to different concentrations of manganese sulphate.



Different letters indicate significant difference between groups, same letters indicate nonsignificance between groups as per Tukey's analysis

Table 4.1 Pearson's correlation of Snails exposed to different concentrations of iron exide.

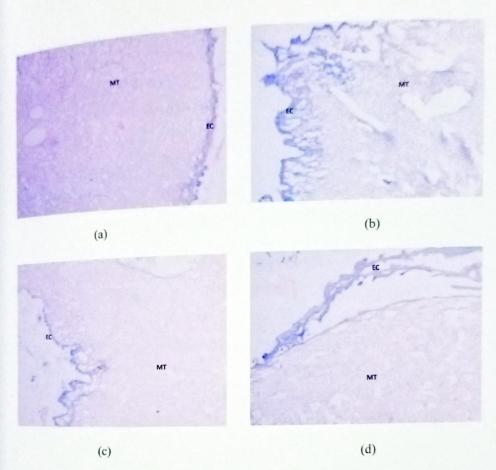
	Protein	Carbohydrate	Condition Index	Micronucleus test
Protein	-	-0.2274	-0.4739	0.5
Carbohydrate	-0.2274	-	0.3642	-0.7345**
Condition Index	-0.4739	0.3642	-	-0.6521*
Micronucleus test	0.5	-0.7345**	-0.6521*	-

p<0.05*, *p*<0.01**

 $\label{thm:concentration} \begin{tabular}{ll} Table 4.2 \ Pears on `s \ correlation \ of \ Snails \ exposed to \ different \ concentrations \ of \ manganese \ sulphate. \end{tabular}$

	Protein	Carbohydrate	Condition Index	Micronucleus test
Protein	-	0.6342*	0.05597	-0.01669
Carbohydrate	0.6342*	-	0.6547*	-0.5127
Condition Index	0.05597	0.6547*	-	-0.8717***
Micronucleus test	-0.01669	-0.5127	-0.8717***	

p<0.05*, p<0.001***



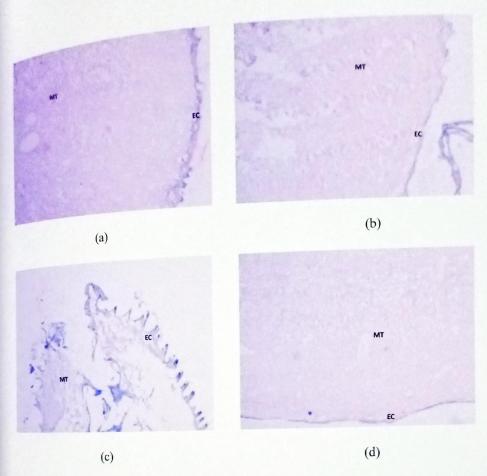
Effect of ferric oxide on histology of the foot tissue of Littorina scutulata (Magnification 1000x).

Fig. (a): Control snail foot section showing normal muscular tissue (MT) and an intact spithelial covering (EC).

Fig. (b): Exp. 1 snail foot section showing large vacuolated muscular tissue (MT) and a ruptured epithelial covering (EC).

Fig. (c): Exp. 2 snail foot section showing vacuolated muscular tissue (MT) and a thin epithelial covering (EC).

Fig. (d): Exp. 3 snail foot section showing vacuolated muscular tissue (MT) and a ruptured epithelial covering (EC).



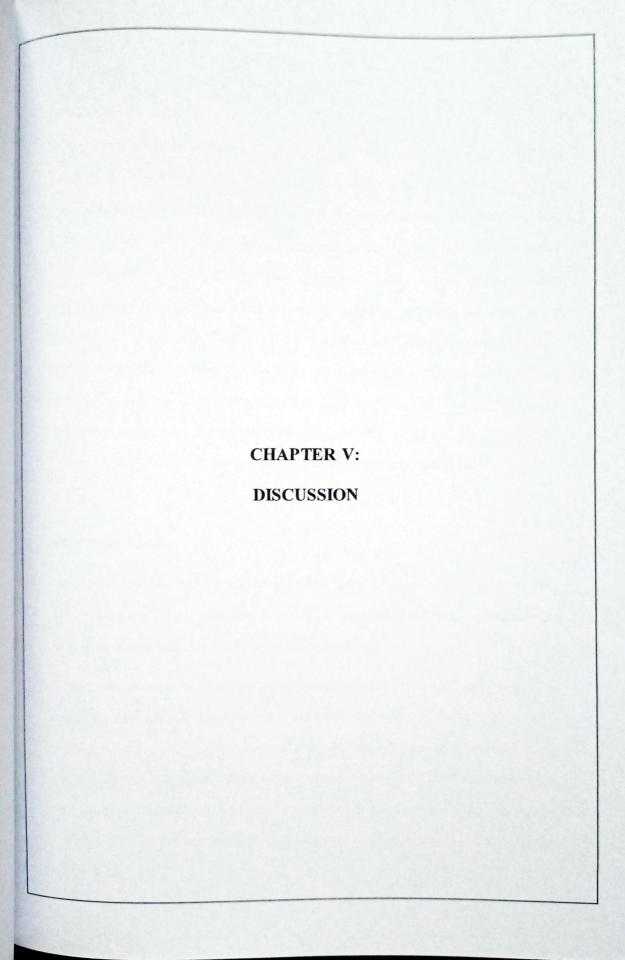
Effect of manganese sulphate on histology of the foot tissue of Littorina scutulata (Magnification 1000x).

Fig. (a): Control snail foot section showing normal muscular tissue (MT) and an intact epithelial covering (EC).

Fig. (b): Exp. 1 snail foot section showing ruptured muscular tissue (MT) and a thin damaged epithelial covering (EC).

Fig. (c): Exp. 2 snail foot section showing highly ruptured muscular tissue (MT) and a thin epithelial covering (EC).

 Fig (d): Exp. 3 snail foot section showing lightly damaged muscular tissue (MT) and a damaged thin epithelial covering (EC).



5. DISCUSSION

5.1 Field Studies

In the present study, water, sediment and snail sample were analysed for presence of iron. Sampling was carried out at study site Nanachepani, Varconda in August 2022. Snail sample along with water and sediment sample were collected to estimate the concentration of iron. Estimation results showed highest iron concentration in water sample followed by sediment and snail sample. Similar findings were obtained by Larba et al., (2014), wherein concentration of heavy metals (Fe, Mn, Pb and Cd) were analysed in both, soil sample and in land snail *Helix aspersa* at heavy metal soil contamination in Northeast Algeria. Both these results approve the hypothesis that snails are good bioindicators of metal pollution in the terrestrial as well aquatic environment. Another study by Adebowake et al.,(2022) revealed that heavy metal concentrations (Pb, Cr, Cd, As, Ni) in African Giant Land Snail (Archachatina marginata) in wild was higher than that of the domesticated one.

5.2 Exposure Results

Exposure studies were carried out by maintaining snails under laboratory conditions. After fully acclimatization of the snails, they were exposed separately to different concentrations of ferric oxide and manganese sulphate for an acute period of 96hrs.

In the present study, the condition index of exposed snails decreased with increase in concentration of iron and manganese. A similar observation was reported by Sheir in 2015, wherein, freshwater snail, *Lanistes carinatus* were exposed to Metals concentrations (Zn, Fe, Mn and Ca), it was observed that CI of the exposed snails reduced significantly especially on the 1st day (17.5 % reduction). Leung and Furness showed that when *N. lapillus* was exposed to dissolved Cd (500 mg/L) for 20 days, the CI decreased significantly, from 30 to 25. This

difference may have been related to the difference in the waterborne and diet-borne Cd toxicity.

In the present study organisms exposed to trace metals showed moderately higher concentration of protein and carbohydrate than that of the control group. Moolman et al., (2007) in his studies found out that, the amounts of carbohydrate and protein reserves allocated over the 2-week exposure period were significantly lower in the metal exposures compared to the control. According to Smolders et al., (2003), toxicant stress preferentially causes a depletion of the more readily available carbohydrate and lipid reserves instead of protein. Although lipids are known as more efficient energy-storage products than either proteins or carbohydrates, the total lipid reserves of both gastropod species were not affected. Protein is also a prominent source of energy and as a metabolic reserve has the potential to indicate metabolic disruptions.

In the present study, genotoxicity in the form of micronuclei was observed, which was potentially due to metal exposure. Similar study carried out by Yadav et al., (2009) showed significant increase over and above negative control in the frequency of micronuclei in fishes (Channa punctata), exposed to metal compounds Hg(II), As(III) and Cu(II), with genotoxicity being As>Hg>Cu. Findings depict genotoxic potential of these metal compounds even in sublethal concentrations. Another study by Kandroo et al., (2015), found that MN frequencies in mercuric chloride exposed snails, increased with increase in exposure time period. A negative correlation between micronucleus test and condition index was observed through correlation analysis, the results of this study are similar to that of Touahri et al., (2016), where in it was concluded that condition index and micronucleus are both affected by variability of intrinsic and extrinsic factors.

Smils exposed to higher concentration of iron and manganese showed moderate to high damage in the epithelial covering and highly ruptured vacuolated muscular tissue was also observed, this observations can be compared to studies done by Bighiu et al. (2017) wherein sea small *Theodoxus fluviatilis* were exposed to metal concentration of Cu, Zn and Pb, at leisure boat harbours (marinas) for two weeks, as a result haemocyte infiltration, and loose connective tissue were the main alterations occurring in the foot tissue of the exposed snails. A similar result was reported by Hanaa et al., (2018) in which Snails' histopathological examination showed degeneration, edema, hypertrophy and vacuolation in head-foot muscles, degeneration and necrotic changes in the digestive gland, irregular shape in mature ova and reduction in sperm number in hermaphrodite gland.

CHAPTER VI: CONCLUSION

6. CONCLUSION

The study showed presence of high concentration of iron in water, sediment and in the model organism land snail *Cyclophorous volvulus* collected from the sampling site. The possible reason for the high concentration of these metals could be due to the on-going construction work in the vicinity of the sampling site.

Toxicological tests such as protein estimation, carbohydrate estimation, micronucleus test and condition index along with histopathological studies were carried out on the subsequent model organism - sea snail *Littorina scutulata* which was collected from Dona Paula and acclimatized in laboratory conditions. The results of toxicological tests help monitor the health of the organism exposed to the metals as well as the pollution level at the sampling site. Snails exposed to metal concentration in laboratory conditions showed varying toxicity responses. This study revealed the negative effects of exposure to moderate-high concentrations of metals, providing a valuable reference for studying the impact of metals on aquatic as well as terrestrial organisms. Thus, snail can be used as a potential bioindicator of trace metal pollution in aquatic as well as terrestrial environments.

Further studies of longer duration are required to understand how heavy metals affects the health and basic functioning of the snail, as well as the toxicokinetics, and defence mechanism existing in them.

CHAPTER VII:
REFERENCES

7. REFERENCES

- Abdallah, A. T., & Moustafa, M. A. (2002). Accumulation of lead and cadmium in the marine prosobranch Nerita saxtilis, chemical analysis, light and electron microscopy. Environmental Pollution, 116(2), 185-191.
- Achuba, F. I. (2008). African land snail Achatina marginatus, as bioindicator of environmental pollution. North-West J. Zoo, 4, 1-5.
- Alonso, M. L., Benedito, J. L., Miranda, M., Castillo, C., Hernández, J., & Shore, R. F. (2002). Interactions between toxic and essential trace metals in cattle from a region with low levels of pollution. Archives of Environmental Contamination and Toxicology, 42, 165-172.
- Attri, K., & Kerkar, S. (2011). Seasonal assessment of heavy metal pollution in tropical mangrove sediments (Goa, India). Journal of Ecobiotechnology, 3(8).
- Azam, I., Afsheen, S., Zia, A., Javed, M., Saeed, R., Sarwar, M. K., & Munir, B.
 (2015). Evaluating insects as bioindicators of heavy metal contamination and accumulation near industrial area of Gujrat, Pakistan. Biomed research international, 2015.
- Baroudi, F., Al Alam, J., Fajloun, Z., & Millet, M. (2020). Snail as sentinel organism for monitoring the environmental pollution; a review. Ecological indicators, 113, 106240.
- Beeby, Alan & Richmond, Larry. (2002). Evaluating Helix aspersa as a sentinel for mapping metal pollution. Ecological Indicators. 1, 261-270.
- Bighiu, M. A., Watermann, B., Guo, X., Almroth, B. C., & Eriksson-Wiklund, A. K.
 (2017). Mortality and histopathological effects in harbour-transplanted snails with different exposure histories. *Aquatic Toxicology*, 190, 11-20

- Brown, R. J., & Milton, M. J. (2005). Analytical techniques for trace element analysis: an overview. Trac Trends in Analytical Chemistry, 24(3), 266-274.
- Cho, Y. J., Getachew, A. T., Saravana, P. S., & Chun, B. S. (2019). Optimization and characterization of polysaccharides extraction from Giant African snail (*Achatina fulica*) using pressurized hot water extraction (PHWE). Bioactive Carbohydrates and Dietary Fibre, 18, 100179.
- Cruz, T. C., Nayak, G. N., Tiwari, A. K., & Nasnodkar, M. R. (2020). Assessment of
 metal pollution and bioaccumulation of metals by edible bivalve *Polymesoda erosa* in
 the Zuari Estuary, west coast of India. Marine Pollution Bulletin, 158, 111415.
- D'costa, A. H., Shyama, S. K., MK, P. K., & Furtado, S. (2018). The Backwater Clam
 (Meretrix casta) as a bioindicator species for monitoring the pollution of an estuarine
 environment by genotoxic agents. Mutation Research/Genetic Toxicology and
 Environmental Mutagenesis, 825, 8-14.
- D'Costa, A. H., Shyama, S. K., MK, P. K., Verenkar, V. S., & Tangsali, R. B. (2020).
 Genotoxic effect of manganese and nickel doped zinc ferrite (Mn 0.3 Ni 0.3 Zn 0.4 Fe
 2 O 4) nanoparticle in Swiss albino mouse *Mus musculus*. Indian Journal of
 Experimental Biology (IJEB), 59(01), 25-32.
- D'Costa, A., Shyama, S. K., & Kumar, M. P. (2017). Bioaccumulation of trace metals
 and total petroleum and genotoxicity responses in an edible fish population as
 indicators of marine pollution. Ecotoxicology and environmental safety, 142, 22-28.
- Da Silva, R. F. B., Viña, A., Moran, E. F., Dou, Y., Batistella, M., & Liu, J. (2021).
 Socioeconomic and environmental effects of soybean production in metacoupled
 systems. Scientific Reports, 11(1), 18662.
- Dias, H. Q., & Nayak, G. N. (2016). Geochemistry and bioavailability of mudflats and mangrove sediments and their effect on bioaccumulation in selected organisms

- within a tropical (Zuari) estuary, Goa, India. Marine pollution bulletin, 105(1), 227-236.
- El-Khayat, H. M., Abd-Elkawy, S., Abou-Ouf, N. A., Ahmed, M. A., & Mohammed, W. A. (2018). Biochemical and histological assessment of some heavy metals on *Biomphalaria alexandrina* snails and *Oreochromis niloticus* fish in Lake Burullus, Egypt. Egyptian Journal of Aquatic Biology and Fisheries, 22(3), 159-182.
- Emam, A. N., Girgis, E., Khalil, W. K., & Mohamed, M. B. (2014). Toxicity of
 plasmonic nanomaterials and their hybrid nanocomposites. In *Advances in molecular*toxicology (Vol. 8, pp. 173-202).
- Fu, J., Wang, Y., Zhang, A., Zhang, Q., Zhao, Z., Wang, T., & Jiang, G. (2011). Spatial distribution of polychlorinated biphenyls (pcbs) and polybrominated biphenyl ethers (pbdes) in an e-waste dismantling region in Southeast China: Use of apple snail (Ampullariidae) as a bioindicator. Chemosphere, 82(5), 648-655.
- Füreder, L., & Reynolds, J. D. (2003). Is Austropotamobius pallipes a good bioindicator?. Bulletin Français de la Pêche et de la Pisciculture, (370-371), 157-163.
- Gerhardt, A. (2002). Bioindicator species and their use in biomonitoring.
 Environmental monitoring, 1, 77-123.
- Gheoca, V., Benedek, A. M., & Schneider, E. (2021). Exploring land snails' response
 to habitat characteristics and their potential as bioindicators of riparian forest quality.
 Ecological Indicators, 132, 108289.
- Gupta, S. K., & Singh, J. (2011). Evaluation of mollusc as sensitive indicator of heavy metal pollution in aquatic system: a review. IIOAB J, 2(1), 49-57.
- Hassan, N. H., & Ab Lah, R. (2020). Relationship of Condition Index and Reproductive Status of Black Snail, Fannus ater in Merchang Lagoon. Universiti Malaysia Terengganu Journal of Undergraduate Research, 2(1), 1-8.

- Holt, E. A., & Miller, S. W. (2011). Bioindicators: Using organisms to measure environmental impacts. Nature Education Knowledge 3 (10): 8.
- Itziou, A., & Dimitriadis, V. K. (2011). Introduction of the land snail Eobania
 wermiculata as a bioindicator organism of terrestrial pollution using a battery of
 biomarkers. Science of the total environment, 409(6), 1181-1192.
- Kong, I. C., Bitton, G., Koopman, B., & Jung, K. H. (1995). Heavy metal toxicity testing in environmental samples. Reviews of Environmental Contamination and Toxicology: Continuation of Residue Reviews, 119-147.
- Lee Jr, R. E., & Von Lehmden, D. J. (1973). Trace metal pollution in the environment.
 Journal of the Air Pollution Control Association, 23(10), 853-857.
- Lepp, N. W. (Ed.). (2012). Effect of heavy metal pollution on plants: metals in the environment (Vol. 2). Springer Science & Business Media.
- Li, J., Lusher, A. L., Rotchell, J. M., Deudero, S., Turra, A., Bråte, I. L. N., ... & Shi,
 H. (2019). Using mussel as a global bioindicator of coastal microplastic pollution.
 Environmental pollution, 244, 522-533.
- Madejón, P., Arrébola, J., Madejón, E., Burgos, P., López-Garrido, R., Cárcaba, A., ...
 & Murillo, J. M. (2013). The snail *Theba pisana* as an indicator of soil contamination by trace elements: potential exposure for animals and humans. Journal of the Science of Food and Agriculture, 93(9), 2259-2266.
- Massadeh, A. M., Alomary, A. A., Mir, S., Momani, F. A., Haddad, H. I., & Hadad, Y.
 A. (2016). Analysis of Zn, Cd, As, Cu, Pb, and Fe in snails as bioindicators and soil samples near traffic road by ICP-OES. Environmental Science and Pollution Research, 23, 13424-13431.

- Moolman, L. J. H. J., Van Vuren, J. H. J., & Wepener, V. (2007). Comparative studies on the uptake and effects of cadmium and zinc on the cellular energy allocation of two freshwater gastropods. *Ecotoxicology and Environmental Safety*, 68(3), 443-450.
- Nagi, H. M., Jagtap, T. G., Abdulsalam, K. M., & Jumbi, A. CONCENTRATION OF SOME TRACE METALS IN THE MANGROVE AREA OF MANDOVI ESTUARY, GOA, INDIA.
- Naik, A. P., Shyama, S. K., & D'Costa, A. H. (2020). Evaluation of genotoxicity, enzymatic alterations and cadmium accumulation in Mozambique tilapia *Oreochromis mossambicus* exposed to sub lethal concentrations of cadmium chloride.
 Environmental Chemistry and Ecotoxicology, 2, 126-131.
- Nasnodkar, M. R., & Nayak, G. N. (2017). Chemical speciation and bio-availability
 of selected trace metals in the mudflat core sediments of the tropical estuaries, India.
 Environmental Earth Sciences, 76, 1-11.
- Neeratanaphan, L., Sudmoon, R., & Chaveerach, A. (2014). Genetic erosion in the freshwater snail *Filopaludina martensi* is affected by lead and cadmium. Applied Ecology and Environmental Research, 12(4), 991-1001
- Newman, M. C., Mulvey, M., Beeby, A., Hurst, R. W., & Richmond, L. (1994). Snail (Helix aspersa) exposure history and possible adaptation to lead as reflected in shell composition. Archives of Environmental Contamination and Toxicology, 27, 346-351.
- Otitoloju, A. A., & Don-Pedro, K. N. (2006). Determination of types of interactions exhibited by binary mixtures of heavy metals tested against the hermit crab,
 Clibanarius africanus. Toxicological & Environmental Chemistry, 88(2), 331-343.
- Otludil, B., Cengiz, E. I., Yildirim, M. Z., Ünver, Ö., & Ünlü, E. (2004). The effects of endosulfan on the great ramshorn snail *Planorbarius corneus* (Gastropoda, Pulmonata): a histopathological study. Chemosphere, 56(7), 707-716.

- pereira, F., Kerkar, S., & Krishnan, K. P. (2013). Bacterial response to dynamic metal concentrations in the surface sediments of a solar saltern (Goa, India). Environmental monitoring and assessment, 185, 3625-3636.
- Phillips, D. J. (1977). The use of biological indicator organisms to monitor trace metal pollution in marine and estuarine environments—a review. Environmental Pollution (1970), 13(4), 281-317.
- Praveen Kumar, M. K., d'costa, A., & Shyama, S. K. (2017). Genotoxic biomarkers as indicators of marine pollution. Marine Pollution and Microbial Remediation, 263-270.
- Radwan, M. A., El-Gendy, K. S., & Gad, A. F. (2010). Biomarkers of oxidative stress in the land snail, Theba pisana for assessing ecotoxicological effects of urban metal pollution. Chemosphere, 79(1), 40-46.
- Salánki, J., Farkas, A., Kamardina, T., & Rózsa, K. S. (2003). Molluscs in biological monitoring of water quality. Toxicology Letters, 140, 403-410.
- Salih, A. H. S. H., Hama, A. A., Hawrami, K. A., & Ditta, A. (2021). The land snail, Eobania vermiculata, as a bioindicator of the heavy metal pollution in the Urban Areas of Sulaimani, Iraq. Sustainability, 13(24), 13719.
- Sanches Filho, P. J., Caldas, J. S., da Rosa, N. N., & Pereira, F. O. P. (2017). Toxicity test and Cd, Cr, Pb and Zn bioccumulation in Phalloceros caudimaculatus. Egyptian journal of basic and applied sciences, 4(3), 206-211.
- Shaw, M. J., & Haddad, P. R. (2004). The determination of trace metal pollutants in environmental matrices using ion chromatography. Environment International, 30(3),
- Sheir, S. K. (2015). Impacts of short-term exposure of Caselio (plant fertilizer) on The freshwater snail, Lanistes carinatus (Ampullariidae, Mollusca). Research Journal of Pharmaceutical, Biological and Chemical Sciences, 6(6), 235-245.

- Srivastava, A. K., & Singh, V. K. (2020). Snails as biological monitor (bioindicator).

 Asian Journal of Advances in Research, 6-12.
- Stabnikova, O., Stabnikov, V., Marinin, A., Klavins, M., & Vaseashta, A. (2022). The role of microplastics biofilm in accumulation of trace metals in aquatic environments. World Journal of Microbiology and Biotechnology, 38(7), 117.
- Subramanian, A., Kunisue, T., & Tanabe, S. (2015). Recent status of organohalogens, heavy metals and pahs pollution in specific locations in India. Chemosphere, 137, 122-134.
- Van Straalen, N. M. (1998). Evaluation of bioindicator systems derived from soil arthropod communities. Applied soil ecology, 9(1-3), 429-437.
- Vasconcelos, P., Lopes, B., Castro, M., & Gaspar, M. B. (2008). Comparison of indices for the assessment of reproductive activity in *Hexaplex trunculus* (Gastropoda: Muricidae). Marine Biology Research, 4(5), 392-399.
- Wamnebo, M. I. (2017). Gastropods as a bioindicator and biomonitoring metal pollution. Aquacultura Indonesiana, 18(1), 1-8.
- Wong, C. S., Li, X., & Thornton, I. (2006). Urban environmental geochemistry of trace metals. Environmental pollution, 142(1), 1-16.
- Xiao, R., Bai, J., Gao, H., Wang, J., Huang, L., & Liu, P. (2012). Distribution and contamination assessment of heavy metals in water and soils from the college town in the Pearl River Delta, China. CLEAN–Soil, Air, Water, 40(10), 1167-1173.
- Yadav KK, Trivedi SP. Sublethal exposure of heavy metals induces micronuclei in fish, Channa punctata. Chemosphere. 2009 Dec;77(11):1495-500.
- Yamada, M. O., Fujimori, K., Yamada, G., Satoh, H., Tohno, S., Tohno, Y., ... &
 Minami, T. (2003). A special comment on trace metals in the viscera of *Turbo*

marmorahas, a toxic snail, on Ryukyu Island. Biological trace element research, 95,

• Yap, C. K., & Cheng, W. H. (2013). Distributions of heavy metal concentrations in different tissues of the mangrove snail Nerita lineata. Sains Malaysiana, 42(5), 597-603.