ASSESSMENT OF HEAVY METAL ACCUMULATION IN THE TISSUE OF SHELLFISH

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NIKITA SAMAL

22P0500012

ABC ID: 905008747562

202208792

Under the supervision of

Mrs. DVITI LAVESH VOLVOIKAR

School of Biological Sciences and Biotechnology

Biotechnology Discipline

GOA UNIVERSITY

Date: 8th April 2024

SBSB: Biotech... Taleigao Plateau, Goa 403206 GOA UNIVERSITY

Examined by:

Seal of the school

DECLARATION

I hereby declare that the data presented in this Dissertation report entitled, **"ASSESMENT OF HEAVYMETAL ACCUMULATION IN THE TISSUE OF SHELLFISH"** is based on the results of investigations carried out by me in the Marine Biotechnology at the School of Biological Sciences and Biotechnology, Goa University under the Supervision of Mrs Dviti Volvoikar and the same has not been submitted elsewhere for the award of a degree or diploma by me. Further, I understand that Goa University or its authorities / College will be not be responsible for the correctness of observations / experimental or other findings given the dissertation.

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N.Samal.

Ms Nikita Samal

22P0500012

M.Sc. Marine Biotechnology

School of Biological Sciences and Biotechnology

Date: 8/04/2024

Goa University

CERTIFICATE

This is to certify that the dissertation report "**ASSESMENT OF HEAVYMETAL ACCUMULATION IN THE TISSUE OF SHELLFISH** " is a bonafide work carried out by Ms Nikita Samal under my supervision in partial fulfilment of the requirement for the award of the degree of Master of Science in the Marine Biotechnology, at the School of Biological Sciences and Biotechnology, Goa University.

Date: 08/04/2024

Mrs. Dviti L Volvoikar

School of Biological Sciences and Biotechnology,

Goa University

SBSB: Biotechnology * GOA UNIVERSITY * Taleigao Plateau, Goa 403206

Department stamp

Prof. Bernard F. Rodrigues

Sr. Professor and Dean

School of Biological Sciences and Biotechnology

Date: 8/4/24

Place: Goa University

Dean of School of Biological Sciences & Biotechnology Goa University, Goa-403206

PREFACE

The coastal ecosystems, harboring a rich diversity of flora and fauna, are vital components of our planet's ecological balance. Among these ecosystems, estuarine and marine environments serve as crucial habitats for various marine organisms, including shellfish, which play significant roles in the marine food web. However, the sustainability of these ecosystems faces unprecedented challenges due to human activities, notably industrialization and urbanization, leading to the discharge of pollutants into the environment. Heavy metals are one of the most concerning pollutants in aquatic environments. These metals, originating from both natural and anthropogenic sources, pose serious threats to marine life and human health due to their persistence and toxicity. Shellfish, being filter feeders, are particularly susceptible to accumulating heavy metals from their surrounding environment, making them valuable indicators of environmental contamination. This study aims to comprehend the geographical and temporal fluctuations in heavy metal concentrations among several clam species living in rural and urban regions through a thorough examination. As pollution levels rise, heavy metal contamination is a regular problem. The general population, however, is ignorant about the amount and acceptable limits of heavy metals in the diet. The current study aims to evaluate the levels of heavy metals in crabs, mussels, and clams in urban and rural regions.

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LIST OF ABBREVIATIONS

Abbreviation	Entity
°C	Degree Celsius
µg	Microgram
µL	Microliter
mL	Millilitre
mg	Milligram
kg	Kilogram
g	Gram
cm	Centimetre
SD	Standard deviation
ppb	Parts per billion
AAS	Atomic absorption spectrophotometer
Cd	Cadmium
Pb	Lead
Ni	Nickel
Cu	Copper
Fe	Iron

ABSTRACT

Heavy metal pollution of the aquatic environment is caused by rapid industrialisation and sophisticated agriculture practices. Aquatic creatures are eventually the route via which heavy metals enter the human body. Seafood contamination is one of the main effects of marine pollution, and it has sparked worries because of the possible health dangers to humans. This current study investigated seasonal bioaccumulation of heavy metals (Cd, Cu, Fe, Ni, Pb, and Zn) in 4 seafood species, including 3molluscs (*Perna viridis, Paphia malabarica, Meretrix casta),* and 1 crustacean species (*Scylla serrata*). Samples were collected from rural and urban areas of Goa namely Shiridao, Chorao Island, Cortalim, Margoa, Amona, and Madkai during the December 2023 and April 2024. The *Perna viridis* were collected from Karwar as Goa Market gets this supply in Goa Cusine from Karwar. The edible samples were analysed for heavy metal concentrations after acid digestion using a flame atomic absorption spectrophotometer.

According to a recent study, urban areas have higher pollution levels than rural areas, as indicated by the accumulation of heavy metals in shellfish. Specifically, the current study revealed that iron (Fe) and copper (Cu) accumulation was significantly higher in urban regions. Furthermore, in some areas, cadmium (Cd) and lead (Pb) accumulation exceeded the permissible limits. To address this issue, continuous monitoring is necessary, along with raising public awareness and implementing strict government policies to mitigate metal pollution in the studied site.

CONTENTS

CHAPTER 1:

INTRODUCTION

1.1 Background:

Natural processes such as weathering and rock erosion release heavy metals into the environment. Numerous lithogenic and human processes cause these metals to find their way into marine and coastal habitats (Tanhan et al., 2023). In many developing nations, mining is one of the numerous economic activities that are widely practiced. Pollutants from mining effluents, primarily heavy metals, could build up in aquatic creatures and make their way into the human food chain. (Bonsignore et al., 2018 According to Gwyneth Grace E. Bernales, drinking water or eating certain foods may expose people to heavy metals. Heavy metals are present in the environment because of both human and natural action (Grace Bernales et al., 2022). Goa situated on the western coast of India, harbours diverse marine ecosystems, including estuaries like the Mandovi estuary, which are susceptible to anthropogenic pollution. (Rainbow & White, 1989)

There are many causes contribute to the presence of heavy metals, such as mining, sewage discharge, urban runoff, industrial effluents, soil erosion, crustal weathering, pesticides and disease control agents (used on crops) etc. (Mitha et al., 2021). While many biological processes in plants and animals depend on these metals, sometimes they have an added benefit because of their capacity to orchestrate chemical reactions and go through oxidation-reduction, which lets them get around control mechanisms like transport, homeostasis, compartmentalization, and binding to essential cell components (Grace Bernales et al., 2022).

Native metals cause cellular malfunction in human and eventually toxicity when they connect to protein areas that are not intended for them, displacing native metals from their typical binding locations. According to the US Food and Drug Administration (2015), breathed or consumed by humans, minute amounts of heavy metals can cause organ and systemic toxicity. Moreover, many rural regions, particularly those that area coastal or where fishing is the main source of food and money, appear to be unaware of the existence of these threats (Grace Bernales et al., 2022). Certain metals and heavy metals (Co, Cr, Cu, Fe, Mn, Mo, Ni, Se, and Zn) are vital components of many critical enzymes and play crucial roles in a variety of oxidation-reduction processes. Nevertheless, an excessive concentration of these metals can cause harm to cells and tissues. Some metals, such As, Pb, Cd, and Hg, are thought to be non-essential and perhaps hazardous at low concentrations since they have no known biological activities (Bonsignore et al., 2018). Even in tiny concentrations, the non-essential elements cadmium and lead are harmful. Toxic metals found in seafood, such as Cd, Co, Cr, Ni, and Pb, can have negative health effects on humans, including increased risk of cancer, circulatory system issues, neurological disorders, and renal damage (Mitha et al., 2021).. At a certain range of cellular concentrations, other metals like Cu, Fe, Mn, Ni, and Zn are necessary for normal human cellular activities (Mitha et al., 2021).

While a low intake would result in deficiency illnesses, an excessive intake of critical metals can also have hazardous consequences (Tanhan et al., 2023). There has been much written on the use of bivalves as bio monitors of heavy metal pollution. This is because of their traits, which are beneficial for biomonitoring from an ecological and biological perspective (Ponnusamy et al., 2014). Numerous studies have been conducted on bivalves, with a focus on mussels and oysters, to monitor metal pollution in coastal systems and determine the bioavailability of pollutants. Consuming seafood has several health advantages for consumers and is an excellent source of high-quality protein, amino acids, vitamin D, and polyunsaturated fatty acids (PUFAs). It is also likely a significant contributor to well health (Saher & Kanwal, 2019).

On the other hand, mussels and oysters are sessile species that inhabit the surface of rocks and reefs, where they mostly consume plankton and suspended materials. Hence, rather than being sediments, they might be thought of as bio-indicators of the ambient pollution state of water. Benthic organisms, like crabs and clams, are often found in sediments where they feed mostly on organic matter, benthic diatoms, and zoo benthos. Because these pollutants are more likely to be concentrated in sediments, they tend to collect heavy metals more effectively than sessile species (Liu et al., 2017). Bivalve mollusks have been proposed as a possible bio monitoring for metallic pollution in maritime environments (Ponnusamy et al., 2014).

Heavy metals have the potential to build up to hazardous amounts and harm the ecosystem in certain situations. Accordingly, heavy metals that enter the food chain because of pollution pose a risk to consumers as chemical risks (Sivaperumal et al., 2007). When present in large amounts, the necessary metals can potentially have harmful consequences. Meals intended for human consumption should only contain a small number of metals that have been shown to be dangerous (Mensah et al., 2009).

Many diets include shellfish, but it's crucial to understand the possible dangers of heavy metal poisoning in these seafood varieties. Goan cuisine is inextricably linked to seafood, and the species chosen for this study are ones that are regularly eaten. Because these mussels serve as indicators of pollution, this study is important to understanding the degree of contamination occurring in water bodies as it increases daily. Mollusks' filter-feeding process allows them to bioaccumulate residues of heavy metals. This is problematic since they are regarded as important food sources. Thankfully, regulatory bodies like the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) have set acceptable thresholds for heavy metal content that people can eat (Grace Bernales et al., 2022).

1.2 Aim and objective:

Even though the concentrations of heavy metals in Goa's waters are known, research on certain regions and species has not yet been done. As a result, it is imperative to investigate the levels of heavy metals in commercially significant shellfish species that are routinely harvested for human consumption.

This study aims to achieve the following objectives:

1. Sample collection, identification, and digestion of the sample.
2. Estimation of the concentration of lead (Pb), nickel (Ni), cadmium (Cd), iron (Fe) and copper (Cu) in the flesh of shellfish in selected samples collected from Goa and Karwar.
3. Comparative assessment of heavy metal contamination in rural or urban areas.

The species and location selected differ from those from previous studies. In earlier studies, only total mercury and methyl mercury analyses for the following species were carried out: clams (*Meretrix* species) and mud crabs (*Scylla serrata*); no additional analyses have been carried out. No research has yet been done in the regions of Amona and Siridao.

Essential components of this issue include informing people about the safety of seafood if the WHO/FAO heavy metal guideline falls below an acceptable level.

Therefore, such research may be done in particular intervals to get the most recent data.

CHAPTER 2:

LITERATURE REVIEW

Shellfish serve as important bioindicators for assessing the accumulation of heavy metals in marine ecosystems due to their sedentary nature and ability to accumulate contaminants from surrounding waters (Vijayalakshmi et al., 2019). Bivalves, including *Paphia malabarica*, are commonly studied shellfish species in the region due to their widespread distribution and ecological significance. They exhibit the capability to accumulate heavy metals such as cadmium (Cd), mercury (Hg), lead (Pb), arsenic (As), and chromium (Cr) in their tissues, reflecting environmental contamination levels accurately (Vijayalakshmi et al., 2019; Khandeparker & Anil, 2019). Studies conducted in the Mandovi estuary of Goa have reported spatial and temporal variations in heavy metal concentrations in shellfish tissues (Khandeparker & Anil, 2019).

2.1 Bivalves as heavy metal pollution bioindicators:

Bivalves, a diverse group of mollusks including clams, mussels, and oysters, play a crucial role in marine ecosystems and are widely recognized as bioindicators of environmental health (Pereira et al., 2020). The use of bivalves as bioindicators stems from their ability to accumulate contaminants from their surrounding environment, including heavy metals, organic pollutants, and pathogens (Liu et al., 2019).

Bivalves accumulate contaminants through various mechanisms, including filtration of water for food and respiration, absorption through gills and tissues, and ingestion of sediment-bound contaminants (Campana et al., 2018). Numerous monitoring programs worldwide utilize bivalves as bioindicators to assess ecosystem health and monitor pollution levels in coastal and estuarine environments (Pereira et al., 2020). The use of bivalves as bioindicators offers several advantages, including their widespread distribution, ease of sampling, and cost-effectiveness compared to traditional monitoring methods (Liu et al., 2019).

2. 2 Guidelines for managing heavy metal pollution in aquatic ecosystem:

Government regulations and policy interventions play a crucial role in controlling heavy metal pollution in aquatic environments. Legislation such as effluent discharge standards, water quality guidelines, and seafood safety regulations help limit metal inputs and ensure compliance with safe levels of contamination (Hassan et al., 2018). Pollution prevention measures focus on reducing metal emissions at the source through improved industrial practices, wastewater treatment technologies, and pollution control measures (Khan et al., 2019). Implementing best management practices in industries, agriculture, and urban areas can significantly reduce metal pollution inputs into aquatic ecosystems. (Meshram et al., 2014)

Various remediation technologies are available for mitigating heavy metal contamination in aquatic environments. These include phytoremediation, bioremediation, sediment dredging, and chemical treatments aimed at immobilizing or removing metals from contaminated sediments and waters (Cundy et al., 2020). These technologies help restore ecosystem health and reduce metal bioavailability to seafood. Raising public awareness about the risks of heavy metal contamination in seafood is essential for promoting consumer knowledge and behavior change. (El-Shenawy, 2004)

Educational campaigns, seafood advisories, and labeling initiatives can empower consumers to make informed choices and reduce exposure to contaminated seafood (Chen et al., 2019). Addressing heavy metal contamination in seafood requires collaborative efforts between governments, industries, research institutions, NGOs, and local communities. Partnerships aimed at sharing knowledge, resources, and expertise can facilitate the development and implementation of effective mitigation strategies. (Shue et al., 2014)

2.2.1 Demystifying heavy metal limits in seafood

Regulatory agencies worldwide establish limits for heavy metal concentrations in seafood to protect public health and ensure food safety. These guidelines serve as a reference for international trade and help ensure the safety of seafood consumed globally. (Anagha et al., 202).

The permissible limits for heavy metals in seafood can vary depending on the regulatory body and the specific type of seafood. Provisional tolerable weekly intake (PTWI) values may not be available for all heavy metals, and some metals may not have weekly tolerable intake values due to their toxicity. Iron is considered an essential nutrient, and there is typically no PTWI established for it.

However, excessive intake of iron from supplements or contaminated food sources can lead to toxicity. The World Health Organization (WHO) recommends a provisional tolerable monthly intake (PTWI) of 7 µg/kg body weight for cadmium (WHO, 2018). The European Food Safety Authority (EFSA) has set a tolerable weekly intake (TWI) of 2.8 µg/kg body weight for nickel (EFSA, 2015). The Codex Alimentarius Commission of the Food and Agriculture Organization/World Health Organization (FAO/WHO) establishes maximum copper levels in specific fish and fisheries products.

As per the Codex Alimentarius Commission (2020), finfish fillets have a maximum copper content of 30 mg/kg. Additionally, the FAO/WHO Codex Alimentarius Commission establishes maximum zinc levels in specific fish and fisheries products. Zinc, for instance, has a maximum limit of 100 mg/kg in finfish fillets (Codex Alimentarius Commission, 2020). The WHO recommends a provisional tolerable weekly intake (PTWI) of 25 µg/kg body weight for lead (WHO, 2010). The WHO recommends a provisional tolerable weekly intake

(PTWI) of 1.6 µg/kg body weight for methyl mercury, a form of mercury commonly found in seafood (WHO, 2010). The WHO has set a Provisional Tolerable Weekly Intake (PTWI) of 15 µg/kg body weight for inorganic arsenic (WHO, 2011).

2.3 How accumulation of heavy metals occurs in shellfish:

There are two primary pathways by which heavy metals enter the aquatic food chain: non-dietary pathways via permeable membranes like the muscle and gills, and direct ingestion of food and water through the digestive system.(Author et al., 2015) In fact, oysters that develop at the bottom might collect Cd up to ten times more than oysters that grow at the same location but in baskets submerged in the water's top (Batvari et al., 2016). According to published research, the concentration of Cd tends to drop in surface water and increase in deeper seas. (Djedjibegovic et al.2020) Given that gastropods are often buried in fine sediments, their living conditions and the presence of metallothioneins, proteins that bind to Cd and aid in the production of shells, may be contributing factors to the buildup of Cd in their bodies.(Ponnusamy et al., 2014)

2.3.1 How the heavy metals reach the human body:

Heavy metals can enter the human body from inhalation, skin contact, or the gastrointestinal system. Eating vegetables, coming into touch with them, and breathing in contaminated dust are the major ways that humans are exposed to heavy metals. (Kumar & Achyuthan, 2007) The digestive tracts of fish and shellfish, as well as non-dietary pathways including gills and muscles, are potential entry points for heavy metals into seafood. (Bhatkhande & Nasnodkar, 2022) When plants and animals that humans consume come into touch with water, soil, or the seafloor, heavy metals have the potential to infiltrate the food chain. (Mishra et al., 2019) Water is contaminated by industrial pollution and naturally occurring off-gassing of mercury from the earth's crust. The mercury that enters the streams is methylated by bacteria and algae, which subsequently passes the methylation process to fish and shellfish, and ultimately to people.(Ragi et al., 2017)

2.3.2 Diseases caused by the accumulation of heavy metals:

Numerous illnesses, such as skin lesions, internal malignancies, neurological issues, lung disease, peripheral vascular disease, hypertension, cardiovascular disease, and diabetes mellitus, can be brought on by prolonged exposure to heavy metals.(Sivaperumal et al., 2007) Lead and mercury are two heavy metals that can have serious side effects, including as renal failure, bloody diarrhea, and discomfort in the abdomen during colic. (Kesavan & Vijay Kumar, 2013) Arsenic, cadmium, chromium, lead, and mercury are considered priority metals of public health relevance due to their high degree of toxicity. Even at lower exposure levels, these metallic elements are known to causes organ damage and are classified as systemic toxicants. Certain regions of the brain may experience cell death as a result of heavy metal accumulation in the blood. (Sarkar et al., 2008)

There are some techniques used to detect heavy metals in water, sediments and in live organisms like fish, mollusc and crustaceans. Several techniques are employed for detecting heavy metals in shellfish tissue. (Saher & Kanwal, 2019) Among these, instrumental analytical methods such as atomic absorption spectrometry (AAS), inductively coupled plasma mass spectrometry (ICP-MS), and inductively coupled plasma optical emission spectrometry (ICP-OES) are commonly utilized due to their high sensitivity and precision. These techniques enable the quantitative analysis of various heavy metals, including but not limited to cadmium (Cd), lead (Pb), mercury (Hg), arsenic (As), and chromium (Cr), in shellfish samples. (Ramkumar et al., 2024)

2.3.3 Previous studies:

In Goa's Mandovi estuary, the clam species Paphia malabarica exhibited seasonal variations in heavy metal accumulation, as reported by Krishna Kumari et al. (2006b). Their study found high levels of lead (Pb) during December to April, while cadmium (Cd) levels remained uniform throughout the year. Furthermore, elevated concentrations of iron (Fe), zinc (Zn), and copper (Cu) were observed from September to January.

Shenai-Tirodkar et al. (2016) investigated Oyster species including Crassostrea sp., C. gryphoides, and Saccostrea sp. from Chicalim Bay, Nerul Creek, and Chapora Bay. They identified high levels of cadmium (Cd) in oyster gills during autumn and winter, attributed to increased surface area and mucus adsorption.

Ray et al. (2019b) analyzed total mercury and methyl mercury in benthic fishes like Perna viridis and pelagic fishes in Goa's Mandovi and Zuari rivers. They found higher mercury concentrations in benthic fishes (19-261 µg/kg) compared to pelagic fishes (18-155 µg/kg).

Bhutia et al. (2023) studied Cassosstrea sp. and Polymesoda sp. from lower Chapora Estuary, middle Zuari Estuary, and Moira River. They noted significantly high iron (Fe) levels in all body tissues and elevated zinc (Zn) levels in gills of the bivalves.

Rodrigues et al. (2021b) examined Saccostrea cucullata from Zuari, highlighting high manganese (Mn), zinc (Zn), and copper (Cu) accumulation in soft tissues compared to hard shells.

In the Sunderban mangrove wetland, Sarkar et al. (2008) found copper (Cu) and zinc (Zn) levels exceeding permissible limits in Sanguinolaria acuminata, Anadara granosa, Meretrix meretrix, and Pelecyora trigona during January and February.

Sankar et al. (2006) reported higher copper (Cu), zinc (Zn), manganese (Mn), nickel (Ni), and chromium (Cr) levels in freshwater environments compared to brackish water and marine environments in Kerala's Calicut region from April to September.

Kumar & Achyuthan (2007) investigated heavy metal accumulation in prawn, Solenocera crassicornis crab, Scylla serrata, and mussel, Perna viridis, along the East Coast, highlighting zinc (Zn) as the predominant metal in crab hepatopancreas and gills.

Yogeshwaran et al. (2020b) analyzed zinc (Zn), copper (Cu), cadmium (Cd), and lead (Pb) in Scylla serrata from Punnakayal, Tuticorin, reporting higher Zn and Cu levels compared to Cd and Pb

Cheung et al. found elevated levels of cadmium (Cd), copper (Cu), and chromium (Cr) in Pacific oysters from Hong Kong's Lau Fau Shan market during autumn and winter.

Saher & Kanwal (2019) reported higher zinc (Zn) concentrations in all species and lead (Pb) and cadmium (Cd) levels exceeding permissible limits in Karachi fish harbor species.

Bonsignore et al. (2018b) analyzed various metals in fish species from the Tuscany coast, while Liu et al. (2017) assessed heavy metal contamination in shellfish from China's Laizhou Bay, noting high cadmium (Cd) levels.

Rainbow & White (1989b) studied zinc (Zn), copper (Cu), and cadmium (Cd) concentrations in amphipods from Scotland's Marine Biological Station, highlighting a decrease with increasing amphipod dry weight.

These studies provide valuable insights into the spatial and temporal variability of heavy metal contamination in shellfish species across different geographical regions.

CHAPTER 3:

MATERIALS AND METHOD

3.1: Study area:

Goa has an extensive coastline stretching along the Arabian Sea, offering a wide variety of habitats for shellfish. This includes sandy beaches, rocky shores, estuaries, and mangrove forests. Each of these habitats presents unique conditions for shellfish growth and exposure to heavy metals (Mitha et al., 2021). Goa is home to various shellfish species, including clams, mussels, oysters, and crabs, which are an integral part of the coastal ecosystem and are often consumed by locals and tourists. Studying different species of shellfish can help assess the variability in heavy metal accumulation patterns and the potential risks to human health (Shenai-Tirodkar et al., 2016).

For investigating heavy metal accumulation in shellfish, samples were collected from the rural and urban areas of Goa and Karwar for a period of 5 Months from December 2023 to April 2024 (post-monsoon). The present study aimed to determine the concentration of heavy metals in shellfish species and assess human health risks by consuming these shellfish. The sampling locations were Chorao Island, Siridao, Cortalim, Amona, Margao, and Karwar; samples were collected with the help of local fishermen. Immediately after collection, the specimens were brought into the laboratory and cleaned using distilled water. Four seafood species were collected: green mussel (*Perna viridis*), Clam (*Paphia malabarica*), Clam (*Meretrix casta*), and Mud Crab (*Scylla serrata*) from both rural and urban areas. The samples were cleaned and stored at -20ºC until further analysis.

3.2 Identification of Shellfish:

The sample identification and dissection were done with the help of the Zoology Faculty. The Scylla serrata identified by the chelipeds (claw-legs) are massive, smooth, and longer than the other legs. The male’s abdomen is narrow, with segments 3-5 fuses. The shell color varies from a deep, mottled green to a very dark brown. The green mussel identification was done by the feature such as the smooth periostracum (outer shell) is dark green, becoming browner towards the point of attachment (umbo). *Meretrix* species were identified using morphological characteristics such as a large clam with a thick shell covered by thin, delicate, straw-colored or grey periostracum and a greyish-blue or bluish-brown band on its posterodorsal margin (Linnaeus, 1758). The short neck clam has a shell that is triangular to oval in shape with rounded anterior and posterior margins. Shell is more or less long and smooth but the outer surface of shell has concentric ridges. Hinge area is short and has narrow diverging teeth. The pallial sinus is not too deep and is 'U' shaped. (I D E N T I F I C A T I O N, 2010)

3.3 Digestion of the sample and AAS analysis:

After cleaning the samples the average length was taken for each sample by using graph paper. The samples were taken in triplicate each set containing 6 species. The average wet weight of each set was measured, and after that, they were dried at 70 ± 2 ºC in a hot air oven for 24 hours. (Tanhan et al., 2023) The samples were then processed for further analysis.

The shellfish's muscle tissue (soft body parts) was weighed, and 0.2g of soft tissue was taken and homogenized without adding water. A 4 ml mixture of concentrated nitric acid (HNO3) and perchloric acid (HClO4) in a 3:1 ratio was added to the above homogenized soft tissue. The mixture obtained was kept for digestion for about a day to get a clear solution that showed a yellow peal color. These clear solutions were then filtered through 10µm Whatman’s filter paper. One mL of the filtered solution was collected in a fresh reagent bottle and the final volume was made up to 100 ml by diluting with distilled water (Tanhan et al., 2023), (Krishna Kumari et al., 2006a), (Grace Bernales et al., 2022).

This diluted solution was stored in two 50 ml capacity centrifuge tubes to be analyzed for heavy metal using a flame atomic absorption spectrophotometer (AAS) (Perkin Elmer PinAAcle 900F made in india).

Five heavy metals i.e., Cd, Pb, Ni, Cu, and Fe were chosen to be assessed, in selected species. Most heavy metals exist in combination with other organic materials in soil or plant matter; therefore, acid helps in breaking existing bonds between the metals and the matrix from which they are to be extracted. Most metals, however, require acid digestion for solubility purposes. (Patchaiyappan et al., 2023)

CHAPTER 4 :

RESULT AND DISCUSSION

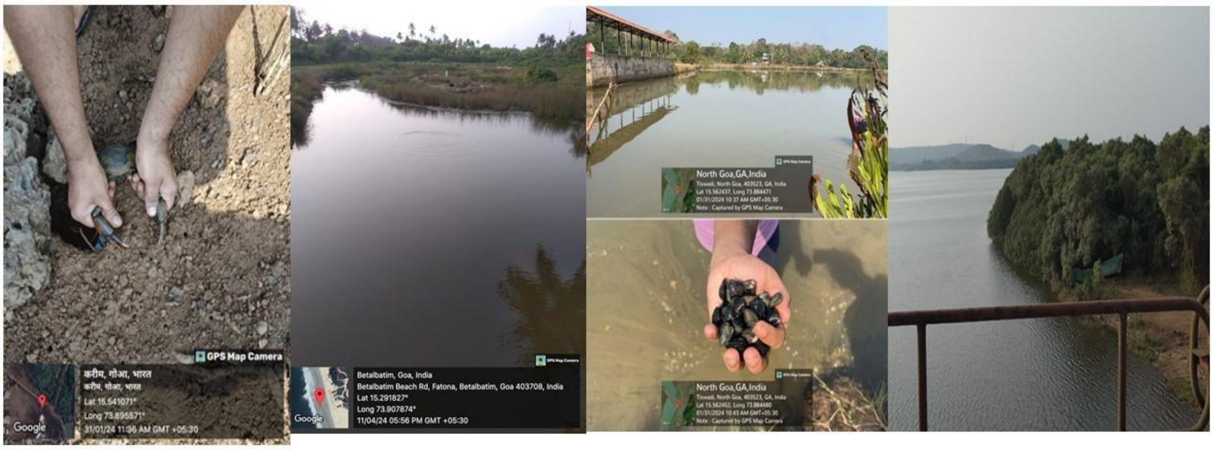
4.1 Study area:

Samples were collected from different areas of Goa, and one sample was collected from Karwar. Table No.1 below shows six sampling sites along with their coordinates; the sampling sites were divided into rural areas and urban areas. The species and the site chosen in this study are different from those in previous studies. No studies have been done before in Siridao, Amona, and Betal Batim.

Table 1: Location co-ordinates of sampling site

SAMPLES	**RURAL**	**URBAN**
Green Mussel- ***Perna viridis***	Margoa (Betal batim) Latitude -15.292155^0 Longitude -73.906412^0	Karwar Latitude -14.85194^0 Longitude -74.13064^0
Clam ***Paphia malabarica***	Siridao Latitude –15.43584^0 Longitude -73.860698^0	Amona Latitude -15.529421^0 Longitude -73.986226^0
Clam ***Meritrix casta***	Chorao Latitude –15.56244^0 Longitude -73.884471^0	Amona Latitude -15.529421^0 Longitude -73.986226^0
Mud Crab - ***Scylla serrata***	Chorao Latitude- 15.54125^0 Longitude - 73.90016^0	Cortalim Latitude -15.40125^0 Longitude -73.907859^0

The chosen sampling site pictures are depicted in Fig.1. Two species, *Meretrix casta* and *Scylla serrata*, were collected from Chorao. Two species belonging to *Meretrix meretrix* and *Paphia malabarica* were obtained from Amona. *Perna viridis* was obtained from Betal Batim and Karwar. *Paphia malabarica* and *Scylla serrata* were collected from Siridao and Cortalim, respectively.



Chorao – Scylla serrata ***Betal batim – Perna viridis*** ***Chorao – Meretrix casta*** ***Amona- Meretrix meretrix Paphia malabarica***

Fig 1: Sampling sites for different species

After collecting the samples, they were cleaned using tap water, and the length of each species was measured. The average length of the sample was calculated. Fig 2: Illustrates the measurement of the length of the samples collected across various sampling sites.

The samples were collected from Siridao (*Paphia malabarica*, n=18), Chorao (*Scylla serrata*, n=6) (*Meretrix casta*, n=18), Betal batim, Margao (*Perna viridis*, n=18), Amona (*Paphia malabarica*, n=18), (*Meretrix meretrix*. n=18), Karwar (*Perna viridis*, n=18). Cortalim (*Scylla serrata*, n=6). Seafood is an inseparable part of Goan cuisine; the species selected for this study are known to be consumed in daily life.

Fig 2: Measurement of the length of the samples collected across various sampling sites.

4.2 Species identification and dissection:

This dissertation aimed to understand clams, green mussels, and crabs by exploring their morphological characteristics, taxonomic relationships, and anatomical structures through comprehensive species identification and dissection techniques. After that, the soft tissue from the sample was extracted and kept in three sets (each set had 6 samples) for further analysis. Figure 3 illustrates the steps followed in the dissection of *Paphia malabarica*, *Perna viridis*, *Scylla serrata*, and *Meritrix meretrix*.

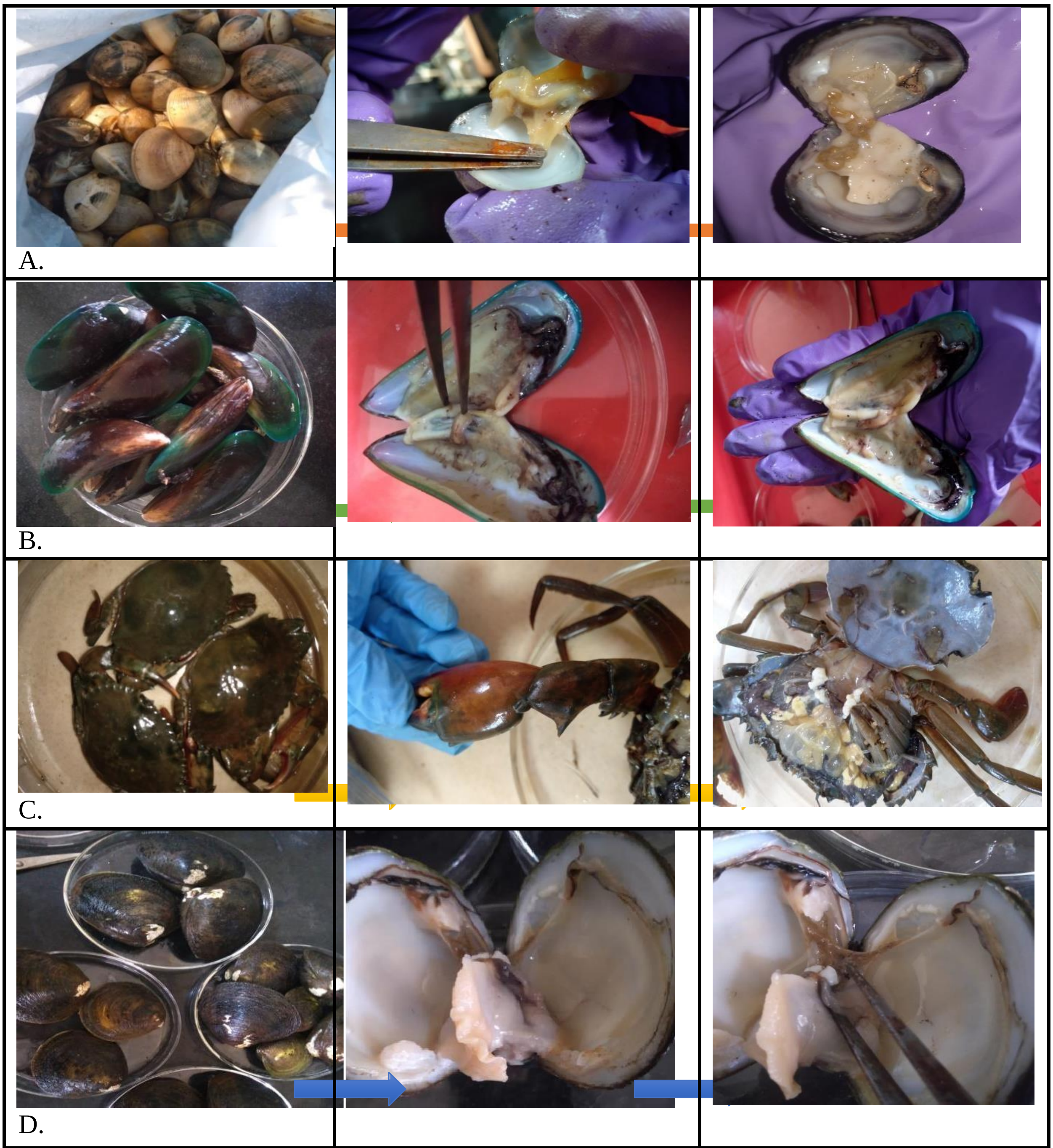


Fig 3. Dissection of the shellfish: A) *Paphia malabarica* B) *Perna viridis* C) *Scylla serrata* D) *Meritrix meretrix.*

The average wet weight and dry weight were measured for each of the measures. International organizations such as FAO/WHO set standard values for metal content on a wet weight basis. To compare international standards, the mean ratio (wet to dry weight) is used to convert metal content to µg/g wet weight. Regulatory standards and guidelines for heavy metal concentrations in food products are typically stated in terms of dry weight. To ensure compliance with legal requirements and assess food safety, the measured concentrations were converted from wet weight to dry weight. This enables a direct comparison of the values obtained in our study with regulatory limits. Table 2, 2.1, 2.2, 2.3 Illustrates the dry weight, wet weight and average length of the each sample.

Table 2: Illustrates the dry weight, wet weight and average length of the *Perna viridis* and *Scylla serrata*

RURAL	6 Species /SET		2 species/SET	
	Perna viridis		***Scylla serrata***	
	Wet wt. (in g)	Dry wt. (in g)	Wet wt. (in g)	Dry wt. (in g)
SET -1	45.16	7.84	21.94	6.21
SET- 2	42.78	6.26	11.878	3.13
SET -3	48.53	8.2	11.769	3.09
Average weight	**45.49**	**7.43**	**15.19**	**4.14**
Average Length	**7.2 cm**		**10.3 cm**	

Table 3: Illustrate the dry weight, wet weight and average length of the *Meritrix casta* and *Paphia malabarica*

	6 Species/SET			
RURAL	***Meritrix casta***		***Paphia malabarica***	
	Wet wt. (in g)	Dry wt. (in g)	Wet wt. (in g)	Dry wt. (in g)
SET -1	5.5	1.16	8.51	1.87
SET- 2	10.78	2.26	9.23	1.96
SET -3	6.98	1.47	8.62	1.85
Average weight	**7.75**	**1.63**	**8.78**	**1.89**
Average Length	**2.5 cm**		**3.4 cm**	

Table 4: Illustrate the dry weight, wet weight and average length of the *Perna viridis* and *Scylla serrata*

6 species/SET			2 species/SET	
URBAN	***Perna viridis***		***Scylla serrata***	
	Wet wt. (in g)	Dry wt. (in g)	Wet wt. (in g)	Dry wt. (in g)
SET -1	42.88	6.73	10.28	3.35
SET- 2	47.45	8.02	20.10	5.78
SET -3	45.08	7.47	12.83	3.92
Average weight	**45.13**	**7.40**	**14.43**	**4.35**
Average Length	**7.7 cm**		**9 cm**	

Table 5: Illustrate the dry weight, wet weight and average length of the *Meritrix meretrix* and *Paphia malabarica*

6 species/SET				
URBAN	***Meretrix meretrix***		***Paphia malabarica***	
	Wet wt. (in g)	Dry wt. (in g)	Wet wt. (in g)	Dry wt. (in g)
SET -1	35.26	6.10	9.75	1.98
SET- 2	59.95	8.92	10.02	2.21
SET -3	31.64	4.63	8.64	1.84
Average weight	**42.28**	**6.55**	**9.47**	**2.01**
Average Length	**7.57 cm**		**3.8 cm**	

The Fig no. 4 below shows the obtained dry weight of the different samples; It is crucial to measure and record accurately the dry weight, wet weight, and average length of shellfish specimens during scientific research and monitoring programs aimed at understanding shellfish biology, ecology, and environmental health. These measurements are critical in obtaining reliable data, essential for effectively monitoring and managing shellfish populations.

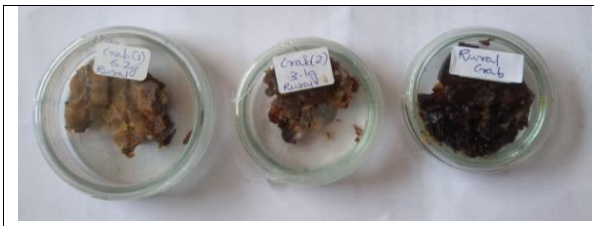
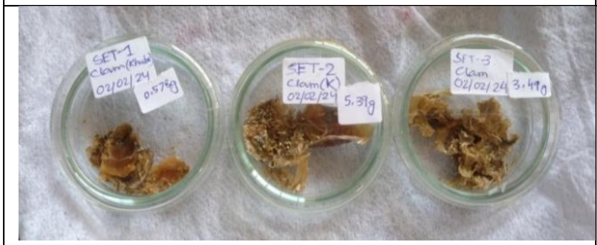
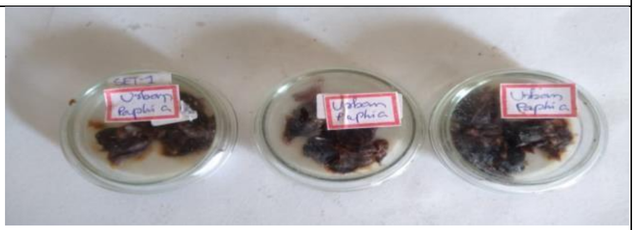
 A. Dry weight of Scylla serrata muscle collected from Chorao	 B. Dry weight of Scylla serrata muscle collected from Chorao
 C. Dry weight of Perna viridis collected from Betul	 D. Dry weight of Perna viridis collected from Karwar
 E. Dry weight of Meritrix casta collected from Chorao	 F. Dry weight of Meritrix casta collected from Amona
 G. Dry weight of Paphia malabarica collected from Shiridao	 H. Dry weight of Paphia malabarica collected from Amona

Fig 4. The weight recored after dryed shellfish muscle at 70 ± 2^0 C for a day

<u>4.3 Acid digestion of sample and AAS analysis:</u>

The process of analysing muscle tissue using Atomic Absorption Spectrometry (AAS) involves breaking down the tissue's organic matrix to release heavy metals and dissolve them for subsequent quantification by AAS. Many heavy metals are present in insoluble or bound forms within biological tissues, making them difficult to analyse directly using AAS. Acid digestion helps convert these insoluble metal species into soluble forms through chemical reactions, such as dissolution and complexation. Once the metals are in a soluble form, they can be accurately measured using AAS, allowing for precise determination of their concentrations in the sample.

For acid digestion, 3g of soft tissue was taken and digested in 60 ml of concentrated nitric acid and perchloric acid in a ratio of 3 1. There is a specific wavelength to detect the specific metal. Tanhan et al. (2023) reported that all metals were examined using the absorbance mode at their respective ideal wavelengths, which were Cd at 228.8 nm, Cu at 324.7 nm, Fe at 248.3 nm, Ni at 232.0 nm, and Pb at 283.3 nm. The calibration curve for each individual metal was used to determine its concentration.

The steps followed for acid digestion of the samples are summarised in Fig 5.

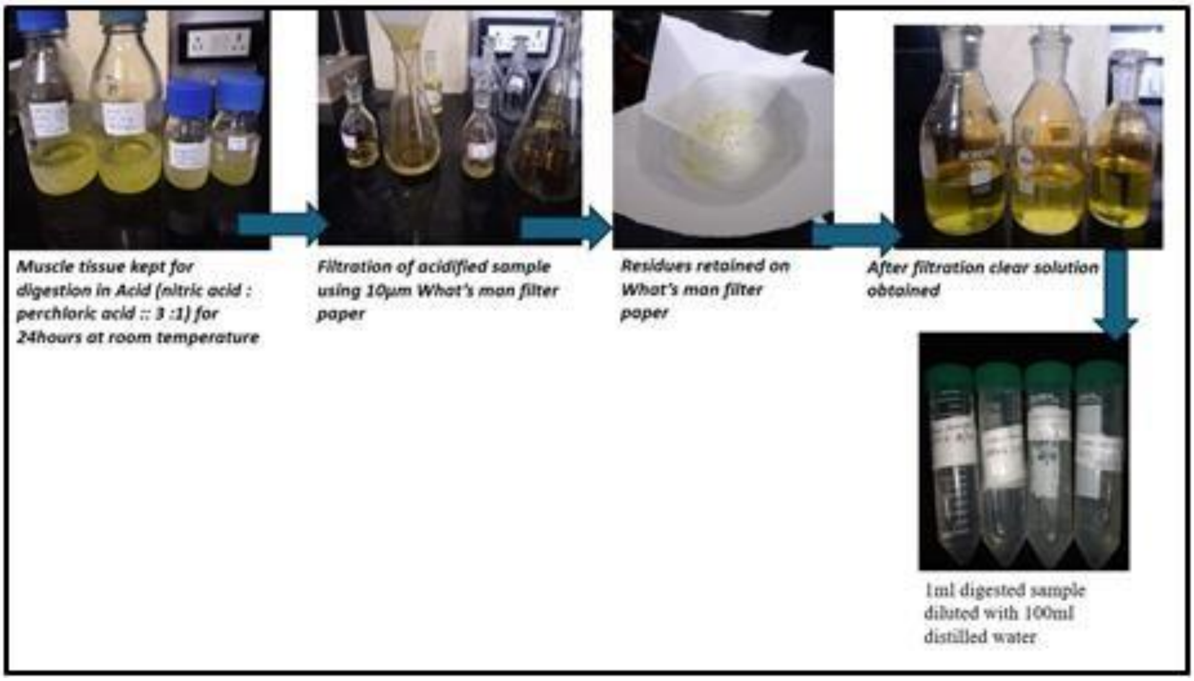


Fig. 5 Muscle tissue kept for digestion in Acid (nitric acid: perchloric acid: 3:1) for 24hours at room temperature, 2.Filtration of acidified sample using What's man filter paper, 3. Residues retained on What's man filter paper, 4. After filtration clear solution obtained. 5. 1ml digested sample diluted with 100ml distilled water

4.3.1 AAS result calculation and analysis:

Many regulatory standards, guidelines, and scientific literature express contaminant concentrations in terms of micrograms per gram (µg/g) or milligrams per kilogram (mg/kg) for easier comparison and standardisation. By converting concentrations from parts per billion (ppb) to µg/g, AAS results can be directly compared to regulatory limits, reference values, and thresholds established for environmental, food safety, and human health purposes.

Table no. 6: Calculation of average,standard deviation (SD) and conversion of metal concentration from ppb to µg/g in *Meretrix casta*

Meritrix casta		Unit in ppb			RURAL	
Sr.No.	Parameter	SET-1	SET-2	SET-3	Mean ± SD	µg/g
5	Cadmium (Cd)	0.568	0.524	0.506	0.533 ± 0.03	0.017
4	Copper (Cu)	9.368	7.521	6.778	7.889 ± 1.33	0.26
2	Iron (Fe)	170	50	30	83.33 ± 75.71	2.78
1	Lead (Pb)	1.532	1.433	1.751	1.572 ± 0.16	0.052
3	Nickel (Ni)	0.439	0.058	0.30	0.265 ± 0.19	0.008

Table no. 7: Calculating average and standard deviation (SD) as well as converting metal concentration from ppb to µg/g in *Paphia malabarica.*

Paphia malabarica		Unit in ppb			RURAL	
Sr.No.	Parameter	SET-1	SET-2	SET-3	Mean ± SD	µg/g
1	Cadmium (Cd)	0.510	0.542	0.577	0.543 ± 0.03	0.018
2	Copper (Cu)	-	-	-	-	-
3	Iron (Fe)	90	20	-	55 ± 49.49	1.833
4	Lead (Pb)	1.551	1.907	3.369	2.28 ± 0.96	0.076
5	Nickel (Ni)	2.242	1.467	0.927	1.54 ± 0.66	0.051

Table no. 8: Calculating average and standard deviation (SD) as well as converting metal concentration from ppb to µg/g in *Scylla serrata*

Scylla serrata		Unit in ppb			RURAL	
Sr.No.	Parameter	SET-1	SET-2	SET-3	Mean ± SD	µg/g
1	Cadmium (Cd)	0.050	0.172	0.225	0.149 ± 0.08	0.005
2	Copper (Cu)	15.83	16.37	16.67	16.29 ± 0.42	0.543
3	Iron (Fe)	80	40	70	63.33 ± 20.81	2.11
4	Lead (Pb)	1.349	0.948	0.741	1.013 ± 0.31	0.034
5	Nickel (Ni)	-	-	-	-	-

Table no. 9: Calculating average and standard deviation (SD) as well as converting metal concentration from ppb to µg/g in *Perna viridis*

Perna viridis		Unit in ppb			RURAL	
Sr.No.	Parameter	SET-1	SET-2	SET-3	Mean ± SD	µg/g
1	Cadmium (Cd)	0.041	0.016	0.07	0.042 ± 0.027	0.001
2	Copper (Cu)	**33.35**	**31.32**	**32.12**	**32.26 ± 1.02**	1.075
3	Iron (Fe)	120	50	230	133.33 ± 90.7	4.44
4	Lead (Pb)	1.193	1.341	1.105	1.21 ± 0.11	0.04
5	Nickel (Ni)	0.930	0.138	0.984	0.68 ± 0.47	0.022

Table no. 10: Calculating average and standard deviation (SD) as well as converting metal concentration from ppb to µg/g in *Meretrix meretrix*

Meretrix meretrix		Unit in ppb			URBAN	
Sr.No.	Parameter	SET-1	SET-2	SET-3	Mean ± SD	µg/g
1	Cadmium (Cd)	1.249	1.116	1.181	1.182 ± 0.01	0.039
2	Copper (Cu)	12.06	11.28	10.75	11.36 ± 0.66	0.378
3	Iron (Fe)	98.12	98.43	98.88	98 ± 0.38	3.267
4	Lead (Pb)	3.136	2.762	3.104	3.001± 0.2	0.10
5	Nickel (Ni)	2.612	2.352	2.0263	2.66 ± 0.34	0.07

Table no. 11: Calculating average and standard deviation (SD) as well as converting metal concentration from ppb to µg/g in *Paphia malabarica*

Paphia malabarica		Unit in ppb			URBAN	
Sr.No.	Parameter	SET-1	SET-2	SET-3	Mean ± SD	µg/g
1	Cadmium (Cd)	-	-	-	-	
2	Copper (Cu)	46.28	47.32	47.68	47.09 ± 0.72	1.56
3	Iron (Fe)	69.31	69.3	69.33	69.32 ± 0.015	2.31
4	Lead (Pb)	1.413	1.478	1.632	1.51 ± 0.11	0.001
5	Nickel (Ni)	2.647	2.593	2.531	2.59 ± 0.05	0.002

Table no. 12: Calculating average and standard deviation (SD) as well as converting metal concentration from ppb to µg/g in *Scylla serrata*

Scylla serrata		Unit in ppb			URBAN	
Sr.No.	Parameter	SET-1	SET-2	SET-3	Mean ± SD	µg/g
1	Cadmium (Cd)	0.726	0.695	0.720	0.714 ± 0.01	0.023
2	Copper (Cu)	119.5	120.4	121.6	120.5 ±1.06	4.016
3	Iron (Fe)	90.12	90.11	90.09	90.11 ± 0.015	3.003
4	Lead (Pb)	2.693	2.976	2.951	2.87 ± 0.15	0.095
5	Nickel (Ni)	1.974	1.556	1.428	1.653 ± 0.28	0.055

Table no. 13: Calculating average and standard deviation (SD) as well as converting metal concentration from ppb to µg/g in *Perna viridis*

Perna viridis		Unit in ppb			URBAN	
Sr.No.	Parameter	SET-1	SET-2	SET-3	Mean ± SD	µg/g
1	Cadmium (Cd)	0.477	0.326	0.311	0.371 ± 0.09	0.012
2	Copper (Cu)	59.27	60.32	60.87	60.16 ± 0.81	2.005
3	Iron (Fe)	170.11	170.21	10.32	170 ± 0.13	5.66
4	Lead (Pb)	4.863	5.069	4.784	4.906 ± 0.14	0.163
5	Nickel (Ni)	3.864	3.356	4.489	3.903 ± 0.56	0.301

4.3.2 : Estimation of the concentration of Lead (Pb), Nickel (Ni), Cadmium (Cd), Iron (Fe) and Copper (Cu) in the flesh of shellfish:

In this assessment of different heavy metal concentrations in different shellfish species, we found that the iron content was higher than other metals. However, Fe content was found to be less in Scylla serrata, which was collected from Cortalim. Iron (Fe) and Zinc (Zn) are known to be essential trace elements for biota. Molluscs are known to have a high content of Fe as it is a constituent of goethite (α-FeOOH) for the proper functioning of radula (Tanhan et al., 2023). . The maximum permissible concentration for Fe is 100 mg/kg. (Hossain et al., 2022),(WHO Task Group on Environmental Health Criteria for Mercury--Environmental Aspects. et al., 1989).

Fig No.6 illustrates the conc of Heavy metals (µg/g) detected in *P. malabarica* species isolated from Siridao (rural) and Amona (urban). The concentrations of various metals were determined in *Paphia malabarica* samples collected from two different locations. The metal concentrations in the specimen collected from the Amona site, revealed 0.039 µg/g of Cadmium, 0.387 µg/g of Copper, 3.26 µg/g of Iron, 0.1 µg/g of Lead, and 0.077 µg/g of Nickel. In contrast, specimens collected from the Siridao site exhibited metal concentrations of 0.018 µg/g of Cadmium, 1.83 µg/g of Iron, 0.076 µg/g of Lead, and 0.05 µg/g of Nickel. Nickel concentration was found below the permissible limit.

The metal concentrations analyzed in six clam (in one set of sample) specimens collected from Siridao and Amona were within the permissible limits except copper concentration. Copper concentration was found to be 49.92 µg/g which crossing the permissible limit of 30 µg/g.

Krishna Kumari et al. (2006b) observed seasonal fluctuations in heavy metal accumulation in *Paphia malabarica* clams collected from Mandovi river. They noted elevated lead (Pb) levels during December to April, while cadmium (Cd) concentrations remained consistent throughout the year. Additionally, higher concentrations of iron (Fe), zinc (Zn), and copper (Cu) were detected from September to January.

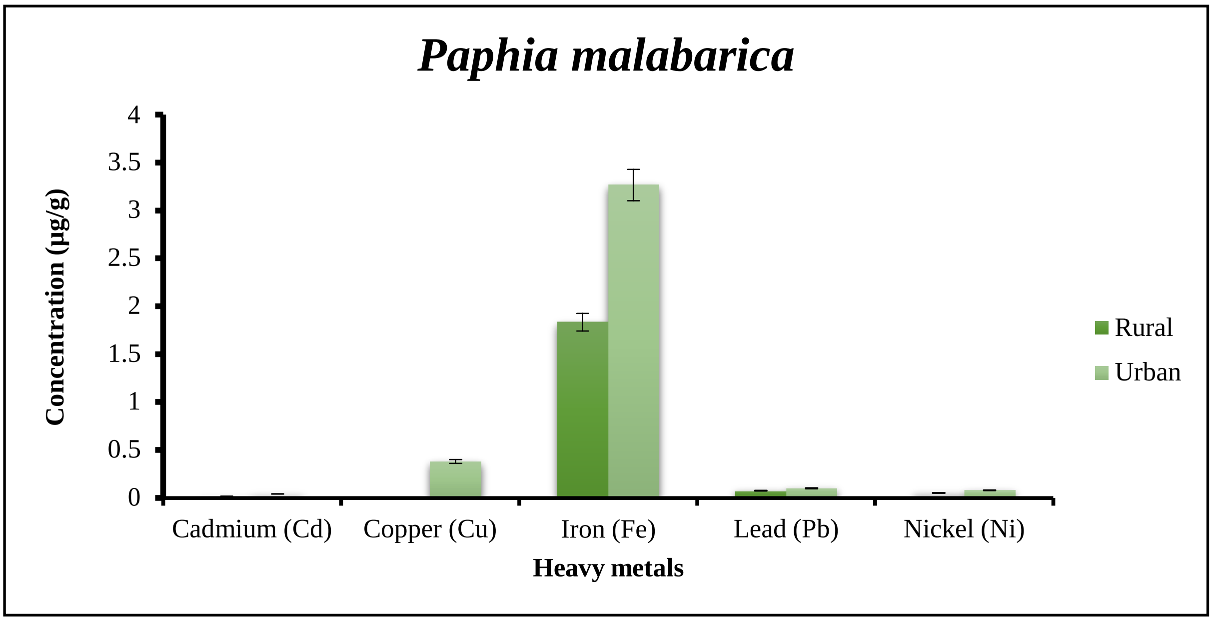


Fig 6: Heavy metal accumulation in *Paphia malabarica* collected from Siridao and Amona

Heavy metal concentrations were detected in *Scylla serrata* samples obtained from two distinct locations i.e. Chorao and Cortalim.. In specimens collected from Chorao, the concentrations were found to be 0.005 µg/g of cadmium, 0.543 µg/g of copper, 2.11 µg/ g of iron and 0.03 µg/g of lead with no nickel content detected.

Conversely, specimens from Cortalim exhibited the following concentrations: 0.023 µg/g of cadmium, 4.01 µg/g of copper, 3.03 µg/g of iron, 0.09 µg/g of lead and 0.05 µg/g of nickel. The sample weighed 14.43 grams, obtained from 2 crabs, was found to have copper and lead concentrations exceeding the permissible limits. The copper concentration was recorded at

57.96 µg/g, while the lead concentration was 1.38 µg/g. On the other hand, the iron content from the 2 mud crabs was 43.34 µg/g. Fig No.7 illustrates the conc of Heavy metals (µg/g) detected in *Scylla serrata* species isolated from Chorao (rural) and Cortalim (urban). Remarkably, the copper content was higher than the concentration of iron. Conversely, in *Scylla serrata* specimens from Chorao, all metal concentrations were found to be within the permissible limits.

Yogeshwaran et al. (2020b) analyzed zinc (Zn), copper (Cu), cadmium (Cd), and lead (Pb) in *Scylla serrata* from Punnakayal, Tuticorin, reporting higher Zn and Cu levels compared to Cd and Pb.

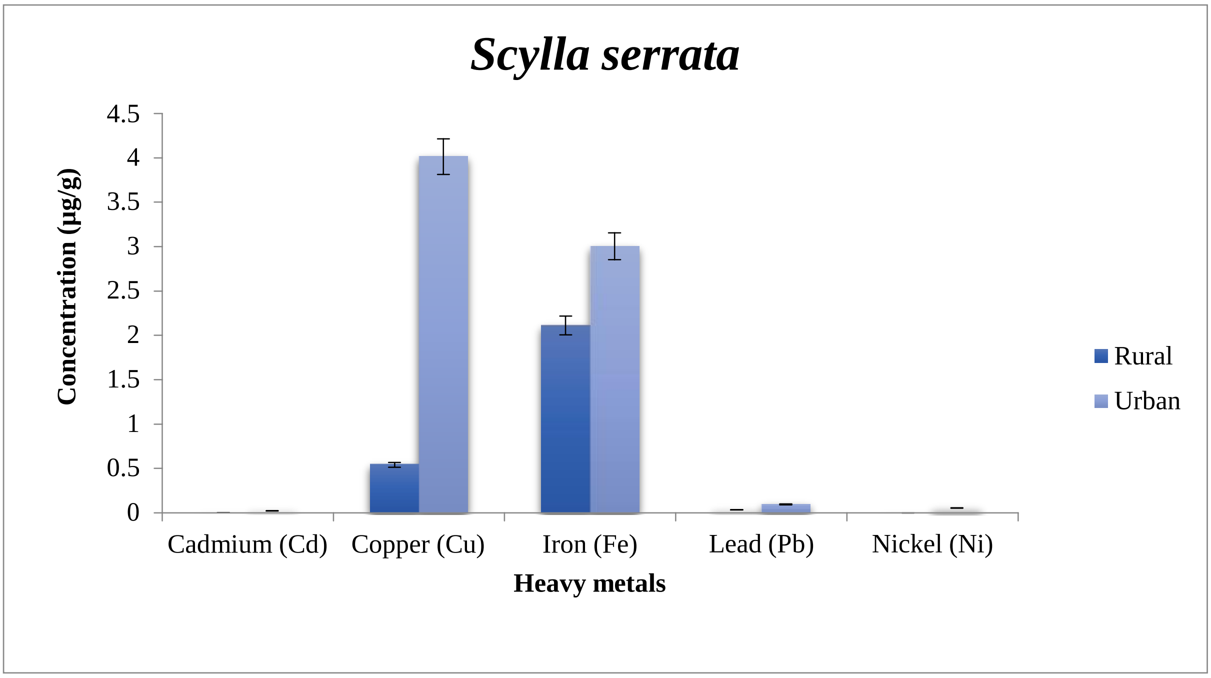


Fig 7: Heavy metal (µg/g) accumulation in *Scylla serrata* collected from Chorao and Cortalim

Metal concentrations detected in *Perna viridis* specimens collected from Betal Batim were as follows: 0.001 µg/g for cadmium, 1.07 µg/g for copper, 4.44 µg/g for iron, 0.04 µg/g for lead and 0.023 µg/g for nickel. Fig No.8 illustrates the conc of Heavy metals (µg/g) detected in *Perna viridis* species isolated from Betal batim (rural) and Karwar (urban).

In a sample comprising of 6 green mussels (one set) weighed 45.49 grams, the copper concentration measured was 48.922 µg/g, while the lead concentration was recorded as 1.83 µg/g. The concentrations of copper and lead were found to exceed the permissible limits. Additionally; the iron concentration in the sample was found to be 202.18 µg/g.

The metal concentrations detected in *Perna viridis* collected Karwar were found to be higher than those observed in samples collected from Betal Batim. Specifically, in Karwar, the following concentrations were recorded: 0.012 µg/g for cadmium, 2.005 µg/g for copper, 5.66 µg/g for iron, 0.16 µg/g for lead, and 0.13 µg/g for nickel.

Moreover, in a sample comprising 6 green mussels weighing 45.13 grams, the concentrations of cadmium, copper, and lead exceeded the permissible limits. The cadmium concentration measured 0.5 µg/g, the copper concentration was 90.49 µg/g, and the lead concentration reached 7.37 µg/g. Additionally, the iron concentration in the sample was 255.74 µg/g.

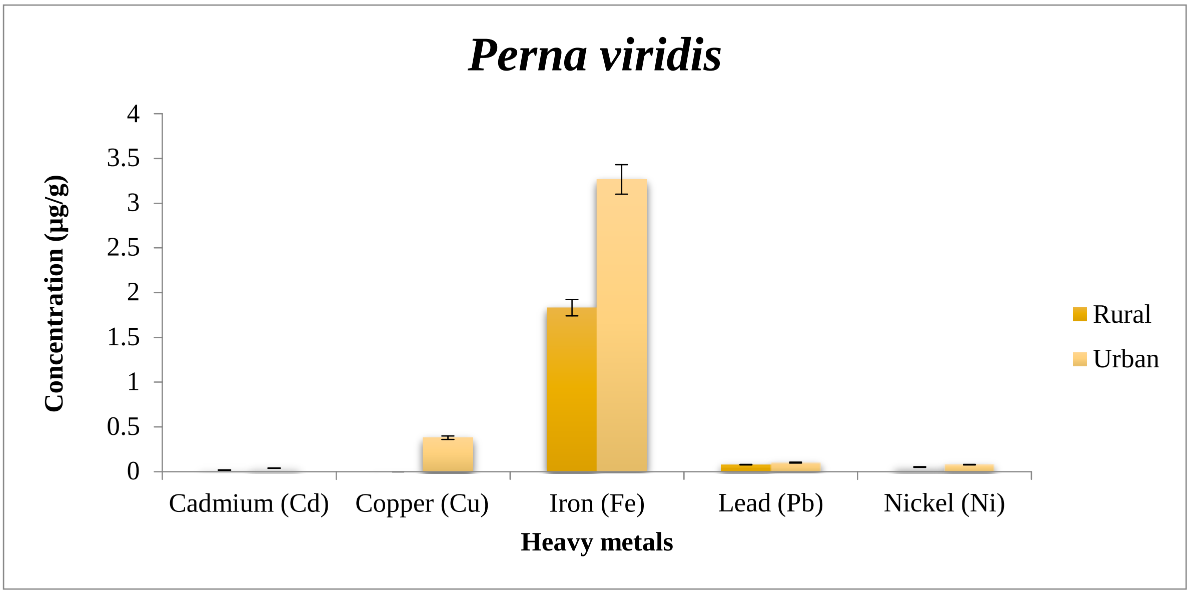


Fig 8: Heavy metal (µg/g) accumulation in *Perna viridis* collected from Betal batim and Karwar

The metal concentrations recorded in *Meretrix casta* (3 gm of sof tissue) specimens collected from Chorao were found to be 0.017 µg/g of cadmium, 0.26 µg/g of copper, 2.77 µg/g of iron, and 0.05 µg/g of nickel. The sample used for of 6 clams weighed 7.75 grams, all metal concentrations were found to be below permissible limits. Fig No.9 illustrates the conc of Heavy metals (µg/g) detected in *Meretrix casta* and *Meretrix meretrix* collected from Chorao (rural) and Amona (urban) respectively.

Metal concentrations were detected in *Meretrix meretrix* (3g of soft tissue) specimen collected from Amona. The concentrations recorded were 0.017 µg/g for cadmium, 0.26 µg/g for copper, 2.77 µg/g for iron and 0.05 µg/g for nickel.

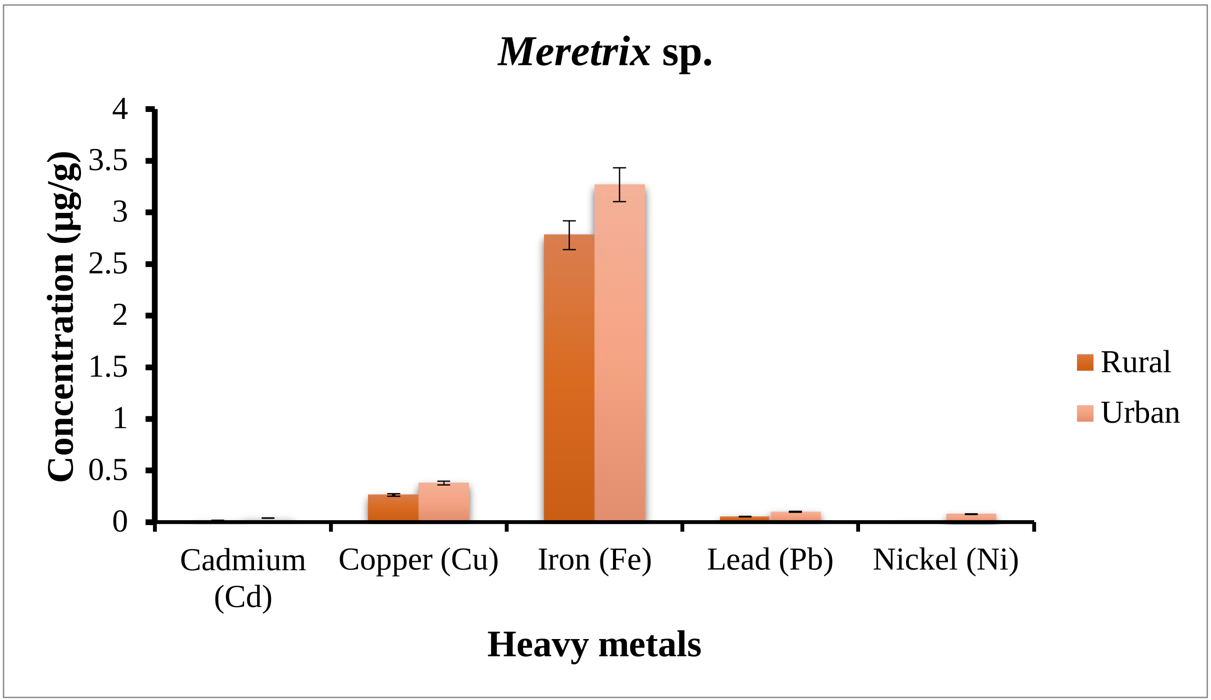


Fig 9: heavy metal (µg/g) accumulation in *Meretrix casta* and *Meretrix meretrix* collected from Chorao and Amona respectively

4.3.2: Comparative assessment of heavy metal contamination in rural or urban areas :

Urban areas tend to have higher levels of cadmium (Cd) compared to rural areas. For instance, when muscle tissue samples were examined, a cadmium concentration of 0.039 µg/g was found in a 3g sample. Fig No.10 illustrates the Comparative assessment of Cadmium concentration found in shellfish collected from rural and urban area.

However, in 6 clams, the concentration recorded was 1.66 µg/g, which exceeds the acceptable limit for cadmium (0.5 µg/g) as found in *Meretrix meretrix* species isolated from Amona. Furthermore, *Perna viridis* specimens collected from Karwar displayed a cadmium concentration of 0.558 µg/g which exceeds the permissible limit of 0.5 µg/g.

Previous studies have shown that urban areas tend to have higher concentrations of cadmium than rural regions. For example, significant levels of cadmium were found in oysters (*Saccostrea cucullata*) from the Deltaic Sunderbans, ranging from 10 to 40 mg/kg (Sarkar et al., 1994), and in clams (*Paphia malabarica*) along the Goa coast, ranging from 1.4 to 8.4 mg/kg (Kumari et al., 2006). The consumption of cadmium at levels ranging from 0.43 to 0.71 µg/kg/day has been associated with adverse effects, particularly among vulnerable populations such as women with low iron reserves, individuals with renal impairment, smokers, and children.

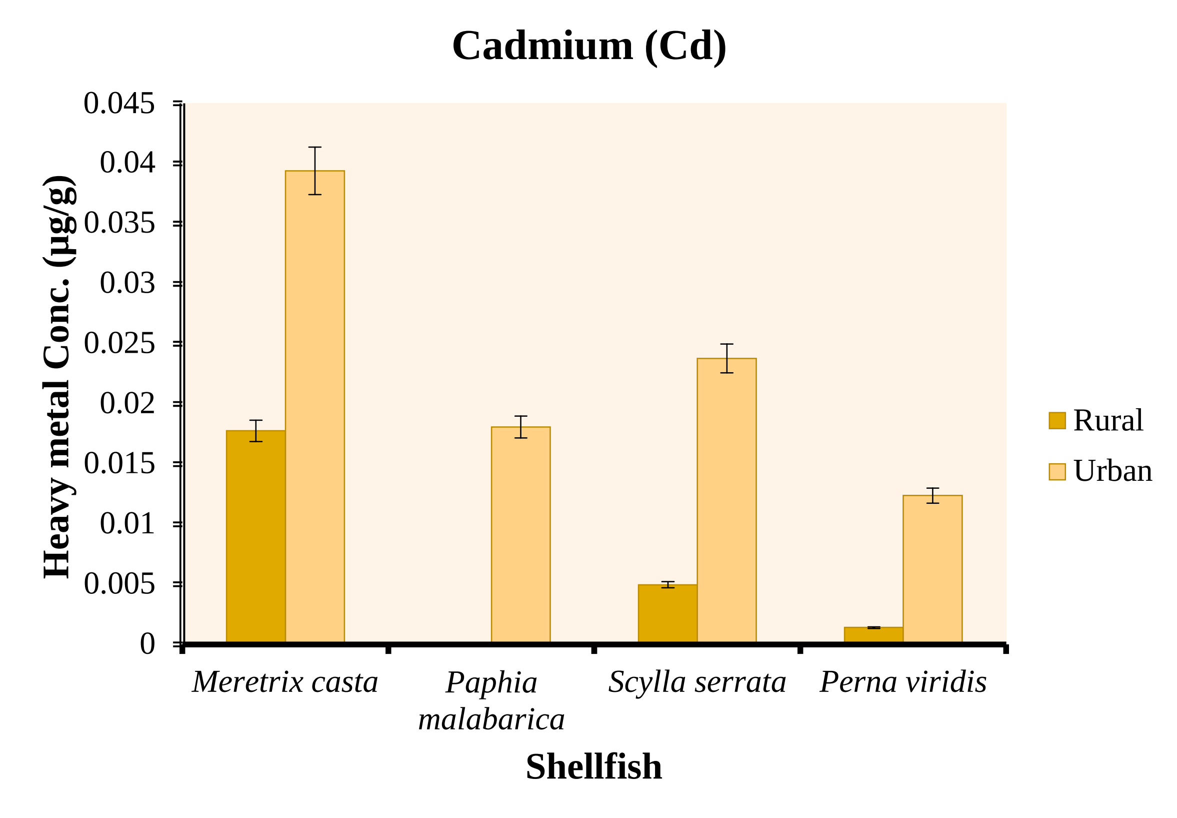


Fig 10: Comparative assessment of Cadmium concentration found in shellfish collected from rural and urban area

Urban areas tend to have higher copper (Cu) concentrations than rural areas. For example, an analysis of muscle tissue from *Scylla serrata* showed a copper concentration 4.016 µg/g in a 3g sample. In addition, Cu concentrations of 48.92 µg/g were recorded in 6 *Paphia malabarica* specimens, 57.95 µg/g in 2 mud crabs, and 90.49 µg/g in 6 green mussels. All of these concentrations were known to exceeded the permissible limit. Fig no. 11 Illustrate the Comparative assessment of Copper concentration found in shellfish collected from rural and urban area.

The estuary ecosystem is facing contamination from remnants of copper-rich antifouling paint, which originate from fishing vessels, barge construction, and tourism boats (Alagarsamy, 2006). Studies have also found that *Saccostrea cucullata* tissue from the

Deltaic Sundarbans has elevated copper levels ranging from 170 to 610 mg/kg (Sarkar et al., 1994), leading to shellfish mortality (Hung and Han, 1990). Consumption of shellfish containing such high copper concentrations, such as oyster tissue, clams, and crabs, can lead to health issues in humans including jaundice, cirrhosis of the liver, and stunted development (Ray et al., 2019a).

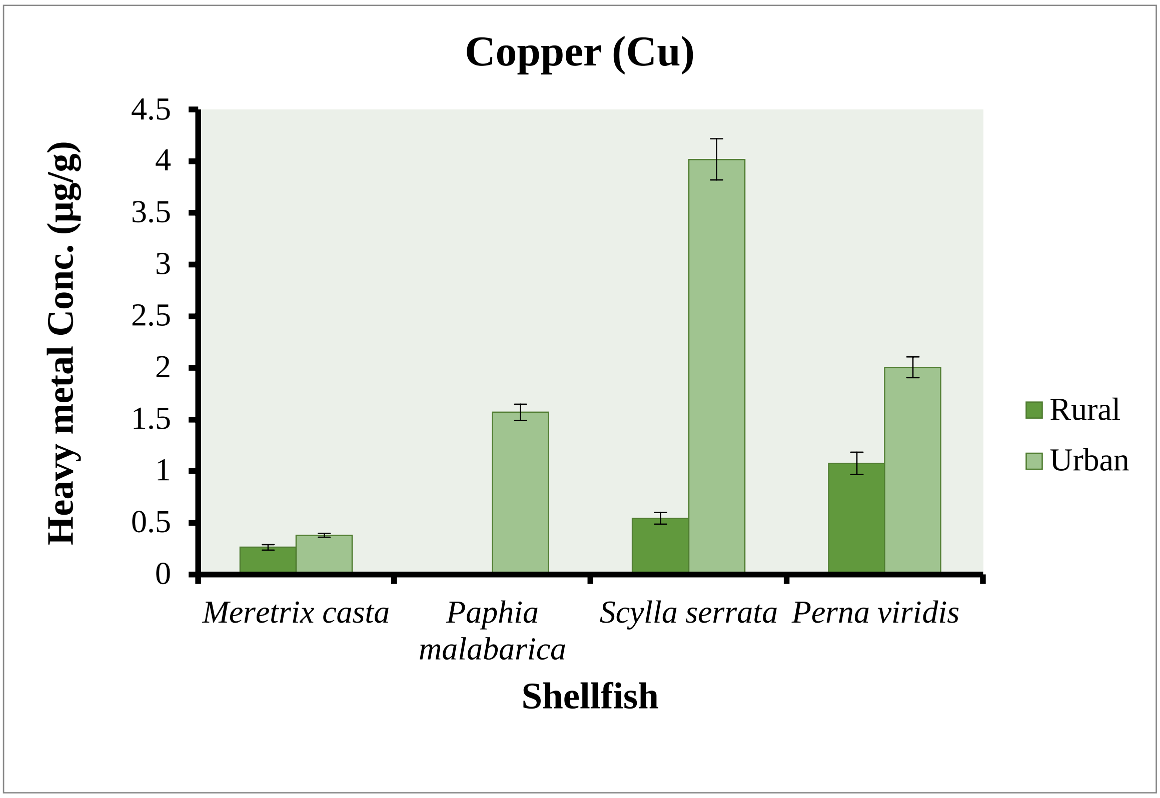


Fig 11: Comparative assessment of Copper concentration found in shellfish collected from rural and urban area

Lead (Pb) concentrations are usually higher in urban areas than rural regions. However, one exception is *Paphia malabarica*, which is mostly found in rural areas. Lead concentrations below the permissible limit were found in nearly all species investigated, except for *Scylla serrata* and *Perna viridis* collected from rural areas.

The highest lead concentration in *Perna viridis* collected from the urban area was found to be 0.16 µg/g wheresas 0.1 µg/g of lead was recorded in *Meretrix* species. Fig no. 12 illustrate the Comparative assessment of Lead concentration found in shellfish collected from rural and urban area.

Heavy traffic load of motor vehicles, boats, ships, combustion of fossil fuel, organic waste discharge in the vicinity of sampling sites as reported by (Rainbow & White, 1989a) could be the reasons for the Pb concentrations in seafood. Lead levels were found to be highest (1.70 mg/kg) in *S. cucullata* during the post-monsoon season and lowest (0.10 mg/kg) in Chicalim Bay during the pre-monsoon season (Rodrigues et al., 2021a).

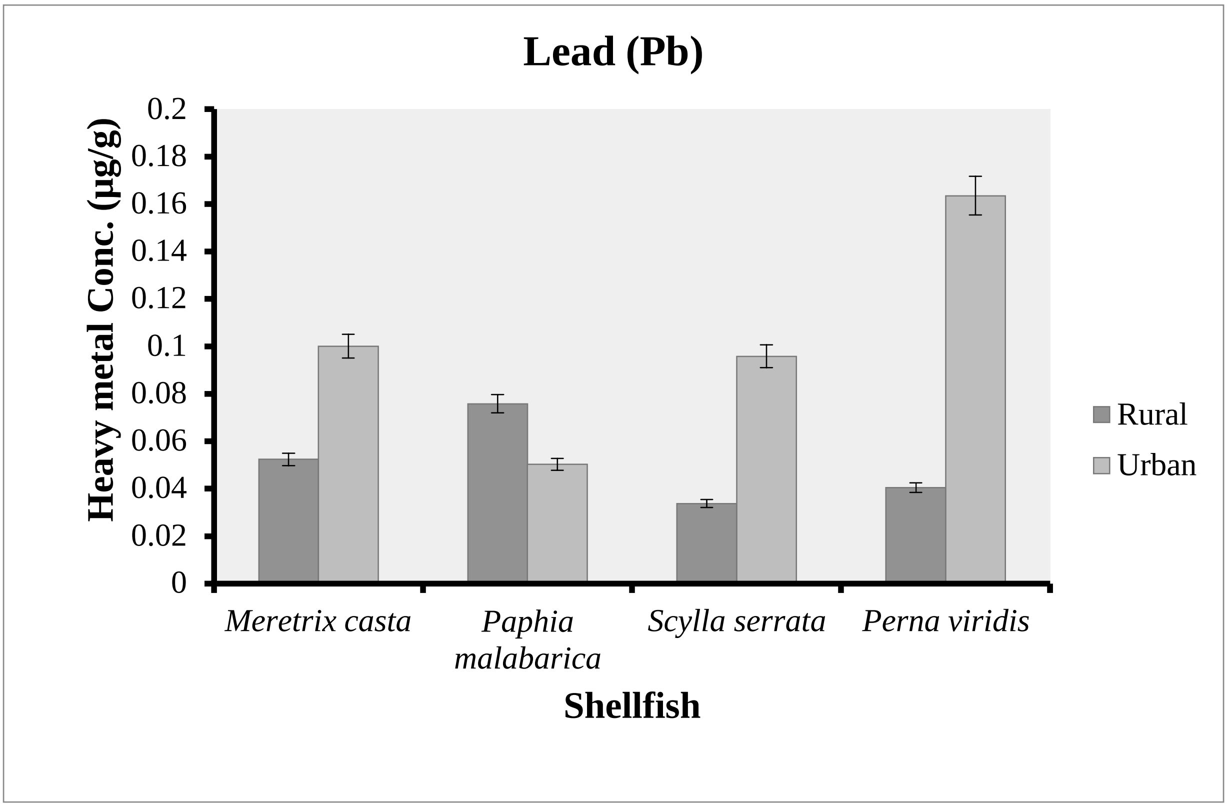


Fig 12: Comparative assessment of Lead concentration found in shellfish collected from rural and urban area

Nickel concentration was found to be higher in specimens studied from urban regions as compared to those collected from rural regions. No nickel was detected in *Scylla serrata* specimens collected from rural regions. Fortunately, nickel concentrations in all areas remained below permissible limits 80 µg/g. Fig no. 13 illustrate the Comparative assessment of Nickel concentration found in shellfish collected from rural and urban area

The study found that the nickel concentration in all areas was lower than the acceptable limit, typically between 70 to 80 mg/kg, according to the USFDA guidelines of 1993. This is because shellfish feed on plankton and detritus, which are low in nickel content (Hossain et al., 2022) . Moreover, shellfish produce proteins known as metallothioneins, which regulate metal levels in cells and prevent toxicity by binding to metals like nickel. This mechanism allows shellfish to trap nickel and other metals effectively, preventing their build-up in critical tissues. It's worth noting that nickel does not have a strong binding affinity in shellfish.

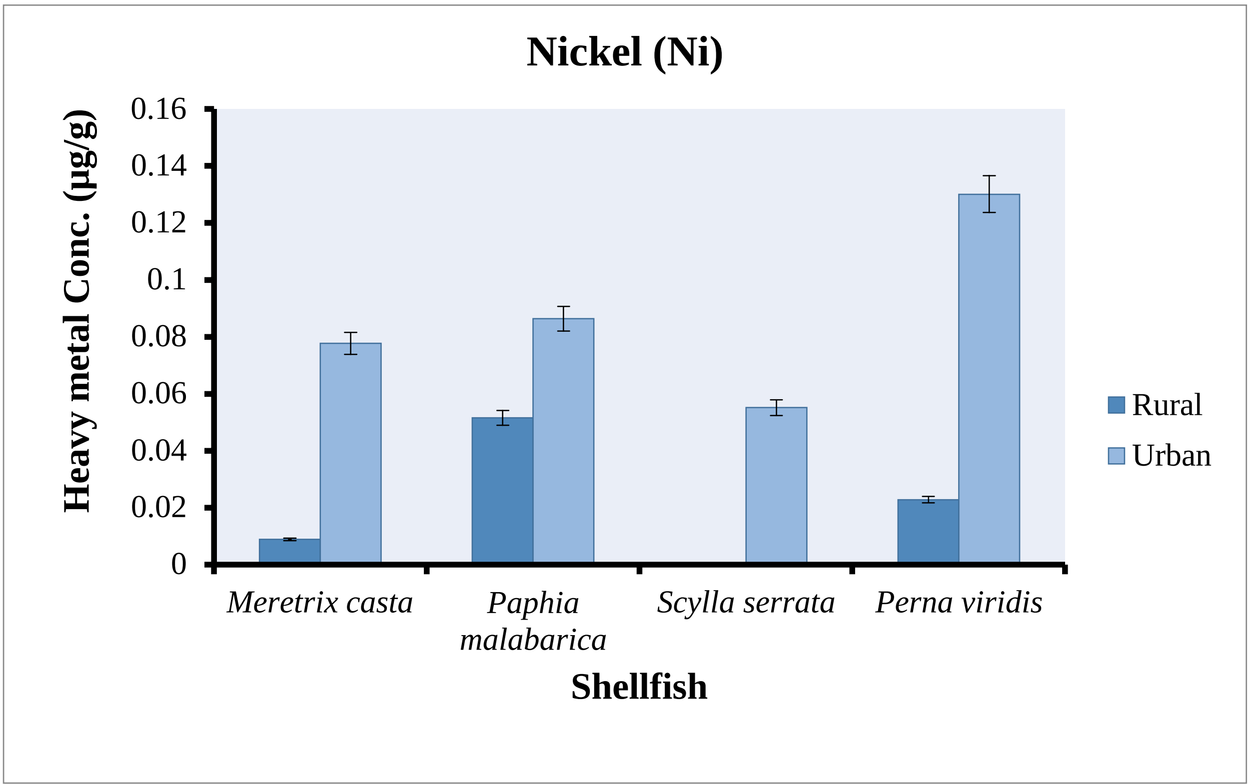


Fig 13: Comparative assessment of Nickel concentration found in shellfish collected from rural and urban area

It was found that the concentration of iron was significantly high in *Perna viridis* collected from Betal batim. The iron concentration recorded in 3g tissue samples was 4.44 µg/g; in 6 samples, it was as high as 202.17 µg/g. Interestingly, when compared to urban areas, the iron levels were found to be higher in *Perna viridis, Scylla serrata and Meretrix meretrix*. This was evident from the fact that in six green mussels, the iron concentration was found to be 255.74 µg/g, 43.34 µg/g in *Scylla serrata*, and 138.11 µg/g in *Meretrix meretrix*. Fig 14 Illustrate the Comparative assessment of iron concentration found in shellfish collected from rural and urban area.

Iron (Fe) was found to be the most abundant trace element in all the tissue samples, with its concentration varying from 2.44 to 227.72 mg/kg wet weight. (Krishna Kumari et al., 2006) These high levels of iron indicate environmental stress, most likely caused by human activities such as urban expansion, agricultural practices, and industrial operations. While iron is naturally present in marine environments, human-induced factors like agricultural runoff and industrial discharges can contribute to heightened iron levels in coastal waters. It is crucial to differentiate between natural and anthropogenic sources of iron when measuring its concentrations in shellfish. This distinction is essential to understand the environmental impacts and ensure accurate evaluations.

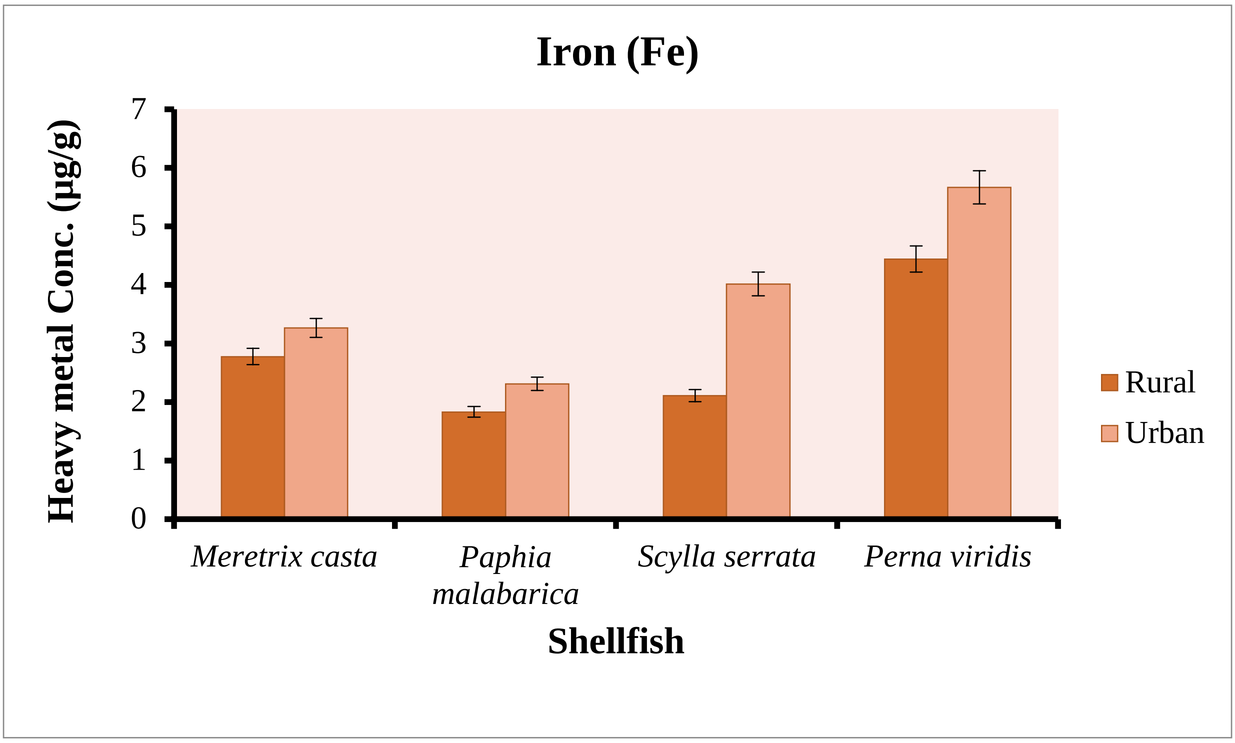


Fig 14: Comparative assessment of Iron concentration found in shellfish collected from rural and urban area

The concentrations of heavy metals in marine species can vary due to various factors, such as feeding strategies, metabolic activities, and the affinity of certain organs for specific metals (Yogeshwaran et al., 2020b). When marine species are exposed to heavy metals, particularly Cd, Cu, and Zn, they produce metallothionein, a low-molecular-weight protein rich in cysteine. Metallothionein (MT) is responsible for regulating the intracellular balance of essential and non-essential metals and their detoxification (Saher & Kanwal, 2019).

CHAPTER 5:

CONCLUSION AND SUMMARY

Based on the results of our study we can conclude that the specimens collected from urban sampling sites recored more heavy metal accumulation as comared to specimens obtained from rural areas. Furthermore, concentrations of Fe, Cu and Cd in Shellfish were found to be above the limits recommended by international authorities for safe consumption by humans. Identifying the source(s) of these metals is important, and measures should be taken to reduce the metal pollution in seafood. The present work calls for continuous monitoring, people awareness, and a stringent government policy to control metal pollution in the coastal waters along the Goa coast. (Bonsignore et al., 2018b)

The bivalves can assimilate and accumulate metals in their hard shell as well as in soft tissues. (Tanhan et al., 2023). Though certain metals are essential micronutrients to bivalves, their accumulation above the threshold limit can have implications for the growth of bivalves (Racheal J. Rodrigues). Studies on heavy metal contamination in shellfish species have some interests and assessments based on two main factors: first is their accumulation capability reflecting heavy metal bioavailability in the environment and biota, and second is also a key factor to assess the health risks related to contaminated shellfish consumption.

Knowledge of the respective habitat (status and conditions) of any shellfish species is mandatory in order to identify the bioaccumulation of heavy metal in any shellfish species (Hajeb and Jinap 2009) that mainly includes the anthropogenic activities carried out near the habitat is also one of the conspicuous factors that affect bioaccumulation in shellfish (Zheng et al., 2007). Their feeding method can contribute to a more significant accumulation of heavy metals than other marine species. Molluscs, especially the filter-feeding animal bivalves, are well-known for their active ingestion of heavy metal-bound organic and inorganic matter.

FUTURE PROSPECTS

In future assessments of heavy metal accumulation in shellfish tissue from Goa, it is imperative to incorporate comprehensive sediment and water quality studies in the respective areas. By examining sediment and water samples alongside shellfish tissue, researchers can gain a holistic understanding of heavy metal distribution and sources in the coastal ecosystem. This integrated approach allows for the identification of potential sources of contamination, such as industrial discharges, urban runoff, and agricultural activities. Moreover, assessing the bioavailability and transfer of heavy metals from sediment and water to shellfish tissue provides insights into ecological risks and potential human health impacts. Implementing longitudinal studies across different seasons and locations within Goa can further elucidate temporal and spatial variations in heavy metal concentrations, aiding in the development of effective management strategies for safeguarding both environmental and public health.

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APPENDIX

Table 1: Acid Ratio Preparation for Heavy Metal Analysis

Name of acid	Concentration	Acid volume (ml/g)
Nitric acid	Concentrated	15ml
Perchloric acid	Concentrated	5ml