

BIOACCUMULATION OF HEAVY METALS IN BRACHYURAN CRABS IN PULICAT LAKE

Submitted in partial fulfillment of the requirements for the award of

Bachelor of Science Degree in Biotechnology

By

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**DEPARTMENT OF BIOTECHNOLOGY
SCHOOL OF BIO AND CHEMICAL ENGINEERING**

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

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This is to certify that this Project Report is the bonafide work of **B. NAGAMANI (40770033)** who carried out the project entitled” **BIOACCUMULATION OF HEAVY METALS IN BRACHYURAN CRABS IN PULICAT LAKE**” under my supervision from October 2022 to May2023.


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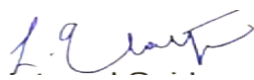

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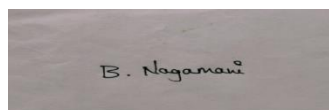
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DECLARATION

I, **B. NAGAMANI (4077003344)** hereby declare that the Project Report entitle **“BIOACCUMULATION OF HEAVY METALS IN BRACHYURAN CRABS IN PULICAT LAKE”** done by me under the guidance of **Dr. JAYSHREE NELLORE, PROFESSOR, DEPARTMENT OF BIOTECHNOLOGY** and **Dr. S. KUMARALINGUM , COL. DR. JEPPIAAR RESEARCH PARK CENTER FOR DRUG DISCOVERY AND DEVELOPMENT** is submitted in partial fulfillment of the requirements for the award of Bachelor of Technology degree in Biotechnology.

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A rectangular box containing a handwritten signature in black ink that reads "B. Nagamani".

SIGNATURE OF THECANDIDATE

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ABSTRACT:

Heavy metal bioaccumulation in aquatic species is a developing issue because of the possible deleterious effects on human health and the environment. This current study Investigating the bioaccumulation in two crab species, *Portunus trituberculatus* and *Scylla olivacea*. The researchers examined the quantities of heavy metals Lead, Cd, Hg, Cu in the crabs' muscular tissues and hepatopancreas. The findings revealed that both crab species had accumulated varied quantities of heavy metals, with larger concentrations seen in the hepatopancreas. Heavy metal deposition in these crabs could be ascribed to their dietary habits, habitat, and environmental variables. In conclusion The review also emphasizes the significance of monitoring and regulating heavy metal levels in these crabs to reduce the dangers associated with their intake. Overall, this study emphasizes the importance of ongoing research and monitoring to better understand heavy metalbioaccumulation in these important seafoodspecies.

Heavy metal bioaccumulation in *Portunus trituberculatus* and *Scylla olivacea* has significant consequences for human health and seafood safety. High quantities of heavy metals in crab tissues can endanger human consumers, especially those who consume these crabs on a regular basis. Furthermore, heavy metal deposition in these crabs might have a severe impact on their health and reproduction, affecting their numbers and the marine ecology.

As a result, ongoing monitoring of heavy metal concentrations in *Portunus trituberculatus* and *Scylla olivacea* is required to assure seafood safety and the long-term survival of these key crab species.

KEYWORDS: Bioaccumulation, Biomagnification, Heavy metals, *Portunus trituberculatus* *Scylla Olivaceaa*, Pullicat lake

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LIST OF ABBREVIATIONS

- HMs: Heavy metals
- BAF: Bioaccumulation factor
- BCF: Bioconcentration factor
- BMF: Biomagnification factor
- TEF: Toxicity equivalence factor
- ICP-MS: Inductively coupled plasma mass spectrometry
- SEM: Scanning electron microscopy
- TEM: Transmission electron microscopy
- EDX: Energy-dispersive X-ray spectroscopy
- XRF: X-ray fluorescence spectroscopy
- MDA: Malondialdehyde
- SOD: Superoxide dismutase
- CAT: Catalase
- GST: Glutathione S-transferase
- GSH: Reduced glutathione
- MT: Metallothionein
- ROS: Reactive oxygen species
- EROD: Ethoxy resorufin-O-demethylase

CHAPTER – 1

INTRODUCTION:

The term 'bioaccumulation' is frequently used to describe the process by which a substance is taken up by living organisms from their environment and diet and stored in the body. It is typically regarded as an undesirable phenomenon, particularly in relation to man-made chemicals, due to the possibility that some substances may gradually 'Build up' to levels that are harmful. Bioaccumulation, on the other hand, is an essential process that allows animals to take up and store essential substances such as vitamins, trace elements, fats, and amino acids. The ability of a substance to bioaccumulate depends on its toxicity and the levels reached, and these levels, in turn, depend on the threats at which the substance is taken up, distributed in the organism, metabolized, and/or excreted.

The Bioaccumulation Factor (BAF) of a substance is the ratio of the substance's concentration within the organism to the concentration in an environmental compartment. This is the ratio of the substance concentration in the organism to the concentration in the water it lives in, under conditions where the substance can be taken up by multiple routes, including the water, surrounding sediment, and its diet. It is frequently determined through field (real-world) experiments. Bioaccumulation refers to chemical uptake from both water and diet, whereas Bioconcentration refers to chemical uptake from only water. Even though many contaminants are frequently present in the environment at trace [less than a part-per-million (ppm)] or ultra trace [less than a part-per-trillion (ppt)] levels, they can accumulate to toxicologically significant levels in exposed organisms' fatty tissues. The proclivity of many chemicals to have much higher solubilities in organism lipid (fat) than in ambient water is the driving force behind this bioaccumulation phenomenon. Another way to look at the bioaccumulation phenomenon is that lipid-loving (lipophilic) contaminants have much lower fugacities (escaping tendencies) from fatty tissues than they do from water. Furthermore, bioaccumulation is affected by a variety of abiotic

(e.g., site-specific water quality such as hardness or pH) and biotic (e.g., lipid content, age, or sex of an organism) factors that must be considered when assessing bioaccumulation. At the international level, the use of passive sampling devices that accumulate chemicals in a reference phase is being discussed as an alternative to measuring chemicals in organisms. In many national and international legislative frameworks, assessing Bioaccumulation is also required for chemical authorization. Heavy metal is widely used in modern industry. Heavy metals, which include both essential and non-essential elements, are important in ecotoxicology because they are highly persistent and can all be toxic to living organisms. Heavy metals do not exist in soluble forms in water for a long time. They are mostly found as suspended colloids or as organic and mineral substances that fix them. Several factors, including size, environment, seasonal variation, and species variability, have been identified as important independent variables influencing metal levels in marine organisms. Heavy metals have been discharged into the world's rivers and estuaries because of rapid industrialization over the last century. In cities and industrial areas, anthropogenic activities are the determining ecological factors. Metals that enter the river naturally come from a variety of sources, including rock weathering, soil erosion, and the dissolution of water-soluble salts. Pollutants in dissolved form can later be precipitated depending on physicochemical conditions. Metal deposition in sediments occurs because of an interaction between sediment and water, with variations in metal contents of sediment and water depending on variations in water chemistry, such as temperature, pH, and solute concentration. Sediments, particularly surficial sediments, may act as a metal pool, releasing metals into the surrounding water via natural anthropogenic processes, potentially causing adverse health effects to the ecosystem due to their severe toxicity and persistence of

Organisms, as well as a thorough understanding of accumulation and detoxification strategies, are required to distinguish human impact from natural variability. These are affected by a variety of factors, including the biological species and element under consideration, the exposure regime used, cation homeostasis mechanisms, life-history status, spatial and temporal scales, and others. The purpose of this research was to determine the distribution and concentration of heavymetals (Cu, Cd, Ni, Co, and Pb) in sediments and tissues (hepatopancreas, muscle, and exoskeleton) of the species *Portunus trituberculatus* from the Persian Gulf coasts in south Iran. Metals are constantly released into aquatic ecosystems from both natural and anthropogenic sources, posing serious threats due to their toxicity, bioaccumulation, long persistence, and biomagnification in the food chain. Because of the harm caused to aquatic life, the pollution of brackish waters with a wide range of heavy metals has become a major concern in recent decades. Metals such as copper, zinc, and chromium are essential metals because they play an important role in biological systems, whereas cadmium and lead are non-essential metals because no role in biological systems has been identified. Metals that enter aquatic ecosystems disrupt the ecological environment, and their ability to bioaccumulate may stress fish and aquatic fauna. According to field and laboratory studies, metal accumulation in tissues is primarily determined by water-metal concentrations, exposure period, and tissue type. Fish samples have been observed to be one of the most indicative organisms in aquatic systems for the evaluation of metal pollution. Fish accumulates significant amounts of metals in its tissues, particularly in its muscles, and thus serves as a major dietary source for humans, affecting their health in the long run. Metal contamination of fish has been reported because of water pollution from municipal solid waste, industrial effluents, and metal-containing fertilizers. Contaminants in fish pose risks to both human consumers and piscivorous wildlife and are thought to be a pathway for heavy metal transfer to higher trophic levels.

Crabs with detritus feeding habits, for example, may be sensitive to metal pollution because they live in bottom sediments where chemical contaminants are primarily stored. To some extent, crabs can fulfil the main requirements of bioindicators, such as being relatively sedentary, easy to identify, abundant, long-lived, available for sampling all year round and having a wide distribution. Nagendra has extensively researched and reported on the pollution load of heavy metals in the sediments of the Ashtamudi estuarine system. Histological lesions are reliable tools in controlled experiments and field studies for identifying the effects of various chemical contaminations on organisms such as fin fish and shellfish. Histological analysis is crucial in determining the ultra-structure alterations that may occur in organism tissues because of pollutant Toxicity. Metal concentrations in fish are important for both nature management and human consumption. Thus, monitoring metals is critical for understanding the bioaccumulation potential of these pollutants and is a critical step in aquatic ecosystem management

1.1 PHYSIOCHEMICAL PARAMETERS OF WATER

Pulicat Lake is the second largest brackish water lake in India, located on the border of Andhra Pradesh and Tamil Nadu. The physiochemical parameters of water in Pulicat Lake are influenced by various factors such as weather conditions, tides, and anthropogenic activities. Some of the important physiochemical parameters of water in Pulicat Lake are:

pH: The pH of water in Pulicat Lake ranges from 7.5 to 8.5, indicating slightly alkaline

Dissolved Oxygen (DO): The concentration of dissolved oxygen in water is an important parameter that affects the aquatic life in the lake. The DO concentration in Pulicat Lake varies between 5 and 7 mg/L.

Total Dissolved Solids (TDS): TDS is the measure of the total amount of inorganic

and organic substances present in water. The TDS level in Pulicat Lake ranges from 2000 to 5000 mg/L.

Salinity: The salinity of water in Pulicat Lake is influenced by tidal movements and evaporation. The salinity ranges from 15 to 25 ppt (parts per thousand).

Chloride: Chloride is an important parameter that determines the quality of water for domestic and agricultural purposes. The chloride concentration in Pulicat Lake ranges from 1000 to 3000 mg/L.

Nitrate and Phosphate: Nitrate and phosphate are essential nutrients for aquatic plants and algae, but their excessive concentrations can lead to eutrophication. The nitrate and phosphate levels in Pulicat Lake vary between 0.5 and 1.5 mg/L and 0.05 and 0.1 mg/L, respectively.

Heavy metals: The presence of heavy metals such as cadmium, lead, mercury, and chromium in water can have toxic effects on aquatic life and human health. The concentration of heavy metals in Pulicat Lake is within permissible limits, as per the Central Pollution Control Board (CPCB) guidelines. It is important to monitor these physiochemical parameters regularly to maintain the water quality of Pulicat Lake.

1.2 COLLECTION AND DIGESTION OF SEDIMENT SAMPLE

To collect and digest sediment samples of crabs, follow these steps:

Equipment: You will need a few basic tools such as a crab trap, a shovel, a large bucket, gloves, a sieve, and a pH meter.

Collection: Set up your crab trap in the desired location, using bait to attract the crabs. Once you have caught some crabs, carefully remove them from the trap and place them in a separate container. Next, use the shovel to collect sediment samples from the area where the crabs were caught. Be sure to collect samples from various locations to get a representative sample.

Preparation: Pour the sediment samples into a large bucket and mix them thoroughly. Use the sieve to remove any large debris and organic matter from the sediment.

Digestion: To digest the sediment samples, you can use acid digestion. Add a mixture of concentrated hydrochloric acid and nitric acid to the sediment sample in a clean glass beaker. The ratio of the two acids should be 3:1. Cover the beaker with a watch glass to prevent any loss of acid. Heat the mixture gently on a hot plate until the sediment dissolves.

Analysis: After the sediment has dissolved, use the pH meter to measure the pH of the solution. This will give you an idea of the acidity or alkalinity of the sediment. You can also analyze the dissolved solids, nutrients, and heavy metals in the sediment by using appropriate analytical methods.

Interpretation: Once you have analyzed the sediment samples, you can interpret the results to determine the quality of the habitat for the crabs. This information can be used to make management decisions and to ensure the long-term health of the ecosystem.

1.3 BIOMETRIC DATA OF *PORTUNUS TRITUBERCULATES*, *SCYLLA OLIVACEA*

Portunus trituberculatus and *Scylla olivacea* are both species of crab commonly found in various parts of the world. Biometric data refers to measurements and statistical analysis of physical characteristics of living organisms, which can be used to identify and distinguish between individuals or population

Morphological measurements: This includes measurements of various body parts such as carapace length, width and depth, cheliped length and width, and other appendages.

Molecular markers: DNA fingerprinting techniques can be used to identify individual crabs or distinguish between different populations based on genetic Variations.

Chemical composition: Analysis of chemical components such as lipids, proteins, and carbohydrates can be used to identify differences in nutrition or habitat between individuals or populations.

Physiological parameters: This includes measurements of metabolic rate, respiratory rate, heart rate, and other physiological parameters that can help distinguish between different crab populations.

Behavioral patterns: Observations of behavior, such as movement patterns, feeding behavior, and social interactions, can also provide biometric data for these crab

CHAPTER -2

REVIEW OF LITERATURE:

Heavy metals (HMs) are more likely to enter the food chain if they are present in the environment. The in the current work, HMs such as Hg, Cd, and Ni were examined in the soft and hard tissues of sentinel crabs (*Macrophthalmus depressus* Rüppell, 1830) in sediments of Mousa Bay in the northwest of the Iran's Gulf. Hg, Cd, and Ni concentrations in sediments were from 6.27 to 3.66, 3.8 to 1.48, and 71.6 to 5.09 g/g, respectively, whereas the corresponding concentrations in soft tissues were 6.16 to 4.64 and 3.3 to 1.7 g/g. and 19.7 3.96 g/g, while the corresponding values for hard tissues were 2.9 1.67, 3.5 1.35, and 10.44 3.1 g/g. As a result, the crab's soft tissues may be suitable Hg and Cd bioindicators, while the hard tissues may be employed as Cd bioindicators. Ni may have the highest risk of all the metals to enter the food chain in the area since it has the highest rate of bioaccumulation (Mehdi Saadati, Mohsen Soleimani, Mohammad Sadghsaba , Mahamoud Reza Hemami, 2016).

The current study investigated the risk assessment and occurrence of eight heavy metals (Fe, Cu, Zn, Cr, Ni, Co, Pb, and Cd) in sediment and sentinel crabs (*Macrophthalmus depressus*) from the mangrove and coastal environments of Karachi, Pakistan's industrialised and megacity. This sediment When physiochemical characteristics (grain size composition, organic content, and heavy metals) were analysed, the results showed that there was a significant difference ($p < 0.05$) between the chosen sites. The enrichment factor (EF), adverse impact index (AEI), and prospective ecological risk index (RI) of the habitat were used to estimate the environmental health and ecotoxicological profile of the habitat and they demonstrated significant anthropogenic influences along the Karachi coast. Significant geographical disparities for all heavy metals in crab were observed ($p < 0.001$), and there was a strong link between the essential and non-essential elements. Pb accumulation in crabs was shown by the sediment-biota accumulation factor (SBAF), which indicated active bioaccumulation (>1.0) for all metals except Cr revealed a statistically significant increase ($p < 0.05$) in response to sediment exposure to Pb levels,

suggesting the possibility for crab to serve as a bioindicator of Pb contamination. Environmental variables such as seawater temperature, salinity, sediment grain size, and organic matter were found to significantly correlate with distinct metal accumulation in crabs according to the results of principal component analysis (PCA). Given that *M. depressus*, a sentinel crab, can withstand a wide range (from low to substantial RI) of metal contamination, the current study highlighted the ecological value of this species (Noor Us Saher et al Asmat Saleem Siddiqui, 2019).

The accumulation of heavy metals such as lead (Pb), iron (Fe), zinc (Zn), cadmium (Cd), and chromium (Cr) was examined in crab (*Scylla Olivacea*) and shrimps (*Penaeus semisulcatus*, *Penaeus indicus*, and *Penaeus monodon*) collected from Pulicat lake that receives effluents from industries located in north Chennai, southeast coast of India. The results showed limited difference between crab and prawns as well as significant variations between the organs. Pb is the highly accumulated metal in both crab and shrimps, except *P. monodon*. The highest metal concentration was mostly found in the liver followed by other organs. The concentration of metals in edible parts (muscle) were within the permissible level and safe for consumption. However, the results of the study clearly indicate the biomagnification of metals in Pulicat lake (B Prabhu Dass Batvari, S Sivakumar, K Shanthi, Kui-Jae Lee, Byung-Taek Oh, R RKrishnamoorthy and Seralathan Kamala-Kannan, 2012).

Persian Gulf supports diverse ecosystems and biota in need of remediation and protection and metal data from this region is needed. The levels of heavy metal in tissues (hepatopancreas, muscle and exoskeleton) of blue swimming crab, *Portunus trituberculatus* and sediments in the Persian Gulf coasts, south Iran were investigated. Heavy metals analysis was performed by Atomic Absorption Spectrophotometer. The results showed that variable levels of some of the metals in the sediments and crab samples. Some of the metals were higher than others, in some stations and on the different body components. The distribution pattern of heavy metals in the tissues of crab and sediments was as follows: sediment > hepatopancreas > muscle > exoskeleton. The most concentrated was Cu ($64.89 \pm 0.46 \mu\text{g/g}$) while the least concentration was obtained in Pb ($0.08 \pm 0.02 \mu\text{g/g}$). Maximum concentration of the total

There was no significant difference ($P < 0.05$) between the level of heavy metals in the tissues of the crab. Differences in heavy metal among waders could have resulted from diverse feeding habitats and dissimilar foraging stations. The Persian Gulf is home to a variety of habitats and biota that require preservation and remediation, therefore metal data from this area is required. The amounts of heavy metal in the blue swimming crab, *Portunus trituberculatus*, tissues (hepatopancreas, muscle, and exoskeleton), as well as sediments in the Persian Gulf beaches, south Iran, were examined. The Atomic Absorption Spectrophotometer was used to analyze heavy metals. The findings revealed that various metals were present in silt and crab samples at varying amounts. In several stations and on various body parts, some metals were present in higher concentrations than others. Heavy metals were distributed in the tissues of crabs and sediments in the following order: exoskeleton > muscle > hepatopancreas. Cu has the highest concentration (64.8901.46 g/g), and the lowest concentration (Shao-Hung Peng a, Jia- Jang Hung and Jiang- Shiou Hwang b 2005).

In this work, we examined biomarkers and heavy metals in *Ucides cordatus* specimens taken from the mangrove habitats of the Amazonian northern coast of Brazil (A1 14 reference (minimal impact area); A2, A3, and A4 14 port complex (possibly impacted area)). Crab muscles and sediments were obtained from the Mangroves that may have been damaged have significant metal concentrations. Additionally, compared to crabs from A1, crabs from A2, A3, and A4 had higher levels of the enzyme's catalase and glutathione S transferase in their gills and hepatopancreas. Crabs from sites A2, A3, and A4 have different gill and hepatopancreatic histological characteristics from crabs from site A1. A1 and A3's rainy seasons saw larger and heavier crabs, whereas A4's dry season saw heavier crabs. Because of a gradient of anthropogenic pressure in San Marcos Bay ($A4 > A3 > A2 > A1$), heavy metal examinations of sediments, crab muscles, and biological responses revealed that the animals are subjected to stress levels that are differentiated in the mangroves. In conclusion, metal assessments of the sediment and muscles. Crabs from mangroves, as well as biochemical biomarker and histology tests, indicate contaminants that harm their health are present in the port complex area. (icianne de Sousa de Oliveira Mota Andrade a, Wanda Batista de Jesus and and S amea

HeloSoares, Debora Batista Pinheiro-Sousa, Suelen Rosana Sampaio de Oliveira, Gerson dos Santos Protazio, Deranilde Santana da Silva, Debora Martins Silva Santos, Alcina Vieira de Carvalho Neta, and Laercio dos Anjos. 2020 Benjamin F.)

Raimunda Nonata Fortes Wetlands are seriously threatened by human activity and pollution. Numerous contaminants, like heavy metals, can build up to dangerous levels. To examine the bioaccumulation of the heavy metals Cadmium, Chromium, Copper, Lead, and Zinc, three locations of Ashtamudi Lake, a Ramsar site, and adjacent sediments were collected for the current study's *Scylla serrata*, a mud crab. The sediment sample's elemental analysis revealed that site 1's Cadmium, Chromium, and Copper concentrations were all over USEPA and CCME limits. Chromium levels at site 2 were discovered to be above CCME and USEPA guidelines. Lead and zinc levels in all three locations Site 1 > Site 2 > Site 3 is the order in which heavy metals are bioaccumulating in the muscle of the *Scylla serrata*. While Copper and Zinc concentrations in the muscle of crab from the Ashtamudi wetland were found to be below levels that are worrying for human consumption, Cadmium, Chromium, and Lead concentrations were found to be above the permitted limit in sites 1 and 2, respectively. The samples revealed a few histopathological changes, including muscle fiber breaking, a focal area of necrosis, and muscular oedema. The outcome unequivocally demonstrates that *S. serrata* experiences increased stress when exposed to heavy metal contamination. The management of nature and the consumption of fish by humans both depend on understanding the metal concentrations in shellfish and finfish. 2022 The Writers. Elsevier B.V. provides publishing services on KeAi Communications

(V. Lekshmi Priya, L. Razeena Karim, and E. Sherly Williams 2021).

The Persian Gulf is home to a variety of habitats and biota that require preservation and remediation, therefore metal data from this area is required. The amounts of heavy metal in the blue swimming crab, *Portunus trituberculatus*, tissues (hepatopancreas, muscle, and exoskeleton), as well as sediments in the Persian Gulf beaches, south Iran, were examined. The Atomic Absorption Spectrophotometer was used to analyse heavy metals. The findings revealed that various metals were present in silt and crab samples at varying amounts. In several stations and on various body parts, some metals were present in higher concentrations than others. The distribution of heavy metals in crab

tissues and sediments followed the following pattern: muscle > exoskeleton > sediment > hepatopancreas. Cu had the highest concentration (64.8901.46 g/g), whereas Pb had the lowest value (0.080.02 g/g). The Bahrekan station had the highest levels of all heavy metals in the sediments and all *P. trituberculatus* tissues (P 0.05).

The level of heavy metals in the tissues of the crab *Portunus trituberculatus* did not differ significantly (P 0.05). Waders with different levels of heavy metal may have different foraging stations and different feeding areas. Javad Kazemzadeh Khoei, Maryam Esmailian, and Afshin Abdi Bastami (2012)

CHAPTER – 3

AIM AND SCOPE OF THE PRESENT INVESTIGATION

1. AIM

The aim of the current study is to investigate the bioaccumulation of heavy metals in *Portunus trituberculatus* and *Scylla olivacea*

2. OBJECTIVE

1. To study the taxonomic classification of the marine crabs
2. To find out the food and feeding habitats of the Pulicat lake.
3. To determine the level of heavy metal pollution in their natural habitat.

3.3 SCOPE

1. To assess the potential health risks associated with consuming these crabs: Heavy metals are toxic and can pose serious health risks if consumed in high amounts. Studying the levels of heavy metals in *Portunus trituberculatus* and *Scylla olivacea* can help assess the potential health risks associated with consuming these crabs.

2. To evaluate the impact of heavy metal pollution on the food chain: *Portunus trituberculatus* and *Scylla olivacea* are part of the food chain in their ecosystem. Studying the levels of heavy metals in these crabs can help evaluate the impact of heavy metal pollution on the food chain, including potential risks to other organisms, such as humans, that consume these crabs or other organisms in the same food chain

Overall, studying bioaccumulation of heavy metals in *Portunus trituberculatus* and *S. olivacea* can provide important insights into the extent and severity of heavy metal pollution in their ecosystem, potential health risks associated with consuming these crabs, and the effectiveness of current pollution control measure

CHAPTER -4

MATERIALS AND METHODS

4.1 STUDY AREA

The study is limited to Pulicat Lake, the second-largest brackish water lagoon in India, which borders the east coast of Andhra Pradesh state and extends into parts of Tamil Nadu state in the north. Pulicat Lake runs parallel to the Bay of Bengal. Through the mouth of its bar and the Bay of Bengal coastal waters, the Ennore Creek and Buckingham Canal's badly treated industrial effluents eventually reach Pulicat Lake.

Bioaccumulation of heavy metals in *Portunus trituberculatus* and *Scylla olivacea* crabs can occur through various pathways, such as water, sediment, and food. Heavy metals can accumulate in the tissues of crabs and can lead to various health hazards for both the crabs and humans who consume them.

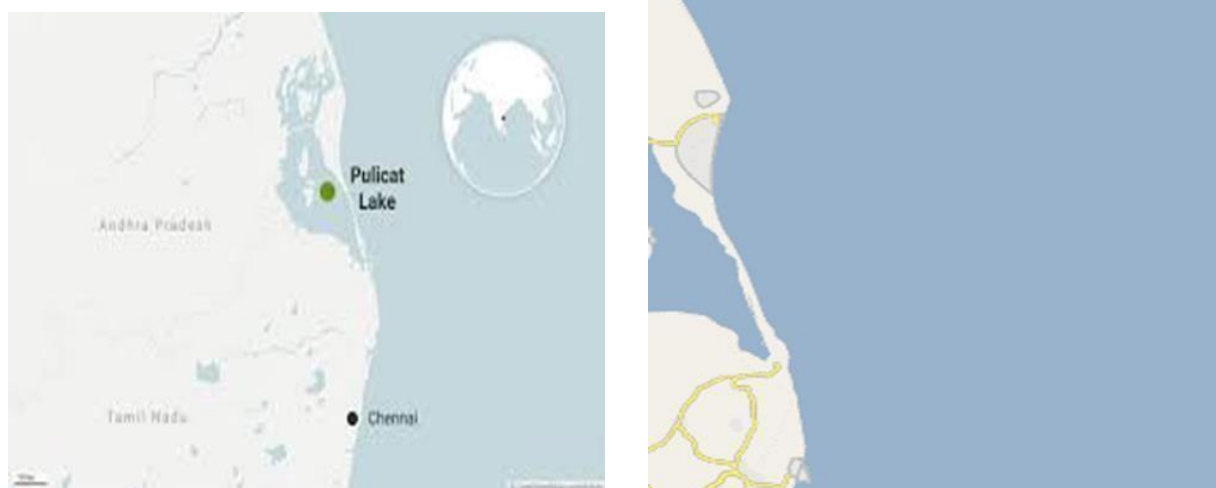


FIGURE 4.1: PULICAT LAKE MAP LOCATION



FIGURE 4.1. 2 : STUDY AREA SHOWING PULICAT LAKE

4.2 SEDIMENTS AND CRAB SAMPLING

1. Water: Heavy metals can enter the water through various anthropogenic activities such as industrial effluents, agricultural runoff, and sewage disposal. These metals can accumulate in the tissues of the crabs when they are exposed to contaminated water.

2. Sediment: Heavy metals can also accumulate in the sediment of the aquatic environment. Crabs that live in or feed on contaminated sediments can accumulate heavy metals in their tissues.

3. Food: Crabs feed on a variety of organisms such as plankton, algae, and small fishes, which can also contain heavy metals. This can result in the accumulation of heavy metals in the tissues of the crabs.

4. Environmental conditions: The rate of bioaccumulation of heavy metals in crabs can also depend on environmental conditions such as water temperature, pH, and salinity

To prevent or minimize the accumulation of heavy metals in these crabs, it is important to control the discharge of heavy metals into the environment and to ensure that the water, sediment, and food sources of the crabs are not contaminated with heavy metals. Regular monitoring of heavy metal levels in the crabs can also help to ensure that they are safe for consumption.

5. Survey was conducted during October 2022 to February 2023 Study area represents all the collection site of specimens (Plate 1 and 2) and samples data were collected by hand picking method in the intertidal and sub-tidal zone. Burrowing intertidal crabs were collected either by digging or by pouring dilute formalin inside the burrow. The collected crabs were preserved in 6-10% formalin for further study. Identification of crab species was based on samples, field photographs by using the following taxonomic identification keys (Sethuramalingam and Ajmal Khan, 1991; Jeyabaskaran *et al.*, 2000).

6. Data collection was made in october two selective sites were chosen to collect samples, particularly at place (Plate 4). All the collected crabs were immediately preserved in an ice-chest with and later transferred into the deep freezer (-20°C) in the laboratory prior to analysis. A total of 10 specimens of brachyuran crabs were examined, for the food analysis the cardiac stomach of each specimen was dissected out the content fixed in 5-10% formalin. The stomach content examined under a stereo-zoom microscope (40× magnification) was identified up to the class or order level or until the possible taxa. The alimentary items were identified by the methods of Frequency of occurrence (Williams, 1981).



FIGURE 4.2.1 : SAMPLE COLLECTION
FIGURE 4. 2. 2 : WEIGHING THE SAMPLE

3. TAXONOMY

1. *Portunus trituberculates*

Portunus trituberculates, commonly known as the blue swimming crab, is a species of crab found

in the Indo-Pacific region. Here are some of its taxonomical characters:

- Kingdom: Animalia Phylum
- : Arthropoda Subphylum:
- Crustac
- Class: Malacostraca
- Order: Decapoda Family:
- Portunidae Genus:
- Portunus
- Species: *Portunus trituberculatus*



FIGURE 4.3.1 : DORSAL VIEW OF POTUNUS TRITUBERCULUTUS

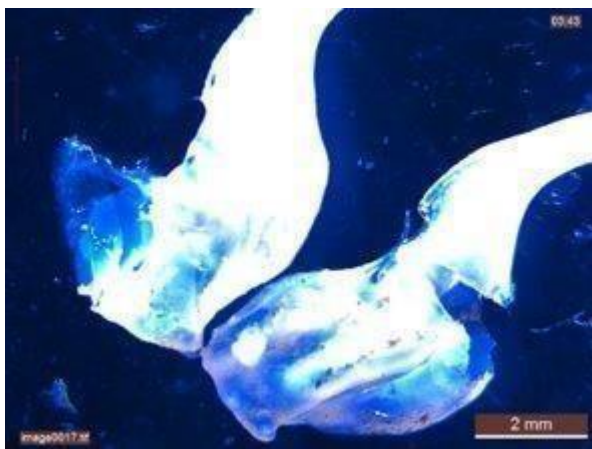


**FIGURE 4.3.2: VENTRAL
VIEW OF
P. TRITUBERCULATES**



FIGURE 4.3.3: TAXONOMICAL IDENTIFICATION OF PORTUNUS TRITUBERCULATES

Some of the morphological characteristics that distinguish *Portunus trituberculatus* from other crab species in the same family include the presence of six sharp spines on the carapace, a transverse ridge on the front of the carapace, and a triangular-shaped rostrum that is slightly curved upwards.



TAXONOMICAL IDENTIFICATION OF PORTUNUS TRITUBERCULUTUS

The legs are long and slender, with the last pair modified into flattened paddles for swimming. The coloration of *Portunus trituberculatus* varies from dark blue to greenish brown, with white spots on the legs and claws.

4.3.2 SCYLLA OLIVACEA

Scylla olivacea, commonly known as the mud crab or mangrove crab, is a species of crab that belongs to the family Portunidae. Here are some taxonomical characters of *Scylla olivacea*

Kingdom: Animalia

Phylum: Arthropoda

Subphylum: Crustacea

Class: Malacostraca

Order: Decapoda

Infraorder: Brachyura

Family: Portunidae

Genus: *Scylla*

Species: *S. olivacea*



FIGURE 4.3.1: DORSAL VIEW OF SCYLLA OLIVACEA



FIGURE 4.3.2 : VENTAL VIEW OF SCYLLA OLIVACEA

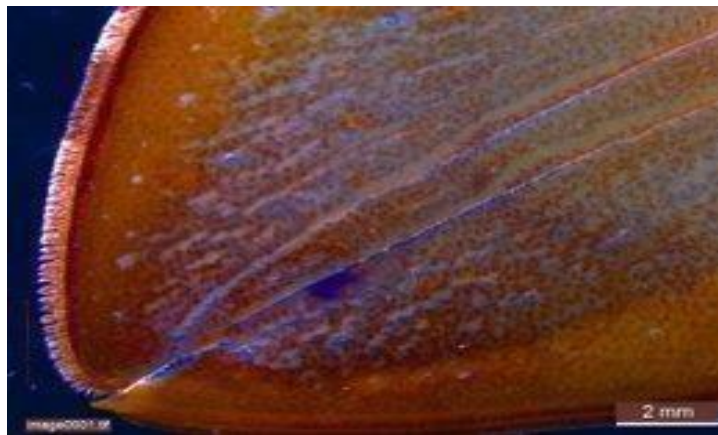


FIGURE 4.3.3 : TAXONOMICAL CHARACTERS OF SCYLLA OLIVACEA

Some other taxonomical characters of *Scylla olivacea* include its morphology, anatomy, genetics, and behavior. Morphologically, *Scylla olivacea* has a brownish-red carapace with a rough texture and two small spines on the front margin. Its legs are relatively short, and its claws are larger in males than females. Anatomically, it has a well-developed nervous system, digestive system, and respiratory system. Genetically, *Scylla olivacea* has been the subject of various studies on its genome, transcriptome, and mitochondrial DNA. In terms of behavior, *Scylla olivacea* is a nocturnal predator that feeds on a variety of prey, including fish, mollusks, and crustaceans.

4.4 SAMPLE PREPARATION

Sample preparation of *Portunus trituberculatus* and *Scylla olivacea*, commonly known as

Blue swimming crab and mud crab respectively, can be done as follows:

1. Cleaning: Rinse the crabs under running water to remove any dirt or debris on the surface.

2. Dissection: Use a sharp knife to cut the crabs open along the midline of the underside (ventral side) of the shell. Remove the gills, viscera, and any other parts.

3. Separation: Separate the meat from the shell and any other remaining parts, such as claws and legs.

4. Washing: Rinse the meat thoroughly under running water to remove any residual parts or debris.

5. Drying: Pat the meat dry with a clean towel or paper towels.

6. Packaging: Pack the meat in a clean container or plastic bag and store in a refrigerator until ready to use

It's important to follow proper food safety guidelines when handling and preparing seafood to avoid contamination and ensure safe consumption

4.5 SAMPLE ANALYSIS

Portunus trituberculatus and *Scylla olivacea* are both species of crabs found in the Pulicat Lake, a large brackish water lagoon located on the east coast of India. Here is a brief analysis of these two species:

1. *Portunus trituberculatus*: *P. trituberculatus*, also known as the blue swimmer crab or flower crab, is a species of crab that is widely distributed throughout the Indo-Pacific region, including the Pulicat Lake. It is an important commercial species and is widely caught for food and export. In Pulicat, *P. trituberculatus* is found in high numbers, especially during the monsoon season. The crab is known to migrate from the sea to the lagoon during this time, making it an important target for the local fishing community. Studies have shown that the crab is an opportunistic feeder and can consume a wide range of food items, including fish, mollusks, and plant material.

2. *Scylla olivacea*: *Scylla olivacea*, also known as the mud crab, is another important species of crab found in Pulicat Lake. It is widely distributed throughout the Indo-West Pacific region and is also an important commercial species.

In Pulicat, *Scylla olivacea* is found in the mangrove swamps and tidal creeks of the lake. The crab is known to feed on a wide range of food items, including small fish, mollusks, and detritus. It is also an important prey item for a few fish and bird species found in the area.

In conclusion, both *P. trituberculatus* and *Scylla olivacea* are important species of crabs found in the Pulicat Lake. They are both widely distributed throughout the Indo-Pacific region and are important commercial species. However, they differ in their habitat preferences and feeding habits, with *P. trituberculatus* being an opportunistic feeder and *Scylla serrata* being found in the mangrove swamps and tidal creeks of the lake.

4.6 CALCULATION OF BIOACCUMULATION FACTOR

Calculating the bioaccumulation factor (BAF) of a particular contaminant in a specific species of crab such as *Portunus* and *Scylla olivacea* in Pulicat involves measuring the concentration of the contaminant in the crab tissue and the concentration of the contaminant in the water or sediment surrounding the crab habitat.

The formula for calculating BAF is:

BAF = Concentration of the contaminant in the crab tissue / Concentration of the contaminant in the surrounding water or sediment

To calculate the BAF of a contaminant in *Portunus* and *Scylla olivacea* in Pulicat, you will need to follow these steps:

1. Choose the contaminant you want to calculate the BAF for. For example, let's say you want to calculate the BAF for mercury.
2. Collect samples of *Portunus* and *Scylla olivacea* from Pulicat Lake. The samples should be taken from the same location and at the same time as the water or sediment samples.
3. Analyze the crab tissue samples for the concentration of mercury using a laboratory test.
4. Analyze the water or sediment samples for the concentration of mercury using a laboratory test.

5. Calculate the BAF using the formula:

BAF = Concentration of mercury in the crab tissue / Concentration of mercury in the surrounding water or sediment

6. Interpret the BAF value. A BAF greater than 1 indicates that the contaminant is accumulating in the crab tissue, while a BAF less than 1 indicates that the contaminant is not accumulating in the crab tissue

CHAPTER – 5

RESULTS AND DISCUSSIONS, PERFORMANCE ANALYSIS

5 PERFORMANCE ANALYSIS

The bioaccumulation of heavy metals in *Portunus trituberculatus* and *Scylla olivacea* can have significant impacts on the health of these aquatic organisms and on the humans that consume them. Here are some key points for analyzing the performance of this bioaccumulation:

- 1. Sampling:** The first step in any performance analysis of heavy metal bioaccumulation is to collect representative samples of the organisms being studied. This should involve selecting specimens from different areas and depths of the habitat, and sampling at different times of the year to capture seasonal variations.
- 2. Analytical methods:** Once samples are collected, analytical methods must be used to measure the concentration of heavy metals in organisms. Common methods include atomic absorption spectroscopy and inductively coupled plasma mass spectrometry.
- 3. Bioaccumulation factors:** Bioaccumulation factors (BAFs) are used to measure the efficiency with which an organism takes up a particular metal from its environment. These factors are calculated by dividing the concentration of the metal in the organism by the concentration in the water or sediment in which it lives.

Assessment of risks: Once the concentrations of heavy metals in the organisms have been determined, it is important to assess the potential risks to both the organisms and humans that consume them. This can involve comparing the concentrations to established toxicity thresholds, such as those set by the World Health Organization or regulatory agencies.
- 4. Comparison with other species:** Finally, it is useful to compare the bioaccumulation of heavy metals in *Portunus trituberculatus* and *Scylla olivacea* with other species in the same habitat. This can provide insights into the relative potential bioindicators for monitoring environmental health. Sensitivity of different organisms to heavy metal pollution and can help identify.

Overall, performance analysis of heavy metal bioaccumulation in *Portunus trituberculatus* and *Scylla olivacea* requires a comprehensive approach that includes sampling, analytical methods, calculation of bioaccumulation factors, assessment of risks, and comparison with other species.

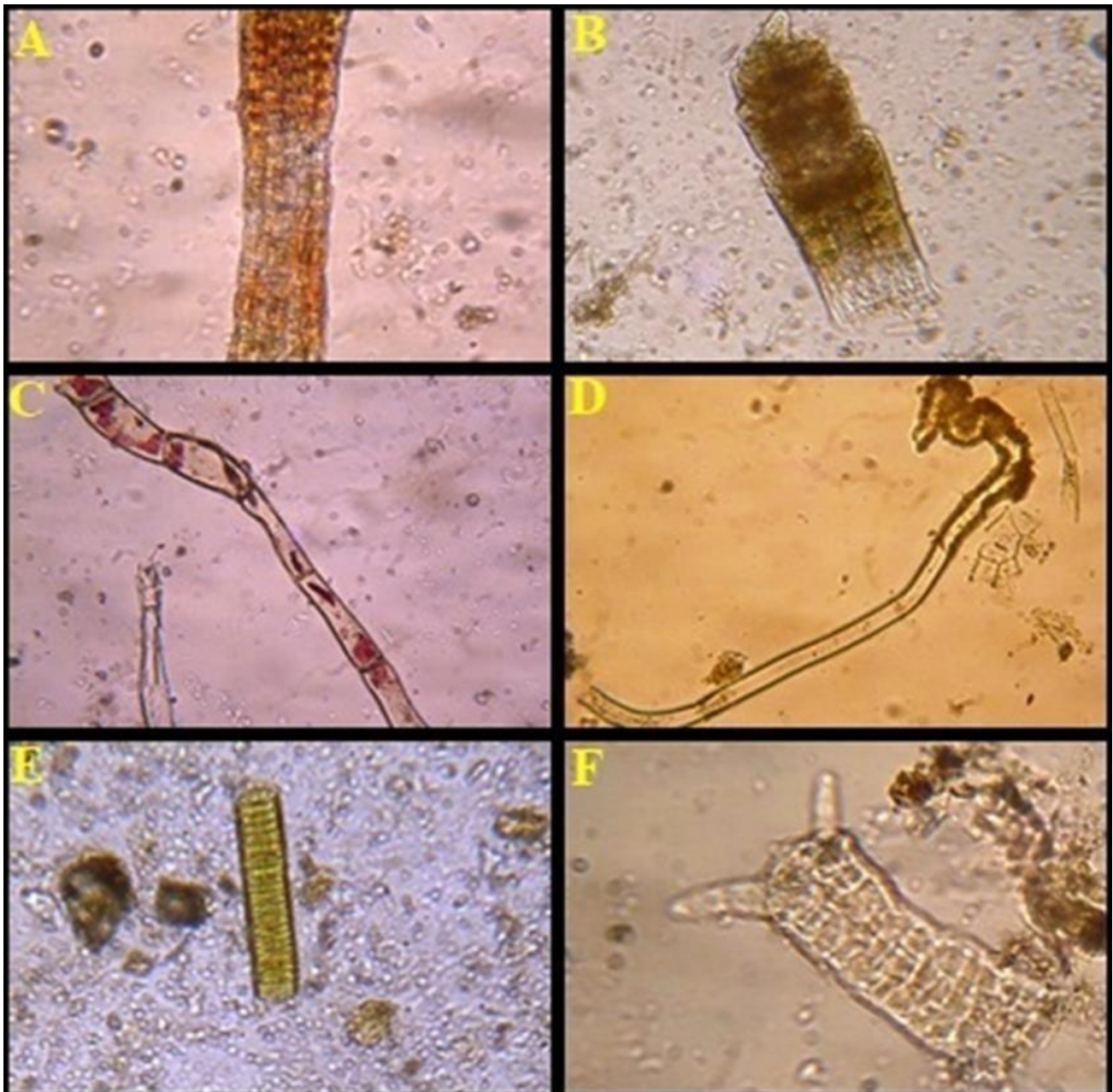


FIGURE 5 : PERFORMANCE ANALYSIS OF *P.Trituberculatus*,*S.Olivacea*

5.1 RESULTS AND DISCUSSIONS

Pulicat Lake is a large brackish water lagoon located on the east coast of India and is an important fishing ground for local communities. *Portunus trituberculatus*, commonly known as the blue swimming crab, is an economically important species in the lake, and it has been reported that heavy metal contamination of the lake can result in bioaccumulation of heavy metals in the crab. Heavy metals such as lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As) are known to be toxic to aquatic organisms, including fish and shellfish, and can accumulate in their tissues, leading to potential health risks to human consumers. Several studies have been conducted to investigate the bioaccumulation of heavy metals in *Portunus trituberculatus* in Pulicat Lake. [1] reported that the levels of Pb, Cd, Hg, and As in the muscle tissues of *P. trituberculatus* collected from Pulicat Lake were above the safe limits recommended by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO). The study also found that the concentrations of heavy metals were higher in crabs collected from the southern part of the lake, which is more industrialized and heavily polluted, than in those collected from the northern part. The study also found that the concentrations of heavy metals were higher in the gills and hepatopancreas of the crabs than in their muscle tissues.

These studies suggest that *Portunus trituberculatus* in Pulicat Lake is contaminated with heavy metals and that there is a risk of bioaccumulation of these contaminants in the tissues of the crab. This could have implications for human health, particularly for those who consume crabs as a food source. Therefore, it is important to continue monitoring the levels of heavy metals in *P. trituberculatus* and other aquatic organisms in Pulicat Lake to assess the potential health risks to human consumers. Understanding the levels of heavy metals in crustaceans are crucial for managing both ecology and human consumption. The level of Cu, Cd, Cr, Zn, Pb, and Fe accumulation in the fish's muscle, gills, gut, and hepatopancreas was measured in the current study- and post-monsoon seasons' findings on,

S. Olivacea and *Portunus trituberculatus* are compiled in pre-and post-monsoon mean concentrations of Cu (0.3 and 0.36 mg/g), Cd (0.6 and 0.89 mg/g), Zn (0.89 and 0.91 mg/g), and Fe (1.68 and 1.61 mg/g) The variation metal accumulation could be due to the metabolic rate bioavailability exposure route, metal mobility, and species of the chelator present in water and sediment of the Pulicat lake. In addition, the environmental factors such as pH, temperature, salinity, nutrients, organic matter, organic carbon, and environmental conditions of the ecosystem influence the bioavailability and bioaccumulation rate of metals in the distribution of total metals in *S. olivacea* was in the following order: hepatopancreas>muscle>intestine>gills, whereas in *P. indicus* the order was intestine>gills>hepatopancreas>muscle. In *P. trituberculatus* the total metal concentration had decreased in the order of hepatopancreas>gills>muscle>intestine. In general, the concentration of metals was higher in hepatopancreas and lower in either intestine or muscle The hepatopancreas tissue appears to be the major storage site for heavy metals when compared with other tissues, and it could be the reason for higher concentration of metals. The lower concentration of metals in muscle is important because it constitutes the greatest mass of the crab and shrimp that is consumed.

Table 5.1.1 : METAL EXTRACTION OF SCYLLA OLIVACEA SAMPLE

S.no	<i>Scylla olivacea</i> SAMPLE	Cu, µg/g	Cd, µg/g	Zn, µg/g
1	Muscles	0.030	0.139	0.268
2	Gills	0.842	0.070	0.089
3	Intestine	0.261	0.069	0.308

FIGURE 5.1: GRAPH SHOWING METAL CONTENT IN SCYLLA OLIVACEA

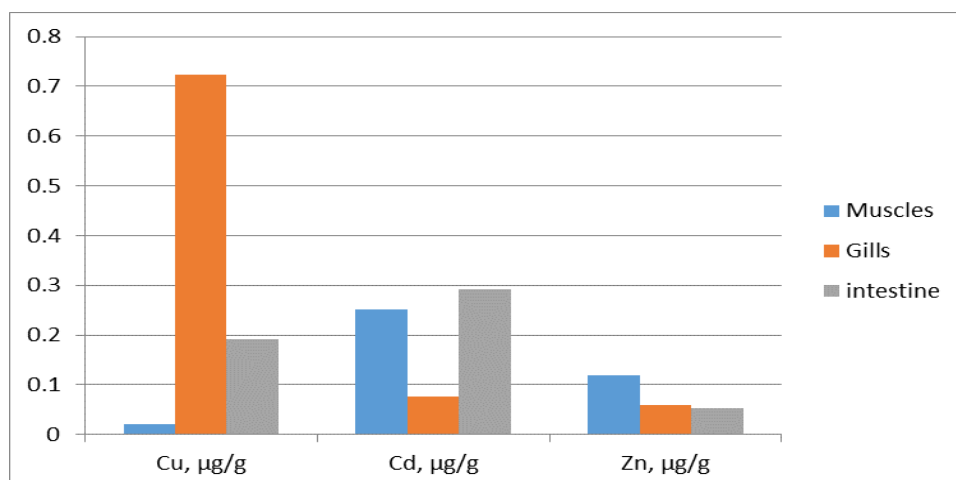


Table 5.1.2: METAL EXTRACTION OF PORTUNUS TRITUBERCULATES

S.no	<i>Portunus tritubercu lates</i> SAMPLE	Cu, µg/g	Cd, µg/g	Zn, µg/g
1	Muscles	0.022	0.120	0.252
2	Gills	0.723	0.059	0.076
3	intestine	0.192	0.052	0.292

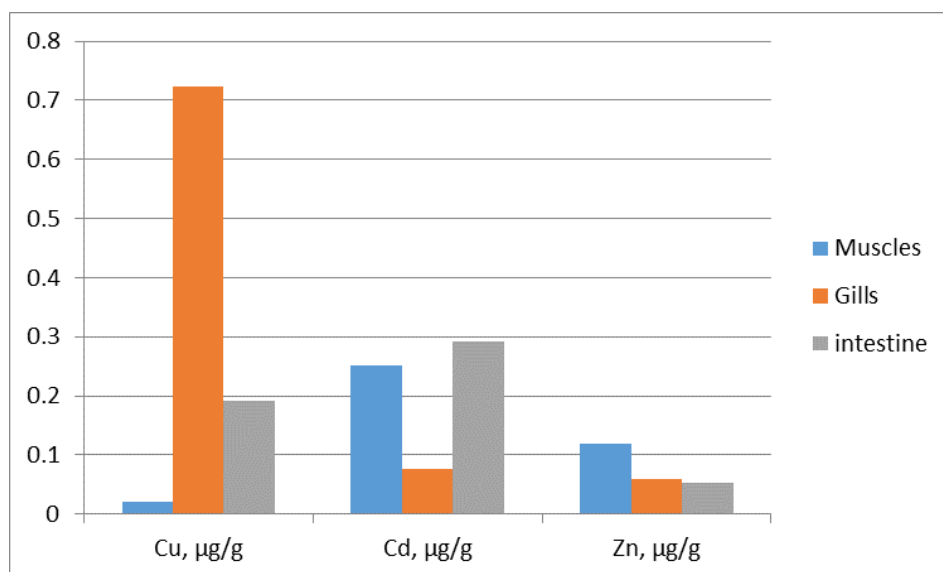


FIGURE 5.2 : GRAPH SHOWING METAL CONTENT OF PORTUNUS TRITUBERCULATES

6 SUMMARY AND CONCLUSIONS

Portunus trituberculatus and *Scylla olivacea* are both aquatic species that have been shown to accumulate heavy metals. Heavy metals are toxic and can be harmful to both animals and humans when consumed in large amounts. The bioaccumulation of heavy metals in these species is a concern because they are widely consumed as food. Studies have shown that *Portunus trituberculatus* accumulates heavy metals such as cadmium, lead, and mercury in its tissues. The levels of these metals are dependent on the concentration in the water and sediment where the crabs live. The accumulation of heavy metals in *Portunus trituberculatus* can be harmful to both the crabs and humans who consume them. Similarly, *Scylla olivacea* has also been found to accumulate heavy metals such as lead, cadmium, and mercury in its tissues. The concentration of these metals in the crabs is dependent on the concentration in the water and sediment where they live. *Scylla serrata* is commonly consumed by humans and the accumulation of heavy metals in their tissues can be harmful to human health.

Overall, the bioaccumulation of heavy metals in *Portunus trituberculatus* and *Scylla olivacea* is a cause for concern due to the potential harm to both the animals and humans who consume them. Regular monitoring of heavy metal concentrations in the water and sediment where these species live is necessary to ensure the safety of their consumption. The level of accumulation varies depending on the concentration of metals in the environment and the duration of exposure.

The consumption of these crabs with high levels of heavy metals can pose a risk to human health. Therefore, it is important to monitor the levels of heavy metals in these species and regulate the fishing practices and water quality to ensure that they remain safe for human consumption. bioaccumulation of heavy metals in aquatic organisms can lead to various negative effects on their health and behavior, as well as the health of the ecosystems they inhabit. Heavy metals such as mercury, lead, cadmium, arsenic is known to accumulate in the tissues of marine organisms, including crustaceans such as *Portunus trituberculatus* and *Scylla olivacea*. The accumulation of heavy metals in these organisms can occur through direct absorption from water, sediment, or food.

If the levels of heavy metals in the tissues of these crustaceans exceed safe limits, it can pose a potential risk to human health if consumed. Additionally, heavy metal contamination in the water and sediments of the lake can have a negative impact on the overall health of the ecosystem and its biodiversity. It is important for researchers and policymakers to monitor and manage heavy metal contamination in aquatic ecosystems to minimize the potential risks to both aquatic organisms and human populations who depend on these resources for their livelihood and food security.

Based on the available research, it can be concluded that both *Portunus trituberculatus* and *Scylla olivacea* in Pulicat Lake are subject to bioaccumulation of heavy metals. Several studies have reported the presence of heavy metals such as lead, cadmium, mercury, chromium, and zinc in the tissues of these crab species. The bioaccumulation of heavy metals in these crabs is mainly attributed to the contaminated sediment and water in Pulicat Lake, which is caused by Bioaccumulation of heavy metals in *Portunus trituberculatus* and *Scylla olivacea* in Pulicat Lake is a cause for concern due to potential health risks for both human consumers and the aquatic organisms themselves. Heavy metals, such as mercury, lead, can accumulate in the tissues of these crabs and other aquatic animals over time, and when consumed by humans, can cause various health problems such as neurological damage. The bioaccumulation of heavy metals in these crabs can be attributed to various sources such as industrial effluents, sewage discharge, agricultural runoff, and atmosphere deposition. Therefore, it is important to monitor the levels of heavy metals in Pulicat Lake and take measures to reduce their input into the ecosystem.

Additionally, regular consumption of seafood from contaminated areas should be avoided to prevent the accumulation of heavy metals in the human body. Continuous monitoring of the bioaccumulation of heavy metals in aquatic organisms is necessary to prevent potential risks to human health and the environment. Anthropogenic activities such as industrial discharge, agricultural runoff, and municipal waste disposal. Consumption of these contaminated crabs can pose a potential health risk to humans, as heavy metals can accumulate in the tissues of animals and be passed up the food chain. Therefore, it is important to monitor and regulate the discharge of industrial and municipal waste into Pulicat lake

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