MANGROVE GASTROPODS AS BIOMONITORS FOR HEAVY METAL CONTAMINATION

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FACULTY OF SCIENCE
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MANGROVE GASTROPODS AS BIOMONITORS FOR HEAVY METAL CONTAMINATION

ABSTRACT

A process called biomonitoring can be used to analyze the factors that affect the ecosystem. In this study, gastropods and sediments were used to observeits impact on the study sites under the Klang Islands Mangrove Forest Reserve (KIMFR): Pulau Klang (Klang Island), Pulau Carey (Carey Island) and Telok Gong representing the natural mangrove, agricultural and industrial environments, respectively. Environmental monitoring was performed using gastropods and sediments that were collected and tested for heavy metals including arsenic (As), lead (Pb), cadmium (Cd), and mercury (Hg). The inductive coupled plasma mass spectrometry (ICP-MS) was used for heavy metal testing in gastropods whereas inductive coupled plasma optical emission spectrometry (ICP-OES) was used for sediment. The gastropods collected were observed to identify their species composition and abundance, and correlate heavy metals with the species. Telok Gong was found to be more diverse with nine species, followed by Carey Island and Klang Island with eight and five species respectively. Klang Island had less species richness, but more abundance. Ten gastropod species was discovered from the study sites: *Nerita lineata, Cerithidea quadrata, Chicoreus capucinus, Telescopium telescopium* (TG, KI & CI), *Cassidula aurisfelis* (TG & KI), *Ellobium aurisjudae, Littorina scabra, Littorina melanostoma* (TG & CI), *Thais tissoti* (CI), and *Cerithidea cingulata* (TG). Due to a scarce in availability and size, only six species were selected for heavy metal testing: *Chicoreus capucinus, Telescopium telescopium, Nerita lineata, Cassidula aurisfelis, Cerithidea quadrata,* and *Ellobium aurisjudae.* Concentration results of heavy metals from the separation of gastropod body and shell showed that As and Hg were more concentrated on the gastropod body whereas Cd and Pb were concentrated on the gastropod shell. *Chicoreus capucinus* showed a high concentration of As whereas *Ellobium aurisjudae* showed a higher concentration of Cd compared to other species. *Cassidula aurisfelis* showed a higher concentration of Pb where as *Telescopium telescopium* showed higher a concentration of Hg. It was assumed that these species could be used as biomonitors for specific metals. However, each species has its own ability to retain heavy metals hence, future studies for specific metals and gastropods should be conducted to confirm the species' effectiveness as biomonitors. The heavy metal comparison showed As and Pb were more concentrated in sediments than in gastropods. However, the concentrations of Cd and Hg were similar which were < 0.5 µg/g in both gastropods and sediments. This may be due to the metals' affinity towards the samples. The data comparison for the locations showed that Telok Gong had higher concentrations of As and Pb in both sediment and gastropod because it is surrounded by industries. However, Pb detected in gastropod at Klang Island showed higher value than Telok Gong. Sediment quality index (CBSOG SQG (2003) showed moderate pollution of As at Telok Gong and low pollution at the other sites. The values of Cd, Pb and Hg were relatively low for the three sites. The noticeable amount of As and Pb in gastropods indicate that the sites were polluted from adjacent areas like industries and shipping ports.

Keywords: Biomonitor, Mangrove, Sediment, Metals, Gastropods.

GASTROPOD PAYA BAKAU SEBAGAI BIOPEMANTAU UNTUK PENCEMARAN LOGAM BERAT

ABSTRAK

Proses yang dipanggil biomonitor boleh digunakan untuk menganalisis faktor-faktor yang memberi kesan kepada ekosistem. Dalam kajian ini, gastropod dan sedimen digunakan untuk meneliti impak terhadap kawasan kajian di Hutan Simpan Paya Bakau Kepulauan Klang: Pulau Klang, Pulau Carey dan Telok Gong, yang masing-masing berfungsi sebagai habitat bakau semulajadi, serta kawasan pertanian dan perindustrian. Pemantauan persekitaran dijalankan dengan menggunakan gastropod dan sedimen yang dikumpul dan diuji untuk logam berat seperti arsenik (As), plumbum (Pb), kadmium (Cd), dan merkuri (Hg). Kaedah plasma gandingan aruhan-spektrometer jisim (ICP-MS) digunakan untuk menguji logam berat pada gastropod, manakala kaedah plasma gandingan aruhan–spectrometer pancaran optikal (ICP-OES) digunakan untuk menguji sedimen. Gastropod yang dikumpul diteliti untuk menentukan komposisi dan jumlah spesis, serta menghubung kait logam berat dengan spesis tersebut. Telok Gong didapati mempunyai spesis yang lebih pelbagai dengan sembilan spesies, diikuti dengan Pulau Carey dan Pulau Klang yang masing-masing mempunyai lapan dan lima spesies. Walaupun Pulau Klang mempunyai kekayaan spesis yang kurang, tetapi mempunyai bilangan spesies lebih banyak. Sepuluh spesis gastropod diperoleh dari lokasi kajian: *Nerita lineata, Cerithidea quadrata, Cassidula aurisfelis, Chicoreus capucinus, Telescopium telescopium* (TG, KI & CI), *Cassidula aurisfelis* (TG & KI), *Ellobium aurisjudae, Littorina scabra, Littorina melanostoma* (TG & CI), *Thais tissoti* (CI) dan *Cerithidea cingulate* (TG). Disebabkan kekurangan bilangan dan saiz sampel, hanya enam spesis telah dipilih untuk pengujian logam berat: *Chicoreus capucinus*, *Telescopium telescopium*, *Nerita lineata*, *Cassidula aurisfelis*, *Cerithidea qudrata* dan *Ellobium aurisjudae*. Keputusan penumpuan logam berat daripada pemisahan badan dan cangkerang gastropod menunjukkan As dan Hg lebih tertumpu pada badan gastropod, manakala Cd dan Pb lebih tertumpu pada cangkerang gastropod. *Chicoreus capucinus* menunjukkan kepekatan As yang tinggi, manakala *Ellobium aurisjudae* menunjukkan kepekatan Cd yang lebih tinggi berbanding spesies yang lain. *Cassidula aurisfelis* menunjukkan kepekatan Pb yang lebih tinggi, manakala *Telescopium telescopium* menunjukkan kepekatan Hg yang lebih tinggi. Telah diandaikan bahawa spesis ini boleh digunakan sebagai biomonitor untuk logam tertentu. Walau bagaimanapun, setiap spesis ini mempunyai kemampuan tersendiri untuk menyimpan logam berat; maka, kajian lanjut berkaitan logam tertentu dan gastropod perlu dilaksanakan untuk mengesahkan keberkesanan spesies tersebut sebagai biomonitor. Perbandingan logam berat menunjukkan As dan Pb lebih tertumpu di dalam sedimen berbanding di dalam gastropod. Namun, kepekatan Cd dan Hg adalah sama iaitu< 0.5 µg/g pada kedua-dua gastropod dan sedimen. Hal ini mungkin disebabkan tarikan logam berat tersebut terhadap sampel. Perbandingan data untuk lokasi-lokasi kajian menunjukkan bahawa Telok Gong mempunyai kepekatan As dan Pb yang lebih tinggi pada kedua-dua sedimen dan gastropod kerana dikelilingi oleh industri. Walaubagaimanapun, Pb yang terdapat pada gastropod di Pulau Klang menunjukkan kepekatan yang lebih tinggi daripada Telok Gong. Indeks kualiti sedimen (CBSOG SQG (2003) menunjukkan kadar pencemaran yang sederhana di Telok Gong dan kadar pencemaran yang rendah di lokasi yang lain. Nilai Cd, Pb dan Hg adalah secara relatifnya rendah di ketiga-tiga lokasi kajian. Kandungan As dan Pb yang ketara pada gastropod menunjukkan bahawa lokasi kajian tersebut dicemari dari kawasan bersebelahan seperti industri dan pelabuhan perkapalan

Kata kunci: Biomonitor, Paya Bakau, Sedimen, Logam, Gastropod.

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°C	:	Degree Celsius
g/cm3	:	Gram per cubic centimeter
ha	:	Hectare
Kiloton year-1	:	Kiloton per year
Km2	:	Square kilometer
<	:	Less than
μg	:	Microgram
μg/g	:	Microgram per gram
μg/kg	:	Microgram per kilogram
m	:	Meter
mm	:	Millimeter
μm	:	Micrometer
ml	:	Milliliter
>	:	More than
%	:	Percentage
ppm	:	Parts per million
∑	:	Sum of
As	:	Arsenic
Cd	:	Cadmium
CBSOG SQC (2003)	:	Consensus-Based Sediment Quality Guidelines 2003
DMHg	:	Dimethyl mercury
FAO	:	Food and Agriculture Organization

FIMS : Flow Injection Mercury system

Hg : Mercury

H2S : Hydrogen sulphide

HgCl4& HgCl3 : Mercury chloride

H2O2 : Hydrogen peroxide

HNO3 : Nitric acid

ICP-MS : Inductively coupled plasma mass spectrometry

ICP-OES : Inductively coupled plasma optical emission spectrometry

ISQG : Interim Sediment Quality Guideline

KIMFR : Klang Islands Mangrove Forest Reserve

MeHg : Methyl mercury

Pb : Lead

SQC : Sediment Quality Guideline

SPSS : Statistic Package of Social Science

US EPA 1986 : United States Environmental Protection Act 1986

US EPA6020A : United States Environmental Protection Act Analytical method 6020A

US EPA7471A : United States Environmental Protection Act Analytical method 7471 A

WHO : World Health Organization

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CHAPTER 1:INTRODUCTION

1.1 Mangroves and Mangrove Forest in Selangor

Mangroves provide shelters for coastal regions thereby preventing erosions from oceanic waves and wildlife influences (Chong, 2006). Given the mangroves’ varying chemical and physical compounds, they also serve as sink for pollutants (Yusnus et al., 1970; Praveena et al. (2010)).

Mangroves act as a buffer against storms and offer protection for both the shipping and aquaculture industries. Mangrove forests were extensive along the coast of Selangor during the 1960s. By the year 2000, about 60% of the mangroves were reclaimed for various development projects. A few areas in the Selangor mainland still have substantial area of mangroves (Sasekumar, 2005). Selangor’s mangrove forest supports a high diversity of animals that live within the forest and in waterways. Within the forest, crustaceans, molluscs and sipuncula are dominant. In the waterways, fishes and prawns spend part of their life cycle there (Chong et al., 1990).

1.2 Biomonitors

Markert et al. (1999) defined biomonitoring as a technique for spotting the effect of external influences on ecologies and their growth over a long duration, or to determine variances between one site and another. The term ‘metal biomonitor’ is used to represent species with heavy metals in their tissues; thus, they become a determinant of bioavailability of metals in their established habitat (Rainbow, 1995). Relevant organisms must be selected to guarantee that they meet the bioindicator and biomonitor requirements. A bioindicator is a living organism that gives us an idea on the health of an ecosystem. Some organisms are very sensitive to pollution in their environment; so, if pollutants are present, the organism may change its morphology, physiology or behavior, or it could even die. Biomonitors are organisms that accumulate contaminants in their tissues and can be used to yield a relative measure of the total amount of contaminants in the environment integrated over a period of time. They respond simultaneously to different stressors providing quantitative information on the quality of the environment. Tanabe et al. (2000) recommended on using mussels, as well as other bivalves to biomonitor the aquatic metal pollution due to their several advantages over other organisms such as being abundant, are sedentary, having tolerance, are reasonably long-lived and having reasonable size. Several research projects were also done using intertidal gastropods, but mussels were more commonly used; this is because the stages of implementation done by the U.S. Mussel Watch Program were found to be very useful (Goldberg, 1975). Naturally, different gastropod species can show distinct accumulative capacities for different metal compounds, which can provide many potential bioindicators for the biomonitoring of aquatic metal pollution e.g., insects, mollusks, fish and birds (Zhou et al., 2008).

1.3 Heavy Metal Pollution

Metals are chemical elements that are unable to degrade; hence, they can only be transferred from organism to organism. Because of this, they are good bioindicators that can be used a baseline in the law of conservation. MacFarlane (2002) reported that the continuous dominance of metals in mangrove environments is resulted from urbanization, agricultural overspill and industrial pollution.

1.4 Sediments

Norasyikin (2008) asserted that sediments can hold metal in a dissolved form for long periods of time through physical manners such as rainfall, chemical processes such as absorption, and biological means such as biotic interaction. Contaminants are introduced to the aquatic ecology via various ways such as sewage release, ocean and lake disposals, non-point sources, contaminated spills and aerial accretion.

1.5 Problem Statement

Mangrove ecosystems in Peninsular Malaysia are being threatened by many types of pollutants, especially heavy metals. Mangrove environments are subjected to exploitation and inept management practices such as land reclamation as well as weak forestry, aquaculture and agricultural schemes (Ong, 1993). Molluscs and crustaceans are important organisms in mangrove areas, playing important ecological roles including being major food items for fish and birds (Zgozi, 2000). Although there are many research and analyses done on heavy metals in Peninsular Malaysia, there is still a lack of research conducted using gastropods in the islands chosen for this study. Therefore, it is essential to attain a deeper understanding of the mangrove ecosystem for future research and sustainable development and management. This work is done to analyze heavy metals in gastropods and sediments that function as biomonitors in three different types of mangrove habitats: natural forest (Klang Island), agriculture area (Carey Island) and industrial area (Telok Gong).

1.6 Research Objectives

1. To enumerate the species composition and its abundance as well as the diversity of the gastropods in three different anthropogenic activity locations: Telok Gong (industrial area), Carey Island (agriculture area) and Klang Island (natural mangrove habitat).
2. To identify the differences in concentration of heavy metals (As, Pb, Cd, and Hg) in selected gastropod soft bodies and shells as well asthe differences in sediments and gastropods in the three locations: Telok Gong, Carey Island and Klang Island.
3. To determine whether the selected mangrove gastropods have the capacity to function as biomonitors by observing the accumulation of heavy metals (As, Pb, Cd, and Hg) in each species.

CHAPTER 2:LITERATURE REVIEW

2.1 Mangrove Ecosystem

Mangroves are important habitat for several molluscs, insects, mammals, fishes, reptiles, birds, crustaceans, amphibians, and minuscule living organisms existing in the muddy and salty environment. These organisms interact in a myriad of ways, including multifaceted interdependence, predation patterns, commensalism, and parasitism (Spalding et al., 2010). These interactions form mangrove food webs which serve as a key component in energy flow and nutrient cycling. Molluscs and crustaceans are important organisms in mangrove areas, playing their vital ecological roles including as adiet for fishes and birds (Zgozi, 2000). These ecological interactions of mangroves are driven by their physical structure. The solid complex structures of the roots provide habitations for crustaceans, fishes and mollusks. The degree of dependence of the organisms on the mangrove environment varies greatly for each organism. However, only some animals inhabit in the mangrove environment such as gastropod *Nerita* species, fiddler crab and mud lobster. Other animals are opportunists like migratory birds such as the Kingfisher and Sandpiper that may benefit from mangroves but are found elsewhere. There are also organisms that exploit the mangrove environment as a life cycle habitat for breeding and as a nursery.

2.2 Biomonitors

A biomonitor has the ability to measure and analyze the accumulated metal concentration absorbed by organisms in their tissues over a specified period (Luoma & Rainbow, 2008). Biomonitors signify the extent of environmental changes through physiological, behavioral and biochemical reactions given by the changes in extent of lysosome latency and scope of growth (Phillips & Rainbow, 1993).

According to Rainbow (1995), biomonitors are able to ascertain geographical as well as sequential variations in the bioavailabilities of heavy metals in a marine setting, thus, contributing chronological gauges of those sections on the total ambient metal load that are directly relevant to ecotoxicology. Rainbow (1995) emphasized that having a basic knowledge of biology, ecology and physiology is crucial as it is a requirement prior to selecting an organism as a biomonitor. Understanding the facts on the absorption of metals in various species provides details on the quantum of accumulated metal. It was also stressed regarding the importance of correct use of species and that the robustness of the species alone is inadequate for the organisms to be used as biomonitors. Thus, data from other studies worldwide must be compared. There are other criteria for selecting organisms such as being sedentary, easy to identify, abundant, long lived, available for sampling throughout the year, big size, tolerance from exposure to environmental variations like salinity, and having a strong net accumulator.

Biomonitors are defined as a net accumulation of traceable metals in the body of living organisms (Rainbow, 2002). The metals accumulated come from all sources of metal intake such as diet in the case of aquatic invertebrates. The collected metal concentration in a biomonitor is referred to the incorporated amount of all bioavailable sources of metal to be monitored. It is viewed that organisms vary in terms of their uptake route for the same metal. As such, a comparative bioavailability of a specific metal on an organism can be determined.

Species with long lifespans are likely to reflect bioavailabilitie sover a certain period of time. Bioavailability of metals is the extent to which bioaccessible metals adsorb onto or absorb into and across biological membranes of organisms, which is expressed as a fraction of the total amount of metal the organism is proximately exposed to (at the sorption surface) during a given time and under defined conditions. For example, macrophytic algae lacks the roots of angiosperms like sea grasses and takes up metals only from solution (so long as fronds are not in direct contact with sediment). Such algae are therefore excellent biomonitors of the bioavailable metal in solution in the water column. From the foregoing, Rainbow (2006) opined that biomonitors should be resilient and capable of tolerating higher metal bioavailabilities, as to indicate physico-chemical disparity in habitation settings.

The biomonitoring of aquametal pollutions should compare bioindicators to biomonitors of different aquatics inhabitants such as gastropod, fish, zooplankton, insect, algae, macrophyte, bivalve mollusc, amphibian, etc. (Zhou et al., 2008). A bioindicator is a living organism that gives us an idea of the health of an ecosystem. Some organisms are very sensitive to pollution in their environment, so if pollutants are present, the organism may change its morphology, physiology, or behaviour, or it could even die. Biomonitors are organisms that accumulate contaminants in their tissues and can be used to yield a relative measure of the total amount of contaminants in the environment integrated over a period of time. They respond simultaneously to different stressors, providing quantitative information on the quality of the environment. Benthic macroinvertebrates like aquatic insects, crustaceans, worms, and mollusks can be used as bioindicators. Some organisms can also be used as biomonitors and bioindicators like molluscs e.g. mussels.

2.3 Bioaccumulation and Its Patterns

Bioaccumulation can be defined as an essential procedure by which living organisms are affected by chemicals. Intensification of chemical concentration in an organism may occur over time due to the chemicals present in its surrounding. Bioaccumulation is the extent by which the absorption of toxins by organisms is greater than the toxins lost from their system. Bioaccumulation processes involve absorption, storage and elimination (Kamrin, 1997). According to Latini et al. (2005), bioaccumulation is caused by the dynamic concentration balance between contact from the external environment and the absorption, elimination, storage, and degradation within an organism.

Aquatic organisms have different ways of absorbing metals (Rainbow, 2002). At worst, the concentrated metals absorbed by an organism may have no major excretion. However, there might also be an equilibrium rate of absorption and excretion, resulting in no net accumulation. This process is often referred to regulation as the metal content that is regulated to all organs in the whole body. Regulation of the whole body is limited to significant and insignificant metals. There can only be regulations of trace metal concentrations in the whole body of invertebrates such as decapods crustaceans (Rainbow, 2002). Barnacles are typical cases of powerful accumulators for many trace metals such as zinc. The zinc that is taken up from the solution of barnacles accumulates without a major secretion (Rainbow & White, 1989). Apart from that, there is limited secretion of zinc absorbed from diet (Rainbow & Wang, 2001). Hence, the concentration of zinc in the body would rise throughout the life span of the barnacle, to which it would attain an elevated value when compared to the local Zn bioavailability. This makes them outstanding biomonitors (Phillips & Rainbow, 1988; Rainbow, 1998). Similar generalizations were made when copper is accumulated by crustaceans.

2.4 Heavy Metal Pollution

Heavy metals are elements which have a density above 5 g/cm^3. Industrial metal pollutants that are often found in the estuarine systems include copper (Cu), zinc (Zn) and lead (Pb) (Mills et al., 1982). Heavy metals are released into the surroundings viz anthropogenic activities like industrialization, smelting and fuel combustion. All solid and liquid wastes stemming from industrial activities have contaminating chemicals such as sulphides, chromium salts, and other toxic heavy metals (Tariq et al., 2006). There can be substantial metal deposits in surface sediments that are amassed in poised particles, dissolved in water lines, and assimilated by intertidal organisms. High concentration of heavy metals can be harmful to animals in the coastal regions and impact the health of the ecosystem and its many components. Since heavy metals are toxic and have adverse effects to environmental health, continuous monitoring must be done on the development of infrastructures and human activities in the coastal regions. This is to avoid heavy metal pollution on wildlife to secure food safety and human health (Ismail, 2011). Heavy metal pollutants are less noticeable compared to other sources of aquatic pollution; however, their high toxicity level would adversely affect the ecosystem (Edem et al., 2008; Shanmugam et al., 2007).

Heavy metals such as lead, cadmium, mercury and chromium, are unnecessary in the metabolism of plants and animals whereby they are toxic even in low concentrations. If the concentration levels of these heavy metals exceed a critical threshold either in the cells or organs of an organism, the organism will experience a number of symptoms and at higher doses, death. For example, although cadmium is not a crucial element, it is still regarded as a concern to organisms (Kabata-Pendias, 1992). It is easily accumulated and absorbed by plants which consequently increase the tendency for food contamination (Baker, 1981; McGrath et al., 1997).

Avila-Pérez et al. (1999) have analyzed the concentrations of heavy metals in aquatic environments and the results depicted major metal contaminations of lead, mercury, iron, and chromium. While temporal and spatial distributions of the entire metal level were discovered, no structured model of a specific metal concentration was identified. Metal density that is above 5 g/cm^3 is certain for five main types of heavy metals namely, lead, mercury, arsenic, chromium, and cadmium. Nevertheless, cadmium, arsenic and lead are heavy metals of particular interest because of their wide-spectrum of negative influences on living organisms. There are many chronic effects observed in living organisms exposed to cadmium such as kidney damage and lung emphysemain birds and mammals, effects on growth and replication in microorganisms, and sublethal effects reported on the growth and reproduction of aquatic invertebrates (WHO, 1992). Arsenic has been found to greatly affect the vital organs causing degenerative changes at the molecular and genetic levels. When rats were fed on 3% of *Sargassum fusiforme*, which contained large amounts of inorganic arsenic, accumulation ofarsenic was observed in the blood and tissues accompanied with high body temperature (Singh et al., 2015). Toxicity of cadmium and lead for four species of insects (*Pteronar cysdorsata, Hydropsy chebetteni, Brachycentrus* sp., and *Ephemerella* sp.), one snail (*Physa integra*) and one amphipod (*Gammarus pseudolimnaeus*) were determined during a 28-day exposure. The cadmium-exposed snails and lead-exposed amphipods were eleven and four times lower than the 7th and 4thday (96 h) value of the metals respectively (Spehar et al., 1978).

2.5 Sources and Route of Metal Pollution

Major sources and route of metal pollution (Potters, 2013):

- **Source:** The metals Cd, Cu, Fe, Zn, Ni, and As come from volcanic activities, forest fires, dust particles, and anthropogenic discharges such as from vehicle exhausts and coal power stations. Some metals come from the erosion of metal-containing rocks or a certain surface runoff carrying along the metal particles deposited from the surrounding on that particular surface. Anthropogenic emission in presently diminishing emissions of natural sources an example is shown for atmosphere (see Table 2.1) particularly for Pb, Zn, Cd, Hg, and Cu.

- **Route:** Pollution that comes from anthropogenic activities enables the flow of these metals into rivers and oceans. The metals penetrate the environment via aerosol drop (which may eventually evaporate allowing the metal particle to float in the air) or incorporated into dust or particulate matter. Atmospheric deposition may transpire through precipitation (wet deposition) or as dry matter (dry deposition). This causes atmospheric metal pollution to contaminate the oceans.

  Rivers are the second route for metal exposure in the environment. Metals always form distinct complexes in rivers (owing to speciation) and precipitate into sediments. Nevertheless, when the riverbed is dredged, trawled or subjected to extreme weather, these sediments can be discarded. This is a perfectly natural method and is not regarded as pollution even though the impacts may be similarly serious towards living organisms in the water. Some examples for the cause of pollution arecontaminated waste disposal and sewage or agricultural toxic waste.

  Metals can also be absorbed by organisms through various routes such as inhalation from the atmosphere, absorption via skin, ingestion of seawater, and ingestion of food. In these pathways, invertebrates are also involved through the food chain, where the metals accumulated in them will be passed to other fish species and then to higher hierarchy mammals, which include humans in the final stage (see Figure 2.1).

Table 2.1: Metal emissions into the atmosphere (in kiloton.year-^{1})

Metal	Natural sources (eg)	Anthropogenic sources (e.g.)
Arsenic	12 (Volcanic activity)	18 (Mining, metal smelting)
Cadmium	1.3 (Volcano, forest fire)	7.6 (waste incineration)
Copper	28 (decaying vegetation)	35 (wood production)
Lead	12 (earth crust)	332 (lead melting, gasolines)
Nickel	30 (weathering of rocks)	56 (tobacco smoking)
Zinc	45 (soil)	132 (power plant emission)

Source: Data taken from Duce et al. (1991)

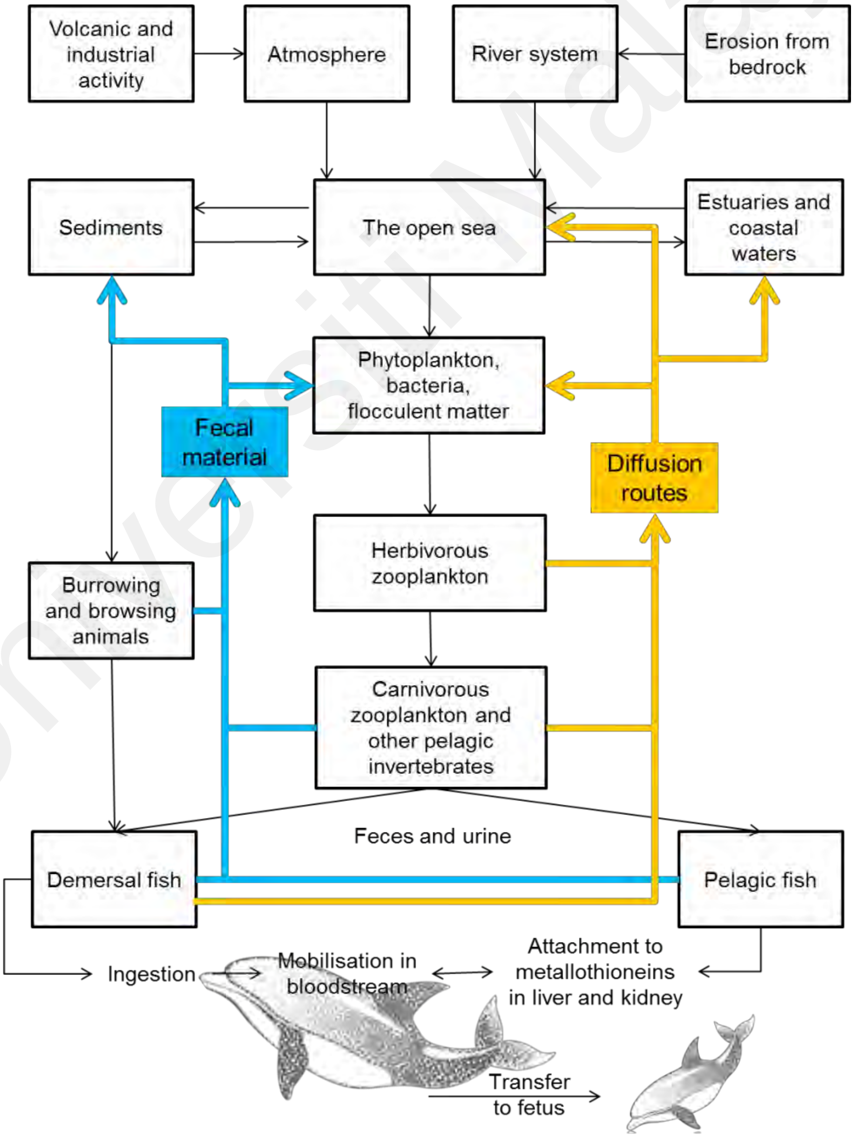


Figure 2.1: Routes of metal contaminants in the food chain based on Gaskin (1982)

2.6 Heavy Metals: Lead, Cadmium, Mercury, Arsenic

2.6.1 Lead

Lead (Pb) is a common example of anthropogenic metal pollution. It is a soft and malleable metal that becomes bluish-white when freshly cut, to which it would eventually turn greyish when exposed to air. Lead is a valuable component for construction, lead-acid batteries, bullets, weights, pewter, fusible alloys, and as a radiation guard. Various health issues can arise from the over exposure to lead. This metal can affect the neural pathways leading to blood poisoning. Pb concentration has risen rapidly during the industrial age especially as an anti-knocking agent added to vehicle fuel. However, according to U.S. Environmental Protection Agency [US EPA] (1986), following the removal of lead from gasoline and petrol, human blood lead levels dropped rapidly (Potters, 2013). Lead is mainly accumulated in the exoskeleton, gills and digestive gland of aquatic invertebrates such as freshwater gastropods (Fantin et al., 1982)

2.6.2 Cadmium

Gunwal et al. (2014) defined cadmium as a soft, ductile, silver-white or bluish-white metal. It takes place with zinc sulphide ores or as a pollutant to copper ores and lead. This material is mainly applied in rechargeable nickel-cadmium batteries, pigments, coatings, stabilizers as well as alloys. Other than mining and industrial processing wastes, Cd pollution normally comes from burning coal (0.25–0.5 ppm) and oil (0.3 ppm), wearing down of car tyres (20–90 ppm), corrosion of galvanized metal (impurity: 0.2% Cd), phosphate fertilizers (phosphate rock 100 ppm), sewage sludge (30 ppm), atmospheric deposition, domestic wastewater and industrial discharges (Figure 2.2).

Cadmium is extremely toxic; its toxicity has severe effect on the environment. Cadmium is not biodegradable, so it remains in circulation after being released to the environment (Kermani et al., 2010). Compared with other heavy metals, cadmium compounds are fairly water-soluble thus, more flexible in the soil. They are usually more bioavailable and more likely to bioaccumulate (Nordic Council of Ministers, 2003).

Through the food chain, the effects of high-level Cd include decreased growth, kidney damage (800 µg/g dry weights induce renal damage in humans), cardiac enlargement, hypertension, fetal deformity, and cancer. Nonetheless, the actual impact of Cd on animals is relatively minor. A few studies have confirmed the connection between the exposure to Cd and other disease symptoms. As some marine mammals and invertebrates have efficient detoxification mechanism, they are able to tolerate high levels of heavy metal exposure. However, if the heavy metal load exceeds a certain threshold in each organism, it may affect the organism extensively. For example, cadmium levels up to 2,000 µg Cd per g dry weight were discovered in arctic ringed seals, but displayed no obvious health effects (Sonne-Hansen et al., 2002). The same applies tohigh levels of cadmium discovered in baleen whales. There is proof that Cd in silver sea bream can trigger endocrine disruption (Woo & Man, 2011). Cd had led to itai-itai disease in Japan. Itai-itai disease was first noticed in the Junzu River basin region in Toyama at central Japan around the 1930s. However, it was not identified as a cadmium poisoning disease until the 1960s. Later, it was confirmed that the disease was caused by pollution from the Kamioka mine (owned by the Mitsui Mining & Smelting Company Ltd.) located in the upstream region of the river (Kaji, 2012).

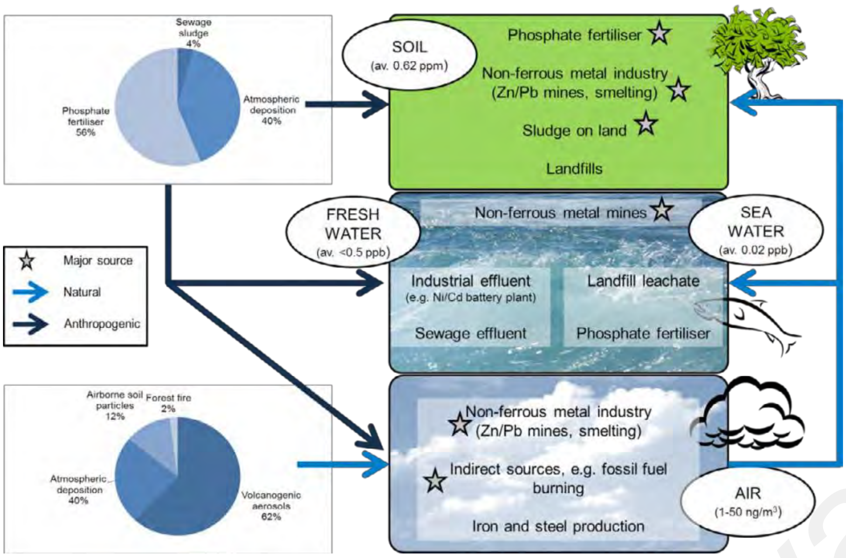


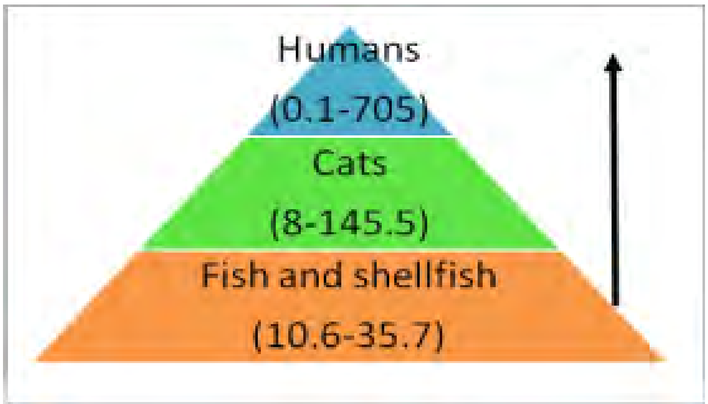
Figure 2.2: Influx of Cd into aquatic ecosystems based on Pan et al. (2010)

2.6.3 Mercury

Mercury (Hg) is usually found in various forms throughout the world, such as mercuric sulphide (most common), metallic mercury, organic, and inorganic mercury compounds (Gupta, 2012). Elemental or metallic mercury is described as a liquid whitish metal found at room temperature. It presents an odorless and colorless gaseous substance when heated. Mercury is incorporated into its organic forms in the food chain whether as methylmercury (MeHg) or dimethylmercury (DMHg). This normally takes place between grazing zooplankton. Apart from that, Hg highly resembles lipids thus, enabling cell membranes to interfere with cell metabolism (Boening, 2000; de Pinho et al., 2002).

Mercury is biomagnified along the food chain up to marine mammals from the organisms stated in Figure 2.3 (Coelho et al., 2010).

Figure 2.3: Mercury in tissue samples from Minamata. All concentrations are given in ppm



Source: (Allchin, 1999)

Allchin (1999) stated that at least in one instant, the bioaccumulation of mercury can be found to be deadly on a big scale such as in the Minamata disaster in 1956. The penetration of Hg into the local food chain is because methylmercury was discharged from the industrial water waste of a chemical plant, the Chisso Corporation. Then, the metal accumulated in the gastropod shellfish and fish in the local bay which serve as food for the local population. The resulting severe pollution has caused a number of neurological problems. For instance, hearing, speech and vision impairments, muscle weakness, and ataxia which resulted in coma, insanity, paralysis, and death. It also caused developmental and behavioral abnormalities and impaired reproduction.

In some production processes and applications, the revelation of these harmful effects has resulted in a gradual substitution of Hg. Nevertheless, the manufacturing of reference calomel electrodes, mirror telescopes, and fluorescent lamps still requires the use of the metal. Its input into the ocean has slowed down in the last decade as a result of reduced use of the metal. To date, approximately 75% of Hg (3600–4500 tons) in the marine setting originates from natural sources such as eroding ores.

In seawater, dissolved mercury ions occur as $HgCl_4$ or $HgCl_3$. They can be effortlessly absorbed in the sediments and suspended particulate matter. In the sea, they also produce complexes with organic molecules such as cysteine residues on proteins or humic acids.

Hg may be present in its metallic form or as sulphide under anaerobic conditions (Kirk et al., 2012).

Hg^{2+} is further methylated to MeHg and DMHg by bacteria and algae in the photic zone of the ocean. In the dark sediments, an analogous reaction ensues. Organisms at the lowest trophic level take up the methylated forms of mercury more readily. Then, the mercury compounds start to bioaccumulate as depicted by the Arctic environment (Figure 2.4).

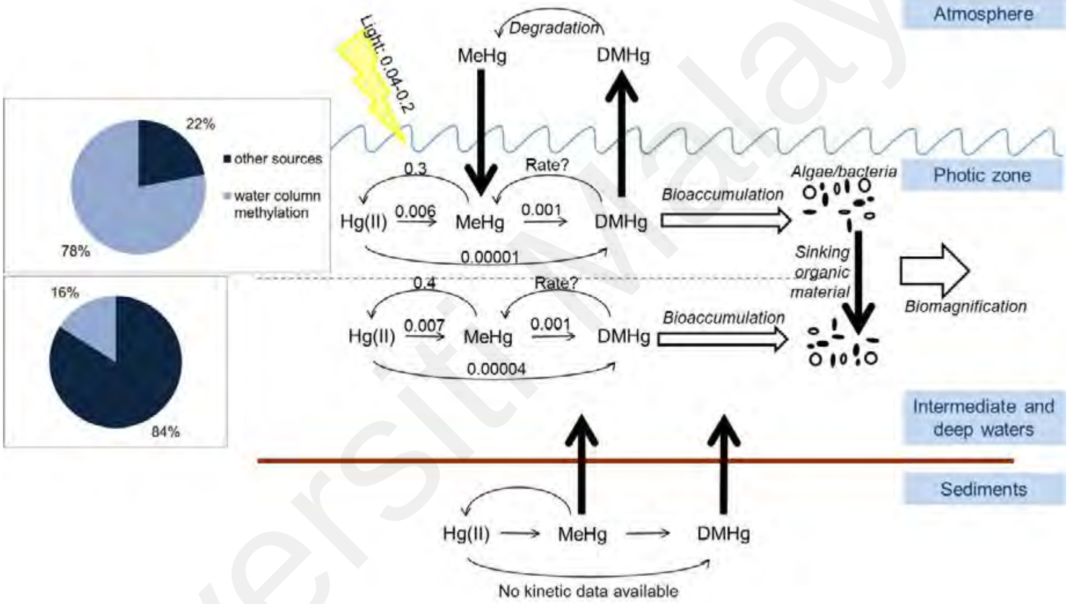


Figure 2.4: Mercury cycling in the Arctic Ocean based on Kirk et al. (2012)

Figure 2.4 depicts the various processes that influence the concentration of MeHg in the water level of the Arctic Ocean, the different process routes of Hg methylation and demethylation (thin arrows) processes whereby each are regulated by their corresponding rate constants (k, expressed in d^{-1}; values shown above the arrows), and the accompanying biogeochemical fluxes (thick arrows). The pie charts show the ratios of MeHg in photic zone and deeper waters which are assumed to have come from the Hg(II) methylation in the water column (Kirk et al., 2012).

2.6.4 Arsenic

Arsenic (As) is an element that relatively occurs among all living tissues and non-living matter such as soil, air and water). In the earth's crust, the abundance of arsenic is at the 20th rank, in seawater, it is at the 14th rank and in the human body, and it is at the 12th rank. Arsenic is a carcinogen and a teratogen, which means that it is capable of passing through the placenta as well as leading to the malformations and fetal death in several mammals. At the same time, low quantity of arsenic can lead toto the growth and development of plant and animal species (Eisler, 1988).

Almost all end products of arsenic that are locally produced are utilized as algaecides, insecticides, fungicides, herbicides, wood preservatives, and stimulants for the growth of animals and plants. Small quantities are employed in the production of textiles, glass, and veterinary and medical applications (National Research Council, 1977; U.S. EPA, 1980). Woolson (1975) reported that the significant anthropogenic basis of arsenic in the environment is found in agricultural settings.

Eisler (2007) stated that arsenic concentrations are typically low (< 1.0 mg/kg fresh weight) in the majority of living organisms. However, they are higher in marine life and organisms from regions that are organically arseniferous or near manufacturing industries and agronomic usage of arsenic. While arsenic is bioconcentrated in organisms, it does not get biomagnified in the food web. This may be because the metal is more concentrated in one organism at the base tropic level, and arsenic has a strong bond with the tissues of the organism and does not get dispersed to other organism's tissues. This is proven by Hepp et al. (2017) who stated thatthe invertebrate abundance and richness were lowest at the highest impacted location. Arsenic in biofilm and in invertebrates increased with the arsenic content in the water. The highest arsenic accumulators were bryophytes (1760µgg^{-}

^{1}), followed by the biofilm (449μgg^{-1}) and shredder invertebrates (313μgg^{-1}); predators had the lowest arsenic concentration. It was found that although arsenic is bioaccumulated, mainly by food ingestion, it is not biomagnified through food webs and is not carried from the aquatic to the terrestrial environment when insects leave the stream water.

The toxins in inorganic arsenic compounds are greater than those in organic compounds. Arsenic is consumed via various means, including inhalation, ingestion, penetration of the skin, and absorption through mucous membranes, whereby each cell absorbs the arsenic via an active phosphate transport system.

Eisler (2007) asserted that the means for arsenic toxicity differs critically among biological species, although all appear to cause comparable indicators of contamination. Arsenic exposure to humans and wildlife can occur via food, water and air. This leads to damages to various human organs such as the heart, liver, brain, and kidney. Furthermore, arsenic exposure is associated to respirational cancers, epidermoid carcinomas of the skin and precancerous dermal keratosis.

Blackfoot disease (BFD) is an endemic peripheral vascular disease confined to the southwestern coast of Taiwan which is caused by arsenic pollution. Sporadic cases of BFD occurred as early as the early 20th century, and peak incidence was noted between 1956 and 1960, with prevalence rates ranging from 6.51 to 18.85 per 1,000 populations in different villages (Tseng, 2005).

2.7 Heavy Metal in Study Areas (Klang)

Port Klang is situated within three international ports bordered by mangrove forests. This region is an attraction for fisheries, tourism, transportation, and navigation (eg: navigating river depths and water velocities). Nevertheless, there is poor understanding regarding Port Klang's heavy metal concentration in the sediments and organisms. Thisarea is impacted by human activities such as shipping and coastal development (Sany et al., 2013). The sediment qualities in this region have been affected by strong coastal currents that are regulated by monsoonal seasons. Study by Sany (2012) revealed that the surface sediment was fairly contaminated by Hg, Pb and As in the Klang Strait. Only Cd had a higher contamination level. In addition, living organisms in this strait have had higher risks of Cd and Hg. Sediments at the entrance of the Klang River poses higher ecological risk.

A study by Yuswir et al. (2014) was done to establish the heavy metal bioavailabilityand presence of Cd, Cu, Co, Cr, and Zn on health assessment in urban surface soil via the Physiologically Based Extraction Test in vitro human digestion model. Heavy metal bioavailability was done to show the fraction of the total amount of metal the organism is proximately exposed to during a given time and under defined conditions. The results revealed heavy metal concentrations in the following order: Zn, Cu, Co, Cd, and Cr. Bioavailability results on heavy metal concentration using ICP-OES was adopted to ascertain the proportional hazard. The proportions of risk were < 1 for the surface soil samples. Conclusively, the soil samples of Klang district are safe for humans.

Port Klang coastal sediments were examined to assess the spatial distribution of heavy metals As, Cu, Cd, Cr, Ni, Pb, Zn, Pb, Mn, Al, and Fe (Sany et al., 2013). Collection of the sediment samples was done from 21 locations encompassing West Port, North Port, and South Port. The geo-accumulation indicators were aimed at assessing the status of pollution on the basis of the background values. Their results indicated that metal concentrations of As, Cd and Pb were reasonably greater than the background values. This is a significant environmental concern for this area. Moreover, the level of toxins implied that the living organisms are exposed to higher risk of As (Sany et al., 2013). Sany (2012) attributed the contamination risk in the Klang Strait to human activities and natural processes.

Sany et al. (2011) analyzed the heavy metals Cu, Cr, Zn, Hg, Pb, Cd, Ni, and As in the West Port of Peninsular Malaysia throughout the dry and wet seasons. Except for copper and chromium, the metal contents in the dry season were higher than in the rainy season. The average metal concentration in the two seasons (α level = 0.05) was very similar. Based on the Interim Sediment Quality guidelines (ISQG), arsenic depicted the highest pollution level, while metal concentrations are fairly contaminated.

2.8 Importance of Heavy Metal Study

Tchounwou et al. (2012) reported that the use of heavy metals in domestic, agricultural, technological, medical, and industrial applications had resulted in their exposure to the surrounding and thus increase the risk of ecological and human health. The toxicity of heavy metal depends on certain factors such as exposure route, genetics, chemical species, and an individual’s nutritional status. Arsenic, cadmium, lead, and mercury have high toxin contents and are of public health concerns. Their effects on humans, even consumed at low dosage, can damage the internal organ. Therefore, the heavy metal study is crucial as to reduce their pollution risks to environment and human. Based on Jantataeme et al. (1996), the exposure of lead (Pb) towards snails for more than 24 hours showed an increase in their mortality rate due to the increased lead residues in their system as a result of increased exposure time. This shows exactly how an environment may manipulate the number of species population andshows the importance of heavy metal study.

2.9 Heavy Metals in Sediments

The mangrove trees function as land builders by holding sediment above ground due to their root structure. Bird and Barson (1977) presented that the sedimentation rate in mangrove areas is in the range from 1 to 8 mm a year. According to an assessment done in 1961, around 60% of the southern Thai coastline used to be occupied by mangroves. In the past three decades, these mangrove areas have been reduced to about 50% with less than 10% left on the east coast. Coastal erosion and accretion occur irregularly along the coast. This shows the importance of mangroves in preventing soil erosion (Thampanya et al., 2006).In another view, mangrove forests are not the cause, but the result of sedimentation in a confined coastal area (Woodroffe, 1992).

Suspended sediments are brought back into the environment through discharges from the river, and through the dumping of dredged substances and resuspending bottom sediment via ships and waves (Wolanski & Gibbs, 1995). The methods of transporting sediments are similar to hydrodynamic processes in mangrove areas (Ayukai & Wolanski, 1997). The floating sediments will then be trapped in the mangroves when transported by tidal flows (Wolanski et al., 1980).

In an aquatic system, sediments are a vital sink for metals. Sediments contain absorbed heavy metals that would be suspended into the water column by tidal currents. More than 90% of heavy metals are likely to be suspended in an aquatic environment (Calmano et al., 1993). It became crucial to analyze the probable environmental impacts of heavy metal contamination due to related biotoxicity and high incidence of bioaccumulation found in the food chain (Chon et al., 2010; Kishe & Machiwa, 2003; Pan & Wang, 2011). Sediments represent a toxin pool. Consequently, analyzing metal contamination will be influenced by the historical disparity between lithogenic and anthropogenic efforts in an aquatic ecosystem (Marchand et al., 2006; Olubunmi & Olorunsola, 2010).

Metal absorption in mangrove sediments produces a harmful impact on microbial activities, plant growth, and soil fertility. The sternness of this impact is influenced greatly by the quality of metals and sediments (Haris & Aris, 2012). Toxins in heavy metals rely on the concentration of metal and other materials, their component forms, and the application of physico-chemical parameters which include dissolved oxygen, organic carbon, temperature, sediment grain size, pH, and salinity (Rai et al., 1981; Wang et al., 2002).

Sediments act as sink for many anthropogenic chemical pollutants including sewage from agricultural, industrial, urban, and recreational activities (Apitz et al., 2005). Trace elements are natural contaminants that are present in aquatic areas and turn into toxins when they occur beyond certain thresholds of bioavailable levels (Rainbow, 1995). The concentration oftrace elements (Cd, Cu, Ni, Pb, Zn, and other metals) are frequently higher than the level of sediments at the background, which are influenced by human activities such as construction, industry, transportation, mining, and agriculture (Ismail, 1993; Lim & Kiu, 1995; Shazili et al., 2007). Previous research had linked high sediment absorption of inorganic elements in aquatic and coastal regions with the increase in agricultural activities, industrial growth, urbanizations, etc. (Naji et al., 2010; Cuong et al., 2008). Pollution in aquatic and coastal regions contributes to ecological hazards and health threats. Irrespective of the kind of environment, sediments are known as a sink of pollutants that may influence the aquatic systems (Ngiam& Lim, 2001). Furthermore, aquatic organisms such as fish and invertebrates feed from the sediment surface that may contain pollutants. Consequently, these pollutants are then transferred through the trophic level (Rainbow, 1995).

Heavy metal absorption in the soil entails multifaceted chemical and biological networking. Metal retention can be categorized into five major activities which include complex organics, cation exchange and precipitation as sulphides and as oxides, oxyhydroxides, and carbonates (Dunbabin & Bowmer, 1992). Precipitation as sulphides is exceedingly essential in swampy mangroves since the ecosystem is periodically exposed to air and water during a particular tidal cycle. As metal sulphides enter anaerobic environments, the sulphate-reducing bacteria will generate H_2S (Lacerda et al., 1993). Furthermore, metal fixation below the sediments will be resulted from the coagulation of particulate and colloidal issues (Dunbabin & Bowmer, 1992). Zheng et al. (2008) examined the basis and allocation of Hg, Pb, Cd, Zn, and Cu in the surface sediments of the following rivers: Wuli, Cishan and Lianshan River. The researchers further examined the toxin risk of heavy metals. They applied the Sediment Quality Guideline (SQG) indices and their outcomes indicated that Hg pollution in the Wuli River was derived from earlier sediment pollution from the chloro-alkali industry. Pb, Cd, Zn, and Cu pollutions originated from atmospheric deposition and unidentified sources. The Cishan River sediment contamination by heavy metals originated from the zinc plants. Meanwhile, the Lianshan River pollution came from the sewage or wastewater. Hg and Cd were the major pollutants to the Wuli and Lianshan Rivers, while only Cd had become toxic to the Cishan River.

Regular assessments on heavy metals have been carried out in Malaysia since the early 1980s and showed that there is an elevation of sedimental contamination from heavy metals, particularly in areas where the source of pollutants is located. A study done by Ismail (2011) stated that the level of trace elements in intertidal sediments around Peninsular Malaysia could vary between 0.1–340 µg/g (As), 2–330 µg/g (Cr), 1–670 µg/g (Cu), 0.2–610 µg/g (Zn), n.d.–45 µg/g (Cd), 0.5–250 µg/g (Pb), and n.d.–50 µg/g (Ni) respectively. Commonly, with the exception of several areas, the heavy metal concentration in intertidal sediments around Peninsular Malaysia is still small. Trace elements have been discovered that polluted regions near to the pollutant sources and have non-uniform distribution across the coastal regions. This is approved by the works of Naji et al. (2010), Ismail (1993) and Ismail et al. (1989). Yap et al. (2002) have also found Cu and Pb in intertidal and offshore sediments on the west coast of Peninsular Malaysia. The total amount of Cu and Pb from the zone was similar to previous reports from other regions in Malaysia. Sediments with higher level of metals offshore are impacted from sea-based activities, while intertidal sediments elevations are from land-based activities.

A study done by Sany (2012) revealed that Klang Strait sediments are moderately polluted with Hg, Pb and As. Cd was expected to be higher in terms of its contamination level. This is because Cd showed the highest sink factor for sediment and high ecological risk assessment value. This may be due to the high concentration in the water compared to the sediment. In addition, living organisms in the strait had higher risk of Cd and Hg pollution. The risk index classification showed that only sediments from the Klang River entrance might represent a higher ecological risk. Although there were several surveys on heavy metal contents in marine sediments of Malaysia (e.g. Yap et al., 2002), the data obtained from the mangroves are still limited.

2.10 Heavy Metals in Mollusc (Tissues and Shell)

In the 1980s, biomonitors using *P. viridis* were implemented in the coastal waters of Asia Pacific (Nicholson & Lam, 2005; Tanabe et al., 2000), Malaysia (Ismail et al., 2003b; Yap et al., 2003), Hong Kong (Nicholson & Szefer, 2003), Singapore (Bayen et al., 2004), and China (Klumpp et al., 2002).

To assess the pollution of metals in an aquatic setting, molluscs are mostly used. In the Straits of Johor, Hadibarata et al. (2012) used intertidal bivalves *Perna viridis*. They observed the variation in the change of land use. Satellite images from 1991, 2000, 2005, and 2008 were used to compare the differences between selected water quality parameters and heavy metal (Cd, Pb, and Zn) content in both water and the *P.viridis.* This was done to see the difference before and after the increase in land use activities. The samples were collected and analyzed for pH, temperature, dissolved oxygen, ammoniacal nitrogen, and heavy metal (Cd, Pb, and Zn) content. Their findings showed an increase in pollutants, nutrients, and heavy metals which reduced the water quality. Thedegradation of water quality level was due to land use. This showed a significant impact of *P. viridis* since they can accumulate high concentration of pollutants (metals).

Amin et al. (2006) used snail *N. lineata* as indicators to monitor metal pollution in two study areas: intertidal zone of Johor, Malaysia and Dumai, Indonesia. The concentrations of Cd, Cu, Pb, Zn, Ni, and Fe were determined in the marine gastropod *N.lineata*. Their results showed that metal concentrations in the shell, operculum and soft tissue of the *N. lineata* varied at different sampling stations. Samples from Johor accumulated higher heavy metal concentrations compared to samples from Dumai, except for Cu and Ni in the shell and operculum, which exhibited a vice versa situation. Higher concentrations of metals were recorded in samples collected from the stations close to the industrial and anthropogenic activities in both Dumai and Johor area.

High levels of bioaccumulation of copper (Cu) and mercury (Hg) werediscovered from the aquatic gastropod snail. The freshwater snail, *Bellamya bengalensis*, presented the highest level of copper rather than mercury. Hg in tissues on exposure to $HgCl_2$ increases with prolonged exposure period when compared with the control group. Impressively, the snails have the ability to control and excrete heavy metal from their body (Mahajan, 2014). Phillips and Depledge (1986) in their research of the spindle shell *Hemifusus ternatanus*, found that it had accumulated large quantities of arsenic in its tissues. Their analysis indicated that most of the arsenic was found in the soft parts compared to the operculum and shell, whose metal concentrations are found in inorganic form. This is possibly because the arsenic had been used up by *H. ternatanus* which isobtained from saltwater and food due to biotransformation of accumulated inorganic arsenic that occurred in the gill and other tissues. The ingestion of gill tissues rather than its foot tissue may cause toxicity.

Yap et al. (2009) reported in their study that in the southwestern intertidal area of Malaysia, the soft tissues of the mudflat snail, *T.telescopium,* contained high concentrations of Cu and Zn. The digestive caecum of the soft tissues showed a higher concentration of Zn, while the shells indicated a higher Pb concentration. The researchers indicated that the shells and soft tissues of the*T. telescopium* are a possible indicator for Cu, Zn, and Pb. Ismail and Safahieh (2005) also conducted studies of heavy metal accumulation in *T. telescopium* soft tissues in Lukut River, Negeri Sembilan, while Amin et al. (2005) conducted a research in an intertidal mudflat using *T.telescopium* shell, operculum and soft tissue in Dumai, Indonesia. Their presence in intertidal mudflats near mangrove fields and riverbanks is an input tool for assessing heavy metals in the mangrove ecosystem from inland across the river systems. Their results showed that *T. telescopium* contains elevated heavy metal concentration within the ranges of 0.33–

0.69 µg/g, 9.38–52.29 µg/g, 1.73–10.78 µg/g, and 14.69–69.87 µg/g dry weight for Cd, Cu, Pb, and Zn respectively.

Another mollusc is the intertideal gastropod *N. lineata* which can also be proposed as a useful biomonitors for heavy metals. *N. lineata* is generally discovered in mangrove trees, rocky shores, intertidal mud, and sandy beaches as opposed to other gastropods. This species is sometimes discovered aggregated on the intertidal rocky shores and close to the roots of mangroves. Ismail et al. (2003a) recorded high metal concentrations in this snail, which was tested along the coastal regions of Selangor and intertidal Sepang River. Their research revealed that these snails have the capability of being a biomonitoring agent in their own habitat. Subsequently, Yap and Cheng (2009), and Amin et al. (2009b) had conducted an in-depth research that assessed the influence of heavy metal on *N. lineata* to determine if the snail can potentially be used as an indicator of heavy metals. These studies were done at both sides of the Straits of Malacca i.e., the coastal areas of Dumai, Indonesia, and the western shorelines of Peninsular Malaysia. The results of the studies suggested identical patterns of previous findings by Ismail et al. (2003a) where heavy metal accumulation was consistently elevated in regions near various anthropogenic activities compared to other areas.

Berandah et al. (2010) studied the gastropod *C. capucinus* collected from the Sungai Janggut mudflat, Kuala Langat, Selangor and assessed the heavy metal concentration. Evaluation on the biota-sediment accumulation factor (BSAF) values were done to determine the gastropod's ability to take in heavy metals from the surrounding. The concentration was highest for Cu that was discovered in the caecum (194 ± 24.4 µg/g dw), Cd in the digestive gland (32.9 ± 0.000 µg/g dw), and Fe in the operculum (971 ± 2.50 µg/gdw). High concentrations of Ni and Pb were observed in the shell; where as high levels of zinc were examined in the tissues. However, BSAF highest values were reported in the caecum for Cu (101.2), Zn (27.4), and Cd (53.1), whereas for the shell was Pb (32.6) and Ni (8.88). The gastropod was reported as macro concentrator organs because the values for BSAF are above two. The purpose of obtaining the BSAF values was to estimate the proportion in which metals occur in the organism and in associated sediment. Based on the values calculated, the different parts of the gastropod could be classified into groups such as macro concentrator (BSAF >2), micro concentrator (1< BSAF <2) or deconcentrator (BSAF<1) as proposed by Dallinger (1993)

Blood cockles, *Anadara granosa*, are another useful species as biomonitors for heavy metals. Several studies on heavy metals in *A. granosa* in the western coastlines of Peninsular Malaysia showed various reports (Alkarkhi et al., 2008; Yap et al., 2007; Yusof et al., 2004). Ismail (2006) and Amin et al. (2009a) monitored heavy metals in the western coast of the Peninsular Malaysia using intertidal molluscs.

Said et al. (2013) conducted a study on the sea grass bed in Tanjung Kupang, a western part of the Johor Straits, and Merambong Island, where metal concentrations (As, Cd, Cu) in the seawater and soft tissues of the sea snail *Strombus canarium* using ICP-MS were analyzed. Their findings showed high concentrations of Cu in *S. canarium*. The Cu had accumulated in the soft tissues of the gastropod and seawater due to the *S. canarium's* capability of absorbing Cd into its system through seawater. As such, this gastropod was found to be a potential indicator for Cd contamination in the Straits of Johor. Nonetheless, the metal concentration in the *S. canarium* was is still in the acceptable threshold as suggested by the World Health Organization, Food and Agriculture Organization.

Palpandi et al. (2010) stated that the concentrations of heavy metals in molluscs were mainly in the soft tissues. Palpandi et al. (2010) further highlighted the exceptional regularity of the mollusc's shell which is made up of a microlaminate composite of mineral and biopolymers, making the shell strong. Thus, gastropod shells are a useful bioindicator to determine the extent of biotransformation in the marine food chain regarding the risk of heavy metals. Palpandi et al. (2010) also studied the degree of Cu, Zn, Fe, and Mg on the shells of four gastropod species (*Turbo bruneus*, *Littorina scabra, Nodilittorina pyramidalis*, and *Morula funiculata*) from Kanyakumari in the southeastern coast of India. The accumulation of trace metals was sorted as Mg>Fe>Zn>Cu. The accumulation of Mg and Fe in the *T. bruneus'* shell was higher than in the other species. However, the concentrations of Zn and Cu were higher in the *M. funiculate*, whereas the minimum accumulation of all the metals studied was recorded in the shells of the *L. scabra*.

Badran (1999) measured heavy metals (Mg, Sr, Mn, Fe, and Zn) in individual shells of different gastropod *Nerita*s pecies (*N. albicilla*, *N. undata*, *N. polita*, *N. Costata*) collected from Phuket Island, Thailand. Out of the four species, *N. albicilla* had had the highest heavy metal concentration. Kupekar and Kulkarni (2014) studied the marine gastropod shells of the *Hemifusus pugilinus* and *Bursa spinosa*. In both species, there were higher concentrations of Mn, Cu and Fe. Cd and Pb were also in noticeable amounts in the gastropod shells. The order of heavy metal accumulation in the *H.pugilinus*' shell was Mn>Cu>Zn>Fe>Cd>Pb, while in the shell of the *B. spinosa* was Mn>Cd>Cu>Zn>Fe>Pb. The gastropod shells provide safety storage for the heavy metals, which is important in protecting the vital organs. This protection could enhance the continuous existence of a species in polluted environments. Hence, in order to determine pollution changes in the environment, gastropod shells can be used as bioindicators. Yap et al. (2003) also claimed that there were high levels of Cd and Pb in the shell of the *P. viridis*.

CHAPTER 3:METHODOLOGY

3.1 Study Sites

Klang Islands Mangrove Forest Reserve (KIMFR) consists of Pulau Carey, Pulau Klang, Che Mat Zain, Pulau Ketam, Selat Kering, Jugra, Pulau Gedong, and Telok Gong (Ahmad et al., 2009). This research was conducted on three islands i.e., Klang Island, Carey Island and Telok Gong (Figure 3.1). Klang Island (N 03° 01 ' 12.3″, E 101° 20 ' 09″) is covered by natural mangroves, while Carey Island (N 02° 49 ' 41.0″, E 101° 21 ' 56.7″) is covered by oil palm plantation. Telok Gong (N 02° 56 ' 54.1″, E 101° 22 ' 14.0″) is considered the most impacted site surrounded by factories, logistics hubs, warehouses, and port. Sampling collections were conducted on threeoccasions. The first was done on 6thApril 2016 in Carey Island, followed by 24thApril 2016 in Telok Gong. The final sampling was done on the 10thMay 2016 in Klang Island. The sampling was done during dry season. All gastropods collected were within a range of 1-13 cm in length.

Figure 3.1: The sampling locations of mangroves in Klang Islands

3.2 Field Sampling and Preparation

Soil sampling collection was done for the four replicates of the samples from each island using a soil sampler about 15cm deep that was labeled, air dried and stored. The gastropods were randomly hand picked from five plots of each mangrove forest, approximately 200m from the shoreline, kept in a sterilized polyethylene bag and labeled. The collected samples were kept in an ice box, frozen at 0°C before dissection. There were ten species found from the three locations mentioned (*Nerita lineata, Cerithidea quadrata, Cassidulaa urisfelis, Chicoreus capucinus, Telescopium telescopium, Ellobium aurisjudae, Thais tissoti, Littorina scabra, Littorina melanostoma,* and *Cerithidea cingulata*), but only six species were selected for analysis based on the following criteria:

1. Local abundance of species (*N. lineata, C. aurisfelis*)

2. Big body size (*C. quadrata*, *C. capucinus*, *T. telescopium*, *E.aurisjudae*). Big body size will providea highnumber of samples since it will have higher volume of soft body and shell.

Species Identification

The species were identified based on the taxonomic handbook (Oliver, 2004). The characteristics used for identification included shell type, color, shape and texture.

Diversity Analysis

Species richness was calculated manually by calculating the number of types of species.

Species diversity was calculated using the Shannon-Weiner index, H' (Shannon, 1948), H' = $-\Sigma$ (p_i ln p_i), where pi=proportion of the total number of individuals in the i-th species.

The evenness of species was derived from the formula J' = H'/H'max, where H' is the number derived from the Shannon-Wiener index and H'max is the maximum possible value of H' (Pielou, 1966).

The Sørensen index S, was used to discover the similarity of species in the two locations (Sørensen, 1948); S=2 j / a + b, where a = number of species in locality A (first locality), b = number of species in locality B (second locality), and j = number of common species from both localities.

3.3 Analysis Procedure for the Heavy Metals (As, Cd, Pb, Hg)

The following procedure was conducted after the sample collection and identification from each island. For the analysis of the six selected gastropods (*N. lineata*, *C. quadrata*, *C. aurisfelis*, *C. capucinus*, *T. telescopium*, and *E. aurisjudae*), their soft bodies and shells were dissected and separated using sterilized steel forceps and scissors which were then weighed, labeled, grinded, and kept frozen in a freezer at 0°C. The separated fresh weight sample was sent for heavy metal determination. Gastropods Soft Body.Heavy metals are a major interest in bioavailability studies as listed by the US Environmental and Protection Agency (EPA) which include aluminum, beryllium, arsenic, cadmium, copper, chromium, mercury, nickel, lead, selenium, and antimony. From these, As, Cd, Pb, and Hg were randomly picked and tested in this study as they have the potential for human exposure which may increase health risk.

The dissected gastropod bodies and shells under went an acidification process similar to the method used by Rahimah (2012) where the soft body and shell were redistilled separately with HNO_3 and H_2O_2. Each mixture was added to 5 ml of 30% H_2O_2 after being heated and cooled for an hour. Subsequently, the solution was gradually heated to a boil point (approximately 10 minutes) before adding 5 ml of HNO_3. This solution was subsequently lowered to 10 ml by boiling and cooling. The solutions were kept in polyethylene bottles mixed with reagent water into different volumes within the expected range of the inductively coupled plasma mass spectrophotometer (ICP-MS) for analysis. The standard procedure of USEPA 6020A was used for ICP-MS. The heavy metal concentrations (Cd, Pb, Hg, and As) were examined through the inductively coupled plasma (ICP-MS) expressed in µg/g.

For sediment analysis, the fresh sediment samples were equally divided into two sections (A and B). An amount of 1.5 g of sediment A was weighed and put into a beaker prior to the digestion process. Several types of acids were added into the sediment sample which included 4 ml of diluted nitric acid solution (comprised of 2 ml of nitric acid 65% and 2 ml of deionized water), 2 ml of nitric acid 65%, and 2 ml of hydrochloric acid 37%. The mixture of sediment and acids was then heated using a hot block at 85°C for 30 minutes. After heating process was completed, the soil sample was then placed in a desiccator to cool. The sample's volume was then marked up to 50 ml by adding deionized water (Rahimah, 2012).

Each digested sample was aspirated into the inductively coupled plasma optical emission spectrometer (ICP-OES). The standard procedure of USEPA6010A was used for the detection of Pb, Cd and As by ICP-OES and USEPA7471A was used for the detection of Hg by CVASS (FIMS-400). Ten gram of sediment B was weighed to determine its dry weight and was placed in a crucible cup prior to heating in an oven for

2 hours under a temperature of 103°C–105°C. After the completion of the heating process, the sediment sample was placed in a desiccator for cooling before it was weighed again. The results obtained by both procedures were used for further statistical analysis. The readings obtained were utilized to ascertain the concentration of the heavy metals in terms of dry weight, which was assessed via the following formula:

$$\text{Heavy metal concentration} = (R1 \times \text{Mark-up Vol}) / (W \times DWF)$$

Where:

R1 = Instrument reading

Mark - up volume = 50 ml

W = Sample weight

DWF = Dry weight factor

In order to calculate the dry weight factor (DWF), 10 g of soil was weighed and placed in a crucible cup prior to heating in an oven for 2 hours at 103°C - 105°C. Once the heating process was completed, the soil sample was then placed in the desiccator to cool down before being weighed again. The DWF was determined using the following formula:

$$DWF=1- (W1-W2)/(W1-W_{cup})$$

Where:

W1= Weight of sample and crucible before heating

W2= Weight of sample and crucible after heating

Wcup=Weight of crucible cup

3.4 Statistical Analysis

Microsoft Excel and SPSS version 22 were used to conduct the statistical analysis. Pearson's chi-squared test was attempted to determine if there was any association of the species abundance with the locations. Moreover, this test was done to prove the null hypothesis (there is no relation between the abundance of species and locations), while the alternate hypothesis was to show that there is a relation between the species abundance with each location. Non-parametric, Wilcoxon Signed Ranks Test was done to compare the difference in heavy metal betweensoft body and shell. Since the data was not normally distributed, Spearman’s correlation was adopted to show the connection between heavy metal accumulation in the body and the shell for each selected six species. Due to the abnormal distribution of the variables, generalized linear model, compare means and Bonferroni post-hoc test was used to compare metal concentration and mean difference among the six selected species. Owing to the non-normal distribution of the variable, the generalized linear model was employed for the analysis. The term Generalized Linear Model (GLM) is defined as a conventional linear regression model for a continuous response variable for continuous or categorical predictors. It has the capacity to assess non-normal distribution data. GLMs rely on an assumed relationship known as the link function between a linear predictor function of the explanatory variables and the mean response variable. The main importance of GLM is that it can handle a larger class of error distribution and deliver more effective ways of ensuring linearity and constraining the predictions to be within a range of possible values based on the link function. GLMs offer a comprehensive modeling concept that includes the most significant models for handling non-normal error structures. Compared means were employed to compare heavy metals among land-use types and to compare between the gastropods and the sediments; the Mann-Whitney U test was also used for the sediment and gastropod comparison. All the values of gastropod species were combined to obtain results. This analysis stated that the estimates could vary for different species, with respect to the material. Thus, it is a non-parametric alternative test to the independent sample t-test. For the Mann-Whitney U test, the values need to be measurable on an ordinary scale. The test is used to test the null hypothesis based on both samples from the same basic set or same median value.

CHAPTER 4: RESULTS AND DISCUSSION

This chapter demonstrates the results and discussion of heavy metals found in the gastropods and sediments in the three different locations with different types of land-use. There are four subsections in this chapter: 1) The gastropod species abundance, composition and diversity, 2) comparison in concentration of heavy metals between gastropod body and shell, 3) comparison of heavy metals between gastropods and sediments, and 4) comparison of heavy metal concentration among species.A lot ofstudies have shown that heavy metal contamination in coastal habitats is strongly linked to enhance economic growth in the past decade; therefore, considering the surrounding locations, the contamination levels can be ascertained (Pan et al., 2011). The concentration of heavy metals in sediments was compared to established sediment quality guidelines.

4.1 Species Composition and Diversity

Normally, individual organisms can be reorganized, but the larger units are utilized to establish the diversity of life, including their species and subspecies, which are not easily identified. The species are grouped into families, orders, kingdoms, and genera. Ecologists can also categorize the species into other varying structures like ecosystems and communities. The species originate in the taxonomy of basic rank of classification, with respect to the International Commission of Zoological Nomenclature. The main use of this taxonomy is to obtain information on the individual specimen from the collection. Generally, species can be defined as a group of individuals, where the purpose of studying local populations and communities are analyzed. It carries the three connotations of concept, category and taxonomy. Table 4.1 shows the 10 species recorded in this study and their abundance in different locations which are *N. lineata*, *C. quadrata*, *C. aurisfelis*, *C. capucinus*, *T. telescopium*, *E. aurisjudae*, *T. tissoti*, *L. scabra*, *L. melanostoma*, and *C.*

cingulata. The sample size calculated was 203 ± 5%. Among the total 10 species collected, 43% of the species are from Telok Gong, 38% from Carey Island, and 19% from Klang Island. From the analysis, Telok Gong has higher species richness with nine species, while Carey Island has eight, and Klang Island has five species. Although there are more species richness in Telok Gong than the others two locations, the total abundance seems to be low in Telok Gong. This may be due to indirect effects of metal pollution, that is, changes in species interactions towards the environment rather than by the changes in abiotic conditions. Another reason is due to species tolerance (Nahmani and Lavelle., 2002; Grześ, 2009). The trend that was noted in the three study sites was that a low species abundance has a high species richness, and vice versa.

Though there were other researches done in Port Klang, there is still not much background information on the population of species present in these islands. These species compositions provide the baseline information for future studies, should the present environment be affected further due to anthropogenic activities. However, the species composition is not complete since the samples collected were only from nearby shorelines. As illustrated in Table 4.1, Telok Gong is more diverse with an H'-value of 1.564 containing at least nine species (*N. lineata*, *C. quadrata*, *C. aurisfelis*, *C. capucinus*, *T. telescopium*, *E. aurisjudae*, *L. scabra*, *L. melanostoma*, *C. cingulata*) and a total of 93 gastropods found in that location. Carey Island, with an H'-value of 1.519, has eight species (*N. lineata*, *C. quadrata*, *C. capucinus*, *T. telescopium*, *E. aurisjudae*, T. *tissoti*, *L. scabra*, *L. melanostoma*) with a total of 138 gastropods. Klang Island, with an H'-value of 1.129, has five species (*N. lineata*, *C. quadrata*, *C. aurisfelis*, *C. capucinus*, *T. telescopium*) with a total of 198 gastropods. However, Carey Island is more even with a J'-Value of 0.730 representing the total evenness in that location, followed by Telok Gong with a J'-value of 0.712, and Klang Island with a J'-value of 0.702. Generally, all locations were quite even since their values were close to 1. Carey Island and Telok Gong consisted of almost similar species as the S-value was closer to 1 (0.824). This was followed by Klang Island and Telok Gong (0.714), whereas Klang Island and Carey Island showed the least similarity (0.651).

Table 4.1: Species composition, abundance, diversity index, evenness, richness, and similarity index with location

Species name	*N.lineata*	*C.quadrata*	*C.aurisfelis*	*C.capucinus*	*T.telescopium*	*E.aurisjudae*	*T.tissoti*	*L.scabra*	*L.melanostoma*	*C.cingulata*	Total	Species richness	Shannon wiener index H'	Evenness J'	Sørenson's Index S		
Telok Gong (43%)	11	6	42	21	2	8	0	1	1	1	93	9	1.564	0.712	0.824		0.714
Carey Island (38%)	65	10	0	29	3	3	18	9	1	0	138	8	1.519	0.730		0.615	
Klang Island (19%)	111	17	51	1	18	0	0	0	0	0	198	5	1.129	0.702			
Total no of species	187	33	93	51	23	11	18	10	2	1	429						

It is highly crucial to note that the abundance of species can assist in future studies to ascertain any loss or growth of a species due to its environment. According to Sasekumar et al. (1984), many sediment epifaunal gastropods are found grazing on the sediment surface or on low tree trunks where algae and mangrove detritus are usually abundant. Such species of gastropods are *Cerithidea obtusa*, the genus *Nerita* sp., and several members of the *Ellobiidae* family. Many of the gastropods are air-breathers (ellobiids) or partial-air breathers (periwinkles). By moving up the tree trunks during high tide, mobile gastropods like *Cassidula nucleus, Cerithidea* can also avoid predation by marine fish like trunk catfish (*Arius* spp.) that prefer ingress into the shore at high tides. In accordance with Sasekumar (1974), the 10 gastropods collected for this study belong to two types: soil-surface fauna (*C. capucinus*, *T. telescopium*, *E. aurisjudae*, *C. quadrata*, *C. aurisfelis*, *N. lineata*, *T. tissoti*, *C. cingulata*) and tree fauna (*N. lineata, L. scabra*, *L. melanostoma*) (see Figure 4.1).

Figure 4.1: Ten species identified at the study site with their length range

Table 4.2 demonstrates the comparison using Pearson's chi-squared test of different species from the three sites: Telok Gong, Carey Island and Klang Island. In this evaluation, Pearson's chi-squared test estimated the significant difference among the species according to abundance and locations. No significant p-value was shown when there was an absence of species in the locations, except for *C. aurisfelis*. The chi-squared assumptions were assessed and it showed that the assumption was violated because the sample size was small. There is difference in the abundance between table 4.2 and 4.1 because chi square test showed error.

Table 4.2: Comparison of species with its location showing abundance and % in parenthesis*

Species	**Telok Gong**	**Carey Island**	**Klang Island**	**p-value**
N.lineata	3	4	5	0.725
C. quadrata	4	3	4	1
*C. aurisfelis***	5	0	4	0.006
C. capucinus	4	2	1	0.301
T. telescopium	1	1	2	1
E. aurisjudae	2	2	0	0.451
T. tissoti	0	2	0	0.286
L. scabra	1	2	0	0.725
L. melanostoma	1	1	0	1
C. cingulata	1	0	0	1

*Pearson's chi-squared test
N (N = Number representation)

The results showed that Telok Gong and Carey Island seem to have more diverse species, although they are an industrial and agricultural area respectively. Such areas constantly release unwanted metals and chemicals into the environment. This raises the question of how these various species are still available in Telok Gong and Carey Island, regardless of their primary functions as an industrial and agricultural area compared to Klang Island's natural mangrove habitat where by Klang Island has a higher abundance but lower diversity compared to Telok Gong and Carey Island. There are also some species that are absent in Klang and Carey Island though these sites are less impacted compared to Telok Gong. Based on Table 4.1, there is a higher number of individual species abundance that came from *N.lineata*. Hence, looking in to it shows that this species survives more in a good environment. Compararively, there is a low abundance of this species in Carey Island and even lower abundance in Telok Gong. This shows that this species is affected by the pollution in Telok Gong and Carey Island. This same trend is seen for the species in *C. qudarata, C. aurisfelis* and *T. telescopium*. This can lead to an extended scope for future research to look into the pollution rate and species diversity in the Klang Islands. However, complexity in community formation is strongly related to complication in the environment. This fact complicates the comparison of diverse values from diverse environments (Gray et al., 1997).

4.2 Comparison of Heavy Metal Concentration between the Gastropod Body and the Gastropod Shell

This section refers to the second objective of this work which shows either the body or the shell of gastropods that attained higher metal concentration.

Based on Table 4.3, both heavy metals in the gastropod body and shell were significantly different ($p < 0.05$). All species were combined to determine the result for this section. Accordingly, the mean concentration of heavy metals indicates that the concentration of arsenic in the body (0.766 µg/g) is higher than in the shell (0.077 µg/g) of the gastropod. The result of Asmetal in the gastropod body was supported based on Phillips and Depledge (1986), where analysis was done with the gastropod *H.ternatanus* collected from fish trawlers at the Hong Kong seathat showed the presence of most arsenic in the soft parts compared with that in the operculum and shell.

Cadmium showed a higher mean concentration in the gastropod shell (0.066 µg/g) than in the body (0.041 µg/g), and lead in the shell (0.275 µg/g) showed a higher concentration compared to its body (0.126 µg/g). This shows that cadmium and lead are found more in the shell than in the body. This finding was supported by the previous studies where cadmium and lead were found to be higher in gastropod shell than body (Kupekar and Kulkarni, 2014; Yap et al., 2009). Heavy metals built up in the gastropod shell and soft tissues. However, the shell could be of better use as a bioindicator since they would be available in larger amounts compared to the soft tissues of the gastropod. The shells are useful in influencing the degree of biotransformation in aquatic food webs. Kupekar and Kulkarni (2014) opined that heavy metal accumulation would differ as per the size and habitat distribution of the gastropod species. Generally, the tissues of macro benthos would accumulate heavy metals. Nevertheless, the existence of heavy metals in the gastropods' shell designates that it is a safer place for storing heavy metals. This type of storage is responsible in protecting the vital organs. Thus, this modification could assist species to survive in polluted areas. However, since each metal has its own affinity towards the shell and soft body, other metals could be accumulated more in the soft body than in the shell (Berandah et al., 2010).

Mercury was significantly different ($p < 0.05$) and more concentrated in the body (0.011 μg/g) of the gastropod than in the shell (0.002 μg/g). Although there is significantly more mercury in the body, both values in the body and shell, in response to mercury, seemed to be very low compared with other metals (As, Cd and Pb). The concentration might differ, and firm conclusions cannot be made because this result is a combination of the six species that were tested and analyzed, and that all three locations might show distinctions in the bioavailability of the metals. Nonetheless, according to Kupekar and Kulkarni (2014), Phillips and Depledge (1986), and Yap et al. (2009), most of the results from this research are supported by them although the species used were different from this study.

Table 4.3: Comparison of mean concentration of heavy metals between body and shell based on Wilcoxon Signed-Rank Test

SAMPLE SIZE (N=24)	Body (μg/g)	Min	Max	Shell (μg/g)	Min	Max	p-Value
Arsenic	0.766	0.233	3.290	0.077	0.016	0.234	< 0.05
Cadmium	0.041	0.000	0.147	0.066	0.000	0.250	< 0.05
Lead	0.126	0.000	0.800	0.275	0.000	1.300	< 0.05
Mercury	0.011	0.000	0.096	0.002	0.000	0.010	< 0.05

4.2.1 Comparison of Body and Shell Concentration According to Each Species

Figure 4.2 compares the mean value of different species with respect to the body and shell of different heavy metals tested (arsenic, cadmium, lead, and mercury). All species showed higher content of As in body than in shell. From the results, *C. capucinus* has the highest mean value for arsenic concentration in the gastropod body compared with other species. Moreover, the arsenic concentration in the gastropod body has ahigher mean value for all species. This was supported by Bayen et al. (2004) where the *P.viridis* tissue had the highest concentration of arsenic than other metals in all of their stations in Singapore (13–32 μg g^{-1}dw). This exceeded the maximum permissible value in Singapore of 1 μgg^{-1}ww for molluscs (Government of Singapore, 1990). However, this did not exceed the limit of the U.S. Food and Drug Administration (1995) where it was observed as non-hazardous.

Cadmium in the body has a lesser mean value than cadmium in the shell for all the six species. Heavy metals like Cd are accumulated in the gastropod shell via a process of replacement of the calcium ions in the crystalline phase of the shell (Foster & Chacko, 1995).

The lead in the body is lesser than lead in the shell for all species, except for *T. telescopium* and *E. aurisjudae,* indicating that the accumulation of heavy metals in gastropod tissue is species specific. Lead was greater in the shells of the *Villorita cyprinoides* compared in the soft tissue (Babukutty and Chacko, 1992).Another experiment by Berandah et al. (2010) using *C. capucinus* showed that higher lead presence was detected in the gastropod shell than in the tissues.A research by Erving (2017) at the Red Sea in Egypt showed that there was a higher presence of lead in the body than in the shell of the species *Echinolittorina subnodosauorium* and *Planaxis* *sulcatus*, which show a similar result with *T. telescopium* and *E. aurisjudae*. In addition, the lead has a higher mean value next to the arsenic.

Mercury in the body has a higher mean value than mercury in the shell for all six species. Elevated Hg concentrations were observed in all body parts (arms, mantle and viscera) of the gastropods in Turkey (Belivermiş et al., 2019).

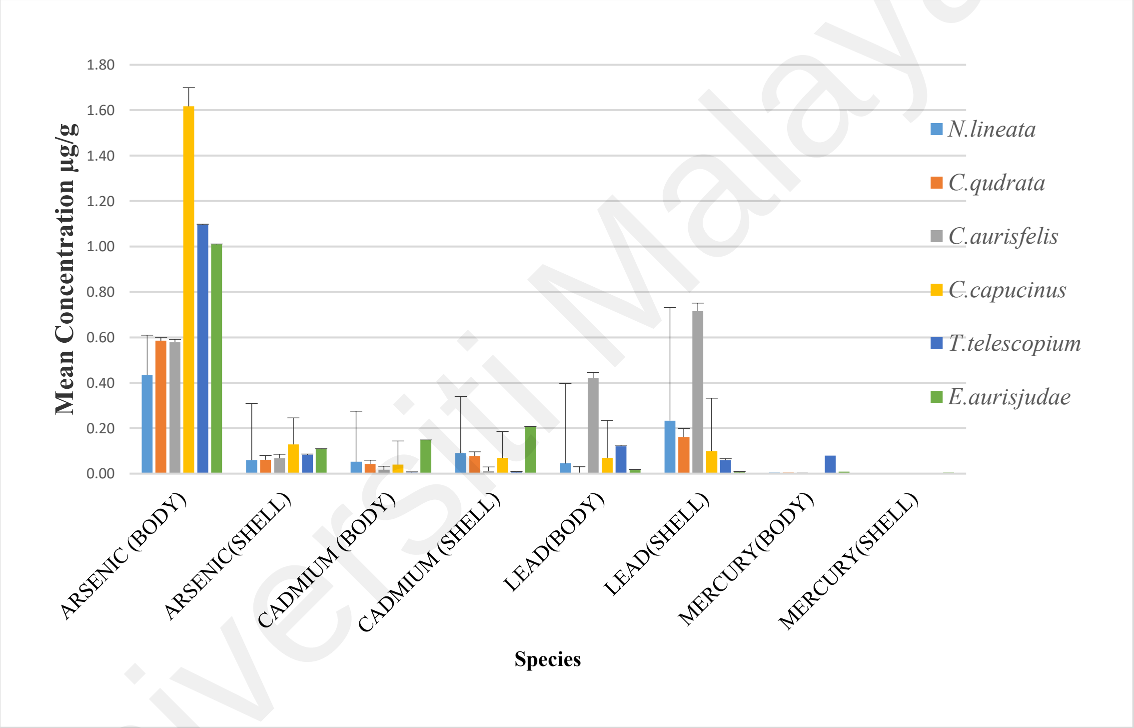


Figure 4.2: Mean of heavy metal concentration in body and shell of the six selected gastropod species. Error bars denote SD, N=24.

4.2.2 Spearman's Correlation

The correlations of heavy metal content in the selected six species were examined. The correlation data of the same heavy metal between the shell and body of the same species were presented in Table 4.4 for the species *N. lineata, C. qudrata, C. aurisfelis, C. capucinus, T. telescopium* and *E. aurisjudae* respectively. The sample size used was (N=24).

The correlation of arsenic in the body and shell showed positive and negative correlation without any significance for all species, except for the *T. telescopium* which showed a significant positive correlation of 0.01 level with r = 1.00. The correlation of cadmium in the body and shell depicted a significant positive relationship only for *E. aurisjudae* (r =1.00, p = 0.01). There was no significant relationship in any of the six species for the correlation between lead shell and lead body value. *N. lineata* depicted a significant positive correlation for mercury in the shell and mercury in thebody (r = 781, p = 0.05).

The results showed that only some species display significant correlation between the same heavy metal in the shell andbody. No same species showed significance for other corresponding metals such as the *C. capucinus* which showed only correlation for arsenic. This shows that the accumulation of each metal (As, Cd, Pb, Hg) differs for the same species. There is no significant correlation for each metal except for few a species. This is similar to Koide et al. (1982) that indicated there were strong correlations between metals in the shell but not in the soft tissues in general. Presumably, this pertains to differences in the biochemical behavior of the metals in the period between the uptake by the organism and the release to the environment or to the shell.

Nevertheless, by observing the relationships for other species, it can be seen that only *C. capucinus, E. aurisjudae*, *N. lineata* showed significant correlation which depicted that these species might show differences in concentration of the respective specific metal between shell and body. Future studies on covariance might be needed for these species, which can show a better understanding on the relationship of the metals between shell and body.

Table 4.4: Spearman's correlation analysis for heavy metal between the body and shell

Spearman's Rho	Species name	Arsenic in body	Cadmium in body	Lead in body	Mercury in body
Arsenic in shell	*N. lineata*	0.357			
	C. quadrata	0.400			
	C. aurisfelis	–0.700			
	C. capucinus	1.000**			
	T. telescopium	–0.600			
	E. aurisjudae	–0.500			
Cadmium in shell	*N. lineata*		0.633		
	C. quadrata		0.738		
	C. aurisfelis		0.700		
	C. capucinus		0.800		
	T. telescopium		–0.137		
	E. aurisjudae		1.000**		
Lead in shell	*N. lineata*			0.000	
	C. quadrata			–0.775	
	C. aurisfelis			0.600	
	C. capucinus			–0.400	
	T. telescopium			–0.464	
	E. aurisjudae			–0.500	
Mercury in shell	*N. lineata*				0.781*
	C. quadrata				0.943
	C. aurisfelis				0.363
	C. capucinus				–0.333
	T. telescopium				0.319
	E. aurisjudae				–0.866

** Major correlation is seen at the 0.01 level (two-tailed).
* Major correlation is seen at the 0.05 level (two-tailed).

4.3 Comparison of Concentration of Heavy Metals between the Gastropod and the Sediment in the Study Sites

Figure 4.3 illustrates the mean concentration of the total heavy metal in the gastropod (combined value of body and shell) and in the sediment. The results obtained for the gastropod excluded the species factor since the values were not normally distributed as certain species were lesser in number or not present in one or two locations (e.g., *E.aurisjudae*). Overall, there are significant and higher heavy metals in the sediment than in the gastropod ($p < 0.05$). Table 4.5 indicates that there are substantial differences in the heavy metal mean scores, which showed that themeasures of the heavy metal score change were different based on the location of the gastropod and sediment samples. For the three different locations (Telok Gong, Carey Island and Klang Island), differences in concentration were found in the measures of heavy metals for the gastropod and sediment samples. Telok Gong has a higher lead concentration (17.750 µg/g), followed by arsenic (12.588 µg/g) in the sediment. Conversely, the metal concentration indicates that arsenic and lead are lesser in the gastropod thanin the sediment. The other two heavy metals (cadmium and mercury) have minimum concentrations in both gastropod and sediment with a< 0.4 µg/g mean concentration in all locations. Carey Island showed a higher Pb (11.126 µg/g) concentration, followed by arsenic (8.218 µg/g) in the sediment. However, in the gastropod, the metal concentration indicates that the concentration of arsenic (0.658 µg/g) is higher than cadmium (0.276 µg/g). The heavy metals lead and mercury in the gastropod as well as cadmium and mercury in the sediment have a minimum concentration of < 0.2 µg/g.

The third location, Klang Island, showed a higher lead concentration (17.066 µg/g), followed by arsenic (6.640 µg/g) in the sediment. In the gastropod, lead also has a higher concentration (0.768 µg/g) than arsenic (0.624 µg/g). The other two heavy metals (cadmium and mercury) have a minimum concentration of < 0.04 µg/g in both the sediment and gastropod. The sequence for heavy metal concentration in sediment for Telok Gong, Carey Island and Klang Island is Pb>As>Cd>Hg. The sequences of heavy metal concentration in the gastropod samples are significantly different for each site whereby ($p < 0.001$): in Telok Gong, the sequence is As > Pb > Cd > Hg, in Carey Island, As > Cd > Pb > Hg, and in Klang Island, Pb > As > Cd > Hg. There is a difference in the sequence of metal in the different locations due to their surrounding land-use as supported by Bayen et al. (2004) who showed high levels of Pb near the shipping yards and lanes than other locations. Figure 4.3 shows the high accumulation of Pb and As in the sediment than the other two metals.

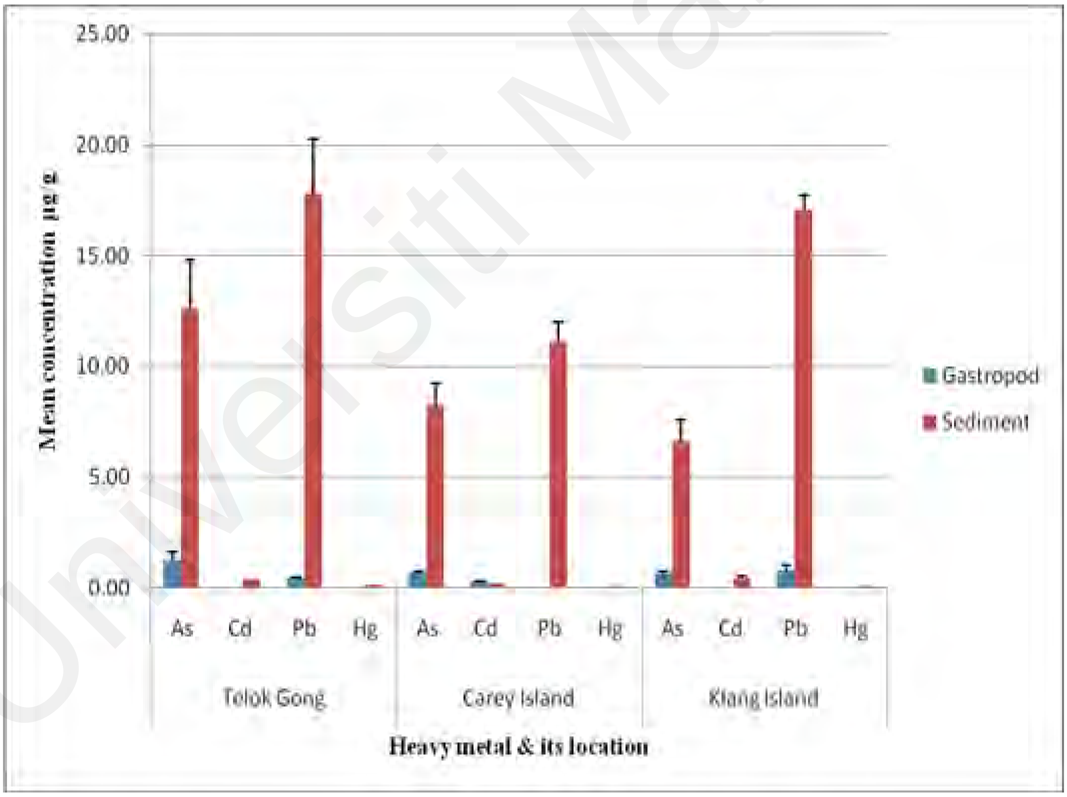


Figure 4.3: Comparison of heavy metal concentration in gastropod and sediment among sampling locations

Table 4.5: Comparative analysis between the gastropod and sediment samples among various locations

Sample	Location	Measures	Arsenic	Cadmium	Lead	Mercury
GASTROPOD(N=24)	TELOK GONG	Mean	1.24	0.03	0.39	0.01
		Std. Deviation	1.05	0.01	0.17	0.021
		Minimum	0.55	0.02	0.22	0.00
		Maximum	3.52	0.05	0.65	0.06
	CAREY ISLAND	Mean	0.65	0.27	0.03	.013
		Std. Deviation	0.28	0.07	0.037	0.00
		Minimum	0.37	0.17	0.01	0.01
		Maximum	1.12	0.35	0.13	0.02
	KLANG ISLAND	Mean	0.62	0.01	0.76	0.01
		Std. Deviation	0.39	0.00	0.73	0.03
		Minimum	0.28	0.01	0.14	0.00
		Maximum	1.53	0.02	1.98	0.10
SEDIMENT(N=12)	TELOK GONG	Mean	12.58	0.37	17.75	0.09
		Std. Deviation	4.44	0.02	5.00	.014
		Minimum	5.92	0.36	10.31	0.09
		Maximum	15.00	0.40	21.20	0.12
	CAREY ISLAND	Mean	8.21	0.17	11.12	0.06
		Std. Deviation	2.01	0.06	1.74	0.02
		Minimum	5.89	0.10	9.85	0.05
		Maximum	10.58	0.24	13.67	0.09
	KLANG ISLAND	Mean	6.64	0.41	17.06	0.07
		Std. Deviation	1.87	0.21	1.31	0.02
		Minimum	4.42	0.18	15.60	0.05
		Maximum	8.45	0.70	18.31	0.10

There is a difference in the sequence of metal concentration between the sediment and gastropod. This may be due to the gastropods' metabolism activities such as food intake, individual variations, salinity, temperature, spawning, and serial factors (Hamed and Emara, 2006). According to the mean value, the sediment has a higher concentration for all the heavy metalscompared tothe gastropod. According to Hamed and Emara (2006), it can be deduced that the sediments function as a reservoir for all pollutants in the ecosystem and for dead organic matter.

A study by Hossen et al. (2015) on clam species from the diverse coasts in Selangor discoveredthat the cadmium and lead mean values are 1.75–4.43 µg/g and 1.61–8.21 µg/g respectively. However, this study had lower values for cadmium and lead in the gastropod ranging from 0.01–0.27 µg/g to 0.03–0.76 µg/g respectively. Meanwhile, a study by Hadibarata et al. (2012) on mussels showed lower values for Cd (0.00026 µg/g) and Pb (0.00689 µg/g).

Nevertheless, only arsenic and lead have significant differences in concentration between the gastropod and the sediment. This gives the assumption that the heavy metals could be easily absorbed in the sediment than in the gastropod. The cadmium and mercury mean concentration difference between the gastropod and the sediment is minimal, showing that the absorption of cadmium and mercury is almost similar for both gastropod and sediment. The level of metal concentrations between the gastropod and the sediment varies. This may be due to the metal affinity absorbed in the sediment and gastropod. Generally, the concentration in the gastropod can be used to decide whether the animal is affected, or if the food chain will be affected. As for the concentration in sediment, it can be used to assess whether the environment is polluted or not. In a study, the concentration of metals slightly relied on different clam species, but mostly, they depended on the site locations. Thus, although the species play a role according to the location, only it's surrounding provide more accurate results (Hossen et al., 2015). The mean concentration difference showed that gastropods have some ability to maintain the metals accumulated in them. Generally, this result relates to thesecond objective as it shows the differences for the metals in each location, gastropod and sediment, especially for arsenic and lead. In terms of sediment, Telok Gong showed higher concentrations of As and Pb compared to other locations, whereas in terms of gastropod, Pb is shown to be higher in Klang Island. The high accumulation of Pb in the gastropod in Klang Island may be due to the physiochemical factors like salinity and the temperature of sediment in that location. This was proven by Denton and Burdon-Jones (1981) where at 36% and 20% salinity, the lead uptake was marginally increased, and the accumulationby all tissues was significantly greater at a higher temperature (30 °C) for black lip oyster; *Saccostrea echinata.*

The statistical test with the use of Mann-Whitney U test (Table 4.6) showed similar results as in Figure 4.12. Table 4.23 shows a comparative analysis of gastropod and sediment samples for different materials. The sample size N for gastropod was 24 and an average of 4 to 5 individual species contributed to the sample size for all the analysis. The sediment sample size was N=12. The p-value of the gastropod and sediment analysis is < 0.001 and the mean difference of heavy metals for the gastropod and sediment is high, whereby the sediment has a higher value than the gastropod.

Table 4.6: Comparative statistical analysis of gastropod and sediment samples

SAMPLE		**N**	**MEAN**	**STD. DEVIATION**	**Z***	**P-VALUE**
Arsenic	Gastropod	24	0.84	0.70	–4.83	< 0.001
	Sediment	12	9.14	3.78		
Cadmium	Gastropod	24	0.10	0.12	–3.72	< 0.001
	Sediment	12	0.32	0.16		
Lead	Gastropod	24	0.40	0.51	–4.83	< 0.001
	Sediment	12	15.31	4.21		
Mercury	Gastropod	24	0.01	0.02	–4.44	< 0.001
	Sediment	12	0.08	0.02		

*MANN-WHITNEY U TEST

Table 4.7 displays the value of heavy metals in the sediments in this study. Since there is no specific sediment quality guideline provided by the Malaysian authority, the comparison of sediment quality was done using international quality guidelines. However, there have been studies conducted in the Klang Island which are also included in Table 4.7. In comparison with the sediment quality guidelines, Table 4.7 displays the concentrations of lead (Pb) in the three locations, which were found to be in the non-polluted range, as denoted by the EPA Sediment quality, Contaminated Sediment Standing Team (2003) (Consensus-Based Sediment Quality Guidelines (CBSOG SQG), New York Sediment Criteria (New York State Department of Environmental Conservation Division of Fish, Wildlife and Marine Resources, 1993), and Sediment Quality Criteria Guideline (1992) (as cited in Sany et al., 2011). The present study conducted at the three locations showed comparatively low values than the studies by Sany et al. (2011), Sany et al. (2013) and Yap (2005).

According to the NYSC (1993), EPA SQ, and SQG (1992), cadmium is in the low effect range. However, the CBSOG SQG (2003) indicated that all three locations are moderately polluted by cadmium. This may be due to the fact that Carey Island is a palm oil agricultural area. The use of fertilizers and pesticides containing phosphate, where some sources of cadmium are present as well as rainwater runoff from the estate soil might have spread to other areas in that location. Telok Gong also showed this effect because it is an industrial area along a fishing village, though formerly, it was a traditional agriculture locale. According to Haris and Aris (2012), a concentrated value of mercury was found in the sediment of Lumut Strait along the mixing area of the Langat River, where the concentration was higher along the shores of stations PK 1, PK 2 and PK 28. Haris and Aris (2012) results is used as a comparison in this study to show the relation between the results as well as to determine the cause of the metal. Telok Gong, which is alongside the Lumut Straits, confirms the sources of heavy metals since it is an industrial area. The Langat River, which runs alongside the agricultural area of Carey Island, also leads towards the metal concentration, since effluents and wastewater in the river may have contributed to the mercury concentration in the water that was subsequently transported to the estuary. Haris and Aris (2012) also proved that boats and ferries at the Klang River contributed to the deposition of mercury at PK 7, resulting in a higher concentration in its surface sediment. This suggests the effects of the heavy metals that were tested in this study at the selected locations, which coincides with Haris and Aris (2012).

These locations are highly interconnected because they belong to the Klang Islands Mangrove Forest Reserve (KIMFR). A study by Sany et al. (2011, 2013) on sediments showed that the present cadmium level is below its reported values; though Carey Islandshowed similar levels of cadmium as reported by Yap (2005). According to the CBSOG SQG (2003), the arsenic levels in Carey Island and Klang Island are indicative of these areas being non-polluted where as Telok Gong is moderately polluted. Nevertheless, based on the SQG (1992) (as cited in Sany et al., 2011) and NYSC (1993), this research indicates that the value is between the range of low and severe effects for all three locations. Therefore, it is assumed that the locations are moderately polluted. Moderate pollution at Telok Gong is highly likely due to it being an industrial locality (medium and heavy industries) that is close and accessible to the West ports. This area was previously known for its large expanses of tapioca, coconut, cocoa, and oil palm plantations, but was replaced with high and wide steel structure warehouses (Telok Gong, 2018). Many medium and heavy industries like logistics, electronics production factories, power plant, ship building, and repair companies as well as the high number of heavy vehicle transports release gas emissions. The levels of arsenic in all three locations are comparatively low, in accordance with Sany et al. (2011, 2013) and Yap (2005).

Mercury values as compared to the guidelines, showed that all three locations are non-polluted and in the low effects range, as per the SQG (1992), CBSOG SQG (2003) and NYSC (1993). Comparing this to Sany et al. (2011), the mercury value is low; however, only a difference of ± 0.2 μg/g can be seen between the values in the present study and from Sany et al. (2011). The values of lead and cadmium from the research on Larut River and Sangga Besar River (Rahimah, 2012) showed a concentration that is two times higher, with lead having a much higher concentration than cadmium.

Commonly, the quality guidelines stipulated that the sediment showing heavy metals in these three mangrove sites are relatively low for Cd, Pb and Hg. For As, the CBSOG SQG (2003) showed that Carey Island and Klang Island are non-polluted, while Telok Gong is moderately polluted. Telok Gong risks an increasing level of pollution in the future if the exposure to metal is prolonged since it is a developing industrial site. This might have moderate effects on the organisms present in the area. The sediments seem to have higher mean concentration for As and Pb compared to that in the gastropods. The values of As and Cd in this study are similar to that in Yap (2005).

Table 4.7: Comparison of average heavy metal concentration in sediments obtained fromthe research analysis (with sediment quality guidelines)

Elements Sediment Quality Standard/Previous study	**Pb** µg/g			**Cd** µg/g			**As** µg/g			**Hg** µg/g		
Present study	**Telok Gong**	**Carey Island**	**Klang Island**	**Telok Gong**	**Carey Island**	**Klang Island**	**Telok Gong**	**Carey Island**	**Klang Island**	**Telok Gong**	**Carey Island**	**Klang Island**
	17.750 ± 5.007	11.126 ± 1.749	17.066 ± 1.313	0.378 ± 0.021	0.175 ± 0.066	0.420 ± 0.132	12.588 ± 4.447	8.218 ± 2.010	6.640 ± 1.766	0.098 ± 0.015	0.063 ± 0.021	0.080 ± 0.022
EPA (Environmental Protection Agency) Sediment Quality proposed												
Non-Polluted	< 40			N/A			N/A			N/A		
Slightly polluted	40–60											
Severely polluted	> 60			> 6			N/A			N/A		
CBSOG SQG*(2003)												
Non-Polluted	< 40			< 0.99			< 9.8			< 0.18		
Moderately Polluted	40–70			0.99–3			9.8–21.4			0.18–0.64		
Heavily Polluted	> 70			> 3			> 21.4			> 0.64		
New York Sediment Criteria												
Lowest effects range	32			0.6			6			0.15		

Table. 4.7, continued

Elements Sediment Quality Standard/Previous study	**Pb** µg/g			**Cd** µg/g			**As** µg/g			**Hg** µg/g		
Present study	**Telok Gong**	**Carey Island**	**Klang Island**	**Telok Gong**	**Carey Island**	**Klang Island**	**Telok Gong**	**Carey Island**	**Klang Island**	**Telok Gong**	**Carey Island**	**Klang Island**
	17.750 ± 5.007	11.126 ± 1.749	17.066 ± 1.313	0.378 ± 0.021	0.175 ± 0.066	0.420 ± 0.132	12.588 ± 4.447	8.218 ± 2.010	6.640 ± 1.766	0.098 ± 0.015	0.063 ± 0.021	0.080 ± 0.022
Severe effects range	110			9			33			1.3		
Sediment Quality Criteria Guideline (1992)**												
Low effects range (ISQG-low)	31			0.6			6			0.2		
High effects range (ISQG-high)	250			10			33			2		
Heavy metal status in the West Port of Malaysia, Sany et al. (2011)	51.74			1.72			59.62			0.299		
Heavy metals background value in Klang Strait (Yap, 2005)	39.8			0.18			18.79			N/A		

Table. 4.7, continued

Elements / Sediment Quality Standard/Previous study	**Pb** µg/g			**Cd** µg/g			**As** µg/g			**Hg** µg/g		
Present study	**Telok Gong**	**Carey Island**	**Klang Island**	**Telok Gong**	**Carey Island**	**Klang Island**	**Telok Gong**	**Carey Island**	**Klang Island**	**Telok Gong**	**Carey Island**	**Klang Island**
	17.750 ± 5.007	11.126 ± 1.749	17.066 ± 1.313	0.378 ± 0.021	0.175 ± 0.066	0.420 ± 0.132	12.588 ± 4.447	8.218 ± 2.010	6.640 ± 1.766	0.098 ± 0.015	0.063 ± 0.021	0.080 ± 0.022
The average concentration of heavy metal in Klang Strait, Sany et al. (2013)	59.45			0.82			60.36			N/A		
Larut River: Rahimah (2012)	48.9 ± 3.20			2.48 ± 0.42			N/A			N/A		
Sangga Besar River Rahimah (2012)	34.92 ± 3.40			1.15 ± 0.17			N/A			N/A		

* Consensus-Based Sediment Quality Guidelines (CBSOG) SQG (2003)
** Interim Sediment quality criteria guideline (ISQG) (1992)
Source: Sany et al. (2011)

4.4 Heavy Metal Concentrations among Gastropod Species

The species selected are irrespective of their locations since the data was not normally distributed as certain species was not available or only available in smaller quantities compared to those in adjacent selected locations (e.g., *E. aurisjudae* was not available in the Klang Island) (Table 4.1). The analysis was done for the species and heavy metals. This section also depicts three sub-sections showing which species contains high concentration of heavy metals using the individual results of body and shell values. The third sub-section depicts which species contains the highest concentration using the combined results of the body and shell values.

The post-hoc test results found that the body values (Table 4.11–4.14) showed significant difference $p < 0.05$ for each metal corresponding only with specific species. Considering that the body values of the heavy metal arsenic showed a significant difference in mean of the *C. capucinus* with *N. lineata* and *C. aurisfelis* (Table 4.11) and relatively displayed in Figure 4.4, this showed that the *C. capucinus* had a higher metal concentration. For other metals like cadmium, lead, and mercury, this showed similar significant mean differences and high heavy metal concentration corresponding only with the specific species. For cadmium, *E. aurisjudae* corresponds with *C. aurisfelis* and *T. telescopium*, for lead, *C. aurisfelis* corresponds with *N. lineata, C. quadrata* and *C. capucinus*, and mercury, *T. telescopium* corresponds with *N. lineata*, *C. quadrata, C. aurisfelis, C. capucinus*, and *E. aurisjudae*. Cadmium showed a high concentration for *E. aurisjudae* (Table 4.12, Figure 4.5), lead showed for *C. aurisfelis* (Table 4.13, Figure 4.6), and mercury showed for *T. telescopium* (Table 4.14, Figure 4.7).

According to the shell values in Tables 4.15 and 4.17, there is a significant difference ($p < 0.05$) of the post-hoc test results only for metal arsenic and lead. This significance is shown only for specific species. According to the shell values (Figures 4.8–4.11) the heavy metal *C. capucinus* had high metal concentration of arsenic; for cadmium and mercury, the species *E. aurisjudae* showed a high concentration; for lead, *C. aurisfelis* showed a high concentration. This result can help to determine which species and its body part (either shell or soft body) could be used for heavy metal determination of specific metal in future studies.

As a summary, Table 4.8 shows which species contains high concentration of each metal using separate result from body and shell values. All metals, except mercury, indicated that only specific species possess a high concentration of specific metal only. The results referring to both body and shell values showed the same species having high concentration of the specific metal (e.g., arsenic showed for *C. capucinus* according to both body and shell value). However, the heavy metal mercury showed that two different species contain high concentration based on the results from the body and shell values. This is because there could be a variation in the affinity of this metal towards different parts of the gastropod which is supported by Berandah et al. (2010). Each different parts of the gastropod may play a different function; it may be metabolic or physiological roles. This may influence the distribution of metals in the different parts of the organism. There is no prior evidence for the result found for specific species that shows high accumulation of metal for the specific metal in this study. However, there are prior evidences seen for the same species with different metal such as *C. capucinus*, which showed an accumulation of lead in the shell and cadmium in its caecum (Berandah et al., 2010). *T. telescopium* had a high amount of lead in its body according to Ezraneti et al. (2017), and mercury in the shell according to Male et al. (2014). Elevated level of Hg was seen in *C. aurisfelis* (Wolswijk et al., 2020). In the future, if the same test was conducted in different locations with the same type of species and the same metals as in this study, a more detailed observation could be attained on the use of specific species for specific metals accumulating with high concentration.

Table 4.8: Species containing high concentration of metal according to body and shell value

Heavy Metal	**High concentration in species according to body value**	**High concentration in species according to shell value**
Arsenic	*C. capucinus* (1.617 µg/g)	*C. capucinus* (0.129 µg/g)
Cadmium	*E. aurisjudae* (0.147 µg/g)	*E. aurisjudae* (0.206 µg/g)
Lead	*C. aurisfelis* (0.420 µg/g)	*C. aurisfelis* (0.715 µg/g)
Mercury	*T. telescopium* (0.080 µg/g)	*E. aurisjudae* (0.010 µg/g)

4.4.1 Heavy Metal Concentration in the Gastropod

The two tables show the p-value acquired from the GLM. Table 4.9 shows the result according to body values while Table 4.13 shows according to shell values.

Table 4.9 indicates that there are significant differences in the heavy metal score ($p < 0.05$) among the species, which showed that the change in mean values of the heavy metal were different among the different species according to the body value.

Table 4.9: Analysis of generalized linear model for heavy metals in species with respect to the body values

ELEMENT	WALD CHI-SQUARE	P-VALUE
As	17.611	0.003
Cd	14.076	0.015
Pb	36.023	< 0.001
Hg	245.042	< 0.001

Table 4.10 indicates that there are major distinctions in the heavy metal score ($p < 0.05$) only for As and Pb among the species, which showed that the changes in mean values of the heavy metal were dissimilar among the different species in the gastropod with respect to shell value.

Table 4.10: Analysis of generalized linear model for heavy metals in species respect to the shell values

ELEMENT	WALD CHI-SQUARE	P-VALUE
As	11.110	0.049
Cd	9.540	0.089
Pb	24.424	< 0.001
Hg	2.496	0.777

4.4.1.1 Mean Concentration of Arsenic in Gastropod Body

The Bonferroni post-hoc test was done to determine whether the specific means were different. Figure 4.4 shows the mean concentrations of arsenic in the selected six species. *C. capucinus* has a higher concentration of arsenic (1.61 µg/g). Table 4.11 indicates that arsenic concentration was significantly different between *N. lineata* and *C. capucinus*, with the former being 1.18 µg/g lower than the latter. A major distinction was also seen between *C. aurisfelis* and *C. capucinus*. *C.aurisfelis* was lower 1.03 µg/g than *C. capucinus* whereas no major distinction observed between other species (p> 0.05).

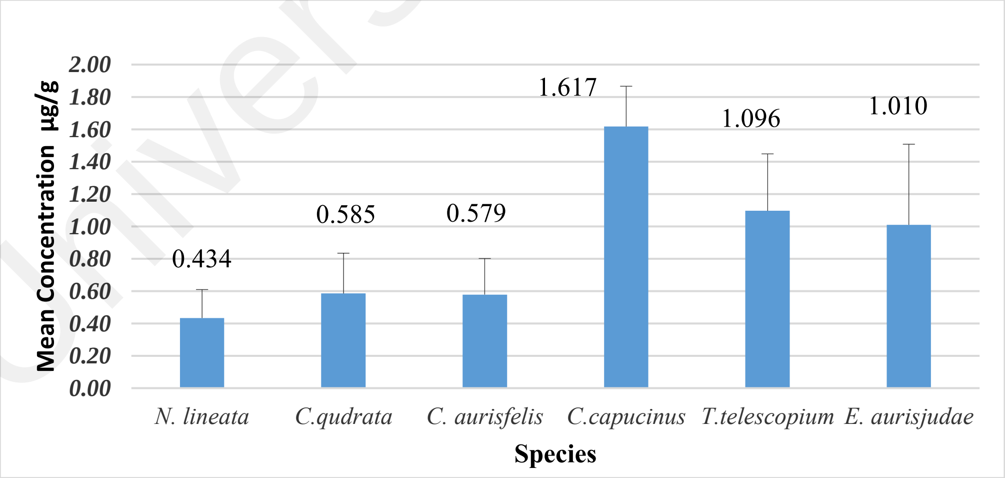


Figure 4.4: Arsenic in gastropod body

Table 4.11: Arsenic in gastropod body

(I) Species	(J) Species	Mean Difference (I-J)	SE	P-Value	95% Wald CI for Difference	
					Lower	Upper
N. lineata	*C. quadrata*	–0.15	0.30	1.00	–1.04	0.74
N. lineata	*C. aurisfelis*	–0.14	0.28	1.00	–0.97	0.68
N. lineata	*C. capucinus*	–1.18	0.30	0.00 [a]	–2.07	–0.28
N. lineata	*T. telescopium*	–0.66	0.39	1.00	–1.81	0.49
N. lineata	*E. aurisjudae*	–0.57	0.52	1.00	–2.12	0.97
C. quadrata	*C. aurisfelis*	0.00	0.33	1.00	–0.97	0.98
C. quadrata	*C. capucinus*	–1.0	0.35	0.05	–2.06	0.00
C. quadrata	*T. telescopium*	–0.51	0.43	1.00	–1.77	0.75
C. quadrata	*E. aurisjudae*	–0.42	0.55	1.00	–2.05	1.20
C. aurisfelis	*C. capucinus*	–1.03	0.33	0.02 [a]	–2.01	–0.05
C. aurisfelis	*T. telescopium*	–0.51	0.41	1.00	–1.74	0.70
C. aurisfelis	*E. aurisjudae*	–0.43	0.54	1.00	–2.03	1.17
C. capucinus	*T. telescopium*	0.52	0.43	1.00	–0.74	1.78
C. capucinus	*E. aurisjudae*	0.60	0.55	1.00	–1.02	2.24
T. telescopium	*E. aurisjudae*	0.08	0.61	1.00	–1.70	1.87

a. Significance at $p < 0.05$

4.4.1.2 Mean Concentration of Cadmium in Gastropod Body

The Bonferroni post-hoc test was also employed toestablish the reason for thedissimilaritiesof the specific means. Figure 4.5 displays the mean concentrations of cadmium in the selected six species. *E.aurisjudae* has the highest concentration of cadmium (0.147 µg/g). Table 4.12 shows that cadmium concentration was significantly different for *C. aurisfelis* with *E. aurisjudae* showing a mean difference of – 0.013 µg/g, this depicts that *E. aurisjuda e*has a higher concentration. There was also a huge distinction between *T. telescopium* and *E. aurisjudae*. The mean difference between both these species indicates a negative of 0.14 µg/g. This shows that *E. aurisjudae* has a higher concentration than other species. A small distinction was seen among other species ($p > 0.05$).

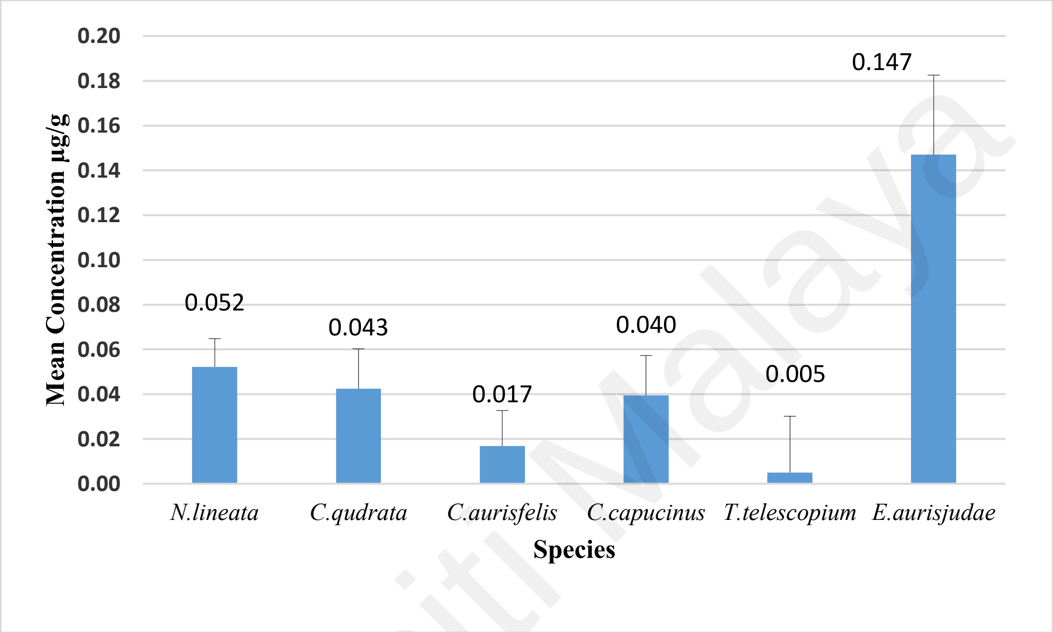


Figure 4.5: Cadmium in gastropod body

Table 4.12: Cadmium in gastropod body

(I) Species	(J) Species	Mean Difference (I-J)	SE	P-Value	95% Wald CI for Difference	
					Lower	Upper
N. lineata	*C. quadrata*	0.01	0.02	1.00	–0.05	0.07
N. lineata	*E. aurisjudae*	0.03	0.02	1.00	–0.02	0.09
N. lineata	*C. capucinus*	0.01	0.02	1.00	–0.05	0.07
N. lineata	*T. telescopium*	0.04	0.02	1.00	–0.03	0.13
N. lineata	*E. aurisjudae*	–0.09	0.03	0.18	–0.20	0.01
C. quadrata	*E. aurisjudae*	0.02	0.02	1.00	–0.04	0.09
C. quadrata	*C. capucinus*	0.00	0.02	1.00	–0.07	0.07
C. quadrata	*T. telescopium*	0.03	0.03	1.00	–0.05	0.12
C. quadrata	*E. aurisjudae*	–0.10	0.04	0.12	–0.22	0.01
C. aurisfelis	*C. capucinus*	–0.02	0.02	1.00	–0.09	0.04

Table 4.12,Continued

C. aurisfelis	*T. telescopium*	0.01	0.03	1.00	–0.07	0.09
C. aurisfelis	*E. aurisjudae*	–0.13	0.03	0.01[a]	–0.24	–0.01
C. capucinus	*T. telescopium*	0.03	0.03	1.00	–0.05	0.12
C. capucinus	*E. aurisjudae*	–0.10	0.04	0.10	–0.22	0.00
T. telescopium	*E. aurisjudae*	–0.14	0.04	0.01[a]	–0.27	–0.01

a. Significance at $p < 0.05$

4.4.1.3 Mean Concentration of Lead in Gastropod Body

The Bonferroni post-hoc test was conducted to ascertain how the specific means were dissimilar. Figure 4.6 exhibits the mean concentrations of lead in the six chosen species. *C.aurisfelis* has a higher concentration of lead (0.42 µg/g). Table 4.13 signifies that lead concentration was very different between *N. lineata* and *C. aurisfelis* whereby the difference in mean was – 0.37 µg/g. *C. aurisfelis* with *C. quadrata* shows a mean difference of – 0.41 µg/g. The negative value shows that the other corresponding species has a lower concentration than *C. aurisfelis*. There was also a significant difference showing *C. aurisfelis* with 0.35 µg/g higher than *C.capucinus*, while no major difference was seen among other species ($p > 0.05$).

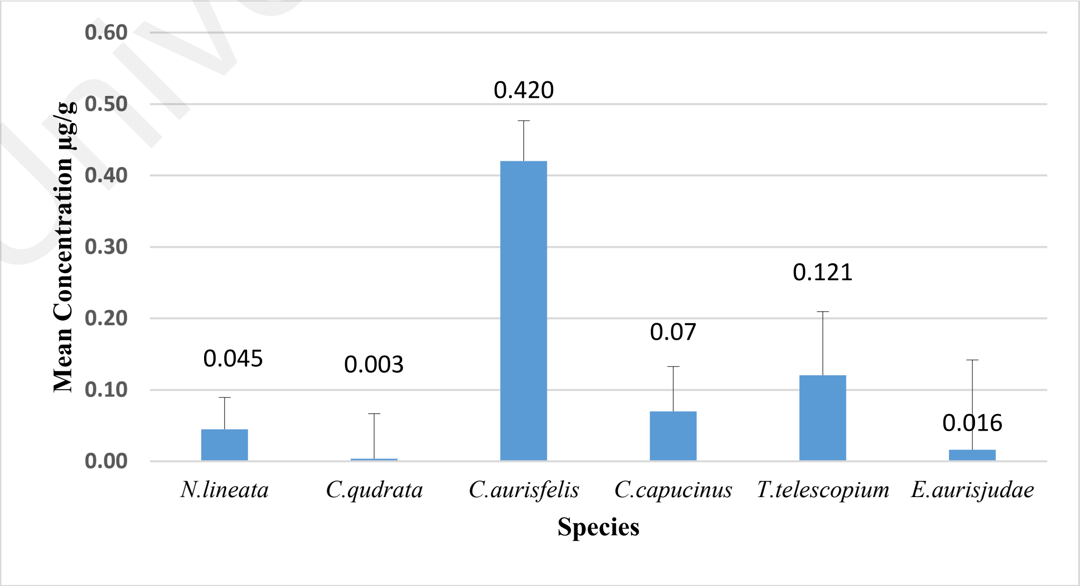


Figure 4.6: Lead in gastropod body

Table 4.13: Lead in gastropod body

(I) Species	(J) Species	Mean Difference (I-J)	SE	P-Value	95% Wald CI for Difference	
					Lower	Upper
N. lineata	*C. quadrata*	0.04	0.07	1.00	–0.18	0.26
N. lineata	*C. aurisfelis*	–0.37	0.07	0.00 [a]	–0.58	–0.16
N. lineata	*C. capucinus*	–0.02	0.07	1.00	–0.25	0.20
N. lineata	*T. telescopium*	–0.07	0.09	1.00	–0.36	0.21
N. lineata	*E. aurisjudae*	0.02	0.13	1.00	–0.36	0.42
C. quadrata	*C. aurisfelis*	–0.41	0.08	0.00 [a]	–0.66	–0.16
C. quadrata	*C. capucinus*	–0.06	0.08	1.00	–0.32	0.19
C. quadrata	*T. telescopium*	–0.11	0.10	1.00	–0.43	0.20
C. quadrata	*E. aurisjudae*	–0.01	0.14	1.00	–0.42	0.40
C. aurisfelis	*C. capucinus*	0.35	0.08	0.00 [a]	0.10	0.59
C. aurisfelis	*T. telescopium*	0.30	0.10	0.06	–0.00	0.60
C. aurisfelis	*E. aurisjudae*	0.40	0.13	0.05	0.00	0.80
C. capucinus	*T. telescopium*	–0.05	0.10	1.00	–0.37	0.26
C. capucinus	*E. aurisjudae*	0.05	0.14	1.00	–0.35	0.46
T. telescopium	*E. aurisjudae*	0.10	0.15	1.00	–0.34	0.55
a. Significance at $p < 0.05$						

4.4.1.4 Mean Concentration of Mercury in Gastropod Body

The post-hoc test was performed to determine the difference between the specific means. Figure 4.7 shows the mean concentrations of mercury in the selected six species. *T. telescopium* has a higher concentration of mercury (0.08 µg/g). Table 4.14 indicates that the mercury concentration was significantly different between *N. lineata* and *T. telescopium*, with a negative mean difference of 0.07 µg/g. Similarly, there was also a significant difference between *C. quadrata* and *T. telescopium*, *C. aurisfelis* and *T. telescopium*, and *C. capucinus* and *T. telescopium*, with a mean difference of –0.07 µg/g, and *T. telescopium* showing a 0.07 µg/g higher than *E. aurisjudae*. Nevertheless, there are no major distinctions observed among other species ($p > 0.05$).

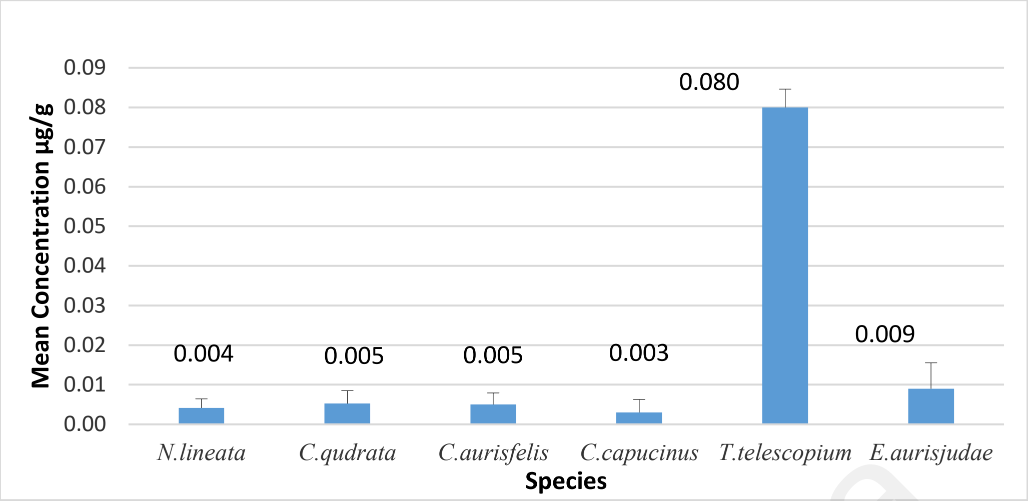


Figure 4.7: Mercury in gastropod body

Table 4.14: Mercury in gastropod body

(I) Species	(J) Species	Mean Difference (I-J)	SE	P-Value	95% Wald CI for Difference	
					Lower	Upper
N. lineata	*C. quadrata*	–0.00	0.00	1.00	–0.01	0.01
N. lineata	*C. aurisfelis*	–0.00	0.00	1.00	–0.01	0.01
N. lineata	*C. capucinus*	0.00	0.00	1.00	–0.01	0.01
N. lineata	*T. telescopium*	–0.07	0.00	0.00 ^{a}	–0.09	–0.06
N. lineata	*E. aurisjudae*	–0.00	0.00	1.00	–0.02	0.01
C. quadrata	*C. aurisfelis*	0.00	0.00	1.00	–0.01	0.01
C. quadrata	*C. capucinus*	0.00	0.00	1.00	–0.01	0.01
C. quadrata	*T. telescopium*	–0.07	0.00	0.00 ^{a}	–0.09	–0.05
C. quadrata	*E. aurisjudae*	–0.00	0.00	1.00	–0.02	0.01
C. aurisfelis	*C. capucinus*	0.00	0.00	1.00	–0.01	0.01
C. aurisfelis	*T. telescopium*	–0.07	0.00	0.00 a	–0.09	–0.05
C. aurisfelis	*E. aurisjudae*	–0.00	0.00	1.00	–0.02	0.01
C. capucinus	*T. telescopium*	–0.07	0.00	0.00 a	–0.09	–0.06
C. capucinus	*E. aurisjudae*	–0.00	0.00	1.00	–0.02	0.01
T. telescopium	*E. aurisjudae*	0.07	0.00	0.00 a	0.04	0.09

a. Significance at $p < 0.05$

4.4.1.5 Mean Concentration of Arsenic in Gastropod Shell

Bonferroni post-hoc test was used to determine the differences in the specific means. Figure 4.8 shows the mean concentrations of arsenic in the selected six species. *C. capucinus* has a higher concentration of arsenic (0.129 µg/g). Table 4.15 indicates that the arsenic concentration was significantly different between only *N. lineata* and *C. capucinus*, with a difference in mean of – 0.06 µg/g lower than the latter. Other than that, there was no major distinction seen among other species ($p > 0.05$).

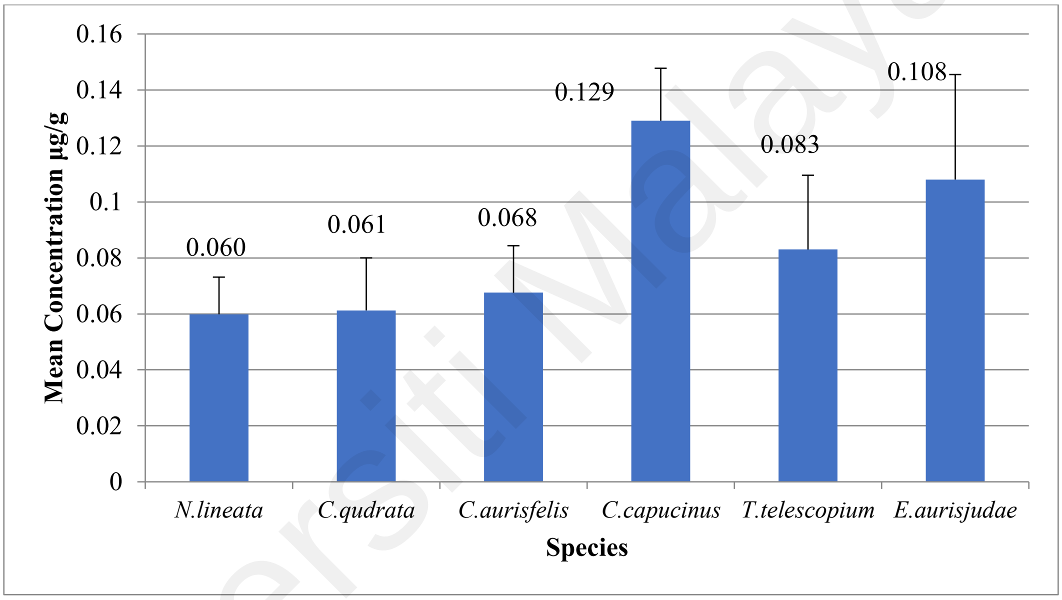


Figure 4.8: Arsenic in gastropod shell

Table 4.15: Arsenic in gastropod shell

(I) Species	(J) Species	Mean Difference (I-J)	SE	P-Value	95% Wald CI for Difference	
					Lower	Upper
N. lineata	*C. quadrata*	–0.00	0.02	1.00	–0.06	0.06
N. lineata	*C. aurisfelis*	–0.00	0.02	1.00	–0.07	0.05
N. lineata	*C. capucinus*	–0.06	0.02	0.03 [a]	–0.13	-0.00
N. lineata	*T. telescopium*	–0.02	0.03	1.00	–0.11	0.06
N. lineata	*E. aurisjudae*	–0.04	0.04	1.00	–0.16	0.06

Table 4.15, Continued

C. quadrata	*C. aurisfelis*	–0.00	0.02	1.00	–0.08	0.06
C. quadrata	*C. capucinus*	–0.06	0.02	0.16	–0.14	0.01
C. quadrata	*T. telescopium*	–0.02	0.03	1.00	–0.11	0.07
C. quadrata	*E. aurisjudae*	–0.04	0.04	1.00	–0.17	0.07
C. aurisfelis	*C. capucinus*	–0.06	0.02	0.22	–0.13	0.01
C. aurisfelis	*T. telescopium*	–0.01	0.03	1.00	–0.10	0.07
C. aurisfelis	*E. aurisjudae*	–0.04	0.04	1.00	–0.16	0.08
C. capucinus	*T. telescopium*	0.04	0.03	1.00	–0.04	0.14
C. capucinus	*E. aurisjudae*	0.02	0.04	1.00	–0.10	0.14
T. telescopium	*E. aurisjudae*	–0.02	0.04	1.00	–0.16	0.11
a. Significance at $p < 0.05$						

4.4.1.6 Mean Concentration of Cadmium in Gastropod Shell

The Bonferroni post-hoc test was done to determine how the specific means were different. Figure 4.9 displays the mean concentrations of cadmium in the selected six species. I it is shown that *E. aurisjudae* has a higher concentration (0.206 µg/g) of cadmium in the shell. Table 4.16 indicates that cadmium concentration has no major distinction among the species ($p > 0.05$).

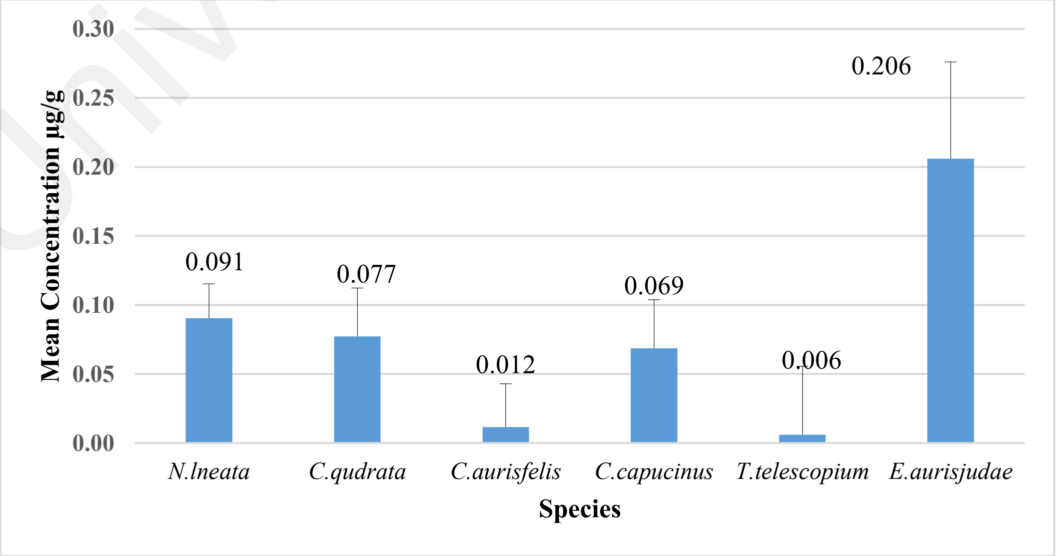


Figure 4.9: Cadmium in gastropod shell

Table 4.16: Cadmium in gastropod shell

(I) Species	(J) Species	Mean Difference (I-J)	SE	P-Value	95% Wald CI for Difference	
					Lower	Upper
N. lineata	*C. quadrata*	0.01	0.04	1.00	–0.11	0.13
N. lineata	*C. aurisfelis*	0.07	0.04	0.72	–0.03	0.19
N. lineata	*C. capucinus*	0.02	0.04	1.00	–0.10	0.14
N. lineata	*T. telescopium*	0.08	0.05	1.00	–0.07	0.24
N. lineata	*E. aurisjudae*	–0.11	0.07	1.00	–0.33	0.10
C. quadrata	*C. aurisfelis*	0.06	0.04	1.00	–0.07	0.20
C. quadrata	*C. capucinus*	0.00	0.05	1.00	–0.13	0.15
C. quadrata	*T. telescopium*	0.07	0.06	1.00	–0.10	0.25
C. quadrata	*E. aurisjudae*	–0.13	0.08	1.00	–0.35	0.10
C. aurisfelis	*C. capucinus*	–0.05	0.04	1.00	–0.19	0.08
C. aurisfelis	*T. telescopium*	0.00	0.05	1.00	–0.16	0.17
C. aurisfelis	*E. aurisjudae*	–0.19	0.07	0.17	–0.42	0.03
C. capucinus	*T. telescopium*	0.06	0.06	1.00	–0.11	0.24
C. capucinus	*E. aurisjudae*	–0.13	0.07	1.00	–0.36	0.09
T. telescopium	*E. aurisjudae*	–0.20	0.08	0.29	–0.45	0.05
a. Significance at $p < 0.05$						

4.4.1.7 Mean Concentration of Lead in Gastropod Shell

The Bonferroni post-hoc test was conducted to establish how the specific means were dissimilar. Figure 4.10 displays the mean concentrations of lead in the selected six species. *C. aurisfelis* has a higher concentration of lead (0.715 µg/g). Table 4.17 indicates that lead concentration was significantly different between *N. lineata* and *C. aurisfelis*, with a mean difference of –0.48 µg/g, and *C. quadrata* and *C. aurisfelis*, with the former being 0.55 µg/g lower than the latter. There was also a significant difference between *C. capucinus* and *C. aurisfelis*. C. *aurisfelis* showed a 0.61 µg/g higher mean valuethan *C. capucinus*. It also showed a mean of 0.65 µg/g higher than *T. telescopium*, while no major distinctions were seen among other species (p> 0.05).

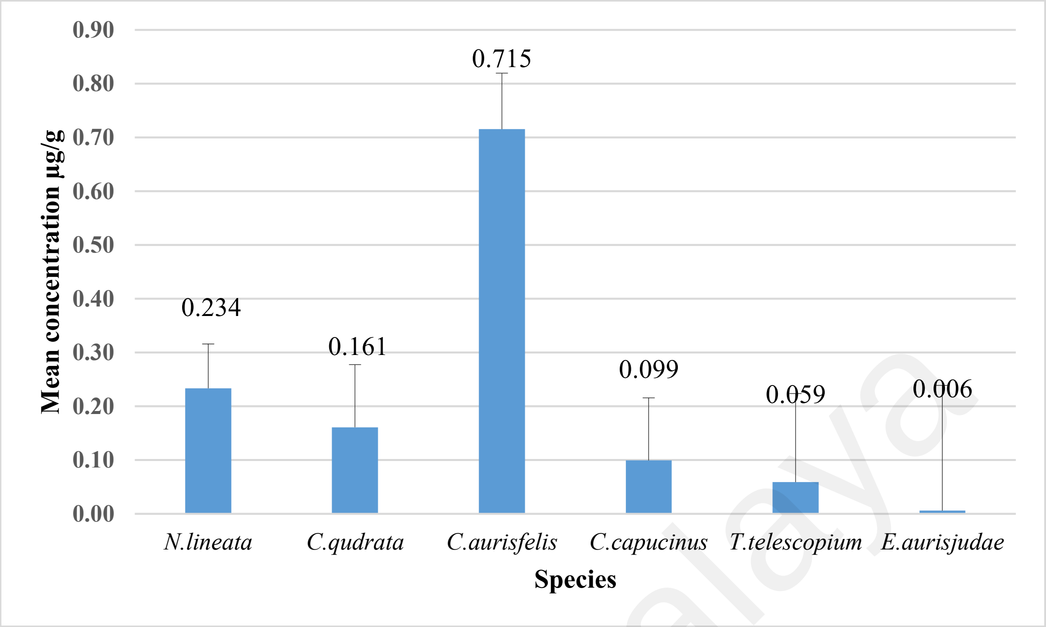


Figure 4.10: Lead in gastropod shell

Table 4.17: Lead in gastropod shell

(I) Species	(J) Species	Mean Difference (I-J)	SE	P-Value	95% Wald CI for Difference	
					Lower	Upper
N. lineata	*C. quadrata*	0.07	0.14	1.00	–0.34	0.49
N. lineata	*C. aurisfelis*	–0.48	0.13	0.00 [a]	–0.87	–0.09
N. lineata	*C. capucinus*	0.13	0.14	1.00	–0.28	0.55
N. lineata	*T. telescopium*	0.17	0.18	1.00	–0.36	0.71
N. lineata	*E. aurisjudae*	0.22	0.24	1.00	–0.49	0.95
C. quadrata	*C. aurisfelis*	–0.55	0.15	0.00 [a]	–1.01	–0.09
C. quadrata	*C. capucinus*	0.06	0.16	1.00	–0.42	0.54
C. quadrata	*T. telescopium*	0.10	0.20	1.00	–0.49	0.69
C. quadrata	*E. aurisjudae*	0.15	0.26	1.00	–0.60	0.91
C. aurisfelis	*C. capucinus*	0.61	0.15	0.00 [a]	0.15	1.07
C. aurisfelis	*T. telescopium*	0.65	0.19	0.01 [a]	0.08	1.22

Table 4.17, Continued

C. aurisfelis	*E. aurisjudae*	0.70	0.25	0.08	–0.03	1.45
C. capucinus	*T. telescopium*	0.04	0.20	1.00	–0.55	0.63
C. capucinus	*E. aurisjudae*	0.09	0.26	1.00	–0.67	0.85
T. telescopium	*E. aurisjudae*	0.05	0.28	1.00	–0.78	0.89
a. Significance at $p < 0.05$						

4.4.1.8 Mean Concentration of Mercury in Gastropod Shell

The Bonferroni post-hoc test was established to determine how the specific means were dissimilar. Figure 4.11 displays the mean concentrations of mercury in the selected six species. *E. aurisjudae* is shown to have a high concentration of mercury in the shell. The mercury concentration indicated in Table 4.18 does not have any major distinctions among the species ($p > 0.05$).

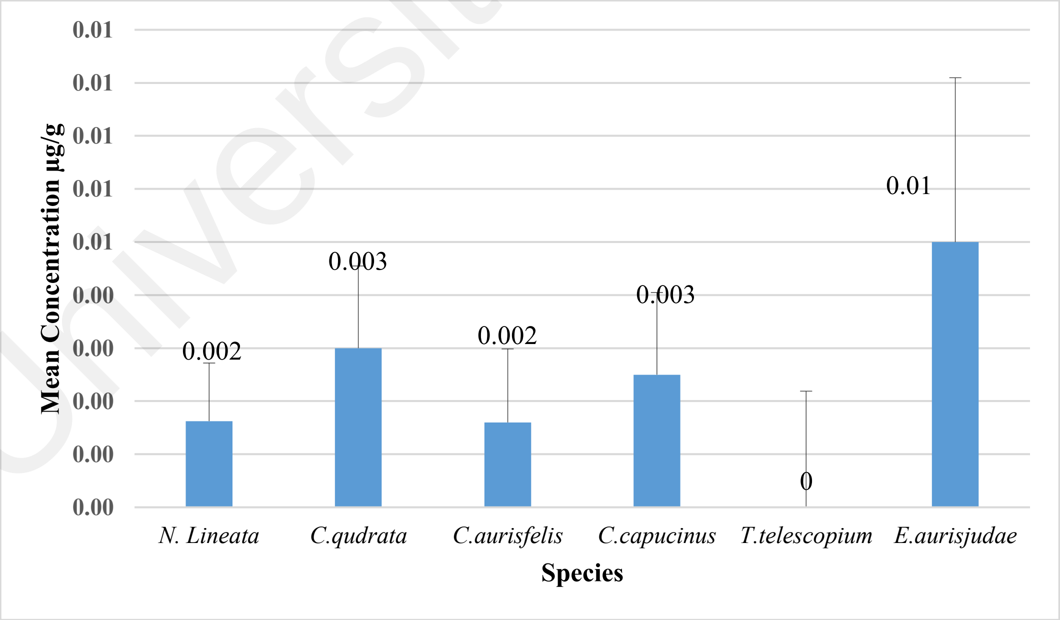


Figure 4.11: Mercury in gastropod shell

Table 4.18: Mercury in gastropod shell

(I) Species	(J) Species	Mean Difference (I-J)	SE	P-Value	95% Wald CI for Difference	
					Lower	Upper
N. lineata	*C. quadrata*	–0.001	0.002	1.00	–0.007	0.004
N. lineata	*C. aurisfelis*	0.000	0.002	1.00	–0.005	0.005
N. lineata	*C. capucinus*	–0.001	0.002	1.00	–0.006	0.005
N. lineata	*T. telescopium*	0.002	0.002	1.00	–0.006	0.009
N. lineata	*E. aurisjudae*	–0.003	0.003	1.00	–0.013	0.006
C. quadrata	*C. aurisfelis*	0.001	0.002	1.00	–0.005	0.007
C. quadrata	*C. capucinus*	0.001	0.002	1.00	–0.006	0.007
C. quadrata	*T. telescopium*	0.003	0.003	1.00	–0.005	0.011
C. quadrata	*E. aurisjudae*	–0.002	0.003	1.00	–0.012	0.008
C. aurisfelis	*C. capucinus*	–0.001	0.002	1.00	–0.007	0.005
C. aurisfelis	*T. telescopium*	0.002	0.003	1.00	–0.006	0.009
C. aurisfelis	*E. aurisjudae*	–0.003	0.003	1.00	–0.013	0.007
C. capucinus	*T. telescopium*	0.003	0.003	1.00	–0.005	0.010
C. capucinus	*E. aurisjudae*	–0.003	0.003	1.00	–0.013	0.008
T.telescopium	*E. aurisjudae*	–0.005	0.004	1.00	–0.016	0.006

a. Significance at $p < 0.05$

4.4.2 General Comparison of Metal Concentration in Species

This section contains a general comparision of the metal concentration in each species. The discussion was made by combining the data of the gastropod body and shell values.

According to Figure 4.12, the actual mean concentration for each species differs. The sequence of metal accumulation for *N. lineata, C. capucinus*, *T. telescopium*, and *C. quadrata*is as follows: As> Pb > Cd > Hg. These species accumulated arsenic more compared to other metals. On the other hand, *C. aurisfelis* tended to have more lead than other metals as per the following sequence: Pb > As > Cd > Hg. *E. aurisjudae* contained more arsenic compared to other metals and the sequence of its metal accumulation is As>

Cd > Pb > Hg. These trendsare significantly different ($p < 0.05$) according to Table 4.9 and 4.10, but there is less significance seen for cadmium and mercury.

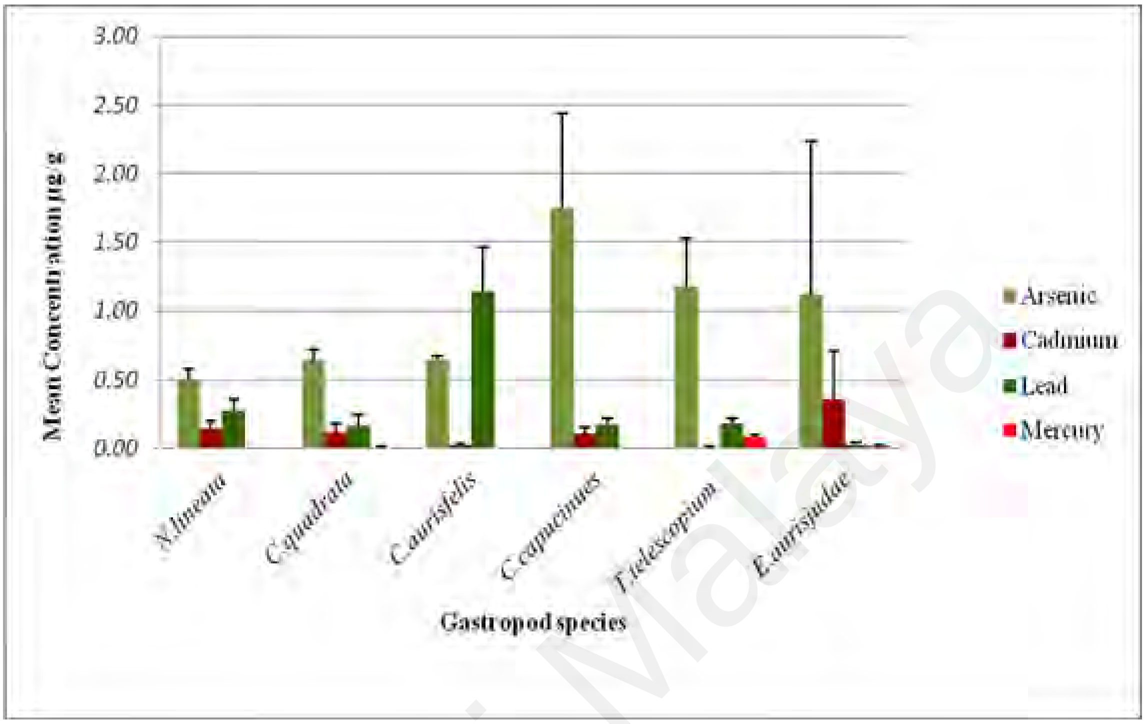


Figure 4.12: Combined body and shell values for mean concentration of heavy metals in each species

When all the species were observed with regards to their mean concentration of their respective metals, there seemed to be a higher level of arsenic concentration in all of the species, except for *C. aurisfelis*, which it accumulated a higher lead concentration. This dictates some variations in the metal affinity and accumulation pattern of lead and arsenic, which differs from species to species. The species might have the ability to remove arsenic from its system through regulation pattern, and in contradiction, it might not be able to remove lead from its system. Each species has its own strength and metabolism, therefore, there could be changes in the metal accumulated in them. The invertebrates may balance the metal accumulated in them through what is termed as ‘regulation’ (i.e., metal uptake and excretion rates processes). It is normal for regulation to occur at the tissue and organ level in various invertebrates. Trace metal accumulators regulate its concentration and store them in one or more specific areas in the tissues such as the muscles (Rainbow, 2002). By comparing the lead concentration, only *C. aurisfelis* has a higher concentration. Therefore, it could be used as a biomonitor for lead in the future as macro concentrators were particularly suggested as suitable biomonitors (or biomonitoring organ/material) Bohac (1999). However, there is still limited research regarding this species as a biomonitor for lead. Nonetheless, Ismail et al. (2003a), Badran (1999), Berandah et al. (2010), Yap and Cheng (2009), and Yap et al. (2009) have studied the use of the *Nerita* species and *T. telescopium* for lead, and *C. capucinus* for cadmium as biomonitors for metals. In this study of arsenic, the species *C. capucinus* accumulated more than other species; this shows that it may be used as a biomonitor since it has the ability to retain more arsenic compared to other species. Again, this too needs further studies. *E. aurisjudae* seemed to attain higher accumulation of cadmium among other species. This clearly shows that this species may have the ability to retain the metal cadmium in its body system. Different species show different retention ability of metal, which is supported by Phillips and Rainbow (1988), where the barnacle results showed a relatively higher cadmium bioavailability at North Point and in Victoria Harbour, whereas *P. viridis* showed the highest cadmium level at Rennies Mill. This indicates that the barnacles and *P. viridis* vary in terms of their net uptake of cadmium, in which the bioavailability of cadmium to barnacles is different from that of green-lipped mussels.

All species seem to show low mean concentration of mercury. However, *T. telescopium* seemed to have a slightly higher concentration than others. This indicates that it might have the ability to be a biomonitor for mercury. Nevertheless, since mercury levels are very low, a conclusion cannot be made. This study shows that each species has its own metal retaining metabolism, and to discover the specific biomonitor for a specific metal, further studies are needed on each of these species by using accumulation and depuration technique. Kanakaraju and Anuar (2009) attempted to determine accumulation factors. *N. lineata* was studied for metal pollution biomonitoring. The experiment was done by collecting and removing lead (Pb) through the exposure of *N. lineata* to Pb concentrations (1–4 ppm) under laboratory-controlled conditions. The Pb exposure time with a steady rise indicatedthat it has a higher retention in the tissues of *N. lineata*; however, depuration brought about a decrease in the concentration over time. This finding suggested that *N. lineata* can withstand high Pb exposure and is deemed appropriate for biomonitoring in aquatic environments.

Lau et al. (1998) suggested the use of accumulative indices to offer indications on the appropriateness of a specific organism to be used as a monitoring agent for a particular metal in an aquatic habitat. The mollusc species demonstrated different preferences for the uptake of different metals. Variations in the heavy metal contents in the shell and tissues of the same species were observed. These indices provide details on the uptake of metals in different parts of the organism, which helps in the selection. Ismail and Safahieh (2005), Ismail et al. (2003a), Badran (1999), Yap and Cheng (2009), Yap et al. (2009), and Berandah et al. (2010) used the following as biomonitors: *Telescopium* species for Cu, Zn and Pb; *Nerita* species for Mg, Sr, Mn, Fe, Zn, and As; and *C. capucinus* for Cd, Cu, Fe, Ni, Pb, and Zn. The distribution of Cu, Zn and Pb concentrations in soft tissue, such as the mantle, foot, cephalic tentacle, gill, muscle, digestive caecum, and shell of the mudflat snail (*T. telescopium*) were concluded from eight geographical sites of the south-western intertidal regions of Peninsular Malaysia (Yap et al., 2007).

There are significant and non-significant trace metals, but the body regulation is limited to the essential ones, with only peculiar invertebrates conducting body regulation of trace metal concentration (Rainbow, 2002). This shows that if the metal is non-essential, it can be totally eliminated from the body because the accumulated trace metals in an animal's system can be toxic. As, Cd, Pb, and Hg are non-essential metals as these metals could be a problem for the organism which have an adverse impact on its metabolism. When the heavy metal level in the cells or organs exceeds a critical limit, the organism will experience a number of symptoms and eventually result to its death. Nevertheless, for this research, it was found that the mean concentration of the gastropods analyzed in this study showed minimal concentration; therefore, it might not have much effect. However, extended exposure to these metals, which increases by time, may have an adverse effect on their body. In general, the highest accumulated metals in each species will lead to biomagnifications through the food chain which include fish, higher mammals and humans. These intertidal gastropods are non-commercial gastropods, but they are prey for fish and have influence on the food chain. The accumulation pattern should be considered in future studies for each species along with time intervals to know the bioavailability of metals and metal retention on their body as the uptake rate could be balanced by the excretion rate (Rainbow, 2006). Nevertheless, in some species might have the ability to excrete non-essential metals from their systems. However, if it is an essential metal, gastropods have the ability to retain it. The results showed the presence of non-essential metals in the gastropods. Thus, non-essential metal accumulation and metabolization by gastropods without affecting their system are questionable, keeping in mind that they would have certain threshold limits.

CHAPTER 5: CONCLUSIONS

Based on the results, Telok Gong was more diverse, with nine species found in the location, followed by Carey Island with eight specie, and Klang Island with only five species. Klang Island was found to have more abundance, but with less richness of species compared to Telok Gong and Carey Island; this shows the effect of pollution on the abundance of species in Telok Gong and Carey Island.

The results further indicate that arsenic and mercury were more concentrated in the gastropod bodies than in the shells, while cadmium and lead were more concentrated in the gastropod shells than in the bodies; this may be due to the metal affinity of each heavy metal. According to each metal concentration, *C. capucinus* showed the highest concentration for arsenic, while *E. aurisjudae* showed a higher concentration of cadmium. *C. aurisfelis* showed the highest concentration for lead and *T. telescopium* showed a higher concentration of mercury. However, each species has its own ability to retain metals in their system, thus, firm conclusions cannot be made on the exact use of the specific species for specific metals. Nonetheless, there are some studies on the use of *T. telescopium*, *C. capucinus*, and *Nerita* species as biomonitors. Specific study on chronic and lethal effects foreach of the above species could be conducted and their full capabilities as effective biomonitors can be ascertained.

The results obtained indicated that the sediment contains higher concentrations of arsenic and lead than in the gastropod. The other two heavy metals; cadmium and mercury, have similar concentration for both the gastropod and sediment. The sequence of metal concentration in the sediment based on location is as follows: in Telok Gong, the sequence is Pb>As >Cd>Hg, while in Carey Island, the sequence is Pb>As>Cd> Hg, and in Klang Island, Pb>As>Cd>Hg. The sequence of heavy metal concentrations in gastropods sample is as follows: in Telok Gong, the sequence is As > Pb > Cd >Hg, while in Carey

Island, the sequence is As > Cd > Pb > Hg, followed by Klang Island, Pb > As > Cd > Hg. There is a distinction in the sequence of metals between sediment and gastropod and this may be due to the metabolic activity of the gastropod and the metals' affinity to be incorporated to the gastropod's system, where there could be an elimination of heavy metals through excretion from their system. When comparing all three locations, Telok Gong has a higher As and Pb concentration. The values of Cd, Pb and Hg are relatively low for the three study sites, as per the Sediment Quality Guideline. The values of As showed that Carey Island and Klang Island are not polluted, where as Telok Gong is moderately polluted. These conclusions are made based on the Contaminated Sediment Standing Team (2003). There is a chance for elevated levels of heavy metal in the future in Telok Gong since it is a developing industrial area. This might have a moderate effect on the organisms present in that location. Comparison with previous studies showed that the values from this study are comparatively low. The results showed that the accumulation of As and Pb is in noticeable amounts in all the gastropods, but Cd and Hg was found in lesser amounts.

Based on the background of the study sites, Telok Gong has the highest concentration of all the heavy metals since it is surrounded by industries, which explains the reason for high concentration in the gastropod and sediment. The high concentration of cadmium in the gastropod at Carey Island compared to the other two locations proved that the background of the site, which is an agricultural area that utilizes fertilizers and pesticides containing some amount of cadmium, has an impact on the gastropod. Klang Island displayed a contradiction in the result since it was regarded as a natural habitat, but showed prominent concentration of heavy metals (As, Pb). This indicate that there are some sources of pollutant coming from the surrounding. The result clearly showed that the heavy metals come from nearby industries and shipping ports which might have disturbed the organisms and consequently, the food chain.

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1. **Farhathul, M., & Rozainah, M. Z.** (2016). *Mangrove gastropods as biomonitors for heavy metal contamination***.** Poster presented at 21st Biological Sciences Graduate Congress (BSGC) 2016, 15-17th December 2016, University of Malaya, Kuala Lumpur, Malaysia.