ISSN:2521-0912 (Print)
ISSN: 2521-0513 (Online)

Contents List available at RAZI Publishing

Journal CleanWAS

Journal Homepage: http://www.razipublishing.com/journals/journal-cleanwas/

https://doi.org/10.26480/jcleanwas.01.2017.06.16

Study on accumulation of Fe, Pb, Zn, Ni and Cd in *Nerita lineata* and *Thais bitubercularis* from Tanjung Harapan and Teluk Kemang, Malaysia.

Tengku Hanidza Tengku Ismail[1]* Nur Azyan Fathiah Adnan[1] and Mohd Armi Abu Samah[2]

Department of Environmental Sciences[1] Faculty of Environmental Studies, University Putra Malaysia. 43400 UPM Serdang. Selangor. Malaysia
*thanidza@upm.edu.my

Department of Chemistry[2] Kulliyyah of Science International Islamic University malaysia 25200 Kuantan Pahang marmi@iium.edu.my

ARTICLE DETAILS

Article history:

Received 27 September 2016
Accepted 13 December 2016
Available online 10 January 2017

Keywords:

Solid waste; sustainable management; environmental awareness; 3R's concept; Libya

ABSTRACT

The presence of Cd, Cu, Zn, Ni, Fe, Al, Zn, Mn, Cr, and Sn were attributed to metal industries. Cu contamination was associated with piggery industry. Shipping activities contribute to elevated levels of Pb, Cu and As. Elevated levels of metals in the sediments are attributed to anthropogenic activities. Samples were collected in April 2012 and analysed using inductively coupled plasma mass spectrometry (ICP-MS). Fe is the most abundant metal in the tissue and shell compared to the rest of the metals. The concentrations of heavy metals in the soft tissues of *Nerita lineata* taken from Tanjung Harapan follow this order: Fe > Zn > Ni > Cu > Cd while in *Thais bitubercularis*, the metal concentrations were higher following the order of Fe > Zn > Cu > Ni > Cd. The samples taken from Teluk Kemang were higher and exhibited different trend for both organisms. Results from this study are useful for further exploration of T*hais bitubercularis* as accumulators of Cu, Cd, and Zn. For recommendation, more studies on monitoring the concentration level of heavy metals in marine environment should be done regularly and increase numbers of samples use to biomonitor the heavy metals in marine environment as it is important to have information or data regarding the quality of marine environment in order to control pollution such as water pollution from being contaminated with heavy metals. This is essential as the pollutants emit in the marine environment may affect marine lives as well as human's health

1. INTRODUCTION

The presence of heavy metals along the Straits of Melaka is known to be elevated due activities such as shipping activities, runoffs from rivers and mangrove forests, industrial and domestic discharges which has threatened the marine environment as well as human health. [1] extensively reviewed the status of heavy metal pollution in the Malaysian aquatic environment. The manufacturing sectors have been identified as the main contributor to heavy metal pollution. The presence of Cd, Cu, Zn, Ni, Fe, Al, Zn, Mn, Cr, and Sn were attributed to metal industries. Cu contamination was associated with piggery industry. Shipping activities contribute to elevated levels of Pb, Cu and As. Elevated levels of metals in the sediments are attributed to anthropogenic activities. The river sediments along Juru River (Penang) have elevated levels of Pb, Zn, and Cu which was due to discharge from sewage treatment plants. The levels were five, four, and two times, respectively, higher that the natural value. The Langat River (Negeri Sembilan) was heavily polluted with Zn and Cd. Areas along Tanjong Karang and the Johor Strait, the concentration of Pb and Zn were more than doubled than the global shale value, which was contributed to the usage of unleaded petrol (Pb) and tires (Zn). [2] showed evidence of temporal and spatial distribution of heavy metals (As, Cd, Cr, Cu, Ni, Pb, Hg, and Zn), in water and in sediments of Port Klang. Their findings indicated that the concentrations of As, Cd, Hg, and Pb in sediment and As, Cd, Hg, Pb, Cr, and Zn in water were significantly higher than the background values. The main sources of heavy metal contamination in this area were industrial wastewater and port activities.

Analyses of sediments and marine organisms allow the determination of the concentration of heavy metals in the aquatic environment. Bivalves (such as Perna viridis and Saccostrea sp) and gastropods (Thais sp.) are commonly used as bioindicators for heavy metal (especially for Cd, Cu, Pb, and Zn). One of the characteristics of molluscs is they are able to concentrate toxic substances to a considerable level that exceed the levels that is safe for human consumption. The abundance of Nerita lineata and Thais bitubercularis along the shores of Peninsular Malaysia and the ability to bioaccumulate heavy metals made them a potential biomonitor organisms for marine pollution [3]. Earlier reports by [1] indicated that the levels of heavy metals in the fish, bivalves and shrimp were within the safe limits of the Malaysian Food Act of 1983. The levels of Pb and Cd were elevated in the Thais sp. as a result of transfer metals from the rock oysters (Saccostrea sp.) on which they feed on. In some a parts of the east coast of the Peninsula, the concentration of Cd were higher compared to the west coast areas (1.42 - 28.6 μg/g vs 0.05-1 μg/g, respectively). Since these places are located away from industrial activities, it is possible that Cd is naturally occurring, at a higher level. [4] collected Nerita lineata, from various sites along the west intertidal area of Peninsular Malaysia and analyzed for Cd, Ni, Cu, Pb, and Zn. Their results indicated that this organism is a good biomonitor for Cu, Pb, and Ni. The mean concentrations (mg/g dry weight) were 3.15 (Cd), 5.59 (Cu), 49.78 (Fe), 24.18 (Ni), 48.86 (Pb) and 7.86 (Zn) in the shells and 1.03 (Cd), 2.65 (Cu), 566.63 (Fe), 5.85 (Ni), 92.72 (Pb) and 92.75(Zn) in the soft tissues. In both studies, there was no significant correlation between the concentration of heavy metals in the sediments and the soft tissues of Nerita lineata. Studies on the bioaccumulation of metals are limited. As reported by Shazili et al (2006), the bio-concentration factors (BCF) of 132 –12900 ×103 for Cu, 580–76000 for Pb and 481–17200 for Cr were estimated in rock oysters from Kalong Bay, Kemaman and values of 0.9 × 10-3 - 1.9 × 10-3 for Pb, 8.0 × 10-3 for Cu and 2.6 × 10−3 to 3.3 × 10−3 for Cd from Crassostrea belcherici (oyster) cultured at the Merbuk estuary. Another indicator of bioaccumulation property is the biota–sediment accumulation factors (BSAFs) which is the ratio of biota to sediment contamination concentration. Studies in gastropods showed that BSAF values >2 are classified as macroconcentrators, values 1<BSAF<2 are microconcentrators, and BSAF value <1 are deconcentrators.[5] indicated that the soft tissues of Nerita lineata were microconcentrators of Cu and Zn while the shell and the operculum are classified as macrocentrators for Cd, Ni, and Pb.

1.1 Accumulation of heavy metals in shells

Apart from soft tissues, according to [6], heavy metals may also be able to accumulate in the calcareous of the molluscs shells but the relationship between heavy metals in both part of molluscs which are the soft tissues and the shells are not much known. [7] stated that contaminants can be relocated to the shells as a result from the detoxification mechanism. The shell is able to play a role as toxic waste dump in order to eliminate toxic chemicals from the tissue as well as from the food chain itself [8]. Based on some studies, [9] stated that molluscs shells can be able to be used for biomonitoring materials for heavy metals. Shell as biomonitoring also has advantages over soft tissues as according to [10], metal concentrations in shells show less variability than in the soft tissues and shells is able to give indication about the level of the contamination that is more realistic than the soft tissues. Besides, [8] stated that the rises of the concentrations of environment affect the rises of certain elements in molluscs's shells. Seawater comes in direct contact with the outer layer of the shells unlike the soft tissues that located inside the shells [9].

1.2 Sediment

Sediments can be defined as mixtures of certain components which consist of different species as well as organic debris and between the different phases, and metals can be partitioned among the phases. Sediments are able to capture hydrophobic chemicals pollutants that enter the water bodies [11]. The sediments will eventually release the chemical pollutants such as heavy metals slowly back into the water column. Besides, sediments are also known to be responsible of nutrients and pollutant transportation in aquatic environment [12]. Sediments are also known to be the metal repository and a small parts of materials flow into the coastal waters [13]. There are several factors that affect the accumulation and distribution of heavy metals in sediments of the marine ecosystems such as the composition of mineralogical, texture of the sediment, physical transport, oxidation and reduction state as well as processes of adsorption and desorption. Sediments can be a good indicator for medium and also for long period of time of metal loads as sediments is consider as among the major sinks of trace metals in aquatic environment. Apart from that, sediments also collect as samples as it can be used to determine the main sinks for accumulation of heavy metals as well as sediments are persistent in marine environment. Sediments may also play essential role as potential non-point source of heavy metals that may as well indicate the water quality system. Anthropogenic activities such as urban and industrial activities have been known to be one of the contamination sources of metals that affect the marine environment as well as the coastal ecosystems. Therefore, analysis of sediments is important as it is capable to show the current environmental quality. Apart from that, there are a lot of studies conducted around the world that indicate heavy metals have contaminated the marine sediments. Sediments also have been widely practiced as one of the media for monitoring contamination in marine environment. In addition, sediments is normally used as one of the monitoring tool because it is economically to be used and besides that, sediments is increasingly being implemented in environmental assessment of aquatic systems at the early phases.

1.3 Heavy metals

Anthropogenic activities which include industrial activities become more increasingly near coastal areas thus, contribute to the increase of metal concentrations in marine environment and this has threatened the marine environment as well as human health. If heavy metals are consumed over the limit, may lead to harmful effects. According to [14], a lot of the marine organisms accumulate heavy metals in their soft tissue and body concentrations. Certain metals such as Cd (cadmium), Cu (copper), Zn (zinc), and Pb (lead) have been known to accumulate in the aquatic food chain and are distributed in coastal environment due to natural source and anthropogenic activities [15]. Therefore, in this study, the metals are accumulated readily in the soft tissues of molluscs. Heavy metals that are analysed in this study are copper (Cu), zinc (Zn), cadmium (Cd), lead (Pb), iron (Fe) and nickel (Ni). Basically, there are several processes or pathways of the emission of heavy metals to the environment which include to surface water for example runoff, to the air such as combustion and to the soil [16]. According to [17], heavy metals or trace metals are referring to trace elements that biologically and industrially essential and heavy metals is terms used for elements with an atomic density greater than 6 g/cm^3. Apart from that, metallic chemical element which possess relatively high density and very toxic or poisonous at low concentrations are also can be considered as heavy metals. One of the characteristic of heavy metals is heavy metals do not break down thus they may give several effects to the biosphere for a long period of time, where heavy metals are non-degradable thus, can accumulate in the environment even though they are emitted in smaller quantities. On top of that, several factors such as ability to concentrate along the food chain, persistence and toxicity make heavy metals as among the serious pollutants in the environment.

1.4 Lead (Pb)

Lead is a member of Group 14 (IVA) in the periodic table and lead naturally is a mixture of stable isotopes which are 208Pb (51–53%), 206Pb (23.5 – -27%), 207Pb (20.5 – 23%), and 204Pb (1.35 – 1.5%) (ATSDR, 2007). Lead is consider as non-essential and accumulating metal and is potential to be toxic if ingested in excess. Apart from that, even though in traces, lead is among the non-essential metals that are consider to be toxic. Due to human activities, for over the past three centuries, environmental levels of lead rise more than 1,000-fold and during years of 1950 until 2000, it has been greatest increasing which reflected the rising of leaded gasoline being use worldwide. According to [18], lead is very toxic and anthropogenic is the predominant source of lead to the ocean. Factors such as atmospheric deposition and superficial soil erosion contribute for the lead to reach the aquatic system and the mechanism which is the absorption into the sediments and/or suspended particles regulates lead concentration in aquatic environment. The effect of lead in terms of health is it can give adverse impacts as it can damage the kidneys, nervous system as well as reproductive system especially in children as it is very toxic. Dullness, poor attention span, epigastric, vomiting, convulsions, coma, death and others are usually being faced if chronic lead toxicity in humans occurs. According to [16], lead in human bodies is accumulated in the skeleton and the elimination from the body is slow which is principally via urine.

1.5 Cadmium (Cd)

Higher level of cadmium has been reported in marine phosphates and phosphites as well as near coastal areas and cadmium exist naturally in ocean water with average levels less than 5 to 110 ng/L [19]. Cadmium is known as among the most biotoxic elements and as well as a top pollutant. In addition, cadmium is carcinogenic and might cause damage to body cells and cadmium is non-essential metals. Cadmium is a member from group II B with atomic weight of 112.41 and it is one of the industrial pollutants which affect humans' organs. Human activities such as processes of preparation of alloy and electroplating involve the use of cadmium which can lead to groundwater and soil contamination that results in accumulation of cadmium in marine plankton and vertebrates as well as in plants. Besides, examples of human activities that involve cadmium is also recorded by[16], where this metals are used in PVC products as stabilizers, certain alloys, in rechargeable nickel-cadmium batteries and in phosphate fertilizer. Cadmium that enters the sea are about 50% of it comes from anthropogenic activities which consist of industrial waste as well as fertilizers containing phosphate from the animal source. According to [19], destruction of lung epithelial cells that can lead to tracheobronchitis, and pneumonitis in humans and animals may occur if acute inhalation exposure to cadmium is faced at concentration more than about 5 mg/m^3 and long-term of impairment of lung function may also occur from a single, high-level of cadmium exposure. Another effects of long-term cadmium exposure is it can cause skeletal damage for instead itai-itai disease or also known as ouch-ouch which is the combination of osteomalacia and osteoporosis that happened in Japan in year 1950s [16].

1.6 Nickel (Ni)

Nickel which is the 24th most abundant element possess properties where it is very desirable in combining with other metals in order to form mixtures named alloys which can be used in making metal coin, jewelry and stainless steel [19]. Heavy metals such as nickel or its compounds are used by many types of industries such as mining, chemical and metal-finishing. Ni^{2}+ is used in electroplating, batteries manufacturing, mine, metal finishing and forging and others. Negative effect of nickel to humans health is allergic reaction and if exist in large amount, nickel may cause lung and nasal sinus cancers. Nickel can be also found from automobile repairs work for examples in workshop.

1.7 Copper (Cu)

Copper which is reddish in colour metal can occurs naturally such as in water, sediment as well as rock and in year 2000, about 640,000,000,000 grams of copper were emitted into the environment by industries such as mining industries [19] and it is one of the essential metals to life. Natural abundance of copper that occurs in the earth is approximately 2.5 x 10-4 mg/l in the sea and 60 mg/kg in the earth's crust. Copper is one of the essential trace elements but if it is in high levels, it may cause harm to the human health and copper is known to be toxic to aquatic organisms even though its concentration is very low. Copper can be found in some molluscs as well as in some arthropods as it is a functional part of the respiratory protein haemocyanin but it is potential to be toxic when further accumulation of these essential trace metals in metabolically available form. Copper is widely used worldwide in many types of aspects which comprise in industrial application such as in metal finishing and also in agriculture where copper are used as fungicides and algicides [19]. Besides, copper can be usually being found near industrial activities, landfills as well as waste disposals. Although copper is one of the essential metals and it is important for health, higher doses of expose to copper may cause harm for instead, drinking water containing copper can cause nausea, vomiting and stomach cramps while liver and kidney damage and possibility of death may occur if copper is intentionally uptakes [19].

1.8 Zinc (Zn)

Zinc is blue-white, lustrous metal that is occur naturally in the earth's surface rocks which burns in air with a bluish-green flame and there are approximately fifty five mineralized forms of zinc [19]. Natural background of total zinc concentrations in seawater is 0.002–0.1 µg/litre while in sediments the zinc concentration is up to 100 mg/kg dw and the reason of the increased level are natural occurrence of the metals itself and also anthropogenic activities. Zinc is among the metal in which has most potential impact of entering the environment due to agricultural activity. Zinc which is one of the essential metals apart from copper are among metals that has contribute to the contamination of marine ecosystems. Since zinc is one of the essential metals, thus it is able to be regulated by the mussel when concentration of zinc in the environment is high while in low concentration in the environment, the variability of concentration of zinc in the environment can be partially reflected. Excess of zinc can lead to toxicity and results in certain biochemical effects. Zinc in water comes from both natural processes such as weathering and also from the effects of anthropogenic activities such as according to [19], mine drainage, municipal and industrial effluents and also urban runoff.

1.9 Iron (Fe)

[20] also stated that iron is include as essential metals apart from copper (Cu) and zinc (Zn). Iron (Fe) may have high natural background levels and it can be found anywhere include in plant and man-made products.

2.1 Study area

The study areas in this study consist of two different areas which are Tanjung Harapan in Selangor and Teluk Kemang in Negeri Sembilan. Both study areas are located in Peninsular Malaysia

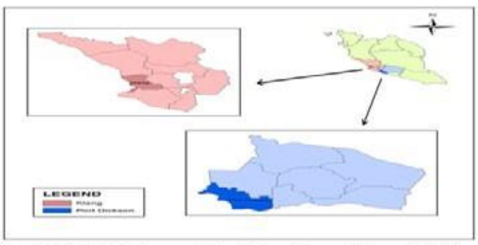

Figure 3.1: District of study areas which are Tanjung Harapan in Klang and Teluk Kemang in Port Dickson.

2.2 Tanjung Harapan

Tanjung Harapan or as known as Esplanade by the local people in years 1990s is located in Selangor, Malaysia which is at Northport of Port Klang where the Port Klang is situated on the west coast of Peninsular Malaysia. Port Klang is the largest port and the busiest port in Malaysia and the Northport which is one of the ports in Port Klang is the largest container port serving Malaysia. Besides, Port Klang is the the 13th busiest transshipment port in 2004 and the 16th busiest container port in 2007 in the world as well as it is the 26th busiest port in by total cargo tonnage handled in year of 2005. As Port Klang lies on the Klang River on the Strait of Malacca so does Tanjung Harapan. According to [21], the Straits of Malacca are an important economic component as well as the trade link as 75% of Malaysia trade passing through this Straits and this increasing of industrial development in various East Asian countries has result in shipping traffic thus, also increase the level of pollution in the water of the Straits of Malacca. Regarding to this matter, it is vital to monitor the accumulation of heavy metals as it is one of the serious pollutant related. Due to the Tanjung Harapan location which is at the Northport of Port Klang, this area is well-known of having a lot of anthropogenic activities which includes, industrial activities such as shipping, factories and warehouses. Besides, Tanjung Harapan also acts as a centre of attraction for tourism like restaurants and fishing ponds. Tanjung Harapan physical appearance can be described as facing the sea, mix substratum and it has rocky barrier along the shore whereas the description of this area is that Tanjung Harapan is a town/city and it is located to a port as well as it is considered as industrial area.

2.2 Teluk Kemang

Teluk Kemang is one of the tourism places in Port Dickson, Negeri Sembilan which is also located in Peninsular Malaysia. There are seven large outfalls, two unlined channels charging waste or water that is polluted and nine small drains within two kilometres of the Teluk Kemang. Being located on the Strait of Malacca, Teluk Kemang is also being slightly affected by shipping activities impact. Teluk Kemang is one of the centres of attraction for recreational activities and beach activities in Negeri Sembilan. A lot of hotels and resorts can be seen along this area. Apart from that, boats can also be seen along the coastal. There are a lot of studies being conducted by researches about marine ecosystems in Teluk Kemang as to determine the effects of level of pollution in this area to marines' organism. Teluk Kemang physical appearance can be described as facing the sea, sedimentary rock and sandy area meanwhile the description recorded for this area is that Teluk Kemang is a town/city and it is near to a port.

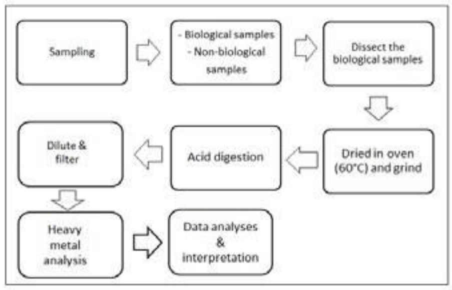

Figure 1: Flow chart of the methodology of this study.

3.1 Material and Methodology

3.2 Sample

3.2.1 Nerita lineata

Mollusca is the phylum of Nerita lineata. This species is under the class of gastropoda and it is the family of Neritidae. N. lineata is among the commonest herbivorous snails in the monsoon drains and mangroves and at night, this species grazes algae growing on rocks during the low tide and they can be found above water during the high tide and are normally inactive during day time [22]. This species is belong to subclass of prosobranchia and order of archaeogastropoda which they often grazes on algal films. They can be found near the food source such area that expose to sunlight and water exchange occur. Because of their shape, this species are well-known by the locals as 'siput timba' or the bucket snail. The distribution of N. lineata usually found in tropical intertidal area such as rocky shores, intertidal mudflats as well as on the mangrove trees [5]. As one of the herbivorous gastropods, they are usually be found at area where there are enough light penetrates for the plant food growth for example on the shore. According to [4], this species commonly is found behind the rock in rocky beaches in order to prevent themselves from heat stress. The morphology of N. lineata can be easily identified by their pronounced spiral cords that are thick and large and they are the largest species of the local neritas. N.lineata uses their calcareous operculum as their defense strategy and they have flattened disc-like whitish egg capsules that laid on hard surfaces [23] such as rocks. The shells of this species can be easily distinguished as they has grey shell with black grooved lines and their body size ranges from 2-3 cm [22] The importance of molluscs is they are common to be found and play role in food as well as non-food resources therefore, it is essential determine the heavy metal accumulation in this species as they act as source of food to human being. N. lineata is one of the intertidal molluscs that can be abundantly found on the west coast of Peninsular Malaysia thus, are suitable as biomonitoring indicator. Some studies that has been studied previously shown that intertidal molluscs can be among the good biomonitoring organisms. According to [24] and [25], a good biomonitor should be abundant in numbers, sedentary in order to represent the geographical condition of the study area, can be easily collected and have long life span of about one year and above.

3.2.2 Thais bitubercularis

Thais bitubercularis is a predatory gastropods that can be found on rocky shores.This species is belonging to class of neogastropoda which make it as one of the group of highly specialized predators. This family of muricidae is carnivorous where they attack other molluscs for feeding. Oysters and barnacles are also known as the source of food for these snails. They have a radula which is used as drill to penetrate the thick shells of their prey. They can be found in intertidal area among the rocks and many of this genus are in tropical as well as in warm seas. Species of Thais spp. can be used as indicator as they are widely distributed along Malaysian coastline. Some of the characteristics of marine invertebrates that are useful in monitoring the levels of heavy metals in seawater are the marine invertebrates able to integrate temporal variations in the concentration of heavy metals as well as they are able to concentrate several metals from the surrounding mediums. The morphology of Thais bitubercularis can be distinguished as they are spirally ridged that is quite sharp, has spines on shoulder and has dark brown-grey axial stripes. As the Thais bitubercularis is under class of gastropoda, they possess strong shells that functions to protect them from predators, abrasion by the sands as well as protecting them from fluctuating salinity. According to [26], analysing of sediments, water as well as marine organisms allow the pollution levels of heavy metals in the aquatic environment to be estimated and among the constituents of marine environment, the levels of heavy metals in molluscs and other invertebrates are usually considerably higher. Apart from that, molluscs have been known as biomonitoring as well as bioindicators subjects because they are easily available to be collected and they are abundant in both aquatic and terrestrial ecosystems. One of the characteristics of molluscs is they are able to concentrate toxic substances to a considerable level that exceed the levels of the environmental which lead to the molluscs to be hazard to human in term of consumption as they act as food to human.

3.2 Sampling and storage

A sampling was conducted in Tanjung Harapan in Selangor and in Teluk Kemang in Negeri Sembilan on April 2012. About 30 – 40 of each species of Nerita lineata and Thais bitubercularis were collected by hand picked from the two different study areas for the analysis of heavy metals in the organisms. Sediments from both study areas were also collected for the analysis. These samples were transported to the laboratory and brought back in iced box and kept frozen in order for further used for dissection and analysis.

3.3 Sample preparation

3.3.1 Biological samples (Nerita lineata and Thais bitubercularis)

The gastropods were dissected and the shells were separated from the soft tissues. These biological samples were then dried in an oven at 60°C until the constant dry weights of the samples were obtained. The samples were dried approximately at least 3 days to constant weight dry. Afterwards, the samples were grinded by using acid-washed pestle and mortar.

3.3.2 Non-biological samples (Sediments)

Sediments from both sites were dried in an oven at 60°C until the constant weights of the samples were obtained which approximately 3 days. The samples were then grinded by using acid-washed pestle and mortar. Then, the grinded samples were sieved through a 0.5-mm stainless steel sieve and shaken vigorously in order to produce homogeneity.

3.4 Sample digestion

3.4.1 Biological samples (Nerita lineata and Thais bitubercularis)

About 0.5-0.7 g of dried tissues [20] from each species of the gastropods were weighted. The dried tissues were placed in acid-washed digestion tubes. The tissues were digested in 10 ml of concentrated nitric acid in each digestion tube (AnalaR grade, BDH 69%). All the digestion tubes containing the tissues and concentrated nitric acid were placed in a hot-block digester at temperature of 40°C for 1 hour. Afterwards, the temperature of the digestion block was increased to 140°C for 3 hours in order for the samples to be fully digested [27]. Each sample was diluted to 40 ml with double distilled after they were cooled. The digested samples were shacked by using a vortex mixer and filtered through Whatman No. 1 filter papers and they were stored in acid-washed pill boxes for further use for heavy metal analysis.

3.4.2 Non-biological samples (Sediments)

About 1 g of dried non-biological samples which are the samples were weighted. The samples were placed in the digestion tubes that had been acid-washed. Afterwards, the samples were digested by using a ratio of 4:1 according to [27] where 8 ml of concentrated nitric acid (AnalaR grade, BDH 69%) was mixed with 2 ml concentrated perchloric acid (AnalaR grade, BDH 60%) and then the samples were put in the digestion tubes. All the digestion tubes were placed in a hot-block digester at temperature of 40°C for 1 hour and the temperature of the digestion block was increased to 140°C for 3 hours. The same methodology were used which was the samples were each diluted to 40 ml with double distilled water and were shacked using a vortex mixer then they were filtered through a Whatman No.1 filter papers. The digested sediments were stored into acid-washed pill boxes until heavy metals determination.

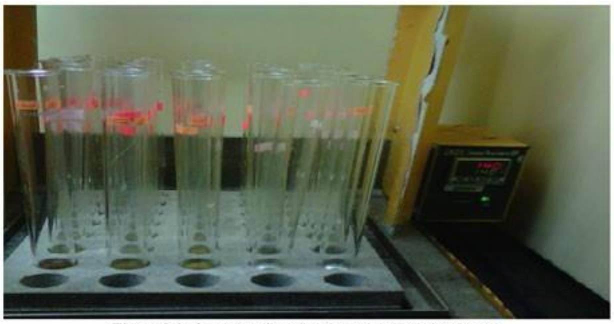

Figure 3.3: Samples digestion by using digestion block.

3.5 Determination of lead (Pb), cadmium (Cd), nickel (Ni), copper (Cu), zinc (Zn) and iron (Fe)

Inductively coupled plasma mass spectrometry (ICP-MS) was used in analyzing the heavy metals (Pb, Cd, Ni, Cu, Zn, Fe) in the digested samples in order to get precise determination of the heavy metals. The instrument was calibrated with blank and standards containing each element at 10 ppb, 20 ppb, 50 ppb, 100 ppb and 200 ppb. This is to generate a calibration curve with correlation coefficient of 0.999 or better. All the samples were determined based on the calibration curve. The data obtained from ICP-MS were presented in mg/kg dry weight basis.

Figure 3.4: Digested samples for the determination of heavy metals by using ICP-MS.

3.6 Quality control

In order to avoid potential contamination, all glassware and equipment used were acid-washed. The acid washed was prepared from 5% of nitric acid and all the glassware and equipment were soak in the acid washed overnight.

3.7 Statistical analysis

The data recorded were analyzed by using software of Microsoft EXCEL 2010 edition. Biota–sediment accumulation factors (BSAFs) were calculated for the selected metals in the molluscs. BSAF is the ratio of biota to sediment contamination concentration and can be calculated based on the formula suggested by [20].

$BSAF = Ct / Cs$

Where Ct is the mean metals concentration (mg/kg dry weight) in the organism tissue while Cs is the mean metal concentration in the sediment (mg/kg dry weight). Pearson's correlation coefficients between the selected heavy metals in the soft tissues and shells with the sediments were calculated using Statistical Analysis Software (SAS). The significance among the mean concentration of the heavy metals between locations as well as types and parts of the molluscs were determined by Tukey test.

4.1 Results and discussions

4.2 Metals concentration in molluscs in Tanjung Harapan

4.1.1 Concentration of Iron (Fe)

Iron (Fe) was found highest in the soft tissues of T. bitubercularis compare to other parts in both molluscs with value of 420.930 mg/kg in sample 2 whereas the lowest concentration of iron (Fe) was found in the soft tissues of

N. lineata in sample 1 with value of 168.749 mg/kg. Based on Figure 4.1.1.1, the average value of iron (Fe) concentration in N. lineata was 202.990 mg/kg which was greater than its shells while in T. bitubercularis the average metal concentration was found with 355.240 mg/kg in its shells which was lower than the concentration of iron (Fe) in its soft tissues. Figure 4.1.1.1 showed the concentration of heavy metals in all three samples of both molluscs.

T. bitubercularis was found to have highest concentration of iron (Fe) in its soft tissues due to the facts that iron (Fe) the essential elements that is very important for the metabolic activities in organisms especially in molluscs. Nevertheless, in N. lineata, the concentration of iron (Fe) in its soft tissues was found to be the lowest than other parts of both molluscs. This is probably because different species has different tendency to accumulate the metals. From the results, overall, it can be seen that T. bitubercularis possessed higher concentration of iron (Fe) as compare in N. lineata. 4.1.2 Concentration of Nickel (Ni)

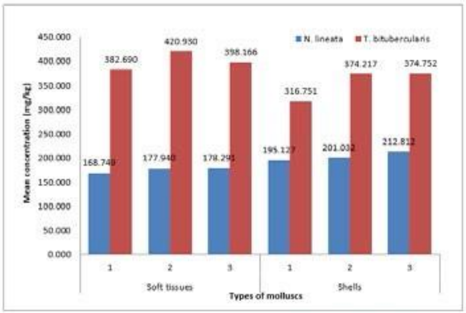

Figure 4.1.1.1 The concentration of iron (Fe) in molluscs in Tanjung Harapan.

From Figure 4.1.2.1, concentration of nickel (Ni) was found highest in the shells of T. bitubercularis in sample 3 with value of 4.180 mg/kg while the lowest concentration of nickel (Ni) can be found in the same types of molluscs in its soft tissues in sample 1 with value of 3.529 mg/kg. In N. lineata, the concentration of nickel (Ni) was found higher in its soft tissues with average value of 3.719 mg/kg compare to its shells which the average value was 3.635 mg/kg.

As in Teluk Kemang, T. bitubercularis in Tanjung Harapan also possessed highest nickel (Ni) concentration in its shells. This is because of shells which is the outer layer of the molluscs comes in direct contact with the seawater than the soft tissues [28], which made it has highest concentration of the metal. Besides, the shells of T. bitubercularis is more bigger in surface area than in the shells of N. lineata thus this can also affect the concentration of metals accumulated in the T. bitubercularis's shells as compare to N. lineata. This also explained about the huge difference of the metals found between the soft tissues and the shells of T.bitubercularis.

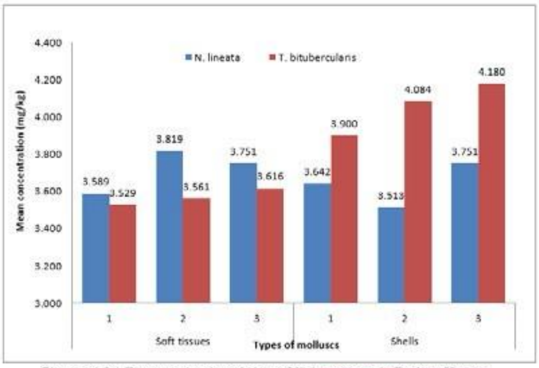

Figure 4.1.2.1 The concentration of nickel (Ni) in molluscs in Tanjung Harapan.

4.1.3 Concentration of Copper (Cu)

From Table 4.1.3.1, copper was found to be higher in T. bitubercularis than in N. lineata for both tissues and shells. The highest concentration of copper was found in the soft tissues of T. bitubercularis in sample 2 with 243.548 mg/kg while the lowest in the shells of N. lineata in sample 2 with 0.106 mg/kg. Copper was found abundantly high in concentration in the soft tissues of T. bitubercularis.

The concentration of copper (Cu) were found higher in the soft tissues of N. lineata and also in T. bitubercularis compare to in their shells as copper can be found in some molluscs and in some arthropods as it is a functional part of the respiratory protein haemocyanin. Thus, contribute to the level of concentration of copper (Cu) in soft tissues of both molluscs. T. bitubercularis is a a predatory gastropods that can be found on rocky shores where they attack other molluscs for feeding. Therefore, they tend to accumulate higher metals in their bodies as these metals that were consumed are able to biomagnifies along the food chain compare to N. lineata which is commonest herbivorous snails that graze on algal films.

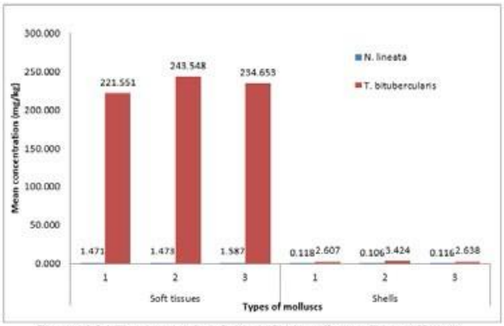

Figure 4.1.3.1 The concentration of copper (Cu) in molluscs in Tanjung Harapan.

4.1.4 Concentration of Zinc (Zn)

Since zinc (Zn) and copper (Cu) are among the essential metals, the concentration of zinc was found to be similar in ways that it can be found higher in the soft tissues of N. lineata and T. bitubercularis rather than their shells. The highest concentration of zinc (Zn) can be found in the soft tissue of T. bitubercularis in sample 1 with 395.200 mg/kg whereas the lowest concentration of zinc (Zn) was in the shell of N. lineata in sample 2 with value of 0.785 mg/kg. Zinc (Zn) also plays important roles as enzymatic proteins as well as respiratory pigments by their presence in the essential biomolecules. This explains about the high concentration of this heavy metal in the molluscs's soft tissues than in their shells. Besides, zinc (Zn) can be found abundantly in Tanjung Harapan as zinc in water comes from both natural processes such as weathering and also from the effects of anthropogenic activities such as mine drainage, municipal and industrial effluents and also urban runoff.

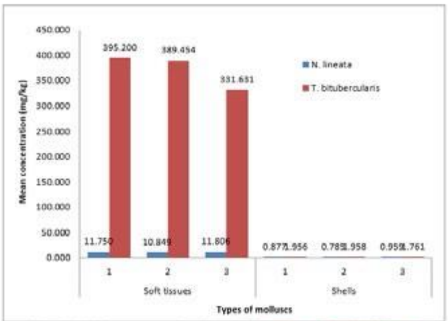

Figure 4.1.4.1 The concentration of zinc (Zn) in molluscs in Tanjung Harapan.

4.1.5 Concentration of Cadmium (Cd)

Based on Figure 4.1.5.1, concentration of cadmium (Cd) was found higher in the soft tissues of both N. lineata and T. bitubercularis. The highest concentration of cadmium (Cd) was in the soft tissues of T. bitubercularis in sample 3 with value of 0.992 mg/kg whereas the lowest concentration of this heavy metal was found in the shells of N. lineata in sample 2 and sample 3 with value of 0.003 mg/kg.

From the results obtained, soft tissues was proved to be a good bioindicator for cadmium (Cd) in both molluscs and many previous studies also stated that soft tissues of molluscs are widely used in biomonitoring of heavy metals in marine environment. It is also important to determine the accumulation of heavy metals in the soft tissues of molluscs as some molluscs are edible which is they can be eaten by human. Thus, knowing the levels of concentration of heavy metals in it can prevent the metals from affecting human health apart from allow us to determine the quality of the marine environment.

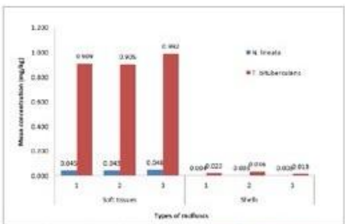

Figure 4.1.5.1 The concentration of cadmium (Cd) in molluscs in Tanjung Harapan.

4.1.6 Concentration of Lead (Pb)

Based on Figure 4.1.6.1, concentration of lead (Pb) was found higher in both N. lineata and T. bitubercularis compare to their shells. The highest concentration of lead (Pb) was found in the soft tissues of T. bitubercularis in sample 3 with value of 0.266 mg/kg whereas the lowest concentration of lead (Pb) was found in the shells of N. lineata in sample 2 with value of 0.061 mg/kg. In N. lineata, the concentration of lead (Pb) was lower in the shells than in the soft tissues where in the soft tissue the average concentration was 0.145 mg/kg of lead (Pb) meanwhile in the shells of T. bitubercularis the average concentration was 0.081 mg/kg of lead (Pb) concentration.

As been explained, soft tissues of molluscs are better in accumulation of the metals compare to the shells and studies that had been done by other researchers are mostly used soft tissues of molluscs and only few of them used the shells of molluscs in determining the concentration of metals in molluscs. Concentration of lead (Pb) was higher as by referring to previous studies, there is elevated level of lead being recorded in coastal areas that near to ports and industrial estates and this is similar to Tanjung Harapan which is located in the Northport of the Port Klang where shipping activities are carry out. The difference in the level of concentration between the two molluscs is because both of them are from different species and thus possessed difference ability of accumulates difference metals.

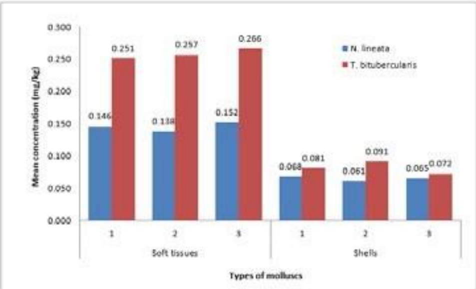

Figure 4.1.6.1 The concentration of lead (Pb) in molluscs in Tanjung Harapan.

4.2 Metals concentration in molluscs in Teluk Kemang

4.2.1 Concentration of Iron (Fe)

From Figure 4.2.1.1, concentration of iron (Fe) was found highest in the shell of T. bitubercularis in sample 3 than in other parts of the two species studied with value of 593.601 mg/kg. The lowest concentration of iron (Fe) in both species and in both parts of the species can be found in shell of N. lineata in sample 2 and in the soft tissues of N. lineata in sample 3 with value of 325.563 mg/kg. For concentration of iron (Fe) in soft tissue of N. lineata the average value was 369.890 mg/kg and in soft tissue of T. bitubercularis the average value was 436.638 mg/kg. T. bitubercularis possessed high iron (Fe) in their shells than in the shell of N. lineata probably because T. bitubercularis is a family of muricidae which are carnivorous where they attack other molluscs for feeding and oysters and barnacles are known as the source of food for this snails compare to N. lineata which is a herbivorous snails that grazes algae growing on rocks [22]. Thus, these heavy metals tend to accumulate more on the outer part or known as the shells of the T. bitubercularis compare to the shell of N. lineata.

In N. lineata, iron (Fe) was found to be slightly higher in their soft tissues than in their shells with average value of 369.890 mg/kg in the shells and the difference between the iron (Fe) concentration in their shells and soft tissues was only 5.476 mg/kg. This is because iron (Fe) is an essential element which is important for many enzymes and respiratory pigment thus, it is normal for the organisms to possess that much metals concentration in their bodies. Besides, iron (Fe) is one of the essential elements that is very important for the metabolic activities in organisms especially in molluscs. Apart from that, iron (Fe) have high natural background levels and it can be found anywhere include in plant and this N. lineata graze on algae found on the rocks and this explained the concentration levels of iron (Fe) in the soft tissue of this species.

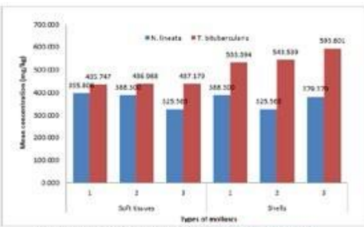

Figure 4.2.1.1 The concentration of iron (Fe) in molluscs in Teluk Kemang

4.2.2 Concentration of Nickel (Ni)

Based on Figure 4.2.2.1, in both of the molluscs which are N. lineata and T. bitubercularis, the concentration of nickel (Ni) were found higher in the shells compare to the soft tissues of both molluscs. The highest concentration of nickel (Ni) was found in shell of T. bitubercularis in sample 3 with value of 5.969 mg/kg while the lowest was found in the soft tissues of T. bitubercularis in sample 1 with value of 2.886 mg/kg. The concentration of nickel (Ni) was found higher in the shells of both N. lineata and T. bitubercularis because shells are known to be capable to play a role as toxic waste dump in order to eliminate toxic chemicals from the tissue as well as from the food chain itself [8]. Therefore, this explained about the differences of nickel (Ni) concentration in the shells of both molluscs. In addition, according to [9], seawater comes in direct contact with the outer layer of the shells unlike the soft tissues that located inside the shells thus probability of shells accumulated more heavy metals are high. T. bitubercularis had highest concentration of nickel (Ni) probably because of the surrounding environmental factors of Teluk Kemang where there are human activities occur that contribute to the accumulation of nickel (Ni) in the shells such as recreational and tourism activities where there are many visitors visit this place.

Based on the observation, there are some cigarette left at some of the coastal area in Teluk Kemang where the cigarette smoke are known as the major sources of nickel thus, contribute to the concentration of nickel (Ni) in shells of T. bitubercularis. On top of that, the concentration of nickel (Ni) was found to be the highest as this heavy metals may leach into the seawater through waste water due to improper treatment processes of the waste water. In Teluk Kemang, there are a lot of resorts and hotels along the coastal as well as restaurants that may leach out this heavy metals into the water without proper treatment.

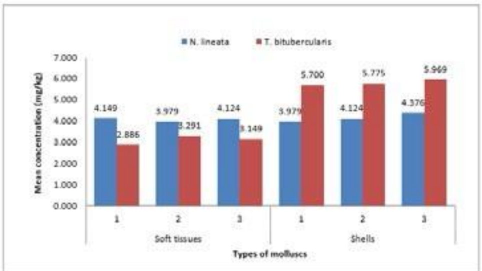

Figure 4.2.2.1 The concentration of nickel (Ni) in molluscs in Teluk Kemang

4.2.3 Concentration of copper (Cu)

From Figure 4.2.3.1, T. bitubercularis possessed higher concentration of copper (Cu) in their soft tissues and shells compare to those in N. lineata. Based from the results obtained, the highest concentration of copper (Cu) was found in the soft tissues of T. bitubercularis in sample 3 with value of 55.193 mg/kg. Meanwhile, the lowest concentration of copper (Cu) was in the shells of N. lineata in sample 3 with value of 0.214 mg/kg.

Copper can be found in some molluscs as well as in some arthropods as it is a functional part of the respiratory protein haemocyanin. Thus, making this heavy metal abundantly accumulated in the soft tissues of the molluscs than in their shells. Copper can also come from anthropogenic activities occur nearby such as industrial activities as well as waste disposal. Different species of molluscs have different level if accumulated the metals and based on the results obtained, T. bitubercularis was found to have better accumulated copper (Cu) in their soft tissues than N. lineata in Teluk Kemang.

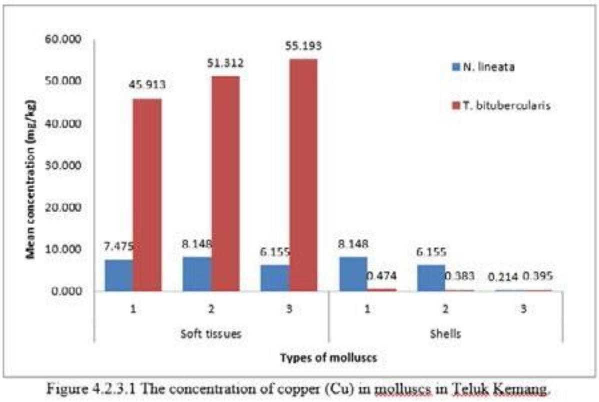

Figure 4.2.3.1 The concentration of copper (Cu) in molluscs in Teluk Kemang.

4.2.4 Concentration of zinc (Zn)

Figure 4.2.4.1 show the concentration of zinc (Zn) in molluscs in Teluk Kemang where the highest concentration of zinc (Zn) was found in the soft tissues of T. bituberculris in sample 3 with value of 267.740 mg/kg whereas the lowest concentration of zinc (Zn) was found in the shells of N. lineata in sample 3 with value of 0.436 mg/kg.

This is same as copper concentration in Teluk Kemang where the heavy metal concentration is higher in the soft tissue of T. bituberculris and lowest in the shell of N. lineata. This is probably because both heavy metals are essential metals that usually being found. According to [19], the source of zinc in water comes from both natural processes such as weathering and from the effects of anthropogenic activities such as mine drainage, municipal and industrial effluents and also urban runoff. Teluk Kemang is known as one of the centre of attraction in Negeri Sembilan where a lot of hotels, resorts and restaurant exist in this area where can potentially leach out heavy metals such as zinc (Zn) in the water bodies nearby.

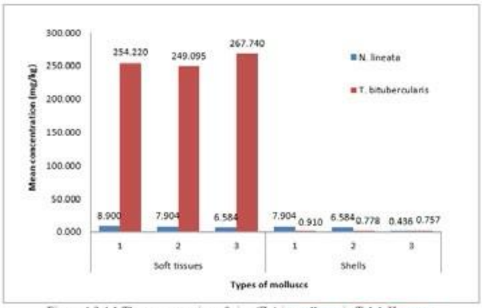

Figure 4.2.4.1 The concentration of zinc (Zn) in molluscs in Teluk Kemang

4.2.5 Concentration of cadmium (Cd)

Concentration of cadmium (Cd) was found numerously highest in the soft tissues of T. bituberculris in sample 3 compare to N. lineata and other parts of both molluscs with value of 1.195 mg/kg. On the other hand, the lowest concentration of cadmium (Cd) in both molluscs was found in the shells of N. lineata in sample 3 with value of 0.004 mg/kg. There was huge difference between the concentration of cadmium (Cd) in the T. bituberculris's soft tissues and in the shells.

As for cadmium (Cd), it was found highest in the soft tissues of T. bituberculris probably because this species is capable to accumulate cadmium (Cd) much better in their soft tissues than in their shells as according to [14], a lot of the marine organisms accumulate heavy metals in their soft tissue and body concentrations. N. lineata also possessed higher concentration of cadmium (Cd) in their soft tissues than in their shells. From the observations made in Teluk Kemang, there are some old pipes at the shore of Teluk Kemang and by referring to [16], this metals which is cadmium (Cd) are used in PVC products as stabilizers thus, can indirectly contribute to the level of concentration of cadmium (Cd) in the water. This also proved that surrounding activities in this area may influence the quality of the marine environment and in this context is in term of the level of concentration of heavy metals in the molluscs.

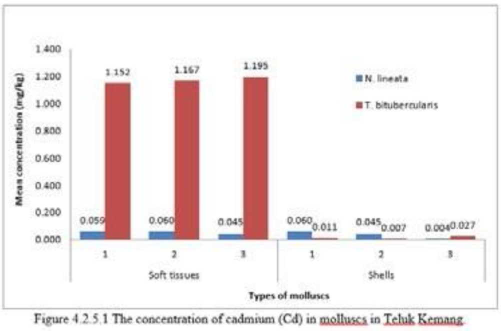

Figure 4.2.5.1 The concentration of cadmium (Cd) in molluscs in Teluk Kemang

4.2.6 Concentration of lead (Pb)

Based on Figure 4.2.6.1, concentration of lead (Pb) was found higher in the soft tissues of both N. lineata and T. bituberculris compare in their shells. The highest concentration of lead (Pb) was found in the soft tissues of T. bituberculris in sample 2 with value of 0.177 mg/kg whereas the lowest concentration of this heavy metal was also found in the shells of this species which was T. bituberculris in sample 3 with value of 0.026 mg/kg.

Lead (Pb) was found abundantly in both soft tissues of N. lineata and T. bituberculris because of this heavy metal was leach out from the anthropogenic activities carry out in Teluk Kemang. Although, Teluk Kemang is not located very near to a port compare to in Tanjung Harapan, this area is also can be consider as the pathway of ships as it is situated along the Straits of Malacca and there are also small tourism boats along the coastal as Teluk Kemang is one of the famous recreational and tourism areas in Negeri Sembilan, Malaysia. As lead (Pb) is used in the leaded gasoline that is used by ships and boats as fuels, thus this contribute to the increase in concentration of lead (Pb) in the marine environment.

Different types of molluscs has different capable of accumulate metals and this is similar in N. lineata and T. bituberculris as both molluscs tend to accumulate different concentration of lead (Pb) in their soft tissues and shells. From the results collected, T. bituberculris accumulate more lead (Pb) in its soft tissues but lowest concentration of this heavy metal was recorded in its shells. T. bituberculris possess strong shells that functions to protect them from predators, abrasion by the sands as well as protecting them from fluctuating salinity thus making them as one of the marine organism that has long life span thus can accumulate more metals compare to N.lineata. This characteristic makes them suitable as biomonitoring organisms for heavy metals accumulation.

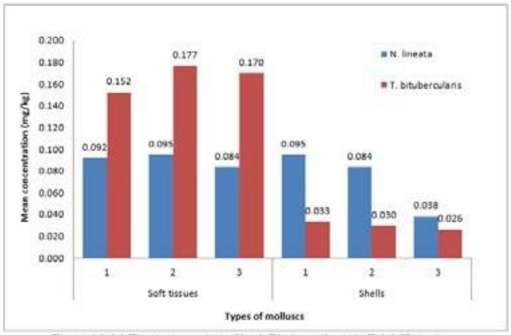

Figure 4.2.6.1 The concentration of lead (Pb) in molluscs in Teluk Kemang

4.3 Comparison of biota–sediment accumulation factors (BSAFs) of heavy metals in soft tissue of molluscs and sediments

4.3.1 Tanjung Harapan

Table 4.3.1.1 showed the comparison of biota–sediment accumulation factors (BSAFs) of iron (Fe), nickel (Ni), copper (Cu), zinc (Zn), cadmium (Cd) and lead (Pb) in soft tissue of molluscs and sediments of Tanjung Harapan. Based on the results collected, there are differences in BSAFs between the two species of molluscs namely, N. lineata and T. bituberculris and the sediment. The highest BSAF was copper (Cu) in T. bituberculris with value of 91.186 while the lowest BSAF was iron (Fe) in N. lineata with value of 0.028. The descending form of BSAF for N. lineata were as followed ; Cd > Ni > Zn > Cu > Pb > Fe while for T. bituberculris were as followed ; Cu > Cd > Zn > Ni > Pb, Fe. BSAFs values of cadmium accumulated in soft tissues of T. bituberculris and N. lineata, copper and zinc accumulated in soft tissues of T. bituberculris were found to have BSAFs values of more than 2. Based on Table 4.5.1.1, BSAFs values that were less than unity or 1 showed that the capacity of molluscs to accumulate the heavy metals is low to that of the sediment.

Copper (Cu) in T. bituberculris was found as the highest BSAF among the two species of molluscs with value of 91.186 as this metal can be found in some molluscs as well as in some arthropods as it is a functional part of the respiratory protein haemocyanin. Besides, in Tanjung Harapan, there are many industrial activities and it is located in Northport where shipping activities take place and these activities may leach out copper into the water bodies. When BSAF were calculated, it was found that iron (Fe) in N. lineata had the lowest BSAF value compare when the iron (Fe) value was directly determined from the metal concentration in the molluscs and sediments which the value was 0.028. This showed that, the high level iron (Fe) may come from high natural background levels in the samples analyzed in this study. Apart from that, this result is probably because iron (Fe) is very important for the metabolic activities in organisms especially in molluscs thus it had been recorded as having high metal concentration in the sample analyzed.

Table 4.3.1.1 Comparison of BSAFs of heavy metals in soft tissue of molluscs and sediments of Tanjung Harapan

Heavy metals	Mollusca	Metals concentration in soft tissues (mg/kg)	Metals concentration in sediments (mg/kg)	Biota-sediment accumulation factors (BSAFs)
Fe	*N. lineata*	174.993	6187.408	0.028
	T. bituberculatis	400.595		0.065
Ni	*N. lineata*	3.719	2.633	1.412
	T. bituberculatis	3.569		1.355
Cu	*N. lineata*	1.510	2.558	0.590
	T. bituberculatis	233.251		91.186
Zn	*N. lineata*	11.468	17.299	0.663
	T. bituberculatis	372.095		21.510
Cd	*N. lineata*	0.045	0.018	2.583
	T. bituberculatis	0.935		53.265
Pb	*N. lineata*	0.145	3.973	0.037
	T. bituberculatis	0.258		0.065

4.3.2 Teluk Kemang

From table 4.3.2.1, showed the comparison of biota–sediment accumulation factors (BSAFs) of iron (Fe), nickel (Ni), copper (Cu), zinc (Zn), cadmium (Cd) and lead (Pb) in soft tissue of molluscs and sediments of Teluk Kemang. Based on the results collected, there are differences in BSAFs between the two species of molluscs namely, N. lineata and T. bituberculsris and the sediment. The highest BSAF was cadmium (Cd) in T. bituberculsris with value of 135.162 while the lowest BSAF was iron (Fe) in N. lineata with value of 0.038. The descending form of BSAF for N. lineata were as followed ; Cd > Cu > Ni > Zn > Pb > Fe while for T. bituberculsris were as followed ; Cd > Zn > Cu > Ni > Pb > Fe.

BSAFs values of cadmium and nickel accumulated in soft tissues of T. bituberculsris, copper accumulated in soft tissues of T. bituberculsris and N. lineata, zinc accumulated in soft tissues of T. bituberculsris were found to have BSAFs values of more than 2 which showed the storing of that particular heavy metals in the molluscs soft tissues in respect to sediments. Nevertheless, soft tissues that accumulated large amount of metals do not show the actual or bioavailable fractions in the surrounding sediments of a substrate.

Cadmium (Cd) in T. bituberculsris was found to have the highest value of BSAF among the two species of molluscs. This is due to human activities in Teluk Kemang. Based on the observation made along the coastal of Teluk Kemang, there is some kind of old PVC pipes near the shore. As according to [16], where these metals are used in PVC products as stabilizers, certain alloys, in rechargeable nickel-cadmium batteries and in phosphate fertilizer. Therefore, this explains the BSAF value of the cadmium (Cd) in Teluk Kemang. Lowest BSAF value among all the molluscs studied were iron (Fe) in N. lineata. As mentioned, iron (Fe) had the lowest BSAF value compare when the iron (Fe) value was directly determined from the metal concentration in the molluscs and sediments due to this metals can be found naturally in the molluscs and the human activities around this area does not much affected the metals concentration in the molluscs and the sediments.

Table 4.3.2.1 Comparisons of BSAFs of heavy metals in soft tissue of molluscs and sediments of Teluk Kemang

Heavy metals	Molluscs	Metals concentration in soft tissues (mg/kg)	Metals concentration in sediments (mg/kg)	Biota–sediment accumulation factors (BSAFs)
Fe	*N. lineata*	369.890	9718.753	0.038
	T. bituberculsris	436.638		0.045
Ni	*N. lineata*	4.084	3.273	1.248
	T. bituberculsris	3.109		0.950
Cu	*N. lineata*	7.259	2.560	2.836
	T. bituberculsris	50.806		19.847
Zn	*N. lineata*	7.796	9.971	0.782
	T. bituberculsris	257.018		25.776
Cd	*N. lineata*	0.055	0.009	6.323
	T. bituberculsris	1.171		135.162
Pb	*N. lineata*	0.090	1.823	0.049
	T. bituberculsris	0.166		0.091

4.4 Comparison of biota–sediment accumulation factors (BSAFs) of heavy metals in shells of molluscs and sediments

Biota- sediment accumulation factors (BSAFs) is used to determine the ratio of biota to sediment contamination concentration. BSAF values can be divided as follows for different parts of gastropod which include different part of tissues and also the shells.

4.4.1 Tanjung Harapan

Table 4.4.1 Biota–sediment accumulation factors (BSAFs)

Biota–sediment accumulation factors (BSAFs) groups	Biota–sediment accumulation factors (BSAFs) values
Macro concentrator	BSAF > 2
Micro concentrator	1 < BSAF < 2
Deconcentrator	BSAF <1

Source : Berandah *et al.* (2010)

Biota–sediment accumulation factors (BSAFs) of shells of two different species of rocky shores snails namely, N. lineata and T. bituberculsris were calculated from the results collected. The highest value of BSAF in Tanjung Harapan was nickel (Ni) in T. bituberculsris's shell with value of 1.540 while the lowest BSAF value was lead (Pb) in N. lineata's shell with value of 0.016. Table 4.6.2.2, stated the biota-sediment accumulation factors (BSAFs) of heavy metals in shells of molluscs and sediments of Tanjung Harapan. Based from both results of BSAFs of both molluscs in Tanjung Harapan, for the shells of N. lineata, the descending form of BSAFs values are as follow ; Ni > Cd > Zn > Cu > Fe > Pb. Meanwhile for T. bituberculsris, the descending form begin from Ni > Cd > Cu > Zn > Fe > Pb. BSAFs values of more than 1 but less than 2 were found in the shells of T. bituberculsris for accumulation of nickel (Ni), cadmium (Cd) and copper (Cu) as well as in N. lineata for accumulation of nickel (Ni). These BSAFs values ranges are consider as microconcentrator. Other than this, the BSAFs values were less than 1 which was considered as deconcentrator. From the results, it was found that none of them were macroconcentrator which is BSAF value more than 2. BSAF values might be lower than 1 if the organism metabolizes the chemical or it might be because the system has not yet reach steady state.

Table 4.4.1.1 Comparisons of BSAFs of heavy metals in shells of molluscs and sediments of Tanjung Harapan

Heavy metals	Mollusca	Metals concentration in shells (mg/kg)	Metals concentration in sediments (mg/kg)	Biota-sediment accumulation factors (BSAFs)
Fe	*N. lineata*	202.000	6187.408	0.033
	T. bituberculsris	355.240		0.057
Ni	*N. lineata*	3.635	2.633	1.381
	T. bituberculsris	4.054		1.540
Cu	*N. lineata*	0.113	2.558	0.044
	T. bituberculsris	2.890		1.130
Zn	*N. lineata*	0.874	17.299	0.051
	T. bituberculsris	1.892		0.109
Cd	*N. lineata*	0.003	0.018	0.190
	T. bituberculsris	0.026		1.472
Pb	*N. lineata*	0.065	3.973	0.016
	T. bituberculsris	0.081		0.020

4.4.2 Teluk Kemang

From Table 4.4.2.1, for the shells of N. lineata, the descending form of BSAFs values are as follow; Cd > Cu > Ni > Zn > Pb > Fe whereas for T. bituberculsris, the descending form was Ni > Cd > Cu > Zn > Fe > Pb. The shell of N.lineata can be considered as macroconcentrator because the BSAF value for the accumulation of cadmium (Cd) was more than 2 which was 4.174. Macroconcentrator may be considered as good biomonitor (Bohac, 1999). Most of the shells of the molluscs were found to have BSAFs values less than unity. BSAFs values are different for each species of molluscs and also different in Tanjung Harapan and Teluk Kemang. Based on the results obtained, it was found that between the shells of these N. lineata and T. bituberculsris collected from Tanjung Harapan and Teluk Kemang, N. lineata from Teluk Kemang is the only one that recorded to have BSAF value more than 2 for the accumulation of cadmium (Cd). This showed that, Teluk Kemang possessed high accumulation of cadmium (Cd) compare to Tanjung Harapan. From observation made in Teluk Kemang while sampling the samples, there was old PVC pipes along the coastal. As according to [16], cadmium (Cd) is used in PVC products as stabilizers thus, this explains the results obtained. BSAFs values may only be used to determine the accumulation of metals capacity as well as classifications between the different tissues in the organisms.

Table 4.4.2.1 Comparisons of BSAFs of heavy metals in shells of molluscs and sediments of Teluk Kemang

Heavy metals	Mollusca	Metals concentration in shells (mg/kg)	Metals concentration in sediments (mg/kg)	Biota-sediment accumulation factors (BSAFs)
Fe	*N. lineata*	354.414	9718.753	0.037
	T. bituberculsris	556.845		0.057
Ni	*N. lineata*	4.160	3.273	1.271
	T. bituberculsris	5.814		1.776
Cu	*N. lineata*	4.839	2.560	1.890
	T. bituberculsris	0.418		0.163
Zn	*N. lineata*	4.875	9.971	0.490
	T. bituberculsris	0.815		0.082
Cd	*N. lineata*	0.036	0.009	4.174
	T. bituberculsris	0.015		1.687
Pb	*N. lineata*	0.072	1.823	0.040
	T. bituberculsris	0.030		0.016

4.5 Significant mean concentrations of heavy metals (Fe, Ni, Cu, Zn, Cd and Pb).

4.5.1 Locations (Tanjung Harapan and Teluk Kemang)

Mean concentrations of heavy metals namely, iron (Fe), nickel (Ni), copper (Cu), zinc (Zn), cadmium (Cd) and lead (Pb) in Tanjung Harapan were found to have significantly differences with those heavy metals in Teluk Kemang. This showed that the concentrations of the accumulation of heavy metals in these two different study areas are different. This is because both study areas have been influenced with different anthropogenic activities nearby that emit different concentration of heavy metals in the marine environment. From Table 4.7.1, iron (Fe) in Teluk Kemang was found as the highest concentration of heavy metals compare to the others with 431.950 mg/kg. Iron (Fe) was found as the highest in heavy metals in Teluk Kemang as according to [20], iron is one of the essential metals. Besides, iron (Fe) may have high natural background levels and it can be found anywhere include in plant and man-made products. This heavy metal which is iron (Fe) was abundantly found in Teluk Kemang than in Tanjung Harapan is mainly because in Teluk Kemang there are a lot of rocks including sedimentary rocks and the rocks colored are oranges, yellow and red which is believes to contain iron oxides. Iron can be found in large amount in earth's crust between 2 to 3 percent in sedimentary rocks. Thus, this may influence the mean concentration of iron (Fe) in Teluk Kemang.

Another element that was higher in Teluk Kemang than in Tanjung Harapan was nickel (Ni) with 4.292 mg/kg and cadmium (Cd) with 0.319 mg/kg. Nickel (Ni) can be found more in Teluk Kemang as along this coastal there are a lot of hotels and resorts as well as restaurant that might emit waste into the water where nickel in water comes from human activities such as domestic waste water. Cadmium (Cd) in Teluk Kemang was found to be significantly different and higher with value of 0.319 mg/kg than in Tanjung Harapan. This happened because this heavy metals comes from PVC products as stabilizers, certain alloys and in rechargeable nickel-cadmium batteries [16] where based on the observation while sampling in Teluk Kemang, there are old pipes at the coastal of Teluk Kemang that may affect the emission of this metals in seawater.

Zinc (Zn), copper (Cu) and lead (Pb) in Tanjung Harapan were significantly different and higher than those in Teluk Kemang. Zinc is one of the essential metals apart from copper and they are among of the metal that has contributed to the contamination of marine ecosystems. Zinc (Zn) can also come from anthropogenic activities such as from municipal and industrial effluents. Although both study areas are industrial areas, Tanjung Harapan is known as a more develops industrial area compare to Teluk Kemang. The same thing goes with the concentration of copper as this metals usually be found near industrial activities, landfills and waste disposals. Among the heavy metals studied, lead (Pb) was found as the lowest concentration in both areas but it is significantly different between both areas. Lead (Pb) was higher in concentration in Tanjung Harapan than in Teluk Kemang because by referring to previous studies, there is elevated level of lead being recorded in coastal areas that near to ports and industrial estates in which Tanjung Harapan is situated in Northport near Port Klang, Selangor

Table 4.5.1.1 Mean concentrations of heavy metals (Fe, Ni, Cu, Zn, Cd and Pb) between locations

Location	Mean concentrations of heavy metals (mg/kg)					
	Fe	Ni	Cu	Zn	Cd	Pb
Tanjung Harapan	283.450 b	3.745b	59.441a	96.582a	0.252b	0.137a
Teluk Kemang	431.950 a	4.292a	15.830 b	67.651 b	0.319a	0.090b

Means with the same letter are not significantly different

4.5.2 Species of molluscs and their different parts (soft tissues and shells)

N. lineata and T. bitubercularis were found to be significantly different in each of the heavy metals which are in iron (Fe), nickel (Ni), copper (Cu), zinc (Zn), cadmium (Cd) and lead (Pb) based on Table 4.5.2.1. This is because each species have different characteristics and morphology that can affects the accumulation of metals either in their soft tissues or in their shells. T. bitubercularis was found to accumulate higher concentration of heavy metals than N. lineata. Both molluscs accumulated higher concentration of iron (Fe) compare to the other heavy metals where T. bitubercularis was found to have higher concentration of iron (Fe) than N. lineata with value of 437.330 mg/kg.

T. bitubercularis accumulated higher concentration of heavy metals than N. lineata probably because it is predatory gastropods and the family of T. bitubercularis is muricidae which are carnivorous. They attack other molluscs for feeding. This in turn increase the concentration of metals accumulated in T. bitubercularis through biomagnification that occurs along the food chain where top predators accumulate more contaminant in their tissues. Species which are carnivorous tend to bioaccumulate higher level of metals compare to herbivorous or omnivorous. This is proved based on previous studies such as [10]. The different parts of molluscs which consist of soft tissues and shells were found to be significantly different in concentration of heavy metals between them. For instead, iron (Fe) concentration in soft tissues was significantly different in iron (Fe) in the shells. Iron (Fe) and nickel (Ni) were found higher in the shells of the molluscs compare in their soft tissues whereas other heavy metals were found higher in the soft tissues than in the shells. Iron (Fe) and nickel (Ni) were higher in concentration in the shells as shells act as the outer layer that comes indirect contact with the seawater [9] making the metals accumulated more in the shells. Apart from that, iron (Fe) is known as the essential elements that are vital for the metabolic activities in organisms especially in molluscs.

Table 4.5.2.1 Mean concentrations of heavy metals (Fe, Ni, Cu, Zn, Cd and Pb) between types/ species of molluscs.

Types/ species of molluscs	Mean concentrations of heavy metals (mg/kg)					
	Fe	Ni	Cu	Zn	Cd	Pb
N. lineata	278.070 b	3.900b	3.431b	6.278b	0.035b	0.093b
T. bitubercularis	437.330 a	4.137a	71.841 a	157.995 a	0.537a	0.134a

Means with the same letter are not significantly different

Table 4.5.2.2 Mean concentrations of heavy metals (Fe, Ni, Cu, Zn, Cd and Pb) between parts of molluscs (soft tissues and shells).

Parts of molluscs	Mean concentrations of heavy metals (mg/kg)					
	Fe	Ni	Cu	Zn	Cd	Pb
Soft tissues	345.530 b	3.620b	73.207 a	162.09 4a	0.552a	0.165a
Shells	369.870 a	4.416a	2.065b	2.139b	0.020b	0.062b

Means with the same letter are not significantly different

4.6 Relationship between metals concentration in organisms and sediments

A correlation matrix can indicate the relationship between two items which in this study is between metals concentration in organisms with the metals concentration in the ambient sediments. Correlation coefficient indicates the strength of the relationship between the independent variables and the dependent variables while the significance of the relationship is indicate by the probability level. From Table 4.6.2, the correlation coefficient of concentration of heavy metals in the soft tissues and shells of T. bitubercularis with those in the sediments in Tanjung Harapan does not showed significant correlation at $P<0.05$ as the size of the sample (N) in this study is small. Table 4.6.4 which show the correlation coefficient of concentrations of heavy metals in the soft tissues and shells of N. lineata with those in the sediments in Teluk Kemang also does not showed any significant correlation at $P<0.05$.

Based on the results obtained, the no significant correlation indicate that the changes in sediment metal loading is not the only factor which is able to give affects to the bioavailable metals [13] which consist of Fe, Ni, Cu, Zn, Cd and Pb to T. bitubercularis in Tanjung Harapan and to N. lineata in Teluk Kemang. This is probably because the molluscs do not come in direct contacts with the contaminated sediment as they were protected by their shell [13] and also by their physical surrounding habitat. For instead, T. bitubercularis in Tanjung Harapan were found on rocks and their shells closure prevent the direct contact with the sediments..

Table 4.6.2 shows correlation coefficient of concentrations of heavy metals in the soft tissues and shells of T. bitubercularis with those in the sediments in Teluk Kemang. Significant correlation showed in nickel for soft tissues of T. bitubercularis and the ambient sediments ($r = -0.999$, $P<0.05$). Nickel in sediments was found to be highly negatively correlated with the soft tissues indicates that the changes of metal in the sediment result in an identical changes in metal in the soft tissues but the changes are in opposite direction. Highly positive correlation also showed in cadmium for shells of T. bitubercularis and the ambient sediments ($r = 0.999$, $P<0.05$) in Teluk Kemang. This means, the changes of metal in the sediments result in an identical changes in metals in the shell of T. bitubercularis and showed there are stronger relationship between them.

From Table 4.6.3 which shows the correlation coefficient of concentrations of heavy metals in the soft tissues and shells of N. lineata with those in the sediments in Tanjung Harapan, the significant correlation showed in cadmium for shells of N. lineata and the ambient sediments ($r = 1.000$, $P<0.05$). This stronger relationship is the perfect positive correlation which means that changes of metal in the ambient sediments result in an identical change in metal in the shells. Therefore, the use of the shells of N. lineata as a biomonitor of cadmium (Cd) in Tanjung Harapan is supported as well as the use of the soft tissues of T. bitubercularis as a biomonitor of nickel and the use of the shells of T. bitubercularis as a biomonitor of cadmium in Teluk Kemang.

Table 4.6.1 Correlation coefficient of concentrations of heavy metal (Fe, Ni, Cu, Zn, Cd and Pb) in the soft tissues and shells of T. bitubercularis with those in the sediments in Tanjung Harapan.

	Sediments					
	Fe	Ni	Cu	Zn	Cd	Pb
Soft tissues						
Fe	0.558					
Ni		0.280				
Cu			-0.982			
Zn				-0.926		
Cd					-0.464	
Pb						-0.759
Shell						
Fe	-0.049					
Ni		-0.047				
Cu			-0.918			
Zn				-0.956		
Cd					-0.249	
Pb						0.968

Notes: *Correlation is significant at P<0.05.

Table 4.6.2 Correlation coefficient of concentrations of heavy metal (Fe, Ni, Cu, Zn, Cd and Pb) in the soft tissues and shells of T. bitubercularis with those in the sediments in Teluk Kemang

	Sediments					
	Fe	Ni	Cu	Zn	Cd	Pb
Soft tissues						
Fe	0.804					
Ni		-0.999*				
Cu			-0.732			
Zn				0.663		
Cd					0.829	
Pb						-0.947
Shell						
Fe	0.992					
Ni		-0.340				
Cu			0.980			
Zn				0.448		
Cd					0.999*	
Pb						0.356

Notes: *Correlation is significant at P<0.05.

Table 4.6.3 Correlation coefficient of concentrations of heavy metal (Fe, Ni, Cu, Zn, Cd and Pb) in the soft tissues and shells of *N. lineata* with those in the sediments in Tanjung Harapan

	Sediments					
	Fe	Ni	Cu	Zn	Cd	Pb
Soft tissues						
Fe	-0.073					
Ni		-0.631				
Cu			0.067			
Zn				0.771		
Cd					-0.115	
Pb						-0.954
Shell						
Fe	-0.684					
Ni		0.942				
Cu			0.960			
Zn				0.968		
Cd					1.000*	
Pb						-0.373

Notes: *Correlation is significant at P<0.05.

Table 4.6.4 Correlation coefficient of concentrations of heavy metal (Fe, Ni, Cu, Zn, Cd and Pb) in the soft tissues and shells of *N. lineata* with those in the sediments in Teluk Kemang.

	Sediments					
	Fe	Ni	Cu	Zn	Cd	Pb
Soft tissues						
Fe	-0.982					
Ni		0.865				
Cu			-0.134			
Zn				-0.012		
Cd					-0.983	
Pb						-0.337
Shell						
Fe	0.096					
Ni		-0.486				
Cu			0.433			
Zn				-0.285		
Cd					-0.876	
Pb						0.105

Notes: *Correlation is significant at P<0.05.

4.7 Comparisons of metals concentration in molluscs with guidelines and previous
studies

From Table 4.7.1, heavy metals concentration in soft tissues of T. bitubercularis from Tanjung Harapan and Teluk Kemang were found over the maximum permitted proportion of Malaysian Food Act 1983 for the accumulation of copper and zinc. Soft tissues of T. bitubercularis from Teluk Kemang were also found exceeded the Malaysian Food Act 1983 for the accumulation of cadmium. Based on the research by [5], the accumulation of cadmium in N. lineata in Tanjung Harapan also exceed the maximum permitted proportion of Malaysian Food Act 1983 but from this study, stated that N. lineata from Tanjung Harapan does not accumulate cadmium more than the guidelines. This showed that from year 2006 to year 2012, the level of cadmium in Tanjung Harapan has slightly decreased. Soft tissues of T. bitubercularis from Tanjung Harapan and Teluk Kemang exceeded the maximum permitted proportion of Malaysian Act 1983 for copper and zinc where the heavy metals concentration in the soft tissue of the molluscs were found higher in Tanjung Harapan than in Teluk Kemang probably because of the human activities around the sampling sites and slightly difference in geographical areas or habitat of the molluscs as well as the characteristics of the molluscs themselves. Copper can be found in some molluscs as it is a functional part of the respiratory protein haemocyanin whereas copper and zinc are known as essential elements that incorporated into vital biomolecules which play important roles in enzymatic proteins as well as respiratory pigments. Apart from that, since T. bitubercularis is belong to class of neogastropods, according to [30] this molluscs have higher concentration of cadmium compare to grazers where this is because of animal prey of this class of neogastropods. Molluscs that are found at sheltered shore during sampling possessed higher concentrations of metals than those collected from shore that is much being expose where this showed that metal bioavailabilities in that sheltered shore were elevated than in exposed shore [30]. This is true for T. bitubercularis collected from Teluk Kemang where it exceeded the maximum permitted proportion by Malaysian Food Act 1983. Human activities that emit untreated industrial waste into water bodies also contribute to cadmium concentration.

Table 4.7.1 Mean concentrations of heavy metals in soft tissues of molluscs (mg/kg)

Sources	Molluscs (soft tissues)	Heavy metals concentrations (mg/kg)					
		Fe	Ni	Cu	Zn	Cd	Pb
Tanjung Harapan (This study)	*N. lineata*	118.744	3.719	1.510	11.468	0.045	0.145
	T. bitubercularis	400.595	3.569	233.251	372.095	0.935	0.258
Teluk Kemang (This study)	*N. lineata*	369.890	4.084	7.259	7.796	0.055	0.090
	T. bitubercularis	436.638	3.109	50.806	257.018	1.171	0.166
Tanjung Harapan (Yap *et al.*, 2009)	*N. lineata*	-	-	14.9	92.7	1.63	18.1
Pontian (Yap *et al.*, 2009)	*N. lineata*	-	-	12.50	84.70	0.70	7.42
Malaysian Food Act, 1983 (maximum permitted proportio)	Molluscs	-	-	30	100	1	-

5.0 Conclusion

Findings from this study showed that the concentrations of heavy metals in the soft tissue were higher than the shells for Nerita lineata and Thais bitubercularis . Fe is the most abundant metal in the tissue and shell compared to Cu, Zn, Ni, Cd, and Pb. Similar pattern was bserved in the sediments samples. The concentrations of heavy metals in the soft tissues of Thais bitubercularis taken from Tanjung Harapan were higher than Nerita lineata following the order of: Fe > Zn > Cu > Ni > Cd. The metal concentrations in Nerita lineata follow this order: Fe> Zn > Ni > Cu > Cd. The samples taken from Teluk Kemang were lower and exhibited different trend for both organisms. For Nerita lineas, the concentrations were Fe > Cu > Zn > Ni > Cd > Pb while in the Thais bitubercularis the order was Fe> Zn> Cu > Cd > Ni > Pb. For the sediment samples, different pattern was observed. There was evidence of spatial difference where Fe is detected in large amount compared to other metals for both locations. The Fe concentration taken from Teluk Kemang was higher than Tanjung Harapan. The heavy metal accumulation capacity, indicated by the biota–sediment accumulation factors (BSAFs), showed that only Cd has the potential to be accumulated in Nerita lineata whereas for the Thais bitubercularis Cu, Cd, and Zn were accumulated in the soft tissues. Results from this study are useful for further exploration of Thais bitubercularis as accumulators of Cu, Cd, and Zn. For recommendation, more studies on monitoring the concentration level of heavy metals in marine environment should be done regularly and increase numbers of samples use to biomonitor the heavy metals in marine environment as it is important to have information or data regarding the quality of marine environment in order to control pollution such as water pollution from being contaminated with heavy metals. This is essential as the pollutants emit in the marine environment may affect marine lives as well as human's health

References

[1] Shazili, N.M.M., Yunus, K., Ahmad, A. S., Norantonina Abdullah, N. & Abd Rashid, M.K. (2006) Heavy metal pollution status in the Malaysian aquatic environment. Aquatic Ecosystem Health & Management, 9:2, 137-145.

[2] Sany, S.B.T., Salleh, A., Sulaiman, A.B. Sasekumar, A., Rezayi, M., Tehrani, G.M. (2013). Heavy metal contamination in water and sediment of the Port Klang coastal area, Selangor, Malaysia. Environ Earth Sci (2013) 69:2013–2025

[3] Cheng, W. H., Yap, C. K.*, Ismail, A. & Abdul Rahim, I.(2012). Distribution and Concentrations of Ni in Tissues of the Gastropod Nerita lineata Collected from Intertidal Areas of Peninsular Malaysia. Pertanika Journal of Tropical Agriultural Science. 35 (4): 711 - 724

[4] Cheng. H., Yap, C. K. (2009). Heavy metal concentrations in Nerita lineata: The potential as a biomonitor for heavy metal bioavailability and contamination in the tropical intertidal area. Research Gate.1-8

[5] Yap, C. K. & Cheng, W. H. (2009). Heavy metal concentrations in Nerita lineata: the potential as a biomonitor for heavy metal bioavailability and contamination in the tropical intertidal area. Marine Biodiversity Records, 2(1). 1-9.

[6] Bertine, K.K. & Goldberg, E.D., (1972). Trace elements in clams, mussel and shrimp. Limnology and Oceanography. 17,877-884.

[7] M.C. Lin, C.M. Liao, (1999) 65Zn(II) accumulation in soft tissue and shell of abalone Haliotis diversicolor supertexta via the alga Grarcilaria tenuistiptata var. liui and the ambient water, Aquaculture 178 89–101.

[8] Walsh, K., Dunstan, R.H., Murdoch, R. N., Conroy, B.A., Robert, T.K. & Lake, P., (1994). Bioaccumulation of pollutants and changes in population parameters in the gastropod mollusk Austrocochlea constricta. Archieve Environmental Contamination Toxicology, 26, 367-373.

[9] Yap, C.K., Ismail, A., Tan, S.G., & Rahim, A., (2003). Can the shell of the green-lipped mussel Perna viridis from the west coast of Peninsular Malaysia be a potential biomonitoring material for Cd, Pb and Zn. Field and laboratory studies. Estuarine, Coastal and Shelf Science, 57, 623-630.

[10] Cravo, A., Bebiano, M. J. & Foster, P., (2004). Partitioning of trace metals between soft tissues and shells of Patella aspera. Environment International, 30, 87-98.

[11] McCready S., Birch G.F and Long E.R. (2006). Metallic and organic contaminants in sediments of Sydney Harbour, Australia and vinicity – A chemical dataset for evaluating sediment quality guidelines. Environment International, Vol. 32, No. 4, 2006, pp. 455-465.

[12] Osman A.G.M and Kloas W. (2010). Water quality and heavy metal monitoring in water, sediments and tissues of the African Catfish Clarias gariepinus (Burchell, 1822) from the River Nile, Egypt. Journal of Environmental Protection, 2010, Vol. 1. 389 – 400.

[13] Yap, C.K., Ismail, A., Tan, S.G. & Omar, H. (2002). Concentrations of Cu and Pb in the offshore and intertidal sediments of the west coast of Peninsular Malaysia. Environment International, 28, 467-479.

[14] Rainbow, P.S. (1990). Heavy metals in marine invertebrates. In heavy metals in the marine environment, 67-79.

[15] Kamaruzzaman, B.Y., Ong, M.C., Noor Azhar, M.S., Shahbudin, S. & Jalal, K.C.A. (2008). Geochemistry of sediment in the major estuarine mangrove forest of Terengganu region, Malaysia. American J. Applied Sciences 5(12): 1707-1712.

[16] Jarup, L. (2003). Hazards of heavy metal contamination. Br.Med. Bull.68, 167-182.

[17] Alloway BJ (1995). Heavy Metals in Soils 2nd edition, Blackie academic and Professional, Glasgow, p. 500.

[18] Flegal, R. A., and C. C. Patterson, (1983). Vertical concentration profiles of lead in the central Pacific at 15øN and 20øS, Earth Planet. Scœ Lett., 64, 19-32.

[19] ATSDR, Agency for Toxic Substances and Disease Registry (2008). Toxicological profile for chromium. Atlanta, GA: US Department of Health and Human Service, Public Health Service. 1600 Clinton Road N.E, E-29 Atlanta, Georgia 30333 (6-9): 95-134.

[20] Berandah, F. E., Kong, Y. C. & Ismail, A. (2010). Bioaccumulation and Distribution of Heavy Metals (Cd, Cu, Fe, Ni, Pb and Zn) in the Different Tissues of Chicoreus capucinus Lamarck (Mollusca: Muricidae) Collected from Sungai Janggut, Kuala Langat, Malaysia. EnvironmentAsia. 3(1): 65-71.

[21] Chua, T.E., Lorre, I.R., Ross, S.A., Bernad, S.R., Gervacio, B. & Ebarvia, M.C., (2000). The Malacca Straits. Marine Pollution Bulletin, 41, 160-178.

[22] Yap, C.K., Cheng, W. H., Ismail, A., Ismail, A.R. & Tan, S.G. (2010). Biomonitoring of heavy metal (Cd, Cu, Pb, and Zn) concentrations in the west intertidal area of Peninsular Malaysia by using Nerita lineata. Toxicological & Environmental Chemistry, 91:1, 29-41.

[23] Lim, K.K.P., Murphy, D.H., Morgany, T., Sivasothi, N., Ng. P.K.L., Soong, B.C., Tan, H.T.W., Tan, K.S. & Tan, T.K. (2001). Volume 1: The ecosystem and plant diversity and volume 2: animal diversity. In a guard to mangroves of Singapore. BP Guide to Nature Series published by the Singapore Science Centre, sponsored by British Petroleum. Singapore: Raffles Museum of Biodiversity Research, The National University of Singapore & The Singapore Science Centre.

[24] Bu-Olayan, A.H. & Subrahmanyam, M.N.V., (1997). Accumulation of copper, nickel, lead and zinc by snail, Lunella coronatus and pearl oyster. Pinctada radiate from the Kuwait coast before and after the pulf war oil spill.

[25] Hartley, D.M. & Johnston, J.B. (1983). Use of the fresh water calm Corbiculu manilensis as a monitor for organochlorine pesticides. Bulletin of Environmental Contamination and Toxicology, 31, 33-40.

[26] Hamed, M.A. & Emara, A.,M.,(2006) Marine mollusks as bomonitors for heavy metal levels in the Gulf of Suez, Red Sea. Journal of Marine Systems, 60, 220-234.

[27] Yap, C.K., Ismail, A., Tan, S.G. & Omar, H. (2002). Concentrations of Cu and Pb in the offshore and intertidal sediments of the west coast of Peninsular Malaysia. Environment International, 28, 467-479.

[28] Yap, C. K. & Cheng, W. H. (2013). Distributions of heavy metal concentrations in different tissues of the mangrove snail Nerita lineate. Sains Malaysiana, 42(5), 597–603.

[29] Cravo, A., Bebiano, M.J. & Foster, P., (2004). Partitioning of trace metals between soft tissues and shells of Patella aspera. Environment International. 30, 87-98.

[30] Blackmore, G., 2001. Interspecific variation in heavy metal body concentrations in Hong Kong marine invertebrates. Environmental Pollution, 114, 303-311.

[31] Blackmore, G., 2001. Interspeci c variation in heavy metal body concentrations in Hong Kong marine

[32] invertebrates. Environmental Pollution, 114,