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Heavy Metals Bioaccumulation Assessment in *Acanthopleura gemmata* from Fort Jesus Mombasa

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Abstract: *The availability and the levels of heavy metals contamination was assessed in marine mollusk species Acanthopleura gemmata from Fort Jesus, Mombasa Kenya. The levels of heavy metal contamination in flesh and shells of Acanthopleura gemmata were compared with that of marine algae U. Lactuca and U. Reticulata species, water and sediment in its environment. The heavy metals investigated included Fe, Cu, Mn, Zn, Pb, Cr and Cd and were assed using atomic absorption spectroscopy (AAS). The results of this study indicated that the concentrations of all the heavy metals were high in the flesh samples of Acanthopleura gemmata as compared to the algae, shells, water and sediments samples. The concentrations of heavy metals varied with the size of the organism. The smallest Acanthopleura gemmata had the high concentrations of Zn: 347.75 ± 31.66 μgg^{-1}, and Cd: 13.92 ±1.24 μgg^{-1}. The largest Acanthopleura gemmata on the contrary had high concentrations of Fe: 3182.01±41.30 μgg^{-1}, Pb: 67.43±0.81μgg^{-1} and Cu: 53.07±2.87μgg^{-1}. Cr and Mn were below detection limit in flesh samples but the concentrations were 25.71±5.72 μgg^{-1} for Mn and 29.11±8.75 μgg^{-1} for Cr in the shells. The concentration of Cd was below detection limit in algae, water and sediment samples. The findings from this study indicated that Acanthopleura gemmata are good bioaccumulators of heavy metals as compared to water and sediments. The results further showed that metal bioaccumulation is dependent on both the size of the Acanthopleura gemmata and the type of heavy metal*

Keywords: *Chitons, heavy metals, bioaccumulation, AAS, Fort Jesus*

I. Introduction

Heavy metals are metals with density above 5g/cm^3 that can be toxic to the environmental plants and organisms [1]. Heavy metals are broadly classified into essential and nonessential metals. Metals like Cd and Pb are non-essential and toxic at low concentrations while Cu, Zn and Mn are essential metals and are toxic at high concentrations levels [2].These metals occur naturally in the environment. However, human activities have increased their concentrations to levels that can be harmful to both the marine organisms and humans [3], [4], [5].

Heavy metals bioaccumulate in the environment because they are nonbiodegradable [1], [5]. They can bioammulate to concentrations that are harmful to both man and organisms in the environment. Anthropogenic activities such as discharge of industrial waste, sewage waste and solid waste into the environment has been the major contributor to the high levels of heavy metals in the environment in Mombasa city [6], [7], [8].

Heavy metals occur in nature in several particulate forms. They can be bound into particles, precipitated as metal coatings on particles, incorporated in organic matter like algae, can exist as colloids, aggregates or held in crystalline detrital particles. They may occur as dissolved free ions, complexation, chelated with certain inorganic ligands or organic ligands like amines and proteins [1], [2]. The bioavailability of heavy metals in the marine environment is determined by the environmental variables in the ecosystem. These include salinity, pH, redox potential, temperature, organic particulate matter and biological activity [1], [2].

Monitoring of heavy metals pollution is important so as to protect the natural resources and human lives [3]. Heavy metals have been associated with many human disorders like impaired neuropsychological functions and growth retardation amongst other causes, gastrointestinal, asthma, heart and liver problems and reproductive problems amongst others [2], [9], [10], [11], [12].

The use of biomonitors has proved to be effective in the environmental assessment of heavy metals [13]. Marine mollusks such as bivalves and chitons have been used as biomonitors because they are low in the food chain in the marine environment; they live in the same environment from young to adult stage and can accumulate high metals concentrations more than their immediate environment [14], [15], [16], [17], [18], and [12].Bioaccumulation of metals depend on size, sex as well as location of mollusks [20], [21], [22]. *Acanthopleura gemmata* (Figure 1) is a species of mollusks from the class of polyplacophora and are found in rocky shores, commonly known as chitons [23], [24]. The *Acanthopleura gemmata* have minimal movement and exhibit homming behavior. They adhere to rocks using pressure they exert on their ventral foot and belt and they feed on marine algae using their radula [23], [18].

Figure 1: *Acanthopleura gemmata*

Mombasa is the second largest City in Kenya. It is highly affected by large population growth, urbanization and industralisation [25]. These have brought about production of large amounts of waste daily in form of solid waste, industrial and municipal waste [8]. There has been a challenge of proper waste disposal and has resulted in detrimental effects to the environment. Solid waste and sewage waste are disposed off along the shores and has led to pollution of the marine environment [26]. This waste carry toxic chemical amongst them is heavy metals [27], [28], [29], [7].

Therefore, this study aimed at assessing heavy metals bioaccumulation in *Acanthopleura gemmata* and comparing the concentrations with marine algae, water and sediment samples from the environment.

II. Materials And Methods

The current study was carried out in Fort Jesus in Kenya. Fort Jesus is located at Tudor Creek on the North West of Mombasa Island [30].

It is a rocky shore and mainly a tourist's attraction site; the area hosts a museum and also used for water sports events. However it has a municipal sewage disposal point that flows into the ocean.

Figure 2: Sewage disposal at Fort Jesus

2. 1 Sample collection, preparation and analysis

Sampling was done at low tide during the dry season twice. The *Acanthopleura gemmata* sampled ranged from the largest size to the smallest size found at the shore. The samples were collected based on their position on the rocky shore between the upper and lower eulittoral zone at the shore.

The *Acanthopleura gemmata* were handpicked and stored in Ziploc bags. The *Acanthopleura gemmata*were classified into three groups (Small, medium and large). The samples were cleaned with distilled water then the shells were separated from the flesh. The samples were then dried at 60°C, then ground using a mortar and pestle. 0.2g of homegenised flesh sample was accurately weighed using Shimadzu electronic weighing balance (Model TX423L) and transferred into a clean beaker then 10 ml of concentrated nitric acid was added. The sample was heated at a temperature ranging from 70°C to 90°C, until all tissue was digested. The temperature was then gradually increased to 135 °C. Then drops of H_2O_2 were added for further oxidation. Samples were then filtered with filter paper (GF/A) after cooling. Then sample were diluted to 50 ml with double distilled water [15]. This was done in triplicate.

Sampling of water and sediment was done according to EPA [31]. Water samples were put in 100 mL bottles and acidified on the spot. The sediment was scooped using a shovel. Algae samples were handpicked. All samples were stored in plastic Ziploc bags then stored in ice for transportation. Once in the laboratory, the rock and algae samples were cleaned dried at 60°C then ground using a motar and pestle.

Digestion of Shell and sediment samples was done in triplicate according to the United States Environmental Protection Agency (USEPA) method 3050A. Water samples were digested according to USEPA method 3005B and algae samples were digested as described by Sekabira et al. [32]. Analysis was done by GF – 990 Shimadzu flame atomic absorption spectrometer. The method was validated using a certified reference material (CRM)"IAEA (International Atomic Energy Agency) 433"and IAEA 436, for trace elements and methyl mercury in Marine sediments also used by Mwatsahu [33] (Table 1 and 2).

All samples were analysed using GF – 990 Shimadzu flame atomic absorption spectrometer.

Table 1: Mean concentration levels of heavy metals in CRM IAEA 433, and measured values

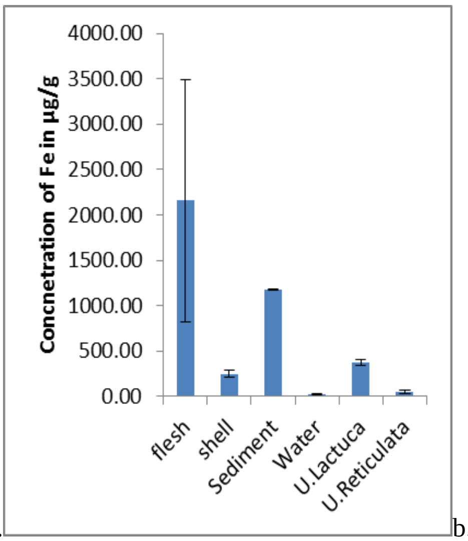
Metal	CRM (µg/g)	Measured (µg/g)	Mean recovery value%
Cu	30.8± 2.6	30.44± 2.4	98.83
Zn	101± 8	100.45 ± 10.33	99.45
Mn	316± 16	315.55± 21.7	99.86
Cd	0.153± 0.033	0.148 ± 0.014	96.73
Cr	136 ± 10	134.87± 10.99	99.65
Pb	26± 2.7	25.91± 2.7	99.65

Table 2: Mean concentration levels of heavy metals in CRM IAEA 436, and measured values

Metal	CRM (µg/g)	Measured (µg/g)	Mean recovery value %
Cu	1.73±0.19	1.69±0.13	98.84
Zn	19.0±01.3	18.88±1.44	99.37
Mn	0.238±0.042	0.236±0.64	99.15
Cd	0.052±0.007	0.051±0.006	98.07
Cr	0.194±0.058	0.193±0.071	99.48

III. Results And Discussion

The levels of heavy metals in *Acanthopleura gemmata* was higher for all metals except for Mn as compared to the other samples while sea water showed the lowest concentration levels in all metals. Fe had the highest concentration levels in all samples compared to the other metals. Fe was found to have the highest concentration in the flesh then the sediment samples. The concentration levels of Fe in *Acanthopleura gemmata w*as between 3182.01±41.30µg/g and 643.03±124.89µg/g for flesh samples while in the shells the concentration range was between 277.70±54.19 µg/gand 201.63±9.09µg/g.



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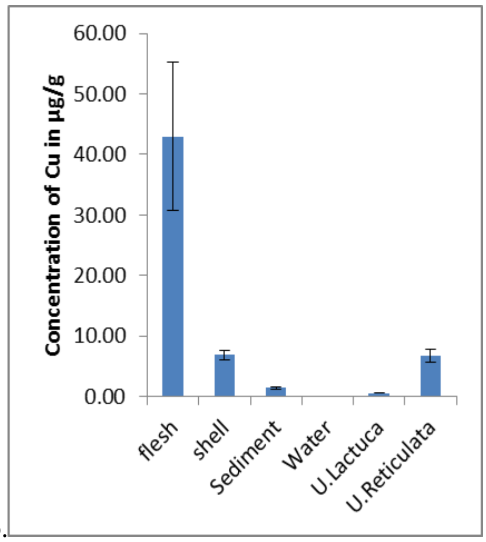

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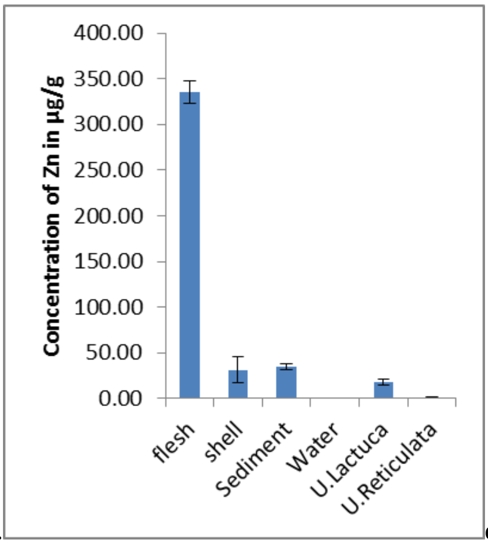

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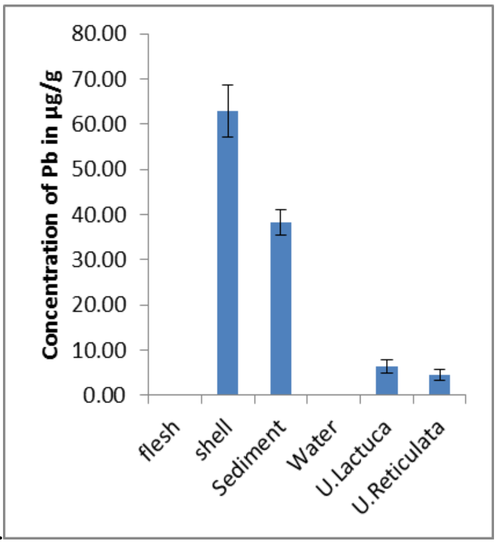

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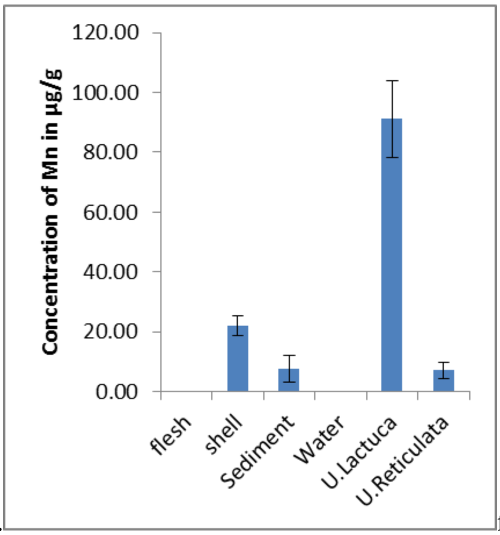

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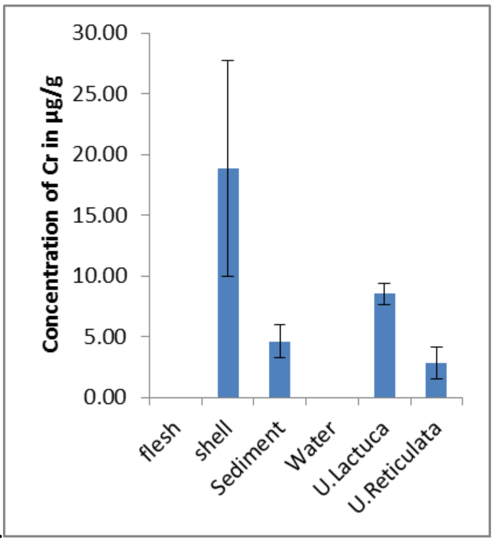

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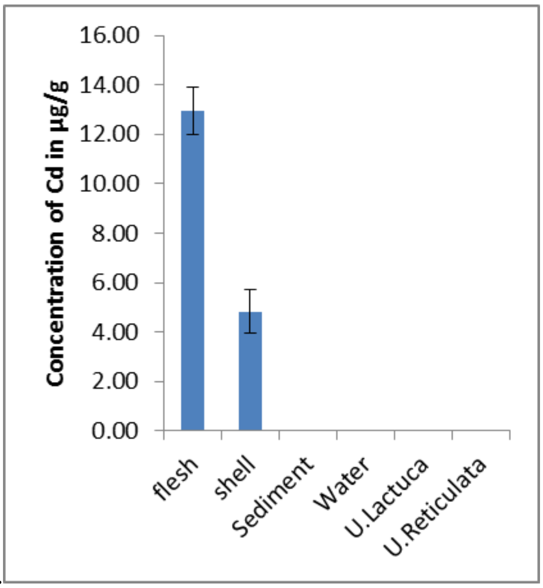


Figure 3 a., b., c., d., e., f., g.,: A comparison of concentration of metals between *Acanthopleura gemmata*and sediment, water and algae samples

Fe is an abundant metal in the earth's crust it is also bonded in sediments. Fe also had the highest concentration levels in the water, algae and sediment sample. Iron can be transported from different places like cultivated land and rock quarries. It can be transported through erosion during rainy season and storm water runoff [34].The high concentration levels of Fe could also be as a result of municipal waste disposal or the nature of study area. The study site had so much debris of rusted metals; these metals are due to the historic background. This area was used as a shipping dock, a prison and was also a war zone by the Portuguese.

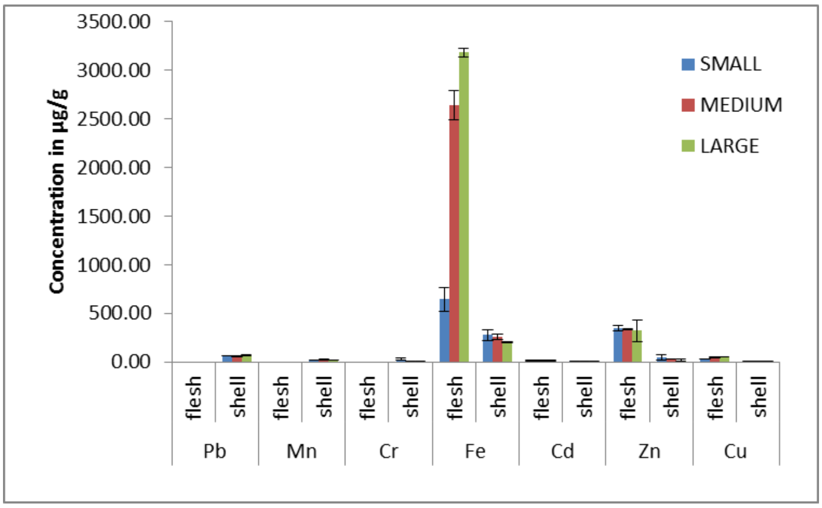
Fe, Cu and Zn are important for metabolic functions in the marine organisms, therefore the high concentrations of these metals in the *Acanthopleura gemmata* could be as a result of high usage in the organisms. Moreover, the high levels of iron in *Acanthopleura gemmata*could also be because they posses' radulae made of magnetite (iron oxide), which they use to scrap off algae from the rocks [18].

Very low concentrations were observed for Cr and Mn in the flesh of *Acanthopleura gemmata*. However, the levels were high in the shells, *U.lactuca* and sediment samples. These metals in the environment are minimised due to good clean up by industries before waste release to the environment. *U.lactuca* like many marine algae, tend to accumulate high metal concentrations from sea water at high concentration levels more than water in their environment thus reflecting the heavy metals concentration in sea water [35].

The shells and sediment samples had high concentration levels of Pb. Pb is not metabolized in the flesh because it is toxic. It however, has a tendency of competing with calcium carbonate in the shell in form of aragonite which is isostructural leading to the formation of lead carbonate. Therefore Pb can replace the calcium in the shells especially under low pH [36], [20]. Furthermore, sediments act as sinks for most toxic metals like Cr, Pb and Cd. They can be bound into particles or precipitated as metal coatings on particles [1], [27].

Acanthopleura gemmata had high concentrations of Cd for both flesh and shell samples. The concentration levels was higher in the flesh sample at 13.92µg/g this is higher than the allowed limits by WHO where the maximum value should be 1 µg/g on edible shell fish [37].High levels of Cd can cause increased mortality, growth reduction and also affects the respiratory and muscle functions. It has been observed to accumulate in the liver and kidney of marine organisms [38].

Weight gain and age have been suggested to affect metal concentration in marine mollusk species [15]. This is true for Fe, Cu and Zn which showed a strong correlation (0.93-0.99) between concentration of metals in the flesh samples and the size of the mollusks. This means that the concentration levels of metals in the flesh samples increased with increase in size of the mollusks. Therefore bioaccumulation of this metals is high in the larger organisms compared to the small organisms.



However Cd, showed a low negative correlation of -0.52 meaning that the metal concentration levels is not dependant on size gain of the organisms. Furthermore, this metal has no purpose in the metabolic processes in the organisms body [1]. Pb, Cr and Mn concentration had no relationship with the size of organism. This is because their concentration levels in the flesh of the *Acanthopleura gemmata*were below detection limit in the flesh samples.

In the shell samples Cd and Zn had a positive strong correlation of 0.97 and 0.99 respectively, while Cr and Fe had a negative correlation of -0.89 and -0.93 respectively between concentration and size. This shows that these metals concentration levels is affected by weight gain for Cd and Zn while Cr and Fe concentration decrease with size of shell. Cu, Mn and Pb concentration level had very low correlation with size of shell.

IV. Conclusions

The findings from this study indicated that *Acanthopleura gemmata* are good accumulators of heavy metals as compared to water and sediments. The results further showed that metal bioaccumulation is dependent on both the size of the *Acanthopleura gemmata* and the type of heavy metal. Sediment samples had the highest concentration of all metals except Mn and Cu. Of the algae species analysed *U.lactuca* had the highest concentration of all the metals except for Cu.

Based on these findings, continuous monitoring of pollution of the environment is important so as to protect the marine environment. The use of *Acanthopleura gemmata*as biomonitors can be implemented because they can accumulate high metals level. Findings in this work could be a baseline for future assessments of heavy metals pollution along the Kenyan coast. A study can be done to determine the efficiency of Chiton shells in the removal of toxic metals from the environment.

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References

[1]. Duffus J. H., (2002) "Heavy Metals" – A meaningless term. Pure and Applied Chemistry, 74: 793-807.
[2]. Förstner U. and Wittmann G. T. W., (2012) Metal Pollution in the Aquatic Environment. Springer Science & Business Media, second edition Science – P 1-25.
[3]. UNEP/GPA (2006), The State of the Marine Environment: Trends and Processes. UNEP/GPA,The Hague.
[4]. Baby j., Raj J. S., Biby E. T., Sankarganesh P., Jeevitha M. V., Ajisha S. U. and Rajan S. S., (2010) Toxic Effects of Heavy Metals on Aquatic Environment. International Journal of Biological and Chemical Science 4(4): 939-952
[5]. Naser A. H., (2013) Assessment and Management of Heavy Metal Pollution in the Marine Environment of the Arabian Gulf: A Review. Marine Pollution Bulletin 72: 6–13.
[6]. Pheby D., Grey M., Giusti L. and Saffron L., (2002) Waste Management and Public Health: the State of the Evidence. A Review of the Epidemiological Research on the Impact of Waste Management Activities on Health. South West Public Health Observatory.
[7]. Matoka C. M., Omolo S. O. and Odalo J. O., (2014) Heavy Metal Bioaccumulation as Indicators of Environmental Pollution and Health Risks. Journal of Environmental Science, Toxicology and Food Technology, 8(2):24-31.
[8]. .Government of Kenya, (2009) State of the Coast Report, Towards Integrated Management of Coastal and Marine Resources in Kenya. National Environmental Management Authority, (NEMA) Nairobi 88pp.
[9]. WHO, (1981) Environmental Health Criteria. Manganese.
[10]. WHO, (1988) Environmental Health Criteria. Chromium.
[11]. WHO, (1998) Environmental Health Criteria. Copper.
[12]. WHO, (2001) Environmental Health Criteria. Zinc.
[13]. Yarsan E., Yipel M., (2013) The Important Terms of Marine Pollution "Biomarkers and Biomonitoring, Bioaccumulation, Bioconcentration, Biomagnification". Journal of Molecular Biomarkers & Diagnosis S1: 003. doi:10.4172/2155-9929.S1-003.
[14]. Ali A. A. and Bahroon A. A., (2000) Bioaccumulation of Iron and Lead in the Soft Tissues of Some Marine Mollusks and Associated Sediment at Labour Island, Aden, Yemen. University of Aden Journal of Natural and Applied Science, 4(1): 81-88.
[15]. Otchere F.A., (2003). Heavy Metals Concentrations and Burden in the Bivalves (*Anadara (Senilia) senilis, Crassostreatulipa and Pernaperna*) from Lagoons in Ghana: Model to Describe Mechanism of Accumulation/Excretion. African Journal of Biotechnology, 2(9): 280-287.
[16]. Ugolini A., Ruwa R. K., Baroni D. and Borghini F., (2006) *Talorchestiamartensii* (Weber) (Amphipoda, Talitridae) as a Bioindicator of Heavy Metals Contamination of Kenyan Sandy Shores. Biologia Marina Mediterranea 13(2):256-257..
[17]. Zhou Q., Jianbin Z., Jianjie F., Jianbo S. and Guibin J., (2008) Biomonitoring an appealing tool for assessment of metal pollution in aquatic ecosystem. Analytica chimica Acta 606: 135-150.
[18]. Shaw J., Clode P., Brooker L. and Maker G., (2009) The Chiton Fauna of the Swan River Estuary and their Potential Role as Indicators of Environmental Contamination. Swan Canning Research and Innovation Program, Final Report
[19]. Singh K.Y. T., Krishnamoorthy M. and Thippeswamy S., (2012) Seasonal Variations of Cu, Pb, Fe, Ni and Cr in the Edible Wedge Clam, Donax faba (Mollusca, Bivalvia) from the Padukere Beach. Journal of Theoretical and Experimental Biology, 8:3 and 4: 95-1 Shaw J., Clode P., Brooker L. and Maker G., (2009) The chiton fauna of the Swan River Estuary and their potential role as indicators of environmental contamination. Swan Canning Research and Innovation Program, Final Report.
[20]. Hashem A. M., (2005) Distribution and relationships of heavy metals in giant clam (*Tridacna* maxima) and associated sediments from different sites in the Egyptian red sea coast. Egyptian journal of Aquatic research, 31(2): 45-59.
[21]. Burger J. and Gochfeld M., (2006) Locational Differences in Heavy Metals and Metalloids in Pacific Blue Mussels *Mytilus [edulis] trossulus* from Adak Island in the Aleutian Chain, Alaska. Science of the Total Environment, 368: 937 - 950.
[22]. Amisah S., Adjei-Boateng D., Obirikorang K. A. and Quagrainie K. K. (2009) Effects of Clam Size on Heavy Metal Accumulation in Whole Soft Tissues of *Galatea paradoxa* (Born, 1778) from the Volta Estuary, Ghana. International Journal of Fisheries and Aquaculture, 1(2):014-021.
[23]. Kaas P. and Belle R. A., (1985) Monograph of Living Chitons (Mollusca: Polyplacophora). E.J. BRILL Publishers 3: 23-24
[24]. Schwabe, E. (2015). Acanthopleura gemmata (Blainville, 1825). In: MolluscaBase (2015). Accessed through: Odido, M.; Appeltans, W.; BelHassen, M.; Mussai, P.; Nsiangango, S.E.; Vandepitte, L.; Wambiji, N.; Zamouri, N. Jiddou, A.M. (Eds) (2015) African Register of Marine Species at http://www.marinespecies.org/afremas/aphia.php?p=taxdetails&id=211919 on 2015-11-21
[25]. Kenya Bureau of National Statistics (KSNB), (2009) www.knsb.or.ke/index.php?option=com_content&view=article&i=176&Itemid=645
[26]. Mwaguni S., Munga D. (1997), Land Based Sources and Activities Affecting the Quality and Uses of the Marine Coastal and Associated Freshwater Environments along the Kenya Coast. Coastal Development Authority, Mombasa.
[27]. Kamau J. N. (2002) Heavy Metal Distribution and Enrichment at Port-Reitz Creek, Mombasa. Western Indian Ocean Journal of Marine Science 1: (1)65-70.

[28]. Mwashote B. M., (2003) Levels of Cadmium and Lead in Water, Sediments and Selected Fish Species in Mombasa, Kenya. Western Indian Ocean Journal of Marine Science, 2(1): 25-34

[29]. Okuku E. O., Ohowa B., Mwangi S.N., Munga D., Kiteresi L., Wanjeri V. O., Okumu S. and Kilonzo J., (2011) Sewage pollution in the Coastal waters of Mombasa City, Kenya: A norm Rather than an Exception. International Journal of Environmental Resources, 5(4): 865-874.

[30]. Mohamed O. S., Neukermans G, Kairo J. M., Dahdouh-Guebas F. and Koedam N., (2009) Mangrove Forests in a Peri-urban Setting: the Case of Mombasa (Kenya). Wetlands Ecology and Management, 17:243–255.

[31]. EPA (2000) A Guide to the Sampling and Analysis of Waters, Wastewaters, Soils and Wastes, P 441.

[32]. Sekabira H., Oryem – Origa G., Mutumba K. and Basamba T. A., (2011). Heavy Metal Phytoremediation by *Commelinaheghalensis* (L) and Cyanodondactylon (L) Growing in Urban Stream Sediments. International Journal of Plant Physiology and Biochemistry, 3(8):133-142.

[33]. Mwatsahu S., (2013) Is an Assessment of Heavy Metals in Water, Sediments, and Fauna of Selected Areas along the Kenyan Coastline. A thesis submitted for Msc. Science in Applied Analytical Chemistry in the School of Pure and Applied Science of Kenyatta University.

[34]. Adu O. K., (2010) An Assessment of Heavy Metals Contamination of Sediments and Tissues of the clam *galatea paradoxa* (born 1778) in the Volta Estuary, Ghana. MPHIL Thesis, Fisheries and Water Shed Management.

[35]. Abuagla Y. (2013). Marine Algae as Biomonitors for Heavy Metals Pollution at the Red Sea Coast:A review Red Sea University Journal. Refereed scientific biannual Journal Vol.(3)

[36]. Prakash N. T., Naidu T. S. and Rao K. S. J., (1994a) Metal content in selected tissues and shells of Pernaviridis (L) from east coast of India. Chemistry and Ecology, 9: 1-6.

[37]. FAO/WHO (1984). List of maximum levels recommended for contaminants by the joint FAO/WHO Codex *Alimentarius Commission*, 3:1-8

[38]. Chiarelli R. and Roccheri C. M., (2014) Marine Invertebrates as Bioindicators of Heavy Metal Pollution. Open Journal of Metal, 4: 93-106