IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 12, Issue 8 Ver. I (August. 2018), PP 56-63
www.iosrjournals.org

Analysis of Bio-Accumulation of Heavy Metals in Seaweeds *Ulva rigida* and *Halimeda opuntia* in Validation of Their Safety For Use in Aquaculture Feeds in Kenya

[1]Mutia, G.M*. [2]Mtolera S. P. Matern
[1]*Department Of Fisheries Management And Aquaculture Technology, South Eastern Kenya University, Kenya*
[2]*Institute Of Marine Sciences, Zanzibar, Tanzania*
Corresponding Author: Mutia, G.M

Abstract: *With capture fisheries output being threatened by overfishing and dwindling stocks, a long term vision for the supply of fish to the worlds growing population is aquaculture. However, the issue of fish feeds remains one of the challenges facing the aquaculture sector where the sources for most of the ingredients used for fish feed formulation are the same as those used for human consumption. Hence, a strategic focus for aquaculture must be to derive new sources, primarily taken from outside the human food chain, and to derive them mainly from primary producers for example marine algae (seaweeds). However, the use of seaweeds as animal feed is, among others, determined by their heavy metal status. U. rigida and H. opuntia are some of the seaweed species primarily used in artisanal fishery at the south coast of Kenya as fishing baits and have the potential for use as supplement ingredients in the formulation of fish feed for the widely farmed Nile tilapia. For purposes of determining their safety for use in fish feeds, a study was carried out to determine the concentration levels of Arsenic, Cadmium, Lead and Mercury. The heavy metals were investigated with respect to seasonal and site variations. Seaweed sample analysis was done following Atomic Absorption Spectroscopy. The values obtained were compared to the World health Organization (WHO) recommended levels for heavy metals in food and feed supplements. The concentration levels were significantly different ($p<0.05$) in the two seaweed species, varied with seasons and sites and compared variably with the WHO recommended levels.*

Key words: *Aquaculture, Fish feeds, Halimeda opuntia, Heavy metals, Seaweeds, Ulva rigida*

Date of Submission: 03-08-2018 Date of acceptance: 21-08-2018

I. Introduction

Aquaculture is one of the rapidly growing sectors which is an essential source of food, livelihood and income for millions of people around the world. According to (1) aquaculture production globally was estimated at 97.2 million tonnes in 2014 with a value of USD 157 billion. One of the major contributors to the drastic growth of the aquaculture sector is the development of the fish feed industry. The quality of feed contributes significantly to the level of productivity and the quality of the carcass. Good quality feeds lead to good productivity and first-rate fish and fishery products. The composition of the final product is particularly important in aquaculture given that fish consumption has been recommended as an excellent source of proteins that are preferable than terrestrial sources. In this sense, aquaculture nutrition plays an important role in the production of a valuable food for human consumption. However, the fish feed sector is faced with challenges of sources of ingredients especially sources of crude protein and lipids that are similar to those in the human food industry. This competition has led to the search for other sources of crude protein and lipids that are not in the human food industry (2). Therefore, seaweeds have been fronted as good sources of crude protein that are not in competition with the human food industry especially in Kenya. The usefulness of seaweeds as a dietary ingredient for fish feeds has been investigated by (3). The authors suggested that seaweeds such as *Gracilaria bursa-pastoris*, *Ulva rigida* and *Gracilaria cornea* have great potential as alternative ingredients in diets for European sea bass juveniles and milk fish. Additionally, studies and feeding trials on the utilization of seaweeds as a source of protein in fish feeds is an ongoing activity worldwide.

Seaweeds are one of the important contributors to primary productivity in the coastal ecosystems. A number of seaweed species are of high economic value for their significant quantities of lipids, first class proteins, essential vitamins, carbohydrates and minerals (4). They have been shown to be useful as dietary ingredients in marine and freshwater fish feeds (3). However, they also have the capacity to accumulate heavy metals dissolved in seawater due to the ability to bind metals to molecular groups present in their tissues (5). The accumulation of heavy metals in the thallus of the seaweeds reflects the bioavailability of the metals in the respective sites as well as the capacity of the seaweeds to take them up (6). Heavy metal contamination is therefore an aspect that can affect the safety of edible seaweeds as a food or as an ingredient in animal feed formulations.

Some seaweed species have relatively long life cycles and they can reflect the short term fluctuations in the heavy metal concentration of their environment. *Ulva* and *Halimeda* species are considered as good indicator species as they can accumulate many types of metals in their tissues (7) hence the need to determine their safety for use in aquaculture feeds. The presence of Arsenic, Pb, Hg and Cd in the environment is a major concern because of their toxicity to flora and fauna (8). The levels of arsenic have been shown to be higher in the aquatic environment than in most areas of land as it is fairly water soluble and may be washed out of arsenic-bearing rocks. Seaweeds in particular are known to contain high concentrations of arsenic in comparison to terrestrial plants owing to the ability of marine plants to concentrate the arsenic they derive from sea water (9). Higher levels of arsenic are thus expected in the aquatic food chain than in terrestrial food chain and these higher levels result in the potential for elevated dietary exposure through diets rich in fish and other food harvested or farmed at sea (9). Hence this study was done to ascertain how much of Arsenic, Pb, Hg and Cd the three seaweeds have bio-accumulated at the Kenya coast in comparison to the acceptable limits.

II. Materials And Methods

2.1 Study Area

The study was carried out at Diani beach (4^{0}27'S and 39^{0}59'E) and Gazi bay (4^{0}25'S and 39^{0}30'E) (Fig 1) located at the south coast of Kenya. Gazi Bay is a tropical, semi-enclosed, shallow bay characteristic of the creeks and bays along the East African coastline. It's an estuarine ecosystem that is drained by two main rivers, Kidogoweni River on the North-West and Mkurumuji River on the South-West side of the bay that bring in fresh water into the bay. Diani on the other hand is a typical coastal shore exposed to the open ocean. It is long and narrow, separated from the main body of the Indian Ocean by a fringing coral-reef platform about 0.9 km wide consisting of mainly dead coral with rubble and living coral. *H. opuntia* and *U. rigida* species of the class chlorophyceae were collected from the two sites. Sample collection was done monthly for one year considering the wet and dry months.

2.2 Collection and processing of seaweed samples

U. rigida and *H. opuntia* were handpicked from the sites (ensuring that a complete plant was collected as far as possible along with the hold fast). The seaweed samples were kept in polyethylene bags and containers, properly labelled, and immediately transported in a cool box to the laboratory where they were stored at -18 ºC until further analysis. The samples were cleaned of epiphytes, epifauna, pebbles and other molluscan shells in seawater and washed at least five times in running fresh water, gently blotted dry and fresh gross weights recorded to an accuracy of 0.1 g. At least 10 samples were randomly selected and pooled for each species, which included both vegetative and reproductive material. Dry weights were recorded after drying the samples at 70^{o}C (in hot air oven) to a constant weight.

2.3 Digesting of Seaweed for Heavy Metal Analysis

This was done following AOAC methods (10). A porcelain crucible with a capacity of 50ml was oven dried for approximately 30 minutes at 105^{0}C. The crucible was then cooled in a desiccator and weighed. Approximately 2 g of seaweed powder of each of the 2 seaweed species was weighed accurately into the crucible and dried in an oven at 105 °C overnight (16 hours). Then, the crucible and contents were cooled in a desiccator, weighed and then charred slowly under a watch glass on a hotplate for about one and half hours. The crucible and contents were then placed in a cool muffle furnace <100 °C) and the temperature raised slowly (about 100 °C per hour) to 420 ± 5 °C. After muffling, the crucible and contents were cooled and weighed to determine the weight of crude ash. The crucible was covered with a watch glass and the ash was moistened with 1-2 drops of distilled water. 3 ml of 5 M hydrochloric acid was pipetted under the lip of the watch glass with care to avoid any loss by effervescence. The covered crucible was then warmed on a boiling water bath for 30 minutes. The cover was rinsed and removed, 0.2 ml 15 M nitric acid added and the solution evaporated to dryness. The crucible was then placed in an oven at 105^{0} C for 1 hour to complete the dehydration. The dried salts were moistened with 2 ml 5 M HCl. 10 ml of distilled water was added and warmed on a boiling water bath until all salts were in solution (about 10 minutes).The solution was filtered through a Whatman No. 44 filter paper into a 250 ml volumetric flask and the insoluble residue was transferred using a rubber tipped glass rod. The filter paper was rinsed with warm 0.02 N HCl followed by distilled water. The filter paper was discarded and the solution made to volume with distilled water. The absorbance was then determined using a computerized Hitachi Model 170-10 type atomic absorption spectrophotometer. Blank solutions were prepared similarly. The methodology was tested using standard solutions for each of the four heavy metals.

2.4 Determination of physico-chemical parameters

Air/water temperature, pH and salinity were measured in-situ using a thermometer, pH meter (HANNA Instruments, Canada, Model no. HI 8424), and salinometer (Sper Scientific LTD, USA, Model no. 300011) respectively. Three replicate measurements were taken at each site, where the seaweeds were collected at a depth of between 0.1 -0.3m during the low spring tides. Precipitation data for the period of study was obtained from the Kenya Meteorological Department. Major seawater nutrients determined were nitrate and phosphate. Three replicate water samples for nutrient analysis were collected in polyethylene bottles (prewashed in acid) along the transect line sampling points and stored frozen prior to analysis. All chemicals used were of analytical grade and all the glassware were acid pre-washed before use. For all the analysis, procedural blanks were included. The accuracy and the consistency of the analytical procedure were determined by analyzing check standards which had an absorbance at the middle range of the calibration curve.

2.5. Statistical Data Analysis

Data analysis was done using computerized statistical programme (STATISTICA 8.0, 2007). The data were subjected to student's t-test for independent samples and the significant differences in heavy metal concentration level accepted at $p \leq 0.05$.The heavy metal contents below the sensitivity threshold of the instrument were recorded as not detectable as they could not be measured.

III. Figures And Tables

Fig 1 shows the two study sites Gazi bay and Diani beach. Fig 2 shows the variations in concentration levels of Cd, As, Pb and Hg in the species *H. opuntia.* There were significant difference in concentration of the four heavy metals at Gazi bay and Diani beach *(*$p \leq 0.05$). Gazi bay registered the highest concentration levels of the four heavy metals. The highest concentration levels of Cd were recorded in October and February both at 0.4±0.03 mg kg^{-1} DW. Arsenic levels were highest in December at 6.89±0.11 mg kg^{-1} DW, Pb in December at 4.08 mg kg^{-1} DW while that of Hg was highest in November at 4.08±0.36 mg kg^{-1} DW. Fig 3 shows the concentration levels in the species *U. rigida* with respect to sites and months. With the exception of arsenic, the other three heavy metals were not detected in *U. rigida*. The highest arsenic levels were recorded in samples collected from Diani beach at 5.95±0.18 mg kg^{-1} DW. TABLE 1 and 2 shows the correlation coefficient between physico-chemical variables and heavy metal concentration in *H. opuntia* and *U. rigida.*

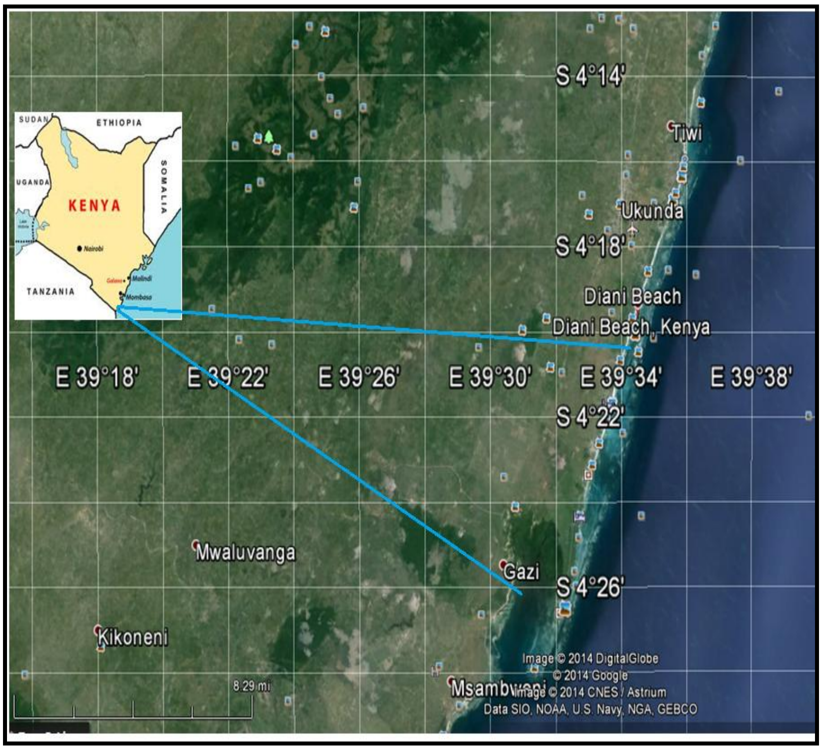

Figure 1: map of the south coast of Kenya showing the two sampling sites Gazi bay and Diani beach.

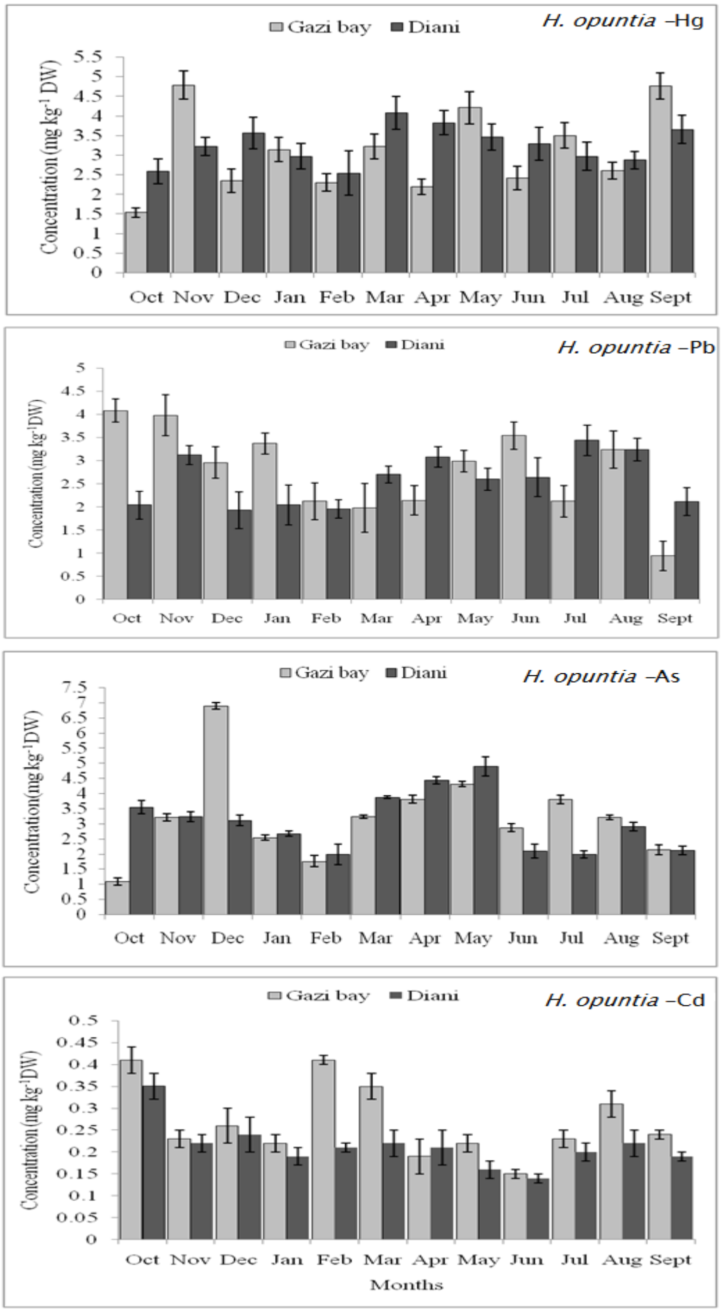

Figure 2: variations in concentration levels of cadmium, arsenic, lead and mercury in *H. opuntia* with respect to sites and months

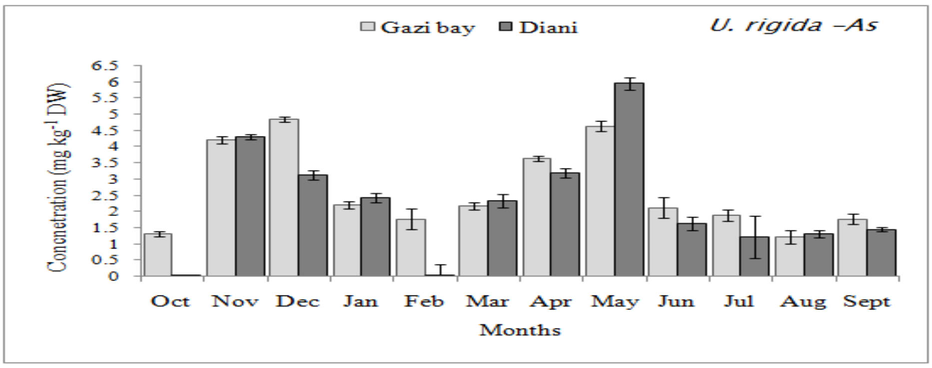

Figure 3: variations in concentration levels of arsenic in *U. rigida* with respect to sites and months

Table 1: Correlation coefficient of physico-chemical parameters and heavy metal concentration in *H. opuntia*

Environmental variables in Gazi bay	Arsenic	Cadmium	Lead	Mercury
Salinity	-0.211	-0.314	-0.131	0.178
Water temperature	-0.119	0.265	-0.511	0.028
pH	-0.372	0.040	-0.529	-0.192
Nitrates	0.456	-0.360	0.021	0.279
Rainfall	0.344	-0.024	0486	0.132
Phosphates	0.344	-0.426	-0.201	0.243

Environmental variables in Diani	Arsenic	Cadmium	Lead	Mercury
Salinity	-0.768*	0.271	0.073	0.073
Water temperature	0.271	0.283	-0.529	-0.529
pH	-0.376	0.444	0.105	0.105
Nitrates	0.346	-0.407	0.133	0.133
Rainfall	0.620*	-0.464	0.269	0.268
Phosphates	0.626*	-0.328	0.357	0.357

Environmental variable	Gazi bay	Diani
Salinity	-0.499	-0.481
Water temperature	0.094	0.291
pH	-0.768*	-0.624*
Nitrates	0.395	0.443
Rainfall	0.276	0.546
Phosphates	0.074	0.461
Biomass	0.448	0.175

Table 2: Correlation coefficient of physico-chemical parameters and arsenic concentration in *U.* rigida

Site		Changes in physico-chemical parameters					
Gazi bay	Month	Salinity	water temp (^{0}C)	pH	Rainfall (mm)	Nitrates (mg/L)	Phosphates (mg/L)
	Oct	27.7±0.3	29.1±1.00	8.0±0.15	141	0.0213±0.0020	0.008±0.0005
	Nov	27.3±3.8	30.4±0.90	7.8±0.09	181	0.0257±0.0012	0.008±0.0005
	Dec	28.7±1.8	30.8±1.40	7.8±0.06	67	0.0233±0.0019	0.005±0.0041
	Jan	34.3±0.7	32.7±0.85	7.8±0.42	17	0.0150±0.0015	0.007±0.0005
	Feb	33.3±1.3	33.4±0.68	8.1±0.07	14	0.0147±0.0012	0.006±0.0005
	Mar	33.7±0.3	33.4±0.70	8.1±0.15	30	0.0183±0.0003	0.012±0.001
	Apr	27.0±1.0	29.8±2.22	7.9±0.12	158	0.0283±0.0032	0.012±0.003
	May	26.7±0.3	28.6±1.48	7.8±0.07	311	0.0367±0.0029	0.013±0.001
	Jun	33.3±1.7	28.6±0.81	8.0±0.09	108	0.0263±0.0058	0.011±0.001
	Jul	34.7±0.3	29.6±0.06	8.1±0.09	56	0.0217±0.0007	0.009±0.0005
	Aug	34.0±0.6	29.2±0.12	8.1±0.03	81	0.0220±0.0015	0.009±0.0015
	Sept	33.7±0.7	30.4±0.22	8.0±0.03	76	0.0270±0.0006	0.009±0.0005
Diani	Oct	32.3±0.7	29.1±0.03	8.0±0.03	112	0.0170±0.0006	0.004±0.0005
	Nov	33.3±0.7	30.9±0.13	7.9±0.06	118	0.0203±0.0007	0.005±0.0005
	Dec	33.3±0.3	31.7±0.37	7.9±0.13	69	0.0193±0.0007	0.002±0.0010
	Jan	34.0±0.6	31.7±0.35	8.2±0.01	37	0.0100±0.0000	0.002±0.0005
	Feb	34.0±0.6	32.7±0.35	8.3±0.03	18	0.0110±0.0010	0.002±0.0005
	Mar	32.7±0.7	33.1±0.06	8.3±0.03	49	0.0113±0.0007	0.002±0.0005
	Apr	32.0±0.6	30.7±0.33	7.9±0.07	184	0.0153±0.0007	0.005±0.0010
	May	32.0±1.0	29.7±0.35	8.0±0.09	238	0.0287±0.0003	0.010±0.0005
	Jun	33.0±1.0	28.4±0.32	8.0±0.01	84	0.0237±0.0015	0.002±0.0010
	Jul	34.0±0.0	28.0±0.03	8.3±0.03	64	0.0153±0.0012	0.002±0.0005
	Aug	34.3±0.7	28.0±0.03	8.3±0.06	60	0.0143±0.0003	0.003±0.0005
	Sept	34.0±0.6	28.7±0.33	8.3±0.03	54	0.0143±0.0007	0.002±0.001

Table 3: The mean (±SE) results of physico-chemical parameters in sea water from Gazi bay and Diani beach

IV. Discussion

The findings in this study indicate that *H. opuntia* collected from Diani beach and Gazi bay has accumulated varying levels of the four heavy metals total arsenic, lead, mercury and cadmium. *U. rigida* on the other hand had arsenic as the only heavy metal detected. Thus, significant differences in As, Pb, Cd and Hg between the two seaweed species, the months and sites and sites of collection were recorded. This is in agreement with (11) and (12) who found significant variations in heavy metal concentration among different species of seaweeds, seasons of research and sites of seaweed collection. The differences in heavy metal concentration could be attributable to the structural differences between the two seaweed species as noted by (13) and (14) and the physico-chemical parameters such as salinity, temperature, pH, light, nutrient concentration.

It has been shown that unlike several other bio-indicators of heavy metal bio-accumulation such as filter-feeding animals, seaweeds accumulate only heavy metal ions that are dissolved in the seawater (15) and (16). Hence, the finding of the heavy metals As, Pd, Hg and Cd particularly in *H. opuntia* reflects the bioavailability of the metals in Gazi bay and Diani beach as well as the capacity of the seaweed species to take them up (17). Furthermore, the variations in As, Pb, Hg and Cd in the two seaweed species can also be attributed to the ability of seaweeds of binding metals to molecular groups present in their tissues (18). The results also indicate that total arsenic was detected in the two seaweed species *H. opuntia* and *U. rigida*. This is supported by (19) who pointed out that arsenic is a naturally occurring metalloid element in the earth's crust and is very widely distributed in the aquatic and terrestrial environments. Other studies have proposed that the total arsenic in the earth's crust ranged from 45 to 3275 mg kg^{-1} (20) hence the possibility of its availability in aquatic environments and bio-accumulation in seaweeds. Essentially, seaweeds have been shown to be primary accumulators of arsenic in the marine environment and an important stage of its metabolism through the marine food chain (21).

The differences could also be a reflection of the effects of seaweed morphology in heavy metal accumulation as well as the variations in the affinities of different seaweed species for different heavy metals (22). In this case, it is noted that the jointed calcareous *H. opuntia*, appear to have accumulated higher levels of arsenic, lead, mercury and cadmium in comparison with sheet-like species such as *U. rigida* which had undetectable levels of the heavy metals. This corroborates (23) findings that showed individual plants differ in terms of their arsenic uptake capacity. There significant differences in total arsenic content observed between the wet and dry months is in agreement with (24) who observed that seasonal variability in heavy metal accumulation may result from either internal biological cycles of the organism or from changes in the availability of the metals in the environment of the organism. (25) noted this phenomenon and attributed this to be as a result of precipitation that facilitates the dilution of sediment solutions and seawater especially within the intertidal zone during the wet season.

With regard to mercury, seaweeds have been shown to have a relatively high affinity for the heavy metal. In the current study, while Hg was not detectable in *U. rigida*, the highest concentration was recorded in *H. opuntia*. The high Hg found corroborates with (7) study, who found the highest concentration of mercury in *H. opuntia* among several seaweed species studied. The ability of *H. opuntia* to concentrate high levels of heavy metals including mercury was also noted by (26) who attributed the capability to its cell structure and high affinity for metals. The results indicate significant difference between the wet season and dry season in mercury content in *H. opuntia* which was attributed to the significant positive correlations between mercury concentration and precipitation.

The concentration of lead recorded in the current study was relatively lower in comparison to the findings of (27) who recorded 4.2 mg kg^{-1} DW in other organisms such as the gills of fish species *Siganus sutor* collected from Gazi bay during the dry season and 5.6 mg kg^{-1} DW during the wet season in the same species. Some authors have detected much higher contents in red and brown seaweed than those found in this study at 14.2 mg kg^{-1} DW as recorded by (28) while (29) found lower Pb contents below 0.57 mg kg^{-1} DW. The significant difference in Pb concentrations between wet and dry seasons could be attributable to the significant correlations with precipitation, salinity and pH. Precipitation is an important factor in aquatic environments as they may receive Pb from polluted air or land through surface runoff or percolating ground waters (30).

With reference to cadmium, the highest level recorded in *H. opuntia* is comparable to the findings of (7) who recorded levels of 0.14 mg kg^{-1} DW in *H. opuntia*, *Chaetomorpha linum* (0.06 mg kg^{-1} DW) and *Laurencia obtusa* (0.08 mg kg^{-1} DW) from Saudi Arabia. (27) recorded cadmium concentration ranging from 0.2 to 0.83 mg kg^{-1} DW in *S. sutor* from Gazi bay while in sediments the value ranged from not detectable to 1.0 mg kg^{-1} DW. Significant differences concentration between the dry and wet season as well as sites indicates that the biological availability of a heavy metal is influenced by its ambient environment.

The total arsenic content found in *U. rigida* and *H. opuntia* was within the permissible for arsenic in sea foods and products as relates to the quality criteria laid down by France at 3.0 mg kg^{-1} DW as well as that which is applied by the Joint FAO WHO Expert Committee (JFWEC) also at 3 mg kg^{-1} DW. *H. opuntia* was found to have mercury, lead and cadmium within permissible limits of < 1 mg kg^{-1}, <5.0 mg kg^{-1} and <0.5 mg kg^{-1} respectively as compared to the Joint FAO WHO Expert Committee (JFWEC) during the study period.

V. Conclusion

The results have shown that the two seaweed species collected from Gazi bay and Diani have bio-accumulated varying levels of arsenic, mercury, cadmium and lead that varies with the species, geographical location and months. The concentration values correlated significantly with the ambient environmental factors. Seaweeds like *H. opuntia* which seems to accumulate relatively high levels of the four heavy metals studied in comparison to the other species may prove useful in the removal of heavy metals from aquaculture effluents but not as an ingredient in fish feeds due to its seemingly high affinity for heavy metals. *U. rigida* on the other hand could be utilized as an ingredient in fish feed formulation.

Acknowledgements

The authors acknowledge the Western Indian Ocean-Regional Initiative for Science and Education (WIO-RISE) network at the Institute of Marine Sciences, Zanzibar (IMS) for the financial support without which these studies would not have been possible and the University of Nairobi for providing the laboratory facilities for analysis.

References

[1]. The State of World Fisheries and Aquaculture contributing to food security and nutrition for all. Rome, FAO 2016, pp 200
[2]. J.N. Kumar, B. Meghan, R.N. Kumar, Distribution and biochemical constituents if different seaweeds collected from Okha coast, Gujarat, India, Indian journal of geomarine sciences 46 (02), 2017, 349-357.
[3]. L.M.P. Valente, A. Gouveia, P. Rema, J. Matos, E.F. Gomes and I. S. Pinto, "Evaluation of three seaweeds Gracilaria bursa-pastoris, Ulva rigida and Gracilaria cornea as dietary ingredients in European sea bass (Dicentrarchus labrax) juveniles, Aquaculture, 252, 2006, 86-91.
[4]. D.I, Sanchez-Machado, J. Lopez-Hernandez, and P. Paseiro-Losada,2002, High-performance liquid chromatographic determination of -tocopherol in macroalgae, Journal of Chromatography, 976, 2002, 277-284.
[5]. R. L.Veroy, N. J. Montano, M. L. B. Deguzman, E. C. Laserma and G. J. B Cajipe, Studies on the binding of heavy metals to algal polysaccharides from Philippines seaweeds, carrageenan and the binding of Lead and cadmium, Botanica Marina, 23, 1980, 59-62.
[6]. C. S. Karez, V. F. Magalhaes, W.C. Pfeiffer and F.G.M. Amado, Trace metal accumulation by algae in Sepetiba Bay, Brazil, Environmental Pollution, 83**,** 1994, 351–356
[7]. M. E. El-Nagaar and E. Al-Amoudi, Heavy metal levels in several species of Marine algae from the red sea of Saudi Arabia. Journal of King Abdulaziz University of Sciences, 1, 1989, 5-13.
[8]. C. S. Lobban, P. J. Harrison and M. J. Duncan. The physiological ecology of seaweeds Cambridge University Press, New York, p 242, 1985.
[9]. J.A Norman, C.J. Pickford, T.W and M. Waller, Human intake of arsenic and iodine from seaweed based food supplements and health foods available in the Uk, Food additives and contaminants 5, 2009, 103-109
[10]. AOAC, (Association of Official Analytical Chemists) (1995). Official Method of Analysis, 12th edn, Association of official Analytical Chemists, Washington DC, pp 832.
[11]. S. Topcuoglu, K.C, Guven, N. Balkis, and C. Kirbasoglu, Heavy metal monitoring of marine algae from the Turkish coast of the Black Sea, Chemosphere Biomaterials, 52, 2003, 683-1688.
[12]. N. Jothinayagi and C. Anbazhagan, Heavy Metal Monitoring of Rameswaram Coast by Some Sargassum sp", American-Eurasian Journal of Scientific Research, 4, 2009, 73-80
[13]. G. W. Garnharm, G. A Codd and G. M. Gadd,(1992), Kinetics of uptake intracellular locations of cobalt, manganese and zinc in the estuarine green alga Chlorella salina, Applied Microbiology and Biotechnology, 37, 1992, 270.
[14]. N. Favero, F. Cattalini, D. Bertaggia, and V. Albergoni, Metal accumulation in a biological indicator (Ulva rigida) from the Lagoon of Venice (Italy), Archives of Environmental Contamination and Toxicology, 31, 1996, 9–18.
[15]. S. N. Luoma, G. M. Bryan, and L. Langston, Scavenging of heavy metals from particulates by brown seaweeds, Marine pollution Bulletin, 13, 1982, 394-6.
[16]. S. N. Luoma, Bioavailability of trace metals to aquatic organisms—A review, The Science of the Total Environment, 28, 1983: 1-22.
[17]. C. S. Karez, V. F. Magalhaes, W. C. Pfeiffer and F. G. M. Amado, Trace metal accumulation by algae in Sepetiba Bay, Brazil, Environmental Pollution, 83, 1994: 351–356.
[18]. R. L. Veroy, N. J. Montano, M. L. B Deguzman, E. C. Laserma and G. J. B. Cajipe, Studies on the binding of heavy metals to algal polysaccharides from Philippines seaweeds, carrageenan and the binding of Lead and cadmium, Botanica Marina, 23, 1980, 59-62.
[19]. A.M.A. Abdallah, M.A. Abdallah and A. I Beltag, contents of heavy metals in marine seaweeds from the Egyptian coast of the Red sea, Chemistry and Ecology, 5, 2005, 399-411
[20]. Y. Jang, Y, Somamanna and H. Kim, source, distribution, toxicity and remediation of arsenic in the environment-a review, International Journal of Applied Environmental Sciences, 2, 2016, 559-581
[21]. C. Almela, M. J. Clemente, D. Velez and R. Montoro, Total arsenic, inorganic arsenic, lead and cadmium contents in edible seaweed sold in spain". Food and Chemical Toxicology, 44, 2006, 1901–1908.
[22]. T. Sawidis, M. T. Brown, G. Zachariadis and I. Sratis, Trace metal concentrations in marine macroalgae from different isotopes in the Aegean sea, Environment International, 27, 2001, 43-47.
[23]. S. W. Al-Rmalli, R. O Jenkins and P. I. Haris, Dietary intake of cadmium from Bangladeshi foods". Journal of Food Sciences, 77, 2012, 26-33.
[24]. A. Farkas, J. Salanki and A. Specziar, Age and size-specific patterns of heavy metals in the organs of freshwater fish Abramis brama L. populating a low contaminated site, Water Research, 37, 2003, 959-964.
[25]. M. I. Yahaya, S. Mohammed and B. K. Abdullahi, Seasonal Variations of Heavy Metals Concentration in Abattoir Dumping Site Soil in Nigeria, Journal of Applied Science and Environment Management, 13, 2009, 9 – 13.

[26]. C. V. G. Phipps, P. J. Davies and D. Hopley, The morphology of Halimeda banks behind the Great Barrier Reef east of Cooktown, Queensland, Proceedings of 5th International Coral Reef Symposium, 5, 1985: 27
[27]. B. M. Mwashote, Levels of Cadmium and Lead in Water, Sediments and Selected Fish Species in Mombasa, Kenya, Western Indian Journal of Marine Sciences, 2, 2003, 25-24.
[28]. J. J. Ortega-Calvo, C. Mazudos, B. Hermosin and C. Saiz-jimenez, chemical composition of spirulina and eukaryotic algae food products marketed in spain, Journal of Applied Phycology, 4, 1993, 425-435
[29]. C.Van Netten, C. S.A. Hoption., D. R. Morley and J. P. Van Netten, Elemental and radioactive analysis of commercially available seaweed, The Science of Total Environment, 255, 2000, 169-175.
[30]. E. O. Lawson, Physico-chemical parameters and heavy metal contents of water from the mangrove swamps of Lagos lagoon, Lagos, Nigeria, Advances in Biological Research, 5, 2011, 08-21.

1Mutia, G.M. " Analysis Of Bio-Accumulation Of Heavy Metals In Seaweeds Ulva Rigida And Halimeda Opuntia In Validation Of Their Safety For Use In Aquaculture Feeds In Kenya." IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT) 12.8 (2018): 56-63.