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Bioaccumulation of Metals in Mangrove Snail (*Cerithidea obtusa*) from Southwest Johor, Malaysia

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Abstract Pollutants are persistent in the environment, taint the food chain, and harm people's health because of their toxicity. In this study, mangrove snails called Cerithidea obtusa (*C. Obtusa*) were gathered from Southwest Johor, Malaysia, and the amounts of macro and micro elements Ca, Fe, K, Mg, Na, As, Mn, and Zn in their soft tissues, shells, and related surface sediments were analysed. The Neutron Activation Analysis (NAA) method was used to calculate the elements concentration. The mean concentrations of Ca, Fe, K, Mg, Mn, Na, As, Mn and Zn were found to be followed by the order Fe > Na > K > Mg > Ca > Na > Mn > Zn > As in the sediment, while Ca > Na > K > Mg > Fe > Zn > Mn > As and Ca > K > Na > Mg > Fe > Mn > Zn > As in the soft tissue and shell of *C. Obtusa* respectively. The Enrichment Factor (EF) of Ca, K, Mg, Na, As, Zn, and Mn ranged from 0.58 to 2.58, 0.82 to 1.04, 1.14 to 2.34, 2.32 to 4.18, 1.31 to 3.45, 1.11 to 1.52, and 0.23 to 0.38, respectively. Mn had the lowest EF (0.23), and 4.18 had the greatest (Na). Most of the elements showed geoaccumulation index (Igeo) < 0 except for As and Na. Based on the Biota-Sediment Accumulation Factor (BSAF), the shell of *C. Obtusa* acts as a deconcentrator for all the elements except for Ca.

Keywords Pollution, Enrichment, Geoaccumlation, Neutron Activation

1. Introduction

Environmental pollution is a major challenge in today's human society. Environmental pollution is an environmental threat and a major concern. In recent years, there has been an increase in the pollution of the marine environment as a result of the exponential growth in the human population and the subsequent increasing trend of anthropogenic pressure, which poses a threat to all aquatic ecosystems [1-3]. Human activities have led to unprecedented levels of environmental pollution in many coastal and river regions worldwide [4,5]. Pollutants are persistent in the environment, taint the food chain, and, because of their toxicity, they can result in a variety of health issues to the consumers. Living organisms are actually threatened by prolonged exposure to these metals in the environment [6]. The marine food web, the habitat, and a species' place of origin, however, all have an impact on how contaminants are expelled and bioaccumulated in aquatic animals [7].

Elements that are significant or only found in trace amounts in soil, water, air, plants and animals are commonly known as major and trace elements (Na, Mg, Ca, Fe, Al, K, and Ba, Co, Cs, Ga, Sc, Sb, Ta, V) [8]. Important elements, including K, P, Na, Mg and Ca, play a physiological role in skeletal structure, colloidal system maintenance and acid-base equilibrium management [9]. While these components are important and necessary for the biological functioning of plants and animals, elevated levels can be harmful because they can interfere with metabolic processes [10]. Additionally, non-essential metals, such as hazardous metals released into water from microscopic suspended particles, might affect the feeding rate and metabolic efficiency of bivalve molluscs [11]. The ecology of the species, eating habits, environmental variables, and circumstances all have an impact on their abundance in fish tissue [1]. However, the excretion and bioaccumulation of toxins in aquatic animals are all influenced by the marine food web, the environment and the place of origin [1,12].

The majority of research conducted in Malaysia focuses on the levels of potentially harmful metals such as Zn, Cu, Ni, Mn, Cr, Cd, Pb, and As in sediment or the muscle tissue of bivalves captured in mangrove swamp habitats [13-16]. Only a small number of researchers have studied the content of macro elements such Ca, Mg, Na, K, and Fe in sediments or the muscle of bivalves [17,18]. Finding out whether macro elements can affect the bioaccumulation of major and trace elements is equally important. Furthermore, this kind of research could help us better understand some mangrove ecosystems, which are thought to be under constant anthropogenic strain.

There is a lack of information on studies of bioindicators based on pollution of macro elements and micro elements in the mangrove study area in Malaysia. The objective of the current study is to determine the distribution of macro and micro components in the soft tissues and shells of the mollusc, *C. Obtusa* as well as, in the sediments from its environment. Additionally, to evaluate the possible use of this capability for biomonitoring of pollution in the study region and compare the levels of macro and micro components in the study environment with those in other regions of the world.

2. Materials and Methods

As indicated in Figure 1, the samples for this study were taken from river estuaries in the mangrove region of southwest Johor, Malaysia, between latitudes N 05°20' 24.7" and N 01°15'58" and longitudes E 103°25'30.6" and E 103°58'14.2". High and low tides occur in the sampling geographic areas which often have similar weather conditions. Activities near sampling stations are listed in Table 1.

Due to their widespread distribution and abundance in the study area, bivalve mollusks (*C. obtusa*) were chosen to evaluate the bioaccumulation of macro and micro components in their shell and soft tissues. In order to eliminate variations in metal content caused by a species' size or reproductive stage, only commercially acknowledged normal sizes for each species were collated [13-14]. About 40–50 individual bivalve mollusks of similar length (approximately length, 5.0 cm) were employed in this experiment. Mangrove surface sediments weighing 500–600 g were randomly collected at a depth of approximately 5.0 cm to examine the potential for a relationship between components in bivalve mollusks and their environment.

All samples were taken to the lab, rinsed with tap water to remove any remaining snail-associated soil, and then placed in a marked, clear plastic bag. The shell is then delicately crushed to extract the soft tissue from it without damaging it. Each location's surface sediments, shells, and soft tissues were ground into a powder using mortal and pastel, sifted through a stainless steel aperture measuring 63 µm, and heated at 80°C for at least 72 hours to a constant dry weight. Prior to being placed in plastic pillboxes, the mixture was rapidly mixed [1,15].

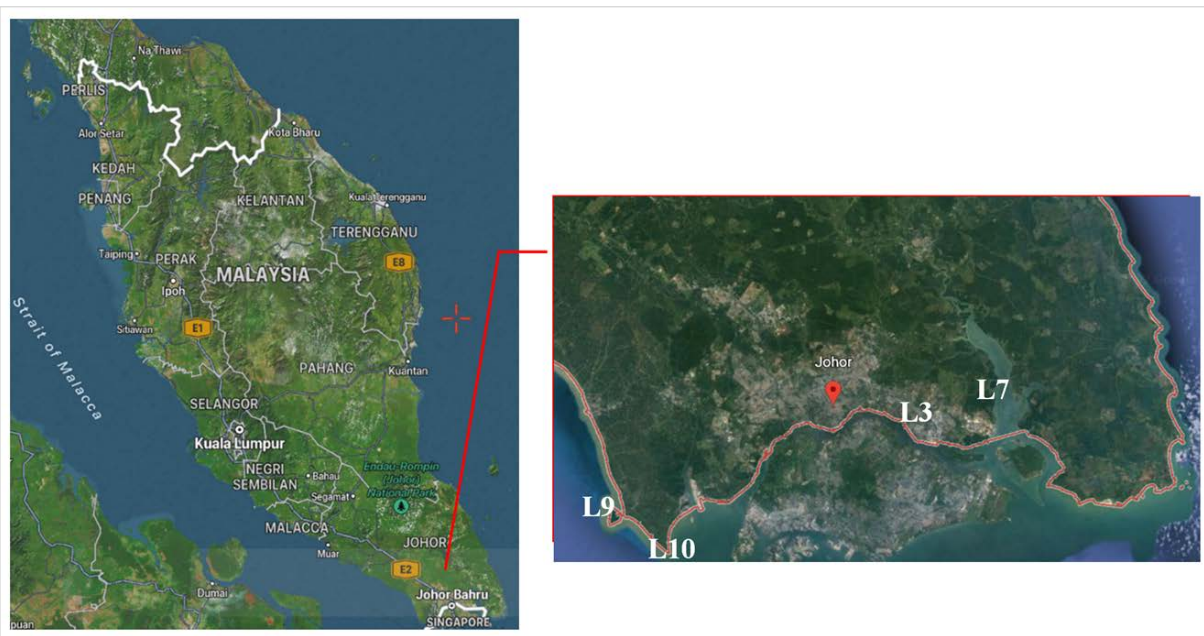

Figure 1. Sampling stations

Table 1. The description of sampling location

ID	Location Name	GPS Reading	Description of nearby activities
L3	Sungai Pasir Gudang, Johor	N 01°24'3.99" E 103° 57' 26"	Shipping Area, Intertidal Area
L7	Sungai Kim Kim, Johor	N 01°26'40.2" E 103° 58'14.2"	Residential, Intertidal Area
L9	Pulau Kukup, Johor	N 01°19'18.7" E 103° 25' 30.6"	Fish cage, shrimp Pond, Tourism spot
L10	Johor Tanjung Pia	N 01°15'58" E 103° 30'39"	Shipping, Tourism spot

Preparation of Samples for INAA

A special effort was made to maintain the samples and the workspace clean during the sample preparation process, which was carried out under carefully monitored conditions. Additionally, to prevent contamination, tongs and gloves were always used for handling. The samples were prepared for assessing NAA in accordance with the prescribed process (USEPA, 2001; IAEA-TECDOC-1360, 2003. For irradiation of the samples, each sample was weighed at 100 mg for short-term irradiation and 200 mg for long-term irradiation, and then placed in a polyethylene vial. For short radiation, the samples were irradiated for 1 minute, counting time 5 minutes and 20 minutes after cooling period of 20 minutes and 24 hours, respectively. On the other hand, for long radiation, the samples were irradiated for 6 hours, counting time 1 hour after cooling period of 3 - 4 and 21 – 28 days, respectively. Standard reference samples such IAEA SL-1 (lake sediment) were used, and sediment samples were processed in a manner similar to the samples of interest. Furthermore, as multi-element comparators for soft tissue bivalve molluscs, standard reference samples SRM 1566b (Oyster tissue) and SRM 2976 (Mussel Tissue) were employed [19,20].

Method to Evaluate Pollution Level

The geoaccumulation index (I_{geo}) and the enrichment factor were used to determine the contamination level (*EF*). In this work, the traditional *EF* was estimated using a formula created by Buat-Menard and Chesselt [21] using Fe as a normalizer.

$$EF = [(C_n/C_{Fe})_{\mathrm{sample}}]/[(C_n/C_{Fe})_{\mathrm{crust}}]$$

where $(C_n/C_{Fe})_{sample}$ refers to the ratio of metal to Fe in sediments, $(C_n/C_{Fe})_{crust}$ refers to the ratio of metal to Fe in the earth's crust. Buat-Menard and Chesselt [21] proposed seven categories for *EF*. No enrichment if $EF < 1$, minor enrichment if $EF < 3$, moderate enrichment if *EF* falls between 3 and 5, $5 < EF$: moderately severe enrichment if *EF* falls between 5 and 10, severe enrichment if *EF* falls between 10 and 25, very severe enrichment if *EF* falls between 25 and 50 and extremely severe enrichment if $EF > 50$.

The values for I_{geo} were determined using the Muller [23] formula as follows:

$$I_{geo} = \log_2 [C_n/(1.5 \text{ x } B_n)]$$

where B_n is the baseline values applied to the UCC and C_n is the heavy metal concentrations detected in the sediments as mentioned by Wadepohl [24]. To reduce the effects of any background value fluctuations that might be brought on by lithologic variations in the sediments, a factor of 1.5 was added into the I_{geo} equation [22]. Seven I_{geo} categories were used by Muller [23] to categorise the severity of elemental pollution. If $I_{geo} < 0$, indicate no pollution, no pollution if $I_{geo} < 1$, no pollution to moderate pollution if $I_{geo} < 2$, medium pollution if $I_{geo} < 4$, serious pollution if $I_{geo} < 5$, extremely severe pollution, if $I_{geo} > 5$.

The ratio of the metal in the living organism and associated sediment (*BSAF*) was calculated using the equation [38]:

$$BSAF = C_p/C_q$$

where C_p and C_q are the mean metal concentrations in various snail components (tissues and shell) and the associated sediment, respectively. According to the classification suggested in, snail tissues can be classified as macroconcentrators if the *BSAF* value is larger than 2, microconcentrators if the value is between 1 and 2, and deconcentrators if it is less than 1 [39].

3. Results and Discussion

For sediment and soft tissue bivalve mollusc, the multi-element comparators IAEA SL-1 (Lake Sediment) and SRM 1566b (Oyster tissue)/SRM 2976 (Mussel Tissue) were employed, respectively. Table 2 displays the macro and micro element measured values for SL-1, SRM 1566b, and SRM 2976 as well as the findings of the certified reference material's analytical testing. For SL-1, the range of recoveries using the INAA method is 78.4 to 136.1, and for SRM 1566b and SRM 2976, the range is 76.1 to 131.98, respectively. The INAA method yields acceptable recovery discrepancies between measured and certified samples. The INAA method's measured and verified recoveries fall within the permitted range.

Table 3 shows the average elemental concentrations in the sediment samples. Between the four sampling sites, there were no appreciable differences in the mean concentration of macro and trace metals in the subsurface layer (t-Test, $p > 0.05$). The most prevalent macro element is iron (Fe). The concentration of the elements was in the following order: Fe > Na > K > Mg > Ca > Na > Mn > Zn > As. The nature of the organic molecules in mangrove (clay substrate) could lead to a high concentration of Fe compared with other elements [25,26]. Fe may have precipitated as sulphide, which is prevalent in mangrove sediments, which would explain the higher quantity of Fe. In the mangrove, Fe and Mn combine to create complexes with organic substances. environments, which are significantly concentrated in the organic components (Fe more efficiently than Mn). Na and K levels in this region are enriched as a result of tidal infiltrations of sea water into the mangrove system. It appears that montmorillonite is the main source of Na in mangrove habitats when considering the involvement of clay minerals in the fixation of Na. Because the former cation can more quickly fix with clay minerals, Na values are higher than K values [27]. For the open exchange sites in clay minerals, Na ions compete much more successfully than K ions. Despite K's importance as a nutrient, the mangrove species exhibit a remarkable affinity for it when it comes to the synthesis of carbohydrates and the growth of tissues. K levels in the sediments are likewise lowered by this process, albeit less so than Na. In this context, it is obvious that the increased K content may have come from the mangrove vegetation, including local litter. The weathering of orthoclase-, microcline-, and biotite-rich rocks is the source of K for the sediments. It's possible that the mangrove flora's elimination of K is the cause of the significantly lower concentration of K over Na. The increased activity of calcareous species like crab, molluscs, mussels, etc. in this high-productive site may be the cause of the elevated value of Ca in mangrove sediments. The greater amounts of these metals in agricultural area discharges that contain fertiliser, pesticide, and rodenticide residues would mostly account for the increased zinc content [28]. Due to their geographic location, the amount of metals that accumulated in sediment varied. Additionally, the discharge of various volumes of sewage and municipal trash may have had an impact on the variation in the pattern of accumulation.

Table 4 (a) and (b) shows the average elemental content in the soft tissue and shell of *C. Obtusa,* respectively. Between the sampling sites, there were no appreciable differences in the concentration of macro and trace metals in the soft tissue and shell of *C. Obtusa* (t-Test, $p > 0.05$). On the other hand, there is a noticeable variation between the Ca in the shell and the soft tissue of *C. obtusa*. The concentration of the elements was in the following order: Ca > Na > K > Mg > Fe > Zn > Mn > As. Contrarily, the order of the elements in the shell of the C. *Obtusa* was Ca > K > Na > Mg > Fe > Mn > Zn > As. It was found that the concentration of Ca is the highest in both soft tissues and shell of *C.Obtusa*. Ca has a vital role in the physiology of bivalves as the primary portion and the calcium carbonate granules in the soft tissues [29-31]. Furthermore, due to the substance's low affinity for chelating substances, the chemical is more readily available to the cell.

Table 2. Mean of measured and certified values of SL-1, SRM 1566b and SRM 2976 (mg/kg dry weight)

Element	SL-1		Recovery(%)	SRM 1566b/SRM 2976		Recovery(%)
	Measured	Standard		Measured	Standard	
Ca	133823	163000	82.1	8413	7600	110.7
Fe	67939	67400	100.8	263.6	205.8	128.1
K	19735	14500	136.1	6292	6520	96.5
Mg	8859	11300	78.4	825.7	1085	76.1
Na	1733	1700	102	6570	6887	95.4
As	25.45	27.6	92.2	6.49	7.65	84.9
Mn	3370.1	3460	97.4	43.6	33	131.98
Zn	246.69	223	110.6	1329	1424	93.31

Table 3. Concentrations of heavy metals (mg/kg dry weight) in sediment

Element (mg/kg)	Sungai Pasir Gudang, Johor				Sungai Kim Kim, Johor				Pulau Kukup, Johor				Tanjung Pia, Johor			
	min	max	ave	std	min	max	ave	std	min	max	ave	std	min	max	ave	std
Ca	3720	51531	19961	27345	-	-	-	-	3672	7266	5294	1822	-	-	-	-
Fe	21642	23473	22856	830	28166	29425	28607	583	26032	28114	27073	1472	29433	34580	32429	2157
K	12815	13668	13347	372	15288	15955	15562	289	14459	18819	15927	1969	14711	15264	14938	289
Mg	16190	18107	17025	982	14357	16073	14812	841	12347	15681	13425	1525	11039	13340	11875.9	1008
Na	14842	15361	15123	245	11884	14655	13472	1304	19284	26087	21132	3310	25944	28450	27595	1429
As	21.09	22.08	21.72	0.46	24.22	25.73	24.71	0.69	10.24	12.22	11.21	0.81	11.30	12.05	11.72	0.31
Mn	95.30	125.44	113.34	14.10	132.26	145.06	139.84	5.48	165.35	213.93	186.13	23.54	129.65	147.41	136.72	8.42
Zn	62.67	79.60	69.94	7.07	78.67	85.06	81.77	2.65	75.74	92.07	83.08	6.84	62.40	79.46	72.35	7.69

Table 4(a). Concentrations of heavy metals (mg/kg dry weight) in soft tissue of *C. obtusa*

Element (mg/kg)	Sungai Pasir Gudang, Johor				Sungai Kim Kim, Johor				Pulau Kukup, Johor				Tanjung Pia, Johor			
	min	max	ave	std	min	max	ave	std	min	max	ave	std	min	max	ave	std
Ca	20852	22073	21515	513	451	19484	11349	8214	10331	21692	16333	4806	17221	17753	17510	229
Fe	964	1265	1135	128	1470	1769	1601	127	5619	7394	6284	806	635	673	653	16
K	8604	9306	8906	304	10783	11201	11015	189	11946	13919	12658	875	10308	11411	10696	509
Mg	6887	8178	7585	622	5858	6271	6010	181	5858	6271	6010	181	7093	8767	7754	723
Na	10140	10842	10596	314	15114	15951	15574	349	15114	15951	15574	349	23650	27334	25537	1505
As	7.58	7.91	7.77	0.14	10.08	10.51	10.27	0.18	9.42	11.07	10.43	0.72	9.57	11.25	10.35	0.76
Mn	133.78	160.97	143.83	12.09	196.16	211.92	204.36	6.30	438.53	537.10	485.69	43.30	461.37	578.51	524.16	62.17
Zn	276.62	302.81	291.53	10.90	318.39	350.10	327.34	15.25	220.56	250.12	233.23	12.75	241.62	270.94	256.32	14.71

Table 4(b). Concentrations of heavy metals (mg/kg dry weight) in shell of *C. obtusa*

Element (mg/kg)	Sungai Pasir Gudang, Johor				Sungai Kim Kim, Johor				Pulau Kukup, Johor				Tanjung Pia, Johor			
	min	**max**	**ave**	**std**	**min**	**max**	**ave**	**std**	**min**	**max**	**ave**	**std**	**min**	**max**	**ave**	**std**
Ca	297308	375408	354069	37880	346072	537036	403445	89523	364357	407291	389515	18842	331074	370221	347779	17959
Fe	896.4	1092.7	1008.4	92.7	-	-	-	-	205.5	324.3	264.9	84.0	98.0	302.2	209.2	85.1
K	-	-	-	-	-	-	-	-	516.7	574.7	545.7	41.0	-	-	-	-
Mg	-	-	-	-	-	-	-	-	300.0	877.8	503.0	324.9	299.2	697.0	461.2	208.9
Na	5233	5783	5533	279	5386	5862	5609	238	6515	7069	6754	284	5711	5729	5720	12
As	0.338	0.480	0.421	0.074	-	-	-	-	-	-	-	-	-	-	-	-
Mn	23.17	36.91	31.26	5.80	5.62	10.05	8.28	2.34	15.58	23.91	20.27	3.87	14.50	15.62	15.21	0.50
Zn	8.69	18.91	11.85	4.79	-	-	-	-	3.83	6.29	5.06	1.06	-	-	-	-

Contrarily, the levels of Fe in *C. Obtusa's* soft tissues and shell were significantly lower than those in the sediment. The body's enzyme system depends on Mg, which is essential for human nutrition. The Mg functions in all cells of soft tissues and helps to maintain bone health. It is also a cofactor for enzyme systems and is advised in diets since it is essential for energy metabolism and the production of proteins. However, the levels of Na in *C. Obtusa*'s soft tissues and shell were lower than those in the sediment. The main cation in extracellular fluid and one that controls its volume is Na [32].

The EF values for all sampling sites are presented in Figure 2 using Fe as the normalising element. According to sediment samples, the EF of Ca, K, Mg, Na, As, Zn, and Mn ranged from 0.58 to 2.58, 0.82 to 1.04, 1.14 to 2.34, 2.32 to 4.18, 1.31 to 3.45, 1.11 to 1.52, and 0.23 to 0.38, respectively. Mn had the lowest EF (0.23), and 4.18 had the greatest (Na). The geo-accumulation index has been calculated, and Figure 3 shows the results. According to the findings, 98 percent of samples fell into classes 0 and 1, which can be categorised as unpolluted with the exception of Na and As. It was discovered that all of the analysed areas can be classified as minor enrichment with major and trace elements based on enrichment factor except for Na where it is classified as minor to moderate enrichment. The I_{geo} value of Na for all of the areas under study ranged from 1 to 2, indicating considerable pollution that may be brought on by anthropogenic causes. Alternately, it's possible that seawater tidal infiltrations into the mangrove system can be prevented by taking into consideration how clay minerals help fix Na and keep it bound to the clay surfaces. BSAF of As, Zn, Mn, Ca, Fe, K, Mg and Na in the soft tissues and shell of C. Obtusa varied in the range 0.36 – 0.93, 2.81- 4.17, 1.27-3.83, 1.08 – 3.09, 0.02 – 0.23, 0.67 – 0.79, 0.41 – 0.65, 0.70 – 1.16 and 0.02, 0.06 – 0.17, 0.06 – 0.28, 17.74 – 73.58, 0.01 – 0.04, 0.03, 0.04, 0.21 – 0.42 respectively as illustrated in Table 5. The shell of *C. Obtusa* acts as a deconcentrator for all the elements except for Ca, according to the BSAF value. It is widely acknowledged that calcium is the most prevalent metal in both shells and soft tissues. Additionally, the availability of the chemical to the organism is increased by its low affinity with chelating compounds. Therefore, the amount of Ca acquired by aquatic molluscs is a reflection of the calcium content of the aquatic environment. In this study, we discovered that calcium was the primary component of the shell. Due to the fact that bivalve molluscs are abundant, easy to sample, have limited mobility, and have a widespread geographic distribution, they are practical, astonishingly effective, and well-liked bioindicators of coastal environmental contamination [32-34]. However, the concentration of trace elements in tissues is a result of a delicate balance between several activities, such as absorption, transformation, detoxification, and excretion. As a result, even among creatures with similar ecological environments, the amount of the accumulated components is frequently species-specific [35-37].

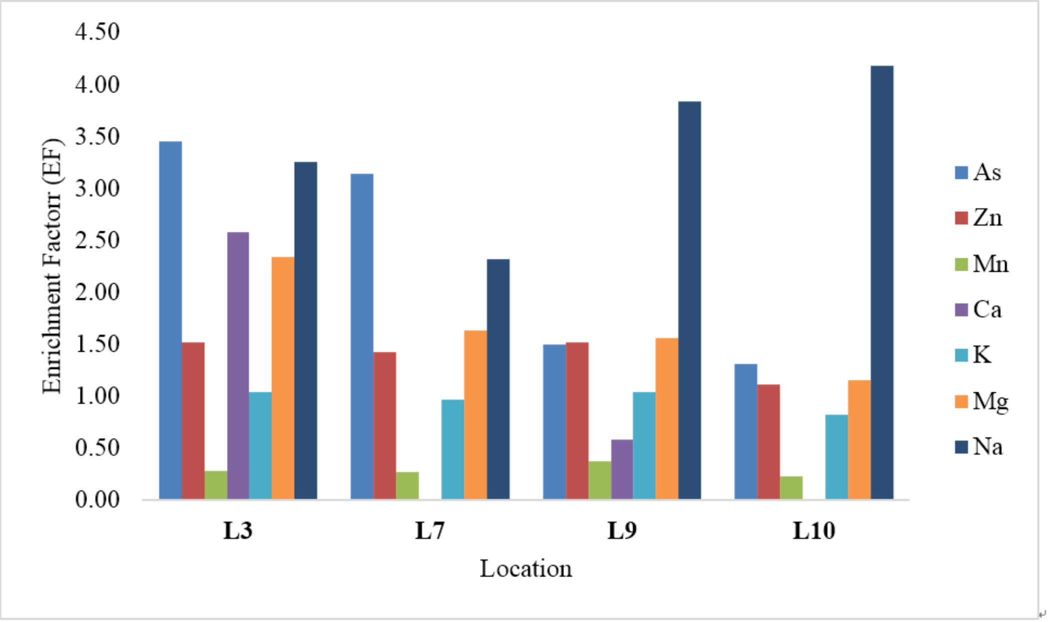

Figure 2. The Enrichment Factor (EF) of four locations

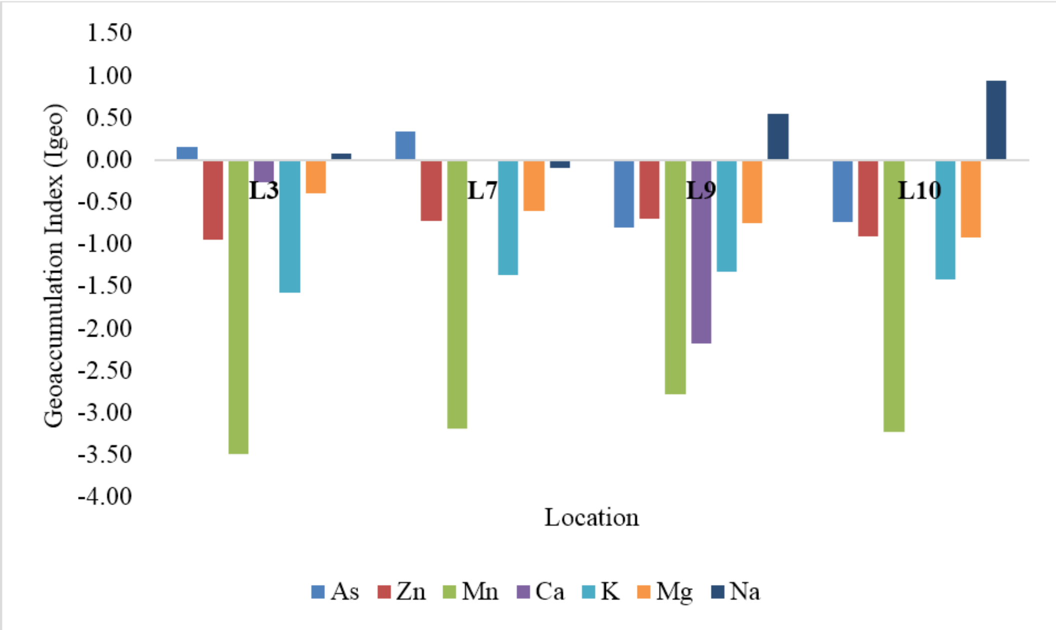

Figure 3. The Geoaccmulation Index (Igeo) of four locations

Table 5. BSAF of elements in the shell and soft tissues of the *C. Obtusa*

	Different Part	As	Zn	Mn	Ca	Fe	K	Mg	Na
L3	Tissues	0.36	4.17	1.27	1.08	0.05	0.67	0.45	0.70
	Shell	0.02	0.17	0.28	17.74	0.04	nd	nd	0.37
L7	Tissues	0.42	4.00	1.46	nd	0.06	0.71	0.41	1.16
	Shell	nd	nd	0.06	nd	nd	nd	nd	0.42
L9	Tissues	0.93	2.81	2.61	3.09	0.23	0.79	0.62	1.07
	Shell	nd	0.06	0.11	73.58	0.01	0.03	0.04	0.32
L10	Tissues	0.88	3.54	3.83	nd	0.02	0.72	0.65	0.93
	Shell	nd	nd	0.11	nd	0.01	nd	0.04	0.21

nd – not detected

4. Conclusions

Eight element concentrations in the tissue and shell of C. obtusa species and related sediment samples were investigated. The bioaccumulation of heavy metals in C. obtusa's soft tissue and shell was high for some of elements, particularly for Ca at sites L3 and L9, which may be related to their habitat and the most common metal in both soft tissues and shells. The concentations of Ca and Zn in soft tissues were significantly higher than in sediments. Based on the pollution index value, the studied area has moderately enriched concentrations of As and Na at L3 and L7 and L9 and L10, respectively. This might be due to as a result of anthropogenic activity close to the sampling location. Future ecotoxicological studies aiming to establish *C. Obtusa* as a trustworthy species for heavy metal biomonitoring and bioindicators will benefit from our findings. Bioaccumulation of metals in sediment or in living mangrove organisms change time to time. Data captured in this manuscript is based on the sample collected in 2019 at the particular location. The data in the study will be a reference material for future research. Data collected over time can be used to analyze how the bioaccumulation of metals varies with time.

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