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Choose of Heavy Metals Pollution Biomonitors: A Critic of the Method that uses Sediments total Metals Concentration as the Benchmark

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ABSTRACT: The study aimed to come up with a list of specific macroalgae species, which could be used to biomonitor specific metal elements in the coastal waters of East Africa. Water extraction, EDTA, aqua regia extraction and optimized BCR 3-step sequential extracts were used to mimic bioavailable metals under various environmental conditions. The results indicated that *Ulva lactuca* could be used as a biomonitor to predict BCR 3-step sequential bioavailable Al, Cd, Co, Fe, Mn, Zn and Ni whereas *Sargassum* species could be used as a biomonitor for BCR 3-step sequential bioavailable Co, Cu, Fe, Mn and Zn. In *Sargassum spp*. only Co showed significant correlation with concentration in sediment's aqua regia extracted metals whereas in *Ulva lactuca* only Al, Co, Cu and Fe showed significant correlation with total metals extracted through aqua regia procedure. This study therefore recommends the use of *Ulva lactuca* and *Sargassum* for biomonitoring of Al, Cd, Co, Fe, Mn, Zn, Ni and Co, Cu, Fe, Mn, Zn respectively. The study recommends the use of labile fraction of BCR sequential extraction for screening of macroalgae to be used for heavy metal pollution monitoring in East Africa region.

Key words: Biomonitor, Bioavailable, EDTA extraction, Sequential extraction, Aqua regia extraction, and Heavy metals

INTRODUCTION

Oceans receive trace metals from both natural processes and land based activities. Heavy metals are of concern because they are persistent compounds with high toxicity, which can efficiently be bioaccumulated in the organisms and biomagnified along the food chain with a resultant severe threat to oceanic and human health (Lin, 1992; Ahmad *et al.*, 2010). Chemical analysis of the sediment though commonly carried out in metals determination, it has limitations in that it cannot provide reliable evidence of the integrated influence and possible toxicity of such pollutants to the organisms and ecosystem (Zhou *et al.*, 2008; Adjei-Boateng *et al.*, 2010).

The use of biomonitors provides information on the quantities of pollutants that have been sequestered in the organisms and corresponding effects induced. This fact has made marine macroalgae to be used extensively in many coastal waters around the world as biomonitors of metal contamination in (e.g. Fowler, 1979; Phillips, 1990, 1993; Rainbow and Phillips, 1993). An important assumption underlying use of seaweeds as biomonitor is that metal concentrations in the seaweeds are directly proportional to the bioavailable metal concentrations in environment (Bryan and Hummerstone, 1973; Morris and Bale, 1975; Forsberg *et al.*, 1988; Ho, 1990; Say *et al.*, 1990; Barreiro *et al.*, 1993; Haritonidis and Malea, 1995).

Most studies have used total metals concentration for screening of biomonitors despite the fact that total metal concentrations neglect the fact that bioaccumulation of metals is not only dependent on the specific metal, the biological species under consideration and the environmental compartment in which the organisms reside but also the prevailing environmental conditions. A single extraction procedure may provide a good estimate of bioavailable metals for uptake by a certain species whereas a different extraction procedure may provide a better estimate for a different species (Peijnenburg and Jager, 2003). This has a consequence in the choice of macroalgae as a biomonitor given that the metals

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bioaccumulation by macroalgae will depend on environmental conditions that determine the amount of available metals for uptake. The objective of this study was therefore to identify seaweed that could be used for biomonitoring of specific heavy metals in coastal waters East Africa by applying a number of metals extraction procedures.

MATERIALS & METHODS

Sediments samples were collected from Mikindani, Mtwapa, Chale and Gazi areas along the Kenyan Coast (Fig. 1) and Msimbazi in Tanzania. The sampling sites were selected on the basis of their proximity to urban areas or industrial activities. According to this criterion, the sites close to the urbanized or industrialized area were Mikindani, Mtwapa and Msimbazi where as the sites considered to be relatively pristine were Gazi and Chale. It should be noted that in this study, the term "relatively pristine sites" is used in comparative terms since in reality there are very few places (if any) in the world that are completely unaffected by some sought of chemical contamination. In this study, Gazi and Chale were included as reference sites to provide background concentration.

Sampling was conducted in months of August and September 2006. Three random sediment samples (top 10 cm) were collected at each site using plastic hand corers (Ø= 8cm), and extraneous material (such as debris and stones) removed. The samples were pooled at each location, mixed by hand, air-dried. In the laboratory, about 1 kg of each sediment sample was freeze-dried. The samples were further mixed, ground in a mortar and sieved through a 500 μm sieve.

Fig. 1. Map showing sampling stations

To a mimic different environmental concentration of metals that could be present under the various environmental conditions. Three replicate subsamples were taken for each location for four types of metal extractions; (i) extraction using clean artificial seawater, (ii) a standard EDTA extraction method, (ii) BCR three-step sequential extraction and (iv) aqua regia extraction for total metal concentrations Water-soluble fraction was obtained by shaking of the homogenized sediments and freshly prepared seawater in clean polypropylene bottles at a ratio of 1:1 as summarized in Table 1. EDTA extractions were performed according to the standard protocol described by The European Institute for Reference Material and Measurements (Geel, Belgium) for certification of EDTA extractable Cd, Cu, Cr, Ni, Pb and Zn in the amended soil material CRM 483 as summarized in Table 1. For internal analytical accuracy check, three replicate samples of certified reference material (CRM 483) were also analyzed alongside the sediments samples. Recoveries of metals in the certified reference material were between 75-83% of the certified values. Slight deviations observed in recoveries maybe attributed to the fact that the reference material used was a few years older and may have lost some stability.

Table 1. Analytical procedure for EDTA, Water and aqua regia extractions

Extraction procedure	Chemical reagents and experimental conditions
EDTA	To 2.5g subsample add25ml of 0.05M EDTA (pH 7), shake for 1hour at 30 rpm and 20°C.
Water extractible	To 20g subsample add 20 ml of sea water, shake for 24 hours at 130rpm.
Aqua regia	To 0.5gsubsample add 1.5 ml HNO_3 (69%) and 4.5 ml HCl (37%) anddigest at 90, 200, 350 and 500 watts for 5, 3, 5 and 5 minutes respectively.

Sequential extraction procedure is summarized in Table 2. The operationally defined fractions were: acid extractable (F1); reducible phase (F2); and oxidizable phase (F3). Concentrations of all elements released by the three steps protocol were summed to form a sequentially extracted 'labile' fraction. Samples of Freshwater Sediments Reference Material (BCR 701) were included with each batch of samples (through the three steps) for verification of the measurements. Additionally, representative aliquots of each sample were subjected to a single aqua regia digestion in a mixture of HNO_3 and HCl as described in Table 1 and digested in a digestion microwave oven (model ETHOS 900, Milestone, Shelton, CT, USA). The samples were digested at 90, 200, 350 and 500 watts for 5, 3, 5 and 5 minutes respectively. For each batch of samples that were digested in the microwave, three replicates of both certified reference material (CRM 141R) and procedural blank samples were included for quality check. Products of digestion were transferred into polypropylene vials, diluted to 50 ml with Milli-Q water, and stored at -20°C until analysis. Recovery of metals in the reference materials were between 95-107% of the certified values in the aqua regia extractions and between 97-105%, 81-92% and 83-97% in the F1, F2 and F3 fractions respectively.

Table 2. Analytical procedure for European Community Bureau of Reference (BCR) three steps sequential extraction

Operational phase definition	Chemical reagents and experimental conditions
Exchangeable and carbonate (F1)	Step 1: 1g subsample, 40ml of 0.11M CH_3COOH, shake for 16h at a speed of 400rpm and at 20°C
Reducible (F2)	Step 2: To step 1 residue, add 40ml0.5M$NH_2OH.HCl$ (pH 2 adjusted with HNO_3), shake for 16h (at 20°C; shake at a speed of3000rpm)
Oxidizable (F3)	Step 3: To step 2 residue, add 10ml of 8.8MH_2O_2 (pH 2-3), digest for 1h at 20°C, heat to 85°C digest for 1h, add 10mlH_2O_2, digest at 85°C for 1h, add 50ml1MCH_3COONH_4 (pH2) and shake for 16h

From each site, sufficient quantities of *Ulva lactuca* and *Sargassum spp* were also collected. Seaweeds that were heavily covered with epiphytes and sediments were rejected during sampling. Samples were carefully washed with seawater at the sampling sites and transferred (by species) into clean polythene bags and transported to the laboratory. For each species and location, 3 x 0.5g sub-samples of dried material were carefully weighed and put in digestion vessels to which 1.5 ml of highly purified concentrated HNO_3 (70%) and 4.5 ml of HCl (37%) were added. Samples were then extracted in closed bombs using a laboratory microwave oven model ETHOS 900 (Milestone, Shelton, CT, USA). Three replicate sample of reference material (CRM 279, *Ulva lactuca*) and

reagent blank were also included for analytical quality check. The digestion program consisted of heating the samples at 90, 200, 350 and 500 watts for 5, 3, 5 and 5 minutes respectively. Once the digestions were complete, the solutions were diluted by adding MilliQ water to make the volume to 50 ml. The final solutions were stored at -20°C while awaiting metals determination.

Metal concentrations in the solutions were analyzed with an Inductively Coupled Plasma Mass Spectrometer (ICP-MS, Varian, Australia). Yttrium was used as an internal standard to correct for signal interference due to differences in solution matrix between calibration solutions and samples.

RESULTS & DISCUSSION

Different extraction methods yielded different quantities of metals (Table 3). In general, aqua-regia extraction liberated the highest quantity of metals, whereas among the partial extractions, sequential extraction yielded the highest quantity of metals followed by EDTA extraction while the least quantity of metals was extracted by water extraction (Table 3 and Fig. 3).

Average heavy metal concentrations in the sediments from the investigated sites obtained through water extraction are presented in Table 3. In all the sites, residual fraction held the highest concentrations of all elements as compared to the concentration of elements in water extractible fraction. Residual fraction with regards to water extractible metals refers to the difference in concentration between aqua regia and water extracted fractions of an element. The proportion of metal fractions extracted into water for each element was never exceeded 2% (typical values were less than 1%) of total metals from sediments except for the isolated cases of Mn and Co in Gazi. Generally, Mn was the most abundant element in water extractible fraction followed by Co and Cd (Fig. 2, Table 3). Water extraction yielded the least concentration of metal as compared to the other extraction methods (Table 3, Figure 2). The results of the water extractions indicate that most elements were less extractable using only water as a medium. The relatively higher abundance of Mn, Co and Cd in the water extractible fraction could be attributed to weak adsorption of these elements on the sediments particles (Tessier *et al.*, 1979). The higher relative abundance of Al and Cd observed in Gazi implies that the high bioavailability would probably result into higher than expected accumulations of these metals in marine plants despite the fact that the location is located in a remote mangrove area with no known pollution sources.

Average heavy metal concentrations in the sediments from study sites obtained through EDTA extraction are presented in Table 3. Generally, the most abundant metals in EDTA extractible fraction were Cd and Mn. Most elements in all the sites were predominant in the residual fraction with a relative abundance between 45.5- 98.0% of the aqua regia extracted sediments' metals apart from Mn in Gazi which was more abundant in EDTA extractable fraction (54.51%) as compared to residual fraction (Fig. 2). Residual fraction with regards to EDTA extractible metals refers to the difference in concentration between aqua regia and EDTA extracted fraction of an element. Comparison of relative concentration of metals in mobilizable fractions using EDTA extractions procedures are shown in (Table 4). EDTA extraction yielded intermediate amount of metal in comparison to the other partial extraction methods used (Table 3 and Fig. 2). In this study, the differences between EDTA extractible metals and the residual metals were found to be statistically significant ($P<0.05$) for all the sites. The significant differences observed between EDTA extractible metals and the residual metals shows that EDTA is useful in providing a contrast between anomalous and background samples. This is in agreement with the findings of Elliot and Shastri, (1999) that EDTA is known to extract only non-silicate metals from sediments i.e. the exchangeable, bound to organic matter and the carbonated phases and it is ineffective in removing metals from the detrital fractions. This proves that EDTA extraction can be used to provide a good idea about potentially bioavailable metals from sediments. Cd, Mn and Cu were the most abundant metals in the EDTA extracts, showing that EDTA was more effective in extracting these metals.

Average heavy metal concentrations in sediments obtained through BCR sequential extraction are presented in Table 3. When considering labile fraction (sum of F1 + F2 + F3), the most mobilizable elements were Cd, Mn and Co (Table 3, Fig. 2). Generally, most of the metals were more abundant in the residual fraction than in the labile fraction apart from Co in Gazi (64.96%) that was more abundant in the labile fraction as compared to the residual fraction (Fig. 2). Residual fraction with regards to sequential extractible metals refers to the difference in concentration between aqua regia and labile fraction of an element. BCR sequential extraction (which was used in this study to mimic any possible field condition that could control metals solubility) clearly showed that most of the metals were held up in residual fraction of the sediments. This further proves that not all metals are available in nature for uptake by macroalgae. Availability of metals is known to be controlled by the prevailing environmental

Table 3. The concentration of metals (µg/g) in the various extractable fractions

Metal	*Aqua regia (Total)*	*Labile*	*EDTA*	*WE*
GAZI				
Al	334.947	162.378	52.113	0.584
Cd	0.027	0.013	0.011	0.0004
Co	0.072	0.047	0.028	0.003
Cr	1.576	0.324	0.138	0.02
Fe	638.582	167.796	77.248	1.311
Mn	4.623	1.936	2.52	0.486
Ni	0.757	0.218	0.163	0.008
Zn	3.821	1.298	1.02	0.005
MIKINDANI				
Al	10129.4	1892.98	6.952	0.036
Cd	0.09	0.031	0.024	0
Co	11.859	3.304	1.183	0.009
Cr	37.973	4.892	0.07	0.028
Cu	23.092	5.935	4.024	0.001
Fe	22013.5	3633.41	446.128	1.305
Mn	248.854	97.292	62.553	0.82
Ni	21.524	7.567	1.135	0.012
Zn	94.376	28.432		0.011
MTWAPA				
Al	7473	1837.153	67.04	0.001
Cd	0.096	0.031634	0.037	0.000
Co	8.920	3.138526	1.185	0.002
Cr	37.24	9.173364	0.075	0.031
Cu	20.68	5.366608	4.354	0.001
Fe	15003	2480.711	369.9	1.409
Mn	150.45	52.19441	55.37	0.146
Ni	14.56	4.51225	0.897	0.008
Zn	80.52	21.33409		0.003
MSIMBAZI				
Al	6838	1594.023	33.13	0.012
Cd	0.212	0.066277	0.080	0.000
Co	7.245	2.2577	1.063	0.025
Cr	17.67	6.426221	0.210	0.010
Cu	20.48	8.807258	5.530	0.002
Fe	11987	1457.592	381.5	2.643
Mn	185.6	58.47317	61.21	5.382
Ni	14.99	2.482542	0.939	0.015
Zn	106.9	28.90342		0.020
CHALE				
Al	3296	566.301	129.4	0.116
Cd		0.004644		
Co	0.500	0.23782	0.077	0.003
Cr	9.576	4.241614	0.227	0.025
Cu	2.188	0.156	0.365	0.001
Fe	2787	599.0232	129.6	1.256
Mn	20.67	8.064862	8.547	1.131
Ni	6.306	1.193789	0.328	0.010
Zn	11.35	1.082377	0.683	0.003

Fig. 2. The relative concentration (log_{10} scale) of mobilizable metals yielded by various extraction methods

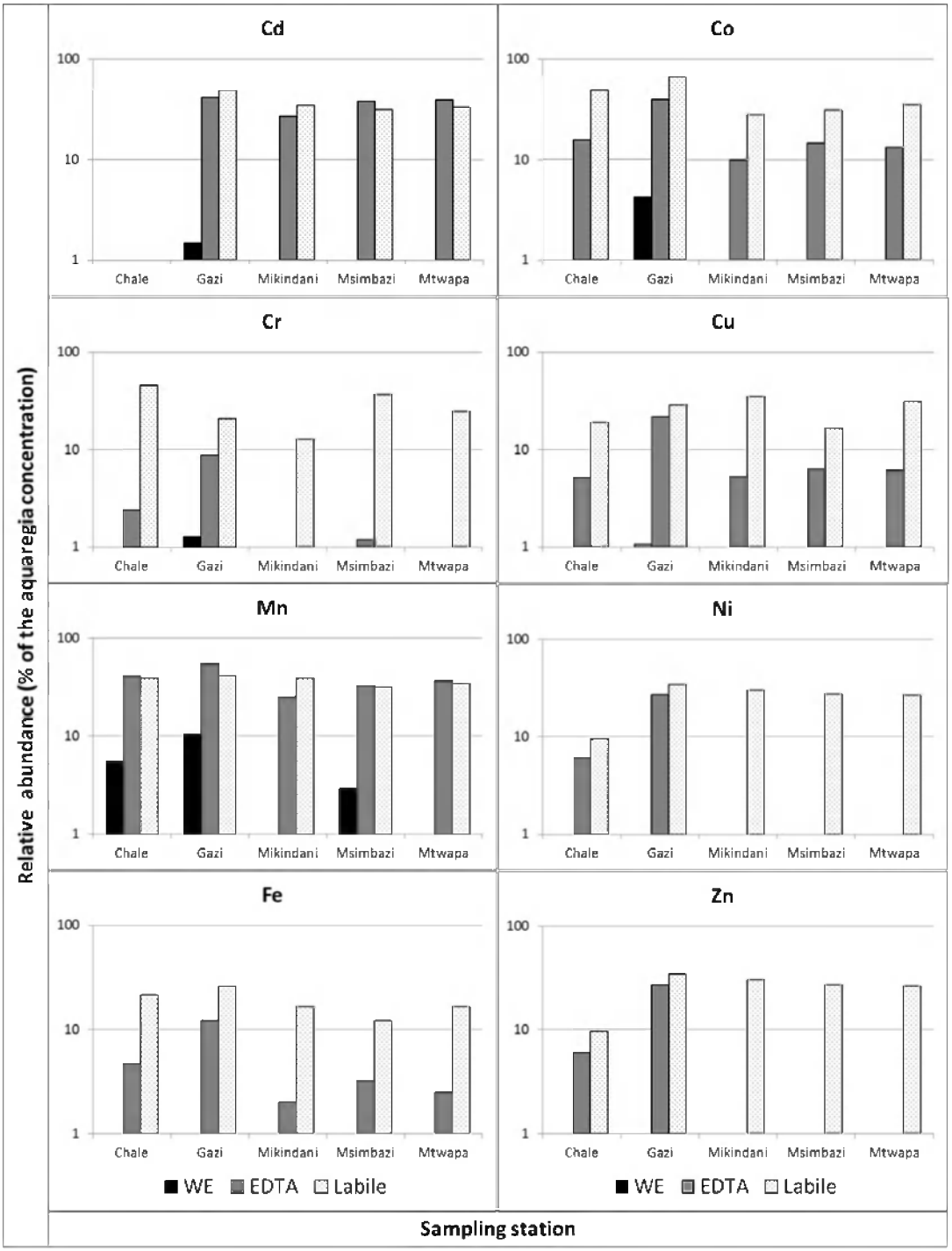

Fig. 3. Tissue metals concentrations of *Sargassum spp* from Chale (S.CH), Gazi (S.GA) and Mtwapa (S.MT) and *Ulva lactuca* from Chale (U.CH), Mikindani (U.MK) and Msimbazi (U.MS)

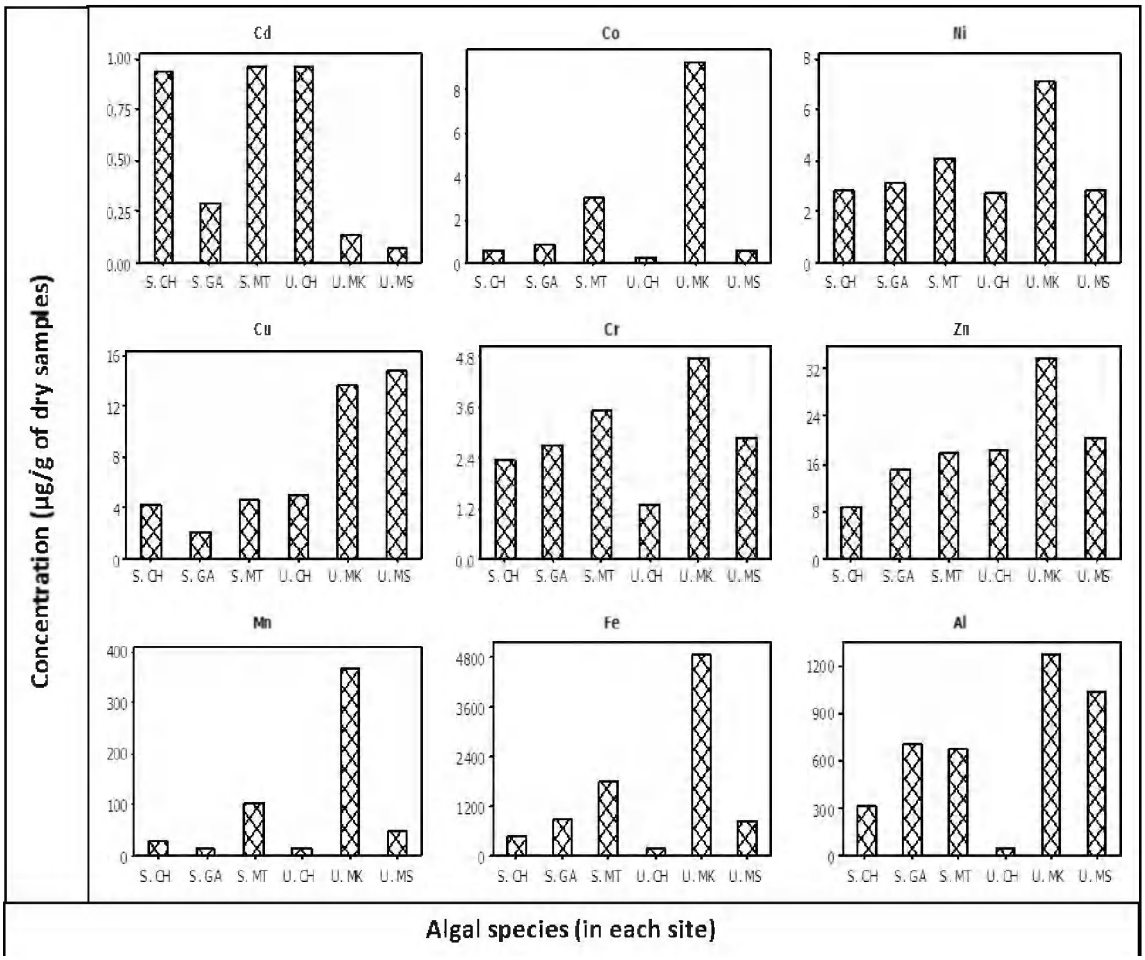

Table 4. Correlation matrix of marine plants tissue metals concentration and the environmental concentration (estimated by different extraction procedures)

Element	Aqua regia (Total)	Labile	EDTA	WE
U. lactuca				
Al	0.870*	0.931**		
Cd		0.753*		
Co	0.779*	0.743*		
Cu	0.749*		0.799**	
Fe	0.782*	0.940**	0.705*	
Mn		0.787*		
Ni		0.862**		
Zn		0.828*		
Sargassum spp				
Co	0.908**	0.911**	0.861**	
Cu		0.666*		
Fe		0.651*	0.578*	1.000*
Mn		0.833**		
Zn		0.684*		

* Statistically significant ($p<0.05$); ** ($p<0.01$)

conditions. Kerez *et al.,* (1994) identified some physicochemical parameters that influence metal solubility as pH, salinity, temperature, light, particulate matter, and organic matter. When considering sequential extraction mobilizable (labile) fractions, Cd and Mn were the most abundant elements thus probably the most mobilizable elements. Despite the fact that Gazi was considered in this study to be a relatively pristine site with very low levels of total metals (than the other sites), a substantial amount (41.9-65% of the total) of most elements (Al, Cd, Co and Mn) in this site were in the mobilized fraction. As stated elsewhere by Fernandes, (1997) the occurrence of metals in more easily leached phases would characterize samples collected at polluted sites. The case of Gazi is actually unique due to very low levels of total extractible metals. The higher bioavailability could thus be attributed to naturally occurring process such as weathering and the elemental composition of the parent rock and particles grain size or the prevailing environmental condition that might be favouring desorption as compared to sorption processes.

Average heavy metal concentrations in the sediments from the study sites obtained through aqua regia extraction are presented in Table 3. In all the sampling sites, Fe was present in the highest concentration with a range of 638.52-22,013.5 µg/g whereas Cd had the least concentration with a range of 0.027- 0.212 µg/g. Mikindani had the highest concentration of aqua regia extracted (total) metals whereas Chale had the least concentration of all the elements (Table 3). The difference (in terms of the concentrations of total heavy metals in the sediments) between the Gazi and Chale in one hand and Mtwapa, Msimbazi and Mikindani sites on the other hand could be explained by proximity to pollution point sources this is in agreement with the findings of previous works by Biney *et al.,* (1994) in some selected sites in Africa. *Ulva lactuca* collected from Mikindani bioaccumulated higher levels of Co, Ni, Zn, Cr, Mn, Fe and Al as compared to macroalgae collected from the other sites. Comparison of the bioaccumulation potential for *U. lactuca* and *Sargassum spp* was done using Chale data since both species were found in Chale (i.e. exposed to the same levels of metals). It was evident that *U. lactuca* was only more efficient in bioaccumulating Cd and Cu whereas *Sargassum spp* was more efficient in bioaccumulating Al, Co, Cr, Fe and Mn. Both *U lactuca* and *Sargassum spp* were found to be good bioaccumilators of Cd that was present in concentrations below detection limits of the analytical technique but was efficiently bioaccumulated to level of 0.955 µg/g and 0.939 µg/g in *Ulva lactuca* and *Sargassum spp* respectively. This clearly shows that these macroalgae can indeed be used in monitoring pollutants with concentrations below the detection limits of analytical equipment.

Generally, metal tissue concentration of specific metals in the considered plants followed the following order Fe>Al>Mn>Zn>Cu>Co>Ni>Cr>Cd (Fig. 3). The trends for the specific species were as follows: Fe> Al> Mn> Zn>Ni> Cu> Cr> Co> Cd and Fe> Al> Mn> Zn> Cu> Co> Ni> Cr> Cd for *Sargassum spp.* and *Ulva lactuca* respectively (Fig. 3). Fe, Mn and Zn, which are essential metals, were among the most abundant metals in plant tissues. Other essential metals such as Ni, Cu and non- essential metals such as Cd, Cr were also present in the tissues (Fig. 3).

Al, Cd, Co, Fe, Mn, Zn and Ni in *Ulva lactuca* showed significant correlation with concentration in sediment's labile fraction yielded through BCR extraction whereas, only Al, Co, Cu and Fe in *Ulva lactuca* showed significant correlation with total metals extracted through aqua regia procedure. Only Cu and Fe showed significant correlation with EDTA extracted metals. Water extractable metals showed no significant correlation with the metals bioaccumulated in *Ulva lactuca* (Table 4).

Co, Cu, Fe, Mn and Zn in *Sargassum spp.* showed significant correlation with concentration in sediment's labile fraction yielded through BCR extraction whereas only Co showed significant correlation with concentration in sediment's aqua regia extracted metals. Only Cu and Fe showed significant correlation with EDTA extracted metals while water extractable metals showed significant correlation with only Fe bioaccumulated in *Sargassum spp* (Table 4).

The high levels of some metals in the plant reflect the high bioavailability of these metals in the study area, and the capacity of the alga to take them up and sequencer them (Karez *et al.,* 1994). The discriminatory uptake of the essential metals could also be due to the important enzymatic functions that they play in these plants. Metals concentration in macroalgae from the impacted areas (Mikindani, Mtwapa and Msimbazi) were higher than for those from a relatively pristine sites (Gazi and Chale), proving that *U. lactuca* and *Sargassum spp* bioaccumulation potential for metals was dependent on the level of metals in the environment. This finding supports the finding of Forsberg *et al.,* 1988 which reported that algae have been considered as a valuable indicator for the assessment of heavy metals in coastal areas because of their faithful accumulation capacity. Haritonidis *et al.,* (1993) and Rijstenbil *et al.,* (1993) further attributed the ability of macroalgae to bioaccumulate heavy

metals to the formation of thiols and peptides when found under environmental stress *Ulva lactuca* from Mikindani had relatively higher concentration of bioaccumulated metals than the macroalgae from the other sites. The high bioaccumulation capacity of *Ulva lactuca* has been reported elsewhere by Ho, (1990a), who attributed the high bioaccumulation capability to the cells in the thallus that are structurally uniform and physio- logically active in uptake of metals. The ability of *Ulva lactuca* to show wide range in bioaccumulation depending on the availability of metals (as seen in this study between bioaccumulated metal in Mikindani *Ulva* verses *Ulva* in Chale) has made *U. lactuca* to be widely used as a heavy metal indicator for metals such as Fe, Mn, Pb, Cu, Zn (e.g. Talbot and Chegwidden, 1982; Ho, 1990a). Brix *et al.,* (1983) also recommended the use of *U. lactuca* as a suitable indicator organism because of its reasonable size; it is sedentary nature, ease of collection and high bioaccumulation capacity for metals.

The relative abundance of metals in the two macro-algal species reflects uptake that is proportional to either the total levels of metals in the sediment or the available metals estimated mostly by BCR extraction and in a single case by EDTA extraction. Labile fraction showed significant relationships with a total of 12 elements bioaccumulated in *U. lactuca* (7) and *Sargassum spp* (5) whereas aqua regia extracted metals showed only 5 significant relationships with *Ulva* (4) and *Sargassum* (1). Ideally based on the results of this study, *Sargassum spp* could have been disqualified as a potential biomonitor of metals if only total metal (aqua regia extracts) were considered. Labile fraction has therefore proved to be an effective method that can be used to determine the macroalgae that could be used for monitoring specific elements even in cases that total metals have failed.

The results of this study have pointed out some of the weakness in using total metals concentration (aqua regia extracts) for determining the macroalgae to be used for biomonitoring. For instance the use of total concentration could have limited the use of *U. lactuca* to monitoring of Al, Co, Cu and Fe, this could have left out Cd, Mn , Zn and Ni that can also be effectively be monitored by *Ulva lactuca*. The worst case is that of *Sargassum spp*, in which the use of total metals could have recommended the use of *Sargassum spp* for monitoring only Co (if not rejecting its use all together) and this could have left out metals such as Cu, Cu, Fe, Mn and Zn that could be monitored effectively by *Sargassum spp*. *Ulva lactuca* can therefore be used for biomonitoring in East Africa coast to give an indication of the concentration of labile Al, Cd, Co, Fe, Mn, Zn and Ni whereas *Sargassum spp* can be used to give an indication of labile Co, Cu, Fe, Mn and Zn.

CONCLUSION

From the results of this study, it could be concluded that:

1. The role of *Ulva lactuca* and *Sargassum spp* from the study areas as biomonitors and conduits for transfer of heavy metal to upper trophic levels is significant.
2. *Ulva lactuca* can be used as a biomonitor in East African coast to give an indication of pollution status of Al, Cd, Co, Fe, Mn, Zn and Ni. In sites where *Ulva lactuca* is not available, *Sargassum spp* could be used to give an indication pollution status of Co, Cu, Fe, Mn and Zn. Using the two species as a complements is also encouraged. It should however, be noted that such indications are based on bioavailable metals in sediments and not total amount of sediments
3. The use of total metal concentration for screening of potential biomonitor should be discouraged since the concentrations are not environmentally relevant and as such possible potential biomonitor candidates could be locked out. Where its use is necessary, such cases should also consider using BCR sequential extraction concentrations as a complimentary tool.
4. The use of water extraction and EDTA extracts for screening of potential biomonitors is strongly discouraged.

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