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Assessment of Heavy Metal Contamination in Red Seaweeds from the Coastal Areas of Zanzibar by Using EDXRF Technique

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Authors' contributions

This work was carried out in collaboration between both authors. Authors SAS, SKS and MAK have designed the study, performed the statistical analysis and literature searches and wrote the first draft of the manuscript. Authors AAM and FOM supervised the analyses of the samples, reviewed the first draft and wrote the final manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The content of Mn, Zn, Ni, Cu, Fe, and Pb in 10 samples of *Kappaphycus alvarezii* (cottonii) and 10 samples of *Eucheuma denticulatum* (spinosum) from 15 different sites in the intertidal coastal area of Zanzibar were determined by using EDXRF. The heavy metals assessed in both species decreased in the order of Fe >Mn > Zn >Pb> Cu > Ni. In general, the cottonii samples in Unguja had significantly higher ($p \leq 0.05$) concentrations of Fe, Zn, Ni, Cu and Pb than the spinosum samples, but the latter had significantly higher levels of Mn. Similarly, the cottonii samples in Pemba had significantly higher ($p \leq 0.05$) concentrations of Ni, Cu and Pb than the spinosum samples, but the latter had significantly higher levels of Fe and Zn. The concentrations of heavy metals in cottonii and spinosum in the present study were noticed lower compared to publish results. The results from this study revealed that, the heavy metals contaminations in cottonii and spinosum along the coastal area in Zanzibar were mainly caused by effluents that directly flow into

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marine environments. Hence, serious measures should be taken to reduce the flow of waste effluents into the marine ecosystem.

Keywords: Heavy metals; seaweed; zanzibar; EDXRF.

1. INTRODUCTION

Heavy metals are released to the environment through various industrial activities, domestic wastewater, geological weathering of the earth's crust, shipping and harbor activities and atmospheric deposition [1]. These metals can enter into the aquatic environment and deposit into marine organisms via the bio-accumulation and bio-concentration through the food chain; they then become toxic when their accumulation reaches substantial levels [2]. The contamination of aquatic environments by these metals due to anthropogenic activities poses major effects on marine plants. The adverse effects of heavy metals, such as Mn, Hg, Zn, Cd, Cu and Pb, have raised public safety concerns because of their high toxicity, non-degradability and persistence in the environment [3-5]. Some metals, such as Cd, Pb and Hg, show high toxicity even at trace levels and, thus, rank amongst the priority metals that are of great significant to public health [3]. Heavy-metals pollution transcends boundaries, and the metals may discharge into the marine environment through various anthropogenic activities [6,7]. Heavy-metal pollution could negatively affect marine species, including seaweeds, and poses considerable environmental risks [8,9]. Increasing significance of heavy-metal contaminants disturbing environment is especially evident in aquatic systems [10].

Seaweeds are considered bio-indicators of heavy-metals contamination in marine ecosystems and can be used to detect mineralisation and the anthropogenic impact of coastal marine communities [11-13]. Recent studies have shown that varying concentration of heavy metals contaminants can be observed in marine plants species, including seaweeds [14-16]. Khalid et al. 2014 and Qari, 2015 separately reported the presence of heavy metals in seaweed by using Atomic Absorption Spectroscopy (AAS) [17,18]. Mutia et al. 2018 also revealed the presence of As, Pb, Cd and Hg in two seaweed species namely, *Ulvarigida* and *Halimedaopuntia*, by using AAS [19]. In Zanzibar, studies on the accumulation of heavy-metals in seaweeds are scarce. Thus, the aim of this study is to assess the concentrations of heavy metals in *Kappaphycus alvarezii* (cottonii) and *Eucheuma denticulatum* (spinosum) cultivated in Zanzibar by using energy dispersive X-ray fluorescence (EDXRF). This technique is a suitable for multi-element analysis; it requires no chemical pre-treatment and only small sample amounts. Therefore, the method can minimise the occurrence of sample contamination and allows the simultaneous detection of a wide range of elements.

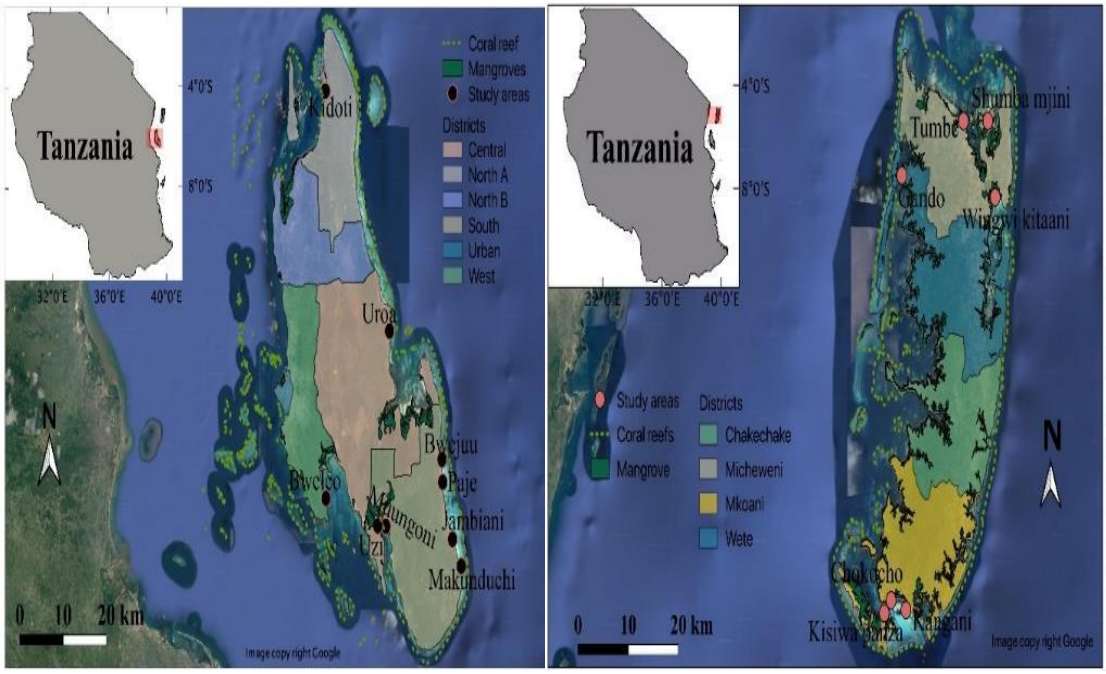
2. MATERIALS AND METHODS

2.1 Description of the Study Area

The samples analysed in this study were random collected from the intertidal coastal areas in Zanzibar's Islands with have a population of approximately 1.3 million [20]. It comprises Unguja and Pemba as main islands and numerous smaller isles, the first being the biggest of the two. Many of Zanzibar's resident live near the marine environment, and seaweed farming is one of the major economic activities. The sampling areas in this study included Chokocho likokuu, Chokocho kisiwa panza, Kangaani kuukuu, Gando, Wingwi kitaalani, Micheweni shumbamjini, Tumbe, Makunduchi, Bweleo, Paje kikwaju jeuri, Muungoni duta, Jambiani mbuyuni, Kidoti Bondeni, Bwejuu and Uroa (Fig. 1). The sampling areas were selected according to the richness of Spinosum and Cottonii species.

2.2 Sampling and Sampling Preparation

Ten samples of each Spinosum and Cottonii were collected randomly from 15 different sites in the intertidal coastal areas of Zanzibar. The collected samples were washed with sea water to remove epiphytes and coarse debris and then placed in labelled plastic bags. Upon arrival at the laboratory, the samples were rinsed with distilled water to remove any traces of elements resulting from contamination. Thereafter, all samples were first air dried for 72 hours and then were oven-dried for 24 hours at 70^0C to a constant weight. The dried samples were then grind into a fine powder using pestle and mortar. The powdered materials were sieved through a 2 mm polystyrene sieve prior to their use.



(a) **(b)**

Fig. 1. Map of Zanzibar showing the sampling points (a) Unguja (b) Pemba

2.3 XRF Preparation

Exactly 4 g (dry weight) of each sample was mixed with 0.9 g of cellulose binder, placed into a bowl together with four spherical balls (radius, 3 mm) and then fixed to pulveriser for further grinding and homogenisation. The Pulverized machine was set at a speed of 150 rpm for 10 minutes. A hydraulic RetschTM machine was used to compress the samples into pellets through application of 15 tons of pressure. The pellets were labelled and taken to the EDXRF machine (Model: Xepos, Serial No. 4R0138) for analysis. The EDXRF system was operated using X-lab ProTM computer software, which considers matrix effects. Here, X-rays are generated by the X-ray tube built into the EDXRF. The instrument use three different secondary targets to increase the excitation sensitivity of elements, the Molybdenum secondary target for (K-Line Cr-Y) and L-Line from (Hf-U), Aluminium oxide (Al2O3) polarization target (For L-Lines Zr-Ce) and for High Oriented Pure Graphite (HOPG) Bragg crystal (K-Lines from Na-V). The spectra running time was 30 minutes per sample.

The concentrations of elements in the samples were calculated by using the built-in software called X-lab ProTM with the Turboquant (Tq 9232) algorithm for matrix effect correction [21]. The software corrects for matrix effects (M_i) and interference effects (K_i) effects according to the fundamental parameter methodology. The software corrects for the background effect on a spectral line intensity (I_i), which is given as counts per second (cps). After all corrections are made, the software converts the intensity of the fluorescent radiation into an element concentration using Equation (1) below [22].

$$C_i = K_i \times I_i \times M_a \quad (1)$$

where C_i is the concentration of a given element i, M_a is the correction factor for matrix effects. K_i is the constant of proportionality, I_i is the intensity of the fluorescent radiation from the element i.

2.4 Statistical Analysis

One-way ANOVA was used to evaluate the differences among seaweed species. Prior to ANOVA, the homogeneities of the variances were verified using Levene's test. A *t*-test was used to statistically compare the mean concentrations of elements collected from the two seaweed species. A probability level of $p < 0.05$ was considered statistically significant. All

data were presented as arithmetic mean with standard deviation attached. All statistical analyses were made using the software Excel 2013 and SPSS Version 23, and figures were produced using Origin Version 8.5 software.

3. RESULTS AND DISCUSSION

3.1 Heavy-Metal Concentrations in Spinosum

The heavy metals in spinosum samples collected from the intertidal coastal areas of Unguja and Pemba and their mean concentration were assessed. Samples from Unguja showed mean concentrations of Mn, Zn, Ni, Cu, Fe and Pb in spinosum were 44.94 µg/g, 8.16 µg/g, 1.76 µg/g, 2.97 µg/g, 788.67 µg/g and 3.83 µg/g, respectively. The heavy metals detected found in the order of Fe > Mn > Zn > Pb > Cu > Ni. The ranges of mean concentration values of these metals were: 12.8−44 µg/g for Mn, 1.3−8.16 µg/g for Zn, 1.24−1.76 µg/g for Ni, 1.99−2.97 µg/g for Cu, 180.71−788.67 µg/g for Fe and 1.45−3.83 µg/g for Pb. Out of these metals examined, Fe showed the highest concentration (180.71−788.67 µg/g), whereas Ni revealed the lowest mean concentration (1.24−1.76 µg/g). By comparison, spinosum samples from Pemba showed mean heavy-metal concentrations in the range of 10.98−53.06 µg/g for Mn, 2.33−11.97 µg/g for Zn, 1.04−1.89 µg/g for Ni, 1.42 −2.65 µg/g for Cu, 230.20−1696.86 µg/g for Fe and 1.44−2.79 µg/g for Pb. Moreover, the mean concentrations of heavy metals detected in spinosum decreased in the order of Fe > Mn > Zn > Pb> Cu > Ni. The *t*-test was used to compare the mean concentrations of heavy metals recorded in spinosum and cottonii samples from Unguja and Pemba. In this test, significance was set at $p \leq 0.05$.

In general, samples from Pemba had significant higher ($p \leq 0.05$) mean concentration of Fe and Zn, whereas samples from Unguja had significantly higher concentrations of Mn, Ni, Cu and Pb (Fig. 2). Spinosum showed high mean concentrations of Mn, followed by Zn, Pb and Cu, and low mean concentrations of Ni; this findings agrees with the result reported by [23]. Moreover, the mean concentrations of Pb and Cu were lower than those reported in the study of Dadolahi-Sohrab et al. 2011 [24]. The lower values observed in our study may be attributed to difference species of red seaweed. Specifically, Dadolahi-Sohrab et al. 2011 [24] used *Acanthophora specifera,* whereas the present study used *E. denticulatum* to assess heavy metals levels.

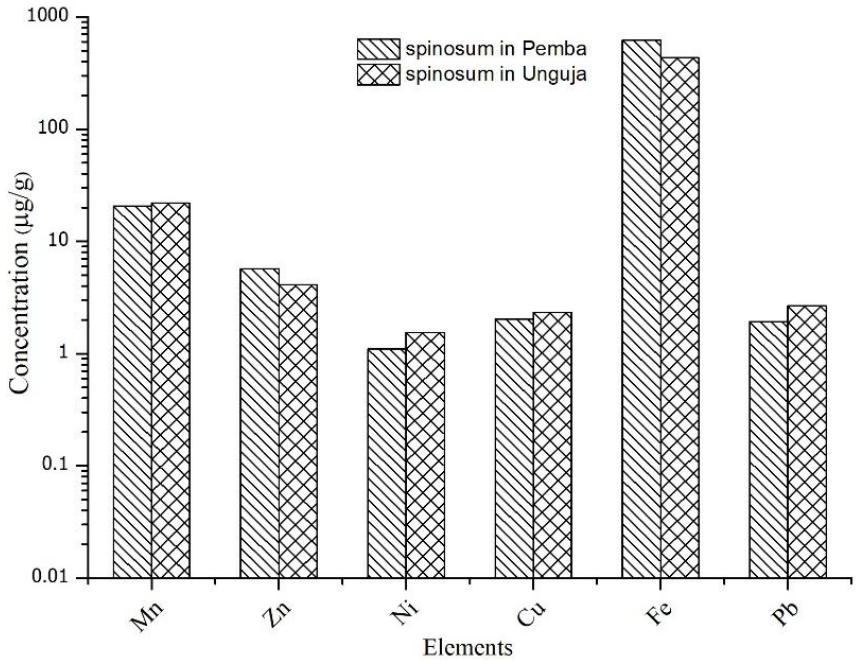


Fig. 2. Comparison of heavy-metals concentrations in spinosum samples collected from Unguja and Pemba

3.2 Heavy-Metals Concentrations in Cottonii

The concentrations of Mn, Zn, Ni, Cu, Fe and Pb in cottonii samples collected from Unguja and Pemba are given in Tables 1 and 2. The range of heavy-metal contaminations in samples collected from Unguja varied as follow: 8.27−19.48 µg/g for Mn, 4.7−10.62 µg/g for Zn, 1.78−2.16 µg/g for Ni, 2.34 −3.36 µg/g for Cu, 425.54−666.77 µg/g for Fe and 1.83−4.62 µg/g for Pb; in Pemba, these heavy-metal contaminants showed the following variations: 11.41−32.09 µg/g for Mn, 2.61−6.32 µg/g for Zn, 1.37−1.92 µg/g for Ni, 2.56 −3.24 µg/g for Cu, 401.89−828.44 µg/g for Fe and 3.75−4.18 µg/g for Pb. The distribution of heavy metals decreased in the order of Fe > Mn > Zn > Pb> Cu > Ni. Samples collected from Unguja had significant higher ($p \leq 0.05$) mean concentrations of Zn, Ni and Cu compare with samples collected from Pemba, but the latter had significantly higher mean concentrations of Mn, Fe, and Pb (Fig. 3). Interestingly, the concentrations of Pb, Cu and Ni in samples collected from Pemba and Unguja were lower compared with those reported by Dadolahi-Sohrab et al. 2011 [24]. The higher concentrations of Cu and Zn in the samples collected in Unguja and Pemba may be attributed to the discharge of municipal wastewater to the marine environment.

The concentrations of Mn in the cottonii samples in the present study were in the range of 11.41−32.09 µg/g in Unguja and 18.27−19.48 µg/g in Pemba. As indicated in Table 1, 32.09 µg/g for Mn was detected at in samples collected from Muongoni duta in Unguja, whilst 19.49 µg/g of Mn in samples collected from chokocho likokuu in Pemba, as shown in Table 2. Higher concentrations of Mn in plants are a common feature for maintaining osmosis balance, ion regulation and enzyme catalysis [25]. Homaidan et al. 2011 [26] reported high concentrations of Mn in seaweeds. While this element is important as a micronutrient for plants growth, it is toxic at concentrations exceeding the amount needed for normal growth. In this study, 4.18 µg/g and 4.62 µg/g of Pb were found in cottonie samples from Muungoni duta and Kangaani kuukuu, respectively. The abundance of Pb is due to lubricant oils used by diesel boat engines [27]. Average of Pb concentrations in the cottonii samples decreased amongst sampling site in the order of Muongoni duta > Uzi > Bweleo (Table 1) and Kangaani kuukuu > Chokocho kisiwa panza > Chokocho likokuu (Table 2).

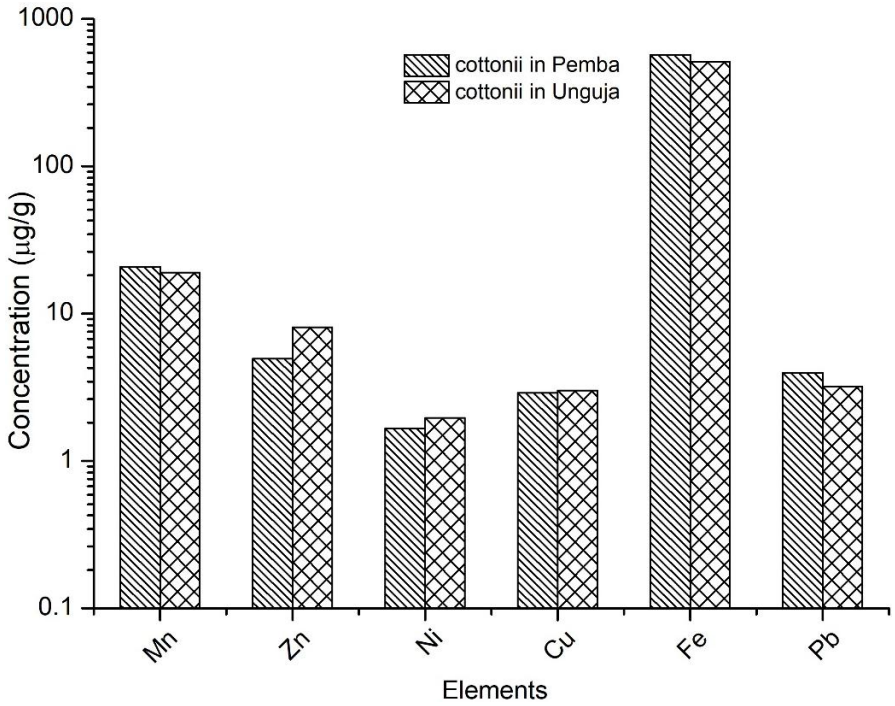


Fig. 3. Comparison of heavy-metal concentrations in cottonii collected from Unguja and Pemba

Table 1. Mean ± SD concentrations (µg/g) of heavy metals in cottonii collected from Unguja

Site	Elements					
	Mn	Zn	Ni	Cu	Fe	Pb
Bweleo	11.41±1.09	2.61±0.73	1.37±0.97	BDL	401.89±12.56	BDL
Muongoni duta	32.09±7.75	6.32±0.90	1.68±0.26	2.56±0.11	828.44±11.81	4.18±0.13
Uzi	19.03±7.20	5.92±0.84	1.94±0.01	3.40±1.09	487.18±9.88	3.75±1.36

Note: BDL=below detection limit

Table 2. Mean ± SD concentrations (µg/g) of heavy metals in cottonii collected from Pemba

Site	Elements					
	Mn	Zn	Ni	Cu	Fe	Pb
Chokocho kisiwa panza	19.22±6.84	8.91±0.58	1.78±0.28	2.34±0.41	446.81±10.82	3.13±1.01
Kangaani kuukuu	18.27±4.04	4.70±0.52	2.16±0.44	3.36±0.50	425.54±9.58	4.62±0.29
Chokocho likokuu	19.48±5.16	10.62±0.69	1.94±0.37	3.33±0.89	666.77±13.07	1.83±1.29

3.3 Heavy-Metal Concentrations in Spinosum and Cottonii

Heavy metals concentrations in the spinosum and cottonii were compared, as presented in Fig. 4. The *t*-test showed that cottonii samples collected from Unguju had significantly higher ($p \leq 0.05$) mean concentrations of Fe, Zn, Ni, Cu and Pb than spinosum sample. However, the latter had a significant higher ($p \leq 0.05$) mean concentration of Mn.

Samples of cottonii collected from Pemba had significantly higher ($p \leq 0.05$) mean concentrations of Ni, Cu, Mn and Pb compared with samples of spinosum; however, the latter had significantly higher mean concentrations of Fe and Zn as shown in Fig. 5. Figs. 4 and 5 reveal that the mean concentrations of Fe were much higher compared with those of Zn. Specifically, the respective mean concentrations of Fe in the cottonii and spinosum samples collected from Unguja were 63.5 and 105.7 times higher than those of Zn.

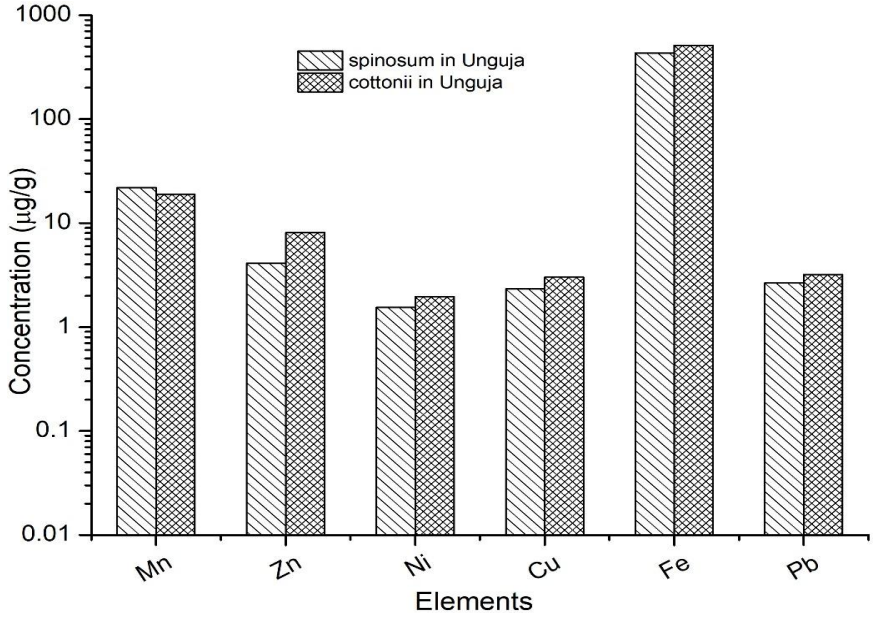


Fig. 4. Comparison of heavy-metal concentrations in cottonii and spinosum collected from Unguja

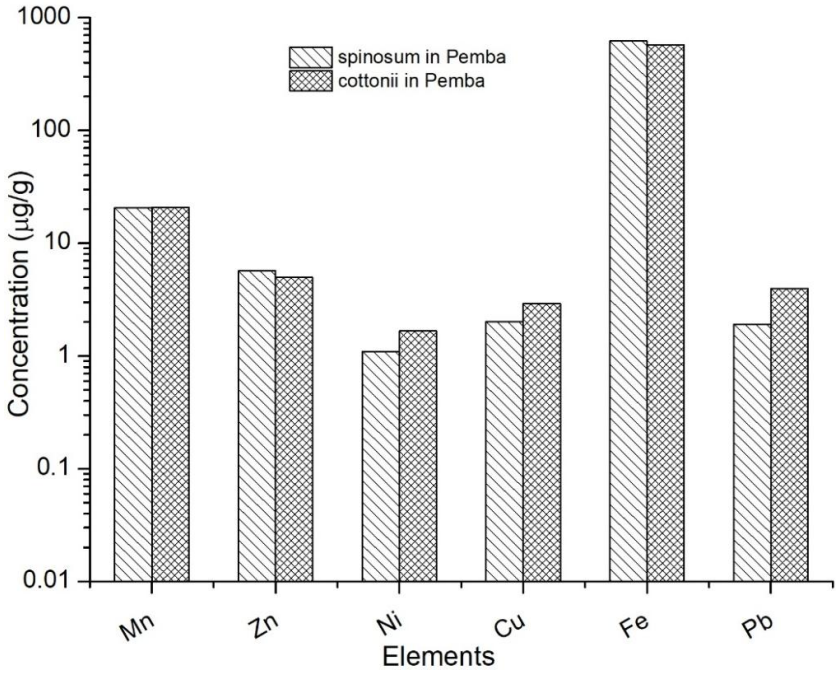


Fig. 5. Comparison of heavy-metal concentrations in cottonii and spinosum collected from Pemba

The concentrations of Pb in samples of cottonii collected from Unguja and Pemba were much higher than those in samples of spinosum, likely because of the lubricating oil used in diesel boat engines [27]. The levels of heavy-metal contaminations in both samples may be attributed to waste effluents directly flowing into the marine environment. Waste effluents from various anthropogenic activities have been shown to contain several heavy metals including Fe, Zn, Ni, Cu and Pb [28-30]. Ni and Mn, were not reported in any of the literature reviewed, however, they were found in low concentrations in both samples.

4. CONCLUSION

This study illustrates the application of the EDXRF nuclear technique to assess heavy-metal contamination in red seaweed comprising cottonii and spinosum species collected from 15 sites in the intertidal coastal areas of Zanzibar. The *t*-test was used to compare the mean concentrations of heavy metals recorded in samples of spinosum and cottonii from Unguja and Pemba. In this test, significance was set as $p \leq 0.05$ and the results obtained agree with those of other studies. The levels of heavy-metal contaminations in cottonii and spinosum in this study were noticeably lower compared with published results. Samples of cottonii in Unguja had significantly higher ($p \leq 0.05$) mean concentrations of Fe, Zn, Ni, Cu and Pb than samples of spinosum, but the latter had significantly higher concentrations of Mn. Similarly, samples of cottonii in Pemba had significantly higher ($p \leq 0.05$) mean concentrations of Ni, Cu and Pb than samples of spinosum, but the latter had significantly higher mean concentrations of Fe and Zn. In generally, the heavy-metals contaminations detected in cottonii and spinosum along the coastal area in Zanzibar were mainly caused by waste effluents flowing directly flow into the marine environment. Therefore, serious measures should be taken to reduce the flow of waste effluents into the marine ecosystem.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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