***Original Research Article***

# Assessment of heavy metal pollution in red seaweed collected from intertidal coastal areas in Zanzibar by using EDXRF technique

.

## ABSTRACT

Few studies have been reported the heavy metals concentrations in red seaweed comprises cottonii and spinosum species throughout the world. This study was designed to assess heavy metals (Mn, Zn, Ni, Cu, Fe, As and Pb) contaminations in cottonii and spinosum species commonly farmed in Unguja and Pemba, Zanzibar. The species were collected from intertidal coastal areas and concentration of the assessed metals were carried out using Energy Dispersive X-ray Fluorescence (EDXRF). The samples of cottonii in Unguja had significantly ($p \leq 0.05$) higher concentrations of Fe, Zn, Ni, Cu and Pb than in spinosum, while the samples of spinosum had significantly higher levels of As and Mn. Likewise, the samples of cottonii in Pemba had significantly ($p \leq 0.05$) higher concentrations of Ni, Cu and Pb than in spinosum, whereas the samples of spinosum had significantly higher levels of Fe, As and Zn. The concentrations of heavy metals in cottonii and spinosum in the present study were noticed lower compared to publish results. However, the heavy metals contaminations in cottonii and spinosum along the coastal area in Zanzibar were mainly caused by effluents that directly flow into marine environments.

*Keywords: Heavy metals; Seaweed; Zanzibar; EDXRF*

## 1. INTRODUCTION

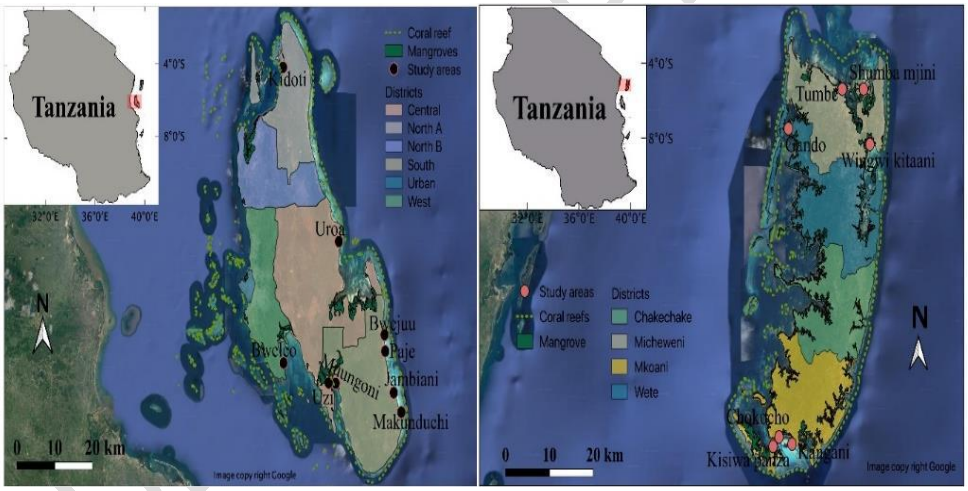
Heavy metals are released into the environment following various industrial activities, domestic wastewater, geological weathering of the earth crust, shipping and harbour activities, and atmospheric deposition [1]. The metals can enter into the aquatic environment and deposited into marine organism via the effects of bioaccumulation and bio-concentration through the food chain process and become toxic when accumulation reaches a substantially high level [2]. The contamination of these metals into aquatic environment posing a major effects of marine plants which is directly related to anthropogenic activities. The adverse effects of heavy metals such as As, Mn, Hg, Zn, Cd, Cu and Pb have raised public concern about the safety due to their toxicity level, non-degradable and persistent in the environment [3-5]. It has been also reported that metals such as As, Cd, Pb and Hg have a high degree of toxicities even at trace levels and ranks among the priority metals that are of public health significant [3]. Heavy metals pollution does not respect any boundaries and may discharge into marine environment by various anthropogenic activities [6, 7]. The contamination of the marine ecosystem by heavy metals pollution negatively affects marine species including seaweeds and may pose considerable environmental risks and concerns [8, 9]. Although seaweeds provide essential nutritional elements, the increasing significance of heavy metals disturbing the environment is especially evident in aquatic systems [10].

Seaweeds were identifying as bio-indicators of heavy metals contamination in the marine ecosystem for detecting mineralization and anthropogenic impact of coastal marine communities [11-13]. Studies have shown that heavy metals pollution can be found in varying concentrations in marine plants species including the seaweeds [14-16]. Moreover, in the studies of Khalid et al. 2014 and Qari, 2015 reported separately the presence of heavy metals in seaweed by using Atomic Absorption Spectroscopy (AAS) [17, 18]. Mutia et al. 2018 has also found the presence of As, Pb, Cd and Hg in two seaweed species namely as Ulva rigida and Halimeda opuntia by using AAS [19]. In Zanzibar, a very limited number of studies have been publish about the accumulation of heavy metals of seaweeds. Ther aim of this study was to assess the heavy metals in Eucheuma denticulatum (cottonii) and Eucheuma spinosum cultivated in Zanzibar islands by using Energy dispersive X-ray fluorescence (EDXRF). The EDXRF has been shown to be a suitable technique for multi-element analysis, no chemical pre-treatment and small amount of sample is required, minimising sample contamination and the wide range of elements which may simultaneously be detected.

## 2. MATERIAL AND METHODS

### 2.1 Description of the study area

The samples analysed in this study were collected from the intertidal coastal areas in Zanzibar Islands with a population of about 1.3 million Peoples [20]. Zanzibar comprises the Zanzibar Archipelago in the Indian Ocean, 25-50 kilometres off the eastern coast of Mainland Tanzania. In the western part of the Indian Ocean, between latitudes 50 and 60 south of the equator and between 390 and 450 east of the Greenwich Meridian. It comprises Unguja and Pemba as main islands and numerous small isles, the first being the biggest of the two. In Zanzibar, peoples lived near to marine environment and seaweed farming is one of the major significant economic activities. The seaweed farming activities mainly take place along intertidal coastal regions and sampling areas includes; Chokocho liko kuu, Chokocho kisiwa panza, Kangaani kuukuu, Wingwi kitaalani, Micheweni shumba mjini, Tumbe, Makunduchi, Bweleo, Paje kikwaju jeuri, Muungoni duta, Jambiani mbuyuni, Kidoti Bondeni, Bwejuu and Uroa (Figure. 1). These sampling areas have been selected based on the richness of the Spinosum and Cottonii species, proximity to small scale industrial activities and effluents that directly flow into marine environments.



(a) (b)

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

### 1.2 Sampling and Sampling Preparation

Ten samples of Spinosum and ten samples of Cottonii were collected randomly from 15 different intertidal coastal areas in Zanzibar. The collected samples washed up on the seas 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 $^{0}$C to obtain a constant weight. The dried samples were then grinded into fine powder using pestle and mortar. The powdered materials in each sample species were sieved through 2-mm polystyrene sieve.

### 1.3 XRF preparation

A dry weight of 4 g of each sample with 0.9 g of cellulose binder was put into a bowl together with four spherical balls each with 3 mm radius and fixed to pulveriser which was further grinded and homogenised. The Pulverized machine was set at a speed of 150 revolutions per minutes (rpm) for 10 minutes. By using hydraulic RetschTM machine, the samples were compressed into pellets through application of pressure of 15 tones. The pellets were labelled and taken to the EDXRF machine for analysis. The model of the system is Xepos with serial No. 4R0138 and is operated by using X-lab ProTM computer software in which matrix effects was counted for. In this model, X-rays are generated by the X-ray tube built in 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.

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

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

where Ci is the concentration of a given element i, Ma is the correction factor for matrix effects. Ki is the constant of proportionality, Ii is the intensity of the fluorescent radiation from the element i.

### 1.4 Quality control

Quality control was carried out using NIST standard reference material Trace and Minor Elements in Tomato Leaves (SRM 1573a) analysed with samples. As Table shows, the analysed elements were within the range of standard reference materials.

**Table 1: The comparison between the experimental values (mgkg$^{-1}$) of standards reference material (SRM 1573a Tomato leaves) and reference values.**

| Elements | Experimental values | Refence values |
|---|---|---|
| Mg | 11550 | 12000 |
| Al | 549 | 598 |
| K | 25086 | 27000 |
| Ca | 49351 | 50500 |
| Cr | 1.44 | 1.99 |
| Mn | 275 | 246 |
| Fe | 309 | 368 |
| Cu | 4.17 | 4.7 |
| Zn | 28 | 30.9 |
| Rb | 16.6 | 14.89 |
| Sr | 77 | 85 |

### 1.5 Statistical analysis

One-way ANOVA was used to evaluate the differences among vegetables 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 groups (farm site and local market). 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 metals concentration in spinosum

The heavy metals in spinosum samples collected from intertidal coastal areas in Unguja and Pemba and their mean concentration values in (µg/g) were found. The results at Unguja showed that the mean concentration values of Mn, Zn, Ni, Cu, Fe, As 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, 10.59 µg/g and 3.83 µg/g respectively. The heavy metals detected found in the order of Fe > Mn > As > Zn > Pb > Cu > Ni. The ranges of mean concentration values of these metals were: Mn (12.8−44) µg/g, Zn (1.3−8.16) µg/g, Ni (1.24−1.76) µg/g, Cu (1.99−2.97) µg/g, Fe (180.71−788.67) µg/g, As (5.46−10.59) µg/g and Pb (1.45−3.83) µg/g. Out of seven metals examined, the highest mean concentration of Fe (180.71−788.67) µg/g was observed and the lowest mean value of Ni (1.24−1.76) µg/g was recorded. Likewise, the results at Pemba showed that the mean values of heavy metals in samples of spinosum ranged from 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, 7.26−11.25 µg/g for As and 1.44−2.79 µg/g for Pb. Moreover, the results showed that the mean concentrations of heavy metals detected in spinosum found in the order of Fe > Mn > As > Zn > Pb > Cu > Ni. The t-test from IBM SPSS software version 23 was used to statistically compare the mean concentrations of heavy metals recorded in samples of spinosum and cottonii from Unguja and Pemba. In this test, the significant value of less than a 0.05 probability was taken.

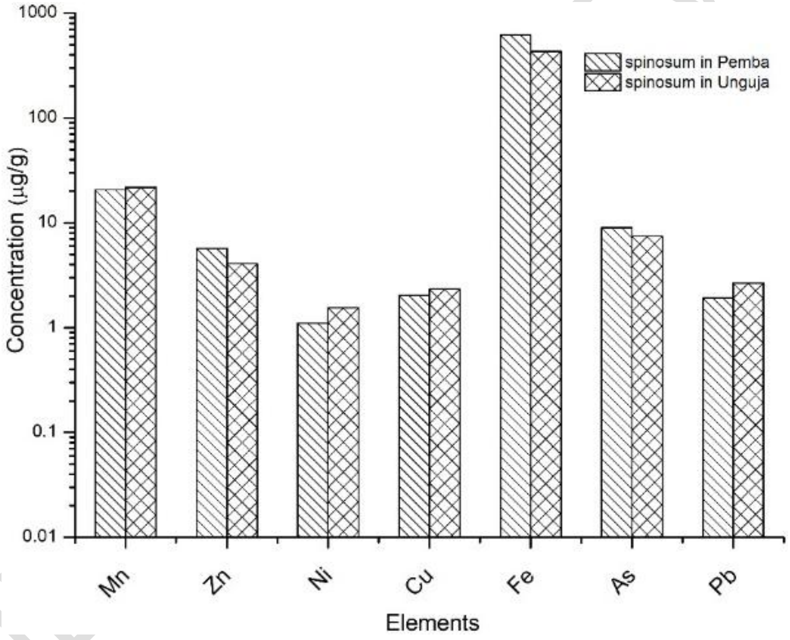


**Fig. 2. A histogram showing comparison of heavy metals concentrations in samples from spinosum at Unguja and Pemba.**

Samples from Pemba had significant (p ≤ 0.05) higher mean concentration of Fe, Zn and As, while samples from Unguja had significantly higher concentrations of Mn, Ni, Cu and Pb (Figure 2). Besides Fe, the highest mean concentration values recoded in spinosum was for Mn followed by As, Zn, Pb and Cu and the minimum value was observed for Ni which conforms to the result reported by [23]. Moreover, the mean values of Pb and Cu concentrations were lower than reported in the study of Dadolahi-Sohrab et al. 2011[24]. The lower values in our study might be attributed to the difference in the red seaweed species. Dadolahi-Sohrab et al. 2011 [24] used acanthophora specifera species which is also red seaweed while in this study used spinosum to assess heavy metals levels. The total arsenic value ranged from 0.114−236 µg/g, for 953 dried seaweed food samples throughout the world was reported by WHO technical report series 959 [25]. In the present study, the highest contents of As found in spinosum and cottonii were 6.77 µg/g and 11.25 µg/g, these values were lower compared to reported value by WHO.

### 3.2 Heavy metals concentration in cottonii

The concentrations of Mn, Zn, Ni, Cu, Fe, As and Pb in the samples of cottonii collected from Unguja and Pemba are given in Tables 2 and 3. The levels of heavy metals in Unguja were found to vary from 18.27−19.48 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, 3.5−6.16 µg/g for As and 1.83−4.62 µg/g for Pb; while in Pemba, the levels were ranged from 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, 4.97−6.77 µg/g for As and 3.75−4.18 µg/g for Pb.

The heavy metals distribution pattern decreased in the order: Fe > Mn > As > Zn > Pb > Cu > Ni. Samples from Unguja (S1, S2 and S3) had significant ($p \leq 0.05$) higher mean concentrations of Mn, Zn, Ni, Cu, Pb and Fe, while samples from Pemba (S4, S5 and S6) had significantly higher mean concentrations of As (Figure 3). However, the concentrations of Pb, Cu and Ni from samples in Pemba and Unguja were lower compared with the study of Dadolahi-Sohrab et al. 2011 [24]. Higher concentrations of Cu and Zn particular in samples S1, S2 and S3 have been attributed to the discharge of municipal wastewater to the marine environment.

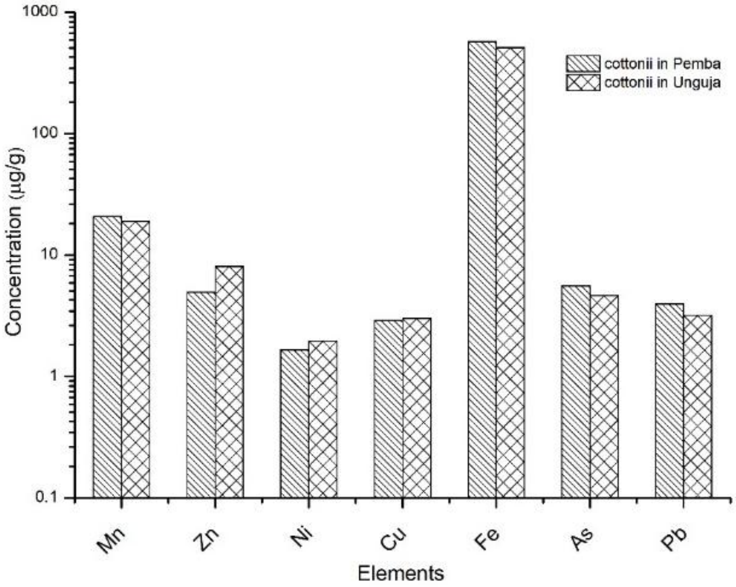


**Fig. 3. A histogram showing a comparison of heavy metals concentrations in samples from cottonii at Unguja and Pemba.**

The concentrations of As in samples of cottonii in the present study lie between 3.50− 6.16 µg/g in Unguja and 4.97−6.77 µg/g in Pemba. As indicated in Table 2, the highest levels of 6.16 µg/g was detected at Uzi in Unguja, while 6.77 µg/g was detected at chokocho kisiwa panza in Pemba as shown in Table 3. The values of As concentrations in samples of cottonii were strongly supported by the findings ranged from 1.72−9.46 µg/g which was reported by Ryu et al., 2009 [26].

**Table 2: Mean ± SD concentrations (µg/g) of heavy metals in cottonii from Bweleo (S4), Muongoni duta (S5) and Uzi (S6), Unguja.**

| Site | Elements | | | | | | |
|---|---|---|---|---|---|---|---|
| | Mn | Zn | Ni | Cu | Fe | As | Pb |
| S4 | 11.41±1.09 | 2.61±0.73 | 1.37±0.97 | BDL | 401.89±12.56 | 6.77±0.54 | BDL |
| S5 | 32.09±7.75 | 6.32±0.90 | 1.68±0.26 | 2.56±0.11 | 828.44±11.81 | 5.01±0.40 | 4.18±0.13 |
| S6 | 19.03±7.20 | 5.92±0.84 | 1.94±0.01 | 3.40±1.09 | 487.18±9.88 | 4.97±1.13 | 3.75±1.36 |

**Note:** BDL=below detection limit

**Table 3: Mean ± SD concentrations (µg/g) of heavy metals in cottonii from Chokocho kisiwa panza (S1), Kangaani kuukuu (S2) and Chokocho liko kuu (S3), Pemba.**

| | Elements | | | | | | |
|---|---|---|---|---|---|---|---|
| **Site** | **Mn** | **Zn** | **Ni** | **Cu** | **Fe** | **As** | **Pb** |
| SI | 19.22±6.84 | 8.91±0.58 | 1.78±0.28 | 2.34±0.41 | 446.81±10.82 | 4.27±1.38 | 3.13±1.01 |
| S2 | 18.27±4.04 | 4.70±0.52 | 2.16±0.44 | 3.36±0.50 | 425.54±9.58 | 3.50±1.03 | 4.62±0.29 |
| S3 | 19.48±5.16 | 10.62±0.69 | 1.94±0.37 | 3.33±0.89 | 666.77±13.07 | 6.16±1.13 | 1.83±1.29 |

Figures 4 (a) and (b) compare the mean concentrations of metals in cottonii and spinosum collected from Pemba and Unguja. The t-test shows that samples of cottonii in Unguju had significantly ($p \leq 0.05$) higher mean concentrations of Fe, Zn, Ni, Cu and Pb than in spinosum. The samples of spinosum had significantly higher mean concentrations of As and Mn as shown in Figure 4 (a). Likewise, samples of cottonii in Pemba had significantly ($p \leq 0.05$) higher mean concentrations of Ni, Cu and Pb than in spinosum. Samples of spinosum had significantly higher mean concentrations of Fe, As and Zn as shown in Figure 4 (b). In both samples from Unguja and Pemba, mean concentrations of Fe were much higher compared to Zn; for instant, the mean concentrations of Fe in samples of cottonii and spinosum from Unguja were 63.5 and 105.7 times higher than that of Zn.

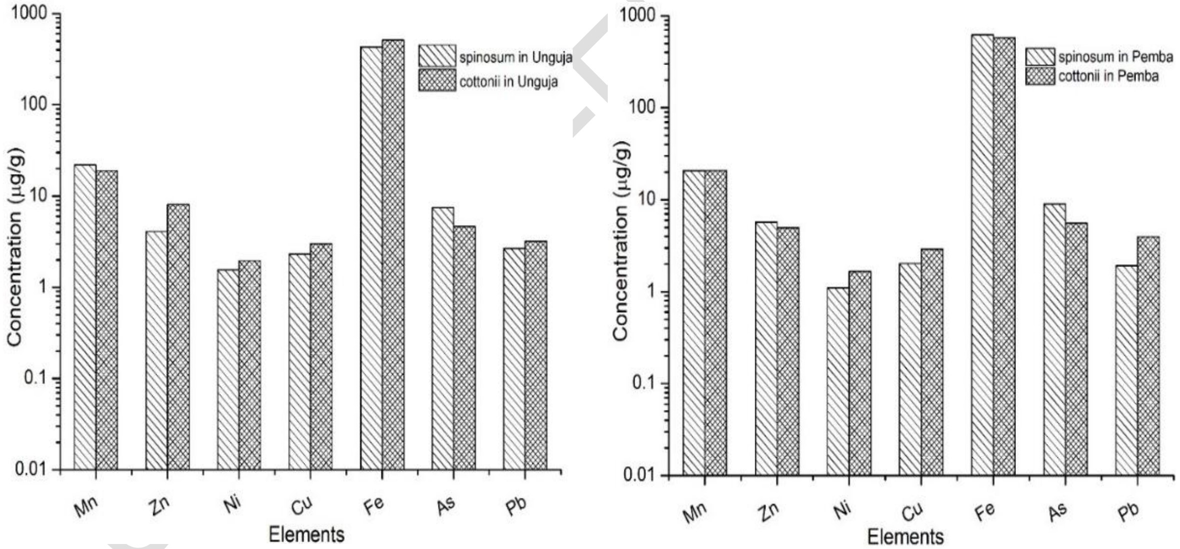


**Fig. 4. The histograms showing the comparison of heavy metals concentrations in samples from cottonii and spinosum at Unguja and Pemba.**

In this study, the heavy metals of these species of red seaweed commonly farmed in Zanzibar were detected. The levels of Pb in samples of cottonii collected from Unguja and Pemba were much higher compared to spinosum, this was because of the lubricating oil from diesel and engine from boat [27]. Generally, the levels of heavy metals contaminations in both samples along coastal area in Zanzibar might be caused by waste effluents that directly flow into the marine environment. Thus, the waste effluents from various anthropogenic activities were shown to contain heavy metals including Fe, Zn, Ni, Cu, As 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.

## CONCLUSION

The present study illustrates the application of the EDXRF technique in assessment of heavy metals contamination in red seaweed comprises cottonii and spinosum species collected from intertidal coastal areas in Zanzibar. The t-test from IBM SPSS computer software version 23 was used to statistically compare the mean concentrations of heavy metals recorded in samples of spinosum and cottonii from Unguja and Pemba. In this test, the significant value of less than a 0.05 probability was taken. The levels of heavy metals contaminations in cottonii and spinosum in this study were noticed generally lower compared to published results. However, samples of cottonii in Unguju had significantly ($p \leq 0.05$) higher mean concentrations of Fe, Zn, Ni, Cu and Pb than in spinosum while the samples of spinosum had significantly higher concentrations of As and Mn. Likewise, the samples of cottonii in Pemba had significantly ($p \leq 0.05$) higher mean concentrations of Ni, Cu and Pb than in spinosum, whereas the samples of spinosum had significantly higher mean concentrations of Fe, As and Zn. The concentrations of Pb in samples of cottonii collected from Unguja and Pemba were much higher compared to spinosum, this might be caused by lubricating oil from diesel and engine from the boat (Aballah et al., 2005). Generally, the heavy metals contaminations detected in cottonii and spinosum along the coastal area in Zanzibar were mainly caused by waste effluents that directly flow into marine environments. Thus serious measures should be taken to reduce the flow of pollutants into the marine ecosystem.

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## COMPETING INTERESTS

Authors have declared that no competing of interests exist.

## AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Author SAS, SKA and MKA 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.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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