DETERMINATION OF CONCENTRATION OF HEAVY METALS IN FISH FROM SEA PORT OF ZANZIBAR BY ENERGY DISPERSIVE X-RAY FLUORESCENCE (EDXRF)

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ABSTRACT

Seafood is the major source of food for a large number of people residing in the coastal areas of Zanzibar. It has been reported that the seafood are a connecting link for the transfer of toxic heavy metals in human beings. The present study assessed the metal concentration upon sample species of fish along the coast Sea Port of Zanzibar. Fish samples (namely changu, sardine, baracout and tuna fish) were the ideal species for the assessment study on effects of heavy metal contamination in aquatic organisms. They were collected at the Sea Port and the concentrations of the assessed metals were determined using Energy Dispersive X-ray Fluorescence (EDXRF). Concentrations of Fe, Pb, Cr, Ni, As, Cu and Zn were found to be higher in sardine whilst Hg was found to be higher in changu specie. Concentrations of Cd and Mn were found to be below the detection limits in all sample species but higher in mussels. Whereas Hg was only detected in changu species. Comparing the data from this study to data from other studies in other regions, the concentrations of Fe, Cr and As in different species of fishes collected was quite higher than the values reported in the literature. The results of this study indicated that As, Hg and Cr were higher in fish than WHO/FAO (2004).

Key words: EDXRF, X-Rays, Fish, Pollution studies, Environment

INTRODUCTION

Heavy metals are naturally occurring elements that have a high atomic weight and a density at least 5 times greater than that of water. Their multiple industrial, domestic, agricultural, medical and technological applications have led to their wide distribution in the environment; raising concerns over their potential effects on human health and the environment. Because of their high degree of toxicity, arsenic, cadmium, chromium, lead, and mercury rank among the priority metals that are of public health significance. These metallic elements are considered systemic toxicants that are known to induce multiple organ damage, even at lower levels of exposure (Tchounwou et al. 2012).

Fish has long been a favorite meal and is a major source of food for most people of Zanzibar. Apart from the beneficial effects, there are also some factors that contribute to risk from fish consumption, mainly due to the potential adverse effects of heavy metals (Wong, 2001). Heavy metals can be classified as potentially toxic (aluminum, arsenic, cadmium, lead, mercury) (Wuana and Okieimen 2011) and essential (copper, zinc, selenium)(Camara, 2001). The most nonessential heavy metals of particular concern to fish are cadmium (Cd), lead (Pb) and mercury (Hg) (Tao, 2000). Heavy metals are considered the most important form of pollution of the aquatic environment because of their

toxicity and accumulation by marine organisms. Toxic elements can be very harmful, even at low concentration, when ingested over a long time period. Their effects are not immediate and show up after many years (Asaolu, 2002). The distribution of metals varies between fish species, depending on age, development status and other physiological factors (Catsiki, 1999).

It is reported that, many marine organisms have the ability to hold toxic materials that are in small concentrations in the water to very high concentrations in their body without harming them but the toxicity affect humans who feed on them (Gümgüm, 1994). It is also reported that small amounts of absorbed heavy metals are held in the body either temporarily or permanently. Consequently, small fish in polluted water will become enriched with the accumulated substances (Hashmi, 2002). To understand the toxicity of heavy metals, the concentrations of Zinc, Nickel, manganese, Lead Cadmium, Cobalt and Iron in marine fish species were determined from Parangipettai Coast, India (Raja, 2009) and Ureje Dam, South-Western Nigeria where correlation of metal concentration with weight and length has been discussed (Adefemi, 2008). It was found that heavy metals accumulation exceeded the recommendation set by WHO in food fish and was not fit for human consumption. The analysis carried out (Mohamed and Makame, 2015) for heavy metal concentrations in the soil, which later absorbed by plant reveals a significant deposition of lead. Heavy metals in soil may be passed over to the food chain through fish and consequently affect the human health and that of ecosystem (Rahman et.al. 2010). There has also been a report (Kruitwangen et al. 2008, Mohamed and Makame 2015) on elevated concentrations of Zn and Pb in the fish samples collected on mangrove ecosystems at Chwaka in Zanzibar.

In Zanzibar, large fish found in the sea port feed on polluted seaweeds (algae) and small fish which appear to swim about the whole area of the sea. Fish feeding on polluted algae and other small fish living in contaminated sea water will have toxic concentrations in their bodies (Powell, 2006). Hence, to assess the current concentration of heavy metals in fish is important for evaluating the possible risk on fish and ultimately on the fish consumers.

MATERIAL AND METHODS

Samples Collection

A total of 20 fish samples representing 4 species of different sizes commonly found at the study area were collected. The samples selected for analysis were: sardine, tuna fish, changu (Figure 1) and baracouta.



Figure 1: Picture showing fish sample collected from Zanzibar Sea Port

Samples Preparation

Prior to the analysis, the fish were removed scales, fins, heads, tails, and bones then washed with distilled water to remove any traces of elements resulting from contaminations. Then the flesh of fish samples were dried in an oven separately to constant weights at a temperature around 65-80 ˚C for several hours, so as to ensure all moisture content is removed. The samples were then ground at a time using a grinder to fine powder. The powdered materials

in each sample species were sieved using 2 mm sieve.

XRF Sample Preparation

A dry weight of 12.00 g of each sample with 2.7 gram 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 Fritsch Pulverisette™ (Industriiesstrasse, 8-55743 I dar-Okerstein, Germany) Pulverized machine was set at a speed of 150 revolutions per minutes (rpm) for 30 minutes. The analyte was placed into a polished lapped thrust piece with the smooth surface and fixed into hydraulic Retsch™ Retsch GmbH Retsch-Allee 1-5, 42781 Haan,Germany) press (Figure 2). Five replicate pellets from each sample were prepared. The pellets (Figure 3) were made by applying an average pressure of 12.5 tons. The pellets were labeled and taken to the Spectro Xepos™ (Spectro analytical instruments GmbH, Boschstr. 10, 47533 Kleve, Germany) EDXRF machine (Figure 4) for measurement and analysis.



Figure 2: The Retsch™ Press for compressing the powder into a pellet

Determination of Elemental Composition

Elemental compositions of the samples were determined from spectra of the respective samples using the X – lab pro™ software. The spectrum shown in Figure **5** is the typical of spectra obtained in this study for sardine. The spectra were used to calculate the element concentrations in a given sample and the detection limit (DL) of the instrument for the respective element.

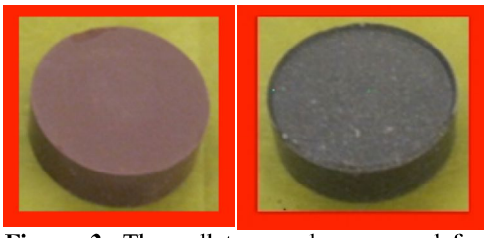


Figure 3: The pellets samples prepared for EDXRF analysis



Figure 4: External view of the Spectro Xepos™ EDXRF system

RESULTS AND DISCUSSIONS

Detection Limit (DL)

The Detection limit (DL) is defined as the lowest concentration level that can be determined to be statistically significant from an analytically blank (Koleleni and Kandoro, 2006). It specifies the minimum concentration that can be determined by the instrument. DL depends on the capability of the analyzing tool to distinguish the peak

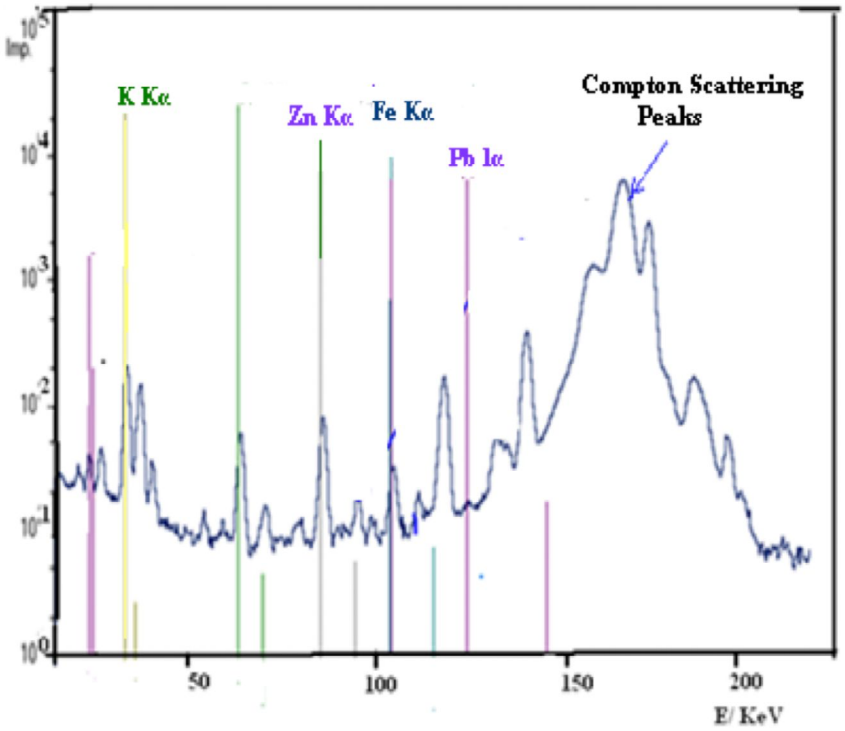


Figure 5: A typical spectrum of Pb, Fe and Zn in Sardine obtained from the EDXRF

intensity (I_i) from the fluctuations of the background intensity (I_b) due to counting statistics or background noise. The DL varies from element to element and depends upon several factors. Some elements become very radioactive and can be determined at very low levels (sub ppb). Usually the detection limit depends upon the "other" elements in the sample - the matrix. If an element in the sample becomes radioactive, besides the element of interest, the background noise may be too high to determine the desired element at low levels. (Synovec, 1985). In this study the method incorporated in the X-Lab Pro software package was used to calculate DL of each element. The DL was determined by the X lab pro software using the relation given in equation (1) by Rousseau (2005):

$$DL = \frac{3 \times C_i}{I_i - I_b} \sqrt{\frac{I_b}{T_b}} \quad \text{_____(1)}$$

Where, T_b is the time used to measure the background intensity. The results obtained are given in table 1. It can be noticed that, DL of the same element in different samples is not the same. For instance, the DL of Cd in fish is 3.8(µg/g).

Table 1: The DL (in µg/g) of various elements in Fish

Atomic number, Z	Element	Detection Limit (µg/g)
23	V	5.5
24	Cr	5.2
25	Mn	3.8
26	Fe	2.6
27	Co	1.7
28	Ni	1.0
29	Cu	1.4
30	Zn	1.0
33	As	0.3
48	Cd	3.8
80	Hg	0.6
82	Pb	0.5

CONCENTRATION HEAVY METAL IN FISH SAMPLES

The variation of lengths and weights of collected fish species are shown in Table 2. It can be observed that change in lengths was found to be proportional to the weights. For a very small change in lengths, a corresponding change in weights is very large, for example, at 10.5 cm length, weight is 102.0 g and in case of 11.0 cm length, weight is 100.5.0 g for sardine specie samples. Therefore, the observation of this study is related to many previous literatures which stated that, the occurrence of trace metals in fish species was related to lengths, weights and age of the fish (De marco, 2006).

Distribution of Heavy Metals in collected fish species.

The EDXRF results of the fish samples collected from Sea Port of Zanzibar are discussed in this section. The values were mean of the five measurements and the errors presented in the values were the Standard Error of the Mean (SEM). The concentration levels of heavy metals were given in µg/g. The results of this research work are presented in Table 3 to 4. The Tables present the levels of tested Heavy Metals concentration of Cr, Mn, Fe, Ni, Cu, Zn, Cd, Pb, As, Hg and Fe. The variation of the detected metals concentration in collected fish species was determined and then the obtained data were recorded as shown in the Tables. Furthermore, the results show the change in concentrations of different sizes of collected fish samples with respect to lengths and weights. The values show that as length and weight increase, the concentrations increase gradually. All tested heavy metals were detected in different sizes of fish sample at different concentrations depending upon the size of fish. The data were compared with research studies reported in literature from different countries and the corresponding international standards permissible limits for these metals in marine resources such as WHO, FAO, USEPA and EC.

The mean values of metals concentration for

Table 2: Variation of Lengths with weights of collected samples of fish species

Sample No.	Change		Sardine		Baracouta		Tuna fish	
	Length (cm)	Weight (g)	Length (cm)	Weight (g)	Length (cm)	Weight (g)	Length (cm)	Weight (g)
1	7.2	150.0	6.0	80.0	14.5	192.0	9.2	181.7
2	8.5	175.0	8.0	89.0	18.0	211.7	10.6	200.0
3	10.0	212.5	10.5	102.0	21.6	230.3	12.0	226.3
4	11.0	233.0	11.0	100.5	26.0	248.8	13.5	235.0
5	13.4	245.0	12.5	119.0	28.3	260.0	15.0	253.0

changu specie

Among the assessed metals, Cr, Mn, Fe, Ni, Cu, Zn, Cd, Pb, As and Hg were detected in changu specie with concentrations range of (16.1 - 20.7) μg/g, (0.37 - 0.70) μg/g, (105.8 - 128.4) μg/g, (0.60 – 0.80) μg/g, (1.2 - 2.01) μg/g, (22.2 - 3.7) μg/g, (0.8 - 0.86) μg/g, (0.6 - 7.4) μg/g and (1.2 - 2.4 μg/g. The present results (Table 3) show that metals Fe, Zn, Cr and As were found at greatly higher concentrations than the other heavy metals. The general observed trends for the metals for this sample specie was Fe > Zn > Cr > As > Cd > Hg > Cu > Pb > Ni > Mn. Moreover, the highest mean of range concentrations (μg/g) of Fe (105.8 -128.4), Zn (22.2 - 32.7), Cr (16.1 - 20.7) and As (6.60 - 7.40) were observed while the lowest mean of range concentrations (μg/g) of Mn (0.37 - 0.70), Ni (0.60 - 0.80) and Pb (0.60 - 0.86) were obtained.

Table 3: The mean values of metals (μg/g) for five samples of changu specie. The error in the measurements were less than 3%.

Elements	1	2	3	4	5
Cr	16.1	19.0	19.5	20.2	20.7
Mn	BDL	BDL	BDL	BDL	BDL
Fe	105.8	109.9	115.2	119.2	128.4
Ni	BDL	BDL	BDL	BDL	BDL
Cu	1..60	1.80	1.90	1.93	2.01
Zn	22.2	28.1	28.3	29.0	32.7
Cd	BDL	BDL	BDL	BDL	BDL
Pb	0.60	0.65	0.70	0.83	0.86
As	6.60	6.83	6.70	6.70	7.40
Hg	1.17	1.46	1.70	2.20	2.43

The mean results of metals concentration for Sardine specie

The concentration of heavy metals detected in Sardine fish is given in Table 4. The detected heavy metals were Cr, Mn, Fe, Ni, Cu, Zn, Cd, Pb, As and Hg with the maximum mean values of 23.07, 2.97, 317.1, 87.47, 7.27, 52.11, 2.55, 2.01, 5.05 and 5.05 (μg/g) respectively were observed, whereas the minimum mean values of 20.9, 1.58, 306.7, 86.2, 6.42, 48.5, 1.6, 1.37, 4.33 and 0.02 (μg/g) were noted. The general observed trends for the metals for this sample specie is Fe > Ni > Zn > Cr > Cu> As > Cd > Pb > Hg. The maximum ranges of mean concentrations of Fe (306.7 - 317.1) (μg/g), Ni (86.17 - 87.47) (μg/g), Zn(48.5 - 52.11) (μg/g) and Cr (20.9 - 23.07) (μg/g) was recorded while the lowest range of mean concentration Hg (0.02 - 0.13) (μg/g) was noted.

The mean results of heavy metals concentration for Baracouta specie

The heavy metals in baracouta samples and their mean concentration values were found. The result shows the levels of Cr, Mn, Fe, Ni, Cu, Zn, Cd, Pb and As were recorded with maximum mean values of 24.2 μg/g, 1.43 μg/g, 95.8 μg/g, 1.73 μg/g, 1.65 μg/g, 33.7 μg/g, 2.10 μg/g, 1.03 μg/g and 5.05 μg/g respectively. The general observed trends for the metals for this sample specie is Fe > Zn > Cr > As > Cd > Ni > Mn > Cu > Pb. The ranges of concentrations (μg/g) of metals found in this specie sample were: Cr(19.4 - 24.2) μg/g, Mn(2.01 - 1.43) μg/g, Fe(79.2 - 95.8) μg/g, Ni(1.13 - 1.73) μg/g, Cu(1.33 - 1.65)μg/g, Zn(28.4 - 33.7)μg/g, Cd(1.83 - 2.10) μg/g, Pb(0.61 - 1.03) μg/g and As(4.20 - 5.05) μg/g. The highest mean concentration value with range of Fe (79.2 - 95.8) μg/g was recorded while the lowest mean value Pb (0.61 - 1.03) was observed. It can be noted that the element Cu was not detected for the sample with lower weight. Moreover, Mn and Cd were found to BDL whereas Hg was not detected in this sample.

The mean results of heavy metals concentration for Tuna fish

The heavy metals concentrations observed in this specie were Cr, Mn, Fe, Ni, Cu, Zn, Cd and Pb with the ranges of concentrations of (14.2 - 20)µg/g, (0.05 - 0.17) µg/g, (91.6 - 99.0)µg/g, (0, 37 - 0.86)µg/g, (4.10 - 4.93)µg/g, (31.4 - 49.8)µg/g, (0.03 - 0.17)µg/g and (0.06 – 0.33) µg/g respectively. The general observed trends for the metals for this sample specie is Fe > Zn > Cr > Cu > Ni > Pb > Mn > Cd. The results show that Fe, Zn, Cr and Cu are more concentrated in this sample species with the range concentrations (µg/g) of (91.6 - 99.0), (31.4 - 49.8), (14.2 - 20.1) and (4.10 - 4.93) respectively while the minimum concentrations of Cd, Mn, Pb and Ni, were observed with the range concentrations (µg/g) of (0.03 - 0.17), (0.05 - 0.17) (0.06 – 0.33) and (0. 37 - 0.86) respectively. As and Hg were not detected.

Table 4: The mean results of metals concentration (µg/g) for samples of sardine. The errors in the measurements were less than 3%.

Elements	1	2	3	4	5
Cr	20.9	21.2	21.5	21.7	23.07
Mn	BDL	BDL	BDL	BDL	BDL
Fe	306.7	307.3	307.6	307.7	317.1
Ni	86.2	86.1	86.2	87.3	87.5
Cu	6.4	6.7	6.8	6.87	7.2
Zn	48.5	52.3	51.6	51.7	52.1
Cd	BDL	BDL	BDL	BDL	BDL
Pb	1.4	1.8	1.8	1.92	2.01
As	4.33	4.47	4.89	5.01	5.05 ± 1.12
Hg	BDL	BDL	BDL	BDL	BDL

Comparison of Metal Concentrations (µg/g) in fish Species Samples

The range concentration of heavy metals detected in fish species samples were found. In general, all species had higher concentration of Fe compared to other elements. maximum metals concentrations detected in fish species were ranged from Fe: 95.7 µg/g (baracouta) to 317.1 µg/g (sardine), Zn: 32.7 µg/g (changu) to 52.1 µg/g (sardine), Cr: 20.1 µg/g (tuna fish) to 24.2 µg/g (baracouta), Cu: 1.6 µg/g (baracouta) to 7.2 µg/g (sardine), Pb: BDL (tuna fish) to 2.01 µg/g (sardine) and As: BDL (tuna fish) to 7.40 µg/g (changu). There was a great variation in Ni concentrations among above mentioned species .The mean concentration of Ni was found to be the highest in sardine (67.5µg/g) and baracouta (1.7µg/g), whereas it was found below detection limit in changu and tuna fish. The level of Hg was only detected in changu specie with the range concentration of 1.17µg/g to 2.43µg/g, whereas Mn and Cd were both BDL in all tested fish species. Co and V were not detected at all. It can be noticed that, the maximum concentration of Cu was noted in Sardine and tuna fish species with the range of (6.4 to 7.2)µg/g and (4.1 to 4.9)µg/g respectively, while highest concentration of Ni was detected in sardine only. The maximum concentration of As was observed in changu, sardine and baarcouta species, and was not detected in tuna fish specie (see figure 6 and 7). Therefore, different levels of metals were observed in different species or within the same species. The differences were explained due to the fact that the concentration of metals depends on species (that is, degree particular specie picks up the matter from the sediment and water during feeding).

The bottom feeders are known to concentrate more metal levels than the surface feeders (Tuzen, 2003). Moreover, ecological factors such as place of development and nutrient availability may also cause the inconsistency of metal concentration in fish.

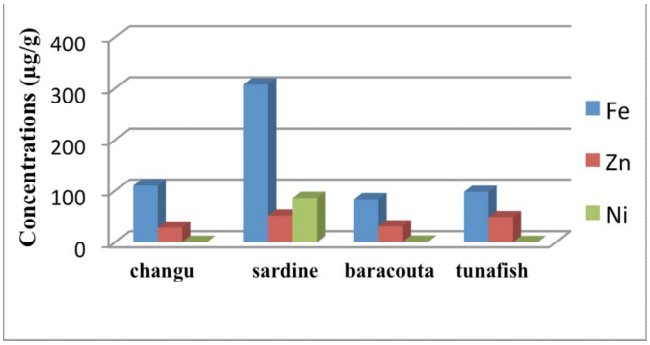


Figure 6: The levels of Fe, Zn and Ni in fish samples

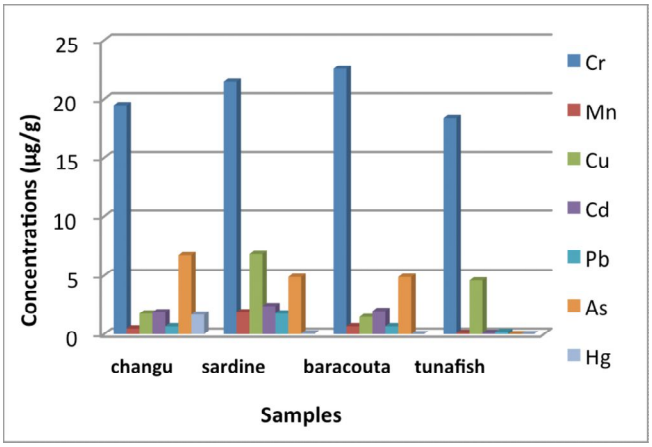


Figure 7: The levels of Cr, Mn, Cu, Cd, Pb, As and Hg in fish samples

The metal concentrations in fish observed in this study compared with literature data from other sea world regions

The mean concentration values of metals found in this study were compared with the mean values of elements reported in the literatures. The concentration of Mn and Cd were found below the detection limit of all species samples analyzed in this study. The elements were reported to exist in the range of: 0.17-1.6 µg/g for Cr, 3.62-73.4 µg/g for Fe, 0.42-11.55 µg/g for Cu, 0.02-1.43 µg/g for Cd, 3.98-19.39 µg/g for Zn, 1.51-76.0 µg/g for Mn, 4.05 - 6.07 µg/g for Ni, ND – 0.21 µg/g for As, 0.38 –2.10 µg/g for Pb and 0.08 - 0.26 µg/g for Hg. When compared to previous studies from different sampling areas, the results of this study show that Fe (87.5–311.9) µg/g,, Cr (17.2–22.3) µg/g, As (BDL–7.0) µg/g and Ni (BDL–86.8) µg/g were more concentrated in tested fish sample species. However, the observations of Cu and Pb were nearly similar to the observations of other workers (Kalay,1999 and Asaolu, 2001). Hence, fish caught in Sea Port of Zanzibar had high concentrations of Fe, Cr, As and Ni.

Comparison of the mean concentrations of toxic metals in Fish species with the Maximum Tolerable Limits (MTLs)

In this study, the contamination levels of heavy metals detected in fish species samples were compared to the permissible limits recommended by Food and Agriculture Organization and World Health Organization (FAO/WHO, 2004). The toxic metals detected ranged from: Cr (17.2 µg/g for tuna fish to 22.3 µg/g for sardine), Ni (1.4 µg/g for Barcouta to 86.8 µg/g for sardine), Pb (BDL for tuna fish to 1.7 µg/g for sardine) and

As (BDL for tuna fish to 7.0 µg/g for changu). The higher content of Hg (1.8 µg/g) was only detected in changu specie. However, all these values were considered too high when compared with the values of the permissible limit for human consumption, as recommended by the Food and Agricultural Organization FAO / WHO (2004) ; USEPA, 1984; EC, 2006 and FDA, 2007. Therefore, the high values obtained for Cr, Ni, Pb and As indicated that these heavy metals were highly accumulated in the Sea Port of Zanzibar.

CONCLUSIONS

This study has identified the presence of toxic elements in fish species found in the Sea port of Zanzibar. With regards to the objectives of this research and the results obtained, this current study will provide useful information and a baseline for future along with continuous studies on the heavy metals concentrations in fish species in Sea Port of Zanzibar.

When comparing heavy metal concentration in fish species, the mean concentration of Ni(86.8)µg/g, Fe(311.9)µg/g, Zn(50.3)µg/g, Cu(6.8)µg/g and Pb(1.7)µg/g were found to be higher in Sardine whereas Hg (0.75)µg/g and As(7.0)µg/g were higher in changu species. The toxic heavy metals Cd and Mn were below detection limits in all measured fish species whereas Co and V were not detected in all species. The different levels of observed metals in different species or within the same species were explained due to the fact that the concentration of metals depends on species (that is, degree of particular specie picks up the matter from the sediment and water during feeding). The bottom feeders are known to concentrate more metal levels than the surface feeders. Moreover, ecological factors such as place of development and nutrient availability may also cause the inconsistency of metal concentration in fish. In contrast, the levels of heavy metal concentration obtained in studied samples appeared within the values reported in other countries. However, when compared to the permissible limits recommended by international agencies, the heavy metals concentration were found to be higher than the recommended maximum level allowed in food by FAO/WHO(2004), which were Cd(0.05) µg/g, Pb(0.5) µg/g and As(0.1) µg/g. The mean values of Cu, Cr and Pb in fish species were below the FAO/WHO threshold limits. The mean value of Hg (0.75) µg/g in changu specie was above the threshold limit of EC (2006) and WHO (1984).

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