

# 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 seafoods are a connecting link for the transfer of toxic heavy metals in human beings. The present study assessed the metal concentrations 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 species. Concentrations of Cd and Mn were found to be below the detection limits in all sample species but higher in mussels. 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 were 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).

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**Keywords:** EDXRF, X-Rays, Fish, Pollution studies, Environment

## INTRODUCTION

Heavy metals are naturally occurring elements that have high atomic weights 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 favourite 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 risks from fish consumption, mainly due to the potential adverse effects of heavy metals (Wong 2001). Heavy metals can be classified as potentially toxic (aluminium, arsenic, cadmium, lead, mercury) (Wuana and Okieimen 2011) and essential (copper, zinc, selenium) (Camara 2001). The most non-essential 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 concentrations, 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 correlations of metal concentrations with weight and length have 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 by Mohamed and Makame (2015) for heavy metal concentrations in the soil, which are later absorbed by plants revealed 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 have also been reports by Kruitwangen et al. (2008), and 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 fishes 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 concentrations of heavy metals in fish is important for evaluating the possible risks on fish and ultimately on the fish consumers.

## MATERIALS 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 scales, fins, heads, tails, and bones of fish samples were removed and then washed with distilled water to remove any traces of elements resulting from contaminations. Then the flesh parts 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™ (Industriestrasse, 8-55743 I dar-Okerstein, Germany) pulverizer 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 labelled and taken to the Spectro Xepos™ (Spectro analytical instruments GmbH, Boschstr. 10, 47533 Kleve, Germany) EDXRF machine (Figure 4) for measurement and analyses.



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



**Figure 3:** The pellets samples prepared for EDXRF analysis.



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

### 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 sardines. 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.

## RESULTS AND DISCUSSIONS

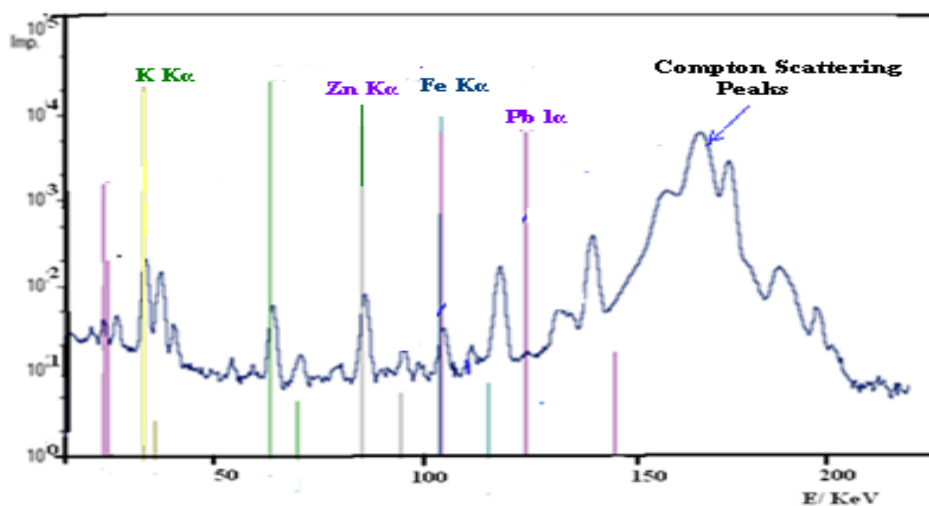
### Detection Limit (DL)

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

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

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  $\mu\text{g/g}$ .



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

**Table 1:** The DL (in  $\mu\text{g/g}$ ) of various elements in fish

Atomic number, Z	Element	Detection Limit ( $\mu\text{g/g}$ )
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 metals in fish samples

The variations of lengths and weights of collected fish species are shown in Table 2. It can be observed that changes in lengths were 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 species samples. Therefore, the observations of this study are 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 concentrations of heavy metals were given in  $\mu\text{g/g}$ . The results of this research work are presented in Tables 3 to 4. The tables present the levels of tested heavy metals concentrations of Cr, Mn, Fe, Ni, Cu, Zn, Cd, Pb, As, Hg and Fe. The variations of the detected metals concentrations in collected fish species were determined and then the obtained data were recorded as shown in the tables. Furthermore, the results showed the changes in concentrations of different sizes of collected fish samples with respect to lengths and weights. The values showed that as length and weight increase, the concentrations increase gradually. All tested heavy metals were detected in different sizes of fish samples

at different concentrations depending upon the sizes of fishes. 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.

**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

**The mean values of metals concentration for changu species**

Among the assessed metals, Cr, Mn, Fe, Ni, Cu, Zn, Cd, Pb, As and Hg were detected in changu species 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, respectively. 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 trend for the metals for the sample species 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 concentrations for sardine species**

The concentrations of heavy metals detected in sardine fish are 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 trend for the metals for in sardine

species samples was 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) were recorded, while the lowest range of mean concentrations of Hg (0.02–0.13 µg/g) were noted.

**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

#### Mean results of heavy metals concentration for baracouta species

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

#### Mean results of heavy metals concentrations for tuna fish

The heavy metals concentrations observed in this species 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 trend for the metals for this sample species was Fe > Zn > Cr > Cu > Ni > Pb > Mn > Cd. The results showed that Fe, Zn, Cr and Cu were 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.

#### Comparison of metal concentrations (µg/g) in fish species samples

The range concentrations of heavy metals detected in fish species samples were found. In general, all species had higher concentrations of Fe compared to other elements. Maximum metals concentrations detected in fish species

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 were great variations in Ni concentrations among above mentioned species. The mean concentrations of Ni were found to be the highest in sardine (67.5 µg/g) and baracouta (1.7 µg/g), whereas it was found below detection limit in changu and tuna fish. Hg was only detected in changu species with the range concentrations of 1.17 µg/g to 2.43 µg/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 concentrations of Cu were 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 species (see Figures 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 concentrations of metals depend on species (that is, degree particular species 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 concentrations in fish.

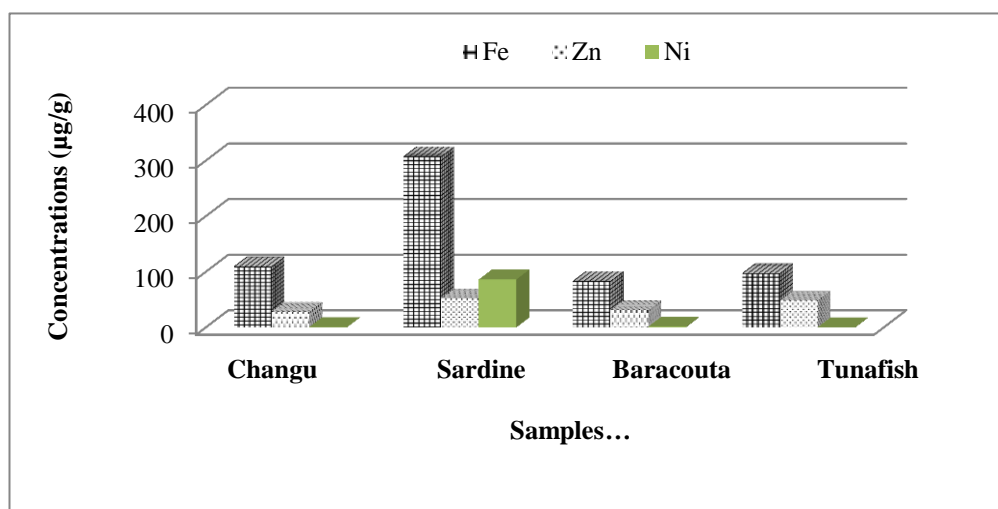
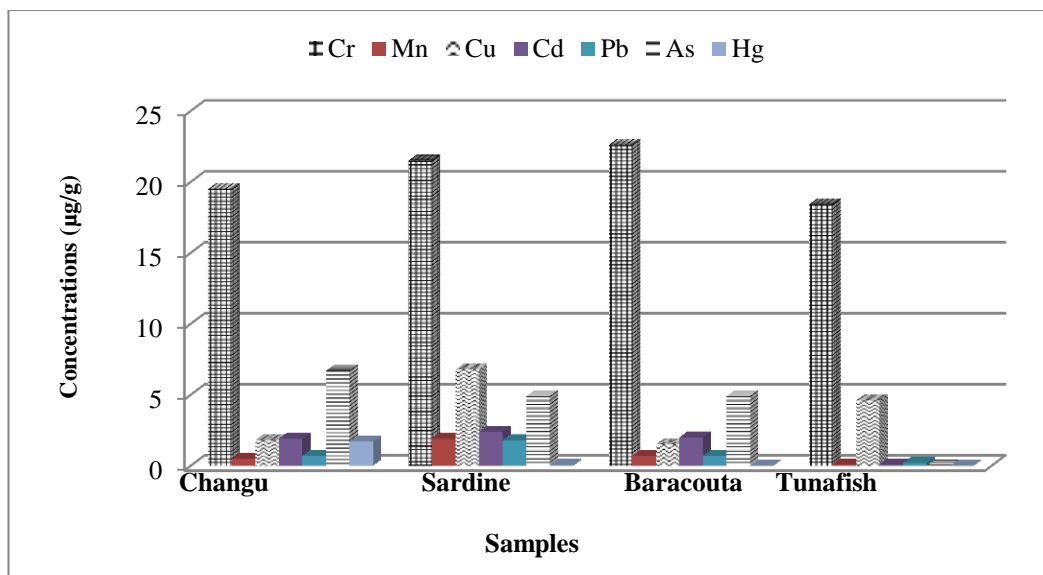


Figure 6: The levels of Fe, Zn and Ni in fish samples.



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

#### **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 concentrations of Mn and Cd were found below the detection limit in all species samples analysed 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 showed 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 researchers (Kalay 1999, 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 as follows: 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 highest content of Hg (1.8 µg/g) was only detected in changu species. However, all these values were considered too high when compared with the values of the permissible limits 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 has provided 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 concentrations in fish species, the mean concentrations 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 concentrations of metals depend on species (that is, degree of particular species 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 concentrations in fish. In contrast, the levels of heavy metal concentrations 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 concentrations were found to be higher than the recommended maximum levels allowed in food by FAO/WHO (2004), which are 0.05 µg/g for Cd, 0.5 µg/g for Pb and 0.1 µg/g for As. 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 species was above the threshold limit of EC (2006) and WHO (1984).

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