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Review

Analysis of heavy metals in the shellfish from the neighbourhood of the sea port of Zanzibar

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Abstract

Shellfish is one of the major sources of food for coastal population of Zanzibar. Toxicity of shell fish by heavy metals is of concern to consumers. This study aimed at assessing the metal concentrations of the shellfish along the coast in the neighbourhood of Sea Port of Zanzibar. Shellfish (namely oysters, mussels, clams and crabs) were collected for the assessment on effects of heavy metal contamination among the aquatic organisms. The concentrations of the shellfish were determined using Energy Dispersive X-ray Fluorescence (EDXRF). The concentrations analysed include Cr, Mn, Fe, Ni, Cu, Zn, Cd and As. The range ofCd, Cr, Ni, Mn and Zn were found to be higher in clams while Fe, Pb and Cu were found to be higher in crabs. The values of Cr, Cu, Zn, As and Cd in different shellfish species appear to be higher than the values reported in the literature.

Keywords: X-ray fluorescence, shellfish, heavy metal analysis, mussels, oysters and clams

INTRODUCTION

Shellfish (such as mussels, oysters and clams are consumed as food) is no longer caught along many shores because it is simply too polluted with sewage or toxic chemical wastes that have discharged from the land nearby (Pina, 1998). People who eat poisoned shellfish are at a risk of paralytic shellfish poisoning (Nasser, 2000). Adverse effects on the coastal environment include heavy metals, organic pollution and oil spills. The discharge of these wastes without adequate treatment often contaminate the coastal water with conservative pollutants (such as heavy metals), many of which accumulate in the tissues of resident organisms like fishes, oysters, crabs and seaweeds (Leous, 2005). Several studies have been carried out to analyze heavy metals in different species of shellfish. Among of these studies are those carried out by Reinfelder (1997); Chang, Chong, Soto, Roja and Wang (2001). The finding was that, marine organisms such as oyster and mussel could accumulate pollutants differently due to species-specific ability/capacity to regulate or accumulate trace metals.

Shellfish are consumed by most people of Zanzibar. In Zanzibar Sea Port, the shellfish grow in locations where the seepage from pit latrines, septic tanks and input of untreated wastewater from Zanzibar municipal are directly disposed to coastal areas (Mohammed, 2002). Therefore, there is a high possibility for the shellfish to be contaminated. Hence, this study was aimed to assess the

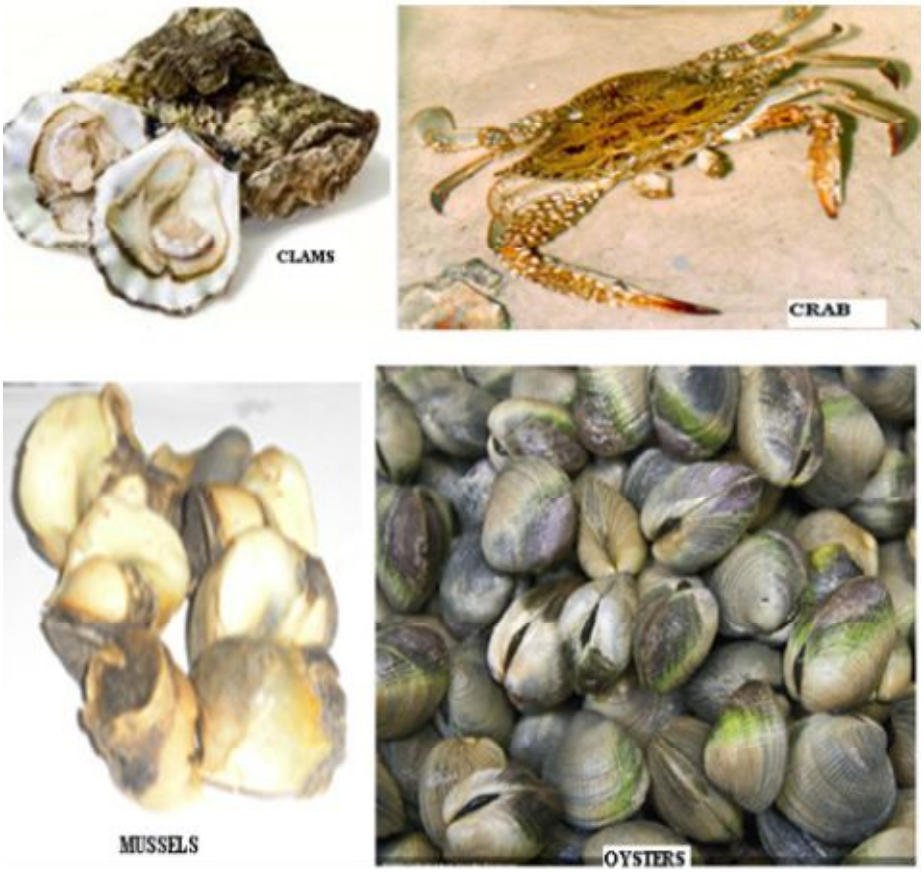


Figure 1. Pictures showing shellfish sample collected from Zanzibar Sea Port

current concentration of heavy metals in shellfish which is important for evaluating the possible risk of shellfish consumers.

Samples Collection

A total of 20 shellfish samples (figure 1) representing 4 species of different weights were collected at the sea port. The selected sample species were mussels, oysters, crabs and clams. The samples were then packed into labelled ethylene bags to the laboratory for analysis.

Samples Preparation

Prior to the analysis, the soft tissues of the Shellfish were separated from the shells of each sample specie. Then the soft tissue 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 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 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 press machine. Five replicate pellets from each sample were prepared. The pellets were made by applying an average pressure of 12.5 tons. The pellets were labelled and taken to the EDXRF machine for measurement and analysis.

Determination of Elemental Composition

Elemental compositions of the samples were determined from spectra of the respective samples using the X – lab pro^{TM} software. The spectrum shown in Figure 2 is the typical spectra obtained in this study for crab. The spectra ware used to calculate the element

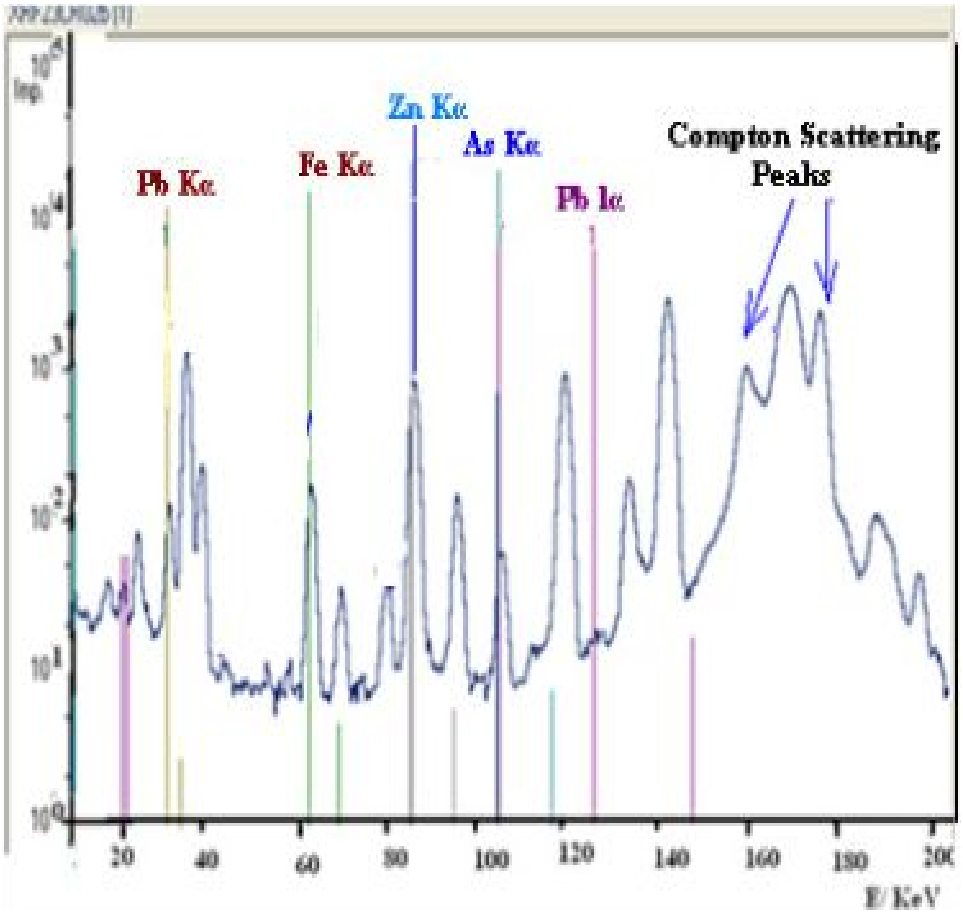


Figure 2. A typical spectrum of Pb, As, Fe and Zn in Crab obtained from the EDXRF

concentrations in a given sample and the detection limit (DL) of the instrument for the respective element.

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 has 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 ppt). 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 turboqunt 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 by Rousseau (2005).

Elemental Concentration of Shellfish Samples

Table 1 shows the weights of the analyzed samples of four commonly edible shell fish species, namely Mussels, Clams, Oysters and Crabs collected from Sea Port of Zanzibar

Distribution of Heavy Metals in different collected shellfish species

The concentrations of heavy metals were analyzed in tissues samples using EDXRF. The EDXRF results of the shellfish samples were given in Table 3 - 6. The values are mean of the five measurements and the errors presented in the values are the Standard Error of the Mean (SEM). The concentration levels of heavy metals

Table 1. The measured weights of different collected samples of shellfish

Sample No.	Weight (g)			
	Oysters	Mussels	Crabs	Clams
1	90.0	240.0	120.0	80.0
2	100.0	280.4	140.0	90.0
3	120.0	320.4	160.0	110.0
4	125 .0	340.0	190.0	130.0
5	130.0	350.5	200.0	150.0

Table 2. Mean of concentration (µg/g) of heavy metals in oysters. Errors in the measurements are less than 5%

Elements	1	2	3	4	5
Cr	10.2	11.8	12.1	12.8	13.2
Mn	BDL	BDL	BDL	BDL	BDL
Fe	386.6	387.3	388.2	390.5	392.7
Ni	BDL	BDL	BDL	BDL	BDL
Cu	17.5	18.6	19 0	19.6	20.0
Zn	369.5	369.9	371.5	372.0	372.5
Cd	BDL	BDL	BDL	BDL	BDL
Pb	BDL	BDL	BDL	BDL	BDL
As	22.1	22.9	23.0	23.2	23.4
Hg	BDL	BDL	BDL	BDL	BDL

Table 3. Mean of concentration (µg/g) of heavy metals in mussels. Errors in the measurements are less than 5%

Elements	1	2	3	4	5
Cr	9.8	10 6	11.4	11.5	11.6
Mn	BDL	BDL	BDL	BDL	BDL
Fe	213.7	216.6	223.7	224.1	266.0
Ni	BDL	BDL	BDL	BDL	BDL
Cu	11.0	11.61	13.14	13.42	.13.62
Zn	472.8	472.9	473.1	474.5	473.7
Cd	152.1	158.8	159.2	161.1	161.2
Pb	LOD	LOD	LOD	LOD	LOD
As	90.3	90.77	91.0	94.1	98.7
Hg	LOD	LOD	LOD	LOD	LOD

are given in µg/g. Change in weights in each species is found to be proportional with the measured concentrations. The values of sampled shellfish were then compared with the available literature data of other areas and with corresponding international standards permissible limits for marine resources.

Concentration of Heavy Metals in Oysters

The mean concentration values of heavy metals in oyster tissue are presented in Table 2. The maximum accumulation of heavy metals recoded were Fe, Zn, As and Cr. The concentrations metals were found in the order of Fe > Zn >As > Cr and were ranged as follows Fe (386.6 - 392.7), Zn (392.7 -372.5), As (22.1 - 23.4) and Cr (10.2 - 13.). The levels concentrations of Mn, Ni, Cd, Pb and Hg were found below detection limits whereas Co and V were not detected. The highest recorded value among the metals was Fe which varied from 386.6µg/g to 392.7 µg/g and the lowest recorded value among heavy metals was Cr which varied from 10.2 µg/g to 13.2 µg/g.

Concentration of Heavy Metals in Mussels

The result of metal concentrations detected in the mussels samples has given in Table 3. The maximum concentration of Cr, Fe, Cu, Zn, Cd and As were noted with highest concentrations while Mn, Ni, Pb, Hg, Co and V had BDL. Concentrations in mussels' species ranged as follows: Cr (9.8 -11.6), Fe (213.7 - 266.0), Cu (11.0 - 13.62), Zn (472.8 - 473.7), Cd (152.1 -161.2) and As (90.3 - 98.7). Irrespective of species, heavy metals accumulated in the mussels muscle in the order Zn > Fe > Cd > As > Cu > Cr. The concentration of heavy metals

Table 4. Mean concentrations (µg/g) of heavy metals in crabs. Errors in the measurements are less than 5%

Elements	1	2	3	4	5
Cr	BDL	BDL	BDL	BDL	BDL
Mn	BDL	BDL	BDL	BDL	BDL
Fe	678.1	693.3	851.9	855.2	855.7
Ni	BDL	BDL	BDL	BDL	BDL
Cu	45.4	45.6	50.4	62.2	62.6
Zn	414.8	417.2	1046	1048	1050
Cd	BDL	BDL	BDL	BDL	BDL
Pb	BDL	BDL	1.20	1.42	1.67
As	31.16	31.40	31.70	35.7	39.47
Hg	BDL	BDL	BDL	BDL	BDL

Table 5. Mean of concentrations (µg/g) of heavy metals in clams. Errors in the measurements are less than 5%

Elements	1	2	3	4	5
Cr	12.3	12.4	17.2	20.8	21.1
Mn	235.7	27 8.9	283.3	290.2	293
Fe	223.9	234.0	239.1	241.8	242.0
Ni	2.13	2.50	2.77	3.26	3.30
Cu	47.2	47.6	48.0	48.1	48.1
Zn	4271.	5036	5038	5070	5090
Cd	4.43	5.10	5.27	6.30	6.61
Pb	BDL	BDL	BDL	BDL	BDL
As	1.92	2.21	2.50	3.20	3.26
Hg	BDL	BDL	BDL	BDL	BDL
Co	ND	ND	ND	ND	ND
V	ND	ND	ND	ND	ND

in the tissues varied significantly. The highest recorded value among heavy metals was Zn which varied from 472.8µg/g to 473.7 µg/g and the lowest recorded value among heavy metals was Cr which varied from 9.8 µg/g to 11.6 µg/g.

Concentration of Heavy Metals in Crabs

The concentration of heavy metals detected in crabs' samples has given in Table 4. The contamination of Fe, Cu, Zn and As were noted with different concentrations while Mn, Cd, Cr, Ni, Pb, Hg, Co and V were BDL. The metals accumulated were in the order Zn > Fe > Cu > As >Pb while their concentrations were ranged as follows: Zn (414.8 -1050) Fe (678.1 - 855.7), Cu (45.4 - 62.6), As (31.16 - 39.47) and Pb (1.20 -1.67) µg/g. The concentration of heavy metals in the tissues varied significantly. The highest recorded value among heavy metals was Zn which varied from 414.8 µg/g to 1050.0 µg/g and the lowest recorded value among heavy metals was Pb which varied from 1.20 µg/g to 1.67 µg/g.

Concentration of Heavy Metals in Clams

The concentration of heavy metals detected in the samples has given in Table 5. The results show that Cr, Mn, Fe, Ni, Cu, Zn, Cd and As were more concentrated in this sample species with the range concentration of (12.3 -21.1), (235.7 -293), (223.9 9 -242.0), (2.13 -3.30), (47.2 - 48.1) and (4271 -5090), (4.43 - 6.61) and (1.92 - 3.26)µg/g respectively. The accumulation of heavy metals was observed in the order of Zn >Mn> Fe >Cu > Cr > Cd > As. The highest recorded value among the heavy metals was Zn which varied from 4271 to 5090µg/g and the lowest recorded value among heavy metals was As which varied from1.92µg/g to 3.26µg/g. The elements Pb and Hg were BDL.

Comparison of Metal Concentrations (µg/g) in shellfish Species Sample

A comparison of metals values for the shellfish samples analyzed in this work were presented in Table 6 below. Among the analyzed metals, Fe, Zn, and Cu and As were only noted to be higher contaminated in all the species and they were in the order of Zn > Fe > As > Cu with the concentrations range of (369.5-5090)µg/g, (213.7-855.7)µg/g, (3.26-98.7)µg/g and (11.0-62.6)µg/g respectively. The highest value of Cd metal was observed in Mussels and Calms with the range of concentrations of (152.1- 161.2) and (6.30 – 6.61)µg/g

Table 6. Comparison of Metal Concentrations (µg/g) in shellfish Species. Errors in the measurements are less than 5%

Element	Oysters	Mussels	Crabs	Clams
Cr	10.0 -13.2	9.8-11.6	BDL	20.8-21.1
Mn	BDL	BDL	BDL	290.2-293.0
Fe	386.6-392.7	213.7-266.0	678.1-855.7	241.8-242.0
Ni	BDL	BDL	BDL	3.26 -3.30
Cu	17.5-20.0	11.0-13.62	45.4-62.6	48.1-48.1
Zn	369 -372.5	472.8-369.5	414.8 – 1050	5070 -5090
Cd	BDL	152.1-161.2	BDL	6.30-6.61
	BDL	BDL	BDL-1.67	BDL
As	22.1-23.4	90.3-98.7	31.16-39.47	3.20 -3.26

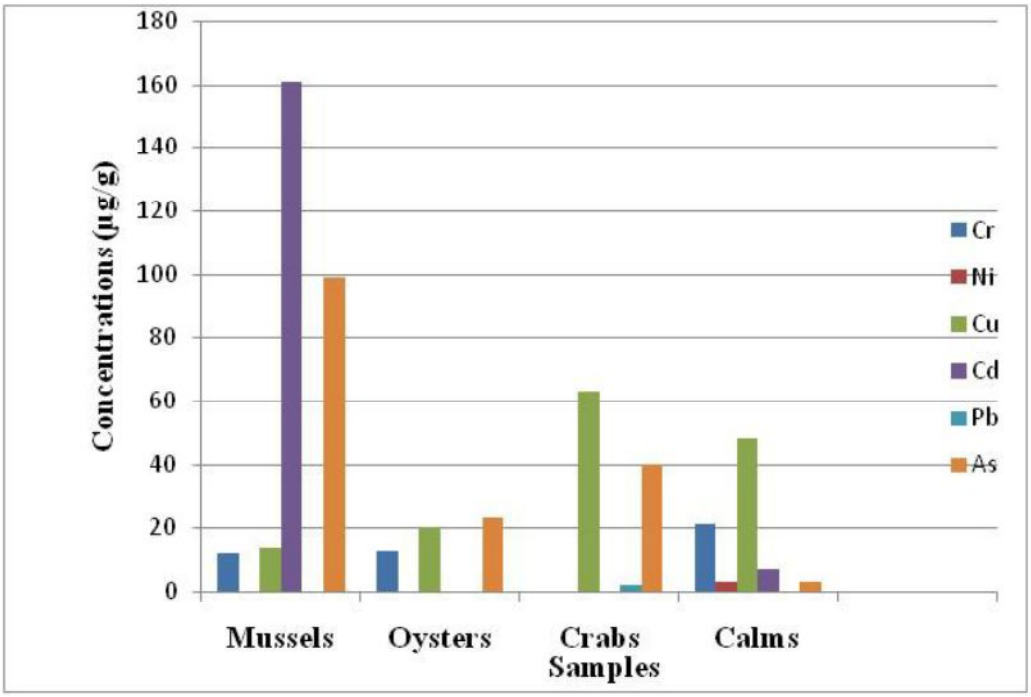


Figure 3. Level of Heavy Metals Conc. (µg/g) in Shellfish.

respectively, it value was BDL in Oysters and Crabs. The maximum value of Mn (290.2 – 293.0)µg/g and Ni (3, 26 – 3.30)µg/g were only detected in Calms whereas in Oysters, Mussels and Crabs were BDL. The Pb element which was only .noted in Crabs (with weight > 140.0g) with the range concentration of (1.42 - 1.67)µg/g, it was found BDL in Oysters, Mussels and Clams (see figure 3). The results show that Crabs and Calms exhibited highest considerable range concentrations of Zn (590.0 - 5070)µg/g whereas Fe (678.1 – 855.7)µg/g was more concentrated in Crabs (Figure 4). The As which is one of the metals contaminated in all the shellfish samples, was more observed in Mussels with the range concentrations of (90.3 – 98.7)µg/g The elements Hg, Co and V were all BDL. From the result obtained, it can be noted that levels of metals detected in shellfish species were different. The finding was that, marine organisms such as oysters, crabs, clams and mussels could accumulate pollutants differently due to species-specific ability/capacity to regulate or accumulate trace metals (Reinfelder 1997; Chang, Chong, Soto, Roja and Wang 2001), and that, Mussels are biofilter organisms which retain small particles from sea water, so the presence of some pollutants in mussel tissue indicate a contamination of marine environment.

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

Literature data and data from this study were given in Table 7 to compare element concentration levels in shellfish. The sampled oysters were compared with the

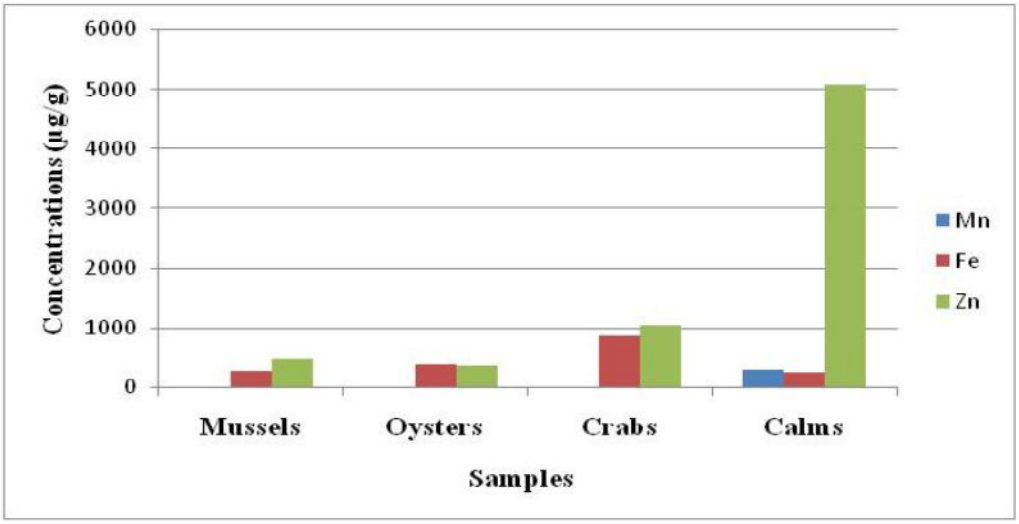


Figure 4. The level of Zn, Fe and Mn conc. (µg/g) in shellfish

Table 7. A comparison of the metal concentrations in shellfish found in this study with literature data from other sea world regions

Oyster

Location	Cr	Fe	Cu	Cd	Zn	Mn	Ni	As	Pb	Reference
Trinidad	0.64		52.0	0.52	52.0		0.31			Bekele,2005
Nigeria	0.09	51.5	106.0	0.82	616	1.30	0.10	1.55	0.09	Avong,2009
Mexico	6.43			2,23					5.84	Gonzá, 2009
Zanzibar	**11.7**	**389.4**	**18.7**	**BDL**	**370.7**	**BDL**	**BDL**	**22.8**	**BDL**	**This Study**

Mussels

Location	Cr	Fe	Cu	Cd	Zn	Mn	Ni	As	Pb	Reference
M'negro	1.5	4160	10.0	2.70	380	20.0	9.1	-	10.0	Sandro, 2010
Spain	24.0	445	18.4	0.45	266	10.0	10.0	-	3.2	Besad, 2002
Italy	-	4000	137	0.8	125	78.0	4.5	-	5.5	Roselli, 2009
Zanzibar	**10.8**	**240**	**12.3**	**156.4**	**473.5**	**BDL**	**BDL**	**95**	**ND**	**This Study**

Crabs

Location	Cr	Fe	Cu	Cd	Zn	Mn	Ni	As	Pb	Reference
Malaysia	-	-	11.7	0.22	-	-	-	-	28.5	Zaleha, 2010
Nigeria	-	2145	-	-	413	2600	-	160	230	Avong, 2009
Qunsland	0.08	3.80	5.2	0.001	17.7	0.50	0.02	3.0	0.09	Munro, 1999
Zanzibar	**BDL**	**766.9**	**54.0**	**BDL**	**732**	**BDL**	**BDL**	**35.4**	**0.8**	**This Study**

Clams

Location	Cr	Fe	Cu	Cd	Zn	Mn	Ni	As	Pb	Reference
Nigeria	-	-	1.77	3.35	4.95	0.15	0.1	0.45	0.19	Falusi, 2007
Egypt	0.28	64.3	1.74	0.22	9.35	77.50	1.25	-	0.72	Khalid, 2005
Malaysia	-	227.4	-	4.25	44.7	31.7	-	-	36.4	Devagi, 2008
California	37.0	756.0	1.77	0.14	62.8	-	28.6	1.59	0.25	Jannifer, 2004
Zanzibar	**21.0**	**241.9**	**47.7**	**6.45**	**5080**	**291.0**	**3.25**	**3.20**	**ND**	**This Study**

BDL for: Cr = 9.0µg/g; Cd = 4.1 µg/g; Mn = 5.1 µg/g; Ni =2.1 µg/g; Pb = 1.0 µg/g.

Table 8. Comparison of mean concentrations (µg/g) of toxic metals in shellfish species with the MTLs set by different International Organizations.

SS and IOs	Cr	Cd	Pb	Ni	Hg	As
Oysters	11.7	BDL	BDL	BDL	ND	22.8
Mussels	10.8	156.4	ND	BDL	ND	94.8
Crabs	BDL	BDL	0.8	BDL	ND	35.4
Claims	21.0	6.45	ND	3.25	ND	3.2
FAO,2004	1.0	0.05	0.5	--	-	0.1-5.0
WHO,2004	1.0	0.05	1.0	0.5-1.0	-	-
USFDA,2007	-	4.0	1.7	-	1.0	86
EC,2006	-	0.5-1.0	1.50	-	0.5	-

Note: SS = Sample Species; IOs = International Organizations
BDL for Cd = 4.1 µg/g; µg/g; Cr = 9.0 µg/g; Pb = 1.0 µg/g; Ni = 2.1 µg/g

available literature data of other areas of Trinidad, Nigeria and Mexico. It can be noted that the heavy metal concentrations of Cr, Fe and As were generally higher in this study while Cu and Zn were similar to the metal concentrations in oysters from the reported literature. However, the sampled mussels were compared with the literature of Montenegro, Spain, Turkey and Italy. The metal concentrations of Cd, Zn and As were higher while Cr, Fe and Cu were similar to the metal concentrations in mussels from the reported literature. Furthermore, when the detected metals in crabs were compared to previous studies, the results show that Cu (54.0µg/g) and Zn (732.4 µg/g) have the highest mean concentrations whereas Fe, Pb and As were in similar range to the findings of Falusi, (2007); Munro, (1999) and Avong, (2009). The values of Cd, Mn, Ni and Cr reported by authors above were below detection limits while in this study the values were 4.1, 5.1, 2.1 and 1.0 µg/g respectively. On other hand, the metal levels found in clams from this study indicate that Cu(47.7) µg/g, Cd(6.45) µg/g, Zn(5080) µg/g, Mn(291.0) µg/g, and As(3.2) µg/g were higher than those reported by Falusi, (2007), Khalid, (2005), Devagi, (2008), Devagi, (2008) and Jannifer, (2004) whereas Cr(21.0) µg/g, Fe(241.9) µg/g and Ni(3.25) µg/g were similar to the metal concentrations reported for other area such as California.

Comparison of mean concentrations (µg/g) of toxic metals in shellfish species with the MTLs set by different International Organizations

Among the different metals analyzed Cd, Cr, Pb, As, Ni and Hg are classified as chemical hazards and maximum residual levels have been prescribed for human (FAO, 2004; EC, 2006; FDA, 2007). In this study, the contents of Cr(BDL–21.0)µg/g, Cd(BDL–156.4)µg/g, Ni (BDL-3.25)µg/g and As(3.20–94.8)µg/g in the samples were much higher than the permitted levels (FAO/WHO,2004 and USFDA, 2007). Pb concentration (BDL – 0.8)µg/g is much less than the permitted levels, whereas Hg was not detected. Therefore, the analyzed shellfish species accumulated highest concentrations of Cr (in oysters and crabs), Cd, Ni (in claims) and As (in mussels) metals that exceeded the limits designated by the mentioned International Organizations, (Table 8).

CONCLUSION

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

Among the analyzed metals in shellfish species, Fe, Zn, and Cu and As were noted to be higher contaminated with concentrations range of (369.5-5090)µg/g, (213.7-855.7)µg/g, (3.26-98.7)µg/g and (11.0-62.6)µg/g respectively. It was noted that Clams exhibited highest considerable range concentrations of Zn (590.0 - 5070)µg/g whereas Crabs were more contaminated with Fe (678.1 – 855.7)µg/g. The As was more observed in Mussels with the range concentrations of (90.3 – 98.7)µg/g. The levels of metals detected in shellfish species were different. The finding was that, marine organisms such as oysters, crabs, clams and mussels could accumulate pollutants differently due to species-specific ability/capacity to regulate or accumulate trace metals. The elements Hg, Co and V were all below detection limits.

Generally, when comparing the shellfish species, the mean concentration of Zn(5080)µg/g was found to be higher in shellfish (clams), Cd(156.4)µg/g and As(94.5) µg/g were more detected in mussels whereas. The toxic heavy metals Cd and Mn in all measured oysters were below detection limit. However, these limits are within the range of the concentration of the elements reported in literature. In contrast, the levels of heavy metal

concentration obtained in studied samples were 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 shellfish samples were below the FAO/WHO threshold limits while As(9.8) µg/g in mussels, Cd and Ni in all shellfish samples were above the FAO/WHO (2004) limits.

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