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Some metal concentrations in the edible parts of *Tridacna maxima*, Red Sea, Egypt

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Abstract The concentrations of Ca, some essential (Fe, Zn, Mn, and Cu) and non-essential nutritive elements (Cd, Pb, and Ni) were measured in the edible parts (mantle and adductor muscles) of Tridacna maximx collected from south Quseir City (Red Sea). The general trend of metal contents of the different parts follows the order; Ca > Fe > Zn > Pb > Mn > Cu > Cd > Ni. The tissues before cooking recorded the highest average concentrations of Cu, Pb, Fe, Cd, Mn, and Ni (2.658, 5.250, 34.375, 1.464, 3.207, and 0.886 ppm, respectively) relative to tissues after cooking and the water of cooked tissues (WCT). The total cooked tissues recorded higher average contents of Zn and Ca (17.282 and 1,114.679 ppm) than the uncooked tissues. Calcium recorded the highest concentration in the ECT of adductor and mantle muscles (2,081.126 \pm 177.39 and $1,893.326 \pm 394.28$ ppm). Mantle recorded higher concentrations of Pb, Mn, Ni, and Ca (7.489 \pm 4.65, $4.241 \pm 1.13, 0.980 \pm 0.60, \text{ and } 1,039.362 \pm 177.42 \text{ ppm},$ respectively) than adductor muscle before cooking. Ca concentration levels in the WCT increased after cooking tissues especially in adductor muscles. This may attributed to the liberation of larger amount of calcium in ionic form in water. The clams may have intracellular regulatory

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Home Economic Department, Faculty of Education, South Valley University, Red Sea Branch, Hurghada, Egypt mechanisms to keep their concentrations in equilibrium, subsequently; the recorded metal concentrations are in the safe limits for human consuming, where these concentrations did not exceed the clam's capacity of regulation.

Keywords Heavy metals · Edible parts · *Tridacna maxima* · Red Sea

Introduction

Bivalves are valuable sentinel organisms (Farrington et al. 1982, 1983) as they greatly concentrate many chemical pollutants from seawater and sediment. Bivalve clams are used as bio-indicators and sedentary filter feeders as they usually have the ability to accumulate heavy metals in their tissues without metabolizing them appreciably (Gunther et al. 1999; Nasci et al. 1999; Olivier et al. 2002; Blaise et al. 2002; Fung et al. 2004; Zhou et al. 2003). The bioavailability, clam sizes, hydrodynamics of the environment, changes in tissue composition, and reproductive cycle influence the metal concentrations (Boyden and Phillips 1981). In comparison to fish and crustacean, bivalves have very low level of activity of enzymes capable of metabolizing persistent organic pollutants and reflect the magnitude of environmental contamination more accurately (Phillips 1980; Kumari et al. 2006). Most metals are generally concentrated many times over within organism's soft tissues especially mantle and during the period of shell growth, the level in the soft parts as well as shell increased appreciably (Carriker et al. 1982; Thorn et al. 1995; Huanxin et al. 2000; Yasoshima and Takano 2001). Otchere (2003) pointed out that, element concentrations in mollusks in the same location differ between different species and individuals due to species ability to regulate and accumulate metals. Szefer et al. (1999) reported that, the soft tissues and byssi of *Mutilus edulis* are good bio-indicators for identification of coastal areas exposed to metal contaminations.

Tridacna maxima is the most wide-ranging giant clam species, being found from the east coast of Africa to as far east as the Red Sea and eastern Polynesia. It is recognizable by its brightly colored mantle (blue, green, and brown) and boring habit (Ellis 1999, 2000). Giant clams flesh have been traditionally used as subsistence food source (Ellis 1999). Adductor muscles and mantles are consumed as food in raw, cooked, or dried forms. Giant clam shells are sold as souvenir items (Leung et al. 1993).

Many authors studied the metal levels in the edible mollusks; in tissues and shells of *Cerithidea cingulata*, *Crassostrea madrasensis*, and *Meretrix meretrix* (Kesavan et al. 2010), tissue of *Mytilus edulis* (Pellerin and Amiard 2009; Szefer et al. 1999), and *Paphia malabarica* (Kumari et al. 2006). The concentration level of some elements in Tridacnidae were studied in tissues (Ishii et al. 1992; Adjei-Boateng et al. 2011; Madkour et al. 2011) and shells (Madkour 2005).

The present study aims to evaluate the concentration levels of Ca, some essential nutritive elements (Fe, Zn, Mn, and Cu) and non-essential nutritive (Cd, Pb, and Ni) in the most edible parts (mantle and adductor muscles) of the common giant clam *Tridacna maxima* compared to the metal safe limits to be used as food for human.

Materials and methods

Materials

Thirty specimens of giant clam *Tridacna maxima* were collected from inshore zone of the Red Sea in area extended between 10 ($26^{\circ}0'42.78''$, $34^{\circ}16'24.81''$) and 17 km ($25^{\circ}58'9.76''$, $34^{\circ}21'56.43''$) south of Quseir city (Fig. 1). The investigated site is away from the direct human impacts as well as the flash floods. The maximum recorded depth in this site does not exceed 5 m.

Elements determination in the soft tissues (adductor and mantle muscles)

The mantle and adductor muscles of each specimen were separated from the animal tissues then subdivided into two parts. The first part (mantle and adductor) was freezed, while the second part (mantle and adductor) was dried directly at 70 °C for 48 h. For each sample, the first parts of mantle and adductor muscles were cooked in distilled water (200 ml for each sample) for about 50–60 min. The cooked parts (4.4519 \pm 1.07 and 7.7958 \pm 1.97 g) of adductor and mantle muscles, respectively (\pm standard deviations, SD) were dried at 70 °C for 48 h to remove the moisture content. The dried parts (Table 1) are ranged between 1.12 and 2.33 g (adductor muscle with average 1.5276 \pm 0.36) and between 1.78 and 3.88 g (mantle



Fig. 1 Area of study on the Red Sea map

muscles with average 2.484 ± 0.63). The freshly dried parts and the dried parts of the cooked samples were ground using an agate mortar for 5 min to <80 mesh in order to estimate the complete homogeneity of the samples. Generally, about 0.5 g of all powdered samples (cooked and uncooked) were digested in 10 ml of hot conc. HNO₃ to near dryness and then diluted to 25 ml with de-ionized water (Chester et al. 1994). The waters of cooked tissues (WCT) were evaporated to dryness then digested in 10 ml of hot conc. HNO₃ to near dryness and then filtered and diluted to 25 ml with de-ionized water (Mohammed and Dar 2010). The digested mantles and adductors as well as the WCTs were used to determine the metal contents $(\pm SD)$ in the selected parts and liberated metals from the cooked parts. Calcium, some essential nutritive elements (Fe, Zn, Mn, and Cu) and non-essential nutritive (Cd, Pb, and Ni) were determined in the digested samples using atomic absorption spectrophotometer (AAS model GPC A932 Ver 1.1). The measurement accuracies were checked by applying three replicates in each measurement and the recorded metal concentrations in the uncooked and cooked tissues were expressed in µg/gm dry wt. and in ppm for the WCT.

The statistical analyses of the different samples (mantles and adductors) before and after cooking as well as the WCT were applied using the multiple comparisons (Bonferroni) of the ANOVA test using SPSS (Ver. 11). The standard deviation was calculated using software surfer 8.

Results

Heavy metal concentration of giant clam *T. maxima* before cooking

Iron recorded the highest concentration of the essential elements before cooking in the mantle $(36.859 \pm 20.44 \text{ ppm})$, while the adductor muscle recorded $31.891 \pm 20.59 \text{ µg/gm}$ dry wt., followed by Zn which recorded 14.497 ± 3.97 and $14.394 \pm 2.37 \text{ µg/gm}$ dry wt. (in the adductor and male muscles, respectively). Generally, mantle recorded the higher mean concentrations of metals Pb, Mn, Ni, and Ca $(7.489 \pm 4.65, 4.241 \pm 1.13, 0.980 \pm 0.60, \text{ and } 1,039.362 \pm 177.42 \text{ µg/gm}$ dry wt., respectively) than the adductor muscle (Table 2) while adductor muscle recorded a relatively high concentration for Cu, Zn, and cd $(3.061 \pm 1.51, 14.497 \pm 3.97, \text{ and } 1.856 \pm 0.92 \text{ µg/gm}$ dry wt., respectively).

Heavy metal concentration of giant clam *T. maxima* after cooking

The metal concentrations are almost the same pattern after cooking where, Cu, Fe, Pb, Mn and Ca recorded the highest concentrations (2.240 \pm 0.71, 33.504 \pm 7.38, 3.862 \pm 5.66, 4.026 \pm 0.99 and 1,122.285 \pm 200.13 µg/gm dry wt., respectively) in mantle tissues (Table 2) while the adductor muscle recorded a slightly high metal concentrations for Zn

	Adductor muscle		Mantle tissue		
	After cooking (wet weight)	Dry weight	After cooking (wet weight)	Dry weight	
Average	4.452 ± 1.07	1.528 ± 0.36	7.796 ± 1.97	2.484 ± 0.63	
Range	3.336-6.560	1.121-2.331	5.450-11.900	1.781-3.879	

Table 1 Average wet and dry weights of adductor and mantle muscles after cooking $(\pm SD)$

SD standard deviations

Table 2 Metal concentration (±SD) of Tridacna maxima tissues (adductor and mantle before and after cooking) and WCT

Elements	Tissue before cooking		Tissue after cooking		WCT	
	Add. muscle	Mantle	Add. muscle	Mantle	Add. muscle	Mantle
Cu	3.061 ± 1.51	2.255 ± 1.02	1.520 ± 0.25	2.240 ± 0.71	1.289 ± 0.40	1.706 ± 0.50
Zn	14.497 ± 3.97	14.394 ± 2.37	17.328 ± 4.95	17.236 ± 2.16	6.644 ± 3.44	5.574 ± 1.61
Pb	3.011 ± 2.31	7.489 ± 4.65	2.290 ± 1.75	3.862 ± 5.66	2.362 ± 0.79	2.620 ± 0.87
Fe	31.891 ± 20.59	36.859 ± 20.44	19.479 ± 11.15	33.504 ± 7.38	10.667 ± 12.24	31.382 ± 9.63
Cd	1.856 ± 0.92	1.071 ± 1.05	1.009 ± 0.80	1.002 ± 0.41	0.000	0.184 ± 0.31
Mn	2.172 ± 1.30	4.241 ± 1.13	1.775 ± 4.74	4.026 ± 0.99	1.209 ± 0.95	3.632 ± 1.48
Ni	0.792 ± 1.22	0.980 ± 0.60	1.112 ± 1.09	0.810 ± 1.09	0.855 ± 0.41	0.758 ± 0.58
Ca	715.492 ± 383.84	$1,\!039.362 \pm 177.42$	$1,\!107.072\pm133.79$	$1,\!122.285\pm200.13$	$2,\!081.126 \pm 177.39$	$1,\!893.326\pm 394.28$

and Ni (17.328 \pm 4.95 and 1.112 \pm 1.09 µg/gm dry wt., respectively). Cd recorded nearly equal level in both adductor and mantle muscles (1.0 µg/gm dry wt.).

A general trend in metal concentration of adductor and mantle muscles were observed for Ni, Cu, Cd, Pb, Mn, and Fe; where Fig. 2 illustrated that, metals concentration of tissues before cooking is slightly higher than the cooked tissues, but the Zn and Ca concentrations increases in tissues after cooking (Fig. 2). Heavy metal concentration in WCT

The WCT showed different patterns in the metal concentrations, mantle recorded higher Cu, Cd, Pb, Mn, and Fe concentrations $(1.706 \pm 0.50, 0.184 \pm 0.31, 2.620 \pm 0.87, 3.632 \pm 1.48$, and 31.382 ± 9.63 ppm, respectively) than those of the adductor muscles (Table 2; Fig. 2). Inversely, the adductor muscles recorded relatively high Zn, Ni, and Ca concentrations (6.644 ± 3.44,





 0.855 ± 0.41 , and $2,081.126 \pm 177.39$ ppm, respectively). Generally, Fe recorded the highest values among the essential and non-essential elements for tissues (before and after cooking) and the WCT.

Metal concentrations of total tissue before and after cooking and the WCT

The average concentration of heavy metals in the total tissue of *T. maxima* before and after cooking as well as the WCT showed a general trend in their concentration and accumulation (Table 3) as following graduation for the essential (Cu, Fe, Mn, and Zn) and non-essential elements (Ni, Cd, and Pb) as well as Ca as a major constituting element: Ca > Fe > Zn > Pb > Mn > Cu > Cd > Ni. Generally the average concentration of Cu, Pb, Fe, Cd, Mn, and Ni showed higher values (2.658, 5.250, 34.375, 1.464, 3.207, and 0.886 µg/gm dry wt., respectively) in the total tissues before cooking than the cooked tissues and the

Table 3 Average metal concentrations of the total tissues and WCT

Elements	Tissue before cooking	Tissue after cooking	WCT
Cu	2.658	1.880	1.498
Zn	14.446	17.282	6.109
Pb	5.250	3.076	2.491
Fe	34.375	26.492	21.025
Cd	1.464	1.005	0.092
Mn	3.207	2.901	2.421
Ni	0.886	0.960	0.807
Ca	877.427	1,114.679	1,987.226

WCT. While the cooked tissues showed higher concentrations for Zn and Ca (17.282 and 1,114.679 μ g/gm dry wt.) than uncooked tissues.

Calcium is one of the major constituting element for the giant clam shells, where it recorded the highest concentration in the WCT of mantle and adductor muscles $(2.081.126 \pm 177.39 \text{ and } 1.893.326 \pm 394.28 \text{ ppm})$ with average of the total WCT 1,987.226 ppm followed by the cooked tissues (mantle 1,122.285 \pm 200.13 µg/gm dry wt. and adductor muscle 1,107.072 \pm 133.79 µg/gm dry wt.) with mean of 1,114.679 µg/gm dry wt. of the total tissue. The tissues before cooking recorded the lowest concentrations $(715.492 \pm 383.84 \ \mu g/gm \ dry \ wt.$ for adductor muscle, $1,039.362 \pm 177.42 \,\mu\text{g/gm}$ dry wt. for mantle, and 877.427 µg/gm dry wt. for the total tissues). ANOVAmultiple comparison test (Bonferroni test) illustrated that, there are many significantly differences in the heavy metal concentrations between the uncooked and the cooked tissues for Cu, Zn, and Ca at P values of 0.023, 0.006, and 0.014, respectively (where P is significant at 0.05). On the other hand, another significant differences were observed (Table 4) between the cooked tissues and their WCTs for Zn at 0, Cd at 0.002, and Ca at 0 (where P = 0.05).

Discussion

The trace metals can be divided into essential elements and non-essential elements, the essential elements occur normally in all organisms. The high doses of the essential elements can be poisonous and causes hazardous effects on organisms (Kesavan et al. 2010). The non-essential

Dependent Variable	(I) Action	(J) Action	Mean difference (I – J)	Sig.
Cu	Tissue before cooking	Tissue after cooking	0.78	0.023*
Zn	Tissue before cooking	Tissue after cooking	-2.84	0.006*
Pb	Tissue before cooking	Tissue after cooking	1.88	0.201
Fe	Tissue before cooking	Tissue after cooking	7.88	0.303
Cd	Tissue before cooking	Tissue after cooking	0.46	0.128
Mn	Tissue before cooking	Tissue after cooking	0.30	1.000
Ni	Tissue before cooking	Tissue after cooking	-0.07	1.000
Ca	Tissue before cooking	Tissue after cooking	-237.25	0.014*
Cu	Tissue after cooking	WCT	0.381	0.739
Zn	Tissue after cooking	WCT	11.173	0.000*
Pb	Tissue after cooking	WCT	0.883	1.000
Fe	Tissue after cooking	WCT	5.468	0.966
Cd	Tissue after cooking	WCT	0.911	0.002*
Mn	Tissue after cooking	WCT	0.480	1.000
Ni	Tissue after cooking	WCT	0.154	1.000
Ca	Tissue after cooking	WCT	-872.548	0.000*

Table 4ANOVA-test ofmultiple comparisons(Bonferroni test) of the edibletissues and WCT

* The mean difference is significant at the 0.05 level

elements do not have any positive effects on organisms and they are harmful already in low doses. Adjei-Boateng et al. (2011) illustrated that, metal concentrations in the clam tissues were highly variable over the sampling period and seemed to be influenced by the reproductive cycle of the clam. On contrary, Denton et al. (1999) pointed out, the metal concentration in the soft tissues of the studied mollusks are relatively low especially for lead, nickel and cadmium.

The elements (Cu, Fe, Mn, and Zn) are essential nutrition elements for most marine organisms (Kesavan et al. 2010), where copper is essential for respiration and other enzymatic functions as well as the importance of other elements, where the high doses causes changing to poisonous and can cause hazardous effects on organism. During the present study, the concentrations of the essential elements were low and within the safe limit proposed by many authors (Table 5). The low concentrations may be due to the availability of these metals in the environment (Richardson et al. 2001; Palpandi et al. 2010), where the studied site was not impacted with exception of presence of few small fishing boats in the area (not exceed 5). The effectiveness of metal uptake may differ in relation to ecological need and metabolism of animals and concentration of the heavy metals in water, food, and sediment as well as some other factors such as salinity, temperature and interacting agents (Roesijiadi and Robinson 1994). The present results suggested that, the level of contamination of these metals does not exceed the clam's capacity of regulation (Amiard et al. 1987; Durou et al. 2005).

In the present study, the recorded concentrations of the Fe and Zn, as essential elements, were within the safe limits proposed by FAO/WHO (1984); Eisler (2010a, b); Ishii et al. (1992) where the biological and physiological roles of these clam may be the main reason for their concentrations variability (Phelps et al. 2003; Ferreira et al. 2004). The clams also have intracellular regulatory mechanisms (Luoma and Rainbow 2008; Ferreira et al. 2004; Luoma and Rainbow 2008; Madkour et al. 2011) to keep their concentrations in the clam tissues in equilibrium, where the concentration levels of Fe and Zn in the present study do not exceed the clam's capacity of regulation (Wang et al. 2002). The non-essential elements (Pb, Cd, and Ni) do not have any positive effects on organisms and they may harmful in low concentrations. They can inhibit an essential elements and cause enzymatic disturbances to the body. The safe limits of these heavy metals were proposed by FAO/WHO (1984) for some bivalves and gastropods reached to 10 ppm for Pb and 0.5 ppm/day for a meal contain Cd and 80 ppm for Ni. On the other hand, the safe limits for these elements in the edible bivalves including Tridacna for human consuming were 10 ppm for Pb (according to Eisler 2010a, b; WHO, World Health Organization 1999), 2.4–3.5 ppm for Cd (Ishii et al. 1992), and 80 ppm for Ni (Sankar et al. 2006; Eisler 2010a, b). The recorded concentrations in the present study illustrated that, these elements were within the acceptable safe limit and permissible for human consuming as a food (Amisah et al. 2011) with exception of Cd which recorded a relatively higher concentration (0.09–1.46 ppm) in T. maxima than the recorded values of FAO/WHO (1984); Gregori et al. (1996) and Blasco et al. (1999) in some bivalves but lies within the safe limits of other bivalves including Tridacnidae. However, the main reason for the moderately low concentration of most metals may be related to the intracellular regulatory mechanisms that keep their concentrations in the clam tissues in equilibrium and not increase (Madkour et al. 2011) and may be due to the change in the metabolic rates of bivalves and their bioavailability to accumulate heavy metals (Otchere 2003). Moreover, the present study is in accordance to (Vazquez et al. 1993) who suggested that, the metal levels in the surroundings are low and are not interfering with normal metabolic processes of T. maxima.

The risk associated with the consumption of *T. maxima* were ascertained by comparing the studied metals concentrations of the tissue before and after cooking to the WHO, World Health Organization (2000) safety reference standards and other references listed in Table 5. The recorded heavy metals in present study revealed that, the metal concentrations were within the permissible limits for human consumption. Cooking process may play the major role in the recorded differences of metal concentrations before and after cooking as well as in the WCT; so, the increasing in water concentrations may be attributed to the metals liberation from the cooked tissues.

The high concentrations of calcium in the tissues may be related to the biological and physiological processes of the animal tissues in the formation of calcium carbonate of the shell because of the inorganic phase of calcium carbonate contributes 98 % of the shell mass (Palpandi et al. 2010). On the other hand, the WCT recorded very high Ca concentrations that may reflect the bioaccumulation in tissues the marine organisms such as, bivalves, gastropods, corals, and fish (Kesavan et al. 2010).

The concentration levels of Cu, Pb, Fe, Cd, Mn, and Ni are decreased in the cooked tissues may be due to the ability of these metals to liberate in the WCT. Inversely, Ca and Zn were increased because of the metals tend to concentrate in the cooked tissues where the cooking process may play a vital role in condensing these two essential elements for the animal growth and nutritional. Moreover, Ca and Zn may have the ability to associate with non-metal phosphorus in a phosphate form $[Ca_3 (Po_4)_2]$ and Zn₃ $(Po_4)_2$]. The increased concentrations after cooking may be

Table 5 Comparison of the mean concentration of the present study and the safe limits of metals in the edible bivalves including Tridacna spp.

	Results of the present study (average in tissue)			Safe level of the	References	
	Before cooking	After cooking	WCT	edible bivalves		
Cu	2.66	1.88	1.50	10*	Gregori et al. 1996	
				20	FAO/WHO 1984	
				15	Eisler 2010a, b	
				10-20	Denton et al. 1999	
				3.2-3.9	Ishii et al. 1992	
Zn	14.45	17.28	6.11	50*	Gregori et al. 1996	
				40	FAO/WHO 1984	
				50	Eisler 2010a, b	
				2.4	Rayment and Barry 2000	
				100	Eisler 1981	
				12–14	Ishii et al. 1992	
Pb	5.25	3.38	2.49	10	FAO/WHO 1984; Eisler 2010a, b; WHO 1999	
				1.63-5.5	Denton et al. 1999	
				3.1-4.6	Ishii et al. 1992	
Fe	34.37	26.49	21.02	20-40	Eisler 2010a, b	
				10–34	Ishii et al. 1992	
Cd	1.46	1.0	0.09	1*	Gregori et al. 1996; Blasco et al. 1999	
				2*	Butler and Timperley, 1996	
				1.3*	Rayment and Barry 2000	
				3.7*	USEPA 1994	
				0.5	FAO/WHO 1984	
				1–2	Eisler 2010a, b	
				0.3-0.5	WHO 1999	
				2.4–3.5	Ishii et al. 1992	
				10	Denton et al. 1999	
Mn	3.20	2.90	2.42	5	Eisler 2010a, b	
				3.3–11	Ishii et al. 1992	
Ni	1.13	0.96	0.81	80	FAO/WHO 1984; Sankar et al. 2006; Eisler 2010a, b	
				1.5-2.9	Ishii et al. 1992	
				0.4–3.6	Denton et al. 1999	
				1.6	Rayment and Barry 2000	
Ca	877.43	1,114.68	1,987.23	1,620–2,790	Ishii et al. 1992	

* Measuring the fresh wet samples

related to the metal containing tissues (Ishii et al. 1992). The increase in the Ca concentration in the WCT of adductor muscles than the mantles after cooking may be attributed to the liberation of larger amount of calcium from adductor muscles in ionic form in water with higher ratios than mantle. In addition to the used water (distilled water) contents of calcium that causes their elevation with relatively high values. The recorded significance decrease of Cu (P = 0.023) in the cooked tissues is attributed to the liberation of few amount from this element in ionic form, while the significance increase of Zn and Ca (P = 0.006 and P = 0.014, respectively) in the cooked tissues may be due the probability of formation of calcium phosphate and zinc phosphate, phosphorous was found in complicated

bonds of phosphate form where $[Ca_3 (Po_4)_2 \text{ and } Zn_3 (Po_4)_2]$ as illustrated by Ishii et al. (1992).

Finally, the heavy metals in the giant clams *T. maxima* are in the safe acceptable limits and can be regulated by their soft tissues. Therefore, the giant clams should be cultured for human exploiting, where the adductor muscle and mantle are consumed as food in row, cooked, or dried forms (Leung et al. 1993).

Conclusion

Adductor and mantle muscles before cooking recorded higher concentrations of Cu, Pb, Fe, Cd, Mn, and Ni than after cooking and the WCTs, inversely the cooked tissues recorded higher Zn and Ca concentrations than uncooked tissues.

General trend of metal contents in the different tissue parts follows the order of Ca > Fe > Zn > Pb > Mn >Cu > Cd > Ni. The metal concentrations of Ca and Zn increased in tissues after cooking. This is may be related to the ability of these metals to diffuse in or from the cooked tissues and the used water. These metals may associated with non-metal phosphorus; probably forming an insoluble divalent metal phosphate [Ca₃ (Po₄)₂ and Zn₃ (Po₄)₂].

The recorded metal concentrations in the edible parts of *T. maxima* were in the safe acceptable limits of consuming subsequently, we recommended by wide scale of culturing for human exploiting.

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