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Original Research

Heavy Metals Residues in Bivalve Mollusks in Fayoum Province and their Potential Health Hazards

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Abstract

Bivalves Mollusks have a potential benefit which include protection from anemia due to its content of iron and vit B12 in addition they pose a threat in promoting the ability to be contaminated by different heavy metals residues as a result of mining, industrial production untreated sewage sludge. Hg, Pb, Cd, As and Cr are the main five toxic heavy metals that induce human poisoning. For evaluation of Hg, Pb, Cd, As and Cr residues in Bivalve Mollusks in Fayoum province and their potential hazards, a total of 64 random samples of Bivalve Mollusks (Callista Florida species) were collected from the fish markets of Fayoum City, (Wadi Elrayan), Egypt The results revealed that the mean residues of Hg, Pb, Cd, As and Cr were 0.75, 0.77, 0.089, 7.285 and 0.011 mg/kg/ww, respectively. All samples examined for Hg and As exceeded the PML while Pb and Cd lied with the PML The collected samples were subjected to soaking in running water for 30 minutes; soaking in 5% acetic acid solution for 30 minutes and boiling with 5% acetic acid solution for 15 minutes .The heights reduction % of heavy metals residual levels were recorded after treatment in boiling 5% acetic acid treatment with the following reduction %; Hg (80%), Pb (67.6%), Cd (77.5%), As (44.35%) and Cr (75.76%). The assessment of Hazard quotient (HQ) and hazard index (HI) value was exceeded 1 which indicates a potential risk to human health and TR was > 1x10-4 which indicates a carcinogenic risk to the local consumers and will face high chronic risk if they consume Bivalve Mollusks on regular basis in their diet.

KEYWORDS
Hg, Pb, Cd, As, Cr, Bivalve Mollusks, THQ, HI, TR

INTRODUCTION

Massive increases in human exposure to heavy metals have been brought about by the industrial activities of the previous century. The most frequent heavy metals that cause human poisonings are mercury, lead, chromium, cadmium, and arsenic. Bivalve mollusks are a diverse species, and it was estimated that about 100,000 described species virtually inhabit the entire world seas and oceans. Bivalve mollusks shellfish are soft bodied invertebrates belonging to phylum Mollusca (Gosling, 2003). They are low in calories but high in protein and rich in vitamins B12, C, D, A, omega-3polyunsaturated fatty acids and a good source of essential amino acids (Venugopal and Gopakumar, 2017; Anita *et al*., 2018) in addition to iron, selenium, magnesium, and zinc. It is naturally low in carbohydrates content as well as total and saturated fat.

Bivalves' mollusks have a potential benefit which include protection from anemia due to its content of iron and vitamins B12 and in the same time poses a threat in promoting the ability to be contaminated by different heavy metal residues as a result of mining, increase industrial activities of the last century, untreated sewage sludge. Important biological elements of oceanic habitats, bivalves have the ability to bioaccumulate pollutants while feeding through water filtering, serving as environmental watch dogs (Pavón *et al*., 2022). Hg, Pb, Cr, Cd and As are the main five toxic heavy metals that induce human poisoning (Balali–Mood *et al*., 2021). Increased levels of hazardous metals are frequently found in marine creatures from coastal locations linked to industrial discharge (Zaki 2004; Suresh *et al*., 2013). These five heavy metals (Pb, Hg, Cd, As, Cr) are among the priority metals with significant public health implications due to their high level of toxicity and are classified as group 1 carcinogens by the International Agency for Research on Cancer (IARC, 1980) and this group is used when there is sufficient evidence of carcinogenicity in humans. Even at modest exposure levels, they are all known to cause damage to various organs since they are systemic toxins. These metals are also categorized as "known" or "probable" human carcinogens by the United States Environmental Protection Agency (USEPA, 1989). Epidemiological and experimental studies demonstrated an association between exposure and the incidence of cancer in humans and animals (Naggar *et al*., 2016). In the early 20thcentury, bivalve mollusks were proposed as promising bioindicator shellfish species which tend to identify the chemical contaminants in water due to their high bioaccumulation capacity (Zuykov *et al*., 2013). The variations in the environmental pollution in various places, even within the same country, may be reflected the differences in the heavy metals' concentrations among different localities. (Muhammad *et al*., 2011; El

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Nemr, 2012; Innocent, 2014; Ikenaka *et al*., 2015; Dar *et al*., 2018).

Thus, this study was conducted to determine the residual level of Hg, Pb, Cd, As and Cr in the soft edible tissue of market Mollusks species collected from Faiyum Governorate, (Wadi Elrayan area), Egypt. Moreover, the health risk assessment for the examined heavy metals was determined through calculation of the target hazard quotient (THQ), Hazard Index (HI) and Cancer target risk (TR). Moreover, the effect of different treatment methods which easy to be done by consumer (5% acetic acids and boiling) in order to reduce the heavy metal residual levels in Bivalve Mollusks.

MATERIALS AND METHODS

Samples collection

A total of 64 random samples of Bivalve Mollusks were collected from Fayoum City fish markets, which harvested from Wadi Elrayan area (Wadi Elrayan is a unique nature protectorate in Faiyum Governorate, which include a waterfall and two artificial lakes and were created to absorb the excess of the agricultural drainage water. The collected samples were subjected to heavy metal assessment. The samples were transferred cooled directly in ice box without delay to the Food Control Laboratory, Faculty of Veterinary Medicine, Zagazig University, for the heavy metal analysis. (Images of Bivalve Mollusks Callista Florida species)

Preparation of samples

All Bivalve Mollusks samples were surfaces cleaned from any dirt's by washing it by bi-distilled water and the shells were opened and the flesh content was removed weighted and analyzed. The 64 Bivalve Mollusks samples were divided into four groups, 16 samples for each. The first group was analyzed without any treatment as control group, while the second group was put in 200 ml glass container and soaking in running non chlorinated water for 30 minutes. The third group was soaked in 5% acetic acid solution with intermittent shaking for 30 minutes. The fourth group was boiled in 5% solution of acetic acid for 15 minutes in glass container. After treatment 2 gram of each sample was kept separately in polyethylene bag and kept in deep frozen state until analysis.

Methods of analysis

Quantitative determination of heavy metals was carried out by "Buck scientific 210VGP Atomic Absorption Spectrophotometer" at the Faculty of Veterinary, Zagazig University.

Estimated daily intake (EDI)

EDI for heavy metals was obtained using the following equation described by the Human Health Evaluation Manual (US Environmental Protection Agency, (USEPA, 2010):

EDI = C x FIR / BW

Where C is the concentration of the metal in the sample (µg/g wet weight); FIR ingestion rate, which was estimated at 48.57 g/day (FAO, 2003); BW is the body weight of Egyptian adults, which was estimated at 70 kg.

Non-carcinogenic risk

The Target Hazard Quotient (THQ) is commonly used in assessments of heavy metal noncarcinogenic risk as a combined risk index (USEPA, 2011).

THQ= EF × ED × C × FIR / RfD × BW × AT × 10^{-3}

Where EF represents the exposure frequency (350 days/year), ED is the exposure duration (70 years), C represents the individual heavy metal concentration in bivalves (mg/kg/ wet weight), , FIR is the daily intake of bivalves (g/day), BW represents the average weight of an adult (kg) , AT represents the mean exposure duration for noncarcinogens (25550 days) and RfD stands for the oral reference dose; were, 0.001 (Cd), 0.003 (Cr), 0.3 (Hg), 0.0003 (As) µg/kg/day and 0.0015 (Pb) (EFSA, 2010).

Hazard index (HI) to estimate the risk of mixed contaminants was also calculated by using the following equation:

HI = Σ HR*i*

Where i represents each metal. When HR and /or HI of >1 indicates that there is a potential risk to human health and whereas a result of ≤1 indicates no adverse health risk effects.

Cancer target risk (TR)

Carcinogenic risks on individual developing cancer over a lifetime were Calculated by the following (USEPA, 2006).

TR= EF ×ED×C× FIR/ RFD× AT

Here, EF = exposure frequency (350 days/year),

ED =exposure duration (30 years), AT = average time for carcinogens (365 days/year×70 years), CSF = oral carcinogenic slope factor (USEPA, 2010).

CSF for As, Cd, and Pb and Cr are 1500, 380, 8.5×10^{-3} and 5.0×10^{-1}, µg/ kg/ day respectively (OEHHA, 2009; USEPA, 2015).

Statistical analysis

The SPSS computer software, version 25, was used for all statistical analyses (Inc., 1989-2017) to determine variance, normality, and homogeneity; the Kolmogorov-Smirnov tests were performed. The obtained data were statistically evaluated to identify significant differences between treatments (one-way ANOVA test), followed by pairwise comparisons using Duncan's test at $p< 0.01$ for normally distributed data, respectively (Pb and Cr). For non-normally distributed data (Hg, Cd, and As) and reduction percentage, a Kruskal-Wallis H test was performed. The correlation coefficient was calculated to examine the relationship between the interventions applied.

Log reduction: Log reduction = $\log_{10}$ (A/B)

Where A is the control and B is the treatment

Reduction %= [(Co - Cf)/ Co] × 100, where Co and Cf are the initial and equilibrium concentration (mg/kg/ww) of metal ions in solution, respectively.

RESULTS AND DISCUSSION

The results obtained in Table 1 revealed that the mean±SE for Hg, Pb, Cd, As and Cr residues, in control Bivalve Mollusks flesh group the results were 0.75±0.063, 0.77±0.108, 0.089±0.027, 7.285±0.769 and 0.011±0.003 mg/kg/ww, respectively. For Hg concentration the results were higher than those reported by Baudrimont *et al*. (2005); EC (2006); Páez-Osuna and Osuna-Martínez (2015); Atia *et al*. (2018) and Barchiesi (2020). The higher obtained results were attributed to the high source Hg pollution. The Commission Regulation (EC) No 1881/2006 stated the recommended residual level in Bivalve not more than 0.5 mg/kg/wet. Results from this study revealed that all heavy metals values in examined Bivalve Mollusks lied within the permissible limits except mercury (Hg) and (AS). Aquatic ecosystems are exposed to Hg from both natural and artificial sources. The Hg contamination is attributable to human activities such as coal burning, mining, and industrial operations. These activities release mercury into the environment, which coastal food systems.

For Pb concentration, the results were significantly higher than those reported by Olmedo *et al.* (2013); Silva da *et al.* (2016), and Atia *et al.* (2018) and were significantly lower than those reported by Vázquez-Boucard *et al.* (2014), and Eltanani (2021). The EC (2006) stated the recommended residual level in Bivalve not more than 1.5 mg/kg/wet. Results from the current study According to ES (2010), the obtained results for Pb values in examined Bivalve Mollusks soft tissue samples lied within the permissible limits.

For Cd concentration, results from this study were significantly higher than those reported by Atia *et al.* (2018), and Silva da Araújo *et al.* (2016) and were lower than those reported by Eltanani (2021). The EC (2006) stated the recommended residual level in Bivalve not more than 1 mg/kg/wet. According to ES (2010), the obtained results for Cd values in examined Bivalve Mollusks soft tissue samples lied within the permissible limits.

For AS, Results from the present study were significantly lower than those reported by Atia *et al.* (2018); Rattanachongkiat *et al.* (2004) and Krishnakumar *et al.* (2016). The obtained results were comparable with the levels reported by Kucuksezgin *et al.* (2006). The EC (2006) stated the recommended residual level in Bivalve not more than 0. 5 mg/kg/wet. According to ES (2010), the obtained results for all heavy metals values in the examined Bivalve Mollusks flesh samples lied within the permissible limits

Table 1. Mean values of the effect of different treatments for normally distributed data on Pb and Cr.

Treatments	Pb (mg/kg/ww)			Cr (mg/kg/ww)		
	Mean±SE	Min.	Max.	Mean±SE	Min.	Max.
Control	0.768±0.108[a]	ND	1.31	0.011±0.003[a]	ND	0.03
Running water	0.638±0.131[ab]	ND	1.26	0.006±0.003[ab]	ND	0.03
Acetic acid	0.448±0.094[ab]	ND	0.93	0.001±0.001[b]	ND	0.01
Acetic acid with boiling	0.280±0.058[b]	ND	0.67	0.004±0.002[ab]	ND	0.02

Different superscript letters within a column donate significant differences (P <0.01) between treatments. (Duncan's test at p< 0.01) ND: The lower detected limits was 0.000l mg/kg/ww

Table 2. Descriptive statistics of Hg, Cd and As among the different treatments.

	Treatments	Mean±SE	Min.	Max.
Hg	Control	0.750±0.063	0.38	1.22
	Running water	0.339±0.052	ND	0.72
	Acetic acid	0.325±0.065	ND	0.72
	Acetic acid with boiling	0.150±0.047	ND	0.55
Cd	Control	0.089±0.027	0.03	0.36
	Running water	0.064±0.007	0.03	0.11
	Acetic acid	0.028±0.006	ND	0.07
	Acetic acid with boiling	0.020±0.005	ND	0.06
As	Control	7.285±0.769	2.94	13.04
	Running water	6.185±0.497	3.54	9.93
	Acetic acid	4.795±0.499	2.28	8.39
	Acetic acid with boiling	4.054±0.593	0.46	8.05

ND= not detected

Table 3. Mean Rank Log values of the effect of different treatments for non-normally Distributed data Hg, Cd and AS.

Treatments	Hg	Cd	As
Control	52.88	42.5	42.38
Running water	30.25	40.63	39.13
Acetic acid	28.75	24.25	26
Acetic acid with boiling	18.13	18.63	22.5
Kruskal-Wallis H, degree of freedom= 3 ($\chi^2(3)$)	29.81	23.76	13.10
Asymp. Sig.	0.00	0.00	0.00

A Kruskal-Wallis H test showed that there was a statistically significant difference.

Table 4. Correlation Coefficient between the different treatments on the reduction effect of heavy metals in bivalve Mollusks.

	Running Water	Acetic Acid	Acetic Acid with Boiling
Hg	-0.10	-0.12	-0.446**
Pb	0.14	-0.11	-0.337**
Cd	0.13	-0.21	-0.278*
As	0.13	-0.17	-0.334**
Cr	0.04	-0.281*	-0.12

**: Correlation is significant at the 0.01 level (2-tailed); *: Correlation is significant at the 0.05 level (2-tailed) except arsenic (As), where its residual values exceeded the permissible limits. Bivalve mollusks concentrate arsenic in seawater, but it exists in the organic forms (mainly arsenobetaine and arsenocholine, also referred to as "fish arsenic") are generally considered to be nontoxic and are excreted in urine within 48 hours of ingestion which have not been shown to produce adverse effects in humans consuming these seafoods and is also rapidly excreted (ATSDR, 2013). Aquatic ecosystems are exposed to arsenic (As) from the mining of non- ferrous metals, the extractions of minerals, the burning of fossil, fuels and waste, other sources include burning municipal and industrial trash, and using wood preservatives. The possible sources of As in the environment could be originated from mineral weathering, arsenic-based pesticides and fertilizers as well as production of paints, dyes and soap.

For Cr is one of the most prevalent and pervasive contaminants in the aquatic environment and its particles reach the aquatic environment through effluents released from the textile, tannery, mining, dyeing, printing, and photography industries. Due to its ease of passage across cellular membranes and subsequent reduction to trivalent form, chromium is regarded as the most hazardous type. Trivalent chromium interacts with various macromolecules, including genetic material exposing the harmful and mutagenic changes (Zuykov *et al.*, 2013; Bakshi and Panigrahi, 2018). According to the reports by WHO (1985) and FEPA (1991) the maximum allowable limit of chromium in fish food is 0.05–0.15 mg/kg body weight. The obtained result was lower than WHO (1985); FEPA (1991) and Bakshi and Panigrahi (2018).

Effect of different treatment on the reduction levels of heavy metals residues

The results obtained in Table 1 and Fig.1 showed that the mean values±SE for Hg, Pb, Cd, As and Cr residues in the second group after immersing in running water treatment were 0.3±0.052, 0.64±0.131, 0.064±0.007, 6.185±0.497 and 0.008±0.005 mg/kg/ww respectively. The mean±SE values for Hg, Pb, Cd, As and Cr residues in the third group Bivalve Mollusks flesh samples after immersing in 5%Acetic acid treatment for 30 minutes were 0.325±0.065, 0.448±0.094, 0.028±0.00, 4.795±0.499 and 0.001±0.0009 mg/kg/ww respectively. The mean±SE values for Hg, Pb, Cd, As and Cr residues in examined Bivalve Mollusks flesh sample after Boiling in 5% solution of acetic acid group for 15 minutes were 0.15±0.0047, 0.28±0.0568, 0.02±0.005,4.054±0.593 and 0.004±0.002 mg/kg/ww respectively. The data illustrated also in Table 1, showed that the lowest significant (P <0.01) concentration of Pb and Cr (mg/kg/ww) for normally distributed data which include Pb and Cr as compared to control was detected in treatment with Acetic acid and boiling, and treatment with Acetic acid only, respectively. Additionally, the other treatments showed a trend toward decrease in both Pb and Cr concentrations. Data

Table 5. Average Mean rank Log values of examined Heavy metals residues after different treatments.

Treatments	Hg	Pb	Cd	As	Cr
Running water	17	19.13	10.75	20.25	20
Acetic acid	23.36	23.5	15.5	25.25	24.75
Acetic acid with boiling	30.13	30.88	24.33	28	22.13
Kruskal-Wallis H, degree of freedom= 2 ($\chi^2(2)$)	7.67	5.78	9.98	2.52	1.08
Significant level ≤	0.02	0.05	0.01	0.28	0.58

A Kruskal-Wallis H test showed that there was a statistically significant difference in the effect on heavy metals except As between the different treatments.

Table 6. Mean values of Reduction % of Heavy metals after different treatments.

Treatments	Hg	Pb	Cd	As	Cr
Running water	54.83	26.17	28.17	15.1	59.6
Acetic Acid	56.67	48.17	69.01	15.22	91.92
Acetic Acid with boiling	80	67.57	77.46	44.35	75.76

Table 7. According to the standard Permissible limits (ES 2010).

HM	Samples within MPL		Samples exceed MPL	
Hg (0.50mg/kg)	No	%	No	%
Pb (1.5 mg/kg)	0	0	64	100
Cd (1 mg/kg)	64	100	0	0
AS (0.50mg/kg)	64	100	0	0
Cr (0.05–0.15 mg/kg)	0	0	64	100
	64	100	0	0

Table 8. Risk assessment parameters values of examined heavy metals.

HM	EDI	TR*	THQ**	HI**
Hg	5.20E-01	--***	1.11E+03	
Pb	5.30E-01	2.03E-01	7.45E-02	
Cd	6.00E-01	2.61E+01	3.00E-02	1.78E+01
As	5.05E+00	5.65E+01	1.73E+02	
Cr	8.00E-03	8.50E-02	2.00E-02	

*If TR > 1x10-4 it indicates a carcinogenic risk. 1 × 10-6 < TR< 1 × 10-4, it indicates an acceptable level of carcinogenic risk, and TR < 1 × 10-6, it indicates a negligible carcinogenic risk.
**value of THQ or HI .When HR and /or HI of >1 indicates a potential risk to human health, whereas a result of ≤1 indicates no adverse health risk effects.
*** Not consider creating cancer.

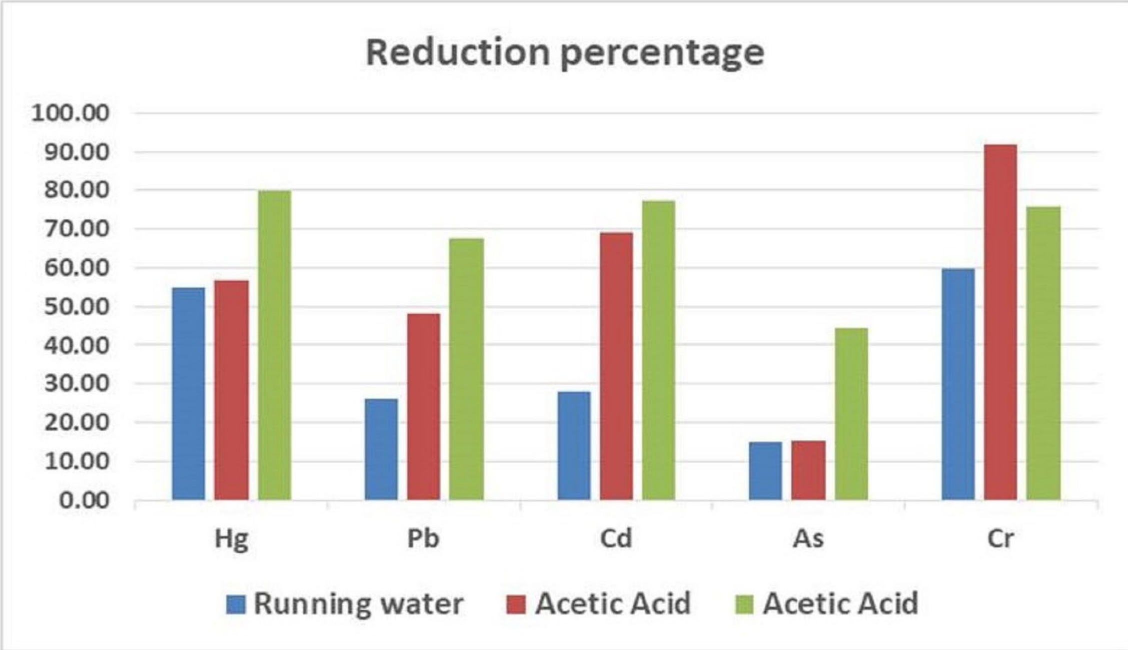

Fig. 1. Average of heavy metals reduction values among the different treatments.

presented in Table 2, showed that Kruskal-Wallis H test revealed a significant difference between the different treatments in the mean rank concentration of Hg, Cd and As χ^2 (3) = 29, 81, 23.763 and 13.104 (P < 0.0001, 0.0001 and 0.004), respectively and they were ranked in a descending order as control> running water > acetic acid > acetic acid with boiling. The results given in Table 3, a Kruskal-Wallis H test revealed that the different treatments significantly reduced Hg, Pb, and Cd levels, which followed the same pattern of rank: acetic acid with boiling > acetic acid > running water. On the other hand, a non-significant mean rank was identified between the different treatments in the reduction of As, as well as Cr heavy metals. The data in Table 4, indicated the overall mean reduction percentages in the heavy metals' residues by the various treatments. According to the results, acetic acid with boiling had the highest reduction effect on Hg, Pb, Cd, and As, followed by acetic acid and finally running water, while Cr was efficiently removed by using Acetic acid, followed by Acetic acid with boiling and then running water. The results given in Tables 4,5 and 6, showed that, when compared to the other treatments, using running water for heavy metal reduction had the least effect, Acetic acid with boiling, on the other hand, had a strong heavy metals reduction impact, as evidenced by a significant negative correlation (P<0.05) with Cd (r = -0.278) and a highly significant negative correlation (P<0.01) with Hg, Pb, and As (r = -0.446, -0.337, and -0.334, respectively). The primary impact of acetic acid treatment was noticed on Cr, which was significantly negative correlated (P<0.05), these result could be supported by the significant negative correlation between acetic acid with boiling and reduction and removal impact of applying acetic acid was on Cr, they had an inverse relationship. The heights reduction % of heavy metals residual levels were recorded after treatment in boiling 5% acetic acid treatment with the following reduction percentage; Hg (80%), Pb (67.6%), Cd (77.5%), As (44.35%) and Cr (75.76%).

Health risk assessment of heavy metals

The results obtained as given in Table 8 revealed that THQ for Hg, Pb, Cd , As and Cr were1.11E+03 , 7.45E-02, 3.00E-02, 1.73E+02 and 2.00E-02 ,respectively while the Hi value was 1.78E+01.The TR assessment were 2.03E-01, 2.61E+01, 5.65E+01 and 8.50E-02 for Pb, Cd , As and Cr respectively. The TR for Hg not recorded to be carcinogenic (ATSDR, 2013). Noncancer risk of heavy metals for evaluation of Hg, Pb, Cd, As and Cr residues in Bivalve Mollusks and their potential hazards consumers was evaluated. The risk assessment followed the guidelines recommended by the United States Environmental Protection Agency (USEPA, 1989). For noncarcinogenic effects, the EDI was compared with the recommended reference doses (RFD) (4E03, 1E03, 3E04, 3E03 and 5E04 mg/kg/d for Pb, Cd, As, Hg, respectively (USEPA, 2010). The results obtained revealed that the HQ and HI values were exceeded 1 which indicates a potential risk to human health and there is a risk that non-carcinogenic effects will occur with a probability tending to rise as HQ and HI rise (USEPA, 2019). USEPA (2011) states that 10 -6 (1 in 1,000,000) to 10 -4 (1 in 10,000) indicate a range of allowable estimated lifetime carcinogen risks. Heavy metals with risk factors less than 10-6 may be excluded from further evaluation. The risk involved with a target metal's carcinogenic effect is given as the increased probability of contracting cancer over a 70-year lifetime (USEPA, 1989; NYSDOH, 2007). The obtained TR was > 1x10-4 which indicates a carcinogenic risk to the local consumers and will face high chronic risk if they consume Bivalve Mollusks (Callista Florida species) on regular basis in their diet.

CONCLUSION

It could be concluded that the Bivalve Mollusks which collected from Fiayom province constitute a public health problem due to contamination with the five estimated heavy metals due to risk involved with a target metal's carcinogenic effect is given as the increased probability of contracting cancer. Regarding the effect of the different treatments used in reduction of heavy metals, we highly recommend that to reduce the daily consumption of Bivalve Mollusks and advice consumers with washing it with running water followed by efficient cooking with 5% Acetic acids.

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CONFLICT OF INTEREST

The authors declare that they have no financial and personal relationships with other people or organizations that could influence their work.

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