Journal of Agricultural Sciences (Tarim Bilimleri Dergisi) 2024, 30 (3) : 546 – 561 DOI: 10.15832/ankutbd.1374919

Journal of Agricultural Sciences
(Tarim Bilimleri Dergisi)

J Agr Sci-Tarim Bili
e-ISSN: 2148-9297
jas.ankara.edu.tr

JAS

Health Risk Assessment of Metals via Consumption of Rapa Whelk (*Rapana venosa*) from the Black Sea

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ARTICLE INFO
Research Article
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Received: 12 October 2023 / Revised: 02 January 2024 / Accepted: 25 January 2024 / Online: 23 July 2024

Cite this article
Bayrakli B, Yigit M, Altuntas M, Maita M (2024). Health Risk Assessment of Metals via Consumption of Rapa Whelk (*Rapana venosa*) from the Black Sea. *Journal of Agricultural Sciences (Tarim Bilimleri Dergisi),* 30(3):546-561. DOI: 10.15832/ankutbd.1374919

ABSTRACT

The present study investigated the bioaccumulation of metals in raw, heat treated -and sterilized Rapa whelk, and evaluated the consumer risks for human consumption. All of the metals, with the exception of Mn, were found to be lower than the permissible FAO standards. A remarkable amount of metal was released into the boiling water (Al, Cr, Cu, Fe, Hg, Pb, Sb, Se, Zn) after heat treatment and hypochlorite solution (Al, As, Cu, Hg, Mn, Pb, Se, Zn). After sterilization, the levels for As, Mo, Cd, Sb, Cr, Zn, Se, Cu, and Hg in Rapa whelk were reduced by 47.4%, 40.1%, 24.9%, 20.3%, 17.5%, 4.5%, 3.6%, 0.93%, and 0.68%, respectively. The metals in Rapa whelk exposed to hypochlorite immersion were found to be below permissible upper limits. The target hazard quotients for the non-carcinogenic risks of consuming sterilized Rapa whelk were below "1" (THQ<1), showing "no potential health risks" for adult men, women and children when consuming sterilized Rapa whelk. Indeed, Rapa whelk could be a good source of Cr, Cu, Fe, Mn, Mo, Se, and Zn to meet the daily recommended quantities in food, when consumed regularly. However, the cancer risks of As, Cd, Ni, and Pb proved to be over "acceptable levels"; hence, the safe consumption limits determined in this study are advisable when consuming Rapa whelk.

Keywords: Cancer risk, Hazard index, *Rapana venosa*, Toxic metals, Trace elements

1. Introduction

The precise line between whether a metal should be considered beneficial or harmful is dependent on dose limits and exposure frequencies (Alkan et al. 2016; Leonard et al. 2022; Yildiz et al. 2023). Trace elements such as copper, manganese, zinc, iron are essential for the metabolic activities in humans and other living organisms, which however can be toxic and cause severe damage at high levels of bioaccumulation in tissues (Çağlak & Karsli 2014; Makedonski et al. 2017). Metals such as arsenic, lead, cadmium and mercury with high bioaccumulation potentials in aquatic animals are certainly toxic (Duyar et al. 2023; Mol et al. 2019), with possible impacts on central nervous system functions, damage on blood composition, kidneys, lungs, and liver with severely reduced energy levels (Hajeb et al. 2014).

The sea snail "Rapa whelk" is a good bioindicator for metals, and its bioaccumulation is highly dependent on the level of metal pollution in the ecosystem (Liang et al. 2004; Terzi & Civelek 2021), possibly because of their low motion on the sea floor (Hwang et al. 2017). Despite being a seafood with notable health benefits, when Rapa whelk is consumed at high intake levels it can be seriously detrimental to health (Stancheva et al. 2012). Therefore, monitoring demersal marine species for possible environmental contaminations (Hwang et al. 2017) is crucial for any signs of toxic contamination and to ensure seafood safety. Since raw meat of Rapa whelk spoils quickly under inappropriate temperature conditions, heat treatment and sterilization are common applications prior to cold storage and marketing (Bayrakli et al. 2016). inconvenient conditions, heat treatment and sterilization are common preprocesses in Rapa whelk marketing prior to cold storage (Bayrakli et al. 2016). Joyce and Bo highlighted that cooking methods such as boiling, steaming, and frying can alter the toxicity level of metals in meat through various mechanisms, including the evaporation of water and volatile components, solubilization of the element, and metal binding to other macronutrients present in the food (Joyce & Bo 2016).

This study aimed to investigate consumer risks for adult men, women and children via consumption of Rapa whelk exposed to heat treatment and sterilization, and to evaluate possible reduction levels of metals in Rapa whelk through boiling and hypochlorite soaking.

2. Material and Methods

2.1. Study area and sampling

The Rapa whelk samples were collected from the nature from the southern coast of the Black Sea (Türkiye; 41°44´18.87"N, 35°55´09.69"E) (Figure 1) and immediately transferred to the commercial processing facilities (Sadiklar Seafood Company) in Dikmen-Sinop, Türkiye.

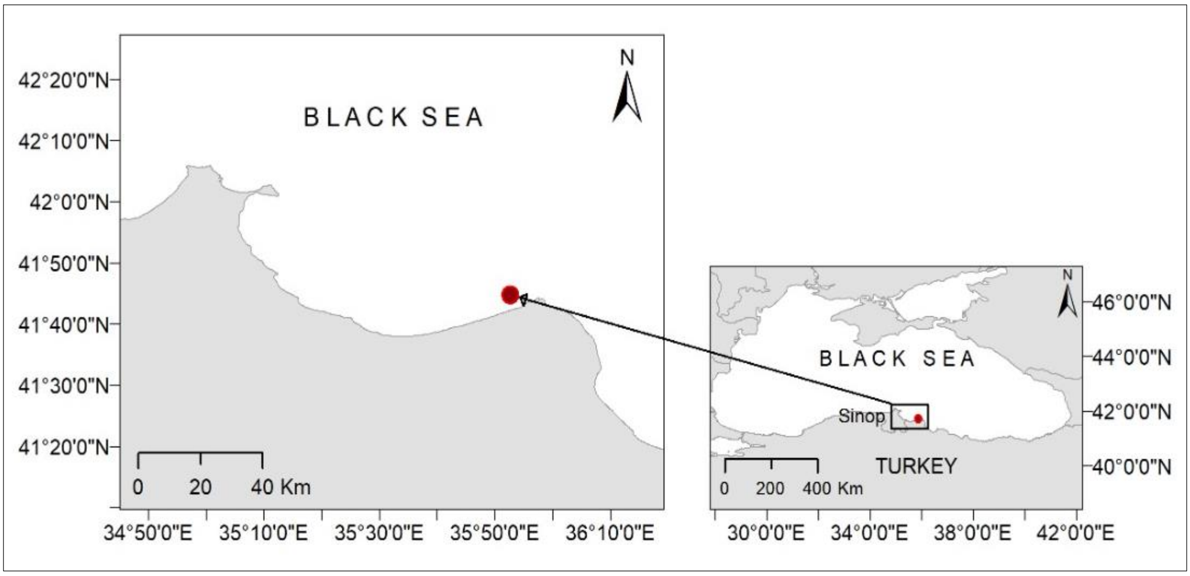

Figure 1- Study area, southern coast of the Black Sea

In total, 40 individuals of Rapa whelk were randomly selected from the harvest batch, and the shells of 20 individuals were carefully cracked using a hammer to obtain the raw meat with the precaution of preventing metal contamination. This group served as an untreated control group with no further processing. Wet weight of raw Rapa whelk was measured in a range between 13.90-38.02 g (mean weight 22.10 ± 7.88 g). The other group of 20 individuals was consecutively exposed to heat treatment and sterilization. Initially, the Rapa whelk was kept in an industrial boiler (2-ton) at 130 °C for 5 minutes; afterwards, the meat was separated from the shells using a fork. It is important to note that metal contamination was disregarded during this fork separation process. The operculum and intestines were removed, and the edible parts were pressure-washed. After heat treatment via boiling, the meat was sterilized in tubs containing 1% sodium hypochlorite (NaOCl) solution for 30 minutes, and then homogenized with a blender, shocked in plastic bags at -80 ℃ and cold-stored at -20 ℃ for further analyzes. Metal analyses in raw, heat treated, -and sterilized Rapa whelk were performed in order to evaluate a possible reduction of the metals in the meat. Additionally, the possible transfer of metals from the meat into the boiling water (heat treatment phase) or hypochlorite solution (sterilization phase) were also evaluated prior to -and after the treatments. Thereafter, all samples were transferred to the laboratories of the Department of Fisheries, Vocational School at Sinop University (Türkiye) under cold-chain within three hours of transport time.

2.2. Analyses of metal contents

All metal analyses in the present study were performed in triplicates following the EPA Method 200.3. Samples weighing 1.5 g were digested in Teflon vessels including a mixture of concentrated supra pure grade HNO_3 and H_2O_2 (7:1) according to (HPR-FO-67) temperature and pressure profile using a microwave digestion system (Milestone SK10). After adding the acid, Teflon bombs were closed and heated at 200 ºC for 15 minutes and kept at same temperature for another 15 minutes. The digested solution was transferred into 50 mL polypropylene falcon tubes and made up to 50 mL by adding pure water. Standard Reference Material (CRM) and blank solutions were prepared using the same procedures. The CRM materials UME CRM 1201 and SEM 2016 mix were used to evaluate the precision and accuracy of the analyses. Inductively Coupled Plasma Spectrometry (ICP-MS Agilent 7700X) was used to measure the concentration for Arsenic (As), Aluminum (Al), Copper (Cu), Iran (Fe), Manganese (Mn), Mercury (Hg), Cadmium (Cd), Lead (Pb), Zinc (Zn), Selenium (Se), Chromium (Cr), Nickel (Ni), Molybdenum (Mo), Sb (Antimony) and Cobalt (Co) through multi-element techniques. During these experiments, all glassware and Teflon bombs were soaked overnight in 10% HNO_3, rinsed twice with distilled water and air dried to avoid contamination before use.

2.3. Estimations of consumer health risks

The health risks from metals via oral intake have been assessed as non-carcinogenic -and carcinogenic risks for adult men (AM, 70 kg, aged 19-30), adult women (AW, 57 kg, aged 19-30), and children (C, 36 kg, aged 9-13), according to reference classification by IOM (2006). The total mollusc consumption in Europe is around 1.7 kg/year per capita (4.66 g/day per person), that is nearly 7% of total seafood consumption of 23 kg per person in Europe (Failler et al. 2007). The estimated daily intake (EDI, mg/kg body weight/day) of metals was calculated by the formula according to Yigit et al. (2018a b), and Bayrakli (2021; Duyar et al. 2023).

$$\mathrm{EDI} = \frac{\mathrm{MC\ x\ MS}}{\mathrm{BW}}$$

Where; MC, metal concentration (mg kg^{-1} ww) in meat; MS, meal size (kg/day), average daily intake of molluscs in the EU; BW, body weight (kg) for adults (men 70 kg, women 57 kg), or children (37 kg)

2.4. Non-carcinogenic risk

Non-carcinogenic risks were evaluated through a comparison of the exposure level with a reference dose suggested for the same exposure duration. Target hazard quotient (THQ), representing non-carcinogenic risk, is the ratio of estimated daily intake (EDI) and reference dose (RfD), an assumption of human exposure below which that substance is unlikely to pose measurable health risks. If the exposure level exceeds this threshold, there may be potential noncancerous health effects. Risk evaluation for As has been performed with the assumption that the toxic inorganic arsenic was 3% of the total (FSA 2004). The THQ caused by a single element within a single exposure route over a lifetime duration of 70 years has been estimated through the following equation(US-EPA 1989):

$$THQ = \frac{\mathrm{EDI}}{\mathrm{RfD}}\ x\ 10^{-3}$$

Where; EDI, estimated daily intake (mg/kg body weight/day); RfD, reference doses (mg kg−1 day−1) for metals (EFSA, 2010; US-EPA, 2013)

There is no potential harmful effect when THQ is below “1” (THQ<1) (US-EPA, 1989), but a cumulative reaction of health effects may occur when the value is over “1” (THQ>1) (Hallenbeck 1993). For this reason, the arithmetic sum of each THQ is the total target hazard quotients (TTHQ), represented as hazard index (HI). When the hazard index exceeds unity, there may be potential health risks. For the evaluation of potential risks from multiple metals, noncancerous hazard index (HI) was estimated using the equation below (US-EPA 1989):

$$\mathrm{HI\ (TTHQ\ \Sigma n)} = (\mathrm{EDI}_1/\mathrm{RfD}_1) + (\mathrm{EDI}_2/\mathrm{RfD}_2) + (\mathrm{EDI}_3/\mathrm{RfD}_3) + \dots\dots\dots + (\mathrm{EDI}_n/\mathrm{RfD}_n)$$
$$\mathrm{HI} = \Sigma_n\ \mathrm{THQ}_m$$

Where; EDIn, estimated intake level for the nth metal, n=15 in the present study.

2.5. Carcinogenic risks for toxic metals

The carcinogenic risk defines the incremental probability of any kind of cancer in the lifetime of an individual as a result of exposure to potential carcinogens. The cancer slope factor (CSF) converts EDI averaged over a lifetime of exposure directly to the incremental risk of an individual developing cancer. Among the measured metals, As, Cd, Ni, Pb are known to potentially cause cancer in humans (IARC 2012). The lifetime cancer risk (CRR) was estimated using the equation below (US-EPA 1989; 2010):

$$CRR = EDI\ x\ CSF$$

Where; EDI, estimated daily intake (mg/kg/day); CSF, cancer slope factor (As: 1.5mg/kg/day, Cd: 6.3mg/kg/day, Ni: 0.84mg/kg/day, Pb: 0.0085mg/kg/day) (Liang et al. 2017).

Despite the fact that methylmercury (MeHg) is classified as possibly carcinogenic to humans (IARC, 2012), the CSF for Hg has not been issued by the US-EPA; therefore, the cancer risks for Hg were not estimated here. The level of cancer risk for carcinogens ranges between 10^{-4} and 10^{-6}, that is a lifetime cancer risk level of 1/10.000, and 1/1.000.000, respectively. This means that a risk factor lower than 10^{-6} is acceptable and can be ignored, but a risk factor exceeding 10^{-4} is considered to be unacceptable. Hence, an average cancer benchmark of 10^{-5} was considered as the threshold in this study (US-EPA 2010).

2.6. Safe consumption limits

The safe daily consumption limit (SDC) was calculated using the following formulae (US-EPA 2000):

$$SDC = \frac{\mathrm{RfD\ x\ BW}}{\mathrm{MC}}$$

Where; RfD, reference doses (mg kg^{-1} day^{-1}) for metals (EFSA 2010); BW, body weight (kg) for adults (men 70 kg, women 57 kg), or children (37 kg); MC, metal concentration (mg kg^{-1} ww) in meat

The safe weekly consumption rate (SWC, meals per week) was calculated by converting the SDC into MS, that is 0.227 kg/day for adult men and women, and 0.114 kg/day for children (US-EPA 1989, 2000). The safe weekly consumption (SWC, meals per week) was calculated according to the equation reported by (Yigit et al. 2018a):

$$SWC = \frac{\mathrm{SDC\ \ x\ 7}}{\mathrm{MS}}$$

Where; SDC, safe daily consumption rate (US-EPA 2000); MC, analyzed metal concentration (mg kg^{-1} ww) in meat

2.7. Compensation of daily requirements for essential trace elements

The percent compensation of minimum daily requirements (CDR_{min}) for essential trace elements in mollusc consumption was calculated using the following formulae according to Yiğit et al. (2020):

$$CDRmin = \frac{EDI\ \ x\,100}{EAR}$$

Where; EDI, estimated daily intake (mg/kg/day); EAR, estimated average daily requirement for a healthy human (IOM 2006).

2.8. Statistical analysis

Statistical analyses were conducted using metal analyses data from Rapa whelk samples during the pre-processing treatment prior to cold-storage. The statistical significance of the data was evaluated by ANOVA (Analysis of Variance). The Tukey comparison test was used to determine the differences between the experimental groups. The statistical analyzes were performed using the PAST computer program - 1.95 version (Hammer et al. 2001). The results were accepted as significantly different at P<0.05 level. Linear relationships between the metal elements were appraised using Pearson's rank correlation test. As a result of this evaluation, a Principal Component Analysis (PCA), which allows for dimension reduction in the data set, was applied in case of a high degree of correlation between the metal elements. The PCA was performed using 2 components for the examination of percentages of variance explanations. Standardized loadings based upon the correlation matrix for the PCA are presented in Table 1. Rv4.0.5 statistical software was used in the PCA analyses, and again the level of significance was considered as P<0.05.

Table 1- Standardized loadings based upon correlation matrix for Principal Component Analysis

Al	0.94	0.28	0.97	0.0285	1.2
Cr	–0.55	0.79	0.93	0.0715	1.8
Mn	0.96	0.22	0.98	0.0220	1.1
Fe	1.00	0.03	0.99	0.0079	1.0
Co	0.91	–0.19	0.87	0.1337	1.1
Ni	0.91	0.38	0.98	0.0176	1.3
Cu	0.46	0.78	0.82	0.1769	1.6
Zn	0.10	0.88	0.78	0.2218	1.0
As	–0.47	0.81	0.88	0.1210	1.6
Se	–0.26	–0.44	0.26	0.7386	1.6
Mo	–0.23	0.89	0.85	0.1548	1.1
Cd	–0.49	0.58	0.58	0.4233	1.9
Sb	0.67	0.37	0.59	0.4105	1.6
Hg	0.92	0.07	0.86	0.1412	1.0
Pb	0.64	–0.42	0.59	0.4075	1.7

PC: Principal Component

3. Results

3.1. Statistical evaluation

The Pearson correlation test results (Figure 2) and the correlogram (Figure 3) indicated strong positive significant correlations between Cu-Zn, Cd-As, As-Mo, Hg-Al, Hg-Fe, Mn-Fe, Mn-Al, Mn-Ni, Fe-Al, Fe-Ni, and Al-Ni. Moreover, strong negative significant correlations were found between Cr-Co, Zn-Se and Cu-Se (Figure 2, Figure 3). There were no relations between Cu-Cd, Zn-Hg, As-Se, Mo-Hg, Se-Hg, and Se-Co (Figure 3).

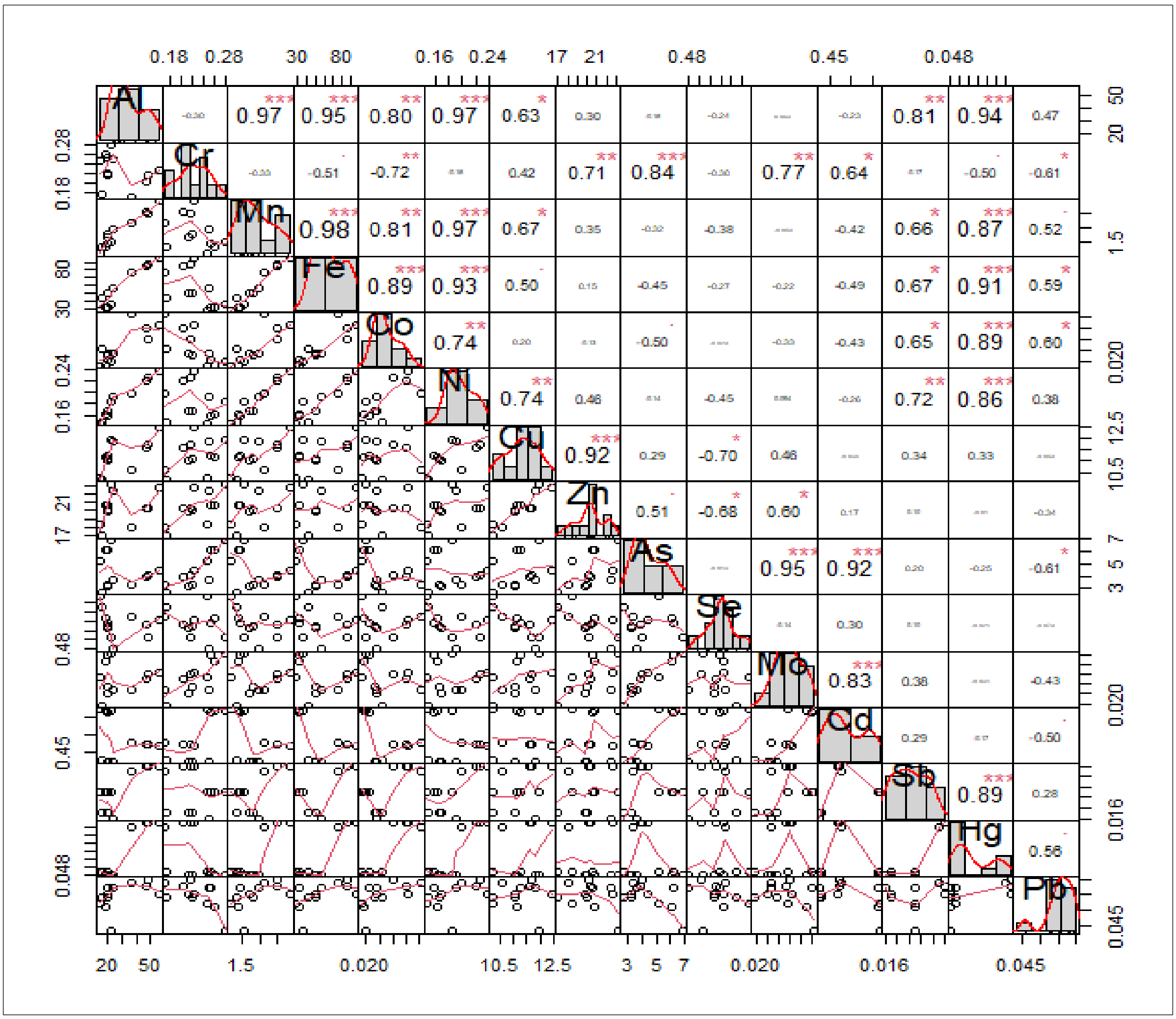

Figure 2- Pearson correlation results (top diagram) with bivariate scatter (bottom diagram) and density (diagram) plots (Each significance level is associated to a symbol: p-values (0, 0.001, 0.01, 0.05, 0.1, 1) <=> symbols (“*”, “**”, “*”, “.”, " “)**

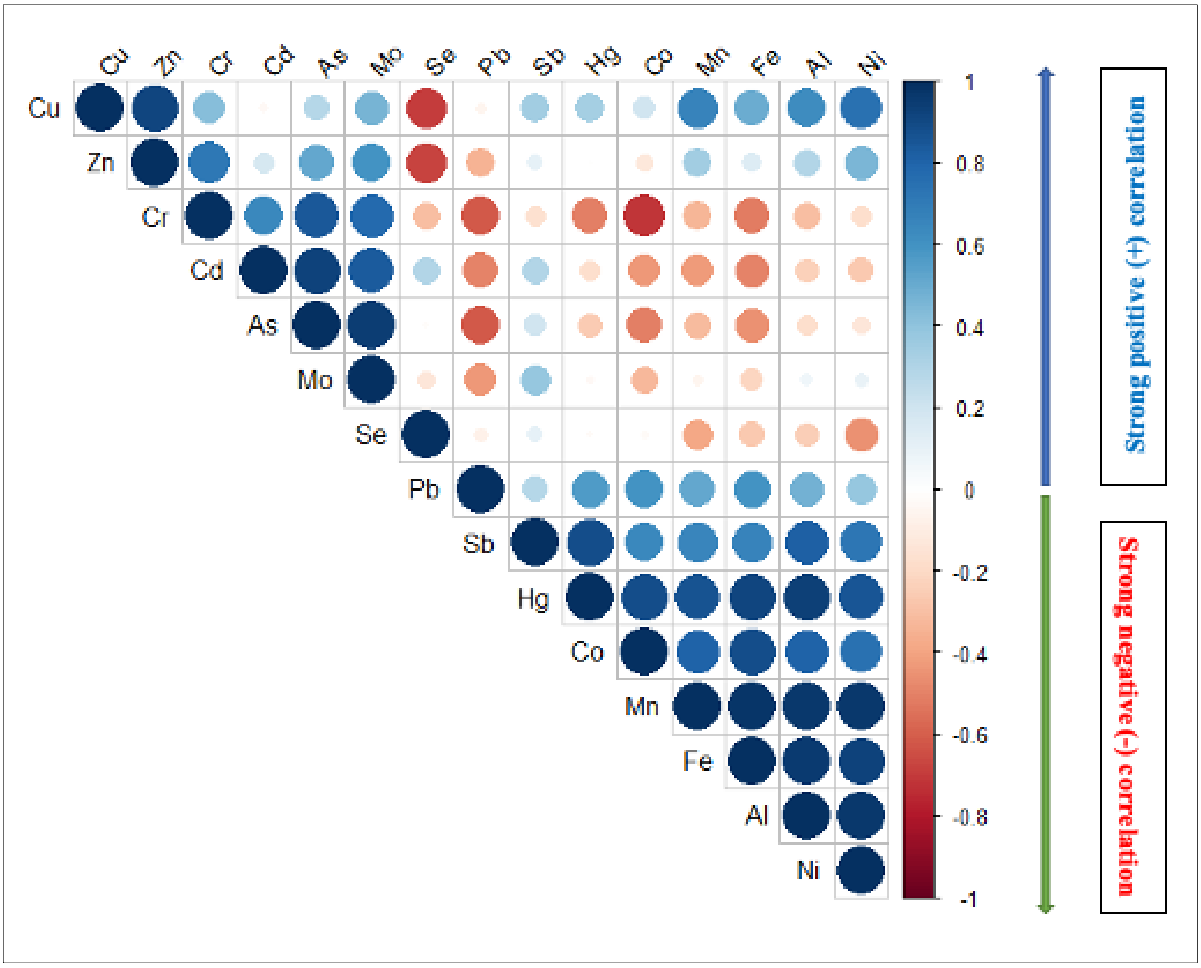

Figure 3- Correlogram of Pearson correlation results for each metal elements

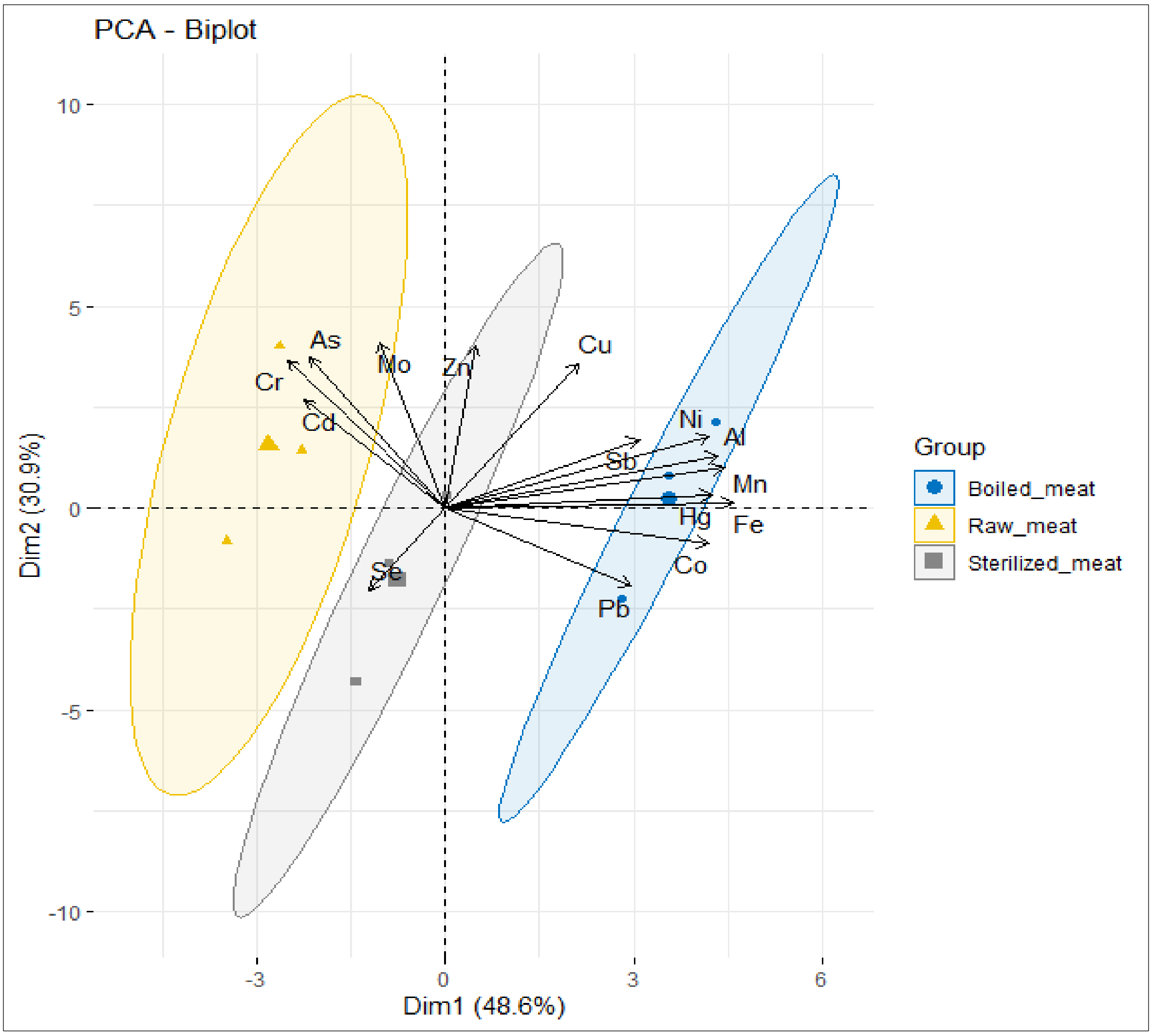

Figure 4- Biplot for principal component analysis of metal element levels represented

The PCA analyses performed between the metal elements showed that the size of the data could be represented by two components. The PC-1 and PC-2 principal components explained 49% and 31% (80% of total) of the total variation in the data, respectively (Table 2). The metals Al, Mn, Fe, Co, Ni and Hg were positively and strongly explained by PC1, while Cr, Cu, Zn, As and Mo were positively and strongly explained by PC2. Mo and Se were poorly explained as negatively by PC1. Co, Se and Pb were negatively and poorly explained by PC2. Fe and Hg are the weakest metals explained by PC2 compared to the other metals. Se was negatively and poorly explained by both the PC1 and PC2 components. It was observed that the proportion of common variance (community) was high for all metals except Se, and it was revealed that there was a high level of correlation between metals with a common variance close to "1" (one) and other metals (Table 1). The biplot of the principal components were plotted based on raw, heat treated, -and sterilized meat groups. The levels of As, Cd, Cr and Mo in raw meat were higher than the other metals. Concentrations of Al, Co, Fe, Hg, Mn, Ni, Pb and Sb were quite high compared to other metals in heat treated meat. Additionally, there was a high degree of correlation between these metals and the variation in metals was explained by PC1. Concentrations of Cu, Se and Zn were high in the sterilized meat group. Se is negatively correlated with Cu and Zn. The variation in Se cannot be also fully explained by both major components compared to variations among other metals (Figure 4).

Table 2- Accounted variance for each principal component (PC)

Variations	*PC-1*	*PC-2*
SS loadings	7.30	4.63
Proportion Variance	0.49	0.31
Cumulative Variance	0.49	**0.80**
Proportion Explained	0.61	0.39
Cumulative Proportion	0.61	1.00

3.2. Concentrations of metal in rapa whelk versus international standards

Metal concentrations in raw, heat treated, -and sterilized Rapa whelk are shown in Table 3. Remarkable variations have been observed in heavy metal levels among groups at different processing stages. Considering all the metal levels in raw Rapa whelk meat collected from the same marine area, average metal levels decreased in the order of Fe>Zn>Al>Cu>As>Mn>Cd>Se>Cr>Ni>Pb>Hg>Mo>Sb>Co; whereas metal levels in the heat treated and sterilized meat showed a declining order as Fe>Al>Zn>Cu>As>Mn>Se>Cd>Ni>Cr>Hg>Pb>Co>Mo>Sb, and Fe>Al>Zn>Cu>As>Mn>Se>Cd>Cr>Ni>Pb>Hg>Mo>Co>Sb, respectively. All average values (mg/kg wet weight basis) of the elements analyzed in the Rapa whelk meat, with the exception of Mn, were lower than the permissible FAO standard's upper limits in marine products for healthy food (FAO 1983) (Table 3).

Table 3- Metal contents and data on metal reduction in Rapa whelk meat after heat treatment (boiling) and sterilization (hypochlorite immersion) in comparison to permissible upper limits of FAO standards. Metals with no reduction but increment after sterilization are grey highlighted

Elements	***Permissible limit (mg/kg ww)***	***Metal contents in Rapa whelk (mg/kg)*** *means ± SD, ww (wet weight) basis (min – max range in parenthesis)*			***Metal Reduction***			
					after heat treatment *(boiling)*		***after sterilization*** *(hypochlorite immersion)*	
	FAO (1983)	***Raw meat***	***Boiled meat***	***Sterilized meat***	***weight (mg/kg)***	***percent (%)***	***weight (mg/kg)***	***Percent (%)***
Al	NA	18.65 ± 3.27^{a} (14.16 - 21.86)	46.71 ± 8.32^{b} (35.98 - 56.26)	20.08 ± 3.52^{a} (15.66 - 24.27)	*-28.06*	*-150,48*	*-1,43*	*-7,67*
As	10.0	6.10 ± 0.69^{b} (5.24 - 6.92)	3.94 ± 0.45^{a} (3.34 - 4.44)	3.21 ± 0.41^{a} (2.67 - 3.66)	2.17	35.53	2.89	47.44
Cd	1.0	0.566 ± 0.003^{c} (0.563 - 0.569)	0.472 ± 0.004^{b} (0.468 - 0.479)	0.425 ± 0.003^{a} (0.423 - 0.429)	0.09	16.62	0.14	24.86
Co	0.26	0.019 ± 0.001^{a} (0.018 - 0.021)	0.035 ± 0.004^{b} (0.030 - 0.041)	0.024 ± 0.001^{a} (0.023 - 0.026)	*-0,02*	*-81,90*	*-0,01*	*-25,47*
Cr	1.0	0.261 ± 0.014^{a} (0.247 - 0.281)	0.203 ± 0.024^{b} (0.170 - 0.223)	0.215 ± 0.031^{ab} (0.175 - 0.251)	0.06	22.01	0.05	17.50
Cu	30	11.15 ± 0.69^{a} (10.27 - 11.94)	11.58 ± 0.76^{a} (10.60 - 12.46)	11.05 ± 0.67^{a} (10.21 - 11.84)	-0.43	-3.86	0.10	0.93
Fe	100	32.44 ± 3.35^{a} (28.33- 36.54)	85.91 ± 7.76^{c} (76.04 - 95.50)	49.98 ± 5.23^{b} (43.37 - 56.16)	*-53,47*	*-164,80*	*-17,54*	*-54,07*
Hg	1.0	0.048 ± 0.0004^{a} (0.048 - 0.049)	0.061 ± 0.0003^{b} (0.060 - 0.061)	0.048 ± 0.001^{a} (0.048 - 0.049)	-0.01	-25.56	0.0003	0.68
Mn	1.0	1.38 ± 0.18^{a} (1.14 - 1.55)	2.52 ± 0.31^{b} (2.14 - 2.89)	1.69 ± 0.26^{a} (1.34 - 1.96)	*-1,14*	*-82,16*	*-0,31*	*-22,67*
Mo	NA	0.042 ± 0.005^{b} (0.035 - 0.046)	0.033 ± 0.004^{b} (0.027 - 0.036)	0.025 ± 0.005^{a} (0.019 - 0.029)	0.01	21.52	0.02	40.09
Ni	80	0.163 ± 0.016^{a} (0.147 - 0.184)	0.220 ± 0.016^{b} (0.198 - 0.237)	0.169 ± 0.018^{a} (0.149 - 0.192)	*-0,06*	*-34,97*	*-0,01*	*-3,76*
Pb	2.0	0.051 ± 0.0061^{a} (0.043 - 0.057)	0.058 ± 0.002^{a} (0.055 - 0.060)	0.055 ± 0.001^{a} (0.054 - 0.057)	*-0,01*	*-14,45*	*-0,01*	*-8,94*
Sb	1.0	0.021 ± 0.0001^{a} (0.0207 - 0.021)	0.026 ± 0.0006^{b} (0.025 - 0.026)	0.017 ± 0.0002^{a} (0.016 - 0.017)	-0.01	-23.09	0.004	20.27
Se	2.0	0.546 ± 0.037^{a} (0.506 - 0.594)	0.532 ± 0.021^{a} (0.503 - 0.550)	0.526 ± 0.037^{a} (0.479 - 0.571)	0.01	2.46	0.02	3.63
Zn	40	20.73 ± 1.95^{a} (18.43 - 23.20)	20.30 ± 1.93^{a} (17.99 - 22.73)	19.80 ± 2.24^{a} (16.95 - 22.41)	0.43	2.07	0.94	4.52

FAO: Food and Agriculture Organization–UN; NA: not available

Table 5- Estimated daily intake per meal size, safe daily consumption rate (SDC), safe weekly consumption (SWC), target hazard quotient, total target hazard index, and cancer risk rate for the studied metals in adult men (AM), adult women (AW), and children (C) consuming boiled -and sterilized Rapa whelk based on cancer slope factor and reference dose

Element	***RfD*** *(mg/kg/ day)*	***CSF*** *(mg/kg/ day)*	***EDI (mg/kg/day, ww)***			***SDC (kg/day)***			***SWC (meals/week)***			***THQ***			***Cncer Risk (CRR)***		
			AM	***AW***	***C***	***AM***	***AW***	***C***	***AM***	***AW***	***C***	***AM***	***AW***	***C***	***AM***	***AW***	***C***
As	0.0003	1.5	0.00021	0.00026	0.00042	0.22	0.18	0.11	6.73	5.48	6.89	0.021	0.026	0.042	$3.2x10^{-4}$	$3.9x10^{-4}$	$6.2x10^{-4}$
Cd	0.001	6.3	0.000028	0.000035	0.000055	0.16	0.13	0.08	5.07	4.13	5.20	0.028	0.035	0.055	$1.8x10^{-4}$	$2.2x10^{-4}$	$3.5x10^{-4}$
Ni	0.02	0.84	0.000011	0.000014	0.000022	8.27	6.73	4.25	255.0	207.7	261.2	0.0006	0.0007	0.0011	$1.2x10^{-5}$	$1.5x10^{-5}$	$2.3x10^{-5}$
Pb	0.002	0.0085	0.0000037	0.0000045	0.0000071	2.53	2.06	1.30	78.17	63.65	80.05	0.0018	0.0023	0.0036	$3.1x10^{-8}$	$3.8x10^{-8}$	$6.1x10^{-8}$
Al	1.0	N/A	0.0013	0.0016	0.0026	3.49	2.84	1.79	107.5	87.54	110.1	0.0013	0.0016	0.0026	For carcinogenic exposure; no cancer risk if value: > 0.000001 (10^{-6}) cancer risk if value: < 0.000001 (10^{-6}) benchmark: < 0.00001 (10^{-5}) ((US-EPA, 2010)		
Co	0.0003	N/A	0.0000016	0.0000020	0.0000032	0.86	0.70	0.44	26.49	21.57	27.12	0.0054	0.0067	0.0105			
Cr	0.003	N/A	0.000014	0.000018	0.000028	0.98	0.79	0.50	30.10	24.51	30.82	0.0048	0.0059	0.0093			
Cu	0.04	N/A	0.00074	0.00090	0.00143	0.25	0.21	0.13	7.82	6.36	8.00	0.018	0.023	0.036			
Fe	0.7	N/A	0.0033	0.0041	0.0065	0.98	0.80	0.50	30.23	24.62	30.96	0.0048	0.0058	0.0092			
Hg	0.0001	N/A	0.0000032	0.0000039	0.0000062	0.15	0.12	0.07	4.48	3.65	4.59	0.032	0.039	0.062			
Mn	0.14	N/A	0.00011	0.00014	0.00022	5.78	4.71	2.97	178.4	145.2	182.7	0.0008	0.0010	0.0016			
Mo	0,005	N/A	0.0000017	0.0000020	0.0000032	14.0	11.4	7.21	432.1	351.8	442.4	0.0003	0.0004	0.0006			
Sb	0.0004	N/A	0.0000011	0.0000014	0.0000022	1.68	1.37	0.86	51.80	42.18	53.04	0.0028	0.0034	0.0054			
Se	0.005	N/A	0.000035	0.000043	0.000068	0.67	0.54	0.34	20.52	16.71	21.02	0.0070	0.0086	0.0136			
Zn	0.3	N/A	0.0013	0.0016	0.0026	1.06	0.86	0.55	32.71	26.63	33.50	0.0044	0.0054	0.0085			
HAZARD INDEX (HI)												**0.134**	**0.165**	**0.261**			

RfD (mg kg^{-1} day^{-1}): reference dose (EFSA 2010; US-EPA 201); CSF: cancer slope factors for As, Pb (US-EPA 1996), and Cd, Ni (Y. Liang et al. 2017); EDI (mg kg^{-1} day^{-1}): Estimated daily intake per meal size; ww (wet weight) basis; THQ: target hazard quotient; CRR: cancer risk rate; HI: hazard index representing arithmetic sum of THQs

3.3. Concentrations of metal in raw, heat treated -and sterilized meat of Rapa whelk

The levels of As, Cd, Cr, and Mo were highest in the raw meat of Rapa whelk (6.10 ± 0.69, 0.566 ± 0.003, 0.261 ± 0.014, and 0.042 ± 0.005 mg/kg, respectively), compared to those exposed to heat treatment -and sterilization (3.94 ± 0.45, 0.472 ± 0.004, 0.203 ± 0.024, 0.033 ± 0.004, mg/kg, and 3.21 ± 0.41, 0.425 ± 0.003, 0.215 ± 0.031, 0.025 ± 0.005 mg/kg, respectively). The decrease of levels of As, Cd, and Cr after both heat treatment and sterilization were statistically significant ($P<0.05$), but the decline in Mo was significant in the sterilized treatment group ($P<0.05$) compared to the raw meat. Concentrations of Cu, Pb, Se, and Zn did not alter ($P>0.05$) when the Rapa whelk was exposed to heat treatment (11.58 ± 0.76, 0.058 ± 0.002, 0.532 ± 0.021, and 20.30 ± 1.93 mg/kg) or sterilization (11.05 ± 0.67, 0.055 ± 0.001, 0.526 ± 0.037, and 19.80 ± 2.24 mg/kg) and remained similar to the non-treated meat (11.15 ± 0.69, 0.051 ± 0.0061, 0.546 ± 0.037, and 20.73 ± 1.95 mg/kg). In contrast however, elevated levels of Al, Co, Fe, Hg, Mn, Ni, and Sb were recorded in Rapa whelk exposed to heat treatment, which thereafter declined to significant lower levels when further treated with sterilization through hypochlorite immersion ($P<0.05$). Overall, As, Mo, Cd, Sb, Cr, Zn, Se, Cu, and Hg concentrations in the meat reduced by 47.44%, 40.09%, 24.86%, 20.27%, 17.50%, 4.52%, 3.63%, 0.93%, and 0.68%, respectively after the final processing phase of sterilization through hypochlorite immersion (1% NaOCl solution) (Table 3). The mean concentrations of metals found in the boiling water after heat treatment, and in the hypochlorite solution after sterilization, are given in Table 4. Overall, it was evidenced that a remarkable amount of Al, Cr, Cu, Fe, Hg, Pb, Sb, Se, and Zn were released into the boiling water during the heat treatment, and Al, As, Cu, Hg, Mn, Pb, Se, and Zn were concentrated in hypochlorite solution during the sterilization process.

3.4. Non-carcinogenic and carcinogenic consumer risks

The EDI per meal size (mg kg^{-1} day^{-1}), SDC (kg day^{-1}), SWC (meal/week), THQs and HI, and CRR for the studied metals in adults (men and women) and children upon consuming Rapa whelk, along with reference dose and cancer slope factor are presented in Table 5.

3.5. Compensation of minimum daily requirements for essential elements via Rapa whelk consumption

The compensation level of minimum daily requirements for the essential elements through Rapa whelk intake in human being has been given in Table 6. The findings show that 0.041%, 0.105%, 0.055%, 0.005%, 0.005%, 0.078%, and 0.014% of the daily requirements for Cr, Cu, Fe, Mn, Mo, Se, and Zn for adult men could be compensated by eating Rapa whelk, whereas these levels were found to be 0.070%, 0.129%, 0.050%, 0.008%, 0.006, 0.096%, 0.024%, and 0.121%, 0.265%, 0.112%, 0.013%, 0.012%, 0.195%, 0.037%, for adult women and children, respectively.

Table 6- Percent compensation of minimum daily requirements (CDRmin) for essential trace elements in adult men (AM), adult women (AW), and children (C) via consuming sterilized Rapa whelk, based on estimated daily average requirement levels according to IOM (2006)

Element	*EAR (mg/day, person ww)*			*EDI (mg/kg/day, ww)*			*CDR_{min} (%)*		
	AM	*AW*	*C*	*AM*	*AW*	*C*	*AM*	*AW*	*C*
Cr	0.035	0.025	0.023	0.000014	0.000018	0.000028	0.041	0.070	0.121
Cu	0.70	0.70	0.54	0.00074	0.00090	0.00143	0.105	0.129	0.265
Fe	6.00	8.10	5.80	0.0033	0.0041	0.0065	0.055	0.050	0.112
Mn	2.30	1.80	1.75	0.00011	0.00014	0.00022	0.005	0.008	0.013
Mo	0.034	0.034	0.026	0.0000017	0.0000020	0.0000032	0.005	0.006	0.012
Se	0.045	0.045	0.035	0.000035	0.000043	0.000068	0.078	0.096	0.195
Zn	9.40	6.80	7.00	0.0013	0.0016	0.0026	0.014	0.024	0.037

EAR: estimated average daily requirement (mg/day) for a healthy human; ww (wet weight) basis; EDI (mg kg^{-1} day^{-1}): Estimated daily intake per meal size; ww (wet weight) basis; CDR_{min} (%): compensation of minimum daily requirement calculated according to Yigit et al.(2020).

4. Discussion

Both heat treatment and sterilization directly affected the metal levels in Rapa whelk. The levels of As, Cd, Cr, Mo, Se, and Zn reduced by 35.53%, 16.62%, 22.01%, 21.52%, 2.46%, and 2.07%, respectively in heat treated meat compared to the raw Rapa whelk, which is in line with the findings of Laparra et al. (2004). The reduction of metal levels in seafood after boiling can be attributed to solubilization in boiling water, as a result of broken chain between As and the food molecules as suggested by Hajeb et al. (2014). Results of the present study are also in close agreement with Ersoy (2011) and Devesa et al. (2001), who presented considerable reduction of Cr and Ni in fish exposed to microwave cooking. This was in line with Jorhem et al. (1994), who underlined severe decline in Cd, Ni, Co levels in crayfish after heat treatment. Further support was found in a study by and Atta et al. (1997), with a reduction in Cd, Cu, Pb, Zn levels in Tilapia niloticus following heat treatment. Perelló et al. (2008) reported decreased levels of Hg and As in hake fish after heat treatment, which supported our results in this study for As, but Hg showed a decline after further treatment of sterilization. The reduction of some metals in cooked food might be attributed to the solubilization of metals from the soft tissue into the boiling water, as was earlier reported for the As solubilization from rice to water (Sengupta et al., 2006). In contrast, however, increased level of As in fish and molluscs after heat treatment was reported by Devesa et al. (2001). It seems there are inconsistencies among different reports regarding the effects of heat treatment on metals in seafood. This was the case for Al, Co, Fe, Hg, Mn, Ni, and Sb, which increased by heat treatment; however, As, Cd, Cr, Mo, Se, and Zn levels in heat treated Rapa whelk meat in the present study reduced remarkably. It has been reported that several factors could play important roles in the cooking process (Houlbrèque et al. 2011), such as time, temperature, and cooking medium (Hajeb et al. 2014). Juniawanti (2020) found that the duration of treatment may play a critical role. There 2020 study reported a 12%, 76%, and 83% reduction for Pb in Kupang shellfish after heat treatment for 15, 20, and 45 minutes, and over 83% after 60 minutes of boiling. In the present study, the initial preparations of samples prior to the analyses were conducted at the facilities of a commercial seafood processing company, where stainless steel metal benches and forks were used in the removal of meat from the shell. It is possible that a certain amount of metal contamination occurred during this process. While the degree of possible contamination was not determined here, it is still advisable to use non-metal materials during preprocessing in order to minimize any possible metal contamination. The decline in Al, Co, Fe, Hg, Mn, Ni, and Sb levels in this study may be attributed to the loss of moisture as has been earlier underlined by Protasowicki et al. (2008), or to the transfer of some metals from the shell into the meat. The shell of Rapa whelk is an efficient accumulator for metals (Richardson et al. 2001), and can be used as an indicator for metal accumulation records in the aquatic environment (Protasowicki et al. 2008).

Eventhough increased Hg was found in heat treated fish, the bio-accessibility of Hg was reduced by 24% compared to the raw fish (Maulvault et al., 2011). This was also reported for other metals with increased Cd, Se, and Zn levels in heat treated mussels, which however showed less bio-accessibility for these metals compared to raw mussels (Metian et al., 2009). Similarly, Houlbrèque et al. (2011) found increased Cd with significantly lowered bio-accessibility in mussels after heat treatment. It is interesting to underline that the inclusion of black coffee, black tea or green tea into the boiling media might decrease the bio-accessibility of Hg by up to 50-60% in raw fish, underlining a combined effect of heat treatment and the incorporation of tea or coffee for the reduction of Hg bio-accessibility in seafood (Ouédraogo & Amyot 2011). In the present study, the boiling process resulted in concentrating Al, Cr, Cu, Fe, Hg, Pb, Sb, Se, and Zn in the boiling water, which was in agreement with Metian et al. (2009), who reported that the heat treatment process concentrated the elements in the mussel soft tissue, and an important part of the elements were released into the boiling water, that eventually decreased their bio-accessibility when consumed. Hence, considering the findings in the present study regarding increased levels of Al, Cr, Cu, Fe, Hg, Pb, Sb, Se, and Zn in the boiling water, it could be possible that the bio-accessibility of these metals have been reduced as well, which however needs further clarification.

According to the findings in this study, the global intake of metals could be reduced by around 20% in Cd, Mo, Cr, and up to 35.5% in As, when the boiling water of Rapa whelk is discarded before eating. This is in close agreement with Metian et al. (2009), who noted that the consumption of heat treated mussels might contribute to a reduction in Cd intake by 65%, globally. Further studies are required to investigate possible reductions in metals from seafood with norm-exceeding levels to lower contents that may allow “safe food marketing” with reduced consumer risks in heat treated products.

Several processing methods applied in the industry, such as skinning, trimming, fat removal or cooking (frying, microwaving, breading) may not always reduce metal concentrations in fish meat. For instance, Burger et al. (2003) found that the Hg in heat treated fish was higher than that of raw fish. Further, the heavy metals of As, Cd, and Hg could not be removed from the meat by common cooking methods such as frying, microwaving, or boiling (Morgan et al. 1997; Perelló et al. 2008). Various different kinds of solutions and complex agents such as acid and alkaline solutions, cysteine and homocysteine have been used for metal removal from seafood (Hajeb & Jinap 2009; 2012; Okazaki et al. 1984; Schab et al. 1978). In the present study, it was noteworthy that Rapa whelk released significant amounts of elements (Al, As, Cu, Hg, Mn, Pb, Se, Zn) into the hypochlorite solution during the sterilization process. Some earlier investigations provided evidence for reduced metal concentrations from fish by cysteine, the most reactive amino acid with Hg (Aizpurfa et al. 1997; Hajeb & Jinap 2012; Okazaki et al. 1984). A removal of 40% Hg from minced shark meat treated by salt solution (0.1 M NaCl) was reported by Aizpurfa et al. (1997). Similarly, Lipre (1980) found a 40% to 44% removal of Hg from cod fillets using 0.1% and 1% cysteine solutions for 24 h, respectively. And Danesh (1974) reported a 50% removal of Hg, Pb, and Cd from seafood through washing with 1% cysteine hydrochloride solution followed by heating at 100 to 132 ℃ for 1 to 30 min that enabled the evaporation of heavy metal ions. Yannai & Saltzman (1973) removed Hg from raw tuna by 55% in 2-2.2 pH and 79% at 1.5 pH though 0.33% cysteine hydrochloride immersion.

Further, Suprapti et al. (2016) found that soaking mussels in an acetic acid solution of 25% concentration for 90 min reduced Pb, Cr, and Cd to nearly half their original levels. Semenov et al. (2001) underlined that 0.1% citric acid solution can be used for Hg removal. The authors noted that Hg removal from fish was higher in a shorter soaking time, likely due to the reductive property of citric acid over time. Despite the increase of some metals after heat treatment in the present study, the levels for As, Cd, Mo, Sb, Cr, Se, Zn, Cu, and Hg reduced by 47.44%, 24.86%, 40.09%, 20.27%, 17.50%, 3.63%, 4.52%, 0.93%, and 0.68%, respectively, when the heat treated meat was immersed for 5 minutes in hypochlorite solution (1% NaOCl), which was comparable with findings from earlier investigations. Nevertheless, the discrepancies between removal efficiency among earlier studies could be attributed to the type of leaching agents, application times, pH and several other conditions, or a combination of all.

In the present study, the biplot analysis for the principal component of metals plotted by raw, heat treated, and sterilized meat groups showed a strong correlation between metals with high level of common variance close to "1". From the results of principle component analyses it was noteworthy to see that the processing treatments affected metal distributions with principal elements of As, Cd, Cr, Mo in raw meat, Al, Co, Fe, Hg, Mn, Ni, Pb, Sb in heat treated meat, and Cu, Se, Zn in sterilized meat groups. This is a clear indication of lowering trends of toxic elements in raw meat towards essential elements in sterilized meat of Rapa whelk. Indeed, trace elements such as Cu, Mn, Zn, Fe are essential for human health, supporting metabolic activities; however, these trace elements can be toxic when consumed at high doses (Makedonski et al. 2017). In contrast however, metals such as As, Pb, Cd and Hg have high bioaccumulation capability in aquatic animals and are certainly toxic with severe health risks in human (Mol et al. 2019).

Considering non-carcinogenic health risks for children, adult men, and adult women, the THQs for all investigated metals remained below safety limit "1" (US-EPA 1989), showing that none of these metals pose non-carcinogenic risks for the three population groups when consuming sterilized Rapa whelk from the Turkish Black Sea coast. This is in line with the findings for Rapa whelk from the Bulgarian Black Sea coast (Peycheva et al. 2017). Major risk contributors among the cumulative metal concentrations in the present study were Hg, Cd, As, and Cu with HI rates of 23.9%, 23.6&, 25.8%; 20.9%, 21.2%, 21.1%; 15.7%, 15.8%, 16.1%; and 13.4%, 13.9%, 13.8% for adult men, women, and children, respectively. In accordance with this study, highest HI contribution with 23% in fish was reported for Cd by Akoto et al. (2014). The EDIs in Rapa whelk collected from the Bulgarian Black Sea coast were much lower than the recommendations by international organisations (Peycheva et al. 2017), which was in agreement with the findings of the present study. Mean EDIs for the investigated metals in Rapa whelk in this study were far below the RfD provided by EFSA (2010), with the exception for As (0.0003 mg/kg/day), that was very close to the RfD for adult men (0.00021 mg/kg/day), women (0.00026 mg/kg/day), but higher for children (0.00042 mg/kg/day). Hence for the children, it is advised to handle with caution. Assuming that the toxic inorganic arsenic was 3% of the total, EDIs and hazard risks for the toxic inorganic arsenic were estimated by multiplying the total arsenic with 0.03 (3% toxic inorganic arsenic of the total), following the suggestions of the FSA (2004). The element As is naturally found in the earth's crust together with other elements. The arsenic combined with elements such as oxygen, chlorine, and sulfur, is referred to as "inorganic arsenic". When the arsenic combined with carbon and hydrogen is referred to "organic arsenic", which is found in seafood (fish and shellfish) and are less toxic or relatively nontoxic to human health. Overall lower EDIs found for the investigated metals compared to the RfDs (except As), strongly indicates that consumers would not experience significant health risks when eating Rapa whelk from the Turkish coast of Black Sea. A direct comparison of EDIs among various studies might result in erroneous predictions, due to variations in parameters such as exposure time, frequency, consumption rate, average body weight (Peycheva et al. 2017) or even a combination of all these factors. Hence, comparing EDIs with international reference values for specific conditions might be suggested.

For carcinogenic risks, estimations were made only for the following five metals: As, Cd, Ni, Pb, because of a lack of carcinogenic slope factors for the other metals investigated. The estimated CRRs of As for all three populations were higher than the acceptable range from 10^{-6} to 10^{-4}, with 3.2×10^{-4} for men, 3.9×10^{-4} for women, and 6.2×10^{-4} for children. The CRRs of Cd, Ni, and Pb were found to be 1.8×10^{-4}, 2.2×10^{-4}, 3.5×10^{-4} for men, 1.2×10^{-5}, 1.5×10^{-5}, 2.3×10^{-5} for women and 3.1×10^{-8}, 3.8×10^{-8}, 6.1×10^{-8} children, respectively. These values were overall higher than the acceptable range given by US-EPA (2010), and ranked in the order of As>Cd>Ni>Pb, showing that that As was the main contaminant creating a relatively high CRR among these metals.

In regards to the reference dose levels (RfD) of the international guidelines (EFSA 2010) for different body weight, age and gender groups, the highest level of safe daily consumption (SDC) per person adult men, women and children were found to be 14.01, 11.41, 7.21 kg/day for Mo; 8.27, 6.73, 4.25 kg/day for Ni; and 5.78, 4.71, 2.97 kg/day for Mn, respectively, which were followed by the remaining metals in the order of Al>Pb>Sb>Zn>Fe>Cr>Co>Se>Cu>As>Cd>Hg. The safe weekly consumption rates (SWC) per person followed the same trend with the highest values for Mo (432.0, 351.8, and 442.4 kg/day), and lowest for Hg (4.48, 3.65, and 4.59 meals/week) in adult men, women and children, respectively. This means that consuming Rapa whelk at these levels would not pose any significant health risk to consumers in all three populations. According to the results in this study, the level of safe consumption rates (SCR) were 32 to 3006 times higher than the "actual daily mollusc consumption" per person in European countries, which corresponds to 4.66 g capita per day (Failler et al. 2007). Similar to the findings in the present study, Yigit et al. (2018b) reported reasonably high levels of SCR (152.8, 64.3, 43.1, 12.9 kg/day person for Cu, Zn, Fe, and Mn, respectively) in Mediterranean mussels (*Mytillus galloprovincialis*) that did not have any hazardous effect on humans when consumed.

Regards the compensation levels of elements needed for a healthy human body, an adult man, woman or a child could only meet about 0.041-0.121% of the minimum daily requirement for Cr, 0.105-0.265% for Cu, 0.05-0.112% for Fe, 0.005-0.013% for Mn and Mo, 0.078-0.195% for Se, and 0.014-0.037% for Zn by consuming Rapa whelk, based on the estimated daily average requirement levels provided by IOM (2006). This was in line with Yigit et al. (2018b), who indicated that an individual would compensate only 0.013-0.94% of the minimum daily requirement for Cu, Zn, Fe, or Mn when consuming Mediterranean mussels collected near a Cu-alloy cage farm.

5. Conclusions

The present study reveals that levels of Hg, Cu, Se, Zn were reduced by 0.68 to 4.52% in Rapa whelk, whereas Cr, Sb, Cd were lowered by around 20%. Over a 40% reduction of Mo and As in Rapa whelk was achieved through heat treatment coupled with sterilization. Metals in Rapa whelk exposed to hypochlorite immersion were below permissible upper limits without any risks to consumers. THQs were below "1", an indication of "no potential health risks" for human when consuming sterilized Rapa whelk. However, As, Cd, Ni, and Pb should be considered with caution, since these were close to or over the acceptable range provided by international organisations. Hence, it is highly advisable not to exceed the safe consumption limits for Rapa whelk estimated in this study. Further, regular the consumption of Rapa whelk would contribute the minimum daily requirement levels for Cr, Cu, Fe, Mn, Mo, Se, and Zn. These findings indicate that the per capita consumption of seafood in Turkey is below the global average (6.5 kg/year) (TUIK 2023).

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