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Toxic Elements in Edible Mollusks from Igneada Coasts of the Black Sea, Turkey

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Abstract

In this present work, the amounts of toxic elements were determined in the soft tissues of mollusks (*Mytilus galloprovincialis* and *Rapana venosa*) collected from Igneada shores of the Black Sea where suggested as Marine Protection Area (MPA). *M. galloprovincialis* accumulated the highest amounts of Cd in winter, while the highest amounts of Cd and Hg were detected in *R. venosa* in winter and autumn. The concentrations of toxic elements found in the soft tissues of mollusks varied for Cd: 0.07-0.14, Hg: 0.03-0.44 and Pb: 0.09-0.21 mg/g dry wt. The estimated levels of all non-essential metals in the present work were lower than the limits permitted by European Community Regulation (EU) and the Turkish Food Codex (TFC). The estimated weekly intakes (EWI) and daily intakes (EDI) of all the through consumption of these seafood by Turkish people in the Igneada coasts of the Black Sea were quite below the permissible tolerable weekly/daily intakes for 70 kg person (PTWI / PTDI) set by FAO/WHO. As results, it can be concluded that no hazard effects on people health would be raised at present from the consumption of these mollusks' species.

Keywords: Black Sea, Igneada, Toxic Elements, Mytilus galloprovincialis, Rapana venosa.

1. Introduction

The environmental trouble in the Black Sea resulting from anthropogenic forcing and accompanied by natural variability and climatic changes, is displayed by dramatic changes in its ecosystem and resources (Sezgin et al., 2010). Environmental problems of the Black Sea are well concerned to the unique characteristics of the marine environment (Mee, 1992). In a very recent review, Bat et al. (2018a) pointed out that the heavy pollution in the Black Sea with industrial, agricultural and municipal discharges from rivers is main sources. These heavy metals may be taken up by living organisms especially Mollusks (Bat & Arici, 2018), deposited in the sediments (Bat & Özkan, 2015) or remain for some period in the water itself.

Benthic assemblages are the main components of the Black Sea coastal marine life. Because of its commercial significance and the relation to human health, the regional and national administrations show a clear tendency to monitoring mollusks (Bat & Arici, 2018; Bat et al., 2018b). Thus trace metal concentrations (Cd, Hg and Pb) in *M. galloprovincialis* and *R. venosa* of Igneada coasts of the Black Sea were studied. These toxic elements were chosen for the work because they have been widely used as indicators for the bioaccumulation of trace metals. They are non-essential metals and known to have detrimental effects on many biological systems. Amounts of non-essential metals in marine organisms have been found to depend mainly on the local environmental factors. *M. galloprovincialis* and *R. venosa* are widely consumed species by local population. Samples were collected from Igneada shores located in the north-western part of Turkey, being a small town within the district of Demirköy in Turkey's Kirklareli Province. Igneada lies on the Black Sea coast with a beach of 40-50 m width and 10 km length and is exposed to different types and degree of urban and industrial pollution from Danube River. The main goal of this survey was to determine the non-essential metals (Cd, Hg, Pb) contamination in commonly consumed Mollusca species from Igneada coasts of the Black Sea and the potential health risk to consumers.

2. Material and Methods

2.1. Water sampling

The water sampling was conducted between November 2012 and May 2013 covering 4 seasons. Physicochemical properties of water bodies were assessed using the YSI 6600 V2 Water Quality Probe. Seasonal changes in temperature, salinity, pH and Dissolved Oxygen values were measured throughout the year.

2.2. Collection of animals

The study focused on Igneada coasts of the Black Sea because of its importance to fishing and the suggestion from previous MISIS project of possible indicated Marine Protected Area (MPA). The locations of Igneada coast of the Black Sea is given in Figure 1 along with the sampling locations. Edible Mollusca samples the veined rapa whelk *Rapana venosa* (Valenciennes, 1846) and the Mediterranean mussel *Mytilus galloprovincialis* Lamarck 1819 were collected in 3 sampling sites in the shore waters of Igneada by snorkel diving seasonally between November 2012 and May 2013.

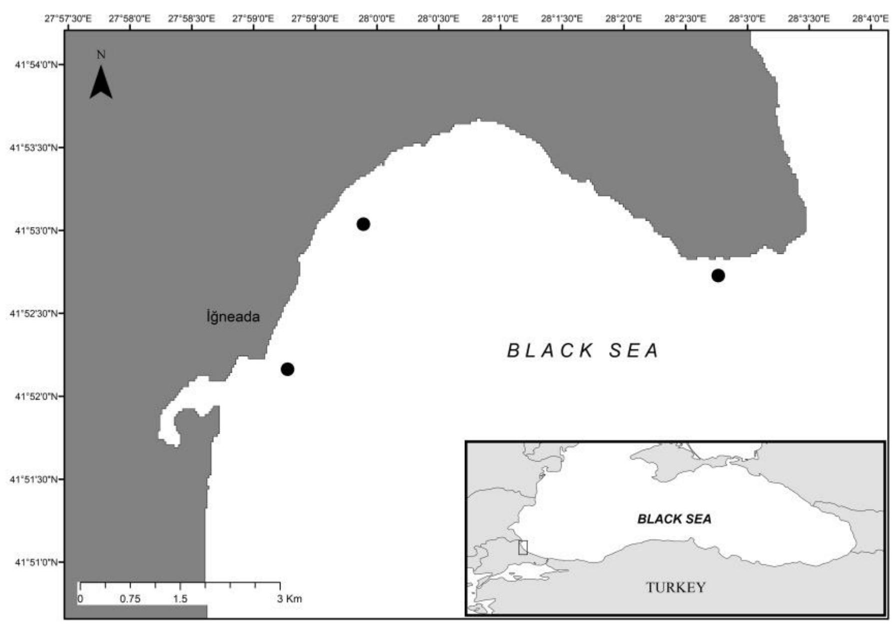

Figure 1. Map showing the location of sampling stations in Igneada coasts of the Black Sea.

2.3. Measurements of the basic biological parameters

Mollusca species were first identified, and then the total lengths (mm) and the body-wet weights (g) of each specimen were measured.

2.4. Preparation of animals

After collection, these animals were washed with seawater to remove any debris on them and separately put in tanks with aerated clean seawater for 48 hours to empty gut contents. The treatment carried out on the samples followed the methods described by Bernhard (1976). They were then weighed and edible soft tissue samples were taken using plastic knives. Each tissue samples for both animals were placed in polyethylene zippered bags and frozen at -21℃ until metal analysis.

2.5. Analytic procedure

For metal analysis, the tissue samples digested with Suprapur® HNO3 (nitric acid) using a Milestone Systems, Start D 260 microwave digestion system. In parallel, blanks and certified reference material samples were made similarly to verify the accuracy and precision of the method using the Standard Reference Material (SRM) 2976 (mussel tissue) from National Institute of Standards and Technology (NIST). The results between the certified and the analytical values were in good agreement, together with the Relative Standard Deviation (RSD) percentage which was about ±5% for all elements. Tissues were analyzed in triplicate by an ICP-MS, Agilent Technologies, 7700x and the results were expressed as mg kg^{-1} dry weight (wt.).

2.6. Calculation of Intake Levels

The mean heavy metals daily intake was calculated according to the following formula:

Intake level = mean toxic element content X daily consumption of mollusks per person/ body weight

Estimated weekly intake (EWI) values were calculated from estimated daily intakes (EDI) values. According to health risk, the tolerable daily/weekly intakes were estimated by means of references for edible soft tissues of mollusks consumed by local people. The daily quantity of mollusk species consumed is 1g /person (FAO, 2010). Intake estimates were expressed as per unit body weight (mg/kg body wt. /weekly and daily).

3. Results

The measured values of physicochemical variables of the water are given in Table 1.

Table 1. Mean values of physicochemical properties of water bodies of seasons.

Seasons	Temperature (℃)	Salinity (‰)	pH	DO (mg/L)
Winter	14.95±0.3	17.91±0.2	8.28±0.1	8.83±0.6
Spring	18.25±0.7	16.40±1.5	8.50±0.1	9.88±0.7
Summer	23.94±0.9	15.53±0.6	6.92±0.1	8.02±0.5
Autumn	17.96±0.8	18.28±0.1	6.77±0.1	8.16±0.3

The temperatures recorded at the sample locations ranged from 9.6-24.7℃. The water temperatures of Igneada coast were within Water Pollution Control Regulations of 2872 the Official Gazette Environment Law (<25℃) for water (Official Gazette of Republic of Turkey, 1983). Salinities were between 14.9 and 18.3‰. The pH levels of the water of Igneada coast in November 2012-May 2013 ranged from 6.8-8.6, the values were within the standards maximum 9 set by the Turkish Environmental Regulation (2004-2005), for General Quality Criteria of Marine Water for pH levels. The dissolved oxygen (DO) level in Igneada coast of the Black Sea ranged from 7.4-10.5 mg/L between November 2012 and May 2013. It is considered suitable for marine life.

The mean lengths and total weights, together with number of specimens and wet weight to dry weight ratios of the species sampled between November 2012 and May 2013 are given in Table 2.

Table 2. Biological characteristics of the species analysed

Species	Total length (mm)	Total weight (g)	Wet wt. to dry wt. ratio in soft part	n
Rapana venosa	65±2	60±3	4.4	48
Mytilus galloprovincialis	50±5	11±3	7.6	48

The results of the analysis of Cd, Hg and Cd in the veined rapa whelk and the Mediterranean mussel are presented in Figures 2-4. The amounts for studies elements were not statistically different from the localities, therefore mean values were given.

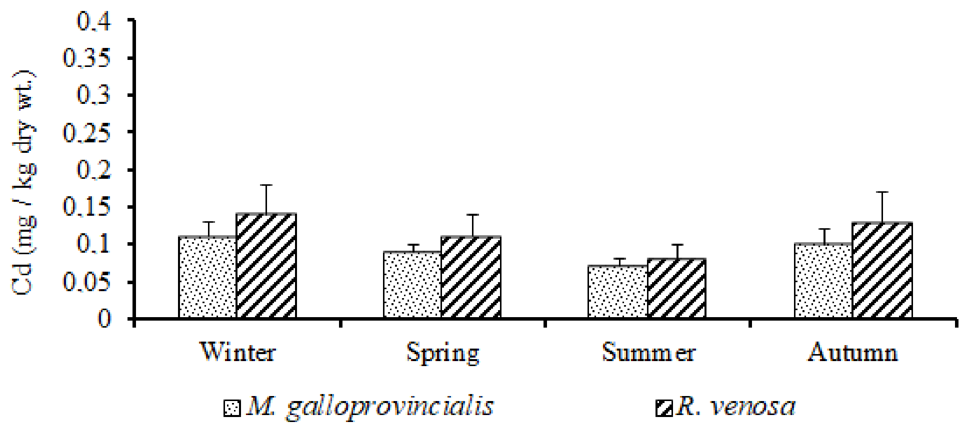

Figure 2. The means with standard errors (vertical line) of Cd concentrations (mg/kg dry wt.) in the soft tissues of mollusks (*Mytilus galloprovincialis* and *Rapana venosa*) collected from Igneada shores of the Black Sea between November 2012 and May 2013.

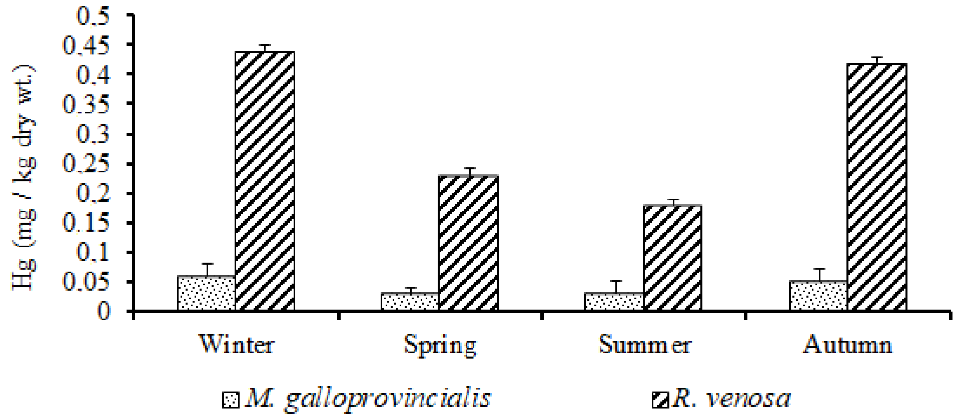

Figure 3. The means with standard errors (vertical line) of Hg concentrations (mg/kg dry wt.) in the soft tissues of mollusks (*Mytilus galloprovincialis* and *Rapana venosa*) collected from Igneada shores of the Black Sea between November 2012 and May 2013.

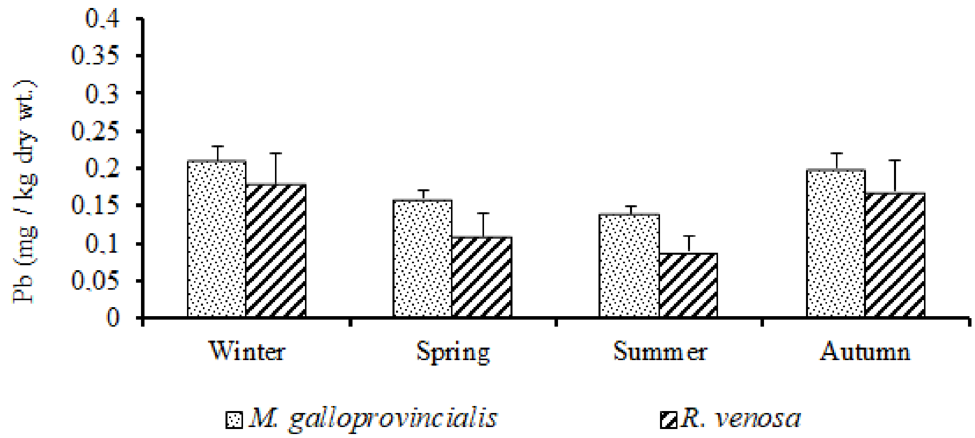

Figure 4. The means with standard errors (vertical line) of Pb concentrations (mg/kg dry wt.) in the soft tissues of mollusks (*Mytilus galloprovincialis* and *Rapana venosa*) collected from Igneada shores of the Black Sea between November 2012 and May 2013.

The EDI and EWI values given in Table 3 were estimated by assuming that a 70-kg person will consume 1 g the veined rapa whelk or the Mediterranean mussel /day which are 7 g seafood/week (FAO, 2010). The tolerable weekly intake of these toxic elements as PTWI (Provisional Tolerable Weekly Intake), are set by the Food and Agriculture Organization/World Health Organization (FAO/WHO) Joint Expert Committee on Food Additives (JECFA) (Council of Europe, 2001; FAO/WHO, 2010 and 2011; WHO, 1989, 2000 and 2004).

Table 3. Estimated Weekly Intakes (EWI) and Estimated Daily Intakes (EDI) of heavy metals in edible tissues of *R. venosa* and *M. galloprovincialis* from Igneada shores of the Black Sea, Turkey.

Metals	PTWI[a]	PTWI[b]	PTDI[c]	EWI[d]		EDI[e]	
				R. venosa	*M. galloprovincialis*	*R. venosa*	*M. galloprovincialis*
Cd	0.007	0.49	0.07	0.00098	0.00077	0.00014	0.00011
Hg	0.004	0.28	0.04	0.00308	0.00042	0.00044	0.00006
Pb	0.025	1.75	0.25	0.00126	0.00147	0.00018	0.00021

[a]PTWI (Provisional Tolerable Weekly Intake) in mg/week/kg body wt.
[b]PTWI for 70 kg adult person (mg/week/70 kg body wt.)
[c]PTDI (Permissible Tolerable Daily Intake) (mg/day/70 kg body wt.)
[d]EWI (Estimated Weekly Intake) (mg/week/ kg body wt.)
[e]EDI (Estimated Daily Intake) (mg/day/ kg body wt.)

4. Discussion

In this work, Cd and Hg levels were higher in *R. venosa* than those in *M. galloprovincialis*, whereas Pb was high in the Mediterranean mussel. The difference in Cd, Hg and Pb amounts between the veined rapa whelk and the Mediterranean mussel may be the result of different rates of these metals uptake because of dietary differences between species. The veined rapa whelks are carnivorous. The Mediterranean mussels are suspension feeders and their main predators are the veined rapa whelk in the Black Sea. In 1947, a native of Japanese waters *R. venosa* was noticed in the Black Sea where it has become abundant. It has already destroyed enormous banks of oysters in the Black Sea. When oysters are not available the Black Sea specimens of this voracious predator can keep alive by devouring smaller mollusks. If different food organisms accumulate the metals differently, then seasonal changes in a very large quantity of foods could affect metal level of their predators. It is also known that *R. venosa* can withstand very unfavorable conditions, such as lowered salinity and variable temperatures, and is extremely fertile. Evidently this unwelcome visitor has discovered a perfect refuge for itself in its new environment.

Differences in weights may show differences in the concentrations of the metals in body tissues. The differences of toxic element levels between the two species may also be due to differences in size. The large size veined rapa whelks are likely heavier than the Mediterranean mussel of similar size, and thus may have had a longer time to accumulate Cd and Hg. In case of Pb, *R. venosa* is heavier than *M. galloprovincialis* and both mollusks have taken the same amount of a pollutant, then *R. venosa* would have lower concentration of the pollutant due to biological dilution. Alternatively, the bulk of the Pb in the sea water can be combined with particulate matter and quickly moved to the seabed sediments, reaching the sea bottom taken by the Mediterranean mussel. Metal concentrations in biota including Mollusca species depend on many factors such as season, length and weight, and physicochemical properties of the water bodies affect the bioaccumulation of metals. Temperature for instance can affect metal uptake by mollusks through increased metabolism and physiological parameters. In this work, seasonal effects included high amounts of toxic elements in mollusks collected in the autumn and winter, which were attributed to increased rainfall that washes metal-contaminated soils into the coastal water bodies. Similarly, Ünsal and Besiktepe (1994) observed the highest Hg concentration in *M. galloprovincialis* in autumn. They indicated that when the Mediterranean mussels were captured in 1991 the rainfall maintained a high level and resulted in high industrial discharge of contaminant into the sea throughout the winter and spring.

The variation of the toxic elements in the soft tissues of the mollusks during the sampling seasons may result from the leaching of these elements from contaminated soils or sediments and subsequent transport to the coastal area. The high rainfall for occurs during the autumn and winter. Cd, Hg and Pb are non-essential metals for organisms and their concentrations may increase due to reduced activity of Mollusca during the winter months and elimination later when activity increases. It is obvious that pollutants discharged by the River Danube into the western Black Sea and carried by currents in a southern direction have a significant role in the pollution occurring at Igneada. The toxic element in *R. venosa* (Table 4) and *M. galloprovincialis* (Table 5) were compared among the Turkish Black Sea coasts.

Table 4. Toxic element amounts (mg kg^{-1} dry wt.) in *Rapana venosa* collected from the Turkish Black Sea coasts.

Location (Year)	Cd	Hg	Pb	Method**	References
Bosphorus (1988)	4.9±1.3	<0.3	3.5±1.3	No	Topçuoglu et al.(1994)
Sinop (1997)*	0.03–0.22	N.M.	0.08–0.46	Yes	Bat et al.(2000)
Thrace Coasts (2012)	0.1–1.6	0.4–0.7	0.1–0.7	No	Mülayim & Balkıs(2015)
Igneada (2012)	0.1	0.6	0.1	No	Mülayim & Balkıs(2015)
Sinop (2013)	2.9-4.4	<0.05	<0.05	Yes	Bat & Öztekin(2016)
Igneada	0.08-0.14	0.18-0.44	0.09-0.18	Yes	This work

*wet wt.
** To allow depuration for elimination of the gut contents

Table 5. Toxic element amounts (mg kg^{-1}) in *Mytilus galloprovincialis* samples collected from the Turkish Black Sea coasts.

Location (Year)	Cd	Hg	Pb	Method**	References
Igneada (1992)*	N.M.	0.03-0.06	0.12	No	Ünsal et al.(1993)
Sinop (1992-1993)*	0.03-0.24	N.M.	0.11-1.18	Yes	Bat et al.(1999)
Pazar (1998)	2.36±0.64	N.M.	4.8±1.25	No	opçuoglu et al.(2003)
Western Black Sea (2000)	0.05-0.08	0.07-0.09	0.04-0.08	No	Altug & Güler(2004)
Rize (2001)	>0.02	N.M.	5.3±0.91	No	Topçuoglu et al.(2003)
Sinop (2010)	0.27-0.98	N.M.	2.10-4.10	Yes	Bat et al.(2012)
Sinop (2013)	0.4-0.77	<0.05	0.15-0.70	Yes	Bat & Öztekin(2016)
Sinop (2014)*	0.03±0.01	0.02±0.009	0.08±0.03	Yes	Bat et al.(2018c)
Igneada (2015)*	<0.02-0.09	<0.05	<0.05-0.18	Yes	Bat et al.(2018b)
Igneada	0.07-0.11	0.03-0.06	0.14-0.21	Yes	This work

*wet wt.
** To allow depuration for elimination of the gut contents
N.M.: Not measured in the study

It can be seen that there are variations of accumulation metals between regions. This may be due to their different geographic locations. Comparison of the results should be made with caution because of the differences in sampling procedures and methodology discussed well by Bat & Raffaelli (1999) and Bat el al. (1999). In many studies (Tables 4 and 5) no time was allowed for depuration of the animals to clear their guts. Many studies (Elwood et al., 1976; Flegal et al., 1977; Chapman et al., 1980; Hare et al., 1989; Bat & Raffaelli, 1999; Bat et al., 1999) have been demonstrated that the absence of clearing of gut contents have shown different results in metal accumulation. Moreover sizes of the animals were different. Phillips (1976) pointed out that smaller and lighter specimen of mussels were found to contain significantly higher heavy metal concentrations including Cd and Pb than larger and heavier specimen in the marine environment. On the other hand, Phillips and Rainbow (1994) emphasized that if growth is fast compared with metal accumulation, the observed concentration of the metal will decrease with age and weight, even though the overall metal content increased.

PTWI is the maximal level of a pollutant to which a person can be exposed per week over a lifespan without an unacceptable risk of health effects (WHO, 1996; Council of Europe, 2001; FAO/WHO, 2010; EFSA 2010, 2012a,b,c). The results of the present work are significantly below the recommended values of FAO/WHO (see Table 3). Therefore the non-essential metals in mollusks do not toxic any apparent threat to the population and these species are healthy for consumption.

5. Conclusion

Mollusca samples collected from Igneada shores of the Black Sea have been found to be contaminated with Cd, Hg and Pb. The differences in Cd, Hg and Pb amounts between species may be the result of different feeding habits, different size, or both. However the means for Cd, Hg and Pb were well below the health criteria limits (Commission Regulation, 2006; Official Gazette of Republic of Turkey, 2009). It is well concluded that consumption of these mollusks from Igneada shores as seafood may not possible hazard to people at the time of the work. The results once confirmed the potential of Mollusca species to be used as bio-indicators of metal pollution.

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