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Trace Elements Risk Assessment for Consumption of the Bivalve Species *Chamelea gallina* (L., 1758) (Veneridae) and *Donax trunculus* L., 1758 (Donacidae) along the Black Sea Coastline of Bulgaria

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Abstract: The main purpose of the study was to identify the levels of trace metals (Fe, Mn, Cu, Zn, Ni, Cd, Pb and Cr) in bivalve species *Chamelea gallina* and *Donax trunculus* from the marine coast of Black Sea, Bulgaria. Compared to the permissible limits set by the EU and the US FDA, all trace metal concentrations found in the bivalve species from the Bulgarian coastal waters of the Black Sea were lower. The public health risks associated with the consumption of *Chamelea gallina* and *Donax trunculus* in relation to reported trace metal concentrations were evaluated. The target hazard quotients (THQ) of all elements were below 1, showing the absence of health hazard for the population when consuming these bivalve species. Target risk (TR) of Ni and Pb indicated that consumption over a long period would not result in a carcinogenic effect.

Key words: Trace elements, Black Sea, bivalves, human health risk

Introduction

The bivalve species are recognised as commercially important and high quality fishery resources in various coastal regions of the world. The alimentary and health benefits relating to the bivalve consumption suggested that these species could be a valuable part of the human diet. Bivalves are spread worldwide and are often influenced by anthropogenic pollution. As filter-feeding organisms, bivalves play an important role as indicators for the coastal ecosystem pollution. The bivalve soft tissues are used as bioindicators of different environmental contaminants, especially trace elements such as Fe, Mn, Cu, Zn, Ni, Cd, Pb and Cr. Their examination may provide accurate data for microelements concentrations in the marine environment.

The Black Sea has connections with the Azov Sea through the Kerch Strait and with the Marmara Sea through the Bosphorus. Its unique characteristics determine its specific hydrological and hydrochemical regime, including in relation to the fade of the heavy metals in its waters (Bat & Arici 2018). Water pollution has been increasing due to less self-cleaning ability caused by the weak natural circulation. The most impact source of pollution in the Black Sea is the industrial waste emitted by all the coastal countries (Russia, Ukraine, Romania,

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Bulgaria, Georgia and Turkey). The major rivers Dnieper, Dniester and Danube mouth into the sea and discharge pollution from industrial, agricultural and domestic origin from the entire Black Sea basin (Bat & Arici 2018).

Besides the well-known Mediterranean mussel *Mytilus galloprovincialis* Lamarck, 1819, there are two further prospective bivalve species dominating in the Bulgarian Black Sea benthic zone, which are known as "white clams" – *Donax trunculus* L., 1758 and *Chamelea gallina* (L., 1758). *Donax trunculus* dominates in fine sands (1–9 m depth), where it is exposed to more aggressive abiotic factors making it a more adaptive species. *Chamelea gallina* prefers deeper sand bottoms (up to 25 m) due to its lower adaptive ability (compared to wedge clam). Since 2012, these white clam species have been commercially exploited in the Bulgarian Black Sea part and their catches reached 506 tonnes in 2019 (Gumus et al. 2020). However, information on the trace element contents in these two species from the Black Sea is relatively scarce. Consequently, various pu-

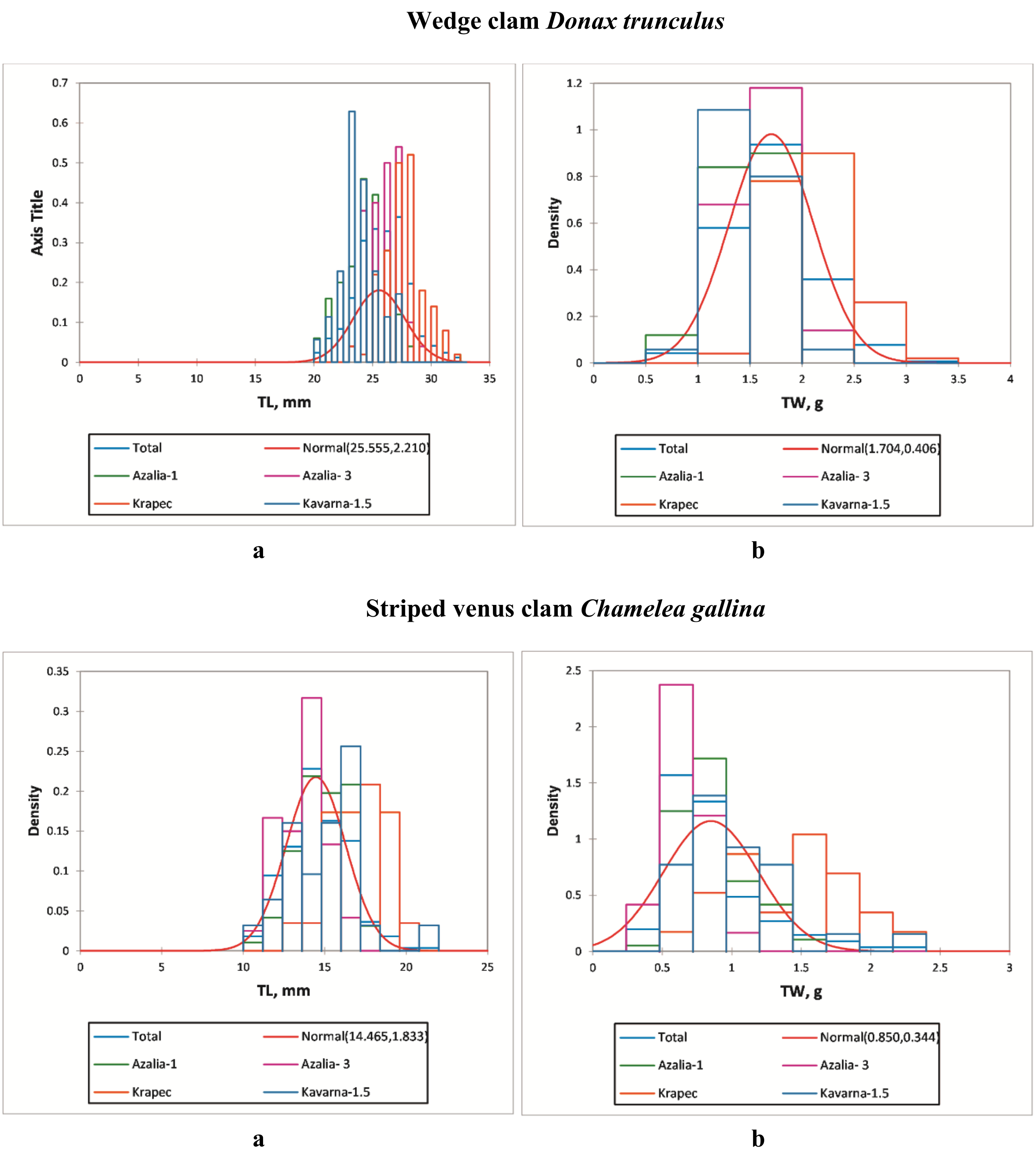

Fig. 1. Histogram of the length (TL, mm) (a) and weight (TW, g) (b) classes distribution of *Donax trunculus* and *Chamelea gallina*

tative health risks associated with the bivalve consumption requires assessing the trace element contents in order to determine the health hazard for the consumers of these bivalves.

The aim of the present study is to assess the total concentrations of eight trace elements (Cd, Cr, Cu, Fe, Ni, Pb, Mn and Zn) in the striped venus clam (*C. gallina*) and wedge clam (*D. trunculus*) collected from the Bulgarian part of the Black Sea. We evaluate the potential risks to public health using a model calculating the estimated daily intake (EDI), target hazard quotient (THQ), hazard index (HI) and carcinogenic risk (TR).

Materials and Methods

The abundance of *C. gallina* and *D. trunculus* was assessed through dredging activities: (i) 0.3 knots speed, 5 min dragging duration with small drag, and (ii) 0.55 knots, 15 min dragging with a bigger dredge in the shelf area within 1–8 m isobaths. Samples were collected from four points between the village of Krapec to the St. Konstantin and Elena Resort (43.587 N 28.609 E; 43.434 N 28.37 E; 43.246 N 28.02 E; 43.247 N 28.022 E).

The abundance of *D. trunculus* was also assessed by bottom trawling using a beam trawl (0.3 knots speed and 5 min trawling duration in the coastal area, within 1–3 m isobaths). Samples were collected from area of Azalia Resort zone on two points (43.24642 N 28.01972 E, 43.24781N 28.02058 E, 43.24661N 28.02167E, 43.24569N 28.02111E).

For each specimen, the main biometric parameters were measured individually: weight with the shell (Total Weight, TW) in grams (g) and length of the shell (Length of the Shell, SL) in mm. The XLSTAT software product was used to display the linear-weight histograms of the samples. The statistical data about the different length and weight classes presented in the histograms (Fig. 1) include lower and upper limits, frequency, relative frequency and density.

Approximately 2–4 kg of bivalves with comparable shell length were collected, placed into bags and brought to the laboratory in iceboxes. Around two hundred specimens of each species were taken randomly for determination of sample mean. The average length of the bivalves was 3.28 ± 0.32 cm for *D. trunculus* and 2.1 ± 0.18 cm for *C. gallina*. The samples were washed with cold distilled water and were randomly divided into ten groups. Each group consisted of 60 mussels with 20 individuals for each replicate (n=3). The samples were washed with Milli-Q water, brushed and shucked. The bivalves were then placed in a steam cooker and were steam-cooked at 90 °C for 10 min in core, placed on a filter paper to absorb the excess moisture and then the flesh was removed using a Teflon knife.

Each tissue of *C. gallina* and *D. trunculus* sample (c. 1 g wet weight) was placed in Teflon digestion vessels. Acid wet digestions using 8 cm^3 HNO_3 (65% w/v) and 2 cm^3 H_2O_2 (30 %w/v) were performed using a microwave closed-vessel digestion system MARS 6 (CEM Corporation, USA) subject to a 3-stage program according to the procedure stated by Peycheva et al. (2021a). The concentrations of Cd, Cr, Cu, Fe, Ni, Pb, Mn, Zn, K, Ca, Mg and Na in the samples were determined using ICP-OES Spectrometer (Optima 8000, Perkin Elmer, USA) with certain parameters (Peycheva et al. 2021b).

To evaluate the human exposure to toxic metals and essential elements in the bivalve species under analysis, an estimation of human health risk was determined by using estimated daily intake (EDI), the non-carcinogenic target hazard quotient (THQ), hazard index (HI), and lifetime carcinogenic risk (TR) coefficients. For the calculation of those indices, the algorithm described by Peycheva et al. (2021a, 2021b) was used.

All analyses were performed in triplicate and the results were expressed as mean values ± standard deviation (SD). T-test was used to compare the results for toxic and essential elements. Differences at $p \leq 0.05$ were considered significant (Graph Pad Prism 6).

Results

The mean concentrations (mg/kg ww) of toxic elements in the studied edible portion of *Chamelea gallina* and *Donax trunculus* are presented in Table 1.

Table 1. Toxic elements composition (mean ± standard deviation in mg/kg) of *Donax trunculus* and *Chamelea gallina*

	Wedge clam *D. trunculus*	**Striped venus clam *C. gallina***	**Regulations**
Cd	0.119 ± 0.009	0.257 ± 0.046	1.0 mg/kg*
Ni	0.308 ± 0.797	0.676 ± 0.203	-
Pb	< LOD	0.62 ± 0.035	5.0 mg/kg**

* obtained from Zhelyazkov et al. (2018); ** obtained from Commission European (2006); < LOD – bellow detection limit

Table 2. Essential elements content (mean ± standard deviation) and nutritional contribution of *Donax trunculus* and *Chamelea gallina*

		Wedge clam *Donax trunculus*			**Striped venus clam *Chamelea gallina***		
	RDA[a] or TDI[b] (mg/day)	mg/kg	EDI	% DRI	mg/kg	EDI	% DRI
Cr	0.05 (WHO 2008)	0.542 ± 0.045	0.006	12.38	0.761±0.463	0.009	17.35
Cu	2 (EU 2008)	10.467± 2.016	0.120	7.48	3.060 ± 0.644	0.035	2.19
Fe	12.5 (NRC 1989)	129.930 ± 8.036	1.485	18.56	254.107 ± 104.209	2.904	36.30
Mn	1.8-2.3[c] (IM 2001)	9.494 ± 1.343	0.109	6.03	13.065 ± 4.795	0.149	8.30
Zn	12 (NRC 1989)	26.137±5.138	0.299	2.49	19.651 ± 2.565	0.225	1.87

*significant difference ($p < 0.05$) (raw vs. steamed); [a]RDA, recommended daily allowances; [b]TDI, tolerable daily intake; [c]AI, adequate intake, EDI – estimated daily intake; % DRI – calculated percent from the daily recommended intake

Table 3. Target hazard quotient (THQ) and target cancer risk (TR) values of toxic and essential elements of bivalve sampled from the Black Sea, Bulgaria.

		Cd	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Zn**	**HI[*]**
RfDo[a]		0.001	0.003	0.040	0.700	0.140	0.0002	0.0035	0.300	
THQ	*D. trunculus*	0.001	0.002	0.003	0.002	0.0008	0.00003	n.d	0.001	0.010
	C. gallina	0.003	0.003	0.001	0.004	0.0011	0.0004	0.0002	0.013	0.013
TR	*D. trunculus*		$3.1 x10^{-6}$				$6.1 x10^{-6}$	n.d.		
	C. gallina		$4.3x10^{-6}$				$1.3 x10^{-5}$	$6.4 x10^{-9}$		

[a]RfDo (oral reference dose in mg/kg/d) were obtained from the EPA Region III Risk-Based Concentrations summary table (USEPA 2020), with the exception of Pb (Hang et al. 2009). The body weight used for calculation is 70 kg; [*]HI – Hazard Index

The essential metal concentration and the nutritional contribution of clam species are given in Table 2. Target hazard quotient (THQ) and target cancer risk (TR) values of toxic and essential elements of bivalve sampled from the Bulgarian Black Sea coast are given in Table 3.

Discussion

In the present study, the range of cadmium (Cd) concentration among the studied bivalves was 0.090–0.142 mg/kg ww for *D. trunculus* and between 0.105 and 0.396 mg/kg ww for *C. gallina.* In a previous study from the Sea of Marmara, Turkey (Őzden et al. 2010), Cd concentration ranged between 0.204 ± 0.001 mg/kg and 4.426 ± 0.012 mg/kg for *C. gallina* and between 0.024 ± 0.002 mg/kg and 0.159 ± 0. 003 mg/kg for *D. trunculus*. From the Veneto Region (north-western Adriatic Sea, Italy) Bille et al. (2015) reported mean 0.06 ± 0.05 mg/kg ww for *C. gallina* and 0.03 ± 0.03 mg/kg ww for *D. trunculus*. For *Mytilus galloprovincialis* from Sicilian coast, Italy, Cammilleri et al. (2020) reported and up to 0.201 ± 0.025 mg/kg ww. The values reported by us do not exceed the limit of 1.0 mg/kg set by Commission Regulation (EC) No 1881/2006 (EC 2006).

Nickel (Ni) is harmful to the human body since it can lead to several adverse effects such as dermatitis, nausea, coughing and even lung cancer (Cempel & Nikel 2006). The concentration of Ni in our samples was higher in *C. gallina*, which followed the same pattern as in our previous studies (Peycheva et al. 2021a). The values were lower than the level allowed by the joint FAO/WHO/Expert Committee on Food Additives (JECFA 1993). There is no maximum permitted limit set by the EU concerning bivalve species. Őzden et al. (2009) found that nickel concentration in *D. trunculus* (7.505 ± 0.516 mg/kg) was higher than in *C. gallina* (1.618 ± 0.005 mg/kg) from the Sea of Marmara. Nickel concentration in *M. galloprovincialis* sampled from Boka Kotorska Bay, Montenegro (Adriatic Sea) ranged between 0.27 mg/kg ww to 1.14 mg/kg ww (Perošević et al. 2018). It may be concluded that Ni levels in the collected bivalve species from Bulgarian waters can be considered safe for consumers.

Lead (Pb) is a toxic metal that leads to decreased intelligence quotient (IQ) in children and occurrences of anaemia in humans (García-Leston et al. 2010). Lead levels in the current study were under detection limit for the samples of *D. trunculus* and between 0.028 mg/kg ww and 0.086 mg/kg ww for the samples of *C. gallina*. European commission (EC, 2006) has set maximum levels of 1.5 mg/kg Pb in bivalve molluscs, which is below the recorded values for the two species analysed by us. In another study of Black Sea, Zhelyazkov et al. (2018) found Pb levels ranging from 0.157 mg/kg up to 0.414 mg/kg ww in *M. galloprovincialis*. The highest Pb concentrations, 1.342 mg/kg and 0.916 mg/kg, were found in *C. gallina* and *D. trunculus*, respectively, collected from Sea of Marmara (Őzden et al. 2009). Peycheva et al. (2021a) reported Pb levels in three bivalve species (*Donax trunculus, Mytilus galloprovincialis* and *Chamelea gallina*) collected from the Black Sea (Bulgaria) from 2021 as follows: 0.06 ± 0.13 for *D. trunculus,* 0.13 ± 0.03 for *M. galloprovincialis* and 0.69 ± 0.06 for *C. gallina*.

Chromium (Cr) is an essential element and together with its compounds is thought to have beneficial effects on glucose metabolism, insulin sensitivity, lipid profile and inflammation. Chromium-deficient diets may cause severe atherosclerosis, heart disease and type 2 diabetes (Heshmati et al. 2017). In the present study, the range of Cr levels was 0.060–0.542 mg/kg ww for *D. trunculus* and 0.052-0.184 mg/kg ww for *C. gallina.* The data found here are within the range of values reported for bivalve species from other marine basins (Astorga Espana et al. 2007, Celik & Oehlenschlager 2005, Jureša & Blanuša 2003, Jovic & Stanković et al. 2014, Özden et al. 2010) and are below the range stated by several other authors (Liu et al. 2019, Türkmen et al. 2005, Usero et al. 2005).

Copper (Cu) is considered to be essential since it has numerous roles in the human body such as an activator of redox enzymes, antioxidant defence and immune function (Bost et al. 2016). However, high intakes of Cu, especially with the diet, may lead to adverse health problems (Heshmati et al. 2017) and the average consumption of Cu should not exceed 2 mg per day. Copper concentration varied in this study and it was significantly higher in *D. trunculus*. Őzden et al. (2010) measured the copper content in different session, being between 0.839 ± 0.049 mg/kg ww and 3.116 ± 0 .052 mg/kg ww in *M. galloprovincialis* and between 0.586 mg/kg ww and 3.547 mg/kg ww in *C. gallina* (Őzden et al. 2009). Cu in *M. galloprovincialis* from Casablanca coast showed values from 13.08 up to 22.8 μg/g dw (Mejdoub et al. 2018).

Iron (Fe) is one of the most critical trace elements in biological systems, being a vital constituent of enzymes and metalloproteins in biological systems (Abbaspour et al. 2014). Additionally, Fe is the most abundant metal in the tissues of clams as shown by all the analyses. The minimum and maximum Fe levels were between 68.48 mg/kg ww (for *D. trunculus*) and 254.107 mg/kg ww (for *C. gallina*). In our previous study of molluscs collected from the Bulgarian part of the Black Sea, Fe-concentration of the samples was 59.69 ± 4.18 ww for *Mytilus galloprovincialis*, 126.44 ± 58.74 mg/kg ww for *D. trunculus* and up to 483.74 ± 25.72 mg/kg ww for the striped venus clam *C. gallina* (Peycheva et al. 2021a). The iron content of *C. gallina* was significantly lower than that of *D. trunculus* during all seasons ($p < 0.05$) in the samples from Sea of Marmara, Turkey (Őzden 2010). A joint effort of FAO/WHO (1999) has set a limit for iron intake based on body weight. For an adult humans weighing 60 kg, the provisional tolerable daily intake (PTDI) for iron is 48 mg (Joint FAO/WHO Expert Committee 1999).

Manganese (Mn) is of great importance to the human body since it takes part in the metabolism of carbohydrates, lipids, amino acids and proteins (Aschner & Aschner 2005, Heshmati et al. 2017). The average Mn levels in *C. gallina* and *D. trunculus* were 13.065 ± 4.795 and 9.494 ± 1.343, respectively, with samples from *C. gallina* being significantly higher than those from *D. trunculus* ($p = 0.007$). Manganese concentrations in muscle tissue of bivalves have been reported in the range 1.456–4.390 mg/kg in *C. gallina* and 4.201–16.687 mg/kg for *D. trunculus* from Sea of Marmara (Özden et al. 2009). They were 0.855-11.306 mg/kg in *M. galloprovincialis* from the Sea of Marmara (Özden et al. 2010) and 13.08–22.8 μg/g dw for *M. galloprovincialis* from Casablanca coast (Mejdoub et al. 2018). The concentrations of Mn in our work were within the range stated by previous studies.

Zinc (Zn) is classified as an essential element because is needed by over 300 enzymes. Zinc function in these metalloenzymes is to participate in catalytic functions, regulatory functions, and maintenance of the stability of the structure (Briffa et al. 2020). Zinc is implicated in DNA and RNA synthesis, together with cell proliferation (Briffa et al. 2005). The FAO/WHO (1983) set a limit for the daily human intake 30 mg/kg for Zn. The established maximum level for zinc in Bulgarian legislation, above which bivalve consumption is not permitted, is 200 mg/kg (Anonymous 2004). In the present study, the highest and lowest Zn concentrations were found for *D. trunculus* (26.14 mg/kg ww and 11.63 mg/kg ww, respectively). Őzden et al. (2009) reported Zn levels as follows: from 9.85 mg/kg ww (winter) up to 11.08 mg/kg ww (summer) for *C. gallina* and from 10.23 mg/kg ww (summer) up to 17.94 mg/kg ww (winter) for *D. trunculus* (from the northern part of the Sea of Marmara). In *M. galloprovincialis*, the reported values were between 14.8 mg/kg ww and 24.5 mg/kg ww at three locations in Boka Kotorska in four seasons in 2005 (Peroševic et al. 2018). In other studies from the Black Sea (Bulgaria), the minimum and maximum concentration of Zn in tissues of molluscs was 19.75 ± 0.38 mg/kg ww for *C. gallina* and 27.46 ± 2.53 mg/kg ww for *D. trunculus* (Peycheva et al. 2021a, 2021b), which is in agreement with the data in the present study.

Nutritional contributions of bivalves in terms of essential elements are presented in Table 2. The estimated daily intake (EDI) for the analysed elements was calculated using the mean concentration of essential element (mg/kg) obtained for each clam and considering a meal of 0.8 g of molluscs per day (FAO 2020). *Chamelea gallina* is a good source of Fe, with a level of 0.8 g meal reaching 36.30 % of the required DRI. Additionally, the same species have the maximum percentage of Cr (17.4 % of DRI) and Mn (8.30 % of DRI).

The target hazard quotients (THQ) is a measure, which assesses the risk associated with the intake of contaminated *C. gallina* and *D. trunculus*. The values below 1 (THQ < 1) reveal a lower level of exposure, which is associated with a daily exposure at this level is not likely to cause harmful effects for human health during a lifetime in population (Bogdanovic et al. 2014). According to the present study, there were no THQ or HI values exceeding the limit value concerning the consumption of *C. gallina* and *D. trunculus* from the Bulgarian Black Sea. The results suggest that there is no significant health risk to people consuming white clams obtained from the Bulgarian Black Sea waters.

TR values are calculated for intake of those toxic metals, which are considered carcinogenic (Cr, Ni and Pb) according to IARC (2012). Based on the US EPA methods, cancer risk lower than 10^{-6} is considered to be negligible, $>10^{-4}$ is considered unacceptable and in the range 10^{-4}–10^{-6} is considered acceptable (USEPA 1989, 2010). With respect to Cr, Ni and Pb, both the minimum and maximum values of TR do not exceed the technical guidelines for the risk assessment of contaminated sites.

Conclusion

This survey was conducted to determine and compare the concentrations of different metals in the bivalve species wedge clam (*D. trunculus*) and striped venus clam (*C. gallina*) collected from the Bulgarian shore of the Black Sea. The concentration of essential (Cr, Cu, Fe, Zn) and toxic (Cd, Ni, Pb) elements were below the limits for bivalve molluscs stated by Commission Regulation (EC) No 1881/2006 and other known world human health safety organization such as FAO/WHO and FDA (FAO 2000, FAO/WHO 2000, FDA 2001). The estimated daily intakes of all toxic and essential metals were below the acceptable daily intakes established by the joint FAO/WHO Expert Committee on Food Additives. The THQ, HI and TR values confirmed that there was no risk associated with human consumption of wedge clam (*D. trunculus*) and striped venus clam (*C. gallina*) from the Bulgarian Black Sea in reasonable amounts (JECFA 1993).

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