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TOXIC METALS IN THE WARTY CRAB IN THE SOUTHERN BLACK SEA: ASSESSMENT OF HUMAN HEALTH RISK

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The present study was performed to assess Cd, Pb, and Hg contaminations and human health risk in the warty crab *Eriphia verrucosa* (Forskål, 1775) in Akliman shores of Sinop Peninsula of the Black Sea. Heavy metals analysis was performed by inductively coupled plasma mass spectrometry. Among studied toxic metals, Pb had the highest mean concentration in *E. verrucosa*. The highest mean concentration of Pb (0.2 mg per kg of wet weight) was observed in male samples of the warty crab. However, higher concentrations of Cd and Hg (0.11 and 0.019 mg per kg of wet weight, respectively) were observed in females of *E. verrucosa*. The mean Cd values found in the warty crabs were higher in May and June than those in July and August. On the other hand, Pb values were recorded in July and August. The mean Hg values were not different between months except July and August for male samples of *E. verrucosa*. Foraging seasons of these crabs are different, which can lead to differences in prey size and ultimately metals intake. However, the results show that a toxic heavy metal concentration in edible tissues of crab from the southern Black Sea was within the permissible limits given by national and international food codices. Target hazard quotient (THQ) for each metal and hazard index (HI) were calculated to evaluate non-carcinogenic human health risks. Estimated THQs of Cd, Pb, and Hg suggest that these metals in the warty crab do not pose any apparent threat to humans, when the HI value is below the value of 1. The result of the analysis has shown that the warty crab *E. verrucosa* can be used as bioindicator as it contains variable levels of the metals observed. Since consumption is the main source of heavy metal intake by humans, monitoring studies are needed to protect public health and take preventive measures.

Keywords: heavy metal, Black Sea, *Eriphia verrucosa*, hazard index, target hazard quotient

The Black Sea takes up a variety of contaminants from agricultural, mining, touristic, domestic, and other anthropogenic activities via direct dumping from major rivers and in other ways [1]. Toxic metals such as mercury, cadmium, and lead, among others, are of great importance, since their anthropogenic contribution outweighs the one which is provided through life span and likewise because they show much toxic properties along the food chain. Toxic metals eventually enter seafood, and their bioaccumulation and magnification can cause physiological and morphological alterations not only in marine coastal animals but in people as well [2]. With the ever-increasing contamination of the coastal ecosystem, risk of toxic metal contamination of seafood is increasing day by day.

Many crustacean species were used as organisms for biomonitoring toxic metals in contaminated marine coastal ecosystems due to a number of suitable characteristics such as their convenient size, ease of sampling, abundance, ease of handling in the laboratory, and ability to accumulate metals. Therefore, it is of great interest to carry out investigations on metal concentrations in crustaceans [3].

People in Sinop city of the southern Black Sea consume a considerable amount of seafood. Since seafood is an important diet for humans, its quality and safety aspects are of particular interest. Over the past many decades, the amounts of heavy metals in seafood have been studied off the coasts of the Black Sea. Heavy metals are known to accumulate in benthic organisms and increase in the food chain. Since consumption is the main source of heavy metal intake by humans, the major interest is in the edible commercial species like the warty crab. It is necessary to have data on the levels of heavy metals in the warty crab in order to assess whether there is a health hazard.

The aim of this study is to evaluate the levels of Cd, Pb, and Hg in the warty crab *Eriphia verrucosa* (Forskål, 1775) in Akliman shores of Sinop Peninsula of the Black Sea.

MATERIAL AND METHODS

E. verrucosa is a type of crab which is locally called "küflü" and consumed extensively by people in spring and summer. All the warty crab samples were captured in 2017. This crab usually lives in shallow water under rocks and between seagrasses. *E. verrucosa*, which is a characteristic form for the hard substratum of the upper-infralittoral at depths of 1–8 m, has an average length of (10 ± 1.5) cm (Fig. 1).

Fig. 1. *Eriphia verrucosa* in Akliman shores of Sinop Peninsula of the Black Sea

The meat was removed from the shell and washed with double-distilled water. Then the samples were frozen at the temperature of −21 °C and stored in polyethylene bags until analysis. Water temperatures at the time of sampling in May, June, July, and August were of +15.8, +21.5, +25.6, and +26.2 °C, respectively. A total of 40 warty crabs, 20 males and 20 females, were used for the heavy metal analysis. Ten individuals were analyzed in each sampling month.

Warty crabs is active at dawn and at night. Diet of *E. verrucosa* consists of molluscs, crustaceans, and worms. Chelipeds (claws) are not equal to each other and are strong. Warty crab catches prey with its claws and cuts it into suitable pieces with cutter plates in its mouth. It should be noted that the size of the claws has an influence on the prey: with increasing size, crab can hunt bigger organisms.

Location of sampling areas is given in Fig. 2. Akliman is located on the outer harbor side of Sinop Peninsula. The distance to the city center is of 11 km. The coast length is of approximately four thousand meters. The slope of the beach is quite low. Sampling covered areas of direct or indirect influence of urban releases and touristic and fishing activities, those located near the mouths of Karasu and Sirakaraagaçlar streams which carry domestic and agricultural discharges to the Akliman coasts of Sinop province as well as a locality not under the influence of industrial releases. However, under the influence of the prevailing winds, pollutants reach the shore by the discharge. The sampling station was chosen to reflect progression of contamination, ecological particularity, and human activities in the area. Tourist activities are very intense in Akliman area, especially during summer months.

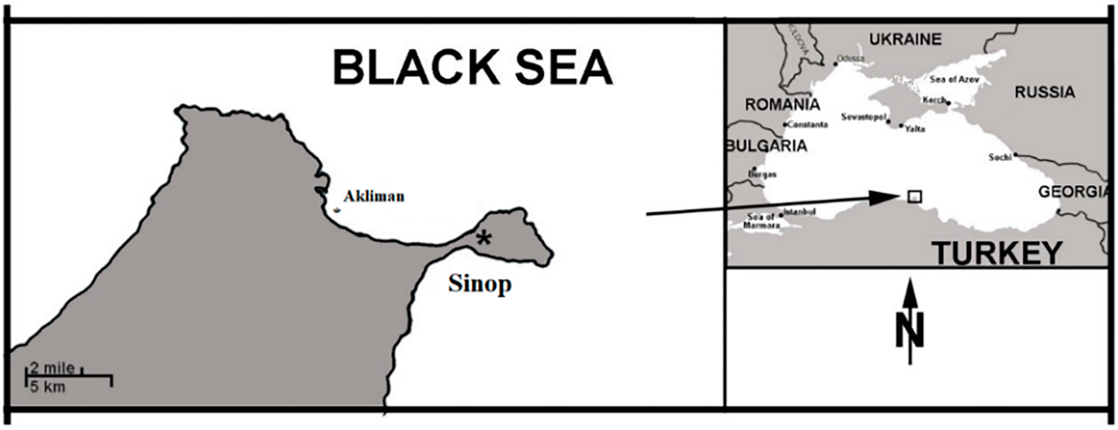

Fig. 2. Sampling area

About 1 g of edible tissues was wet digested with Suprapur® HNO_3 (nitric acid) using a microwave digestion system (Start D 260, Milestone Systems) for analysis and evaluations of concentrations of three non-essential heavy metals. Cd, Pb, and Hg were determined using inductively coupled plasma mass spectrometry (7700x ICP-MS, Agilent).

The element standard solutions used for calibration were prepared by diluting a stock solution of 1000 mg per L (Cd, Hg, and Pb) supplied by Merck (Germany). Standard reference material of lobster hepatopancreas TORT-3 for the metals was used to validate the analysis. Recovery percentages results ranged 95 to 102 %, indicating the accuracy of the results (Table 1).

Table 1. Reference concentration values of standard reference material TORT-3 (mg per kg)

	Cd	Hg	Pb
Certified	42.3	0.137	0.225
Found	41.47	0.140	0.237

Toxic metals in warty crab tissues were expressed as milligram of metal per kilogram of wet weight sample. The sensitivity of the method was determined according to the detection limits established for the spectrometer, which were of < 0.001 µg per L for Pb and Cd and of < 0.01 µg per L for Hg. The operating conditions of ICP-MS set for the analysis of the metals are shown in Table 2.

Assessments of hazard index of toxic metals in the warty crab. The estimated daily intake (hereinafter EDI) depends on both the metal level and the quantity of seafood consumption. The EDI of toxic metals was computed using Equation (1) below modified from [15]:

Table 2. ICP-MS operating conditions for the metal analysis

Operating conditions	Values
Plasma mode	normal, robust
RF power (W)	1550
Sampling depth (mm)	8
Nebulizer (ml per min)	0.2
Spray chamber temperature (°C)	+2
Carrier gas flow (L per min)	0.95
Dilution gas flow (L per min)	0.15
Extraction lens 1 (V)	0
Kinetic energy discrimination (V)	4
Cell gas (He) flow (ml per min)	4
Background on-mass (cps)	< 2
Integration time (μs)	100

$$EDI = \frac{F_{ir} \times C_m}{W_{ab}} \times 10^{-3}\,, \tag{1}$$

where F_{ir} is the seafood ingestion rate (g·person^{-1}·day^{-1}), which was considered to be of 15 g·person^{-1}·day^{-1} in Turkey [17], and this value was calculated as 105 g a week;

C_m is the level of toxic metal in the warty crab (mg per kg of wet weight);

W_{ab} is the mean adult body weight (70 kg).

The estimated weekly intake (hereinafter EWI) values were calculated from EDI values. Intake estimates were determined as per unit body weight (mg·week^{-1}·kg^{-1} of body weight).

Target hazard quotient (hereinafter THQ) [15], which is a proportion of the estimated exposure (EDI) to the oral reference dose (hereinafter R_fD), is used to evaluate the potential non-carcinogenic risk to humans from the sensed contaminated crab, and is shown by Equation (2):

$$TQH = \frac{EDI}{R_f D}\,, \tag{2}$$

where the R_fD values for Hg and Cd are of 0.0003 and 0.001 mg·kg^{-1}·day^{-1}, respectively [20].

R_fD is not available for Pb [5]. The U. S. Department of Health and Human Services Public Health Service [16] pointed out that it would be unsuitable to develop R_fD for inorganic Pb and its compounds, because some of the sanitary impacts related to the exposure to Pb occur with blood Pb levels as low as to be essentially without a threshold [21]. Therefore, the R_fD value for Pb in this study was of 0.0035 mg·day^{-1}·kg^{-1} of body weight as used by many researchers [5, 10, 11].

Exposure to more than one contaminant may induce contribution and/or interactive impacts; hereby, accumulative health effect from plural contaminants' exposure was determined by summing THQ value of individual contaminant and clarified as hazard index (hereinafter HI) as shown in Equation (3):

$$HI = THQ_{Cd} + THQ_{Pb} + THQ_{Hg}\,. \tag{3}$$

HI value of > 1 shows the possibility of reverse health effects and commits the necessity for bearing a further appraisal and likely remedial action. However, HI of < 1 shows no feasible health consequence from exposure of the examined contaminants at existing consumption rate.

Statistical analysis. Statistical analysis was carried out using IBM SPSS Statistics V21. One-way ANOVA and Duncan multiple range test were used to calculate a significant difference in the concentration of different studied metals with respect to different sexes and months. The significance was set at 5 % confidence level.

RESULTS

Mean values of toxic metal concentrations (mg per kg ± SD wet weight) found in the warty crab *E. verrucosa* in Akliman shores of Sinop Peninsula of the Black Sea are presented in Fig. 3.

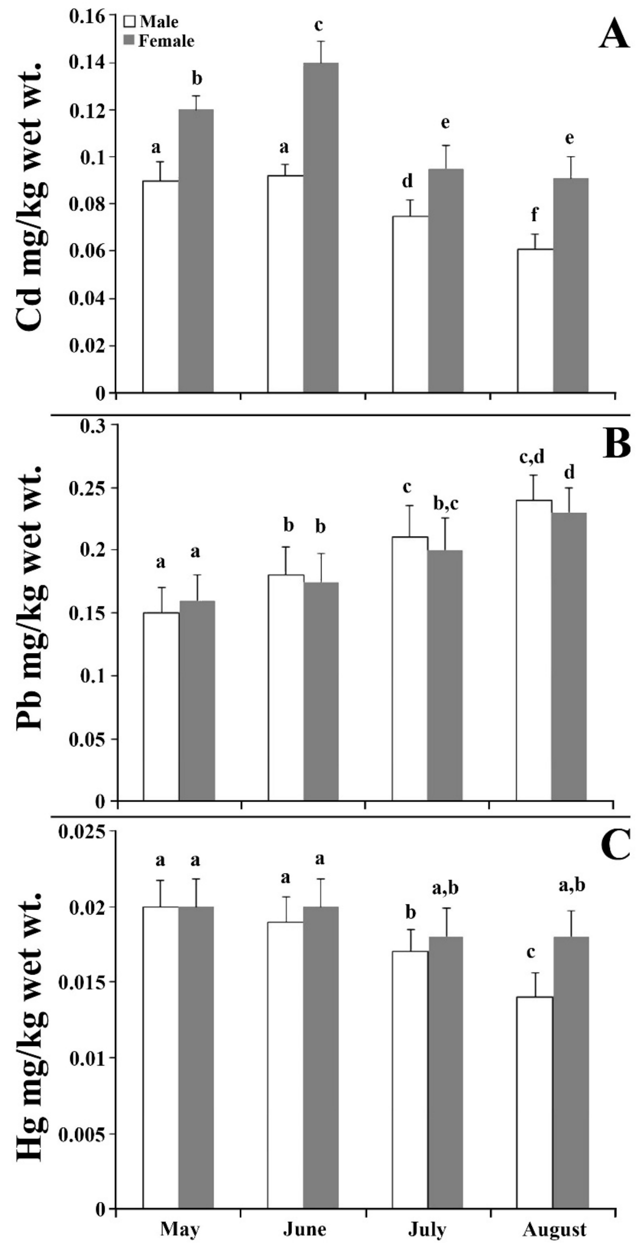

Fig. 3. Mean (± SD) Cd (A), Pb (B), and Hg (C) levels in *E. verrucosa* (N = 40) among sexes and months. Different letters beside vertical bars indicate that the values are significantly different ($p < 0.05$)

The risk to human health as a result of consuming the warty crab was evaluated by calculating EDI, EWI, provisional tolerable weekly/daily intake (hereinafter PTWI and PTDI, respectively), THQ, and HI. The maximum toxic metal values in the edible tissues of *E. verrucosa* were used to evaluate the human health risk from the consumption. EDI, EWI, PTWI, PTDI, R_fD, THQ, and HI of these metals are shown in Table 3.

Table 3. Human health risk parameters in the warty crab collected from the Black Sea

Health risks parameters	Toxic heavy metals		
	Cd	Pb	Hg
Estimated daily intake, EDI, $mg \cdot day^{-1} \cdot kg^{-1}$ of body weight	2.39×10^{-5}	4.18×10^{-5}	4.07×10^{-6}
Estimated weekly intake, EWI, $mg \cdot week^{-1} \cdot kg^{-1}$ of body weight	1.67×10^{-4}	2.93×10^{-4}	2.85×10^{-5}
Provisional tolerable weekly intake, PTWI, $mg \cdot week^{-1} \cdot kg^{-1}$ of body weight	7×10^{-3}	2.5×10^{-2}	4×10^{-3}
Provisional tolerable weekly intake, PTWI, mg per week per 70 kg of body weight	0.49	1.75	0.28
Provisional tolerable daily intake, PTDI, mg per day per 70 kg of body weight	0.07	0.25	0.04
Oral reference dose, R_fD, $mg \cdot day^{-1} \cdot kg^{-1}$ of body weight	1×10^{-3}	3.5×10^{-3}	3×10^{-4}
Target hazard quotient, THQ	2.39×10^{-2}	1.19×10^{-2}	1.36×10^{-2}
Hazard index, HI	4.94×10^{-2}		

DISCUSSION

The non-essential metals such as Cd, Pb, and Hg are highly toxic contaminants, and their uptake and bioaccumulation in coastal ecosystems may cause serious effects straight on food chain as well as on human beings. Among toxic metals studied, Pb had the highest mean concentration in *E. verrucosa*. Higher mean concentration of Pb (0.2 mg per kg of wet weight) was observed in male samples of the warty crab (Fig. 3B). However, the highest concentrations of Cd and Hg (0.14 and 0.019 mg per kg of wet weight, respectively) were observed in females of *E. verrucosa* (Figs 3A and 3C). The mean Cd values found in the warty crabs were higher in May and June than those in July and August ($p < 0.05$). On the other hand, Pb values were recorded in July and August ($p < 0.05$). The mean Hg values were not different between months ($p > 0.05$) except July and August for male samples of *E. verrucosa*. High metal levels may change within individuals depending on feeding habits of crabs, age, size, and length as well as on their habitats and metabolisms. *E. verrucosa* is carnivore and mainly feed on benthic organisms such as mussels, and this can be one of the reasons for their metal bioaccumulation. In this study, female individuals with eggs were found in May and June. The warty crabs were collected from the cliffs and habitats where the sea meadows were abundant. Individuals collected from rocks can take up metals from food and water, while those from seagrass habitat can also accumulate metals from sediment. As known, sediments are the final destination where heavy metals sink [6]. The increased amounts of toxic metals in sediments are the result of higher levels of these metals in surrounding water, which is easily adsorbed on the surface of the sediment and poses an ecological risk, especially to benthic species. In summer, increase in tourist activities and excess of domestic wastes pollute the coastal areas. Although the central population of Sinop is 65 thousand, it reaches 400 thousand, especially in July and August. Akliman coast is also used extensively as a picnic area. During these periods, this coast is exposed to contamination.

Although heavy metal studies related to *E. verrucosa* were conducted in the early 1990s, very little literature is available. These data were recently reviewed by Bat and Arıcı [3]. According to these studies, Cd, Pb, and Hg values in *E. verrucosa* were determined as 0.06–5.04, 0.06–0.8, and 0.02–0.022 mg per kg of wet weight, respectively [4, 7, 12, 14]. Data obtained in the current study were compared with the literature data, and Hg values were similar while Cd and Pb values were very low.

Durmus et al. [9] studied the edible tissues of *E. verrucosa* recorded on the Ordu coasts in the Black Sea with regard to toxic heavy metals Cd and Pb. Their values were of 0.17–0.32 and 0.13–0.36 mg per kg of wet weight, respectively. In our study, when the maximum values were compared, the values on Sinop coasts were lower than those on Ordu coasts. It is concluded that this was due to the difference between the regions [9]. This may also be caused by the contaminants carried by the streams of various sizes on the southeastern coast of the Black Sea [1].

In the present study, Cd, Pb, and Hg concentrations in *E. verrucosa* edible tissues were found to be below the permissible limit which was of 0.5 mg per kg of wet weight for these metals studied in Crustaceans [8, 18, 19]. Our research also aimed to assess whether there is an impact on the health of people consuming these warty crabs. In this sense, the current study has been carried out in accordance with the Marine Environment Policy Marine Strategy Framework Directive [13]. Marine Strategy Framework Directive Descriptor 8 "Concentrations of contaminants are at levels not giving rise to pollution effects" and Descriptor 9 "Contaminants in seafood for people consumption do not exceed amounts established by Community legislation or other relevant standards" are aiming the subject of marine contamination. For this purpose, EDI, EWI, PTWI, PTDI, THQ, and HI calculations were performed. The amount of fish consumed in Turkey is much higher compared to amount of other seafood. Crustacean species are mostly consumed in the coastal cities, and Sinop is one of them. Cd, Pb, and Hg are non-essential toxic metals in organisms; the presence of these metals in seafood may cause risk to human health via consumption. Values given in the present study are significantly lower than the recommended values of Turkish Food Codex [18, 19]. Estimated THQs of Cd, Pb, and Hg suggest that these metals in the warty crab do not pose any apparent threat to humans, where the HI value was of < 1 (U. S. Environmental Protection Agency) as shown in Table 3.

Conclusion. The toxic and non-essential metals in warty crab samples have been analyzed. According to the results obtained, Cd, Pb, and Hg are present in *E. verrucosa*, but in low concentrations. The concentrations of Cd, Pb, and Hg in *E. verrucosa* samples from Akliman shores of Sinop of the Black Sea did not exceed the permissible limits set for metals by EU Commission Regulation and Turkish Food Codex. EDI, EWI, PTWI, PTDI, THQ, and HI of these metals were estimated taking into account the mean concentration value of metal in all the warty crab individuals and the average consumption of them per day for adults. Since HI is of < 1 described by U. S. Environmental Protection Agency, *E. verrucosa* is considered safe for consumption. It is suggested that further investigation should be carried out of the parameters in coastal waters as well as sediment analysis which may help find the cause of each metal bioaccumulation in these species. This kind of biomonitoring studies is necessary to assess the risk to human health due to dynamic character of the marine ecosystems and their constant exposure to toxic metals as mentioned in Marine Strategy Framework Directive.

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ТОКСИЧНЫЕ МЕТАЛЛЫ В КАМЕННЫХ КРАБАХ ЮЖНОЙ ЧАСТИ ЧЁРНОГО МОРЯ: ОЦЕНКА РИСКА ДЛЯ ЗДОРОВЬЯ ЧЕЛОВЕКА

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Проведена оценка загрязнения тяжёлыми металлами — Cd, Pb и Hg — каменных крабов *Eriphia verrucosa* (Forskål, 1775), обитающих на Аклиманском побережье Синопского полуострова Чёрного моря, и потенциальных рисков для здоровья человека. Анализ содержания тяжёлых металлов выполнен методом масс-спектрометрии с индуктивно-связанной плазмой. Среди изученных токсичных металлов свинец имел самую высокую среднюю концентрацию в тканях *E. verrucosa*. При этом максимальные концентрации Pb отмечены в тканях самцов краба (0,2 мг·кг$^{-1}$ сырой массы), тогда как у самок зарегистрированы более высокие, чем у самцов, концентрации Cd и Hg (0,11 и 0,019 мг·кг$^{-1}$ сырой массы соответственно). Средние значения содержания Cd в каменных крабах были выше в мае и июне, чем в июле и августе. Pb отмечен только в июле и августе. Средние значения содержания Hg в образцах самцов *E. verrucosa* были примерно одинаковыми в разные месяцы, за исключением июля и августа. Сезоны активного питания самцов и самок каменных крабов отличаются, что может приводить к различиям в размере пищевых объектов и в итоге — в уровнях накопления металлов. В целом концентрация токсичных тяжёлых металлов в съедобных тканях каменных крабов из южной части Чёрного моря находилась в допустимых пределах, установленных национальным и международным стандартами для пищевых продуктов. Для оценки рисков неканцерогенной природы для здоровья человека определены целевой коэффициент опасности (target hazard quotient) для каждого металла и индекс опасности (hazard index). По рассчитанным целевым коэффициентам опасности Cd, Pb и Hg можно заключить, что эти металлы в каменном крабе не представляют явной угрозы для людей, если, по результатам измерений, значение индекса опасности ниже 1. Результаты анализа показали, что *E. verrucosa* могут быть использованы в качестве биоиндикатора, так как рассмотренные металлы содержатся в этих каменных крабах в разных количествах. Поскольку пищевой путь поступления тяжёлых металлов является для человека основным, необходимо проводить дальнейшие мониторинговые исследования, чтобы защитить здоровье населения.

Ключевые слова: тяжёлый металл, Чёрное море, *Eriphia verrucosa*, индекс опасности, целевой коэффициент опасности