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To cite this article: Büşra Parlak Somuncu, Murat Topbaş, Meriç Albay, Derya Çamur, Hüseyin İltter, Ferruh Niyazi Ayoğlu, Ahmet Altın, Murat Can, Yusuf Demirtaş, Bilgehan Açıkgöz & Fatih Aydın (2025) Essential and toxic elements in nail samples from fishermen in Turkey, *Toxicological & Environmental Chemistry*, 107:1, 155-177, DOI: [10.1080/02772248.2025.2450445](https://doi.org/10.1080/02772248.2025.2450445)

To link to this article: <https://doi.org/10.1080/02772248.2025.2450445>



Published online: 15 Jan 2025.



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Essential and toxic elements in nail samples from fishermen in Turkey

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ABSTRACT

Toxic substances in seafood can accumulate in consumers and cause serious health problems. The purpose of this research was to evaluate the levels of 11 essential and toxic elements (arsenic, copper, mercury, zinc, iron, cadmium, chromium, lead, nickel, selenium and titanium) in nail samples from fishermen who frequently consumed seafood and control groups who consumed them less frequently. This study was conducted in the Marmara Sea coastal cities of Istanbul, Kocaeli, Tekirdağ and Yalova. 352 individuals were enrolled, 263 fishermen and 89 controls. A questionnaire was administered to the individuals, then fingernail samples were collected. Nail samples were digested by the addition of HNO₃ and H₂O₂, then the element levels was measured by ICP-MS. Zinc and iron levels in fishermen's nails were higher than in controls. Positive correlations were observed between levels of arsenic, mercury, iron, cadmium, chromium, lead, nickel, selenium, and titanium in the fishermen's nails and monthly seafood consumption. Mean nail zinc and copper levels were lower than expected in both groups. Other elements were at expected levels. In conclusion, consuming seafood caught in this region does not result in element exposure sufficient to represent a risk, but may increase in the burdens of some elements.

ARTICLE HISTORY

Received 4 June 2024
Accepted 2 January 2025

KEYWORDS

Essential element; toxic element; seafood consumption; nail; fisherman; public health

Introduction

Seafood is abundant in high-quality protein, essential fatty acids, vitamins, and essential elements such as selenium, zinc, and iron. Numerous studies have demonstrated the beneficial health impacts of consuming seafood, resulting in a worldwide rise in its consumption recently (Domingo 2016; FAO 2020).

The health effects of essential elements, with their structural and functional roles in biological systems, vary in association with their levels in organisms. Insufficient intake results in deficiency symptoms, while high intake can be toxic to metabolisms (Goldhaber 2003). However, elements such as mercury, cadmium, arsenic, and lead can lead to toxicity in humans even at very low concentrations (Duruibe, Ogwuegbu, and Edwurugwu 2007). The International Agency for Research on Cancer classifies arsenic, cadmium, hexavalent chromium, and nickel compounds as Group 1 carcinogens, inorganic lead as Group 2A, and methylmercury, titanium dioxide, metallic nickel, and nickel alloys as Group 2B (IARC 2024).

The levels of these elements, which can be naturally present in the environment, can reach very high concentrations due to anthropogenic factors such as industrial, domestic, and agricultural wastes, drugs, mining activities, and refineries (Tchounwou et al. 2012). Aquatic ecosystems in particular are at a greater risk of chemical pollution than other ecosystems since many pollutants eventually find their way to water (Copat et al. 2018). Studies have shown that chemical pollution in the seas is rising on a continual basis, in Turkey and internationally (Otansev et al. 2016; Wilhelmsson et al. 2013). Chemical pollution in aquatic ecosystems increases the element burden in seafood, and these elements can reach humans through the food chain. The accumulation of elements in organisms because they cannot be metabolized and due to their bioaccumulation and biomagnification properties is coming to represent a growing threat to human health (Burger and Gochfeld 2005; Naser 2013).

These factors all underline the importance of determining the levels of elements in humans. Numerous biological materials can be employed for evaluating the element burden in the body. Nail specimens can reveal long-term element exposure by permitting retrospective analysis. They can also be obtained non-invasively and stored at room temperature, making them easily used and preferable to many other biological materials (Rodushkin and Axelsson 2000a).

Twenty percent of the population of Turkey, a country surrounded on three sides by four different seas, and more than 50% of its industrial plants, are found around the Sea of Marmara (Türkmen, Tepe, and Türkmen 2008). The Sea of Marmara is becoming increasingly polluted

due to rapid population growth, unbalanced urbanization, intensive industrialization and maritime traffic in the region. Istanbul and the Gulf of Izmit in particular have been subjected to rapid pollution in the last 50 years. In addition, the fact that the Sea of Marmara lies between the Black Sea and the Mediterranean results in a continuous current, and the effects of this pollution can therefore be seen even in places with smaller populations and fewer industrial establishments (Pekey et al. 2004; Taşdemir 2002). A study of copper, zinc, nickel, chrome, and lead levels in water samples from the Sea of Marmara revealed moderate pollution by at least one element in each area, and that the Sea of Marmara had been subjected to greater pollution in recent years compared with other studies from the same region (Otansev et al. 2016). Pekey et al. determined arsenic, copper, zinc, and cadmium pollution in surface sediment specimens from the Gulf of Izmit (Pekey et al. 2004), while Yaşar et al. observed antimony, mercury, and cadmium pollution in the interior and central parts of the gulf (Yaşar, Aksu, and Uslu 2001). This chemical pollution in the Sea of Marmara may impair the quality and quantity of marine products, leading to a decrease in their consumption in the region, and severe health problems in individuals consuming them (Taşdemir 2002). In a study evaluating the elemental burden in seafood obtained from the Sea of Marmara, it was found that estimated weekly intake of arsenic, cadmium, lead, and mercury in muscle tissues of *Trachurus mediterraneus* and *Merlangius merlangus* exceeded the provisional tolerable weekly intake in some regions (Köker et al. 2021). Similarly, Keskin et al. found that mercury levels in some fish species and cadmium levels in mussels exceeded the threshold limits set out in the Turkish Food Code Index Regulation (Keskin et al. 2007). Türkmen et al. reported higher chromium levels in some fish species than the threshold values specified by the World Health Organization (Türkmen, Tepe, and Türkmen 2008), while Aksu et al. determined higher lead levels in some fishery products than those permitted by Türkiye and European countries (Aksu et al. 2011). A study using hair samples from fishermen consuming large quantities of seafood from the Marmara region reported higher levels of arsenic, chromium, nickel, strontium, and zinc than in a control group (Demirtaş et al. 2024), while another study involving complete blood specimens reported higher mercury and lead levels compared to a control group (Çamur et al. 2021). These studies show the critical importance of determining the levels of these elements in individuals consuming products obtained from these seas and examining the relationship between these levels and seafood consumption.

Studies investigating the relationship between seafood consumption and element levels in humans are frequently limited to just a few

elements, particularly mercury. However, increasing environmental pollution and pollutant diversity associated with growing industrial activities make the evaluation of larger numbers of elements essential (Martín et al. 2015).

The purpose of this research was to evaluate the levels of 11 essential and toxic elements (arsenic, copper, mercury, zinc, iron, cadmium, chromium, lead, nickel, selenium and titanium) in nail samples from fishermen in four cities on the coast of the Sea of Marmara who frequently consumed seafood and a control group who consumed it less frequently, and to determine the correlation between nail element levels and monthly seafood consumption in fishermen.

Materials and methods

Study design and participants

This research was conducted within the framework of the Scientific and Technological Research Council of Türkiye's (TÜBİTAK) 'The Heavy Metal-Trace Element Burden in Seafoods and Fishermen and Risk Mapping' project (no. 116S520) performed between July 2017 and August 2019.

The study was conducted in the coastal cities of Istanbul, Kocaeli, Tekirdağ, and Yalova along the Sea of Marmara, areas affected by residential and industrial pollution. (Figure 1). The research population consisted of fishermen frequently consuming seafood in these four cities and a control group that consumed them less frequently. Since the number of individuals actively engaged in fishing cannot be established, the size of the research population is unknown. A study conducted in Spain, which established that the level of mercury in hair was 5.38 mg/kg in the general population, was used as a reference for calculating the sample size (Raposo et al. 2014). The standard deviation, which is 20% of this value, and the average hair mercury value, expected to be 10% higher in fishermen, were assumed. Sample size was calculated using the Power and Precision software program with 80% power. The analysis determined that at least 64 fishermen and 20 controls should be taken from each province.

Three hundred fifty-two individuals, 263 in the fishermen group (74.8%) and 89 in the control group (25.3%) took part in the study. Fishermen's cooperatives in each were visited for the purpose of establishing contact with fishermen. The study included male fishermen who consumed seafood at least twice a week, were aged 25 or older, had actively engaged in fishing for five years or longer, and consented to participate in the study. The control group consisted of men living in the same cities as the fishermen, consuming seafood less than once every



Figure 1. The Sea of Marmara and the regions where the research was conducted.

15 days, with similar ages to those of the fishermen (± 2 years), and not engaged in fishing.

Sampling and analytical methods

A questionnaire, prepared based on previous literature, was administered to the participants through face-to-face interviews. Subsequently, fingernail specimens were collected. The questionnaire employed as a data collection tool investigated sociodemographic and individual characteristics such as age, marital status, and education. This section also investigated health-related characteristics such as smoking and alcohol use, and the presence of joint prosthesis and amalgam dental fillings. The second section investigated the type, frequency, and amount of seafood consumed. Fingernail specimens were collected using metal-free scissors from at least four fingers, although we attempted to employ ten fingers. These specimens were then placed

into sealed plastic bags and stored in a refrigerator at -18°C until pre-washing and analysis.

The analyses were conducted at the Bülent Ecevit University Science and Technology Application and Research Center. Following the method recommended by Rodushkin and Axelsson, the nail specimens were initially washed with distilled water for 15 minutes in an ultrasonic bath. After the initial rinsing with distilled water, the specimens underwent a sequential washing process with acetone, water (three times), and acetone again. Subsequently, they were transferred into glass containers. Subsequently, they were dried overnight in a stove at 50°C (Rodushkin and Axelsson 2000a). The specimens were then labeled and placed into sealed bags. Prior to analysis the specimens were subjected to microwave solubilization. For that purpose, shredding was performed by adding 0.5 mL 65% supra-pure HNO_3 and 0.5 mL 30% supra-pure H_2O_2 to 0.05 g nail specimens placed into the dry, clean Teflon chopping chambers of a microwave. Solubilization was carried out in the microwave according to the parameters outlined in Table 1. The containers were then taken out from the device and allowed to cool to room temperature. The solubilized specimens were then prepared for analysis in an inductively coupled plasma-mass spectrometry (ICP-MS) device by being placed into 15-ml falcon tubes which were then filled with 10 ml ultra-pure water.

Element levels in the nail specimens were measured using an ICP-MS device. The device's operating conditions during the analysis were RF power 1000 W, nebulizer gas flow rate 0.99 mL/min, auxiliary gas flow rate 1.2 mL/min, lens voltage -9.75 V , and oxide rate 0.021%. The device's set-up solution and stock standards were employed in the device calibration (As^{75} , Cu^{63} , Zn^{66} , Se^{82} , Cd^{111} , Hg^{202} , Pb^{208} , Cr^{52} , Fe^{56} , Ni^{60} , and Ti^{114}). All solutions and reagents were prepared using ultra-pure water with a resistivity of $18.3\text{ M}\Omega\text{-cm}$. Ultra-pure nitric oxide and hydrogen peroxide were utilized in the specimen preparation procedures. The analyses were conducted in accordance with the ICP-MS method described by Rodushkin and Axelsson, and the results were expressed as $\mu\text{g/L}$ (ppb) (Rodushkin and Axelsson 2000b). Five-point calibration curves were established for element measurements. To ensure the accuracy of analysis, the certified reference material (CRM) (NCS ZC 81002b) were used as the quality control sample that were purchased from National Center for Standard Substances of China (Beijing, China). Recoveries of As, Cu,

Table 1. Microwave device working conditions.

Temperature (T)	Pressure (bar)	Increase rate(s)	Duration (min)	Power (W)
150	50	10	10	70
220	50	5	20	80
200	50	5	5	80
150	50	1	5	60
100	50	1	1	0

Zn, Se, Cd, Hg, Pb, Cr, Fe, Ni, and Ti were 82.7%, 74.9%, 82.8%, 98.3%, 100.2%, 103.1%, 105.6%, 91.5%, 107.5% and 87.5% respectively. The results were evaluated as dry weight. Limit of detection (LOD) and limit of quantification (LOQ) values in the nails are shown in [Table 2](#).

Statistical analysis

The research data were analyzed on SPSS version 23.0 software. Descriptive statistics were presented as arithmetic mean, median, standard deviation (SD), minimum, and maximum values for measurement variables and as number (n) and percentage (%) values for qualitative variables. Normality of distribution was assessed using the Kolmogorov–Smirnov test. Student's *t*-test was applied in the comparison of measurement variables between two independent groups when normal distribution conditions were met, or with the Mann–Whitney U-test when such conditions were not met. The chi-square test was applied in the comparison of qualitative variables in independent groups. Spearman's correlation analysis was employed to determine the relationship between two measurement variables. *p* values <0.05 were regarded as statistically significant.

Ethics approval and consent to participate

Permission for the research was obtained from the Bülent Ecevit University Clinical Research Ethical Committee (meeting no, 2018.12 dated 06.06.2018). The study aim and methods were explained in detail, and individuals agreeing to take part provided written consent by signing informed consent forms.

Results

The study involved a total of 352 individuals, including 263 fishermen and 89 controls. There was no significant age difference between the two

Table 2. Limit of detection (LOD) and limit of quantification (LOQ) values in nail samples.

Elements	LOD (µg/g)	LOQ (µg/g)
Arsenic (As)	0.007	0.012
Cadmium (Cd)	0.001	0.005
Chromium (Cr)	0.033	0.120
Copper (Cu)	0.010	0.025
Iron (Fe)	0.014	0.080
Lead (Pb)	0.011	0.038
Mercury (Hg)	0.003	0.005
Nickel (Ni)	0.010	0.024
Selenium (Se)	0.010	0.032
Titanium (Ti)	0.015	0.043
Zinc (Zn)	0.021	0.092

groups. However, the fishermen had a lower level of education than the individuals in the control group ($p < 0.001$). Higher levels of smoking and alcohol consumption were determined in the fishermen than in the control group ($p < 0.001$ for both). No significant difference was determined between the fishermen and control group in terms of the presence of joint prosthesis or amalgam tooth filling. The sociodemographic and health characteristics of the fishermen and control group are shown in Table 3.

The fishermen had consumed 9340.4 ± 6644.5 g seafood in the previous month, and the controls 326.4 ± 316.9 g ($p < 0.001$). Median frequencies of seafood consumption in the previous month were 3-6 meals a week among the fishermen and one meal a month in the controls, the difference being statistically significant ($p < 0.001$).

Among the 11 essential and toxic elements measured in nail specimens, zinc and iron levels were found to be higher in the fisherman group compared to the control group. Nail zinc levels were 112.78 ± 41.18 $\mu\text{g/g}$ in the fishermen group and 98.87 ± 36.59 $\mu\text{g/g}$ in the control group ($p = 0.005$), while nail iron levels were 78.98 ± 44.85 $\mu\text{g/g}$ in the fishermen group and 62.87 ± 38.53 $\mu\text{g/g}$ in the control group ($p = 0.004$). In contrast, arsenic, nickel, and chromium levels were higher in the control group (respectively; $p = 0.002$, $p = 0.008$, $p = 0.002$). No significant difference was determined between the groups in terms of the other elements. Element levels detected in nail samples from the fishermen and control groups are shown in Table 4.

No significant differences were determined in nail mercury levels between the fishermen and control groups when the members were

Table 3. The sociodemographic and health characteristics of the fishermen and control group.

	Fishermen (<i>n</i> = 263)		Control (<i>n</i> = 89)		<i>p</i> value
	<i>n</i>	%	<i>n</i>	%	
Age (years) (<i>mean</i> \pm <i>SD</i>)	53.5 \pm 13.3		52.0 \pm 12.8		0.331
Marital status					
Married	215	81.7	77	86.5	0.384
Single	48	18.3	12	13.5	
Education					<0.001
Elementary school graduate or lower	132	50.2	32	36.0	
Middle school graduate	48	18.3	5	5.6	
High school graduate	63	24.0	16	18.0	
University graduate or above	20	7.6	36	40.4	
Smoking status					<0.001
Smoker	163	62.0	37	41.6	
Former smoker	60	22.8	23	25.8	
Non-smoker	40	15.2	29	32.6	
Alcohol use					<0.001
User	126	47.9	14	15.7	
Former user	12	4.6	1	1.1	
Non-user	125	47.5	74	83.1	
Joint prosthesis					
Yes	12	4.6	2	2.2	0.531
No	251	95.4	87	97.8	
Amalgam tooth filling					
Yes	52	19.8	20	22.5	0.694
No	211	80.2	69	77.5	

subdivided into those with and without amalgam dental filling. Similarly, no significant differences were observed between the fisherman and control groups' nail cadmium and lead levels when the non-smoker/former smokers and smokers were evaluated separately. However, nail chromium levels were higher in the control group when the participants with joint prosthesis were evaluated ($p=0.003$). No significant difference was observed between the nail chromium levels of the participants with joint prosthesis in the two groups. Hg, Cd, Cr and Pb levels of fishermen and control groups according to some characteristics ($\mu\text{g/g}$) is shown in Table 5.

Correlations between the fishermen's nail element levels and their monthly seafood consumption were also examined. Positive correlations were observed in the fishermen group between the amount of seafood consumed monthly and nail iron ($r=0.138$; $p=0.003$), titanium ($r=0.181$; $p=0.003$), cadmium ($r=0.175$; $p=0.005$), chromium ($r=0.158$; $p=0.011$), selenium ($r=0.158$; $p=0.011$), arsenic ($r=0.154$; $p=0.013$), lead ($r=0.147$; $p=0.018$), nickel ($r=0.147$; $p=0.018$), and mercury ($r=0.128$; $p=0.039$) levels. Correlations between the fishermen's monthly seafood consumption and nail element levels are shown in Figure 2.

Table 4. Element levels in nail samples from the fishermen and control groups ($\mu\text{g/g}$).

	Fishermen (n=263)		Control (n=89)		p value
	Mean \pm SD	Min - Max	Mean \pm SD	Min- Max	
Arsenic (As)	0.276 \pm 0.128	0.053-0.994	0.336 \pm 0.166	0.062-0.943	0.002
Cadmium (Cd)	0.133 \pm 0.089	0.006-0.390	0.130 \pm 0.067	0.008-0.308	0.858
Chromium (Cr)	0.856 \pm 0.397	0.259-2.422	1.043 \pm 0.525	0.294-3.108	0.002
Copper (Cu)	10.21 \pm 3.14	3.80-21.90	10.50 \pm 2.73	5.80-16.60	0.345
Iron (Fe)	78.98 \pm 44.85	11.00-196.00	62.87 \pm 38.53	8.00-188.00	0.004
Lead (Pb)	2.160 \pm 1.145	0.200-4.730	2.356 \pm 1.142	0.440-4.490	0.153
Mercury (Hg)	0.053 \pm 0.043	0.004-0.273	0.045 \pm 0.040	0.003-0.252	0.069
Nickel (Ni)	0.501 \pm 0.377	0.020-2.580	0.641 \pm 0.512	0.030-3.080	0.008
Selenium (Se)	0.782 \pm 0.215	0.290-1.500	0.820 \pm 0.211	0.380-1.260	0.145
Titanium (Ti)	4.991 \pm 3.135	0.360-13.540	5.075 \pm 2.470	0.360-11.000	0.443
Zinc (Zn)	112.78 \pm 41.18	34.00-207.00	98.87 \pm 36.59	36.00-197.00	0.005

Table 5. Hg, Cd, Cr And Pb levels of fishermen and control groups according to some characteristics ($\mu\text{g/g}$).

Element	Fishermen group			Control group			p value
	n	Mean	SD	n	Mean	SD	
Mercury (Hg)							
No amalgam filling	211	0.051	0.040	69	0.046	0.044	0.061
With amalgam filling	52	0.060	0.055	20	0.042	0.020	0.674
Cadmium (Cd)							
Non-smoker/former smoker	100	0.131	0.089	52	0.136	0.065	0.668
Smoker	163	0.135	0.089	37	0.121	0.070	0.470
Chromium (Cr)							
No joint prosthesis	251	0.859	0.400	87	1.043	0.531	0.003
With joint prosthesis	12	0.787	0.349	2	1.071	0.495	0.235
Lead (Pb)							
Non-smoker/former smoker	100	2.168	1.151	52	2.217	1.151	0.746
Smoker	163	2.155	1.144	37	2.553	1.115	0.059

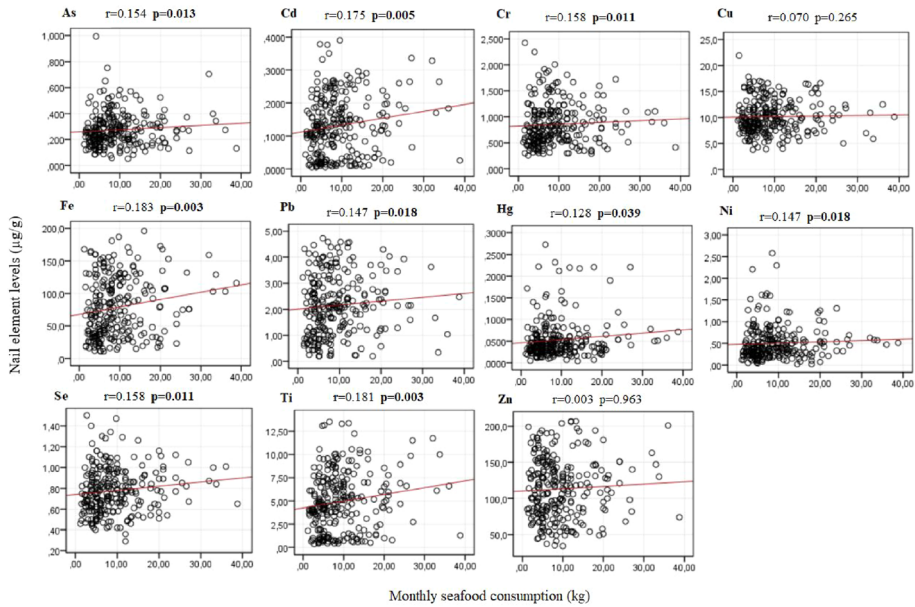


Figure 2. Correlations between the fishermen's monthly seafood consumption and nail element levels.

Discussion

The increase in the diversity of chemical pollution in the environment together with the increase in industrial activities necessitates the use of a wide element panel in monitoring studies. The present research determined the levels of 11 different elements in nail specimens from a fisherman group frequently consuming seafood and a control group with a lower level of such consumption.

Fishermen generally consume seafood they catch themselves in the Sea of Marmara, while participants from the control group can consume seafood from different seas and fresh waters. Correlations between element levels and the amount of seafood consumption were evaluated only in the fishermen group in this study, since we thought that this would better reflect the element burden involved in exposure to marine products from the Sea of Marmara. This analysis revealed positive correlation between the amount of seafood consumed and fishermen's arsenic, mercury, iron, cadmium, chromium, lead, nickel, selenium, and titanium levels. This finding suggests that the consumption of products from that sea can lead to an increase in the burden of several elements.

Exposure to mercury in the general population frequently develops in association with the consumption of seafood from waters with high methylmercury levels (ATSDR 2024). Studies have reported higher mercury levels in biological materials from groups with high seafood

consumption compared to groups lower seafood consumption (Al-Majed and Preston 2000; Andrew et al. 2019; Bates et al. 2007; Johnsson et al. 2004; Kim and Lee 2010). Similarly, two studies from Turkey showed that mercury levels were higher in people with higher in people who consume seafood more frequently (Doğan-Sağlamtimur and Kumbur 2010; Vural and Ünlü 1996). In this study, it was determined that there was no significant difference between the groups' nail mercury levels. However, a positive correlation was observed between the fishermen's nail mercury levels and monthly seafood consumption. Nail mercury levels exceeding $1\ \mu\text{g/g}$ are regarded as an indicator of high exposure to mercury, that level was not exceeded in any of the participants in the present study (Mayo Clinic Laboratories 2024a). This shows that frequent seafood consumption was not associated with high dose mercury exposure in our participants. However, mercury levels in fishermen increased in line with the amount of seafood consumed, showing that greater consumption can lead to an increase in the mercury burden. One of the principal sources of mercury exposure in humans is the use of amalgam dental fillings (ATSDR 2024). The fact that no difference was observed between the fishermen and control groups in terms of the use of amalgam dental filling, and that this finding remained unchanged following stratification based on amalgam use, shows that the finding obtained is not associated with amalgam use.

The cadmium burden may rise in individuals who regularly consume seafood (ATSDR 2012). Studies from the USA and Japan have shown an association between blood cadmium levels and the amount of such products consumed (Hovinga, Sowers, and Humphrey 1993; Ilmiawati et al. 2015). However, other studies from Spain, Brazil, and South Korea have reported no significant difference in cadmium levels according to amounts of seafood consumed (Anwar 2005; Freire et al. 2015; Gonzalez-Reimers et al. 2014; Moon et al. 2014; Takeda et al. 2017). The study by Ayyub et al. highlighted that the WHO criterion for cadmium in nails is $0.2\ \mu\text{g/g}$ (Ayyub et al. 2013). No significant difference was observed in nail cadmium levels between the groups in this research. In addition, the groups' mean nail cadmium levels met WHO criteria. However, a positive correlation was determined between nail cadmium levels and the amount of seafood consumed monthly among the fishermen. This is an important finding in terms of reflecting a cadmium burden that may develop in association with higher consumption. Smoking causes an increase in cadmium levels in biological materials (Telisman et al. 1997; Wolfspurger et al. 1994). However, our analyses revealed no difference between the groups among participants who did not smoke or who had quit, thus eliminating the possibility that the results obtained were due to smoking.

Lead is an element that is widely present in nature and that is highly toxic to humans (Jaishankar et al. 2014). Studies have shown that lead levels in seafood in areas close to industrial facilities exceed normal limits (Aksu et al. 2011; Has-Schön, Bogut, and Strelec 2006). A study from the USA reported higher lead levels in individuals with greater seafood consumption, while research from Brazil, Spain, Pakistan, and Lebanon showed higher levels in biological materials from groups that frequently consumed seafood (Anwar 2005; Gonzalez-Reimers et al. 2014; Hovinga, Sowers, and Humphrey 1993; Salameh, Bouchy, and Geahchan 2008; Takeda et al. 2017). A study from the province of Mersin in Türkiye also observed higher hair lead levels in individuals regularly consuming fish compared to those who never consumed it, and concluded that this was at a level such as to represent a health risk (Doğan-Sağlamtimur and Kumbur 2010). While nail lead levels are expected to be below 4 µg/g in the general population, levels above 10 µg/g may indicate lead exposure (Mayo Clinic Laboratories 2024b). No difference in nail lead levels was observed between the groups in the present research. It was also found that nail lead levels in both groups were within the range expected in the general population and that none of the participants were at risk of high dose lead exposure. Although the absence of a difference in nail lead levels between the groups suggests that seafood consumption is not linked to lead levels in humans, the positive correlation observed between the amount of seafood consumption and nail lead levels in the fishermen shows that greater consumption can increase the lead burden. Lead levels in blood samples from smokers can increase due to the presence of lead in cigarette smoke (ATSDR 2020). However, no significant difference was found between the groups in the present research according to stratification based on smoking.

The main source of arsenic, another element toxic to humans, is water and foodstuffs, particularly seafood (ATSDR 2007). Studies from such different countries as Norway, Croatia, Italy, Egypt, and Puerto Rico have reported a positive relationship between arsenic levels in biological materials and amounts of fish consumption (Brantsæter et al. 2010; Mansilla-Rivera et al. 2014; Meltzer et al. 2002; Miklavčič et al. 2013; Saad and Hassanien 2001; Slotnick et al. 2007). On the other hand, studies from Nevada, Japan, and Pakistan have reported similar nail arsenic levels in groups with different levels of seafood consumption (Anwar 2005; Calderon et al. 2013; Tabata et al. 2006). A level of arsenic in the fingernails of more than 1 µg/g is considered to be an indicator of high dose exposure (ATSDR 2007). Although the arsenic level in the control was higher than that in the fishermen group in the present research, nail levels in both groups were consistent with that in the general population, and no participant was identified as being at risk of high-dose exposure.

The higher nail arsenic level in the control group suggests the involvement of different environmental exposure factors in those individuals. The fact that nail arsenic levels in this study were not higher in the fishermen group and that these individuals' nail arsenic levels were consistent with that of the general population shows that seafood consumption does not lead to an arsenic burden capable of constituting a risk. On the other hand, the positive correlation between monthly seafood consumption and nail arsenic levels in the fishermen group represents evidence that such consumption can increase arsenic intake.

Excessive intake of iron, an essential element due to its functions in metabolism, can result in toxic effects (Abbaspour, Hurrell, and Kelishadi 2014). Seafood is one of the main sources of iron (IOM 2003). However, a study from Spain reported no relationship between the frequency of fish consumption and hair iron levels (Gonzalez-Reimers et al. 2008). Conversely, in this study, nail iron levels were found to be higher in the fishermen group compared to the control group. Moreover, fishermen's nail iron levels rose in line with the amount of seafood consumed per month. The higher nail iron levels in the fishermen may be associated with the essential element iron being abundantly present in seafood. This would seem to be confirmed by the positive correlation between nail iron levels and the monthly amount of seafood consumed. However, nail iron levels in the fishermen and control groups were in a similar range to that reported by Rodushkin and Axelsson in the general population (Rodushkin and Axelsson 2000b). All these findings show that seafood consumption can increase iron intake, but not to the extent that it poses a risk of exposure.

Foodstuffs, and particularly seafood, are the main source of the essential element zinc (ATSDR 2005). While a study from Spain observed a higher hair zinc levels among frequent fish-consumers compared to individuals who consumed it less frequently (Gonzalez-Reimers et al. 2014), studies from Greece and Turkey have reported no difference in zinc levels between groups with varying frequencies of fish consumption (Arvanitidou et al. 2007; Voskaki et al. 2010; Doğan-Sağlamtimur and Kumbur 2010). Average nail zinc levels in the general population are estimated to be in the range of 129-179 $\mu\text{g/g}$ (ATSDR 2005). In the current research, nail zinc levels were found to be higher in the fishermen group. However, despite the higher nail zinc level in the fishermen group, the values in both groups were lower than that expected in the general population. In addition, no association was found between the amount of seafood consumed in the fishermen group and nail zinc levels at correlation analysis. Considering that levels of zinc, another essential element, were lower in both study groups than in the general population, it may be concluded that seafood consumption produces no burden in

terms of exposure, and that, on the contrary, zinc intake was inadequate in both groups.

Although the amount of copper ingested through seafood represents only a very small part of copper intake in humans, the copper burden in seafood from contaminated seas is increasing (Stern et al. 2007). In a study from Spain in 2008, no association was found between the frequency of fish consumption and hair copper levels (Gonzalez-Reimers et al. 2008). However, in another study from 2014, those authors reported higher hair copper levels in a group that frequently consumed fish compared to a group with less frequent consumption (Gonzalez-Reimers et al. 2014). On the other hand, a study from Greece reported higher serum copper levels among frequent fish consumers compared to those who consumed it rarely (Arvanitidou et al. 2007; Voskaki et al. 2010). In a study of human tissues, the average copper level of nails was found to be $18.1\mu\text{g/g}$ (ATSDR 2004). No difference in nail copper levels was observed between the groups in the present study. Similarly, correlation analysis also revealed no association between the amount of seafood consumed monthly and nail copper levels in the fishermen group, and the levels in both groups were lower than those in the general population. These findings all show that the consumption of seafood does not constitute a risk in terms of copper exposure and that, on the contrary, intake of copper, like zinc an essential element, is insufficient.

The effect of seafood on chromium levels in humans has not to date been adequately investigated, although a study from Tunisia reported similar blood chromium levels in groups with different frequencies of consumption (Khlifi et al. 2014). Chromium levels in the present study were higher in the control group than among the fishermen. However, nail chromium levels in the fishermen increased in line with the amount of seafood consumed. The average nail chromium levels in the normal population can vary between $0.52\text{-}1.3\mu\text{g/g}$ and it is seen that the nail chromium levels of both groups are consistent with the literature (ATSDR 2012). According to all the findings, although consumption of seafood may increase chromium levels, this increase is not at a risky level. In addition, different sources may increase the chromium levels in the control group. Chromium levels can rise in individuals who with joint prosthesis due to the possibility of chromium being present in them (ATSDR 2012). When the participants' nail chromium levels were stratified according to prosthesis use, the high chromium level in the control group was maintained among the individuals without joint prosthesis, while the difference between the two groups disappeared among those with such prosthesis. These findings show that nail chromium levels may have increased in association with factors other than joint prosthesis and seafood consumption.

A study from Tunisia reported no difference in blood nickel levels among groups with different seafood consumption frequencies (Khlifi et al. 2014). A study from Spain determined a positive correlation between seafood consumption frequency and urine nickel levels in children and adolescents living in the industrialized province of Huelva, while no such relationship was reported in other, less industrialized regions of Andalusia. These results show that seafood can lead to nickel exposure on a regional basis (Aguilera et al. 2010). Nail nickel levels in this study were higher in the control group than in the fishermen group. This shows that other factors capable of causing nickel exposure apply to the individuals in the control group. Nonetheless, it has been shown that nail nickel levels vary between 1.1-3.9 $\mu\text{g/g}$ in data from different countries, and our study found that the nail nickel levels of the two groups were consistent with this (ATSDR 2005). Based on these findings, it may be concluded that the participants were not at risk in terms of nickel exposure. However, nail nickel levels rising in line with the amount of monthly seafood consumption shows that such consumption can affect degrees of exposure to this element.

Foodstuffs, particularly seafood, are the principal source of intake of the essential element selenium (Filippini et al. 2018). Studies from different countries have shown a positive correlation between selenium levels and seafood consumption (Birgisdottir et al. 2013; Brantsæter et al. 2010; Miklavčič et al. 2013; Rasmussen et al. 2009). However, while some studies have reported no association between selenium levels and seafood consumption (Bárány et al. 2003; Buscemi et al. 2014; Fang et al. 2012; Skalny et al. 2019), a study from Italy observed a negative correlation between nail selenium levels and fish and marine product consumption (Filippini et al. 2017). Studies have found that the normal nail selenium level is between 0.54-1.56 $\mu\text{g/g}$ (ATSDR 2003). No difference was determined between the groups' nail selenium levels in this research. Conversely, correlation analysis revealed a positive correlation between the monthly amount of seafood consumption and nail selenium levels. The two groups' nail selenium levels were also consistent with the previous literature. These findings suggest that seafood consumption may contribute to selenium intake, but does not lead to a risk in terms of exposure.

The relationship between titanium levels in humans and seafood consumption has not been sufficiently investigated. One study involving children reported a positive correlation between fish consumption and hair titanium levels (Oyoo-Okoth et al. 2013). However, a study of women from China reported no correlation between hair titanium levels and fish or meat consumption (Li et al. 2016). No difference was determined in terms of nail titanium levels between the fishermen and control groups in the present study. In addition, the fact that the two groups' nail

titanium levels were consistent with the previous literature suggests that exposure to titanium associated with seafood consumption is not at a sufficient level to pose a risk (Rodushkin and Axelsson 2000b). Nonetheless, the correlation between the amount of seafood consumed monthly and nail titanium levels in the fishermen shows that such consumption can raise titanium intake.

Conclusions

Zinc and iron levels in nail specimens were higher in the fishermen group in this research, while arsenic, chromium, and nickel levels were higher in the control group. The amount of seafood consumed monthly was positively correlated with the fishermen's iron, titanium, cadmium, arsenic, lead, mercury, chrome, and selenium levels in nail specimens.

The results of this research show that the consumption of seafood from this region does not result in sufficient elemental exposure to pose a risk. Considering that the fishermen generally consumed seafood they had caught themselves, greater consumption of these products may lead to an increased burden for some elements in fishermen. Identifying sources of increased pollution in the Sea of Marmara and taking the requisite precautions will make it possible to prevent elemental exposures. These measures will both reduce existing pollution and also prevent possible future pollution.

Acknowledgments

We would like to thank all participants who took in this research and all health personnel who contributed.

Disclosure statement

No potential competing interest was reported by the authors.










Funding

This work was supported by the Scientific and Technological Research Council of Turkey (TÜBİTAK) and was conducted as part of the 'Heavy Metal-Trace Element Load and Risk Mapping in Seafood and Fisheries' project (no. 116S520).

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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