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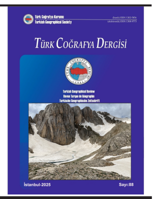

Potentially toxic element pollution records in the Mediterranean mussel (*Mytilus galloprovincialis*) along the Çanakkale Strait, Türkiye

Çanakkale Boğazı boyunca Akdeniz midyesinde (Mytilus galloprovincialis) potansiyel toksik element kirliliği kayıtları, Türkiye

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A B S T R A C T / Ö Z

In this study, the Mediterranean mussel *Mytilus galloprovincialis*, which can accumulate potentially toxic elements (PTEs) in its tissues, was used as a bioindicator. The main objective of the study was to determine the records of PTEs such as Cr, Pb, Cd, Ni, Co, Fe, Cu, Hg, As, Mn, and Zn in mussel samples collected from five stations along the eastern shores of the Çanakkale Strait. PTEs in the samples were examined using Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES), and common and different sources of the pollutants were addressed using correlation and principal component analysis (PCA). The obtained data indicate the presence of As well above the limit values at all sampling stations. Correlation and PCA analyses indicate strong positive correlations between some PTEs, such as Cr-Ni and Fe-Cu, indicating that PTEs originate from common anthropogenic sources. Cd is negatively correlated with As and Zn, pointing to a different source. According to ICP-OES analyses, the PTEs at the stations studied are ranked according to average values as follows: Al>Fe>As>Mn>Cu>Zn>Cr>Ni>Pb>Cd>Hg. The PTEs that pose the most risk are As, Pb, Ni, Cr, Cu, and Zn. Ferry transport, chemicals used in agricultural activities, and municipal waste are likely to be the main contributors to the PTE load.

Bu çalışmada, dokularında potansiyel olarak toksik elementleri (PTE) biriktirebilen Akdeniz midyesi Mytilus galloprovincialis biyoindikatör olarak kullanılmıştır. Çalışmanın ana amacı, Çanakkale Boğazı'nın doğu kıyılarındaki beş istasyondan toplanan midye örneklerinde Cr, Pb, Cd, Ni, Co, Fe, Cu, Hg, As, Mn ve Zn gibi PTE'lerin kayıtlarını belirlemektir. Örneklerdeki PTE'ler, İndüktif Eşleşmiş Plazma-Optik Emisyon Spektrometresi (ICP-OES) kullanılarak incelendi ve kirlilik kaynaklarının ortak ve farklı özellikleri, korelasyon ve temel bileşen analizi (PCA) kullanılarak ele alındı. Elde edilen veriler, tüm örnekleme istasyonlarında sınır değerlerin oldukça üzerinde As varlığını göstermektedir. Korelasyon ve PCA analizleri, Cr-Ni ve Fe-Cu gibi bazı PTE'ler arasında güçlü pozitif korelasyonlar olduğunu göstermektedir. Bu da PTE'lerin ortak antropojenik kaynaklardan kaynaklandığını göstermektedir. Cd, As ve Zn ile negatif korelasyon göstererek farklı bir kaynağa işaret etmektedir. ICP-OES analizlerine göre, incelenen istasyonlardaki PTE'ler ortalama değerlere göre şu şekilde sıralanmıştır: Al>Fe>As>Mn>Cu>Zn>Cr>Ni>Pb>Cd>Hg. En fazla risk oluşturan PTE'ler As, Pb, Ni, Cr, Cu ve Zn'dir. Feribot taşımacılığı, tarımsal faaliyetlerde kullanılan kimyasallar ve belediyelere ait atıklar, PTE yükünün ana kaynakları olmalıdır.

1. Introduction

More than 80% of wastewater generated by human activities is discharged into rivers and oceans without treatment, significantly contributing to pollution (Lin et al., 2020). Potentially toxic elements (PTEs), on the one hand, lead to water pollution (Chowdhary et al., 2022). There is a significant relationship between PTE-induced water pollution and gastrointestinal diseases, skin disorders, cancer, and childhood illnesses (Lin et al., 2020). Comprehensive experimental studies on the effects of pesticides on the biodiversity of aquatic communities have documented that these chemicals produce different effects on various species (Relyea, 2005). Experimental studies directly applied to *M. galloprovincialis* have shown that these chemicals pose significant ecotoxicological risks to mollusk cells and tissues (Matozzo et al., 2019). In Tyler-Walters et al. (2022), lethal and sub-lethal effects of pesticides and hydrocarbons on mussels, which are sensitive to these contaminants, were discussed in detail with respect to larval development, byssus thread production, growth, and metabolism.

In addition to industrial, urban, and agricultural waste and pollutants, fertilizers applied in agriculture and pesticides used in pest control also cause water pollution (Parris, 2011). These polluted groundwater and surface waters naturally mix with rivers, lakes, and marine environments. Therefore, the consumption of contaminated mussels inhabiting coastal areas that store PTEs for long periods may lead to health problems (Yiğit et al., 2018). Mussels are widely used as bioindicators and biomonitors (Rayment & Barry, 2000; Storelli et al., 2000; Kouali et al., 2020; Mora et al., 2004). These organisms, which can rapidly reach sufficient size and weight for analysis, are frequently preferred in pollution studies due to their strong shells and resistance to external threats.

The widespread use of *M. galloprovincialis*, also known as the black mussel or Mediterranean mussel and commonly consumed along the Turkish coasts, has increased in ecological and biological pollution analyses due to its abundance (Uysal, 1970; Bat et al., 1999, 2012; Çayır et al., 2012; Bilgin & Uluturhan, 2015; Balcıoğlu & Gönülal, 2017; Demir & Akkuş, 2018; Çolakoğlu et al., 2019; Gökkuş & Berber, 2019; Çınar et al., 2020; Tosun et al., 2021; Türkoğlu et al., 2023; Doğruyol et al., 2024).

The coasts of the Çanakkale Strait (ÇS) are under intense pressure from pollution loads caused by maritime traffic, intensive agricultural activities in surrounding deltas, waste from residential areas, and pollution originating from tourism destinations. Various studies have been conducted on the pollution caused by this anthropogenic pressure throughout the strait's waters and coastal-river sediments (Ilgar & Güven, 2007; Koçum & Dursun, 2007; Ustunada et al., 2011; Özden, 2013; Özden & Tunçer, 2015; Ilgar, 2017; Akarsu et al., 2022; Çavuş et al., 2023; Oran & Erginal, 2023). This study presents current data on PTE concentrations in mussel samples collected from five localities along the eastern coast of the strait, including statistical analyses.

2. Materials and Methods

The study area comprises the eastern coasts of the ÇS, an international waterway between the Mediterranean and the Black Sea, extending along the Biga Peninsula (Figure 1). The ÇS is located between 40°02′–40°30′ North latitude and 26°10′–26°45′ East longitude. The coastline extends 78 km along the western shore (Gelibolu Peninsula) and 94 km along the eastern shore (Biga Peninsula), with the narrowest point being 1,200 meters, the deepest point 8,275 meters, an average depth of 60 meters, and a maximum depth of 106 meters (Ilgar, 2002). The area exhibits a transitional zone between Mediterranean and Black Sea climate types. According to 92 years of meteorological data recorded at the Çanakkale Meteorological Station from 1929 to 2021, the average temperature is 15.22°C, and the annual average precipitation is 625 mm.

Mussel samples were collected from five stations: Çardak coastal spit (S1), Çardak lagoon mouth (S2), Kemiklialan village fishing pier (S3), Kepez port (S4), and the coastal area of central Çanakkale (S5). Sampling locations are shown in Figure 1. The mussel samples, stored in polyethylene bags, were analyzed using ICP-OES at the laboratory of Burdur Mehmet Akif Ersoy University, Türkiye. To determine the total PTEs (Cr, Pb, Cd, Ni, Fe, Cu, Hg, As, Al, Mn, and Zn) concentration in the organism, and considering the natural variability of PTE accumulation across different tissues, the entire soft tissue was used instead of a specific part (Rainbow, 1998). During sample preparation, 0.5 g of the sample from each station was digested in 5 mL of 65% HNO_3 and 3 mL of 40% HF. ICP-OES analysis was performed using the Perkin Elmer ICP-OES Optima 8000 and Milestone Stard-D devices. PCA was used to reduce the dimensionality of the dataset and identify the major components contributing to the variance. Pearson correlation analysis (Wilks, 2011) was applied to evaluate the strength and direction of relationships between variables.

3. Results and Discussion

3.1. PTE Contents in Mussels

The ICP-OES analysis results of the mussel samples are presented in Table 1, expressed in mg/kg. According to average values, the PTEs are ranked in descending order as follows: Al>Fe>As>Mn>Cu>Zn>Cr>Ni>Pb>Cd>Hg.

S1 was taken from the beach facing the strait at the Çardak coastal spit located in the northeastern part of the ÇS. In samples collected from mussels colonizing a tire at the ferry pier, the order of abundance of PTEs is as follows: Fe>Al>Cu>Cr>Ni>Zn>Pb>As>Cd>Mn>Hg. In this sample, notable PTEs are Pb of 1.7 mg/kg and Ni of 3.1 mg/kg. Especially, the Ni concentration of 3.1 mg/kg is well above averages, while Pb reached the highest value among all samples. These data indicate that station S1 is exposed to contamination pressure mainly from Ni and Pb, and relatively from Cr and Cu. The pollution at the tent camping area on the coastal spit and the use of insecticides and pesticides in this area to control flies and insects have previously been reported to pose an ecological risk (Çalışkan et al., 2011).

In S2, collected near the channel where the waters of Çardak Lagoon mix with the waters of the ÇS, PTEs are ranked in descending order as Fe>Al>As>Cu>Zn>Cr>Ni>Pb>Mn>Cd>Hg. The

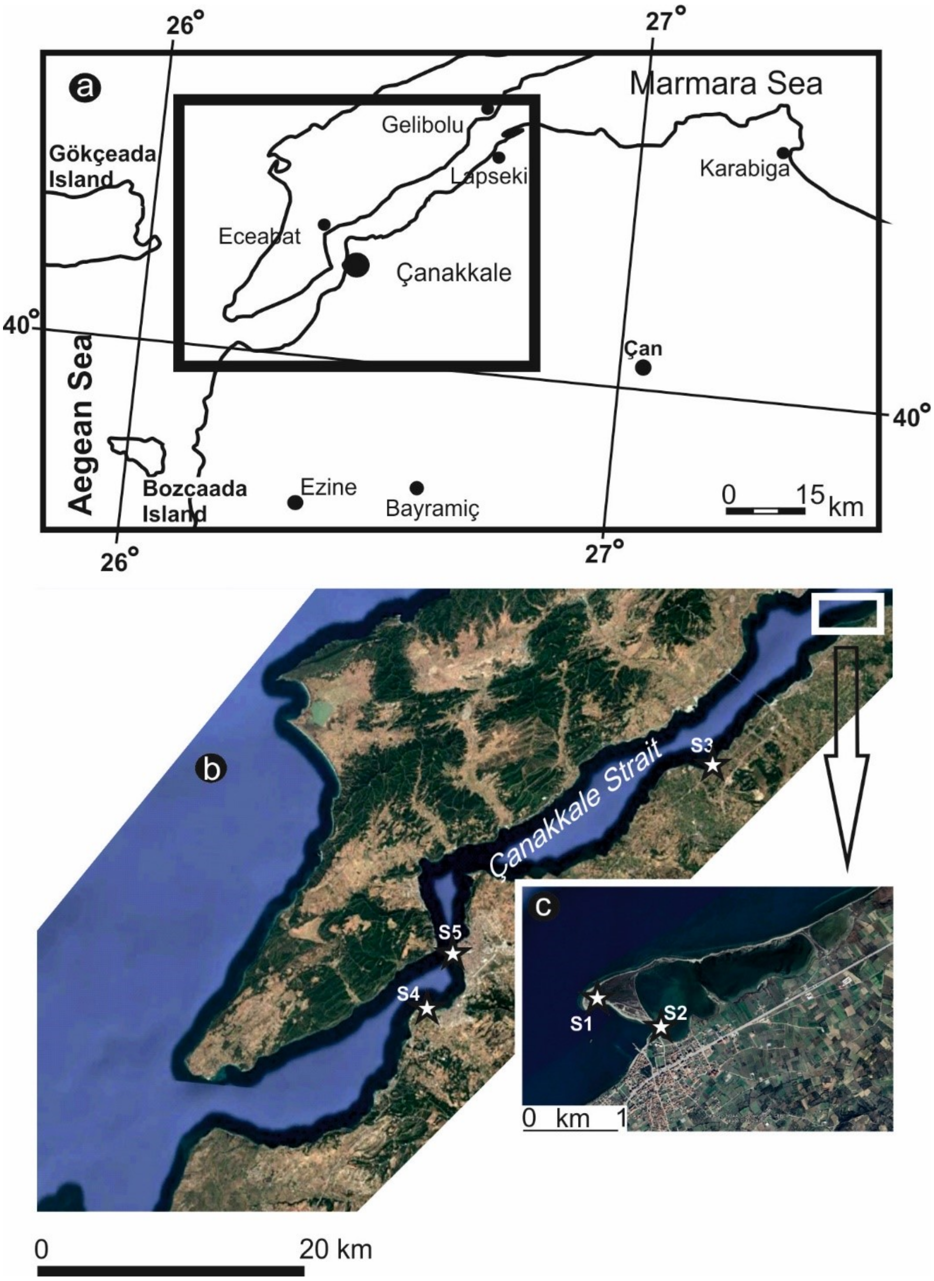

Figure 1. Location map of the study area (a) and sampling points on the Google Earth satellite image (b, c). Stars and numbers indicate the sampling sites.

Table 1. ICP-OES analysis results obtained from M. galloprovincialis samples.

Elements (mg/kg) Sample No	Cr	Pb	Cd	Ni	Fe	Cu	Hg	As	Al	Mn	Zn
S1	4.6	1.7	0.5	3.1	94.8	7.3	0.1	0.69	48.7	0.44	2.3
S2	1.4	0.8	0.2	1.1	18.7	2.9	-	9.78	13.1	0.32	2.7
S3	3	1.7	0.1	0.4	62.8	5.5	-	6.85	102	3.85	4.2
S4	8.8	1.7	0.1	4.9	68.5	4.7	-	11.3	75.5	4.9	5.1
S5	1.2	1.4	0.1	0.6	17.1	3.7	-	13.1	26	16.1	6
Average	3.8	1.46	0.2	2.02	52.38	4.82	0.02	8.34	53.06	5.12	4.06

most important PTE at this station, As, is at a very high concentration of 9.78 mg/kg, which is well above the overall average of 8.34 mg/kg. The Cd and Hg enrichment observed in sediments of Çardak Lagoon by Kükrer et al. (2019) was not detected in this sample.

In S3, taken from the fishing pier of Kemiklialan Village, PTEs are ranked in descending order as Al>Fe>Cu>Zn>Mn>As>Cr>Pb>Ni>Cd>Hg. The detection of 6.85 mg/kg As and 1.7 mg/kg Pb in S3 is important in terms of toxic risk. Compared to all stations, Al was found at a very high concentration of 102 mg/kg.

S4, located south of Çanakkale, was collected from the coastal area near Kepez Port, where Kepez Stream flows into the sea. This area is under pressure from the densely populated Kepez settlement, summer residences located on the delta, intensive fruit cultivation, and coastal fishing activities. PTEs are ranked in descending order as follows: Al>Fe>Cr>As>Zn=Mn=Ni>Cu>Pb>Cd>Hg. As exhibits with a value of 11.3 mg/kg. Ecological risk analysis of sediment samples taken from the section of Kepez Stream near the port showed moderate enrichment for Zn, As, and Co; significant enrichment for Pb; very high enrichment for Cr; and extreme enrichment for Ni, which poses a high potential ecological risk (Çavuş et al., 2023). Therefore, the mussel PTEs data show similarities with the As and Zn concentrations in Kepez Stream sediments. On the other hand, wastewater from ships and oil spills may have contributed to the input of lead into the water (Boran et al., 2018). Especially when compared with studies on the same mussel species in this coastal zone, an increase has been observed compared to the Ni and Cr values obtained by Çayır et al. (2012) and the Pb and Cu values by Demir et al. (2018). This explains the continuing increase of pollution pressure even today.

S5, taken from the mouth of Sarıçay, a pollution-loaded stream flowing into the ÇS and passing through the densely populated city of Çanakkale, has a somewhat different PTE distribution as follows: Al>Fe>Mn>As>Zn>Cu>Pb>Cr>Ni>Cd>Hg. Here, the concentration of As (13.127 mg/kg) is notable. Recent ecological risk analyses conducted on the sediments of Sarıçay have identified moderate risk for Cd and very high risks for Ni and Pb (Akarsu et al., 2022). The presence of Pb enrichment in the mussel PTE data is also an important point, as it explains metal pollution alongside As. The Cu (7.7 mg/kg) and Zn (6.2 mg/kg) values determined in this sample are the highest compared to all stations. This highlights Sarıçay's function as a conduit for PTEs derived from urban wastewater sources.

The common finding in all samples is the presence of As at levels significantly above the limit values. Microbiological studies on the bacterial load of samples taken from the same mussel species on both sides of the ÇS have shown that mussels from stations similar to those in this study have a high bacterial load (Şener et al., 2013; Tosun et al., 2021). The relationship between the pathogens identified in the surface waters of Sarıçay and the wastewater used for irrigation purposes (Yüksek, 2003) has revealed the impact of domestic discharges and sewage waste on the deterioration of water quality. The relationship of pollution in the inland waters of Çanakkale with industry, agriculture, and wastewater has also been previously reported (Akbulut et al., 2006).

3.2. Correlation and PCA Analysis of PTEs

Pearson correlation analysis was performed to evaluate the relationships among the analyzed PTEs accumulated in the black mussel samples. This analysis particularly reveals the presence of strong and significant correlations between certain PTEs. Notably, there is a high positive correlation between Cr and Ni ($r = 0.933$, $p < 0.05$) and between Fe and Cu ($r = 0.935$, $p < 0.05$). According to this correlation, these PTEs are likely derived from a common source. Additionally, the high correlations of Pb with Fe ($r = 0.775$), Cu ($r = 0.776$), and Al ($r = 0.778$) indicate that they move together (Table 2).

According to the correlation analysis, the important PTE, Cd, shows strong negative correlations with As ($r = -0.868$) and Zn ($r = -0.774$). In other words, Cd behaves differently from the other PTEs and is likely derived from a different source. A high positive correlation between Mn and Zn ($r = 0.880$, $p < 0.05$) also indicates that they have a common origin or source.

Table 2. Pearson correlation coefficients among PTEs in *M. galloprovincialis* samples.

Correlations											
	Cr	Pb	Cd	Ni	Fe	Cu	Hg	As	Al	Mn	Zn
Cr	1										
Pb	.063	1									
Cd	.037	.111	1								
Ni	.933*	.415	.254	1							
Fe	.664	.775	.582	.600	1						
Cu	.395	.776	.679	.349	.935*	1					
Hg	.b	.b	.b	.b	.b	.b	.b				
As	-.099	-.358	-.868	-.144	-.777	-.861	.b	1			
Al	.523	.778	-.229	.199	.612	.515	.b	-.227	1		
Mn	-.259	.102	-.525	-.296	-.506	-.353	.b	.656	-.162	1	
Zn	.097	.262	-.774	-.053	-.374	-.371	.b	.776	.175	.880*	1

*. Correlation is statistically significant at the 5% significance level (two-tailed test).
. Could not be calculated because at least one variable was constant.

On the other hand, due to the low amount of Hg and insufficient data, a correlation coefficient could not be obtained.

PCA, a multivariate technique used both to reduce dimensionality and to understand and cluster patterns among variables, was applied following the correlation analysis. Figure 2 shows two principal components explaining a total variance of 78.94% (PC1: 50.69%; PC2: 28.25%). In the PC plane, the PTEs Cu, Fe, Ni, Cr, Pb, and Al have positive loadings along the PC1 axis and are grouped together. As indicated by the correlation analysis, these metals likely originate from a common anthropogenic source, possibly from industrial activities or marine pollution sources such as ship traffic in the ÇS. Notably, Fe and Al, which may also originate from suspended particles, show strong correlations with toxic metals (Cu, Ni, Cr, Pb), indicating that both anthropogenic and lithological sources contribute simultaneously to metal accumulation in mussels.

As, Mn, and Zn are positioned negatively along the PC1 axis, indicating that they likely originate from sources distinct from the main anthropogenic metals, such as fertilizers and pesticides used in agricultural activities. The position of Cd along the PC2 is noteworthy; as it seperates from other PTEs likely due to distinct behavior. Its levels in mussel tissues were very low, which may be due to limited bioavailability of sediment-bound Cd, physiological or adaptive mechanisms restricting uptake, or the relatively short exposure period of mussels. Comparative experimental studies between artificial mussels and natural mussels also support the limited accumulation of Cd due to the adaptive mechanisms of mussels (Gonzalez-Rey et al., 2011).

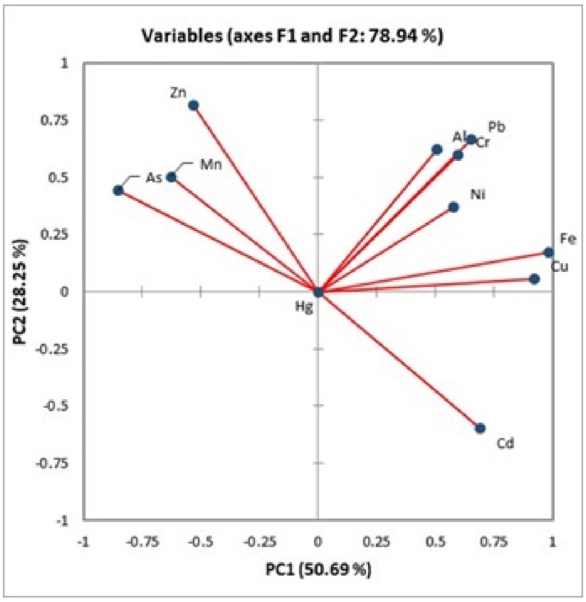

Figure 2. PCA of PTEs accumulated in black mussel samples.

4. Conclusion

In this study, where the Mediterranean mussel was used as a bioindicator, PTEs in samples collected from five stations on the eastern coast of the ÇS were analyzed by the ICP-OES method. The presence of As in all obtained samples is notable. Correlation and PCA analyses particularly show strong positive relationships in terms of the transport dynamics of anthropogenic PTEs. Especially, the fact that As levels exceed limit values at all stations stands out. As, Pb, Ni, Cr, Cu, and Zn pose potential ecological risks with contributions from ferry transportation, agricultural pesticides, and urban waste.

Confilict of Interest: The authors declare that there is no conflict of interest.

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