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# Heavy metals ın the Medıterranean mussel (*Mytilus galloprovincialis* lamarck, 1819) from sınop coasts (Black Sea, Turkey)

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**Abstract**
*Mytilus galloprovincialis is* a commonly used bioindicator species for assessing environmental pollution. The objective of this study was to investigate the concentrations of heavy metals, including Cadmium (Cd), Copper (Cu), Zinc (Zn), Lead (Pb), Arsenic (As), and Mercury (Hg), in the soft tissues of *Mytilus galloprovincialis* obtained from the inner harbor and pier areas influenced by land-based pollutants in the Sinop Peninsula of the Black Sea. Mussel samples were collected from the Sinop Black Sea coast at 0-5 m (surface) between June and August 2022. The study revealed that Hg had the lowest metal concentration, at 0.13 mg/kg. Zn exhibited the highest concentration, with a value of 262.58 mg/kg, followed by Cu (54.69 mg/kg), Pb (7.48 mg/kg), and As (5.41 mg/kg). The metal concentrations were ranked as Zn > Cu > Pb > As > Cd > Hg. The obtained metal values were compared against various standards. It was observed that the Zn, Cu, Pb, As, and Cd levels in mussel meat exceeded the specified standard limits. These findings underscore the need to accurately identify the sources of land-based pollutants from coastal areas in the Black Sea and promptly implement appropriate measures and controls.

**Keywords:** Heavy metals, Sinop coasts, Black Sea, *Mytilus galloprovincialis*, monitoring, bioindicator

## 1. Introduction

The Black Sea ecesystem has been seriously damaged as a result of domestic and industrial lodas arise from land-based sources and rivers. As a result of massive amounts of domestic and industrial effluents are transported by rivers and discharged into the Black Sea. Consequently, organic and inorganic pollutants are accumulated here (Gökkurt Baki and Baki, 2022; Vasconcelos *et al*., 2011) [1, 2]. However the toxicity of many shemicals is relatively unknowmn, particularly to marine fish (Gökkurt Baki *et al*., 2015) [3]. The toxicity of heavy metals can vary
depending on the dosage, manner of exposure, and the amonut and type of the heavy metal in addition to the nutrition of the relevant living organism, which depend on various factors such as age, sex and genetics (Gökkurt Baki, 2021) [4].

The utilization of aquatic organisms as bioindicators for heavy metal pollution is widespread in current research, with algae and molluscs commonly employed as indicators (Jitar *et al*., 2013) [5]. These organisms function as biofilter organisms, capable of retaining small particles from the water. Consequently, pollutants in mussel tissues indicate contamination in the marine environment (Jitar *et al*., 2013) [5].

Certain non-essential metals, such as cadmium (Cd), lead (Pb), and mercury (Hg), possess high toxicity and can cause severe health issues for humans, even at trace levels. Conversely, elements like chromium (Cr), copper (Cu), iron (Fe), nickel (Ni), and zinc (Zn) are considered essential as they play roles in biological systems and are part of human nutrition (Mertz, 1981) [6]. However, elevated levels of these essential elements beyond specific accumulation thresholds can also have toxic health effects (Barone *et al*., 2018; Copat *et al*., 2018; Kouali *et al*., 2022) [7-9].

Mussels are widely recognized as sentinel organisms for bioindication of coastal water pollution owing to their exceptional ability to accumulate pollutants from seawater (Rainbow and Phillips, 1993; Mol and Üçok Alakavuk, 2011; Adams and Rowland, 2003; Kapranov *et al*., 2021) [10-13].

The mussel *Mytilus galloprovincialis* Lamarck is a major component of the littoral fauna in the Black Sea and marine mussels are sedentary organisms and easy to collect a large number of organisms from the location at a certain period of the year (Bat *et al*., 2012) [14].

Mussels, like many other marine organisms, can absorb both essential and non-essential elements from their surroundings into their tissues (Fowler, 1990; Rainbow and Phillips, 1993) [15, 10]. Consequently, accumulating these elements leads to significantly higher concentrations in mussels' soft tissues than in their surroundings (Casas *et al*., 2008) [16]. When accumulated beyond metabolic requirements, even essential elements can be harmful to organisms (Rainbow, 2002) [17], and certain non-essential metals, such as mercury, cadmium, and lead, exhibit high toxicity even at relatively low concentrations (Bat *et al*., 1999; Çevik *et al*., 2008; Türk-Çulha *et al*., 2011, Tepe and Süer, 2016) [18-21], potentially affecting normal physiological functions, including fertilization, even at sub-toxic doses (De Guglielmo *et al*., 2019; Kapranov *et al*., 2021) [22, 13].

The present study aimed to determine the concentrations of heavy metals (Cd, Cu, Zn, Pb, As, and Hg) in the bivalve species *Mytilus galloprovincialis* collected from the coasts of Sinop in the Black Sea. *Mytilus galloprovincialis* was selected based on its economic value and role as a pollution indicator due to its lifestyle. Furthermore, the findings of this study are significant for establishing a data infrastructure and facilitating planning for future studies.

## 2. Materials and Method

### 2.1 Study Area

The study area's coordinates, specifically the Port and Pier stations, are depicted in Figure 1. Mussel samples were collected from the Sinop Black Sea coast, at a depth range of 0-5 m (surface), from June to August 2022.

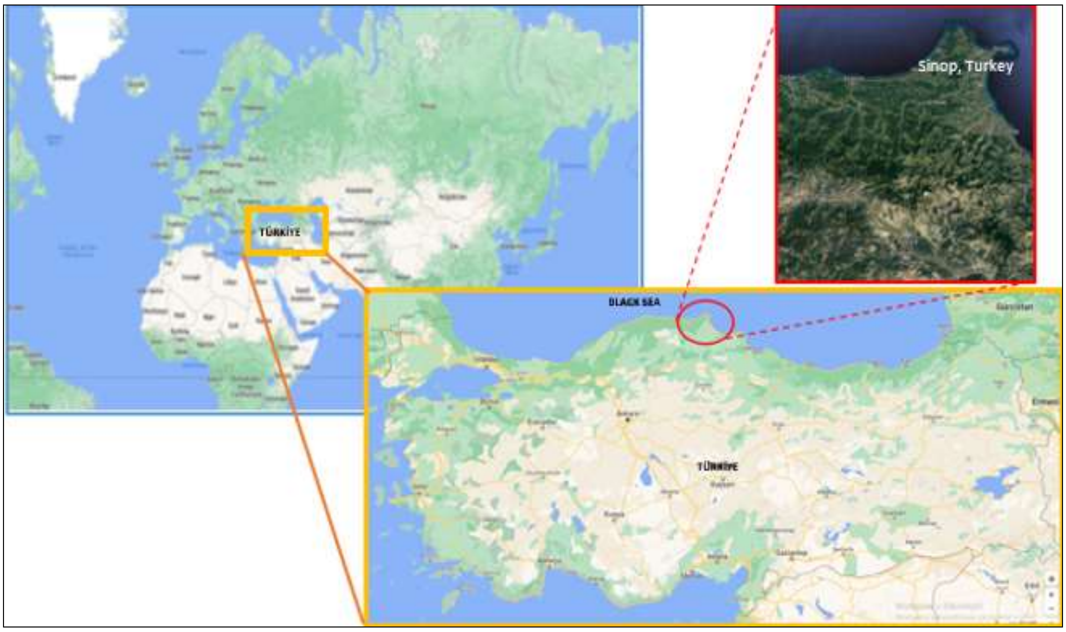


**Fig 1:** The map of the study area

The province of Sinop encompasses a coastline stretching approximately 187 km along the Black Sea. It is well-documented that the coasts of Sinop are subject to the influence of land-based pollutants, including urban wastewater discharges, the presence of an outdated solid waste landfill site, and the impacts stemming from various diffuse sources such as agricultural areas. To evaluate the repercussions of these specific pollutant factors on the aquatic ecosystem, the bioindicator species *Mytilus galloprovincialis* was selected due to its life and feeding habits. The water quality parameters of the sampling area are provided in Table 1.

**Table 1:** Physical and chemical values of the sampling areas (June-August).

| | Temperature | Conductivity (MSV/cm) | TDS* | Salinity | DO** | pH | ORP*** |
|---|---|---|---|---|---|---|---|
| Sampling Station Data | 24.81±0.28 | 32.34±0.12 | 21.02±0.08 | 17.68±0.08 | 7.03±0.20 | 7.40±0.09 | 229.83±5.73 |

*TDS:Total dissolved solids, **DO: Dissolved Oxygen, ***ORP: Oxidation-Reduction Potential

### 2.2. Sampling and sample preparation, and analysis

A total of 188 samples of *Mytilus galloprovincialis* were collected for this study between June and August 2022. Mussels of comparable sizes were selected from various stations to ensure the representativeness of the samples. The collected mussels were thoroughly cleaned, rinsed, and transported to the laboratory for further analysis (Figure 2). After measurement and weighing, the shell parts were separated, and the edible portions were stored in a deep freezer until analysis. The concentrations of heavy metals (Zn, Cd, Cu, Pb, As, and Hg) in all samples were determined using an ICP-MS Spectrophotometer following microwave digestion.

**Fig 2:** *Mytilus galloprovincialis*

The mussels samples exhibited an average length of 5.31±0.04cm, an average weight of 15.15±0.33g, an average meat weight of 2.78±0.07g, and an average meat yield of 18.79±0.44%. The length-weight relationship is illustrated in Figure 3.

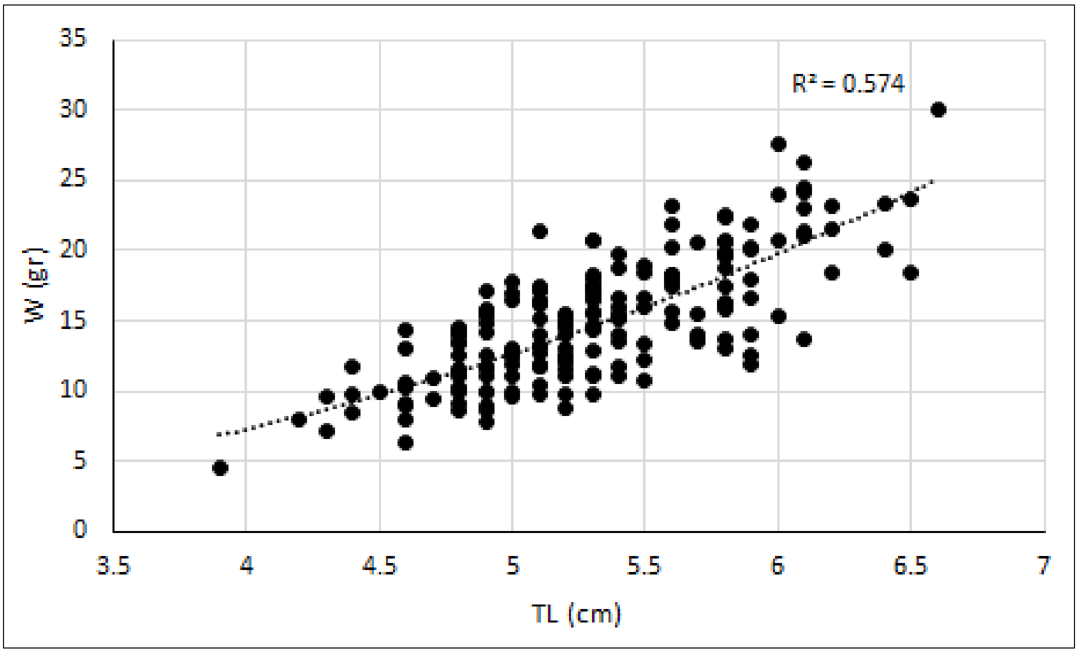


**Figure 3.** The length-weight relationship of mussel samples.

The shell parts of the mussel samples, intended for weight and size measurements, were carefully extracted and stored in a freezer at -18°C until the analysis of the edible parts. For the analysis of heavy metals (Zn, Cd, Cu, Pb, As, and Hg) in all samples, an ICP-MS Spectrophotometer was utilized following microwave digestion (Table 2). The metal concentrations were determined by analyzing three replicates, and the reported results represent the average values obtained from these replicates.

**Table 2:** Analysis Methodology for Mussel Samples

| Parameter | Method | References |
|---|---|---|
| Mercury (Hg), Arsenic (As), Cadmium (Cd), Copper (Cu), Zinc (Zn), lead (Pb) | ICP-MS after microwave digestion | EPA Method 6020B |

### 2.3. Statistical analyses
The statistical analysis was conducted using the SPSS Version 21.0 software. All analyses were performed with three replicates. The data were presented as mean values ± standard error, and statistical significance was determined at $p<0.05$.

## 3. **Results and Discussions**
The average size and weight values of the sampled mussels are given in Table 3.

**Table 3:** Biological Characteristics of Mussels and related specifications

| Name | Scientific name | Shell-on mussel weight (g) | Tissue weight (g) (weight ratio in soft part) | Shell-on mussel length (cm) |
|---|---|---|---|---|
| Mussel | *Mytilus galloprovincialis* | 15.15±0.33 | 2.78±0.07 | 5.31±0.04 |

The concentrations of trace metals in *M. galloprovincialis* soft tissues collected from the Sinop coast of the Black Sea were arranged in increasing order (µg.g$^{-1}$ dry weight). The specific concentrations of heavy metals were as follows: mercury (Hg) with a value of 0.13±0.01 µg.g$^{-1}$, cadmium (Cd) with a value of 2.42±0.08 µg.g$^{-1}$, arsenic (As) with a value of 5.41±0.52

µg.g$^{-1}$, lead (Pb) with a value of 7.48±0.54 µg.g$^{-1}$, copper (Cu) with a value of 54.69±3.30 µg.g$^{-1}$, and zinc (Zn) with a value of 262.58±12.09 µg.g$^{-1}$ (Table 4). Among the determined concentrations of heavy metals, copper (Cu), lead (Pb), arsenic (As), and cadmium (Cd) exhibited the highest values, following zinc (Zn).

**Table 4:** The Mean Values (Milligrams per Kilogram, Wet Weight) and Standard Deviations of Heavy Metals in Mussels (*M. galloprovincialis*), Sampled in the Black Sea

| | Cd | Cu | Zn | Pb | As | Hg |
|---|---|---|---|---|---|---|
| *Mytilus galloprovincialis* | 2.42±0.08 | 54.69±3.30 | 262.58±12.09 | 7.48±0.54 | 5.41±0.52 | 0.13±0.01 |

All the obtained results were compared with standards such as Turkish Food Codex TFC (2008) [23], EPA (1988) [24], FAO (1983) [25], and EC (2006) [26] (Table 5).

**Table 5:** A comparison of the obtained data with standard limits was performed in the study (Concentrations are in mg.kg$^{-1}$ dry weight)

| | Cd | Cu | Zn | Pb | As | Hg | |
|---|---|---|---|---|---|---|---|
| *Standards (limits)* | 0.05 | 30 | 30 | 0.30 | | 0,50 | TFC (2008) [23] |
| | 0.50 | | | 0.50 | | 0.50 | FAO (1983) [25] |
| | 0.05 | | | 0.30 | | 0.50 | EC (2006) [26] |
| | | | | | 0.0175* | | EPA (1988) [24] |
| | | | | | 0.01 0.6-2500** | | Neff (1997) [27] |
| | 2.42 | 54.69 | 262.58 | 7.48 | 5.41 | 0.13 | Present study |

*concentrations is in mg.l$^{-1}$ in clean coastal and ocean waters
**µg.g$^{-1}$ (ppm) in whole or muscle tissues

In the present study, the element Zn exhibited the highest metal concentration. Zinc is an essential element for living organisms, but exceeding the recommended limit values can have toxic effects on human and other organisms' health. The average value for zinc in the collected mussel samples from the Sinop coast was 262.58±12.09 mg/kg$^{-1}$ ($p<0.05$). This value exceeds the recommended zinc limits of 30 mg kg$^{-1}$ by FAO (1983) [25] and 50 mg kg$^{-1}$ by Anon (2002) [28] and MAFF (1995) [29]; Mol and Üçok-Alakavuk (2011) [11]. Another essential element for living organisms, copper (Cu), also plays a significant role. FAO (1983) [25] has set the limit value for copper at 30 mg.kg$^{-1}$. The determined value for Cu in this study was 54.69 mg.kg$^{-1}$ ($p<0.05$), surpassing the limits set by FAO (1983) [25]. This suggests that the concentration of Cu in the aquatic ecosystem may threaten human health. Human activities are likely the source of Cu input into the ecosystem. Therefore, it is crucial to take immediate action to prevent the introduction of land-derived points, diffuse sources of waste into surface waters, and promote the adoption of waste reduction/prevention technologies.

Furthermore, certain elements like Cd, As, and Pb may not always be present in aquatic organisms. Even at low concentrations, these elements can be hazardous to the organisms and the species that consume them, including humans.

Accumulation of cadmium in the human body can lead to kidney dysfunction, skeletal damage, and reproductive deficiencies (EC, 2001) [30]. Since food is the primary source of cadmium intake for humans, Anon (2002) [28] and (EC, 2001) [30]. EC (2001) [30] have set the limit for mollusks at 1.0 mg kg-$^{1}$. According to European Union standards, the limit for mollusks is 1.5 mg.kg$^{-1}$ (EC, 1997) [31]. This study measured the average Cd concentration as 2.42±0.08 mg.kg$^{-1}$ ($p<0.05$), surpassing the specified limit value.

The legal limit for mercury in fish products is 0.5 mg kg$^{-1}$ (Anon, 2002; EC, 2001; EC, 1993) [28-32]. The average mercury concentration determined in the mussel samples in this study was 0.13±0.01 mg.kg$^{-1}$ ($p<0.05$), which falls below the set limits.

The lead (Pb) content in the examined mussel samples was determined to be an average of 7.48 mg.kg$^{-1}$ ($p<0.05$). Lead absorption poses a significant risk to public health; therefore, the limit for lead in fishery products FAO/WHO (2004) [33] and mollusks Anon (2002) [28] is set at 0.5 mg.kg$^{-1}$. However, EC (2001) [30] allows up to 1 mg.kg$^{-1}$ Pb in bivalve mollusks, and the European Communities (EC, 1997) [31] permits up to 2 mg.kg$^{-1}$ Pb. The obtained Pb concentration in this study exceeds all the specified limit values.

Arsenic exhibits complex marine biogeochemistry, which has significant implications for its toxicity to marine organisms and their consumers, including humans. This study measured the average arsenic concentration as 5.41±0.52 mg.kg$^{-1}$ ($p<0.05$). The concentration of total arsenic in clean coastal and ocean waters is typically 1 to 3 mg/L, with a mean of approximately 1.7 mg/L (Andreae, 1979; Andreae and Andreae, 1989; Li, 1991; Neff, 1997) [34-36, 27], which is about 100 times higher than the U.S. EPA human health water quality criterion (fish consumption) value of 0.0175 mg/L. Additionally, Neff (1997) [27] stated in their study that total arsenic concentrations in the whole body or muscle tissues of marine organisms worldwide can generally range from 0.01-0.6 mg/g dry weight up to 2500 mg/g dry weight (ppm), depending on the method used (Table 6).

**Table 6:** Concentrations of total arsenic in the whole or muscle tissues of marine organisms from throughout the world. Concentrations are in µg.g$^{-1}$ dry weight

| Taxon | Number of samples | Range | Geometric mean | Reference |
|---|---|---|---|---|
| Bivalve | 151 | <0.6-214 | 10.44* | Neff (1997) [27] |
| | 188 | | 5.41** | Present study |

**Concentrations are in mg.kg$^{-1}$ dry weight; *Concentrations are in µg.g$^{-1}$ dry weight

The accumulation of arsenate from seawater solution is generally limited in most marine animals (Wrench *et al.*, 1979; Sanders *et al.*, 1989) [37-38]. However, concentrations of total arsenic exhibit significant variation in the tissues of marine plants and animals, regardless of their trophic position within the local food web (Langston, 1984; Jonhnsons and

Braman, 1975; Hanaoka *et al*., 1988) [39-41].

## 4. Conclusion

In this study, mussel (*Mytilus galloprovincialis*) samples collected from the coasts of Sinop in the Central Black Sea (port and pier) were analyzed for levels of Zn, Cu, Pb, Cd, As, and Hg. The results revealed that the levels of Zn, Cu, Pb, Cd, and As exceeded the limit values set by standards, while the Hg level was below the limit value. This finding highlights the importance of monitoring commercially important products like mussels, collected and consumed without proper control, to safeguard consumer health. This study aimed to analyze mussels that could be safely collected and consumed.

The Black Sea has been subjected to pollution effects for many years due to contamination from surrounding countries and land-based pollutants, exhibiting characteristics of an inland sea. The species investigated in this study, *Mytilus galloprovincialis*, is economically preferred by consumers but is vulnerable to pollutant effects due to its sessile nature. Consequently, the elevated levels of heavy metals detected in the mussels can be attributed to the structural condition of the sampling area, the pollutants it is exposed to, and the species characteristics. Although the Turkish coasts of the Black Sea have advantages in industrial and mining activities, they have reached a critical point where intensive agricultural activities and pollutants from other countries risk public health. To mitigate pollution from both point sources and diffuse sources, it is crucial to expedite the adoption of zero waste practices and waste prevention technologies, accompanied by increased control and inspections. Shifting away from the conventional approach of waste generation followed by waste management and embracing industrial pollution prevention techniques is imperative.

Urgent measures are needed to halt the harmful effects of anthropogenic pollutants and human activities on the Black Sea ecosystem. Actions should be implemented to minimize all non-natural inputs into the Black Sea. The data from this study and other research endeavors play a vital role in fostering societal trust by providing information about economically significant species and products for human consumption. Promoting and conducting more scientific studies are essential for sustainable planning and management.

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