Metal Bioaccumulation of *Mytilaster lineatus* (Gmelin, 1791) Collected from Sinop Coast in the Southern Black Sea

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**ABSTRACT**

**Objective:** Metal bioaccumulation of the bivalve *Mytilaster lineatus*, based on different seasons and stations was investigated in Sinop Peninsula of the Black Sea. Soft tissues of mussels from Gazibey Rock and Içliman were taken during the spring and the summer of the year 2016 and were analyzed for Hg, Cd, Pb, Cu and Zn.

**Materials and Methods:** The soft tissues of the mussels digested with Suprapur® HNO₃ (nitric acid) using a Milestone Systems, Start D 260 microwave digestion system. The accuracy and precision of the method was verified by the simultaneous analysis of the certified reference materials NIST 2976 (mussel tissue) for the samples. The Inductively Coupled Plasma–Mass Spectrometer (ICP-MS), Agilent Technologies, 7700x was used for metal analyses.

**Results:** Results showed that higher concentrations of almost all metals in Içliman than those in Gazibey Rock. However, no significant differences were detected between seasons except for both Cu and Zn. The metal amounts of *M. lineatus* ranged between 0.024-0.035, 0.054-0.072, 0.13-0.25, 0.64-0.85 and 6-20 mg kg⁻¹ wet wt. for Hg, Cd, Pb, Cu and Zn, respectively.

**Conclusion:** The measured metals in both sampling areas did not exceed the threshold set by the European Commission and Turkish Food Codex. The results of this study also confirmed the potential of *M. lineatus* to be used as bio-indicators of heavy metal pollution.

**Keywords:** Heavy metals, bioaccumulation, bio-indicator, *Mytilaster lineatus*, Black Sea

**INTRODUCTION**

Large amounts of contaminants have been released into the Black Sea over the last several decades (1). Among these contaminants, heavy metals have long been recognized as one of the major problems of the Black Sea (2). Heavy metals are largely loaded to the coastal zone of the Black Sea from rivers, domestic sources, agricultural, fisheries and touristic activities (1). Metal pollution in the Black Sea coasts has been intensively studied in recent years because of the fact that these heavy metals are persistent toxic, prone to bioaccumulation and hazard a risk to people and coastal ecosystems (3).

The comprehension of the bioaccumulation fact of heavy metals in the living biota is of an extreme complexity. This is because of the complex of different parameters which influence this bioaccumulation like the physico-chemical characteristics of surrounding waters of the studied area, the chemical properties of the pollutants and the biologic factors of the organism (4,5).

Marine mussels are a very large group of Mollusca ubiquitous in the seas. They are very important organisms on the rocky and gravel bottoms of the Black Sea and are important prey source for a variety of marine organisms, including fish, crabs and humans and their utilizing contribute to economies (6).

*Mytilaster lineatus* is a genus of marine mussels from the waters of the Atlantic Ocean and Mediterranean Basin. *M. lineatus* is widely distributed in the Black Sea and is...
one of the most dominant mollusc species of Sinop Peninsula fauna (7). However, data regarding the biology, ecology and evolution of *M. lineatus* is still limited. It inhabits rocky substrata in shelf waters ranging from the shallow to the deeper continental slope. It is known that mussels feed on suspended matter filtered by water. Diatoms (such as *Coscinodiscus* sp., *Chaetoceros* sp., *Nitzschia* sp. and *Pleurosigma* sp.), microscopic algae and detritus are usually found in the contents of their stomach (8). In recent years, therefore, the studies on mussels belonging to the Mollusca phyla have been investigated, with a focus on heavy metal contamination. Heavy metals are accumulated by mollusks with no mobility which tend to be highly vulnerable because they feed on suspended organic particles which is bond of various toxic metals. Thus, they often contain high levels of heavy metals when compared with other marine organisms (9-13).

The main studied tissue for metal content in mussels is the whole soft edible part due to its relevance for human health. There is no pollution source in Gazibey Rock, whereas Içliman receives domestic waste. In addition, fishing activities are active in Içliman (14,15).

Moreover, there is no available data on metal bioaccumulation in *M. lineatus* in the Black Sea. Thus, the present study constitutes using *M. lineatus* as bio-indicator species for the first time, a contribution to investigate the heavy metal accumulation in this species in the Black Sea.

**MATERIALS AND METHODS**

The area of Sinop Inner Harbour has been chosen as the research area. In the selection of the stations namely Gazibey Rock and Içliman, where the mussels were abundantly identified (Figure 1).

Gazibey Rock is a small island with a maximum depth of 24 meters. While the north facing side descends vertically, the other side slopes and gradually deepens. During the research, the samples were taken from the northern part of the rocks of Gazibey.

The average depth at Içliman station, which is a natural harbour, is 15 meters. The ground has a fairly flat and sandy structure. The floor of the selected area is covered with dead mussel shells, pebbles, algae and marine litter due to the use of port.

**Preparation of Mussel Samples**

*M. lineatus* has a wide distribution on the Sinop shores and tolerance range for different salinities and temperatures and has sufficient size, sessile life form and is robust in laboratory conditions, making bivalves the prime candidate for studying the bioaccumulation of heavy metals. *M. lineatus* individuals (Figure 2) were collected randomly during each sampling occasion from Gazibey Rock and Içliman by SCUBA diving at the depth of 20 and 10 meters, where the mussel settlements were most dense, respectively. The samples were put in plastic tanks filled with seawater from each station, then immediately transported to the laboratory. About 20 individuals were chosen randomly according to their shell length measurements. There was no significant difference among the shell lengths between different samplings (*p* >0.05, one-way ANOVA). For each sampling, specimens were pooled together after a 48 h depuration of their gut contents in filtered clean seawater (11-13). The shells were opened and soft tissues were carefully taken (16). The soft parts of the specimens were put in sterilized petri dishes, weighed and stored at a temperature of -21°C until metal analysis (17,18).

**Determination of Metals**

The soft tissues of the mussels digested with Suprapur® HNO₃ (nitric acid) using a Milestone System, Start D 260 microwave digestion system. The accuracy and precision of the method was verified by the simultaneous analysis of the certified reference materials NIST 2976 (mussel tissue) for the samples. The certified reference values were 61.0 ± 3.6 for Hg, 0.82 ± 0.16 for Cd, 1.19 ± 0.18 for Pb, 4.02 ± 0.33 for Cu and 137 ± 13 mg/kg. The results of the elements for the standard reference material were found as 63.2 ± 3.8 mg/kg (104%) for Hg, 0.79 ± 0.12 mg/kg (96%) for Cd, 1.12 ± 0.13 mg/kg (94%) for Pb, 3.92 ± 0.28 mg/kg (98%) for Cu and 129 ± 8.0 mg/kg (94%) for Zn. The results indicated good agreement and all analyses were considered satisfactory, with the Relative Standard Deviation (RSD) percentage which was between 94 and 104 % for the metals. The ICP-MS, Agilent Technologies, 7700x was used for metal analyses.
In addition, some physical and chemical parameters (salinity, pH, conductivity, total dissolved solids, temperature and dissolved oxygen) of sea water were measured at the same time.

Statistical Analysis
In order to distinguish if the observed differences among datasets were statistically significant, analysis of variance (ANOVA) using SPSS 21.0 statistical software was used to investigate the effects of seasons of collection and sampling site on the variations in metal concentrations in the bivalves. The Tukey’s (HSD) test was used as post hoc test to indicate the pairs of data which have significant differences (19). The results are expressed in mg kg\(^{-1}\) of wet weight.

RESULTS

Sea Water Analysis
Sea water samples were taken from the surface of the stations at the time of sampling and the parameters determined as a result of analysis (salinity, pH, conductivity, total dissolved solids, temperature and dissolved oxygen) are given in Table 1 according to the spring and summer.

| Table 1. Physico-chemical parameters of sea water samples |
|---------------------------------------------------------|
| Parameters | Gazibey Rock | Içliman |
|-------------|-------------|--------|
|             | spring      | summer | spring       | summer |
| Salinity (%o) | 16.8±0.2  | 17.3±0.2  | 17.1±0.1  | 17.2±0.2  |
| pH          | 7.91±0.3  | 8.11±0.3  | 8.18±0.1  | 8.21±0.2  |
| Conductivity (uS/cm) | 27426±14  | 28143±13  | 27382±22  | 28876±31  |
| TDS (g/L)   | 18.25±0.1  | 18.1±0.1  | 18.38±0.1  | 18.91±0.1  |
| Temperature (°C) | 21.1±0.2  | 21.7±0.1  | 22.2±0.1  | 23.4±0.2  |
| Dissolved oxygen (mg/L) | 4.91±0.1  | 4.72±0.1  | 4.84±0.1  | 4.65±0.1  |

Heavy Metals in *Mytilaster lineatus*
Mussels tolerate salinity changes from 5 up to 40‰. There is not much growth in low and extreme salinity values in surrounding waters. Water temperature is an important factor that affects the general condition and distribution of the mussel. Generally suitable for feeding, and growing of the mussels that can tolerate temperature changes up to 2-30°C, the optimum temperature is 8-26°C (6). The results from Table 1 indicated that physico-chemical parameters of sea water were considered suitable for mussel life.

The concentrations of heavy metals in the soft tissues of the mussels collected from the Sinop shores of the Black Sea during the spring and the summer of the year 2016 are shown in Figures 3-7.
Results showed that the levels of metals in the mussels from Icliman were higher than those in the mussels from Gazibey Rock (p<0.05). Essential metal levels were higher than non-essential metals in both sampling areas. Concerning the effect of seasons on Hg, Cd and Pb bioaccumulations slightly higher levels of the heavy metals in spring was observed than those in summer. This was vice versa for both Cu and Zn. However, the differences in metal levels between spring and summer were not significant in each sampling area except for Zn (p<0.05).

The concentrations of heavy metals in soft tissues of the mussels ranged between 0.024-0.035, 0.054-0.072, 0.13-0.25, 0.64-0.85 and 6-20 mg kg⁻¹ wet wt. for Hg, Cd, Pb, Cu and Zn, respectively.

**DISCUSSION**

The comparison of results with those of EU Guideline and TFC (Table 2) shows that the concentrations of all metals in both sampling areas did not exceed the threshold set by the guideline categories.

Bat et al. (11) investigated seven heavy metal (Fe, Zn, Mn, Cu, Pb, Cd and Hg) concentrations in *Mytilus galloprovincialis* samples for evaluating marine pollution. In their study high level of these metals found in mussel populations located close to major cities and industrialized areas (11). It was suggested that consumption of about 1 serving of mussels from clean coastal waters per week is enough (11). They also found that there is no health risk for mussel consumers living on the Sinop shores of the Black Sea as we found for *M. lineatus*.

The seasonal variability may result from either internal biological cycles of the aquatic organisms or from changes in the variability of metals in the ecosystem. The seasons of spring and summer are with high biological activities which cause an increment in the metabolic rate and eventually increase the oxygen use and uptake of dissolved metals via the gills. Additionally these seasons could be related to the reproductive period which could lead to considerable energy consumption, decline the detoxification ability and increase the metal bioaccumulation (25,26).

Unfortunately, there is no study with *M. lineatus*. For this reason, we compared the data obtained in our study with other Mollusca species especially *Mytilus galloprovincialis* from other coasts of the Black Sea. Large differences in heavy metal amounts in Bivalve species were found between different localities of the Black Sea.

In Turkish coasts of the Black Sea, the highest values of Zn and Pb in Trabzon and Cu in Artvin were found. The order of the metal values obtained in all studies conducted on the Turkish coast of the Black Sea are listed as Zn> Cu> Pb> Cd> Hg (11). The highest values of Zn, Cu and Pb in mussels were found on the shores of Giresun, Trabzon, Artvin and Rize coasts of the Turkish Black Sea were determined by Baltas et al. (27).

On the Russian coasts of the Black Sea Cu (8.83±2.71 mg/kg dry wt.) and Pb (3.07±0.85 mg/kg dry wt.) values were highest in Blue Bay (28). Zn (106-196 mg/kg dry wt.) and Cd (1.9-3.4 mg/kg dry wt.) values were highest in Inal Bay (29) and Hg (3-83 mg/kg wet wt.) values were observed on the Crimean coasts (30). When the maximum values of the metals are taken into consideration, the order of the metal values obtained in all studies conducted on the Russian coasts of the Black Sea is Zn>Cu>Pb>Cd>Hg.
Cu, Cd and Pb amounts studied on the border between Ukraine and Romania were 0.57-1.31, 0.07-0.23 and 0.07-0.163-83 mg/kg wet wt., respectively (31).

In Romania the highest values of Cu (6.5 mg/kg wet wt.), Pb (11.02 mg/kg wet wt.) and Cd (3.3 mg/kg wet wt.), were observed on Navodari, South Constanta and Vama Veche coasts of the Black Sea, respectively (32). However, Zn (190±18 mg/kg dry wt.) and Hg (33±2 mg/kg dry wt.) values on the highest North Eforie coasts of the Black Sea (33). When the highest amounts of heavy metals are taken into consideration, the order of the metal values on the Romanian coast of the Black Sea is as follows: Zn > Cd > Cu > Pb > Hg.

The highest values of Zn (104.4-239.2 mg/kg dry wt.) and Cd (0.98-2.24 mg/kg dry wt.) were found in the Gulf of Varna (34). In Cape Galata, the highest of both Cu (24.2±2.2 mg/kg dry wt.) and Pb (59.1±6.2 mg/kg dry wt.) were recorded by Gorinstein et al. (35) and Gorinstein et al. (36). The order of the metal values obtained in the studies carried out on the Bulgarian coast is found as Zn > Cu > Cd > Pb.

In general, the amounts of studied heavy metals found in Mytilaster lineatus were lower than that of Mytilus galloprovincialis in the Black Sea coasts.

Molluscs are known to take up and accumulate heavy metals, both essential and nonessential, from the surrounding water and suspended particles. Cu and Zn are essential metals in the organisms. Zn is used as an active centre for metal enzymes and activators of other enzymes systems, while Cu is an integral part of the respiratory pigment haemocyanin. These metals are introduced into the marine coastal environment through different human activities especially urban.

Indicated in many studies (3), bivalves accumulate Cd mostly in hepatopancreas and in kidney in the form of stores in lysosomes and accumulate Cu mostly in hepatopancreas, gonads and gills. In fact, in Mollusca, the blood carries a respiratory pigment depend on Cu, the hemocyanin, which clarifies its circulation in these largely vascularized organs.

CONCLUSION

M. lineatus collected from Sinop shores of the Black Sea during the spring and the summer of the year 2016 have been found to be contaminated with Hg, Cd, Pb, Cu and Zn. Although M. lineatus was not preferred as food such as Mytilus galloprovincialis, in the current study these measured metals were well under the permissible levels. The results of this study also confirmed the potential of M. lineatus to be used as bio-indicators of heavy metal pollution.

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