Assessment of recent and chalcolithic period environmental pollution using *Mytilus galloprovincialis* Lamarck, 1819 from Yarimburgaz Cave, the northern Marmara Sea and Bosphorus coasts

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Yarimburgaz Cave

**Summary** Marine or freshwater mussel species are found in large quantities around populated areas and accumulate metals in aquatic systems. Therefore, these organisms are used to monitor environmental pollution. *Mytilus galloprovincialis* is a generally accepted bioindicator of metal pollution and is used in this study. The aim of this study is to determine the changes in the environmental conditions since antiquity. *M. galloprovincialis* shells were used to monitor Chalcolithic pollution levels in Yarimburgaz Cave, one of the oldest settlements in Europe. Recent samples were collected from 12 stations on the coasts of the Northern Marmara Sea and the Bosphorus between May—September 2004. The environmental pollution substantially changed over the last 7500 years. The comparison of the geochemical characteristics of the environmental pollution observed in the Chalcolithic period and today revealed that pollution from both household and industrial chemicals has increased in Istanbul.

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1. Introduction

Previous studies have indicated that most marine species are not suitable for monitoring metal pollution. Thus, appropriate bioindicator species are required (Ikuta, 1990). Species with shells accumulate metals and trace elements in their shells and flesh, and are used as bioindicators in marine and coastal pollution analyses (Farrington et al., 1987; Maanan, 2007; Phillips, 1980; Widdows, 1985). Sedentary mollusca, such as Mytilus galloprovincialis, are preferred in environmental monitoring studies because they have long life spans, can be easily recognized, can be sampled in large quantities, and can tolerate the pollution (Argese et al., 2005; Goldberg, 1975; Kayhan, 2007; Kayhan et al., 2007; Serafim et al., 2002).

In a seasonal study performed in the Lagoon of Venice, a multiple biomarker approach was adopted to evaluate the natural and anthropic stresses influencing the biological responses of the mussel (M. galloprovincialis) and to assess the effects of the spatial rather than temporal variations (Da Ros et al., 2000, 2002; Nasci et al., 2002; Nesto et al., 2004). Bocchetti et al. (2008) investigated the natural variability of several biomarkers in Tapes philippinarum and M. galloprovincialis sampled from the Northern Adriatic, in which these organisms are sentinel species that are important for future environmental impact assessments. The results indicated a significant influence of the seasonal variability on several biomarkers and species-specific differences. This influence should be considered to discriminate anthropogenic disturbances.

Several compounds of environmental interest were investigated using a toxicity test for the early life stage of bivalve mollusca. Three pesticides (carbofuran, atrazine, and malathion), for which literature data are available only for species of oysters, were tested with M. galloprovincialis (Losso et al., 2004).

Rivaro et al. (2000) showed minimal evidence for a seasonal pattern, except for Cu, Zn, and V. Concentrations of these metals were a function of the sampling site and the reproductive cycle. Additionally, transplanted mussels can identify local anthropogenic sources of metals. In general, areas of known pollution display high concentrations of these metals; however, select areas not substantially influenced by human activities also showed high concentrations of several metals. Valve activity was measured in the Mediterranean mussel M. galloprovincialis in response to sub-lethal concentrations of four metals (Hg, Cu, Zn, and Cd) and two phosphate industry effluents from the Atlantic coasts of Morocco. Valve movements were monitored using a proximity inductive sensor which could display all activity from the full closure to wide opening of the shell valves (Fdl et al., 2006). Taleb et al. (2007) noted that the difference in the variations of the annual physical parameters found in Oran Harbor and found in the Maarouf corresponds to the influence of the domestic and industrial sewage discharged in the Algerian western coastal area. The damage caused to the lysosomal membrane and ADN appears to be a universal marker for the effects of stress on marine organisms such as bivalves.

In a monitoring study conducted by Risso-de Faverney et al. (2010), artificial tire reefs were deposited in a marine protected area (Vallauris-Golfe Juan Bay, France) located along the NW Mediterranean coast, and the potential toxic effects of the tire material were investigated by transplanting marine mussels (M. galloprovincialis). The metal accumulation (cadmium, copper, and zinc) and biomarker response in mussel tissues indicated a clear separation. The organisms were found to be significantly more affected by tire reefs than other anthropogenic pollutants.

In this study, the shells of the Mediterranean mussel, M. galloprovincialis, obtained from Yarimburgaz Cave excavations were used to detect paleo-environmental conditions. To determine the current environmental pollution levels with respect to past levels, the toxic, non-toxic and radioactive trace element content of these cave shells were compared to recent shells collected from the Sea of Marmara.

1.1. Regional geology of Yarimburgaz Cave

The study area, known as the Yarimburgaz Cave district, is located 2 km north of Altınsehir. The oldest rocks in this region are reefal limestones and clayey limestones of the Middle Eocene (Lutetian) age. Poorly cemented sediments from the Miocene (Sarmatian) age containing pebbles, sands, and clay are found on top of these formations (Dığış, 1986; Meric et al., 1991) (Fig. 1).

The Altınsehir formation (Fig. 2) has two members: the reefal limestone on the top (Yarimburgaz member) and the clayey limestone on the bottom (ikizelli member). Yarimbuzgaz limestone (Tayk) (Middle Eocene) corresponds to deposits from the reef, and the Ikizelli member (Tai) corresponds to the deposits of the fore reef. Among the two, the hard and resistant Yarimbuzgaz limestone developed karstic features.

Yarimbuzgaz Cave, which is one of the karstic formations, has revealed one of the earliest known horizons of human history. Mollusca were found in a grayish clayey lance of soil within layer 3. This pocket of earth was almost completely compacted with molluscs fragments and yielded some shards and bone. Mollusca were noted elsewhere within the deposit of layer 3. Accordingly, this mollusc bearing pocket of earth may also indicate a short break in occupation (Meric et al., 1991).

Salvage excavations executed in this partly destroyed cave in 1986 revealed archeological levels dated to prehistoric periods (6500–7000 BP). The material yield of the assemblage enables an inference of the subsistence patterns and the environmental conditions of the period (Meric et al., 1991).

1.2. Archeological and paleontological features of Yarimbuzgaz Cave

Being one of the oldest human footprints, Yarimbuzgaz Cave archeological findings are important for Istanbul and for the Near East and Europe (Steiner et al., 1998). The cave is found on Catalca Peninsula, located 22 km west of Istanbul and 1.5 km north of Lake Kucukcekmece. The entrances of the cave are narrow holes on the surface (Fig. 2). Geomorphological studies showed that the cave was formed by a water system, carving the karstic Eocene limestone (Fig. 1). The cave has two sections, the upper cave and lower cave, with entrances at 18.60 m and 11.46 m above sea level, respectively (Özdoğan et al., 1991). Both of the entrances face the Sazilider Valley. The main corridor of the lower cave is separated into two branches after the 240th m. One branch
orients north, and the other points in the northeast direction. Both branches have a dead end. The longest branch is 600 m long. To determine the thickness of the cave walls, 5 m was dug, and the last 3 m was found to be culturally sterile. However, the main rock was not reached (Arsebük, 1993; Arsebük et al., 1990; Arsebük and Özbaşaran, 1995). A small spring named the “Little Danube Stream” is located near the cave.

Neolithic and Chalcolithic cultures detected in Yarimburgaz Cave were one of the first cultures to farm. Additionally, they are important in terms of their relations with the Balkans and Europe. The drawings of ships found on the walls

Figure 1  (A) Local topography map of the terrain surrounding Yarimburgaz Cave showing the valley interrupted by the Sazlidere River (from Farrand and McMahon, 1997). (B) The geological map of Yarimburgaz Cave (Kucukcekmece) and its surroundings (modified from Dقبول, 1986).
environmental pollution. The natural dyes are less toxic and more hypoallergenic.

Layers found in Yarimburgaz Cave have been formed slowly, and no distinct cultural or paleontological boundaries have been noted between layers (Steiner et al., 1998). Tools and other cultural artifacts found in select layers covering a long period of time indicate that the cave has been used by humans. After a short break, stratification continued within the fourth cultural layer, or the Yarimburgaz layer, in 6800 BP. This culture is characterized by pottery with intricate carvings and incised decorations. The cave was uninhabited for three more cultural stages until 6300 BP.

Studies of the mollusca from Yarimburgaz Cave indicated that in 7000 BP, the sea was close to the cave. A close shoreline was likely present in the Holocene and displayed similar characteristics with other locations in the Middle-Late Pleistocene (Meriç et al., 1988). The age of the mollusc shells found in a hand-made clay pot from the third Chalcolithic period was found to be 6880 ± 80 years old (GrN. 15528) through a 14C test (Meriç et al., 1991) (Fig. 3).

Ecological data suggests that the shoreline displayed cove characteristics in the Chalcolithic period. Later, the cove was filled with materials carried by the freshwater systems found to the north, forming the Kucukcekmece lagoon. Further rearrangements transformed the lagoon into a lake. The shoreline of the lake was suggested to be nearer to the cave approximately 7000 BP (Meriç et al., 1991).

During the Middle Pleistocene period, the Yarimburgaz layers were formed during use of the cave by humans. Teeth samples of an extinct cave bear were found in the layers belonging to the middle Pleistocene, and an electron spin resonance test revealed an age of 270,000–390,000 ± 40,000–60,000 years (Arsebük, 1995a, 1995b). In a multidisciplinary study (Steiner et al., 1998), a paleoeocological and taxonomical approach was performed using isotopic osteometric methods. The cave was found to be inhabited by cave bears (Spelearctos Ursus) during the middle and last ice age. The cave was near the coast of an estuary.
2. Material and methods

2.1. Sample collection site

*M. galloprovincialis* shells found in the third layer of Yarimburgaz Cave (Fig. 3) were analyzed for metals and trace, radioactive and rare earth element contents. The shells were compared with recent samples collected from 12 different stations located on the northeast Marmara and Bosphorus between May 21—November 13, 2004 (Fig. 4, Table 1).

2.2. Chemical analysis of *M. galloprovincialis* shells

Firstly shells collected (Fig. 5) from Marmara and the Bosphorus were washed with purified sea water and kept at 40°C in an oven for drying.

For metal, trace, radioactive, and rare earth elements analyses were made. And the shells were pounded in an Agate mortar until a homogeneous mixture of powder was obtained. Each sample was separately packaged at the Istanbul University Marine Science and Management in the Laboratory of Marine Geology and sent to Acme Analytical Laboratories, Vancouver, Canada. The metal, trace, radioactive, and rare earth elements (REEs) analyses were performed by Acme Analytical Laboratories (Vancouver, Canada) in January 2008. The analysis was performed for 0.2 g samples using standard protocols (Method Cod: P150 Pulverize to 150 Mesh 13; Method Cod: 4B (Full Suite) LiBO₂/Li₂B₄O₇ fusion ICP-MS analysis). Rare earth and refractory elements are determined by ICP mass spectrometry following a lithium metaborate/tetraborate fusion and nitric acid digestion of a 0.2 g sample. In addition a separate 0.5 g split is digested in Aqua Regia and analyzed by ICP Mass Spectrometry to report the precious and base metals (in highlight).

Elementary analysis: MDL (method detection limits) [µg g⁻¹]: Ba, Be, Sn, and Zn, 1; Co and Th, 0.2; Cs, Ta, Hf, Nb, Rb, U, Zr, Y, La, Ce, Mo, Cu, Pb, Ni, Cd, Sb, Bi, Tl, and Ag, 0.1; Ga and Sr, 0.5; V, B; W, As, and Se, 0.5; Pr, Eu, and Ho, 0.02; Nd, 0.3; Sm, Gd, Dy, and Yb, 0.05; Tb, Tm, Lu, and Hg, 0.01; Er, 0.03; Au, [ng g⁻¹] 0.5.

<table>
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<tr>
<th>Sample no.</th>
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<th>Coordinates</th>
<th>Latitude</th>
<th>Longitude</th>
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<td>28°43.994'E</td>
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<td></td>
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<tr>
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<td>28°19.017'E</td>
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<tr>
<td>2</td>
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<td>41°5.727'N</td>
<td>29°3.328'E</td>
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<tr>
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<td>İstinye</td>
<td>41°6.728'N</td>
<td>29°3.651'E</td>
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<tr>
<td>4</td>
<td>Anadoluvalcagi</td>
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<td>29°5.164'E</td>
<td></td>
</tr>
<tr>
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<td>Pasabahce</td>
<td>41°7.053'N</td>
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<td>29°3.915'E</td>
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<tr>
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<td>12</td>
<td>Dil Iskelesi</td>
<td>40°46.370'N</td>
<td>29°30.671'E</td>
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</table>

Figure 4  Location map of samples Yarimburgaz Cave, Northern Marmara Sea, and Bosphorus coasts.
The quality control report shows that the 4B method was assigned the number of VAN 07002440.1 in the Acme Analytical Laboratories. Reference materials of “STD SO-18 Standard” were applied for Ba, Be, Co, Cs, Ga, Hf, Nb, Rb, Sn, Sr, Ta, Th, Ti, V, W, Zr, Y, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, and Lu, whereas the “STD DS7 standard” was applied for Mo, Ni, Cu, Zn, Pb, Bi, Sb, As, and Cd. The 1DX method and a reference material “STD DS7” was applied for Ag, Au, Hg, Tl, and Se (Table 2).

2.3. Statistical analysis

The descriptive statistics analysis, t-test, ANOVA (analysis of variance), factor analysis, and correlation analysis were performed using Windows PASW Statistics 18.0 (SPSS (Hong Kong) Ltd., Hong Kong) and Statistics 6.0 (Statistics for Windows is developed by SPSS Inc.). To reduce the background noise, all of the chemical elements in the shells were considered in the statistical analyses. A correlation matrix
| No. | Sample ID     | Ba [μg g⁻¹] | Nb [μg g⁻¹] | Rb [μg g⁻¹] | Sr [μg g⁻¹] | Th [μg g⁻¹] | Zr [μg g⁻¹] | Y [μg g⁻¹] | La [μg g⁻¹] | Ce [μg g⁻¹] | Pr [μg g⁻¹] | Nd [μg g⁻¹] | Sm [μg g⁻¹] | Eu [μg g⁻¹] | Tb [μg g⁻¹] |
|-----|---------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| 1   | Selimpasa     | 17          | 0.2         | 0.3         | 1464        | 0.3         | 1           | 0.4         | 1           | 1.9         | 0.26        | 1.1         | 0.16        | 0.05        | 0.02        |
| 2   | Baltalimanı   | 2           | <0.1        | 0.2         | 1291        | <0.2        | 0.4         | <0.1        | 0.2         | 0.3         | 0.06        | <0.3        | <0.05       | <0.02       | <0.01       |
| 3   | İstinye       | 5           | <0.1        | <0.1        | 1238        | <0.2        | 0.3         | <0.1        | 0.2         | <0.1        | 0.04        | 0.3         | <0.05       | <0.02       | <0.01       |
| 4   | Anadolucağı   | 2           | <0.1        | <0.1        | 1238        | <0.2        | <0.1        | <0.1        | 0.2         | 0.3         | 0.05        | <0.3        | <0.05       | <0.02       | <0.01       |
| 5   | Pasabahçe     | 3           | <0.1        | <0.1        | 1113        | <0.2        | 0.2         | <0.1        | 0.3         | 0.01        | <0.3        | <0.05       | <0.02       | <0.01       | <0.01       |
| 6   | Kanlica       | 6           | <0.1        | 0.3         | 1251        | <0.2        | 3.5         | 0.2         | 0.2         | 0.3         | 0.05        | <0.3        | 0.05        | <0.02       | <0.01       |
| 7   | Kuzukusu      | 5           | <0.1        | 0.2         | 1273        | <0.2        | 0.3         | <0.1        | 0.2         | <0.1        | 0.04        | <0.3        | <0.05       | <0.02       | <0.01       |
| 8   | Beylerbeyi    | 9           | 0.1         | 0.2         | 1168        | <0.2        | 0.2         | 0.2         | 0.2         | 0.1         | 0.04        | <0.3        | <0.05       | <0.02       | <0.01       |
| 9   | Kuzguncuk     | 2           | <0.1        | 0.1         | 1026        | <0.2        | 0.2         | <0.1        | 0.2         | <0.1        | 0.02        | <0.3        | <0.05       | <0.02       | <0.01       |
| 10  | Uskudar       | 4           | <0.1        | 0.3         | 1105        | 0.2         | 1           | 0.2         | 0.3         | 0.05        | 0.4         | <0.3        | <0.05       | <0.02       | <0.01       |
| 11  | Tübıtk-MAM    | 11          | <0.1        | 0.4         | 1108        | <0.2        | 2.9         | 0.1         | 0.5         | 0.4         | 0.06        | <0.3        | <0.05       | <0.02       | <0.01       |
| 12  | Dil İskalesi  | 16          | <0.1        | 0.3         | 1298        | <0.2        | 3.6         | 0.2         | 0.3         | 0.06        | 0.3         | <0.05       | <0.02       | <0.01       | <0.01       |
|     | Standard      | 518         | 21.2        | 28.6        | 451.6       | 10.5        | 294.2       | 33.2        | 12.7        | 27.2        | 3.53        | 14.1        | 2.93        | 90          | 52          |
|     | (STD ISO-18)  | 529         | 21.9        | 29          | 912.9       | 10.5        | 297.3       | 33.7        | 12.9        | 28          | 3.58        | 14.6        | 3.91        | 91          | 54          |
|     | Standard      | 600         | 15          | 140         | 400         | 12          | 180         | 3.5         | 40          | 70          | 9           | 30          | 7           | 1.2         | 1           |
|     | (STD ISO-18)  |             |             |             |             |             |             |             |             |             |             |             |             |             |             |
|     | Standard      |             |             |             |             |             |             |             |             |             |             |             |             |             |             |
|     | (STD DS7)     |             |             |             |             |             |             |             |             |             |             |             |             |             |             |
|     | Shale         |             |             |             |             |             |             |             |             |             |             |             |             |             |             |
|     | (Krauskopf, 1979) |         |             |             |             |             |             |             |             |             |             |             |             |             |             |
|     | Seawater      |             |             |             |             |             |             |             |             |             |             |             |             |             |             |
|     | (Krauskopf, 1979) |         |             |             |             |             |             |             |             |             |             |             |             |             |             |
|     | Crust         |             |             |             |             |             |             |             |             |             |             |             |             |             |             |
|     | (Krauskopf, 1979) |         |             |             |             |             |             |             |             |             |             |             |             |             |             |
|     | Shale         |             |             |             |             |             |             |             |             |             |             |             |             |             |             |
|     | (Krauskopf, 1979) |         |             |             |             |             |             |             |             |             |             |             |             |             |             |
|     | Seawater      |             |             |             |             |             |             |             |             |             |             |             |             |             |             |
|     | (Krauskopf, 1979) |         |             |             |             |             |             |             |             |             |             |             |             |             |             |

Table 2: The geochemical parameters of shell samples at the northern Marmara Sea and the Bosphorus on coasts with Yarımburgaz Cave (with standards, and according to Krauskopf values of crust, shale and seawater).
(Pearson) was calculated for the transformed Chalcolithic era shells and recent shells elemental contents of Ba, Nb, Rb, Sr, Th, Zr, Y, La, Ce, Pr, Nd, Sm, Mo, Cu, Pb, Zn, Ni, As, Cd, Sb, Hg, Se, and Au.

2.4. Taxonomy of *Mytilus galloprovincialis* Lamarck, 1819

Mytilidae family (Mollusca, Bivalvia) has a wide distribution range. *M. galloprovincialis* Lamarck, 1819 is an important member of this family and is distributed in the Mediterranean and Black Sea. On the Turkish coastline, *M. galloprovincialis* is commonly found on the Black Sea coasts, the Sea of Marmara, the Biga Peninsula, the Gulf of Edremit and the coasts of Ayvalik. However, on the Mediterranean coasts, this mollusc is rarely observed. *Mytilid* shells have microstructures. The species living in subtropical regions have shells with two or three layers of aragonite and calcite, whereas the others have two layers of aragonite and nacre (*Gosling, 1992*).

3. Results

The measured values of Be, Co, Cs, Ga, Hf, Sn, Ta, U, V, W, Gd, Ho, Tb, Er, Lu, and Th in *M. galloprovincialis* shells were found to be below the MDL (method detection limits). Some rare earth elements (REEs) in the Lanthanide series, such as Eu, Tb, Dy, Er, and Lu were not found in the recent shells, but were measured in specimens from the Chalcolithic period in sample of Yarimburgaz Cave. Nevertheless, several elements (Mo, As, Cd, Sb, Hg, and Se) were not detected in specimens from the Chalcolithic period in this sample, but were measured in recent shells.

The measured values of Ba, Sr, Zr, Y, La, Ce, Pr, Nd, and Sm in samples from Yarimburgaz Cave were found to be higher than the values of recent shells. However, several metal, trace, and rare earth elements which are potential pollutants (Ag, As, Cd, Sb, Br, Ca, Co, Cs, Fe, Na, Sr, and Zn) were observed at higher quantities in recent samples. Pb, Zn, Ni, As, Au, and Se values measured in the samples from the middle of the Bosphorus Strait were found to be the highest of all samples measured.

In this study, the distribution of Ba and Sr (Fig. 6) in Yarimburgaz Cave shells was higher than in recent shells. Ba values were similar in recent shells from sample 12 and in ancient shells from sample of Yarimburgaz Cave. The lowest Ba content was noted in samples 2, 4, and 9. The highest Sr content was noted in the sample of Yarimburgaz Cave. The lowest Sr value was noted in sample 9 and the highest in sample 12.

The radioactive elements (Th: 0.3 μg g⁻¹, Y: 0.4 μg g⁻¹) were measured at higher levels in Yarimburgaz Cave shells than in recent shells. Th was measured only in sample 10 (Uskudar) shells (0.2 μg g⁻¹). The Y values were determined in samples 6, 8, 10, and 12 (0.2 μg g⁻¹) and in 11 (0.1 μg g⁻¹).

The distribution of trace elements is displayed in Fig. 7. Nb was found only in sample of Yarimburgaz Cave and 8 (Beylerbeyi), with a higher value in sample of Yarimburgaz Cave shells. Zr was not detected in sample 4 (Anadolu-kavagi) and was at its highest value in samples 6, 11, and 12. As was not detected in sample of Yarimburgaz Cave and was in the range of 0.5–1.8 μg g⁻¹ in recent shells.
Au was detected in shells from the Chalcolithic period and from recent shells in samples 1 (Selimpasa), 4 (Anadolukavagi), 5 (Pasabahce), 6 (Kanlica), 7 (Kucuksu), and 8 (Beylerbeyi). The Au content from recent shells in samples 1 and 4 are similar to sample of Yarimburgaz Cave. The highest value was noted in sample 7 (Kucuksu) (Table 2).

The REE Eu, Tb, Dy, and Lu were only detected in Yarimburgaz Cave. La, Ce, Pr, Nd, Sm, and Y values are lower in the recent shells than in Yarimburgaz Cave (Fig. 8). The La value ranges over 0.2–0.6 µg g⁻¹ in the recent shells, but is 1 µg g⁻¹ in Yarimburgaz Cave. The Ce value is highest (1.9 µg g⁻¹) in Yarimburgaz Cave, ranging over 0.1–0.4 µg g⁻¹ in the recent shells. Nd was detected in recent shells in samples 2 (Balatalimani) and 10 (Uskudar), and the Sm values is lower in sample 6 (Kanlica) than in shells from the Chalcolithic period. Y was detected off of the Bosphorus on the Asian side and eastern Marmara coast in samples 6, 8, 10, 11, and 12.

The heavy metals Cd and Hg were found only in sample 8 (Table 2). Cu, Pb, Zn, and Ni were measured in all samples. Mo, As, Cd, Sb, Hg, and Se were not observed in the Yarimburgaz Cave but were measured in some of the recent samples. The lowest value of Zn was observed in sample 3 (Istinye). Cu, Pb, and Ni were measured at high values in recent shells (except sample 11). The Ni value ranged over

Figure 6  Distribution of Ba (A) and Sr (B) elements in samples from Yarimburgaz Cave, the northern Marmara Sea, and Bosphorus coasts.

Figure 7  Distribution of trace elements in samples from Yarimburgaz Cave, the northern Marmara Sea, and Bosphorus coasts.

Figure 8  Distribution of rare earth elements in samples from Yarimburgaz Cave, the northern Marmara Sea, and Bosphorus coasts.
4.6–8.3 μg g⁻¹ and the highest value was for recent shells in sample 8 (Beylerbeyi). The Ni values were also low in the Chalcolithic period sample and in the recent sample 11 (Tubitak-MAM) (Fig. 9).

The measured Zn values of the other recent samples 1, 7, and 9 were close to but lower than the value of sample of Yarimburgaz Cave. In sample 5, the highest toxic metals were found to be Pb and Zn; whereas in sample 8, Ni and Zn; in sample 1 (Selimpasa), Cu and Ni; and in sample 4 (Anadolu-kavagi), Pb display the highest metal content (Fig. 9).

The toxic metalloids were determined only in the recent shells and were in the range of 0.1–0.7 μg g⁻¹ for Mo, 0.5–0.8 μg g⁻¹ for Se, and 0.1–0.4 μg g⁻¹ for Sb. The maximum value of Mo and Sb were also determined in sample 1 (Selimpasa). The Se content was determined in the Bosphorus on the Asian side and in eastern Marmara coast in samples 5, 6, 7, 8, 9, 10, 11, and 12.

The Chalcolithic period shells and Selimpasa shells did not display Cd, Hg, and S. The values of Au are similar in all shells. Only Chalcolithic period shells displayed REEs such as Nb, Th, Y, Nd, Sm, Eu, Tb, Dy, and Lu. Only recent shells displayed Mo, As, and Sb. Other REEs and trace elements (Ba, Rb, Sr, Zr, La, Ce, Pr, Pb, and Au) are higher in the Chalcolithic period shell. Zn is higher in the Chalcolithic period shell, whereas other metals such as Cu, Pb, and Ni are higher in the recent shells.

A significant and positive Pearson correlation was found between the following: Nd and Th (r = 0.99, n = 23, α = 0.01) in all shells, whereas no correlation was noted between Nb and Th (r = –0.98, α = 0.01). The correlation between Cd and Hg was negative (r = –1, n = 23, α = 0.0).

The relationship between the recent shells (r = 1, n = 12, α = 0.01) was significantly positive, and a full linear relationship was obtained. A significant and strong positive correlation was found between the Chalcolithic period shells and recent shells (r = 0.99, n = 13). When the Chalcolithic period shells serve as a control group, a strong correlation is noted between Kuzguncuk and Selimpasa (r = 0.97), between Kuzguncuk and Kucuksu (r = 0.97), and between Istin et and Tubitak-MAM (r = 0.87).

The Cu and Pb and the Ni and Hg display a negative relationship, whereas the Pb and Zn, the Ni and Cd with Sb (r = 0.71, α = 0.05), and the Pb and As (r = 0.95, α = 0.05) display a positive correlation. The Zn (9.23) is an effective descriptive statistic within the group of heavy metals.

For the first sampling date, the variations of each metal concentration in the whole shell were tested by a one-way analysis of variance considering the site as a variable. When the ANOVA was significant, post hoc pairwise comparisons between the stations were conducted using Scheffe’s test to determine which values differed significantly. We correlated the Chalcolithic period with recent shells and elemental groups using ANOVA. The group value was p = 1, showing no statistically significant difference between the relationships (p > 0.05). With all shells from the one-way ANOVA, the Chalcolithic period and the recent shells were found to display no significant difference (F = 0.008, p = 1; p > 0.05).

However, the one-way ANOVA table shows that the degree of significance is p = 0.00 among recent shells. The one-way ANOVA showed that the relationship between recent shells is significant at p = 0.000 (p < 0.05).

The groups were compared to determine the differences with each other. According to Tukey’s test results in the table of Multiple Comparisons, the difference between the Chalcolithic era shells (X: 65.37) and the recent shells (values in the range of X: 45.54–58.58) were determined to be statistically significant. The Chalcolithic era shells (X = 65.37) was found to be significantly different from sample 9 (Kuzguncuk) (X = 45.54) and 10 (Uskudar) (X = 49.38).

For the recent shells in Dil Iskelesi, the shells (X = 58.58) showed significant differences from the other recent shells (values in the range of X: 45.54–57.44). Nevertheless, the Dil Iskelesi shells (X = 58.58) showed significant differences from Kuzguncuk (X: 45.54), Selimpasa (X: 57.44) and Uskudar shells (X: 49.38).

The Kaiser-Meyer-Olkin (KMO) test was performed to determine the reliability, displaying correlation eligibility values close to 1 (KMO measure of sampling adequacy 0.91). Six factors explain 91.13% of the total variance in practice. The first factor explains 37.82% of the variance. After rotation equalizing the relative importance of the factors, the contribution of factor 1 falls to 34.64%. The component values of each item was examined according to a Principal Component Analysis (PCA) component matrix divided into six components. Therefore, Ba, Nb, Sr, Th, Y, La, Ce, Pr, and Nd explain the first factor; Rb, Zr, Zn, As, Cd, and...
and Se explain the second factor; Rb, Mo, Cu, and Sb explain the third factor; Ni and Cd explain the fourth factor; Pb explains the fifth factor; Pb and Zn explain the sixth factor. The variables influencing the factors were found when examining the component values of the Rotated Component Matrix used in the creation of the structure. The condition index of the shells was also included in the PCA. Data were normalized using a log(x + 1) transformation. Factors 3 and 4 have been mutually replaced. Factor 5 included Sr and Au. For another evaluation, Ba (variable) is effective in factors 1 and 2, Nd is in the first factor, Zr is in the second factor, Cd is in the third factor, Mo is in the fourth factor, Au is in the fifth factor, and Cu is in the fifth factor. Therefore, the factors contain effective and positive effects that do not differ.

4. Discussion

The *Mytilus galloprovincialis* shells were determined to be a combination of aragonite-calcite. The shell was composed of Ca (104.37, 16 μg g⁻¹), Mg (74 μg g⁻¹), and Sr (36.17 μg g⁻¹) (Ökten, 2009). The Mg, Sr, Ba, and Si contents are high because they are structural elements of the shell. The abundance of these metals, therefore, exceeds other metals. Additionally, Mg, Ba, and Sr are important for the development, growth, and reproduction of the organism and are related to the temperature and salinity of the environment.

Nb is mainly found in niobite [(Fe, Mn)(Nb, Ta)₂O₆] and niobite tantalite [(Fe, Mn)(Ta, Nb)₂O₆] minerals in nature. Ba, Sr, La, and Pr were found in all of the shells analyzed, whereas Rb, Zr, Ce, Nd, and Sm were not observed in several of the recent samples (Table 2). The rare earth elements La and Pr were found in sample of Yarımburgaz Cave at its highest value. The measured values of these elements in coasts Marmara Sea as in samples 1 (Selimpasa), 11 (Tubitak-MAM) and 12 (Dil Iskelesi) were nearly identical.

High Ba content in sediments is normal. However, the high values of Au and Sr suggest that the sediment can be related to hydrothermal outlets. The most common and most widely used source of barium is naturally occurring sedimentary barite mines. The sediment has a layered structure because of the transportation by seas or rivers. Hot water outlets are usually seen in the region. The observed non-parallel relationship between the Ba, Pb, and Zn may indicate an anthropogenic origin of these elements (Apaydın and Erseçen, 1981; MTA, 2000).

An increase in the environmental pollution in the last 7500 years is prominent in the study field (Özdögan, 1999; Özdoğan and Koyunlu, 1986). Marine pollution constitutes a major environmental problem in Turkey, which is surrounded by three seas. Marine transportation, tourism, disposal of industrial and domestic wastes without purification, petroleum derivatives released by accidents, and accidental chemical spills by water systems are the main causes of marine pollution that adversely affect the marine life.

The system in Marmara and the Bosphorus display unique hydrographic and ecological characteristics. These locations experience different pollution inputs from domestic, industrial, maritime transport (including tanker accidents) and from the Black Sea and Danube River. However, these pollutants flow to coasts Marmara Sea from the Black Sea and the Aegean Sea, notably from the increased concentrations of these pollutants in the Black Sea coasts.

As a result of urbanization and industrial activities, the limits of pollutants have been exceeded (Dethlefsen, 1988; Hammand and Bellies, 1980; Kaya et al., 1998; Sani, 1984). The Golden Horn and Gulf of Izmit is affected by physical and chemical pollutants, whereas, Bosphorus is polluted mainly by domestic and industrial wastes (Başarin et al., 2000; Çağatay et al., 2006; Göksu et al., 2005; Topcuoğlu et al., 2004). In these studies, fish, algae species, and the organic parts of the mussels have been used for to investigate the metals in the sea water.

Metals, such as Hg, Cd, Pb, Cu, Ni, Zn, Cr and As, accumulate in the food chain and display toxic effects on the biota. *M. galloprovincialis* is a filter feeder, filtering organic material and phytoplankton and accumulating toxic materials in its tissues and shell. In this study, the distribution of the elements in the shells was determined to be higher than the average of the sea water (Krauskopf, 1979) (Table 2).

The distribution of trace elements and REEs were affected by the geochemistry in the bottom sediments and in seawater of the terrigenous (terrestrial and anthropogenic) material in the Bosphorus and the Marmara Basin. In the Marmara Sea, the small and terrestrial inputs are higher. The trace elements (Ba, Sr, etc.) and lithophile elements (K, Rb, Li, La, Nb, etc.) originate from terrigenous sources. These metals are thought to originate from terrigenous erosion material, transported by the stream drainage network as suspended matter. The domestic and industrial discharges into the sea result in excessive organic matter in the sediments of the sea floor and a high metal pollution. Therefore, the form of the metal will change and precipitate, settling into the sediment in the basin. Thus, the bottom water is affected and can contaminate the shells.

The comparative toxicities of some of the metal and trace elements found in sea water can be shown as follows as from highest to lowest: Hg > Cd > Zn > Pd > Pb > As > Cr > Sn > Zn. The metal atoms can combine with organic molecules and be released to the environment when the organic molecules are degraded (Balkis and Algan, 2005).

Atayeter (1991) showed that the Fe, Cu, Zn, Pb, and Al concentrations in the gills and digestive systems of the *M. galloprovincialis* specimens collected from Anadolukavagi (Bosphorus, Istanbul) vary throughout the year. In August, September, and November, the maximum accumulation of Zn is observed, followed by Fe, Cu, and Pb. However, the maximum accumulation was observed in Pb during January and February. Şentürk (1993) analyzed the Hg, Cd, and Pb concentrations in mollusc specimens collected from various parts of the Sea of Marmara, and found that the average concentrations of Hg, Cd, and Pb were 0.46 μg g⁻¹, 0.25 μg g⁻¹, and 0.304 μg g⁻¹, respectively. These concentrations are below the acceptable limits suggested for marine products. The Cu, Zn, Pb, and Cd concentrations of the soft tissues of *M. galloprovincialis* specimens collected from Sinop (Black Sea) were significantly different from the values observed in the coastal waters (Bat and Gündoğdu, 1999).

The Zn, Cu, Mg, and Fe contents of the inner and outer parts of the *M. galloprovincialis* shells have been compared in the samples from Napoli Bay, and only the Mg values have been found to vary (Cotugno et al., 1983). In a study performed on the coasts of NW Spain, the lead and nickel
concentrations observed in the shells and in the soft tissues of *M. galloprovincialis* have been compared with the values observed in the sediment. The shell of *M. galloprovincialis* was more reliable for monitoring the metal levels when compared to the soft tissues (Puente et al., 1996).

Mauri and Baraldi (2003) monitored the changes in metal concentrations in the shells and soft tissues. They transferred *M. galloprovincialis* specimens from the open sea to the Venice Lagoon, in which the pollution was well documented. They measured the Cr, Pb, Cu, Zn, Mn, and Fe concentrations periodically in the shell and other tissues. Initially in the lagoon and in the soft tissue of *M. galloprovincialis*, no difference was observed for Pb and Zn observed, and Cu, Cr, Mn, and Fe values displayed low levels. After a month, these metal levels increased in the *M. galloprovincialis*, but after two months, the values decreased. Except for the metal content of Cu in the shell, the Zn and Pb contents were determined to have decreased. They showed a considerable change in the metal concentrations three months after transfer.

The metal concentrations in the gills were found to be higher when compared to the other parts of the *M. galloprovincialis* specimens collected from the Danube Delta (Black Sea) (Roméo et al., 2005). A multivariate analysis (Duncan) showed that the highest accumulating metal in the shell was Fe, and the least accumulating metals were Cu, Mn, and Co (Tosyali, 2005).

Aksu (2005) and Aksu et al. (2007) studied Cd, Cu, Ni, Pb, Zn, Mn, Fe, Cr [μg g⁻¹], and Hg [ng g⁻¹] in the edible parts of *M. galloprovincialis* specimens collected from the Bosphorus, Anadolukavagi, Beykoz, Uskudar, Baltalimanı, Buyukdere, and Ortakoy, in May, August, November 2003, and February 2004. The sampled specimens were separated into two groups according to their sizes: large (>10 cm) and small (<10 cm). The metal concentrations were higher in the small sized group. The distribution of metals was also found to differ between the stations. Cd, Mn, Ni, Pb, and Zn were higher in Beykoz samples. Cr and Hg were higher in Ortakoy samples. Cu was highest in the small sized group in Ortakoy and in the large sized group in Beykoz. However, Fe was the highest in the small sized group in Beykoz and in the large sized group in Buyukdere. The highest concentrations of the toxic and non-toxic metals have been observed during August and November 2003.

Another recent study by Yabanlı et al. (2015), studied the low levels of toxic metals (Cd, Hg, Pb, Cr, Ni) found in tissues of *M. galloprovincialis*, comparing with the other studies including the inner part of the Gulf of Izmir. And this specimen can be used as a sensitive biomonitor for

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![Figure 10](image-url)  
**Figure 10** Distribution of metals in samples of the part eaten (after Aksu’s (2005) data) with shells (our study).
the availabilities of studied elements in the inner Gulf of Izmir.

Cd, Cr, Ni, Pb, and Hg measurements were compared with the values [μg g⁻¹] reported in Aksu et al. (2007) obtained in May and August 2003 from Anadoluakavagi, Uskudar, Istinye (Buyukdere), and Batalımanı (Fig. 10). In the shells, Hg [ng g⁻¹] was only found in the Beylerbeyi samples, whereas in the organic material, Hg [ng g⁻¹] values were found to be high in all samples, notably in the Ortaköy sample. The Ni concentration was the highest in the shells, and the Cu was highest in the organic material. Pb was found to be lower both in the shells and in the organic materials when compared to other metals. Zn was measured at a minimum level in the shells; however, the Zn content was relatively higher in the organic material. The metal distribution agreed in all of the samples in the organic material, but not in the shells. Therefore, a linear relationship was not observed in the distribution of metals between the shell and the organic material (Fig. 10).

5. Conclusions

The results show that the recent M. galloprovincialis shells and the samples from the Chalcolithic period display considerable differences in the geochemical characteristics. The rare earth elements Eu, Tb, Dy, Er, and Lu were only observed in the Chalcolithic period shells, whereas the trace and rare earth elements Ba, Sr, and As were measured in all samples. The source of the Th is likely to be a type of granitic and gneissic rocks. A non-linear relation was observed in Ba, Pb, and Zn concentrations in the shells, suggesting an anthropogenic origin for these elements. A linear relation was observed neither between the metal concentrations nor in the metal contents of the shell with the soft tissues of the recent samples. Differences in the Cd, Pb, As, Sb, and Se contents were noted in the Chalcolithic period shells and recent shells. The source of metal pollution as a result of domestic and industrial activities can be explained by anthropogenic inputs. The resulting statistical value (p = 1) shows no statistically significant difference between the relationships noted in the shells.

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