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Macrobenthic Invertebrates of the Hersek Lagoon (Marmara Sea, Turkey) under Pollution Pressure

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Abstract: The Hersek Lagoon is a small lagoon located in the Marmara Sea. It is a breeding and feeding area for many bird species, which makes it an internationally important wetland despite its small size. However, it is under pressure due to the deterioration of the natural water regime and increasing pollution. Although macroinvertebrates are important indicators in the environmental monitoring, no study has been conducted on the macroinvertebrate fauna of this lagoon. The present study was conducted to evaluate the relationship between environmental parameters and benthic macroinvertebrates. Totally, 34 species were identified in the lagoon, two of which (*Hydroides dianthus* (Verrill, 1873), *Lekanesphaera monodi* (Arcangeli, 1934)) are reported from the Sea of Marmara for the first time. Three alien species were detected in the lagoon: *Ruditapes philippinarum* (A. Adams & Reeve, 1850), *Polydora cornuta* Bosc, 1802 and *Hydroides dianthus* (Verrill, 1873). Diversity index values at all stations showed that macrobenthic fauna is poor in terms of species diversity. Low levels of dissolved oxygen and the high total organic carbon content throughout the lagoon sediment seem to negatively affect the benthic fauna, especially at stations far from the open sea. The fact that the dominant species in the lagoon are resistant to organic pollution also supports this view.

Key word: Wetlands, macrobenthic invertebrates, biodiversity, lagoon ecosystem, ecology, organic enrichment

Introduction

Wetlands are ecologically efficient ecosystems with high biodiversity and production; which are home to many migratory animals (GARRIDO et al. 2011). Lagoons located between wetlands are defined as coastal ponds that are partially connected with the sea and, as they are situated between land and sea, they are influenced by both ecosystems (NEWTON & MUDGE 2003). They have high production due to the nutrient-rich waters of mouthing streams (GILABERT 2001). Lagoons are also suitable areas for the breeding of invertebrates, fish and birds due to their rich vegetation (GARRIDO et al. 2011). For these reasons, they are among the most important ecosystems in global scale, both ecologically and economically.

Turkey has 72 coastal lagoons, 12 of which are located in the Marmara region (FAO 2015). Our study was conducted in the Hersek Lagoon, which is located in the Izmit Bay and is under intense pressure by industrial activities. It is a small and shallow coastal lagoon with an area of 14 km²

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and is connected with the Marmara Sea (BERTRAND et al. 2011, FAO 2015). The lagoon and the creek that used to supply water to it (Yalak Dere) are no longer connected as a result of tectonic movements; currently, the lagoon is fed only by the Marmara Sea, rainfall and runoff (UZUN 2014). The lagoon is shallow, with a depth of 0.3–0.7 m (MISCHKE et al. 2012). Izmit Bay, where the lagoon is located, is highly affected by domestic and industrial pollution (GÜVEN et al. 1997, AKKOYUNLU et al. 2002, TOLUN et al. 2008).

Benthic invertebrates are among the most useful bioindicators for monitoring marine ecosystems due to their rapid response to human-induced and natural pressures (PEARSON & ROSENBERG 1978, POCKLINGTON & WELLS 1992, DAUER 1993, PAN-CUCCI-PAPADOPOULOU et al. 1999, ÇAGLAR 2008).

Although the Hersek Lagoon is a small area, it is a critical wetland ecosystem in terms of both wildlife and recreational potential. The lagoon has bird areas that are important according to international criteria and is itself a wetland of international importance. It was accepted as a 1st degree protected area by the Ministry of Forestry and Water Affairs, General Directorate of Nature Conservation and National Parks (DALKIRAN & BAKI 2011). However, no detailed study of the benthic community has been conducted previously in the Hersek Lagoon.

Our study aims to evaluate the macrobenthic invertebrates of the Hersek Lagoon both qualitatively and quantitatively through the estimation of its species diversity, abundance and dominant species. In addition, information about the presence of indicator species as well as the physical and chemical parameters provides the scientific data about the ecological status of this lagoon, which is under intense pollution pressure.

Materials and Methods

Study area and sampling

Sampling was conducted in July 2017 at six stations in the Hersek Lagoon (Fig. 1). Due to the very shallow and muddy structure of the lagoon, samples were taken from 20 x 25 cm areas with a Surber net and were sieved with 0.5 mm mesh. The sampled invertebrates were stored in a 4 % formaldehyde solution prepared with seawater. Sampling was performed in triplicate at each station. Species were identified using FAUVEL (1927), PERRIER (1930), RIEDL (1963), NAYLOR (1972), LOCARD (1982), BELLAN-SANTINI et al. (1982, 1989, 1993, 1998), BUCQUOY et al. (1882-1898), D'ANGELO & GARGIULLO (1987), TENEKIDI (1989), POPPE



Fig. 1. Map of the sampling area. Coordinates of the stations: Station 1: 40°42'53.1"N, 29°30'46.5"E; Station 2: 40°43'09.5"N, 29°30'31.9"E; Station 3: 40°43'14.5"N, 29°30'22.4"E; Station 4: 40°42'58.9"N, 29°31'34.5"E; Station 5: 40°43'13.3"N, 29°31'12.6"E; Station 6. 40°43'21.2"N, 29°30'55.1".

& GOTO (1991, 2000), COSSIGNANI et al. (1992), ARDUINO et al. (1995), WILKE & AARTSEN (1998), HOUART (2001), GIANNUZZI-SAVELLI et al. (2002, 2003). Nomenclature follows WoRMS (2020). We determined the number of individuals belonging to each species. We measured the temperature, salinity, dissolved oxygen and pH values of the water using YSI multiparameter device.

Samples taken from the upper layer of sediment with the help of a plastic shovel were brought to the laboratory in bags in cooler containers. Samples were stored in a freezer at -20°C until analysis. The total amount of organic carbon was determined using the Walkey-Blake method (GAUDETTE et al. 1974, LORNING & RANTALA 1992). The methods described by GALEHOUSE (1971) and MCMANUS (1991) were used to determine the mud percentage of the sediment samples.

Statistical analyses

We determined the frequencies of each species using the SOYER's (1970) Frequency index (Fi). We used the following formula while calculating this index: $Fi = N_A / N \times 100$. In this formula, N_A is the number of samples containing the species A and N is the total number of samples. The species were placed into three groups: "Constant" (Fi > 50 %), "Common" (50 %> Fi' > 25 %) and "Rare" (Fi < 25 %).

The dominance was calculated using the dominance index formula (BELLAN-SANTINI 1969): DI= $N_t / N_n x 100$, where N_t is the individual number of the t species and N_n is the number of individuals of all species.

The homogeneity and regularity of the distribution of the number of individuals among the species in the study area were evaluated using the Evenness (Pielou) Index (PIELOU 1966). The Pielou regularity index (J') was calculated with the help of the formula: $J' = H'/H_{max}$, where H' is Shanon-Weaver Diversity Index value calculated at the station and H_{max} is the highest calculated value of the Shanon-Weaver Index.

We calculated the Shannon-Weaver Diversity Index (H') using the composition of species and individual numbers at the sampling stations (ZAR 1984). The Shannon index formula was published by Shannon in 1949 and is an index applied to the biological system (KREBS 1989). These index values range from 0 to 5 and may exceed 5 in rare cases. Values above three indicate the balance in habitat and community (KOCATAŞ 1992).

$$H' = -\sum_{i=1}^{K} P_i \log_2 P_i P_i = f_i \div n$$

where k is the species number, fi is the number of the individuals of the i species and n is the total number of individuals in the sample.

Similarities between stations were determined using the Bray-Curtis Similarity Index, applied by using the software PRIMER, v. 6. The multidimensional scaling (MDS) analysis was used to visualise similarities between species distributions among stations. For these analyses, a log (x + 1) transformation were applied to the raw data. The contribution of each species to the similarities and differences at station groups was calculated using SIMPER analysis (CLARKE & WARWICK 2001). Spearman's Rank Correlation Coefficient (SIEGEL 1956) was used to determine the correlation between species and individual numbers and other abiotic parameters such as the total organic carbon (TOC), dissolved oxygen, salinity and mud percentage.

Results

Macrozoobenthic species diversity

We found 32 species of 26 families belonging to the phyla Annelida, Arthropoda, Cnidaria, Mollusca and Sipuncula (Table 1). The most dominant group was the Crustacea (35.5 %) with 12 species, followed by Annelida with 11 species (32.4 %) and Mollusca with nine species (26.5 %). Two invertebrate species, i.e. *Hydroides dianthus* (Verrill, 1873) and *Lekanesphaera monodi* (Arcangeli, 1934), are recorded from the Sea of Marmara for the first time (Table 1).

Ruditapes philippinarum (A. Adams & Reeve, 1850), *Polydora cornuta* Bosc, 1802 and *Hydroides dianthus* are alien species for the coast of Turkey (Çınar et al. 2005).

The number of species at each station varied from three (Station 3) to 26 (Station 4, see Table 1). The abundance of each species at each station is presented as the average of three replicate samples. In total, we obtained 76713 individuals during the study. The species represented by many individuals were Monocorophium insidiosum (Station 4) with 34146 individuals, followed by Polydora cornuta (Station 4) with 6014 individuals. At the Station 4, the highest numbers of both individuals and species were recorded. The two above-mentioned species also had the highest dominance index: M. insidiosum - 59.17 % and P. cornuta - 10.11 %. They were followed by Ecrobia ventrosa (8.25 %) and Abra alba (6.9%). The prevalence of macrobenthic invertebrates was evaluated using the Soyer's frequency index, resulting in 15 species (E. ventrosa, Tritia neritea, C. glaucum, A. alba, M. insidiosum, Gammarus crinicornis, G. aequicauda, Gammarus sp., Nereiphylla rubiginosa, P. cornuta, Hediste diversicolor, Capitella telata, C. capitata, Capi*tella* sp. and Oligochaeta sp.) being categorised as "constant". Other five species (Ruditapes philippinarum, Mytilus galloprovincialis, Sphaeroma serratum, Microdeutopus gryllotalpa and Hydroides dianthus) were "common". The remaining 12 species were "rare" (Table 1).

The analysis on the benthic invertebrate diversity of the stations revealed low values of the Sha**Table 1.** Macroinvertebrate species in the Hersek Lagoon and their density (average number of individuals per m²), biotic variables and diversity index values across sampling stations. Abbreviations: FI, Frequency Index; DI, Dominancy Index; J', Pileou Regularity Index; H', Shanon–Weaver Diversity Index.

| Spacing | | Stations | | | | | | ы |
|--|--------|----------|------|---------|---------|---------|-----------|-------|
| Species | 1 | 2 | 3 | 4 | 5 | 6 | F1 | |
| Phylum Annelida | | | | | | | | |
| Class Clitellata | | | | | | | | |
| Oligochaeta (gen. sp.) | 6.7 | 20 | 33.3 | 186.7 | | 33.3 | 83 | 0.37 |
| Class Polychaeta | | | | | | | | |
| Capitella capitata (Fabricius, 1780) | 53.3 | | | 26.7 | 40 | 6.7 | 67 | 0.17 |
| Capitella teleta Blake et al., 2009 | 120 | | | 26.7 | 80 | 13.3 | 67 | 0.31 |
| <i>Capitella</i> sp. | 20 | | | 13.3 | 33.3 | 6.7 | 67 | 0.1 |
| Glycera fallax Quatrefages, 1850 | | | | | 13.3 | | 17 | 0.02 |
| Glycera tridactyla Schmarda, 1861 | | | | | 6.7 | | 17 | 0.01 |
| Harmothoe antilopes McIntosh, 1876 | | | | 6.7 | | | 17 | 0.01 |
| Hediste diversicolor (O. F. Müller, 1776) | 320 | | | 1293.3 | 333.3 | 540 | 67 | 3.25 |
| Hvdroides dianthus (Verrill, 1873) | | | | 280.0 | 13.3 | | 33 | 0.38 |
| Nereiphylla rubiginosa (de Saint-Joseph, 1888) | 13.3 | | | 13.3 | 20 | 6.7 | 67 | 0.07 |
| Polydora cornuta Bosc. 1802 | 393.3 | 406.7 | | 6013.3 | 753.3 | 180 | 83 | 10.11 |
| Subphylum Crustacea | | | | | , | | | |
| Class Malacostraca | | | | | | | | |
| Order Amphipoda | | | | | | | | |
| Ampithoe ramondi Audouin, 1826 | | | | | | 6.7 | 17 | 0.01 |
| Cryntorchestia cavimana (Heller, 1865) | | | | | 13.3 | 017 | 17 | 0.02 |
| Gammarus aequicauda (Martynov 1931) | | | | 146 7 | 67 | 13 3 | 50 | 0.22 |
| Gammarus crinicornis Stock, 1966 | | | | 973.3 | 80 | 20 | 50 | 1.40 |
| Gammarus insensibilis Stock, 1966 | | | | 380 | 00 | 20 | 17 | 0.33 |
| Gammarus sp | | | | 2020 | 26.7 | 20 | 50 | 2 70 |
| Protohvale (Protohvale) schmidtii (Heller, 1866) | | | | 67 | 20.7 | 20 | 17 | 0.01 |
| Microdeutonus gryllotalna Costa 1853 | | | | 2366.7 | 113.3 | | 33 | 0.33 |
| Monocoronhium insidiosum (Crawford, 1937) | | | | 34146 7 | 8026.7 | 3140 | 50 | 59.17 |
| Order Isonoda | | | | 54140.7 | 0020.7 | 5140 | 50 | 57.17 |
| Idotea halthica (Pallas 1772) | | | | 67 | | | 17 | 0.01 |
| Lekanesnhaera monodi (Arcangeli 1934) | | | | 13.3 | | | 17 | 0.02 |
| Sphaeroma serratum (Fabricius, 1787) | | | | 15.5 | 13.3 | 20 | 33 | 0.02 |
| Phylum Chidaria | | | | | 15.5 | 20 | 55 | 0.05 |
| Class Anthazoa | | | | | | | | |
| Class Anunazoa | | | | 20 | | 12.2 | 50 | 0.05 |
| Actinita Sp. | | | | 20 | | 13.5 | 50 | 0.05 |
| Class Bivelvie | | | | | | | | |
| Class Bivalvia | 1720 | 572.2 | 72.2 | 190 | 1640 | 1100 | 100 | 6.0 |
| Abra alba (W. Wood, 1802) | 240 | 52.2 | /3.3 | 180 | 1040 | 80 | 82 | 0.9 |
| Medialug advigtious Lemonal, 1810 | 340 | 33.5 | | 40 | 435.5 | 80 | 03 | 0.01 |
| Modioius adriaticus Lamarck, 1819 | | | | 2667 | 0./ | | 1/ | 0.01 |
| Mytitus guitoprovinciaits Lamarck, 1819 | | | | 200.7 | 26.7 | | 22 | 0.51 |
| Rualtapes philippinarum (Adams & Reeve, 1850) | | | | 0./ | 26.7 | | 33 | 0.04 |
| Class Gastropoda | | | | (7 | | | 17 | 0.01 |
| Bittium reticulatum (da Costa, 1//8) | 000 | 200 | 02.2 | 6./ | 40.00 7 | 2267 | 1/ | 0.01 |
| Ecrobia ventrosa (Montagu, 1803) | 980 | 380 | 93.3 | 5/3.3 | 4066.7 | 226.7 | 100 | 8.25 |
| Parthenina terebellum (Philippi, 1844) | | | | 2067 | 0./ | 22.2 | 17 | 0.01 |
| Irilla neritea (Linnaeus, 1/58) | 146./ | | | 306./ | 115.5 | 55.5 | 0/ | 0.78 |
| Phylum Sipuncula | | | | | 150.0 | | 1- | 0.00 |
| <i>Nephosoma</i> sp. | 4112.2 | 1.422.2 | 200 | 40000 | 173.3 | - 4 - 0 | 17 | 0.23 |
| Iotal number of individuals | 4113.3 | 1433.3 | 200 | 49320 | 16186.7 | 5460 | | |
| Standart deviation of av. individual number | 335.8 | 130.8 | 20.3 | 5799.9 | 1504.1 | 557.8 | | |
| J' | 0.7 | 0.8 | 0.9 | 0.4 | 0.5 | 0.5 | | |
| $H'(\log 2)$ | 2.4 | 1.8 | 1.5 | 1.8 | 2.2 | 2 | | |



Fig. 2. Similarity between stations as revealed by the Bray–Curtis Similarity Index and Multidimensional Scaling Analysis (MDS).

non-Weaver Diversity Index at all the stations in the Hersek Lagoon (Table 1). The similarity analyses (Bray-Curtis analysis and MDS) revealed three major groups accounting for 60 % similarity among the stations (Fig. 2). The first group consisted of stations 2 and 3 (with similarity between them 63.07 %). The second group included stations 1 and 6 (similarity 69) %), and the third group consisted of stations 4 and 5 (similarity 66.73 %). The SIMPER analysis of each of these three groups showed a similarity within the first group of 22.86 %, with E. ventrosa (11.43 %), A. alba (8.98%) and species of Oligochaeta (100%) contributing to this similarity. The similarity within the second group was 41.37 %, with species contributing to it being A.alba (22.98 %), H. diversicolor (6.69 %), E. ventrosa (4.74) and P. cornuta (3.76%). Within the third, the similarity was 31.98 %, due mostly to *M. in*sidiosum (24.52 %), P. cornuta (2.30 %), E. ventrosa (1.75 %) and *H. diversicolor* (1.02 %).

Physical and chemical parameters

The water temperature in the Hersek Lagoon was 26.58-30.42°C, salinity 26.3-33.56 PSU, dissolved oxygen 3.93-13.95 (mg/l) and pH 7.46-8.94. The organic carbon content in the sediments was 1.69-5.5 %. The mud percentage was 19.7–91.11 % (Fig. 3). We evaluated the relationship between ecological parameters and the number of species and individuals using the Pearson Correlation index (Table 3). When examining the influence of the physical and chemical parameters on the species number, we found strong negative correlations between the species number and organic carbon (r = -0.91, P < 0.01), between the species number and the salinity (r =-0.82 P, <0.05) and between the species number and the mud percentage (r=-0.89, P < 0.05). There was a strong positive correlation between the species number and dissolved oxygen (r= 0.93, P < 0.01).

| Stations | T (°C) | S (‰) | DO (mg l ⁻¹) | рН | TOC (%) | MP (%) |
|----------|--------|-------|--------------------------|-----|---------|--------|
| 1 | 27.1 | 26.3 | 4.36 | 7.8 | 3.50 | 53.1 |
| 2 | 29.3 | 32.4 | 4.92 | 7.5 | 3.80 | 91.1 |
| 3 | 30.4 | 33.6 | 3.93 | 7.7 | 5.50 | 91.1 |
| 4 | 27.2 | 26.7 | 13.95 | 8.2 | 2.10 | 35.4 |
| 5 | 26.6 | 27.2 | 11.75 | 8.2 | 1.85 | 20.3 |
| 6 | 29.6 | 28.4 | 11.60 | 8.9 | 1.69 | 19.7 |

Table 2. Physical and chemical parameters of sea water and sediment in the Hersek Lagoon. Abbreviations: T, temperature; S, salinity; DO, dissolved oxygen; TOC, total organic carbon; MP, mud percentage.

Table 3. Pearson Correlation Coefficient (r) of abiotic and biotic parameters in water and sediments from the Hersek Lagoon. Abbreviations: T, Temperature; S, Salinity; DO, Dissolved oxygen; TOC, Total organic carbon; MP, Mud percentage; NS, number of species; Ni, number of individuals. Legend: *Correlation is significant at the 0.05 level (1-tailed). **Correlation is significant at the 0.01 level (1-tailed).

| | Т | S | DO | рН | тос | MP | Ni |
|----------|-------|-------|--------|------|--------|------|------|
| Salinity | .852* | | | | | | |
| DO | 443 | 599 | | | | | |
| pН | 037 | 463 | .736 | | | | |
| ТОС | .568 | .765 | 874* | 736 | | | |
| MP | .579 | .828* | 838* | 833* | .920** | | |
| NS | 730 | 819* | .929** | .624 | 890* | 906* | |
| Ni | 561 | 538 | .764 | .234 | 519 | 462 | .764 |

Discussion

In this study, we provide information about the benthic invertebrate fauna of the Hersek Lagoon, where no macrobenthic study has been previously performed. As stated in the findings, L. monodi and H. dianthus constitute new records for the Marmara Sea and H. dianthus is an alien species in Turkey. It is widely distributed in various natural habitats on the eastern coast of North America, including open coasts and partially brackish coves, lagoons and harbours (LINK et al. 2009). In Turkey, it has only been reported from the Aegean Sea coast (Ç1NAR et al. 2006). The other alien species identified in this study are P. cornuta and R. philippinarum. While H. dianthus and P. cornuta are transported by shipping activities, the pathway of introduction of R. philippinarum is through aquaculture (Çınar et al. 2005). It has been reported from the coasts of the Aegean, Mediterranean and Marmara Seas in Turkey (ALBAYRAK et al. 2001, ÖZTURK et al. 2014). Polvdora cornuta is a common invasive species widely distributed from the North Atlantic to Australia (ÇINAR et al. 2005). It has been reported from all seas surrounding Turkey except the coastal waters of the Black Sea (KARHAN et al. 2008, DAĞLI & ERGEN 2008, Cinar et al. 2014).

Monocorophium insidiosum, the most dominant species in our study, is a detritivorous amphipod that lives in a wide salinity range (15–40 PSU). It forms tubes and is common in lagoons (DIVIACCO 1983, PRATO & BIANDOLINO 2006, OLIVER et al. 2006). This species is also known to be an indicator of pollution (ANGER 1977). Other species with high dominance in this study area were *P. cornuta*, *E. ventrosa* and *A. alba. Polydora cornuta* especially is an opportunistic species with high reproductive capacity (TAKATA et al. 2011).

We observed the highest number of species and individuals at Stations 4 and 5, while the lowest number was at the Station 3. Due to their locations within the lagoon, Stations 4 and 5 have more interaction with seawater, while the Station 3 lies far from the sea. Besides rain and seawater, the lagoon has no additional water source (UZUN 2014). For this reason, the number of species and individuals decreases with increasing distance from the sea. Similar results have been found in hypersaline lagoons (GORDON 2000). Dissolved oxygen values decrease at stations, which are far from the sea. This is because oxygen-rich sea waters, while they are the major water source, do not reach the most distant areas within the lagoon due to limited water mixture (Uzun 2014).

Dissolved oxygen content decreases with the increase of the total organic carbon content (TOC) in the sediment and there is a resulting significant

decline in the number of species. There is a very strong positive correlation between mud percentage of sediment and TOC. As the mud percentage increases, grain size decreases and the accumulation surface increases. The correlation between the TOC and the accumulation of pollutants in sediment had been described (HYLAND et al. 2005). ALBAYRAK et al. (2006) found four critical TOC value ranges in the Sea of Marmara: low (1-5.9 mg/g), medium (6-11.9 mg/g), high (12-21.9 mg/g) and very high $(\geq 22 \text{ mg/g})$. According to these values, the Hersek Lagoon, with values between 16.9 and 55 mg/g, fits between high and very high levels. Organic matter enrichment can indicate pollution but coastal lagoon sediments also typically have high organic matter content (FALCO et al. 2004). Corroborating previous studies, we also observed a decrease in the overall number of species in the sediment but an increase in the abundance of the tolerant species (HYLAND et al. 2005, FUJIBAYASHI et al. 2019). In this study, we observed that the number of species in the lagoon is low and there was a high proportion of tolerant species. Increased TOC is known to cause physiological stress in benthic organisms (PEARSON & ROSENBERG 1978). Organic degradation increases alongside an increase in TOC, while oxygen levels drop due to increased biological oxygen demand and the relevant by-products of the organic degradation process (ammonia and sulphides) are produced (HYLAND et al. 2005). On the other hand, it has been reported that species richness is lower in sediment with low organic matter content due to the lack of nutrients (FUJIBAYASHI et al. 2019). We observed the negative consequences of high carbon accumulation in the Hersek Lagoon. However, the benthic fauna structure is under the influence of many factors, including currents, contaminants, substratum structure and predators; it is, therefore, impossible to say that TOC is the primary factor affecting species richness in the lagoon (HYLAND et al. 2005).

Conclusion

Macroinvertebrates are important components of the food chain, providing food for a wide range of organisms, from fish to aquatic birds. Because the lagoon is a home for many bird species, perhaps most notably flamingos, it is critical to keep the benthic environment healthy. Due to its biological diversity and economic importance, more studies focusing on protective measures to secure the future and sustainability of the lagoon are necessary. This study constitutes an important step in this direction and will serve as a resource for future investigations. Acknowledgments: We are grateful to Dr. Serhat Albayrak, Dr. Senem Çağlar, Dr. Turgay Durmuş, Dr. Mert Kesiktaş, MSc. Eren Nural, MSc. Berivan Elif Arslan and MSc. Elif Yücedağ. This study was supported by the Research Fund of Istanbul University (project number BAP 26111).

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