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Original Research

Monitoring the Pollution Level in Istanbul Coast of the Sea of Marmara Using Algal Species *Ulva lactuca* L.

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Abstract

Heavy metal pollution in coastal regions has become a serious problem due to significant environmental degradations. In the present study, an algal species, *U. lactuca*, was investigated to assess the impact of heavy metal pollution on the six different locations along the coastal line of the Sea of Marmara in Istanbul. Heavy metal and mineral nutrient elements such as Ca, Cd, Cu, Fe, K, Mg, Mn, Pb, and Zn were determined using ICP-OES. The following lowest and highest concentrations (mg/kg; dw) were measured in *Ulva* samples: 1,751.66-2,465.68 for Ca, 0.45-3.22 for Cd, 6.67-18.32 for Cu, 553.32-989.33 for Fe, 3,264.48-4,301.68 for K, 961.50-1,614.53 for Mg, 8.27-25.32 for Mn, 4.93-19.32 for Pb, and 15.16-41.30 for Zn. The data revealed that *U. lactuca* is capable of accumulating considerable amounts of metals. Besides, metal deposition in the plant did not alter the mineral nutrient uptake pattern extensively, indicating relatively less contamination risk in the area. Overall, the comparison of heavy metal contents with seawater and sediment samples in the *Ulva* species showed that *U. lactuca* is a suitable plant for biomonitoring studies.

Keywords: sea lettuce, chlorophyta, heavy metal pollution, mineral nutrient uptake

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Introduction

Anthropogenic and industrial-based activities release high amounts of heavy metals into aquatic environments, which significantly disturbs marine life due to their toxic and accumulative natures [1-3]. Recently, heavy metal-related pollution in coastal regions has become a serious problem because of its significant environmental degradation [4]. Thus, environmentally friendly and efficient alternative technologies are required to clean the heavy metal-polluted waters [5].

Despite its imprecise definition, metals of higher than 5 g/cm^3 densities are regarded as heavy metals [6]. Some heavy metals in nature, such as Cd, Hg, and Pb, are not required by living organisms, therefore their presence, even in minor amounts, is toxic [7]. However, certain doses (trace amounts) of some heavy metals such as Cu and Cr (for plants), and Fe, Mn, Mo, Zn, and Ni are required by plants for healthy life cycles [8-10]. The over-accumulation or toxicity of heavy metals could cause serious consequences for biological systems by disturbing cell functions, finally leading to the formation of various symptoms or death of an organism [11]. Biologically, heavy metals could induce the production of ROS species, which interferes with functions of DNA, proteins, and lipids, and causes the oxidative stress leading to cell death [12-14].

Some organisms are considered as biomonitors or bioindicators to evaluate certain characteristics of the biosphere [15-16]. Using biological species in biomonitoring aquatic systems could allow for evaluation of the presence of contaminants and/or their effects on nearby living ecosystems. In addition, analysis of aquatic components such as water or sediment by relevant biomonitors could provide an overall perception about the contaminants rather than only a small fraction of eco-toxicological indications. Therefore, it considerably reduces or eliminates the requirement of complex investigations on chemical speciation of contaminants [4].

Green algae species show a widespread distribution in various freshwater ecosystems with significantly different physical and chemical parameters. They are very relevant organisms for aquatic biomonitoring studies because of their heavy metal uptake capacity, affinity of multiple elements, sedentary lifestyle, considerable biomass, and easy characterization [17-18]. Algae could bind the aquatic free metal ions via carbohydrate, protein, or lipid molecules in the cell wall surface; the density of these molecules varies depending on the nature and concentrations of the metal ions [2, 19-20]. Metal ion concentration and biomass, temperature, pH, cations/anions, and metabolic stage of algae significantly affect metal adsorption [21-23].

Ulva lactuca L., which is a member of Ulvaceae family, is a well-known and approved biomonitoring plant, and many studies are available indicating the use of this species for monitoring aquatic pollutants in various marine systems [13, 17-18, 24-29]. This species has widespread distribution along the shores of Turkey and grows well along the coastal lines of the Sea of Marmara. It could accumulate high nutrient levels from contaminated or polluted environments [25]. Therefore, the present study aimed to obtain further information about the use of *U. lactuca* as a biomonitoring algae species along the Istanbul coast of the Sea of Marmara, Turkey, and to provide information about marine environmental quality as well as degree of pollution.

Materials and Methods

Study Area

According to the 2014 consensus, Istanbul is one of the largest cities of Turkey with about 15 million inhabitants [30]. It is also the cultural, economic, and financial center of Turkey. It is located in northwestern Turkey in the Marmara region within an area of 5,343 km^2 [31-32]. In addition, it employs around 27% of Turkey's industrial workforce and provides 40% of Turkey's industrial workspace [33]. The Sea of Marmara, which is entirely located within Turkey's border as an inland sea, connects the Black and Aegean Seas and separates the Asian and European parts of Turkey. It covers an area of 11,350 km² (280 x 80 km) and is 1,370 m at its deepest point [34-35].

The Sea of Marmara has faced heavy pollution since the 1980s. The main sources of pollution are especially heavy industrial facilities in the Gulf of Izmit near Istanbul and waste from such densely populated cities as Istanbul and Izmit to the northeast. Today, 0.3 million m^3 industrial and 2.1 million m^{3}of domestic waste is produced daily [33].

In the present study, *U. lactuca* samples were collected from six different locations along the coastal line of the Sea of Marmara in Istanbul. These locations include the Buyukada, Fenerbahce, Maltepe, Bakirkoy, Beykoz, and Beylikduzu coasts (Fig. 1).

Plant Sample Analysis

Plant samples (eight individuals) were collected from six different locations approximately 50-100 cm depth during the third and fourth weeks of February 2014. Samples were washed with distilled water to eliminate the dust particles as specified in standardized procedure and then oven-dried at 80°C for 24 h. 0.2 g of each sample was transferred in Teflon vessels and then 8 mL of 65 wt% HNO_3 solution (Merck) was added to each sample. Samples were dissolved in a Berghof-MWS2 microwave oven set on 5 min at 145ºC, 5 min at 165ºC, and 20 min at 175ºC. Later, samples were filtered with filter papers of 1-2 μm average pore diameter, and then transferred to volumetric falcon tubes to which up to 50 mL ultrapure water was added. For each sample, Ca, Cd, Cu, Fe, K, Mg, Mn, Pb, and Zn concentrations were measured using inductively coupled plasma optical emission spectroscopy (ICP-OES, Perkin Elmer-Optima 7000 DV).

Fig. 1. Study areas in Istanbul Province, Turkey: 1) Buyukada Coast, 2) Fenerbahce Coast, 3) Maltepe Coast, 4) Bakirkoy Coast, 5) Beykoz Coast, and 6) Beylikduzu Coast.

Statistical Analysis

For statistical analysis, one-way analyses of variance (ANOVA) with Tukey's post-hoc HSD were performed using IBM SPSS Statistics 20 software. The statistical significance levels were indicated as $^{**}P<0.01$ and $^{*}P<0.05$.

Results and Discussion

The mineral nutrient and heavy metal contents of *U. lactuca* samples are given in Table 1. The average of highest and lowest concentrations of each element are:

- Ca: the average highest concentration (2,465.684 mg/kg) was measured in Buyukada samples while the average lowest concentration (1,751.661 mg/kg) was in Beylikduzu
- Cd: the average highest concentration (3.218 mg/kg) was found in Beylikduzu samples while the average lowest concentration (0.451 mg/kg) was in Buyukada
- Cu: the average highest concentration (18.315 mg/kg) was obtained in Beylikduzu samples while the average lowest concentration (6.671 mg/kg) was in Buyukada
- Fe: the average highest concentration (989.328 mg/kg) was observed in Beylikduzu samples while the average lowest concentration (553.318 mg/kg) was in Buyukada
- K: the average highest concentration (4,301.681 mg/kg) was measured in Beylikduzu samples while the

Table 1. Mineral element and heavy metal concentrations (mg/kg dw.) of *U. lactuca*. Statistical analyses such as one-way analysis of variance (ANOVA) with Tukey's post-hoc HSD were performed. The mean differences are significant at $p<0.01$ (**) and $p<0.05$ (*) levels. The letters a, b, c, and d indicate different averages within the same line, which are significant in terms of averages ($p<0.05$).

	Buyukada	Fenerbahce	Maltepe	Bakirkoy	Beykoz	Beylikduzu
Ca	2,465.684±33.231^{a}	2,159.529±28.556^{*ab}	2,066.894±23.504^{*b}	1,864.715±17.226^{**bc}	1,808.659±16.164^{**c}	1,751.661±15.319^{**c}
Cd	0.451±0.012^{d}	1.008±0.015^{*c}	1.489±0.029^{*c}	2.221±0.038^{*b}	2.596±0.041^{*b}	3.218±0.053^{*a}
Cu	6.671±0.101^{d}	10.917±0.131^{*c}	13.007±0.155^{*b}	15.282±0.167^{**b}	16.339±0.185^{*b}	18.315±0.201^{**a}
Fe	553.318±10.981^{c}	686.148±13.057^{*b}	721.229±16.122^{*b}	775.318±19.154^{*b}	882.157±23.185^{*a}	989.328±29.210^{*a}
K	3,264.478±39.315^{c}	3,555.217±41.526^{*b}	3,601.253±40.669^{*b}	3,821.644±43.457^{*b}	3,900.335±45.854^{*b}	4,301.681±41.746^{*a}
Mg	1,614.533±14.521^{a}	1,401.121±13.78^{8**ab}	1,361.524±15.959^{*b}	1,111.625±10.013^{*b}	1,035.006±13.338^{*c}	961.497±8.615^{**c}
Mn	8.267±0.092^{d}	13.329±0.172^{*c}	15.208±0.201^{*c}	18.224±0.245^{*b}	20.339±0.287^{*b}	25.318±0.314^{*a}
Pb	4.927±0.070^{d}	7.628±0.095^{*c}	8.918±0.104^{**c}	12.355±0.196^{*b}	16.297±0.221^{*a}	19.315±0.246^{*a}
Zn	15.157±0.222^{c}	22.229±0.519^{*b}	26.871±0.735^{*b}	31.882±.811^{*b}	35.328±0.924^{*a}	41.297±1.015^{*a}

average lowest concentration (3,264.478 mg/kg) was in Buyukada

- Mg: the average highest concentration (1614.533 mg/kg) was determined in Buyukada samples while average lowest concentration (961.497 mg/kg) was in Beylikduzu
- Mn: the average highest concentration (25.318 mg/kg) was found in Beylikduzu samples while average lowest concentration (8.267 mg/kg) was in Buyukada
- Pb: the average highest concentration (19.315 mg/kg) was obtained in Beylikduzu samples while average lowest concentration (4.927 mg/kg) was in Buyukada
- Zn: the average highest concentration (41.297 mg/kg) was observed in Beylikduzu samples while average lowest concentration (15.157 mg/kg) was in Buyukada.

In most of the locations, such as the Buyukada, Fenerbahce, Maltepe, Bakirkoy, and Beykoz coasts, mineral nutrient and heavy metal contents in *U. lactuca* samples decreased in the following order: K>Ca>Mg>Fe>Zn>Mn>Cu>Pb>Cd. However, in Beylikduzu it was K>Ca>Mg>Fe>Zn>Mn>Pb>Cu>Cd (Table 1).

Many studies have reported some members of the *Ulva* genus for their biomonitoring potential [13, 17, 26-28]. For example, *U. lactuca* was reported as a biomonitor plant for Cd, Fe, Mn, and Pb elements, while *U. rigida* was for Cd, Pb, and Zn [36-37]. *U. clathrata, U. linza*, and *U. flexuosa* species were reported for Cd, Cu, Pb, and Zn metals [38]. In a recent study, *U. lactuca* was demonstrated as an effective bio-sorbent for the removal of Cu, Cd, Pb, and Zn from synthetic solutions [39]. Many other studies have also reported similar results; therefore, to avoid repetitive arguments with close interpretations, a comparison table was formed to show the heavy metal contents in *U. lactuca* from various locations, and the results obtained from the present study (Table 2). Based on comparisons of present study results with the available literature of *U. lactuca* demonstrating that Cd, Cu, Fe, and Zn in Vadinar, India [13]; Cd and Pb in the Red Sea, Egypt [24]; Cu and Mn in Malta Lake, Poland [28]; Fe and Mn in Mikindani, Kenya [27]; and Mn in Chincoteague Island, Virginia and Msimbazi, Tanzania [17, 27]; Mn and Zn in Nielba River, Poland [28]; and Zn in Canakkale and Izmir, Turkey and Sikka, India [4, 13] were relatively higher than the findings of the present study.

A greater amount of heavy metal pollution in Beylikduzu Coast samples could be explained by the influence of the connection between Kucukcekmece Lagoon and the sea. Particularly, in recent years, the pollution rate of Kucukcekmece Lagoon has significantly increased due to anthropogenic factors. Overurbanization in the vicinity of the lagoon and unprocessed industrial wastes caused an increase in heavy metal entries to the lagoon and consequently to the sea through the connection [44]. Excessive heavy metal content at Beykoz Coast may

Table 2. Heavy metal concentrations (mg/kg dw.) of *U. lactuca* in different regions in the last decade.

Species	Study Area	Cd	Cu	Fe	Mn	Pb	Zn	Reference
Ulva lactuca	Egyptian Red Sea	5.30	8.30	-	16.90	37.20	40.70	[24]
Ulva lactuca	Chincoteague Island, Virginia	-	0.69	-	50.45	-	12.95	[17]
Ulva lactuca	Assateague Island, Maryland	-	0.65	-	22.40	-	6.86	[17]
Ulva lactuca	Black Sea, Turkey	0.023	3.93	784.0	19.10	0.648	22.40	[25]
Ulva lactuca	Canakkale, Turkey	0.054	8.51	197.6	-	0.0056	50.10	[4]
Ulva lactuca	Izmir, Turkey	0.014	13.90	129.6	-	0.0175	67.80	[4]
Ulva lactuca	Jijel, Algeria	0.041	2.37	-	-	1.001	5.40	[26]
Ulva lactuca	Chale, Kenya	0.955	5.15	350.0	20.55	-	18.50	[27]
Ulva lactuca	Mikindani, Kenya	0.150	13.60	4000	325.0	-	32.50	[27]
Ulva lactuca	Msimbazi, Tanzania	0.110	15.80	900.0	40.80	-	17.30	[27]
Ulva lactuca	Itaparica Island, Penha Beach, Brazil	0.265	3.13	-	10.60	0.729	10.20	[18]
Ulva lactuca	Nielba River, Poland	-	11.37	-	323.85	-	157.9	[28]
Ulva lactuca	Malta Lake, Poland	-	18.47	-	549.90	-	36.34	[28]
Ulva lactuca	Sikka, India	2.89	14.20	948.0	7.70	3.50	146.0	[13]
Ulva lactuca	Vadinar, India	3.60	21.50	1256	7.60	0.80	340.0	[13]
Ulva lactuca	Vadinar, India	2.76	16.00	1439	7.80	1.00	326.0	[13]
Ulva lactuca	Magallanes, Chile	-	6.97	720.0	27.20	-	16.20	[29]

be attributed to uncontrolled wastewater. The municipality has made efforts to solve this problem by constructing the Kavacık-Pasabahce-Beykoz sewer system. For the same reason, the Atakoy Advanced Wastewater Treatment Plant was constructed along the Bakirkoy Coast in 2010. In this way, wastewater discharge to Bakirkoy has been prevented and hence pollution along this coast has significantly decreased. The reason for low heavy metal contents at Fenerbahce and Maltepe coasts is a better level of infrastructure systems in these locations, thus avoiding wastewater discharge [33].

The average highest Ca and Mg concentrations were in Buyukada samples, while the average lowest concentrations were in Beylikduzu (Table 1). This observation is just the opposite of that obtained for heavy metals (Cd, Cu, Fe, Mn, Pb, and Zn). Therefore, in *U. lactuca* samples, uptake of Ca and Mg has a negative effect on uptake of other heavy metals [7, 10]. In addition, Cd and Pb concentrations, which are potential toxic elements [40], were investigated to have an insight about the pollution levels on various coasts of Istanbul. The highest concentrations of these heavy metals were mainly observed at Beylikduzu Coast, indicating the pollution level of this coastal region. In a study, sediment samples collected from the Bosphorus were reported to contain high levels of Cd and Pb compared to other investigated regions [41]. In the present study, after the Beylikduzu Coast, the second highest heavy metal contents were measured in the Bosphorus. In addition, many similar studies have reported a high level of heavy metals in sediment and water samples collected from Bakırkoy, Beylikduzu, and Bosphorus locations [35, 42-43].

Overall, the comparison of heavy metal contents with seawater and sediment samples [35, 40, 41-43] in *Ulva* species showed that *U. lactuca* is a good candidate for biomonitoring studies.

Conclusions

The release of heavy metals in coastal regions has been a serious threat to aquatic environments. Although certain heavy metals in trace amounts could be tolerated by plants, they are usually toxic. So their presence and concentrations in water must be regularly monitored in terms of sustainability of aquatic environments. However, this should be done in a more environmentally friendly and efficient way. To achieve this, employing biological species as biomonitors to evaluate the presence of contaminants and/or their effects on the environment has been regarded as an alternative. In this sense, the present work used the *U. lactuca* algal species as a biomonitoring plant to provide insights about the water quality and pollution levels along the Istanbul Coast of the sea of Marmara. The analysis of plant samples using ICP-OES revealed that *U. lactuca* is capable of accumulating metals to some extent, although this deposition did not significantly change the overall mineral nutrient status of plants. This shows that *U. lactuca* could be used as a potential biomonitoring plant. Furthermore, from a biotechnological perspective the engineering of this algal species could produce new plant lines with enhanced metal accumulation capacities.

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