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Accumulation of Heavy Metals in *Cladophora fracta* and *Chaetomorpha ligustica* Species

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ABSTRACT

Besides playing a crucial ecological role, certain types of algae have also become significant indicators of chemical pollutants. In this research, marine algae were examined to evaluate their suitability as biomonitoring instruments for detecting heavy metal pollution in coastal regions. The levels of cadmium (Cd), nickel (Ni), copper (Cu), lead (Pb), and zinc (Zn) were assessed in two types of green algae: *Cladophora fracta* and *Chaetomorpha ligustica*, which were both gathered from the same natural habitat. Samples were collected from the coast of Bostanlı, Izmir (Aegean Sea, Turkey) in August 2023. The collected samples were first subjected to morphological species identification. Inductively Coupled Plasma-Mass Spectro-metry (ICP-MS) analysis was used to measure the accumulated concentrations of heavy metals (Zn Ni, Cu, Pb, Cd) of the separated species. As a result of the analyses, it was determined that the accumulation of heavy metals was higher in *Cladophora fracta* compared to *Chaetomorpha ligustica*. Both species have particularly high accumulation potentials for Zn in their tissues. The heavy metal accumulation concentrations for both species are as follows, respectively: Zn > Ni > Cu > Pb > Cd.

KEYWORDS: Heavy metal, Aegean Sea, pollution, green algae

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1. Introduction

Heavy metal pollution is one of the most significant environmental issues of our time. Various industries such as mining, energy and fuel production, fertilizer and pesticide production and use, iron and steel industry, electrolysis, electro-osmosis, leather industry, electronics manufacturing, aviation, and atomic energy installation produce and discharge wastes containing various heavy metals into the environment. Due to all these activities, severe environmental pollution erupts, which ultimately threatens human health and the ecosystem. There are three types of heavy metals that are a cause for concern: toxic metals (Hg, Cr, Pb, Zn, Cu, Ni, Cd, As, Co, Sn, etc.), precious metals (Pd, Pt, Ag, Au, Ru, etc.), and radionuclides (U, Th, Ra, Am, etc.) (Wang and Chen, 2006).

The increasing human population and coastal expansion contribute to the rise in anthropogenic pollution burden, posing a serious threat to marine and aquatic environments. Polluted water can degrade water quality, thus limiting the use of water bodies for various purposes. It is well known that heavy metal pollution in aquatic environments is a growing problem worldwide and has currently reached an alarming level. Levels of heavy metal pollution in marine ecosystems can be estimated through analysis of aquatic organisms (Morillo et al., 2005). While certain trace elements such as manganese, copper, and chromium are necessary for biological functions, they can become toxic when present in excess amounts. Conversely, elements like arsenic, cadmium, lead, and mercury can be highly toxic even at very low concentrations. (Domingo, 1994).

From both economic and ecological standpoints, there is growing interest in utilizing plants as phytoremediators to purify water in diverse aquatic ecosystems (Eid et al., 2020; Ahmad et al., 2014; Lytle and Lytle, 2001). Many aquatic plant species have demonstrated the capability to uptake zinc and other heavy metals from water, thereby accumulating higher concentrations internally compared to their surrounding environment (Whitton, 1981). Various species are commonly utilized to monitor metal concentrations in different geographical areas, often selected based on their heavy taxonomic distribution (Ho, 1990; Stratis et al. 1996). Metals of interest, such as copper, zinc, cadmium, and lead, have been highlighted (West, 1973). Organisms investigated as potential monitors for trace metals in water include clams (Lord, 1974), as well as clams, mussels, and shrimp (Bertine and Goldberg, 1972), groupers (Taylor and Bright, 1973), and algae (Stokes et al., 1973). It is true that algae primarily bind free metal ions, and the concentrations of these ions are influenced by the nature of suspended particulate matter. This matter is formed by a combination of organic and inorganic complexes, as noted by Luoma (1983), Seeliger and Edwards (1977), and Volterra and Conti (2000).

Macroalgae within the Chlorophyta phylum, an ecologically significant group in aquatic systems, are commonly utilized as indicators of heavy metal pollution, as noted by McCormick and Cairns (1994). *Cladophora* species, as a filamentous green algae, have been proposed as effective biological monitors for species present in natural waters (Whitton, 1970). It is indeed a hardy plant, thriving in diverse climatic regions, and it has been observed that substances are concentrated in the tissues of this plant to a significant extent (Keeney et al., 1976).

The uptake and bioaccumulation of heavy metals by algae have primarily been investigated using atomic absorption spectrophotometry (Stokes et al.1973; Stokes, 1975).

The aim of this study is to present the obtained data and compare them with the results found in the literature. Additionally, by comparing the heavy metal accumulation potentials of two species belonging to the genera *Cladophora* and *Chaetomorpha*, both of which are pollution indicators within the Cladophorales order, their potential applications and futures have been estimated.

These species have the ability to easily adapt to areas contaminated with heavy metals. In this study, heavy metal levels of toxic metals such as Zn, Pb, Ni, Cu, Cd were measured in *Cladophora* and *Chaetomorpha* species. The samples were collected from Bostanlı, İzmir, a region with high terrestrial pressure. The main reasons for selecting these species include their tendency to reach alarming levels due to cultural eutrophication, their wide distribution ranges, and their high biological accumulation capacities attributed to their adaptation to various climatic regions.

2. Material and Methods

2.1. Materials

The materials of this study consist of species belonging to the genera *Cladophora* and *Chaetomorpha* within the order Cladophorales.

***Cladophora fracta* (O.F.Müller *ex* Vahl) Kützing**

The apical cell of the algae has a width of 30 µm and a length of 125 µm. Median cells range in width from 50 µm to 100 µm and in length from 350 µm to 250 µm. Apical cells have oval tips, and the branching pattern is pseudodichotomous. The thallus is slender and filamentous, with a curved shape, and apical branching is prevalent. Various cell shapes were observed, with round cells being predominant. The cells contain numerous chloroplasts arranged reticularly (Figure 1).

***Chaetomorpha ligustica* (Kützing) Kützing**

This species forms a soft and intertwined, bright to light green mass composed of fine filaments, which consist of a series of non-branching cells (Figure 2). The cells are 90 µm in diameter, multinucleate, and the length is approximately 4 times the width.

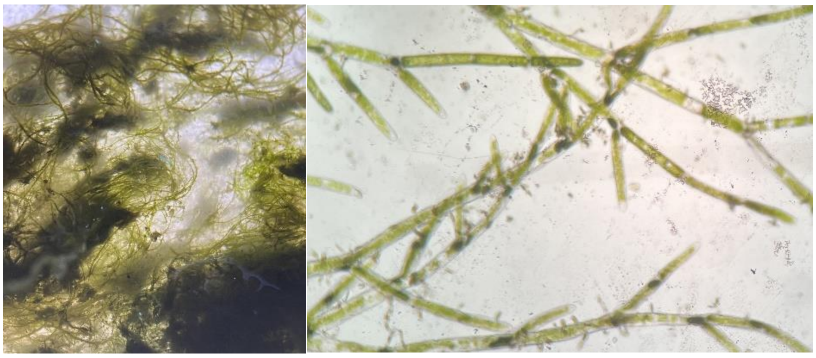

Figure 1. *Cladophora fracta.* a) Habit, b) Filament and cell structure

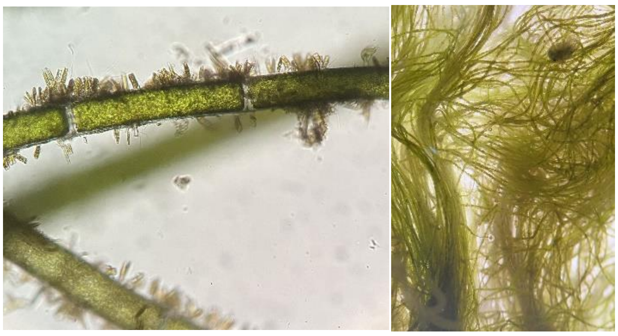

Figure 2. *Chaetomorpha ligustica.* a) Habit, b) Filament and cell structure

2.2. Study Area and Sampling

Samples were collected from the coast of Bostanlı, Izmir (38.456731ºN, 27.090075ºE) (Aegean Sea, Turkey) in August 2023 (Figure 3). The sampling area within İzmir Bay is influenced by the heavy ship traffic in İzmir Port, the discharge of treated water into the bay, and the water carried by the Gediz River.

The samples were directly collected by hand from the sublittoral zone. The collected samples were washed with clean water to separate them from waste and epiphytes. The washed samples were then brought to the laboratory. Samples were examined at the Department of Biology, Manisa Celal Bayar University (Türkiye). Cladophorales species were initially examined under a stereomicroscope and systematically categorized. Subsequently, a more detailed examination was conducted under a microscope, and the apical cell length, width, thallus cell length, branching patterns, and cell shapes of each species were measured. The identification of the samples was made on the basis of the descriptions by Cormaci et al. (2014) and Sfriso (2010).

The species identified were first dried in the sun, and then 0.5 grams of each species were weighed for heavy metal analysis. Approximately 0.5g of homogenized samples were weighed precisely and digested using a mixture of nitric acid, hydrochloric acid, hydrogen peroxide, and hydrofluoric acid in a 3000 Multiwave microwave oven under an appropriate temperature/pressure program. The amounts of Zn, Ni, Cu, Cd, and Pb in the solutions prepared using the microwave digestion system were measured with ICP-MS. The values presented for the samples represent the results of a single measurement.

Figure 3. General view of the research area

3. Results

We compared the heavy metal accumulation potentials of two different species belonging to two different genera, both of which serve as pollution indicators, collected from the same habitat. The levels of cadmium (Cd), copper (Cu), lead (Pb), nickel (Ni), and zinc (Zn) in the collected samples were investigated, and the obtained results were evaluated. Based on the ICP-MS analysis, we found that both species accumulated the highest levels of Zn.

Heavy metal accumulation concentrations in the thallus of *Chaetomorpha ligustica*; Zn: 197,496 µg/g Ni: 36,694 µg/g Cu: 12,452 µg/g Pb:11,514 µg/g Cd:0,097 µg/g (Figure 4).

The order of accumulation potentials and concentrations of other metals was as follows: Zn > Ni > Cu > Pb > Cd.

Heavy metal accumulation concentrations in the thallus of *Cladophora fracta*; Zn: 117,153 µg/g Ni: 80,467 µg/g Cu: 25,054 µg/g Pb:20,076 µg/g Cd:0,613 µg/g (Figure 4).

It has been determined that, generally, *Cladophora fracta* has a higher heavy metal accumulation potential than *Chaetomorpha ligustica*, except for zinc.

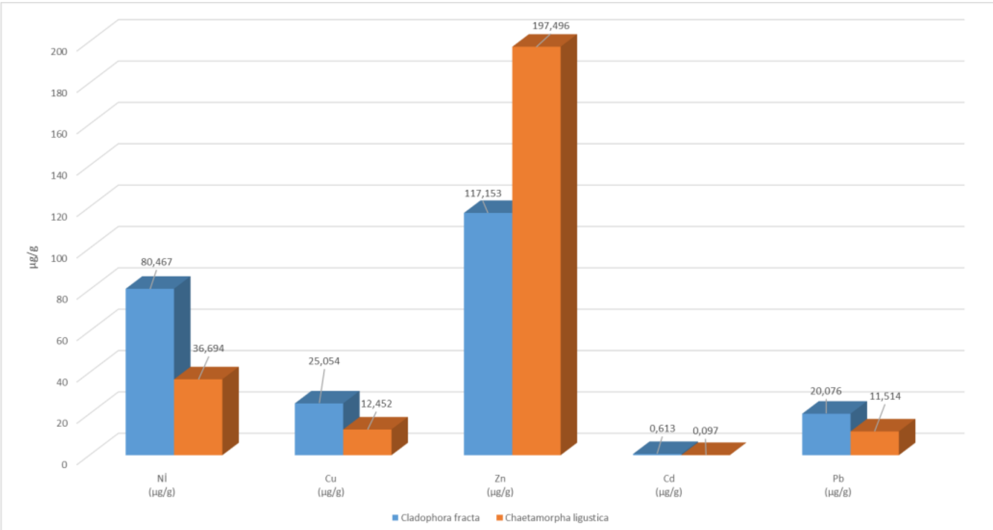

Figure 4. Heavy metals concentrations in *Cladophora fracta* and *Chaetomorpha ligustica* samples

4. Discussion

Chemical pollution in aquatic ecosystems can cause destructive effects. Heavy metals, which are transferred through the food chain, not only contribute to the degradation of aquatic ecosystems but also have adverse effects on human health. It is known that utilizing aquatic organisms for remediation and monitoring of pollution in aquatic ecosystems is possible. Particularly, due to their immobility compared to other organisms, wide distribution ranges, and ease of collection, aquatic plants are more suitable for use in biological remediation and monitoring studies.

In recent years, many studies have been published using different macroalgae as biomonitors and for water remediation purposes (Agarwal et al., 2020).

For many years, algae have been recognized for their role in wastewater treatment and their ability to bind heavy metal cations. This is primarily due to their high negative surface charge, which allows them to attract and absorb positively charged heavy metal ions from water. This property makes algae effective in removing pollutants like heavy metals from wastewater, contributing significantly to water purification processes (Rao, 1986).

The concentrations of heavy metals accumulated in macroalgae vary depending on the geological structure of the sampling area, the season of sampling, morphological characteristics of the algae, their lifespan, physicochemical parameters of their habitat, and interactions with other heavy metals (Sawidis et al., 2001).

Previous studies conducted in the Aegean Sea have reported Pb values for *Cystoseira* species ranging between 0.02-2.5 µg g^{-1} (Sawidis et al., 2001), while Akçalı and Küçüksezgin (2009) reported a value of 0.083 µg g^{-1}. When these values are compared with the results obtained in this study, it is evident that the Pb value found in this study is considerably higher.

Wang and Chen (2009) reported that the capacity of algae to absorb heavy metals is quite high. Vymazal (1987) demonstrated in a study that *Cladophora* species have a rapid ability to absorb Zn.

In benthic macrophytes, Zn concentrations below 100 µg g^{-1} are considered to indicate unpolluted areas, as evaluated by Storelli et al. (2001), while higher concentrations are indicative of anthropogenic pollution. The highest Zn value obtained in this study is 197.496 µg/g, indicating a high anthropogenic impact.

Malea and Haritonidis (1999) stated that algae accumulate essential metals such as Zn at high levels while maintaining toxic metals like Cd, Cu, and Pb at low levels necessary for metabolic activities.

In a study conducted in 2011 to measure the levels of some heavy metals in *Cladophora glomerata* seasonally in Lake Hazar, the Cu concentration was found to be 1.31 mg/g and 1.77 mg/g, and Zn was found to be 9.61 mg/g and 11.8 mg/g (Alp and Ozbay, 2011).

In a study conducted by Akçalı and Küçüksezgin (2009) along the Aegean Sea coast, the order of heavy metal concentrations in *Cystoseira* species was determined to be Pb < Cd < Cu < Zn. In this study, the highest Zn accumulation value for *Cystoseira* species was reported to be 62.48 µg g^{-1}. According to Akçalı and Küçüksezgin (2011), the highest heavy metal concentration measurements in macroalgae species *Enterophmorpha* sp., *Gracilaria gracilis*, and *Ulva* sp. collected from the shores of Bostanlı (Izmir) were reported as follows: Cd (0.49 µg/l), Pb (3.20 µg/l), Cu (5.57 µg/l), and Zn (10.36 µg/l). Çetingül et al. (2000) reported the heavy metal accumulation concentrations of *Cladophora dalmatica* species as Cu 5.93 µg g^{-1} and Zn 136.61 µg g^{-1} in their study conducted in Izmir Bay. Sawidis et al. (2001), their study on *Cystoseria sp.* in the Aegean Sea, reported Pb values ranging from 0.02 to 2.5 µg g^{-1}, whereas the Pb value obtained in this study is significantly higher.

In this study, the heavy metal concentration levels of *Chaetomorpha ligustica* species are as follows: Zn: 197.496 µg/g, Ni: 36.694 µg/g, Cu: 12.452 µg/g, Pb: 11.514 µg/g, Cd: 0.097 µg/g. The heavy metal concentrations in *Cladophora fracta* species are as follows: Zn: 117.153 µg/g, Ni: 80.467 µg/g, Cu: 25.054 µg/g, Pb: 20.076 µg/g, Cd: 0.613 µg/g. When looking at the values from previous studies, it is seen that the special Zn, Pb and Cu values obtained in this study are higher.

Considering the conducted studies, İzmir Bay has significantly high concentrations of heavy metals due to industrial activities, İzmir port, and the inflow of rivers into the bay.

It is understood that *Cladophora* and *Chaetomorpha*, which adapt to the high anthropogenic influence in this region, have high biological accumulation potentials. Considering their wide distribution ranges, ability to thrive in different climatic regions, and dominance in aquatic environments with high chemical pollution, it is thought that these species are more suitable to be used as biomonitoring organisms compared to other species.

5. Conclusion

Rincon et al. (2005) indicated in their study on *Cladophora* species that they have the potential to be used as biological material for remediation purposes, particularly in the removal of heavy metal pollution. In addition to being used as a biomonitoring, their high biological accumulation potentials make them suitable for use in biological purification purposes as well. Brinza et al. (2007) examined the heavy metal accumulation capacities of *Cladophora* species and reported that they have the ability to adsorb a high level of various heavy metal ions, including K, Mg, Ca, Fe, Sr, Co, Cu, Mn, Ni, V, Zn, As, Cd, Mo, Pb, Se, and Al.

Chmielewská and Medved (2001) confirmed the high bioaccumulation capabilities of *Cladophora* sp. for Pb, Cd, Ni, Cr, and V metals in their study aimed at determining their metal accumulation abilities. As a result, it was found that algae contribute to improved water quality through wastewater treatments.

As a result, it has been determined that among the species we have been studying, *Cladophora fracta*, in particular, has a high capacity for accumulating heavy metals. Utilizing the bioaccumulation abilities of macroalgae, it is possible to observe the extent of pollution in aquatic ecosystems resulting from human activities and take parallel measures accordingly.

Due to their tolerance to metals, simple morphology, provision of sufficient tissue for analysis, and sedentary lifestyle, *Cladophora* species can be successfully used in biomonitoring studies. Moreover, their ease of collection and relatively widespread distribution across various coastal regions ensure reliable outcomes (Zbikowski et al., 2007).

Compliance with Ethical Standards

Conflict of interest

The authors declare that they have no competing interests.

Author contribution

All authors' contributions are equal for the preparation of research in the manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

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