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Monitoring Heavy Metal Pollution with *Padina pavonica* (Linnaeus) Thivy, 1960: Summer Season Samples from Akliman and Karakum

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Abstract: This study investigates the bioaccumulation of cadmium (Cd), mercury (Hg), lead (Pb), manganese (Mn), iron (Fe), and zinc (Zn) in *Padina pavonica*, a brown algaealga recognized as a bioindicator of marine pollution. Samples of seawater, sediment, and macroalgae were collected from two coastal sites, Akliman and Karakum (Sinop Province, Black Sea), during the summer of 2022 to assess spatial variations in metal concentrations.

The findings revealed location-dependent differences in metal accumulation in *P. pavonica*, with the highest Fe levels recorded in Karakum (1608.9 mg kg^{-1}), followed by Akliman (903.7 mg kg^{-1}). In Akliman, the descending order of metal concentrations was Fe > Mn > Zn > Pb > Cd > Hg, whereas in Karakum, Zn concentrations exceeded those of Mn. Seawater analyses revealed relatively high levels of Pb and Mn in Akliman, pointing to possible anthropogenic inputs. The Biota Concentration Factor (BCF) for Mn was particularly high, demonstrating substantial uptake by the macroalgae. Moreover, the Biota-Sediment Accumulation Factor (BSAF) values exceeded 2 for both Cd and Zn, signifying substantial accumulation in the algal tissue relative to sediment concentrations. These findings provide basic information on the ecological condition of the coastal waters and demonstrate that *P. pavonica* could serve as an effective biomonitor for heavy metal pollution.

Keywords: Environmental monitoring, macroalgae, Sinop, *Padina pavonica*, pollution

Padina pavonica (Linnaeus) Thivy, 1960 ile Ağır Metal Kirliliğinin İzlenmesi: Akliman ve Karakum'dan Yaz Mevsimi Örnekleri

Özet: Bu çalışma, deniz kirliliğinin bir biyogöstergesi olarak kabul edilen kahverengi makroalg *Padina pavonica*'da kadmiyum (Cd), cıva (Hg), kurşun (Pb), mangan (Mn), demir (Fe) ve çinko (Zn) metallerinin biyobirikimini incelemektedir. Metal konsantrasyonlarındaki mekânsal değişimleri değerlendirmek amacıyla 2022 yazında Sinop ili (Karadeniz) kıyılarında yer alan Akliman ve Karakum bölgelerinden deniz suyu, sediman ve makroalg örnekleri toplanmıştır.

Elde edilen bulgular, *P. pavonica*'da metal birikiminin örnekleme alanına bağlı olarak değiştiğini göstermiştir. En yüksek Fe seviyesi Karakum'da (1608,9 mg kg^{-1}) ve ardından Akliman'da (903,7 mg kg^{-1}) kaydedilmiştir. Akliman'daki metal birikimi sıralaması Fe > Mn > Zn > Pb > Cd > Hg şeklindeyken, Karakum'ta Zn konsantrasyonları Mn'den daha yüksek bulunmuştur. Deniz suyu analizlerinde Akliman bölgesinde Pb ve Mn düzeylerinin nispeten yüksek olduğu belirlenmiş, bu durum potansiyel insan kaynaklı kirlilik etkenlerine işaret etmektedir. Mn için Biyota Konsantrasyon Faktörü (BCF) oldukça yüksek bulunmuş, bu da makroalg tarafından önemli miktarda alındığını göstermiştir. Ayrıca, Cd ve Zn için Biyota-Sediman Akümülasyon Faktörü (BSAF) değerlerinin 2'nin üzerinde olması, bu metallerin *P. pavonica* dokularında sedimana kıyasla önemli ölçüde biriktiğini göstermektedir. Bu bulgular, kıyı sularının ekolojik durumu hakkında temel bilgiler sunmakta ve *P. pavonica*'nın ağır metal kirliliği için etkili bir biyogösterge olarak kullanılabileceğini ortaya koymaktadır.

Keywords: Çevresel izleme, makroalg, Sinop, *Padina pavonica*, kirlilik

Article Info (Research)

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Citation: Arıcı, E. (2025), Monitoring Heavy Metal Pollution with *Padina pavonica* (Linnaeus) Thivy, 1960: Summer Season Samples from Akliman and Karakum, MEMBA Journal of Water Sciences 11, (3) 388-397.
DOI: 10.58626/memba.1760671

Submission Date: 7 August 2025
Acceptance Date: 11 September 2025
Publishing Date: 30 September 2025

1. Introduction

Heavy metal pollution has become a significant environmental issue, particularly in coastal marine ecosystems where industrial, agricultural, and urban activities are intense. These toxic substances not only degrade water quality but also threaten marine biodiversity and ecosystem functioning (Bat et al., 2018; El-Sharkawy et al., 2025). Coastal zones are especially vulnerable, as they often act as sinks for various anthropogenic pollutants. Among these contaminants, heavy metals are of particular concern in marine ecotoxicology due to their persistence, bioaccumulative nature, and potential toxicity even at low concentrations (Akçalı and Küçüksezgin, 2011).

Biological monitoring methods provide an effective and practical approach for assessing heavy metal pollution. Among the various organisms used, marine macroalgae stand out as bioindicators due to their sessile life, wide distribution, high metal accumulation capacity, and ease of sampling and analysis (Chakraborty et al., 2014; Bat et al., 2020). Macroalgae can accumulate metal ions at concentrations several orders of magnitude higher than those found in ambient seawater by interacting with surrounding particulate and dissolved matter (Bryan and Langston, 1992).

Padina pavonica (Linnaeus) Thivy, 1960, a calcified brown macroalga commonly known as peacock's tail, typically is widely found in shallow, rocky coastal regions of temperate and subtropical zones. Due to its sensitivity to environmental changes and ability to accumulate heavy metals, *P. pavonica* is frequently used as an effective bioindicator for monitoring heavy metal pollution in marine environments (Gil-Díaz et al., 2014).

Studies on *P. pavonica* in different regions show that heavy metal accumulation can vary depending on regional, ecological, and anthropogenic factors. Research carried out along the coasts of Sicily, Greece, Egypt, and Slovenia has shown that this species can be used as a good bioindicator of heavy metal pollution (Conti et al., 2010; Malea and Kevrekidis, 2014; El Zokm et al., 2021; Orlando-Bonaca et al., 2021). In Türkiye, particularly along the Aegean and Mediterranean coasts, *P. pavonica* has been used to study heavy metal levels in coastal ecosystems and demonstrated the impact of human activities (Akçalı and Küçüksezgin, 2011; Alagöz Erguden et al., 2021). However, there are still very few studies using this species on the Black Sea coastm (Arıcı 2017).

The Black Sea is a semi-enclosed basin characterized by limited water circulation and a stratified water column, resulting in widespread anoxic conditions in its deeper layers. Heavy metals, introduced mainly through industrial discharges, urban wastewater, and riverine inputs, pose a serious and persistent threat to regional ecosystems (Bat et al., 2018). Located on the northern coast of Türkiye, Sinop Province has experienced increasing urbanization and seasonal population growth in recent years, leading to intensified anthropogenic pressures on its coastal and marine ecosystems. Within Sinop, the coastal regions of Akliman and Karakum are heavily influenced by human activities including tourism, fisheries, and coastal development, all of which may contribute to elevated heavy metal inputs (Bat et al., 2014). Although heavy metal pollution in the Sinop region has been investigated in macroalgal assemblages and other matrices (water and sediment) along the Turkish Black Sea coast, no published study has comparatively and quantitatively examined heavy metal accumulation in *P. pavonica* collected from the Akliman and Karakum coasts. Monitoring heavy metal accumulation in *P. pavonica* from these areas provides information on the current ecological status and helps in identifying potential environmental risks (Arıcı, 2017). Therefore, *P. pavonica* not only reflects the ecological integrity of coastal ecosystems but also serves as practical bioindicator for determining and monitoring pollution levels along the Black Sea coastline.

This study aims to determine the levels of heavy metal accumulation in *P. pavonica,* as well as in the surrounding water and sediment collected from the Akliman and Karakum coasts of Sinop Province, thereby providing a comparative assessment of the ecological status of these regions and offering possible environmental risks along the Sinop coastline.

2. Materials and Methods

2.1. Study Area Description

Fieldwork involving the collection of *Padina* samples (Figure 1) was conducted during the summer of 2022 along the coastal regions of Akliman and Karakum in Sinop, a northern Turkish province bordering the Black Sea (Figure 2). Akliman is located about 10 km west of central Sinop. It is a popular recreational zone, especially in the summer months, and is subject to moderate anthropogenic pressure through tourism-related activities. Karakum is a densely developed area with high levels of human activity. It is a popular destination that includes hotels, restaurants, and residential buildings located close to the coastline. These locations were selected based on the abundance of *P. pavonica*, which grows naturally attached to rocks in the intertidal zone.

Figure 1. Field sampling of *Padina pavonica*

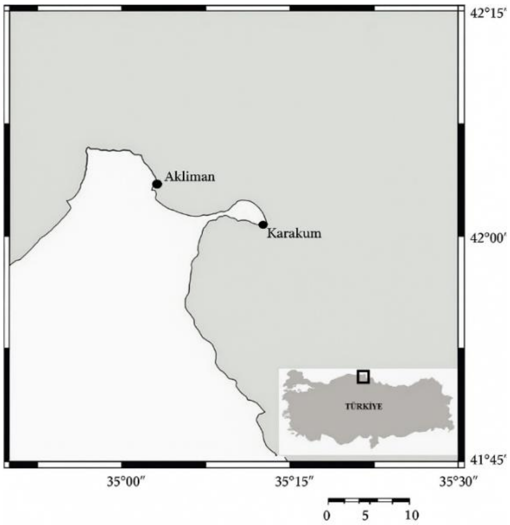

Figure 2. Sampling stations

2.2. Sampling Procedure

Approximately 0.5 kg of macroalgae samples were collected by hand from the intertidal zones of the Akliman and Karakum shores. The samples were labeled and transported to the laboratory. Seawater samples were collected using pre-cleaned 250 mL polyethylene bottles from just below the surface and stored in ice-filled coolers for transportation. Sediment samples were taken from the upper 0–5 cm layer using a sediment core, placed in plastic bags, and kept at 4°C until analysis. Water temperature, salinity, pH, and dissolved oxygen were measured in situ using a YSI Pro Plus Water Quality Probe. All samples were labeled, and transported to the laboratory within 24 hours for further physicochemical and heavy metal analysis.

2.3. Sample Preparation

Macroalgae samples were rinsed with tap water followed by bi-distilled water and dried in an oven at 60°C for 24 hours. The dried samples were ground and stored in airtight containers until analysis. Seawater samples were filtered through 0.45 µm membrane filters to remove suspended particles. Surface sediment samples were dried at 105°C for 24 hours, homogenized, and sieved through a 63 µm mesh; the sieved fraction was prepared for elemental analysis.

2.4. Metal Analysis

Metal concentrations in macroalgae, seawater samples and surface sediment were determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Agilent 7700X). Prior to analysis, algal and sediment samples were digested using a microwave digestion system (Milestone Systems, Start D 260) with Suprapur® HNO_3, following the Aquatic Plant HPR-FO-08 method for algae and the Seawater Sediment HPR-EN-33 methodology for sediments. The metals analyzed included Cd, Hg, Pb, Mn, Fe, and Zn.

2.5. Quality Control and Quality Assurance (QA/QC)

To ensure the accuracy and reliability of the analytical results, certified reference materials (CRMs) were used as standards: BCR 279 (Sea lettuce) for macroalgae samples, NIST SRM 2702 for marine sediment, and TUBITAK UME CRM 1201 for seawater.

The measured concentrations of metals from all CRMs (BCR, NIST, TÜBİTAK) showed recovery rates between 95.7% to 105.77%, indicating acceptable method accuracy (Table 1).

2.6. Data Analysis

To compare heavy metal concentrations across different environmental compartments and sampling sites, the samples were analyzed to identify any significant variations using SPSS Version 21.0 software (IBM, USA). The threshold for significance was established at 0.05, with p-values below this considered statistically meaningful. Data were tabulated using Microsoft Excel 2019 (Microsoft, USA). All results are reported as mg kg^{-1}.

2.7. Biota-Sediment Accumulation Factor (BSAF)

To evaluate the bioaccumulation potential of metals in macroalgae relative to sediment concentrations, the Biota-Sediment Accumulation Factor (BSAF) was calculated. BSAF is a dimensionless index commonly used in environmental monitoring and risk assessment to quantify the extent to which metals are transferred from sediments to biological tissues (Kleinov et al., 2008)

The BSAF was calculated using the following equation:

$$BSAF = Cm / Cs \quad (1)$$

where: C_m = concentration of the metal in macroalgae (mg kg^{-1} dry weight), C_s = concentration of the metal in sediment (mg kg^{-1} dry weight)

Organisms with BSAF values greater than 2 are identified as macro-concentrators, due to their high potential to accumulate metals or metalloids within their tissues. Values greater than 1 are classified as a concentrator, whereas values below 1 suggest limited uptake or stronger retention within the sediment matrix.

2.8. Bioaccumulation Factor (BCF)

To assess the extent to which metals are accumulated in macroalgae from the surrounding aquatic environment, the Bioaccumulation Factor (BCF) was calculated. A BCF value exceeding 5000 indicates that the substance is highly bioaccumulative (Geyer et al., 2000).

The BCF was determined using the following formula:

$$BCF = Cm / Cw \quad (2)$$

where: C_m = metal concentration in macroalgae (mg kg^{-1} dry weight), C_w = metal concentration in seawater (mg L^{-1})

Table 1. Comparison of experimental measurements with certified reference values for selected metals

	Metals	**Certified values**	**Found values**	**Recovery (%)**
BCR (mg kg^{-1})	Cd	0.274 ± 0.022	0.27 ± 0.03	98.54
	Hg	0.05*	0.05 ± 0.003	100
	Pb	13.48 ± 0.36	12.9 ± 0.5	95.7
	Mn	-	-	-
	Fe	2400*	2380	99.17
	Zn	51.3 ± 1.2	50.6 ± 2	98.64
NIST (mg kg^{-1})	Cd	0.817 ± 0.011	0.81	99.14
	Hg	0.4474 ± 0.0069	0.44	98.35
	Pb	132.8 ± 1.1	134.2	101.05
	Mn	1757 ± 58	1760	100.17
	Fe	-	7.36×10^3	-
	Zn	485.3 ± 4.2	472.9	97.44

	Cd	3.95 ± 0.15	3.80 ± 4.7	96.2
	Hg	-	0.10 ± 4.9	-
TUBITAK (μg kg^{-1})	Pb	14.7 ± 0.4	14.5 ± 1.3	98.64
	Mn	14.5 ± 0.5	14.5	100
	Fe	45.6 ± 2.7	45.2 ± 0.9	99.12
	Zn	104 ± 5	110 ± 8	105.77

3. Results

Summer season samples collected in 2022 from Akliman and Karakum were used. In the sampling areas, seawater temperature ranged between 23.9–24.5 °C, salinity between 18.4–18.6, pH between 8.56–8.64, and dissolved oxygen between 3.74–4.16 mg L^{-1}.

Among the *P. pavonica* samples collected from the sampling stations, Pb and Mn concentrations were notably higher at the Akliman site (48.5 mg kg^{-1} and 71.7 mg kg^{-1}, respectively). Cd and Hg levels were higher at Karakum, with concentrations of 0.45 mg kg^{-1} and 0.0018 mg kg^{-1}, respectively, and Zn concentration was also found to be higher at Karakum, reaching 65.2 mg kg^{-1}, compared to 44.8 mg kg^{-1} at Akliman. The analysis of seawater samples revealed spatial variations in heavy metal concentrations. Regarding essential heavy metals, Mn concentrations were higher in Akliman (12.4 μg L^{-1}) than in Karakum (9.3 μg L^{-1}), whereas Fe and Zn were more abundant at Karakum, with concentrations of 324.56 μg L^{-1}and 177.5 μg L^{-1}, respectively, compared to 242.4 μg L^{-1} and 156.5 μg L^{-1} at Akliman. The results of the surface sediment analysis showed that Cd levels were similar at both sites. Pb and Mn concentrations were higher at Akliman, measured at 8.38 mg kg^{-1} and 301.1 mg kg^{-1}, respectively, compared to 5.54 mg kg^{-1} and 215.3 mg kg^{-1} at Karakum. All results were given in Table 2.

Table 2. Metal concentrations of macroalgae, seawater and sediment

Metals	Macroalgae (mg kg^{-1})		Seawater (μg L^{-1})		Sediment (mg kg^{-1})	
	Akliman	Karakum	Akliman	Karakum	Akliman	Karakum
Cd	0.27	0.45	0.12	0.14	0.08	0.09
Hg	0.0008	0.0018	0.06	0.012	0.009	0.0019
Pb	4.85	1.13	1.56	0.73	8.38	5.54
Mn	71.7	48.16	12.4	9.3	301.1	215.3
Fe	903.7	1608.9	242.4	324.56	3200.5	5040.8
Zn	44.8	65.2	156.5	177.5	7.55	8.42

The highest BCF values were recorded for Mn at both stations, reaching 5782.26 at Akliman and 5178.49 at Karakum, suggesting strong bioconcentration potential of Mn in *P. pavonica*. Similarly, Fe exhibited high BCF values at both sites, particularly in Karakum (4957.25). *P. pavonica* exhibits a tendency to accumulate Zn and Cd from sediments compared to other metals, as indicated by BSAF values exceeding the threshold of 2, thereby classifying it as a macro-concentrator for these elements. In contrast, the BSAF values for Hg, Pb, Mn, and Fe remained well below 1, suggesting either low bioavailability of these metals in the sediment or their stronger retention within the sediment matrix, limiting their transfer to algal tissues (Table 3).

Table 3. BCF and BSAF values

Metals	BCF		BSAF	
	Akliman	Karakum	Akliman	Karakum
Cd	2250	3214.29	3.38	5
Hg	133.33	150	0.89	0.95
Pb	3108.97	1547.95	0.58	0.2
Mn	5782.26	5178.49	0.24	0.22
Fe	3727.14	4957.25	0.28	0.31
Zn	286.26	367.32	5.94	7.74

The distribution of metals across sampling sites exhibit the highest amount of Fe concentration in sediment samples, while Hg was found at low levels across all matrices. Generally, higher metal concentrations in seawater and macroalgae were observed at the Karakum station compared to Akliman (Figure 3). The plot highlights differences in metal accumulation between the two locations and bioaccumulation metrics.

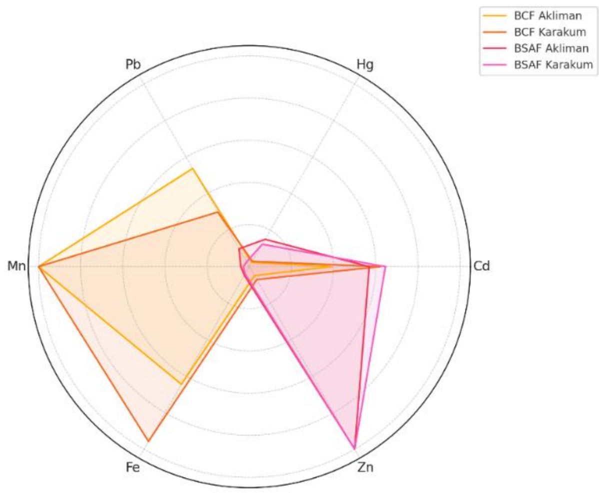

Figure 3. Radar plots of the BCF and BSAF values for metals in sampling regions

4. Discussion

Marine macroalgae, particularly brown algae, are widely recognized as useful bioindicators due to their high metal accumulation capacity, wide distribution, and ability to reflect changes in environmental quality over time. A correlation between seawater and sediment metal concentrations is important for monitoring environmental pollution (Bat et al., 2020).

The macroalga *Padina pavonica* (Linnaeus) serves as an efficient indicator organism in marine pollution studies, particularly for tracing heavy metal concentrations (Gil-Díaz et al., 2014). In the present study, although Zn concentrations were higher than Mn at the Karakum station, the average metal concentration in *P. pavonica* followed the order: Fe > Mn > Zn > Pb > Cd > Hg. This distribution shows Fe and Mn as the most abundant elements in the algal tissue, which is consistent with their natural abundance and essential roles in metabolic processes (Malea and Kevrekidis, 2014). However, presence of Pb, a toxic and non-essential metal, draws particular attention. Pb accumulation in macroalgae can vary depending on environmental exposure, as this metal is primarily introduced into aquatic ecosystems through anthropogenic activities, including industrial discharges, urban runoff, and maritime traffic (Diganta et al., 2023). High Pb levels (20.22 ± 0.44 µg g^{-1} dw) have previously been reported in *P. pavonica* samples collected from Mersin Bay (Alagöz Ergüden et al., 2021). In contrast, different metal accumulation patterns have been observed in macroalgae from the eastern Aegean region as Fe > Zn > Cd > Hg > Pb (Akçalı and Küçüksezgin, 2011). Similarly, both Conti et al., (2010) and El Zokm et al., (2021) observed the metal accumulation order of Zn > Pb > Cd in southern Tyrrhenian waters and Egyptian coastal areas, respectively. Such variations underscore the influence of local environmental conditions, pollution sources, and species-specific uptake mechanisms. In this study, due to these environmental changes, Pb levels were found to be high in both *P. pavonica* and sediment samples from the Akliman station. This may be attributed to the seasonal increase in human activities, particularly tourism and associated waste inputs during the summer months. The results align with the findings of Tüzen et al., (2009), who also reported high Pb accumulation in *P. pavonica* collected along the Sinop coastline, suggesting a persistent and possibly region-specific contamination pattern. Similarly, in the studies conducted in the same region by Topçuoğlu et al., (2002) and Bat and Şahin, (2019), Pb levels were observed to have gradually increased in the sediments of the studied area. Table 4 presents various studies that have investigated heavy metal accumulation in *P. pavonica*.

BCF and BSAF values demonstrate that *P. pavonica* has a strong capacity to bioconcentrate metals for Mn, Fe, Zn, and Cd from its environment. This accumulation pattern means that the species reflects the metals available in water and sediment and also shows site-specific differences in pollution. These results suggest that *P. pavonica* may be better than other algae for use as a biomonitor of these metals.

Table 4. Metal concentrations in *Padina pavonica* (mg kg^{-1}) from different sites, including the present study

Study location	Metals						References
	Cd	Hg	Pb	Mn	Fe	Zn	
Sicily	0.86		2.94			37.3	Conti et al., 2010
Greece	0.16–0.31		4.89–7.59	377–390		87.0–140	Malea and Kevrekidis, 2014
Izmir/Foça	103.7±8.47*	46.6±5.66*	4.73±0.21*		299.4±27.3	75.1±8.27	Akçalı and Küçüksezgin, (2011)
Izmir/Urla	179±17.4*	44.0±2.52*	3.23±0.68*		236.9±41.7	53.4±3.43	Akçalı and Küçüksezgin, (2011)
Marmaris /Turunç	147.6±12.1*	43.4±5.29*	1.22±0.19*		39.1±7.21	26.3±1.14	Akçalı and Küçüksezgin, (2011)
Egypt	1.35		13.36	89.658	940	109.79	El Zokm et al., 2021
Iskenderun Bay	0.24±0.00		5.89±0.02		2777.90±207	25.48±2.17	Alagöz Erguden et al., 2021
Mersin Bay	0.23±0.01		20.22±0.44		634.20±68.3	165.73±3.07	Alagöz Erguden et al., 2021
Slovenia	0.069–0.784	0.019-0.079	3.04–18.2	76.1–239	1410–5820	19.1–60.2	Orlando-Bonaca et al., 2021
Sinop	0.36	0.0013	2.99	59.93	1256.3	55	This study

Values marked with * are expressed in µg/kg.

Sediments are known to function as long-term sinks for metals in aquatic environments (Tessier and Campbell, 1987), while macroalgae reflect more immediate changes in environmental quality, making them effective bioindicators. They respond to current environmental conditions and can reveal correlations between metal concentrations in sediment (Wang and Chen, 2000; Arıcı and Bat, 2020). In this context, *P. pavonica* proved useful for assessing sediment-associated contamination through BSAF analysis. In this study, BSAF values for Cd and Zn were found to exceed 2, indicating accumulation in the algal tissues when compared to sediment concentrations. These results differ from the findings of Amini et al., (2013), who found that BSAF values for Zn, Fe, Cu, and Cd were all below 1 in Iranian coastal areas. This discrepancy may be linked to increased pollution levels during peak seasonal activity in Sinop, particularly due to uncontrolled discharge into nearshore waters. The results of BSAF values confirm that these Cd and Zn are bioaccumulative in *P. pavonica* and demonstrate their utility as indicators of sediment-originated contamination. BCF values showed that Mn had the highest accumulation at both stations, emphasizing its high bioavailability and uptake efficiency in *P. pavonica*, consistent with its essential role in algal physiology and mobility in aquatic systems.

Due to its semi-enclosed nature and stratified water structure, the Black Sea exhibits limited circulation, leading to widespread oxygen depletion in its deeper layers. This unique hydrological setup, combined with heavy metal inputs from industries, urban runoff, and rivers, presents a long-standing environmental challenge for the region's marine ecosystems (Bat et al., 2018). Sinop Province has seen increased urban growth and seasonal population rises in recent years, causing more pressure on its coastal and marine environments. Among its coastal regions, Akliman and Karakum are especially impacted by human activities like recreational tourism, and commercial fisheries, which are potential contributors to increased levels of metal pollution (Bat et al., 2014). In this context, the regular monitoring of heavy metal accumulation in *P. pavonica* along the Sinop coasts would make contribution to identifying regional pollution levels and developing strategies for the protection of Black Sea coastal ecosystems.

5. Conclusion

This study shows that *Padina pavonica* can be used as a useful indicator to detect heavy metal pollution in coastal areas, especially in the Sinop region of the southern Black Sea. Samples collected during the summer of 2022 from Akliman and Karakum showed differences in metal concentrations in macroalgae, seawater, and sediment. Pb and Mn levels were higher at Akliman, while Cd, Hg, and Zn concentrations were greater at Karakum. Mn and Fe had the highest BCF, showing that they are highly available in seawater and easily taken up by *P. pavonica*. On the other hand, Cd and Zn showed high BSAF values, meaning that *P. pavonica* can strongly accumulate these metals from sediment and act as a macro-concentrator. The high Pb levels at Akliman, similar to earlier studies from the area, point to ongoing human impact, likely increased by seasonal tourism and coastal activities. These results underline the importance of regularly monitoring heavy metals in marine biota to follow pollution trends and support regional coastal management strategies.

6. Acknowledgement

This study was not supported by any external funding. The paper was presented at the 10th ASES International Congress on Health, Engineering and Natural Sciences, held in Sinop, Türkiye, in April 2025. I would like to express my gratitude to Ertan Arıcı and Tolga Özkaptan for their support in the collection of samples during the research.

7. Compliance with Ethical Standard

a) Author Contributions

The author contributed to the conceptualization, sample preparation for analysis, data curation, statistical analysis, and writing – original draft. The author also reviewed and edited the manuscript, and approved the final version for publication.

1. EA.: Conceptualization, sample preparation, data curation, writing, reviewing and editing.

b) Conflict of Interests

The author declares no conflict of interest.

c) Statement on the Welfare of Animals

Formal approval is not required for this type of study.

d) Statement of Human Rights

There are no human subjects in this study.

e) Declaration of Not Using AI

The author declares that no generative artificial intelligence was used in the writing of this manuscript.

f) Funding
This study was not supported by any external funding.

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