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Seagrass as a Bioindicator for Heavy Metal Pollution in Semi-Enclosed Marine Ecosystems

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Abstract

This study delves into utilizing Seagrass as a bioindicator for heavy metal detection in semi-enclosed marine ecosystems, with a specific focus on the Jordanian coast of the Gulf of Aqaba. The research evaluates the relationship between human activities and the responses of marine organisms, employing the seagrass species Halophila stipulacea as a key subject. This research examines the ability of seagrass to sense and respond to environmental changes, particularly in terms of trace metal accumulation. These accumulations serve as indicators of the marine environment's health and the extent of human impact. Observations revealed differences in trace metal concentrations across three distinct habitats. Notably, varying levels of Cadmium (Cd) and Chromium (Cr) were found in seagrass leaves, while Copper (Cu) and Iron (Fe) were more prevalent in roots. Increased concentrations of Malondialdehyde (MDA), a marker of environmental stress as indicated by lipid peroxidation (LPO), point to a potential link between human activities, such as boating, and the health of seagrass. These findings underscore the complex interactions between marine biology, environmental management, and the innate abilities of organisms to perceive and adapt to changes in their environment. The study bridges the gap in understanding organismal responses to environmental changes and emphasizes the need for ongoing research. Such research is crucial to comprehend the broader effects of environmental shifts on marine life. By continuously monitoring trace metal levels and understanding the responses of seagrass over time, this study lays the groundwork for innovative conservation and management strategies. These strategies are aimed at protecting vital marine environments from the growing impacts of human disturbances.

Keywords: *Seagrass, Halophila stipulacea, Trace metal, Bioindicator, Gulf of Aqaba*

1. Introduction

Understanding how organisms perceive, process, and respond to environmental stimuli, especially in relation to pollutants, is pivotal in the realm of cognitive ecology [1]. Within the marine ecosystems, such interactions have profound implications for the wellbeing of organisms and how they interpret and navigate their surroundings. The importance of monitoring marine pollution, particularly in assessing trace metals and other contaminants, has been magnified due to rapid industrial advancements. The nexus of the marine environment with humans and organisms necessitates prompt action in this arena [2].

Historically, the cognitive domain of sentinel organisms, or biological indicators, has been employed to quantify the level of biologically available contaminants in aquatic ecosystems. Pioneers in this realm include [3 - 7]. By evaluating the concentrations of pollutants in these organisms' tissues, a holistic, time-integrated view of contaminant bioavailability is established [8; 9]. Prominent works within the Gulf of Aqaba have utilized bioindicators, such as [10 - 15]. Their focus on seagrass (Halophila stipulacea) as a tool for metal pollution biomonitoring has offered invaluable insights. Notably, varying concentrations of metals like Cd, Pb, Cr, Ni, Zn, Mn, and Fe were found across different parts of the seagrass and different locations. Incorporating the lens of environmental management, it's worth noting that lagoon ecosystems, characterized by their shallow waters and barriers such as coral, present unique challenges [16; 17]. Urban sprawl and anthropogenic activities, especially along Aqaba's semi-enclosed lagoons, amplify pollution concerns. Point and non-point pollution sources, predominantly from boat oil tanks and antifouling coating, aggravate the predicament.

The primary goals of this study are to analyze heavy metal concentrations in H. stipulacea and sediment across different locations. This investigation aims to understand the extent of pollution and how the presence of these pollutants might affect the behavioral and physiological responses of marine organisms. Employing H. stipulacea as a prospective bioindicator, the research seeks to provide essential insights into the hazards of heavy metal pollution in marine ecosystems, particularly focusing on the implications for the health and adaptability of marine life in these environments.

2. Materials And Methods

2.1 Study area

The Gulf of Aqaba situated at the eastern fork of the Red Sea (Fig. 1), harbors unparalleled biodiversity and coastlines that spans across four countries. However, its semi-enclosed nature and rampant coastal development, which encompasses industrial and tourism ventures, amplify the vulnerability of the area to metal pollution.

Fig1 : Location of the Gulf of Aqaba

Sample Collection

Samples of seagrass (*H. stipulacea*) were obtained from three locations along the Jordanian coast of the Gulf of Aqaba: the Royal Yacht Club (RY), Tala Bay Marina (TB), and the Marine Science Station (MSS). Upon collection and preparation, the samples were treated with 10 ml of nitric acid and left overnight before being heated. Concentrated nitric acid and hydrogen peroxide were added to the dried samples, which were then dissolved, filtered, and adjusted for volume. The samples were stored at the refrigerator for later analysis using atomic absorption spectrophotometer. Further details about the sample preparation can be found in [18].

Lipid Peroxidation assay

Lipid peroxidation in seagrass samples was quantified using a modified protocol from [19; 20] and related sources with minor alteration. A 0.30 g fresh seagrass tissue was homogenized in 20 mL solution of 0.25% thiobarbituric acid (TBA) in 10% trichloroacetic acid (TCA), using agate mortar and pestle. The homogenate mixture was incubated at 95 °C for 30 min followed by quick cooling and centrifuged at 10,000 g for 10 min. The absorbance of the clear supernatant was read spectrophotometrically at 532 nm, and correction for unspecific turbidity was done by subtracting the absorbance of the sample at 600 nm. A 20 ml of 0.25% TBA in 10% TCA was used as blank. The concentrations of lipid peroxides were quantified and expressed using Beer's law with an extinction coefficient of 155 mM^{-1} cm^{-1}. The Lipid peroxidation content was calculated and expressed as MDA mM g^{-1}, indicating the degree of lipid peroxidation in seagrass.

Statistical Analysis

Non-parametric statistical techniques, such as the Spearman correlation coefficient and Kruskal-Wallis ANOVA, were utilized to analyze the data. The results were reported as mean and standard deviation with a significant level set at $p < 0.05$. Data analysis was performed using Sigma Stat version 3.5 software.

3. Results and Discussion

Concentrations of Trace Metals At Three Sample Sites For Seagrass

The seagrass levels exhibited a significant variation in Cd amounts between sample locations with a p-value less than 0.05, indicating statistical significance. Similar to the leaves, seagrass roots showed a consistent trend in Cd concentrations, with levels ranging from 0.315-0.398 µg/g in TB and RY lagoons, while the MSS had the lowest concentration of 0.314 µg/g. Furthermore, the seagrass roots demonstrated a significant difference in Cd concentrations between sample locations with a P-value less than 0.05. (Fig. 2) illustrates the Cd concentration (µg/g) ±SD in (a) seagrass leaves and (b) seagrass roots across three sites along the Jordanian coast of the Gulf of Aqaba, namely Royal Yacht Club (RYC), Marine Science Station (MSS), and Tala bay (TB).

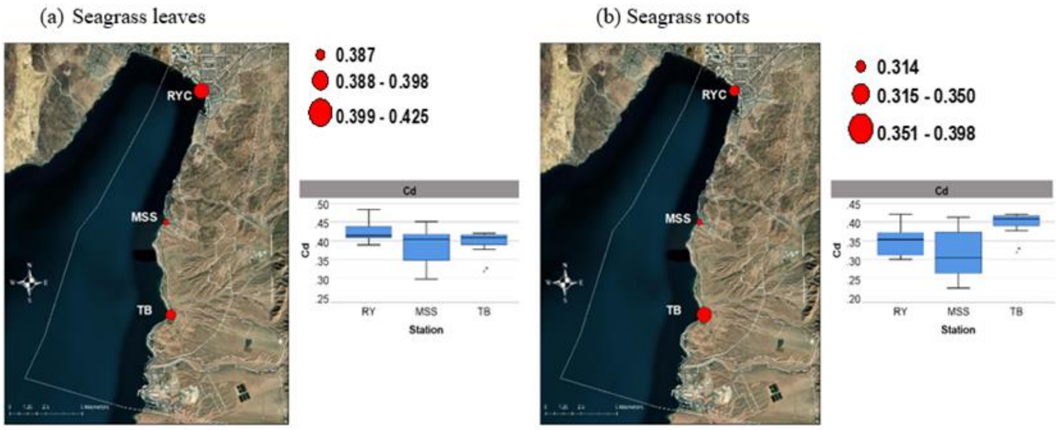

Fig 2 : Cd concentration (µg/g)±SD in (a) seagrass leaves, (b) seagrass roots, along three sites within the Jordanian coast of Gulf of Aqaba; Royal Yacht Club (RYC), Marine Science Station (MSS) and Tala bay (TB)

The concentration of Cr in seagrass leaves was found to be significantly higher in RY and TB, ranging from 5,336-4,314 g/g, compared to the lowest concentration observed in MSS (3,985 µg/g). Furthermore, the Cd concentrations in seagrass leaves exhibited a considerable variation among the sample locations with a significant difference observed with a P-value of less than 0.05 .The trend in Cd concentration in the seagrass roots was similar to that in the leaves, with the highest concentrations observed in the RYC and TB lagoons (ranging from 0.931 to 2.443 µg/g) and the lowest concentration in the MSS (2.14 µg/g). Moreover, the Cr concentrations in seagrass roots also showed a significant variation between sample locations with a P-value of 0.000 as shown in (Fig. 3).

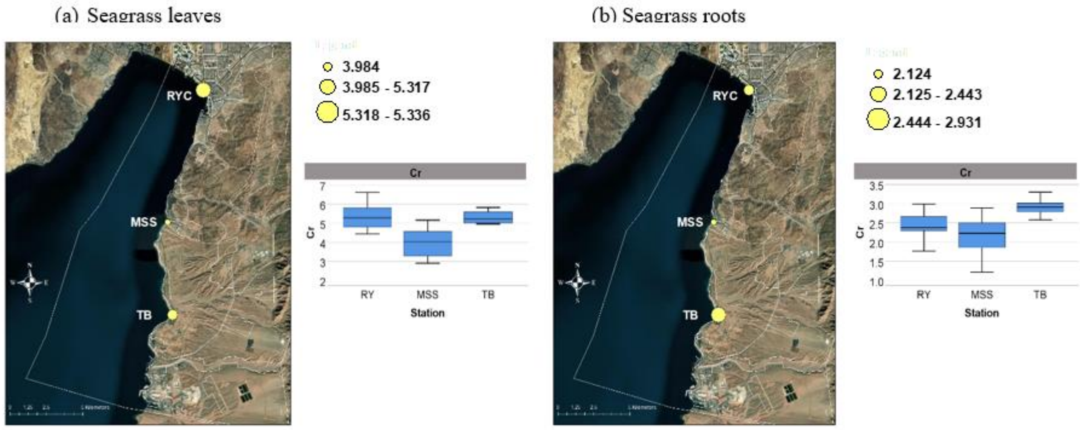

Fig. 3 *: Shows Cr concentration (µg/g) in (a) seagrass leaves, (b) seagrass roots, along three sites within the Jordanian coast of Gulf of Aqaba; Royal Yacht Club (RYC), Marine Science Station (MSS) and Tala bay (TB)*

The leaves and roots of seagrass in MSS exhibited remarkably high concentrations of copper, ranging from 0.573-0.279 µg/g and 3.242-2.88 µg/g, respectively. In contrast, the Cu content in seagrass leaves and roots from RY and TB was exceedingly low, ranging from 0.23-0.278 µg/g and 0.325-2.857 µg/g, respectively. A substantial discrepancy was observed in among different stations, indicating a variation in pollution levels. However, the statistical analysis of the results suggested no significant difference among the three locations with a p value > 0.001, as depicted in (Fig. 4).

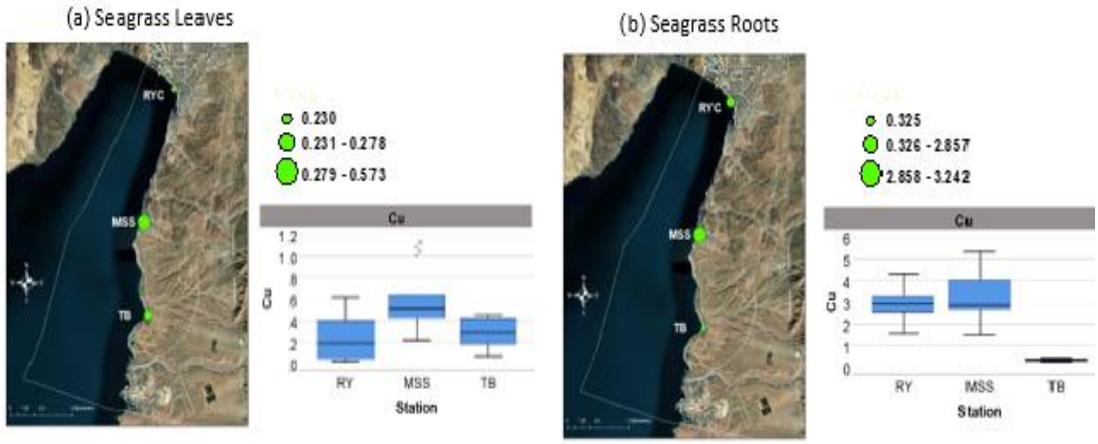

Fig.4 *: Shows Cu concentration (µg/g) in (a) seagrass leaves, (b) seagrass roots, along three sites within the Jordanian coast of Gulf of Aqaba; Royal Yacht Club (RYC), Marine Science Station (MSS) and Tala bay (TB).*

Seagrass leaves from TB and RY showed significantly higher concentrations of Fe, ranging from 274.796-207.813 µg/g, compared to those from MSS, which had lower concentrations of 121.686 µg/g. Notably, the roots of seagrass accumulated remarkably higher Fe concentrations than the leaves, with values of 393,067±26,261 and 201,493±66.93 µg/g, respectively, as illustrated in (Fig. 5).

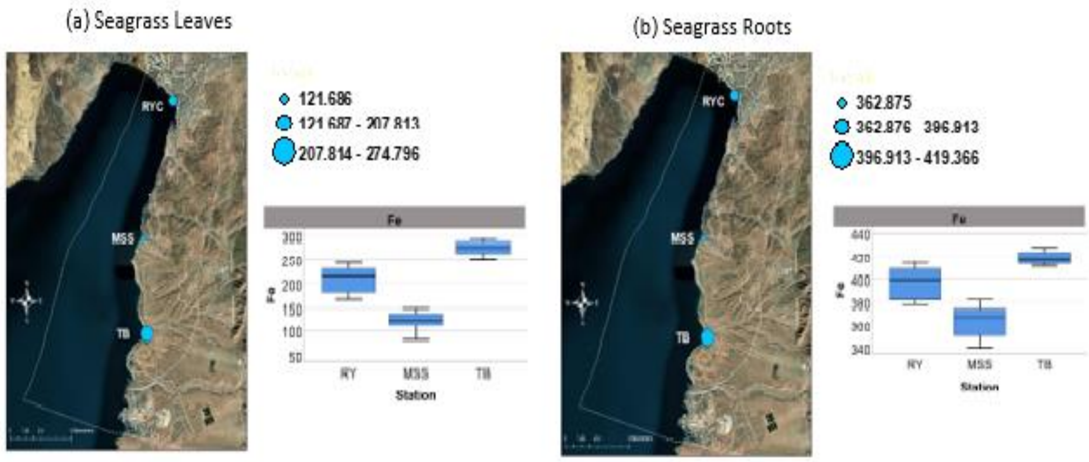

Fig 5 : Shows Fe concentration (µg/g) in (a) seagrass leaves, (b) seagrass roots along three sites within the Jordanian coast of the Gulf of Aqaba; Royal Yacht Club (RYC), Marine Science Station (MSS) and Tala bay (TB)

Concentrations Of Trace Metals in Different Sites

The Royal Yacht Club and Tala Bay sites exhibited substantially higher Fe concentrations, with mean values of 412.3±89.437 and 366.54±127.7 µg/g, respectively, while the Marine Science Station had the lowest mean Fe concentration of 314.46±162.24 µg/g. A statistically significant difference was observed (P=0.0001) in the Fe concentrations among the various sampling sites. MSS had the highest Cu concentrations with a mean of 1.432±0.502 µg/g, followed by Royal Yacht Club with 0.733±0.13 µg/g, while Tala Bay had the lowest Cu concentrations averaging at 0.25±0.039 µg/g. The difference in Cu concentrations among the sites was statistically significant (P=0.0001). The Royal Yacht Club and Tala Bay sites exhibited the highest Cr concentrations, with averages of 7.391±4.272 µg/g and 5.992±1.866 µg/g, respectively, while the Marine Science Station had the lowest average Cr concentrations of 5.473±2.373 µg/g. The variation in Cr concentrations among the sites was statistically significant (P=0.0054). The Cd concentrations averaged between 0.453-0.044 µg/gin Marine Science Station, 0.446-0.038 µg/gin Tala Bay, and 0.396-0.023 µg/g in Royal Yacht Club, while the difference in Cd concentrations between the sites was not statistically significant (P=0.478). (Fig. 6) illustrates the mean concentrations of Fe, Cu, Cr, and Cd at different sampling sites.

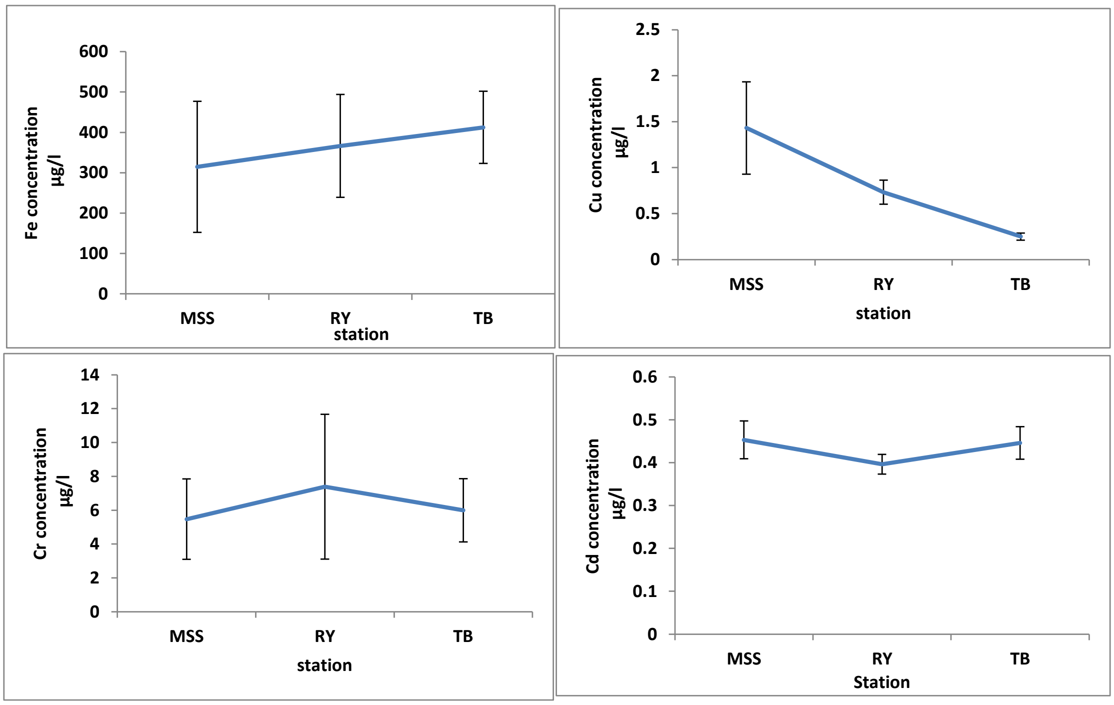

Fig 6 : The mean concentrations ± SD of Fe, Cu, Cr, and Cd at various sampling sites

As part of our analysis, we incorporated the values of heavy metals and the outcomes of lipid peroxidation using similarity diagrams. These factors played a crucial role in our study as they assisted us understand the potential impact of heavy metals on lipid peroxidation. By including us in comprehending the potential impact of heavy metals on lipid peroxidation. Incorporating these factors in our analysis allowed us to obtain a more holistic understanding of the associations between various variables and draw more robust and informed conclusions based on our discoveries.

As shown in (Fig. 7) shows the similarity diagrams of the different elements across three sampling sites which indicate that trace metal concentrations were divided into two distinct clusters, cluster A consists of the Royal Yacht Club and Tala Bay sites, which share 97% similarities of their characteristics and are semi-enclosed lagoons. In contrast, the Marine Science Station, which has an open sample site, is geographically distant from the other two location.

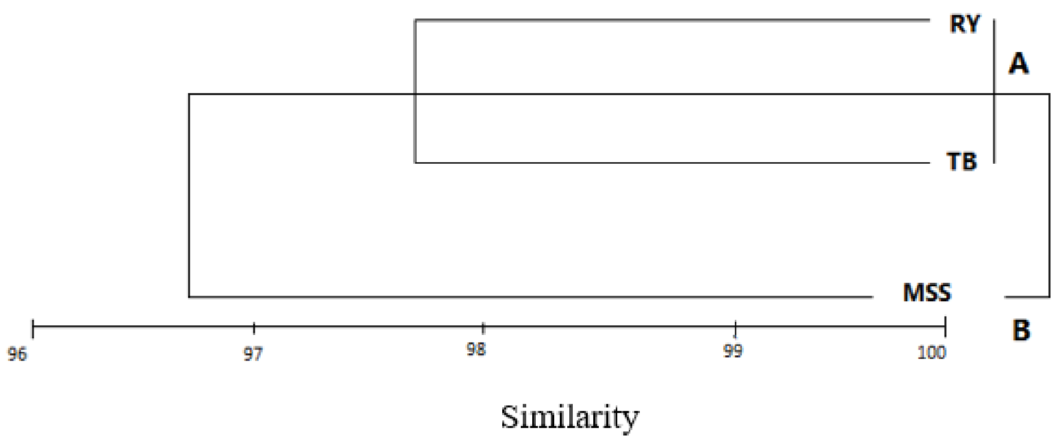

Fig 7 : Similarities dendogram of different elements in various sampling sites

Lipid Peroxidation Assay

Seagrass samples collected from the MSS exhibited normal levels of lipid peroxidation (LPO), with seagrass leaves and roots tissue homogenates having values of 1.989 µM g^{-} and 5.005 µM g^{-} respectively. However, there was a significant increase in MDA levels in tissue homogenates from stressed sites, as indicated in Table 1, when compared to those of the control ($p<0.05$). This increase is reflected in the substantial elevation of MDA values, with RYC having the highest MDA values followed by TB, compared to the control site (MSS). The extensive boat activities within the semi-enclosed area of RYC may contribute to this observation.

Table 1: Lipid peroxidation content (MDA mM g^{-1}) of seagrass tissues (root and leaves) collected from the Gulf of Aqaba under different environmental stressors. All values are reported as mean values (n = 10, TB: Talabay; RYC: Royal Yacht Club; MSS: Marine Science Station).

Name	TB	RYC	MSS
Seagrass (leaves)	0.005448	0.008428	0.001989
Seagrass (roots)	0.005913	0.485905	0.005005

To establish the presence of significant differences in MDA levels among the three sampling sites (TB, RYC and MSS), we employed one-way ANOVA.

Table 2: Pairwise comparisons of mean differences and statistical significance among TB, RYC, and MSS Sampling Sites

Pairwise comparison	Mean difference	Standard error	p-value
RYC - TB	0.4772	0.1209	0.0012
MSS - TB	-0.003	0.1209	0.9999
MSS - RYC	-0.4802	0.1209	0.0009

The post-hoc test revealed significant differences in MDA levels between RYC and TB, and between MSS and RYC, but not between TB and MSS, as shown in Table 2. The statistical analysis strongly indicates that significant differences in MDA levels exist among the three sampling sites, with RYC having the highest MDA levels, followed by TB and MSS. The increase in MDA levels could potentially be linked to the extensive boat activities within the semi-enclosed area of RYC.

Heavy Metals In Seagrass

This study aimed to comprehensively examine the concentrations of trace metals, namely Cd, Cr, Cu, and Fe, in seagrass leaves and roots at three distinct sites along the Jordanian coast of the Gulf of Aqaba: the Royal Yacht Club (RYC), Marine Science Station (MSS), and Tala Bay (TB). The analysis encompassed a critical comparison of trace metal concentrations in seagrass leaves and roots across these three locations, with the objective of unraveling the spatial variation in seagrass metal uptake and accumulation patterns

The study unveiled a significant variation in Cd concentrations in both seagrass leaves and roots among the three sample locations, indicating that the spatial distribution of Cd might be affected by local environmental factors, such as water chemistry, sediment composition, and anthropogenic activities [21]. The semi-enclosed nature of RYC and TB lagoons might promote Cd accumulation, which could explain the higher Cd concentrations observed in these areas compared to the protected area MSS. Similarly, Cr concentrations in seagrass leaves and roots showed significant variation between sample locations, with the highest concentrations in RYC and TB, suggesting a higher level of Cr pollution in these areas due to industrial activities, wastewater discharge, or sediment characteristics in these areas [22]. Conversely, Cu concentrations did not show a significant difference among the three locations, indicating that seagrass is not differentially accumulating Cu across these sites [23]. Our study also revealed that seagrass roots play a major role in Fe uptake and accumulation, with higher Fe concentrations in seagrass leaves and roots from TB and RYC, and the roots accumulating significantly higher Fe concentrations than the leaves. These findings suggest that Fe concentrations in these coastal ecosystems may be influenced by site-specific environmental factors [21]. The similarity diagrams revealed a distinct clustering of trace metal concentrations in seagrass among the sample locations, with RYC and TB forming Cluster A, and MSS being distinct from the other two sites, possibly due to differences in hydrodynamics, water chemistry, and anthropogenic activities in MSS as protected area comparted with other two areas [24].

Effect of environmental stressors on Lipid Peroxidation (LPO)

The results presented in Table 1 demonstrate the levels of MDA in various parts of seagrass samples obtained from three distinct locations: TB, RYC, and MSS. Lipid Peroxidation (LPO) is a natural phenomenon that occurs in plants and animals, but when it is intensified due to stress, it can result in cell damage and mortality. MDA a byproduct of LPO, is regarded as a biomarker of oxidative stress [19]. Our findings indicate that seagrass samples collected from RYC showed the highest levels of LPO, with MDA values of 0.485905 µM g^{-1} for seagrass roots, followed by TB, with MDA values of 0.008428 µM g^{-1} for seagrass leaves. In contrast, the control site, MSS, had the lowest levels of MDA in both seagrass leaves and roots. These findings strongly suggest that seagrass samples from RYC and TB may be undergoing higher levels of oxidative stress as compared to those from the control site MSS the protected site.

Our data strongly suggest that the elevated MDA levels observed in RYC are primarily caused by the extensive boat activities within the semi-enclosed area. The physical damage caused by boats activities can lead to increased turbulence, resulting in the production of free radicals that induce oxidative stress [25]. The results indicate that anthropogenic activities, such as boating, can have severe and negative impacts on seagrass beds. These findings are consistent with the previous studies, such as the one by [20] which also reported the damaging effects of anthropogenic activities on seagrass ecosystems.

However, this study provides crucial insights into the spatial distribution and variability of trace metal concentrations in seagrass along the Jordanian coast of the Gulf of Aqaba. The results emphasize the significance of considering site-specific environmental factors, such as water circulation patterns, sediment composition, and human activities, when evaluating the bioaccumulation of trace metals in seagrass ecosystems [18, 21]. Furthermore, the study highlights the necessity of regular monitoring of trace metal concentrations in coastal environments to better understand their potential ecological impacts and inform management strategies for the conservation and protection of these essential ecosystems [22]. Consequently, the findings presented in this study suggest that seagrass beds in RYC and TB are likely experiencing significantly higher levels of oxidative stress compared to the control site, MSS. Anthropogenic activities, such as boating, are likely contributing to the observed effects.

This study underscores the criticality of comprehending the impacts of human activities on marine ecosystems and the pressing need for conservation efforts to protect seagrass beds and the vital ecosystem services they provide.

4. Conclusion

The interplay between seagrass and its surrounding environment highlights the complex interactions within marine ecosystems. These insights are crucial in understanding the relationship between seagrass and the trace metal concentrations in their habitat. Variations in trace metal accumulation across different sites indicate that both natural and human-induced factors significantly affect how seagrass reacts to these metals. Elevated levels of MDA in seagrass tissues from certain areas may signal the plant's response to environmental stress. Identifying these stress markers offers a clearer picture of the processes governing marine habitats. The knowledge gained from this research is vital for developing adaptive management strategies to preserve these marine ecosystems, taking into account their ecological importance and their role in human maritime activities, communication, and societal interactions.

Recommendations

Adopting an ecological approach that focuses on the response and adaptation of seagrass to environmental changes is recommended for future research. Acknowledging the processes that regulate the interactions between seagrass and their environment is crucial for understanding the overall impact on marine ecosystem health and dynamics. It is important to monitor trace metal levels in these environments over time. Such observations would inform strategies aimed at preserving not only the physical but also the overall integrity of marine ecosystems. Additionally, understanding how trace metals are assimilated and their effects on interactions within the marine food web is essential. This knowledge would improve the accuracy of environmental assessments and support informed decision-making in marine environmental management, enhancing the connection between environmental conservation and the broader interactions in marine habitats.

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