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Research Article

Morphophysiological Traits of Gray Mangrove (*Avicennia marina* (Forsk.) Vierh.) at Different Levels of Soil Salinity

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The traits of gray mangrove (*Avicennia marina* (Forsk.) Vierh.) at different levels of soil salinity were assessed at three sites along the Tubli Bay coastline of Bahrain. Morphophysiological traits include rates of photosynthesis and transpiration, leaf morphology, and leaf pigment content. Results showed that the rates of photosynthesis and transpiration, the intercellular CO_2 concentration, and stomatal conductance were significantly lower ($p \leq 0.05$) in the trees of a low salinity site compared to those of high salinity sites. Pairwise correlation between soil electrical conductivity levels and photosynthesis and transpiration rates was very low, implying an adverse effect of low salinity on both processes. However, the dimensions of leaf area and the sclerophylly index were significantly higher ($p \leq 0.05$) in the trees grown in low salinity site compared to those in more saline conditions. On the other hand, pigments were significantly lower in leaves of trees grown in low salinity site compared to the other two sites. The pairwise correlation between electrical conductivity values and chlorophyll a, b, and carotenoid was 0.51, 0.52, and 0.57 consecutively at ($p < 0.0001$), implying moderate effect of salinity on leaf pigments. This study illustrates the adverse and positive effects of soil salinity on some traits of gray mangrove in Bahrain, which might be considered in ecosystem rehabilitation along other coastlines of the country.

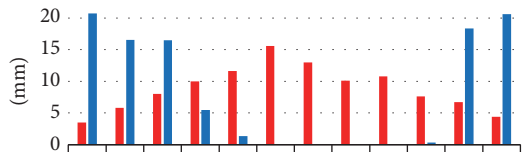
1. Introduction

Gray mangrove (*Avicennia marina* (Forsk.) Vierh.) grows principally in tropical areas. However, its distribution extends to some parts of the temperate regions of the world [1]. In Southwestern Asia, gray mangroves grow in discrete associations along the coasts of the Arabian Gulf as well as the eastern and western shores of the Red Sea [2]. Globally, climatic, edaphic, physiographic, and biotic factors influence the distribution and biodiversity of mangroves and their associated communities [3]. Hence, a tall, dense growth of mangroves is found in high rainfall areas across the tropics; however, dwarf forms of the species typify its physiognomy in the Arabian Peninsula. The shrubby form of mangroves reflects the aridity of the environment, the high seawater salinity, and other local site conditions [4, 5].

The productivity of mangroves reflects their physiological activity, which differs within and between mangrove species [6, 7]. Salinity is one of the most critical factors affecting physiological activities, with a consensus that high salinity interferes with the physiological activities of mangroves. Hypersaline conditions and high solar irradiance loading reduced carbon assimilation rates and suppressed stomatal conductance (g_s) in several mangrove species [8–10]. Equally, an increase was reported in CO_2 assimilation rates in mangroves at low salinity levels [11].

High salinity negatively affected photosynthesis in *A. marina* by reducing g_s, which in turn limits the species' photosynthesis and transpiration rates [12]. On the contrary, low g_s increased water use efficiency (WUE) of mangroves [13]. It was reported that assimilation rates and g_s in mangroves were at their maximum at 50-75 % seawater salinity [14]. Another study stated that photosynthetic performance and chlorophyll content in *R. mangle* and *L. racemosa* were maintained under increased levels of salinity [15]. On the other hand, a negative correlation was reported between high salinity levels and chlorophyll content of mangroves [16, 17].

Leaf morphological and physiological traits are affected by several biotic and abiotic factors. For instance, a change was reported in the total pigments of *A. marina* subjected



	1	2	3	4	5	6	7	8	9	10	11	12
Evap. (mm)	3.5	5.8	8	10	12	16	13	10	11	7.6	6.7	4.4
Rainfall (mm)	21	17	17	5.5	1.4	0	0	0	0	0.3	18	21

Months

Figure 1: Average monthly rainfall and evaporation rates in the study area (mm).

to leaf spot disease (*Alternaria alternate*) [18]. High levels of salinity negatively affected leaf area (LA), leaf specific area (LSA), and leaf weight [10, 19].

The ecophysiological traits, no doubt, are genetically based. However, some plant adaptations have emerged as a response to environmental conditions, yielding site-specific phenotypes. Knowing these characteristics is essential for evaluating species performance in harsh environments [20]. This research was undertaken to assess the impact of soil salinity on some physiological and morphological traits of gray mangrove in the arid environment of Bahrain.

2. Material and Methods

2.1. Study Area. Bahrain is a small island covering 779Km^2 of land area. It is located between latitudes 25° 32 and 26° 20 North and longitudes 50° 20 and 50° 50 East. The climate is arid, characterized mainly by high temperature and low rainfall. Daily evaporation rate has an average of 5.75 mm, giving a mean annual rate of 2099 mm [21] (Figure 1).

Gray mangroves grow principally at three primary locations along the coasts of Tubli Bay in the country. The Bay is a sheltered and very shallow water body measuring 10 Km^2. Three Mangrove sites, Tubli, Sitra-1, and Sitra-2 (Figure 2), were selected along the Bay coasts based on the sites' accessibility and possible soil salinity differences due to the discharge of 100,000m^3 day-1 of treated wastewater [22]. The latter two sites (Sitra-1 and 2) are at a distance of 7 Km from Tubli and within 1 Km of each other. Trees at the chosen sites were of the same age, with mean heights and diameters of 3.3 m and 5.8 cm, respectively, in Tubli stand, whereas the same parameters were 2.6 m and 5 cm in Sitra-1 and 2.2 m and 5 cm in Sitra-2. The mean pH of the Bay seawater is 8.0± 0.2, while salinity is 42.3± 1.46‰. The mean seawater temperature is 26.4±1.4°C. Tides occur twice a day, with heights ranging between 0.18 and 0.51m.

2.2. Methods

2.2.1. Soil Conductivity (EC) and pH. At each study site and along a transect line placed in a seaward landward direction, three soil cores were taken in summer and winter (mid-June and mid-March 2016) at three different depths: 0-5 cm, 10-15 cm, and 15-30 cm. The removed soil cores were placed in sealed bags and transported to the laboratory for analysis. Individual samples were air-dried, homogenized using mortar and pestle, and passed through a 2 mm sieve. EC and pH of soil samples were measured using 1:1 (soil : water ratio, w/v) extracts [23].

2.2.2. Physiological Traits. Using the LI-6400XT Portable Photosynthesis System [24], instantaneous rates of photosynthesis (A) and transpiration (E), along with the corresponding intercellular CO_2 concentration (Ci), g_s, and WUE were measured. The measurements were taken in situ on ten replicates of fully expanded sun leaves at each of the three study sites for five consecutive days in March 2016. During these measurements, average photosynthetic active radiation (PAR) was 1199.3 μmol m^2 s^{-1}, whereas leaf temperature and relative humidity averaged 32°C and 51%, respectively.

2.2.3. Leaf Morphology. Four fully expanded sun leaves were excised randomly from ten randomly selected trees in each mangrove stand. Leaf area was measured using Easy Leaf Area Software [25]. The leaves were washed with distilled water, weighed and placed in deionized water overnight till saturation. Saturation weight was measured, and then the leaves were dried in an oven at 70°C till constant weight. Leaf dry matter (LDM) and specific leaf area (SLA) were determined [26] using the formulas: LDM (g Kg^{-1}) = leaf dry weight/leaf saturated weight, SLA (m^{2}Kg^{-1}) = leaf area/leaf dry weight. Leaf thickness (LT) was computed according to [27] as; LT (μm) = 1/(SLA x LDM). Sclerophylly index (IE) was calculated [28] using the formula: (g dm^{-1}) = leaf dry weight/leaf area. The leaves were classified as sclerophylly when IE > 0.6 [29]. Finally, relative water content (RWC) was calculated according to [29]; as RWC (%) = (fW − DW)/(TW − DW)x100, where FW: fresh leaf weight, DW: dry leaf weight, TW: turgid leaf weight.

2.2.4. Leaf Pigments. Ten fully grown sun leaves were taken from the upper canopy of mature mangrove trees representing three replicates at each site in summer and winter (mid-June and mid-March 2016). One gram of fresh leaf samples was chopped, excluding the main vein, and ground using mortar and pestle. The ground material was dissolved in 100 ml chilled 80% acetone, kept in the dark at room temperature for 10 minutes, and filtered through Whatman paper no.1. The absorbance of the filtrates was measured at 663, 645, and 470 nm. Pigments were determined according to [30, 31] as follows:

Chl (a) = 12.7 (A663)-2.69 (A645) x v/w

Chl (b) = 22.9 (A645)-4.68 (A663) x v/w

Total Chl = 20.2 (A645)-8.02 (A663) x v/w

Carotenoids (CAR) = 4 (A470) x v/w

where A: spectrophotometer absorbance, V: volume of acetone, and W: fresh leaf weight.

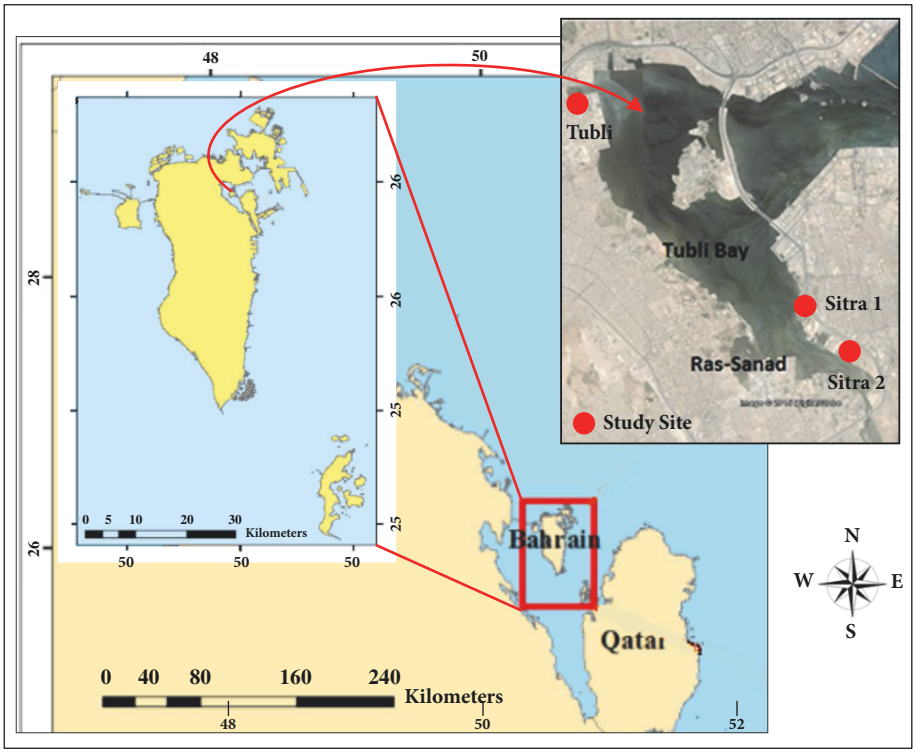


Figure 2: Locations of study sites.

2.3. Statistical Analysis. Differences between the means for the measured parameters were assessed using the least significant difference (LSD at < 0.05) in one and two-way ANOVA, as appropriate. Standard errors of the means for treatments were calculated. The pairwise correlation between factors was assessed at 0.05. The statistical software JMP 11 was used for statistical analysis [32].

3. Results and Discussion

3.1. Soil EC and pH. Average EC of the soil profile at the three sites was lower in winter than in summer, due to low evaporation rates and surface water runoff into the bay. In summer, mean EC was 38% and 29% less at the Tubli site than its values in the Sitra-1 and 2 sites respectively, due to site proximity to the outfall discharging treated municipal wastewater. The mean EC for the soil profile was significantly higher ($p \leq 0.05$) in Sitra-1 than in Tubli. However, no significant differences existed in EC values between Sitra-1 and 2 (Table 1). A similar result was observed in winter, where mean EC of the soil profile at Tubli site was recorded at 13% and 28% less than its averages in Sitra-1 and 2, respectively. Significant differences ($p \leq 0.5$) in EC existed between Tubli site and Sitra-1, but not between Sitra-1 and 2. Also, a decrease in EC values from the top to bottom layers of the soil profile was observed at the Tubli site. However, no general trend of EC values was detected at the other two sites. Significant differences in EC values were observed between the sites, notably at 0-5 cm depth. However, no significant differences were detected in pH between sites in both seasons, although in winter the pH of the soil profile in Sitra-2 was significantly lower than that at the other sites, probably due to accidental release of a pollutant.

3.2. Physiological Traits. Photosynthetic assimilation rates of mangroves ranged from 3.13 to 17.73 μmol m^2 s^{-1}, with an average of 9.48 μmol m^2 s^{-1} (Table 2). On the other hand, E ranged between 1.8 mmol m^2 s^{-1} and 8.2 mmol m^2 s^{-1}. The corresponding C_i values ranged between 27.83 μmol^{-1} and 364.87 μmol^{-1}. On the other hand, g_s values ranged from 70 to 570 mmol m^2 s^{-1}. In this study, the mean maximum A is comparable to values reported for gray and red mangroves [11, 33]. However, [10] reported higher values of g_s for different mangrove species. The rates of A and E, as well as g_s, were significantly lower in Tubli stand compared to the other two stands ($P \leq 0.01$), due to low soil EC at the site. A and E rates were significantly higher, while g_s and C_i were significantly lower is Sitra-2 stand compared to the Sitra-1 stand ($P \leq 0.01$). Given its low soil EC (12 ± 1.6 dS m^{-1}), Tubli trees had the lowest level of physiological parameters of the three sites studied, with EC means at 14 ± 1.1 and 17 ± 1.4 dS m^{-1}, respectively, for sites Sitra-1 and 2. As is the case in our study, a positive effect of high salinity substrate on the photosynthetic assimilation rate of mangroves has been reported by several scholars [34–36]. The authors attributed the positive effect of high salinity on photosynthesis to the increase in the mesophyll and chlorophyll content in the leaves of mangroves. On the other hand, some scholars reported the adverse effect of increasing salinity levels on A, g_s, and C_i of gray mangrove [4, 7, 12, 37]. In our results,

Table 1: Electrical conductivity (dS m^{-1}) and pH of soil profile at the study sites∗.

Stand	Depth (cm)	EC (dS m^{-1}) Summer	EC (dS m^{-1}) Winter	pH Summer	pH Winter
Tubli	0-5	17.57 ± 3.34 bcdef	14.77 ± 2.87 def	8.03 ± 0.07 ab	8.00 ± 0.03 ab
	5-15	17.43 ± 5.84 bcdef	12.03 ± 2.79 ef	8.13 ± 0.09 a	7.95 ± 0.02 abc
	15-30	10.60 ± 0.56 f	10.03 ± 2.87 f	8.04 ± 0.15 ab	7.97 ± 0.03 abc
mean∗		15.2 ± 2.26 b	12.3 ± 1.6 b	8.06 ± 0.057 a	7.97 ± 0.02 a
Sitra (1)	0-5	25.03 ± 3.02 ab	16.77 ± 2.29 cdef	7.84 ± 0.12 bc	8.02 ± 0.03 ab
	5-15	23.77 ± 3.20 abc	12.20 ± 1.88 ef	7.92 ± 0.10 abc	7.73 ± 0.24 cd
	15-30	26.03 ± 1.35 a	13.60 ± 1.19 def	7.99 ± 0.09 abc	8.04 ± 0.02 ab
mean∗		24.9 ± 1.37 a	14.2 ± 1.1 ab	7.91 ± 0.048 a	7.93 ± 0.09 a
Sitra (2)	0-5	18.90 ± 3.87 abcde	17.40 ± 2.06 bcdef	7.95 ± 0.12 abc	7.47 ± 0.02 de
	5-15	26.00 ± 2.83 a	20.47 ± 1.88 abcd	7.96 ± 0.06 abc	7.46 ± 0.03 e
	15-30	19.20 ± 3.75 abcde	13.57 ± 1.63 def	8.12 ± 0.01 a	7.44 ± 0.02 e
mean∗		21.4 ± 2.10 a	17.1 ± 1.40 a	8.01± 0.05 a	7.46 ± 0.01 b

∗Levels not connected with the same letter are significantly different ($P \leq 0.05$).

Table 2: Rates of photosynthesis, transpiration, and other corresponding physiological parameters of *A. marina* at sites with different salinity∗.

Stand	Photo. (A) μmol CO_2 m^{-2} s^{-1}	Intercellular CO2 Concentration (Ci) μmol CO_2 mol^{-1}	Trans. (E) mmol H_2O m^{-2} s^{-1}	Stomatal Cond. (g_s) mmol H_2O m^{-2} s^{-1}	Water Use Efficiency (WUE	Mean EC∗∗
Tubli	7.7 ± 0.36 c	271.8 ± 7.7 b	3.8 ± 0.08 c	165.7 ± 6.75 c	0.0022 ± 8.69e-5 a	12.3±1.6
Sitra (1)	9.6 ± 0.26 b	301.9 ± 4.5 a	4.2 ± 0.11 b	290.5 ± 12.8 a	0.0023 ± 9.23e-5 a	14.2±1.1
Sitra (2)	10.6 ± 0.35 a	277.7 ± 3.2 b	4.9 ± 0.12 a	226.7 ± 8.6 b	0.0021 ± 7.14e-5 a	17.1±1.4
p value	<0.0001	0.0003	<0.0001	<0.0001	0.3024	
mean	9.48 ± 0.2	282.32 ± 2.99	4.39 ± 0.07	225.4 ± 5.98	0.002 ± 4.73e-5	

∗Levels not connected with the same letter are significantly different ($P \leq 0.05$).
∗∗See Table 1.

Table 3: Leaf morphological parameters of Tubli Bay mangroves at the study sites∗.

Stand	Area (cm^2)	LDMC ($g\ kg^{-1}$)	LT (μm)	IE ($g\ dm^{-1}$)
Tubli	8.96 ± 0.2 a	286.47 ± 4.1 a	0.00056 ± 5.8e-6 a	0.71 ± 0.02 a
Sitra (1)	7.45 ± 0.2 c	282.09 ± 4.1 a	0.00055 ± 5.8e-6 a	0.52 ± 0.02 b
Sitra (2)	8.11 ± 0.2 b	290.81 ± 4.1 a	0.00056 ± 5.8e-6 a	0.57 ± 0.02 b
Mean	8.1 ± 0.13	286.4 ± 2.41	0.0005 ± 3.4e-6	0.60 ± 0.01
P value	<0.0001	0.3384	0.2271	<0.0001

∗Levels not connected with the same letter are significantly different ($P \leq 0.05$).

low salinity levels negatively affected A, E, and g_s of gray mangrove, possibly due to a decrease in pigment content [38]. Nevertheless, g_s was significantly higher in Sitra-1 and 2 compared to the Tubli site ($P \leq 0.01$).

The pairwise correlation between EC, A, and E was 0.72 and 0.61, respectively, at ($p < 0.0001$), implying the positive effect of high salinity on both physiological processes of gray mangrove. However, the amplitude of salinity variations in this study did not have any significant impact on WUE, which conforms to [14] but contradicts [12, 13, 33]'s findings.

3.3. Leaf Morphology. LA and IE were larger in leaves of gray mangrove at Tubli site compared with the other two sites (Table 3). LA was 20% and 10% greater in Tubli stand compared to that in Sitra-1 and 2 respectively. There were significant differences in LA and IE between Tubli stand and the other two stands ($p \leq 0.05$). Higher values of LA and IE coincide with lower levels of EC at the Tubli site compared to the other two sites (see Table 1). The finding of lush leaf growth at the low salinity levels agreed with [7, 37, 39–41].

LDM and LT are traits reflecting species' strategy of resource use. No statistical differences were observed in these variables among the trees of the three sites. The nil effect of EC in our investigation on LDM and LT can be attributed to a wide-ranging tolerance amplitude of *A. marina* for salinity [42]. On the other hand, IE value differed significantly between sites, where it was higher in Tubli by 38% and 26% than those of Sitra-1 and 2, respectively. Higher IE in low salinity media, as was the case in our study, contradicts the findings of [43] of a positive correlation between IE and salinity in *A. germinans*.

3.4. Leaf Pigments. At an average RWC= 69.2%, the concentration of Chl (a) in gray mangrove ranged between 0.38 mg g^{-1} and 0.96 mg g^{-1} fresh weight in the summer (Table 4). In the meantime, Chl (b) ranged from 0.11 mg g^{-1} to 0.63 mg g^{-1}. Total Chl varied between 0.48 mg g^{-1} and 1.37 mg g^{-1}, while chlorophyll a/b varied between 1.06 mg g^{-1} and 3.95 mg g^{-1}. CAR ranged between 0.16 mg g^{-1} to 0.33 mg g^{-1}. In winter, at 81.2% mean RWC, the concentration of Chl (a) ranged between 0.26 mg g^{-1} and 0.78 mg g^{-1}, while Chl (b) varied from 0.04 mg g^{-1} to 0.38 mg g^{-1}. Total Chl and Chl (a/b) ranged from 0.42 mg g^{-1} to 0.83 mg g^{-1} and 0.84 mg g^{-1} to 11.15, respectively. CAR ranged between 0.095 - 0.24 mg g^{-1}. The results of this study are comparable with those obtained by [44] for other mangrove species. However, [45] reported higher values for total pigments as well as chlorophyll a/b for the same species. The difference in reported results can be attributed to varying salinity levels and environmental conditions.

Leaf pigments in winter were significantly lower than in summer for all stands ($p \leq 0.05$). In Tubli, Chl a, b, and CAR were lower by 29%, 16%, and 35% respectively in winter than in summer, whereas in Sitra-1 they were more depressed during winter compared to summer by 34%, 33%, and 45%, respectively. Likewise, in Sitra-2 stand, pigments were lower in winter than summer by 17% for Chl (a), 50% for Chl (b), and 22% for CAR. Furthermore, total Chl was significantly higher in the Sitra-1 stand than the other two stands in the summer ($p \leq 0.05$). Similarly, CAR were significantly higher in the Sitra-1 and Sitra-2 stands than in Tubli by 19% ($p \leq 0.05$). In winter, leaf content of Chl (a) was significantly higher ($p \leq 0.05$) in Sitra-2 compared to Tubli and Sitra-1stands. In contrast, Chl (b) was significantly higher ($p \leq 0.05$) in Sitra-1 stand compared to Sitra-2 stand.

The Pairwise correlation between salinity and Chl a, b, and CAR were 0.51, 0.52, and 0.57 consecutively at ($p < 0.0001$), implying a positive effect of salinity on gray mangrove pigments. Our results agree with the findings of [16, 39]. In contrast, higher values of chlorophyll in *A. marina* and *A. germinans* were reported in low salinity conditions [3, 35]. A positive salinity effect of up to 12 dS m^{-1} was reported on pigments of *A. officinalis*, whereas a 31 dS m^{-1} salinity level led to an adverse effect [37]. Similarly, [46] reported a decrease in total pigments of *Ceriops decade* at high salinity levels.

In conclusion, the effect of soil salinity on ecophysiological characteristics of mangroves varied among studies due to variations in site conditions and level of salinity. The current in situ study demonstrates the adverse effect of low soil salinity levels on the physiological traits of the gray mangrove. Low salinity negatively impacted leaf pigments, the rates of photosynthesis, and transpiration, as well as the corresponding intercellular CO_2 and stomatal conductance. However, a favorable effect on leaf area and the sclerophylly index of gray mangrove leaves was noticeable at low salinity levels. The mechanism of mangrove tolerance to salinity is very complex, involving variations among species as well as some traits with different responses. However, gray mangrove is well known as a facultative halophyte which absorbs NaCl preferentially in low salt media [47, 48]. By this regulating mechanism, it maintains turgidity of its leaves, which may have a positive effect on leaf area and the sclerophylly index.

Table 4: Pigments and relative water content of mangrove leaves at the study sites∗.

Site	Season	RWC (%)	Pigments (mg g^{-1} of fresh weight)				
			Total Chl	Chl (a)	Chl (b)	Chl (a/b)	(CAR)
Tubli	Summer	68.63 ± 0.45 b	0.74 ± 0.03 c	0.59 ± 0.03 bc	0.16 ± 0.01 d	3.66 ± 0.06 a	0.21 ± 0.01b
	Winter	81.83 ± 0.89 a	0.55 ± 0.02 d	0.42 ± 0.02 e	0.13 ± 0.02 d	4.26 ± 0.86 a	0.14 ± 0.01 c
Sitra (1)	Summer	69.97 ± 0.39 b	1.07 ± 0.06 a	0.69 ± 0.05 a	0.38 ± 0.026 a	1.86 ± 0.12 b	0.25 ± 0.01 a
	Winter	81.41 ± 0.89 a	0.71 ± 0.02 c	0.46 ± 0.04 de	0.25 ± 0.018 c	1.83 ± 0.23 b	0.14 ± 0.011 c
Sitra (2)	Summer	69.12 ± 0.36 b	0.93 ± 0.06 b	0.62 ± 0.04 ab	0.31 ± 0.03 b	2.08 ± 0.15 b	0.25 ± 0.011 a
	Winter	80.38 ± 0.70 a	0.67 ± 0.03 c	0.51 ± 0.03 cd	0.16 ± 0.01 d	3.30 ± 0.08 a	0.19 ± 0.01b
p value		<0.0001	0.0643	0.1623	0.0034	0.2568	0.0095
mean		75.2 ± 0.4	0.77 ± 0.02	0.55 ± 0.02	0.23 ± 0.01	2.83 ± 0.19	0.19 ± 0.01

∗Levels not connected with the same letter are significantly different ($p \leq 0.05$).

The results of the study imply that low soil salinity caused by treated wastewater could have adverse effects on some physiological traits of the gray mangrove, which may affect tree growth. Restoration projects in the area should take the result of the study into consideration when rehabilitating similar sites.

Data Availability

The primary data used in producing the results of the article is available upon sound request from the corresponding author Dr. Mohammad S. Abido, mohammedsaa@agu.edu.bh.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this work.

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