Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Ecological risk assessment of heavy metal contamination in mangrove habitats, using biochemical markers and pollution indices: A case study of *Avicennia marina* L. in the Rabigh lagoon, Red Sea

Mohammed O. Aljahdali *, Abdullahi Bala Alhassan

Department of Biological Sciences, Faculty of Sciences, King Abdulaziz University, Jeddah, Saudi Arabia

ABSTRACT

Contamination of mangrove ecosystems, including those of the Red Sea area, has caused serious concern globally. Spatial distribution of heavy metals and their bioaccumulation in one of the common mangrove plants of Saudi Arabia, *Avicennia marina* L., was evaluated in 8 stations at the Rabigh lagoon to assess the ecological risks due to heavy metal contamination. Among all the heavy metals, Fe concentration was recorded highest (8939.38 ± 312.63 mg/kg) at station S4. Contamination factor (CF) values for all heavy metals determined in this study were recorded in ascending order as Cu < Cr < Mn < Zn < Fe < Ni < Pb < Cd, with the pollution load index pattern recorded in descending order as S6 > S4 > S3 > S5 > S7 > S1 > S8 > S2. Bio-concentration factor (BCF) was <1 for all the heavy metals and there was a positive correlation between the antioxidants and lead (Pb), which can be a result of the ability of *A. marina* to exclude or detoxify this metal by its mechanism of exclusion or detoxification. A significant correlation existed between the heavy metals concentration in sediment and *A. marina* leaves at one combination or the other, except for Cu and Cd, which do not correlate with any other metal concentration. The information provided in the present study can be used in the monitoring and measurement of heavy metal pollution in marine ecosystems or other aquatic environments, to prevent several ecological risks to the mangrove ecosystem.

© 2020 The Authors. Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

The use and discharge of chemical agents, such as heavy metals, as a result of anthropogenic activities, affects the normal variation and distribution pattern of heavy metals (Chai et al., 2014; Golestaninasab et al., 2014). Assessment of heavy metals to determine ecological risk through measurement of biotic response in plants can provide information on the extent of bioavailability of metals and their influence on the natural state of aquatic ecosystems (Marchand et al., 2016; Bakshi et al., 2017, 2018; Aljahdali and Alhassan, 2020a).

Contamination of ecosystems is on the increase and has become a global problem, due to toxic heavy metal distribution and its abundance and persistence in the aquatic ecosystem (Yuan et al., 2011). Contaminants suspended in the marine environment, and others present in effluent and runoff from the terrestrial environment, end up in the continental shelf and the coastal zones of the world. These have the negative consequences of integrating into the benthic sediment of the aquatic environment and changing the natural status of the ecosystem and its wellbeing (Zhang et al., 2017; Bakshi et al., 2018; Aljahdali and Alhassan, 2020b).

Heavy metals degrade the natural ecosystem, due to their bio-magnification potential at different trophic levels. This can cause ecosystem degradation, loss of biodiversity and health issues in human beings. For example, chronic exposure to heavy metals such as Cd can cause disease conditions, such as lung cancer and dysfunction of the kidney. Therefore, chromium, copper, and lead, if discharged directly into the aquatic environment or coastal sediments can lead to deteriorated conditions. Pb exposure specifically can cause nephropathy and anemia (Fu et al., 2014; Islam et al., 2017; Saha et al., 2017; Rahman et al., 2019).

---

* Corresponding author at: Department of Biological Sciences, Faculty of Sciences, King Abdulaziz University, P.O. Box Box 80203 Jeddah 21589, Saudi Arabia.
E-mail address: moaljahdali@kau.edu.sa (M.O. Aljahdali).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier
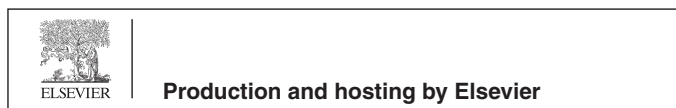
During the past few decades, the coastal area of the Saudi Arabian Red Sea has been the site of anthropogenic activities, particularly industrialization, and as a consequence there has been an increasing input of pollutants such as heavy metals into the region (Badr et al., 2009; Abdel-Hamid et al., 2011). These coastal areas possess numerous lagoons; one of the most important of these is the Rabigh lagoon (22° 45′N 39° 00′E and 23° 00′ N 38° 45′ E, and altitude of 1500 m). A significant aspect of this lagoon is a series of events that might have occurred during the last four decades. In 1987, a rocky embankment was constructed at the mouth of the lagoon. This led to the hypersalinity of the lagoon, with a possible increase in metal concentration in the lagoon and degradation of mangroves. However, the removal of the embankment in 2013 turned what had become a lake back to a lagoon once again (Al-Dubai et al., 2017). Significant changes in physicochemical and ecological variations were apparent in the sediments and mangroves, offering a rare opportunity to gain an understanding of how the ecosystem has been affected (Al-Dubai et al., 2017; Bakshi et al., 2018).

Mangrove ecosystems are composed of diverse communities that survive in different regions of the world, such as the intertidal zones of tropical and subtropical coastal rivers, estuary environments, etc. (Marchand et al., 2016). This ecosystem serves as a habitat for resident and migratory animals and also plays a role in carbon sequestration and protection against coastal erosion. These features make the mangroves unique, and for that reason, pollution of the mangrove ecosystems has recently received increased attention from those concerned with conservation issues. Consequently, there is now an interest in providing or improving conservation strategies associated with mangrove ecosystems (MacFarlane et al., 2007; Liu et al., 2015).

Assessment of ecological risk by determination of heavy metals in benthic sediment and investigation of physiological response against heavy metals in *Avicennia marina* leaves is the main objective of this study. *A. marina* is the only mangrove species found throughout the Rabigh lagoon.

2. Materials and methods

2.1. Study area

The Red Sea contains numerous lagoons on the west coast of Saudi Arabia; among these is the Rabigh lagoon (Fig. 1). The Rabigh lagoon is located on Lat.22° 45′N and Long. 39° 00′E and 23° 00′ N 38° 45′ E.

The lagoon is located northwest of Rabigh city and has an average length of 17 km and an average width of 3 km. The depth of the lagoon has been measured and ranged between 1 m and 11 m. The lagoon is connected to the Red Sea at its mouth, which is located at the northwestern part of the lagoon. It contains islands rich in mangroves and with an abundance of *Avicennia marina*. The tidal range is about 20–30 cm, which is considered to be very low, and the texture of the sediment in the lagoon varies between mud, sandy gravel and sandy mud (Youssef and El-Sorogy., 2016).

However, the dominant type of sediment in the southern half of the lagoon and southeast fringes is mud. The floor of the lagoon is rich in seagrass and an abundance of coral reefs are found in several locations of the lagoon (Youssef and El-Sorogy., 2016).

2.2. Experimental design and sample collection

Eight stations were selected, based on the nature of the catchment, mangrove composition and accessibility. The coordinates of the stations were noted and recorded, using a T10 handheld GPS receiver, and the stations were name-tagged from S1 to S8
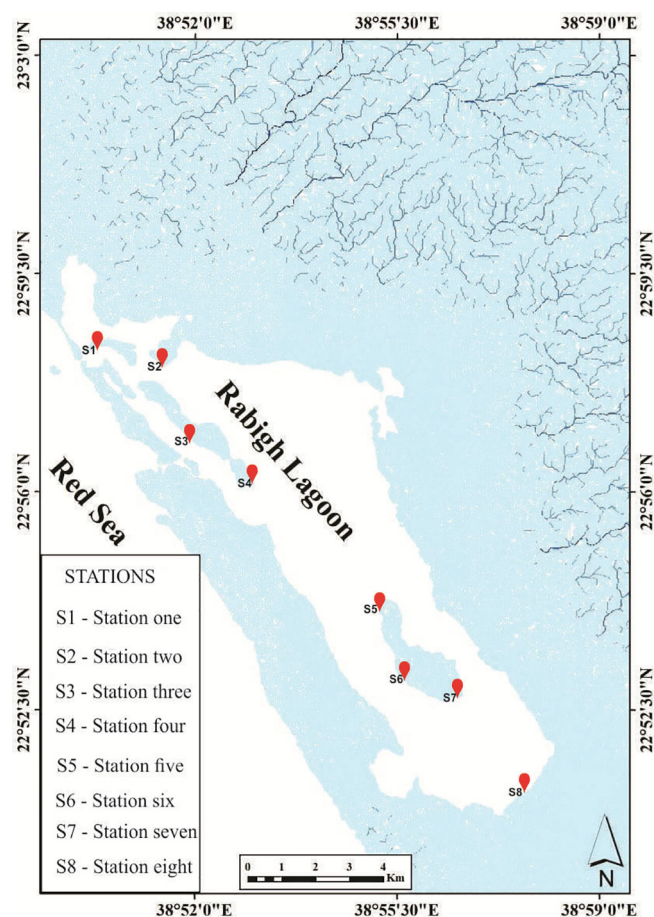

Fig. 1. Rabigh Lagoon, with sampling stations.

(Fig. 1) for proper assessment of heavy metals contamination and its effect on the physiological response of *A. marina* through biochemical analysis. Matured healthy leaves of *A. marina* were sampled from 15 different plants in each station. For each station where plant samples were collected, 15 samples of benthic sediment (0–20 cm) were also taken. The samples were packed in zip-lock plastic bags, stored in an icebox, and transported to the Ecophysiology laboratory, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia, for further sample preparation and analysis.

2.3. Preparation of samples

Preparation of samples for antioxidant analysis was done by washing the freshly sampled leaves, using tap water followed by deionized water. After the leaves were washed, they were placed in an ice-cold mortar and pestle and crushed in that cold condition to maintain the activity of the enzymes, using 0.01(M) chilled phosphate buffer at pH 7. The crushed leaves were then centrifuged for 25 min at 4 °C and 14000 rpm (Kumar et al., 2016; Bakshi et al., 2018).

To estimate antioxidant enzyme activities (Catalase, Glutathione -S- transferase and Superoxide dismutase), the supernatant was used for measurement in an LT-291 Single Beam UV–VIS spectrophotometer.

2.4. Determination of heavy metals in plants and sediments

Leaves and sediments were oven-dried at 40–45 °C for 48 h. and were then ground into a powder with the aid of mortar and pestle,

sieved through a 53 μ sieve mesh, and stored in ziplock bags for further analysis. (Bakshi et al., 2017; Islam et al., 2017). The samples of A. marina leaves and sediment were dried, ground and digested using acid. Next, 0.4 g of dried sediment samples were weighed and digested in an Anton-Paar PE Multiwave 3000 (microwave oven); 8 ml of 1:1 HNO_3:HCl was measured into digestion vessel set at 200 °C and kept for about 1 h (United States, 1997; Bakshi et al., 2018; Aljahdali and Alhassan, 2020b).

For the digestion of plant samples, 0.2 g of initially dried samples were subjected to chemical digestion by adding 6 ml and 2 ml of HNO_3 and H_2O_2 respectively and maintained at 180 °C for 45 min. The resulting digested samples were increased to 50 ml in a measuring cylinder with a very strict specification purified water (Ultra water: 18.2 ms cm^{-1}). The solutions were mixed vigorously and the solutions were left to stand overnight. The following day, Whatman filter paper was used to filter the solution, which was then transferred into glass vials and stored between 4 and 5 °C before subjection to heavy metal analysis, using an atomic absorption spectrophotometer (AAS: UNICAM 696 AA Spectrometer). Eight (8) heavy metals; Fe, Mn, Cu, Zn, Cd, Cr, Pb, and Ni, were determined in this study, in both the sediment and the plant samples.

2.5. Determination of sediment pollution

Four different types of classification systems were used to determine whether the sediment was polluted. These classifications were described by Müller (1969), Abrahim and Parker (2008), Hakanson (1980) and Tomlinson et al. (1980) for Geoaccumulation Index (Igeo), Enrichment Factor (EF), Contamination Factor (CF) and Pollution Load Index (PLI) respectively, to compute the sediment quality of stations where samples were collected. The range that forms the basis for classifying whether or not sediment is polluted, following the systems employed by the authors cited above, is presented in a supplementary data sheet (Table SD1).

2.6. Ecotoxicological risk

The degree of contamination was evaluated using a potential ecological risk index (RI). This was done for specific locations and metals and was correlated with antioxidant enzyme activities in A. marina leaves, to determine oxidative stress caused by metal contamination. The following equation was used for RI computation (Hakanson, 1980):

$$RI = (ErF1 + ErF2 + ErF3 \cdots + ErFn) \text{ and } ErF = Tr^i \times CF^i$$

where ErF = Ecological risk factor, CF^i = contamination factor of metal (i) and Tr^i = toxic response factor of a given metal (i) (Pb = 5, Zn = 1, Cd = 30, Cu = 5, Cr = 2, Mn = 1 and Ni = 5).

A comparison of the results in this study was made with the Sediment Quality Guideline (SQG) as proposed by Long and MacDonald (1998); MacDonald et al. (2000). The SQG, which comprises the Effect Range Low (ERL)/Effect Range Median (ERM) and the Threshold Effect Level (TEL)/Probable Effect Level (PEL), were used to group and track the extent of pollution and risk, based on heavy metal contamination. The concentrations of heavy metals that fall within the range of ERL or TEL signified a decreased adverse effect with low ecological risk, while high concentrations that fall above PEL and ERM signified that the ecological risk was high (Long et al., 1998).

The mean Probable Effect Level quotient (mPELq) (Long et al., 1998) was used in this study to measure the biological impact on the ecosystem by metal pollution or metal as a toxic substance. The following equation was used for the computation of mPELq:

$$mPELq = \frac{\sum_{m=1}^{n} \left(\frac{C_m}{PEL_m} \right)}{n}$$

where C_m = specific metal concentration in benthic sediment, PEL_m = PEL value for metal (m) and n = the total number of metals. The standard classification by Long et al. (2000) (Table SD1) was used to classify the calculated mPELq to determine the tendency of sediment to be toxic due to the concentration of metals that are present.

2.7. Bioaccumulation of metal in mangrove

The bio-concentration factor (BCF), also known as metal phytoextraction efficiency, is the ability of a plant to take up metal from the soil through a process known as bioaccumulation (Zhang et al. 2002; Almahasheer, 2019). To determine heavy metal bioaccumulation in A. marina leaf tissues in this study, BCF was computed, using the following equation as described by Almahasheer (2019); Usman et al. (2013); Macfarlane et al. (2007):

$$BCF_{plant} = \frac{C_{plant}}{C_{sediment}}$$

where C_{plant} is the metal concentration in the plant and $C_{sediment}$ is the metal concentration in sediment. It was also considered that BCF < 1 implies that the mangrove is an excluder; BCF = 1 implies that the mangrove plant is an indicator; and BCF > 1 implies that the mangrove plant falls between being an accumulator and a hyper-accumulator (Baker, 1981; Almahasheer, 2019).

2.8. Analysis of antioxidant enzymes in mangrove A. marina

2.8.1. Assay of catalase (CAT)
Catalase (CAT) activities were measured, as described by Chance and Maehly (1955). CAT activities in A. marina leaves were determined spectrophotometrically following the decrease in absorbance at 230 nm for 2 min. The reaction mixture, containing 0.01 M phosphate buffer, 30 mM hydrogen peroxide and the enzyme extract, were prepared by homogenizing the leaves in phosphate buffer and centrifuging at 5000 rpm. Specific activity was expressed as units/min/mg protein. 1 IU = change in absorbance/min/extinction coefficient (0.021).

2.8.2. Assay of Glutathione – S – transferase (GST)
The activities of Glutathione-S-transferase (GST) were assayed spectrophotometrically at 25 °C, as described by Habig et al. (1974). The medium used for the assay contained 1 mM of 1-chloro 2,4-dinitrobenzene (CDNB), 0.1 M potassium phosphate buffer pH 6.5, 1 mM − 40 mM GSH and 100 μl of leaf extracts in a total volume of 1 ml were used for the assay. GST activity was determined following the production of GS-DNB conjugate by measuring the increment of absorbance at 340 nm per min.

2.8.3. Assay of superoxide dismutase (SOD)
The Superoxide dismutase (SOD) activities were measured according to methods described by Beyer and Fridovich (1987), and as modified by Keyster et al. (2012). 100 mg of A. marina leaves were homogenised by the addition of 500 μl of the buffer, which was composed of 40 mM K_2HPO_4, pH 7.4, 1 mM EDTA, 5% (w/v) and polyvinylpyrrolidone with molecular weight = 40,000. The homogenised plant samples were then centrifuged at 12,000 rpm for 20 min and the resulting supernatants were utilised for determination of SOD activity, using a spectrophotometer. Before measurement of SOD activities spectrophotometrically, 10 μl of the supernatant was measured and added to a mixture of 0.1 mM xanthine, 6.25 nM xanthine oxidase, 50 mM K_2HPO_4, pH 7.8, 0.1 mM EDTA, 0.025% (w/v) Triton X-100, 0.1 mM 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium (WST-1).

The absorbance was read and recorded at 450 nm after the resulting assay mixture was incubated at 37 °C for 20 min. Bovine

serum albumin was used as the standard when determining protein concentrations in the extract (Klein et al., 2018). One unit of enzyme activity was also defined as the concentration of enzyme needed to prevent chromogen production by 50% in one minute under the assay conditions, and specific activity was expressed as units/mg protein.

2.9. Data analysis

Heavy metal concentration in *A. marina*, benthic sediment and antioxidant response in *A. marina* leaves were subjected to different analyses in this study.

Analysis of variance (ANOVA) at $P < 0.05$ was used to determine the mean station variation of heavy metal concentrations in *A. marina* leaves and sediment. Duncan Multiple Range Test (DMRT); a type of post hoc test was used to determine the differences in mean. Before the ANOVA test, the initial data generated were subjected to the Shapiro-Wilk test for normality assessment. Pearson correlation coefficients were used to determine the correlation between heavy metals in sediment and *A. marina* leaves, and also between heavy metal concentrations in *A. marina* leaves and antioxidant enzyme activities. Factor analysis and cluster analysis were used to determine the influence of heavy metals on antioxidant response and the relationship between the stations in terms of heavy metal contamination respectively.

SPSS version 22 and Minitab version 17 Statistics Software Packages were used for the analysis.

3. Results and discussion

3.1. Determination of heavy metals

3.1.1. Heavy metals in sediment

The wide range of variation in mean concentrations of elements in the sediments collected from the lagoon across the stations is represented in Table 1. In this study, Fe concentration was recorded highest (8939.38 ± 312.63 mg/kg) at station 4 (S4) but not significant with station 6 (S6), and was followed by Mn (479.00 ± 39.25 mg/kg) at S4. Other maximum mean concentrations of heavy metals were 218.50 ± 0.50, 134.23 ± 0.02, 20.06 ± 0.

06, 15.00 ± 0.50, 288.50 ± 0.50 and 102.13 ± 19.38 mg/kg for Cu, Zn, Cr, Pb, and Ni respectively. Stations 3 and 6 also records high concentrations, but less than station 4.

The high concentration of heavy metals in some of these stations can be attributed to the minimal circulation between the lagoon and the sea, as there was interference with the circulation in the lagoon, which was observed over the past twenty-five years. Anthropogenic influences, such as effluents and drainages from industrial activities from large factories in the urban environment close to the lagoon, domestic wastes, and other anthropogenic factors are important sources of heavy metals in aquatic ecosystems (Ghosh et al., 2016; Bakshi et al., 2017, 2018; Aljahdali and Alhassan, 2020).

Significant variation in heavy metal concentration across the stations is an indication of non-constant circulation between the lagoon and the sea, and the impact of deposition of materials and rate of erosion at the closed end of the lagoon, due to rainfall (Bakshi et al., 2018; Aljahdali and Alhassan, 2020). Factors such as geomorphology (siltation and intermingling between the lagoon and sea, leading to sedimentation and hydrodynamics in the tides), could be a key reason for an increase in elemental concentrations in stations S3, S4 and S6 (Agah et al., 2016; Bakshi et al., 2018).

3.1.2. Heavy metals in mangrove

Mangroves have the ability and capability to take up or bioaccumulate pollutants, such as heavy metals, and this has been ascertained to be one of the regulatory properties or potentials of this group of plants. The plants do this through the development of different adaptive features, such as morphological, anatomical and physiological features (Bakshi et al., 2017; ELTurk et al., 2018). Analysis of variance (ANOVA at $p < 0.05$) revealed significant variations in heavy metal concentration in *A. marina* plant leaves collected from different stations (Table 1).

The maximum and minimum concentration of Fe in *A. marina* leaves was recorded at station S4 (2897.83 ± 5.08 mg/kg) and S8 (899.25 ± 0.25 mg/kg) respectively. Apart from Fe, which recorded the highest concentration among the heavy metals at station S4, other metals, such as Mn (127.25 ± 0.25) – (2.53 ± 0.02) mg/kg, Cu (60.81 ± 0.05) – (27.84 ± 0.09) mg/kg and Ni (75.85 ± 0.10) – (15.55 ± 0.03) mg/kg with their maximum and minimum concen-

Table 1
Variation in mean concentration of heavy metals (mg/kg) in sediment and *A. marina* leaves.

	Fe	Mn	Cu	Zn	Cd	Cr	Pb	Ni
Sediments								
S1	1551.45 ± 36.05	38.38 ± 30.13	77.25 ± 9.00	34.50 ± 18.75	18.63 ± 0.63	7.75 ± 3.00	133.00 ± 6.25	50.50 ± 1.75
S2	1464.82 ± 2.07	12.25 ± 0.25	76.00 ± 0.50	42.50 ± 5.10	7.50 ± 0.50	8.50 ± 0.50	256.82 ± 0.06	44.88 ± 0.63
S3	5393.90 ± 0.65	120.90 ± 0.40	109.88 ± 0.63	51.04 ± 0.29	4.40 ± 0.15	15.00 ± 0.50	162.75 ± 0.75	27.64 ± 0.11
S4	8939.38 ± 312.63	479.00 ± 39.25	98.25 ± 20.00	103.25 ± 25.75	6.75 ± 1.50	6.63 ± 1.88	150.13 ± 9.38	102.13 ± 19.38
S5	3093.68 ± 4.57	16.23 ± 0.02	73.30 ± 0.20	15.73 ± 0.02	15.73 ± 0.02	14.23 ± 4.98	288.50 ± 0.50	83.50 ± 0.50
S6	8723.25 ± 0.51	375.74 ± 0.01	218.50 ± 0.50	134.23 ± 0.02	20.06 ± 0.06	10.11 ± 0.11	206.40 ± 0.10	72.74 ± 0.01
S7	2711.05 ± 0.55	15.10 ± 0.10	76.76 ± 0.01	14.76 ± 0.01	19.20 ± 0.20	8.26 ± 0.01	273.26 ± 0.10	37.40 ± 0.40
S8	2410.23 ± 0.02	4.60 ± 0.10	44.26 ± 0.01	12.60 ± 0.10	15.35 ± 0.35	12.26 ± 0.01	180.30 ± 0.30	42.60 ± 0.10
ERL			34	150	1.2	81	46.7	20.9
ERM			270	410	9.6	370	218	51.6
TEL			18.7	124	0.68	52.3	30.2	15.9
PEL			108.2	271	4.2	160	112	42.8
Mangrove								
S1	1464.55 ± 1.05	24.25 ± 0.25	35.35 ± 1.10	16.40 ± 0.15	2.77 ± 0.01	6.76 ± 0.01	93.35 ± 0.09	51.25 ± 0.25
S2	903.35 ± 0.10	10.55 ± 0.05	63.70 ± 0.20	33.76 ± 0.01	4.84 ± 0.09	7.60 ± 0.10	100.76 ± 0.01	24.38 ± 0.13
S3	1419 ± 246.50	43.75 ± 5.50	47.25 ± 3.50	47.75 ± 1.25	3.50 ± 0.75	5.38 ± 1.38	103.38 ± 24.88	21.25 ± 12.25
S4	2897.83 ± 0.08	127.25 ± 0.25	60.81 ± 0.05	19.54 ± 0.04	5.53 ± 0.03	3.27 ± 0.02	107.38 ± 0.13	75.85 ± 0.10
S5	922.835 ± 0.08	12.53 ± 0.02	53.53 ± 0.02	10.53 ± 0.03	5.25 ± 0.25	10.78 ± 0.03	124.39 ± 0.13	35.54 ± 0.04
S6	1599.7 ± 0.20	47.26 ± 0.25	45.54 ± 0.04	111.84 ± 0.09	18.25 ± 0.25	9.80 ± 0.05	96.25 ± 0.25	70.25 ± 0.25
S7	1401.13 ± 124.88	40.63 ± 14.13	51.75 ± 1.25	12.50 ± 1.50	10.50 ± 6.00	4.13 ± 0.63	129.75 ± 28.75	33.25 ± 20.75
S8	899.25 ± 0.25	2.53 ± 0.02	27.84 ± 0.09	11.58 ± 0.08	10.40 ± 0.15	5.40 ± 0.15	117.40 ± 0.15	15.55 ± 0.03
F Value	58.797	58.867	305.047	5.246	12.408	139.78	0.986	8.407
P Value	0.000	0.000	0.000	0.000	0.001	0.016	0.501	0.004

trations, were found to have maximum concentration at station S4 and minimum concentration at station S8. Analuddin et al. (2017), reported similar findings and stated that the type of mangrove and the level of heavy metal concentration would affect the heavy metal bioaccumulation potential of mangrove and its ecosystem.

Overall the bio-concentration factor (BCF) of the plant, that is, the ability of plant parts to take up or bioaccumulate metals from the soil was <1 for all the heavy metals except Zn and Cr. Similar findings were reported by Almahasheer (2019), on *A. marina* sampled from the Western Arabian Gulf mangrove. In that report, the BCFs of thirteen (13) heavy metals (Ag, Al, As, Cd, Cr, Cu, Fe, Mn, Mo, Ni, Pb, V, and Zn) were below one. This finding complemented and was in line with our results for BCFs in this study.

The ability of the plant to bioaccumulate metals and other pollutants from the soil and other interactions between plant and soil can be determined using BCFs, which are defined as the ratio of the total concentration of a metal in plants (leaves, root or stem) to the total concentration of a metal in soil or sediment. This is also known as plant metal phytoextraction efficiency (Zhang et al., 2002; Bakshi et al., 2017; Almahasheer, 2019). The low BCF for some heavy metals in this study (Fig. 2) i.e., less than one, can be linked to the ability of the mangrove to exclude or detoxify these metals by a mechanism of exclusion or detoxification (Appenroth, 2010; Almahasheer, 2019). Some of the heavy metals determined in this study, such as Fe, Cu, and Mn, occur naturally and are essential for growth physiology if they do not exceed a limit where they become toxic to the plant. Although some heavy metals like Pb, Cd, and Cr, are toxic to the plant (Shahid et al., 2013; Almahasheer, 2019).
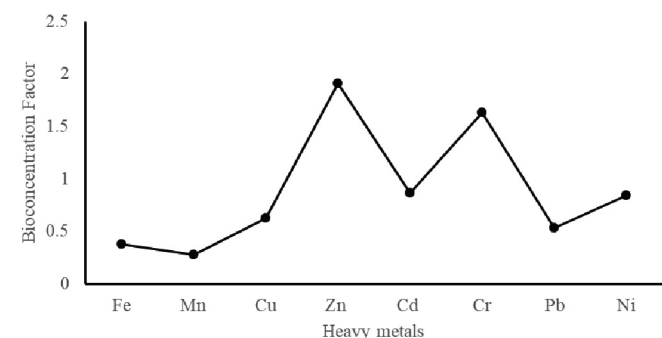

Fig. 2. Bioconcentration factor (BCF) in *A. marina* mangrove (BCF > 1 for Zn and Cr which indicate accumulator to hyper-accumulator of this two metals).

To determine relationships among heavy metals in sediments and mangrove plants, a Pearson correlation matrix was calculated for both heavy metals in sediments and the mangrove plants that were sampled (Table 2). According to the values of the Pearson correlation coefficients, most of the heavy metals determined in sediments for this study correlated significantly with each other.

Significant positive correlations at $p < 0.01$ between Fe and Mn, Cu, Zn; Mn and Cu, Zn, Ni, Fe; Cu and Zn, Fe, Mn; Zn and Fe, Mn, Cu indicate that these metals may have the same origin and have the same factors controlling their distribution and pattern of accumulation. The reason for the strong positive correlation of other metals with Fe could be the tremendous capacity of iron oxides to accumulate some of these metals (Islam et al., 2017; Bakshi et al., 2018). The non –positive correlation of some heavy metals with either Fe, Mn, Cu, Ni or Zn in this study may be due to their relationship to organic detritus, or to different transportation or distribution patterns (Islam et al., 2017).

The present study has revealed correlations between heavy metal concentrations in sediment and mangrove plants. A significant correlation exists between the heavy metals at one combination or another, except for Cu and Cd, where no correlation was found between the sediment and the mangrove plants. A possible reason for both scenarios could be a rise or fall in the capacity of the mangrove plant to take up or bioaccumulate these metals as a response to an increased concentration of these metals in sediment (Chowdhury et al., 2015; Bakshi et al., 2018). *A. marina* can be used as a bio-monitor of environmental pollution in marine ecosystems because of this significant positive correlation between heavy metals in sediments and mangrove plants. However, the variation and the increase in heavy metal concentrations in the sediment of our study area could be the result of anthropogenic activities and lack of proper circulation between the lagoon and the sea (Agah et al., 2016; Bakshi et al., 2018).

3.2. Sediment quality indices

Heavy metal contamination assessment is an important indicator in determining the extent to which an ecosystem is been contaminated through anthropogenic influences.

One of the most commonly used indices in the assessment of sediment contamination and anthropogenic interference is the enrichment factor (EF). The enrichment factor (EF) is determined by setting out the metal to be tested against an element or metal set as a reference due to its low occurrence variability. Such metals are Mn, Ti, Al, and Fe (Reimann and Caritat, 2000; Sutherland,

Table 2
Correlation matrix for heavy metals in sediments and *A. marina*.

	Fe_Sd	Mn_Sd	Cu_Sd	Zn_Sd	Cd_Sd	Cr_Sd	Pb_Sd	Ni_Sd	Fe_PL	Mn_PL	Cu_PL	Zn_PL	Cd_PL	Cr_PL	Pb_PL	Ni_PL
Fe_Sd	1															
Mn_Sd	0.954**	1														
Cu_Sd	0.732**	0.669**	1													
Zn_Sd	0.920**	0.910**	0.834**	1												
Cd_Sd	−0.186	−0.197	0.168	0.009	1											
Cr_Sd	−0.076	−0.301	−0.068	−0.153	−0.092	1										
Pb_Sd	−0.32	−0.41	−0.103	−0.429	0.237	0.141	1									
Ni_Sd	0.579	0.670**	0.287	0.567	−0.001	−0.255	−0.031	1								
Fe_PL	0.486	0.647**	0.19	0.55	−0.076	−0.499	−0.678**	0.444	1							
Mn_PL	0.790**	0.852**	0.315	0.678**	−0.312	−0.355	−0.436	0.535	0.803**	1						
Cu_PL	−0.391	−0.18	−0.247	−0.155	0.19	−0.385	−0.428	−0.014	0.525	−0.061	1					
Zn_PL	−0.042	−0.092	0.197	−0.08	−0.516	0.164	0.211	−0.299	−0.344	−0.224	−0.213	1				
Cd_PL	0.738**	0.576	0.597	0.627**	−0.299	0.238	−0.302	−0.017	0.107	0.444	−0.564	0.193	1			
Cr_PL	−0.695**	−0.615	−0.586	−0.634**	0.57	−0.128	0.497	−0.013	−0.353	−0.544	0.223	−0.429	−0.797**	1		
Pb_PL	−0.169	−0.249	−0.325	−0.309	0.137	0.112	0.425	−0.004	−0.258	−0.136	−0.282	0.012	0.031	0.351	1	
Ni_PL	0.656**	0.615	0.569	0.653**	−0.088	−0.14	−0.362	0.163	0.655**	0.734**	0.018	−0.007	0.504	−0.700**	−0.265	1

Sd – Sediment.
PL – *A. marina* leaves.
** Correlation is significant at the 0.01 level (2-tailed).

2000; Shi et al., 2019). Generally, the EFs values are interpreted in terms of scales. For example, EF values of 0.5–1.5 reflect regional rock compositions; and EF values of >1.5 indicate non-natural weathering processes (e.g. anthropogenic influences) (Sutherland, 2000; Zhang and Shan, 2008; Gu et al., 2012; Shi et al., 2019). In the present study, Fe was chosen as the reference metal for the determination of EFs for other metals. EFs for different heavy metals determined in this study are depicted in Fig. 3a.
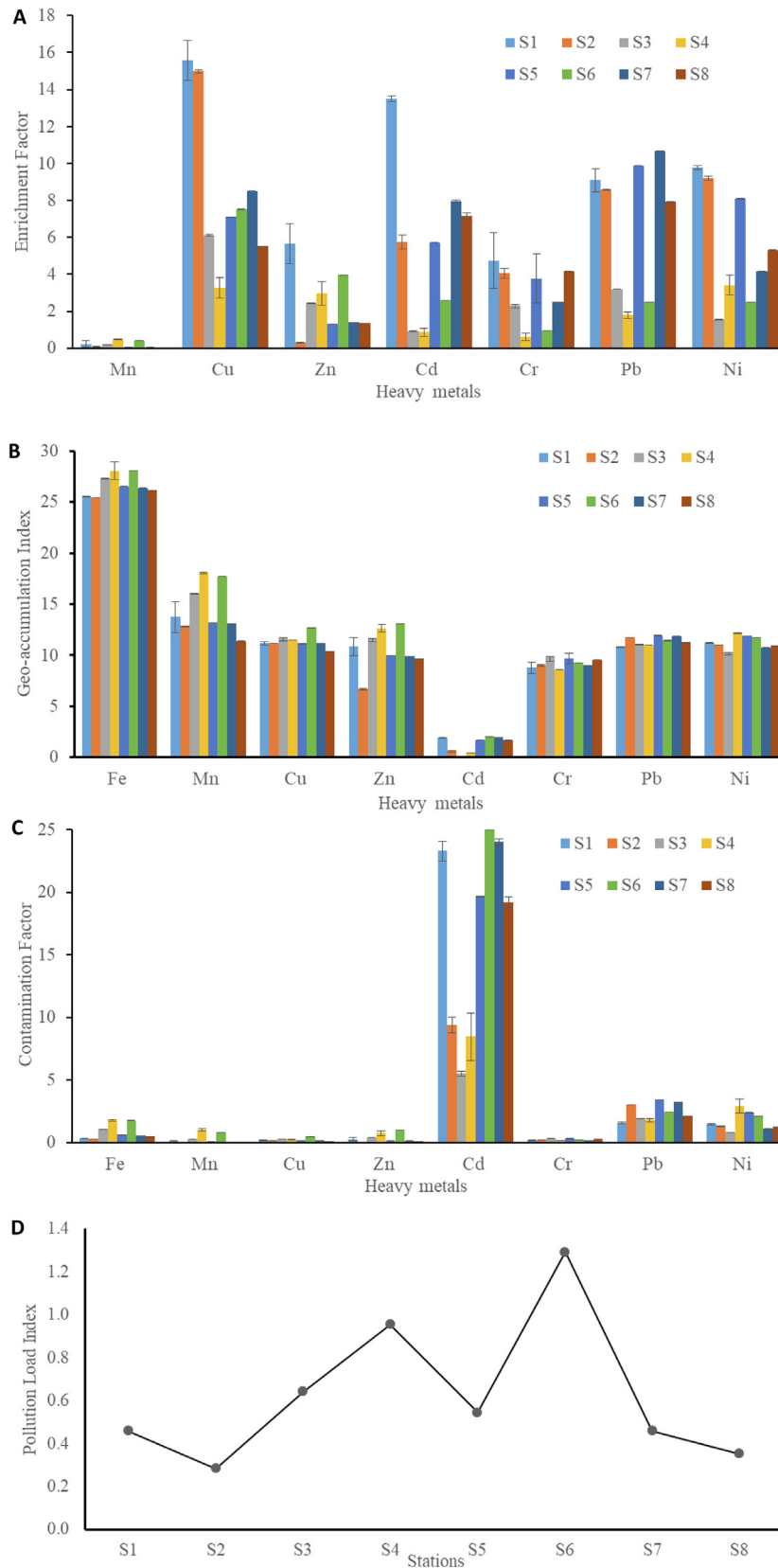
Fig. 3. (A–D) Pollution indices: (A) Enrichment factor, (B) Geo-accumulation Index (C) Contamination Factor and (D) Pollution Load Index.

Maximum EFs for various metals, such as Mn (0.48), Cu (15.56), Zn (5.65), Cd (13.50), Cr (4.75), Pb (10.67) and Ni (9.76) were recorded in S1, S4, and S7, with the majority falling into the S1 category. Most of the values that were ascertained indicate that concentration of these metals in our area of study is due to anthropogenic influences, except for Mn (≈0.5), which measured EF < 1 on the scale, and thus fell within the range of 0.5–1.5, indicating minimal enrichment. Therefore, Mn concentration in this study could be due to regional rock compositions or other natural production of this metal from the benthic region of the lagoon through natural weathering. Cu, Zn, Cd, Pb, and Nickel, however, showed severe enrichment, reflecting the contribution of anthropogenic input at S1, apart from the natural process of weathering (Kumar and Ramanathan, 2015; Bakshi et al., 2017); while Cr showed moderate enrichment.

The geo-accumulation indices (Igeo) for all the heavy metals were computed to determine the level of contamination of sediments, using the seven classes described by Müller (1969). The Igeo value for all the heavy metals across the stations was greater than 5 (extremely polluted), except for Cd, which had a maximum Igeo value of 2 at S6 (moderately polluted) (Fig. 3b). S3 (−0.19), S2 (0.58) and S4 (0.40) all had a Cd Igeo of less than 1, indicating that those stations were either unpolluted or moderately polluted with Cd. Generally, with positive values of Igeo dominating the computation of this index, these results complement the results of EF, since the heavy metals with high EF values also have positive Igeo values, except for Cd at S3. The extreme and moderate contamination status of the metals from these stations may be as a result of industrial and domestic effluent, chemical weathering and other contaminants of anthropogenic sources that end up in the lagoon through surface run-offs during rainfall. However, the uncontaminated status of S3 with Cd may be due to non-anthropogenic interference that has to do with Cd contamination in that station (Ghosh et al., 2016; Mirza et al., 2019).

The observed values of CF for Cu, Zn and Cr were less than one in all the eight stations, indicating a low contamination rate of these metals (Fig. 3c). For Fe, Mn, Cd, Pb and Ni, the values of CF recorded were greater than one, except for a few stations, where Fe and Mn record less than 1 as CF, which is an indication of moderate sediment contamination. Severe contamination (CF > 16) was also recorded for Cd at S1, S5, S6, S7, and S8. However, the trend for CF values of all heavy metals determined in this study was in ascending order, as follows: Cu < Cr < Mn < Zn < Fe < Ni < Pb < Cd.

The PLI values of sediment collected from a particular ecosystem can be used to characterize or make conclusions on the pollution status of that ecosystem. The scale and groupings used in this work were described by Tomlinson et al. (1980). The PLI ranged between 0.28 and 1.29 in S2 and S6 respectively. All PLI values recorded at the eight (8) stations studied did not fall above the baseline (Fig. 3d) except at S6, and this could be a result of the gradual and progressive deterioration of the quality of the aquatic ecosystem. The trend or pattern followed by PLI across the stations is in the following order: S6 > S4 > S3 > S5 > S7 > S1 > S8 > S2.

The different pollution indices (EF, CF, and Igeo) used in this study have shown that Rabigh lagoon sediment is moderate to severely contaminated with heavy metals, and is undergoing moderate contamination and increasing deterioration in sediment quality, especially at S1 and S6. The findings of this work are in line with the report of Bakshi et al. (2018), where the estuarine ecosystem mouth was revealed as having the highest pollution load.

3.3. Ecotoxicological risk

The ecological risk factor (ErF) of the metals studied in this work and their potential ecological risk index (RI) is presented in Fig. 4a. High ErF values were recorded at S1 (90), S6 (97) and S7 (93), and fell within the range that was regarded as a considerable ecological risk (80–159.9) (Hakanson, 1980). S5 (77) and S8 (74) revealed moderate ecological risk, while the value for S2 (38), S3 (22) and S4 (34) fell below the ecological risk category.

RI of sediment samples from the eight (8) stations ranged from a minimum value of 182 to 781 at S3 and S6 respectively. According to the scale of Hakanson (1980), the low potential ecological risk is not evident in all the stations, as the values were all greater than 150. However, the maximum potential ecological risk was observed at S6, S7, and S1, and here attention may be required from stakeholders or environmental managers. The trend for RI was in descending order of magnitude, S6 > S7 > S1 > S5 > S8 > S2 > S4 > S3 and has a similarity with PLI order of magnitude.

Comparisons were made for metal concentrations with TEL-PEL, and ERL- ERM (MacDonald et al., 1996; Long et al., 1998; Long and MacDonald, 1998) to evaluate the ecological risk posed by the metals. The result showed that concentrations of Cu were higher than the ERL and TEL limit at all the stations (Table 1). Zn concentration recorded lower values than ERL and TEL, but much lower than ERM and PEL limits, except at station S6, where there was a concentration higher than the TEL limit. For Cd, concentrations were all above the ERL and TEL limits at all eight stations, but below the ERM at S2, S3, and S4. In contrast, Cr recorded concentrations below TEL and ERL and even far below ERM and PEL limits. Pb and Ni concentrations across the eight stations were higher than TEL and ERL but lower than ERM and PEL values or limits, at S1, S2, S3 for Pb and S1, S2, S3, S8, S7, S8 (ERM) and S3, S7 (PEL) for Ni.

The stations that recorded low-range metal concentrations possessed minimal adverse effects and low ecological risk, while stations that recorded high values (more than PEL and ERM) have a high ecological risk (Long and MacDonald, 1998). The differences recorded in the level of ecological risk at the stations marked in this study may be as a result of anthropogenic sources and activities in the catchment area; natural weathering; chemical properties; and an abnormal degree of circulation between the lagoon and the sea.
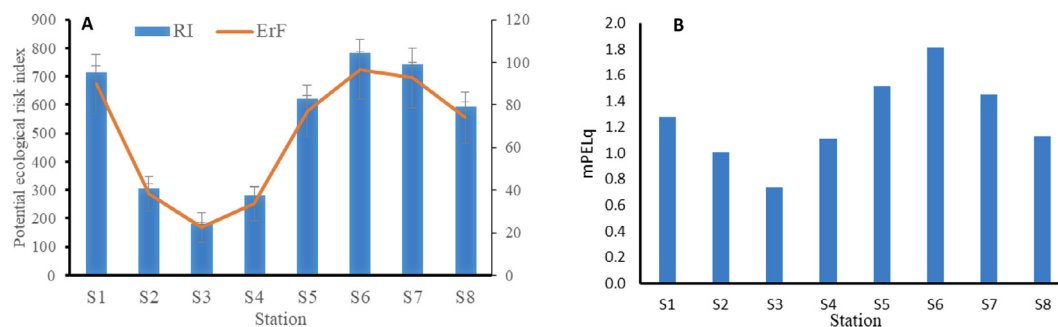

Fig. 4. (A–B) Ecotoxicological Risk in Sediment: (A) Potential ecological risk index in sediment and ecological risk factor in stations (B) mean PEL quotient.

Long et al. (1998) reported estimates of metal toxic effect by computing the mean PEL quotient. In this study (Fig. 4b) mPELq ranged from 1.01 (S2) to 1.82 (S6). According to the classification of average percentage survival of amphipods in marine sediment by Long et al. (2000), S1, S2, S3, S4, S7, and S8 have 21% probability of being toxic, while there is a 49% probability for heavy metal toxicity at S5 and S6 having the maximum score for ecotoxicological risk.

3.4. Analysis of antioxidant enzymes in mangrove

3.4.1. Antioxidant enzyme activities

Catalase (CAT), Glutathione -S- transferase (GST) and Superoxide dismutase (SOD) are some of the important antioxidants that are used as biomarkers of oxidative stress in plants. They can be used to monitor changes in plant anatomy and physiology in case of any environmental stress conditions, by scavenging on reactive oxygen species such as H_2O_2, OH, O_2, etc., produced by organisms such as plants under stressed conditions. The scavenging ability of antioxidants can lead to prevention or reduced membrane lipid peroxidation and improvement of cell membrane stability (Shahid et al., 2014; Asaeda and Barnuevo, 2019).

Antioxidant enzyme activities of CAT, GST, and SOD in this study are presented in Fig. 5. There was variation in mean values of the antioxidants across the stations, and ANOVA at $p < 0.05$ revealed a significant difference in the three (3) antioxidants across the stations. For the three (3) antioxidants, maximum and minimum activities in leaves of A. marina were recorded in S6 and S3 respectively, i.e., CAT (S6:9.20 – S3:5.25 U/mg protein), GST
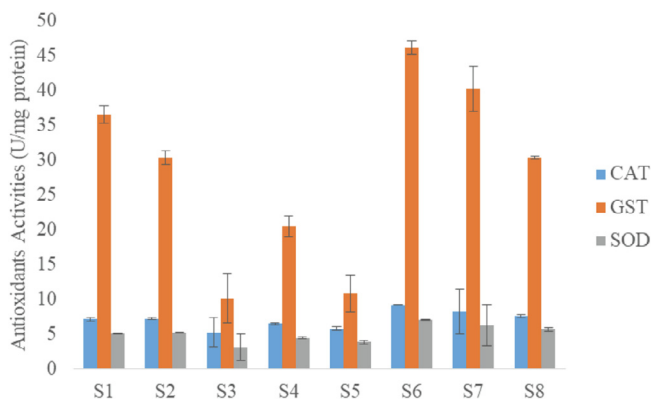

Fig. 5. Antioxidant enzyme activities of CAT, GST, and SOD in A. marina mangrove. CAT – Catalase. GST - Glutathione -S- transferase. SOD - Superoxide dismutase (SOD).

(S6:46.20 – S3:10.13 U/mg protein) and SOD (S6:7.00 – S3:3.06 U/mg protein).

A significant positive correlation ($p < 0.01$) was established between the three antioxidant enzymes with Pb (Table 3). Increased activities of CAT, GST, and SOD at station S6 may be linked to the increased level of reactive oxygen species generation in the mangrove at this station. This may have been due to stress caused by heavy metal contamination of that station. Pb might be one of the heavy metals causing more stress to this mangrove, as it has a significant positive correlation with the three antioxidant enzymes activities (Harish and Murugan, 2011; Asaeda and Barnuevo, 2019; Rahman et al., 2019).

Similar findings have been reported in different studies, indicating an increase in antioxidant enzyme activities with exposure to heavy metals and other pollutants (Zhang et al., 2007; Huang and Wang, 2010; Doğanlar and Atmaca, 2011; Bakshi et al., 2018; Asaeda and Barnuevo, 2019; Ferreira et al., 2019; Aljahdali and Alhassan, 2020a, 2020b).

The biochemical response of A. marina at 8 stations investigated in this study shows the intensity of the level of stress at each of the stations and also indicated specifically the stations that are under the greatest stress caused by metal contamination. The increase in activities of CAT, GST, and SOD in some stations may be as a response to increased ROS generation in the mangrove system under more toxic metal stress in these stations. This can be linked with high PLI and RI values of such stations, as indicated by the ecological risk assessment of sediments sampled from the mangrove ecosystem under investigation in this study (Fig. 5).

The findings established in this study have the possibility of bridging some of the data gaps related to biotic responses of antioxidants as biomarkers of oxidative stress in A. marina mangrove under in-situ metal stress, linked to increased production of ROS. With very few reports on natural populations of A. marina in the Red Sea Rabigh lagoon concerning oxidative stress, resulting from toxic metal exposure, our findings provide baseline data for the preliminary biological behaviour of A. marina when exposed to pollutants, such as heavy metals.

Another study by Bakshi et al. (2018), reported biological responses to metal contamination through antioxidant activities in the Avicennia species of mangroves in India. Mangroves have also been the subject of a study in Australia, which found that ex-situ mangroves (A. marina) in a laboratory revealed a rise in antioxidants, with variations for different metals and metal concentrations (Caregnato et al., 2008).

There was significant variation for all the heavy metals determined in the sediment and mangrove leaves sampled in this study. Wide dissimilarity was evident in the data generated by the present study for metal concentrations and antioxidant responses to

Table 3
Correlation matrix for antioxidant enzyme activity and heavy metal concentrations in A. marina.

	Fe_PL	Mn_PL	Cu_PL	Zn_PL	Cd_PL	Cr_PL	Pb_PL	Ni_PL	CAT	GST	SOD
Fe_PL	1										
Mn_PL	0.803[**]	1									
Cu_PL	0.525	−0.061	1								
Zn_PL	−0.344	−0.224	−0.213	1							
Cd_PL	0.107	0.444	−0.564	0.193	1						
Cr_PL	−0.353	−0.544	0.223	−0.429	−0.797[**]	1					
Pb_PL	−0.258	−0.136	−0.282	0.012	0.031	0.351	1				
Ni_PL	0.655[**]	0.734[**]	0.018	−0.007	0.504	−0.700[**]	−0.265	1			
CAT	−0.386	−0.232	−0.33	0.202	0.09	0.276	0.957[**]	−0.317	1		
GST	−0.378	−0.225	−0.314	0.341	0.096	0.158	0.918[**]	−0.229	0.965[**]	1	
SOD	−0.417	−0.236	−0.377	0.21	0.097	0.279	0.957[**]	−0.337	0.997[**]	0.961[**]	1

Sd – Sediment.
PL – A. marina leaves.
[**] Correlation is significant at the 0.01 level (2-tailed).

Table 4
F values and corresponding p values at 95% confidence interval after ANOVA test for metals and antioxidant enzyme activity in *A. marina*.

|	Fe	Mn	Cu	Zn	Cd	Cr	Pb	Ni
Sediments								
F Value	754.842	114.99	46.274	18.245	101.882	2.079	222.488	13.938
P Value	0.000	0.000	0.000	0.000	0.000	0.0163	0.000	0.001
Mangrove								
F Value	58.797	58.867	305.047	5.246	12.408	139.78	0.986	8.407
P Value	0.000	0.000	0.000	0.000	0.001	0.016	0.501	0.004
Antioxidant activity in mangrove								
	CAT	**GST**	**SOD**					
F Value	0.91	1.02	1.003					
P Value	0.543	0.483	0.492					
Source of variation			Between groups		Within groups			Total
df			7		8			15

A

B
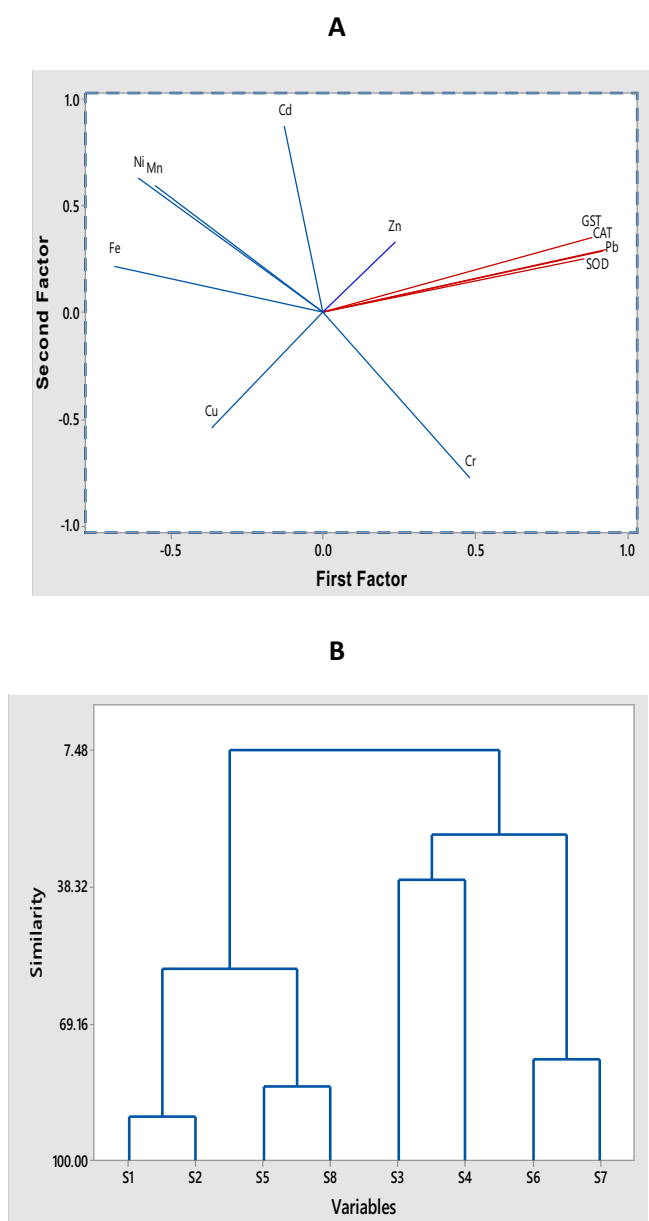
Fig. 6. (A-B) (A) Factor analysis biplot for heavy metals concentration and antioxidants activity (B) Cluster analysis dendrogram for relationship between the 8 stations based on heavy metal concentrations in A. marina. CAT = Catalase, GST = Glutathione –S- transferase, SOD = superoxide dismutase.

oxidative stress in *A. marina*, caused by metal exposure (Table 4). Analysis (Factor analysis) was used to evaluate the influence of metal concentration on the production of antioxidants in *A. marina* (Fig. 6a). Factor 1 was found to account for 44.84% of the total variation in the relationship between the metals and the antioxidants; Factor 2 accounted for 25.98% of total variation and Factor 3 accounted for 14.85% of the total variation. Fe, Mn, Ni, and Cu may display similar geochemical behavior as they were located in the same group and antioxidants formed a group with Pb, which suggested a strong influence of this grouping on the production of these enzymes.

Cluster analysis (Fig. 6b) was performed to establish or determine a clear relationship between the 8 stations based on heavy metal concentrations. An outline of similarities between stations was established, with the formation of different groups of clusters for clear identification of interrelationships (Chung et al., 2011; Rahman et al., 2019). The dendrogram generated from cluster analysis produced four clusters: Cluster 1 (S1 and S2), cluster 2 (S5 and S8), cluster 3 (S3 and S4) and Cluster 4 (S6 and S7). Each cluster signified the relationship between the station contained by it since stations in the same cluster share the same characteristics, in terms of both natural activities, anthropogenic interferences and sources (Varol and Şen, 2012; Rahman et al., 2019).

In summary, this study is to the best of our knowledge, the first to reveal findings on antioxidant activities as biomarkers of oxidative stress caused by metal pollution in *A. marina* sampled from the Rabigh lagoon, after its lack of circulation or proper mixture with the open sea for 25 years.

4. Conclusions

A. marina was evaluated in this study in relation to possible stress caused by heavy metals in eight stations investigated at the Rabigh lagoon, Red Sea. Heavy metal contamination of the benthic sediment using some ecological risk assessment indices (EF, Igeo, and CF) showed that the sediments were moderate to extremely contaminated by heavy metals. These findings were complemented with a high ecological risk factor (ErF) recorded at stations S6 falling in the range of considerable ecological risk and revealing a potentially serious threat of heavy metal contamination at this station. There was a significant correlation between heavy metal concentrations in *A. marina* leaves and mangrove ecosystem sediments, except for Cu and Cd. This is an indication of fluctuation in the bioconcentration factor of both plants and sediment.

In total, the results of our findings established deterioration of the sediment in a gradual pattern which has a potential for negative impacts on the biogeochemical cycle, with potentially lethal consequences for biodiversity survival. Consequently, there is a

need for periodic and regular monitoring of the pollution status in this ecosystem, through the use of biochemical markers in the mangrove *A. marina*.

Acknowledgments

References

Abrahim, G.M.S., Parker, R.J., 2008. Assessment of trace element enrichment factors and the degree of contamination in marine sediments from Tamaki Estuary, Auckland, New Zealand. Environ. Monit. Assess. 136, 227–238.

Abdel-Hamid, A., Hamed, M., Abd El-Azim, H., 2011. Heavy metals distribution in the coral reef ecosystems of the Northern Red Sea. Helgol. Mar. Res. 65, 67–80. https://doi.org/10.1007/s10152-010- 0202-7.

Agah, H., Bastami, K.D., Fumani, N.S., 2016. Ecological risk, source and preliminary assessment of metals in the surface sediments of Chabahar Bay, Oman Sea. Mar. Pollut. Bull. 107 (1), 383–388.

Al-Dubai, T.A., Abu-Zied, R.H., Basaham, A.S., 2017. Present environmental status of Al-Kharrar Lagoon, central of the eastern Red Sea coast, Saudi Arabia. Arab J. Geosci. 10, 305.

Aljahdali, M.O., Alhassan, A.B., 2020a. Metallic pollution and the use of antioxidant enzymes as biomarkers in *Bellamya unicolor* (Olivier, 1804)(Gastropoda: Bellamyinae). Water 12, 202.

Aljahdali, M.O., Alhassan, A.B., 2020b. Spatial variation of metallic contamination and its ecological risk in sediment and freshwater mollusk: *Melanoides tuberculata* (Müller, 1774)(Gastropoda: Thiaridae). Water 12, 206.

Almahasheer, H., 2019. High levels of heavy metals in Western Arabian Gulf mangrove soils. Mol. Biol. Rep. 46 (2), 1585–1592.

Analuddin, K., Sharma, S., Septiana, A., Sahidin, I., Rianse, U., Nadaoka, K., 2017. Heavy metal bioaccumulation in mangrove ecosystem at the coral triangle ecoregion, Southeast Sulawesi, Indonesia. Mar. Pollut. Bull. 125 (1–2), 472–480.

Appenroth, K.J., 2010. What are "heavy metals" in plant sciences?. Acta. Physiol. Plant 32 (4), 615–619.

Asaeda, T., Barnuevo, A., 2019. Oxidative stress as an indicator of niche-width preference of mangrove Rhizophora stylosa. Forest Ecol. Manage. 432, 73–82.

Badr, N., El-Fiky, A., Mostafa, A., Al-Mur, B., 2009. Metal pollution records in core sediments of some Red Sea coastal areas, Kingdom of Saudi Arabia. Env Mon Ass 155, 509–526.

Baker, A.J., 1981. Accumulators and excluders-strategies in the response of plants to heavy metals. J. Plant Nutr. 3 (1–4), 643–654.

Bakshi, M., Ghosh, S., Chakraborty, D., Hazra, S., Chaudhuri, P., 2018. Assessment of potentially toxic metal (PTM) pollution in mangrove habitats using biochemical markers: A case study on *Avicennia officinalis* L. in and around Sundarban, India. Mar. Pollut. Bull. 133, 157–172.

Bakshi, M., Ram, S.S., Ghosh, S., Chakraborty, A., Sudarshan, M., Chaudhuri, P., 2017. Micro-spatial variation of elemental distribution in estuarine sediment and their accumulation in mangroves of Indian Sundarban. Environ. Monit. Assess. 189 (5), 221.

Beyer, W.F., Fridovich, I., 1987. Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. Anal. Biochem. 161, 559–566.

Caregnato, F.F., Koller, C.E., Macfarlane, G.R., Moreira, J.C., 2008. The glutathione antioxidant system as a biomarker suite for the assessment of heavy metal exposure and effect in the grey mangrove, *Avicennia marina* (Forsk.) Vierh. Mar. Pollut. Bull. 56 (6), 1119–1127.

Chai, M., Shi, F., Li, R., Shen, X., 2014. Heavy metal contamination and ecological risk in *Spartina alterniflora* marsh in intertidal sediments of Bohai Bay, China. Mar. Pollut. Bull. 84 (1–2), 115–124.

. Methods in Enzymology: Assay of Catalase and Peroxidases vol. 2, 764–775.

Chowdhury, R., Favas, P.J., Pratas, J., Jonathan, M.P., Ganesh, P.S., Sarkar, S.K., 2015. Accumulation of trace metals by mangrove plants in Indian Sundarban wetland: prospects for phytoremediation. Int. J. Phytoremed. 17 (9), 885–894.

Chung, C.Y., Chen, J.J., Lee, C.G., Chiu, C.Y., Lai, W.L., Liao, S.W., 2011. Integrated estuary management for diffused sediment pollution in Dapeng Bay and neighboring rivers, Taiwan. Environ. Monit. Assess. 173, 499–517.

Doğanlar, Z.B., Atmaca, M., 2011. Influence of airborne pollution on Cd, Zn, Pb, Cu, and Al accumulation and physiological parameters of plant leaves in Antakya (Turkey). Water Air Soil Pollut. 214 (1–4), 509–523.

ELTurk, M., Abdullah, R., Rozainah, M., Bakar, N.K.A., 2018. Evaluation of heavy metals and environmental risk assessment in the Mangrove Forest of Kuala Selangor estuary, Malaysia. Mar. Pollut. Bull. 136, 1–9.

Ferreira, C.P., Lima, D., Paiva, R., Vilke, J.M., Mattos, J.J., Almeida, E.A., Jorge, M.B., 2019. Metal bioaccumulation, oxidative stress and antioxidant responses in oysters Crassostrea gasar transplanted to an estuary in southern Brazil. Sci, Total Environ. 685, 332–344.

Fu, J., Zhao, C., Luo, Y., Liu, C., Kyzas, G.Z., Luo, Y., Zhu, H., 2014. Heavy metals in surface sediments of the Jialu River, China: their relations to environmental factors. J. Hazard. Mater. 270, 102–109.

Ghosh, S., Ram, S.S., Bakshi, M., Chakraborty, A., Sudarshan, M., Chaudhuri, P., 2016. Vertical and horizontal variation of elemental contamination in sediments of Hooghly estuary, India. Mar. Pollut. Bull. 109 (1), 539–549.

Golestaninasab, M., Malek, M., Roohi, A., Karbassi, A.R., Amoozadeh, E., Rashidinejad, R., Ghayoumi, R., Sures, B., 2014. A survey on bioconcentration capacities of some marine parasitic and free-living organisms in the Gulf of Oman. Ecol. Indic. 37, 99–104.

Gu, Y.G., Wang, Z.H., Lu, S.H., Jiang, S.J., Mu, D.H., Shu, Y.H., 2012. Multivariate statistical and GIS-based approach to identify source of anthropogenic impacts on metallic elements in sediments from the mid Guangdong coasts, China. Environ. Pollut. 163, 248–255.

Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione S-tranferases: the first step in mercapturic acid formation. J. Biol. Chem. 249, 7130–7139.

Hakanson, L., 1980. An ecological risk index for aquatic pollution control. A sedimentological approach. Water Res. 14 (8), 975–1001.

Huang, G.Y., Wang, Y.S., 2010. Physiological and biochemical responses in the leaves of two mangrove plant seedlings (*Kandelia candel* and *Bruguiera gymnorrhiza*) exposed to multiple heavy metals. J. Hazard. Mater. 182 (1–3), 848–854.

Harish, S., Murugan, K., 2011. Oxidative stress indices in natural populations of Avicennia alba Blume. as biomarker of environmental pollution. Environ. Res. 111, 1070–1073.

Islam, G.M.R., Habib, M.R., Waid, J.L., Rahman, M.S., Kabir, J., Akter, S., Jolly, Y.N., 2017. Heavy metal contamination of freshwater prawn (*Macrobrachium rosenbergii*) and prawn feed in Bangladesh: a market-based study to highlight probable health risks. Chemosphere 170, 282–289.

Keyster, M., Klein, A., Ludidi, N., 2012. Caspase-like enzymatic activity and the ascorbate glutathione cycle participate in salt stress tolerance of maize conferred by exogenously applied nitric oxide. Plant Signal. Behav. 7, 349–360.

Klein, A., Hüsselmann, L., Keyster, M., Ludidi, N., 2018. Exogenous nitric oxide limits salt-induced oxidative damage in maize by altering superoxide dismutase activity. S. Afr. J. Bot. 115, 44–49.

Kumar, A., Ramanathan, A.L., 2015. Speciation of selected trace metals (Fe, Mn, Cu and Zn) with depth in the sediments of Sundarban mangroves: India and Bangladesh. J. Soils Sedim. 15, 2476–2486.

Kumar, A., Ramanathan, A.L., Prasad, M.B.K., Datta, D., Kumar, M., Sappal, S.M., 2016. Distribution, enrichment, and potential toxicity of trace metals in the surface sediments of Sundarban mangrove ecosystem, Bangladesh: a baseline study before Sundarban oil spill of December, 2014. Environ. Sci. Pollut. Res. 23 (9), 8985–8999.

Liu, J., Ma, K., Qu, L., 2015. Ecological risk assessments and context-dependence analysis of heavy metal contamination in the sediments of mangrove swamp in Leizhou Peninsula, China. Mar. pollut. Bull. 100 (1), 224–230.

Long, E.R., Field, L.J., MacDonald, D.D., 1998. Predicting toxicity in marine sediments with numerical sediment quality guidelines. Environ. Toxicol. Chem. 17, 714–727.

Long, E.R., MacDonald, D.D., 1998. Recommended uses of empirically derived, sediment quality guidelines for marine and estuarine ecosystems. Hum. Ecol. Risk. Assess. 4, 1019–1039.

Long, E.R., MacDonald, D.D., Severn, C.G., Hong, C.B., 2000. Classifying probabilities of acute toxicity in marine sediments with empirically derived sediment quality guideline. Environ. Toxicol. Chem. 19, 2598–2601.

MacDonald, D.D., Carr, R.S., Calder, F.D., Long, E.R., Ingersoll, C.R., 1996. Development and evaluation of sediment quality guidelines for Florida coastal waters. Ecotoxicol. 5, 253–278.

MacDonald, D.D., Ingersoll, C.G., Berger, T., 2000. Development and evaluation of consensus-based sediment quality guidelines for freshwater ecosystems. Arch. Environ. Con. Tox. 39, 20–31.

Macfarlane, G.R., Koller, C.E., Blomberg, S.P., 2007. Accumulation and partitioning of heavy metals in mangroves: a synthesis of field-based studies. Chemosphere 69 (9), 1454–1464.

Marchand, C., Fernandez, J.M., Moreton, B., 2016. Trace metal geochemistry in mangrove sediments and their transfer to mangrove plants (New Caledonia). Sci. Total Environ. 562, 216–227.

Mirza, R., Moeinaddini, M., Pourebrahim, S., Zahed, M.A., 2019. Contamination, ecological risk and source identification of metals by multivariate analysis in surface sediments of the Khouran Straits, the Persian Gulf. Mar. pollut. Bull. 145, 526–535.

Müller, G., 1969. Index of geoaccumulation in the sediments of the Rhine River. Geo. J. 2, 108–118.

Rahman, M.S., Hossain, M.B., Babu, S.O.F., Rahman, M., Ahmed, A.S., Jolly, Y., Akter, S., 2019. Source of metal contamination in sediment, their ecological risk, and phytoremediation ability of the studied mangrove plants in ship breaking area, Bangladesh. Mar. pollut. Bull. 141, 137–146.

Reimann, C., Caritat, P.D., 2000. Intrinsic flaws of element enrichment factors (EFs) in environmental geochemistry. Environ. Sci. Technol. 34 (24), 5084–5091.

Saha, N., Rahman, M.S., Ahmed, M.B., Zhou, J.L., Ngo, H.H., Guo, W., 2017. Industrial metal pollution in water and probabilistic assessment of human health risk. J. Environ. Manage. 185, 70–78.

Shahid, M., Ferrand, E., Schreck, E., Dumat, C., 2013. Behavior and impact of zirconium in the soil–plant system: plant uptake and phytotoxicity. In:

Whitacre (Ed.), Reviews of Environmental Contamination and Toxicology, vol. 221. Springer, New York, pp. 107–127.

Shahid, M., Pourrut, B., Dumat, C., Nadeem, M., Aslam, M., Pinelli, E., 2014. Heavy metal induced reactive oxygen species: phytotoxicity and physicochemical changes in plants. Reviews of Environmental Contamination and Toxicology, vol. 232. Springer International Publishing, pp. 1–44.

Shi, C., Ding, H., Zan, Q., Li, R., 2019. Spatial variation and ecological risk assessment of heavy metals in mangrove sediments across China. Mar. pollut. Bull. 143, 115–124.

Sutherland, R.A., 2000. Bed sediment-associated trace metals in an urban stream, Oahu, Hawaii. Environ. Geol. 39 (6), 611–627.

Tomlinson, D.L., Wilson, J.G., Harris, C.R., Jeffrey, D.W., 1980. Problems in the assessment of heavy-metal levels in estuaries and the formation of a pollution index. Helgoländer Meeresun. 33 (1), 566.

United States Environmental Protection Agency (USEPA), 1997. Method 3051 A. Microwave assisted acid digestion of sediments, sludges, soils and oils. USEPA, U.S. Government Printing Office, Washington D.C. Available at: URL: http://www.epa.gov/SW-846/pdfs/3051a.pdf..

Usman, A.R., Alkredaa, R.S., Al-Wabel, M., 2013. Heavy metal contamination in sediments and mangroves from the coast of Red Sea: *Avicennia marina* as potential metal bioaccumulator. Ecotoxicol. Environ. Saf. 97, 263–270.

Varol, M., Şen, B., 2012. Assessment of nutrient and heavy metal contamination in surface water and sediments of the upper Tigris River, Turkey. Catena 92, 1–10.

Youssef, M., El-Sorogy, A., 2016. Environmental assessment of heavy metal contamination in bottom sediments of Al-Kharrar lagoon, Rabigh, Red Sea, Saudi Arabia. Arab. J. Geosci. 9 (6), 474.

Yuan, G.L., Liu, C., Chen, L., Yang, Z., 2011. Inputting history of heavy metals into the inland lake recorded in sediment profiles: Poyang Lake in China. J. Hazard. Mater. 185, 336–345.

Zhang, H., Shan, B., 2008. Historical records of heavy metal accumulation in sediments and the relationship with agricultural intensification in the Yangtze-Huaihe region, China. Sci. Total Environ. 399, 113–120.

Zhang, C., Shan, B., Tang, W., Dong, L., Zhang, W., Pei, Y., 2017. Heavy metal concentrations and speciation in riverine sediments and the risks posed in three urban belts in the Haihe Basin. Ecotoxicol. Environ. Saf. 139, 263–271.

Zhang, F.Q., Wang, Y.S., Lou, Z.P., Dong, J.D., 2007. Effect of heavy metal stress on antioxidative enzymes and lipid peroxidation in leaves and roots of two mangrove plant seedlings (*Kandelia candel* and *Bruguiera gymnorrhiza*). Chemosphere 67 (1), 44–50.

Zhang, W., Cai, Y., Tu, C., Ma, L.Q., 2002. Arsenic speciation and distribution in an arsenic hyperaccumulating plant. Sci. Total. Environ. 300 (1–3), 167–177.