



Assessment of heavy metals and Molecular Characterization of the Egyptian *Avicennia marina* along the Red Sea Coast

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ABSTRACT

Mangrove plants are the most segregated plants, available only in coastal regions around the globe. *A. marina* is an important mangrove species and has a wide geographical and climatic distribution, which suggests the presence of large amounts of genetic diversity. *A. marina* plant samples from four different locations in the Red Sea area were collected. These samples were screened against 8 ISSR markers reported for molecular characterization of mangrove plants. These markers were found to be polymorphic in nature, thus showed 100% polymorphism.

On the other hand, enzymatic and non-enzymatic antioxidants are important in plant defense against heavy metals. The present study reported the presence of 9 heavy metals in sediments and mangrove plant parts in the four different locations along Egyptian Red Sea Coast. The levels of four antioxidant enzymes in mangrove plants in these locations were detected. Results showed that the following higher and lower mean concentrations of heavy metals in sediments for the four studied areas by ppm: Cr (7.645±0.37-40.202±0.09) > Se (9.319± 0.14- 30.522± 0.25) > Zn (1.656±0.09-5.144±0.03) > Ni (0.01±0.00- 3.161± 0.14) > Au (0.189±0.07- 2.43±0.08) > Cu (0.879±0.03 -1.615±0.20) > Pb (0.29 ± 0.04-1.678±0.04) > Cd (0.161±0.04- 0.373±0.03) > Al (0.018±0.01- 0.228±0.07).

INTRODUCTION

The mangrove ecosystem refers to a tidally influenced wetland ecosystem that supports marine species and its adaptation to adverse environmental conditions, increase the demand to map, manage and monitor this ecosystem (Kamal and Phinn, 2011).

Mangrove trees stretch through the tropics and sub-tropics of the world are as a part of the coastal environment (Tomlinson, 1994; Hogarth, 1999). They represented 75% of tropical coastlines of the world between latitudes 25° N and 25° S (Spalding *et al.*, 1997). The mangrove stands present along the Gulf of Aqaba and the Egyptian-African Red Sea coast cover with a total area exceeding 700 hectares (Gab-Alla *et al.*, 2010). These stands constituted mainly by mangrove of *Avicennia marina*, except a few stands in the southern Sudanese border area where *Rhizophora mucronata* coexists along with *Avicennia marina* (Afele, 2004).

A. marina has always been the species of choice for afforestation drives, because of its fast-growing tendencies, which has the ability to tolerate a range of salinity and adaptability to changing climatic conditions (Gab-Alla *et al.*, 2010). There are about 26 locations of mangrove along Egyptian Red Sea Coasts as shown in (Fig. 1).

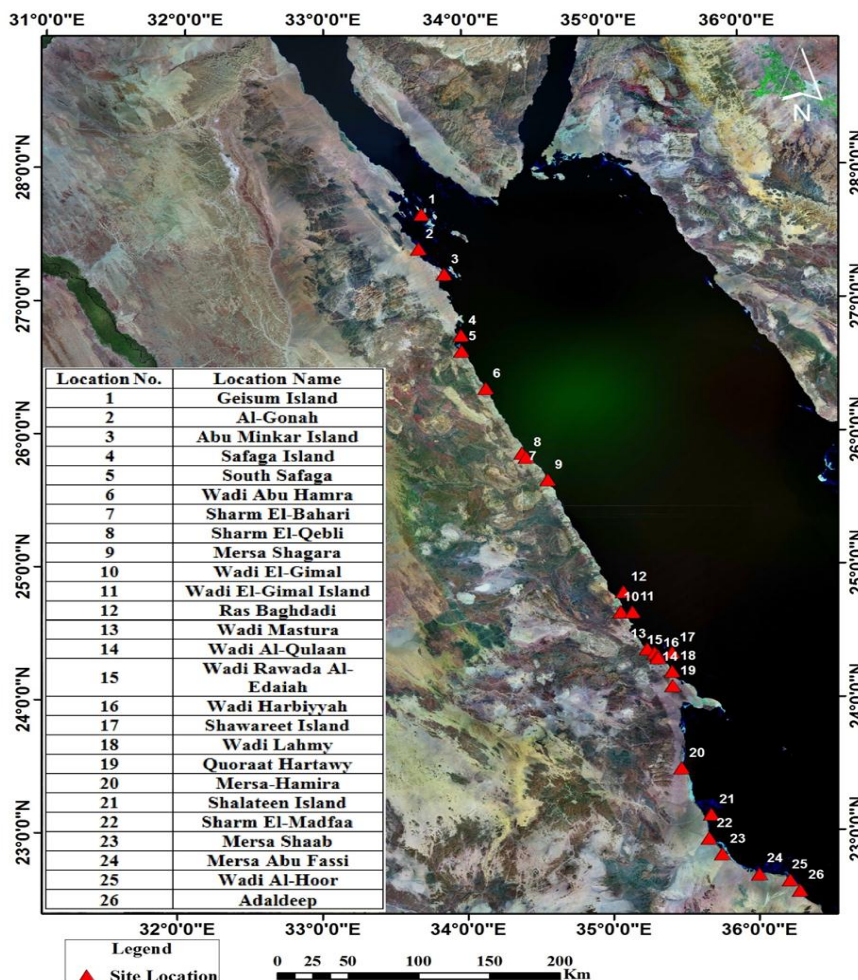


Fig. 1. (LandSat TM) Satellite image showing the locations of mangrove along Egyptian Red Sea Coasts.

Genetic diversity is a characteristic of ecosystems and gene pools that describes an attribute, which is commonly held to be advantageous for sustainability. Williams *et al.*, 1990 stated that the mechanism in which genes flow and the distribution of genetic variation within and among populations have practical importance in the case of conservation strategy, since both factors affect the genetic structure of the populations. Genetic diversity can be estimated by genetic (DNA) markers. The study of genetic diversity is a prerequisite to design the most suitable strategy for the conservation program. Thus, PCR based experiment was adopted ISSR marker was used for understanding the magnitude of polymorphic expression that exists in the studied *Avicennia marina* plant. ISSR-PCR reactions were performed using tested repeated primers.

Inter-simple sequence repeat (ISSR) analysis is a technique commonly used for identification plant species, based on variation found in the regions between microsatellites. ISSRs have often been applied for the comparative study of genetic

variability of mangrove populations across large geographical ranges (Dasgupta *et al.*, 2015).

Jian *et al.* (2004) reported a relatively high genetic diversity at species level among 10 mangrove and non-mangrove populations using inter-simple sequence repeats (ISSR). Li and Chen (2004) estimated the genetic diversity of *Sonneratia alba* which a kind of mangrove from China using eleven ISSR primers that gave 133 discernible DNA fragments of which 103 (77.44%) were polymorphic, indicating a considerable genetic variation at the species level. Moreover, Tan *et al.*, (2005) used inter-simple sequence repeat (ISSR) for studying the genetic diversity within and among populations of a widespread mangrove *Ceriops decandra* populations that collected from Malay Peninsula and North Australia. High genetic variation was reported at the species level. ISSR analysis is a technique common in genetic diversity of mangrove as appearing in most studies, Su *et al.* (2006, 2007) reported the genetic diversity of *Lumnitzera racemosa* and *Lumnitzera littorea* (Combretaceae), an endangered mangrove, both from the Indo West Pacific zone by ISSR technique and Chen *et al.*, (2010) reported the genetic diversity of 7 populations of *Kandelia obovata* from China by using inter-simple sequence repeats (ISSR) technique.

Egyptian mangrove along Red Sea coast was reported to have 2 species of mangrove plants, however, inter-population and intra-population diversity of mangroves have not been studied in details. Hence, the present study was conducted to document the intra-species diversity of *A. marina* using molecular tools.

MATERIALS AND METHODS

Study area and sample collection

The samples of *A. marina* were collected from different four locations that listed in Table 1, The samples are healthy leaves, seeds, pneumatophores (5 to 12 cm in length) and Whole plant (Seedlings) were collected from four different sites between 2017 and 2108.

All study areas have a long series of compact mangrove patches that were clumped with sandy areas. Mangroves were clumped with vigorous growth in 35 km north of El-Quseir (Wadi Abu Hamrah).

The study areas are : Wadi El- Gemal national Park Marsa Alam (latitude 24°39'56.11"N; longitude 35° 06'49.39"E), 35 km north of El-Quseir (Wadi Abu Hamrah) (latitude 26°24'5.75"N ; longitude 34°06'48.23"E), 25 Km South Safaga (latitude 26°37'00.45"N ; longitude 34°00'38.32"E) and 17 Km South Safaga (latitude 26°45'0.93"N; longitude 33°58'27.64"E) showed in Table 1.

Table 1 Coordinates of the four locations of study areas along the Red Sea coast.

Study areas	Location Code	Latitude (N)	Longitude (E)
Wadi El- Gemal national Park Marsa Alam	Area 1	24°39'56.11"	35° 06'49.39"
El-Quseir	Area 2	26°24'5.75"	34°06'48.23"
Wadi Abu Hamrah	Area 3	26°37'00.45"	34°00'38.32"
25 Km South Safaga	Area 4	26°45'0.93"	33°58'27.64"
17 Km South Safaga			

Assay heavy metals of A. marina plant sediment

Analysis of Lead (Pb), Cadmium (Cd), Chrome (Cr), Nickel (Ni), Zinc (Zn), Gold (Au), Copper (Cu), Selenium (Se)& Aluminum (Al) were conducted at Central

Laboratory, 10 samples of sediment of *A. marina* plant were collected from each location of the four locations; then samples were wrapped in plastic. Heavy metals contents in the sediments were determined in all the samples by aqua regia destruction according to the method of Ure (1990) and analyzed with atomic spectrophotometer. After the digestion of samples in acid mixture, the dissolving remaining metal matter and the resulting solution is aspirated into Atomic Absorption Spectrophotometer (AAS). To determine heavy metals, Perkin Elmer model (spectra-AA10, USA) flame atomic absorption spectrometer (AAS) with computer system was employed. Quantitative determination of heavy metals was carried out by "Buck scientific 210VGP Atomic Absorption Spectrophotometer" at the Faculty of Veterinary, Zagazig University. It was conducted by air/ Acetylene flow (5.5/1.11/m) flame (A.A.S). The concentration or the absorption values of heavy metals in blank samples were also calculated and were expressed as mg/g wet weight (ppm).

DNA isolation from A. marina leaves

Total cellular DNA of *A. marina* leaves was extracted using the method of (Murray and Thompson 1980) with some modifications. 150-200mg of plant leaf tissues were ground in liquid nitrogen using Mortar and pestles in 1.5ml Eppendorf tubes along with some liquid nitrogen to avoid thawing of the fine leaf powder 500 μ l of DNA extraction buffer were added. The samples were then vortexed vigorously till complete homogeneity. 150 μ l of chloroform were then added. Samples were shaken well for 10 min at room temperature. Samples were then spun down for 10 min at 14000rpm. 150 μ l from the aqueous phase were then transferred to a new 1.5ml Eppendorf tubes and a similar sample volume of cold Isopropanol (150 μ l) were added. The samples were then good mixed and the DNA fraction was pelleted for 20 min at 14000 rpm, 4°C. The DNA pellet was then washed with 750 μ l of 70% ethanol.

The washed DNA pellet was air dried and resuspended in 30-50 μ l sterile distilled water. For verification of quality and quantity, 2-5 μ l of the total genomic DNA eluate was visualized on a 1% (w/v) agarose gel containing ethidium bromide.

PCR amplification and Phylogenetic analysis ISSR

Isolated genomic DNA was used as template in the amplification reaction. A total of 25 μ l reaction mix was prepared from PCR green master mix 250 μ l solution Tian gene company. Amplification conditions were optimized using (PCR Sense Quest Gradient 96) which is programmed. Primer melting temperatures (Sawayama *et al.*, 2006) were calculated as $4x (G+C) + 2x (A+T)$. PCR amplification was performed for the amplification of template. PCR mixture was incubated in a thermal cycler using the following program: 3 min at 94°C; 40 sec at 94°C, 45 sec at 52°C and 90 sec at 72°C for 35 cycles; 45 sec at 94°C, 5 min at 72°C; soak at 4°C. The products obtained after PCR amplification were electrophoresed in 1% agarose gel in 0.5x TBE buffer at 120V for 1hr.

The obtained bands were compared (in base pairs bp) with the 1kbp molecular marker. Primers used for the molecular characterization were specific for mangrove species. Eight primers were used and are listed in Table 2. The PCR cycle was set for Bands with the same migration were considered homologous fragments, independent of their intensity.

Table 2 List of the ISSR primers used throughout the work

No.	Primer name	Sequence
1	(P1)	(GT)8 TG
2	(P2)	(CAC)3 GC
3	(P3)	(CTG)3 GC
4	(P4)	(CT)6 CC
5	(P5)	ACA CAC ACA CAC ACA CTT
6	(P6)	GGA GAG GAG AGG AGA
7	(P7)	(CTC)6
8	(P8)	(GAA)6

Data Interpretation

The distinct reproducible fragments were scored as present (1) or absent (0) for each ISSR-PCR reaction and were displayed as part of a binary matrix. The molecular data matrices obtained were analyzed and then the tree was created using the software program NTSYS-pc version 2.02.

RESULTS

The samples of *A. marina* were collected from different four locations that listed in Table 1, The samples are healthy leaves, seeds, pneumatophores (5 to 12 cm in length) and Whole plant (Seedlings) were collected from four different sites between 2017 and 2108.

All study areas have a long series of compact mangrove patches that were clumped with sandy areas. Mangroves were clumped with vigorous growth in 35 km north of El-Quseir (Wadi Abu Hamrah). The four locations of mangrove along Egyptian Red Sea Coasts where samples are collected are indicated by red triangles as shown in (Fig. 2).

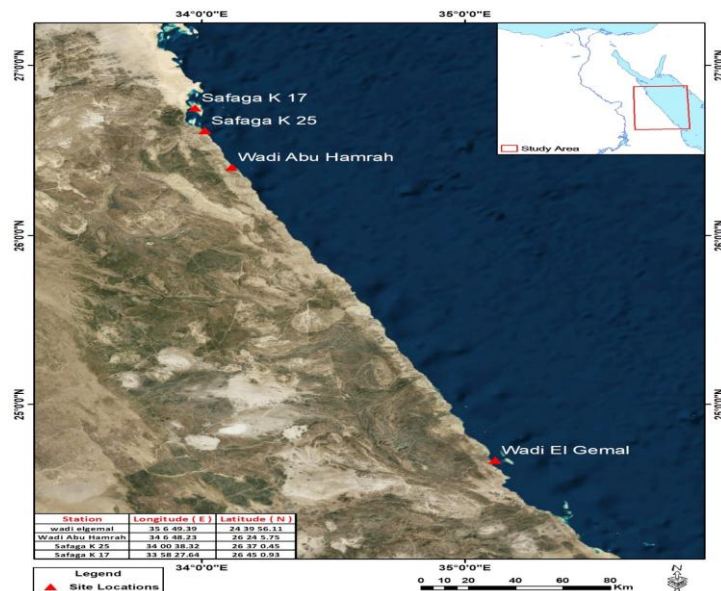


Fig. 2: Satellite image showing the four locations of mangrove along Egyptian Red Sea Coasts. The four red triangles indicating the locations where samples are collected.

Assay heavy metals of *A. marina* plant sediment

Generally, Mangroves play an integral role as a metal accumulator in tropical and subtropical marine ecosystems. Ten sets of sediment samples and portions of *A.*

marina s were collected from four locations to assess the accumulation and ecological risks of heavy metals. Results showed that the following mean concentrations of heavy metals in 10 sediment samples of area 1: Cr (40.202±0.09 ppm) > Se (30.522±0.25 ppm) > Ni (3.161±0.14 ppm) > Zn (3.05±0.07 ppm) > Cu (0.879±0.03 ppm) > Au (0.296±0.03 ppm) > Pb (0.29±0.04 ppm) > Cd (0.169±0.02 ppm) > Al (0.165±0.02 ppm) as showed in (Fig. 3).

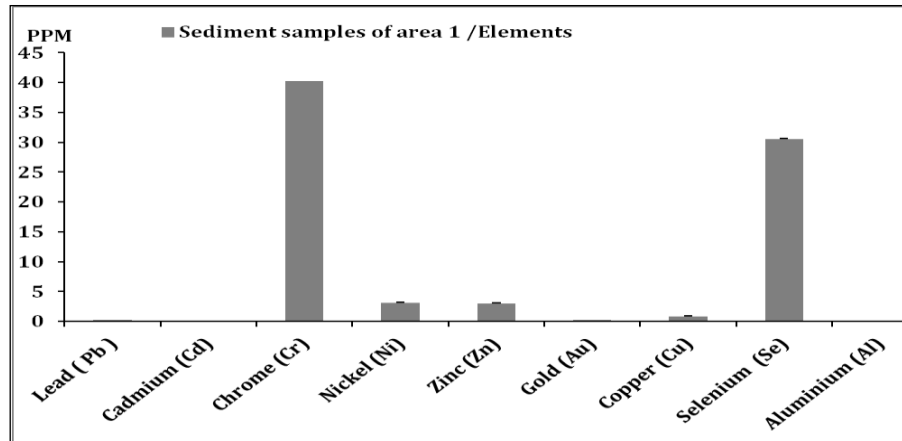


Fig. 3: Average concentrations of measured heavy metals (ppm) in *A. marina* sediment of area 1. Vertical bars show stander error (n=10).

Our results showed that the following mean concentrations of heavy metals in 10 sediment samples of area 2: Cr (10.145±0.04 ppm) > Se (9.319±0.14 ppm) > Zn (5.144±0.03 ppm) > Au (2.43±0.08 ppm) > Ni (2.099±0.07 ppm) > Pb (1.678±0.04 ppm) > Cu (1.505±0.07 ppm) > Cd (0.373±0.03 ppm) > Al (0.168±0.02 ppm) as showed in (Fig. 4).

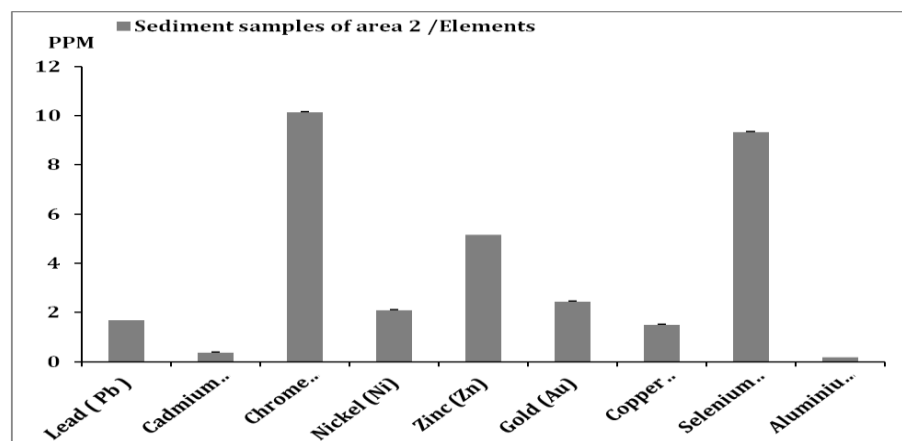


Fig. 4: Average concentrations of measured heavy metals (ppm) in *A. marina* sediment of area 2. Vertical bars show stander error (n=10).

Also, our results showed that the following mean concentrations of heavy metals in 10 sediment samples of area 3: Se (11.113±0.28 ppm) > Cr (7.645±0.37 ppm) > Zn (4.475±0.33 ppm) > Cu (1.615±0.20 ppm) > Pb (1.611±0.42 ppm) > Au (0.189±0.07 ppm) > Cd (0.161±0.04 ppm) > Al (0.018±0.01 ppm) > Ni (0.01±0.00 ppm) as showed in (Fig. 5).

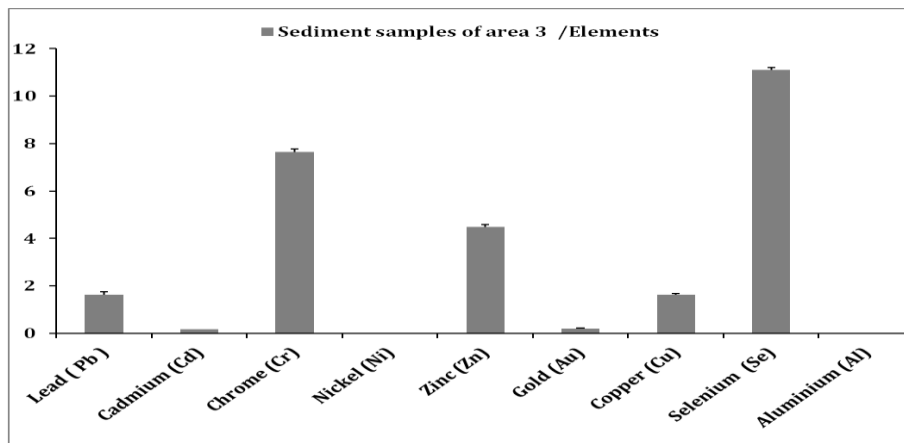


Fig. 5: Average concentrations of measured heavy metals (ppm) in *A. marina* sediment of area 3. Vertical bars show stander error (n=10).

Furthermore, our results showed that the following mean concentrations of heavy metals in 10 sediment samples of area 4: Cr (21.224 ± 0.09 ppm) > Se (14.251 ± 0.11 ppm) > Au (1.9099 ± 0.01 ppm) > Zn (1.656 ± 0.09 ppm) > Pb (1.358 ± 0.04 ppm) > Cu (0.917 ± 0.04 ppm) > Ni (0.623 ± 0.05 ppm) > Cd (0.319 ± 0.05 ppm) > Al (0.228 ± 0.07 ppm) as showed in (Fig.6).

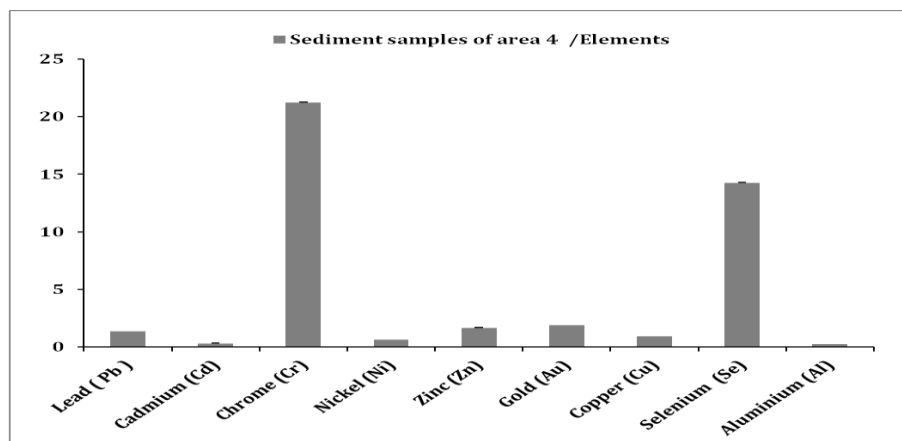


Fig. 6: Average concentrations of measured heavy metals (ppm) in *A. marina* sediment of area 4. Vertical bars show stander error (n=10).

PCR Reaction

In the present study, 18 primers are tested to differentiate between the *A.marina* populations from four different studied area, only 8 primers give PCR product as showed in (Fig. 7). The gradient PCR reaction was set for all the *A. marina* samples that from the four studied area against the 8 primers. The statistical analysis of the PCR results was done and the molecular data matrices obtained were analyzed then the tree was created using the software program NTSYS-pc version 2.02.

Genetic Diversity

In this study, genetic diversity was examined in *A. marina* based on ISSR fingerprinting. In total, the 8 tested primers produced a total of 34 bands for all populations, 20 bands are polymorphic, 14 monomorphic and 7 unique bands Table 3. The highest number of bands produced by 3 primers was 7 produced by primers P1, P2 and P6 and the lowest number of bands was one band produced by the primer P7.

Table 3: Number of polymorphic, monomorphic, unique, non-unique bands and produced by the used ISSR primer in the studied *A. marina* plant from four different locations.

Primer code	Primer sequence	Monomorphic bands	Polymorphism			Polymorphism %
			Polymorphic bands	Unique band	Non-unique	
p1	(GT)8 TG	3	4	3	1	28.5
p2	(CAC)3 GC	2	5	3	2	35.7
p3	(CTG)3 GC	0	2	0	2	14.2
p4	(CT)6 CC	3	1	0	1	7.1
p5	ACA CAC ACA CAC ACA CTT	2	0	0	0	0
p6	GGA GAG GAG AGG AGA	3	4	1	3	28.5
p7	(CTC)6	1	0	0	0	0
p8	(GAA)6	0	4	0	4	28.5

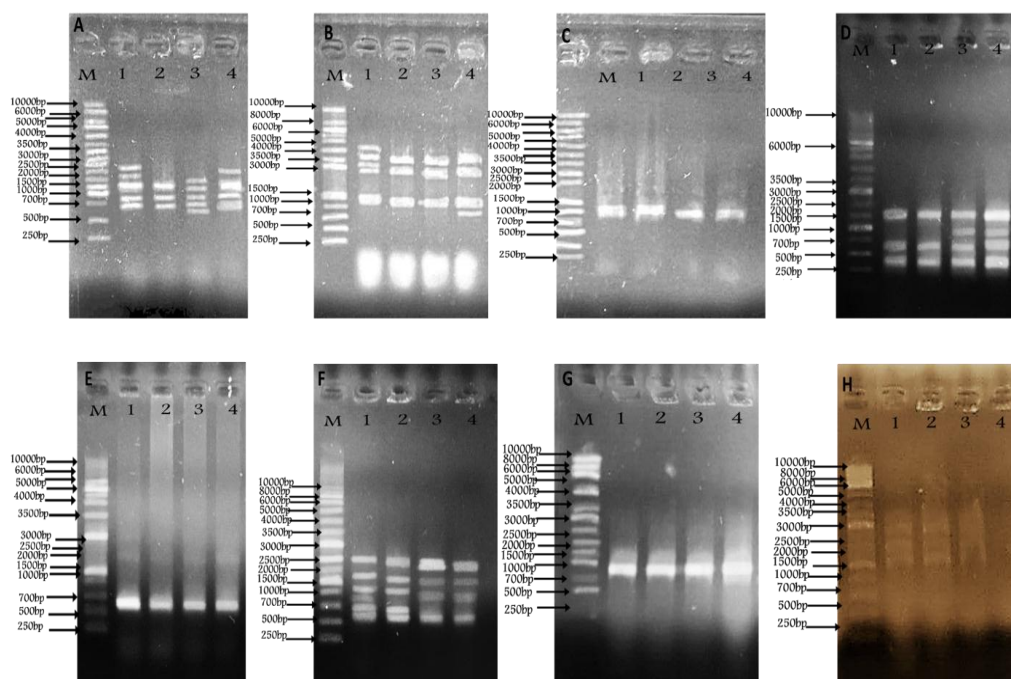


Fig. 7: Photograph illustrating the PCR amplification of ISSR markers using the 8 primers (A: primer 1, B: primer 2, C: primer 3, D: primer 4, E: primer 5, F: primer 6, G: primer 7, H: primer 8) in the examined the *A. marina* populations from four different studied area. M: 1kb ladder, lane 1: area 1, lane 2: area 2, lane 3: area 3, lane 4: area 4.

The lowest percentage of polymorphism was 0 by primers P5 and P7, while the highest percentage was 35.7% in primer P2. The highest polymorphism by (GAA)6 primer was 100% recorded in area 1 and area 2, but the lowest polymorphism was 0% by P1 primer, P4 primer and P8 primer recorded in area 2, area 1&2 and area 3&4 respectively Table 4.

Table 4: Polymorphism by primer for all studied *A. marina* plant from four different locations.

Sequence	% Polymorphism per primer			
	area 1	area 2	area 3	area 4
(GT)8 TG	28.57	0	28.57	14.28
(CAC)3 GC	42.85	14.28	14.28	28.57
(CTG)3 GC	50	50	50	50
(CT)6 CC	0	0	25	25
ACA CAC ACA CAC ACA CTT	0	0	0	0
GGA GAG GAG AGG AGA	28.57	28.57	28.57	14.28
(CTC)6	0	0	0	0
(GAA)6	100	100	0	0

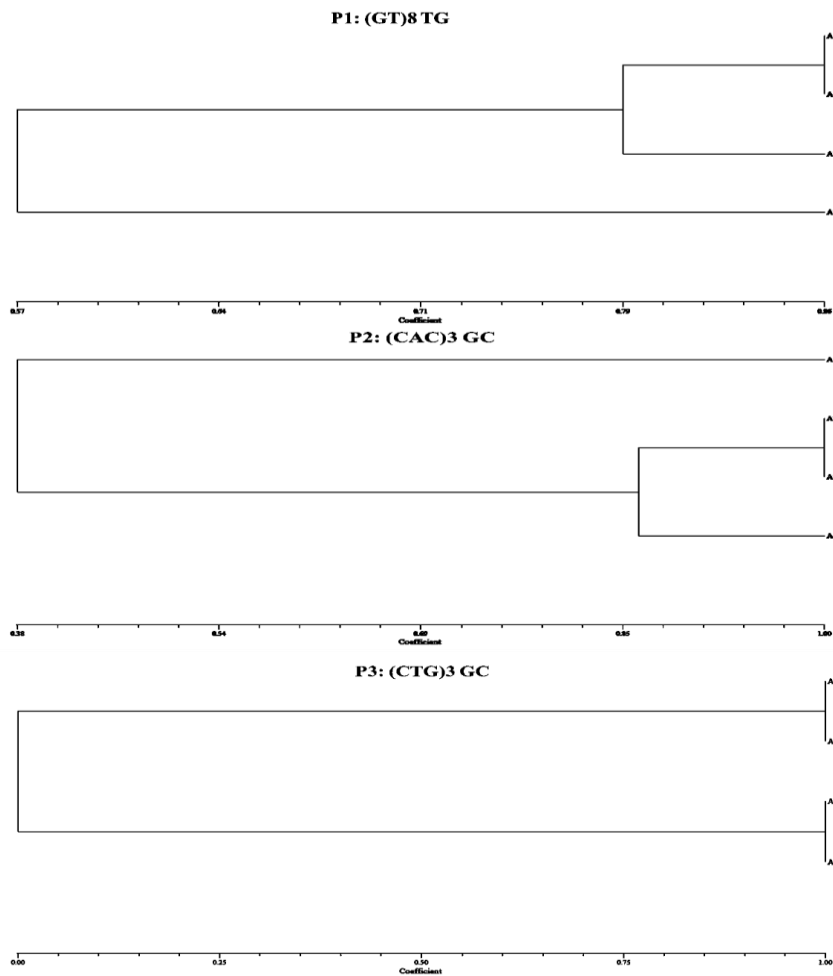


Fig. 8: Distance tree, constructed using the NTSYS-pc version 2.02 software showing the genetic distance between *A.marina* populations from four different locations by using the following primers p1, p2 and p3. A1: area 1, A2: area2 A3: area 3, A4: area 4.

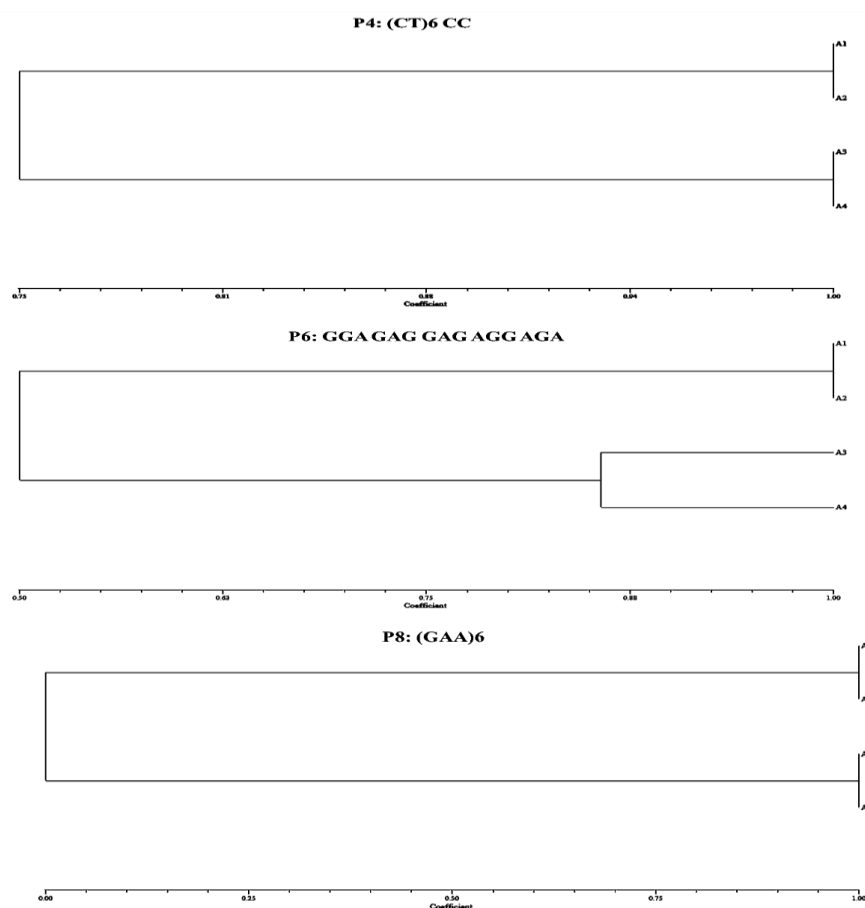


Fig. 9: Distance tree, constructed using the NTSYS-pc version 2.02 software showing the genetic distance between *A.marina* populations from four different locations by using the following primers p4, p6 and p8. A1: area 1, A2: area2 A3: area 3, A4: area 4.

DISCUSSION

Mangroves are physiologically interesting as potential models for stress tolerance and as sources of alternative ideas about physiological strategies relevant at the ecosystem level. Variation in habitat has great impact on the physiological behavior and biochemical expression level of a particular plant species.

Results showed that the following higher and lower mean concentrations of heavy metals in sediments for the four studied areas: Cr (7.645 ± 0.37 to 40.202 ± 0.09 ppm) > Se (9.319 ± 0.14 to 30.522 ± 0.25 ppm) > Zn (1.656 ± 0.09 to 5.144 ± 0.03 ppm) > Ni (0.01 ± 0.00 to 3.161 ± 0.14 ppm) > Au (0.189 ± 0.07 to 2.43 ± 0.08 ppm) > Cu (0.879 ± 0.03 to 1.615 ± 0.20 ppm) > Pb (0.29 ± 0.04 to 1.678 ± 0.04 ppm) > Cd (0.161 ± 0.04 to 0.373 ± 0.03 ppm) > Al (0.018 ± 0.01 to 0.228 ± 0.07 ppm).

The maximum concentrations of the studied metals were chrome and selenium, the medium concentration of the studied metals was zinc, nical and gold, the minimum concentration of the studied metals was copper, lead cadmium and aluminum. This result indicates the revealing a harmful risk to biota in the sediments.

The highest values of Cr (40.202 ± 0.09 ppm) and Se (30.522 ± 0.25 ppm) were observed at station area 1. The highest concentrations of Zn (5.144 ± 0.03 ppm) and Ni (3.161 ± 0.14 ppm) were detected at area 2 and area1 respectively, and the highest value of Au (2.43 ± 0.08 ppm), Cu (1.615 ± 0.20 ppm) and Pb (1.678 ± 0.04 ppm) were recorded at area 2, area 3 and area 2 respectively.

The concentrations of Cd were relatively high with the maximum value (0.373 ± 0.03 ppm) observed at area 2. Also, the concentrations of aluminum were relatively high with the maximum value (0.228 ± 0.07 ppm) observed at area 4. Interestingly, the highest concentrations of most of the heavy metals were observed at stations that were positioned near various anthropogenic influences, such as sewage effluents, refineries, aquaculture facilities and commercial ports. These findings are in agreement with those reported by Badr *et al.*, (2009), who emphasized that the upper 15 cm of sediments contained higher concentrations of pollutants (Cr, Ni, Mn and Zn) along the Red Sea coastal areas of Saudi Arabia. The average concentrations of Cu were lower than the values obtained from previous studies in the Red Sea area. We assume that the major reason for this difference is the presence of many fishing boats that use antifouling paints containing CuSO_4 as a major ingredient, as proposed by Usman *et al.*, (2013).

The mean concentration of Ni was lower than its value in previous studies in the Red Sea. Moreover, the mean concentration of Pb and Cd were comparable with recorded by Abohassan (2013) at both the Al-Shouiba and Yanbou sites. Except for Alzahrani *et al.*, (2018) in which twenty-one sets of sediment samples were collected along the Saudi Arabian coast of the Red Sea, the mean concentration of Cr was typically higher in the current study than values recorded in previous studies carried out along the Red Sea.

From a global perspective, many investigators have discussed the possibility of anthropogenic influences being responsible for the increase of heavy metals in mangrove sediments (Tam and Wong 2000; Cuong *et al.*, 2005; Defew *et al.*, 2005; Harikumar and Jisha 2010; Qiu *et al.*, 2011; El- Said and Youssef, 2013; Li *et al.*, 2016). Both the maximum (3.10 ppm) and the average (0.75 ppm) concentrations of Cd in the investigated area surpassed the averages in shale around the world (Turekian and Wedepohl, 1961). The average concentration observed for Cu was lower the world average concentration and is comparable to the findings of (Preda and Cox, 2002; Harikumar and Jisha, 2010).

The results of this study revealed that the maximum concentrations of Cu, Ni, Pb and Cd exceeded the minimum and average of the common ranges (0.879 ± 0.03 , 0.01 ± 0.00 , 0.29 ± 0.04 and 0.161 ± 0.04 ppm respectively). This indicates the possible litho- genic origin of all of these heavy metals (Cu, Ni, Pb and Cd).

It is known that heavy metals can be introduced into coastal environments from different sources, including natural weathering processes and anthropogenic activities (Sadiq and Zaidi, 1994; Badr *et al.*, 2009). Several previous studies have recorded high concentrations of heavy metals in mangrove sediments and have concluded that anthropogenic activities are a long-term pollution source (Tam and Wong, 2000; Defew *et al.*, 2005). This study revealed that the measured concentrations of Cd, Cr, Cu, Ni and Pb were above the optimum levels in some of the mangrove sediments from the Red Sea as well as other mangrove ecosystems worldwide. The average concentration of Cr was also higher than the average concentrations within the Red Sea coastal area but was lower than other studies, especially in Punta Mala Bay, Panama and Mai Po, Hong Kong (Tam and Wong, 2000; Defew *et al.*, 2005).

Inter-simple sequence repeat (ISSR) analysis is a technique usually used for identification of plant species, based on differences found in the regions between microsatellites. ISSRs have often been applied for the comparative study of genetic variability of mangrove populations across large geographical ranges. ISSR analysis is more reproducible than RAPD and their cost is less than AFLP.

In this study, the eight ISSR primers amplified a total 37 fragments varying from 2 to 7 per primer and ranged from 100 to 4000bp. The highest number of bands produced by 3 primers was 7 produced by primers P1, P2 and P6 and the lowest number of bands was one band produced by the primer P7.

The lowest percentage of polymorphism was 0 by primers P5 and P7, while the highest percentage was 35.7% in primer P2. The highest polymorphism by (GAA)₆ primer was 100% recorded in area 1 and area 2, but the lowest polymorphism was 0% by P1 primer, P4 primer and P8 primer recorded in area 2, area 1&2 and area 3&4 respectively. also, the polymorphism by (CTG)₃ GC primer was 50% recorded in the four studied areas.

Also, the analysis of ISSR marker separate the four locations of isolated *A. marina* into two main distinct clusters.

In the previous reports, Li and Chen (2004) estimated the genetic diversity of *Sonneratia alba* from China using ISSR marker and obtained eleven ISSR primers gave rise to 133 discernible DNA fragments of which 103 (77.44%) were polymorphic, indicating a considerable genetic variation at the species level.

Kader *et al.*, (2012) worked with a set of 10 RAPD and 10 ISSR markers were used to analyze the genetic diversity of the genus *Avicennia* from Sundarbans, India. The study showed that ISSR markers were more efficient than RAPD markers for polymorphism detection, polymorphic bands content per primer and total no of loci detection per primer as they were 75.53%, 11.9% and 15.6%, for ISSR and 69.04%, 9.6% and 13.7% for RAPD, respectively.

A. marina is a dominant mangrove species on along Red Sea coastal. The large population, destruction of their ecosystem, geographical isolation and cross pollination has caused genetic diversity in these plants.

CONCLUSION

Mangroves are defined as woody, evergreen group of plant community; grow on the swampy substrate at tropical and sub-tropical habitats, adjusted to high salinity, periodical tidal influence, strong winds, high temperatures, high precipitation and anaerobic soils. The study of DNA polymorphism of the individual taxa will be providing an advantage for this initiative as the wide genetic plasticity is a prerequisite for sustainability in changed environment. Recent advancement in molecular markers assisted PCR technique will provide the information regarding genetic background of each individual taxon, ultimately leading to valid guided references towards the understanding the inherent nature of the plant itself and beneficial to proper restoration program.

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