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CHEMICAL CONTAMINATIONS AND MICROBIAL DETERMINATIONS OF *AVICENNIA MARINA* (FORSK.)VIERH. IN RED SEA HABITAT, EGYPT

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Abstract:-
Contamination by polycyclic aromatic hydrocarbons (PAH) and heavy metals poses a major threat to Avicennia marina (Forsk.) Vierh.Mangrove plants grown naturally at the Egyptian Red Sea Coast. Also, microbial determination in A. marina habitats is very essential. Samples of water, rhizosediment, sediment and plant parts (shoots & pneumatophores) were collected from three sites in the habitat of A. marina. These sites are: 17 km south of Safaga, Qulaan and Ras-Mohamed. Sediment contents of Cu, Zn, Ni, Mn, Cr and Cd in all sites were lower than sediment quality guidelines (SQG). No microbial pollution was detected in water samples. The average contents of detected hydrocarbons in plant tissues followed the sequence of: Dibutyl Phthalate (1.103) > Diethyl Phthalate (1.02) >Bis (2-Ethylhexyl) phthalate (0.89) > Pyrene (0.394) > Phenanthrene (0.149) > Isophorone (0.098) µg /Kg. In addition, the stimulatory effects of these plants roots to microbial activity in all areas were studied.

Keywords: *Avicennia marina,* polycyclic aromatic hydrocarbons, heavy metals, microbial determinations, Red Sea.

INTRODUCTION:

Along the Red Sea coast, mangroves plants reach their northernmost distribution at Hurghada, Egypt, being mainly composed of A. marina. While, in the most southern part from Mersa El-Madfa (Lat. 23°N) till Mersa Halaib, on the Sudano-Egyptian border, Rhizophora mucronata predominates or dominates with A. marina **(Zahran, 2002)**.The distribution pattern of A. marina and R. mucronata along the Red Sea coast of Egypt indicates overlap in environmental requirements or tolerance of environmental stress **(El-Khouly and Khedr, 2007)**. Although the recent attention of ecological and socio-economic importance, mangrove ecosystems are one of the most threatened tropical environments. In addition to direct clearance, hydrological alterations, climatic changes or insect infestations, chemical pollution could be a significant contributor of mangrove degradation **(Bayen, 2012)**. Anthropogenic pressure from rising populations can adversely affects the mangroves environment. Among organic and inorganic pollutants, heavy metals are one of the main sources responsible for causing a significant negative impact on ecological quality of mangrove **(Defew et al., 2005)**. Petroleum hydrocarbons as priority organic contaminants are extremely complex assemblages of chemical substances coming from various sources **(Abdallah et al., 2015)**. Most hydrocarbons, especially polycyclic aromatic hydrocarbons (PAHs) are genotoxic, mutagenic, carcinogenic and can persist in the environment for many years **(Moles and Norcross, 1998)**. Fertility of the mangrove waters results from the microbial decomposition of organic matters and recycling of nutrients. Among the microbes, the bacterial population in mangroves environment is many fold greater than the fungi. In tropical mangroves environment, bacteria and fungi constitute 91% of the total microbial biomass, whereas algae and protozoa represent only 7% and 2%, respectively **(Alongi, 1988)**. The present study aimed to: (1) investigate polycyclic aromatic hydrocarbons (PAH), heavy metals and microbial contamination in water, sediments and plant parts of A. marina naturally grown through Egyptian Red Sea, Egypt; (2) evaluate the potential ecology risks of heavy metals in the sediments of A. marina habitat; (3) identify the ability of A. marina to accumulate and translocate PAH; (4) identify the heavy metals within the shoots and pneumatophores compartments of A. marina plants; (5) analyze the relationships between hydrocarbon and microbial diversity in sediment of A. marina habitat.

Study area:

Three sites are selected to evaluate the chemical and microbial contamination in A. marina habitat at the Red Sea Coast of Egypt (**Figure 1**). Site (1) is located in 17 km south of Safaga; site (2) is located in Qulaan, 120 km south Marsa Alam; and site (3) is located in RasMohamed at Aqaba Gulf. Samples of water, rhizosediment, sediment and plant parts (shoots & pneumatophores) were collected from A. marina habitat.

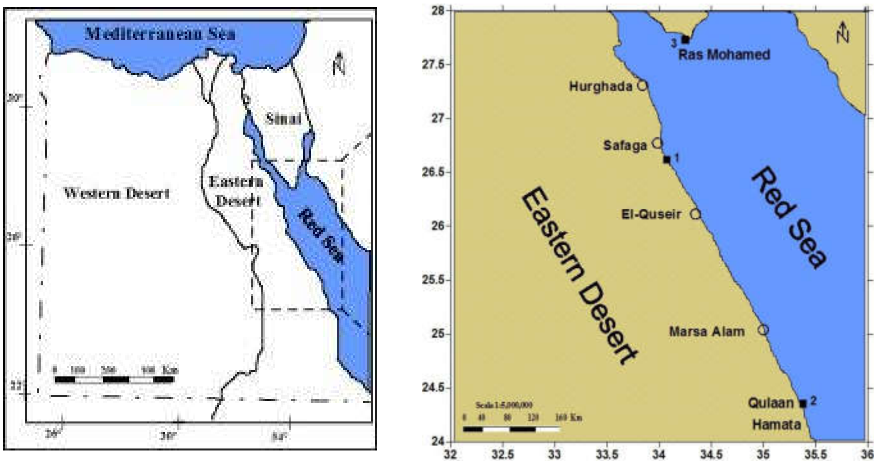

Figure (1): Location map of study area and samples.

Material and Methods:

Water analysis:

Each water sample was divided into four light-proof plastic containers; the first one was preserved in the field via addition of few drops of HNO_3 for heavy metals analysis, the second one was preserved in the field via addition of few drops of H_2SO_4 for COD analysis, the third container was sterilized by ethanol before adding the sample for microbiological analysis, and the fourth one was kept as is for the rest of the analysis. Water samples were filtered prior to analysis. Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD) were analyzed according to **APHA**, (**1998**). Microbiological analyses were performed for assessment of microbial pollution in water samples. The total viable bacterial counts (TVBCs) were determined at 37°C using the spread-plate method **(APHA, 1995)**. The numbers of total coliforms were determined using the most probable number (MPN) method using Mac Conkey broth media.

Sediment analysis:

Two types of sediments were collected at depth (0-30 cm); from the rhizosphere region and far from it. Sediments were air-dried, crushed gently and passed through a 2 mm sieve to eliminate gravel, and debris. Sediment water extracts of 1: 2.5 ratio were prepared and used to determine pH and EC according to the methods described by **Richards (1954)** and **Jackson (1963)**. Rhizosphere sediment samples were analyzed mechanically by the pipette method as outlined by **Kilmer**

and Alexander (1949) using Na-hexametaphosphate as a dispersing agent. The sediment samples were analyzed for total content of the studied elements in the filtered extracts obtained from samples digested as outlined by **Shumo et al. (2014)** using HNO_3 and H_2O_2 mixture. For DTPA-extractable "available" content of heavy metals analyses, sediment samples were extracted according to **Soltanpour and Schwab (1977)** using NH_4HCO_3/DTPA (Diethylene Triamine Penta Acetic Acid) solution.

Plant analysis:
The plant samples are thoroughly washed, air dried at 60ºC and ground prior to digestion according to **Shumo et al. (2014)** as the soil samples.

Enumeration of microorganisms of sediment:
The sediment samples were serially diluted up to 10^{-6} dilution to determine the population of bacteria, fungi, heterotrophic and oil degrading bacteria **(Johnson et al., 1956)**.Total microbial counts were determined according to **Aneja (1993)**. Screening of Total Heterotrophic was determined according to **Mahalakshmi et al. (2011).** Fungal counts were determined using suspension plating method **(Bills et al., 2004)**, Czapek's agar media was used. The colony forming units (C.F.U.) of all fungal isolates were counted. Hydrocarbon-degrading bacteria (DB) were estimated according to **Brown and Braddock (1990).**

Heavy metals analysis:
Eleven heavy metals (Al, Cd, Co, Cr, Mo, V, Fe, Mn, Cu, Ni and Zn) in water, plant and sediment samples were analyzed using Inductively Coupled Argon Plasma, iCAP 6500 Duo, Thermo Scientific, England. Multi-element certified standard solution (1000 mg/L), Merck, Germany was used as stock solution for instrument standardization.

Polycyclic Aromatic Hydrocarbons (PAH) analysis:
Water, sediment and plant samples were refluxed with methanol and KOH then were extracted with n-hexane according to **El-Shahawy (2007)**. Afterwards samples were analyzed on Gas Chromatography with mass spectroscopy (Central lab for drinking water, Dar El-Salam, Cairo, Egypt) according to method of EPA 625. The analysis used GC/MS (GC 3800 vaian) with DB5-MS (30 m X 0.25 mm X 0.25 m) column. The mobile phase was helium with injector temperature 280ºC and injection type split/ splitless. Thermal program: Mass spectroscopy Varian Inc. 1200 with interface temp 250ºC, ion source temp 200ºC and scan range 45-550 m/z.

Statistical analysis:
Results obtained from analyses were subjected to MSTAT-C **(Freed, 1991)** using randomized complete block design "RCBD". Mean values were compared using Duncan new multiple tests as described by **Waller and Duncan (1969)**. Means having the same alphabetical letter in the same column are not significant at P (significance probability value) = 0.05 level. Pearson's Product-Moment correlation analysis of the relationships between hydrocarbon and microbial diversity in sediment of *A. marina* habitat were calculated by program IBM-SPSSstatistic 20.

Results:
1. Water Investigation:
1.1. Chemical Characteristics:
As shown in **Table (1)**, pH values ranged from 7.4 to 8. Ras Mohamed site showed the lowest significant pH value (7.4) meanwhile Safaga and Qulaan sites showed significantly similar higher pH values (7.95 and 8.0, respectively). EC values ranged from 57.2 to 76.2 mS/cm, meanwhile TDS values ranged from 42 to 54 g/L. Ras Mohamed site showed the highest significant EC and TDS values (76.2 mS/cm and 54 g/L, respectively), meanwhile Safaga and Qulaan sites showed significantly similar lower values (57.2 and 58.2 mS/cm; 42 and 44 g/L, respectively). Values of BOD and COD were varied markedly among the sites, where BOD values ranged from 5 to 24 mg/L and COD values ranged from nil to 285.7 mg/L. Qulaan site showed the highest significant BOD and COD values (24 and 285.7 mg/L, respectively). It is followed by Ras Mohamed then Safaga sites. Concentrations of investigated heavy metals followed the following order: Al (N.D-3.81 mg/L) > Fe (0.50-1.44 mg/L) > Mn (0.02-0.142 mg/L) > Zn (N.D-0.0259 mg/L) > Cu (N.D-0.0148 mg/L) > Cd (N.D-0.013 mg/L) > Mo (N.D-0.008 mg/L). Qulaan site was the highest contaminated one and recorded the highest significant concentrations of Al, Cu, Fe, Mn and Zn. It was followed by Safaga site that showed the highest significant Cd and Mo concentrations. Ras Mohamed site were the lowest significant contaminated one except of Mn concentration that was higher than Safaga site.

Table (1): Physicochemical parameters and heavy metals contents of Red Sea surface coastal water samples in *A. marina* habitat.

Parameter	Safaga	Qulaan	Ras Mohamed
pH	7.95 ^{a}	8.0 ^{a}	7.4 ^{b}
EC, mS/cm	57.2 ^{b}	58.2 ^{b}	76.2 ^{a}
TDS, g/L	42 ^{b}	44 ^{b}	54 ^{a}
BOD, mg/L	5 ^{b}	24 ^{a}	8 ^{b}
COD, mg/L	Nil	285.7 ^{a}	8 ^{b}
Al, mg/L	1.9395 ^{b}	3.812 ^{a}	N.D
Cd, mg/L	0.0135 ^{a}	0.0094 ^{b}	N.D
Co, mg/L	N.D	N.D	N.D
Cr, mg/L	N.D	N.D	N.D
Cu, mg/L	N.D	0.0148	N.D
Fe, mg/L	0.5082 ^{b}	1.442 ^{a}	0.4216 ^{c}
Mn, mg/L	0.0209 ^{c}	0.1422 ^{a}	0.0632 ^{b}
Mo, mg/L	0.0087 ^{a}	0.0071 ^{b}	N.D
Ni, mg/L	N.D	N.D	N.D
V, mg/L	N.D	N.D	N.D
Zn, mg/L	0.0071 ^{b}	0.0259 ^{a}	N.D

(Remarks: values with different letters in a column are significantly different at the 0.05 level, n=3, N.D = not detected)

Table (2) shows the detected PAH in coastal surface water. Safaga and Ras Mohamed sites were significantly more contaminated than Qulaan site, where the total detected hydrocarbons values were 4.324, 2.344 and 1.596 µg/L, respectively. Safaga site recorded the highest significant values of Diethyl phthalate and Dibutyl phthalate, meanwhile Bis (2Ethylhexyl) phthalate highest concentration was recorded in Ras Mohamed site.

Table (2): PAH contents and microbial analysis of water samples in *A. marina* habitat.

Site	Diethyl Phthalate (µg/L)	Dibutyl phthalate (µg/L)	Bis (2-Ethylhexyl) phthalate (µg/L)	Total PAH (µg/L)	Total microbial counts×10^4cfu/mL	Total Coliform Counts (MPN/100mL)
Safaga	0.946	1.198^a	2.18^b	4.324^a	139^b	N.D
Qulaan	N.D	0.814^b	0.782^c	1.596^b	183^a	N.D
Ras Mohamed	N.D	0.624 ^{c}	1.72^a	2.344^a	72^c	N.D

(Remarks: values with different letters in a column are significantly different at the 0.05 level, n=3, N.D = not detected)

1.2. Microbial Counts:

Waterborne pathogens are a leading cause of disease and death worldwide, so microbiological analyses of water is essential for the protection of public health in general. Microbial analysis of water samples revealed that, water samples from the three sites free from microbial contamination with coliform bacteria **(Table 2)**. Monitoring microbial water quality has been conducted by measuring indicator bacteria that occupy human intestinal systems that primarily represented fecal coliforms, *Escherichia coli, Salmonella* sp.. These organisms are often used as bio-indicators of water quality since they respond quickly to environmental change and may be effective indicators of alterations in water quality **(Gedamy et al., 2012).** Concerning bacteriological analysis of water samples at three sites, the highest significant count were recorded in Qulaan site (183×10^4 cfu/mL) followed by Safaga (139×10^4 cfu/mL) then came Ras Mohamed with the lowest significant count (72 ×10^4 cfu/mL).

2. Sediments Investigation:

The sediments evaluation is a must as it represents the main accumulator where contaminants may retain for many years.

2.1. Particle size distribution and texture class:

As shown in **Table (3)**, the texture of Safaga and Ras Mohamed sediments were loamy sand, where sand particles recorded the highest content of the investigated samples (87.12 %). Meanwhile sediment of Qulaan was sandy loam in texture, where sand content (79.12%) were lower than Safaga and Ras Mohamed sites. This variation in sand content was accompanied with convenient variation in clay and silt %, where Qulaan site showed higher clay content (10.88 and 10 %, respectively) than Ras Mohamed and Safaga (8.88 and 4%, respectively).

Table (3): Particle size distribution and texture class of the sediment samples in *A. marina* habitat.

Site	Sand, %	Silt, %	Clay, %	Texture class
Safaga	87.12	4	8.88	loamy sand
Qulaan	79.12	10	10.88	sandy loam
Ras Mohamed	87.12	4	8.88	loamy sand

2.2 pH and salinity:

Table (4) shows pH, EC and TDS of studied sediment samples. According to pH, Safaga and Qulaan sediment samples were more alkaline than Ras Mohamed ones, where pH ranged from 9.3 to 9.4, from 9 to 9.1 and from 7.8 to 8.0, respectively. The rhizosphere localities showed higher salinity (EC and TDS values) than non-rhizosphere ones. The sediments salinity order was Ras Mohamed > Qulaan > Safaga ones. The highest saline sample was the rhizosphere of Ras Mohamed where EC value was 9870 µS/cm and the lowest one was in nonrhizosphere of Safaga site where EC value was 5020 µS/cm.

Table (4): pH and salinity of the sediment samples in *A. marina* habitat (water extract 1:2.5):

Site	Localization	pH	EC µS/cm	TDS mg/L
Safaga	Rhizosphere	9.4	5520	3147
	Non-Rhizosphere	9.3	5020	3077
Qulaan	Rhizosphere	9.1	8560	5450
	Non-Rhizosphere	9.1	7730	4505
Ras Mohamed	Rhizosphere	7.8	9870	6367
	Non-Rhizosphere	8.0	7900	4662

2.3 Microbial diversity from sediments of mangrove habitat:

Represented results in **Table (5)** showed that, one gram of the rhizosphere sediment contained 184.5-390 $\times 10^4$ cfu/ g sediment of total microbial counts, 37-103$\times 10^2$ cfu/ g sediment of fungi counts ,109-134 $\times 10^2$ cfu/ g sediment counts of heterotrophic bacteria and 71-95 $\times 10^2$ cfu/ g sediment oil degrading bacteria. While non-rhizosphere sediment recorded 167-204.5 $\times 10^4$cfu/ g sediment of total microbial counts, 24-86 $\times 10^2$ cfu/ g sediment fungi, 78-127134 $\times 10^2$ cfu/ g sediment counts of heterotrophic bacteria and 39-65 $\times 10^2$ cfu/ g sediment oil degrading bacteria.

Table (5): Microbial diversity in rhizosphere and non-rhizosphere sedimentsof A. marina habitat

Site	Localization	Total microbial counts$\times 10^4$cfu/ g sediment	Heterotrophic bacteria$\times 10^2$cfu/g sediment	Total fungi counts$\times 10^2$cfu/ g sediment	Oil degrader $\times 10^2$cfu/ g sediment
Safaga	Non-Rhizosphere	167.0^f	095^e	024^f	39.0^f
	Rhizosphere	184.5^d	109^d	037^e	94.5^a
Qulaan	Non-Rhizosphere	204.5^b	127^c	086^c	65.0^d
	Rhizosphere	390.0^a	160^a	095^b	71.0^c
Ras Mohamed	Non-Rhizosphere	177.0^e	078^f	059^d	43.0^e
	Rhizosphere	220.0^c	134^b	103^a	80.0^b

2.4 Heavy metals contents:

DTPA-extractable contents in sediment expressing the available amount to be consumed by plant were illustrated in **Table (6)**. Safaga site recorded the highest significant values of Co, Cr and Mn. Qulaan site recorded the highest significant values of Cd, Cu, Fe, Mo, Ni, V and Zn. Ras Mohamed site recorded the highest significant values of Al only. The localization of sediment sample (rhizosphere or non-rhizosphere) had no significant superiority in elements containment. Ranges of investigated elements were Al (0.108-0.5022), Cd (0.0066-0.0194), Co (0.0056-0.1326), Cr (0.0851-0.2763), Cu (0.3612-0.6823), Fe (30.81-116.54), and Mn (0.08616.286), Mo (0.0286-0.4697), Ni (0.0593-0.2779), V (0.344-4.89) and Zn (0.308-2.99) mg/Kg. The studied elements average contents followed the sequence of: Fe > Mn > V > Zn > Cu > Al > Cr > Ni > Mo> Co > Cd. Total elements contents in sediment were illustrated in **Table (7)**. The highest significant values of Co, Cu, Ni and Zn were recorded in Safaga site; V in Qulaan site; Cd in Ras Mohamed site; Al, Fe, Mn and Mo in both of Safaga and Qulaan, Cr in both of Safaga and Ras Mohamed. The localization of sediment sample (rhizosphere or non-rhizosphere) had no significant superiority in elements containment. Ranges of investigated elements were Al (625.2-6040), Cd (N.D-1.0), Co (0.33-8.565), Cr (N.D-28.19), Cu (3.95-20.42), Fe (449.66955), Mn (9.26-163.72), Mo (N.D -38.40), Ni (N.D-11.53), V (N.D-178.25) and Zn (N.D32.81) mg/Kg. The average total contents of studied elements differed from the DTPAextractable content and followed the sequence of: Fe > Al > Mn > V > Cr > Zn > Mo> Cu > Ni > Co > Cd.

2.5 Polycyclic aromatic hydrocarbons contents:

Sediment samples hydrocarbons contents were illustrated in **Table (8)**. Safaga site was most noticeably contaminated one as it showed the highest significant values of four from five detected hydrocarbons. The rhizosphere sediment of Safaga site had the highest significant values of Diethyl Phthalate and Pyrene (1.587 and 5.404 µg/Kg, respectively), while the nonrhizosphere sediment in the same site showed the highest significant values of Dibutyl Phthalate and Naphthalene (1.844 and 0.626 µg/Kg, respectively). The rhizosphere sediment in Ras Mohamed site showed the highest significant values of Bis (2-Ethylhexyl) Phthalate (2.164 µg/Kg) and this result agrees with water sample analysis where its highest concentration was recorded in Ras Mohamed site. The localization of sediment sample (rhizosphere or nonrhizosphere) had no significant superiority in hydrocarbons containment. Regarding to the total PAH; rhizosphere sediment of Safaga site showed the highest significant values (8.198 µg/Kg), while the lowest significant value was in non-rhizosphere sediment in Qulaan site (1.740 µg/Kg). The average contents of detected hydrocarbons followed the sequence of: Bis (2Ethylhexyl) phthalate (1.312) >Dibutyl phthalate (1.107) > Pyrene (1.032) > Diethyl phthalate (0.964) > Naphthalene (0.135) µg/Kg.

Table (6): DTPA-extractable contents of heavy metals in sediment samples in *A. marina* habitat (mg/Kg).

Site	Localization	Al	Cd	Co	Cr	Cu	Fe	Mn	Mo	Ni	V	Zn
Safaga	Rhizosphere	0.1521^{b}	0.0145^{b}	0.1326^{a}	0.2763^{a}	0.4176^{c}	43.30^{c}	5.7640^{a}	0.0817^{c}	0.1875^{c}	1.3760^{c}	1.8054^{b}
	Non-Rhizosphere	0.1592^{b}	0.0138^{b}	0.1146^{b}	0.2117^{c}	0.3612^{c}	37.74^{cd}	6.2860^{a}	0.0286^{e}	0.1461^{d}	0.9161^{d}	1.6445^{b}
Qulaan	Rhizosphere	0.1809^{b}	0.0183^{a}	0.0760^{d}	0.1628^{d}	0.6017^{b}	112.27^{a}	0.9995^{bc}	0.2438^{b}	0.2043^{b}	3.5300^{b}	2.617^{a}
	Non-Rhizosphere	0.1080^{b}	0.0194^{a}	0.1049^{c}	0.2235^{b}	0.6823^{a}	116.54^{a}	1.1739^{b}	0.4697^{a}	0.2779^{a}	4.8900^{a}	2.990^{a}
Ras Mohamed	Rhizosphere	0.4479^{a}	0.0076^{c}	0.0078^{e}	0.1004^{e}	0.4228^{c}	87.38^{b}	0.1582^{cd}	0.0500^{d}	0.0815^{e}	0.6821^{de}	0.6317^{c}
	Non-Rhizosphere	0.5022^{a}	0.0066^{c}	0.0056^{f}	0.0851^{f}	0.3688^{c}	30.81^{d}	0.0861^{d}	0.0518^{d}	0.0593^{f}	0.3440^{e}	0.3080^{c}
Average		0.2583	0.0133	0.0735	0.1766	0.4757	71.34	2.4112	0.1542	0.1594	1.9563	1.6661

(Remarks: values with different letters in a column are significantly different at the 0.05 level, n=3)

Table (7): Total contents of heavy metals in sediment samples in *A. marina* habitat (mg/Kg).

Site	Localization	Al	Cd	Co	Cr	Cu	Fe	Mn	Mo	Ni	V	Zn
Safaga	Rhizosphere	4934^{a}	0.1575^{b}	8.565^{a}	26.96^{a}	15.89^{b}	6445^{a}	163.72^{a}	38.40^{a}	11.532^{a}	79.77^{c}	21.85^{b}
	Non-Rhizosphere	5369^{a}	0.1450^{b}	4.915^{b}	19.09^{bc}	20.42^{a}	6035^{a}	163.05^{a}	4.132^{b}	8.135^{ab}	64.07^{d}	32.81^{a}
Qulaan	Rhizosphere	2800^{ab}	0.1200^{b}	3.817^{b}	14.34^{c}	14.97^{b}	3877^{a}	120.77^{b}	19.70^{ab}	6.660^{b}	140.92^{b}	23.50^{b}
	Non-Rhizosphere	6040^{a}	0.1150^{b}	5.185^{b}	24.45^{ab}	17.23^{ab}	6955^{a}	150.35^{a}	32.45^{a}	7.860^{ab}	178.25^{a}	24.81^{b}
Ras Mohamed	Rhizosphere	1324^{b}	N.D	0.6100^{c}	28.19^{a}	9.08^{c}	923^{b}	24.72^{c}	N.D	N.D	0.690^{e}	1.830^{c}
	Non-Rhizosphere	625^{b}	1.00^{a}	0.3300^{c}	N.D	3.95^{d}	449^{b}	9.26^{c}	N.D	N.D	N.D	N.D
Average		*3515.5*	*0.2562*	*3.9037*	*18.841*	*13.591*	*4114.1*	*105.31*	*15.781*	*5.6979*	*77.285*	*17.468*
SQG non-polluted*		/	/	/	< 25.0	< 25	/	< 300	/	< 20.0	/	< 90.0
SQG moderately-polluted*		/	/	/	25-75	25-75	/	300-500	/	20-50	/	90-200
SQG heavily polluted*		/	> 6.0	/	> 75.0	> 50	/	> 500	/	> 50.0	/	> 200.0

(Remarks: values with different letters in a column are significantly different at the 0.05 level, n=3, N.D = not detected, SQG sediment quality guidelines **(Luo et al., 2010)**)

Table (8): Polycyclic aromatic hydrocarbons parameters in sediment samples in *A. marina* habitat (µg /Kg):

Site	Localization	Diethyl phthalate	Dibutyl phthalate	Bis (2-Ethylhexyl) phthalate	Pyrene	Naphthalene	Total detected hydrocarbons
Safaga	Rhizosphere	1.587[a]	0.277[f]	0.746[f]	5.404[a]	0.184[b]	8.198[a]
	Non-Rhizosphere	1.208[b]	1.844[a]	1.668[b]	N.D	0.626[a]	5.346[b]
Qulaan	Rhizosphere	0.958[e]	1.194[c]	1.428[c]	0.786[b]	N.D	4.366[d]
	Non-Rhizosphere	N.D	0.846[e]	0.894[e]	N.D	N.D	1.740[f]
Ras Mohamed	Rhizosphere	1.05[c]	1.354[b]	2.164[a]	N.D	N.D	4.568[c]
	Non-Rhizosphere	0.978[d]	1.124[d]	0.974[d]	N.D	N.D	3.076[e]
Average		*0.964*	*1.107*	*1.312*	*1.032*	*0.135*	*4.549*

(Remarks: values with different letters in a column are significantly different at the 0.05 level, n=3, N.D = not detected)

Table (9): Pearson's correlation between hydrocarbon contents and microbial diversity in sediment of *A. marina* habitat:

Correlation	Diethyl phthalate	Dibutyl phthalate	Bis (2-Ethylhexyl) phthalate	Pyrene	Naphthalene	Total microbial counts	Heterotrophic bacteria	Total fungi counts	Oil degrader
Diethyl phthalate	1								
Dibutyl phthalate	-0.047	1							
Bis (2-Ethylhexyl) phthalate	0.142	0.743*	1						
Pyrene	0.591	-0.779*	-0.505	1					
Naphthalene	0.405	0.470	0.173	0.058	1				
Total microbial counts	-0.950**	-.0176	-0.406	-0.356	-0.333	1			
Heterotrophic bacteria	-0.780*	-0.095	-0.579	-0.353	-0.249	0.838*	1		
Total fungi counts	-0.720	0.077	-0.168	-0.626	-0.661	0.601	0.737*	1	
Oil degrader	-0.292	0.653	-0.019	-0.618	0.496	0.256	0.543	0.322	1

*Correlation is significant at the 0.05 level, ** Correlation is significant at the 0.01 level.

Pearson's Product-Moment correlation analysis of the relationships between hydrocarbon and microbial diversity in sediment of *A. marina* habitat were illustrated in **Table (9)**. A strong positive correlation (p=0.05) were observed between heterotrophic bacteria and both of total microbial count and total fungi count (r = 0.838 & 0.737, respectively). While, a strong negative correlation were observed between Diethyl phthalate and total microbial count at p= 0.01 (r = -0.95) and between Diethyl phthalate and heterotrophic bacteria at p = 0.05 (r = 0.780).

A non-significant (p=0.05) relationship was found between Diethyl phthalate with both of total fungi count and oil degrader by (r = 0.077 and r = 0.653); respectively. Also, nonsignificant (p=0.05) relationship was established between Naphthalene content and oil degrader (r = 0.496). On the other hand, non-significant (p=0.05) relationships were observed between oil degrader and each of total microbial counts, heterotrophic bacteria and total fungi count by (r = 0.256, r = 0.543 & r = 0.322, respectively).

3. Plant Investigation

3.1. Heavy metals contents:

Table (10) shows the content of heavy metals in shoots and pneumatophores of *A. marina.* The highest significant values of Al, Co, Fe, Mn, Ni and V were recorded in Safaga site; Cu in Qulaan site; Cr in Ras Mohamed site; Mo and Zn in both of Safaga and Qulaan sites; Cd in both of Qulaan and Ras Mohamed sites.

Ranges of investigated elements were Al (335.2-7948.3), Cd (N.D-1.0), Co (0.235-5.75), Cr (9.185-104.00), Cu (N.D-93.005), Fe (39.4-8496.7), Mn (2.58-173.5), Mo (N.D-0.9033), Ni (0.46-14.89), V (N.D-36.085) and Zn (5.6-59.81) mg/Kg. The average contents of studied elements followed the sequence of: Fe > Al > Mn > Cr > Cu > Zn > V > Ni > Co > Cd > Mo.

3.2. Polycyclic aromatic hydrocarbons contents:

PAHs in plant samples were illustrated in **Table (11)**. Pneumatophores in Ras Mohamed site had the highest significant values of Dibutyl Phthalate, Bis (2-Ethylhexyl) phthalate and Pyrene (1.322, 1.036 and 0.794 µg/Kg, respectively). Meanwhile pneumatophores in Safaga site had the highest significant values of Diethyl phthalate (1.1 µg/Kg) beside the only record of Phenanthrene (0.896 µg/Kg). Pneumatophores of Qulaan site had the only record of Isophorone (0.59 µg/Kg).

Table (10): Contents of heavy metals in *A. marina* samples (mg/Kg dry weight):

Site	Plant part	Al	Cd	Co	Cr	Cu	Fe	Mn	Mo	Ni	V	Zn
Safaga	Shoot	1228^{b}	0.35^{bc}	0.73^{b}	11.031^{d}	42.855^{b}	2388^{b}	58.33^{b}	0.3766^{ab}	5.056^{a}	3.681^{a}	24.241^{ab}
	Pneumatophores	7948.33^{a}	0.365^{bc}	5.75^{a}	32.628^{c}	50.53^{b}	8496^{a}	173.46^{a}	0.9033^{a}	14.886^{a}	36.085^{a}	56.23^{a}
Qulaan	Shoot	1824.5^{b}	0.9625^{a}	1.045^{b}	11.502^{d}	93.005^{a}	1877^{b}	44.945^{bc}	0.415^{ab}	5.330^{a}	4.687^{a}	29.955^{ab}
	Pneumatophores	436.05^{b}	0.555^{ab}	0.235^{b}	9.185^{d}	50.185^{b}	614.5^{b}	14.387^{cd}	0.575^{a}	5.250^{a}	1.125^{a}	59.81^{a}
Ras Mohamed	Shoot	381.8^{b}	N.D	0.24^{b}	104.0^{a}	N.D	39.4^{b}	9.280^{cd}	N.D	0.460^{c}	N.D	5.600^{b}
	Pneumatophores	335.2^{b}	1.00^{a}	0.44^{b}	92.86^{b}	N.D	418^{b}	2.580^{d}	N.D	7.700^{a}	5.84^{a}	10.98^{b}
Average		*2025.6*	*0.539*	*1.407*	*43.53*	*39.42*	*2305.6*	*50.49*	*0.378*	*6.447*	*8.570*	*31.136*

(Remarks: values with different letters in a column are significantly different at the 0.05 level, n=3, N.D = not detected)

Table (11): Polycyclic aromatic hydrocarbons parameters in *A. marina* samples (µg /Kg dry weight):

Site	Plant part	Diethyl Phthalate	Dibutyl phthalate	Bis (2-Ethylhexyl) phthalate	Pyrene	Phenanthrene	Isophorone	Total detected hydrocarbons
Safaga	Shoot	1.020^{c}	1.102^{d}	1.014^{b}	N.D	N.D	N.D N.D	3.136^{d} 5.012^{a}
	Pneumatophores	1.100^{a}	1.254^{b}	0.970^{c}	0.792^{b}	0.896		
Qulaan	Shoot	1.016^{d}	1.112^{c}	0.902^{d}	N.D	N.D	N.D	3.030^{e}
	Pneumatophores	0.960^{e}	0.902^{f}	0.668^{f}	0.780^{c}	N.D	0.59	3.900^{c}
Ras Mohamed	Shoot	0.956^{f}	0.926^{e}	0.750^{e}	N.D	N.D N.D	N.D N.D	2.632^{f}
	Pneumatophores	1.068^{b}	1.322^{a}	1.036^{a}	0.794^{a}			4.220^{b}
Average		*1.02*	*1.103*	*0.89*	*0.394*	*0.149*	*0.098*	*3.655*

(Remarks: values with different letters in a column are significantly different at the 0.05 level, n=3, N.D = not detected)

Regarding to the total detected hydrocarbons; pneumatophores of Safaga site showed the highest significant values (5.012 µg/Kg) followed by pneumatophores of Ras Mohamed site (4.22 µg/Kg) followed by pneumatophores of Qulaan site (3.90 µg/Kg). Therefore pneumatophores mostly have the ability to accumulate hydrocarbons more than shoot system. The average contents of detected hydrocarbons followed the sequence of: Dibutyl Phthalate (1.103) > Diethyl Phthalate (1.02) >Bis (2-Ethylhexyl) phthalate (0.89) > Pyrene (0.394) > Phenanthrene (0.149) > Isophorone (0.098) µg /Kg.

Discussion:

The reported pH values were more or less in agreement with **Fahmy et al., (2016)** in their study on Egyptian Red Sea coastal water (including Safaga area) where pH values ranged from 8.17 to 8.23, as well as **Isawi (2016)** in her study on Sharm El- Sheikh coastal water where pH were 7.2.

EC and TDS results in water samples were in agreement with **Madkour and Mohammed (2008)** in their study on mangroves habitat in Safaga that reported EC values ranged from 59.5 to 60 mS/cm and TDS values ranged from 37.9 to 38.8 g/L. **Isawi (2016)** attributed the high TDS values of Sharm El- Sheikh coastal water (57.2 g/L) to the rejection of high saline water resulted from tourist resorts desalination units.

BOD and COD results disagree with **Fahmy et al., (2016)** in their study on Safaga surface coastal water that reported BOD and COD values ranged from 1.43 to 1.23 mg/L and 7.54 to 8.32 mg/L, respectively. The noticed relatively high BOD and COD concentrations in Qulaan site may be attributed to the human activities impacts on water body. The highest total microbial count in Qulaan site coincides with the highest BOD and COD values in the same site, which confirms the impact of human activities on water body.

Concerning with heavy metals in water samples, results of Safaga and Qulaan sites were noticeably higher than reported by **Fahmy et al., (2016)** that were Fe (36.12 µg/l) >Zn (12.91 µg/L) >Cu (3.74 µg/L) > Mn (1.98 µg/L) > Ni (1.33 µg/L) > Cr (1.08 µg/L) > Cd (0.44 µg/L). Also the sequence order is totally different. With the excessive growth of industry all over the world, there has been a remarkable increase in industrial waste discharge to aquatic systems, which resulted in

accumulation of heavy metals in environment. Ras Mohamed site were the lowest contaminated one as it is a nature reserve region, and the results agreed with **Isawi (2016)**.
PAH results in water samples were clearly lower than reported by **Abd-ElHadi (2008)** where concentration of total hydrocarbons in Safaga water (11.45 mg/L) were the highest values among Wadi Abo Hamra and Sharm El Bahari sites (2.37 and 0.14 mg/L, respectively). The high concentrations in Safaga area may be attributed to the economic and industrial impacts of Safaga city and Safaga sea port. It is followed by Ras Mohamed site that may be attributed to the tourist activity including marine trips using submarine ships, yachts and boats that lead to some petro-compounds leakage to water body.
Sand particles recorded the highest content of the investigated samples (87.12 %) in Safaga that was in agreement with **Dar (2014)** that reported sand content of 85.6 % in Safaga area. **Mansour et al. (2011)** mentioned that Safaga beach sediments are generally coarse sands mixed with common rock-forming detritus from the surrounding strata. Also in Ras Mohamed, sand recorded the highest content (87.12 %) that was more or less disagree with **Gab-Alla et al. (2010)** in their study in mangroves habitat in Ras Mohamed where the sand content was 79%.
The lower pH values in Ras Mohamed site sediments than the other two sites coincide with lower pH of water samples. This may attributed to the geographical location of Ras Mohamed where it is located at the Red Sea split to the Gulf of Suez and Gulf of Aqaba. Meanwhile, the higher EC and TDS values in Ras Mohamed site sediments than the other two sites coincide with higher EC and TDS of water samples. This again may attributed to the rejection of high saline water resulted from tourist resorts desalination units. The results were in agreement with **Said and Ehsan (2010)** in their study on mangroves in a nearby area (Quseir city).
The results indicated the stimulatory effects of the plants roots, **Corgie et al., (2004)** reported that plant roots provide ideal attachment and supply exudates consisting of amino acids, organic acids, sugars, enzymes … etc. **Khalil et al., (2013)** found that fifteen fungal species belonging to nine genera were identified in mangroves soil which located in coastal area at Red Sea in Egypt, most of the genera detected belonged to the *Ascomycotina* with less extents belonging to the *Deuteromycotina* and *Zygomycotina*. Species were in decreasing order; *Aspergillus* spp., *Cladosporium* spp., *Alternaria* spp., *Penicillium* spp., *Rhizopus* spp., *Absidia* spp., *Acremonium* spp., and *Trichoderma* spp.. **Binet et al., (2000)** reported that microbial communities are larger and more active in planted versus unplanted soil, and the rhizosphere is often enriched in organisms capable of hydrocarbon degradation. **Rugh et al., (2005)** reported the abundance of PAH degrading bacteria in contaminated soil planted with different native Michigan plant species.

Despite the difference in each site specific heavy metals, the sites contamination arrangement of sediment samples followed the same order of water samples, Qulaan > Safaga > Ras Mohamed. This was from the standpoint of the number of the highest significant available metals concentrations. Qulaan site recorded the highest significant values of the majority of available metals contents (7 from 11 heavy metal), this may be attributed to the higher clay and silt % in this site than the other two sites where clay and silt fine particles (<0.063 mm) have the tendency to retain the pollutants more than sand particles (0.063 – 2 mm). Heavy metal concentrations in sediments generally increased with decreasing grain size, because of the affinity of metals to bind with finer particles **(Chen et al., 2016).**
The results of total and available contents in sediments were, more or less, in agreement with **Mansour et al. (2011)** that reported the sequence of Fe > Mn > Ni >Pb> Zn > Cu > Co > Cd in Safaga sediments. The contents of metals in this study were lower than non-polluted levels of sediment quality guidelines (SQG) according to **Luo et al. (2010).**

The results of PAHs in sediments were coinciding with water analysis results where the total PAHs was of the same sequence (Safaga > Ras Mohamed > Qulaan) and therefor accumulated in the sediments with the same sequence. This can be attributed to the economic and industrial activities in Safaga as it is not only as a tourism city but also as a seaport represents a gateway for Duba sea port to some pilgrims or travelers to Saudi Arabia by ferries, meanwhile Qulaan and Ras Mohamed are mainly tourist areas in the first place. The results were obviously lower than reported by **Abd-ElHadi (2008)** where total hydrocarbons in Safaga area ranged from 4.09 to 24.45 mg/Kg. This variation may be due to the difference of locations.
Slow oil degrading activities for both of oil degrader and total fungi count despite of the presence of oil pollutant in sediments may be attributed to the absence of adequate environmental conditions. Biodegradation of petroleum hydrocarbons is a complex process that depends on the nature and on the amount of the hydrocarbons present. One of the important factors that limit biodegradation of oil pollutants in the environment is their limited availability to microorganisms. Petroleum hydrocarbon compounds bind to soil components, and they are difficult to be removed or degraded. Hydrocarbons differ in their susceptibility to microbial attack. Some compounds, such as the high molecular weight polycyclic aromatic hydrocarbons (PAHs), may not be degraded at all **(Das and Chandran, 2011).**

The relation between oil degrader and each of total microbial counts, heterotrophic bacteria and total fungi count may be due to proliferation of heterotrophic bacteria and oil degraders in hydrocarbon polluted sediment. The efficiency depends on the needs and the ability of microorganisms to utilize the hydrocarbon, the favorable oxidation mechanism involved or the type of organism present that can utilize the hydrocarbons **(Ibiebele and Braide, 1987)**. On the other hand, it was observed a negative correlation between total microbial and heterotrophic bacteria with Diethyl phthalate, Dibutyl phthalate, Bis (2-Ethylhexyl) phthalate, Pyrene and Naphthalene. This indicated that low number of total microbial and heterotrophic bacterial populations in the sediment of *A. marina* in presence of high concentrations of each types of studied hydrocarbon. It may attribute to the low dissolved oxygen level and anoxic condition in bottom sediment. **Essien et al. (2008)** recorded relationships between total hydrocarbon content (THC) and hydrocarbon utilizing bacteria (HUB)

in sediments of the estuary mangrove, THCHUB relationship may be affected by several factors as: evaporation, dissolution and photooxidation responsible for the removal of oil in a natural ecosystem.
Concerning with plant samples, Safaga site was more contaminated than the other sites; these findings coincide very well with the results of sediment total metals contents while they are opposite to water and soil available heavy metals. In spite of the higher available heavy metals contents in Qulaan sediment, Safaga plants were more contaminated, this may be explained in the light of the higher sand % of Safaga site that reserves less heavy metals contents than clay and silt due to bigger particles size. Metals have more affinity to bind with finer soil particles **(Chen et al., 2016).**
El-Metwally et al. (2017) reported that Fe and Mn (7.483 and 0.3063 mg/g, respectively) showed the highest values at Safaga than Hurghada and Qusier harbors sediments due to the high terrigenous inputs from the different shipment operations and the wastewater effluents. The plant part had no significant superiority in elements containment in spite of pneumatophores in some cases recorded higher concentrations than leaves and vice versa. These results agree with **Alzahrani et al. (2018)** in his study on *A. marina* along the Red Sea coast of Saudi Arabia that reported similarly equal concentrations of Ni (7.56 and 7.58 µg/g) and Pb (3.79 and 3.67 µg/g) in leaves and pneumatophores, respectively. In other cases leaves recorded higher concentrations than pneumatophores in case of Cu (13.24 and 9.82 µg/g) and Cd (0.18 and 0.07 µg/g), respectively; meanwhile leaves recorded lower concentration than pneumatophores in case of Cr (14.69 and 17.46 µg/g), respectively.
The sequence of heavy metals in plant samples was more or less coinciding with the results of total sediment content and water analyses. The most three abundant metals (Fe, Al and Mn) took the same rank in total sediment contents by the same sequence; whereas Al was the most abundant one in water samples and followed by Fe then Mn.
Moreover, the average values of Cd, Cr, Cu, Ni and Zn (except for Al, Co, Fe, Mn, Mo, V) in plant tissues were higher than those in sediment. These observations reflect the accumulation of water metals in sediment and subsequently inside plant tissues by time. **Guitouni et al. (2016)** and **Alzahrani et al. (2018)** studied the heavy metals in *A. marina* habitats along the Red Sea coast of Saudi Arabia and they concluded that it is very useful to have mangrove plants in coastal regions for the absorption of contaminants and especially of heavy metals and cleaning of the marine environment

Thus *A. marina* accumulates interesting quantities of heavy metals and therefore plays a promising role in cleaning of the coastal environment of these toxic heavy metals. These results were mostly higher than those of *A. marina* in Red Sea Coast of Saudi Arabia where **Abohassan (2013)** reported heavy metals concentrations of Fe (115.06) > Cr (4.35) > Ni (4.07) > Mn (4.00) > Cu (3.37) > Zn (1.63) > Cd (0.07) ppm in the aerial roots and Fe (271.14) > Mn (3.47) > Cu (3.17) > Ni (2.14) > Cd (0.19) ppm in the stem. Also **Guitouni et al. (2016)** in their study on *A. marina* in Saudi Arabia Coast reported lower concentrations than this study, where metals sequence and highest concentrations in the total plant were Zn (11.09) > Cr (3.051) > Ni (1.795) > Fe (1.212) ppm. This is attributed to the presence of higher concentrations of metals in the surrounding sediments in the current study.
Activities related to the use of petroleum, natural gas which produce oils, greases and vapors of sea water pollution lead to the death of a seedling mangrove plant, in addition to thick of petroleum oils which solidifies around the roots of the plant, which prevents breeding and breathing these field observations can be recorded in studied mangroves at km 17 South of Safaga.
Concentration of total PAHs in pneumatophores followed the order of Safaga > Ras Mohamed > Qulaan just like water and sediment samples; while their concentration in shoot system followed the order of Safaga > Qulaan > Ras Mohamed. Regarding to hydrocarbons abundance in plant tissues; Diethyl phthalate, Dibutyl phthalate and Bis (2-Ethylhexyl) phthalate were the most abundant hydrocarbons through the three studied sites where they presented in all shoot and pneumatophores samples. It is worth to mention that these three hydrocarbons were the only existing ones in water samples and the highest abundant hydrocarbons in sediment samples. After those came Pyrene, that existed in only pneumatophores all over the three studied sites. The least abundant hydrocarbons were Phenanthrene and Isophorone that each existed in only one sample namely pneumatophores of Safaga site and pneumatophores of Qulaan site, respectively. The results were obviously lower than reported by **Abd-ElHadi (2008)** where total hydrocarbons in Safaga area ranged from 0.61 to 2.41mg/Kg. This may be attributed to location variation and hydrocarbons sediment content; where **Abd-ElHadi (2008**) reported total hydrocarbons range of 4.09- 24.45mg/Kg in Safaga sediment that is clearly higher than the current study 5.346-8.198µg/Kg **(Table 8)**.
Knopp et al. (2000) reported that the four-ringed PAHs chrysene and dibenzo (ah) anthracene, and the six-ringed PAH indeno (1,2,3-c,d) pyrene are carcinogenic compounds according to the International Agency for Research Cancer (IARC). So mangrove grows in this polluted area under this study containing toxic compounds represent a serous dangerous to grazing animals. **Awad (1989)** concluded that the Red Sea environment is suffering from oil pollution exceeding that which exists in most of the world oceans. Relative oil inputs from all sources are estimated to be each km^2 of the world's oceans receives 9.17 kg of oil annually, while each km^2 of the Red Sea receives 14.61 kg of oil annually. While each km^2 in the world's oceans receives 0.56 kg of oil annually from refinery effluent source; each km^2 in the Red Sea could receive 6.64 kg oil from the same type of source.

Conclusions:
It can be concluded that: (1) Accumulation of metals occurs in sediment and subsequently inside plant tissues by time, thus *A. marina* accumulates great quantities of heavy metals and therefore plays an important role in cleaning of the coastal environment of these toxic heavy metals and represents a serous dangerous to grazing animals. (2) The stimulatory effects of these plants roots to microbial activity in both studied areas can be employ in hydrocarbon remediation processes

in further researches. (3) Slow oil degrading activities for both oil degrader and total fungi count despite of the presence of oil pollutant may attributed to the lack of favorable conditions and suitable managements for bio-remediation.

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