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Microbes as Bioindicators for Contamination of Shatt Al-Arab Sediments in Basrah, Iraq

Maha K. Al-Mishrey[1] iD **, Raghad Shubbar Jaafar[2]*** iD **and Mustafa A. Al-Dossary[3]** iD

[1]Department of Biology, College of Science,University of Basrah,Iraq.
[2]Biological Development Department,Marine Science Center,University of Basrah,Iraq.
[3]Department of Ecology, College of Science, University of Basrah,Iraq.

Abstract

Shatt Al-Arab River in Basrah, Iraq represents an important source of fresh water so the current study aimed to determine the extent of pollution of the Shatt al-Arab River with some heavy metals and hydrocarbons by measuring their concentrations in sediment samples collected from different stations along the river, as well as the adoption of biomarkers represented by bacteria and fungi in determining the extents of such pollution. The results indicate that the river is contaminated with the following metals: : Ni, Cr, Zn, Pb, Cu and Cd. The total mean concentrations of heavy metals in the exchangeable part were high with 55.23, 53.76, 40.52, 33.84, 32.70 and 14.88 µg/g, respectively. The results also indicated a high concentration of hydrocarbons where the total mean was 28.52 µg/g. Bacteria and fungi represent vital indicators of heavy metals and hydrocarbons pollution in the environment. Different types of bacteria (*Sphingomonas paucimobilis, Bacillus subtilis, Pseudomonas aeruginosa, Streptococcus thoraltensis, Staphylococcus lentus, Leuconostoc cremoris and Leuconostoc mesenteroides*) and fungi (*Aspergillus terreus, Aspergillus fumigatus, Aspergillus niger, Curvularia* sp., *Eurotium cristatus, Talaromyces flavus, Mucor* sp., *Rhizopus* sp., *Trichoderma* sp., *Dichotomomyces cejpii,* and *Penicillium* spp.) have been isolated from the same sediment. Isolated of specific genera from bacteria and fungi like these isolated in the present study from the sediment of all studied stations reinforces the rest of the results recorded during the current study and confirms the pollution of the river with the studied pollutants.

Keywords: Shatt Al-Arab River, Bacteria, Fungi, Heavy metals, Hydrocarbons

*Correspondence: raghad.jaafar@uobasrah.edu.iq

INTRODUCTION

Often the environment exposed to major disturbances resulting from the rapid development of industrial and urban activities. These activities lead to human-origin impacts as a result of acute or chronic sources of pollution. Evidence indicates that heavy metal and hydrocarbon pollution have the largest environmental impact[1]. Usually, these types of pollutants find their way into water and have long life, since these pollutants are insoluble[2]. Pollutants tend to settle in the sediment of river, due to the sediments' ability to absorb these substances and deposit them between their particles[3]. Studying the chemical analysis of sediment in the river is therefore a useful way to study environment pollution, that the sediment is considered as recorder for watersheds enteric environment. The sediment that formed during weathering usually changes their composition during adsorption of chemical anthropogenic souces[4].

The influx of pollutants from various waste sources leads to change in the environment, and especially affects the quality of water, sediment and air, which is reflected on the biological components of the environment, such as plants, animals and microorganisms[5]. There are different physical, chemical and biological criterias that can be utilized to determine the quality of the environment[6]. Recently, biological parameters such as bacteria and fungi are widely used to detect the quality of environment as they can give evidence to the presence of pollutants in the environment. In the aquatic environment, these microbial indicators are often associated with the sediment fraction[7]. This association is due to three natural achievements of the sediment which are: 1. providing suitable condition for microbial attachment, 2. representing source of organic matters and nutrients for microbes, 3. and also is considered as a source of protection against sunlight and ultraviolet rays which constitute environmental stress[7]. There are many studies that confirmed the survival of bacteria in sediments for longer life than their age within the water column of both freshwater and marine water. Real association between the number and the type of bacteria through the water column of seawater and sediments have been observed in the previous studies[7,8]. The study conducted found more indicator organisms in sediments than in water and assumed that it is the defense actions for sediments[7].

Many studies have been done to determine the degree of pollution in the environmental ecosystem using microbes[9] conducted ability of community of soil microbial to reflect the amount of pollution in the soil as well as the impact of heavy metal concentration on the soil ecology.[10] Indicated the ability of filimantous fungi to tolerance high levels of heavy metals.[11] isolated different genera of fungi, *Fusarium* sp, *Trichoderma* sp, *Aspergillus* sp and *Rhizoctonia* sp, while the most isolated bacteria was *Bacillus* sp. from heavy metals polluted soil. These species except *Rhizoctonia* sp. responded to the presence of heavy metals in the soil and may thus serve as microbial indicator species. Also different studies indicated the relation between bacteria and fungi and hydrocarbons pollution[12] identified nine bacterial species and seven fungal species from oil polluted site in Effurun, Nigeria. Additionally, another study found five bacteria from diesel contaminated soil, in petroleum filling station at Nadapuram, Kozhikode District, Kerala[13].

For the importance of water environments and because of the different pollutants which enter to such environments from multiple sources, and considering that vital monitoring is the most accurate in giving the clearest envisage of the reality of pollution in an environment, the current study has adopted bioindicators (bacteria and fungi) isolated from the studied environment as an indicator of the pollution of the Shatt Al-Arab River with both heavy metals and hydrocarbons

MATERIALS AND METHODS
Description to the Shatt Al-Arab River

The Shatt Al-Arab river has a total length reach to a about 192Km the first~100Km of the river course situated entirely within the Iraqi territories : only the last~90 km of the river constitutes the border between Iran and Iraq, it widens over its course, expanding from a width of (250-300) m near the Euphrates-Tigris confluence to almost 700 m near the city of Basrah and more than 800 m as it approaches the river mouth An area of 145,190 km2 drains directly to the Shatt Al-Arab region downstream of the Euphrates-Tigris confluence (excluding the Euphrates and Tigris

Basin areas)[14]. The Shatt Al-Arab Delta area is classified as estuarine-deltaic because the river's sediment seeps into a shallow, narrow part of the Arabian Gulf. The Shatt al Arab Delta is 140 km wide and splits into more than 10 branches. The landscape is characterized by green marshy areas, lakes, lagoons and estuaries, bordered by irrigated lands and date palm plantations and surrounded by desert (UN-ESCWA and BGR 2013). Shatt AL-Arab depends on some water sources tributaries for feeding. These tributaries are Al-Hammar, Al-Huyaza and Al-Qurna marshes and Al-Karoon River which flows in its left side. Shatt AL-Arab also affected by the climate tide comes from Arabian Gulf which reduces discharge of Shatt AL-Arab water to the sea. Shatt AL-Arab's two banks are characterized to be low in its flow area reaching Basrah, its full with water channels which are used for irrigation agriculture land[15].

Study Stations

Six sites in Shatt Al-Arab River were chosen to obtain sediment samples as represented in Table 1. Fig. 1 shows the coordinates of the sample locations.

Table 1. The name and the coordination of studied stations

Samples numbers	Sampling station	Latitude	Longitude
1	Al-Qurna	30-58-28.81 N	47-28- 33.81 E
2	Al-Deer	30.7987835 N	47.5742088E
3	Al-Qarma	33.4364631 N	43.9627492 E
4	Al-Ashar	30.31.08.68 N	47.50.46.25 E
5	Abu-Alkasib	302736.98 N	480131.64 E
6	Al-Fao	29.58.57.66 N	48.28.36.62 E

Fig. 1. The location of studied area

Sediments sampling and analysis

Six stations in Shatt Al-Arab River were chosen to collect the sediment samples (Table 1). The sampling period was from 2019 until 2020. Samples were collected to measure the concentration of heavy metals (Cr, Co, Cd, Cu, Pb, Ni, Fe and Zn) in both exchangeable and residual phases. The exchangeable heavy metal ions were extracted from sediment, according to the method from the previous study[16], while the residual heavy metals were extracted according to another study[17]. Flame Atomic Absorption

Spectrophotometer (FAAS) was used to measure the concentration of the studied metals. A method from the previous study[18] was followed to measure the total organic carbon in the sediment. The extraction method of petroleum hydrocarbons followed another study[19].

Isolation and identification of bacteria

One gram of air-dried sediment sample was diluted (using) sterilized deionized water sequently and plated over a nutrient agar plate before being incubated at 30°C for 24 hr. Pure isolated bacterial cultures were identified based on the following characteristics such as colony altitude, size, form, color and texture gram stain reaction then identify by Vitek II (Biomerieux, USA).

Isolation and Identification of fungi

The dilution technique was followed to isolate fungi from sediment. Briefly, 10 g from each sediment sample was added into 100 mL of sterile physiological saline solution, shaken for ten min and diluted up to x10⁴. For each dilution, Approximately 1 ml from each dilution was transferred to a sterile Petri dish, to which 15 ml of sterile medium was added. The components of the Petri dish were mixed well before solidification, and the Petri dish was incubated at 25°C for 2 week. For primary isolation, Malt Extract Agar (MEA), and Potato Dextrose Agar (PDA) media supplemented with chloramphenicol (50 ppm) were used. The plates were then incubated for 5-7 days at 25°C till the growth of fungal colonies appeared. Morphologically distinct colonies were selected and further purified by sub-culturing them on plates containing PDA. The pure isolates were identified according to their micro- and macroscopic structures. The growth pattern, pigmentation and colony morphology were observed during the incubation period for the identification of fungi. The pure isolates were identified up to genus level based on the macro- and the micro-morphological characters following previous studies[20,21].

RESULTS AND DISCUSSION
Hydrocarbons Concentration

There are many sources of hydrocarbon pollution in the environment, as they may be transmitted through the atmosphere or as a result from crude oil and refined products, and also from plant effluent or municipal waste[22]. Sediments are the final settlement for hydrocarbon pollutants, as they latter tend to bind to suspended particles in the aquatic environment. Consequently, the sediment components of these pollutants can determine their various sources, either natural or as a result of human activities[23].

Table 2 shows the means of the total concentration of hydrocarbons in the studied regions where the means were ranged from 19.55 to 37.03 µg/g. The result indicated the highest concentrations of hydrocarbons with the following order: Al-Ashar < Abu-Alkasib < Al-Garma< Al-Deer< Al-Fao < Qurnah. Shat Al-Arab River is one of the main rivers flowing through Basrah and it represents the main waterway in the city. Al-Ashar, Abu-Alkasib and Al-Garma are cities located along the river that runs through the areas that are most densely populated, high commercial and high industrial. Correspondingly, higher potential for hydrocarbons loading from this region was observed.

Table 2. The mean concentration of hydrocarbons with SD

Stations	Mean of concentration (µg/g) With SD
Al-Deer	27.74 ± 0.82
Qurnah	19.55 ± 0.86
Al-Garma	31.70 ± 0.51
Ashar	37.03 ± 0.82
Abu Al-Khaseeb	33.47 ± 0.86
Al-Fao	21.68 ± 0.65
Total of mean	28.52 ± 6.46

Heavy metals in the exchangeable part

Study the exchangeable form of heavy metal in the sediment is a very important approach to assess the river pollution. Sediments usually act as carriers for heavy metals in the hydrological rotation and because metals are distributed into the surrounding waters, they reflect the quality of the water system[24]. The total concentration of heavy metal can not reflect the exact risk of pollution with such pollutants that the fit assessment of heavy metals pollution depends on their bio-availability[25]. The exchangeable concentrations of heavy metals in the studied station are shown in Table 3. The concentrations

showed significant differences between the sampling sites (P≤ 0.05), and the average metal levels are in the following order: Ni > Cr > Zn < Pb < Cu > Cd. Further result indicated that the concentration of studied metals took the following order in related to the studied stations: Al-Ashar < Al-Garma < Abu-Alkasib > Al-Dayr < Al-Fao < Al-Qurna. The result may be due to direct toxic chemicals discharged into the river and the ship-breaking activities.[26]

Heavy metals in the residual parts

The important form of heavy metals in the environment is related to its bioavailability, and heavy metals bioavailability decrease in the order of exchangeable < residual. A residual form of heavy metals represents the geochemical background values for the metals in the sediments, which can not mobilize and not toxic. The high level of residual fraction reflects the good indicator for safe and non toxic environment, which means positive development and long period of metal deposition[27]. Table 4 shows the mean concentration of the studied metals in the residual particles. From results, we can conclude that the composition of the heavy metals followed this order: Ni < Cr > Zn > Pb < Cu < Cd, while the heavy metals concentrations according to stations followed the order of this: Al-Ashar < Al-Garma <

Abu-Alkasib < Al-Fao < Al-Dayr < Al-Qurna. The result showed significant differences between the stations (P≤ 0.05), and this order can be explained by the reduced pollution sources in the last station. The pollution resulting from sewage, industrial, oil pollution (Al-Muftia oil refineries) and municipal wastes were responsible for the high amount of pollution[18]. In general, it is in high amout in comparision with the exchangeable part for all the studied stations, reducing the risk of heavy metals pollution. Table 5 show the the concentration of heavy metals in the Shatt Al-Arab river recorded in previous study through long period of time.

Bacterial & fungal identifications

Microorganisms are used as friendly indicators for pollution in both of aquatic and terrestrial ecosystems as they are easy to be assessed and readily available[37]. Different groups and species of bacteria can be used as bioindicators that the specific presence of some bacterial species in the environment in large numbers indicate the presence of pollutants. In addition, some bacterial behavior can be observed to identify the presence of pollutant. For instance, bacteria produce stress protein when exposed to cadmium[38]. Recently, bacterial markers in aquatic ecosystems are important in bacterial identifications[38]. The present study identified

Table 3. The mean Concentration(μg/g) ± SD of Exchangable part of studied metals in the studied stations

Stations	Ni	Cr	Zn	Pb	Cu	Cd
Al-Deer	50.75 ± 0.45	50.93 ± 0.10	36.81 ± 0.59	30.91 ± 0.55	24.70 ± .20	12.27 ± 0.48
Qurnah	45.17 ± 0.40	45.18 ± 0.39	31.39 ± 0.774	28.79 ± 0.80	18.22 ± 0.020	9.62 ± 0.37
Al-Garma	63.44 ± 0.51	63.32 ± 0.45	50.96 ± 1.24	35.04 ± 0.71	46.22 ± 0.54	16.80 ± 0.52
Al-Ashar	66.22 ± 0.51	58.43 ± 0.49	47.33 ± 1.02	41.06 ± 0.19	47.94 ± 0.31	21.81 ± 0.75
AbuAl-Khaseeb	54.70 ± 0.79	54.66 ± 0.56	40.68 ±0.82	35.00 ± 0.30	36.87 ± 0.58	18.07 ± 0.42
Al-Fao	51.08 ± 0.16	50.06 ± 0.22	35.95 ± 0.46	32.26 ± .55	22.27 ± 0.38	10.72 ±0 .42
Total of Mean	55.23 ± 7.60	53.76 ± 6.08	40.52 ± 6.98	33.84 ± 4.04	32.70 ± 11.99	14.88 ± 4.49

Table 4. The mean Concentration(μg/g) ± SD of Residual part of studied metals in the studied stations

Stations	NI	Cr	Zn	Pb	Cu	Cd
Al-Deer	63.02± 0.21	62.79 ± 0.54	50.56± 0.56	37.76 ± 0.86	24.70 ± 0.20	25.28 ± 0.50
Qurna	61.26 ± 0.31	60.88 ± 0.37	44.13 ± 0.97	35.67 ± 0.50	18.22 ± .02	13.20 ± 0.24
Al-Garma	69.71 ± 0.79	70.78 ± 0.36	62.47 ± 0.95	41.40 ±0.77	58.31 ± 0.59	18.24 ± 0.29
Al-Ashar	73.08 ± 0.45	75.56 ± 0.89	58.33 ± 0.67	46.02 ± 0.64	58.35 ± 3.17	20.80 ± 0.72
AbuAl-Khaseeb	68.22 ± 0.52	59.87 ± 0.82	56.66 ± 1.02	38.35 ± 0.65	46.67 ± 0.49	20.48 ± 0.89
Al-Fao	64.15 ± 1.65	63.70 ± 0.96	55.51 ± 0.72	38.94 ± 1.08	33.86 ± 0.64	14.17 ± 0.40
Total of mean	66.57 ± 4.28	65.60 ± 5.86	54.61 ± 6.08	39.69 ± 3.45	40.02 ± 16.10	66.57 ± 4.26

Table 5. The concentrations of heavy metals (µg / g)with the comparison results of the previous study in Shatt Al-Arab river

Studied Area	Cu	Cd	Pb	Ni	Cr	Co	Fe	Reference
Shatt Al Arab Estuary	29.24	0.27	17.74	104.2	404.10		5210.50	28
Shatt Al-Arab river	61.3	6.39	116.6	-	-		4119.55	29
Shatt Al-Arab river and Shatt Al Basrah	30.15	5.81	40.13	53.80	-		4170.33	30
Shatt Al-Arab river	26.69	-	83.78	-	528.77		1911.03	31
Shatt Al-Arab river	67.3	-	20.5	145	124	-	33718	32
Shatt Al-Arab river	44.11	13.08	104.97	234.64	992.09		20485.79	33
Shatt Al-Arab river	-	-	-	40.942-134.375	-	-	693.245-1159.254-	34
Shatt Al-Arab river	E:9.17-26.49 R:18.47-54.52	E:5.01-13.32 R:6.49-15.98	E:20.15-37.54 R:25.61-73.19	E:30.07-65.93 R:50.47-85.42	E:50.88-65.91 R:55.11-102.87	E:4.46-15.85 R:8.318.00	E:950.78-4987.92 R:770.1520158.26	35
Shatt Al-Arab river	20.27	7.96	74.97	75.70	-	-	4104.61	36
Shatt Al-Arab river	E: 32.70 R: 40.02	E: 14.88 R: 66.57	E: 33.84 R: 39.69	E: 55.23 R: 66.57	E: 53.76 65.60	-	-	Present study

bacteria morphologically (Fig. 2), as well as by using the automated instrument (Vitek II). This instrument gave identifiable accuracy up to 95%

confidence level (Table 6).The results of the present study are in agreement with the findings of the previous studies[39-42].
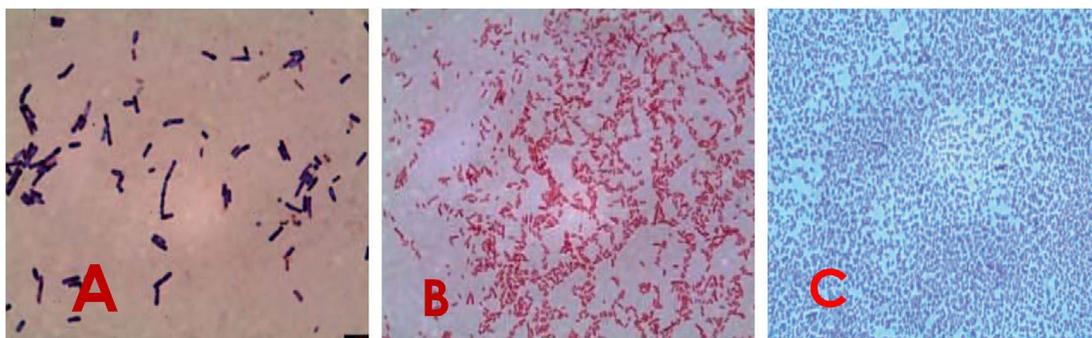

Fig. 2. Microscopic shape of some example of isolated bacteria : A: *Bacillus subtilis*, (gram stains) (1000X). Bacteria appear gram positive bacilli, B: *Sphingomonas paucimobilis*, Bacteria appear gram positive rod , C: *Staphylococcus lentus*, Bacteria appear gram positive cocci.

Table 6. Bacterial species isolated from the sediments of studying station

Bacteria	Gram stain	Shape	Color
Sphingomonas paucimobilis	_	Rod shape	Yellow
Bacillus subtilis	+	Rod shape	Gray-white
Pseudomonas aeruginosa	_	Rode shape	Green
Streptococcus thoraltensis	+	Cocci	Yellow
Staphylococcus lentus	+	Cocci	Yellow
Leuconostoc cremoris	+	Bacilli	White
Leuconostoc mesenteroides	+	Elongated cocci	White

Since fungi can tolerate higher concentration of heavy metals and other pollutant, they play important role as bioindicators for different pollutant in the water ecosystem[43]. In the present study, nine fungal species were isolated from the surface sediments (Table 7, Fig. 3). Most

of the isolated fungi have been studied previously and found to be good bioindicators for heavy metals pollution in water ecosystem[43,44].

Table 7. Fungal species isolated from the sediments of studying station

No.	Type of fungi
1	*Aspergillus terreus*
2	*Aspergillus fumigatus*
3	*Aspergillus niger*
4	*Curvularia* sp.
5	*Eurotium cristatus*
6	*Talaromyces flavus*
7	*Mucor* sp.
8	*Rhizopus* sp.
9	*Trichoderma* sp.
10	*Dichotomomyces cejpii*
11	*Penicillium* spp.

Fig. 3. Microscopic shape of some example of Cultures of some isolated fungi, a: *Aspergillus niger*, b: *Penicillium* sp. (16X)

CONCLUSION

Bacteria and fungi recently are being used as vital indicators for water pollution with heavy metals and other pollutants. They represent the modern and the successful means as they give accurate indication of the water pollution state. In addition to their presence in the environment, it reflects the health status of the environment in terms of the self-treatment possibility of the environment from those pollutants.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All Authors contributed to the design of the research experiment, collecting samples, conducting tests, and producing the results in a manner appropriate for publication. RSJ wrote the manuscript and prepared it for the publication.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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