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Level of Bacterial and Heavy Metals Pollution in Rivers Located in Al-Qurna City, Basrah, Iraq

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Abstract The purpose of the present study was to investigate bacterial and heavy metals pollution in rivers located near Al-Qurna city, Basrah, Iraq. The samples collected from November 2016 to February 2017 from three stations. The heavy metals analysis revealed that the mean concentration of iron, lead and zinc were highest at station 2; 0.35, 0.02 and 1.29 mg/L, respectively. The highest copper concentration was observed in the water sample from sampling station 3 (0.18 mg/L). In this study, 80 samples were collected from three different stations in Al-Qurna city. To find out pathogenic bacteria culturing technique was used followed by staining for identification of bacterial specie. In St. 1 were found 19 samples (23.75%) pathogenic bacteria, 16 samples (20.00%) from St. 2 and 13 samples (16.25%) from St. 3. Four Different bacterial species were reported (*Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes* and *Staphylococcus aureus*). *E. coli* was mostly isolated specie that was identified in 18 samples (22.50%) followed by *P. aeruginosa* 14 samples (17.50%), *S. aureus* 12 samples (15.00%) and *E. aerogenes* 7 samples (8.75%).

Keywords Ecological parameters; Tigris River; Euphrates River

Background

The Tigris and Euphrates are the most historically important rivers of Iraq. Both rivers flow through Syria and Iraq to join one to another in Al-Qurna city to be Shatt al-Arab River, which empties into the Arabian Gulf. Each of them is a vital resource for communities, agriculture and industry in Turkey, Syria and Iraq (Shamout and Lahn, 2015). Due to human and natural activities, contaminant enters the environment. These pollutants cause serious problems and risks to the environment and man himself (Hassan et al., 2010; Mohammad et al., 2012; Shanbehzadeh et al., 2014). Water resources are among the most critical resources and the importance of water resources, particularly surface water (rivers), in meeting the water need of humans, animals and industries underscores the need to protect them against contaminations (Shanbehzadeh et al., 2014). As municipal, industrial and agricultural wastes enter the water, biological and chemical contaminants including heavy metals also enter water resources.

Heavy metals are serious pollutants because of their toxicity, persistence and non-degradability in the environment (Olivares et al., 2005; Morin, 2008). The aim of this study is evaluating the chemical and biological contamination in Rivers near Al-Qurna city from three stations: the first station located in Euphrates River in Shatt Al-Arab South Al-Qurna city, the 2nd and 3rd located in Tigris River North Al-Qurna city and Al-Mudaina city. The concentration of chemical elements in ppm unit detected in the river depend on using atomic absorption, whereas biological method to evaluate bacterial presence (Total Plate Count), aerobic microbes in addition to isolation and identification of some bacteria that present in the three different places. The study analyzed bacterial and heavy metals pollution in Tigris, Euphrates and Shatt Al-Arab Rivers from Al-Qurna city, Basrah, Iraq.

1 Materials and Methods

1.1 Samples collection

80 samples were collected from three stations in rivers of Al-Qurna city (Figure 1) during four months from November 2016 to February 2017: (20 from each station). 100 mL water sample was collected and transferred it into disposable sterilized test tubes. After collection of sample, test tubes were tightly closed to avoid any contamination and protection to make it protected from environmental pathogen contamination.

Figure 1 Three study stations in Al-Qurna city

1.2 Heavy metals detection

Three elements, lead (Pb), Copper (Cu), Iron (Fe) were analyzed by atomic absorption in marine science center, Basrah university. The concentration was determined by ppm.

1.3 Isolation and identification of bacteria

The isolated bacterial species were identified by the following the morphological and biochemical characteristics of the individual colony was recorded. The individual colony was transferred to nutrient agar. The isolates were subjected to following different biochemical test for example Gram staining, Motility test, Indole test, Methyl red test, Voges Proskauer test, catalase test, Nitrate test and Carbohydrate fermentation test as described by (Jolt et al., 1994).

The samples were inoculated in Brain heart infusion broth, incubated at 37 ℃ for 24 h. The growing bacteria on the medium were sub cultivated on MacConkey plates agar, Blood agar plates, nutrient agar, Mannitol salt agar and Thiosulfate-citrate-bile saltssucrose agar (TSBS agar) and Eosin Methylene Blue agar (EMB agar), all plates were incubated in condition at 37 ℃ for 24 h. Microscopic examination such as motility test, morphology, and staining reactions (Monica, 2006) were determined under the compound light microscope. Biochemical tests included Indole test Methyl red (IMVIC) test, Voges-Proskaur test and Citrate utilization test, Triple sugar iron test (TSI) and oxidase test were demonstrated.

2 Results and Discussion

2.1 Chemical analysis of heavy metals composition

Chemical analysis of heavy metals by atomic absorption for samples collected from three rivers located near Al-Qurna city were shown in Table 1.

The heavy metal composition of the water samples from the three stations located near Al-Qurna city showed that the highest level of iron, lead, zinc was observed in the water sample from sampling station 2 with values of 0.35, 0.02 and 1.29 mg/L, respectively. The highest copper concentration was observed in the water sample from sampling station 3 (0.18 mg/L).

Table 1 High concentrations of metals in the water samples obtained from three stations (Rivers) located in Al-Qurna city, Basrah, Iraq (p<0.05)

Sampling stations	Iron	Lead	Copper	Zinc
St. 1	0.24±0.036	0.012±0.003	0.11±0.001	1.25±0.022
St. 2	0.35±0.044	0.020±0.002	0.10±0.001	1.29±0.00
St. 3	0.28±0.023	0.010±0.003	0.18±0.002	1.22±0.016

The results in Table 1 showed there is no significant change in concentration all of iron, lead and zinc in stations 1, 3 in Al-Qurna city. However, at station 2, there is change in concentration of iron, lead, zinc may be due to increased rate of non-treatment industrial waste which discharged to river. These results were agreed with Baudizsova (1997). In case of concentrations of copper there is an increase at station 3 that is due to the flow of the materials from upper regions of the river direct drainage from farmlands, sewage disposal plants. There is slight increase in concentration of iron between three sites due to the anthropogenic activities such as agricultural runoff, urbanization and industrialization.

2.2 Frequency of occurrence of the bacterial isolates

The most bacterial isolates belong to four genera: *Pseudomonas*, *Enterobacter*, *Escherichia* and *Staphylococcus*. A total of 80 samples were collected from three stations in three rivers at Al-Qurna city during study period from 14th November 2016 to 18th April 2017, (20 from each station). After a careful experimental work St. 1 stand top for having most number of coliforms found in drinking water samples with 19 samples (23.75%) followed by St. 2 were total no of coliform identified were in 16 samples (20.00%) and in St. 3 bacterial species were found in 13 samples (16.25%), as shown in Table 2.

Table 2 Total no of infected samples reported monthly

	Nov-16	December	Jan-17	February	Total samples
Station 1	3	4	5	7	18
Station 2	4	2	4	4	16
Station 3	0	3	5	5	13

Four Bacterial species were found (*E. coli*, *P. aeruginosa*, *E. aerogenes* and *S. aureus*) in St. 1. *E. coli* was mostly present specie and it was identified in 26 samples (32.50%) followed by *S. aureus* in 16 samples (20.00%), *P. aeruginosa* in 10 samples (12.50%) and *E. aerogenes* in 7 samples (8.75%) as shown in Table 3.

Table 3 Total no of microorganisms found per month

	Nov-16	December	Jan-17	February	Total samples
Total samples collected	20	20	20	20	80
Total Infected samples	15	14	12	18	58
E. coli	7	5	4	10	26
P. aeruginosa	3	3	1	3	10
E. aerogenes	1	2	4	0	7
S. aureus	4	4	3	5	16

According to Stevens et al. (2003), *E. coli* is main indicator for fecal contamination. Jay (1996) stated that *E. coli* presence is indication of enteric pathogens. According to Baudart et al. (2002), water quality is directly proportional to presence of coliforms in water. According to Baudizsova (1997), *E. coli* should be used as a prime bacteria as indicator for pathogenic contamination of water.

2.3 Biochemical tests

The different species of bacteria were identified using the biochemical tests namely, Motility test, Indole test, Methyl red test, Voges-proskauer test, Citrate utilization test, Catalase test, Oxidase test, Triple sugar Iron test, Urease test and Nitrate test. Broth cultures were observed the colour formation, ring formation and gas production,

which indicated positive and no colour change, no ring formation and no gas production that indicated nagative results

The results of biochemical tests of bacteria were summarized in Table 4. According to the results, species of bacterial commination species were isolated from the studied samples that collected from the Shatt Al-Arab River, south Al- Qurna city to fecal pollution in those areas, as well as to the sewages and lack the good treatment of waste before split the water into the river. These results were agreed with Abed et al. (2016) who diagnosed different bacterial species; which included *Escherichia coli*, *Enterobacter aerogenes*, *Salmonella spp*, *Klebsiella pneumonia*, *Proteus mirbalis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermids*, *E. coli* represented the main bacteria as compared with others bacteria in Euphrates River in Samawah city. Alkanany et al. (2017) who studied bacterial contamination by means of use the indicators bacteria (total and fecal coliforms) for samples of the Shatt al-Arab estuary and he was found highest bacterial contamination levels in Al-Ashar's river while the lowest levels were recorded in Al-Sarragi's river.

In this study, it can be concluded that found the biological pollution in Shatt Al-Arab south Al-Qurna city due to the pollution in al-Qurna area, high percentage of bacteria in al-Qurna area. *E. coli* is the highest bacteria present in the Shatt Al-Arab water due to an organic pollution, increase in concentrations of heavy metals in Tigris River north Al-Qurna city specifically in iron concentrations in Al-Qurna city.

Authors' contributions

All authors in this paper have contributed equally toward the publication of this paper. All authors read and approved the final manuscript.

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Table 4 Biochemical tests of different bacteria isolated from different stations in three rivers in Al-Qurna city

Bacterial Culture Plate (BCP)	Gram staining	Motility	Shape	Indole	Methyl Red	VP	Citrate	Urease	TSI	Catalase	Oxidase	Carbohydrate Fermentation			Nitrate	Identification of Bacteria
												G	F	S		
1	-	-	Rod	+	+	+	+	+	+	+	+	+	+	+	+	*Escherichia coli*
2	+	+	cocci	+	-	-	+	-	+	-	-	+	-	+	-	*Enterobacter aerogens*
3	-	+	Rod	-	-	-	+	-	+	+	+	-	+	+	±	*Pseudomonas aeruginosa*
4	+	+	cocci	-	-	+	+	-	-	+	+	+	+	+	-	*Enterococcus pseudo avium*

Note: VP = Voges Proskauer Test; TSI = Trible Sugar Iron Test; G = Glucose Test; F = Fructose Test; S= Sucrose Test; + = Positive; - = Negative

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