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The Potential Role of Soil Bacteria as an Indicator of Heavy Metal Pollution in Southern, Iraq

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Abstract:

The present study was performed to spotlight the potential role of soil bacteria in the Al-Rumaila oil field as a bioindicator of heavy metals pollution. For this purpose, nine soil samples were collected from different sites, with 20cm depth, to assess the pollution status depending on the total and available concentrations of heavy metals. The result indicates pollution of the studied soils with the following metals: Cd, Cu, Fe, Zn, and Pb. The mean of total concentration for all studied metals was higher than the allowed maximum limit based on the international limit:(3.394, 3.994, 39.993, 8844.979,150.372, and 103.347 µg/g), respectively. While measuring the total Metal concentration is important in determining the degree of pollution in the environment; it cannot be depended to determine their impact on the living organisms. In the present study the means of available concentration of studied metals were as follows: 0.015, 0.787, 0.021, 0.515, and4.304 µg/g. respectively, which were lower than their total concentration. Different types of bacterial genera (*Serratia marcescens, Sphingomonas paucimobilis, Bacillus subtilis, Pseudomonas aeruginosa*, and *Staphylococcus lentus*) were isolated from the same soil. And broadcasts through the results their presence in all studied soils. Therefore, the isolated bacteria play a significant role as an indicator of metal pollution in the soil, which was proved through the result of the Minimum inhibitor concentration (MIC), which indicated a high tolerance ability towered these metals.

Key words: Bacteria, Soil, Bio-Indicators, Heavy Metals, Iraq.

Introduction:

The soil environment can be considered as a landfill for waste generated by the activity of humans. If the amount of waste in the soil is below its tolerance threshold, it seems that the soil environment can be self-repaired or can be rebalanced. The soil pollutants resulting from the waste generated by houses and factories lead to a change in the natural properties of the soil environment. On the living, organisms inhibit these soils. The degree of soil pollutants and the amount of waste affect the number and type of inhibiting microorganisms [1].

The environment's quality is generally evaluated based on the origin of its chemical, physical, and biological parameters [2]. Nowadays, many studies use biological parameters such as bacteria to evaluate soil quality[3]. Bacteria can give clues to the presence of pollutants in soil, and they can be used as a good indicator of the amount of soil pollution[4]. To properly enumerate and manage soil bacterial species, the following criteria must be met: 1) The bacteria must exist where the environmental contaminate is found; 2) The type of bacterial species that is present must be unable to proliferate in the environment; 3) A large number of bacteria must be found; 4) The species should interact with the treatment process that uses natural conditions with similar pollutants; 5) The species should be easy to isolate, count, and diagnose; 6) The assessment technique must be affordable to permit a collection of a large number of samples; 7) The bacterial species living in the soil must be non-pathogenic [5].

Many studies have been conducted to determine the value of an environmental ecosystem using bacteria; [6] found a relationship between *Ramlibacter* and Zn contamination and *Steroidobacter* and Cd contamination. Moreover, [7]

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identified and isolated different bacterial species (*Staphylococcus epidermidis*, *Serratia marcescens*, *Proteus mirabilis*, and *Escherichia coli*) from a heavily polluted water sample. A study by [8] reported the correlation between the bacterial population, their diversity, and soil contaminated with Cd. Heavy metal contamination has been found to harm soil microbes, such as low respiratory rates, which inhibit the microbial activity; therefore, it will have a serious impact on the function of the soil's ecosystem. Finally, [9] indicated the potential use of *Paenibacillus* and *Flavobacterium*, and members of the order *Actinomycetales*, as biomarkers for Cd-stressed soils. The present study aims to assess the heavy metals pollution of the soil in Al-Rumaila oil field and to address the nature and diversity of the associated bacterial community. The potential role of bacteria as bioindicators of these toxic compounds will be investigated in terms of Minimum Inhibitory Concentration (MIC).

Materials and Methods:

Soil Sample Collection

Nine soil samples were obtained from the soil at a depth of 0–20 cm from three different sites in the oil field of AL-Rumaila, (Table 1), Figure 1. The soil samples were labeled and kept in a plastic sac until they were transferred to the laboratory. In the laboratory, the samples were dried by leaving them exposed to air. When they were completely dry, they were ground using a porcelain mortar, then sieved using a 2-mm sieve. The samples were kept in a dry place for subsequent tests[3].

Figure 1. Sampling sites

Table1. Coordination of sampling stations

Stations	Coordination	
1	30°11'45.21"N	47°23'25.96"E
2	30°12'43.55"N	47°24'51.07"E
3	30°13'33.13"N	47°22'29.09"E

Soil Sample Analyses

The soil samples were examined for specific chemical and physical properties, including; pH, EC, total organic carbon (TOC), and total heavy metal concentrations.

pH

The pH of samples was analyzed according to the method described in[10]. Thus, 50 gm of the soil sample was placed in glass beakers, and 100 ml of deionized water (1:2) was added. The mixture was shaken in a shaker, then beakers pliable to stop 1h. A calibrated Lovibond pH 200 meter was used to measure the soil pH (SensoDirect, Germany).

Electric conductivity

A total of 30 g from each soil sample was placed into glass beakers and saturated with distilled water to form a paste. A vacuum pump was used to obtain the soil extract. EC was detailed by using a calibrated Lovibond con200 m (SensoDirect, Germany)[10].

Total organic carbon

The method described in [11] was followed to measure TOC, the total concentration of the organic matter content. First, 2 gm of soil sample was placed in the flask, then 10 ml of 1 N $K_2Cr_2O_7$ added with shaking. Next, 20 ml of concentrated H_2SO_4 was added, and the flask was shaken again for 1 min, and then allowed to stand for 30 min. Then, sulfuric acid was added to the soil suspension, and the flask was stirred again and left to stand for 1 min. Finally, 200 ml distilled water and 10 ml of H_3PO_4 with 1 ml of a diphenylamine were added, and the sample was adjusted titrate using 0.5 N $FeSO_4.7H_2O$ till change the color to red.

The Total Concentration of Metal in the Soil

One gram of soil was digested using the acid mixture (1:1 HCl: HNO_3). Using a hot plate at 80°C, the sample was allowed to evaporate until it was almost completely dry. After that, another soil digestion using the mixture of concentrated $HClO_4$ and HF acids was used to complete the digestion process. The remaining part was dissolved in 20 ml of (0.5 N) HCl and cooled for 10 min [12]. The extractor was transferred to a 25 ml plastic container. This step was repeated twice, and all the supernatants were brought together. Finally, deionized water was used to increase the volume to 25 ml, and the sample was sealed for the analysis of heavy metals. The concentrations of the metals in the soil determined using the equation as follows:

(µg metal / gm = (A *V) /w

Where A = mg/L of metal in processed samples from the calibration curve.

V = final volume of the processed sample in mL.

W = Dry weight equivalent to the sample in gram.

Bioavailability Measurements of Heavy Metals

The DTPA-extraction method was followed to determine the available portions of studying metals, where 10 gr of air-dried soil was transferred into the extraction flask by using 20 ml of buffered (pH7.3) diethylene-triamine pentacetic acid (DTPA).Allowed to stand for 2 hr, the contents under shaking, then filtered during filter paper (Whatman No. 42).The filtration complete to 100 ml using deionized water, kept in a plastic bottle, and processed for metal determination[13]. Atomic flame absorption spectrophotometer (AAS 7000, Shimadzu, Japan) has been used for heavy metal analysis.

Bacterial Isolation

Using sterilized deionized water, one gram of air-dried soil sample was dilution sequency and plate over a nutrient agar plate, where incubated at 30 ° C for 24 hr.

The characterization of bacterial

Pure bacterial cultures were used to identify the bacteria. Different characteristics such as: morphological (colony altitude, size, form, color, texture, and gram stains), and biochemical (Oxidase, VP, and Catalase). And for more confirmation Vitek II (Biomerieux, USA) has been used.

The Study of Minimum Inhibitory Concentration (MIC)

The MIC test was carried out as an initial step to measure the heavy metal tolerance by bacteria. The loopful from overnight culture bacteria aseptically striking on the nutrient agar containing serial concentrations of heavy metals Cd, Cu, Zn, Fe, and Pb, (25, 50, 100, 250, 500, 1000, 1500, 1800, 2000 mg/l,and incubate at 25°C for 48 hr, including an untreated control culture. The minimal concentration of Cd, Cu, Zn, Fe, and Pb that led to inhibit the growth was determined and considered as the MIC. This test was repeated in triplicate, according to [14].

Results and Discussion:

A soil's chemical and physical properties (pH), (EC) and (TOC)

The present study focuses on the important factors, such as, pH, EC, and TOC, which have an effect on the concentration of heavy metals in the soil. Soil pH serves as a useful index of the availability of heavy metals in the soil and their physical property. The present study recorded pH values in the range between 7.50 and 7.89 (Table 2), which indicated that the studied soils have a neutral to sub alkaline nature. This finding is inconsistent with the results reported by [15]. The Iraqi soil contains a high percentage of carbonate, which leads to equalizing the soil's acidity. These findings are in line with [16], who studied soil in Baghdad city. recording causes this results to contain the soil higher quantities of calcium carbonate (lime) and recorded pH ranging between 7 to 8, and causes these results to contain the soil higher quantities calcium carbonate (lime) calcium sulphate. Soil in urban areas with an alkaline reaction has been reported to be a relatively common phenomena [17].

EC expresses the ability of the material to conduct electricity. Soil has the lowest EC value in the Station's soil, 2 (4.30 m/Scm), whereas the highest value was in the soil of the Station 3 (31.00 mS/cm). The differences in the EC values showed significant differences in soluble salt concentration. The level of TOC in stations 1 & 3 was similar, and the least value was in the station 2; (Table 2).

Table 2. pH, EC(m/Scm) and (TOC%)

The Stations	pH	EC (mS/cm)	TOC (100%)
1	7.83	6.72	2.50
2	7.50	4.30	1.92
3	7.89	31.00	2.49

The total concentration of heavy metals

The statistical summary of the present results showed that maximum concentrations of Cd, Cu, Fe, Zn and Pb are, (6.01, 75.18, 10605.5, 254.43 and 199. 40µg / g), respectively. The concentrations of heavy metals were compared with the EPA soil quality guidelines and Canadian soil quality guidelines (Tab.3) because of absence of the formal Iraqi guideline for the acceptable concentrations of heavy metals in the soils.

The maximum concentration of Cu, Fe, Zn and Pb exceeded the reference value and soil quality guidelines. At the same time, for Cd it was lower, and these results indicated that the soil was polluted with the heavy metals. The present recorded heavy metal concentrations were compared with the reference data in urban soils from other Iraqi cities (table 4). As shown in Table 3., the recorded concentration of metals was higher, which reflects the dangerous effects of oil drilling wells in the study stations. The oil extraction operations contributed to raising the concentration of heavy metal compared with "the rest of the sources.

Table 3. Statistical analysis of the total concentrations of heavy metals with the comparison world reference value and guidelines (µg / g).

Standards	Cd (µg/g)	Cu (µg/g)	Fe (µg/g)	Zn (µg/g)	Pb (µg/g)
Mean in the studied area	3.39	39.99	8894.97	150.37	103.34
Maximum	6.01	75.18	10605.5	254.43	199.40
Minimum	1.99	13.16	6982.87	37.11	52.88
World average data[18]	0.4	30	40000	90	35
EPA soil quality guideline [19]	0.6	16	-	110	40
Soil quality guideline of Canada [20]	10	64	-	140	140

Table 4. The Concentrations of heavy metals in some Iraqi soils

Provinces	Cd (µg/g)	Cu (µg/g)	Fe (µg/g)	Zn (µg/g)	Pb (µg/g)	References
Basra	3.394167	39.99333	8894.979	150.3725	103.3475	Present study
Baghdad	0.54	NA	NA	NA	113.98	[21]
Baghdad	1.58	5.25	NA	33.06	8.34	[16]
Dohuk	1.54	35.36	NA	NA	35.36	[22]
Haweja	NA	35.7	NA	51.33	NA	[23]
Fallujah	0.64	2.01	417.70	5.50	3.82	[24]

NA: Not Available

The available concentration of heavy metal

Although it is important to study the total concentration of metals to determine the extent of their contamination, it does not give a clear picture of how far their risks. The actual risk of heavy metals is due to their impact on the soil organisms not to their total concentration. DTPA-extractable metals (µg/g) represent available metals that can be taken by an organism [16]. The mean value of the available concentration of the metal was 0.015 µg/g for Cd and 0.787 µg/g for Cu. For Fe, it was 0.021 µg/g at station one, while it was not detected in the other two stations. The mean was 0.515 µg/g and 4.304 µg/g for Zn and Pb respectively (Table 5). Accordingly, the order of the averages content of DTPA-extractable metals in the analyzed samples were Pb>Cu>Zn>Fe >Cd. The present results showed that the available concentration was less than their total concentration. Metals in the soil are frequently associated with different soil constituents, making them unavailable, in addition to the prominent role of soil characteristics, which affects their availability. The present results are in consistent with the result of other studies [3 25 26].

Table 5. DTPA- extractable metal contents (µg/g) of the studied soil.

Station	Cd (µg/g)	Cu (µg/g)	Fe (µg/g)	Zn (µg/g)	Pb (µg/g)
1	0.010	0.063	0.021	0.206	4.920
2	0.012	2.199	N.D	1.243	4.625
3	0.024	0.100	N.D	0.096	3.369
Mean	0.015	0.787	0.021	0.515	4.304

N.D: -Non-Detection

Isolation and identification of bacteria

Isolated bacteria were identified based on their morphology and biochemical test (Table 6) and for emphasis on the identification, the automated instrument for bacterial identification (Vitek II) has been used, whereas the result which gave the organism were identifiable to the accuracy to a (95%) confidence degree.

Table 6. Some Morphological and Biochemical Characteristics of the isolated bacteria

Bacteria	Gram stain	Shape	Color	Catalase	VP	Oxidase
Serratia marcescens	-	Rod shape	Pink	+	+	-
Sphingomonas paucimobilis	-	Rod shape	Yellow	+		+
Bacillus subtilis	+	Rod shape	Gray-white	+	+	-
Pseudomonas aeruginosa	-	Rode shape	Green	+	-	+
Staphylococcus lentus	+	Coccia	white	+	+	-

Minimum Inhibitory concentration

Studying MIC is considered an initial step to evaluate the susceptibility of bacteria as a bio-indicator agent, that is represented the minimum concentration of metals that inhibit the growth of bacteria [27]. Table 7 represents the MIC values, which were recorded by the isolated bacteria in this study, and the results showed that soil bacteria can be a potential good indicator tool; the MIC for all isolates were high. The low MIC values point to being more toxic metals where maximum values of the MIC indicated to less toxic one [28]. The difference in the MIC value for different metals toward the same bacterial type can be attributed to the pollution with a particular metal which, in turn, raises the level of tolerance of the bacterial community to this metal [28]. The study also showed that the concentrations of metals, many physical and chemical factors might play a prominent role in increasing the susceptibility of bacteria to tolerate different concentrations of metals [29].

Table 7. The minimum inhibitory concentration(ppm)

Bacteria / MIC(mg/l)	Cd	Cu	Zn	Fe	Pb
Serratia marcescen	700	1000	600	2000	500
Sphingomonas paucimobilis	500	250	150	600	2000
Bacillus subtilis	50	150	1900	3000	1800
Pseudomonas aeruginosa	200	300	600	1000	2000
Staphylococcus lentus	150	300	200	2000	1800

Conclusions:

many conclusions can be discerned from the results of the current study, and they are as follows: the oil industry and its expansion in Iraq have a prominent and visible role in increasing soil pollution in those industrial areas with heavy metals accompanying these activities. Determining the total concentration of heavy metals, although important, is a general survey that is unable to give the true picture of the impact of these pollutants on living organisms. To integrate the image of heavy metal pollution, both the total concentration and the available concentration of these pollutants must be measured, so their environmental impacts can be estimated with high accuracy. The use of bacteria as a vital marking of soil pollution with heavy metals is one of the modern and successful means. It gives an accurate indication of the extent of that pollution. In addition to its presence in the environment, it reflects the health status of the environment regarding the possibility of self-treatment of the environment from those pollutants. And what appeared in the results of the current study (MIC study) confirms this, whereas its presence and its display of different MIC results attributes concerning what is proven.

Author's declaration:

- Conflicts of Interest: None.
- I hereby confirm that all the Figures and Tables in the manuscript are mine. Besides, the Figures and images, which are not mine, have been given the permission for re-publication attached with the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in University of Basra.

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بكتريا التربة كمؤشر حيوي لتلوث ترب جنوب العراق بالمعادن الثقيلة

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الخلاصة :

خلصت نتائج الدراسة الحالية الى ان الترب المدروسة من محطات في جنوب العراق ملوثة بالمعادن الثقيلة التالية (الكادميوم و النحاس و الحديد و الزنك و الرصاص), حيث كان متوسط تركيز تلك المعادن اعلى من الحد المسموح به عالميا" وكما مبين في النتائج ادناه (3.394, 3.994, 39.993, 8844.979,150.372, and 103.347 µg/g) وعلى التوالي. يعتبر دراسة التركيز الكلي للمعادن الثقيلة مؤشر جيد لتحديد درجة التلوث الا انه لايمكن ان يعطي صورة واضحة حول تأثراته على الكائنات الحية . سجلت الدراسة الحالية تراكيز منخفضة من التركيز المتوافر للمعادن الثقيلة المدروسة مقارنة" بتركيزها الكلي (0.015, 0.787, 0.021, 0.515, and4.304 µg/) . عزلت أنواع مختلفة من البركتريا من ذات الترب المدروسة (*Serratia marcescens*, *Sphingomonas paucimobilis*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Staphylococcus lentus*) وبذلك يمكن ان يعتبر عزل هذه البكتريا من تلك الترب الملوثة كأحد المؤشرات الحيوية للتلوث بالمعادن الثقيلة والتي اثبت من خلال دراسة MIC (اقل تركيز قاتل للبكتريا)والذي بينت نتائجة قابلية عالية للبكترايا لتحمل تراكيز عالية من تلك المعادن الثقيلة

الكلمات المفتاحية: البكتريا، المؤشرات الحيوية، المعادن الثقيلة، التربة ، العراق.