



Bioremediation to improve the efficiency of recovering soil removed from agricultural use areas

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ABSTRACT: Metals' high toxicity and ability to accumulate in soil and crops seriously threaten global food security. To address this, we need to expedite the restoration of agricultural lands that have been disturbed. Bioremediation, an effective treatment, relies on the ability of microorganisms to remove pollutants. Recent studies have shown that bacteria can serve as environmental sentinels, given their sensitivity to changing conditions and ability to adapt to adverse conditions. This study aimed to create a consortium based on microorganisms isolated from sediments from ten locations on the Euphrates River for applications in agricultural soil restoration areas. Six groups/species of bacteria were isolated, cultivated and evaluated: *Burkholderia cepacia*, *Aeromonas hydrophila* punctate, *Klebsiella pneumonia*, *Aeromonas sorbia*, *Enterobacter aerogens* e *Rhizobium radiobacter* in the removal of salts (Ca, Mg, SO₄, Na and K) and heavy metals (Pb, Co, Cd, Cr, Zn, Fe, Mn, Ni and Cu). All bacteria showed good salt and heavy metal removal rates, where removal rates were obtained for 4 and 7 days ranging between (41.18287-61.31482), (42.03456-2.70738), (46.53214-64.75572), (48.55462-8.30498), (42.94147-64.21361) and (26.65648-70.48373) respectively for the species above.

Keywords: heavy metals; bioremediation by bacteria; contaminated soils; soil salinity; secondary metabolite; sediments of the Euphrates River.

Biorremediação para melhorar a eficiência da recuperação de solos removidos de áreas de uso agrícola

RESUMO: A elevada toxicidade dos metais e a sua capacidade de acumulação nos solos e nas culturas agrícolas representam uma séria ameaça à segurança alimentar mundial. Para resolver este problema, é necessário acelerar o ritmo de restauração das terras agrícolas que foram perturbadas. A biorremediação é um tratamento eficaz e depende da capacidade dos microrganismos de remover poluentes. Vários estudos recentes indicam que as bactérias podem ser sentinelas das mudanças ambientais, dada a sua sensibilidade às mudanças de condições e a sua capacidade de adaptação em condições adversas. Neste estudo objetivou-se criar um consórcio baseado em microrganismos isolados de sedimentos de dez locais do rio Eufrates, para aplicações em áreas de restauração de solos agrícolas. Foram isolados, cultivados e avaliados 6 grupos/espécies de bactérias: *Burkholderia cepacia*, *Aeromonas hydrophila* punctate, *Klebsiella pneumonia*, *Aeromonas sorbia*, *Enterobacter aerogens* e *Rhizobium radiobacter*, na remoção de sais (Ca, Mg, SO₄, Na e K) e metais pesados (Pb, Co, Cd, Cr, Zn, Fe, Mn, Ni e Cu). Todas as bactérias apresentaram boas taxas de remoção de sais e metais pesados, onde foram obtidas taxas de remoção para 4 e 7 dias variando entre (41.18287-61.31482), (42.03456-62.70738), (46.53214- 64.75572), (48.55462- 68.30498), (42.94147- 64.21361) e (26.65648- 70.48373) respectivamente para as espécies supracitadas.

Palavras-chave: metais pesados; biorremediação por bactérias; solos contaminados; salinidade do solo; metabólito secundário; sedimentos do rio Eufrates.

1. INTRODUCTION

The ever-growing anthropogenic load affects the productive properties of agricultural soils and their fertility by causing organic matter reduction, nutrient depletion, heavy metals, pesticides, mineral fertilizers, and polycyclic aromatic hydrocarbons. Poor agricultural practices and management of water and land resources cause a drastic decline in agricultural soil quality and induce disastrous economic losses (ATUCHIN et al., 2023). This situation poses an urgent task of reducing the degradation rate of disturbed farming lands and increasing its restoration rate. This task is one of the 17 main goals of sustainable development defined by the United Nations through 2030 (TAYANG; SONGACHAN, 2021).

Global agricultural communities are particularly concerned with the current heavy-metal contamination of agricultural lands. Heavy metals are a group of metals with a density greater than 5 g/cm³. They persist in nature and consequently tend to accumulate in food chains. Although relatively high levels of these elements occur in a natural environment, their presence as a contaminant in ecosystems results mainly from anthropogenic activities. Some heavy metals such as nickel, iron, copper and zinc are essential to metabolic reactions and are required as trace elements by the organisms (BRIFFA et al., 2020; FU; XI, 2020).

Bacteria are generally the first organisms to be affected by discharges of heavy metals into the environment, increasing

metal-resistant bacteria in these environments (Sher and Rehman, 2019). Resistance to toxic metals in bacteria probably reflects the degree of environmental contamination with these substances and may be directly related to exposure to bacteria (ALTUĞ et al., 2020). Microorganisms have developed several mechanisms to tolerate high concentrations of heavy metals, such as biosorption, bioaccumulation, intracellular sequestration, biotransformation and bioleaching of metals. The ability of metal uptake by those microorganisms (known as biosorption or bioaccumulation) has caught great attention due to its potential to provide an effective and economical means for heavy-metal remediation (TAYANG; SONGACHAN, 2021).

Indicator microbes provide a simpler method because microorganisms have biosorption capabilities and are easy to culture in a short generation time, so their response to toxic substances is quite rapid (ALFADALY et al., 2021). Sediment bacteria are one of the major elements to resist heavy metals and have a high ability to adapt to unfavorable environmental conditions. Sediment bacteria can be used as an indicator organism to several toxic chemicals, including heavy metals and for effective, economical and eco-friendly bioremediation technologies, they have a high tolerance ability for various heavy metals (GONZALEZ HENAO; GHNEIM-HERRERA, 2021).

2. MATERIAL E METHODS

Figure 1 illustrates the current study, from the first step, obtaining the bacteria, until the third step, applying the bacteria and their secondary metabolite to treat contaminated soil.

2.1. Soil pollutants samples

Soil samples were collected from one roadside between Baghdad and Karbala. The sampling site was public ground and uncultivable land, so no specific permission was required during sampling.

2.2. Sediment samples

In the present study, Euphrates River sediment samples were collected between February 2022 and February 2023 from different parts of the Euphrates River between Babylon and Karbala governorates. The following samples were selected depending on the industrial and agricultural activity in the research area near: 1 - Cement factory discharge; 2 - Electrical Power station; 3 - Silk factory discharge; 4 - Beginning Hussaynia river; 5 - Beginning western stream; 6 - Trocar drainage collection; 7 - Al-Hindya hospital; 8 - Old Al-Hindya bridge; 9 - Karbala refinery station; and 10 - Modern Al-Hindya bridge. The samples were taken in two seasons, dry and wet: i) months 1, 2, 11, and 12, were considered to be in the wet season; ii) months 6, 7, 10 and 11 were considered in the dry season, according to Ministry of Transportation/Iraqi Meteorological organization and Seismology. Figure 2 shows the sites studied during two seasons, wet and dry.

2.3. Bacterial examination from sediments

The serial dilution technique was used to isolate and screen bacterial isolates from Euphrates River sediments for ten sites containing industrial, agricultural discharge and sewage water draining directly into the river. 1 mg of the

sediment samples for each site were dissolved adequately in 9 ml sterile water and diluted in 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} and 10^{-5} . From these dilutions, 0.1 ml of the solution was used to spread on the prepared plates. From the bacterial colonies on the agar plates, distinct bacterial isolates were streaked to obtain pure cultures based on the morphology. Inoculated petri dishes were incubated at 37 °C for 24-48 hr (COLE; RANKIN, 2021; JAYARAMAN et al., 2021). The isolated bacterial species were cultured by repeated streaking. They were kept in slants at 4 °C, then a swab was taken from each plate and cultured on the medium of blood and McConkey agar to determine the negative and positive bacteria described in Figure 2, and then the examination carried out with Vitek device (SINGH; HIRANMAI, 2021).

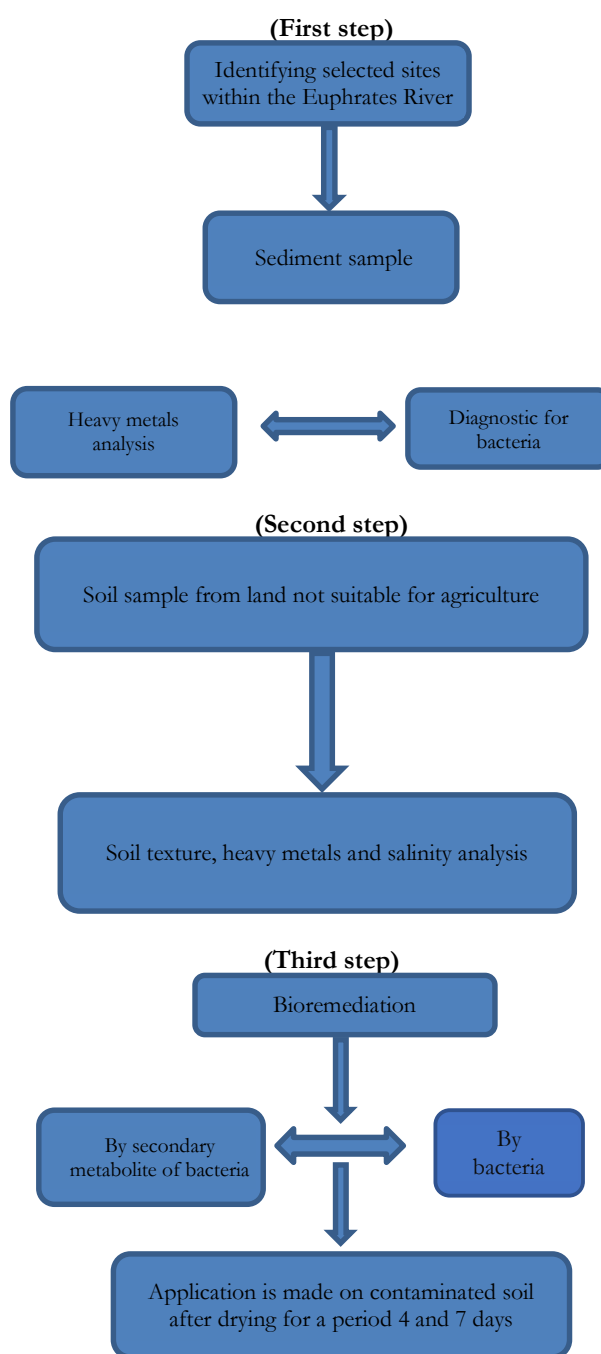


Figure 1. Diagram showing the steps of the current study.
Figura 1. Esquema com etapas do estudo atual.

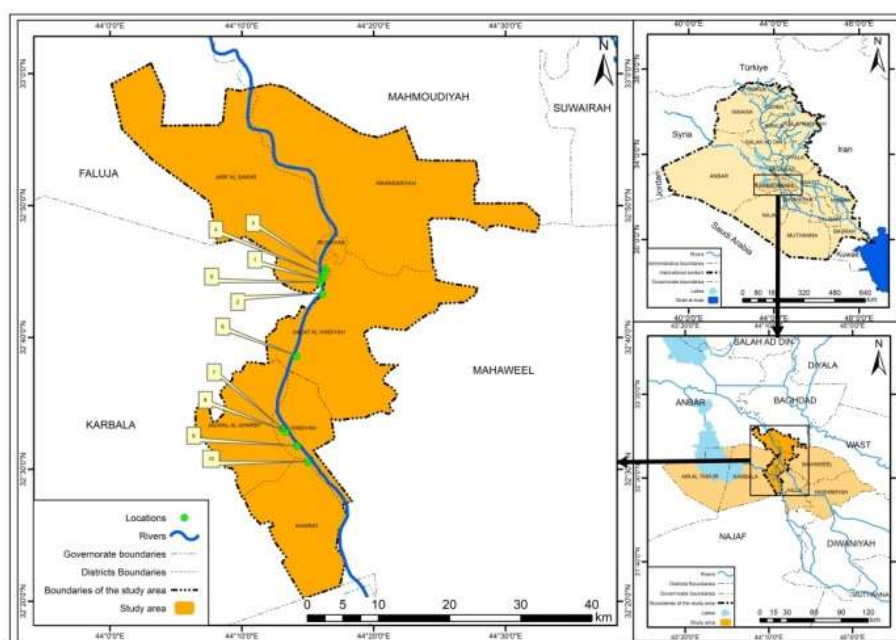


Figura 2: Mapa das áreas de estudo.

Figure 2. Map of study areas.



Figure 3. Bacterial diagnosis.

Figura 3. Diagnóstico bacteriano.

2.4. Preparation of bacteria for remediation

For each type of bacteria, Take 100 Mm from an Eppendorf tube, put it in a test tube containing nutrient broth, and incubate for 24-48 hr at 37 °C. Take 100 Mm from the activated tube, put it in a beaker containing the sterile nutrient media, and incubate for 24 hr (THAI et al., 2023).

2.5. Preparation of pollutant soil for remediation

At the sampling site, two soil cores were collected from the top horizon (5-30 cm depth), which was considered to have a strong salinity and heavy metals. The collected sub-samples were thoroughly mixed with a spade and pooled into one composite sample site. After sampling, the soil samples were transferred to plastic bags, which were sealed and soil analyses were performed at Krishi Vigyan Kendra (KVK), Cachar, which included the determination of soil texture (Ca, Mg, SO₄, Na and K) and heavy metals (Pb, Co, Cd, Cr, Zn, Fe, Mn, Ni and Cu contents (OPEKUNOVA et al., 2019; BARUA et al., 2021; POGŁODZIŃSKI et al., 2021).

2.6. Soil texture

Soil that was previously suitable for agriculture was used, but due to pollutants, it became unsuitable due to the high percentage of salts and metals pollutants in it. The soil

texture was analyzed using sieve analysis with three sieves according to the method mentioned in the source (POGŁODZIŃSKI et al., 2021). In our study, the soil texture contained 22.3% sand, 39.6% silt and loam and 38.1% clay.

2.7. Application to contaminated soil

Soil environmental tests are carried out before treatment. Then, Weigh 5 gm of soil and put it in a petri dish. Put in the plate containing soil for each bacterium. For all six species, the same process is carried out. The plates are incubated for four days, and the same steps are carried out on plates incubated for seven days.

And the efficiency of removal percentage of heavy metals by bacteria was calculated using the equation given below:

$$(R)\% = \frac{C_0 - C_e}{C_0} * 100 \quad (01)$$

where: C₀ and C_e are the heavy metal concentrations measured before and after removal by bacteria (GEBRETSADIK et al., 2020).

2.8. The steps before and after treatment

Take 1.0 gm of the treated soil digest, Heat the digestion solution until it becomes a white residue and bring the volume to 100ml with distilled water filter the solution with filter paper. Measurement by the atomic device (Nwachiri et al., 2020) and. For salinity, Measure the following parameters (Na, K, Ca, Mg, SO₄, Cl) according to (BONMATIN et al., 2003)

2.9. Statistical analysis

Typically, you will perform ANOVA when determining whether three or more means differ. Statisticians refer to the ANOVA F-test as an omnibus test. Two types of statistical analysis by using raincloud plots offer the user maximum utility and flexibility, ensuring that nothing is 'hidden away' and that the reader has all the information needed to assess

the data, its distribution, and the appropriateness of any reported statistical tests in a visually appealing (DENISSEN et al., 2022).

Post hoc tests are an integral part of ANOVA. When you use ANOVA to test the equality of at least three group means, statistically significant results indicate that not all group means are equal. However, ANOVA results do not identify which differences between pairs of means are significant. Post hoc tests explore differences between multiple group means while controlling the experiment-wise error rate (RAHMAN et al., 2021).

3. RESULTS

The following Tables 1 to 6 show the concentration for each parameter before remediation and after four and seven days, respectively, for evaluating the removal percentage for each species of bacteria.

Table 1. Treatment and percentage of removal of *Burkholderia cepacia* Group.

Tabela 1. Tratamento e a porcentagem de remoção de *Burkholderia cepacia* Group.

Parameters	before treat	After four days	%	After seven days	%
Ni	37.942	30.532	19.5308	20.411	46.205
Zn	9.605	5.804	39.573	2.986	68.912
Fe	30.305	19.486	35.699	13.345	55.964
Mn	27.961	23.541	15.808	12.913	53.818
Cr	48.335	37.617	22.174	24.005	50.336
Co	7.111	3.001	57.795	1.655	76.725
Cu	16.284	11.05	32.142	10.542	35.262
Cd	7.915	4.605	41.819	3.957	50.006
Pb	19.157	12.543	34.525	11.004	42.559
Ca	762	227	70.210	159	79.134
Mg	421	327	22.328	186	55.820
Cl	267	97	63.670	45	83.146
K	11.5	3	73.913	1.2	89.565
Na	192	134	30.208	96	50
SO ₄	581	242	58.348	103	82.272
Average			41.183		61.315

Table 2. Treatment and percentage of removal of *Aeromonas hydrophila punctate*.

Tabela 2. Tratamento e a porcentagem de remoção de *Aeromonas hydrophila punctate*.

Parameters	before treat	After four days	%	After seven days	%
Ni	37.942	31.451	17.108	21.681	42.857
Zn	9.605	6.328	34.118	3.941	58.969
Fe	30.305	20.246	33.191	13.061	56.901
Mn	27.961	23.531	15.845	18.003	35.614
Cr	48.335	40.973	15.231	36.046	25.425
Co	7.111	3.210	54.856	1.371	80.719
Cu	16.284	13.452	17.391	12.901	20.775
Cd	7.915	2.825	64.308	1.728	78.168
Pb	19.157	12.136	36.650	8.786	54.137
Ca	762	265	65.223	126	83.465
Mg	421	125	70.309	57	86.461
Cl	267	176	34.082	38	85.768
K	11.5	4.2	63.479	1.5	86.957
Na	192	126	34.375	79	58.854
SO ₄	581	149	74.355	84	85.542
Average			42.035		62.708

Table 3. Treatment and percentage of removal of *Klebsiella pneumonia ssp pneumonia*.

Tabela 3. Tratamento e a porcentagem de remoção de *Klebsiella pneumonia ssp pneumonia*.

Parameters	before treat	After four days	%	After seven days	%
Ni	37.942	21.851	42.40947	14.156	62.69042
Zn	9.605	5.616	41.53045	3.813	60.30193
Fe	30.3045	13.077	56.84799	9.378	69.0541
Mn	27.961	23.092	17.41354	19.624	29.81653
Cr	48.335	43.271	10.47688	33.688	30.30309
Co	7.1105	4.425	37.76809	2.734	61.54982
Cu	16.284	13.92	14.51732	13.127	19.38713
Cd	7.915	2.537	67.94694	0.792	89.99368
Pb	19.157	9.388	50.99441	5.436	71.62395
Ca	762	253	66.7979	167	78.08399
Mg	421	178	57.71971	105	75.05938
Cl	267	123	53.93258	52	80.52434
K	11.5	4.3	62.6087	1.7	85.21739
Na	192	120	37.5	61	68.22917
SO ₄	581	119	79.51807	61	89.50086
Average			46.53214		64.75572

Table 4. Treatment and percentage of removal of *Aeromonas sorbia*.

Tabela 4. Tratamento e a porcentagem de remoção de *Aeromonas sorbia*.

Parameters	before treat	After four days	%	After seven days	%
Ni	37.942	23.726	37.468	19.927	47.481
Zn	9.605	6.568	31.619	3.282	65.830
Fe	30.305	19.653	35.148	14.368	52.588
Mn	27.961	15.527	44.469	9.802	64.944
Cr	48.335	31.001	35.862	18.105	62.543
Co	7.1105	4.517	36.474	2.709	61.901
Cu	16.284	13.068	19.749	12.769	21.586
Cd	7.915	2.927	63.020	1.0440	86.810
Pb	19.157	11.093	42.094	10.165	46.938
Ca	762	116	84.778	59	92.257
Mg	421	127	69.834	43	89.786
Cl	267	171	35.955	50	81.273
K	11.5	3.3	71.304	0.5	95.652
Na	192	101	47.396	59	69.271
SO ₄	581	156	73.150	83	85.714
Average			48.555		68.305

Table 5. Treatment and percentage of removal of *Aeromonas sorbia*.

Tabela 5. Tratamento e a porcentagem de remoção de *Aeromonas sorbia*.

Parameter s	before treat	After four days	%	After seven days	%
Ni	37.942	28.5	24.885	19.654	48.199
Zn	9.605	5.403	43.748	2.997	68.798
Fe	30.304	29.002	4.2980	22.64	25.292
Mn	27.961	25.011	10.550	19.14	31.548
Cr	48.335	37.654	22.098	26.009	46.190
Co	7.1105	3.201	54.982	1.091	84.656
Cu	16.284	12.116	25.596	7.412	54.483
Cd	7.915	2.005	74.668	0.945	88.061
Pb	19.157	12.009	37.313	11.465	40.152
Ca	762	270	64.567	201	73.622
Mg	421	185	56.057	110	73.872
Cl	267	113	57.678	36	86.517
K	11.5	4.3	62.609	1.4	87.826
Na	192	97	49.479	51	73.437
SO ₄	581	258	55.594	113	80.551
Average			42.942		64.214

Table 6. Treatment and percentage of removal of *Rhizobium radiobacter*.
Tabela 6. Tratamento e a porcentagem de remoção de *Rhizobium radiobacter*.

Parameters	before treat	After four days	%	After seven days	%
Ni	37.942	32.828	10.9963	21.021	44.597
Zn	9.605	6.411	318.470	4.397	54.222
Fe	30.304	23.901	43.225	12.838	57.637
Mn	27.961	19.005	64.358	9.803	64.941
Cr	48.335	41.021	-8.319	29.089	39.818
Co	7.1105	3.613	469.545	1.505	78.834
Cu	16.284	14.101	140.623	13.128	19.381
Cd	7.915	2.481	436.121	0.717	90.941
Pb	19.157	9.274	144.730	4.914	74.349
Ca	762	260	-29.265	108	85.827
Mg	421	143	-25.178	87	79.335
Cl	267	176	-52.060	101	62.172
K	11.5	4.6	281.739	1.3	88.696
Na	192	90.1	-27.656	66	65.625
SO ₄	581	214	-30.465	131	77.453
Average			26.657		70.484

4. DISCUSSÃO

The contamination of soil with pollutants has been accelerated by agricultural and industrial development and poses a major threat to global ecosystems and human health. Various chemical and physical techniques have been developed to remediate soils contaminated with heavy metals and salt pollutants. Still, challenges of cost, efficacy, and toxic byproducts often limit their sustainability. Fortunately, bioremediation, achieved through bacteria, has shown great promise for tackling environmental pollution; this technology has been tested in the laboratory without providing appropriate conditions.

Two major groups' bioremediation treatment techniques are used: in situ and ex-situ remediation. The current study used the ex-situ method, which involves using bacteria from the sediments of the Euphrates River to remediate soil contaminated with heavy metals and salts.

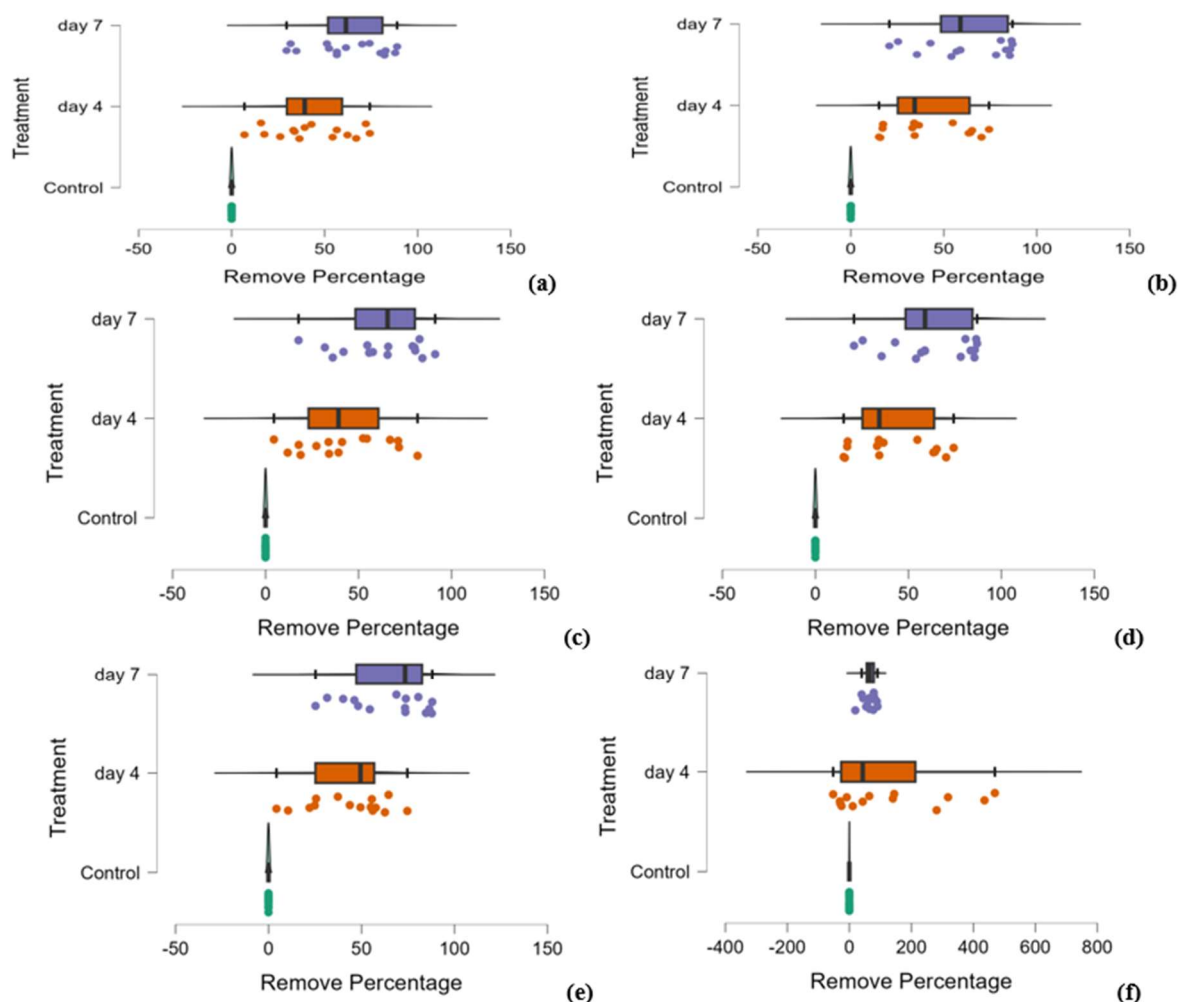


Figure 3. shows the treatment and removal percentage of *Burkholderia Cepacia Group* (a), *Aeromonas hydrophila punctate* (b), *Klebsiella pneumonia ssp pneumonia* (c), *Aeromonas sobria* (d), *Enterobacter aerogens* (e), and *Rhizobium radiobacter* (f).” Note: orange represents thr removal percentage of salinity and heavy metals by bacteria during four days, and blue represents the removal percentage during seven days.”

Figura 3. Porcentagem de tratamento e remoção do *Grupo Burkholderia Cepacia* (a), *Aeromonas hydrophila punctate* (b), *Klebsiella pneumonia ssp pneumonia* (c), *Aeromonas sobria* (d), *Enterobacter aerogens* (e) e *Rhizobium radiobacter* (f).” nota: a cor laranja representa a porcentagem de remoção de salinidade e metais pesados por bactérias durante quatro dias, e a cor azul representa a porcentagem de remoção durante sete dias.”

Six bacterial species (*Burkholderia cepacia* group, *Aeromonas hydrophila punctate*, *klebsiella pneumonia*, *Aeromonas sobria*, *Enterobacter aerogens* and *Rhizobium Radiobacter*) were found during the wet and dry seasons in all sites in the sediments of Euphrates River, which contain high concentrations of heavy metals, this indicates the ability of these bacteria to adapt and resist the environmental changes that occur around them. The genus *Burkholderia* contains over 30 species, which occupy remarkably diverse ecological niches, ranging from contaminated soils to the respiratory tract of humans. The *Burkholderia cepacia* complex is ubiquitous and can be found in soil, water (including sea water), the rhizosphere of plants, humans and various animal species and the hospital environment (TAVARES et al., 2020).

Economic and bio-friendly approaches are needed to remediate contamination from various industries. A novel bacterial strain, *Burkholderia cepacia* complex isolates, has been exploited for various purposes, including biological control of plant pathogens and bioremediation of recalcitrant xenobiotics (CAUDURO et al., 2021). They were obtained at sites 1, 2, 6, 7, and 10 during the wet season and at site 6 during the dry season. Table 1 shows the effectiveness of bacteria in removing salts and heavy metals from the soil during the 4-day and 7-day periods.

Fig 3 illustrates the total mean removal value for salinity and heavy metals, which is 95.740%, and the mean removal value after four days, 41.183% and after seven days, 61.315%.

Aeromonas hydrophila strain bacterium can be found in fresh or brackish water (REN et al., 2019). It was isolated from a Sediments River of sites 1,5 in the wet and dry seasons in sites 1,7. It can decrease salinity and heavy metals and is a heterotrophic bacterium mainly found in areas with a warm climate. It can survive in aerobic and anaerobic environments and digest materials such as gelatin and hemoglobin. It is the best-known species of *Aeromonas* (MEURIAL; KUMAR, 2020). Table 2 shows the effectiveness of bacteria in removing salts and heavy metals from the soil during the 4-day and 7-day periods. It is resistant to most common antibiotics, heavy metals, and cold temperatures, and it survived a wide range of pH values ranging from 3 to 10 and temperatures ranging from 4 to 37 °C (TURE et al., 2022).

As shown in Figure 4, the total mean removal value is 87.589%, the removal percentage for four days is 42.035%, and for seven days, it is 62.707%.

While *Klebsiella pneumonia* is a ubiquitous soil and sediment organism that lives in diverse environments, the species is also known to be involved in nitrogen fixation. The catabolic capacity of *Klebsiella* strains to degrade hydrocarbons, including polycyclic aromatic hydrocarbon (PAH) (CHAN et al., 2021). In our study, it was obtained on sites 2,6 and 8 in the wet season and site 1 in the dry season and was used to treat salinity and heavy metals soil. The results showed that Table 3 shows the effectiveness of bacteria in removing salts and heavy metals from the soil during the 4-day and 7-day periods. Table 3 shows the results. The total removal value is 87.201%, the removal percentage after days is 46.532%, and after seven days is 64.756, as illustrated in Figure 5.

In the present study, a Gram-negative *Aeromonas sobria* was isolated from heavily contaminated sediments in the Euphrates River in sites 1,7,9 and 10 in the dry season; *Aeromonas* species have an extraordinarily efficient ability to transform and remove heavy metals and other contaminants

from polluted areas (QURBANI et al., 2022). For example, species of *Aeromonas* can degrade heavy metals, decolorize triphenylmethane dyes such as malachite green27, and remove nitrates from wastewater (NGO; TISCHLER, 2022). Then, we assessed its ability to uptake heavy metals. The total removal value is 80.626%, the removal percentage after four days is 48.555%, and after seven days is 68.305%; Fig 6 shows the results. These findings suggest that metal-resistant *A. sobria* could be a promising candidate for heavy metal bioremediation in polluted areas (IBRAHIM et al., 2021; YOUNIS; SAEED, 2023); Table 4 shows the effectiveness of bacteria in removing salts and heavy metals from the soil during the 4-days and 7-days periods.

Owing to the variation in survival strategies by microbes to contaminated environments, these microbes resort to different means of adaption such as biosorption, digestion, bioaccumulation, biomineralization and biotransformation to detoxify heavy metals either in-situ or ex-situ (NWAHEHIRI et al., 2020; SAXENA et al., 2022). The uptake of heavy metals by microbes can either be by adsorption (passive) or bioaccumulation (active) (RASTIB et al., 2019; HENAO; HERRERA, 2021). *Enterobacter aerogens* bacteria are characterized by their ability to reduce heavy metals from the environment. The total removal results were 92.113%. After 4 days, 42.941% after seven days, 64.214%, as illustrated in Figure 7. Table 5 shows the effectiveness of bacteria in removing salts and heavy metals from the soil during the 4-day and 7-day periods.

The promising strain that can remove heavy metals from experimental media was selected: *Rhizobium radiobacter*, which uses these pollutants as food sources; the biosorption abilities of *Rhizobium radiobacter* can accelerate ex-situ remediation. The best treatment results were obtained with these bacteria, which are considered better than other species. Table 6 shows the effectiveness of bacteria in removing salts and heavy metals from the soil during the 4-day and 7-day periods, as the total removal rate was 90.470%, after four days 115.791%, and after seven days 65.588%.

Finally, using post hoc comparisons analysis for all results of bacteria and their remediate, there were significant differences $p < 0.001$ between the heavy metals concentrations found in soil samples before and after treatment for four days and between before and after seven days. This indicates the ability and high effectiveness of the bacteria to remove the high rates of pollutants from heavy metals and salts, as illustrated in Figure 10.

Table 7. Posttest Hoc Comparisons – Bacteria Treatment Plants.
Tabela 7. Comparações Posttest Hoc – Estações para tratamento de bactérias.

		Mean Difference	SE	t	Prukey	
Control	day 4	-50.437	3.607	-13.984	1.533×10 ⁻¹⁰	***
	day 7	-64.638	3.607	-17.922	1.533×10 ⁻¹⁰	***
day 4	day 7	-14.202	3.607	-3.938	2.763×10 ⁻⁴	***

*** $p < .001$

Note. P-value adjusted for comparing a family of 3

Note. Results are averaged over the levels of: Bacteria Type

5. CONCLUSIONS

Sediments are a complex and dynamic biogeochemical system comprising tens of thousands to millions of microbes. However, environmental stress may reduce bacterial diversity in the sediments. Therefore, microbes capable of thriving in high concentrations of heavy metals are important as bioremediation agents since they can attain diverse transformations and immobilization practices.

Six heavy metal-resistant bacteria were isolated and characterized in the current research study from sediments of the Euphrates River to remediation roadside soil contaminated with salinity and heavy metals, rendering it unsuitable for cultivation. The present study revealed that these microbes could persist in heavily polluted areas and utilize metal constituents to grow. Therefore, these heavy metals tolerant bacterial species can be exploited to clean up salts and metal-contaminated sites.

6. REFERENCES

- ALFADALY, R.; ELSAYED, A. R.; HASSAN, R.; NOURELDEEN, A. D.; HADEER, G.; AHMED, S. Microbial sensing and removal of heavy metals: Bioelectrochemical detection and removal of chromium (vi) and cadmium (ii). **Molecules**, v. 26, n. 9, e2549, 2021. <https://doi.org/10.3390/molecules26092549>
- ALTUĞ, G.; LŞEN, Ç.; MINE, T.; PELIN, S.; ÇİFTÇİ, K.; SAMET GÜRÜN, S. Antibiotic and Heavy Metal Resistant Bacteria Isolated from Aegean Sea Water and Sediment in Güllük Bay, Turkey: Quantifying the resistance of identified bacteria species with potential for environmental remediation applications. **Johnson Matthey Technology Review**, v. 64, n. 4, p. 507-525, 2020. <https://doi.org/10.1595/205651320X15953337767424>
- ATUCHIN, V.; ASYAKINA, L.; SERAZETDINOVA, Y. R.; FROLOVA, A.; VELICHKOVICH, N.; PROSEKOV, A. Y. Microorganisms for Bioremediation of soils contaminated with heavy metals. **Microorganisms**, v. 11, n. 4, e864, 2023. <https://doi.org/10.3390/microorganisms11040864>
- BARUA, T.; BHUIAN, A.; MAYEEN U.; DEB, N.; SHAHADAT, R.; MD, A. Assessment of Heavy Metal Pollution in Soil of Chattogram Hill Tracts, Bangladesh and Concomitant Health Risk. **Research Square**, v. 1, p. 1-26, 2021. <https://doi.org/10.21203/rs.3.rs-320812/v1>
- BONMATIN, J. M.; MOINEAU, I.; CHARVET, R.; FLECHE, C.; COLIN, M. E.; BENGSCH, E. R. A LC/APCI-MS/MS method for analysis of imidacloprid in soils, in plants, and in pollens. **Analytical Chemistry**, v. 75, n. 9, p. 2027-2033, 2003. <https://doi.org/10.1021/ac020600b>
- BRIFFA, J.; SINAGRA, E.; BLUNDELL, R. Heavy metal pollution in the environment and their toxicological effects on humans. **Heliyon**, v. 6, n. 9, e4691, 2020. <https://doi.org/10.1016/j.heliyon.2020.e04691>
- CAUDURO, G. P.; LEAL, A.; MARMITT, M. de Á.; LETÍCIA, G.; KERN, G.; QUADROS, P.; MAHENTHIRALINGAM, E.; VALIATI, V. New benzo (a) pyrene-degrading strains of the *Burkholderia cepacia* complex prospected from activated sludge in a petrochemical wastewater treatment plant. **Environmental Monitoring and Assessment**, v. 193, p. 1-12, 2021. <https://doi.org/10.1007/s10661-021-08952-z>
- CHAN, H.; XIAO, K.; TSANG, T.; ZENG, C.; WANG, B.; PENG, X.; WONG, P. Bioremediation of crude glycerol by a sustainable organic-microbe hybrid system. **Frontiers in Microbiology**, v. 12, e654033, 2021. <https://doi.org/10.3389/fmicb.2021.654033>
- COLE, S. D.; RANKIN, S. C. Isolation and Identification of Aerobic and Anaerobic Bacteria. **Greene's Infectious Diseases of the Dog and Cat**, p. 19-30, 2021. <https://doi.org/10.1016/B978-0-323-50934-3.00003-3>
- DENISSEN, S.; ENGEMANN, D.; DE, C.; ALEXANDER, C.; LARS BAIJOT, J.; JORNE, P.; IRIS-KATHARINA, G.; MATTHIAS, K.; MICHAEL, D.; MARIE, B. Brain age as a surrogate marker for cognitive performance in multiple sclerosis. **European Journal of Neurology**, v. 29, n. 10, p. 3039-3049, 2022. <https://doi.org/10.1111/ene.15473>
- FAKHRI, V.; JAFARI, A.; VAHED, F.; SU, C.; PIROUZFAR, V. Polysaccharides as eco-friendly bio-adsorbents for wastewater remediation: Current state and future perspective. **Journal of Water Process Engineering**, v. 54, e103980, 2023. <https://doi.org/10.1016/j.jwpe.2023.103980>
- FU, Z.; XI, S. The effects of heavy metals on human metabolism. **Toxicology mechanisms and methods**, v. 30, n. 3, p. 167-176, 2020. <https://doi.org/10.1080/15376516.2019.1701594>
- GEBRETSADIK, H.; GEBREKIDAN, A.; DEMLIE, L. Removal of heavy metals from aqueous solutions using Eucalyptus Camaldulensis: An alternate low cost adsorbent. **Cogent Chemistry**, v. 6, n. 1, e1720892, 2020. <https://doi.org/10.1080/23312009.2020.1720892>
- GONZALEZ HENAO, S.; GHNEIM-HERRERA, T. Heavy metals in soils and the remediation potential of bacteria associated with the plant microbiome. **Frontiers in Environmental Science**, v. 9, p. 1-15, 2021. <https://doi.org/10.3389/fenvs.2021.604216>
- HAIDER, I.; ALI, M.; SANAULLAH, M.; AHMED, N.; HUSSAIN, S.; SHAKEEL, M.; NAQVI, S.; DAR, J.; MOUSTAFA, M.; ALSHAHARNI, M. O. Unlocking the secrets of soil microbes: How decades-long contamination and heavy metals accumulation from sewage water and industrial effluents shape soil biological health. **Chemosphere**, v. 342, e140193, 2023. <https://doi.org/10.1016/j.chemosphere.2023.140193>
- HANSEN, H. K.; GUTIÉRREZ, C.; VALENCIA, N.; GOTSCHLICH, C.; LAZO, A.; LAZO, P.; ORTIZ-SOTO, R. Selection of Operation conditions for a batch brown seaweed biosorption system for removal of copper from aqueous solutions. **Metals**, v. 13, n. 6, e1008, 2023. <https://doi.org/10.3390/met13061008>
- IBRAHIM, U. B.; KAWO, A.; YUSUF, I.; YAHAYA, S. Physicochemical and molecular characterization of heavy metal - tolerant bacteria isolated from soil of mining sites in Nigeria. **Journal of Genetic Engineering and Biotechnology**, v. 19, n. 1, p. 1-13, 2021. <https://doi.org/10.1186/s43141-021-00251-x>
- JAYARAMAN, J.; SIGAMANI, S.; RAMAMURTHY, D. Metal biosorption by magnetotactic bacteria isolated from freshwater sediments and characterization of extracted magnetosomes. **Archives of Microbiology**, v. 203, n. 10, p. 5951-5962, 2021. <https://doi.org/10.1007/s00203-021-02534-w>
- MEURIAL, C. D.; KUMAR, K. Un-culturable microbial community analysis of paddy ecosystem at different depths using Nif H gene DGGE analysis approach.

- Indian Journal of Agricultural Research**, v. 54, n. 6, p. 763-768, 2020. <https://doi.org/10.18805/IJAR.A-5232>
- NATH, S.; SINHA, A.; SINGHA, Y.; DEY, A.; BHATTACHARJEE, N.; DEB, B. S. Prevalence of antibiotic-resistant, toxic metal-tolerant and biofilm-forming bacteria in hospital surroundings. **Environmental Analysis, Health and Toxicology**, v. 35, n. 3, e2020018, 2020. <https://doi.org/10.5620/eaht.2020018>
- NGO, A. C. R.; TISCHLER, D. Microbial degradation of azo dyes: approaches and prospects for a hazard-free conversion by microorganisms. **International Journal of Environmental Research and Public Health**, v. 19, n. 8, e4740, 2022. <https://doi.org/10.3390/ijerph19084740>
- NWAEHIRI, U. L.; AKWUKWAEGBU, P. I.; NWOKE, B. E. B. Bacterial remediation of heavy metal polluted soil and effluent from paper mill industry. **Environmental Analysis, Health and Toxicology**, v. 35, n. 2, e2020009, 2020. <https://doi.org/10.5620/eaht.e2020009>
- OPEKUNOVA, M. G.; OPEKUNOV, A. Y.; KUKUSHKIN, S. Y.; GANUL, A. G. Background contents of heavy metals in soils and bottom sediments north of Western Siberia. **Eurasian Soil Science**, v. 52, p. 380-395, 2019. <https://doi.org/10.1134/S106422931902011X>
- POGŁODZIŃSKI, R.; BARŁÓG, P.; GRZBISZ, W. Effect of nitrogen and magnesium sulfate application on sugar beet yield and quality. **Plant, Soil and Environment**, v. 67, n. 9, p. 507-513, 2021. <https://doi.org/10.17221/336/2021-PSE>
- QURBANI, K.; KHDIR, K.; SIDIQ, A.; HAMZAH, H.; HUSSEIN, S.; HAMAD, Z.; ABDULLA, R.; ABDULLA, B.; AZIZI, Z. *Aeromonas sobria* as a potential candidate for bioremediation of heavy metal from contaminated environments. **Scientific Reports**, v. 12, n. 1, e21235, 2022. <https://doi.org/10.1038/s41598-022-25781-3>
- RAHMAN, A.; JAHANARA, I.; JOLLY, Y. N. Assessment of physicochemical properties of water and their seasonal variation in an urban river in Bangladesh. **Water Science and Engineering**, v. 14, n. 2, p. 139-148, 2021. <https://doi.org/10.1016/j.wse.2021.06.006>
- RASTIB, A.; MEMARIANI, M.; RIAHI, M. Investigation of *Enterobacter aerogenes* effects on heavy oil from biological degradation aspects by GC* GC technique. **International Journal of Petrochemical Science & Engineering**, v. 4, p. 47-52, 2019. <https://doi.org/10.15406/ipcse.2019.04.00102>
- RATHOURE, A. K. Heavy metal pollution and its management: Bioremediation of heavy metal, in Waste management: concepts, methodologies, tools, and applications. **IGI Global**, p. 1013-1036, 2020. <https://doi.org/10.4018/978-1-7998-1210-4.ch046>
- REN, Z.; CAI, Y.; WANG, S.; LIU, S.; LI, ANXIONG, Y.; TANG, J.; SUN, Y.; GUO, W.; ZHOU, Y. First case of *Aeromonas schubertii* infection in brackish water wild Nile tilapia, *Oreochromis niloticus*, in China. **Aquaculture**, v. 501, p. 247-254, 2019. <https://doi.org/10.1016/j.aquaculture.2018.11.036> Get rights and content
- SAXENA, R.; HARDAINIYAN, S.; SINGH, N.; RAI, P. Prospects of microbes in mitigations of environmental degradation in the river ecosystem. In: **Ecological Significance of River Ecosystems**. Elsevier, 2022. p. 429-454. <https://doi.org/10.1016/B978-0-323-85045-2.00003-0>
- SHER, S.; REHMAN, A. Use of heavy metals resistant bacteria - a strategy for arsenic bioremediation. **Applied microbiology and biotechnology**, v. 103, p. 6007-6021, 2019. <https://doi.org/10.1007/s00253-019-09933-6>
- SINGH, S.; HIRANMAI, R. Y. Monitoring and molecular characterization of bacterial species in heavy metals contaminated roadside soil of selected region along NH 8A, Gujarat. **Heliyon**, v. 7, n. 11, e8284, 2021. <https://doi.org/10.1016/j.heliyon.2021.e08284>
- TAHER, A. M.; SAEED, I. O. Bioremediation of contaminated soil with crude oil using new genus and species of bacteria. **Journal of King Abdulaziz University: Marine Sciences**, v. 32, n. 2, e040027, 2022. <https://doi.org/10.1063/5.0094117>
- TAVARES, M.; KOZAK, M.; BALOLA, A.; SÁ-CORREIA, I. *Burkholderia cepacia* complex bacteria: a feared contamination risk in water-based pharmaceutical products. **Clinical Microbiology Reviews**, v. 33, n. 3, p. 1010-1128, 2020. <https://doi.org/10.1128/CMR.00139-19>
- TAYANG, A.; SONGACHAN, L. S. Microbial bioremediation of heavy metals. **Current Science**, v. 120, n. 6, e113891, 2021. <https://doi.org/10.2298/HEMIND200915010V>
- THAI, T. D.; LIM, W.; NA, D. Synthetic bacteria for the detection and bioremediation of heavy metals. **Frontiers in Bioengineering and Biotechnology**, v. 11, e1178680, 2023. <https://doi.org/10.3389/fbioe.2023.1178680>
- TURE, M.; CEBECI, A.; AITINOK, I.; AYGUR, E.; CALISKAN, N. Isolation and characterization of *Aeromonas hydrophila*-specific lytic bacteriophages. **Aquaculture**, v. 558, e738371, 2022. <https://doi.org/10.1016/j.aquaculture.2022.738371>
- YOUNIS, B. M.; SAEED, I. O. Concentration of heavy metals in soil contaminated with crude oil at two Iraqi sites according to environmental indices of pollution. **Nativa**, v. 11, n. 4, p. 558-565, 2023. <https://doi.org/10.31413/nat.v11i4.16521>

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