Evaluation of heavy metal content in water and removal of metals using native isolated bacterial strains

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Abstract. *Saleh LIF, Rashed RO, Muhammed SM. 2021. Evaluation of heavy metal content in water and removal of metals using native isolated bacterial strains. Biodiversitas 22: 3163-3174.* Natural surface water sources are susceptible to chemical contamination, including heavy metals. The goals of this study were to isolate naturally occurring heavy metal-tolerant bacteria by evaluating their ability to remove heavy metals from Tanjaro River and assessing the river's water quality. The potency of bacteria to remove heavy metals (Cd, Pb, Cu, Cr, Ni, Zn, Co, and Fe) from the medium was conducted by inductively coupled plasma-optical emission spectrometry. Results of physicochemical parameters of the water samples were as follows: temperature 11.9–31°C, pH 6.1–8.64, total dissolved solids 268–464 mg.l^{-1}, total hardness 232–485 mg.l^{-1}, and alkalinity 122–324.3 mg.l^{-1}. The overall mean concentrations of the heavy metals were in the order of Zn >Cu> Pb> Ni> Co> Fe> Cr> Cd), with values of 0.086, 0.073, 0.71, 0.068, 0.051, 0.056, 0.031, and 0.024ppm, respectively Moreover, the concentrations were generally exceeded the water quality criteria of WHO in some observations. The isolates included both gram-negative (56.5%) and gram-positive (43.5%) bacteria. The isolates displayed different degrees of resistance to heavy metals with maximum tolerable concentrations (MTCs) ranging from (10-250) ppm. *Bacillus safensis* and *Leucobacter chromiiresistens* were respectively able to tolerate (80, 90 ppm) Cd, (250, 160 ppm) Pb, (210,100 ppm) Cr, (110, 90 ppm) Ni and (160, 170 ppm) Co, while, *Proteus mirabilis* could tolerate 90 ppm Cd. The isolates were able to remove 55.4%Pb, 53.1%Cr, 53.7%Ni, 47.7Fe, 61.4%Co, and 55.8% Cu from a medium supplemented with the mixture of these heavy metals. The finding of this study indicated that Tanjaro River was undergoing frequent changes in water quality. Native bacterial isolates possessed considerable tolerance to selected heavy metals, which provides promising candidates for use in bioremediation.

Keywords: Bacteria, bioremediation, heavy metals, physicochemical parameter, tolerance, water quality

Abbreviations: Cd: cadmium; Co: cobalt; Cr: chromium; Cu: copper; Fe: iron; HCl: hydrochloric acid; HNO$_3$: nitric acid; ICP-OES: Inductively coupled plasma-optical emission spectrometry; L.B: Luria–Bertani; Ni: nickel; Pb: lead; SPSS: Statistical Package for the Social SciencesZn: zinc

INTRODUCTION

Chemical pollution monitoring of surface water helps to determine the level of environmental risk associated with pollutants that are toxic to aquatic organisms and enables the evaluation of their accumulation in the ecosystem (Michalec et al. 2014), since there are no sufficient treatment facilities for the treatment of municipal and industrial waste, effluents are directly discharged into water bodies, causing surface water contamination and reducing water quality (Bui et al. 2016). According to recent studies, the long-term usage of untreated wastewater from industrial sources can degrade water quality and rendering it unsafe for human consumption (Kapahi and Sachdeva 2019). Dumping a significant amount of industrial and household pollutants into rivers make considerable stress on the river's physicochemical and microbiological content (Haque et al. 2019). Industrial effluent containing heavy metals poses a health risk to plants, animals, humans, and aquatic life (Marzan et al. 2017), they not only produce toxic or chronic poisoning in aquatic lives but also pose threat to the environment (Ma et al. 2020). Heavy metals

are naturally occurring elements that have a relatively high atomic weight and density (greater than 5 g.cm^{-3}) compared to water (Afzal et al. 2017). The term heavy metal is collectively used for certain categories of metals, metalloids, lanthanides, and actinides. These compounds can enter natural waters from various sources. Some heavy metals are present at low concentrations but are biologically significant in the aquatic environment, while others pose a critical risk to human health and the environment due to their widespread occurrence as contaminants, accumulation in biota, their carcinogenic, mutagenic, and non-biodegradable nature, and their ability to persist in the environment (Rahman and Singh 2016). The toxicity of metal ions are resulting from their competition with or replacement of a functional metal can cause conformational changes, denaturation and deactivation of enzymes, and the destruction of cell and organelle integrity (El-Shanshoury et al. 2013), they have a variety of negative impacts on biological processes, most of which are related to metal species, solubility, and concentration, effluent properties such as pH, the presence and concentration of other ions and suspended particles

(Bestawy et al. 2013). An increased pollutant load in freshwater increases the nutrient level in the water and can alter the pH and other physicochemical properties of water bodies (Chaurasia and Tiwari 2001). These surface water alterations act as a selective force on bacterial communities, causing them to develop resistance to heavy metals and antibiotics (Aktan et al. 2012). Recently, heavy metal bioremediation using microorganisms has received increasing attention; they can be used for metal remediation by removing, concentrating, and recovering metals from contaminated sites (Irawati et al. 2017). Microorganisms and microbial products can be effective bioaccumulation of metals in both soluble and particulate forms (Shukla et al. 2017). Several microorganisms have developed detoxification and respiration mechanisms that use heavy metals and are thus resistant to them (Marzan et al. 2017). Long-term metal exposure is hypothesized to impose selection pressure on bacteria, favoring the development of tolerable microorganisms with mechanisms to resist heavy metals through efflux, complexation, and reduction of metal ions (Jamil et al 2018). The ability of microorganisms to detoxify metal contamination can be used for bioremediation; isolation and characterization of bacteria from metal-contaminated environments should be carried out in order to identify metal-resistant strain candidates that could be used for heavy metal removal and bioremediation (Rajbanshi 2008). Therefore, the aim of this study was to isolate, molecularly characterize, and analyze the bioremediation potential of heavy metal-tolerant bacteria isolates, as well as determine heavy metal concentrations in water samples from the Tanjaro River.

MATERIALS AND METHODS

Study area

The study was carried out along the Tanjaro River, which is a permanent river situated 7 km southwest of Sulaimani City (Sulaimaniyah), Sulaimani Governorate, Kurdistan Regions, Iraq. The river is created by linking two major streams Kani-Ban and Qiliasan with other small tributaries crossing many urban and agricultural regions (Mustafa 2006). It is used as a source for irrigation and livestock consumption purposes. Finally, it contributes to the Darbandikhan dam, which serves as a supply of drinkable water for the Darbandikhan district, as well as a source of fish, tourism, and hydropower (Rashid 2010). The sources of Tanjaro river pollution are black water and residual materials from motor oil recycling, hospitals, industry, and agriculture run directly into the Tanjaro River through a combined sewerage system (Othman et al 2017; Aziz et al. 2012). Tanjaro river pollution leads to evanescence of rice cultivation and the extinction of fisheries in the River (Rasheed and HamaKarim 2017). The sample locations have geographical coordinates of 35 ° 16 '35 " N 45 ° 51 '9 " E, as shown in (Figure 1).
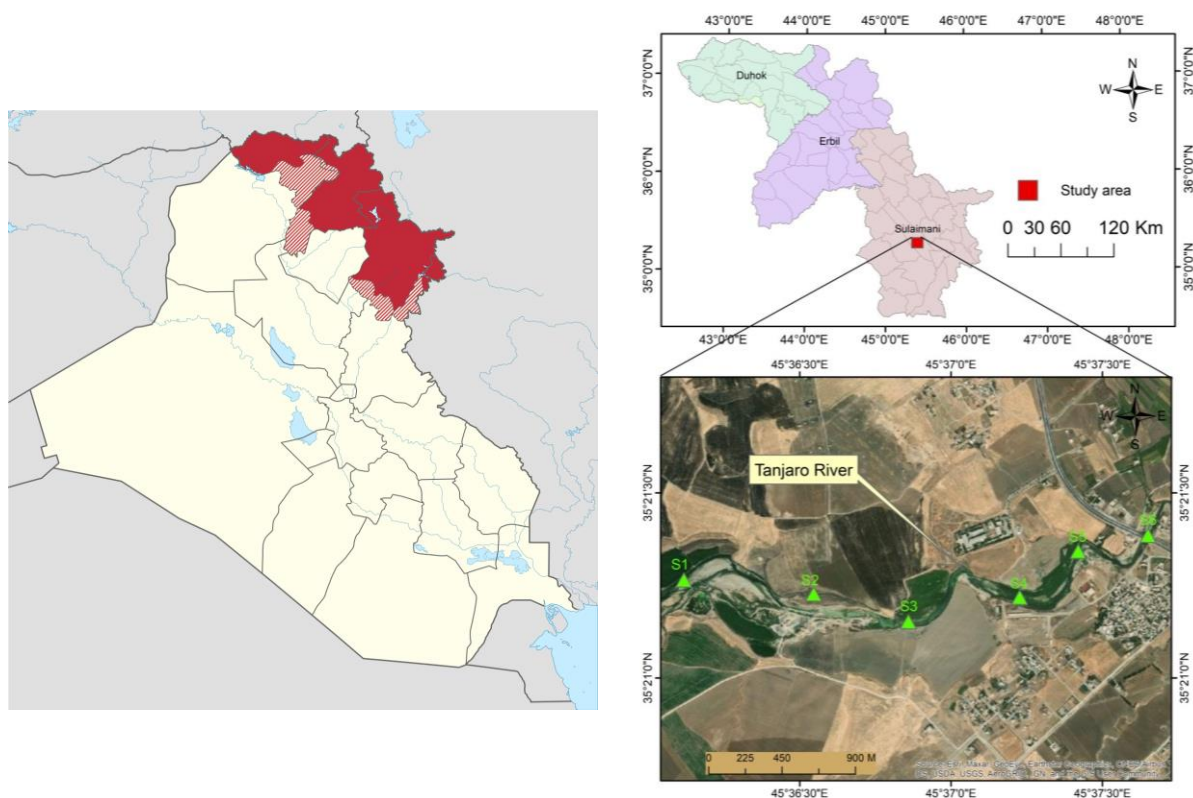

Figure 1. Study area located along the Tanjaro River in Sulaimani Governorate, Kurdistan Regions, F.R. Iraq

Table 1. List of sampling sites and their geographical specification

Coordinates	Sites
35.35444N 45.601955E	S1
35.353762 N 45.609093E	S2
35.352564 N 45.614319E	S3
35.352344 N 45.620424E	S4
35.356574 N 45.622611E	S5
35.356431N 45.627525 E	S6

Sample collection and preparation

A total of 54 water samples were collected over 9 months from January to October 2019. Each month, three replicates of each sample were collected from six sampling sites along the river, designated as S1 to S6 (Table 1). All water samples were analyzed monthly for physicochemical parameters and once per season, the water samples were analyzed for heavy metals and bacterial detection. The sampling, preservation, and transfer of the water samples were conducted according to standard procedures. The acid digestion method was performed for heavy metal detection. The water samples were digested by adding 2 mL of 1:1 HNO_3 and 10 mL of 1:1 HCl then heated until the volume decreased to 25 mL. The volume was then adjusted to 100 mL by adding double-distilled water. The analysis was conducted using an Optima 7300V inductively coupled plasma-optical emission spectrometer (ICP-OES), as described by (APHA 2011).

Physicochemical analysis

Water temperature (T), pH, electrical conductivity (EC), and total dissolved solids (TDS) were analyzed in situ with a portable water quality tester, while total hardness and alkalinity were analyzed according to standard methods (Bartram and Balance 1996).

Preparation of metal solutions

The metals (Cd, Pb, Cu, Cr, Ni, Zn, Co, and Fe) were used as $CdSO_4 \cdot 4H_2O$, $Pb(CH_3COO)_2 \cdot 3H_2O$, $CuSO_4$, $K_2Cr_2O_7$, $Ni(NO_3)_2 \cdot 4H_2O$, $Zn(CH_3CO_2)_2$, $CoCl_3 \cdot 6H_2O$, and $FeCl_3$, respectively. Stock solutions (1000 ppm) were prepared by dissolving metal salts in distilled water. The metallic salts were of analytical grade, and the stock solutions were filter-sterilized with 0.22 μm pore size Millipore membranes and added to 45°C sterilized medium (Silva et al. 2012).

Primary screening of heavy metal-resistant bacteria

Aseptically collected water samples were serially diluted and inoculated on L.B agar medium separately supplemented with 10 ppm of various heavy metal salts ($CdSO_4 \cdot 4H_2O$, $Pb(CH_3COO)_2 \cdot 3H_2O$, $CuSO_4$, $K_2Cr_2O_7$, $Ni(NO_3)_2 \cdot 4H_2O$, $Zn(CH_3CO_2)_2$, $CoCl_3 \cdot 6H_2O$, and $FeCl_3$). The cultures were incubated at 37°C for 24–48 hours. Colonies were selected for further isolation to obtain single colonies. Gram staining was used to examine the morphology of the bacterial cells under a light microscope (Aktan et al. 2012; Ndeddy and Babalola 2017).

Determination of maximum tolerable concentration of heavy metals

The maximum tolerable concentration to eight selected metal salts was carried out using the 96-well microtiter plate method. Bacterial isolates were precultured for 24 hr in liquid L.B medium at 37°C and 120 rpm until reaching an optical density of 0.6 at 600 nm. Next, 50 μl of the preculture was added to 150 μl of LB broth containing 20 ppm of a heavy metal compound as starter. The mixture was transferred into a 96-well microplate and incubated at 37°C for 48 hr, at which point the maximum tolerable concentration (MTC) was determined using a microplate reader. The MTC was defined as the highest concentration of heavy metal that allowed growth after 2 days. Strains showing the highest tolerance to each heavy metal were tested for their heavy metal removal efficacy as described by (Cai et al. 2019; Sultan et al. 2020) with some modifications.

Molecular diagnostic assay

PCR amplification and 16S rRNA sequencing

Bacterial genomic DNA of the isolates was extracted using a Presto mini gDNA extraction kit (Geneaid Biotech Ltd) according to the manufacturer's instructions. PCR analysis was performed with a Mega Cycler PCR using universal bacterial 16S rDNA primers from (Satokari et al. 2001): 7F (5′AGAGTTTGATYMTGGCTCAG-3′) and 1015R (5′ACGGYTACCTTGTTACGACTT-3′) The PCR program was: initial denaturation temperature of 95°C for 5 min, followed by 35 cycles of 95°C for 30 sec, 60°C for 30 sec, 72°C for 3 min, a final extension temperature of 72°C for 5 min, and a 4°C hold. DNA sequencing was performed by the Sanger method using a 3500xl Genetic Analyzer (Applied Biosystems).

Determining of heavy metal removal efficacy

The heavy metal removal potential of the tolerant bacteria was assessed in a batch experiment process. A 500 mL bottle containing 200 mL of L.B broth was prepared separately for each metal (Cd, Pb, Cu, Cr, Ni, Zn, Co, and Fe) and inoculated with 2 mL of 18-h old bacterial culture with OD600 of 0.6. The cell culture was incubated at 37°C and 120 rpm for 24 hr. The culture was then centrifuged (Sigma S-16P) at 5000 rpm for 20 min at 4°C. The supernatant was digested with HNO_3 at 100°C. Heavy metal concentrations in the medium before bacteria inoculation and after the 24 h culturing were detected by ICP-OES (Optima 7300 V). The same treatment without the inoculation of bacterial strains was used as a control for each heavy metal. The results were compared with the control to calculate the heavy metal remediation capacity (%) as follows:

% of heavy metal utilized = Heavy metal utilized / Heavy metal added to the L.B broth ppm ×100

Heavy metal utilized = Heavy metal added to the LB broth – Heavy metal remaining at the end of culture (Afzal et al. 2017; Marzan et al. 2017).

Statistical analysis

The SPSS program (Statistical Package for the Social Sciences) version 23 was used to statistically analyze the results. The analyzed parameters were processed using the variance method (ANOVA) followed by Duncan's test to determine the existence or absence of significant differences between sample events (Ravanbakhsh et al. 2009).

RESULTS AND DISCUSSION

Distribution of physicochemical parameters

The physicochemical parameters (temperature, pH, EC, TDS, alkalinity, and hardness) of the water samples measured during the study period on monthly basis are shown in (Tables 2 and 3), while the heavy metal concentrations (Cd, Pb, Cu, Cr, Ni, Zn, Co, and Fe) in the water samples are given in (Figure 2).

The lowest water temperature was 11.9°C and was recorded in January 2019 at S1, while the highest was 31°C in August at S1 and S6; the yearly mean temperature was 11.2°C. The pH values of the river water ranged from 6.3 to 8.64; the minimum value was recorded at S6 in October, while the maximum was recorded at S2 in August. The results indicated that there were only significant differences among studied sits at ($p < 0.05$). The conductivity of water is affected by suspended matter and also depends upon the amount of ions in the water. The lowest conductivity was 525 $\mu S.cm^{-1}$ at 25°C and was observed in February. From February onwards, the conductivity increased and reach its highest value of 928 $\mu S.cm^{-1}$ at 25°C in September. Conductivity is strongly related to the amount of TDS,

which was confirmed by observing a maximum TDS of 464 mg/l in September. The lowest total hardness value was 232 $mg.l^{-1}$, recorded in April, while the highest was 485 $mg.l^{-1}$ recorded in June at S2. During January and February, the water alkalinity was lower than the permissible level (200 $mg.l^{-1}$). After February, the alkalinity increased to exceed the permissible level. The chloride concentration during the study period ranged from 13.2–77.9 $mg.l^{-1}$.

Metal content of the water samples

The ICP-OES results of heavy metal are shown in (Figure 2). Among the analyzed heavy metals, Zn ions had the highest concentration, while Cd and Cr ions had the lowest concentrations as in the follows order : Zn > Cu > Pb > Ni > Co > Fe > Cr > Cd, with maximum concentrations of 0.086, 0.073, 0.71, 0.068, 0.051, 0.056, 0.031, and 0.024 ppm, respectively.

Isolation of heavy metal-resistant bacteria

During the initial screening process of the Tanjaro River water samples, 200 metal-resistant colonies were observed. After further purification, 39 metal-resistant bacteria that could tolerate heavy metal-containing LB agar were selected for further characterization. These heavy metal-resistant strains included both gram-negative and gram-positive bacteria. Gram staining identified 17 (43.5%) isolates as gram-positive, while the other 22 (56.5%) were gram-negative. The nearest identities of all bacterial isolates and their gram stain properties are presented in (Table 4).
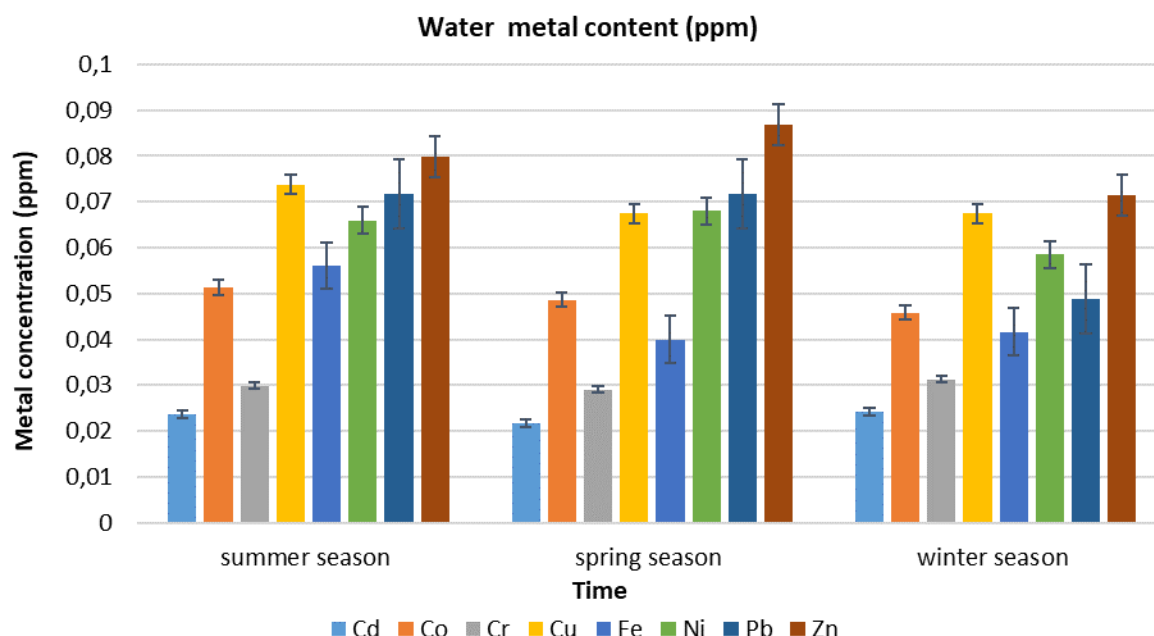

Figure 2. Heavy metal content (ppm) in the water samples

Table 2. Water samples in situ measured parameters during study period

Months/sites	Temperature						PH						EC (µS.cm⁻¹)						Total dissolved solids(mg.l⁻¹)					
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Jan	11.9	12.1	12.1	12.3	12.5	14.3	7.32	7.3	7.34	7.33	7.18	7.16	583	580	576	570	570	589	291	290	289	278	286	295
Feb	15.4	15.4	16.1	16.6	15.8	15	7.28	7.28	7.3	7.2	7.35	7.35	560	562	556	556	555	525	280	280	278	278	277	268
Apr	19	19.9	19.9	20.3	2.3	20.6	6.88	6.94	7.01	7.14	7.23	7.22	555	674	740	804	779	601	272	330	364	397	390	300
May	21.1	21.1	21.5	21.4	22.2	21.4	7.2	7.2	7.27	7.36	7.31	7.27	563	563	567	660	568	559	281	281	283	330	384	279
Jun	26.6	27.3	27.9	28	27.8	25.7	7.4	7.2	7.19	7.1	6.9	7	687	678	657	673	686	693	344	339	328	336	339	348
Jul	30	30	30.1	29	29	30	7.1	7.04	6.8	6.7	6.7	6.5	699	706	702	700	704	706	350	350	346	350	351	350
Aug	31	30	30	30	30.6	31	8.39	8.64	8.54	8.35	8.26	8.39	745	571	748	750	739	745	372	375	374	370	370	372
Sep	27.9	27	36	27.5	28.2	28	6.8	6.9	6.7	6.3	6.8	6.6	928	922	796	794	794	775	464	461	398	397	397	400
Oct	24	24	29	24	25	24	7.1	7.5	6.5	6.3	6.5	6.1	796	794	794	794	922	928	398	397	397	396	461	464
Mean	22.9	23.03	23.3	23.3	23.3	23.3	7.28	7.33	7.18	7.08	7.14	7.07	679.5	692.5	681.7	699.8	701	680.1	339.1	345	339.6	348	361	341.7
±SD	± 1.5	± 1.4	± 1.45	± 1.38	± 1.43	± 1.38	± 0.1	± 0.12	± 0.13	± 0.14	± 0.11	± 0.14	± 29.4	± 27.1	± 22	± 21.4	± 28.2	± 29	± 14.9	± 13.6	± 10.9	± 10.8	± 13	± 14.6

Table 3. Water samples laboratory-measured parameters during study period

	Total hardness (mg.l⁻¹)						Alkalinity (mg.l⁻¹)						Chloride (mg.l⁻¹)					
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Jan	269	282	304	305	285	332	126	122	124	140	126	181	29.9	31	31.6	34	35.5	42.5
Feb	300	258	278	264	248	257.5	127	158	156	129	156	138	21.6	17.7	16.6	17.3	17	21.9
Apr	294	300	264	306	232	266	229	260	225	245	231	218	27.5	36.5	29.4	13.2	21.5	25.7
May	415	355	430	410	485	380	207	208	197	209	184	216.2	24.4	26.1	29	47.2	26.1	43.9
Jun	430	485	310	410	391	390	235.2	228.9	203.5	227.3	209.8	241.6	57.4	58.1	53.88	48.2	51.04	59.5
Jul	322	342	310	371	380	308	205.1	198.7	201.9	208.2	186	203.5	57.4	58.1	53.8	48.2	51	56.7
Aug	364	314	280	326	354	360	241.6	197.1	219.4	240.09	251.2	238.5	75.8	64.5	62.3	67.4	65.9	75.1
Sep	354	368	358	338	322	352	303.6	255.9	248	249.6	287.2	320	73.7	76.5	78.6	76.5	72.3	72
Oct	356	340	324	345	364	320	254.4	244.8	254.4	262.5	298.9	324.3	77.9	76.5	73.7	74	75.1	72.3
Mean ±SD	344.8	338.2	317.8	341.6	340.1	329.5	214.3	213.4	204.1	212.1	210.2	231.2	49.5	49.5	47.7	47.2	45.9	52.2
	± 54.5	± 63.8	± 49.7	± 48.07	± 76.3	± 45.4	± 60.1	± 52.6	± 41.6	± 46.2	± 51.9	± 58.4	± 23.04	± 21.3	± 21.4	± 22.4	± 21.7	± 19.3

Table 4. 16S rRNA identity of bacterial isolates

Code	Bacterial isolates	GenBank acc. no.	Gram reaction
1.	*Bacillus pumilus* strain BP01L	MZ447081	+
2.	*Enterococcus faecalis*-EF02I	MZ447082	+
3.	*Microbacterium maritypicum*-MM03F	MZ447083	+
4.	*Bacillus cereus*-BC04I	MZ447084	+
5.	*Enterococcus gallinarum*-EG05I	MZ447085	+
6.	*Proteus vulgaris*-PV06T	MZ447086	-
7.	*Providencia vermicola*-PV07T	MZ447087	-
8.	*Escherichia fergusonii*-EF08T	MZ447088	-
9.	*Klebsiella quasipneumoniae*-KQ09T	MZ447089	-
10.	*Acinetobacter junii*-AJ10T	MZ447090	-
11.	*Morganella morganii*-MM11T	MZ447091	-
12.	*Pseudomonas aeruginosa*-PA12T	MZ447092	-
13.	*Pseudomonas.aeruginosa*-PA13T	MZ447093	-
14.	*Bacillus cereus*-BC14L	MZ447094	+
15.	*Leucobacter chromiiresistens*-LC15T	MZ447095	+
16.	*Bacillus safensis*-BS16L	MZ447096	+
17.	*Raoultella planticola*-RP17T	MZ447097	-
18.	*Proteus mirabilis*-PM18T	MZ447098	-
19.	*Lysinibacillus fusiformis*-LF19T	MZ447099	+
20.	*Bacillus tropicus*-BT20L	MZ447100	+
21.	*Bacillus zhangzhouensis*-BZH21L	MZ447101	+
22.	*Bacillus zhangzhouensis*-BZH22L	MZ447102	+
23.	*Bacillus safensis*-BS23L	MZ447103	+
24.	*Acinetobacter junii*-AJ24T	MZ447104	-
25.	*Morganella morganii*-MM25T	MZ447105	+
26.	*Pseudomonas taiwanensis*-PT26T	MZ447106	-
27.	*Pseudomonas plecoglossicida*-PP27T	MZ447107	-
28.	*Enterococcus faecalis*-EF28I	MZ447108	-
29.	*Enterobacter tabaci*-ET29T	MZ447109	-
30.	*Enterobacter tabaci*-ET30T	MZ447110	-
31.	*Aeromonas caviae*-AC31T	MZ447111	-
32.	*Microbacterium oxidanse*-MO32I	MZ447112	+
33.	*Pseudomonas aeruginosa*-PA33T	MZ447113	-
34.	*Proteus mirabilis*-PM34T	MZ447114	-
35.	*Enterobacter tabaci*-ET35	MZ447115	-
36.	*Aeromonas caviae*-AC36T	MZ447116	-
37.	*Proteus vulgaris*-PV37T	MZ447117	-
38.	*Bacillus zhangzhouensis*-BZH38L	MZ447118	+
39.	*Bacillus safensis*-BS39L	MZ447119	+

Molecular characterization of metal-resistant isolates

Amplification of the 16S rRNA genes was performed using universal primers. The 16S rRNA gene sequence of each isolated strain (those which showed metal resistance) were searched in the National Center for Biotechnology Information (NCBI) database. Blast queries revealed that the strains belonged to the Bacillaceae, Moraxellaceae, Morganellaceae, Enterococcaceae, Microbacteriaceae, Enterobacteriaceae, Pseudomonadaceae, and Aeromonadaceae families.

Assessment of heavy metal tolerance

The bacteria were evaluated for their ability to resist different concentrations of heavy metals. The MTCs of the bacterial isolates against the tested metal salts are summarized in (Table 5). The MTCs varied from 10–250 ppm. Among the heavy metals, cadmium and copper were highly toxic, while, lead and iron were less toxic to all

strains. All of the detected isolates were resistant to selected metals, but they exhibited different levels of resistance. The isolates presented a diverse metal-resistant phenotype and were resistant to one or more metal ions. *B. safensis*-BS16L and *L.chromiiresistens*-C15T were respectively able to tolerate high Cd (80, 90), Pb (250, 160), Cr (210, 100), Ni (110, 90) and Co (160, 170) concentrations (all values in ppm). In addition, *P. mirabilis*-PM18T could tolerate 90 ppm Cd. The highest levels of resistance were detected for Pb and Fe in all isolates. Although Zn had the highest concentration in the water samples, most of the isolates showed low levels of Zn tolerance.

Heavy metal removal efficacy

The ability of the bacterial isolates to remove heavy metals from the medium was measured by ICP-OES. *P. plecoglossicida*-PP27T and *E. gallinarum*-EG05I removed the highest amount of cadmium (41.9% and 41.1%, respectively), as shown in (Figure 4). *B. safensis*-BS16L removed the highest amount of Pb, Cr, Ni, Fe, and Co (55.4%, 53.1%, 53.7%, 47.7%, and 61.4%, respectively) (Figures 3, 4, and 5). *Enterobacter tabaci*-ET29T removed the highest amount of Cu (55.8%). Among the metals, zinc had the lowest amount of removal, which did not exceed 29.3% (Figure 4).
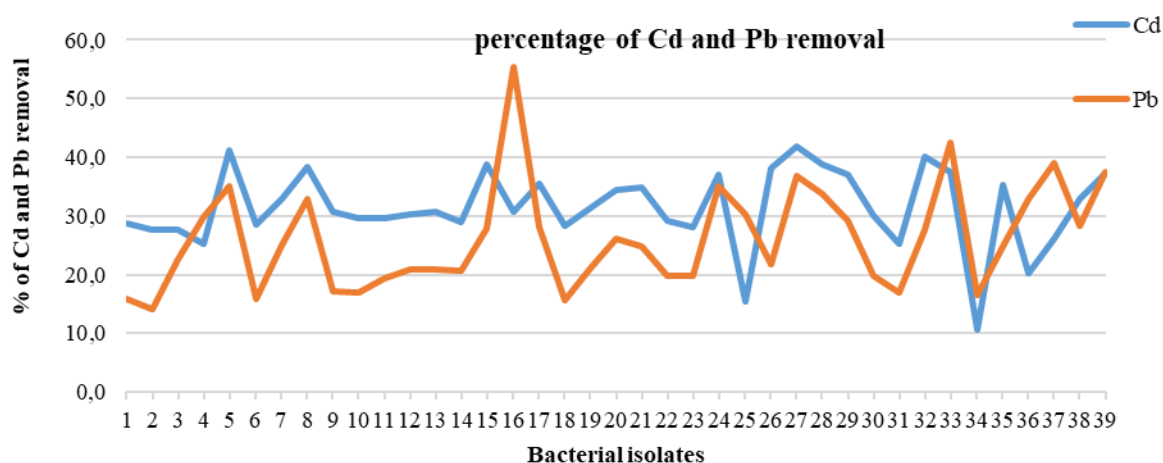
Discussion
Physicochemical characteristics of the water samples

We used a simple portable water quality tester to perform in situ analysis of the water temperature, pH, EC, and TDS. The water temperature is an important factor that affects the concentrations of several inorganic chemical contaminants (WHO 2011) and directly or indirectly influences the biological species that can survive in a given aquatic environment (Iram et al. 2013). No abnormal water temperatures were recorded for the water samples. Statistical analysis indicated that there was only significant variation among different months in the studied area ($P<0.05$). It appears that the coldest temperature value was recorded in January, while the hottest one was during August, similar temperature ranges have been previously documented by (Mustafa 2006).

The water pH is important in assessing water quality because it affects other chemical parameters such as solubility and metal toxicity (Al-Taei et al. 2020). The pH range of surface water according to (WHO 2011) is 6.5–8.5 where keeps most trace elements immobilized. Most of the samples had pH values within this range except samples from S2 and S6. The pH was highest in the sample from S2 (8.64), which is slightly higher than the WHO recommended range for surface water. The pH values of samples collected in August were higher than in the other months, likely because of reduced rainfall and river volume during this time (Besharati et al. 2018), while Mohsenpour et al. (2021) suggested that the elevation in pH level could result from an increase in both photosynthetic activity and sewage disposal with high detergent concentrations.

Table 5. Heavy metal maximum tolerable concentrations (MTCs) of the bacteria isolates

Code	Bacterial isolates	Metal concentration in ppm							
		Cd	Pb	Cu	Cr	Ni	Zn	Co	Fe
1	*Bacillus pumilus* strain BP01L	30	120	40	60	70	50	60	170
2	*Enterococcus faecalis*-EF02I	30	140	30	60	70	30	60	170
3	*Microbacterium maritypicum*-MM03F	20	130	40	60	70	40	60	150
4	*Bacillus cereus*-BC04I	30	130	40	60	70	40	60	150
5	*Enterococcus gallinarum*-EG05I	40	130	40	40	70	40	60	160
6	*Proteus vulgaris*-PV06T	50	100	10	80	40	20	30	150
7	*Providencia vermicola*-PV07T	30	140	40	60	80	50	110	180
8	*Escherichia fergusonii*-EF08T	30	140	60	60	80	60	60	170
9	*Klebsiella quasipneumoniae*-KQ09T	30	130	40	40	70	50	130	160
10	*Acinetobacter junii*-AJ10T	30	140	50	50	70	60	110	160
11	*Morganella morganii*-MM11T	30	140	30	50	60	40	60	160
12	*Pseudomonas aeruginosa*-PA12T	40	130	40	60	70	40	60	140
13	*Pseudomonas.aeruginosa*-PA13T	50	130	40	30	70	60	60	160
14	*Bacillus cereus*-BC14L	20	130	40	60	70	30	20	140
15	*Leucobacter chromiiresistens*-LC15T	90	160	50	100	90	50	170	150
16	*Bacillus safensis*-BS16L	80	250	80	210	110	60	160	250
17	*Raoultella planticola*-RP17T	50	130	40	40	70	50	50	150
18	*Proteus mirabilis*-PM18T	90	130	40	40	70	50	60	150
19	*Lysinibacillus fusiformis*-LF19T	30	130	40	40	70	30	60	150
20	*Bacillus tropicus*-BT20L	30	130	40	30	70	40	30	140
21	*Bacillus zhangzhouensis*-BZH21L	20	120	30	30	70	30	20	150
22	*Bacillus zhangzhouensis*-BZH22L	30	120	20	30	60	20	10	150
23	*Bacillus safensis*-BS23L	40	120	20	30	50	30	40	140
24	*Acinetobacter junii*-AJ24T	40	130	40	30	70	10	10	150
25	*Morganella morganii*-MM25T	40	120	40	30	80	10	80	140
26	*Pseudomonas taiwanensis*-PT26T	40	120	30	100	90	10	80	115
27	*Pseudomonas plecoglossicida*-PP27T	30	130	40	30	70	50	50	150
28	*Enterococcus faecalis*-EF28I	50	120	20	30	40	10	80	140
29	*Enterobacter tabaci*-ET29T	50	130	70	160	90	60	90	260
30	*Enterobacter tabaci*-ET30T	40	130	60	140	60	60	70	170
31	*Aeromonas caviae*-AC31T	50	120	30	100	60	20	50	140
32	*Microbacterium oxidanse*-MO32I	30	120	40	30	70	30	30	140
33	*Pseudomonas aeruginosa*-PA33T	20	130	40	40	70	50	70	160
34	*Proteus mirabilis*-PM34T	40	150	70	80	90	50	30	160
35	*Enterobacter tabaci*-ET35	40	140	50	60	80	60	30	170
36	*Aeromonas caviae*-AC36T	40	150	60	60	80	50	70	150
37	*Proteus vulgaris*-PV37T	40	120	30	30	60	30	40	150
38	*Bacillus zhangzhouensis*-BZH38L	20	120	20	50	80	50	30	150
39	*Bacillus safensis*-BS39L	20	150	60	70	90	50	70	170

Figure 3. Percentage of cadmium and lead removed by isolated bacteria

The ideal pH for bacterial growth is usually between 6.5 and 7.5. The electrical conductivity started from 525 μS.cm^{-1}at 25°C in February which was relatively lower than that recorded by (Rashid 2010) but higher than the results of (Mustafa 2006), then increased as the study period progressed, reaching 928 μS.cm^{-1} at 25°C in September. This may have been due to the changing climate and the increasing temperature, as found by (Al-Ameri 2011). The conductivity is highly dependent on the amount of dissolved solids (such as salt), particulate mobility, and temperature (APHA 2005), and this was confirmed by observing the maximum level of dissolved solids in September, the presence of high concentration of dissolved solid elements could affect water density, freshwater species' osmoregulation, reduces the solubility of gases, and limits the use of water for drinking and irrigation (Azeez 2021), as the water from Tanjaro River is used for irrigation, much of the water will be taken up by the crop and transpired; proportion of the salts will be left behind in the soil and lead to build-up of salts in the root zone of the crop (Rashid 2010). Dissolved calcium and, to a lesser degree, magnesium, which is expressed as an equal amount of calcium carbonate, causes water hardness (WHO 2011). Tanjaro River has hard water according to WHO guidelines, our recorded data exceeded the 200 mg.l^{-1} WHO maximum recommendation. (Ebrahimpour et al. 2010) stated that water hardness affects the solubility and toxicity of heavy metals. Metals are more toxic in soft water than in hard water because their solubility increases with the decreasing of water hardness, heavy metal concentration obtained by (Al-Asadi et al. 2020) is lower than those obtained in our study with higher levels of calcium and magnesium hardness, In our study, the heavy metal concentrations decreased in spring, during which higher levels of hardness were recorded and this confirms the finding of (Aziz et al. 2012) that shows the decrease in metal toxicity with the increasing of water hardness. Water samples had alkalinity values higher than the acceptable level for freshwater 200 mg.l^{-1} (WHO 2006). Throughout the study period, the chloride concentrations were less than the maximum WHO recommended value, The amount of chloride in the water possibly results from the excessive use of chloride as a disinfectant in various water purification and the industrial wastes discharged into the river (Mustafa 2006; Rashid 2010).

Metal content of the water samples

The Tanjaro River is contaminated with heavy metals, wastewater effluent and municipal sewage outlets of the areas and industrial area, Albisaka, Qalawa, Wluba, Shekh-Abbas and Bakrajo boxes are discharged directly to Tanjaro River without any pretreatment that leading to heavy metal accumulation (Majid et al. 2018).

Several variations in heavy metal concentrations were observed between sampling sites. The hydrological formation of the sampling site had a notable impact on water quality. The statistical analysis also revealed that changes in the metal concentrations were primarily influenced by the time of year (Saran et al. 2012). Cd, Pb, and Ni were present in high concentrations in the water samples, while Co, Cu, Cr, Fe and Zn were found within the normal range of (EPA 2003; WHO 2011). The results were lower than those observed by (Mustafa 2006; Rashid 2010) but higher than those obtained by (Rasheed and HamaKarim 2017) at the same river, and those obtained by (Hamdan 2010; Al-Abbawy et al. 2021) at both Shatt-Alarab and Al-Hawizeh Marsh, southern of Iraq, the low observation may be attributed to the fact that most factories stopped operating during their study period. The continuous use of contaminated water for irrigation may cause the accumulation of metals to concentrations that are toxic for plants and animals (Iram et al. 2013).

Isolation of heavy metal-resistant bacteria

Several previous studies indicate that the toxic effect of metal ions exert selection pressure on bacteria whereby those that are resistant to these metals survive (Mustapha and Halimoon 2015). Overall, 39 bacterial strains were able to grow on heavy metal-spiked L.B agar. Gram staining revealed the presence of both gram-positive and gram-negative bacteria. This indicates that both types of bacteria are involved in metal removal; however, a predominance of gram-negative bacteria strains was found among the heavy metal-tolerant strains isolated from the Tanjaro River for all studied metals, which is in accordance with previous findings of (Bennisse et al. 2004; Irawati et al. 2017), which found that the majority of isolates subjected to selection pressures in the presence of toxic compounds were gram-negative. However, another study found different results (Silva et al. 2012). It has been proposed that the cell wall of gram-negative bacteria is an effective barrier against toxic metals and that the cell wall's surface structures interact with metal ions, resulting in their detoxification. By contrast, the peptidoglycan cell wall of gram-positive bacteria absorbs contaminants, overloading the bacterial cell and destroying it (Alegbeleye et al. 2017). The majority of the isolated bacteria belonged to the Bacillaceae and Enterobacteriaceae families (11 species for each family), which is similar to the results obtained by (Besharati et al. 2018; Cai et al. 2019). However, a predominance of Proteobacteria was reported by (Karelova et al. 2011).
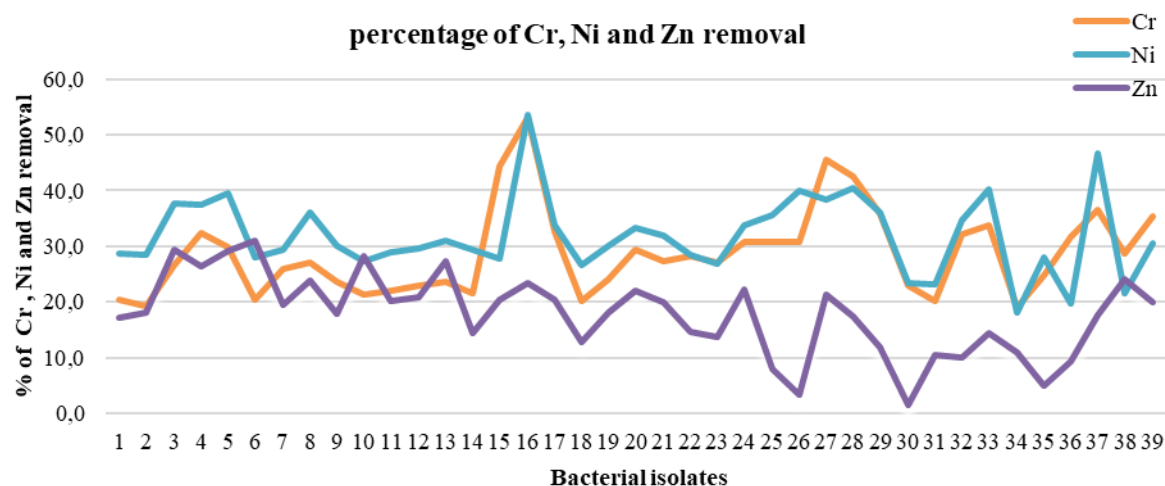
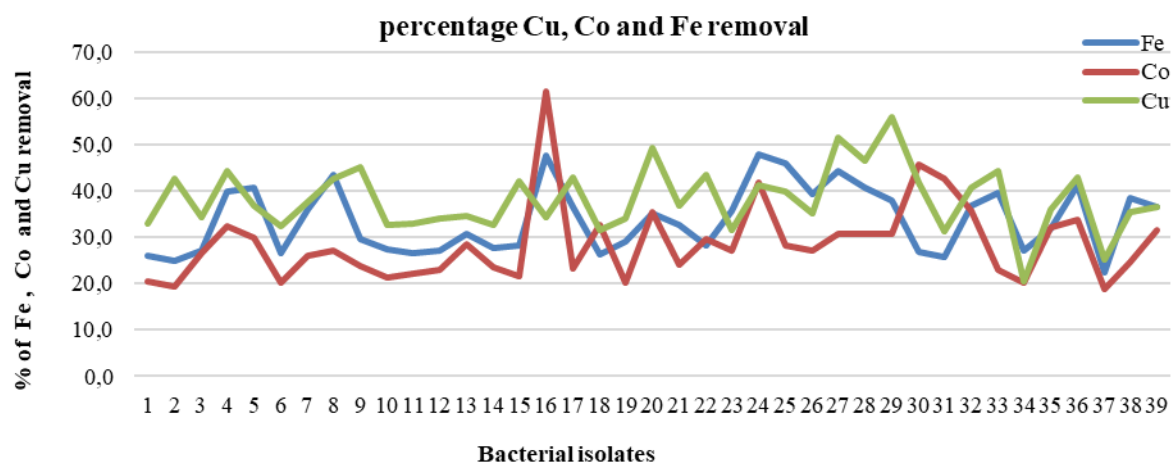**Figure 4.** Percentage of chromium, nickel, and zinc removed by isolated bacteria

Figure 5. Percentage of iron, cobalt, and copper removed by isolated bacteria

Bacterial tolerance to heavy metal salts

The maximum tolerable concentrations of heavy metals among the isolates are summarized in (Table 5). The isolated strains showed different patterns of resistance. This variation in metal tolerance might be due to the presence of different tolerance mechanisms (Irawati et al. 2017). *B. safensis* strain NBRC and *L. chromiiresistens* strain JG showed the highest heavy metal tolerance and were resistant to heavy metals in the order of Pb > Cr > Co > Ni; they were also resistant to higher concentrations than those recorded by (Mandal et al. 2020). Among the investigated heavy metals, iron was the least toxic, whereas cadmium, copper, and zinc were highly toxic to all strains. Similar results were found by (Afzal et al. 2017). The isolates identified in the current study were resistant to high levels of Pb (approximately 250 ppm). This may be attributed to the site where the water samples were taken being polluted with high levels of lead. Othman (2017) stated that lead is one of the heavy metals of special concern in Iraqi Kurdistan because of a number of emission sources, including low-quality petrol, widespread use of leaded paints in industry, unsafe disposal of car batteries and other batteries and lead products into water sources, while (Mustafa 2006) revealed that besides the pollution from sewages, Sulaimani oil refinery wastes are the second most significant source of (pb) pollution in Tanjaro river. This high level of Pb potentially allowing a diverse range of bacteria to adapt to the environment, either through convergent evolution of resistance mechanisms or through plasmid-based transmission of resistance genes. A similar finding was obtained by (Gummersheimer and Giblin 2003). Resistance mechanisms can be encoded in plasmid genes, facilitating the transfer of toxic metal resistance factors from one cell to another. Because heavy metals cannot be degraded or destroyed, their introduction into the environment in various forms can cause significant changes in microbial communities and their activities, compromising their ability to survive (Samanta et al. 2012).

High bacterial metal tolerance is an important factor to be considered for the remediation of heavy metals because it is directly related to the survival and growth of bacteria in metal-contaminated environments (Kang et al. 2016). Generally, the ability of microbes to grow in environments with high metal concentrations is linked to several complex resistance mechanisms and environmental factors, such as microbial surface sorption, enzymatic transformation, precipitation by oxidation/reduction reactions, and biosynthesis of metal-binding proteins or extracellular polymers (Srinath et al. 2002).

Heavy metal removal efficacy

Some microorganisms have evolved detoxification mechanisms to counter the toxic effects of heavy metals (Mustapha and Halimoon 2015). Many microorganisms produce a variety of metabolic enzymes that can safely remove contaminants. This can be achieved either by directly destroying the contaminant or transforming it into a less toxic intermediate (Saran et al. 2012). In this study, we have demonstrated the potential for microbes to be used to remediate sites with metal contamination by removing metals from the contaminated medium. Several metal-tolerant bacterial species were able to remove heavy metals at various concentrations. *B. safensis* and *L. chromiiresistens* were found to tolerate the highest concentrations of Cd, Pb, Cu, and Co, but they were less effective in the bioremediation of cadmium. The highest concentrations of cadmium were removed by *P. plecoglossicida*-PP27T and *E. gallinarum*-EG05I. These results are similar to those of (Yamina et al. 2012). *B. safensis* was able to remove 55.4%, 53.1%, 53.7%, 47.7%, and 61.4% of the Pb, Cr, Ni, Fe, and Co, respectively. However, a previous study done by (Afzal et al. 2017) found that *B. cereus* was the most efficacious strain in terms of metal resistance and metal removal capacity.

Overall, our results showed that the concentrations of cobalt, chromium, copper, iron and zinc in Tanjaro River were all below the allowable limits, in contrast, cadmium, lead and nickel were above the permissible limits set by WHO that are resulting from the impact of sewage and industrial wastewater that directly discharge into the river. Indigenous bacteria could provide new information about the diversity of the species, as well as their role in removing heavy metal from the contaminated area. *B. safensis* and *L. chromiiresistens* show considerable tolerance ability against studied heavy metals with maximum resistance for lead ions. Remarkably, *B. safensis* has been shown to remove Pb, Cr, Ni, Fe and Co, but higher concentration of Cd was removed from the medium by *P. plecoglossicida* and *E. gallinarum.*

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