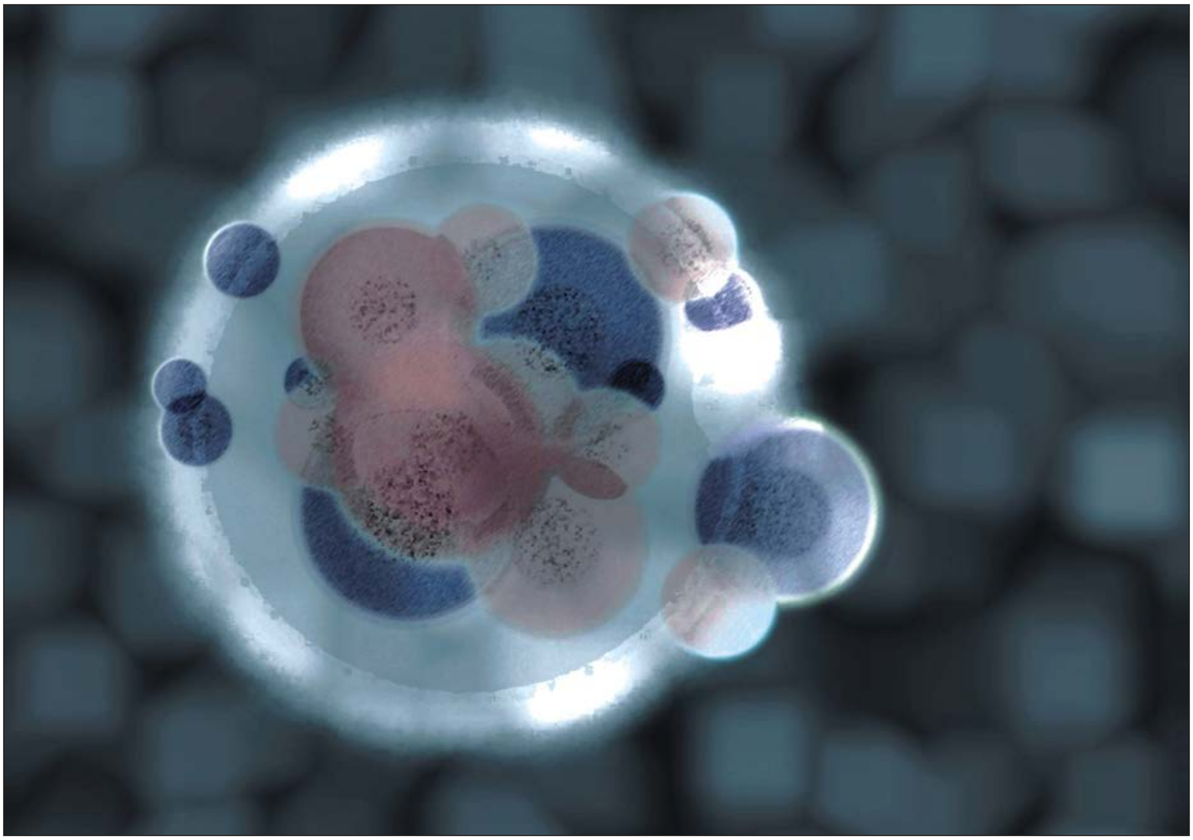

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Dissemination of Heavy Metals and Tolerant Bacteria along Zarqa River (Jordan)

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Abstract: Zarqa River (ZR) is an important river basin in Jordan. However, it is continuously exposed to different types of pollutants including heavy metals. This study was carried out to determine the concentration of the following heavy metals in this vital environment: Pb, Cd, Cu and Zn and to enumerate, isolate and characterize the indigenous bacteria inhabiting this environment to test their heavy metal tolerance and bioremoval ability. Samples in this study include four different types: water, soil, sediment and three local plants (*Nerium oleander*, *Phragmites australis* and *Nicotenia glauca*) from 13 sampling sites assigned as A1-A13 starting from Kherbat Al-Samra treatment plant and ending at King Talal dam. High concentration of Pb was detected in water samples from site A1 (3200 mg L^{-1}) as well as Cd from site A13 (2500 mg L^{-1}). In soil, Zn was found at high concentrations in all sampling sites while Cd was found at high concentration only in site A12. High concentrations of Zn and Cu were detected in sediment samples from sites A1 and A8, respectively. In case of plant samples, Zn was detected at high concentration in *Nicotenia glauca*. A relatively high viable bacterial counts in site A12 soil and sediment samples were detected (2×10^{15} and 1.8×10^{14} CFU mL^{-1}, respectively) and in site A13 water samples (6×10^{17} CFU mL^{-1}). Identified bacteria belong to *Staphylococcus*, *Escherichia*, *Lactobacillus*, *Bacillus*, *Pseudomonas*, *Micrococcus*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Mycobacterium*, *Citrobacter*, *Corynebacterium*, *Acetobacter*, *Serratia* and *Salmonella*. Among them, *Corynebacterium* sp., was the most effective in heavy metal bioremoval.

Key words: Heavy metals, Zarqa river, bacteria

INTRODUCTION

Zarqa River (ZR), with about 73 km in length, is one of the main river basins in Jordan. Recent reports indicate that ZR is excessively exposed to different types of pollution due to intense domestic and industrial activities along the river (IUCN, 2011). ZR pollution is also attributed to discontinuity in water flow and discharge of agricultural wastes (IUCN, 2011). Pollutants can be generally categorized into organic and metallic by-products (e.g., heavy metals). Heavy metals which cannot be degraded biologically or chemically, are known to contribute substantially to ZR pollution (Odat, 2012). Along ZR there are different emission sources of heavy metals increasing their concentrations in the surrounding environment. Because most heavy metals are not required by living organisms, increased concentrations of them may lead to heavy metal poisoning. The negative effect of heavy metal poisoning in humans is well documented in literature (Garbarino *et al*., 1995). Furthermore, the negative effect of heavy metals is extended to natural microbial communities in nature. High concentrations of certain heavy metals may lead to microbial population reduction in terms of volume, diversity and activity (Valsecchi *et al*., 1995; Kandeler *et al*., 2000; Lee *et al*., 2002). An enzyme activity was reported to be significantly reduced upon exposure to high level of metals in soil like dehydrogenase, acid phosphatase and β-glycosidase (Kandeler *et al*., 2000; Lee *et al*., 2002). These findings confirm previous studies reported that microbial activity was negatively affected when the concentration of heavy metals was elevated in the soil, resulting in a reduction in the efficient assortment of soil microbes (Valsecchi *et al*., 1995; Kandeler *et al*., 2000). Nevertheless, microorganisms are believed to have evolved heavy-metal tolerance because of their long exposure to heavy metals on early Earth (Maeir *et al*., 2009). Subsequently, certain genera of microorganisms possess the ability of either to bioaccumulate or even to biotransform heavy metals to non-toxic form. Microbiological approaches can be used in the bioremediation of metal contaminated water, soil and sediment. Proposed methods for metals bioremoval include complexation and precipitation in water, or bioleaching, volatilization and immobilization and complexity in soil and sediments (Maeir *et al*., 2009).

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This study aims to find out and describe the distribution of the following heavy metals in ZR area: cadmium (Cd), cupper (Cu), zink (Zn) and lead (Pb) in water, soil, sediment and three local plants (*Nerium oleander*, *Phragmites australis* and *Nicotenia glauca*). Furthermore, the physicochemical properties of the sampled water, sediment and soil were examined to better understand the factors affecting heavy metal distribution. This study focuses also on the diversity of indigenous bacteria inhabiting the study area with respect to their number and type. The indigenous bacteria were isolated and tested for heavy metal tolerance as well as heavy metal bioremoval.

MATERIALS AND METHODS

Samples collection and preparation: In this study, 13 sites along ZR were selected for sampling. This covers the area from the effluent site at Kherbat Al-Samra Treatment Plant (KSTP) to the inlet of King Talal dam (KTD). Sampling locations are indicated in Fig. 1, where the geographical coordinates of sampling sites are shown in Table 1. Samples were collected during July, 2010.

Four types of samples were collected: surface river water, sediment, soil and three local plants (*Nerium oleander*, *Phragmites australis* and *Nicotenia glauca*). These plants were chosen because they are very abundant in this environment and *Nerium oleander* is well known to be present next to heavily contaminated areas in the beds of the Mediterranean river beds that remain dry most of the year (Kadukova *et al*., 2006). Plant, soil and sediment samples were collected along the river banks.

Water samples were collected in clean sterile polyethylene bottles. Sediment, soil and plant samples were collected in clean plastic bags. Sediment and soil samples were dried and passed through a 2 mm sieve. However, plants were thoroughly cleaned with sterile distilled water to remove soil and any foreign particles. All

Table 1: Geographical coordinates of the sampling sites

Sites	N°	E°
A1	32.14771	36.05181
A2	32.18287	35.99808
A3	32.20526	35.98907
A4	32.20565	35.98330
A5	32.19260	35.96686
A6	32.19382	35.94327
A7	32.19483	35.91589
A8	32.20110	35.90369
A9	32.21415	35.89207
A10	32.21503	35.88445
A11	32.22400	35.96420
A12	32.25630	35.96760
A13	32.29754	36.14560

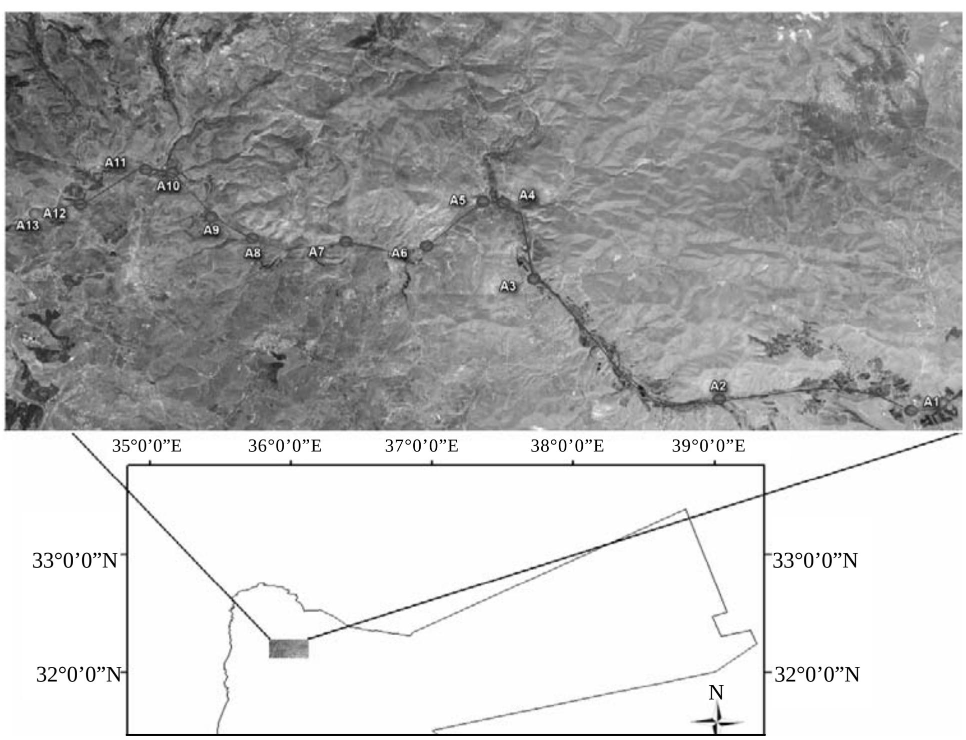

Fig. 1: The stream of Zarqa River and the locations of the sampling sites

plant samples were dried using oven at 80°C for 24 h. This step protects the plant material from microbial decomposition. The plant leaves and roots were then separately milled and sieved through a 1.5 mm sieve. The plant, sediment and soil samples were placed in clean sterile bags.

Physicochemical analysis of samples: Water, sediment and soil samples were analyzed to determine pH, Electric Conductivity (EC), Total Phosphorus (TP), Total Nitrogen (TN), Total Organic Matter (TOM) and grain size. These properties were carried out as described by Allen (1989).

Heavy metals determination

Water samples: The following heavy metals were measured in water samples as described by Karadede and Unlu (2000) using atomic absorption spectrophotometer (Analytic Jena, Inc., AG): Cd, Cu, Zn and Pb.

Sediment and soil samples: Heavy Metals (Zn, Pb, Cd and Cu) were extracted from sediment and soil samples using acidic extraction method modified by Sutherland (2001). In this method, two hundred milligrams (dry weigh) of sediment or soil samples were digested with (10 mL) mixture of HCL: HNO_3: HF (2:2:1, v:v:v) and analyzed by atomic absorption spectrophotometer according to Sutherland (2001).

Plant samples: One gram of the crushed dried samples was digested with 10 mL of a mixture of HCL: HNO_3 (4:1, v:v) (McGrath and Cunliffe, 1985) and heavy metals in the digest were determined using atomic absorption spectrophotometer.

Microbiological analysis

Viable plate count of indigenous bacteria: Colony Forming Units (CFU) mL^{-1} of water sample or soil sample were determined for each sample by the standard viable plate count method. Nutrient agar was used for cultivation. Incubation was done at 37°C. The morphologically different colonies were then sub-cultured several times to get pure cultures. Bacterial isolates were compared based on differences in colonial morphologies (including pigmentation, size, elevation and margins).

Identification of bacteria: All the different bacterial isolates were gram-stained and further characterized by the biochemical tests as described previously by Holt *et al*. (1994).

Microbial tolerance and bioremoval of heavy metals: The isolated bacterial strains were tested for their heavy metal tolerance and bioremoval ability. Isolates were cultivated in nutrient broth and centrifuged. The cells were then washed in Phosphate Buffered Saline (PBS) solution several times. The cells were then exposed to four different heavy metals (copper, lead, zinc and cadmium). Four different heavy metal concentrations (300, 500, 1000 and 1500 mg L^{-1}) of copper, lead and zinc were prepared, whereas the tested cadmium concentrations were (50, 100, 250 and 500 mg L^{-1}). The examination of bacterial tolerance to heavy metals was done by inoculating the bacterial isolates in a mixture of nutrient broth and heavy metals with the above mentioned concentrations. Incubation was done at 37°C. Increase in absorbance of the inoculated nutrient broth was spectrophotometrically measured at wavelength of 600 nm after 24, 48, 120 and 144 h. Heavy metal tolerant bacterial isolates were selected for experiments of heavy metals bioremoval. In heavy metals bioremoval experiments, selected bacteria were tested in nutrient broth amended with (1000 mg L^{-1}) of cadmium, copper, lead and zinc. Heavy metal concentration was measured in the cells before and after the exposure to heavy metal to measure heavy metal bioremoval by bacteria. One milliliter from each culture was retrieved from the culture and centrifuged at 10000xg for 15 min. The supernatant was filtered using a sterile 0.2 μm membrane. Filtrate from the culture medium was diluted with 10% HNO_3 in order to estimate the residual heavy metals. The pelleted cells were weighed and then digested overnight in 1M HCl, sonicated twice for 45 sec and centrifuged at 10000xg for 5 min. For the estimation of accumulated heavy metals inside the cells, the supernatant was collected and diluted with 10% HNO_3. The concentration of heavy metals were determined by atomic absorption spectrophotometer. The metal concentrations were calculated using appropriate blanks. As a result, each of the samples was analyzed in triplicate and the average of three values was used as mean value.

Statistical analysis: Analysis was performed using SPSS software version 16. One-way analyses of variance (ANOVA) was used to determine any statistically significant difference ($p \leq 0.05$).

RESULTS

Physicochemical properties of samples

Water samples: The physicochemical properties of water samples are summarized in Table 2. The pH values ranged from 7.21-8.75 for all thirteen sampling sites. The sites A3, 4, 7 and 8 showed the highest pH value: 8.75, 8.72, 8.42 and 8.52, respectively. These sites showed also the highest Total Phosphorus (TP) and Total Nitrogen (TN) values. The site A3 and A4 are close to KSTP while sites A7, A8 are located close to Jarash-Amman highway.

Table 2: Physicochemical properties of water samples

Site	pH ±SD	EC (meq 100 mL^{-1}) ±SD	TP (mg L^{-1}) ±SD	TN (mg L^{-1}) ±SD	TDS (mg L^{-1}) ±SD
A1	7.21 ±0.015	31.56 ±0.053	7.41 ±0.015	33.79 ±0.090	15.34 ±0.125
A2	7.41 ±0.020	28.57 ±0.460	7.96 ±0.070	33.39 ±0.060	14.70 ±0.100
A3	8.79 ±0.080	25.82 ±0.070	8.33 ±0.030	36.89 ±0.060	13.37 ±0.080
A4	8.72 ±0.050	23.61 ±0.050	8.53 ±0.040	35.82 ±0.070	14.41 ±0.070
A5	8.36 ±0.040	22.40 ±0.060	7.47 ±0.040	35.21 ±0.100	15.78 ±0.070
A6	8.06 ±0.030	15.80 ±0.030	7.25 ±0.030	34.63 ±0.150	15.50 ±0.060
A7	8.42 ±0.030	12.70 ±0.080	8.16 ±0.060	32.74 ±0.170	16.38 ±0.070
A8	8.57 ±0.050	9.70 ±0.040	8.34 ±0.040	31.90 ±0.130	16.88 ±0.070
A9	7.21 ±0.030	5.39 ±0.050	8.09 ±0.040	30.39 ±0.170	17.05 ±0.100
A10	7.81 ±0.080	4.18 ±0.060	7.94 ±0.050	31.39 ±0.170	15.41 ±0.090
A11	8.17 ±0.050	3.81 ±0.020	7.24 ±0.020	30.82 ±0.060	15.81 ±0.090
A12	7.83 ±0.090	2.09 ±0.050	7.41 ±0.070	28.66 ±0.110	12.89 ±0.050
A13	7.94 ±0.020	0.88 ±0.060	8.13 ±0.020	28.79 ±0.060	13.47 ±0.100

EC: Electrical conductivity, TP: Total phosphorus, TN: Total nitrogen, TDS: Total dissolved solids

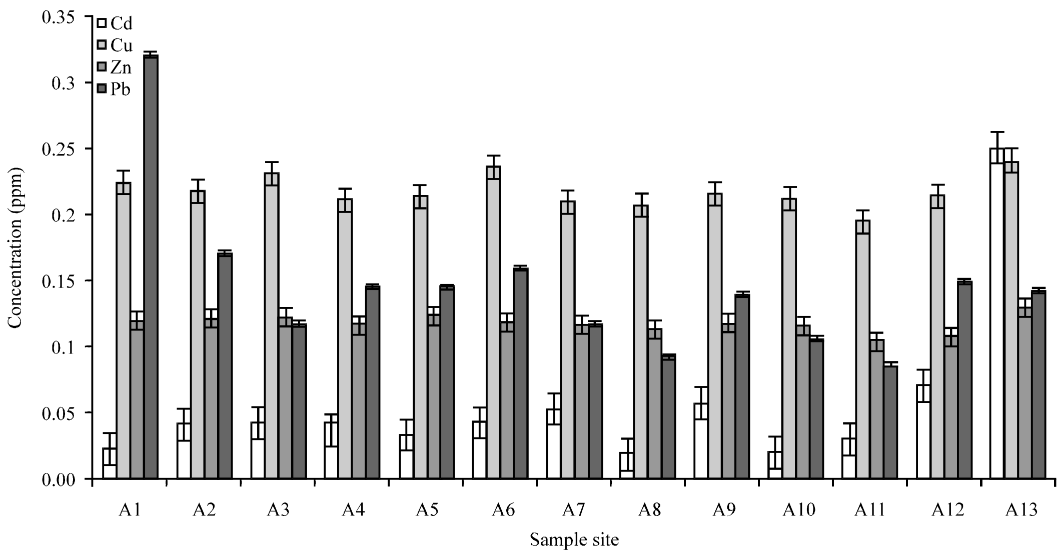

Fig. 2: Heavy metals concentration in water samples retrieved from thirteen sampling sites, Cu: Copper, Pb: Lead, Zn: Zinc and Cd: Cadmium

Soil samples: The average values of the physicochemical properties of soil samples are shown in Table 3. The pH value ranged from 7.35-8.73 and the highest values were measured in sites A3 and 4. TP values ranged from 412-684 mg kg^{-1} and TN values ranged from 508-740 mg kg^{-1}. TOM (%) values ranged from 5.31-6.45 and the highest values were observed in sites A3, 4, 10 and 11, whereas the lowest values were observed in sites A2 and 12. The soil samples in sites A11, 12 and 13 are sandy soil whereas soil samples in sites A1, 2, 3, 7, 8 and 10 are silty.

Sediment samples: Table 4 summarizes the physicochemical properties average values of sediments samples. TN values ranged from 325-1245 mg kg^{-1} and TP values ranged from 412-1312 mg kg^{-1}. TOM values ranged from 2.4-7.7 where the highest values were observed in sites A3, 4, 10 and 11. However, the lowest TOM values were observed in sites A1 and 2. The sediments samples in sites A1 and 12 had a sandy texture where sediments samples in sites A9, 10 and 11 had a clay texture.

Heavy metals concentration

Water samples: Figure 2 illustrates the variation in heavy metals concentration in water samples along the sampling sites of the study area. High concentration of Pb was measured in site A1. High concentration Cd was measured in site A13. Cu and Zn concentrations showed no relative significant variations along the study area.

Soil samples: Heavy metals concentrations in soil samples along the sampling sites of the study area are shown in Fig. 3. High concentrations of Zn were measured in all sampling sites. High concentration of Cd was measured in site A12.

Table 3: Physicochemical properties of soil samples

Site	pH ±SD	EC (meq 100 g^{-1}) ±SD	TP (mg kg^{-1}) ±SD	TN (mg kg^{-1}) ±SD	TDS (mg L^{-1}) ±SD	TOM (%) ±SD	Sand (%) ±SD	Silt (%) ±SD	Clay (%) ±SD
A1	7.35 ±0.05	29.42 ±0.09	417.33 ±1.15	508.00 ±1.00	198.30 ±0.07	5.53 ±0.03	9.3 ±0.030	64.78 ±0.08	25.89 ±0.06
A2	8.05 ±0.02	26.29 ±0.06	462.33 ±0.58	563.50 ±0.50	125.50 ±0.10	5.31 ±0.02	10.4 ±0.100	68.13 ±0.15	21.48 ±0.08
A3	8.57 ±0.02	19.80 ±0.08	512.00 ±1.00	583.33 ±0.58	345.30 ±0.78	6.46 ±0.02	11.2 ±0.060	77.38 ±0.17	11.41 ±0.07
A4	8.73 ±0.04	18.49 ±0.09	589.33 ±1.15	624.33 ±0.58	294.50 ±0.50	5.90 ±0.09	13.8 ±0.100	55.30 ±0.07	30.67 ±0.49
A5	8.63 ±0.05	17.41 ±0.07	622.33 ±1.53	680.00 ±1.00	187.50 ±0.32	5.72 ±0.11	21.6 ±0.250	59.27 ±0.11	19.13 ±0.15
A6	8.15 ±0.06	14.31 ±0.11	649.83 ±0.76	705.00 ±1.00	167.30 ±0.25	5.55 ±0.06	10.6 ±0.100	63.38 ±0.15	25.85 ±0.16
A7	8.36 ±0.04	12.13 ±0.15	684.00 ±2.00	725.33 ±1.53	133.50 ±0.30	5.48 ±0.03	11.7 ±0.360	79.33 ±0.25	8.86 ±0.05
A8	8.06 ±0.04	8.71 ±0.09	638.33 ±1.53	739.67 ±0.58	150.10 ±0.21	5.44 ±0.04	11.5 ±0.320	68.47 ±0.31	19.83 ±0.21
A9	8.36 ±0.05	6.80 ±0.21	586.00 ±1.00	694.00 ±1.00	233.37 ±0.35	5.43 ±0.03	21.3 ±0.200	52.27 ±0.31	26.53 ±0.25
A10	7.42 ±0.02	5.53 ±0.20	529.67 ±0.58	651.67 ±1.53	247.67 ±0.29	5.87 ±0.05	37.36 ±0.24	58.35 ±0.18	4.31 ±0.07
A11	7.64 ±0.04	3.51 ±0.07	473.67 ±0.58	603.00 ±1.00	204.60 ±0.53	6.05 ±0.05	38.37 ±0.31	53.43 ±0.25	8.29 ±0.17
A12	7.94 ±0.05	1.91 ±0.03	438.33 ±1.53	589.67 ±1.53	175.53 ±0.50	5.33 ±0.04	51.53 ±0.31	29.27 ±0.25	19.10 ±0.30
A13	7.55 ±0.05	1.20 ±0.07	411.77 ±0.68	539.83 ±0.76	168.57 ±0.45	5.41 ±0.03	54.70 ±0.36	41.63 ±0.40	3.52 ±0.09

EC: Electrical conductivity, TP: Total phosphorus, TN: Total nitrogen, TDS: Total dissolved solids and TOM: Total organic matter

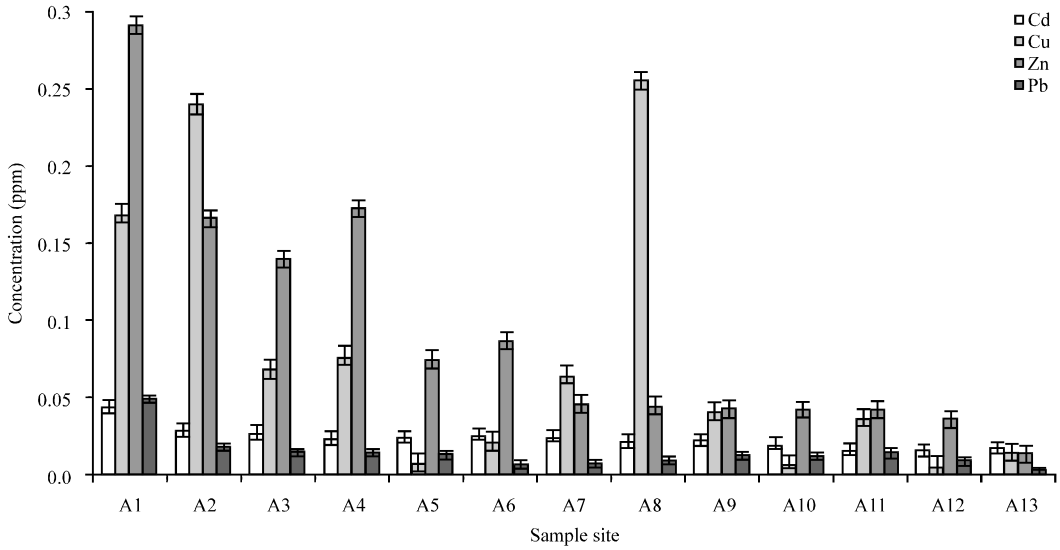

Fig. 3: Heavy metals concentration in soil samples retrieved from thirteen sampling sites, Cu: Copper, Pb: Lead, Zn: Zinc and Cd: Cadmium

Table 4: Physicochemical properties of sediment samples

Site	TOM (%) ±SD	TN (mg kg^{-1}) ±SD	TP (mg kg^{-1}) ±SD	Sand (%) ±SD	Silt (%) ±SD	Clay (%) ±SD
A1	2.39 ±0.05	842.00 ±7.94	911.33 ±3.06	39.42 ±0.45	12.28 ±0.06	48.66 ±0.07
A2	3.50 ±0.05	722.67 ±1.53	844.00 ±2.65	37.19 ±0.03	11.40 ±0.06	51.40 ±0.08
A3	5.80 ±0.04	1129.00 ±4.58	1276.00 ±2.00	16.68 ±0.13	19.80 ±0.05	54.69 ±0.07
A4	6.51 ±0.07	1244.67 ±2.52	1311.90 ±0.85	17.97 ±0.55	18.49 ±0.09	63.53 ±0.15
A5	4.59 ±0.11	1089.33 ±1.53	1190.00 ±2.00	34.89 ±0.05	21.50 ±0.14	43.60 ±0.10
A6	4.32 ±0.07	835.00 ±2.65	933.67 ±1.53	33.41 ±0.11	26.81 ±0.06	39.79 ±0.10
A7	3.60 ±0.09	734.33 ±2.08	827.33 ±1.53	31.81 ±0.11	31.71 ±0.10	36.55 ±0.16
A8	4.12 ±0.04	639.00 ±1.00	623.00 ±1.00	28.93 ±0.15	24.59 ±0.09	46.51 ±0.21
A9	5.39 ±0.05	758.00 ±1.00	733.67 ±0.58	33.21 ±0.10	9.41 ±0.05	57.41 ±0.11
A10	7.70 ±0.07	630.00 ±1.00	744.67 ±1.53	22.89 ±0.07	11.41 ±0.07	65.69 ±0.07
A11	6.51 ±0.08	548.33 ±0.58	638.00 ±1.00	25.07 ±0.40	13.62 ±0.11	61.30 ±0.26
A12	5.20 ±0.11	426.67 ±0.58	539.00 ±1.00	42.26 ±0.14	16.39 ±0.09	41.31 ±0.07
A13	4.30 ±0.08	325.00 ±1.00	412.00 ±1.00	34.53 ±0.21	21.69 ±0.06	43.73 ±0.14

TOM: Total organic matter, TN: Total nitrogen, TP: Total phosphorus

Sediment samples: Heavy metal measurements of sediment samples showed that Zn and Cu were highly accumulated in sediments as compared to soil. High concentration of Zn was measured in site A1 (Fig. 4). High concentration of Cu was measured in site A8. Cd and Pb measurements showed no significant variations in concentrations along the study area (Fig. 4).

Plant samples

Plant leaves: Zn was highly accumulated in *Nicotenia glauca* which is also correlated to high concentration of Zn in soil and sediment. Cd concentration in *Phragmites australis* leaves was found to be the highest as compared to other sampled plants. The results of heavy metal concentrations in plant leaves are shown in Fig. 5.

Plant roots: Zn was also highly accumulated in the roots of *Nicotenia glauca*. Cd concentration accumulated in *Phragmites australis* in higher concentration than other plants roots. The results are depicted in Fig. 6. Plant roots generally accumulated higher concentration of heavy metals in comparison to plant leaves.

Microbiological analysis

Viable plate count of indigenous bacteria: Table 5 shows the number of bacteria expressed as Mean ±SD as Colony Forming Units (CFU) mL^{-1} water or gram soil or wet sediment. The general trend indicates that the viable plate count of bacteria increases as the sampling site deviates from KSTP towards KTD.

Isolation and identification of bacteria: The morphology of bacterial colonies obtained from different soil and water samples varied from circular, filamentous, punctiform to rhizoid while the color of the isolated colonies was diverse: some were yellow, some exhibited brown color while others were creamy yellow, or creamy white, or even bluish green indicating different pigmentation among

Table 5: Viable plate count in water, soil and sediment represented as colony forming unit (CFU) per mL or gram

Sampling site	Water (CFU mL^{-1})	Soil (CFU g^{-1})	Sediment (CFU g^{-1})
A1	$5.5\times10^{8}\pm2\times10^{7}$	$3.5\times10^{8}\pm1\times10^{7}$	$4.0\times10^{8}\pm1\times10^{7}$
A2	$5.0\times10^{10}\pm1.5\times10^{9}$	$2.4\times10^{8}\pm1.5\times10^{7}$	$3.5\times10^{9}\pm5\times10^{7}$
A3	$1.5\times10^{12}\pm1.3\times10^{11}$	$7.5\times10^{10}\pm6\times10^{8}$	$5.0\times10^{12}\pm1\times10^{11}$
A4	$6.0\times10^{11}\pm1.6\times10^{10}$	$2.0\times10^{8}\pm1.4\times10^{7}$	$4.5\times10^{11}\pm6.7\times10^{10}$
A5	$7.0\times10^{11}\pm3.5\times10^{10}$	$1.7\times10^{8}\pm5\times10^{6}$	$5.5\times10^{11}\pm2\times10^{10}$
A6	$2.8\times10^{14}\pm8.2\times10^{12}$	$5.0\times10^{9}\pm1\times10^{8}$	$1.7\times10^{12}\pm1\times10^{11}$
A7	$4.0\times10^{10}\pm5.3\times10^{9}$	$9.0\times10^{9}\pm1.2\times10^{8}$	$6.0\times10^{10}\pm2.1\times10^{9}$
A8	$9.5\times10^{12}\pm2\times10^{11}$	$3.5\times10^{8}\pm2\times10^{7}$	$6.0\times10^{11}\pm1\times10^{10}$
A9	$1.3\times10^{11}\pm5.1\times10^{9}$	$4.0\times10^{8}\pm1\times10^{7}$	$7.0\times10^{12}\pm2.5\times10^{11}$
A10	$5.0\times10^{13}\pm1.5\times10^{12}$	$3.5\times10^{9}\pm2\times10^{8}$	$4.0\times10^{12}\pm1\times10^{11}$
A11	$2.2\times10^{14}\pm7\times10^{12}$	$2.7\times10^{12}\pm5\times10^{10}$	$2.2\times10^{12}\pm1.1\times10^{11}$
A12	$2.5\times10^{15}\pm1.3\times10^{14}$	$2.0\times10^{15}\pm1.5\times10^{14}$*	$1.8\times10^{14}\pm5.5\times10^{12}$*
A13	$6.0\times10^{17}\pm1.1\times10^{16}$*	$1.5\times10^{15}\pm1\times10^{14}$	$1.2\times10^{14}\pm1.1\times10^{13}$

*Represent highest values in each type of samples

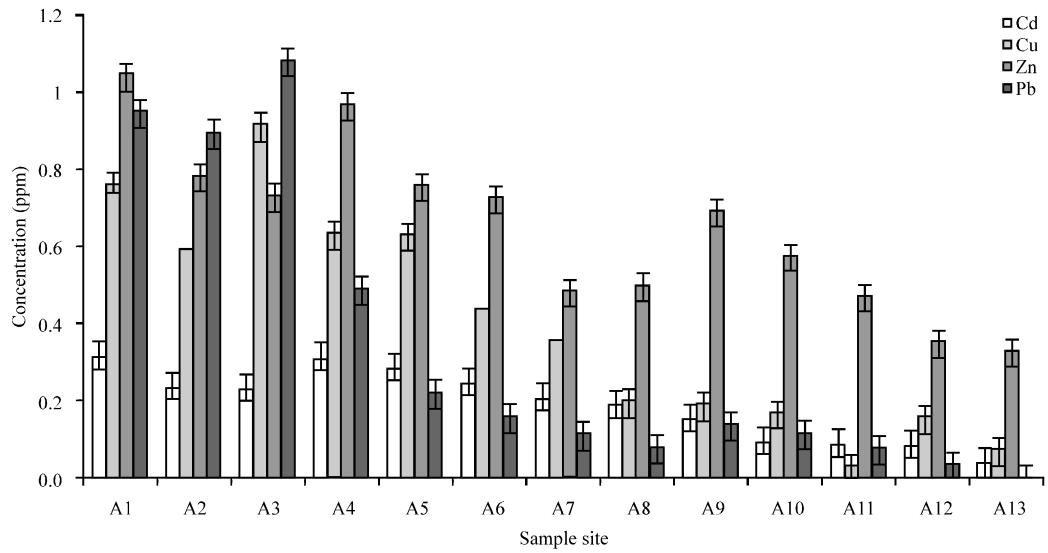

Fig. 4: Heavy metals concentration in sediment samples retrieved from thirteen sampling sites, Cu: Copper, Pb: Lead, Zn: Zinc and Cd: Cadmium

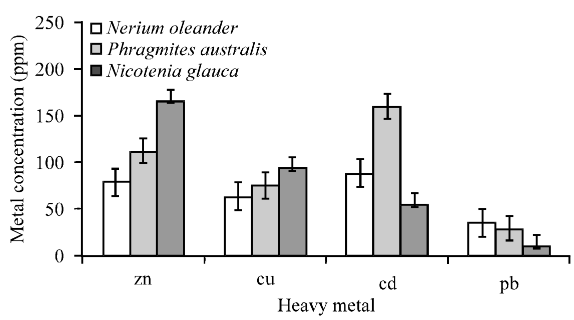

Fig. 5: Heavy metals concentration in the studied plant leaves

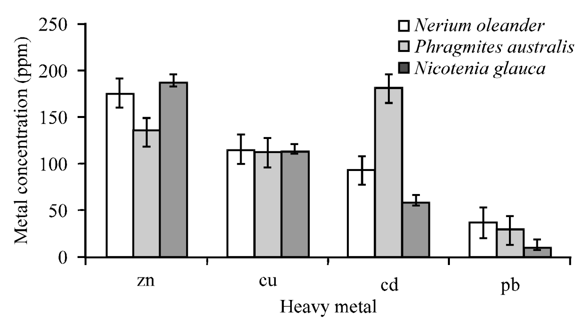

Fig. 6: Heavy metals concentration in the studied plant roots

bacterial isolates. This might reflect the bacterial diversity among the isolates. Gram staining showed that the majority of bacterial isolates were Gram-positive bacilli, others were Gram-positive cocci. Some other bacteria were Gram-negative bacilli. To identify these diverse bacteria, biochemical testes were carried out to identify the genus. As shown in Table 6, the identified bacterial species detected in ZR area belong to the following genera: *Staphylococcus*, *Escherichia*, *Lactobacillus*, *Bacillus*, *Pseudomonas*, *Micrococcus*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Mycobacterium*, *Citrobacter*, *Corynebacterium*, *Acetobacter*, *Serratia* and *Salmonella*.

Table 6: Characterized bacterial genera in ZR area based on biochemical properties and their detection frequency in each type of sample

Bacterial isolates	Soil	Water	Sediment
Gram positive bacteria			
Actinomyces	0	5	3
Bacillus	26	16	7
Corynebacterium	20	8	4
Lactobacillus	0	6	4
Listeria	0	3	2
Micrococcus	16	14	6
Peptococcus	8	2	0
Staphylococcus	19	17	12
Streptococcus	7	3	1
Gram negative bacteria			
Bordetella	3	1	1
Citrobacter	0	2	0
Escherichia	1	3	0
Enterobacter	1	3	2
Klebsiella	1	4	4
Moraxella	8	7	3
Neisseria	11	8	0
Pseudomonas	4	2	0
Salmonella	2	3	2

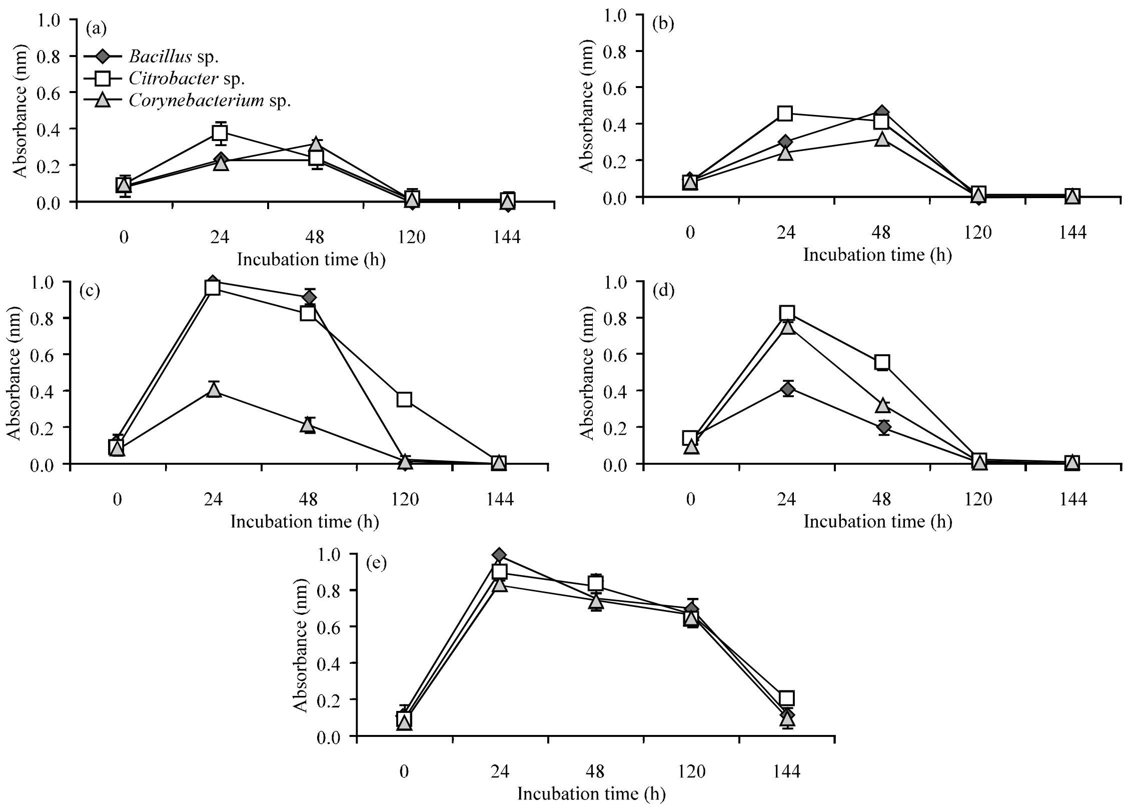

Fig. 7(a-e): Growth of *B acillus* sp., *Corynebacterium* sp. and *Citrobacter* sp., in presence of (a) 500 mg L^{-1} of Zn, (b) 500 mg L^{-1} of Cu, (c) 500 mg L^{-1} of Pb, (d) 100 mg L^{-1} of Cd and (e) Growth of each isolate in heavy-metal-free media was used as control

Microbial tolerance to heavy metals: Growth of the isolated bacteria exposed to different concentrations of heavy metals (300, 500, 1000 and 1500 mg L^{-1} of Cu, Pb and Zn) as well as (50, 100, 250 and 500 mg L^{-1} of Cd) after incubation time of 24, 48, 120 and 144 h was followed. Growth of all bacterial isolates at 1500 mg L^{-1} of heavy metals was negative. However, the highest tolerable concentration for most strains was as following: 500 mg L^{-1} of Cu, Pb and Zn and 100 mg L^{-1} of Cd. Figure 7 shows the growth behavior of *Citrobacter* sp., *Corynebacterium* sp. and *Bacillus* sp. in presence of the four tested heavy metals as compared to biotic control.

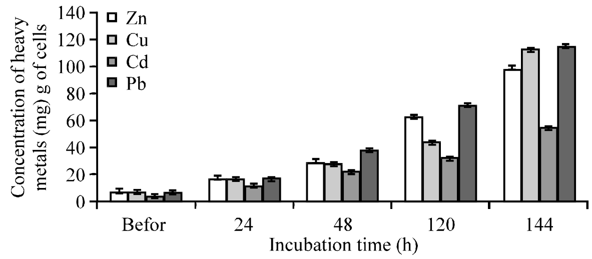

Fig. 8: Intracellular concentration changes of the tested heavy metals in *Bacillus* sp., Cu: Copper, Pb: Lead, Zn: Zinc and Cd: Cadmium

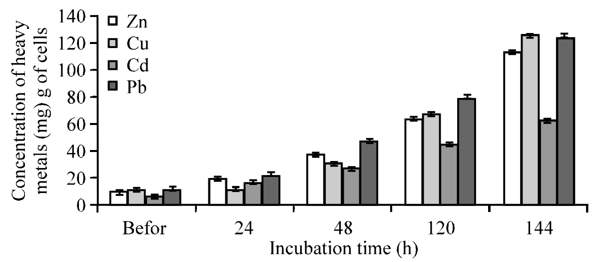

Fig. 9: Intracellular concentration changes of the tested heavy metals in *Citrobacter* sp., Cu: Copper, Pb: Lead, Zn: Zinc and Cd: Cadmium

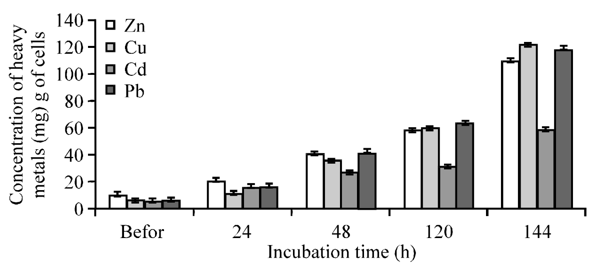

Fig. 10: Intracellular concentration changes of the tested heavy metals in *Corynebacterium* sp., Cu: Copper, Pb: Lead, Zn: Zinc and Cd: Cadmium

Heavy metal bioremoval by bacteria: Three bacterial isolates *Citrobacter* sp., *Corynebacterium* sp. and *Bacillus* sp., were selected for heavy metal bioremoval experiments, based on their heavy metal tolerance capability compared to the other tested bacteria. The tested heavy metals were Zn, Cu, Cd and Pb. Results of showed that intracellular concentration of heavy metals (mg metal per g of cells) increases with incubation time (Fig. 8-10). The highest heavy-metal bioremoval efficiency was observed in case of *Corynebacterium* sp., followed by *Citrobacter* sp. and *Bacillus* sp.

DISCUSSION

Eventhough, some physicochemical properties did not vary significantly from one sampling site to another, other properties varied significantly along the study area. For instance, the tested water samples were found to have a slightly alkaline pH range (7.21-8.75) during the sampling time July (summer season). WHO has recommended maximum allowable range of pH from 6.5-9.2 (De, 2002). Therefore, water samples are considered within the permissible pH range. This slight increase in pH values during summer may be correlated to the high photosynthetic rate of cyanobacteria, algae and plants demanding high concentration of CO_2 and ultimately shifting the equilibrium towards alkaline side (Parashar *et al.*, 2006). In addition, ZR seems to receive a considerable amount of industrial and wastewater discharge that is alkaline in nature. This may also increase the pH values of surface water. The pH values of soil were also found to fall within the alkaline range. This is due to water and pore water trapped in soil and sediment particles which are already alkaline. In respect to the Electric Conductivity (EC), results showed high values of EC (for instance 31.6 meq mL^{-1}) in some sites. Compared to recommended limits by WHO, water has high conductivity values which reflect high amounts of ions that exceed the recommended limit by WHO (1996) Total Dissolved Solid (TDS) ranged from 12.9-17.1 mg L^{-1}. All values of TDS were within the permissible limit of WHO (WHO, 1996). Moreover, the values of Total Organic Matter (TOM) were relatively high (2.4-7.7%) in soil and sediments. This can be explained by the surface runoff and decay of plants especially in winter as well as municipal and industrial wastewater in sediments and consequently in soil. TOM has the cation exchange property and the chelating ability (Manousaki and Kalogerakis, 2011). As a result, TOM exhibit high affinity for many trace elements (Andriano, 1986). In respect to heavy metal distribution along ZR area, it was observed that Cd, Zn and Pb decreased as we move from KSTP to KTD. This indicates that these elements are trapped within sediments and soil. This was correlated with increasing concentration of these elements in sediments and soil. The variation in heavy metal concentration among sampling sites was attributed to the emission source of heavy metals. The pronounced increase in heavy metal concentration in sampling sites A1, 2, 5 and 6 can be explained by the presence of different emission sources. For instance, sites A1 and 2 are located downstream the industrial areas, whereas sampling sites A5 and 6 are located beyond the highway, meaning that these sites are exposed to exhaust automobile emissions.

Metals concentration in soil and sediments showed a proportional correlation with clay and organic matter content. This agrees with what was documented, that the largest part of heavy metals are attached to fine-grained portion (<63 μm), generally because of its large surface area to particle size ratio and its high organic matter content (Moore and Reynolds, 1997; Howard and Vandenbrink, 1999; Krupadam *et al*., 2003; Adamo *et al*., 2005; Cubukcu and Tuysuz, 2007; Mishra *et al*., 2008).

Heavy metal availability to plants is dependent on several factors such as the solubility and heavy metal concentration in soil which is affected by several factors, including the physical and chemical properties, clay content and pH of the soil (Ismail *et al*., 2005). Proportional correlation between heavy metal concentration in the plants *Nerium oleander*, *Phragmites australis* and *Nicotenia glauca* as well as the heavy metals concentration in soil and sediments was established for the studied heavy metals except for Pb. Absorption of heavy metals by plants is a highly selective and regulated process. Plants can take up heavy metals via their roots, stems or leaves. Following absorption, heavy metals can be either accumulated within the absorptive organ or distributed through the plant body and metabolized and this can be influenced by different factors including plant species, type and availability of elements, root exudation capacity, pH, cation exchange capacity, temperature and dissolved oxygen (Cheng, 2003). The determined concentrations of heavy metals indicate that *Nerium oleander* has lower tendency to accumulate metals as compared to *Phragmites australis* and *Nicotenia glauca*. *Nicotenia glauca* accumulated generally the highest levels of metals. The metals were generally accumulated in roots in higher proportions than leaves in all studied plant species. Pb was detected in relatively very low concentration and it was mainly accumulated in the roots and leaves of *Nerium oleander* as compared to the other studied plant species. The detected low levels of Pb that were detected in roots and leaves of the plants species compared to other metals concentrations, can be attributed to its low solubility and strong interactions with soil particles (Babula *et al*., 2008). However, Zn and Cd were highly accumulated in roots and leaves of *Phragmites australis* as compared to the other studied plant species. The metal Cu was highly accumulated in roots and leaves of *Nicotenia glauca* as compared to the other studied plant species.

The majority of studies in Jordan were conducted on *Phragmites australis* as bioaccumulator for heavy metals as a mean of phytoremediation. However, little studies were done on *Nerium oleander* and *Nicotenia glauca*. In a study carried out by Al-Taisan (2009), it was reported that *Phragmites australis* absorbs Zn, Fe and Mn while Ni, Pb and Cd were absorbed in lower quantities. The results demonstrated that *Phragmites australis* is significant as plant filter and can be used to clean soils from contaminants like heavy metals.

Microorganisms, including bacteria, play an essential role in heavy metal impacted environments. Biological methods such as biosorption and bioaccumulation offer a promising substitute to chemical methods (Kapoor and Viraraghavan, 1995). For instance, bacteria may remove, uptake, or transform metals to an inactive form. They may also affect the obtainability of metals in soil and thus improving heavy metal accumulation by plant species (Abou-Shanab *et al*., 2008). However; heavy metals can negatively affect the microbial diversity in any impacted soil if these organisms were not tolerant to the toxic effects of heavy metals (Gadd and White, 1993; Ji and Silver, 1995; Kapoor and Viraraghavan, 1995; Kotrba *et al*., 1999).

Soil samples from sites A1 and A2 contain the highest levels of Cu, Cd and Pb. This was translated into low number of bacteria due to toxicity (Table 5). Moreover, cadmium, lead, copper and zinc, showed an increased toxicity in the following order: lead<zinc< copper<cadmium. These outcomes agree with the results obtained in the study done by Kavamura and Esposito (2010).

Bacteria with heavy-metal tolerance were detected in sites with reduced levels of heavy metals (water, soil and sediment). Levels that are not lethal to bacteria give the chance for adaptation and evolving strategies for heavy metal tolerance and then bioremoval. The identified bacterial genera were arranged in the following descending order according to their common abundance in the studied sites: *Staphylococcus*, *Bacillus*, *Micrococcus*, *Klebsiella*, *Corynebacterium*, *Enterobacter*, *Citrobacter*, *Salmonella*, *Pseudomonas*, *Serratia*, *Peptococcus* and *Neisseria*. No previous studies have reported these genera in ZR in relation to heavy metals except the RSS studies on microbial pollution in respect to *E. coli* and *Salmonella* (RSS, 1984-2004). Indigenous heavy metal-tolerant microorganisms can be suitable as environmental indicators of possible toxicity and are important for investigations concerned of the biochemical and the genetic basis of microbial metal-resistance (De *et al*., 2008). The results revealed that species of the genera *Citrobacter*, *Corynebacterium* and *Bacillus* have the highest tolerance to different heavy metals that grew with the higher concentrations (500 and 1500 mg L^{-1}) of heavy metals. This may pave the way for future applications of these microorganisms to bioremediate heavy metals disseminated along the ZR area.

CONCLUSION

This study indicates that heavy metals (Pb, Cd, Zn and Cu) are disseminated in varying concentrations along ZR. Some heavy metals were detected at high concentrations. This indicates a serious pollution problem in this environment. The microbiological experiments carried out in this study showed that some indigenous bacterial species are heavy metal tolerant and some are able to bioremove heavy metals and that *Corynebacterium* sp., was the most effective in heavy metal bioremoval among the tested bacteria.

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