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METAL POLLUTANTS DISTRIBUTION WITHIN LAKE VICTORIA BASIN AND THEIR INFLUENCE ON THE NATIVE AND TRANSIENT MICROBIAL FLORA

Mutuku Christopher[1], Okemo Paul[2] and Boga Hamadi[3]
[1]Department of Biological Sciences, Chuka University, Chuka, Kenya
[2]Department of Plant and Microbial Sciences, Kenyatta University, Nairobi, Kenya
[3]Department of Botany, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya
E-Mail: sikukuc@yahoo.com

ABSTRACT

Heavy metal concentrations in water and sediments in the rivers draining into the Lake Victoria were determined in this study. The metal levels were compared to the bacterial plate counts and bacterial resistance to commonly used antibiotics assessed. The samples were randomly collected from sewage outfalls and points bordering heavy metal activity settlements. Heavy metal analysis was done using Flame Atomic Absorption Spectrometer (F-AAS). The samples were assayed for native heterotrophic bacteria and coliforms. Sediment samples recorded a higher level of heavy metals with a mean range of 31.97-109.9, lead; 3.48-183.66, zinc; 3.01-17.03, nickel; 1.93-214.61, copper; 17.01-65.91, cobalt; and 2.08-25.89 mg/g chromium compared to water samples whose mean range was as follows;- lead; 0.77-0.94, manganese; 0.10-3.10, zinc; 0.23-1.16, cadmium 0.02-0.04, and copper 0.51-0.57 mg/l. The study showed a significant relationship in the amounts of heavy metals in water samples and the bacterial counts, $r = 0.637$, $p < 0.05$. 53.8% of the isolates showed multidrug resistance. The study showed no significant difference in multidrug resistance between the native heterotrophs and the coliform bacteria ($F = 1.75$ $P = 0.191$ $P > 0.05$). However, multidrug resistance in heterotrophic bacteria (mean 46.52%) was higher than in coliform bacteria (mean 36.36%). Plasmid DNA finger print of the antibiotic resistant isolates showed a positive relationship between the plasmids carried and multidrug resistance. This could suggest that heavy metal pollution in wetlands induces multidrug resistance. The findings point to a potential health threat posed by heavy metal pollution to human and other forms of life in the aquatic ecosystems.

Keywords: heavy metals, pollution, bacterial isolates, antibiotic resistance.

INTRODUCTION

Lake Victoria which is the second largest fresh water lake in the world occupies an area of 68,800 km^2. The Lake is shared by the three East African countries, Kenya (6%), Uganda (51%) and Tanzania (43%). Inflow into the lake occurs through a number of rivers which originate from both lowlands and highlands of Nandi, Kericho and Kisii. These include Rivers Nzoia, Yala, Nyando, Kagera, Kuja, Migori and other inlets draining into the Lake. The area consists of a short rainy season from November to December and a long rainy season from March to May (Calamari *et al*., 1992). The causes of the rising pollution levels in the lake are as many as they are diverse. The lake has for a long time been a sink to excess untreated effluent of both industrial and municipal origin (Nzomo, 2005). The major towns of Kisumu, Eldoret, Kakamega, Homabay, and Kericho have malfunctional sewage plants which discharge inadequately treated sewage in rivers draining into the Lake. Kisumu town has a number of industries including a brewery, tannery, textiles and fish processing industries and the towns sewage plant at Kisat with a design capacity of 9000 cubic meters receives 15000 cubic meters of effluent, much of which flows into the lake without treatment.

Similarly, the large plantations of tea, sugar and coffee together with associated agro industries and a paper mill form important agricultural and industrial activities in the region (Kirugara *et al*., 1996). Among the pollutants discharged into the River ecosystems from agro based and other chemical industries in various forms are heavy metals such as mercury, cadmium, chromium, lead, copper, zinc and arsenic. Metals are important due to their bioaccumulation, toxicity, and health related effects to life in aquatic ecosystems. They produce considerable modifications of microbial communities and their activities (Dolman *et al*., 1994). Toxic metals in the environment lead to selective pressure among the microbial communities resulting in the development of metal resistant populations (Mahler *et al*., 1986). There is evidence of a correlation between tolerance to heavy metals and antibiotic resistance, a global problem currently threatening the treatment of infections in plants, animals and humans (DeRore *et al*., 1994). Studies on chemical pollution in other ecosystems have cited the effects of metal contamination (Fallon *et al*., 1985). The study evaluated the presence and concentrations of heavy metals in water and sediments and their role in the development of antibiotic resistance among environmental bacteria. The findings are vital as the lake and its entire basin serve as the lifeline for about two million people who derive their livelihood directly or indirectly from resources in the lake basin.

MATERIALS AND METHODS

Physico- chemical analysis

Field measurements determined at the eleven sampling river points included water pH, water

conductivity, water temperature, total dissolved solids and dissolved oxygen. The parameters were measured by their respective electrodes using a universal multiline P4 WTW (Weilheim Germany) meter and the mean values recorded.

Metal ion content in water and sediment samples

Water and sediment samples were collected from selected sites in different rivers using 250 ml flasks. The metals were preserved in solution by adding 2 drops of pure nitric acid to each sample. The Lead (Pb), Zinc (Zn), Cadmium (Cd), Copper (Cu) and Manganese (Mn) ion content of the water samples and the Lead (pb), Manganese (Mn), Nickel (Ni), Copper (Cu), Cobalt (Co) and Chromium (Cr) ion content in the sediment samples was determined using the Flame Atomic Absorption Spectrometer (F-AAS noVAA 350). The metal analysis was repeated three times at intervals of 10 days and the mean values were recorded and plotted against the bacterial plate counts.

Bacterial isolation and identification

Water samples for microbial assays were collected just below the water surface into sterile Teflon bottles using aseptic techniques and transported in ice cooler box to the laboratory within 6 hours. 1 ml of each water sample was serially diluted and 0.1 ml sample from the last two diluents (10^4 and 10^5) were plated on nutrient agar plates in duplicates to determine the standard plate count as colony forming units per ml and the most prevalent heterotrophic bacteria selected. Coliforms were isolated by plating on selective and differential media. All the plates were incubated for 18 to 24 hours at 35± 2^{o}C. Distinct and representative colonies from the agar plates were further purified and stored on NA slants in refrigerator. The coliforms were subjected to standard morphological and biochemical tests and identified based on criteria of Prescott and Harley (1996) and Cheesbrough (1990).

Antibiotic susceptibility tests

The bacterial isolates were inoculated into Mueller Hinton broth and incubated for 18 to 24 hours at 35 ± 2^{o}C. Using the methods of Bauer *et al*., (1966), the isolates were assayed for their sensitivities to the following: Ampicillin (25 µg), Cotrimoxazole (25 µg), Augmentin (30 µg), Tetracycline (25 µg), Kanamycin (30 µg), Gentamicin (10 µg), Cefuroxime (30 µg), Chloramphenicol (30 µg), Nalidixic acid (30 µg) and Norfloxacin (10 µg) on Mueller Hinton agar plates. The antibiotic discs were obtained from Abtek Biologicals Ltd, United Kingdom. The agar plates were incubated for 18 to 24 hours at 35 ± 2^{o}C. The diameters of the zones of inhibition were measured to the nearest millimeter for each of the antibiotic tested. The inhibition zone sizes were interpreted using standard recommendations of the National Committee for Clinical Laboratory Standards (NCCLS), (2000). Control plates were incubated without the antibiotic discs.

Plasmid DNA preparation and isolation

Plasmid DNA of the multidrug resistant isolates was prepared and isolated by the alkaline lysis method (Birnboim and Doly, 1979). A single bacterial colony from a 24 h culture was inoculated into 2 ml of Luria-Bertani (LB) broth. The bacterial cells were harvested by centrifuging. The bacterial cells were resuspended in 100 µl of ice-cold cell resuspension buffer consisting of Tris-EDTA. The cells were lysed by adding 200 µl of freshly prepared solution II (NaOH and SDS). 150 µl of ice-cold buffer (Potassium acetate- solution III) was added. The tubes were centrifuged at 12000 rpm for 5 minutes at 4^{o}C and the supernatant transferred to a fresh tube. The DNA was precipitated with 2 voumes of 95% ethanol at room temperature. The pellet of double stranded DNA was rinsed with 1 ml of 70% ethanol at 4^{o}C. The nucleic acid was redissolved in 50 µl of TE (pH 8.0) and the DNA stored at -20^{o}C. The DNA was subjected to 1% agarose gel electrophoresis based on the criteria of Umolu *et al*., (2006). The plasmid sizes were estimated by comparing with previously characterized plasmids of the control strain of *E. coli* V517 used as the genetic marker.

RESULTS

Physico-chemical analysis

The mean range of physico - chemical parameters recorded were as follows, temperature 26.5 – 19.3^{o}C, PH 8.24 - 7.32, electrical conductivity >200 - 0.08 µs, Total dissolved solids 244 - 0.08 mg/l and dissolved oxygen 10.2 - 0.7 mg/l (Table-1).

Table-1. Mean Physico - chemical parameters of the sites (N=3).

	Site	Temp ^0C	PH	Conductivity µs	TDS mg/l	Dissolved O_2 mg/l
1	R. Kisat	25.2	7.6	1.29	1.0	7.2
2	R. Mbogo	22.8	8.17	0.21	150	6.7
3	Chemilil effluent	26.3	7.67	1.0	1.12	0.7
4	R. Nyando -upper	26.5	8.23	0.38	244	8.0
5	R. Yala –upper	19.3	7.91	0.08	0.08	10.2
6	R. Nzoia – upper	22.8	8.02	139	92	9.1
7	R. Nzoia –lower	24.4	8.24	132.7	0.21	9.5
8	R. Woroya	22.4	8.1	102.2	0.52	9.2
9	R. Yala – lower	21.4	7.85	81.2	0.58	0.92
10	R. Nyando -lower	25.9	8.08	>200	151	7.4
11	Hippo Point River	24.4	7.32	115	77	8.5

Metal ion content in water and sediment samples

Sediment samples recorded a higher level of heavy metal concentrations with a mean range of 31.97-109.9, lead; 237.94 - 10225.3, manganese; 3.48-183.66, zinc; 3.01-17.03, nickel; 1.93-214.61, copper; 17.01-65.91, cobalt; and 2.08-25.89 mg/g chromium compared to water samples whose mean range was as follows;- lead, 0.77-0.94; manganese, 0.10-3.10; zinc, 0.23-1.16; cadmium 0.02-0.04 and copper 0.51-0.57 mg/l (Tables 2 and 3).

Table-2. Mean heavy metal concentrations (mg/g) in the sediment samples (N=3).

Site	Lead	Manganese	Zinc	Nickel	Copper	Cobalt	Chromium
R. Kisat	109.91	10225.3	26.05	4.03	1.93	33.98	0
R. Mbogo	37.09	839.2	36.65	9.16	22.72	20.01	2.08
Chemelil effluent	72.99	798.25	183.66	17.03	214.61	65.91	0
R.Nyando-upper	41.88	898.71	71.99	14.98	16.58	55.94	0
R Nyando-lower	44.61	1720.95	53.79	12.24	40.54	49.95	8.04
R.Yala-upper	39.49	892.15	26.45	3.01	8.95	33.98	2.08
R.Yala-lower	37.78	956.83	19.91	17.71	3.93	22.01	19.94
R.Nzoia-upper	32.99	237.66	6.08	9.85	9.51	17.01	2.08
R. Nzoia-lower	31.97	376.94	3.48	14.98	4.35	17.02	25.89
R.Woroya	39.83	350.50	0.007	10.53	32.68	41.97	0

Table-3. Mean heavy metal concentrations (mg/l) in water samples (N=3).

Site	Lead	Manganese	Zinc	Cadmium	Copper
R. Kisat	0.77	1.71	0.26	0.007	0.53
R. Mbogo	0.80	0.52	0.39	0.05	0.55
Chemelil effluent	0.85	3.10	0.55	0.006	0.57
R.Nyando-upper	0.77	0.16	0.23	0.002	0.52
R. Nyando- lower	0.77	0.19	0.23	0.00	0.51
R. Yala – upper	0.94	0.31	1.16	0.00	0.53
R. Yala –lower	0.81	0.44	0.23	0.006	0.52
R. Nzoia-upper	0.83	0.33	0.23	0.04	0.53
R. Nzoia –lower	0.79	0.29	0.23	0.02	0.53
R. Woroya	0.85	0.75	0.25	0.00	0.54
Hippo-point River	0.77	0.10	0.23	0.00	0.52

Bacterial isolation and the standard plate count

The highest total viable counts were observed from River Kisat, stream of effluent from Chemelil sugar factory and River Mbogo (Table-4). 53 species of the most prevalent colonies of native heterotrophs were purified from the agar plates and the coliform bacteria identified based on their IMViC reactions were mainly *E. coli* and *Enterobacter* species.

Table-4. Standard plate counts (cells/ml) from the water and sediment samples.

Site	**Bacterial plate count in water** (cells/ml)	**Bacterial plate count in sediments** (cells/ml)
R. Kisat	4.8×10^6	1.6×10^6
R. Mbogo	1.65×10^6	1.3×10^6
Chemelil effluent	2.8×10^6	5.25×10^6
R. Nyando-upper	0.21×10^6	1.045×10^6
R. Nyando- lower	0.01×10^6	0.61×10^6
R. Yala-upper	0.02×10^6	0.024×10^6
R. Yala- lower	0.03×10^6	0.025×10^6
R. Nzoia- upper	0.086×10^6	0.48×10^6
R. Nzoia- lower	0.002×10^6	0.16×10^6
R. Woroya	0.06×10^6	0.021×10^6
Hippo-point River	0.148×10^6	-

NB (-) No result

Relationship between heavy metal levels and plate counts

The water samples showed a significant relationship between the levels of heavy metals present in the sites and the standard plate counts from the same spots, $r = 0.673$, $p < 0.05$ (Figure-1). The sites with high concentrations of heavy metals also had high levels of bacterial cell counts. The sediment samples however showed no significant relationship between the levels of heavy metals present in the sediments and the bacterial counts from the sediments, $r = 0.175$, $p > 0.05$ (Figure-2) where the sites with high heavy metal content did not necessarily have high levels of bacterial cell counts.

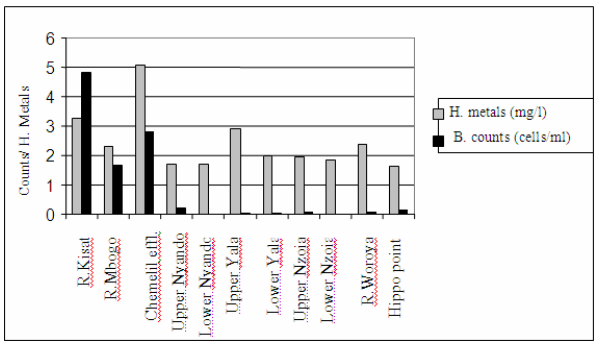

Figure-1. Heavy metal concentrations (mg/l) and bacterial plate counts (cells $\times 10^6$ /ml) in the water.

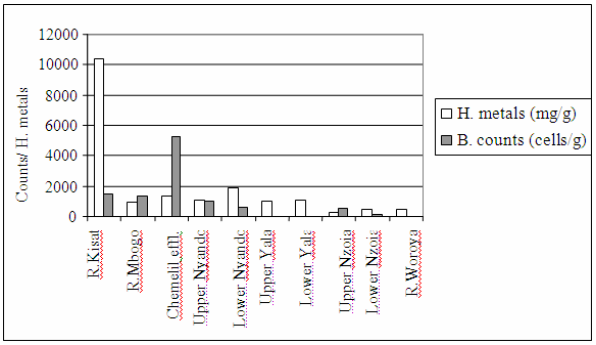

Figure-2. Heavy metal concentrations (mg/g) and bacterial plate counts (cells $\times 10^6$ /g) in the sediments.

Antibiotic susceptibility tests

There was no significant difference ($P > 0.05$) in antibiotics resistance in the two bacterial groups. However, a higher number of native heterotrophic bacteria recorded more resistance than coliform bacteria. The heterotrophs were 100% sensitive to kanamycin and gentamicin but recorded 84.9% resistance to cefuroxime (Figure-3). The most resistant isolates were drawn from both water and sediment samples of River Nzoia, Chemelil effluent, River Mbogo and upper Nyando. The coliform bacteria recorded a remarkable resistance to cotrimoxazole (92.3%) but showed similar sensitivity to kanamycin and gentamicin as the heterotrophs (Figure-4). Coliforms isolated from River Nzoia and Chemelil effluents were the most resistant.

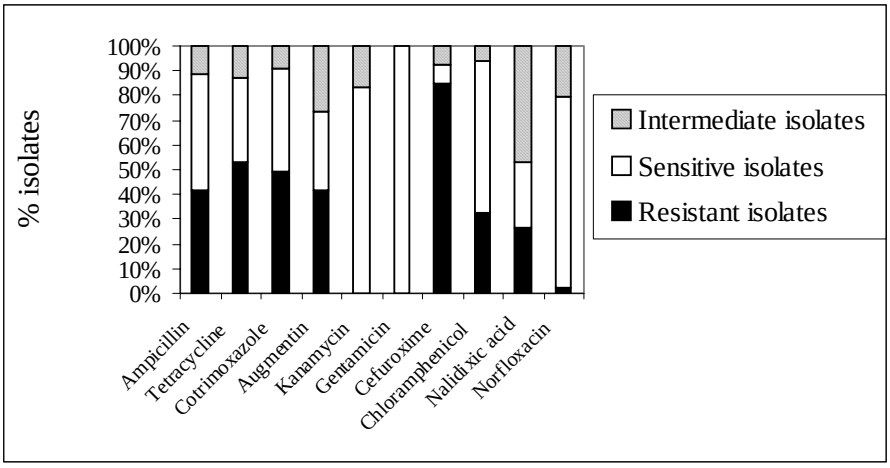

Figure-3. Antibiotic resistance in heterotrophic bacteria.

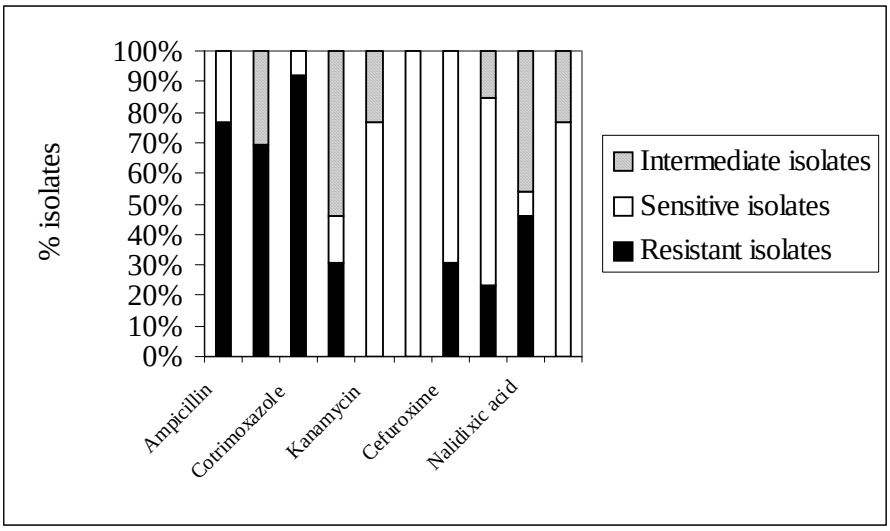

Figure-4. Antibiotic resistance in Coliform bacteria.

Plasmid DNA isolation

Both the coliform and the heterotrophic bacteria yielded plasmids whose molecular sizes ranged from about 28.5 to 0.8 mega Daltons (mDa). *E.coli* species (no.076) from R. Mbogo carried two plasmids at the level of about 3.4mDa and 0.8mDa on the *E.coli* v517 standard marker. Isolate no 009 from R.Nyando yielded a plasmid estimated about 3.6 mDa (Figure-5).

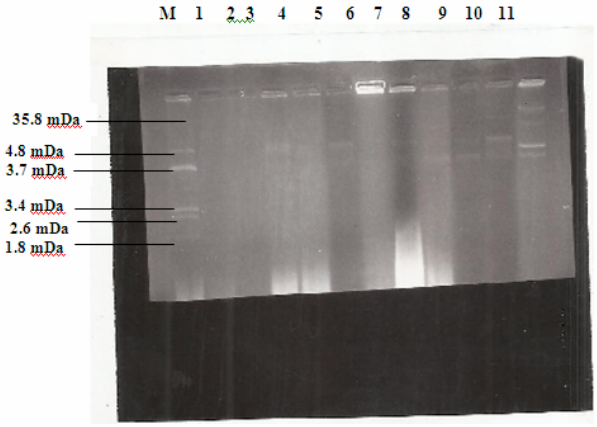

Figure-5. Plasmid profile of the multiple drug resistant bacteria on 1% agarose gel electrophoresis. Lane M is the control *E. coli* strain V517; Lanes 1 to 11 plasmid DNA of the bacterial strains.

DISCUSSIONS

Physico-chemical characteristics influence the growth and diversity of microbial populations. The high water temperature recorded in R. Nyando was due to the high concentration of total dissolved solids due to dumping of industrial wastes from the major towns and residues of chemical herbicides, fertilizers and pesticides used on the adjacent agricultural farms which also influenced electrical conductivity. Turbid waters absorb heat hence are likely to be warmer (Khan and Siddiqui, 1970). The pH of the water in the rivers was generally neutral or slightly alkaline. Neutral pH is suitable for growth of bacteria.

Some metal concentrations detected in the Rivers were above the permissible limit by the World Health Organization (WHO, 1985) and the Kenya Bureau of Standards (KEBS, 1996). The level of Manganese, cadmium and lead were above the permissible limit. Similar studies by Mwamburi and Oloo (1997) found the concentration levels of AL, Mn, and Fe elevated as compared to the W.H.O (1985) drinking water guideline values. Effluent from Chemelil sugar factory and sewage water from River Kisat in Kisumu recorded higher levels of heavy metals. This was attributed to the high mixture of organic and inorganic waste from industrial and municipal wastes discharge, indicating these are hot spots of pollution. The higher concentration of heavy metals in sediments compared to water was due to adsorption in particulate matter. The high viable counts recorded from River Kisat, effluent from Chemelil sugar factory and River Mbogo suggest a more organic pollution in these rivers due to discharge of raw sewage. This increases the biochemical oxygen demand indicating the presence of large numbers of microorganisms. The higher the BOD the higher the usage by aerobic microbes (Vesilind *et al.*, 1990).

This study showed a positive relationship between the amounts of heavy metals in the river water and the bacterial counts, where the sites with high concentrations of heavy metals in the water also had high levels of bacterial cell counts. It is probable that since the heavy metal content was relatively low in water compared to the sediments, some of these levels were appropriate for use by the water microorganisms in their metabolic processes. Eiland (1981) observed that at certain concentrations some metals like copper, zinc, cobalt and nickel are essential for microorganisms since they provide vital cofactors for metallo- proteins and enzymes. However the trend was not observed in sediments possibly due to elevated heavy metal levels in sediments which may have inhibited microbial growth due to toxicity.

Native heterotrophic bacteria recorded a higher incidence of multiple drug resistance compared to Coliforms. However, the incidence of ampicillin, tetracycline, cotrimoxazole and nalidixic acid resistance was higher in coliforms. Heterotrophic bacteria recorded a marked resistance to cefuroxime. Both groups were however highly sensitive to aminoglycosides kanamycin and gentamicin. The results obtained by other workers in similar studies vary. Boon and Cattanach (1999) compared the antibiotic resistant native and feacal bacteria isolated from rivers, reservoirs and sewage treatment facilities. They found that the incidence of resistance to ampicillin, chloramphenicol, kanamycin, nalidixic acid, and streptomycin was greater in native heterotrophic bacteria than *E.coli* isolated from Yarra River in Australia. In contrast Jones *et al*. (1986) reported that *E. coli* Isolated from English lake waters had a greater incidence of antibiotic resistance than native aquatic bacteria.

In the present study some of the antibiotic resistant isolates from R. Mbogo and R. Nyando carried plasmids. Previous studies have demonstrated the role of plasmids in conferring resistance to both antibiotics and metals. Mc Hugh *et al*. (1975) have shown plasmids conferring antibiotic and metal resistance to be present in *Salmonella typhimurium* isolates from human burn wounds. Pickett *et al.*, (1976) demonstrated genetic linkages (presumably by plasmids) between antibiotic resistance in *Enterobacter aerogenes* and tolerance to cadmium and zinc.

CONCLUSIONS

This study established presence of elevated levels of lead, cadmium and manganese in some of the rivers draining into Lake Victoria. These Rivers serve as the sink to excess untreated effluent of both industrial and municipal origin from the major towns. Such metal rich effluent co-selects for antibiotic resistance among the environmental bacteria.

This is evidenced by the presence of plasmids in multidrug resistant isolates suggesting that this characteristic may have been metal induced. This poses a potential public health threat as bacterial strains usually considered harmless could receive R factors that confer multiple drug resistance.

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