International Journal of Pharmacotherapy

www.ijopjournal.com

ISSN 2249 - 7765
Print ISSN 2249 - 7773

STUDY OF METAL TOLERANCE COLIFORM BACTERIA IN THE GOMTI RIVER WATER OF NORTHERN INDIA

Asma Akhter and Mohd Imran*

Department of Biosciences, Integral University, Lucknow-226026, Uttar Pradesh, India.

ABSTRACT

This study was carried out to assess the incidence of metal resistance among coliform bacteria in terms of their viable count (cfu/ml) and minimum inhibitory concentrations (MIC) from the Gomti river water. It was investigated in heavy metal amended Nutrient Agar and Mac Conkey plates at varying concentrations 50 – 800 and 100 – 2000 µg/ml respectively. The results demonstrated that the growth and MIC of coliform were dependent upon the metals type and concentrations level. Reduction in coliform population is evident as metal concentration increased. Minimum MIC level was observed between 50-100 µg/ml against Cr6+ and maximum at between 1600-2000 µg/ml against Cd+, Ni+ and Hg2+ among isolates. Furthermore, increased levels of heavy metal concentration as well as thermotolerant coliforms were found from site A to C in heavy metal resistant (HMR) strains. The isolation rate of heavy metal coliforms strains was highest during the summer and monsoon months.

Abbreviations: Minimum inhibitory concentration, MIC; Mercury, Hg^{2+}; Cromium, Cr^{6+}; Cadmium, Cd^{+}; Nickel, Ni^{+}; Copper, Cu^{2+}; Cobalt, Co^{+}; Zinc, Zn^{2+}; Colony form unit, cfu; Heavy metal resistant, HMR.

Key words: Heavy Metal Resistance; Coliform Bacteria; Growth Response.

INTRODUCTION

Metal contaminants are commonly found in soils, sediments, and water. Concerns about the presence of heavy metals in aqueous effluents are well known and they appear linked to a large variety of industries related to metal finishing, electroplating, plastics, pigments and mining, which threat to the environment. Contamination of the aquatic environment by toxic metal ions is a serious pollution problem; heavy metals may reach watercourses either naturally through a variety of geochemical processes or by direct discharge of municipal, agricultural and industrial wastewater.

At elevated concentrations, soluble metal compounds can be deleterious to human health as well as to aquatic and marine environments [1]. Cadmium, arsenic, mercury, lead and chromium have been known to be extremely toxic at lower concentration. The contamination of the environment with toxic metals has become a worldwide problem, affecting crop yields, soil biomass and fertility, contributing for the bioaccumulation and biomagnifications in the chain. High concentration of all metals like Cr^{6+}, Cu^{2+}, Ni^{+}, Cd^{+} and Zn^{2+} were noticed in River Gomti from 2006–2013.

Coliforms have adapted to the presence of heavy metals in the environment and have developed resistance mechanisms [2]. Although the type of mechanisms may be more or less homologous in all species of bacteria, it is expected to obtain different responses to the same toxic concentration for several bacterial species [3]. Microbial resistance to metal ions is a potential health hazard since these traits are generally associated with transmissible plasmids.

The environmental spread of coliform bacteria provides a useful indication of the prevailing conditions. In this study, the occurrence of heavy metal resistant

Corresponding Author:- **Mohd. Imran** Email: mohd.imran.iu@gmail.com

(HMR) coliform strains along with metal concentration investigated in three sites of the River Gomati, a tributary of the Ganges which flows through the densely populated rapidly industrializing Indo-Gangetic plain.

MATERIALS AND METHODS

Water samples were collected from three sampling station (site A, site B, and site C) of Gomti River Water along with Nadwa Bridge (site A: entry of water in city), Nishatganj Bridge (site B: center of city), where the river is heavily polluted with untreated sewage and domestic wastewater and Gomti Nagar Bridge (site C: city end) at Lucknow city. Water samples were collected 0–20 cm below from the surface using sterile 250 mL bottles. Samples collected for metal concentration analysis were transported to the laboratory within 3 h. All samples were brought to the laboratory in an ice chest, and processed within 4 h of collection. Heavy metal concentration analysis, water parameter determination and thermo and non thermotolerant coliforms (*E.coli* and *Enterobacter*) analysis was done during the monsoon, summer and winter seasons. Heavy metal tolerant coliforms at different concentration were determined in normal weather (March-April).

The pH, temperature and dissolved oxygen, were measured using a Century portable analysis kit. The biochemical oxygen demand, total viable count, total coliform count and thermotolerant coliform count were determined according to Standard Methods for Examination of Water and Wastewater [4].

The strains were identified according to CoWAN and STEEL (Cowan and Steel, 1974) and the Eijkman test was performed [5].

The isolation of coliform was done according to spread plate technique. To determine total counts, plate counts of coliform bacteria were made using Mac Conkey Agar (McC) (Merck), inoculated with appropriate dilution (10^{-1}) from the sample homogenates, and incubated for 24 h at 37ºC. To evaluate the incidence of resistant bacteria, media supplemented with Cd^{+}, Cr^{6+}, Zn^{2+}, Hg^{2+}, Cu^{2+}, Ni^{+}, and Co^{+} were used at their varying concentration 50–800µg/ml. Pigmented colonies were identified as Coliforms and purified after repeated streaking. The purified isolates were preserved at 4 °C in agar slants by repeated sub culturing. Metal analysis of Gomti River water samples site A, B and C in summer, monsoon and winter were measured using a Perkin- Elmer 3110 atomic absorption spectrophotometer, with reference to appropriate standard solutions, and the metal concentrations in the water samples [6] from Industrial Toxicology Research Centre (IITR) Lucknow (accession no. 2234).

The MIC of seven different heavy metals (Cd^{+}, Cr^{6+}, Cu^{2+}, Zn^{2+}, Hg^{2+}, Co^{+} and Ni^{+}) was determined for each isolate using nutrient agar containing each metal in concentrations ranging from 50-2000 µg/ml. Seven different heavy metals were used. An Escherichia coli K-12 strain was used as the control organism as described by [7].

Table 1. Water quality parameters of River Gomati

S. No.	Parameters	Site A			Site B			Site C		
		Summer	Monsoon	Winter	Summer	Monsoon	Winter	Summer	Monsoon	Winter
1.	pH	7.87	7.02	7.54	7.16	7.05	7.73	7.42	7.88	7.25
2.	Temp.	33.42	32.6	23.4	34.78	33.76	23.8	35.65	32.2	26.7
3.	D.O.	7.9	6.8	7.7	4.8	5.6	6.2	0.87	0.94	2.4
4.	B.O.D.	4.2	4.6	4.2	6.9	7.3	7.5	8.1	8.3	8.5
12.	TVC	$2.3x10^5$	$3.4x10^5$	$3.7x10^5$	$6.8x10^6$	$7.4x10^7$	$6.4x10^5$	$8.8x10^7$	$9.8x10^8$	$8.7x10^6$
13.	TC	$1.3x10^3$	$1.56x10^3$	$1.06x10^3$	$5.06x10^4$	$6.06x10^4$	$5.56x10^4$	$7.02x10^4$	$7.28x10^4$	$7.1x10^4$
14.	FC	$1.08x10^3$	$1.16x10^3$	$1.04x10^3$	$2.23x10^3$	$3.06x10^3$	$2.02x10^3$	$3.78x10^3$	$4.14x10^3$	$3.08x10^3$

Temp= temperature (^{0}C), TC= total coliforms/ 100 ml, FC= faecal (thermotolerant) coliforms/ 100 ml, D.O. = Dissolved oxygen, B.O.D. = biochemical oxygen demand, TVC= total viable count/ml, ND= not detected, NA= not available.

Table 2. Identification of coliform bacteria (*E.coli* and *Enterobacter*) isolated from Gomati River water

S.No.	Genus and Species	Site A	Site B	Site C
	Total Strains	147	172	157
A.	**Thermotolerant**			
1.	*Escherichia coli*	62 (42.18%)	75 (43.61%)	71 (45.22%)
B.	**Non-thermotolerant**			
1.	*Escherichia coli*	32 (21.77%)	37 (21.51%)	29 (18.47%)
2.	*Enterobacter aerogenes*	39 (26.53%)	41 (23.84%)	42 (26.75%)
3.	*Enterobacter cloacae*	7 (4.76%)	8 (4.65%)	6 (3.82%)
4.	*Enterobacter liquefaciens*	1 (0.68%)	2 (1.16%)	1 (0.64%)
5.	*Enterobacter species*	6 (4.08%)	9 (5.23%)	8 (5.1%)

Table 3. Water sample heavy metal analysis report of River Gomti:

Metals	TS-1550 (mg/L) (Site A)			TS-1550 (mg/L) (Site B)			TS-1550 (mg/L) (Site C)		
	Summer	**Monsoon**	**Winter**	**Summer**	**Monsoon**	**Winter**	**Summer**	**Monsoon**	**Winter**
Chromium (T)	ND	ND	ND	<0.05	<0.07	ND	<0.09	<0.078	<0.072
Cadmium	0.004	0.005	0.003	0.006	0.008	0.004	<0.014	0.007	0.007
Zinc	0.072	0.081	0.076	0.088	0.058	0.041	0.045	0.047	0.04
Nickel	0.008	0.008	0.007	0.012	0.018	0.006	0.0121	0.0132	0.008
Copper	0.006	0.007	<0.025	<0.025	<0.035	<0.027	0.0078	0.092	<0.028
Cobalt	<0.018	0.006	0.008	<0.025	<0.027	<0.021	0.0081	0.012	<0.02
Mercury	ND	ND	ND	0.005	0.007	0.004	0.0061	0.0062	ND

ND=not detected

Table 4. Viable count of heavy metal tolerant coliform bacteria in the Gomti River water samples:

Metals	Conc.	Site 1 (cfu/ml of water)	Site 2 (cfu/ml of water)	Site 3 (cfu/ml of water)
Control	**No Metal**	2.20±0.03x10^4	3.45±0.05x10^4	9.8±0.07x10^3
Cr^{+6}	50	1.29±0.04x10^4	2.24±0.04x10^4	5.5±0.5x10^3
	100	0.94±0.2x10^4	1.15±0.04x10^4	4.0±0.1x10^3
	200	0.76±0.06x10^4	0.96±0.3x10^4	3.3±0.2x10^3
	400	0.38±0.5x10^4	0.70±0.5x10^4	0
	800	0.14±0.3x10^4	0.54±0.2x10^4	0
Cd^{+}	50	8.7±0.2x10^3	7.0±0.2x10^3	1.7±0.2x10^3
	100	4.5±0.4x10^3	4.7±0.1x10^3	1.1±0.1x10^3
	200	2.6±0.07x10^3	2.6±0.3x10^3	0
	400	0	0	0
	800	0	0	0
Ni^{+}	50	2.48±0.03x10^3	2.10±0.08x10^4	5.0±0.5x10^3
	100	1.49±0.02x10^3	1.40±0.1x10^4	3.3±0.08x10^3
	200	1.18±0.08x10^3	1.15±0.2x10^4	1.5±0.1x10^3
	400	0.68±0.2x10^3	0.8±0.05x10^4	0
	800	0	0	0
Cu2^{+}	50	2.45±0.05x10^4	1.96±0.02x10^4	3.1±0.1x10^3
	100	1.02±0.04x10^4	1.20±0.02x10^4	2.8±0.4x10^3
	200	0.88±0.3x10^4	1.01±0.02x10^4	1.5±0.5x10^3
	400	0.42±0.2x10^4	0.76±0.03x10^4	0.7±0.2x10^3
	800	0	0.44±0.6x10^4	0
Co^{+}	50	1.60±0.02x10^4	1.08±0.002x10^4	2.3±0.2x10^3
	100	1.45±0.02x10^4	1.04±0.03x10^4	1.8±0.3x10^3
	200	0.85±0.4x10^4	0.63±0.1x10^4	1.3±0.07x10^3
	400	0.20±0.2x10^4	0.26±0.05x10^4	0.7±0.1x10^3
	800	0.15±0.4x10^4	0.11±0.04x10^4	0
Hg^{2+}	50	0	0	0
	100	0	0	0
	200	0	0	0
	400	0	0	0
	800	0	0	0
Zn^{2+}	50	2.9±0.4x10^3	2.0±0.1x10^3	1.5±0.4x10^3
	100	1.8±0.5x10^3	0.8±0.3x10^3	1.1±0.3x10^3
	200	0.6±0.2x10^3	0.5±0.2x10^3	0.5±0.2x10^3
	400	0	0	0.1±0.1x10^3
	800	0	0	0

All metal levels have been expressed in µg/ml.
The values represent the mean ±SD.

Figure 1. Map showing sampling sites A, B and C.

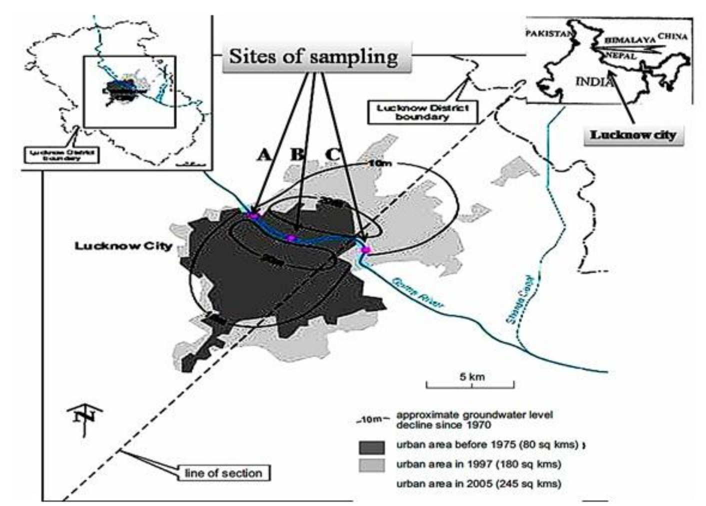
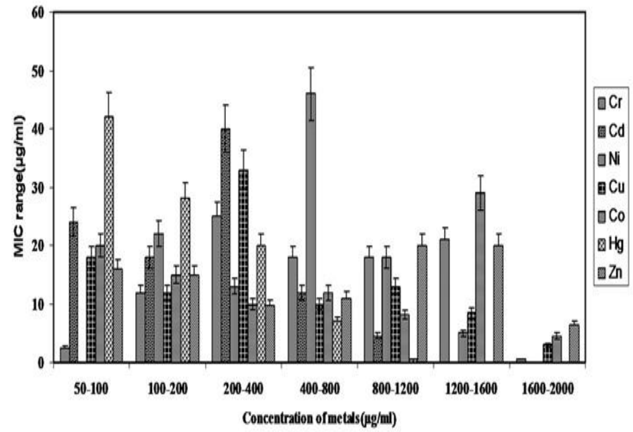


Figure 2. MIC range of coliform isolates against different heavy metal concentration.



The values represent the mean ±SD.

RESULTS AND DISCUSSION

From Table 1 the increase in pollution from site A to site C water is apparent. Table 2 shows the bacterial species identified among the site A, B and C coliform populations isolated. All three sites, thermotolerant *Escherichia* coli and *Enterobacter* appeared with greater frequency.

In the present study, heavy metal tolerant population of coliforms from the river water samples was observed against seven metals (Cr^{6+}, Ni^{+}, Zn^{2+}, Cu^{2+}, Co^{+}, Hg^{2+} and Cd^{+}) at their varying concentrations (50 to 800µg/ml). Viable count of coliforms was higher in non-metal supplemented control plate than metal supplemented plates in site A, B and C respectively. A decrease in viable count was recorded with the increase of metal concentrations tested in all A, B and C sampling sites.

The viable count of coliforms in different concentrations (50-800 µg/ml) of metals ranged from $0.6x10^{3}$ to $1.29x10^{4}$, $0.11x10^{4}$ – $2.24x10^{4}$, $0.1x10^{3}$ – $5.5x10^{3}$ cfu/ml of water in site A, B and C respectively. In case of site-A maximum viable count was recorded against Ni^{+} ($1.49x10^{4}$), followed by Co^{+} ($1.45x10^{4}$), Cu^{2+} ($1.02x10^{4}$), Cr^{6+} ($0.94x10^{4}$), Cd^{+} ($4.5x10^{3}$), Zn^{2+} ($1.8x10^{3}$) at 100 µg/ml respectively. Similar trend of metal toxicity for viable count of coliforms was recorded at 200 – 400 µg/ml of the metals tested. All metals showed no viable count at 800 µg/ml other than Cr^{6+} and Co^{+}.

In case of site B, higher count of coliforms was observed as compared to site A. Maximum viable coliforms count was recorded against Ni^{+} $1.4x10^{4}$ followed by Cu^{2+} ($1.2x10^{4}$), Cr^{6+} ($1.15x10^{4}$), Co^{+} ($1.04x10^{4}$), Cd^{+} ($4.7x10^{3}$) and Zn^{2+} ($0.8x10^{3}$) at 100µg/ml concentration respectively. Similar trend of coliform count was recorded at 200 and 400µg/ml concentration of the metals tested. No viable count was found at 400 µg/ml concentration of Hg^{2+}, Cd^{+}, Zn^{2+} respectively.

In site C: A different trend of metal toxicity was observed as compared to site A and B. Maximum viable count of coliform ranged from $3.3x10^{3}$ – $5.5x10^{3}$ against Cr^{6+} followed by 1.5 $x10^{3}$ – 5.0 $x10^{3}$, 0.7 $x10^{3}$ – 3.1 $x10^{3}$, 0.7 $x10^{3}$ – 2.3 $x10^{3}$ and 0.1 $x10^{3}$ – 1.5 $x10^{3}$ against Ni^{+}, Cu^{2+}, Co^{+} and Zn^{2+} at 50 – 400 µg/ml concentration range respectively. No viable bacterial count was observed against Cd^{+} at above 200µg/ml concentration as compared to other metals tested. The growth of coliforms could not be detected at any concentration (50 – 100µg/ml) of Hg^{2+} amended plates (Table 4). Heavy metal contents in all three sites of the Gomti River are depicted in summer, monsoon and winter (Table 3). Heavy metal analysis in all three weather at different sites showing that the higher concentration was found in summer and monsoon than in winter with in order of site C > site B > site A. This analysis depicts that discharge of municipal, agricultural and industrial wastewater are greater in site B and C in summer and monsoon. The high frequency of thermotolerant *E. coli* site B and C is not unexpected because large numbers of people along with their animals live on the river banks city boundaries in the rural areas. Site B and C coliforms, as expected; include more thermotolerant *E. coli* and most of the MHR coliforms isolated belonged to this group.

In the present study, the level of metal tolerance among 154 isolates was determined in term of Minimum Inhibitory Concentration (MIC) of heavy metals (Cd^{+}, Cr^{6+}, Co^{+}, Hg^{2+}, Zn^{2+}, Cu^{2+} and Ni^{+}). Coliform isolates showed a varied range of their MIC's level against heavy metals tested. All the isolates showed their MIC level in between 50 to 2000 µg/ml concentration of the metals. Maximum (20%) number of coliform isolates showed their MIC range 800-1200µg/ml against zinc and minimum (1 %) against mercury as compared to other metals. Of 29% isolates showed their MIC range 1200-1600 µg/ml against co and minimum (5%) against nickel.

Similarly maximum number of isolates demonstrated their MIC levels in the range of 1600- 2000 μg/ against zinc and minimum against copper by 10% and 4% respectively among all the metals. Maximum number of isolates showed their resistance against Ni^{+} in terms of their MIC levels followed by Cr6^{+}, Zn^{2+}, Cu^{2+}, Co^{+} Cd^{2+} and least to Hg^{6+} in the range of 50-100 μg/ml concentrations tested (figure 1 and figure 2).

In the past few decades, uncontrolled urbanization has caused a serious pollution problem due to the disposal of sewage and industrial effluents to water bodies. Unlike many other pollutants, heavy metals are difficult to remove from the environment [8] these heavy metals such as copper, cadmium, lead, zinc, nickel, mercury and chromium when accumulated in soils, water bodies they can also be present in concentrations toxic to plants, animals, humans and aquatic life [9] Each heavy metal has unique biofunctions or biotoxicities. For example, copper can enhance microbial growth at low concentrations but repress growth at high concentrations and cadmium has high toxicity at low concentrations [10].

Microorganisms have acquired a variety of mechanisms for adaptation to the presence of toxic heavy metals [11].There is increasing evidence for the evolution of metal resistance in natural populations inhabiting contaminated sites [12,13]. Aquatic microbes become resistant to antibiotics and metals as a result of contamination with effluents [14]. Antibiotic resistance in bacteria is more frequently associated and strongly correlated with metal resistance [15]. To survive under metal-stressed conditions, bacteria have evolved several types of mechanisms to tolerate and uptake of heavy metal ions. Therefore this study was performed to determine the HMR populations of coliforms bacteria in the Gomti river water samples receiving long term industrial and domestic wastewater at Lucknow city. Microbial Growth rates in the presence of heavy metals (Cd^{+}, Cr^{6+}, Ni^{+} and Pb^{2+}) were consistently slower than that of the control (similar observation have been reported earlier, [16] Significant variation in the growth pattern (Cfu) was observed for each of the heavy metals used in the study individually. In previous studies Cr (VI) resistant microorganisms have been found capable of growing in at higher concentration (10–1500 mg of Cr (VI)) [17]. Enterobacter cloacae and Klebsiella species have been resistant against Cd^{+}, Cr^{6+} and Pb^{+} at their high concentration supplemented in the medium respectively. In our investigation we observed a varied trend of metal resistance among the –coliforms population in the Gomti river water. Viable count of coliforms was higher in non metal supplemented control plate than metal supplemented plates in site A, B and C respectively. In the growth medium amended with metal concentration of 50μg/ml, no significant negative effects of the metals on the bacterial growth were observed when compared with the control without metal amendment [18]. A decrease in viable count was recorded with the increase of metal concentrations tested in all A, B and C sampling sites. Mercury has shown highest toxicity against coliforms in all water samples from site A, B and C. Other reports are also in agreement of our observation regarding the higher toxicity of mercury [18]. A high range ($1.1x10^{3}$–$2.24x10^{4}$ cfu/ml) of metal resistant coliforms population was recorded in site B as compared to site A ($6.0X10^{2}$ – $1.29X10^{4}$ cfu/ml) and site C ($1X10^{2}$ – $5.5X10^{3}$ cfu/ml). A few or nil number of viable counts of coliforms was recorded at 400 or above in terms of all metals tested from three sampling sites. In site C, a different trend of metal toxicity was observed in terms of viable count of coliforms bacteria as compared to site A and B. Maximum viable count of bacteria ranged from $3.3X10^{3}$ – $5.5X10^{3}$ against Cr^{6+} followed by $1.5X10^{3}$ – $5.0X10^{3}$, $7.0X10^{2}$ – $3.1X10^{3}$, $7X10^{2}$ – 2.3 $x10^{3}$ and 6.1 $x10^{3}$ – 1.5 $x10^{3}$ against Ni^{6+}, Cu^{2+}, Co^{+} and Zn^{2+} at 50 – 400 μg/ml concentration range respectively. Viable count of coliforms showed a toxicity order Cd^{+}> Zn^{2+}> Co^{+}> Cu^{2+}> Ni^{+}> Cr^{6+} which is almost in agreement of toxicity order for E. coli reported by Gulsen and Nuray [19]. In our observation, Viable count of metal tolerant coliforms was observed as proportional to the pollutants levels in sampling sites but it may vary on the interaction affinity of the pollutants specially heavy metal ions with bacterial cell. The lower values of microbial load at higher metal concentrations revealed that the coliform growth was affected due to the presence of heavy metal in the growth medium. The decrease in microbial density caused by a high level of heavy metal contamination found in this study is in agreement with Anyanwu et al. [18]. The total microbial load as shown by the colony forming unit (cfu) represents the coliforms resistant to the heavy metal pollution. It is, therefore, reasonable to assume that these resistant bacteria form a larger part of the total bacterial population at elevated levels of metal contamination of river water. The predominance of coliform bacteria at higher concentration of metal is probably due to their higher level of intrinsic metal resistance [19], Cervantes and Silver [20] explained that many coliforms have genes that control resistances to specific toxic heavy metals and quite similarly to the genes for heavy metal resistances and other ancillary functions. These resistances often are determined by plasmids and transposons that confer highly specific resistances to Cd^{+}, Cu^{2+}, Cr^{2+} and other toxic heavy metals [21]. In this study we observed a higher tolerance level among coliforms against heavy metals which is similar to the previous reports of high resistance level in gram negative bacteria rather than gram positive bacteria. Gunaseelan and Ruban [21] reported a varied range of metal (Hg^{2+}, Cr^{6+}, Cd^{+}, and Zn^{2+}) tolerant gram negative bacterial population isolated from Krishna-Godavari river water. Microorganisms undergo selection pressures in the presence of toxic compounds and develop resistance [22] Bacteria are generally the first organisms to be affected by discharges of heavy metals into the aquatic environment, resulting in an increase of metal resistant bacteria in aquatic environments [23].

Bacterial resistance may be due to the presence of R-plasmid containing genes for both antibiotics & heavy metals [18] Metal tolerance may also be related to the production of capsular polysaccharides usually by Enterobacter group of organisms, which can combine with metals to protect themselves from the toxicity of metals [24] Our research group also working on multiple antibiotic resistant coliforms in contaminated (discharge of municipal, agricultural and industrial wastewater) drinking river water [25,26].

More often the resistance phenomenon is plasmid borne and transferable in nature resulting its spread among the sensitive aquatic bacteria including coliforms. Thus, bacterial tolerance to metal toxicity is a significant environmental phenomenon [27].

Mechanisms of dispersion of the metal resistant bacteria in the natural environment may be via plasmid transfer among the sensitive aquatic bacteria including coliforms [28]. This situation may be therefore posing a threat to natural environments and human health. Presence of metal tolerant bacterium in a given environment may be an indication that such area is affected by heavy metals. Such an area may foster adaptation and selection for HMR organisms [21]. Isolation of bacteria from metal polluted environment would represent an appropriate practice to select metal resistant strains that could be used for heavy metal removal and bioremediation purposes [21].

ACKNOWLEDGEMENTS

We are thankful to Prof. S. W. Akhtar, Vice Chancellor, Integral University, for providing the necessary facility to conduct this research. Authors also like to thank to the Hod's Department of Bio-Sciences & Bio-Engineering, Integral University Lucknow for guidance and their cooperation in regarding the research work.

REFERENCES

1. Srinivasa-Rao K, Anand S, Venkateswarlu P. Adsorption of cadmium (ll) ions from aqueous solution by Tectona grandis L.F (teak larvae powder). *Bioresources*, 5, 2010, 438-454.
2. Bruins MR, Kapil S, Oehme FW, Microbial resistance to metals in the environment. *Ecotoxicol Environ Saf*, 45, 2000, 198-207.
3. Mrvčić J, Prebeg T, Barišić L, Staner D, Bačun-Drùina V, Stehlik-Tomas V. Zinc binding by Lactic acid bacteria. *Food Technol Biotechnol*, 47, 2009, 381-388.
4. Anon. Standard Methods for the Examination of Water and Wastewater 13th ed., American Public Health Association Inc, Washington DC, 1971, 880.
5. Cruickshank R. Medical Microbiology, 11th ed., 1965, E. S. Livingstone, London.
6. AMAAS. Perkin-Elmer, Norwalk, Connecticut, 2009, USA.
7. Ansari MI, Malik A. Biosorption of nickel and cadmium by metal resistant bacterial isolates from agricultural soil irrigated with industrial wastewater. *Bioresour Technol*, 98, 2007, 3149-3153.
8. Ren WX, Li PJ, Geng Y, Li XL. Biological leaching of heavy metals from a contaminated soil by Aspergillus niger. *J Hazard Mater*, 167, 2009, 164-169.
9. Dowdy RH, Volk VV, Movement of heavy metals in soils. In, D.W. Nelsenetal, Editor, Chemical Mobility and Reactivity in Soil Systems. SSSA Spec Publ SSSA, Madison, WI, 11, 1983, 229-240.
10. Karnachuk O V, Kurochkina SY, Nicomrat D, Frank YA, Ivasenko DA, Phyllipenko EA, Tuovinen OH. Copper resistance in strain R2. *Anton Leeuw*, 83, 2003, 99-106.
11. Rajbanshi A. Study on Heavy Metal Resistant Bacteria in Guheswori Sewage Treatment Plant. *Our Nature*, 6, 2009, 52-57.
12. Posthuma L, Van Straalen NM, Heavy metal adaptation in terrestrial invertebrates, a review of occurrence, genetics, physiology and ecological consequences. *Comp biochem Physiol C Toxicol Pharmacol*, 106, 1993, 11-38.
13. Cowan ST, Steel KJ. Manual for the Identification of Medical Bacteria, 1974, Cambridge University Press, London.
14. Trevors JT. Copper resistance in bacteria. *Microbiol Sci*, 4, 1887, 29–31.
15. Bell JB, Elliot GE, Influence of sewage treatment and urbanization on selection of multiple resistance in fecal coliform populations. *Appl Environ Microbiol*, 6, 1983, 227-232.
16. Pal A, Choudhuri P, Dutta S, Mukherjee PK, Paul AK. Isolation and characterization of nickel-resistant microflora from serpentine soils of Andaman. *World J Microb Biot*, 20, 2004, 881-886.
17. McLean J, Beveridge TJ. Chromate reduction by a pseudomonad isolated from a site contaminated with chromate copper arsenate. *Appl Environ Microbiol*, 67, 2004, 1076-1084.
18. Anyanwu CU, Nwankwo SC, Moneke AN, Soil Bacterial Response to Introduced Metal Stress. *Int J Basic Appl Sci*, 11(01), 2011, 109.
19. Gulsen A, Nuray B, Levels of some toxic elements and frequency of bacterial heavy metal resistance in sediment and sea water. *Environ Monitor Assess*, 149(1-4), 2008, 61-69.
20. Cervantes C, Silver S, Plasmid chromate resistance and chromate reduction. *Plasmid*, 27, 1992, 2265-2271.

21. Gunaseelan C, Ruban P, Heavy metal resistance Bacterium isolated from Krishna-Godavari basin, Bay of Bengal *Int J Environ Sci*, 1(7), 2011, 0976-4402.
22. Hideomi N, Ishikawa T, Yasunaga S, Kondo I, Mitsuhasi S, Frequency of heavy-metal resistance in bacteria from inpatients in Japan. *Nature*, 266, 1977, 165-167.
23. Silver S, Bacterial resistances to toxic metal ions- A review. *Genetics*, 179, 1996, 9-19.
24. Bitton G, Freihofer V, Influence of extracellular polysaccharide on the toxicity of copper and cadmium towards *Klebsiella aerogenes*. *Microbiol Ecol*, 4, 1978, 119-125.
25. Akhter A, Imran M, Akhter F, Prevalence of Metal Tolerant Coliforms in the Gomti River Water at Lucknow City. *Bioinformation*, 10(4), 2014,
26. Akhter A, Imran M, Akhter F. Determination of Multiple Antibiotic Resistance (M.A.R) Patterns and M.A.R Indexing Among Metal Tolerant Beta Lactamase Producing E. Coli in Potential Significance of Public Health. *African J microbiol Res*, 8(7), 2014, 619-627.
27. Saikia S, Saikia D, Ramteke PW. Use of microbes from seabird faeces to evaluate Heavy metal contamination in antarctic region. *Appl Ecol Environ Res*, 6(3), 2008, 21-31.
28. Malik A, Metal bioremediation through growing cells. *Environ Int*, 30, 2004, 261-278.