http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 7 (1): 6-9, 2004
ISSN 1028-8880

Resistance of *Bacillus cereus* and *E. coli* Towards Lead, Copper, Iron, Manganese and Arsenic

Rashida Kanwal, Toqeer Ahmed, S.S. Tahir and Naseem Rauf
Microbiology/Analytical Laboratories Pakistan Council of Scientific and Industrial Research, Chairman's Secretariat, Islamabad, Pakistan

Abstract: In present study, drinking ground water of different sectors of Islamabad was analyzed. Twenty-three water samples were collected in sterile bottles from different tube wells located in various sectors of Islamabad. These water samples were observed microbiologically for total viable count (TVC) and coliform count by heterotrophicplate count and MPN method, respectively. Different pathogenic bacteria were isolated and identified. Water samples of tube well number 101 and 138 were found to be contaminated and showed the presence of *E.coli* while the all other tube wells were contaminated with *B. cereus*. These bacterial species were isolated and then the tolerance of these isolated strains were checked in the presence of metals like Pb, Cu, Fe, Mn and As at different concentration (from 0.5 to 10 ppm). Results revealed that these bacteria successfully survive in these toxic conditions. As shown in results that *E.coli* has the ability to survive at 10 ppm of As but *B. cereus* can survive at 10 ppm of Mn and As. While no growth of *E.coli* and *B. cereus* was observed at 10 ppm of other metals like Pb, Cu and Fe.

Key words: Pathogenic bacteria, *E. coli, B. cereus,* toxic metals

INTRODUCTION

Microbial resistance to heavy metals is widespread. This is not surprising, as early in evolutionary history microorganism would have been in contact with toxic concentrations of heavy metals. Geo-chemical events caused the release of heavy metals from earth's crust into the biosphere and still do. The change from a nontoxic to a toxic biosphere altered the redox state and biological availability of number of heavy metals. Resistance to a wide range of toxic metal ions has been reported in bacteria. These include resistances to metals that are purely toxic. Most heavy metals are transition elements with incompletely filled d orbitals. These d orbitals provide heavy-metal cations with the ability to form complex compounds, which may or may not be redox-active. Thus, heavy metal cations play an important role as "trace elements" in sophisticated biochemical reactions. At higher concentrations, however, heavy –metal ions form unspecific complex compounds in the cell, which leads to toxic effects. Some heavy-metal cations, e.g. Hg (II), Cd (II) and Ag(I) form strong toxic complexes, which makes them too dangerous for any physiological function. Even highly reputable trace elements like Zn (II) or Ni(II) and especially Cu(II) are toxic at higher concentrations. Thus, the intracellular concentration of heavy-metal ions has to be tightly Copper resistance in *E.coli* was first identified in enteric bacteria isolated from Australian pig farms. The plasmid born copper resistance genes (pco) was found on the conjugative plasmid and resistance was inducible by cupric salts.

Manganese exists in various oxidation states; from Mn (II) to Mn (VII) every state is possible with the Mn^{2+} cations being the predominant form. Therefore it seems logical that managanese is used by bacteria as an electron acceptor in anaerobic respiration processes. Iron is the only mico-bioelement of the heavy metals. In addition siderophore-mediated uptake of $Fe^{3+,}$ Fe^{2+} is also transported into bacterial cells. Fe^{2+} is similar in ionic diameter and charge to Mg^{2+} Thus it is also accumulated by the fast and unspecific Magnesium transport system in *E. coli*.

Radical character of copper makes it very toxic and many organisms are more sensitive to copper than *E.coli*. Copper toxicity is based on the production of hydrperoide radicals and on interaction with the cell membrane. Because of toxicity, arsenic has no function in trace elements; however bacteria may use it as an electron acceptor for anaerobic respiration. Aerobic bacteria are able to oxidize arsenite again; thus a geomicrobial redox cycle of arsenic exists, similar to the iron and sulfur cycles. Lead (Pb) is not as bad as its reputation. Lead is no transition element, but belong to the element group Iva, C, Si, Ge. Sn. Pb. In seawater it is more rare than mercury (Weast, 1984). Owing to its low solubility its

biologically available concentration is low. Thus lead is not extraordinary toxic for microorganisms. Lead tolerant bacteria have been isolated and the precipitation of lead phosphate within the cells of these bacteria. Thus lead resistance may also be based predominantly on metal ion efflux.

The main objective of this study to analyze the ground drinking waters for the bacterial contamination. In this study, an attempt was made to check the ability of microorganisms (*E. coli* and *B.cereus*) to degrade different concentrations of metals like Pb, Cu, Fe, Mn and As.

MATERIALS AND METHODS

Isolation procedures: Approximately 23 water samples were collected from different tube wells of different sectors of Islamabad. Samples were collected in sterile leak proof bottles following the standard methods of water collection for microbiological analysis between October and January 2003, which are given in Table 1.

Isolation and identification: Isolated colonies were picked up randomly from media plates and pure culture was obtained by further streaking. The isolated cultures were then identified by different bio-chemical tests as given in the earlier literature (Collins and Lyne, 1980, Cappuccino and Sherman, 1986 and Anonymous, 1974, 2001).

The water samples were then examined for total viable count (TVC) by serial dilutions on the Nutrient media plates and *Coliform* count by Most Probable Number (MPN) method.

Isolated and identified bacteria (*E. coli and B. cereus* from tube well number 101 and 158 respectively) were used to check the effect of different concentration like 0.5, 1, 1.5, 2, 2.5, 3, 5, 7, 8 and 10 ppm of different metals such as Lead (Pb), Copper (Cu), Manganese (Mn), Arsenic (As) and Iron (Fe) was checked. Total Viable cells were also checked by making serial dilutions of different concentration of different metals and effect of different concentration of metals was checked on total viable cells.

Temperature tolerance tests were performed to check the ability of microorganisms to grow at different temperatures like from 10 to 50°C.

RESULTS AND DISCUSSION

All water samples, which were collected according to method given in Anonymous (1989) and were examined for different pathogenic bacteria. Water samples were

Table 1: Total bacterial count

Tube well No.	Total viable ount (TVC) per ml	Tube well No.	Total viable count (TVC) per ml
40	$7.2x10^3$	65	302×10^5
63	$7.0x10^2$	41	$8x10^2$
SW II	ND	161	ND
193	$1.0x10^3$	157	ND
Markaz	$5.2x10^3$	100	ND
158	40×10^5	169	ND
62	2x102	103	ND
152	$50\ 10^6$	Main Supply of I-10 Markaz	ND
200	75×10^7	71	$40 x10^2$
201	200	101	$75 x10^2$
204	40×10^4	138	$7.0x10^2$
203	320		

ND = Not Detected

Table 2: Total bacterial count MPN values 100^{-1} ml of sample and 95% confidence limits for various combinations of positive and negative results (when five 10ml, five 1ml and five 0.1 ml test portion are used

Tube well No.	No. of tubes giving a positive reaction 5 of 10 ml	5 of 1 ml	5 of 0.1 ml	MPN (100^{-1} ml)	95% confidence limits Lower	Upper
40	0	0	0	<2	<1	7
63	0	0	0	<2	<1	7
SW II	0	0	0	<2	<1	7
193	0	0	0	<2	<1	7
Markaz	0	0	0	<2	<1	7
158	0	0	0	<2	<1	7
62	0	0	0	<2	<1	7
152	0	0	0	<2	<1	7
200	0	0	0	<2	<1	7
201	0	0	0	<2	<1	7
204	0	0	0	<2	<1	7
203	0	0	0	<2	<1	7
65	0	0	0	<2	<1	7
41	0	0	0	<2	<1	7
161	0	0	0	<2	<1	7
157	0	0	0	<2	<1	7
100	0	0	0	<2	<1	7
169	0	0	0	<2	<1	7
103	0	0	0	<2	<1	7
Supply of I-10 Markaz	0	0	0	<2	<1	7
71	0	0	0	<2	<1	7
101	4	1	1	21	7	63
138	4	0	1	17	5	46

MPN = most probable number

Table 3: Biochemical properties of different identified bacteria

Characteristics	*B. cereus*	*E. coli*
Morphology	Rods, Single or in pairs	Rods, Single
Colonies	Rhizoidal colonies	Round
H_2S Production	-	-
Citrate Utilization	Late +	-
Gram Staining	-	-
Catalase Test	+	+
Growth in 4% NaCl	+	+
Growth in 6.5%NaCl	+	-
Growth at 10°C	+	_
Growth at 37°C	+	+

B. cereus = *Bacillus. cereus* E. coli = *Escherichia. coli* + = Positive result - = Negative result

Table 4: Results of sugar test

Species	Fructose	Galactose	Glucose	Sucrose	Maltose	Mannitol	Lactose	Arabinose	Xylose	Dulcitol
B.cereus	+	-	+	+	-	-	-	-	-	-
E. coli	+	+	+	-	+	+	-	+	+	+

B = Bacillus E. Escherichia + = Positive result - = Negative result

Table 5: growth of bacteria at different concentrations of different metals

Strains	Bacterial count in Blank ml^{-1}	0.5 ppm	1 ppm	1.5 ppm	2 ppm	3 ppm	5 ppm	7 ppm	8 ppm	10 ppm
Growth of bacteria at different concentrations of Pb ml^{-1}										
E. coli	250 10^3	280x10^5	440x 10^5	320x 10^5	200x 10^5	90x 10^5	50x 10^5	300x 10^4	80x 10^2	ND
B. cereus	250x10^3	950x 10^3	500 x10^3	378 x10^3	292x 10^3	260 x10^3	34 x10^3	30x 10^3	ND	ND
Growth of bacteria at different concentrations of Cu ml^{-1}										
E. coli	240x10^7	160x10^7	80x 10^6	150x10^5	24 x10^6	16x 10^6	12x 10^4	2x10^2	ND	ND
B. cereus	285x10^5	510x10^7	44x 10^7	300 x10^3	260x 10^3	142x 10^3	42x 10^3	30x 10^3	30x 10^2	ND
Growth of bacteria at different concentrations of As ml^{-1}										
E. coli	300 x 10^4	290x10^4	280x 10^4	262x10^4	250x 10^4	240x 10^4	235x 10^5	230x 10^4	225x 10^4	220x 10^4
B. cereus	300x10^4	200x10^4	150 x10^4	90 x 10^4	175 x10^3	142x 10^3	103x 10^3	80x 10^3	65x 10^3	40x 10^3
Growth of bacteria at different concentrations of Fe ml^{-1}										
E. coli	225x10^3	350x10^3	340x 10^4	310x10^3	240x 10^3	210x 10^2	140x 10^2	ND	ND	ND
B. cereus	250x10^3	400x10^4	320x 10^3	223x10^3	150x 10^3	50x 10^2	30x 10^2	30x 10^2	20x 10^2	ND
Growth of bacteria at different concentrations of Mnml^{-1}										
E. coli	400x10^4	420x10^4	380x 10^4	280x10^3	215x 10^3	90x 10^3	122x 10^2	90x 10^2	30x 10^2	ND
B. cereus	400x10^4	410x10^4	390x 10^3	200x 10^3	142x 10^3	150x 10^2	50x 10^2	49 x10^3	45 x10^3	40x 10^3

ND = Not detected *B. cereus* = *Bacillus. cereus E. coli* = *Escherichia. coli* ppm = Part per million

tested for TVC by serial dilution on nutrient agar and total coliform by MPN method. Results of TVC and MPN are given in Table 1 and Table 2.

Results showed that all the water samples were found to be contaminated except tube well number SW II, 161, 157, 169, 103 and sample collected from main supply of I-10 Markaz. In all contaminated water samples characteristics growth was observed. Bacterial colonies showed rhizoid growth, which is a characteristic growth of *B. cereus*, var. mycoides.

All the water samples were analyzed for contamination and different bacteria were also identified. In most cases growth of *B. cereus* was observed and only in tube well 101, growth of *E. coli* was observed. All these organisms were identified by different bio-chemical tests, which are mentioned in the material and methods.

The results showed that *E. coli* were Gram's negative rods, occurring single or in pairs and catalase positive whereas B. cereus were also Gram's negative and catalase positive. *B. cereus* was late Citrate positive. Hydrogen sulphide was not produced by both *E. coli.* and *B. cereus* also show a characteristics growth on nutrient media. *E. coli* were distinguished by confirmed test for *E. coli.,* which is given in Bacteriology Manual. Different bacteria have the different ability to grow at different temperature. All the results related to temperature and growth in NaCl (Table 3).

Sugar tests were also performed for identification purpose. Results showed that *B. cereus* ferments fructose, glucose and sucrose, which were positive and all other sugars were negative, as these are not fermented by *B. cereus*. *E. coli* can ferment all other sugars except sucrose and lactose. Results of sugar test are given in Table 4.

Confirmatory Biochemical tests were done in accordance to *Bergey's manual of determinative bacteriology* and Method for examination of water and food by American Public Health Association.

It is clear from this study that microorganisms have the ability to grow in the presence of above-mentioned different concentration of metals. The effect of metals on the growth of bacteria was studied and results are summarized in Table 5.

It is clear from the results that the growth of both *E. coli* and *B. cereus* was observed at lower concentration (1-5 ppm) of all different metals but the growth of *E.coli* and *B. cereus* was higher in case of Arsenic at 5 ppm and even at 10 ppm of Arsenic.

In case of lead (Pb), the growth of E. coli was not observed at 10 ppm and B. cereus was not observed at 8 ppm of lead (Pb). In case of Copper (Cu), growth of E. coli was not observed at 8 ppm of copper (Cu) and B. cereus was not observed at 10 ppm, but it can grow at 9 ppm of copper (Cu). No growth of E. coli was observed at 8 ppm of copper (Cu).

In case of Iron (Fe), growth of E. coli was not detected at 7 ppm and B. cereus at 10 ppm of Iron (Fe), but B. cereus can grow at 9 ppm of Iron (Fe), concentration. In case of Manganese (Mn), higher growth of B. cereus was observed at 10 ppm and no growth of E. coli was

observed at 10 ppm of Manganese (Mn), but it can grow at 9 ppm of Manganese (Mn).

The main objective of this study was to check the quality of drinking ground water, whether it was free of contamination or not. So a complete survey of different sectors of Islamabad was performed. Results indicated that the growth of E. coli was observed in tube well no. 101 and 138 and in most samples of different tube wells, growth of B. cereus was observed. Our complete survey indicated that growth of both these bacteria was observed in water, which was supplied to different sectors of Islamabad, for drinking or other purposes. As these bacterial species are potentially pathogenic and can cause serious diseases, so the water supplied to people, should be properly treated and then supplied. It was also observed that water supplied was without proper treatment with chlorine and tube wells were at contaminated places. By removing these problems, we can get water free of contamination.

REFERENCES

Anonymous, 2001. Compendium of methods for the microbiological examination of food. American Public Health Association. Ed. 4th., pp: 305, 314, 311, 371, 409.

Anonymous, 1974. Bergey's Manual of Determinative Bacteriology. Ed. 8th, Waverly Press, Inc. Mt. Royal and Guilford Aves. Baltimore, Md., U.S.A. 21202, pp: 290-296, 522-530.

Anonymous, 1989. Standard Methods for the examination of water and wastewater. 7th Edn. American Public Health Association 1015 Fifteen-Steer NW Washington, DC 20005, pp: 931-933, 68-80.

Cappuccino, J.G. and N. Sherman, 1986. Microbiology, a laboratory manual. 2nd Ed. Sunny Rockland Community College, State Univ. of New York, pp: 231-233.

Collins, C.H. and P.M. Lyne, 1980. In "Microbiological Methods. Vol. 4. Butter worths Publishers, London, pp: 408.

Goepfert, I.M., W.M. Spira and H.U. Kim, 1972. *Bacillus cereus* Food poisoning organisms. A review. J. Milk Food Technology, pp: 213.

Hill, D., 2001. Basic microbiology for drinking water personnel. 1st edition. AWWA., pp: 23.

Weast, R.C., 1984. CRC Hand book of Chemistry and Physics, 64 Ed., CRC, Boca Raton, Fla., pp: 341-343.