INT. J. BIOL. BIOTECH., 5 (1-2): 107-110, 2008.

RESPONSE OF SOME GRAM POSITIVE AND GRAM NEGATIVE BACTERIA TO LEAD

Shazia Haque [1] and Ehsan Elahi Valeem[2]

[1]*Institute of Environmental Studies, University of Karachi, Karachi-75270, Pakistan.*
[2]*Department of Botany, Govt. Degree Sci. & Com. College Gulshan-e-Iqbal, Block VII, Gulshan-e-Iqbal, Karachi-75300, Pakistan.*
Corresponding author: *valeem@go.com*

ABSTARCT

Toxic effect of various concentrations of Lead (2,4, 8, 10, 16, 20 mg/mL) on some gram +ive and gram –ive bacteria is reported in this study. Lead inhibited the growth of gram +ive *Staphylococcus areus* and *Bacillus cereus* as well as gram –ive bacteria *Salmonella typhi* and *Escherichia coli*. The area of the zone of inhibition was directly proportional to the concentration of Pb. Gram –ive bacteria were more susceptible to Lead than gram +ive bacteria.

Keywords: *Bacillus, Escherichia, Salmonella, Staphylococcus,* Gram +ve, Gram –ve, Lead (Pb)

INTRODUCTION

The effectiveness of heavy metals as germicidal is due to the high affinity of cellular proteins for metallic ions. Blue-green algae and bacteria die due to the cumulative effects of ions within the cell, even if the concentration of ion in solution is minute (Benson, 1999). The oligodynamic action of metals and metal compounds was noted initially by Nägeli (1893) who pointed out that metal and metal compounds confer, in minute quantity of water solutions, the ability to change and finally kill cells in a characteristic way (Busch, 1971; Mckhann *et al.,* 1987). This antimicrobial effect is shown by ions of heavy metals. The exact mechanism of this action is still unknown. McKhann, *et al.,* (1987) showed that minute amounts of certain metals have been found to stimulate the growth of tumors in rabbits. Lead has germicidal ability to exert antimicrobial effect through oligodynamic action (Zentral, 1985; Khan, 1996; Arayne *et al.,* 2002; Sawyer *et al.,* 2003). The ability of Lead to exert a lethal effect upon bacteria can be demonstrated by digging a well filled with Lead solution on an agar plate "seeded" with bacteria (Moussa *et al.,* 2003). After incubation, a zone of inhibition (no growth) surrounds the metal well because the bacteria cells die due to the cumulative effects of ions within the cell, even if the concentration of ions in a solution is small.

In the present study antibacterial effects of Pb have been studied by observing the oligodynamic effect of Pb using gram +ve bacteria viz. *Staphylococcus areus* and *Bacillus cereus* and gram –ve bacteria viz. *Salmonella typhi* and *Escherichia coli.*

MATERIALS AND METHODS

Metal Solution: Standard method for preparation of Lead solution was applied where 1.598 g of Lead Nitrate [Pb $(NO_3)_2$] was dissolved in 200 mL deionised water and 1.5 mL concentrated HNO_3 was added and the solution was diluted to 1000 mL with deionised water. One mL of this stock solution contained 1 mg of Pb. The stock solution was filtered and sterilized and then solutions with 2, 4, 8, 10 and 20 mg/L concentrations Pb were prepared (Khin and Annachhatre, 2004).

Microorganisms: The bacteria employed in this study were *Staphylococcus areus, Escherichia coli, Salmonella typhi* and *Bacillus cereus.* Their cultures were obtained from Microbiology Environmental Research Laboratory of Institute of Environmental Studies, Karachi and were further checked morphologically by gram staining. For the preservation of organisms slant were prepared and for inoculation nutrient broths were used.

.

Media: Nutrient broth was prepared by dissolving 2.4 g of dehydrated Nutrient broth powder in 300 mL distilled water, pH was adjusted to 7. 10 mL of solution were dispensed in each test tube and the test tubes were covered with cotton plug. These test tubes were autoclaved at 121° C and 15 PSI for 30 minutes. Sterility was checked for 24 h in an incubator at 37° C. Nutrient broth was used for the isolation of bacterial culture. Nutrient Agar was prepared by dissolving 8 g nutrient broth powder in 1000 mL distilled water and mixed gently. 18 g Agar powder were added in this 1000 mL. Nutrient broth solution and heating for 15 minutes. 14 mL of this solution were dispensed in each test

tube. These test tubes were autoclaved at 121° C and 15 PSI for 30 minutes. Sterility was checked for 24 h at 37° C. Nutrient agar was used for the streaking, preservation of culture and for pour plate technique.

Table 1. Antimicrobial activity of Pb against some Gram (+) and Gram (-) bacteria.

Type/ Bacteria	Pb Conc. mg/L (ppm)	Area of Inhibition Zone (IZ) Mean (mm^2)*	Standard Error
Gram +ive *Staphylococcus areus*	2	0.00	0.00
	4	94.10	0.79
	8	188.05	2.12
	10	336.78	4.84
	16	524.77	15.87
	20	750.79	5.00
Gram +ive *Bacillus cereus*	2	184.53	15.18
	4	312.48	7.10
	8	486.21	9.59
	10	612.88	10.74
	16	811.00	7.14
	20	1059.85	9.20
Gram –ive *Salmonella typhi*	2	0.00	0.00
	4	171.58	1.10
	8	210.38	9.88
	10	450.00	4.08
	16	610.00	5.77
	20	835.53	1.83
Gram –ive *Escherichia coli*	2	0.00	0.00
	4	0.00	0.00
	8	144.23	1.48
	10	282.88	1.86
	16	450.25	4.03
	20	651.30	0.24

*, after 24 hours of incubation -mean of four replicates.

Experiment: Sterile Petri plates were used for the experiment. Few drops of broth culture were poured in molten agar tube and mixed gently by rolling the tube between the hands. The mixture was then poured in sterile Petri plates and allowed for 20 minutes to solidify. Four wells of 5 mm were dug by well maker and filled with metal solution (0.03 mL) on these agar plates. Each well had an area of 19.6mm^2. After 24 h incubation, the zone of inhibition (no growth area around the wells) was measured.

RESULTS AND DISCUSSION

The toxicity of lead was investigated against four bacterial species – two Gram positive (*Bacillus cereus* and *Staphyllococcus areus*) and two Gram negative (*Salmonella typhi* and *Eschrichia coli).* No detrimental effect was observed up to 2 ppm Pb concentration in case of *S. typhi* and *S. aereus* and up to 4 ppm Pb concentration in case of

E. coli (Table 1). *B. aereus* was much sensitive to Pb, the growth of which was inhibited by Pb even at 2 ppm concentration.

Two way ANOVA for the area of inhibition zone (IZ) induced by Pb in cultures of above bacterial types at 2 to 20 ppm Pb concentration (Table 2A) indicated that Pb concentration as well as bacterial specific nature both had significantly effect ($F = 16.11$, $p < 0.000001$ and $F = 4.48$, $p < 0.0061$, respectively) on the size of the inhibition zone. Interaction between Lead concentration and the bacterial types was, however, insignificant ($F = 0.957$, $p < 0.5085$, NS). DMR test analysis (Table 2B) indicated that the growth of the all bacterial types was inhibited by Lead and the toxicity increased with increasing lead concentration. Among the bacterial types, the inhibition effects of Lead from 2-8 ppm were not significant and didn't differ with each other. The toxicities, however, became significantly pronounced above 10 ppm Pb concentration.

Table 2A. Two-way ANOVA for area of inhibitory zone (IZ) induced by Pb in various bacterial species.

Source	**SS**	**df**	**MS**	**F**	**p**
Lead Concentration	7576228.8	5	1515245.8	16.11	0.00001 ***
Bacterial type	1264161.8	3	421387.3	4.480	0.0061 **
Lead Concentration x Bacterial type	1349858.5	15	89990.6	0.957	0.5085 NS
Error	6774069.6	72	94084.3		
Total	16964318.7	95			

Table 2B. DMR Test for the size of inhibition zone (IZ) induced by six lead concentrations in four bacterial species.

LEAD (N = 16)					**BACTERIA TYPE (N = 24)**				
Rank	TRT #	Mean IZ mm^2	Significant Range	Pb ppm	Rank	TRT #	Mean IZ (mm^2)	Significant Range	Bacteria
1	6	818.7	a	20	1	2	577.8	a	*B. cereus (G+)*
2	4	621.3	a	10	2	1	441.2	ab	*S. areus (G+)*
3	5	599.0	a	16	3	3	379.6	b	*S. typhi (G -)*
4	3	257.2	b	8	4	4	259.4	b	*E. coli (G -)*
5	2	144.5	b	4	$LSD_{0.05} = 176.5\ mm^2$				
6	1	46.1	b	2					
$LSD_{0.05} = 216.2\ mm^2$									

G + = Gram positive; G (-), Gram negative.

The Lead was toxic to each bacterial type tested. Among the species tested, *B. aereus* was the most susceptible species to Pb and *E. coli* the least susceptible one (Table 2B). Pb was somewhat more toxic to Gram positive species than Gram negative species. The response of *S. areus* to Lead was of the intermediate order. our results of oligodynamic action of Lead and its germicidal ability against bacteria are in agreement with previous studies e.g., Zentral (1985), Khan (1996), Arayne *et al., (*2002), and Sawyer *et al.* (2003), etc.

ACKNOWLEDGEMENTS

We would like to thank Prof. Dr. Muhammad Altaf Khan and Dr. Umm-e-Hany for their guidance. The first author appreciates love, patience and encouragement of her father Prof. Dr. Noor-ul-Haque. Thanks are also due to Prof. Dr. D. Khan for data analysis.

REFERENCES

Arayne, M.S., N. Sultana, F. Khanum and M.A. Ali (2002) Antibacterial studies of cefixime copper, zinc and cadmium complexes. *Pak. J. Pharm. Sci*., 15(1): 1-8.

Benson, H. (1999). *Oligodynamic Response of Selected Bacteria. Microbiological Applications.* 7th ed, McGraw Hill, 80 pp.

Busch, A.W. (1971) *Aerobic Biological Treatment of Wastewaters; Principles and Practice. Oligodynamics*. Press Houston, Texas.

Khan, M.A. (1996). *Techniques in Environmental Microbiology and Health.* ISBN 969-8358-01-3 147 pp.

Khin, T. and A. Annachhatre (2004) Novel microbial nitrogen removal processes. *Biotech. Adv*., 22: 519-532.

Mckhann, C.F., H.J. Carlson and H. Douglas (1987) *Oligodynamic Action of Metallic Elements and of Metal Alloys on Certain Bacteria and Viruses: I. In vitro Observations.* Deptt. Paediatrics, Western Reserve Univ School of Medicine, Babies & Children Hospital, Cleveland, Ohio.

Moussa, M.S., H.J. Lubberding, C.M. Hooijmans, M.C.M. Loosdrecht and H.J. Gijzen (2003) Improved method for determination of ammonia and nitrite oxidation activities in mixed bacterial cultures. *Appl. Microbiol. Biotech*., 63: 217-221.

Nägeli, V. and K.W. Nägeli (1893) Über oligodynamische Erscheinungen in lebenden Zellen: Neue Denkschrift *Allgemein Schweiz Gesellsch Naturwiss,* 33(1).

Sawyer, C.N., P.L. Mccarty and G.F. Parkin (2003) *Chemistry for Environmental Engineering and Science.* 5th ed McGraw Hill, Singapore, ISBN: 0-07-123045-9.

Zentral, B.L. (1985) Investigations of oligodynamic action of 17 metals on *Bacillus subtilis*, Enterobacteriaceae, Legionellaceae, Micrococcaceae and *Pseudomonas aeruginosa. Bakteriol. Mikrobiol. Hyg. Abt. B*., 182(1): 95-101.

(*Accepted for publication November 2007*)