

Resistance of Bacteria Isolated from Otamiri River to Heavy Metals and Some Selected Antibiotics

¹I.C. Mgbemena, ²J.C. Nnokwe, ²L.A. Adjeroh and ³N.N. Onyemekara

¹Department of Biotechnology,

²Department of Biology,

³Department of Microbiology, Federal University of Technology, P.M.B.1526, Owerri, Imo State, Nigeria

Abstract: This study is aimed at determining the resistance of bacteria to heavy metals and some antibiotics. The ability of aquatic bacteria isolates from Otamiri River at Ihiagwa in Owerri North, Imo State to tolerate or resist the presence of certain selected heavy metals: Pb⁺, Zn²⁺ and Fe²⁺ and some antibiotics was investigated. Identification tests for the bacteria isolates from Otamiri River revealed them to belong to the genera *Pseudomonas*, *Aeromonas*, *Bacillus*, *Escherichia*, *Micrococcus* and *Proteus species*. The control experiment showed heavy growth of the organisms. These bacteria strains were checked for resistance against heavy metals by culturing them in basal medium in which varying concentrations of heavy metal compounds was incorporated. All the bacteria strains showed resistance against heavy metals with Minimal Inhibitory Concentration (MIC) values ranging from 0.1 to 3.0 mg/L, respectively. Generally, all the organisms had low MIC values for Pb⁺ and high MIC value for Zn²⁺ and Fe²⁺. This indicates average and low toxicity respectively of the heavy metals to the organisms. On the other hand, the isolates also exhibited high tolerance to most of the antibiotics like Gentamycin (77.7%), Rifampicin (66.0%) and Ofloxacin (57.3%). The ability of these organisms to resist the presence of both metals and antibiotics could present some very serious health implication because of the ability of these organisms to pass these resistant genes via R-plasmids to the next cell around will affect a whole bacterial population thereby complicating treatment.

Keywords: Iheagwa, Imo state, *Micrococcus* spp., pollution, tolerance, water, zinc

INTRODUCTION

Water pollution is a major problem in the global context. It has been suggested that it is the leading worldwide cause of deaths and diseases (Larry, 2006) and that it accounts for the deaths of more than 14,000 people daily. The main sources of pollution particularly by heavy metals is usually linked with areas of intensive industry and high automobiles use. The aquatic environment is more susceptible to the harmful effects of heavy metal pollution because aquatic organisms are in close and prolonged contact with the soluble metals (Shoeb, 2006). Heavy metals as natural components of the earth's crust are increasingly found in microbial habitat due to several natural and anthropogenic processes. However, microbes have evolved mechanisms to tolerate the presence of heavy metals either by efflux, complexation or reduction of metal ions or to use them as terminal electron acceptors in anaerobic respiration (Gadd, 1992; Nies and Silver, 1995).

Retaining suitable concentrations of essential metals such as copper, while rejecting toxic metals like lead and cadmium was probably one of the toughest challenges of living cells (Gatti *et al.*, 2000). The first response to toxic metal contamination is a large

reduction in microbial activity (Badar *et al.*, 2000). This is confirmed by the fact that habitats that have had high levels of metal contamination for years still have microbial populations and activities that are smaller than the microbial populations in uncontaminated habitats. Konopka *et al.* (1999) argued that resistance mechanisms do not offer protection at extremely high levels of free metal ions and with a lethal toxic effect. Resistance of essential metals against increased/toxic concentrations of essential metals e.g., Cu, Zn, Ni and Co, confront the cell with a special problem because of their requirement to accumulate some of these cations at trace levels and at the same time to reduce cytoplasmic concentrations from potential toxic levels. Resistant bacterial strains solve these problems by a careful regulation that results from the interaction between chromosomally determined cation transport systems and metal resistance systems that are mostly determined by plasmids (Brown *et al.*, 1999). Many bacterial-resistant systems for toxic metals are encoded by plasmids (Silver, 1996). Plasmids are small circular DNA molecule that can move from one bacterial cell to another (Silver, 1996). However, bacterial plasmids contain genes that provide extra functions to the cells among which resistances to toxic metal is very important. It has been shown that a correlation exists

between metal tolerance and antibiotic resistance in bacteria because of the likelihood that resistance genes to both (antibiotics and heavy metals) may be closely located on the same plasmid in bacteria and the presence of the organisms that possess specific mechanisms of resistance to heavy metals increases destruction or transformation of toxic substances in the natural environment (Philp *et al.*, 2001). Consequently, the range of genes carried on these plasmids (frequently associated with these heavy metal resistant determinants) was shown to extend far beyond those coding for antibiotic resistance (Tsai, 2006). Environmental deterioration often caused by oil spills and discharges resulting from various industrial activities (that may contain a high level of heavy metals) are common phenomenon. Various approaches have been used to detoxify and clean up these metals in any habitat, such as the use of certain chemicals which in turn, cause secondary pollution and physical methods that require large input of energy and expensive materials. Also there is the use of different types of microorganisms such as algae, fungi and bacteria that remove metals from solution (Dubey, 2006). It would necessarily be of immense benefit exploiting microorganisms for this purpose, but the attendant health implications that may result when these organisms develop resistant genes invariably becomes a source of concern for disease treatment and management. The study tends to determine the resistance of bacteria isolated from Otamiri River to Heavy metals and some selected antibiotics.

MATERIALS AND METHODS

Study site: The Otamiri River is the major river that washes through the Ihiagwa Autonomous Community in the Owerri West Local Government Area of Imo State, Nigeria. This river runs from Egbu where it has its major base, through to Nekede, Ihiagwa, Eziobodo, Olakwu, Umuisi, Mgbirichi, Umuagwo and finally to Ozuzu in Etche town of Rivers State where it finally joins the Atlantic Ocean. This river is of a very great significance to the people of Ihiagwa Autonomous Community as it serves as a source of water for domestic use and other purposes especially in those olden days before the introduction of pipe borne water in the community. The major occupations of the people are farming and artisanal activities which involve the release of inorganic compounds such as these heavy metals to the soil. These compounds are easily washed down into adjoining rivers during heavy rain falls.

Test samples: The samples used for this study are water samples collected from Otamiri River in Ihiagwa, Owerri West Local Government Area of Imo State.

Selected heavy metals: The heavy metals are compounds of lead, zinc and iron. These heavy-metal

compounds were purchased from a standard chemical supply store located at number 1 Edede Street/Douglas Road, Owerri, Imo State.

Collection of water samples: The water samples were collected in sterile screw-capped bottles. Three samples were obtained from three different locations (surface water) of Otamiri River in Ihiagwa, Owerri West Local Government Area of Imo State. The samples were transported to the Microbiology Laboratory, Federal University of Technology, Owerri and analyzed within 24 h.

Processing of samples: Serial dilution of the water samples were carried out using sterile distilled water as in Chesbrough (2002). All the different dilutions were properly labelled and used for total plate count.

Isolation of pure cultures: The water samples were examined bacteriologically using culture techniques as in Chesbrough (2002) and Obiajuru and Ozumba (2009). Each water sample was examined by culture technique using streak plate and spread plate technique. A sterile wire loop was used to collect a loop full of each undiluted water sample and inoculated on the surface of nutrient agar, blood agar, MacConkey agar and Eosine Methylene Blue agar. The inoculated plates were subsequently sub-cultured on fresh nutrient agar, MacConkey agar and blood agar plates to obtain pure cultures as well as study their morphological characteristics. They were incubated at 37°C for 24 h. The pure culture isolates were sub-cultured in nutrient agar slant and incubated at 37°C for 24 h and were then stored in the refrigerator until required for further use.

Identification of bacteria isolates: The bacteria isolates were subjected to various tests beginning from the study of their growth morphology on different agar media to different microbiological identification tests such as gram staining and motility tests and biochemical identification tests such as catalase, coagulase, oxidase, citrate utilization, urease, indole production, hydrogen sulphide production, nitrate and nitrite reduction, methyl red, Voges Proskeur and sugar fermentation tests.

Heavy metal resistance tests: Nutrient agar medium containing varying concentrations (0, 0.25, 0.5, 0.75 and 1.0 mg/L, respectively) of the different heavy metal compounds (Zn²⁺, Pb⁺ and Fe²⁺) were prepared. A sterile wire loop was used to collect a loop full of the pure isolate and directly streaked on the surface of the heavy metal incorporated medium. The process was repeated for each microorganism on the media incorporated with the selected heavy metals. The plates were incubated at 37°C for 24 h. After the incubation period, the plates were observed for any kind of growth.

The isolated and distinct colonies on these selective media were subcultured repeatedly on the same media for purification. The pure culture was identified on the basis of their morphology and biochemical characters. The control experiment was carried out by inoculating the pure isolates on basal media without the heavy metals.

Determination of Minimum Inhibitory Concentration (MIC): Minimum Inhibitory Concentration (MIC) of the heavy metal resistant bacteria isolates were determined by gradually increasing the concentration of the heavy metals by 0.25 mg/L each time on the nutrient agar plate until the strains failed to give colonies on the plate. Minimal inhibitory concentration was noted when the isolates failed to grow on the plates after incubation (Rajbanshi, 2008).

Antibiotic susceptibility/resistance: Each isolate from the test samples was examined for antibiotic susceptibility using commercially prepared Antibiotic discs (ABTEC). The antibiotics resistance of the

isolates was determined by Stokes (1975) with the following disks containing Ceftriaxone 30 µg, Rifampicin 20 µg, Ofloxacin 10 µg, Ciprofloxacin 10 µg, Gentamicin 10 µg and Levofloxacin 5 µg, respectively. A standard inoculum of each isolate from overnight culture on nutrient agar was made by inoculating a discrete colony in 10 mL of sterile peptone water and incubating for 3 h at 37°C. Thereafter, 0.1 mL was spread evenly on the surface of solid nutrient agar medium. A sterile forceps was used to place multi-antibiotic discs containing the selected drugs over the surface of each inoculated plate. This procedure was repeated for all the isolates. The plates were labeled and incubated at 37°C for 24 h after which they were examined for growth inhibition. The zone (diameter) of growth inhibition for each antibiotic on the different isolates was measured in millimeters using a transparent metric rule and recorded.

RESULTS

Table 1 presents the results of heavy metal resistance test. There was a decline in the growth of the

Table 1: Heavy metal resistance test

Heavy	Bacteria isolates	Heavy metal concentration in mg/L of medium														
		0.0	0.1	0.25	0.5	0.75	1.0	1.25	1.5	1.75	2.0	2.25	2.5	2.75	3.0	3.25
Lead	<i>Pseudomonas species</i>	++	+	+	+	-	-									
	<i>Aeromonas species</i>	++	+	+	+	-	-									
	<i>Bacillus species</i>	++	+	-	-	-	-									
	<i>Escherichia coli</i>	+	+	+	+	-	-									
	<i>Micrococcus species</i>	++														
	<i>Proteus species</i>	++														
		+														
		++														
		+														
		++														
Zinc	<i>Pseudomonas species</i>	++	+	+	+	+	+	+	+	+	+	-				
	<i>Aeromonas species</i>	++	+	+	+	-	-									
	<i>Bacillus species</i>	++	+	-	-	-	-									
	<i>Escherichia coli</i>	+	+	+	+	+	+	+	+	+	+	+	-			
	<i>Micrococcus species</i>	++	+													
	<i>Proteus species</i>	++														
		+														
		++														
		+														
		++														
Iron	<i>Pseudomonas species</i>	++	+	+	+	+	+	+	+	-						
	<i>Aeromonas species</i>	++	+	+	+	+	+	+	+	+	-					
	<i>Bacillus species</i>	+	++	+++	++	++	++	++	++	++	+	+	+	+	+	+
	<i>Escherichia coli</i>	++	+	+	+	+	+	+	-							
	<i>Micrococcus species</i>	++	+													
	<i>Proteus species</i>	++														
		+														
		++														
		+														
		++														

-: No growth; ++: heavy; +: Scanty growth; ++: Medium growth

Table 2: Result of minimal inhibitory concentrations of bacteria isolates minimal inhibitory concentrations of the bacteria isolates in mg/L

Microorganism	Lead	Zinc	Iron
<i>Pseudomonas specie</i>	0.5	2.00	1.50
<i>Aeromonas specie</i>	0.5	0.50	1.75
<i>Bacillus specie</i>	0.5	0.50	1.75
<i>Escherichia coli</i>	0.5	0.10	3.00
<i>Micrococcus specie</i>	0.1	0.10	1.25
<i>Proteus specie</i>	0.5	2.25	1.50

organisms as concentrations of the metals increased in contrast to the situation in the control i.e., 0.0 mg/L of the metals where there was a profuse/heavy growth by all the organisms. At 0.1 to 0.5 mg/L of lead concentration, there was growth in all the organisms except in *Micrococcus* species while growth ceased from 0.75 to 3.0 mg/L of lead in all the organisms. However, more organisms tolerated the presence of zinc even at high concentrations. *Pseudomonas* species continued to grow up to 2.0 mg/L, *Aeromonas* species and *Bacillus* species did not show any growth in zinc from 0.75 mg/L. *E. coli* and *Micrococcus* species could only survive the presence of zinc at the lowest concentration of 0.1 mg/L while *Proteus* species showed mild resistance up to 2.25 mg/L and failed to grow from 2.5 mg/L. For iron, *Pseudomonas*, *Bacillus* and *Proteus* species showed scanty growth at concentrations of 0.1 to 1.5 mg/L and *Micrococcus* species stopped growing at 1.25 mg/L. *Aeromonas* species grew moderately at 0.1 and 0.25 mg/L and growth was scanty at 0.5 to 1.75 mg/L, it died off at 2.0 mg/L. It was only *E. coli* that showed visible growth in all the concentrations, had profuse growth at 0.1 and 0.25 mg/L, grew moderately at 0.5 to 1.5 mg/L and also showed scanty growth from 1.75 to 3.0 mg/L.

Table 2 shows the minimum values required to inhibit the growth of the microorganisms. The lowest MIC for lead on *Micrococcus* is 0.1 and 0.5 mg/L for other organisms and the MIC for zinc on *E. coli* and *Micrococcus* species is 0.1 and 0.5 mg/L for *Aeromonas* and *Bacillus* and 2.25 mg/L on *Proteus* species. The MIC for iron on *Pseudomonas* and *Proteus* is 1.5 and 1.25 mg/L for *Micrococcus* species, 1.75 mg/L for *Aeromonas* and *Bacillus* species and 3.0 mg/L for *E. coli*.

The results of antibiotics sensitivity tests as shown in Table 3 indicate that the microorganisms exhibited very low minimum inhibition to most of the antibiotics especially to CEF: 6.8%, LEV: 9.7% and CF: 36.9%, respectively. However, majority of the tested organisms were particularly very resistant to most of the antibiotics as in *Pseudomonas* spp. that was 100% resistant to Levofloxacin and 88.9% to Ceftriaxone, *Aeromonas* spp., showed 77.3% resistant to Ciprofloxacin, 100% to LEV and CEF. *Bacillus* spp., was 87.5 and 75% resistant to LEV and CEF, respectively and *E. coli* was 70.6% resistant to CF, 91.2% to LEV and 100% to CEF. *Micrococcus* spp was 60 and 80% resistant to LEV and CEF, respectively while *Proteus* spp was 100% to CF, LEV and CEF and also 66.7% resistant to OFX. These data were deduced from the number of the organisms that survived the effect of the antibiotics.

DISCUSSION

This study was initiated with the aim of identifying indigenous bacteria having potential for heavy metal and antibiotic resistance. Six bacteria species (*Pseudomonas*, *Aeromonas*, *Bacillus*, *Escherichia coli*, *Micrococcus* and *Proteus* species) were isolated from Otamiri River, five of which were rods and the remaining cocci.

The microbial level of resistance or tolerance of each concentration of heavy metal was depicted by the level of growth on the agar. The microbial load decreased with an increase in the concentration (0.25 mg/mL) of heavy metal indicating the toxic effect of the heavy metals on the growth of microorganisms as earlier stated by Badar *et al.* (2000). However, no observable growth of microorganisms at high concentrations explains the theory earlier stated by Konopka *et al.* (1999) that resistance mechanisms do not offer protection at extremely high levels of free metal ions and a lethal toxic effect is observed. Badar *et al.* (2000) stated that bacterial Resistance or Tolerance can be used to minimize the effect of heavy metals on total biological activity of the ecosystem.

Table 3: Antibiotic susceptibility (growth inhibition) pattern of the bacterial species isolated from Otamiri river in Owerri, Nigeria

Isolates	No resistant to antibiotics (%)						
	No examined	CN	CF	LEV	CEF	OFX	RD
<i>Pseudomonas</i> spp.	18	17 (94.4)	9 (50.0)	0 (0.0)	2 (11.1)	13 (72.2)	15 (83.3)
<i>Aeromonas</i> spp.	22	13 (59.1)	5 (22.7)	0 (0.0)	0 (0.0)	9 (40.9)	10 (45.5)
<i>Bacillus</i> spp.	8	8 (100.0)	5 (62.5)	1 (12.5)	2 (25.0)	6 (75.0)	8 (100.0)
<i>E. coli</i>	34	27 (79.4)	10 (29.4)	3 (8.82)	0 (0.0)	18 (52.9)	23 (67.6)
<i>Micrococcus</i> spp.	15	10 (66.7)	9 (60.0)	6 (40.0)	3 (20.0)	11 (73.3)	10 (66.7)
<i>Proteus</i> spp.	6	5 (83.3)	0 (0.0)	0 (0.0)	0 (0.0)	2 (33.3)	6 (100.0)
Total	103	80 (77.7)	38 (36.9)	10 (9.7)	7 (6.8)	59 (57.3)	68 (66.0)

CEF: Ceftriaxone; CF: Ciprofloxacin; LEV: Levofloxacin; OFX: Ofloxacin; RD: Rifampicin; CN: Gentamycin

Generally, contamination with a specific metal increases the level of resistance of the bacterial community to that metal (Badar *et al.*, 2000).

Minimal Inhibitory Concentration (MIC) is the highest concentration of the heavy metal required to inhibit the growth of microorganisms. Thus, lower MIC values indicate more toxic metals and higher MIC values indicate less toxicity. The resistance test indicated that among the three experimented heavy metals, average maximum resistance was shown to iron, showing growth of microorganisms (*Escherichia coli*) up to 3.0 mg/L and minimum tolerance to lead showing no growth above 0.5 mg/L of the heavy metal. Microorganisms showed an average to high resistance of 2.25 mg/L to zinc incorporated basal medium. These could be explained by the fact that generally, zinc and iron have been classified to have low toxicity on microbes while Lead has high toxicity on microorganisms (Nies and Silver, 1995). The high resistance of *Escherichia coli* to Fe²⁺ is probably because the organism possess an additional high affinity ABC-transport system for ferrous iron (Fe²⁺) encoded by *feoABC* gene (Kammler *et al.*, 1993).

Varying microbial resistance levels to heavy metals has been attributed to a variety of resistance mechanisms such as differences in uptake and/or transport of the toxic metal while in other cases, the metal may be enzymatically transformed by oxidation, reduction, methylation or demethylation into chemical species which may be less toxic or more volatile than the parent compound. These mechanisms are sometimes encoded in plasmid genes facilitating the transfer of toxic metal resistance from one cell to another (Silver, 1996).

In a heavy metal contaminated environment, increased uptake of these heavy metals could lead to bioaccumulation of the metals within the organisms. Therefore, removal of these organisms from the environment will help in the bioremediation of these metals. Also the metals could be biotransformed to less toxic or more volatile forms thereby decontaminating the environment (Silver, 1996).

In this study, correlation was found to exist between the resistance of *E. coli* to iron and Proteus to zinc to and antibiotics. The resistance of the organisms to the antibiotics confirms the correlation between resistance metal ions and antibiotics. This has also been reported by several other researchers in bacterial species from different sources (Cenci *et al.*, 1982; Grewal and Tiwari, 1990; Rajbanshi, 2008). Many have speculated and have even shown this to be as a result of the likelihood that resistance genes to both antibiotics and heavy metals could be closely located on the same plasmid in bacteria and are thus more likely to be

transferred together in the environment (Nies, 1999). The most important features of R-plasmid is that they can be transferred to heavy metal resistant bacteria, to confer resistance to several antimicrobial agents (Eugene *et al.*, 2004). This means that if R-plasmid is present in one species in a mixed population of these organisms, other cells will receive the R-plasmid and become resistance to a variety of antimicrobial agents (Peter, 1993). Many antibiotic resistant genes are located on mobile genetic elements (e.g., plasmids, transposons and integrons), some of which are easily exchanged among phylogenetically distant bacteria. Many of these mobile genetic elements encode resistance to multiple antibiotics, heavy metals and other compounds (Tsai, 2006). It is therefore possible that selective pressure by such compound indirectly selects for the whole set of resistances. This also explains the phenomenon of conjugation whereby genetic material is transfer between two bacterial cells (of the same or different species). One cell donates the DNA and the other receives it (Campbell *et al.*, 2008). Transfer of multiple antibiotic resistances by conjugation has become a major problem in the treatment of certain bacterial diseases. Since the recipient cell becomes a donor after transfer of a plasmid it is easy to see why an antibiotic resistance gene carried on a plasmid can quickly convert a sensitive population of cells to a resistant one. And when infections caused by resistant microbes fail to respond to treatment, it may result in prolonged illness and a greater risk of death. Treatment failures also lead to longer periods if infections, which increase the number of infected people moving into the community and thus expose the general population to the risk of contracting a resistant strain of infection.

CONCLUSION

Microbes have adapted to tolerate the presence of metals and at same use them for growth; as such they can be used to clean up metal-contaminated sites. Other implications are not beneficial as the presence of metal resistance mechanisms may contribute to the increase in antibiotic resistance, health risks and so on. In other words, it is important to remember that what we put in the environment can have many effects, not just on humans, but also on the environment and on the microbial community on which all other life depends.

REFERENCES

- Badar, U., R. Abbas and N. Ahmed, 2000. Characterization of copper and chromate resistant bacteria isolated from Karachi tanneries effluents. *J. Ind. Env. Bio.*, 39: 43-54.

- Brown, N.L., D.A. Rouch and B.T. Lee, 1999. Copper resistance determinants in bacteria. *J. Mol. Microb.*, 27: 41-51.
- Campbell, N.A., J.B. Reece, J.B. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky and R.B. Jackson, 2008. *Biology*. 8th Edn., Pearson Cummings, pp: 562.
- Cenci, G., G. Morozzi, F. Scazzocchio and A. Morosi, 1982. Antibiotics and metal resistance of *Escherichia coli* isolates from different environmental sources. *Zentralblatt für Bakteriologie und Hygiene 1. Abteilung Originale C*, 3: 440-449.
- Chesbrough, M., 2002. *Medical Laboratory Manual for Tropical Countries*. Butterworths and Co. Ltd., UK, pp: 21-32.
- Dubey, R.C., 2006. *A Textbook of Biotechnology*. S. Chad and Co. Ltd., India, pp: 569-583.
- Eugene, W.N., G. Denise, C.E. Aderson, N.N. Roberts Jr, S. Pear, T. Martha and H. David, 2004. *Microbiology: A Human Perspective*. Bacteria Pathogens, McGraw-Hill, pp: 616-617.
- Gadd, G.M., 1992. Metals and Microorganisms: A problem of definition. *FEMS Microbiol. Lett.*, 100: 197-204.
- Gatti, D., B. Mitra, B.P. Rosen, 2000. Mini review: *Escherichia coli* soft metal ion translocating ATPases. *J. Bio. Chem.* 275(44): 34009-34012.
- Grewal, J.S. and R.P. Tiwari, 1990. Resistance to metal ions and antibiotics in *Escherichia coli* isolated from foodstuffs. *J. Med. Microbiol.*, 32: 223-226.
- Kammler, M., C. Schon, K. Hantke, 1993. Characterization of the ferrous ion uptake system of *Escherichia coli*. *J. Bacteriol.*, 175: 6212-6219.
- Konopka, A., T. Zakharova, M. Bischoff, L. Oliver, C. Nakastu and R.F. Turco, 1999. Microbial biomass and activity in lead contaminated soil. *J. Appl. Env. Microbiol.*, 65(5): 2256-2259.
- Larry, W., 2006. World Water Day: 'A Billion People Worldwide Lack Safe Drinking Water'. Retrieved from: <http://environmentalout.com/od/environmentalevents/a/waterdaysqa.htm>.
- Nies, D.H. and S. Silver, 1995. Ion efflux systems involved in bacterial metal resistances. *J. Ind. Microbiol.*, 14: 186-199.
- Nies, D.H., 1999. Microbial heavy metal resistance. *J. Appl. Microbiol. Biotechnol.*, 51: 730-750.
- Obiajuru, O.C. and U.C. Ozumba, 2009. *Laboratory Methods for Medical Microbiology and Parasitology*. Eddison Business Concerns, Owerri, pp: 48-49.
- Peter, P., 1993. *Biotechnology: A Guide to Genetic Engineering*. Brow Publishers, USA, pp: 105-108.
- Philp, J.C., R.M. Atlas and C.J. Cunningham, 2001. Bioremediation. *Nature Encyclopedia of Live Science*, pp: 1-10.
- Rajbanshi, A., 2008. Study on heavy metal resistance bacteria in guesswork sewage treatment plant. *Our Nature*, 6: 52-57.
- Shoeb, E., 2006. Genetic basis of heavy metal tolerance in bacteria. *Pak. Res. Repository*, 11: 389-490.
- Silver, S., 1996. Bacterial resistance to toxic metal ions- a review. *J. Env. Health Perspective*, 105(1): 98-102.
- Stokes, E.J., 1975. *Clinical Bacteriology*. 4th Edn., Edward Arnold, London, pp: 216-217.
- Tsai, K.J., 2006. Bacterial heavy metal resistance. *Pak. Res. Repository*, 11: 101-110.