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Bioreduction of Cr (VI) by Heavy Metal Resistant *Pseudomonas* Species

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ABSTRACT

Chromium (VI) contamination has accelerated due to rapid industrialization worldwide. Aim of this study was to check the bacterial species for their tolerance towards multiple metals, antibiotics and further check whether these bacteria are reducing Cr (VI). Bacterial strains were isolated from the industrial area situated at Lagos-Abeokuta Road, Ile Ise Awo, Abeokuta, Ogun State, Nigeria. All of the isolates showed tolerance to lead, zinc and chromium (VI). Bacterial species also showed tolerance towards antibiotics, 100% of strains were resistant to ampicillin, cotrimoxazole, colistin and gentamycin, 66.6% to nitrofurantoin and nalidixic acid, 83.3% were resistant to streptomycin whereas, 50% were resistant to tetracycline. Among all the strains, only two strains *Pseudomonas* strain PH2 and PH4 were chosen for chromium (VI) reduction as these strains showed maximum tolerance towards chromium (VI). Maximum reduction (60%) of chromium (VI) was observed at pH 6 by *Pseudomonas* spp. PH2, which was followed by pH 5 (50%) whereas, pH9 showed least reduction of 12.5%. Similarly, *Pseudomonas* spp. PH4 also reduced chromium considerably at pH 6 (60%), pH 5 (50%), pH 8 (37.5%) and at pH 9 (12.5%) respectively, at a concentration of 100 μg Cr mL^{-1} after 120 h of incubation. The *Pseudomonas* species PH2 reduced chromium (VI) at concentration of 50 μg Cr mL^{-1} (92%), 100 μg Cr mL^{-1} (70%) and 150 μg Cr mL^{-1} (50%), respectively at a pH of 6. Similarly, *Pseudomonas* species PH4 reduced chromium (VI) by 95% at 50 μg Cr mL^{-1}, 70% at 100 μg Cr mL^{-1} and 55% at 150 μg Cr mL^{-1} at a pH of 6. Due to above properties strains could therefore, be used as bioremediators of metals in soils contaminated with heavy metals and can also increase the yield of various crops under heavy metal contamination.

Key words: *Pseudomonas* species, metal tolerance, chromium (VI) reduction

INTRODUCTION

The wide use of chromium (Cr) in industries like leather tanning, metallurgy, electroplating, textile and pigment manufacturing has resulted in release of large quantities of chromium effluent in the World (Wang and Xiao, 1995; Pattanapipitpaisal *et al*., 2001; Sultan and Hasnain, 2007). Chromium occurs mainly in two forms in the environment i.e., trivalent and hexavalent (as chromate and dichromate) and is actively transported to cells (Ortegel *et al*., 2002). Among the different forms of chromium, the hexavalent chromium is the more toxic and carcinogenic due to its high solubility in water, rapid permeability through biological membranes and subsequent interaction with intracellular proteins and nucleic acids (Kamaludeen *et al*., 2003; Ackerley *et al*., 2006). Reduction of Cr (VI) leads to the formation of stables, less soluble and less toxic Cr (III).

Reduction of toxic Cr (VI) to Cr (III) is thus, a useful process for remediation of Cr (VI) affected environments (Jeyasingh and Phillip, 2005). The reduction/detoxification of Cr (VI) by microbes is, however, inexpensive and environmentally safe approach and provides a viable option to protect the environment from chromium toxicity. The reduction of Cr (VI) has been reported in *Bacillus* (Elangovan *et al*., 2006; Chaturvedi, 2011), *Pseudomonas* sp. (Rahman *et al*., 2007), *Escherichia coli* (Bae *et al*., 2005), *Microbacterium* (Pattanapipitpaisal *et al*., 2001), *Ochrobactrum intermedium* (Faisal and Hansnain, 2005) and *Micrococcus* (Sultan and Hasnain, 2005).

Detoxification of chromium by microbes may occur directly or indirectly and is affected by pH, chromate concentration, incubation periods and the types of microbes involved. In the direct mode, the microbes take up chromium and then enzymatically (chromium reductases) reduced chromium (Losi *et al*., 1994). While in the indirect mode, products (reductants or oxidants) of microbes in soil, such as H_2S, reduce chromium by chemical redox reactions (DeFilippi and Lupton, 1992). Furthermore, in growing cultures with added carbon sources as electron donors and in cell suspensions, Cr (VI) reduction can be predominantly aerobic or anaerobic, but generally not both. Interestingly, chromium reductases can catalyze reduction of Cr (VI) to Cr (III) anaerobically (Lovley and Coates, 1997), aerobically (Cervantes *et al*., 2001) and also both anaerobically and aerobically (Marsh and Mclnerney, 2001). The Cr (VI) reductase may be present in the membrane fraction of the cells of PGPR, as found in *Pseudomonas fluorescens* and *Enterobacter cloacae* (Wang *et al*., 1990). Further evidence suggested that cytochrome c548 was involved in the reduction of Cr (VI) by membrane vesicles. In the presence of H2 and excess of hydrogenase, cytochrome C3, a periplasmic protein, in the soluble cell free fraction of *D. vulgaris* (Lovley and Coates, 1997), reduced Cr (VI), 50 times faster than did the Cr (VI) reductase of *P. ambigua* with NADH and NADPH, as electron donor (Horitsu *et al*., 1987). Soluble and membrane associated enzymes of the electron transfer system were found to be responsible for Cr(VI) reduction under anaerobic condition and Cr(VI) serves as the terminal electron acceptor of an electron transfer chain that frequently involves cytochrome b/c (Cervantes and Campos, 2007). The Cr (III) forms an insoluble precipitate, such as $Cr(OH)_3$, which can be removed from wastewater (Jeyasingh and Philip, 2005). The chromium reductase in *P. ambigua* (Campos-Garcia *et al*., 1997) and *Bacillus* sp. (Wang *et al*., 1991) have been purified and characterized. More recently, to clone a chromate reductase gene, novel soluble chromate reductase of *P. putida* was purified to homogeneity and characterized (Puzon *et al*., 2002). The reductase activity was NADH- or NADPH-dependent. Reduction of Cr (VI) by bacterially produced H_2S, followed by precipitation of the Cr (III) formed, is an important mechanism in sulfate-rich soil environment under anaerobic conditions (Losi *et al*., 1994). Hydrogen sulfide, produced in acid sulfate soil under reducing conditions, is easily precipitated as FeS in reduced soils (Eary and Rai, 1991) and sediments. Fe (II) and H_2S, both microbially produced, are effective reductants of Cr (VI) under reduced conditions as is the FeS (Karnachuk, 1995). Therefore, the use of bacteria for reduction/detoxification of chromium is one of the preferred choices and is considered as cost effective approach in bioremediation technologies. The present study was therefore, under taken (1) to determine the resistance pattern of soil bacteria to heavy metals and antibiotics, (2) to check chromium reduction under varying pH and chromium concentration.

MATERIALS AND METHODS

The soil samples used for the isolation of the bacterial strains were collected from the industrial area situated at Lagos- Abeokuta Road, Ile Ise Awo, Abeokuta, Ogun State. The soil samples were collected into a polythene bag and then taken to the laboratory for necessary analysis.

Bacteria were isolated from the contaminated soils of Abeokuta on nutrient agar medium by spread plate technique. One gram of soil sample was added to a flask containing 100 mL of normal saline solution and was serially diluted. A 10 µL of each suspension was spread plated on solid nutrient agar. Plates were incubated at 28±2°C for 24 h and the bacterial colonies were then purified and preserved on nutrient agar slants for further experiments.

The isolated bacterial strains from the contaminated soil were tested for their sensitivity/resistance to three heavy metals viz; lead, chromium and zinc by agar plate dilution method (Holt *et al.*, 1994) using nutrient agar. The freshly prepared agar plates amended with increasing concentration of metals from (0-1000 µg mL^{-1}), were spot inoculated (10 µL) with 10^8 cells mL^{-1}. Plates were incubated at 28±2°C for 72 h and the highest concentration of heavy metals supporting growth was defined as the Maximum Resistance Level (MRL). Each experiment was replicated three times.

To determine susceptibility to antibiotics, the bacterial strains were tested for their sensitivity to ten antibiotics. The reactions to antibiotics were determined by the disc diffusion method (Bauer *et al.,* 1966). The bacterial strains were grown in nutrient broth at 28±2°C for 24 h. A 0.1 mL of the over-night grown culture was spread on the surface of nutrient agar. The antibiotic discs of known potency were then placed on the agar surface and the plates were incubated at 28±2°C for 24 h. The zones of inhibition around the antibiotic discs were measured (in millimeter) against the following antibiotics that were used: tetracycline (25 µg), Colistin (25 µg), streptomycin (25 µg), nitrofurantoin (200 µg), nalidixic acid (30 µg), gentamycin (10 µg), cotrimoxazole (25 µg) and ampicillin (25 µg).

Hydrogen cyanide production by bacterial isolates was detected by the method of Bakker and Schippers (1987). For HCN production, the bacterial strains were grown on an HCN induction medium (30 g tryptic soy broth, 4.4 g glycine, 15 g agar/l) at 28±2°C for four days. For each bacterial isolate, 100 µL of 10^8 cells mL^{-1} was placed in the centre of the petri plates. A disk of Whatman filter paper No. 1 dipped in 0.5% picric acid and 2% Na_2CO_3 was placed at the lid of the petri plates. Plates were sealed with parafilm. After four days incubation at 28±2°C, an orange brown colour of the paper indicating HCN production was observed.

For ammonia production, the bacterial strains were grown in peptone water (g L^{-1}: peptone10 g, NaCl 5 g, pH 7) and incubated at 30±2°C for four days. One milliliter of Nessler reagent was added to each tube and the development of yellow color indicating ammonia production was recorded (Dye, 1962).

To assess the effect of pH on hexavalent chromium [Cr (VI)] reduction *in vitro*, the Nutrient Broth (NB) was amended with 100 µg mL^{-1} of Cr (VI) and the autoclaved medium was adjusted to pH 5, 6, 7, 8 and 9 with 1 M HCL or 1 M NaOH and incubated at 28±2°C for 120 h. Further, to assess the effect of different concentrations (0, 50,100 and 150 µg mL^{-1}) of Cr (VI), the $K_2Cr_2O_7$ were amended in Nutrient broth and incubated at 28±2°C for 120 h. For Cr (VI) reduction, one mL culture from each flask was centrifuged (6000 rpm) for 10 min at 10°C and Cr (VI) in the supernatant was determined by 1, 5-diphenyl carbazide method (Eaton *et al*., 1992) upto 120 h. Briefly, the test samples were acidified (pH 1-2) and 1, 5 diphenyl carbazide (50 µg mL^{-1}) was added and Cr (VI) concentration was detected by UV-VIS spectrophotometer (752N Lamfield medical England) at 540 nm. The spectrophotometer uses CT grating monochromator for its optical system. It has a wavelength range of 195-1020 nm and an accuracy of +/- 2 nm. The light source of this spectrophotometer is coming from imported Philip tungsten lamp, Deuterium lamp. For its display, it uses 4 LCD which has a display area of 0-200% T and -0.3-3 A. It has a spectral band

width of 4 nm, wavelength repeatability of 1 nm. This spectrophotometer has a stray light of 0.3% T at 360 nm; photometric accuracy of +/-0.55 T; photometric repeatability of 0.2% T; stability of +/-0.004 A/h at 500 nm; work pattern of T,A, C; display area of 0-200% T, -0.3-3A and zero setting is automatic.

Data of three replicates were subjected to statistical analysis using pair samples T test with significant level of $p<0.05$. The values indicate the Mean±SD of three replicates.

RESULTS AND DISCUSSION

The selected bacterial strains were tested for their ability to tolerate various concentrations of chromium (VI) and other metals like zinc and lead using agar plate dilution method. Generally, bacterial strains showed a varied level of tolerance to heavy metals (Fig. 1). Among the bacterial strains, *Pseudomonas* sp. PH2 showed highest tolerance to chromium (VI), zinc and copper at concentration 1000 μg mL^{-1}, which was followed by *Pseudomonas* sp. PH4 (1000 μg mL^{-1} for chromium, 800 μg mL^{-1} for zinc and 1000 μg mL^{-1} for lead). There are reports that have shown a high level tolerance to heavy metals by rhizobia (Wani and Khan, 2013). Conflicting reports are, however, available in the literature on the tolerance level of rhizobia, which could possibly be due to the variation in the tolerance ability of bacteria and growth conditions employed (Rajkumar *et al*., 2005). For instance, *Rhizobium leguminosarum* isolated from metal contaminated soil tolerated 92.9 μM of zinc (Delorme *et al*., 2003) while, *Rhizobium* species isolated from nodules of *Trifolium repense* tolerated 300 mg kg^{-1} nickel and showed an effective symbiosis with its legume host, when grown in nickel amended soils (Smith and Giller, 1992). In the present studies, *Pseudomonas* sp. PH2 showed highest tolerance to chromium (VI), lead and zinc at a concentration of 1000 μg mL^{-1}, whereas, *Pseudomonas* sp. PH4 showed highest tolerance to chromium (VI) and lead at concentration 1000 and 800 μg mL^{-1} to zinc. Bacterial strains showed a high tolerance to chromium which was followed by lead and then zinc. The metal tolerant strains were characterized by physiological, morphological and biochemical characteristics. The strain PH1, PH2, PH3, PH4, PH5 and PH6 were characterized as *Pseudomonas* sp.

Nickel and zinc tolerance by *Rhizobium leguminosarum* biovar *trifolii* isolated from sewage sludge treated soil was reported by Purchase and Miles (2001), who observed a metal tolerance of 0.24-0.26 mM Ni^{2+} and 6.0-8.0 mM Zn^{2+}. Similarly, metal tolerance by *Rhizobium*, *Bradyrhizobium* and *Azotobacter* (Pajuelo *et al*., 2008) and varying level of resistance among other PGPR (*Bacillus* and *Pseudomonas*) have also been reported (Yilmaz, 2003; Thacker *et al*., 2007; Wasi *et al*., 2008).

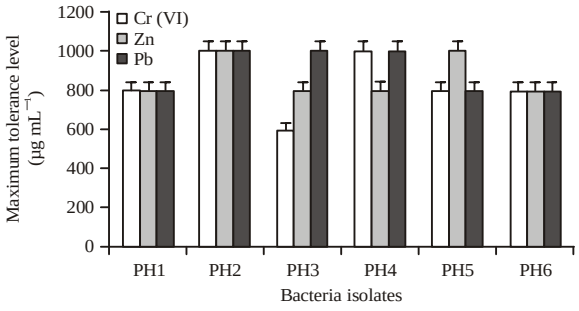

Fig. 1: Metal tolerance level shown by different bacterial isolates

Resistance to antibiotics among metal tolerant rhizobacterial strains differed considerably. Among bacterial species, 100% of strains were resistant to ampicillin, cotrimoxazole, colistin and gentamycin, 66.6% to nitrofurantoin and nalidixic acid, 83.3% were resistant to streptomycin whereas, 50% were resistant to tetracycline. Bacterial resistance to antibiotics is an emerging problem these days. Resistance to antibiotics is acquired by a change in the genetic makeup of microbes, which can occur by either a genetic mutation or by transfer of antibiotic resistant genes between organisms in the environment (Spain and Alm, 2003). With these considerations, the antibiotic resistance among PGPR was studied, which differed from antibiotic to antibiotic for all the PGPR strains. Multiple antibiotic resistances shown by PGPR strains (e.g., *Bacillus* sp. PZ3 and *Pseudomonas* sp. PZ6) might be associated with a high degree of tolerance to metals. In many studies, metal tolerance and antibiotic resistance have been reported (Yilmaz, 2003; Verma *et al*., 2001). It has been suggested that under environmental conditions of metal stress, metal and antibiotic resistant microorganisms will adapt faster by the spread of R-factors than by mutation and natural selection (Silver and Misra, 1988). Similar observations on antibiotics resistance by PGPR strains have been reported (Thacker *et al*., 2007). The variation in the resistance to many tested antibacterial drugs (antibiotics) may possibly be due to the differences in growth conditions and exposure of PGPR to stress conditions or toxic substance as well as presence or absence of resistance mechanisms that could be encoded either by chromosome and/or R-plasmid (Spain and Alm, 2003).

Chromium, a wide spread environmental pollutant is released from various industries including tanneries, metal cleaning and processing, chromium plating, wood processing and alloy formation. Among the different forms of chromium, the hexavalent chromium [Cr (VI)] is the most toxic and carcinogenic (Kamaludeen *et al*., 2003) due to their high solubility in water, rapid permeability through biological membranes and subsequent interaction with intracellular proteins and nucleic acids (Reeves *et al*., 1983). Although the reduction of Cr (VI) causes the chromate toxicity, their further reduction leads to the formation of stable, less soluble and less toxic Cr (III). Reduction of potentially toxic Cr (VI) to Cr (III) is thus, a useful process for remediation of Cr (VI) affected environments (Thacker *et al*., 2007). In this context, the detoxifications of chromium by naturally occurring microorganisms provide a viable option to protect the environment from chromium toxicity. Therefore, the present study was designed to determine the Cr (VI) reducing ability of the metal tolerant strains.

A total of two metal resistant strains were tested for evaluation of their chromium reducing ability under *in vitro* conditions as these strains were highly resistant to chromium (VI). This study was carried out to access the (i) effect of different pH values on the reduction of Cr (VI) and (ii) the effect of chromate concentration on chromium (VI) reduction.

The effect of different pH values on the reduction of chromium (VI) is shown in Fig. 2. Maximum reduction (60%) of chromium (VI) was observed at pH 6 by *Pseudomonas* spp. PH 2, which was followed by pH 5 (50%) whereas, pH 9 showed least reduction of 12.5%. Similarly, *Pseudomonas* sp. PH 4 also reduced chromium considerably at pH 6 (60%), pH 5 (50%), pH 8 (37.5%) and at pH 9 (12.5%) respectively, at a concentration of 100 μg Cr mL^{-1} after 120 h of incubation.

In this study, the chromium reducing ability of PGPR strain was assessed using nutrient broth supplemented with 50, 100 and 150 μg mL^{-1} of $K_2Cr_2O_7$ in order to determine the effect of chromium (VI) reducing ability of the selected culture under *in vitro* conditions (Fig. 3). The time for total reduction of chromium (VI) increased with increase in the concentration of

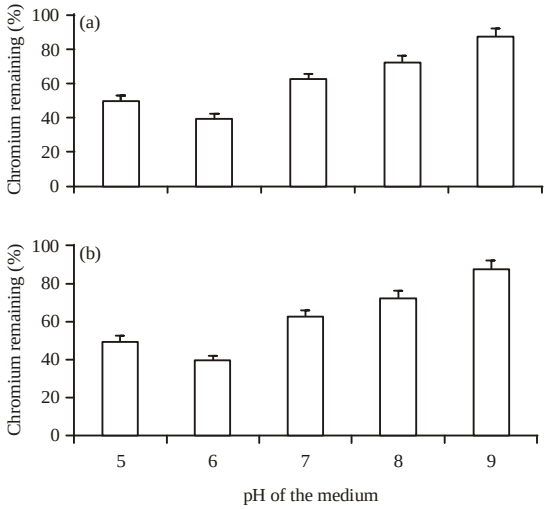

Fig. 2(a-b): Effect of different pH values on chromium (VI) reduction by bacterial isolate (a) pH2 and (b) pH4

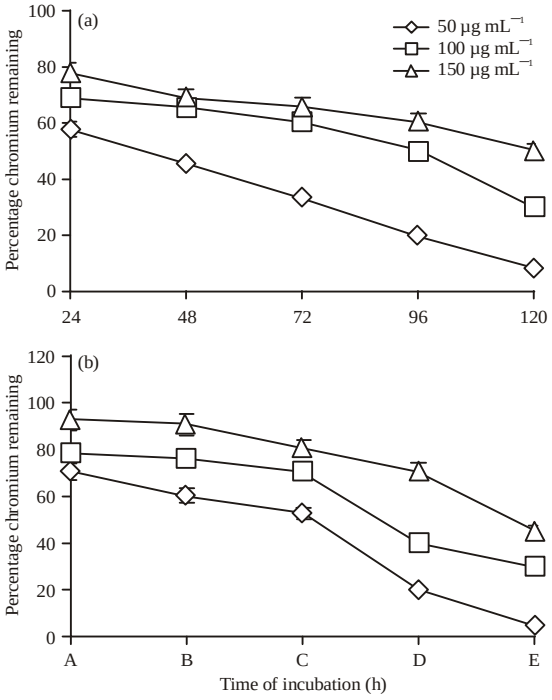

Fig. 3(a-b): Effect of chromate concentration on chromium reduction by bacterial isolate (a) pH2 and (b) pH4

chromium (VI). During this study, the complete reduction of chromium (VI) does not occur even after 120 h of incubation by *Pseudomonas* sp. PH2 (Fig. 3a-b) at 50, 100 and 150 µg mL^{-1} of chromium. The strain PH2 reduced chromium (VI) at concentration of 50 µg Cr mL^{-1} (92%), 100 µg Cr mL^{-1} (70%) and 150 µg Cr mL^{-1} (50%) respectively at a pH of 6. Similarly *Pseudomonas* species PH4 reduced chromium (VI) by 95% at 50 µg Cr mL^{-1}, 70% at 100 µg Cr mL^{-1}

and 55% at 150 μg Cr mL^{-1} at a pH of 6. Our study is in correlation with the study of Yang *et al*. (2009) and Wani *et al*. (2008) who also observed considerable reduction of chromium. In this study, *Intrasporangium* species Q5-1 reduced chromium (VI) by 98% after 84 h of incubation at an initial concentration of 2, 3 or 4 mM of Cr (VI) whereas at an initial concentration of 5 mM Cr (VI), Q5-1 reduced Cr (VI) by 70% after 72 h of incubation. In another study it was found that the *Bacillus* sp. isolated from tannery effluent reduced Cr (VI) by 71.4% at a concentration of 1100 mg L^{-1} of Cr (VI) after 24 h of incubation (Chaturvedi, 2011). Ibrahim *et al*. (2011) found that the *Bacillus* species KSUCr5 reduced 100% of Cr (VI) at an initial concentration of 40 mg L^{-1} within 24 h whereas, 100% chromium reduction was achieved for 60-80 and 100 mg L^{-1} of Cr (VI) after 48 and 72 h, respectively. Furthermore, the strain reduced only 78.2 and 44.2% of Cr (VI) at an initial Cr (VI) concentration of 150 and 200 mg L^{-1}, respectively after 72 h of incubation. The strain KSUCr5 could also reduce Cr (VI) between a pH range of 7-12 with a maximum reduction at pH 10. However, it was found that maximum reduction of Cr (VI) occur at a pH of 7.

CONCLUSION

This study concludes that the bacterial strains not only tolerated heavy metals, antibiotics, produced plant growth promoting substances but also reduced chromium (VI) under different pH and chromium concentration. Due to multifarious properties expressed by the bacterial strains, these strains could therefore, be used as bioremediators of metals in soils contaminated with heavy metals.

REFERENCES

Ackerley, D.F., Y. Barak, S.V. Lynch, J. Curtin and A. Matin, 2006. Effect of chromate stress on *Escherichia coli* K-12. J. Bacteriol., 188: 3371-3381.

Bae, W.C., H.K. Lee, Y.C. Choe, D.J. Jahng and S.H. Lee *et al*., 2005. Purification and characterization of NADPH-dependent Cr(VI) reductase from *Escherichia coli* ATCC 33456. J. Microbiol., 43: 21-27.

Bakker, A.W. and B. Schippers, 1987. Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* SPP-mediated plant growth-stimulation. Soil Biol. Biochem., 19: 451-457.

Bauer, A.W., W.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45: 493-496.

Campos-Garcia, J., G. Martinez-Cadena, R. Alvarez-Gonzalez and C. Cervantes, 1997. Purification and partial characterization of a chromate reductase from *Bacillus*. Revista Latinoamericana Microbiologia, 39: 73-81.

Cervantes, C., J. Campos-Garcia, S. Devars, F. Gutierrez-Corona, H. Loza-Tavera, J.C. Torres-Guzman and R. Moreno-Sanchez, 2001. Interactions of chromium with microorganisms and plants. FEMS Microbiol., 25: 335-347.

Cervantes, C. and G.J. Campos, 2007. Reduction and Efflux of Chromate by Bacteria. In: Molecular Microbiology of Heavy Metals, Nies, D.H. and S. Silver, (Eds.). Springer, Berlin, Germany, ISBN-13: 978-3540697701, pp: 407-420.

Chaturvedi, M.K., 2011. Studies on chromate removal by chromium-resistant *Bacillus* sp. isolated from tannery effluent. J. Environ. Protect., 2: 76-82.

DeFilippi, L.J. and F.S. Lupton, 1992. Bioremediation of soluble Cr (VI) using sulfate reducing bacteria in Allied Signal Research. Proceedings of the National R and B Conference on the Control of Hazardous Materials, San Francisco, February 4, 1992, pp: 1-138.

Delorme, T.A., J.V. Gagliardi, J.S. Angle, P.B. van Berkum and R.L. Chaney, 2003. Phenotypic and genetic diversity of rhizobia isolated from nodules of clover grown in a zinc and cadmium contaminated soil. Soil Sci. Soc. Am. J., 67: 1746-1754.

Dye, D.W., 1962. The inadequacy of the usual determinative tests for the identification of *Xanthomonas* spp. N. Z. J. Sci., 5: 393-416.

Eary, L.E. and D. Rai, 1991. Chromate reduction by subsurface soils under acidic conditions. Soil Sci. Soc. Am. J., 55: 676-683.

Eaton, A.D., L.S. Clesceri and A.E. Greenberg, 1992. Standard Methods for the Examination of Water and Wastewater. APHA/AWWA/WEF., Washington, DC., Pages: 981.

Elangovan, R., S. Abhipsa, B. Rohit, P. Ligy and K. Chandraraj, 2006. Reduction of Cr (VI) by a *Bacillus* sp. Biotech. Lett., 28: 247-252.

Faisal, M. and S. Hasnain, 2005. Bacterial Cr (VI) reduction concurrently improves sunflower (*Helianthus annuus* L.) growth. Biotechnol. Lett., 27: 943-947.

Holt, J.G., N.R. Kreig, P.H.A. Sneath, J.T. Staley and S.T. Williams, 1994. Bergey's Manual of Determinative Bacteriology. 9th Edn., Williams and Wilkins, Baltimore, USA..

Horitsu, H., S. Futo, Y. Miyaazawa, S. Ogai and K. Kawai, 1987. Enzymatic reduction of hexavalent chromium by hexavalent chromium tolerant *Pseudomonas ambigua* G-1. Agric. Biol. Chem., 47: 2417-2420.

Ibrahim, A.S., M.A. El-Tayeb, Y.B. Elbadawi and A.A. Al-Salamah, 2011. Bioreduction of Cr (VI) by potent novel chromate resistant alkaliphilic *Bacillus* sp. strain KSUCr5 isolated from hypersaline Soda lakes. Afr. J. Biotechnol., 10: 7207-7218.

Jeyasingh, J. and L. Philip, 2005. Bioremediation of chromium contaminated soil: Optimization of operating parameters under laboratory conditions. J. Hazrad. Mater., 1: 113-120.

Kamaludeen, S.P.B., M. Megharaj, A.L. Juhasz, N. Sethunathan and R. Naidu, 2003. Chromium-microorganism interactions in soils: Remediation implications. Rev. Environ. Contaim. Toxicol., 178: 93-164.

Karnachuk, O.V., 1995. Influence of hexavalent chromium on hydrogen sulfide formation by sulfate-reducing bacteria. Microbiology, 64: 262-265.

Losi, M.E., C. Amrhein and W.T. Frankenberger Jr., 1994. Environmental biochemistry of chromium. Rev. Environ. Contam. Toxicol., 136: 91-121.

Lovley, D.R. and J.D. Coates, 1997. Bioremediation of metal contamination. Curr. Opin. Biotechnol., 8: 285-289.

Marsh, T.L. and M.J. McInerney, 2001. Relationship of hydrogen bioavailability to chromate reduction in aquifer sediments. Applied Environ. Microbiol., 67: 1517-1521.

Ortegel, J.W., E.D. Staren, L.P. Faber, W.H. Warren and D.P. Braun, 2002. Modulation of tumor-infiltrating lymphocyte cytolytic activity against human non-small cell lung cancer. Lung Cancer, 36: 17-25.

Pajuelo, E., I.D. Rodriguez-Llorente, M. Dary and A.J. Palomares, 2008. Toxic effects of arsenic on *Sinorhizobium-Medicago sativa* symbiotic interaction. Environ. Pollut., 154: 203-211.

Pattanapipitpaisal, P., N.L. Brown and L.F. Macaskie, 2001. Chromate reduction and 16S rRNA identification of bacteria isolated from a Cr(VI)-contaminated site. Applied Microbiol. Biotechnol., 57: 257-261.

Purchase, D. and R.J. Miles, 2001. Survival and nodulating ability of indigenous and inoculated *Rhizobium leguminosarum* biovar *trifolii* in sterilized and unsterilized soil treated with sewage sludge. Curr. Microbiol., 42: 59-64.

Puzon, G.J., J.N. Petersen, A.G. Roberts, D.M. Kramer and L. Xun, 2002. A bacterial flavin reductase system reduces chromate to a soluble chromium (III)-NAD^+ complex. Biochem. Biophys. Res. Commun., 294: 76-81.

Rahman, M.U., S. Gul and M.Z.U. Haq, 2007. Reduction of chromium (VI) by locally isolated *Pseudomonas* sp. C-171. Turkish J. Biol., 31: 161-166.

Rajkumar, M., R. Nagendran, K.J. Lee and W.H. Lee, 2005. Characterization of a novel Cr^{6+} reducing *Pseudomonas* sp. with plant growth-promoting potential. Curr. Microbiol., 50: 266-271.

Reeves, M.W., L. Pine, J.B. Neilands and A. Balows, 1983. Absence of siderophore activity in *Legionella* species grown in iron-deficient media. J. Bacteriol., 154: 324-329.

Silver, S. and K.T. Misra, 1988. Plasmid-mediated heavy metal resistances. Annu. Res. Microbiol., 42: 717-743.

Smith, S.R. and K.E. Giller, 1992. Effective *Rhizobium leguminosarum* biovar Trifolii present in five soils contaminated with heavy metals from long-term applications of sewage sludge or metal mine spoil. Soil Biol. Biochem., 24: 781-788.

Spain, A. and E. Alm, 2003. Implications of microbial heavy metal tolerance in the environment. Rev. Undergraduate Res., 2: 1-6.

Sultan, S. and S. Hasnain, 2005. Chromate reduction capability of a gram positive bacterium isolated from effluent of dying industry. Bull. Environ. Contam. Toxicol., 75: 699-706.

Sultan, S. and S. Hasnain, 2007. Reduction of toxic hexavalent chromium by *Ochrobactrum intermedium* strain SDCr-5 stimulated by heavy metals. Biores. Technol., 98: 340-344.

Thacker, U., R. Parikh, Y. Shouche and D. Madamwar, 2007. Reduction of chromate by cell-free extract of *Brucella* sp. isolated from Cr (VI) contaminated sites. Bioresour. Technol., 98: 1541-1547.

Verma, A., K. Kukreja, D.V. Pathak, S. Suneja and N. Narula, 2001. *In vitro* production of plant growth regulators (PGRs) by *Azotobacter chroococcum*. Indian J. Microbiol., 41: 305-307.

Wang, P.C., K. Toda, H. Ohtake, I. Kusaka and I. Yabe, 1991. Membrane-bound respiratory system of *Enterobacter cloacae* strain HO1 grown anaerobically with chromate. FEMS Microbiol. Lett., 78: 11-15.

Wang, Y.T. and C. Xiao, 1995. Factors affecting hexavalent chromium reduction in pure cultures of bacteria. Water Res., 29: 2467-2474.

Wang, P.C., T. Mori, K. Toda and H. Ohtake, 1990. Membrane-associated chromate reductase activity from *Enterobacter* cloacae. J. Bacteriol., 172: 1670-1672.

Wani, P.A., M.S. Khan and A. Zaidi, 2008. Chromium reducing and plant growth promoting *Mesorhizobium* improves chickpea growth in chromium amended soil. Biotechnol. Lett., 30: 159-163.

Wani, P.A. and M.S. Khan, 2013. Nickel detoxification and plant growth promotion by multi metal resistant plant growth promoting *Rhizobium* species RL9. Bullet. Environ. Contam. Toxicol., 91: 117-124.

Wasi, S., G. Jeelani and M. Ahmad, 2008. Biochemical characterization of a multiple heavy metal, pesticides and phenol resistant *Pseudomonas fluorescens* strain. Chemosphere, 71: 1348-1355.

Yang, J., M. He and G. Wang, 2009. Removal of toxic chromate using free and immobilized Cr(VI)-reducing bacterial cells of *Intrasporangium* sp. Q5-1. World. J. Microbiol. Biotechnol., 25: 1579-1587.

Yilmaz, E.I., 2003. Metal tolerance and biosorption capacity of *Bacillus circulans* strain EB1. Res. Microbiol., 154: 409-415.