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Screening and Characterization of Mercury-Resistant Nitrogen Fixing Bacteria and Their Use as Biofertilizers and for Mercury Bioremediation

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Abstract.- In the current study, mercury-resistant nitrogen fixing bacterial (NFB) strains were isolated by growing them on selective medium (NFM) to be used as biofertilizers and for the bioremediation of mercury from polluted soils and waters. Bacterial strains isolated from the soil around root nodules were checked for resistance to mercury by growing on yeast extract mannitol (YEM) medium supplemented with different concentrations of $HgCl_2$. Mercury resistant bacterial strains were primarily screened by well plate method. Mercury resistant NFB strains were further checked for their H_2S production by growing on lead acetate (LA) medium. Selected nitrogen fixing and mercury resistant bacterial strains were characterized using different biochemical tests and found to belong to genera *Pseudomonas*, *Cronobacter* and *Bacillus*. Quantification of detoxified mercury by selected bacterial strains was done by using dithizone method. *Cronobacter* species were found to be significantly the most efficient in the detoxification of mercury that reached up to 95% ($p<0.05$).

Keywords: *Cronobacter* sp., mercury-detoxification, nitrogen fixing bacteria, H_2S production, biofertilizer.

INTRODUCTION

Mercury, the only metal in liquid form at room temperature and the sixth most toxic element in the list of hazardous compounds is present in the environment for centuries (White *et al.*, 2005). It is released into the environment by different sources and gets deposited in aquatic and terrestrial habitats and thus translocating all along the food chain (Cristol *et al.*, 2008). It is useful in many industrial applications at lower concentrations but at higher concentration it causes acute necrotizing bronchitis and pneumonitis which lead to respiratory failure and ultimately death. Mercury poisoning over a long period of time influences the brain functions and cause disorders in the central nervous system. It also gets deposited in kidneys thus accelerating the process of kidney toxicity, including proteinuria or nephritic disorder (Brodkin *et al.*, 2007; Holmes *et al.*, 2009; Tchounwou *et al.*, 2003; Zahir *et al.*, 2005).

The anthropogenic activities are contributing to mercury contamination day by day by increasing its level in seas, rivers and lakes. Water bodies are getting polluted due to the runoff from rain water, different industries and agriculture lands. The waste water contains trace elements and heavy metals which are non-essentials and highly toxic to plants, animals and human beings (Pacyna *et al.*, 2006; Sharma and Agrawal, 2005). In Pakistan and other developing countries, it is common practice to use water from lakes and rivers for irrigation purpose (Arain *et al.*, 2009) so it is urgent to take some important measures to remove un-necessary and toxic pollutants from effluents before release to the water bodies in order to reduce the risk for environment and humans.

Increasing concentration of mercury in the atmosphere is currently a worldwide problem and to reduce its level in industrial effluents have become a major challenge to be resolved (Wagner-Döbler, 2003). In this regard, various technologies are being developed such as immobilization, ion exchange, carbon absorbents, reverse osmosis, precipitation, electrochemical treatment (Akpor and Muchie, 2010; Sinha and Khare, 2012; Wang *et al.*, 2009) and nanoparticles of gold and manganese oxide (Lisha *et al.*, 2010; Nor Kamarudin and Mohamad, 2010). However, these technologies hold some drawbacks *i.e.*, high cost, low affectivity, non-specific treatment and generate hazardous by-products (Manohar *et al.*, 2002; Zhang *et al.*, 2005). In contrast, bioremediation technique by utilizing

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microorganisms is the best possible mechanism of mercury reduction due to its cost-effectiveness and higher efficiency even in low amounts of metal.

Efforts are being made to utilize the microorganisms to remediate mercury pollutants (Bafana *et al.*, 2010; De *et al.*, 2006). Microorganisms produced hydrogen sulfide (H_2S) which is supposed to be involved in conversion of toxic form (Hg^{2+}) of mercury into nontoxic form (Hg^0). H_2S co-precipitates the Hg^{2+} into non-toxic form (HgS) resulting to overcome the toxic effect of mercury containing organic and inorganic compounds (Amin and Latif, 2013; Ono *et al.*, 1996). Heavy metal resistant microorganisms are involved in various agronomical important processes. In addition to securing plants from harmful effects of metals, the plant growth promoting rhizobacteria play a vital part in augmenting the soil fertility and thus enhancing crop production by supplying essential nutrients and growth regulators (Khan *et al.*, 2007; Zaidi and Khan, 2005). These significant multiple properties of microorganisms make them one of the most suitable choices to use them as a biofertilizer which is alternative of chemical fertilizers and also for the bioremediation of heavy metals. Therefore, in view of the prevailing circumstances of mercury pollution, this study was focused on microorganisms due to their dual advantage exhibiting nitrogen fixation for plant growth promotion and mercury remediation by producing H_2S.

MATERIALS AND METHODS

Isolation of nitrogen fixing rhizobacteria

Root nodules, a habitat of nitrogen fixing bacteria, of different plants such as *Sesbania concolor* (Tingin), *Trifolium alexandrinum* (Berseem), *Iresine herbstii* (Laljhari) and *Dahlia* sp. were collected from Botanical Garden of University of the Punjab, Lahore, Pakistan and stored at 4°C in air tight bags for the usage in further experiments. Nitrogen free medium (NFM), KH_2PO_4, 1.0 g/L, $MgSO_4.7H_2O$, 0.1 g/L, $CaC1_2.2H_2O$, 0.01 g/L, $FeSO_4$, 0.01 g/L, Na-succinate, 1.2 g/L, $NaMoO_4.2H_2O$, 0.005 g/L, $MnSO_4$. H_2O, 0.025 g/L, $ZnS0_4.7H_2O$, 0.007 g/L, $CuSO_4.5H_20$, 0.00125 g/L, $CoS0_4$. $7H_2O$, 0.0014 g/L, H_3BO_3, 0.0003 g/L, pH 6.8, was used for the isolation of nitrogen fixing bacterial (NFB) isolates (Mehnaz *et al.*, 2007; Okon *et al.*, 1977). Rhizospheric soil samples were collected from the root nodules of each plant and 10% soil suspension was prepared in autoclaved distilled water. Soil particles of each sample were allowed to settle down and supernatant was used to prepare tenfold serial dilution. Fifty microlitre of each dilution was spread on each NFM agar plate and allowed to incubate at 37°C for 24 h.

Preliminary screening of mercury-resistant NFB isolates

Bacterial strains showing growth on NFM were further spread on Yeast Extract Mannitol (YEM) agar (0.5g/L K_2HPO_4, 0.2g/L $MgSO_4$,0.1g/L NaCl, 0.5g/L yeast extract, 10g/L Mannitol, 15g/L agar) (Muneer *et al.*, 2013; Cappuccino and Sherman, 2004) supplemented with different concentrations, 10, 20, 50, 100 µg/ml of $HgCl_2$ and incubated at 37°C for 24 h. Bacterial colonies resistant and sensitive to mercury were monitored along with YEM medium without $HgCl_2$ as a negative control after 24 h of incubation.

Screening of mercury-resistant NFB by well diffusion method

Resistance of NFB isolates to $HgCl_2$ was determined by well diffusion/plate method as described by Zeroual *et al.* (2001). The optical density (O.D) of overnight cultures of selected bacterial isolates previously grown on NFM medium and resistant to mercury was determined by spectrophotometer at 600 nm in YEM medium and fixed it at 0.5 by diluting with respective medium. Each bacterial culture (100µl) was spread on YEM agar plates and kept at room temperature for 30 min for the embedment of the microorganisms. Four wells of 5 mm in diameter were made on each plate and fifty microlitre of each concentration (10, 20, 50 and 100 µg/ml $HgCl_2$) was added in wells of each plate separately. The plates were incubated overnight at 37°C and zone of inhibition (mm) around each well was measured. Bacterial isolates were screened out on the basis of their zone of inhibition (mm). Each experiment was conducted in triplicate.

H_2S production by NFB isolates

H_2S production ability of selected bacterial isolates was checked by growing them on lead acetate (LA) medium (3 g/L peptone, 5 g/L yeast extract, 4 g/L glucose, 0.2 g/L ammonium sulphate, 1 g/L lead acetate and 20 g/L agar). Two microlitre overnight cultures (0.5 $O.D_{600nm}$) was spotted on LA medium and incubated for 2-3 days at 37°C as followed by Ono *et al.* (1991). For negative control, selected bacterial cultures were spotted on LA medium without lead acetate.

Biochemical characterization of mercury-resistant NFB

Selected mercury-resistant NFB strains were biochemically characterized by performing various biochemical tests like catalase, cytochrome oxidase, starch hydrolysis, triple sugar iron, gelatin hydrolysis, methyl red (MR), vogesproskauer (VP), Simmon's citrate utilization, indole production and nitrate reduction by following the methods described by Cappuccino and Sherman (2004).

Estimation of mercury detoxification by selected bacterial strains

The overnight culture ($O.D_{600nm} \approx 0.5$) of nitrogen fixing, mercury-resistant and H_2S producing bacterial strains was inoculated in 30 ml of YEM broth supplemented with 20 µg/ml of $HgCl_2$. The flasks were incubated at 37°C for 36 hours on continuous shaking at 150 rpm. The cultures were centrifuged at 12000 *g* for 10 min. The pH of supernatants was adjusted to 1.0 with concentrated sulfuric acid and detoxification of mercury in the medium was quantified using dithizone method as described by Elly (1973) and Humaira *et al.* (2005).

Statistical analysis

All of the data were tested statistically by analysis of variance (ANOVA) using SPSS + Version 20. Each treatment of mercury detoxification was analyzed with three replicates and a standard deviation (SD) was calculated. The data expressed are mean ± SD of each replicate was calculated then means were compared by Duncan's multiple range test.

RESULTS

Mercury resistant NFB

A total of 60 bacterial isolates were isolated from root nodules of different plant species and screened out for their ability to grow on NFM medium. Out of sixty isolates, only ten isolates showed growth on NFM medium. The selected NFB isolates were preserved at -20°C as 30% glycerol stock for further experiments.

Selected NFBs were screened out by growing them on yeast extract mannitol (YEM) medium supplemented with different concentrations of $HgCl_2$ ranging from 10 to 100 µg/ml. Eight NFB were found resistant against different concentration of $HgCl_2$ and were further screened out by well diffusion/plate method. The resistance to mercury by NFB was observed against 20 and 50 µg/ml $HgCl_2$ whereas all bacterial strains were found highly sensitive at concentration of 100 µg/ml $HgCl_2$. No inhibition zones were observed at 10 µg/ml $HgCl_2$. It was observed that the behavior of all bacterial isolates varied against both concentrations of $HgCl_2$ (20 and 50 µg/ml).

H_2S production by mercury-resistant NFB

H_2S production by mercury-resistant NFB isolates was checked on LA medium supplemented with lead acetate. Results of the observations clearly showed that mercury-resistant isolates have ability to produce H_2S and to convert the methyl mercury into ionic mercury (Hg^{2+}) in the cell. H_2S producing strains developed dark color due to the formation of lead sulfates (PbS) on lead acetate medium. Eight bacterial isolates (ZM2, ZM12, ZM24, ZM36, ZM40, ZM45, ZM50 and ZM57) with minimum zone of inhibition produced dark brown (blackish) color colonies and other bacterial isolates produced creamy to light brown color depending upon the sensitivity of the bacterial isolates against $HgCl_2$.

Biochemical characterization

Eight bacterial strains resistant to 20 and 50 µg/ml concentrations of $HgCl_2$ and exhibiting different levels of H_2S production by observing colonies color were subjected to biochemical characterization and identified as *Cronobacter* sp. (ZM12 and ZM36), *Pseudomonas* sp. (ZM24,

Table I.- Biochemical characterization of selected bacterial strains.

Isolates biochemical tests	ZM2	ZM12	ZM24	ZM36	ZM40	ZM45	ZM50	ZM57
Gram reaction	Gm + Rods	Gm - Rods	Gm - Rods	Gm - Rods	Gm + Rods	Gm - Rods	Gm - Rods	Gm + Rods
Catalase	+	+	+	+	+	+	+	+
Oxidase	+	-	+	-	+	+	+	+
Starch hydrolysis	-	-	-	-	-	-	-	-
Gelatin hydrolysis	+	-	+	-	+	+	+	+
TSI	Y/R/-/-	Y/Y/-/-	Y/Y/+/-	Y/Y/-/-	Y/R/-/-	Y/Y/+/ -	Y/Y/+/ -	Y/R/-/-
Methyl red	-	-	-	-	-	-	-	-
Voges proskauer	+	+	-	+	+	-	-	+
Citrate utilization	+	+	+	+	+	+	+	+
Indole production	-	-	-	-	-	-	-	-
Nitrate reduction	+	+	-	+	+	-	-	+
Identification	*Bacillus* sp.	*Cronobacter* sp.	*Pseudomonas* sp.	*Cronobacter* sp.	*Bacillus* sp.	*Pseudomonas* sp.	*Pseudomonas* sp.	*Bacillus* sp.

Key: TSI= Triple sugar iron, R/Y/-/+ , Alkaline slant/Acidic butt/No gas/H_2S production; Gm -, Gram negative; Gm+, Gram positive.

ZM45 and ZM50) and *Bacillus* sp. (ZM2, ZM40 and ZM57) as given in Table I.

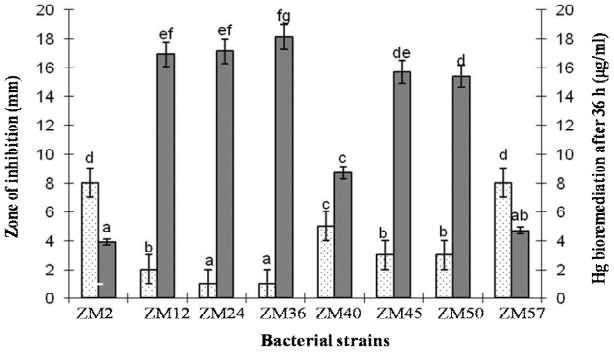

Fig. 1. Correlation between zone of inhibition (mm) by well plate method (dotted bars) and removal of mercury (µg/ml) in culture medium after 36 hours incubation at 37ºC quantified by dithizone method (Black bars). The $p<0.05$ was calculated by ANOVA and different letters indicate significant difference between means of each treatments calculated by DMRT at probability level 0.05.

Estimation of mercury bioremediation by mercury-resistant NFB

The ability of mercury resistant NFB to remediate mercury from the synthetic medium containing 20 µg/ml $HgCl_2$ was determined by dithizone method (Fig. 1). Results indicated that *Cronobacter* species (ZM12 and ZM36) are more efficient to remove mercury from the medium as compared to *Pseudomonas* and *Bacillus* species. It is also clear from the observations that H_2S producing NFB with minimum zone of inhibition are more resistant to mercury and remediate up to 95% of total mercury supplemented in synthetic YEM medium.

DISCUSSION

The present study was designed to screen out mercury resistant and hydrogen sulfide producing NFB from roots of different plants to use them as biofertilizer and for the bioremediation of mercury pollutants. The concentration of mercury into the environment is increasing to dangerous level day by day by the activities of human beings (Pacyna *et al.*, 2006). Various agricultural practices are carried out in developing countries including India and Pakistan that lead to build up of mercury contaminants in the environment; for example extensive use of mercury-based pesticides and fungicides in agricultural fields to prevent fungal diseases (Hussain, 2006; Shoaib *et al.*, 2011). Limited amount of information is available on mercury-resistance in N_2-fixing bacteria (Ghosh *et al.*, 1996). It is note-worthy that nitrogen fixing and mercury-resistant bacterial isolates could volatilize mercury from a site heavily contaminated with $HgCl_2$ (Teng *et al.*, 2015). However, in low amounts of mercury, the mercury-resistant bacteria are capable of detoxifying all of the mercury from the bacterial system (Ray *et al.*,

1989). The microbes residing in the plant roots are involved in establishing a synergistic association with the roots thereby aiding the plants in nutrient absorption and ultimately improving plant growth and soil fertility (Tinker, 1984; Yang *et al.*, 2009). *Enterobacteriaceae* is one of the popular families of species that can be cultivated (Mastretta *et al.*, 2006). The literature study supports our present study as bacteria belonging to *Pseudomonaceae* and *Enterobacteriaceae* were isolated from the soil around the roots of forage crops having associations with plant roots are highly resistant to mercuric chloride (50 µg/ml). Besides their beneficial role in plant growth and development, the endophytic bacteria have a well-known biotechnological importance in improving the applications and efficiency of bioremediation (Weyens *et al.*, 2009). Misra *et al.* (1984) has reported chromosomally determined mercury resistance in *Staphylococcus aureus*.

In the present study mercury resistant gram negative bacteria *Pseudomonas* sp. and *Cronobacter* sp. as well as gram positive bacteria *Bacillus* sp. were isolated from the soil around the roots of different plant species. Many studies have reported the isolation of mercury-resistant bacterial species belonging to the genera *Staphylococcus, Pseudomonas, Escherichia* and *Bacillus* from mercury polluted sites (De *et al.*, 2006; Mirzaei *et al.*, 2008; Poulain *et al.*, 2007). The role of mercury-resistant bacteria, especially *Pseudomonas putida*, in the conversion of toxic state of mercury into less toxic form has been extensively studied for detoxification of mercury contaminated sites either by producing hydrogen sulfide or by expression of *mer* operon (Barkay and Wagner-Dobler, 2005). The direct relationship between H_2S production and bioremediation of mercury was studied in bacteria (Pan-Hou and Imura, 1981) and in yeasts (Amin and Latif, 2011).

In this study, the isolated *Enterobacter*, *Cronobacter*, *Pseudomonas* bioremediate mercury up to 18 µg/ml (approximately 95%) out of total 20 µg/ml mercuric chloride supplemented in YEM medium (p>0.05). Kargar *et al.* (2014) have reported *Pseudomonas* sp. and *Serattia* sp. as the most resistant isolates to antibiotic and mercury and *E. coli* as a highly resistant isolate to mercury. It has been studied from the literature that *Pseudomona*s and *Enterobacter* have been previously reported as nitrogen fixer microorganisms (Mehnaz *et al.*, 2010). *Cronobacter* sp. previously known as *Enterobacter sakazaki*, has been isolated first time from the soil around the root nodules of different plants. The present studies exactly match the previously reported studies that NFB have the potential to detoxify mercury (Tariq and Latif, 2014) and also make beneficial associations with plant roots used as not only for bioremediation of mercury to clean up the environment but also as a biofertilizer to replace the chemical fertilizer.

REFERENCES

AKPOR, O. AND MUCHIE, M., 2010. Remediation of heavy metals in drinking water and wastewater treatment systems: Processes and applications. *Int. J. Physic. Sci.*, **5:** 1807-1817.

AMIN, A. AND LATIF, Z., 2011. Isolation and characterization of H_2S producing yeast to detoxify mercury containing compounds. *Int. Res. J. Microbiol.*, **2:** 517-525.

AMIN, A. AND LATIF, Z., 2013. Detoxification of mercury pollutant by immobilized yeast strain *Candida xylopsoci*. *Pak. J. Bot.*, **45:** 1437-1442.

ARAIN, M., KAZI, T., BAIG, J., JAMALI, M., AFRIDI, H., SHAH, A., JALBANI, N. AND SARFRAZ, R., 2009. Determination of arsenic levels in lake water, sediment, and foodstuff from selected area of Sindh, Pakistan: estimation of daily dietary intake. *Fd. Chem. Toxicol.*, **47:** 242-248.

BAFANA, A., KRISHNAMURTHI, K., PATIL, M. AND CHAKRABARTI, T., 2010. Heavy metal resistance in *Arthrobacter ramosus* strain G2 isolated from mercuric salt-contaminated soil. *J. Hazard. Mat.*, **177:** 481-486.

BARKAY, T. AND WAGNER-DOBLER, I., 2005. Microbial transformations of mercury: potentials, challenges, and achievements in controlling mercury toxicity in the environment. *Adv. appl. Microbiol.*, **57:** 1-52.

BRODKIN, E., COPES, R., MATTMAN, A., KENNEDY, J., KLING, R. AND YASSI, A., 2007. Lead and mercury exposures: interpretation and action. *Can. med. Assoc. J.*, **176:** 59-63.

CAPPUCCINO, J.G. AND SHERMAN, N., 2004. *Microbiology: a laboratory manual*. Pearson education, USA.

CRISTOL, D.A., BRASSO, R.L., CONDON, A.M., FOVARGUE, R.E., FRIEDMAN, S.L., HALLINGER, K.K., MONROE, A.P. AND WHITE, A.E., 2008. The movement of aquatic mercury through terrestrial food webs. *Science*, **320:** 335-335.

DE, J., SARKAR, A. AND RAMAIAH, N., 2006. Bioremediation of toxic substances by mercury resistant marine bacteria. *Ecotoxicology*, **15:** 385-389.

ELLY, C.T., 1973. Dithizone procedure for mercury analysis. *J. Water Pollut. Contr. Fed.*, **45:** 940-945.

GHOSH, S., SADHUKHAN, P., GHOSH, D., MANDAL, A., CHAUDHURI, J. AND MANDAL, A., 1996. Studies on the effect of mercury and organomercurial on the growth and nitrogen fixation by mercury-resistant *Azotobacter* strains. *J. appl. Bact.*, **80:** 319-326.

HOLMES, P., JAMES, K. AND LEVY, L., 2009. Is low-level environmental mercury exposure of concern to human health? *Sci. Total Environ.*, **408:** 171-182.

HUMAIRA, K., MOHAMMAD, J. AND MOHAMMAD, I., 2005. A simple spectrophotometric determination of trace level mercury using 1, 5-diphenylthiocarbazone solubilized in micelle. *Anal. Sci.*, **21:** 507-512.

HUSSAIN, I., 2006. Investigation of heavy metals Cu, Pb, Cd, Mn, Cr, Fe and Ni in Ammi Visnaga. *J. Chem. Soc. Pak.*, **28:** 241.

KARGAR, M., JAHROMI, M.Z., NAJAFIAN, M., KHAJEAIAN, P., NAHAVANDI, R., JAHROMI, S.R. AND FIROOZINIA, M., 2014. Identification and molecular analysis of mercury resistant bacteria in Kor River, Iran. *Afr. J. Biotechnol.*, **11:** 6710-6717.

KHAN, M.S., ZAIDI, A. AND WANI, P.A., 2007. Role of phosphate-solubilizing microorganisms in sustainable agriculture-a review. *Agron. Sustain. Dev.*, **27:** 29-43.

LISHA, K., MALIYEKKAL, S.M. AND PRADEEP, T., 2010. Manganese dioxide nanowhiskers: a potential adsorbent for the removal of Hg (II) from water. *Chem. Eng. J.*, **160:** 432-439.

MANOHAR, D., KRISHNAN, K.A. AND ANIRUDHAN, T., 2002. Removal of mercury (II) from aqueous solutions and chlor-alkali industry wastewater using 2-mercaptobenzimidazole-clay. *Water Res.*, **36:** 1609-1619.

MASTRETTA, C., BARAC, T., VANGRONSVELD, J., NEWMAN, L., TAGHAVI, S. AND LELIE, D.V.D., 2006. Endophytic bacteria and their potential application to improve the phytoremediation of contaminated environments. *Biotechnol. Genet. Eng. Rev.*, **23:** 175-188.

MEHNAZ, S., BAIG, D.N. AND LAZAROVITS, G., 2010. Genetic and phenotypic diversity of plant growth promoting rhizobacteria isolated from sugarcane plants growing in pakistan. *J. Microbiol. Biotechnol.*, **20:** 1614-1623.

MEHNAZ, S., WESELOWSKI, B. AND LAZAROVITS, G., 2007. *Azospirillum canadense* sp. nov., a nitrogen-fixing bacterium isolated from corn rhizosphere. *Int. J. Syst. Evol. Microbiol.*, **57:** 620-624.

MIRZAEI, N., KAFILZADEH, F. AND KARGAR, M., 2008. Isolation and identification of mercury resistant bacteria from Kor River, Iran. *J. biol. Sci.*, **8:** 935-939.

MISRA, T.K., BROWN, N.L., FRITZINGER, D.C., PRIDMORE, R.D., BARNES, W.M., HABERSTROH, L. AND SILVER, S., 1984. Mercuric ion-resistance operons of plasmid R100 and transposon Tn501: the beginning of the operon including the regulatory region and the first two structural genes. *Proc. natl. Acad. Sci.*, **81:** 5975-5979.

MUNEER, B., IQBAL, SHAKOORI, F. R. AND SHAKOORI, A. R., 2013. Tolerance and Biosorption of Mercury by Microbial Consortia: Potential Use in Bioremediation of Wastewater. *Pak. J. Zool.*, **45:** 247-254.

NOR KAMARUDIN, K.S. AND MOHAMAD, M.F., 2010. Synthesis of gold (Au) nanoparticles for mercury adsorption. *Am. J. appl. Sci.*, **7:** 835-839.

OKON, Y., ALBRECHT, S.L. AND BURRIS, R., 1977. Methods for growing *Spirillum lipoferum* and for counting it in pure culture and in association with plants. *Appl. environ. Microbiol.*, **33:** 85-88.

ONO, B., ISHII, N., FUJINO, S. AND AOYAMA, I., 1991. Role of hydrosulfide ions (HS^-) in methylmercury resistance in *Saccharomyces cerevisiae*. *Appl. environ. Microbiol.*, **57:** 3183-3186.

ONO, B., KIJIMA, K., ISHII, N., KAWATO, T., MATSUDA, A., PASZEWSKI, A. AND SHINODA, S., 1996. Regulation of sulphate assimilation in *Saccharomyces cerevisiae*. *Yeast*, **12:** 1153-1162.

PACYNA, E.G., PACYNA, J.M., STEENHUISEN, F. AND WILSON, S., 2006. Global anthropogenic mercury emission inventory for 2000. *Atmosph. Environ.*, **40:** 4048-4063.

PAN-HOU, H.S. AND IMURA, N., 1981. Role of hydrogen sulfide in mercury resistance determined by plasmid of *Clostridium cochlearium* T-2. *Arch. Microbiol.*, **129:** 49-52.

POULAIN, A.J., CHADHAIN, S.M.N., ARIYA, P.A., AMYOT, M., GARCIA, E., CAMPBELL, P.G., ZYLSTRA, G.J. AND BARKAY, T., 2007. Potential for mercury reduction by microbes in the high arctic. *Appl. environ. Microbiol.*, **73:** 2230-2238.

RAY, S., GACHHUI, R., PAHAN, K., CHAUDHURY, J. AND MANDAL, A., 1989. Detoxification of mercury and organomercurials by nitrogen-fixing soil bacteria. *J. Biosci.*, **14:** 173-182.

SHARMA, R.K. AND AGRAWAL, M., 2005. Biological effects of heavy metals: an overview. *J. environ. Biol.*, **26:** 301-313.

SHOAIB, A., QMAR, A. AND AKHTAR, S., 2011. Growth of *Vigna radiata*, *V. mungo* and *V. unguiculata* under abiotic stress of mercury. *Mycopath*, **9:** 1-7.

SINHA, A. AND KHARE, S.K., 2012. Mercury bioremediation by mercury accumulating *Enterobacter* sp. cells and its alginate immobilized application. *Biodegradation*, **23:** 25-34.

TARIQ, A. AND LATIF, Z., 2014. Bioremediation of mercury compounds by using immobilized nitrogen-fixing

bacteria. *Int. J. Agric. Biol.*, **16:** 1129-1134.

TCHOUNWOU, P.B., AYENSU, W.K., NINASHVILI, N. AND SUTTON, D., 2003. Review: Environmental exposure to mercury and its toxicopathologic implications for public health. *Environ. Toxicol.*, **18:** 149-175.

TENG, Y., WANG, X., LI, L., LI, Z. AND LUO, Y., 2015. Rhizobia and their bio-partners as novel drivers for functional remediation in contaminated soils. *Front. Plant Sci*., **6:** 32.

TINKER, P., 1984. The role of microorganisms in mediating and facilitating the uptake of plant nutrients from soil. *Pl. Soil*, **76:** 77-91.

WAGNER-DÖBLER, I., 2003. Pilot plant for bioremediation of mercury-containing industrial wastewater. *Appl. Microbiol. Biotechnol.*, **62:** 124-133.

WANG, J., DENG, B., WANG, X. AND ZHENG, J., 2009. Adsorption of aqueous Hg (II) by sulfur-impregnated activated carbon. *Environ. Eng. Sci.*, **26:** 1693-1699.

WEYENS, N., VAN DER LELIE, D., TAGHAVI, S. AND VANGRONSVELD, J., 2009. Phytoremediation: plant–endophyte partnerships take the challenge. *Curr. Opin. Biotechnol*., **20:** 248-254.

WHITE, D.G., ALEKSHUN, M.N. AND MCDERMOTT, P.F., 2005. *Frontiers in antimicrobial resistance: a tribute to Stuart B.* Levy. ASM Press.

YANG, J., KLOEPPER, J.W. AND RYU, C.M., 2009. Rhizosphere bacteria help plants tolerate abiotic stress. *Trends Pl. Sci.*, **14:** 1-4.

ZAHIR, F., RIZWI, S.J., HAQ, S.K. AND KHAN, R.H., 2005. Low dose mercury toxicity and human health. *Environ. Toxicol. Pharmacol.*, **20:** 351-360.

ZAIDI, A. AND KHAN, S., 2005. Interactive effect of rhizotrophic microorganisms on growth, yield, and nutrient uptake of wheat. *J. Pl. Nutr.*, **28:** 2079-2092.

ZEROUAL, Y., MOUTAOUAKKIL, A. AND BLAGHEN, M., 2001. Volatilization of mercury by immobilized bacteria (*Klebsiella pneumoniae*) in different support by using fluidized bed bioreactor. *Curr. Microbiol.*, **43:** 322-327.

ZHANG, F.-S., NRIAGU, J.O. AND ITOH, H., 2005. Mercury removal from water using activated carbons derived from organic sewage sludge. *Water Res.*, **39:** 389-395.

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