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Bioremediation of Lead from River Water through Lead-Resistant Purple-Nonsulfur Bacteria

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Abstract

Lead toxicity in water is a global problem affecting millions of people. Lead contamination occurs due to industrial use and processing of lead ore. In children lead may be hazardous and results in mental retardation. Removal of this heavy metal from industrial effluents is essential and must be removed from the effluent prior to discharge into the aquatic bodies or river water. The property of some species of bacteria to extract metals from their surrounding has been utilized to purify sewage and industrial effluents. In view of the above, five lead tolerant bacterial strains have been isolated from the heavy-metal contaminated site of the river Ganga of West Bengal, India. Among these isolates, two showed maximum tolerance towards lead. The present study focuses to investigate the capability of lead-resistant bacteria and bioremediation of river water contaminated by lead metal.

Keywords: Bioremediation, River Ganga, Lead, Purple nonsulfur bacteria

1. Introduction

Pollution in industrial area is serious environmental concern in recent times and there is growing interest regarding bacterial resistance to heavy metals is of utmost practical significance. Rivers in urban areas has also been associated with heavy metals problems because of the practice of discharging of small scale industries and untreated domestic wastes which lead to increase in concentration of heavy metals in river water [1]. Heavy metals are not readily degradable in the environment and accumulate in the human bodies to very high toxic levels leading to undesirable effects beyond a certain limit. Metal-resistant organisms may be utilized as potential toxicity indicators to other forms of life. Pb^{++}, Hg^{++}, Cd^{++} are of serious concern due

to their non-biodegradability, high toxicity and occur in a large number of waste waters that contaminate the aquatic environment [2,3]. Bioremediation processes are very attractive over physicochemical methods for heavy metal removal due to low cost and high efficiency at low metal concentration [4]. In the present study, detoxification of lead present in river water was studied by the lead-resistant purple-nonsulfur bacteria isolated from the river Ganga.

2. Materials and Methods

2.1. Determination of lead concentration in water sample

This was performed with the help of Atomic Absorption Spectroscopy (Model no. Varian AA240).

2.2. Isolation of lead-resistant bacteria

Lead resistant bacteria were isolated from river water near effluent-discharge point of Kesoram Rayon Factory, Tribeni, W.B., India. PNSB medium, amended with 10 ppm lead acetate was used. Purified cultures of five lead resistant bacterial isolates were previously grown in PNSB broth amended with 25 ppm lead, tentatively characterized and identified using standard biochemical tests.

2.3. Detoxification of lead

All the 5 PNSB isolates (RPNSB2, RPNSB3, RPNSB5, RPNSB8, RPNSB11) were tested in PNSB broth amended with different concentration of lead (as lead-acetate) at room temperature (28±2˚C). A lead-sensitive PNSB (RPNSB4) and heat-killed bacterial cells were kept as negative control. Subsamples (1ml) were taken every 24 h, centrifuged at 10,000 rpm for 15 min at 24˚C and supernatants filtered through sterilized pre-weighed o.22μm membrane filter. For estimation of the heavy metals from test media, filtrates were diluted 10 fold with 10% HNO_3. The lead concentration was determined by Atomic Absorption Spectrophotometer (Varian AA240).

3. Results and Discussion

A total of six purple non sulphur bacterial (PNSB) strains were isolated from the river Ganga near the effluent discharge point of Kesoram Rayon Factory, Tribeni, West Bengal, India. Out of these, five strains were found to be resistant to lead, which was present in ambient water of the river Ganga in that area. Only one PNSB isolate, PNSB4, showed sensitivity to lead at a concentration 25 ppm. All the isolates have been characterized by morphological and biochemical properties and tentatively identified as *Rhodopseudomonas palustris.*

The lead removal capability of all six isolates was studied at different concentrations of lead (100, 50, 10 ppm) and at different time intervals (24 h, 48 h, 72 h, 96h) (Fig 1-6). PNSB 2 and PNSB3 showed remarkable ability to remove lead from the medium. These isolates could reduce 96% after 4 days. The isolate PNSB 5 could efficiently remove lead from the medium. The bacteria could reduce 80% lead from

the initial concentration of 100ppm in 96 hr. In case of isolate PNSB8 concentration of lead reduced to 22 ppm (78% removal) from an initial concentration of 100 ppm in 96h.The isolate PNSB 11 could efficiently remove lead from the medium. This isolates showed removal of 92% lead from an initial concentration of 100 ppm in 96 h. Among the six bacteria tested for lead uptake PNSB 2 and PNSB 3 could remove maximum quantity of lead followed by PNSB 11, PNSB 5 and PNSB 8. The isolates PNSB 2 and PNSB 3 showed highest resistance to lead. PNSB4 showed no sign of growth in lead amended medium.Such lead resistant bacteria were reported causing bioremediation of lead from contaminated water [5]. The lead biosorption capability was also studied at different concentrations of lead (100, 200, 300, 400 and 500 mg/l) by Murthy *et al* [6].

High pH normally accelerates precipitation of heavy metals [7], particularly for Pb (< 5 μg/L Pb) [8]. Heavy Metals contaminated water was treated by the selected PNSB strains under microaerobic-light and aerobic-dark conditions. Fortunately, removal of heavy metals by PNSB cells did occur under both incubating conditions and in general, the conditions of aerobic-dark produced better removal efficiency than microaerobic-light conditions. In general, actively growing PNSB cells have been used to treat various wastewaters [9].

TABLE 1: Morphological and Biochemical characteristics of the purple non-sulfur bacterial isolates

Characters	RPNSB 2	RPNSB 3	RPNSB 4	RPNSB 5	RPNSB 8	RPNSB 11
Shape	Rod	Rod	Rod	Rod	Rod	Rod
Size(μm)	0.6-0.9 1.2-2.0	0.6-0.8 1.2-2.0	0.5-0.8 1.2-2.1	0.5-0.8 1.2-2.1	0.6-0.9 1.2-2.2	0.5-0.9 1.1-2.0
Gram Character	-ve	-ve	-ve	-ve	-ve	-ve
Motility	+	+	+	+	+	+
Utilization of Gelatine	±	±	±	±	±	±
Nitrate reduction	+	+	+	+	+	+
Growth on 3% NaCl	-	-	-	-	-	-
Utilization of Sodium Thiosulfate	+	+	+	+	+	+
Utilization of Sodium citrate	-	-	-	-	-	-
Utilization of Urea	-	-	-	-	-	-
Starch hydrolysis	-	-	-	-	-	-
Utilization of Sugar						
Glucose	+	+	+	+	+	+
Fructose	+	+	+	+	+	+
Mannitol	-	-	-	-	-	-
Sucrose	+	+	+	+	+	+
Inositol	+	+	+	+	+	+
Rhamnose	+	+	+	+	+	+
Melibiose	-	-	-	-	-	-
Arabinose	-	-	-	-	-	-

Utilization of Substrate						
Succinate	+	+	+	+	+	+
Pyruvate	+	+	+	+	+	+
Malate	-	-	-	-	-	-
Acetate	+	+	+	+	+	+
Benzoate	-	-	-	-	-	-
Utilization of Amino acid						
Arginine	-	-	-	-	-	-
Lysine	-	-	-	-	-	-
Tryptophan	-	-	-	-	-	-
Orinithine	-	-	-	-	-	-

+ indicates presence or positive reaction, - indicates absence or negative reaction

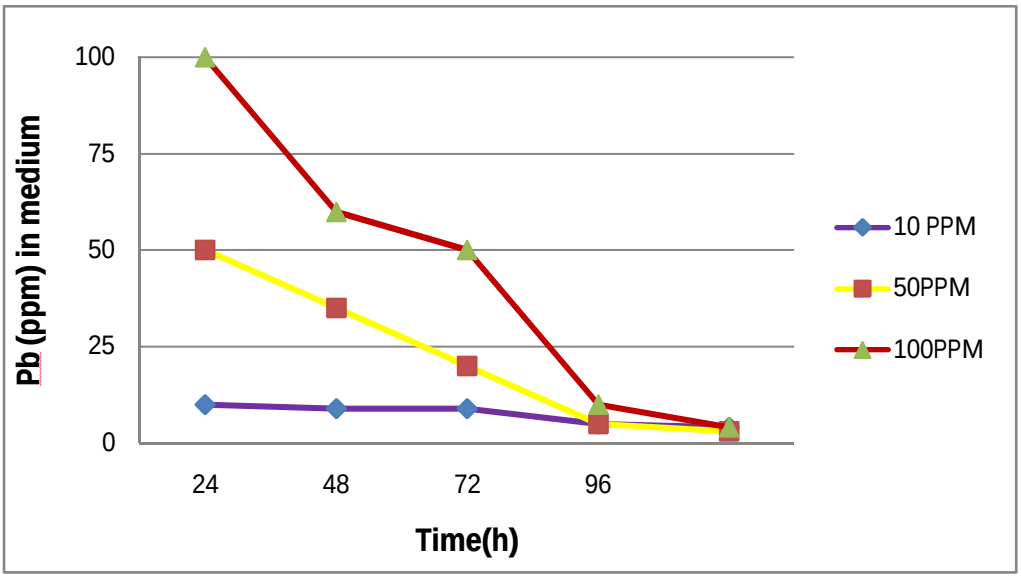

FIGURE 1: Removal of lead by PNSB2 when grown in medium amended with different concentration of lead

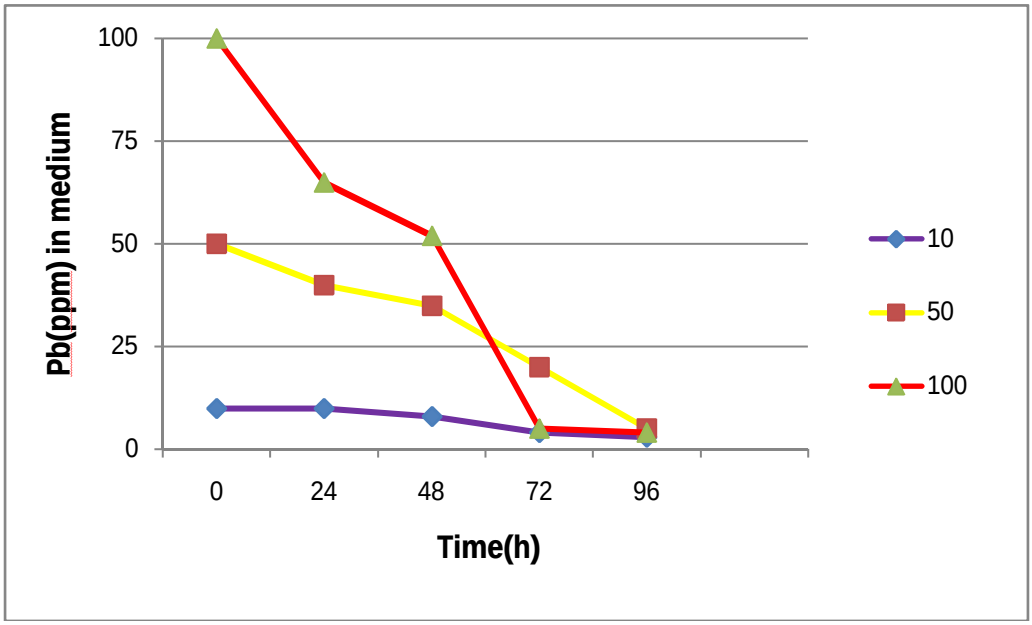

FIGURE 2: Removal of lead by PNSB3 when grown in medium amended with different concentration of lead

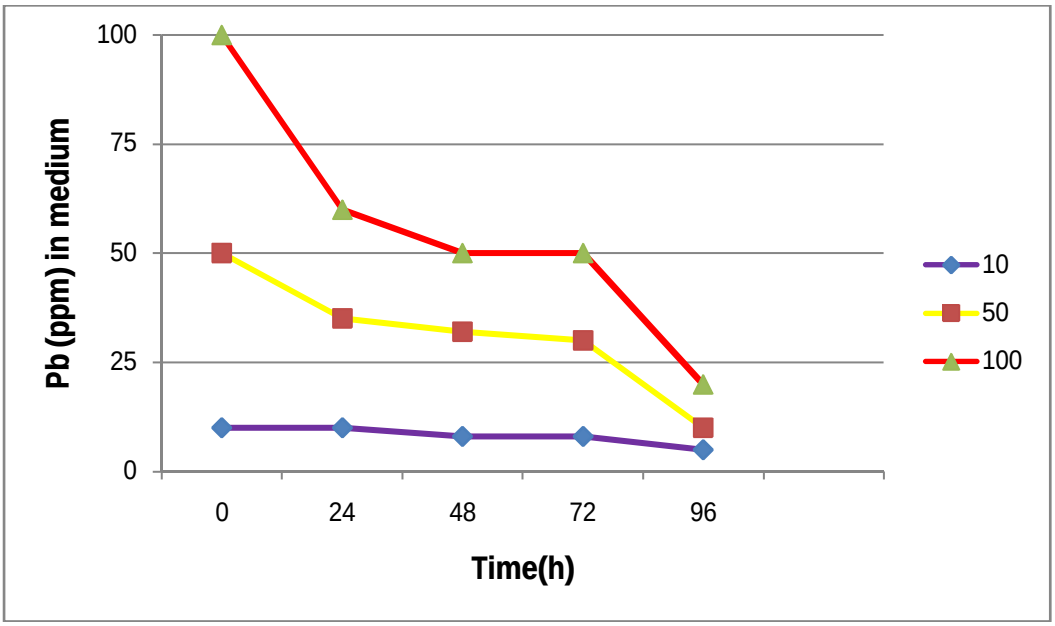

FIGURE 3: Removal of lead by PNSB5 when grown in medium amended with different concentration of lead

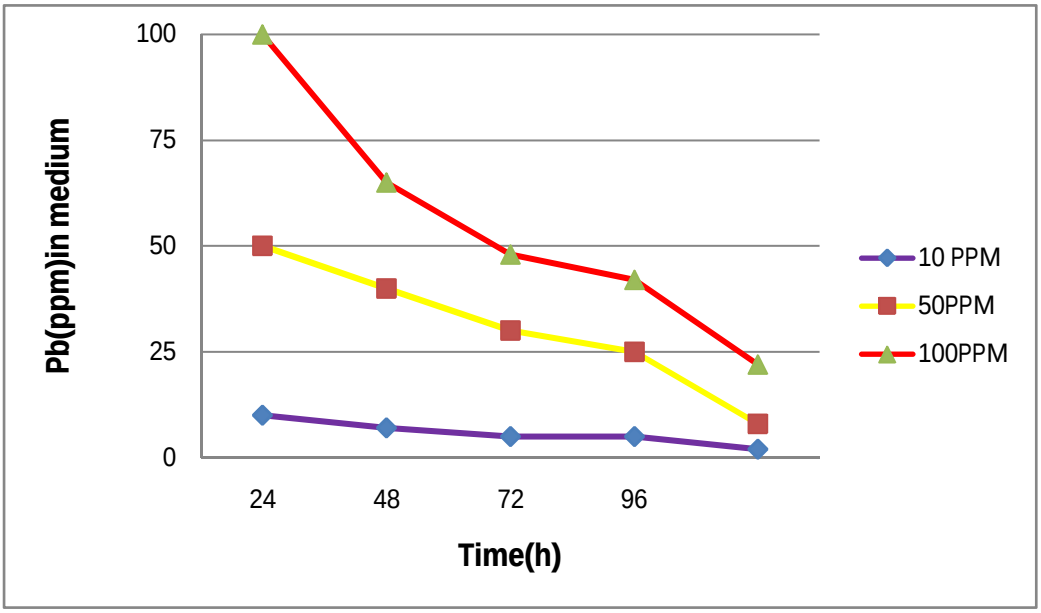

FIGURE 4: Removal of lead by PNSB8 when grown in medium amended with different concentration of lead

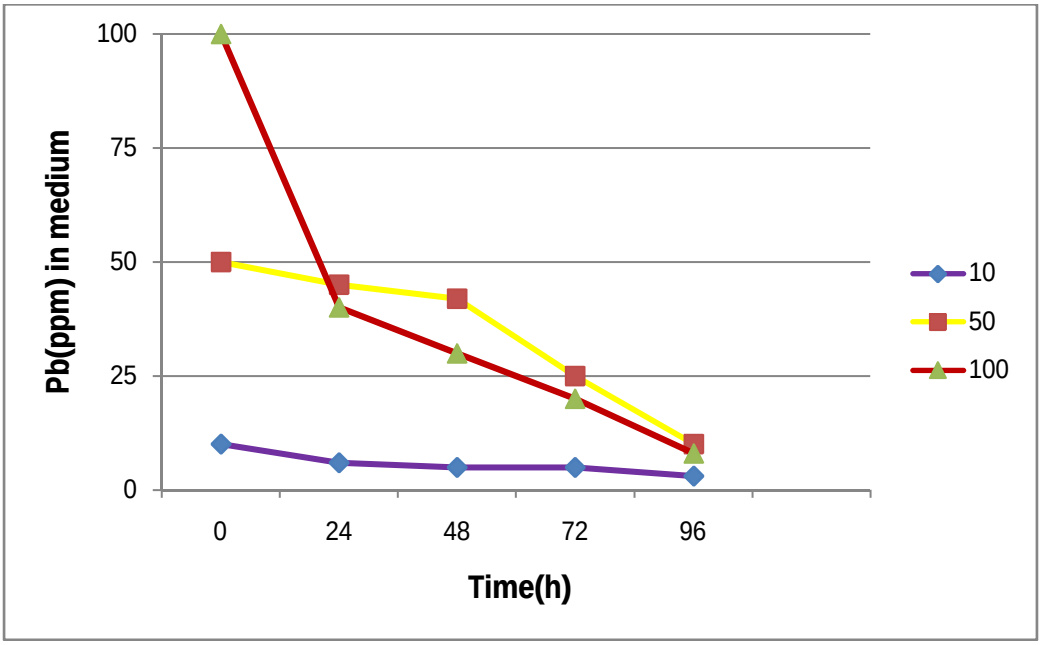

FIGURE 5: Removal of lead by PNSB11 when grown in medium amended with different concentration of lead

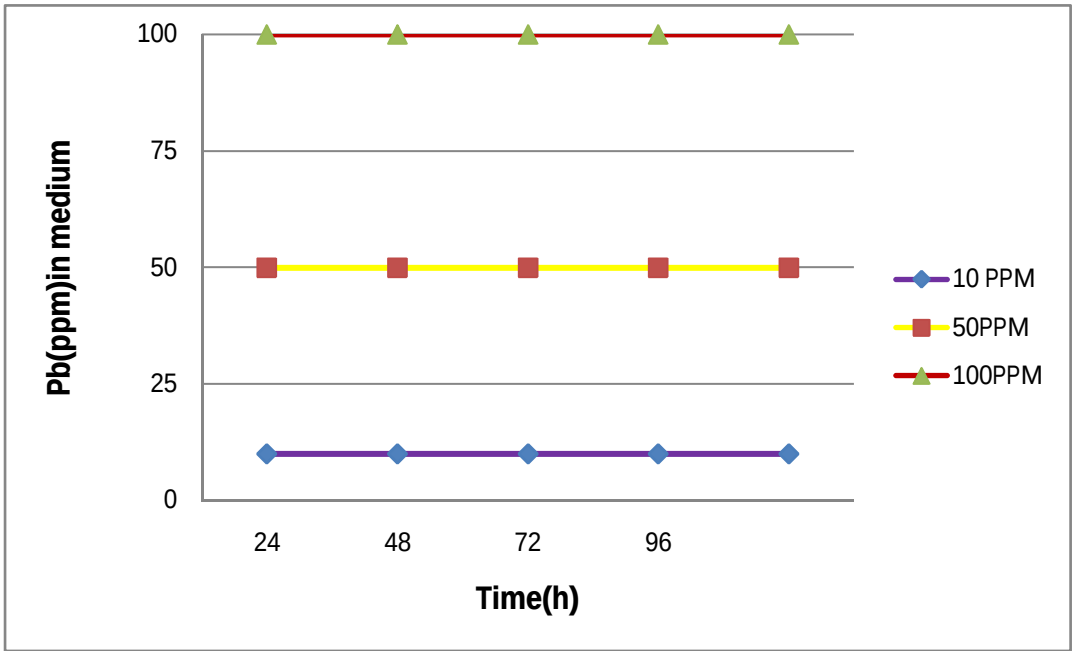

FIGURE 6: Effect of lead on PNSB4 at different concentration (control)

4. Conclusion

The present study revealed that the river water near the effluent discharge of Kesoram Rayon Factory, Tribeni were found to be polluted by the lead along with other heavy metals. The purple non sulphur bacterial isolates PNSB2 and PNSB3 had the high potentiality to remove lead (Pb) from the river water contaminated with heavy metals. Hence, it will be possible in future to use these strains as inoculants for bioremediation of polluted water of river Ganga contaminated with heavy metals such as lead.

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