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Study on the effect of pH and temperature on growth of *Pseudomonas* sp. in the presence heavy metals

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Abstract

The Purpose of present study was to investigate the effect of Cr^{+2} and Pb^{+2} on heavy metal tolerant species of *Pseudomonas* at different pH and temperature for varying incubation periods.It was found that growth of *Pseudomonas* sp. increased in acidic medium (5.0-6.0) in presence of both metals (Cr^{+2} and Pb^{+2}). In presence of chromium metal, *Pseudomonas* sp. showed maximum growth at pH 6.0. At pH 7.0, bacterial growth was gradually declined in Chromium enriched medium while accelerated up to maximum point in presence of Lead. Therefore, 6.0 and 7.0 were considered optimum pH values for highest bacterial growth in Chromium and Lead containing media respectively. In case of temperature, *Pseudomonas* sp. exhibited increase in growth only at 50°C in presence of Chromium and remained suppressed at all other selected temperatures (20°C, 25°C, 37°C and 45°). In presence of Lead, *Pseudomonas* sp. showed significant growth at all selected temperatures (25°C, 37°C, 45°C and 50°C) except 20°C. Optimum temperature values found for bacterial growth in Chromium and Lead medium were50°C and 37°C, respectively.Under optimum conditions, highest growth of *pseudomonas* sp. was observed within three days (72 hours) of incubation for both metals.

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Introduction

Heavy metal contamination is a global environmental problem. The release of metals into the ecosystem either from naturally occurring sources or from anthropogenic activities poses a serious threat to public health due to their persistence, bio-magnification and accumulation within the food chain. Arsenic, copper, cadmium, lead, chromium, nickel mercury and zinc are among the most commonly encountered heavy metals in polluted water Kalavrouziotis *et al.*, 2009; Sengor and Gikas, 2014). The heavy metals are released due to the discharge of effluent into the environment by a large number of processes such as electroplating, leather tanning, wood preservation, pulp processing, steel manufacturing, etc., and the concentration levels of these heavy metals varies widely in the environment. Cadmium and Pb, though non-essential and non-beneficial, are considered highly toxic to plants, animals and microbes (Ajmal*et al.*, 1998). These heavy metals pose a critical concern to human health and environmental issues due to their high occurrence as a contaminant, low solubility in biota and the classification of several heavy metals as carcinogenic and mutagenic (Diels *et al.*, 2002).Pakistan is no exception to it, where land and water bodies near industria l zones are reported to have elevated levels of multiple metal ions (Uzair*et al.,* 2009). Main reason for this metal contamination is that 80% of the land is irrigated by wastewater generated from industry. The Indus River and its tributaries provide water to over 16 million hectares of land, situated in the mainly arid and semi-arid zones of the country. A rapidly growing population, saline groundwater, a poorly performing irrigation distribution system, and recurrent droughts have led to increased water shortages. Under these conditions, the use of untreated urban wastewater for agriculture has become a common and widespread practice [6]. Although Chromium and Lead levels were found to be high in almost all ground water sources, however, extremely high concentrations were found in industrial areas of Karachi (Ensink*et al.,* 2002).

Faisalabad city is the third largest city of Pakistan. It is famous for its different industries, which include paper, leather, textile, sugar, vegetable oil, soaps and detergents and other industries. It is estimated that nearly 1.5 million cubic meters of untreated solid wastes and heavy metals are being disposed off into the natural streams (Rehman, 1993). Besides more than 2000 hectares are irrigated by untreated wastewater which has led to high heavy metal accumulation (Mn, Cu, Co and Zn) in wastewater irrigated agricultural land (Mahmood and Maqbool, 2006). These heavy metals and other pollutants are quite harmful to both the environment and the groundwater because this water is the main source of drinking water. Heavy metals cannot be degraded to harmless products and hence persist in the environment indefinitely. As a result, several methods have been devised for the treatment and removal of heavy metals in contaminated sites. Conventional physico-chemical methods such as electrochemical treatment, ion exchange, precipitation, reverse osmosis and evaporation for heavy metal removal are being economically expensive and have disadvantages like incomplete metal removal, higher reagent, energy requirements and generation of toxic sludge (Kadirvelu*et al.,* 2002). Microbial populations in metal polluted environments adapt to toxic concentrations of heavy metals and become metal resistant (Prasenjit and Sumathi, 2005; Umber, 2008). Different species of *Aspergillus, Pseudomonas, Sporophyticus, Bacillus, Phanerochaete*have been reported as efficient Chromium and Nickel reducers (Yan and Viraraghavan, 2003; Umber, 2008). The response of microorganisms towards toxic heavy metals is of importance in view of their interest in the reclamation of polluted sites.

Chromium and its compounds are widely used in electroplating, leather tanning, cement, dyeing, metal processing, wood preservatives, paint and pigments, textile, steel fabrication and canning industries. These industries produce large quantities of toxic wastewater effluents (Raji and Anirudhan, 1997).

The maximum concentration limit Standard for Cr (VI) for discharge into inland waters is 0.1 mg/l and in potable water is 0.05 mg/l. Lead, a major pollutant that is found in soil, water and air is a hazardous waste and is highly toxic to humans, animals, plants and microbes (Low *et al.*, 2000). Lead resistance has been widely reported and well-studied in both gram-negative bacteria and gram-positive bacteria isolated from Pb-contaminated soils. *Pseudomonas marginalis*shows extracellular Pb exclusion while *Bacillus megatarium*has been reported to demonstrate intracellular cytoplasmic Pb accumulation (Roane, 1999).

Temperature, pH, biomass, heavy metal concentrations are the factors which affect the uptake of heavy metals. Particularly, pH (Gourdon*et al.*, 1990) and heavy metal concentration (Kiran*et al.*, 2005). Metal biosorption performance depends on external factors, such as pH, other ions in solution (which may be in competition), organic material in solution (such as complexing agents), cell metabolic products in solution (which may cause metal precipitation), and temperature (Veglio*et al.*, 1997).

The development of biosorption may provide a basis for a technologyamiedat the removal of heavy metal species from dilute solutions (Volesky, 1993). The objective of this study was to determine characterization of heavy resistant *Pseudomonas*sp isolated from contaminated environment and its ability under various stress conditions for clean-up of industrial wastewater and sewage. This would help us to assess its potential exploitation in metal detoxification and environmental bioremediation.

Materiels and methods

Sample Collection

The bacterial samples, used in this study were taken from the bacterial stock previously isolated from different industrial effluents of Faisalabad (sugar industry, fabric industry and oil industry) and had already been identified and categorized as heavy metal resistant isolates (Umber, 2008). And were kept at Research Laboratory of Zoology Department, GC University, Faisalabad. They were normally grown in Luria Bertani (LB) agar (pH 7.0) at 37 °C.

Purification and Re-identification of Bacterial Isolates

Bacterial colonies were re-identified by morphological and biochemical tests. Colonies were picked and cultured repeatedly on nutrient agar plates to remove contaminations. Finally, pure cultures were obtained and preserved on nutrient agar slants at 8°C. Pure culture of *Pseudomonas* sp. isolated from sugar industry effluent was selected for further investigations because of its high heavy metal resistance (Umber, 2008).

Biochemical tests

Biochemical tests performed for the re-identification of bacteria were Gram's Staining, Catalase Test, Oxidase Test, Indole Test, Motility test, VogasProskauer Test.

Growth of pseudomonas sp.

At various pH values in presence of heavy metals: Bacterial isolates were inoculated into two triplicates of 250ml conical flasks containing 100ml of nutrient broth per flask, 200µg/ml of Chromium in each flask of one triplicate and 200µg/ml of Lead in each flask of second triplicate with one control flask in each case in which metal was absent. Potassium ($K_2Cr_2O_7$) and Lead ($PbCl_2$) salts were the source of metals in growth medium. Value of pH was varied from 5 to 9 (5, 6, 7, 8, and 9). pH of medium was adjusted using 1M solution of HCl and NaOH. Experimentation was conducted on a rotary shaker (160 rpm) at 37°C for 24, 48, 72 and 96 hours of incubation. After incubation period of each 24 hours absorbance of cultures was measured at 600nm using a spectrophotometer.

Growth of pseudomonas sp

At Various Incubation Temperatures in Presence of Heavy Metals: Simultaneously, effect of various temperatures on growth of *Pseudomonas* sp. in the presence of Chromium and Lead at pH 6.0 and pH 7.0 respectively, was studied by incubating the isolates at different temperatures (20, 25, 37, 45 and 50°C). After each 24 hours till 96 hours, absorbance was measured at 600nm using a spectrophotometer.

Statistical analysis

Data was subjected to statistical analysis in which bacterial growth in presence of Cr^{+2} and Pb^{+2} at different pH and temperature were compared using student's t test [20].

Results

Identification of Bacterial Isolates

Pseudomonas sp. was re-identified by using different morphological and biochemical tests (Table 1).

Table 1. Morphological and biochemical studies of Bacteria isolated from different samples of industrial effluents.

Identified Bacteria	Colony morphology	Gram's staining	Catalase test	Oxidase test	Indole test	Motility test	VogasProskaure test
Pseudomonas sp.	Creamy white round	-Ve	+Ve	+Ve	-Ve	+Ve	-Ve

Effect of varying pH on Growth of pseudomonas sp.

n Presence of Chromium (Cr^{+2}): A gradual increase was observed in optical density of culture at pH 5.0 throughout the incubation period of 96 hours as compare to control, indicating a continuous increase in bacterial growth. At this pH, maximum growth was found after 96 hours. Same steady pattern of growth was noticed at pH 6.0 with highest value in 72 hours. *Pseudomonas* sp. growth remained unaffected up to 48 hours at pH 7.0 then declined. Study showed that pH 8.0 and 9.0 tend to inhibit bacterial growth. Highest microbial growth was observed at pH 6.0 therefore it was considered as optimum pH value for growth of *Pseudomonas* sp. at 37°C in presence of Chromium (Fig. 1).

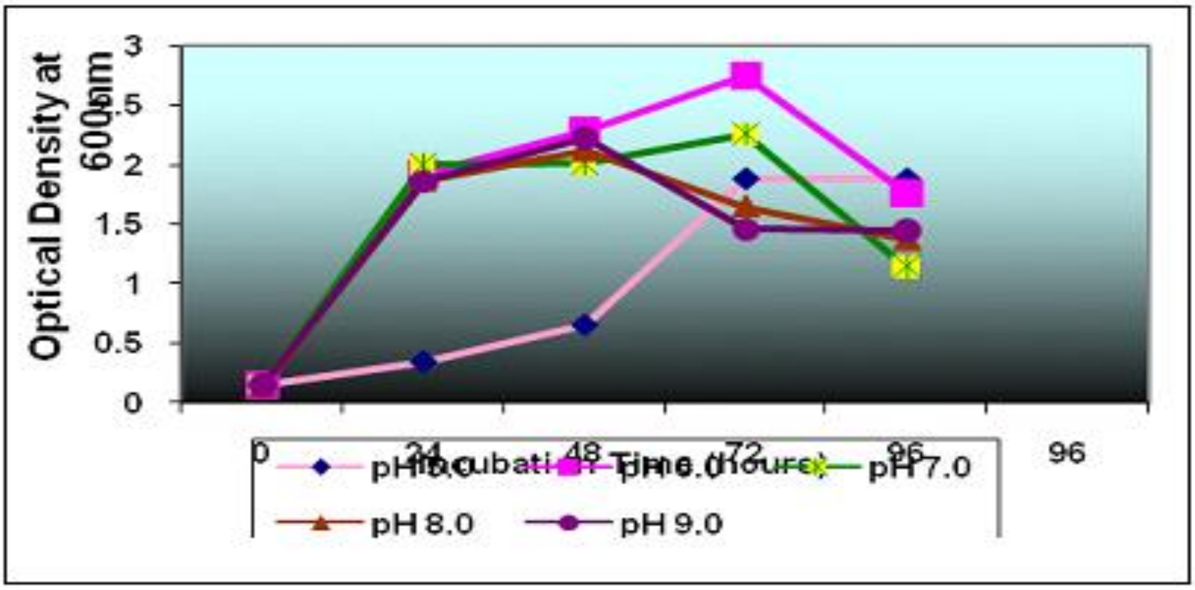

Fig. 1. Effect of pH on growth of *Pseudomonas* sp. in the presence of Chromium at 37°C.

Lead

Pseudomonas sp. grow steadily till 96 hours at pH 5.0. Similar growth pattern was detected at pH 6.0 which attained peak level in 96 hours of incubation. However, maximum proliferation of bacterial sp was noticed in 72th hours at pH 7.0.Lessergrowth was observed between 24 and 48 hours of incubation. Bacterial growth was increased afterwards for pH 8.0 and 9.0.(Table 1and Fig. 2).

Effect of Different Temperature Values on Bacterial Growth in Presence of Chromium (Cr^{+2}) and Lead (Pb^{+2}) at pH 6.0 and 7.0 Chromium

Selected *Pseudomonas* sp. was grown at 20, 25, 37, 45 and 50°C temperatures. Results indicated that *Pseudomonas* sp. growth was remained suppressed at all studied temperatures except 50°C. Bacteria showed maximum growth in 72th hour of incubation (Fig. 3).

Lead

Growth behavior appeared to be abrupt. At 20ºC, *Pseudomonas* sp. did not show significant growth between 24 to 48 hours with a slight proliferation onward. Maximum growth of *Pseudomonas* sp. was occurred in 96and 72 hours at 25ºC and 37ºC respectively. At 45ºC, growth of *Pseudomonas* sp. was increased from 24 to 96 hours gradually. At 50ºC, a steady increase in bacterial growth was observed from 24 to 96 hours with maximum value in first 24 hours.

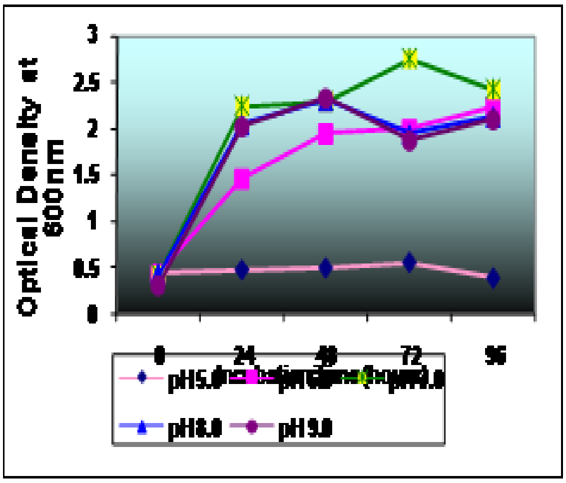

Fig. 2. Effect of various pH values on growth of *Pseudomonas* sp. in the presence of Lead at 37ºC

In present study, 37ºC was considered as optimum growth temperature because at this temperature growth of bacteria was highest (Fig. 4). Detail of results in comparison with control is given in table 3.

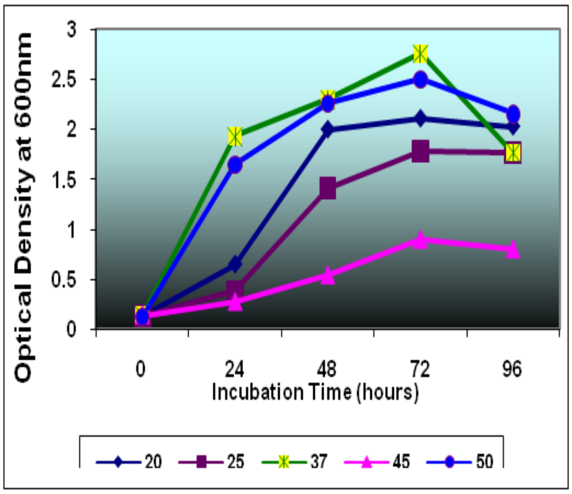

Fig. 3. Effect of various temperatures on growth of*Pseudomonas* sp.in presence of (Cr^{+2}) at pH 6.0.

Comparison of Bacterial Growth for Chromium (Cr^{+2}) and Lead (Pb^{+2}) at Various pH Values in 72th Hour of Incubation

Bacteria showed better growth at low pH in Chromium containing medium as compared to Lead medium. Optimum pH for growth of bacterial isolate was found to be 6.0 in of presence of Chromium enriched medium while it was 7.0 for Lead as shown in Fig.5. In basic medium (8.0-9.0) *Pseudomonas* sp. showed continuous decrease in growth in presence of both heavy metals (Fig.6).

Comparison of Bacterial Growth for Chromium (Cr^{+2}) and Lead (Pb^{+2}) at Various Temperatures in 72th Hour of Incubation

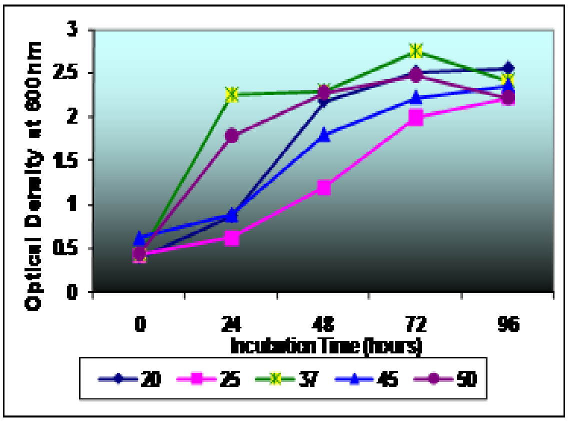

Fig. 4. Effect of various temperatures on growth of *Pseudomonas* sp.in presence of (Pb^{+2}) at pH7.0.

In present investigation, 50ºC and 37ºC was found only favorable temperatures for growth of *Pseudomonas* sp. in presence of Chromium and Lead, respectively.

Discussion

Capacity of living cells to remove metal ions from aqueous solutions is significantly influenced by environmental conditions, such as temperature, pH and biomass concentrations (Chen and Ting 1995). In present research growth of resistant genus *Pseudomonas* sp. was influenced by different environmental conditions i.e. incubation temperature, pH value, and incubation period were studied. Savvaidis*etal.*, (2003); Mortazavi*et al.*, (2005) and Raja *et al.*, (2006) also conducted the same type of studies.

Influence of pH on Growth of Bacteria in Presence of Heavy Metals

Current study revealed that growth of *Pseudomonas spp* in presence of chromium (Cr^{+2}) and lead (Pb^{+2})enhanced with increase in pH of growth medium. Optimum pH for growth of *Pseudomonas* sp. in presence of lead (Pb^{+2}) was 7.0. Same results have also been obtained in couples of other studies by Moghannem*et al*., 2015; Batool*et al*., 2014; Raja *et al*., 2006; AbouZeid*et al.,* 2009 and Shetty *etal*., 2009.]. Results for optimum pH for Chromium metal removal by *Pseudomonas* sp. are in the agreement with Hussein *etal*., (2004).

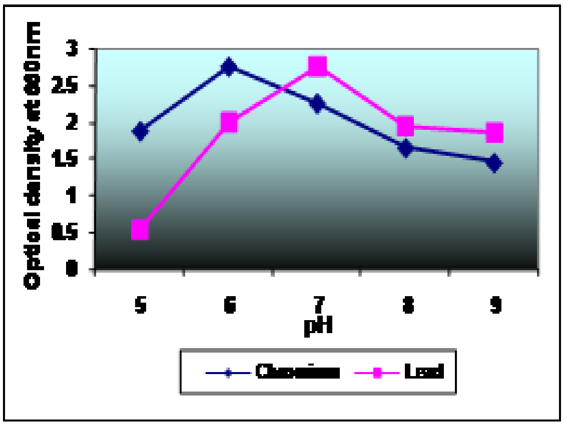

Fig. 5. Comparison between chromium (Cr^{+2}) and lead (Pb^{+2}) for growth of *Pseudomonas* sp.at different pH.

Mortazavi*etal*., (2005) reported that greatest metal removal (Hg^{+2}) by *Pseudomonas* sp. occurred at pH 8.0. AbouZeid*etal*., (2009)reported that pH 8.0 acted as a suppressive level for Pb^{+2} and Cd^{+2} uptakes. Similar finding has been noticed in present study for Lead as well as Chromium at pH 8.0-9.0 and 7.0-9.0, respectively. These results suggest (Liu *et al*., 2009) " the adsorption of metals on the biomass surface is controlled by ionic attraction. At low pH values, inactivated cell surface becomes more positively charged, leading to reduce the attraction between metal ions and functional groups at the cell wall. In contrast, when pH value increases, cell surface becomes more negatively charged and process of retention is favored until a maximum value is reached". However, for values of pH higher than the optimum, formation of hydroxylated complexes of the metal will also compete with the active sites and, as a consequence, retention will be decreased again (Gabr*et al*., 2008).

Influence of Temperature on Growth of Bacteria in Presence of Heavy Metals

Bioaccumulation of Chromium (Cr^{+2}) and Lead (Pb^{+2}) by *Pseudomonas* sp. appears to be temperature dependent too. Temperature of the adsorption medium could be important for energy dependent mechanisms in metal removal by microorganisms. Temperature is known to affect stability of the cell wall, its configuration and can also cause ionization of chemical moieties (Gulay*et al.* 2003). We found that*Pseudomonas* sp. growth decreased in the presence of Chromium at 20°C, 25°C and 45°C but increased at higher temperature thus optimum temperature for growth of bacteria for Cr^{+2} was 50°C. Prasath*etal*., (2010) also reported that maximum metal uptake by *Pseudomonas* sp. was obtained at 50°C. In contrast, according to AbouZeid*etal*., (2009)] suitable temperature for metal uptake by *Pseudomonas* sp. was 30°C. These observations could be explained by the fact that higher temperatures to certain extend lead to acceleration in metabolic activity and energy of the system, which could promote active uptake or attachment of metal to cell surface respectively(Prescott *et al.* 2002).

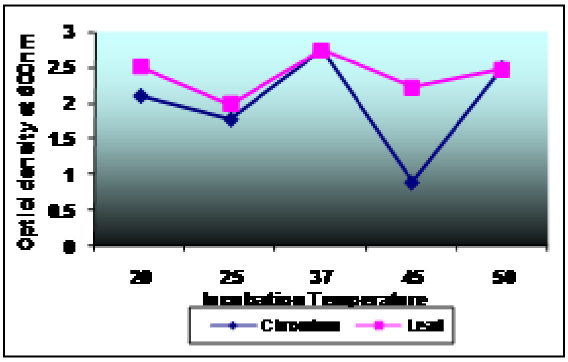

Fig. 6. Comparison between chromium (Cr^{+2}) and lead (Pb^{+2}) for growth of *Pseudomonas* sp.at different incubation temperatures.

Batool*et al.,* (2014); Raja *et al*., (2006) and Bafubiandi*etal*., (2009) reported outcomes similar to ours regarding optimum temperature for growth of bacterial isolate in presence of Pb^{+2}. In this study highest growth of *Pseudomonas sp.* in presence of Lead and Chromium occurred at 37°C and 50°C, respectively so it can be concluded that *Pseudomonas* sp. behave as mesophilic when lead is present in environment while thermophile in presence of Chromium.

In present investigation it was revealed that maximum bacterial growths took place within 72 hours of continuous incubation for both metals (Cr^{+2} and Pb^{+2}) at the most of studied pH and temperature values. [36] also reported same results while [29] found that maximum Pb^{+2} uptake by*Chryseomonasluteola*occurred after incubation period of 2 days, whereas Cd^{+2} uptake by *Pseudomonasmendocina*occurred during 3 days of incubation.

Conclusion

Heavy metal pollution is produced in connection with industrial processes like tanneries. It is suggested that bioremediation could be a good option for cleanup. We confidently propose that investigated bacterial strain can adjust well to Cr (II) and Pb stress. This isolate may play a role in bioremediation processes of heavy metal in polluted areas.

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