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Efficacy of Indigenous *Bacillus* Species in the Removal of Chromium from Industrial Effluent

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Abstract: This study was designed to ascertain the effectiveness of isolated indigenous *Bacillus* species to remove chromium from industrially polluted effluents, through a series of effluent biotreatment regimen. Using microbiological techniques, 6 *Bacillus* strains were isolated, characterized and labeled QIP 1-6. All strains displayed maximum growth on media with a Cr concentration of 400 ppm. Strains QIP 1 and 5 showed maximum resistance to chromium showing moderate growth at 900 ppm of Cr. Following optimization, strain QIP 1 removed 46.81% of the Cr^{6+} and QIP 5 removed 42.50% of the chromium at the same concentration over the same incubation period, as determined through atomic absorption spectroscopy. Following different biotreatment regimens, biotreatment C1-filtered effluent inoculated with strain QIP 1, incubated under optimum conditions, showed maximum Cr^{6+} removal (10.29%) after 48 h. Interestingly, the second highest removal of Cr^{6+} (8.94%) occurred in the untreated, unfiltered raw effluent (D) after incubation for 48 h. Significant ($p<0.05$) strong positive correlation patterns emerged between these two regimen, as well as between regimen B 1-filtered effluent inoculated with strain QIP 1, but incubated under un-optimized conditions-and regimen D. The study revealed that intrinsic bioremediation does occur naturally and that it is quite possible for a consortium of *Bacillus species* to work more effectively at removing the HM from the contaminated sites, than a single isolate, thereby reducing the labor intensive work involved.

Key words: Chromium, removal, effluents, *Bacillus* species

INTRODUCTION

Environmental degradation is a global phenomenon, but is significantly more deleterious in the developing countries, which house the largest populations of human inhabitants, within considerably smaller areas. With such a large populace comes the demand for development, especially in the agricultural sector, the need for economic growth and industrialization, often at the expense of the environment. Over the years, with the active spread and development of the industries, heavy metals, which are either used, or produced, as by-products, by numerous manufacturing, industrial, refining and mining processes, have become ubiquitous, persistent environmental pollutants. In the US, alone, over half of these industrial zones are contaminated with at least one heavy metal, which then leach into the drinking water reservoirs and freshwater habitats, altering the macro, as well as, microbiological communities (Teitzel and Parsek, 2003).

Over the years, with the active spread and development of leather tanneries and textile mills, in Pakistan, heavy metals, produced as by-products by these industries, have become persistent environmental pollutants of the waters and soils of Pakistan. Heavy Metal (HM) contaminated land is becoming an environmental, health and economic issue, as a combination of poorly planned effluent disposal techniques and a rapidly growing population has lead to the gradual accumulation of HM in the waters and soils of Pakistan. Chromium is one particular toxic heavy metals, predominantly used in dyes by tanneries and textile mills, which is the main contaminant in the effluents produced

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by these industries. The EPA in Pakistan has reported that Cr levels in effluents and soils near tanneries and textile mills greatly exceed the safety limit levels standardized by the National Environmental Quality Standards-NEQS (Khan, 2001).

The problem does not remain there, however, as numerous studies have also shown that HM present in soils and waters, gradually accumulate within that system and eventually enter the food chain as they are taken up first by the plants (aquatic and terrestrial), fish and aquatic fowls, birds and other animals that feed on the fish, eventually entering the human food chain (Yilmaz, 2003). As such, it has become imperative that HM, particularly Cr^{6+}, be removed from the environment as quickly and efficiently as possible by employing technologies that do not cause additional, or subsequent problems. Researchers and policy makers alike have now realized that bioremediation-which uses microbes and microbial metabolism-provides a safer, more efficient and less expensive alternative to the physicochemical methods for pollution removal (Khan *et al*., 1997, 2000; Khan, 2001; Franzmann *et al*., 2002; Pandey and Jain, 2002).

This study was designed to ascertain the role of indigenous microbes in removing Cr^{6+}, under laboratory conditions in shake-flask incubators, from media and contaminated wastewater effluents by intrinsic bioremediation. In addition, this study will determine the efficacy of a consortium of bacteria working together, compared to single isolates, in the removal of chromium.

MATERIALS AND METHODS

Bacterial species from the samples were isolated by the serial dilution method. Single colonies were picked and streaked on nutrient agar plates under sterile conditions. Pure cultures were grown on slants by stab and streak method for storage purposes and subsequently for identification and biochemical characterization. The isolated bacterial strains were labeled QIP 1, QIP 2, QIP 3, QIP 4, QIP 5 and QIP 6.

For further identification, characterization and confirmation biochemical tests were employed in accordance to the Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994). These included: Catalase test, Triple Sugar Iron (TSI) Agar Test, Nitrate Reduction Test, Indole Test and Citrate Utilization Test.

Using the method proposed by McLean and Beveridge (2001), Vogel-Bonner (VB) broth was used as the minimal salt media along with glucose as the sole carbon source and K_2CrO_4 salt solution as the HM source. Media with different salt concentrations of 25 50, 75, 100, 200, 300, 400 and 500 ppm in 98 mL distilled water, 2 mL VB and 2 mL 25% (w/v) D-glucose, were prepared under sterile conditions. Controls were also used. Heavy metal analysis was carried out using the Atomic Absorption Spectrometer following digestion of soil and effluent samples (Clesceri *et al*., 1989).

A series of experiments were then carried out to determine the optimum conditions for Cr^{6+} removal, in terms of pH, temperature and glucose concentrations. A comparison of the Cr^{6+} removal from the media, between the most effective strains (QIP 1 and QIP 5) was determined, under the optimized conditions in laboratory conditions.

For the comparative assessment of Cr^{6+} removal, directly from the effluent under laboratory conditions, by the selected bacterial isolates, four sets of biotreatment experiments were designed and conducted. The experiments followed the same regimen, with variations in how the effluent and isolates were treated.

The biotreatment regimens used were as follows:

- Effluent used in this experiment was filtered through 0.2 μm and was not inoculated by the strains (A).
- Filtered effluent was used and was inoculated by the selected strains. Conditions used, however, were not optimized (B1 and B5 i.e., two flasks were prepared and each was inoculated with one of the selected strains).
- Optimized conditions were employed in this experiment of the filtered effluent inoculated with the strains (C1 and C5).
- The effluent was not filtered and was not inoculated by the selected strains (D).

Note: Sample filtration was achieved by filtering the effluent through a 0.2 μm filter paper under aseptic conditions, thereby filtering out all microorganisms.

RESULTS

Bacterial strains were isolated from soil contaminated by industrial effluent of tannery wastewater. Morphological studies of the isolates revealed that all the 6 bacterial strains had identical colonies and the bacteria were determined, through microscopy, to be Gram-positive, spore forming rods. Biochemical confirmatory tests showed that all 6 bacterial strains belonged to the *Bacillus* species.

A series of experiments were designed to determine the tolerance/resistance levels of the *Bacillus* strains against chromium and to ascertain if indeed these strains remove Cr^{6+} ions from the minimal salt media. All six strains, i.e., QIP 1-QIP 6 were cultured on minimal media with varying metal salt concentrations and observed for

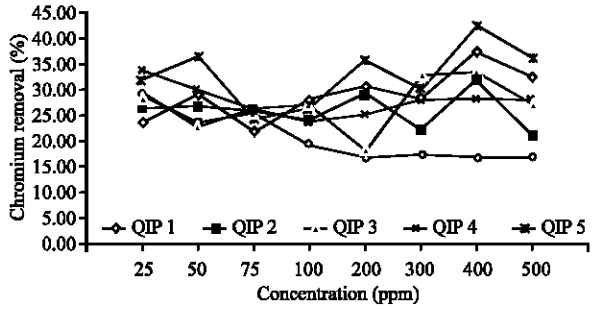

Fig. 1: Comparison of chromium removal (%) by the bacterial isolates at various salt concentrations

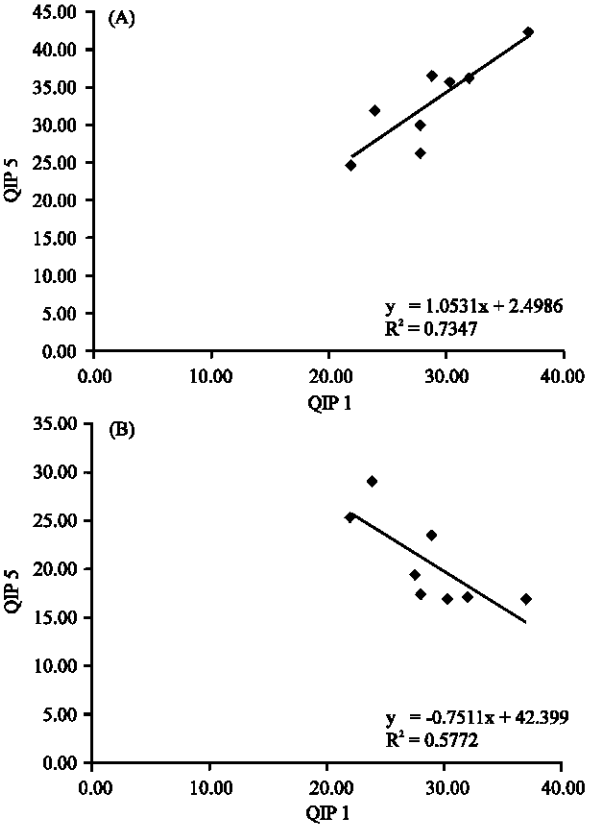

Fig. 2: Significant correlation ($p<0.05$) of Chromium removal by *Bacillus* strains QIP 1, 5 and 6, showing positive correlation between strains QIP 1 and QIP 5 (A) and negative correlation between QIP 1 and QIP 6 (B) at various salt concentrations

six days. *Bacillus* strains QIP 1 and 5 showed maximum resistance to chromium, displaying moderate growth at Cr concentrations as high as 900 ppm and with good growth at 700-800 ppm. All strains displayed maximum growth on media with a Cr concentration of 400 ppm (Fig. 1).

Strains QIP 1 and QIP 5 removed the greatest amount of Cr^{6+} from the media containing 400 ppm metal salt concentration over the stipulated incubation period.

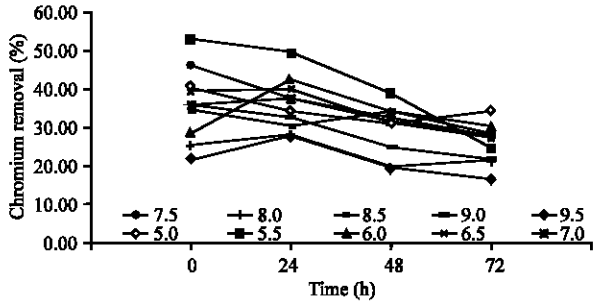

Fig. 3: Effect of pH on chromium reduction (%) by *Bacillus* sp. QIP 1

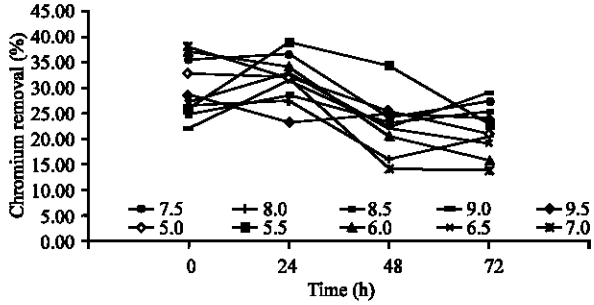

Fig. 4: Effect of pH on chromium reduction (%) by *Bacillus* sp. QIP 5

Strain QIP 1 removed 37.2% of the Cr^{6+}, whereas QIP 5 removed 44.12% of the chromium at the same concentration over the same period of time (Fig. 1).

Correlation analysis between the strains of the *Bacillus* sp. and the removal of Cr^{6+} ions from the media, at various salt concentrations, revealed a significant ($p<0.05$) positive correlation between strains QIP 1 and 5 (Fig. 2). This meant that with the increase in Cr^{6+} ions by strain QIP 1, there was a parallel increase in the metal ion removal by strain QIP 5, thereby complimenting each other action in terms of Cr^{6+} removal. However, when comparing strain QIP 1 with QIP 6, a significant ($p<0.05$) negative correlation was revealed.

On the basis of these experiments, the two strains i.e., QIP 1 and QIP 5 were selected for further testing and optimization of conditions for Cr^{6+} removal.

The optimization parameters used were, pH, temperature and glucose concentration against Cr^{6+} removal using the two *Bacillus* strains QIP 1 and QIP 5. Through these series of experiments it was found that for the optimum removal of Cr^{6+} ions an acidic environment is required. Evident from this study was that *Bacillus* strain QIP 1 and QIP 5 removed the highest percentage of Cr^{6+} ions from the medium at pH 5.5, with QIP 1 removing 53.01% almost immediately and QIP 5 removing 38.85% within 24 h. This suggested that as Cr^{6+} ion removal increased at the specified pH level, in this case at pH 5.5 (Fig. 3 and 4).

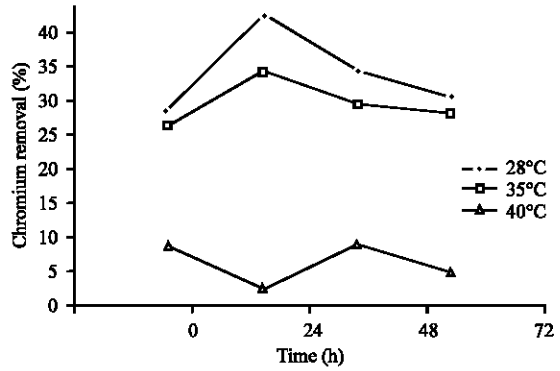

Fig. 5: Effect of temperature (°C) on chromium reduction (%) by *Bacillus* sp. QIP 1

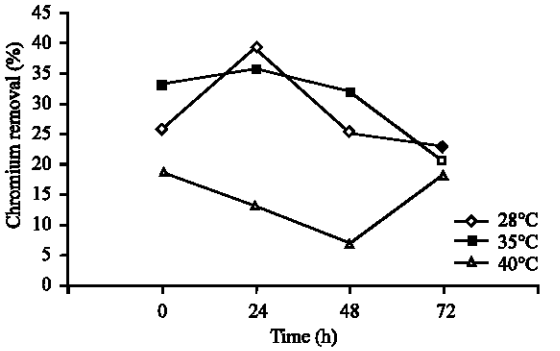

Fig. 6: Effect of temperature (°C) on chromium reduction (%) by *Bacillus* sp. QIP 5

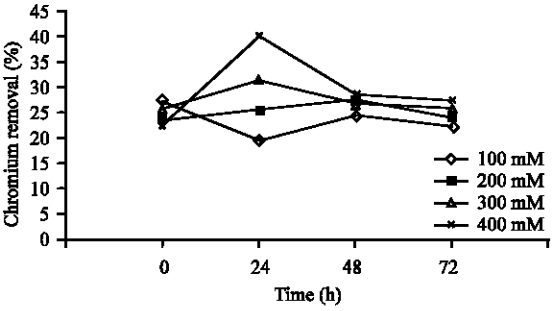

Fig. 7: Effect of glucose concentration (mM) on chromium reduction (%) by *Bacillus* sp. QIP 1

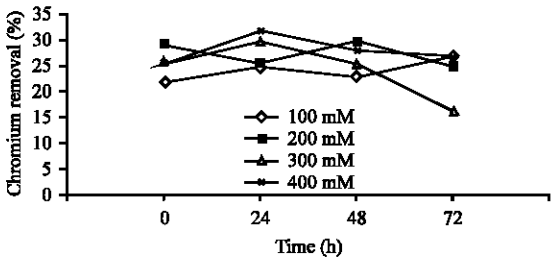

Fig. 8: Effect of glucose concentration (mM) on chromium reduction (%) by *Bacillus* sp. QIP 5

The selected bacilli strains were cultured at temperatures of 28, 35 and 40°C to determine the best temperature for maximum Cr^{6+}removal. Both the strains showed optimum Cr^{6+} removal at 28°C, with QIP 1 removing 42.6% within 24 h and QIP removing 38.85% at the same time interval (Fig. 5 and 6).

To determine the best glucose concentration in which these two selected Bacillus strains (QIP 1 and 5) were cultured on NA with varying glucose concentrations, i.e., 100, 200, 300 and 400 mM. The results of this experiment showed that strain QIP 1 best removed Cr^{6+} ions (40.10%), 24 h after being incubated in media with a glucose concentration of 400 mM. Strain QIP 5 was found to remove 32% of the Cr^{6+} ions, within 24 h also at 400 mM of glucose and 30% after 48 h at 200 mM of glucose (Fig. 7 and 8). This possibly meant that increased Cr^{6+} ion removal at higher concentrations did not necessarily translate to enhanced removal of Cr^{6+} ions at lower concentrations of glucose and vice-versa. However, at higher concentrations, where the glucose levels were less variant, in other words not greatly different, QIP 1 was equally effective in removing Cr^{6+} ions.

Following the determination of the optimum conditions, both these *Bacillus* strains (QIP 1 and QIP 5) were further tested for their ability to adsorb Cr^{6+} ions in the identified "optimized" laboratory conditions. This was achieved by growing strains QIP 1 and 5 in a medium with 400 mM of glucose concentration, Cr^{6+} ion concentration of 400 ppm, at 28°C in pH 5.5, incubated for 120 min. Samples, that were taken from the shake-flask incubator at 10 min intervals, showed that strain QIP 1 adsorbed 46.81% of the Cr^{6+} ions in 60 min, whereas QIP 5 removed 42.5% of the Cr^{6+} ions over the same incubation period. Correlation analysis comparing the efficiency of the two strains (QIP 1 and 5) in removing Cr^{6+} ions revealed a significant ($p<0.05$) positive correlation between the two strains for Cr^{6+} ion removal with time (Fig. 9).

These findings further strengthen the argument that these two strains were the best at removing Cr^{6+} ions, as compared to the other strains isolated. Furthermore, the correlation analysis for this particular experiment complemented the findings of the initial analysis of the preliminary experiments.

The findings of the biotreatment regimens used to ascertain Cr^{6+} removal directly from the effluent showed that under optimized were most effective at removing the HM. Under condition C 1, optimized state inoculated with strain QIP 1, this particular *Bacillus* strain removed 10.29% of the Cr^{6+} ions after 48 h, whereas under condition C 5, optimized state inoculated with QIP 5, 9.33% of Cr^{6+} ions were removed almost immediately and another 9.03% removed after 24 h. These readings were

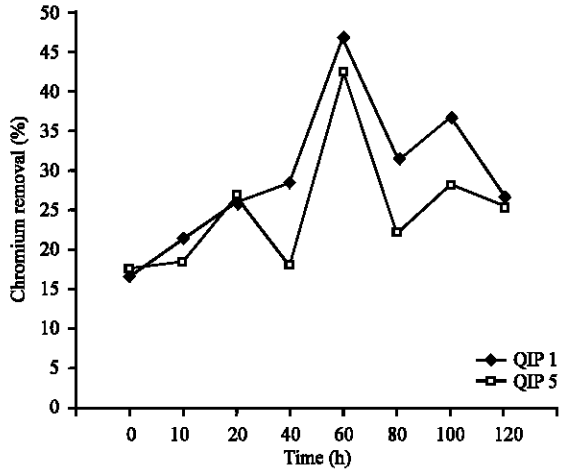

Fig. 9: Chromium adsorption (%) by Bacillus sp. QIP 1 and QIP 5 under optimum conditions

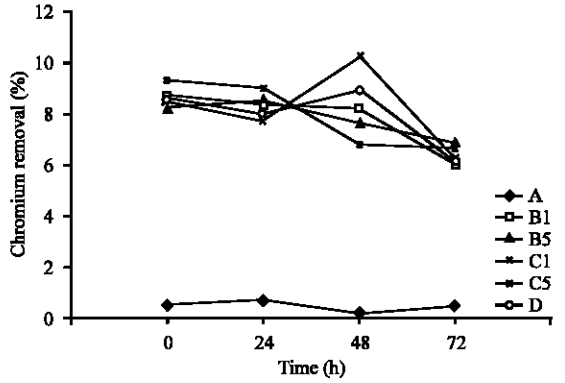

Fig. 10: Chromium reduction (%) patterns following different biotreatment regimes

A Filtered, un-inoculated
B1 Filtered, inoculated with QIP 1; incubated under un-optimized conditions
B5 Filtered, inoculated with QIP 5; incubated under un-optimized conditions
C1 Filtered, inoculated with QIP 1; incubated under optimized conditions
C5 Filtered, inoculated with QIP 5; incubated under optimized conditions
D Unfiltered, un-inoculated

higher than those obtained under un-optimized conditions, as only 8.23% of the Cr^{6+} ions were removed from the media, after 48 h for B 1 (strain QIP 1 under un-optimized conditions) and immediately for B 5 (strain QIP 5 under un-optimized conditions). At the same time, under condition A (filtered and un-inoculated sample) no significant chromium reduction was recorded. However, under condition D, unfiltered and un-inoculated, Cr^{6+} removal was recorded at 8.94% after 48 h (Fig. 10).

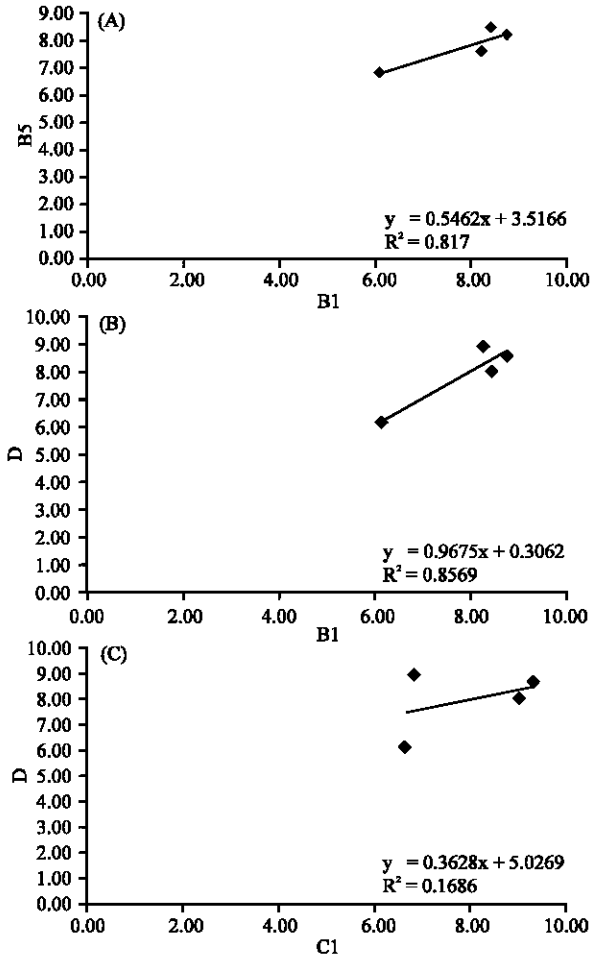

Fig. 11: Significant positive correlation ($p<0.05$) of Chromium removal patterns between specific biotreatment regimen, B1 and B5 (Graph A), B1 and D (Graph B) and C1 and D (Graph C) for the Bacillus strains QIP 1 and 5

B1: Filtered effluent inoculated with QIP 1 strain and incubated under un-optimized conditions
B5: Filtered effluent inoculated with QIP 5 strain and incubated under un-optimized conditions
C1: Filtered effluent inoculated with QIP 1 strain and incubated under optimized conditions
D: Unfiltered and un-inoculated effluent

Under all the conditions, except one (condition A) the net removal of Cr^{6+}, after 72 h of incubation, was between 6.13-6.87%, with very little difference between the optimized and un-optimized conditions.

Statistical analysis of the correlation between the various biotreatment regimes, the strains of the *Bacillus* sp. used and the removal of Cr^{6+} ions also revealed some interesting findings. Significant ($p<0.05$) positive correlation was found between regime B1 and B5, between B1 and D, as well as between C1 and D (Fig. 11).

DISCUSSION

Within all ecosystems, in nature, most everything succumbs to decay, following specific pathways, laws of thermodynamics and, of course, over a specified period of time. Unfortunately, no matter how efficient the natural 'clean-up' system is, the rate of decay is far too slow in comparison to the rate at which humankind is polluting the environment. As a result the 'natural decay' time frame is often too long to cope with the ever-increasing amount of pollutants entering the environment. Furthermore, most of the pollutants are dumped in areas that are not conducive to rapid degradation, thereby leading to a gradual accumulation of contaminants within a specific area. This increasing release of organic and inorganic pollutants by industries and humankind is a cause of concern, both in terms of the preservation of environmental biodiversity, as well as and some may argue more importantly, the many human health-related problems (Shmaefsky, 1999; Pandey and Jain, 2002).

Therefore, researchers developed a feasible method to accelerate the process of decay and removal by encouraging the microbial and associated biota (flora and fauna), within the ecosystem, to degrade, accumulate and/or remove the pollutants from the identified site. This process, aptly known as bioremediation, has been labeled as a cost-effective technology for the treatment of a variety of pollutants and contaminated sites (Atlas and Unterman, 1999). However, like all technologies, there are certain factors that govern the efficacy of bioremediation and these factors are certain limitations of bioremediation (Shmaefsky, 1999).

With the growing demand for food security, intense farming techniques have also been employed and, as a result most of the soils in Pakistan, which were characteristically low in nitrogen, phosphorus and organic matter levels, have now become deficient in micronutrients as well. Like most other developing countries, the levels of inorganic and organic metal contaminants in the soils and waters of Pakistan are also on the rise as a direct result from both agricultural and industrial practices (Khan *et al*., 1997).

Heavy metal contaminated land is becoming an environmental, health and economic issue, in Pakistan as well, as a combination of poorly planned effluent disposal techniques and a rapidly growing population has lead to the gradual accumulation of HM in the waters and soils of Pakistan. The EPA in Pakistan has reported that Cr levels in effluents and soils near tanneries and textile mills greatly exceed the safety limit levels standardized by the NEQS (Khan, 2001).

The presence of chromium in the wastewaters and adjacent soils of textile and tannery mills have been reported by numerous researchers (Anliker *et al*., 1981; Bourghman and Perenich, 1988; Correia *et al*., 1994; Khan, 2001) and in the bioata growing in areas where industrial wastewaters are being dumped, like algae and higher plants, (Srivastava and Prakash, 1991).

With such widespread occurrence of heavy metals and other contaminants in the soils and waters of Pakistan, human exposure to such toxic substances is both unavoidable and prevalent. The health implications are quite serious and devastating, especially for children and pregnant women. Over the years there has been increasing concern amongst researchers, clinicians and civil society groups over the rising levels of heavy metals contaminating the aquatic as well as terrestrial environment. Such toxins tend to persist in the environment, accumulating in the biota, thereby entering the food chain causing deleterious harm to higher animals and humans alike (Zhang *et al*., 1998).

Despite the fact that many may argue on the cost of bioremediation, researchers are in agreement that this process is labor intensive and remediation may take several months before its achieves the desirable effect of lowering the pollutant concentrations to acceptable levels. Furthermore, the long term effects of introducing naturally occurring, yet non-native bioremediation organisms into a specified area are not fully understood and the impact of genetically modified bioremedial organisms is a subject of much debate (Shmaefsky, 1999).

Many studies, including this research work, have shown, under laboratory conditions, the viability of using bioremediation, to remove toxic pollutants from the environment. In addition, short-term studies have also shown that bioremediation does work under various field conditions as well (Pandey and Jain, 2002; Nealson, 2003; Ghazali *et al*., 2004; Narita *et al*., 2004; Rasmussen and Olsen, 2004; Sabaté *et al*., 2004).

This study revealed that indigenous microbes, like *Bacillus* species, through the process of intrinsic bioremediation, can remove toxic HM, like Cr, from the media on which they are grown and can be manipulated in doing so under varying parameters, reflective of the field conditions at the contaminated site. Other researchers have also reported the use of indigenous bacteria, under laboratory conditions, in HM removal, particularly *Bacillus* and *Psuedomonas* species, from contaminated soils and waters (Clausen, 2000; McLean and Beveridge, 2001; Narita *et al*., 2004).

The *Bacillus* species isolated, from soils contaminated by effluents from tannery and textile industries, showed remarkable ability to remove heavy metals, specifically Cr^{6+} ions from media on which they were cultured. The results revealed that through the shake flask transformation reactions, using VB broth as the

minimal salt media, the *Bacillus* strains isolated QIP 1 and QIP 5, removed the maximum amount of Cr, 37.2 and 42.12%, respectively, from the broth containing 400 ppm of the HM in the stipulated time period. Afterwards the reduction ceased and Cr levels in the sample increased. Analysis of the correlation trends between the strains of *Bacillus* used, showed significant ($p<0.05$) positive correlation between strains QIP 1 and 5, whereas a significant negative correlation was seen between strains QIP 1 and 6. Further comparison between strains QIP 1 and QIP 5, under optimized conditions, showed a significantly stronger positive correlation for Cr removal, than under un-optimized conditions, reflected in their R^2 values, 0.76 and 0.73, respectively. Other researchers have also reported Cr removal under the similar conditions and time period, using Bacillus and Pseudomonas strains (Clausen, 2000; McLean *et al*., 2000). Interestingly, all these isolates were also obtained from their respected contaminated sites hence were indigenous to the area.

For the affect of HM salt concentrations on Cr^{6+} by the selected *Bacillus* strains, this study revealed that chromium reduction gradually increased with an increase in HM salt concentrations and peaked at 400 ppm of HM, before decreasing again at 500 ppm. Other researchers have also reported enhanced Cr removal at higher concentrations as compared to at lower concentrations (McLean *et al*. 2000).

The study also revealed another interesting aspect in terms of determining the optimum pH for maximum Cr removal. For both *Bacillus* strains QIP 1 and 5, there was a range of pH values at which maximum Cr was removed. This was dependent on the incubation time. After 24 h, for strain QIP 1, pH 5.5 was the optimum, however, at subsequent time intervals, Cr removal deceased at this pH level and increased at levels 5.5, 6.0 and 6.5 after 72 h. This translated to pH values ranging from 5.0 to 6.5. A similar pattern was revealed for strain QIP 5, however, in this case maximum Cr was recorded at higher pH levels, pH 5.5 and 7.5 after 24 h and at pH 9.0 and 7.5 after 72 h. These results clearly indicate two things, one being that acidic pH is best for chromium reduction, however, the second, more pertinent fact is that optimum pH values may be different for different microorganisms used under different conditions. Researchers have reported pH range from 4 to 7 as being optimal for HM removal rather than pointing to one particular pH value as being the best, while working on HM remedial experiments using different microorganisms (Tsezos, 1990; Brady and Ducan, 1994; Tobin and Roux, 1998).

A comparative study of the variations in Cr^{+6} removal between the two *Bacillus* strains was also studied. The temperature ranges used were 28, 35 and 40°C. The optimum temperature for Cr^{+6} removal was 28°C, which peaked after 24 h and gradually decline over the next 48 h. This meant that the least amount of Cr removal was detected at 40°C, compared to the other two temperatures, even though reduction was observed at all three temperatures.

The findings of this study concur with those reported by other researchers, who worked with different bacterial and fungal strains (Brady and Duncan, 1994; Krauter *et al*., 1996; McLean *et al*., 2000).

For the optimization of glucose concentration to determine which concentration would allow for maximum removal of chromium, the sets of experiments revealed that for strain QIP 1 glucose concentrations of 400 and 300 mM allowed for the highest removal of Cr after 24 and 72 h of incubation, respectively. For *Bacillus* strain QIP 5 a slightly variant result was to be found, in that despite a similar pattern of Cr removal for the first 24 h, maximum removal at glucose concentrations of 400 and 300 mM, yet for glucose concentrations of 400 and 100 mM, maximum Cr removal, from the media, was recorded after 72 h of incubation. Similar findings have been reported by other researchers working with live yeast cells as the bioremedial microorganisms (Krauter *et al*., 1996).

Once the optimal conditions were established, a comparative analysis of the biosorptive ability of both the *Bacillus* strains was ascertained by assessing the amount of chromium they could remove over a measured period of time.

The findings of this study revealed that both the *Bacillus* strains removed Cr quite effectively over the 120 min of incubation, under optimum conditions, in the shake-flask incubator, with the maximum removal occurring after 60 min of incubation. Both QIP 1 and QIP 5 showed maximum chromium at 60 min, with QIP 1 removing 46.81% and QIP 5 removing 42.5% chromium. Correlation analysis also revealed a significant ($p<0.05$) positive correlation between these two strains and a high degree of frequency. These findings are similar to those reported by Badar *et al*. (2001), whose study documented maximum Cu^{2+} and Cr^{6+} removal by *Pseudomonas* and *Bacillus* spp., respectively within 48 h of incubation, with subsequent reduction in HM accumulation after 72 h in both cases.

The most interesting findings, by far, reported in this study, were the results and correlation matrices, observed and computed, for the various biotreatment regimens. The findings of these experiments showed that biotreatment C1 (filtered effluent inoculated with *Bacillus* strain QIP 1 and incubated under optimized conditions) showed maximum Cr^{6+} removal (10.29%) after 48 h of incubation. However, the interesting part of the result was that biotreatment D (unfiltered and un-inoculated), in other words "raw" effluent showed the next best removal rate

(8.94%) after 48 h. This was reflected in the correlation matrices as well, where significant ($p<0.05$) positive correlation between regimens C 1 and D and B 1 and D were computed. The results showed that *Bacillus* strain QIP 5 under C 5 and B 5 conditions removed the maximum amount of Cr from the biotreated effluent, 9.33% immediately under C 5 regimen and 8.50% after 24 h under the B 5 regimen. This translated to the fact that bioremediation was occurring naturally in the polluted soils and effluents and that a consortium of microorganisms were doing an equally good job of removing the HM from the contaminated sites. Of course, they required the necessary time to ensure proper removal, which in all probability was not the case at the contaminated sites.

These findings concur with those reported by other researchers who argue that effective biodegradation may be best carried out by a consortium of microbes (Swenson *et al*., 2000; Franzmann *et al*., 2002).

Hexavalent chromium (Cr VI) is a widely recognized carcinogen associated with various forms of cancer, particularly respiratory tract (Kuo *et al*., 2004) and pancreatic cancers (Alguacil *et al*., 2003). Studies have shown that Cr damages the DNA, causing aberrations, sister chromatid exchanges, gene mutation and cell death and that it's a leading occupational health hazard for workers in the electroplating, tannery and textile industries (Kuo *et al*., 2004). Therefore, with the findings of this study, where a consortium of predominantly *Bacillus* strains have been shown to effectively remove Cr from the environment, it is imperative that such bio-techniques, like intrinsic bioremediation, be promoted and the laboratory-based work be taken forward as *in situ* studies.

Other researchers have also reported on the HM biodegradation properties of a consortium of 6 bacterial strains, predominately of *Bacillus* and *Pseudomonas* spp. (Ghazali *et al*., 2004).

The interpretation of these results quite possibly meant that under un-optimized conditions, *Bacillus* strains QIP 1 and 5 worked equally effectively at removing Cr^{6+} ions. However, more interestingly, the unfiltered effluent, which arguably contained a mixture of microorganisms, worked as effectively as filtered effluent inoculated with strain QIP 1 under optimized conditions.

The findings of this study also lay credence to the argument that a mixture of microorganisms, working together, as found in natural soils and waters, may be more effective at bioremediation, than one specific microorganism working by its lone self. Bioremediation is without doubt an innovative technique that may be regarded as the panacea for the removal of pollutants, like chromium, from the environment to cleanse it of such persistent toxins.

REFERENCES

Alguacil, J., M. Porta, T. Kauppinen, N. Malats, M. Kogevinas and A. Carrato, 2003. Occupational exposure to dyes, metals, ploycyclic aromatic hydrocarbons and other agents and K-ras activation in human exocrine pancreatic cancer. Intl. J. Cancer, 107: 635-641.

Anliker, R., E.A. Clark and P. Moser, 1981. Use of the partition coefficient as an indicator of bioaccumulation tendency of dyestuffs in fish. Chemophere, 10: 263-274.

Atlas, R.M. and R. Unterman, 1999. Bioremediation. In Manual of Industrial Microbiology and Biotechnology ed. Demain, A.L. and J.E. Davies, Washington DC, Am. Soc. Microbiol. Press, pp: 666-681.

Badar, U., R. Abbas and N. Ahmed, 2001. Characterization of copper and chromate resistant bacteria isolated from Karachi tanneries effluents. Ind. Environ. Biotechnol., pp: 43-54.

Bourghman, G.L. and T.A. Perenich, 1988. Fate of dyes in aquatic systems I: Solubility and partitioning of some hydrophobic dyes and related compounds. Environ. Toxicol. Chem., 7: 183-199.

Brady, D. and J.R. Ducan, 1994. Bioaccumulation of heavy metal ions by *Saccharomyces cerevisiae*. Applied Microbiol. Biotechnol., 41: 149-154.

Clausen, C.A., 2000. Isolating metal-tolerant bacteria capable of removing copper, chromium and arsenic from treated wood. Waste Manage. Res., 18: 264-268.

Clesceri, L.S., A.E. Greenberg and R.R. Trussel, 1989. Standard methods for the examination of water and wastewater (17th Edn.). APHA, AWWA, WPCF, Washington. Part, 3000.

Correia, V.M., T. Stephenson and S.J. Judd, 1994. Characterization of textile wastewaters-areview. Environ. Technol., 15: 917-929.

Franzmann, P.D., W.J. Robertson, L.R. Zappia and G.B. Davis, 2002. The role of microbial populations in the containment of aromatic hydrocarbons in the subsurface. Biodegradation, 13: 65-78.

Ghazali, F.M., R.N.Z.A. Rahman, A.B. Salleh and M. Basri, 2004. Biodegradation of hydrocarbons in soil by microbial consortium. Intl. Biodeterioration Biodegradation, 54: 61-67.

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, New York, USA.

Khan, A.G., 2001. Relationships between chromium biomagnifications ratio, accumulation factor and *Mycorrhizae* in plants growing on tannery effluent polluted soils. Environ. Intl., 26: 417-423.

Khan, A.G., A. Bari, T.M. Chaudhry and A.A. Qazilbash, 1997. Phytoremediation-a strategy to decontaminate heavy metal polluted soils and to conserve the biodiversity of Pakistan soils. In: Biodiversity of Pakistan Ed. Mufti, S .A., C.A.Woods and S.A. Hasan, Florida. Pakistan Museum of Natural History, Islamabad and Florida Museum of Natural History, Gainesville, Florida, pp: 133-144.

Khan, A.G., H.D. Fuentes, C.S. Koop, T. Pe and S. Muir, 2000. Phytoremediation of a contaminated mine site using plant growth regulators to increase heavy metal uptake. Waste Treatment and Environmental Impact on the Mining Industry, 1: 427-435.

Krauter, P., R. Martinelli, K. William and S. Martins, 1996. Removal of Cr^{6+} from ground water by *Saccharomyces cervisiae*. Biodegradation, 7: 277-286.

Kuo, H.W., S.F. Chang, K.Y. Wu and F.Y. Wu, 2004. Chromium (VI) induced oxidative damage to DNA: increase of urinary 8-hydroxdeoxyguanosine concentrations (8-OHdG) among electroplating workers. Occup. Environ. Med., 60: 590-594.

McLean, J. and T.J. Beveridge, 2001. Chromate reduction by a Pseudomonad isolated from a site contaminated with chromate copper arsenate. Applied Environ. Microbiol., 67: 1076-1084.

McLean, J.S., T.J. Beveridge and D. Phipps, 2000. Isolation and characterization of a chromium-reducing bacterium from a chromate copper arsenate-contaminated site. Environ. Microbiol., 2: 611-619.

Narita, M., K. Matsui, C.C. Huang, Z. Kawabata and G. Endo, 2004. Dissemination of Tn*MERI1*-like mercury resistance transposons among *Bacillus* isolated from worldwide environmental samples. Microbiol. Eco., 48: 47-55.

Nealson, K.H., 2003. Harnessing microbial appetites for remediation: Demonstration of dioxin dehalogenation by an anaerobic bacterium highlights potential for bioremediation of recalcitrant anthropogenic pollutants. Nature Biotechnol., 21: 243-244.

Pandey, G. and R.K. Jain, 2002. Bacterial chemotaxis toward environmental pollutants: Role of Bioremediation. Applied Envrion. Microbiol., 68: 5789-5795.

Rasmussen, G. and R.A. Olsen, 2004. Sorption and biological removal of creosote-contaminants from groundwater in soil/sand vegetated with orchard grass (*Dactylic glomerata*). Adv. Environ. Res., 8: 313-327.

Sabaté, J., M. Viñas and A.M. Solanas, 2004. Laboratory-scale bioremediation experiments on hydrocarbon-contaminated soils. Intl. Biodeterioration and Biodegradation, 54: 19-25.

Shmaefsky, B.R., 1999. Bioremediation: Panacea or fad? In Access Excellence @ The National Health Musuem. http://www.accessexecellence.org/TSN/ST/st3bg.html

Srivastava, P.N. and A. Prakash, 1991. Bioaccumulation of heavy metals by algae and wheat plants fed by textile effluents. J. Ind. Pol. Control Fed., 7: 25-30.

Swenson, W., J. Arendt and D.S. Wilson, 2000. Artificial selection of microbial ecosystems for 3-chloroaniline biodegradation. Environ. Microbiol., 2: 564-571.

Teitzel, G.M. and M.R. Parsek, 2003. Heavy metal resistance of biofilm and planktonic *Pseudomonas aeruginosa*. Applied Environ. Microbiol., 69: 2313-2320.

Tobin, J.M. and J.C. Roux, 1998. Mucor biosorbent for chromium removal from tanning effluent. Water Res., 32: 1407-1416.

Tsezos, M., 1990. Adsorption by Microbial Biomass as a Process for Removal of Ions from Process, or Waste Solutions. In: Immobilization of ions by biosorption. Eccles, H. and S. Hunt, (Eds.), Chichester. Ellis Harwood, England, pp: 201-218.

Yilmaz, A.B., 2003. Levels of heavy metals (Fe, Cu, Ni, Cr, Pb and Zn) in tissue of *Mugil cephalus* and *Trachurus mediterraneus* from Iskenderun Bay, Turkey. Environ. Res., 92: 277-281.

Zhang, L., L. Zhao, Y. Yu and C. Chen, 1998. Removal of lead from aqueous solution by non-living *Rhizopus nigricans*. Water Res., 32: 1437-1444.