The problem of environmental pollution on account of essential industrial growth in practical terms is the problem of disposal of industrial water, whether solid, liquid or gas. All three types of wastes have the potentiality of ultimately polluting water. Polluted water, in addition to other effects, directly affects soil not only in industrial areas but also in agricultural fields, as well as the beds of rivers, creating secondary sources of pollution. In addition to providing large quantities of water, some effluents also contain considerable amount of essential nutrients which may prove beneficial for plants. (Nagajyothi *et al*., 2008)

Industries are the major sources of pollution in all environments. Based on the type of industry, various levels and quantity of pollutants can be discharged into the environment directly or indirectly (Alao *et al*., 2010). However, the quantity of waste discharge from industries depends on the activities and usage of water.

Heavy metal pollution is a global issue, although severity and levels of pollution differs from place to place. At least 20 metals are classified as toxic with half of them emitted into the environment in concentrations that pose great risks to human health. The common heavy metals that have been identified in polluted water includes arsenic, copper, cadmium, lead, chromium, nickel, mercury and zinc. The release of these metals without proper treatment poses a significant threat to public health because of their persistence, biomagnification and accumulation in food chain. Severe effects include reduced growth and development, cancer, organ damage, nervous system damage, and in extreme cases, death. Exposure to some metals, such as mercury and lead, may also cause development of autoimmunity in which a person's immune system attacks its own cells. This can lead to joint diseases such as rheumatoid arthritis, the kidneys, circulatory system, and nervous system (Rajendran *et al*., 2003; Johnson and Hallberg, 2005; Oelofse *et al*., 2007).
Water pollution is a serious problem globally, involving the discharge of dissolved or suspended substances into ground water, streams, rivers and oceans (Alao et al., 2010). In developing countries of the world, water pollution occurs due to improper management of vast amount of wastes generated by various anthropogenic activities (Alao et al., 2010, Ekhaise et al., 2005). More challenging is the unsafe disposal of these wastes into fresh water reservoirs used primarily for drinking and other household activities (Alao et al., 2010, Kanu et al., 2011).

The review of literature pertaining to the study, "Bioremediation Strategies for Tannery Effluent Treatment" has been presented under the following headings.

2.1 Environmental Pollution and Public Health
2.2 Water Pollution and Toxic Heavy Metals
2.3 Impact of Industrialization on the Biosphere
2.4 Leather Tanning Industry
2.5 Metals let out during the Process of Tanning
2.6 Toxicokinetics of metals
2.7 Phytoremediation and Bioremediation (a promising clean up technology)
   2.7.1 Phytoremedial Plant Brassica juncea
   2.7.2 Bioremediation and Microbial Degradation of Metals
   2.7.3 Basis of Bioremediation
   2.7.4 Factors Required for Bioremediation
   2.7.5 Microbial Populations and Bioremediation Processes
   2.7.6 16s rRNA Sequencing
2.8 Organization of soil
   2.8.1 Soil Bioremediation Strategies
   2.8.2 Metals and Microbes
   2.8.3 Chemical Transformation of Chromium in Soil
2.9 **Interaction Between Chromium and Bacteria**

2.9.1 Bioremediation of Chromium Contaminated Soils

2.9.2 Bacteria in Metal Degradation

2.9.3 Heavy Metal Toxicity Mechanism to Microbes

2.9.4 Plasmid Mediated Metal Resistance

2.9.5 Chromate Reductase

### 2.1 Environmental Pollution and Public Health

Environment is broadly defined as the total planetary inheritance of all resources. These days due to development, forest and grasslands are converted into farms, houses and commercial spaces, raw materials are extracted for energy and commerce, waterways are dammed and diverted, degradation of environment by the release of substances that cause a health hazard or contaminate the soil, water or air is said to be polluting the environment (Yassi and Kjellstrom, 2004).

Environmental protection and rational use of natural resources and other industrial raw materials has become an important sphere of mankind’s advancement in the 20th century. Mankind’s demand for resources and raw materials has intensified the ecological and economic contradictions in the industries (Sen and Chakraborty, 2009).

Environmental pollution is becoming the global problem, over a last few decades a large scale usage of chemicals in various human activities has grown very fast, particularly in countries like India that has to go for rapid industrialization in areas like health and sanitation (Naik et al., 2007).

Environmental degradation is a global phenomenon but is significantly more deleterious in the developing countries, which house the largest population of human inhabitants, within considerably smaller areas. With such a large population comes the demand for development, especially in the agriculture 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 (Qazilbash et al., 2006).

Pollution in general can be seen as something harmful to the wellbeing of the particular environment. Pollution prevention is an aspect of sustainable development as it saves money, reduces liability and promotes working environment. Pollution is the waste which is disposed in the air, water and a land (Sundar, 2010).

The development of industries and services, intensification of agriculture, the enlargement of urbanized area, the development of information technology and the huge increase of transports has favoured human needs. On the other hand, this development has also caused a considerable increase of pollution with consequent damage to the ecosystem (Giordani et al., 2005).

Heavy metals pose a persistent problem at many contaminated sites and are being added to soil, water and air in increasing amounts from a variety of sources, including industrial, agriculture and military activities and domestic effluents. As a result, they are now widely dispersed in the environment in a range of different physiochemical forms (Roundhill, 2001, Abou et al., 2008).

The contamination of environment with heavy metals is a serious problem, since industrial activities and sewage sludge applications have largely contributed to a wide spread of these elements in the terrestrial environment. Therefore, over some years the discarding of solid and or liquid waste products containing heavy metals due to industrial processes has received a lot of attention, and legislation for the protection of the environment has become more rigid (Chen and Hao, 2007).

Actuality of ecological problems is emphasized by mankind’s growing concern for the damage caused to the environment. The main aspect of this concern is linked with the preservation of living being on our planet. The technological and economic aspects deal with the utilization of natural resources and raw materials and their sound exploitation. Advanced technologies are
essential for an integrated scheme for utilization of these resources (Shukla et al., 2010).

2.2 Water Pollution and Toxic Heavy Metals

Pollution of water resources, both surface and underground by indiscriminate discharge of spent wastes of chromium-based industries has become a serious global concern, for it has created an acute scarcity of safe drinking water in many countries (Chandra and Kulsheshtha, 2004).

Ground water is the prime source of drinking water in urban and rural areas of our country. The quality of drinking water in Indian cities has been deteriorated in recent years mainly due to the growth of population and improper disposal of wastewater from industries (Kumar et al., 2004).

Most of the hazards coming to human and ecosystem are mostly due to water pollution. The untreated sewage, industrial effluents and agriculture wastes are often discharged into water bodies. This contaminated water spread wide range of water borne diseases. The agricultural fields around these water bodies are affected (Bajpai et al., 2002).

Natural water in contact with foreign matters during industrial process or domestic use becomes polluted. The effluent generated from industries like tanneries, distilleries, dairies, oil refineries, sugar industries are rich in organic contents (Leghouichi et al., 2008). The effluents with high organic matters are biodegradable and are usually accompanied by very high biochemical oxygen demand values causing environmental pollution (Trivedi, 2004).

Contamination of water supplies and soil by chromium has become a significant problem worldwide. The extensive distribution of this pollutant is due to its use in industrial processes such as leather tanning and metal plating (Morales et al., 2009).

Over the last few decades environmental contamination with heavy metals has increased drastically. Heavy metals found in wastewaters are harmful to the environment and their effect on biological system is very severe. An efficient and
cheap treatment for their removal and reuse of spent metals from wastewater need to be developed. The removal of toxic metals from the environment by microorganisms is an effective means of remediating heavy metal wastes. Microbe based technologies can provide an alternative to conventional methods for metal removal. Chromium is generated from different industries. It occurs in different oxidation states but Cr (III) and Cr (VI) are the most significant. Trivalent chromium occurs naturally in the environment and is an essential nutrient to animals. Hexavalent chromium is a well-known human carcinogen. It can also cause skin ulcer, convulsions, kidney and liver damage. To avoid such toxic effects with Cr (VI), it is necessary to convert it to Cr (III) (Faisal and Hasnain, 2005).

Heavy metals are one of the major water pollutants present in industrial effluents. Water pollution, resulting from an increased concentration of heavy metals is causing serious ecological problems in many parts of the world (Bansal, 2009).

2.3 Impact of Industrialization on Biosphere

Since the beginning of the industrial evolution, pollution of the biosphere with toxic metals has accelerated dramatically (Swaminathan, 2003). Increasing industrialization and population cause increase in living of standards which results in decrease in quality of water. Due to generation of maximum sewage, it flows in open drainage and some percolate in soil. The main sources of pollution are domestic wastewater. The discharge of waste into open drainage through drains enhances the population level (Tiwari et al., 2009).

Industrialization and urbanization has resulted in an increase of pollutants, most of which are being dumped into aquatic system. Industrial effluents containing heavy metal pose a serious threat to the ecosystem. Heavy metals are toxic even in low concentrations and get accumulated in body tissues (Vishnu et al., 2008).

The impact of leather tanning industry on the environment is an ongoing and increasing problem. Water consumption varies greatly between tanneries,
depending on the process involved, the raw material used and the manufactured products. However a large amount of fresh water is used to treat leather and many potentially dangerous chemicals such as chromium, synthetic tannins, oil, resins, biocides and detergents are released (Nazer et al., 2006; Suresh et al., 2001).

The hexavalent chromium is much more toxic to many plants, animals and bacteria inhabiting aquatic environments. Chromium resistant microorganisms from chromium contaminated soil sediments have been isolated by several investigators. The presence of Cr (VI) in the environment exerts selection pressure on microflora. Most microorganisms are sensitive to Cr (VI) toxicity but some group posses’ resistant mechanism to Cr (VI) and can tolerate high levels (Altaf et al., 2008).

The current pattern of industrial activity alters the natural flow of materials and introduces novel chemicals into the environment. The area is polluted as a result of industrial activity; concentrations of toxic substances often exceed the level normally found in soil, water ways and sediment. When toxic substances accumulate in the environment and in food chain, they can profoundly disrupt biological process (Cristina et al., 2008).

The industrial effluent released directly or indirectly into natural water resources, mostly without proper treatment, poses a major threat to the environment. Among the different forms of chromium, the hexavalent chromium Cr$^{6+}$ is the most toxic and carcinogenic due to its high solubility in water, rapid permeability through biological membranes and subsequent interaction with intracellular protein and nucleic acids. The heavy metals in general cannot be biologically transformed to more or less toxic products and hence persist in the environment indefinitely (Wani et al., 2007).

The strong impact of hexavalent chromium on the environment and on human health demands suitable technologies to neutralize the hazards of chromium. The traditional technologies used for the remediation of Cr (VI) contaminated environments are based on physical and chemical approaches, which require large amount of chemical substances and energy. Such
methodologies have proved expensive on a large scale application at contaminated sites and also they have generated hazardous by products (Gonzalez et al., 2003).

2.4 Leather Tanning Industry

Tanning industry in India is one of the well developed industrial sectors. It contributes significantly towards exports, employment generation and occupies an important place in Indian economy. On the other hand, tannery wastes are ranked as the highest pollutants among all the industrial wastes (Camargo et al., 2004).

Tanning is the chemical process. The semi soluble protein called “collagen” is converted into tough, flexible, insoluble and highly durable leather in a succession of many complex stages, consuming high quantities of water (Verma et al., 2008).

Leather refers to animal skin that has been fully tanned. Tanning is the process that chemically alters animal skins, making it supple, strong and resistant to rotting. The leather and leather products sector now represents one of the most important industrial sectors, significantly contributing to the nation’s economy (http://www.tve.org/ho/doc.cfmaid=899).

Leather tanning is the process of converting raw hides or skins into leather. Hides and skin have the ability to absorb tannic acid and other chemical substances that prevent decaying, make them resistant to wetting. Tanning is essentially the reaction of collagen fibers on hide with tannin, chromium, alum or other chemical agents. The most common tanning agents are chromium and vegetable tannins extracted from specific tree barks.

The tanning process transforms hides and skin to leather. The main raw materials are cow hides and goat skins. The structure of the skin consists of interlaced bundles of micelles and fibrils, the collagen. After removal of flesh and fat, the hides are processed with chemicals, which act on microscopic collagen fibers to form a stable and durable mineral. Depending on the desired type of final product, a wide variety of processing steps are undertaken.
A general outline of leather processing is given in Table 1 (UNIDO, 2000). Figure 1 represents the tannery industries in Tamilnadu, Figure 2 and Plate 1 indicates the different stages of processing in a tannery.

Table 1
A general outline of leather processing

<table>
<thead>
<tr>
<th>Processes</th>
<th>Stages of processing</th>
<th>Chemical use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beam house operations</td>
<td>Curing, washing, wetting and soaking</td>
<td>Sodium chloride/sulphide, antiseptics, protolytic/lipolytic.</td>
</tr>
<tr>
<td></td>
<td>Liming, hair burning/unhairing</td>
<td>Calcium oxide/hydroxid sodium sulphide</td>
</tr>
<tr>
<td></td>
<td>Deliming, batting</td>
<td>Sulphuric / formic / lactic acid, ammonium sulphate / chloride, sodium carbonate / bisulfide, sodaash emulsifier, organic solvents, aldehyde, copolymer</td>
</tr>
<tr>
<td></td>
<td>Pickling</td>
<td>Hydrochloric / sulphuric / organic acid, sodium chloride.</td>
</tr>
<tr>
<td>Tanyard operations</td>
<td>Chrome tanning/vegetable tanning</td>
<td>Sodium chloride, chromium salts, vegetable tans</td>
</tr>
<tr>
<td>Dressing operations</td>
<td>Dressing, coloring</td>
<td>Neutralizing agents, retaining agents, dyes(sulphur, acid dye, metal complex, fat liquoring oils)</td>
</tr>
<tr>
<td>Finishing operations</td>
<td>Drying, buffing and finishing</td>
<td>Liquor, resin, pigment, acrylic or polyurethane polymers, waxes, formalin</td>
</tr>
</tbody>
</table>

Chrome tanning is the most common type of tanning where large amounts of chrome powder and liquors are used. A huge quantity of this chromium is discharged along with the effluent (Zahid et al., 2006).
Figure 1

Concentration of tanneries in Tamilnadu
Figure 2
General flow diagram for leather tanning and finishing process
Plate 1
Process of tanning industry

- Tanning Process
- Deharing of Leather
- Leather Finishing Operation
Tannery effluents are ranked as the highest pollutants among all industrial wastes. They are especially large contributor of chromium pollution. For instance, in India alone about 2000-3000 tons of chromium escapes into the environment annually from tannery industries, with chromium concentrations ranging between 2000 and 5000mg/l in the aqueous effluent compared to the recommended permissible discharge limits of 2mg/l (Akan et al., 2009). One of the major emerging environmental problem in tanning industry is the disposal of chromium contaminated sludge produced as a by product of wastewater treatment (Belay, 2010).

The discharge of effluent from this industrial sector is a matter of concern due to its high complexity which causes serious pollution problems (Calheiros, 2007). Chromium when used in the productive cycle is one of the most problematic pollutants discharged by the tanning industry (Mant et al., 2006).

2.5 Metals let out during the Process of Tanning

Metals with atomic masses between 54.63 and 200.59 and special weights of more than 5 g/cm3 are classified as heavy metals (Dias et al., 2002). These elements are natural components of earths’ crust, which are released into the environment because of natural and human activities. In biology, heavy metals are the atoms with toxic effects (such as Al, As, Br, Cd, Cr, Co, Cu, Cd, Fe, Hg, Ni, Mn, Pb, Se and Zn) (Fomina et al., 2005, Cabuk et al., 2005).

The most commonly occurring metals at these sites are lead, chromium, arsenic, zinc, cadmium, copper and mercury. Presence of these metals in ground water and soils may cause a significant threat to human health and ecological systems (Sundar et al., 2010).

2.5.1 Chromium

Chromium is one of the highly toxic ions released into the environment through leather processing and chrome plating industries. There are number of methods available for the removal of chromium VI from industrial wastewater. (Morales et al., 2009).
Chromium is a transition metal that is discharged into the environment through the disposal of waste from industries like leather tanning, metallurgical and metal finishing, textiles and ceramics, pigments and wood preservatives, photographic sensitizer manufacturing, which leads to the contamination of soil. (Vasudevan and Ravinderan, 2008).

Hexavalent chromium is an important heavy metal widely used in the metallurgies, refractory, chemical and tannery industries. Chrome plating the deposition of metallic Cr, impacts a refractory nature to materials rendering them resistant to microbial attack and flexible over extended period of time. More than 1,70,000 tons of Cr wastes are discharged in to the environment annually as a consequence of industrial and manufacturing activities (Parameswari et al., 2009).

Hexavalent chromium has been recognised as one of the most dangerous environmental pollutants due to its ability to cause mutations, irritation, corrosion of skin and respiratory tract to the most microorganisms. It also causes lung carcinoma in humans. Efficient and cheap treatments for heavy metal removal and reusing of spent metals from wastewater need to be developed, microbe based technologies can provide an alternative to convention methods for metal removal (Pal et al., 2005, Faryal et al., 2007).

Concentration of hexavalent chromium should not exceed 0.05 mg/l in drinking water. In Indian context the discharge concentration of chromium should not exceed from 0.1 mg/l as per wastewater discharge standard of Central Pollution Control Board (CPCB, 2000-2001).

Chromium is a common pollutant introduced into natural waters due to the discharge of a variety of industrial wastewater on the other hand, chromium based catalysts are usually employed in various chemical processes, including selective oxidation of hydrocarbons. Chromium III occurs naturally in the environment and is an essential nutrient Chromium (VI) and Chromium (0) generally are produced by industrial processes. The metal chromium which is the Chromium (0) form is used
Although chromium oxidation states range from −IV to +VI, only the +III and +VI states are stable in the natural environment (Gonul et al., 2005, Namasivayam et al., 2007). The chemical and toxicological behaviours of chromium salts depend on the oxidation state of chromium. Chromium (III) is an essential trace element in human nutrition, required for the maintenance of normal glucose, cholesterol, and fatty acid metabolism. Insufficient dietary intake of chromium (III) leads to increase in risk factors associated with diabetes and cardiovascular disease including elevated circulating insulin, glucose, triglycerides, total cholesterol and impaired immune function. On the other hand, water soluble Cr(VI) is extremely toxic and carcinogenic owing to its ability to oxidize other species and has a significant mobility in the environment because of its high solubility in water and weak sorption onto inorganic surfaces (Latif et al., 2007). Although trivalent chromium is considered an essential nutrient for the human body and the toxicity of trivalent chromium is 500–1000 times less to a living cell than hexavalent chromium, exposure to excessive doses of Cr(III) for long periods of time may also cause some adverse health effects. Removal of Cr(VI) from industrial effluents is not only essential because of its toxicity to humans but also because it affects soil fertility by inhibiting biodegradation of organic pollutants due to its ability to inactivate enzymes and precipitating proteins of soil microbial organisms.

One of the important features that distinguish heavy metals from other pollutants is that the Cr (VI) are not biodegradable. Once metal ions enter the environment, their chemical form largely determines their potential toxicity. The presence of toxic heavy metal contaminants in aqueous streams, arising from the discharge of untreated metal containing effluents into water bodies, must be one of the most important environmental issues that deserve due consideration (Rajender et al., 2008).
2.6 Toxicokinetics of metals

2.6.1 Cadmium

It is found in batteries, cigarette smoke, incineration of tyres/rubber/plastic, vending machines and soft drinks. Their toxicity causes liver and kidney damage to the central nervous system and nerve fibre degeneration (Leonard et al., 2004).

2.6.2 Nickel

It is present in hydrogenated fats and oils, stainless cookware, tea and tobacco smoke. Nickel toxicity causes kidney dysfunction, disruption of hormone and lipid metabolism and intestinal cancer.

Nickel reduces photosynthetic activity of plants. Reduction of photosynthetic activity may result both from the disturbance of photochemical and biochemical photosynthetic reduction, and the damage of photosynthetic apparatus at all levels of organization (Sharma and Agarwal, 2005).

Nickel is the potential carcinogen for lung and may cause allergies, lung fibrosis and cancer of respiratory tract in occupationally exposed populations (Kazprazak et al., 2003).

2.6.3 Lead

It is present in auto exhaust, hair dyes, paints, sindhoor (vermillion), glazing of pottery and enamel ware. Lead toxicity causes co-ordination and concentration loss, decreases I.Q and causes infertility. Memory loss, especially long term memory, cause mood swing and sterility. Heavy metal overload in the environment causes growth of stealth pathogen and also combination of certain metals increases toxicity while other combinations reduce it. For example, lead makes 100 times more toxic, while zinc and magnesium reduces cadmium toxicity. One of the major mechanisms behind heavy metal toxicity has been attributed to oxidative stress (Guidotti at al., 2009).

2.7 Phytoremediation and Bioremediation (a promising clean up technology)

The term “Phytoremediation” is used to describe the cleanup of heavy metals from contaminated sites by plants (Diwan et al., 2010).
Phytoremediation, which makes use of plants to decontaminate pollutants, represents a green and environment friendly tool for cleaning the metal polluted soil and water as opposed to the conventional chemical and physical remediation technologies that are generally too costly and often harmful to soil characteristics. The premise of this method is to find out the hyper accumulator, which has greater ability to accumulate the heavy metals (Salt and Kramer, 2000; Trapp and Karlson, 2001). Table 2 indicates the effects of metals on plants.

Environmental pollution affects the quality of pedosphere, hydrosphere, atmosphere, lithosphere and biosphere. Great efforts have been made in the last two decades to reduce pollution source and remediate the polluted soil and water resources. Phytoremediation is most cost effective and with fewer side effects than physical and chemical approaches which has gained increasing popularity in both academic and practical circles. More than 400 plant species have been identified to have potential for soil and water remediation (Lone et al., 2008).

There are different categories of phytoremediation, including phytoextraction, phytofiltration, phytostabilization, phytovolatization and phytodegradation, depending on the mechanism of remediation. Phytoextraction involves the use of plants to remove contaminants from soil. Phytofiltration involves the plant roots or seedling for the removal of metals from aqueous wastes. In phytostabilization, the plants root absorbs the pollutants from soil and keep them in the rhizosphere. Phytovolatization involves the use of plants to volatilize pollutants such as selenium and mercury from the foliage. Phytodegradation means the use of plants and associated microorganisms to degrade organic pollutants (Garbisu and Alkorta, 2001).

Rhizofiltration is the removal of pollutants from the contaminated waters by accumulation into plant biomass. Several aquatic species have been identified and tested for the phytoremediation of heavy metals from the polluted water (Prasad and Freitas, 2003). The roots of Indian mustard are found to be effective in the removal of cadmium, chromium, lead and zinc (Wang et al., 2002). The use of
plants for extraction of contaminants from the environment or for lowering of the toxicity is defined as phytoremediation (Angelova and Ivanov, 2008).

Bioremediation seems to be a good alternative to conventional clean up technologies. The biochemical removal of heavy metals received attention in recent years because of its potential in environmental protection (Elizabeth et al., 2008).

Bioremediation is an environmental friendly and cost competitive alternative to chemical decomposition processes (Patil et al., 2008).

Figure 3 indicates the process of metal uptake and accumulation in plants (Jing et al., 2007)

**Figure 3**

*Metal transfer in plants*

1: A metal iron is sorbed at root surface;

2: Bioavailable metal moves across cellular membrane into root cells;

3: A fraction of the metal absorbed into root is immobilized in the vacuole;

4: Intracellular mobile metal crosses cellular membranes into root vascular tissue (xylem);

5: Metal is translocated from the root to aerial tissues (stems and leaves)
Figure 4
Plant-soil-microbial interactions in the rhizosphere

Table 2
Main effect of heavy metals in plants

<table>
<thead>
<tr>
<th>Metal</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadmium (Cd)</td>
<td>Decreases seed germination, lipid content, and plant growth; induces phytochelatins production</td>
</tr>
<tr>
<td>Chromium (Cr)</td>
<td>Decreases enzyme activity and plant growth; produces membrane damage, chlorosis and root damage</td>
</tr>
<tr>
<td>Copper (Cu)</td>
<td>Inhibits photosynthesis, plant growth and reproductive process; decreases thylakoid surface area</td>
</tr>
<tr>
<td>Mercury (Hg)</td>
<td>Decreases photosynthetic activity, water uptake and antioxidant enzymes; accumulates phenol and proline</td>
</tr>
<tr>
<td>Nickel (Ni)</td>
<td>Reduces seed germination, dry mass accumulation, protein production, chlorophylls and enzymes; increases free amino acids</td>
</tr>
<tr>
<td>Lead (Pb)</td>
<td>Reduces chlorophyll production and plant growth; increases superoxide dismutase</td>
</tr>
<tr>
<td>Zinc (Zn)</td>
<td>Reduces Nickel toxicity and seed germination; increases plant growth and ATP/chlorophyll ratio</td>
</tr>
</tbody>
</table>

(Source: Gardea-Torresdey et al., 2005)
2.7.1 Phytoremedial plant *Brassica juncea*

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae – Plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subkingdom</td>
<td>Tracheobionta – Vascular plants</td>
</tr>
<tr>
<td>Superdivision</td>
<td>Spermatophyta – Seed plants</td>
</tr>
<tr>
<td>Division</td>
<td>Magnoliophyta – Flowering plants</td>
</tr>
<tr>
<td>Class</td>
<td>Magnoliopsida – Dicotyledons</td>
</tr>
<tr>
<td>Subclass</td>
<td>Dilleniidae</td>
</tr>
<tr>
<td>Order</td>
<td>Capparales</td>
</tr>
<tr>
<td>Family</td>
<td>Brassicaceae – Mustard family</td>
</tr>
<tr>
<td>Genus</td>
<td>Brassica L. – mustard</td>
</tr>
<tr>
<td>Species</td>
<td>Brassica juncea (L.) Czern. – brown mustard</td>
</tr>
</tbody>
</table>

Constituents - Seed contains about 20-25% of oil. An essential oil is also produced by the action of water.

Action – Whole plant possesses bitter, aperients and tonic properties. Oil is stimulant and counter irritant. “A hot mustard bath is an emmenagogue”.

The *Brassicaceae* family are distinguished with their ability to accumulate heavy metals in an extremely high degree (Broadley *et al.*, 2001). That is why significant attention is paid to the members of the *Brassicaceae* family, which are distinguished as plant having significant biomass and capacity to accumulate high quantities of heavy metals (Angelova and Ivanov, 2008). Henry (2000) examined plants with high speed of growth from the *Brassicaceae* family for their ability to tolerate and accumulate metals including *Brassica juncea* L., *Brassica nigra* Koch, *Brassica campestris* L., *Brassica napus* L., and *Brassica oleraceae* L. Despite the fact that all of the examined crops from the *Brassicaceae* family accumulates the metals, *Brassica juncea* showed the highest ability to accumulate and transport copper, chromium, cadmium, nickel, lead and zinc towards their stem. *Brassica juncea* showed a significant potential for induced phyto remediation and was suggested that it could be used with soil contaminated with heavy metals.

2.7.2 Bioremediation and Microbial Degradation of Metals

Bioremediation is one of the most promising new technology for treating industrial wastes (solid and liquid) and municipal/urban wastes (sewage, mining...
wastes including effluent containing heavy metals). It could be used as the insitu remediation technology with indigenous microorganisms or could be used in an ex situ mode either in composite piles or bioreactors. By altering the types of microorganism present, nutrients and climatic conditions (pH, moisture, temperature, oxygen levels) and microbial degradation could be enhanced (Zhang, 2003).

2.7.3 Basis of Bioremediation

Bioremediation is based on the idea that all organisms remove substances from the environment to carry out growth and metabolism. Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer et al., 1993). Bioremediation not only involves the degradation of pollutants but also at times it is sufficient to remove pollutants from the environment without degrading bacteria which particularly take up large amounts of metals and minerals to ensure adequate resources for binary fission (Broda, 1992).

2.7.4 Factors required for Bioremediation

The control and optimization of bioremediation processes is a complex system of many factors (Day, 1992). These factors include:

i) The existence of a microbial population capable of degrading the pollutants.

ii) The availability of contaminants to the microbial population (Vidali, 2002).

2.7.5 Microbial Population and Bioremediation Processes

Microorganisms in general play a significant role in bioremediation of heavy metal contaminated soil and wastewater. So far some of the terrestrial microorganisms (Bacillus, Enterobacter, Escherichia, Pseudomonas, yeasts and fungi) transform Cr (VI) effectively. Seng and Bielefeldt (2002) reported the enzymatic biotransformation of chromium (VI) to Cr (III). Some bacterial isolates are resistant to chromium and could be exploited to bioremediate toxic Cr (VI) contaminated environment. According to Polisak et al. (2009), chromium tolerance
or resistance mechanism of microbes is of particular importance for bioremediation and wastewater treatment technology. Nevertheless, no detailed experimental evidence for the reasons behind the resistance or tolerance is available. Till date (i) plasmid mediated (Nies et al., 1998); (ii) genetically mutated (Miranda et al., 2005); (iii) bioadsorption of the cells (Ozdemir et al., 2004); (iv) bioaccumulation (Preetha and Viruthagiri, 2007) and finally (v) biotransformation (Guha et al., 2001) are the rationale suggested for the tolerance exhibited by the organisms.

It is well known that whenever there is a stress, most of the microorganisms exhibit an explicitly different mechanisms, which provide them high tolerance and resistance. In general, the tolerant species may transform the toxic components to non-toxic via enzymatic or non-enzymatic pathway and finally utilize the transformed components as nutrients or ignored them after complexing with other components. Nevertheless, this kind of transformations could not be expected with all microbes and the contaminants.

Certain enzymes produced by microbes attack hydrocarbon molecules causing degradation. The degradation of oil relies on having sufficient microbes to degrade the oil through the microbes’ metabolic pathways (series of steps by which degradation occurs). Fortunately, nature has evolved many microbes to do this job. Throughout the world there are over 70 genera of microbes that are known to degrade hydrocarbons (Glazer et al., 1995). These microbes usually account for less than 1% of natural population of microbes, but could account for more than 10% of the population in polluted ecosystems. If microbes are not present in a system they can be added to promote bioremediation. However, even when these microbes are present, degradation of hydrocarbons can take place only if all the other basic requirements of the microbes are met. Microorganisms could be isolated from almost any environmental condition. Microbes would adapt and grow at sub zero temperatures, as well as extreme heat, desert conditions, in water with an excess of oxygen and in anaerobic conditions with the presence of hazardous compounds or on any waste stream. The main requirements are the presence of an energy source and a carbon source. These microbes because of
their adaptability and other biological systems could be used to degrade or remediate environmental hazards (Zeyaullah et al., 2009).

Bioremediation is a technology that uses metabolic processes to degrade or transform contaminants, so that they remain no longer in harmful form. In some cases, the contaminant is the primary part of the metabolic process, acting as a main source of carbon and energy for the microbial cell. In others, it is transformed into a second substance, serves as a primary energy or carbon source (Agathos and Reineke, 2002).

Microorganisms require carbon as an energy source to sustain the metabolic functions which includes growth and reproduction. The metabolic processes used by bacteria to produce energy require a terminal electron acceptor (TEA) to enzymatically oxidize the carbon source (organic matter to carbon dioxide).

\[
\text{Organic matter} + \text{oxygen} + \text{biomass} \rightarrow \text{carbon dioxide} + \text{water} + \Delta \text{Hf},
\]

Where \( \Delta \text{Hf} \) is the energy generated by the reaction to fuel other metabolic processes including growth and reproduction. In this example oxygen serves as TEA.

Microbes and electron (energy and carbon source) + electron acceptor \( \rightarrow \) more microbes + oxidized end products (destruction complete).

It is relevant here to note that microbial population, oxygen, soil water, pH, nutrients and temperature are important microbial and environmental elements which affect the bioremediation processes. Microbial and environmental factors which affect soil and underground water bioremediation are microbial population, oxygen, soil water, pH, nutrients and temperature. An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Sung and Cheng, 2008).

Bioreduction of Cr (VI) can occur directly as a result of microbial metabolism (enzymatic) or indirectly through a bacterial metabolite such as \( \text{H}_2\text{S} \).
Two enzymatic mechanisms are reported to reduce Cr (VI) both through metabolic activities of the chromate reducing *Bacillus sp*. ES29 (CRB). In aerobic conditions most of the enzyme chromate reductase activity reported is soluble in the cytosol and reduces Cr(VI) to Cr(III) inside or outside the plasma membrane (Camargo *et al.*. 2003). Under anaerobic conditions, CrO$_4^{2-}$ is used as a terminal electron acceptor and is reduced in membrane during anaerobic respiration (Conceicao *et al.*, 2009).

2.7.6 16S rRNA Sequencing

The rRNA is the most conserved (least variable) gene in all cells. Portions of the rDNA sequence from distantly-related organisms are remarkably similar. This means that sequences from distantly related organisms can be precisely aligned, making the true differences easy to measure. For this reason, genes that encodes the rRNA (rDNA) have been used extensively to determine taxonomy, phylogeny (evolutionary relationships) and to estimate rates of species divergence among bacteria. Thus the comparison of 16s rDNA sequence can show evolutionary relatedness among microorganisms. The 16s rDNA sequence has hypervariable regions, where sequences have diverged over evolutionary time. These are often flanked by strongly-conserved regions. Primers are designed to bind to conserved regions and amplify variable regions. The DNA sequence of the16S rDNA gene has been determined for an extremely large number of species (Newman, 1993).

2.8 Organization of Soil

The structural organization of soil particles provides a spatially heterogenous habitat for microorganisms characterized by different substrates, nutrients, oxygen concentrations and water contents as well as variable pH values (Sarssitsch *et al.*, 2001). Soil bacteria degrade organic matter and promote soil moisture retention and fertility which are important for arid ecosystem productivity and stability (Kuske *et al.*, 2002).

2.8.1 Soil Bioremediation Strategies

Contamination of agricultural soils and pastures with heavy metals could enter into human food chains by polluted agricultural and waste products
utilization. Some of these atoms could be absorbed into human body by respiration (such as plumb) and some by nutrition and both are related to the nature of element. Nowadays, the elimination of heavy metals from urban, industrial and agricultural wastes has been changed into a serious discussion as some of these toxic metals are precedent to others, while harder rules are approved for their elimination (Cabuk et al., 2005; Dias., 2002). Non degradability of heavy metals and their tendency to concentrate in the human body diverse them from other toxic pollutants. Based on WHO reports (2005), many people are exposed to health risks caused by heavy metals in different ways (Faryal and Hameed, 2005; Faryal et al., 2007).

The remediation of Cr (VI) contaminated soil today is essentially based on physical and chemical approaches which include excavation or pumping of contaminated material followed by the addition of reducing chemicals that lead to the precipitation and sedimentation of reduced chromium Cr (III), less toxic than Cr (IV) and greatly insoluble. The ability of several microbial groups (bacteria, fungi, microalgae) to reduce Cr (VI) to Cr (III) has been considered of much interest in order to clean up soil/water polluted with chromate. In fact, there is no doubt that the development of an effective biological system to alleviate the environmental problems associated with hexavalent chromium is highly desirable (Reddy et al., 2003).

Heavy metals such as lead, arsenic, cadmium, copper, zinc, nickel, and mercury are discharged from industrial operations such as smelting, mining, metal forging, manufacturing of alkaline storage batteries, and combustion of fossil fuel. Moreover, the agricultural activities like application of agrochemicals, and long-term usage of sewage sludge in agricultural fields also add a significant amount of metals in the soils Various anthropogenic sources of metal contamination of soils have been shown in Figure 5 (Ahemad, M., 2012).

In contaminated soil with hexavalent chromium, some indigenous bacteria are able to reduce Cr (VI), but the rates of the natural attenuation (that is to say
any human interferance) of Cr toxicity are slow and therefore, unacceptable to device remediation strategies (Tokunaga et al., 2003).

2.8.2 Metals and Microbes

Metals play an integral role in the life processes of microbes. Some metals such as Cr, Ca, Mg, Mn, Cu, Na, Ni and Zn are essential as micronutrients for various metabolic activities and for redox processes. Toxicity of metals occur through the displacement of essential metals from their active binding sites or through ligand interactions (Brunis et al., 2002). Usually the microbes have solved this problem by using two types of uptake systems of metal ions. One is fast, non-specific and driven by chemiosmotic gradient across the cytoplasmic membrane of the bacteria. The second type of uptake system has high substrate specificity, is slower and often uses ATP hydrolysis as the energy source and is only produced by the cell in times of need. Though, there are specific uptake systems, high concentrations of nonessential metals may be transported into the cell by a constitutively expressed non-specific system. This open gate is the one reason why metal ions are toxic to microbes. As a consequence, microbes have been forced to develop metal ion homeostasis factors and metal resistance determinants (Nies, 1999).

The mechanisms by which ions bind to the cell surface include electrostatic interactions, Van der Waals forces, covalent bonding, redox interactions and extracellular precipitation or a combination of these processes. The negatively charged groups (carboxyl, hydroxyl and phosphoryl) of the bacterial cell wall adsorb metal cations which are then retained by mineral nucleation (Murthy et al., 2012).

At high concentrations, metal ions can either completely inhibit the microbial population by inhibiting their various metabolic activities or organisms can develop resistance or tolerance to the elevated levels of metals (Khan et al., 2009).

Among bacteria Bacillus sp. has been identified as having a high potential for metal sequestration and also used in commercial biosorbent preparations. The walls of the gram positive bacteria are efficient metal chelators and in Bacillus sp.
the carboxyl group of glutamic acid of peptidoglycan is the major site for metal deposition. Teichoic and Teichronic acids are important binding sites in *Bacillus* sp. These extracellular polymers have been implicated in nutrient storage, adhesion and in barrier formation against environmental toxicants, including metals (Ilhan et al., 2004).

**Figure 5**

**Anthropogenic activities leading to the contamination of soils with heavy metals**

![Figure 5](image)

Figure 6 indicates the various heavy metal toxicity mechanisms to microbes.

**Figure 6**

**Heavy metal-toxicity mechanisms to microbes**

![Figure 6](image)
2.8.3 Chemical Transformation of Chromium in Soil

In soils, chromium although it has several oxidation states occurs mainly with two different oxidation states Cr (III) and Cr (VI) which have opposite chemical and physical characteristics (James, 2002). The organic matter of the soil plays an important role in the reduction of Cr (VI) to Cr (III) by creating reducing conditions by increasing activities of microbial communities, by acting as an electro donator and by indirectly lowering the oxygen level of the soil (oxygen is depleted through an increase of an available carbon source to specific bacterial population is fundamental to alleviate an environment) from hazardous forms of chromium (Cervantes et al., 2001).

2.9 Interaction between Chromium and Bacteria

Soil contamination by heavy metals is often irreversible and may repress or even kill parts of the microbial community and it is generally assumed that the exposure to metals leads to the establishment of a tolerant/ resistant microbial population (Viti and Giovannetti, 2008). The terms resistance and tolerance are
often used interchangeably but their significance is different. Resistance is the ability of a microorganism to survive toxic effects of metal exposure by means of detoxification mechanism produced in direct response to the metal species concerned and defined tolerance as “the ability of a microorganism to survive metal toxicity by means of intrinsic properties and or environmental modification of toxicity” (Fransisco, 2002).

Agricultural soil irrigated with tannery effluents had accumulated multi-fold elevated levels of chromium and other heavy metals. Other workers have also reported high concentration of chromium in the same area (Singh et al., 2004). Heavy metal contamination is known to cause shifts in microbial communities with emergence of bacterial species with elevated metal tolerance (Stepanauskas et al., 2005; Ansari et al., 2008).

### 2.9.1 Bioremediation of Chromium Contaminated Soils

Remediation of soils, water and sediments, contaminated with metal and organic pollutants have been studied extensively in the last two to three decades and several treatment techniques are available for remediation of soil contaminated with chrome wastes. In Tamil Nadu, the problem due to tanneries is very acute in the northern region. This is mainly due to crowding of hundreds of tanneries located in nearby places. Studies reveal that the groundwater Cr (VI) concentrations were > 20 mg/L in ground water samples. The techniques chosen are mainly based on the feasibility and cost at that particular location and the concentration of Cr (VI) present in the polluted soils. Bioremediation have been widely used, because they are economical and also do not generate further waste into the environment (James et al., 1997).

A wide range of microorganisms have been demonstrated to have Cr reducing ability (Kamaludeen et al., 2003). These properties are harnessed in bioremediation, where in the microbial strains are multiplied to desired population and pumped into soil/sediments to promote Cr reduction. The efficiency can be enhanced, if the organic matter content and nutrient availability of the soil are sufficient to promote the growth of the introduced microflora. In *insitu* techniques,
nutrients will be pumped along with aeration to promote Cr reduction. Some of the Cr-reducing bacteria and algae have been efficiently used in the treatment of Cr-rich wastewater (Cifuentes, 1996). However, success was limited in complex soils (Ramasamy et al., 2000).

Bacterial Cr (VI) reduction can occur under both aerobic or anaerobic conditions in presence of different electron acceptors such as oxygen, nitrate, sulphate and ferric iron, but the suitable condition for Cr (VI) bioremediation are aerobic at higher Cr (VI) concentrations and anaerobic at lower Cr (VI) concentrations (Tesena and Bielefeldt, 2002).

The chromium bioremediation approach being cost effective and environmentally friendly in comparison to physical and chemical treatments and is very attractive. Nevertheless to our knowledge, bioremediation strategies for chromate detoxification have yet to be significant on large scale environmental remediation, mostly because the knowledge of microorganism-chromium interactions are to be deepened. However, there is no doubt that a better understanding of the Cr (VI) resistance and Cr (VI) reduction mechanisms which permit specific bacteria to survive and play their role in the presence of high concentration Cr (VI) would result in an adequate biological plan to alleviate the environmental contamination by hexavalent chromium (McLean and Beveridge, 2002).

2.9.2 Bacteria in Metal Degradation

Microorganisms have long been known to accumulate heavy metal ion from the environment. This ability offers an attractive option for the removal and recovery of heavy metal ions from wastewater. Microorganisms are much more efficient at removing metal ions from wastewater and from other pollutants. Removal of many heavy metal ions is commonly reported, while chromium is typically removed at low percentage and is considered as one of the most difficult metals to remove using biomass (Kocberber and Donmez, 2007).

The ability of direct Cr (VI) reduction has been found in many bacterial genera including Pseudomonas sp, Micrococcus, Bacillus sp, Achromobacta,
Microbacterium, Arthrobacter, Corynebacterium. The capability of Cr (VI) reduction is not uncommon in Cr resistant bacteria of soils (Megharaja et al., 2003).

Chromium(III) is rather benign, less mobile, forms water insoluble compounds in aqueous solution and easily absorbed in soils and waters whereas Cr(VI), which is the toxic form of chromium is readily adsorbed and soluble (Zahoor and Rehman, 2009). Subsequently bioreduction of Cr (VI) to Cr(III) is an effective way of combating Cr(VI) pollution and is the most promising practice with proved expediency in bioremediation (Sarangi and Krishnan, 2008). Diverse bacteria have developed several strategies to resist chromate mainly through chromate reduction and chromate efflux. The main role of these strategies is to depress chromate toxicity to cells. Hence, chromate-reducing bacteria are able to reduce bioavailable, highly soluble chromate [Cr(VI)] to thermodynamically stable and less toxic trivalent chromium [Cr(III)] (Cheung and Gu, 2007; He et al. 2011).

The application of bacteria to detoxify metals has been tested in a number of systems, but the viability and metabolic activity of cells are still major limiting factors affecting the bioremoval efficiency of the cellular biomass and enzymes involved (Cheung and Gu, 2007). Cr(VI) reduction at high pH conditions is important for many bioremediation efforts as many effluents released containing toxic metals are under alkaline pH (Ye et al. 2004; Stewart et al. 2007). In addition, high concentration of salts in wastewater treatment systems can be a major problem for conventional biological treatments (Amoozegar et al. 2005; Amoozegar et al. 2007). Therefore, bacteria that can survive under highly alkaline and high salt conditions and can detoxify metals need to be identified.

2.9.3 Heavy Metal Toxicity Mechanism to Microbes

At high concentrations, metal ions can either completely inhibit the microbial population by inhibiting their various metabolic activities or organisms can develop resistance or tolerance to the elevated levels of metals. Unlike many other pollutants, metals can undergo biodegradation and produce less toxic, less mobile and/or less bio-available products, heavy metals are difficult to be removed from contaminated environment. These metals cannot be degraded biologically, and
are ultimately everlasting, though the speciation and bioavailability of metals may change with variation in the environmental factors. Some metals such as, zinc, copper, nickel and chromium are essential or beneficial micronutrients for plants, animals and microorganisms while others (e.g., cadmium, mercury and lead) have no known biological and/or physiological functions. However, the higher concentration of these metals has greater effects on the microbial communities in soils in several ways- (1) it may lead to a reduction of total microbial biomass. (2) it decreases the number of specific populations or (3) it may change microbial community structure. Thus, at high concentrations, metal ions can either completely inhibit the microbial population by inhibiting their various metabolic activities like protein denaturation, inhibition of cell division, cell membrane disruption or organisms can develop resistance or tolerance to the elevated levels of metals (Shukla et al., 2007).

To overcome these toxic conditions microorganisms may tend to respond, using a variety of strategies that ensure their survival and reproduction. In general, while microbial metal resistance includes a variety of strategies to deal with toxic metal concentrations in the environments, these strategies are aimed at either to prevent entry of the metal into the cell or to actively pump the metal out of the cell. Such resistance can be divided into two classes: metal dependent and metal-independent (Murthy et al., 2012).

2.9.4 Plasmid Mediated Metal Resistance

The ability of a genetic marker to transfer from one bacterium to another through conjugation or transformation provides a good presumptive evidence for the involvement of plasmid particularly if the frequency of transfer is high. Moreover, loss of certain genetic markers as a result of treatment of bacterial cell to plasmid curing agents also suggests for the plasmidial nature of the marker (Mesas et al., 2004). Some heavy metal resistance determinants move from plasmid to chromosome (or in the reverse direction). This makes plasmid encoding heavy metal resistance an important aspect of environmental research. The plasmid can be the source of resistance genes for cloning purpose which have
potential use in biotechnology such as the manufacture of biosensors and bioremediation processes. An attempt was made to identify plasmid or chromosome mediated determinants of *P. aeruginosa* to confer resistance to heavy metals and antibiotics (Raja and Selvam, 2009).

A strong selective pressure for transformation of metal- and metalloid-related resistance genes is present in heavy metal contaminated environments (Ryan *et al.*, 2005, Cai *et al.*, 2009). Horizontal gene transfer (HGT) events driven by mobile genetic elements, such as phages, plasmids, insertion sequences, integrons and transposons, have been shown to provide microbes with a wide variety of adaptive traits for microbial survival under hostile environmental conditions (He *et al.*, 2011).

The study of the interactions between heavy metals and microorganisms has been specially focused on bacterial transformation and conversion of metallic ions by reduction in different polluted environments, the selection of metal resistant microorganisms from polluted environments, and the use of resistant microorganisms as indicators of potential toxicity to other forms of life as well as on mechanisms, determinants and genetic transfer of microbial metal-resistance (Murthy *et al.*, 2012).

### 2.9.5 Transformation

The ability to introduce plasmid DNA molecules into the cells has been of central importance to the development of molecular biology. Several methods have been reported in the literature to introduce plasmid DNA into the cells. These methods include chemical treatment, electroporation, use of biolistic gun, polyethylene glycol, ultrasound (Song *et al.*, 2007), microwave (Fregel *et al.*, 2008) and hydrogel (Yoshida *et al.*, 2009). However, the chemical methods have attained much attention in most of the laboratories, due to their accessibility and cost effectiveness. The physiological state of cells that enables them to bind and take up high molecular weight exogenous DNA is called "competence". Uptake of free DNA by *Escherichia coli* cells which have become competent by treatment of chemicals providing Ca$^{2+}$ ions followed by a heat shock pulse was first reported by
Mandel and Higa. Subsequently, several modifications of this method became available for transformation of *E. coli* with plasmid DNA (Singh *et al.*, 2010).

### 2.9.6 Chromate Reductase

Although some heavy metals are required as micronutrients, all heavy metals are toxic in excess. In order to avoid toxicity, metals must be quickly and efficiently eliminated from any cell. In general, there are two basic mechanisms of resistance to heavy metal ions: intracellular complexation of toxic metal ions is mainly used in eukaryotes; whereas, reduced accumulation based on active efflux of the cations is the primary mechanism developed in prokaryotes. In bacteria also binding factors and enzymatic transformations (oxidation, reduction, methylation, and demethylation) play a role as defense mechanisms.

The enzymatic reduction of Cr (VI) involves membrane bound chromate reductase during anaerobic respiration or a soluble cytosolic chromate reductase under aerobic conditions and activity of which is enhanced by NADH or glutathione as enzyme co-factors (Mishra *et al.*, 2012).

Cr(VI) reductase is a central enzyme in the Cr(VI) reduction system ornate in many soils and enteric bacteria which enables them to reduce Cr(VI) to Cr(III), which readily forms an insoluble less toxic chromium hydroxide at neutral pH, and thus, is relevant to an understanding of the detoxification and ultimate remediation of Cr(VI) pollution (Bae *et al.*, 2005).

Figure 8 depicts the structure of Chromate reductase isolated from the bacteria *Gluconacetobacter hansenii* (Jin *et al.*, 2012).