



## BIOLOGICAL STRATEGIES FOR DETOXIFICATION OF HEXAVALENT CHROMIUM

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### ABSTRACT

Hexavalent Chromium is mobile, highly toxic, carcinogenic and mutagenic. It is released into the environment from various industrial processes. In contrast trivalent chromium is less toxic and immobile. Hence, Cr (VI) can be detoxified by converting it to Cr (III). Reduction of Cr (VI) can be achieved by chemical or biological means. Conventional methods for removing metals from contaminated sites include chemical precipitation, oxidation/reduction, ion exchange, filtration, use of membranes, evaporation and adsorption on activated coal, alum, kaolinite and ash. The biological treatment of hexavalent chromium [Cr (VI)] has fascinated people because this alternative is more efficient and less expensive than conventional physicochemical treatments. Microbial reduction of hexavalent chromium has practical importance because biological strategies are part of green technology that is cost effective and eco friendly. The problem of detoxification of chromium can be overcome by bioremediation process which uses plants like hyperaccumulators and different types of microbes for the treatment of contaminants. It helps in complete restoration of the contaminated site. This review focuses on the bioremediation of chromium by plants and microbes like bacteria, fungus, yeast, algae and genetically modified crops.

**KEYWORDS:** Hexavalent chromium, detoxification, heavy metals, microbes, biological



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## INTRODUCTION TO CHROMIUM

Global mechanization, urbanization and various natural processes have led to the increased release of toxic compounds into the biosphere. These hazardous toxic pollutants include a variety of organic and inorganic compounds, which pose a serious threat to the ecosystem. The contamination of soil and water are the major environmental concerns in the present scenario. This leads to a greater need for remediation of contaminated soils and water with suitable approaches and mechanism.<sup>1</sup> Heavy metals, a major category of globally-distributed pollutants are natural elements that have been extracted from the earth and harnessed for human industry and products for millennia. Heavy metal ions are the elements in the d-block of the periodic table or transition metal. Many of them are known to be highly toxic to both humans and other living organisms, with their property of bioaccumulation and to be persistent over times causing remarkable health damage. These heavy metals enter into the natural water bodies through the wastewater discharge from electroplating, leather, tanning, foundry, chemical manufacturing, jewelry works, dye manufacturing, mining, industries of Cd-Ni batteries, phosphate fertilizer, pigments, etc.<sup>2</sup> The term heavy means occurring or produced in large amount or in greater amount than normal and metal is a chemical element that is malleable and ductile usually solids. However, heavy metal exhibits metallic properties and has a specific weight higher than 8 mg/cm<sup>3</sup>. Furthermore, metals belonging to d-block elements of the periodic table have a specific gravity of not less than five times the specific gravity of water and are termed as heavy metals.<sup>3</sup> Chromium is a heavy metal with large industrial applications such as in textile dyeing, chemicals/pigments production, wood preservation, tanning activity and electroplating for surface treatment. Chromium has been ranked as 17th in the Environmental Protection Agency "Top Hazardous Substance Priority List" (Agency for Toxic Substances and Disease Registry (ATSDR), 2011). Chromium was discovered by French chemist Nicholas Louis Vanquelin in 1797. The most common oxidation states of Chromium are Cr (II), Cr (III) and Cr (VI) with Cr (III) being the most stable. The oxidation states Cr (IV) and Cr (V) are relatively rare. Chromium compounds of Cr (VI) oxidation states are powerful oxidizing agents. Many naturally or genetically modified microorganisms possess the ability to degrade, transform or chelate various toxic chemicals and hence provide better strategies to combat environmental pollution. On a regular basis, scientists deploy either natural or modified microbes to remove contaminants, viz., heavy metals, metalloids, radioactive waste, and oil products from polluted sites.<sup>4</sup>

## CHARACTERISTICS

Chrome metal (Chromium 0) is the element that makes steel "stainless". Cr (0) is the metallic form produced in industry and is a solid with high fusion point usually used for the manufacturing of steel and other alloys. Chromium (Cr) is a naturally occurring element with atomic number 24 and atomic mass of 51.996 amu. The

element belongs to the group of transition metals and in the oxidation state elementary presents an electronic configuration (Ar) 4d<sup>5</sup>s<sup>1</sup>. Chromium is naturally present in the environment; it is widespread in rocks, animal, plants and soil. Chromium (Cr) is the seventh most abundant element in the earth's crust. It is released into the environment through naturogenic and anthropogenic sources. Chromium, a steel grey, lustrous, hard and brittle metal, occurs in nature in the bound form that constitutes 0.1 – 0.3 mg/kg of the Earth's crust. In nature, Cr is found in the form of its compounds and the most important chromium ore is chromite, (Fe, Mn) Cr<sub>2</sub>O<sub>4</sub>. Chromium represents an essential micronutrient for living organisms. Cr (III) is an essential trace element known for its particular role in the maintenance of normal carbohydrate metabolism in mammals and yeasts. Moreover, it has also been suggested that Cr (III) is involved in the tertiary structure of proteins and in the conformation of cell RNA and DNA.

## OXIDATION STATES

Chromium exists in several oxidation states ranging from (-II) to (+VI) the trivalent and hexavalent states being the most stable. Both Cr (III) and Cr (VI) differ in terms of mobility, bioavailability and toxicity. Toxic effects of chromium are valence dependent. Trivalent chromium (Cr (III)) is an essential micronutrient for humans and is relatively less soluble.<sup>5</sup> Trivalent chromium (III) is the form of chromium naturally found in the environment. Chromium (III) is required for the body to utilize sugars, proteins and fat properly. Cr (III) is classified as an essential trace element for humans, since it seems to participate in the metabolism of glucose and lipids. However, Chromium seems not to be required by microorganisms or plants. At the extracellular level, Cr (III) is relatively innocuous as a consequence of its insolubility and subsequent inability to cross cell membranes. Inside the cell, Cr(III) may generate toxic effects by its ability to bind to phosphates in DNA. Hexavalent chromium (VI) is released into the environment as a result of industrial activity. Chromium (VI) is not an essential nutrient rather considered the most toxic form of chromium, which usually occurs associated with oxygen as chromate (CrO<sub>4</sub><sup>2-</sup>) or dichromate (Cr<sub>2</sub>O<sub>7</sub><sup>2-</sup>) oxyanions. Chromium has been designated as a priority pollutant by United States Environmental Protection Agency (USEPA) due to its carcinogenicity and mutagenicity. It is toxic to all forms of living systems including microorganisms by causing oxidative stress, DNA damage and altered gene expression. In humans, several traumata are associated with Cr (VI) exposure, including nasal irritation and ulceration, skin irritation, eardrum perforation and lung carcinoma. Chromium poisoning causes skin disorders and liver damage. Its high solubility in aqueous systems, its permeability through biological membranes and subsequent interaction with intracellular proteins and nucleic acids<sup>6</sup> together with carcinogenic and mutagenic effects on living organisms make industrial effluents receive specific treatments for Cr(VI) elimination. Furthermore, Cr (VI) can accumulate in the placenta, impairing fetal development in mammals. In the environment, Cr (VI) contamination alters the structure of soil microbial communities. As a result of reduced

microbial growth and activities, organic matter accumulates Cr (VI) in soil. Cr (VI) is toxic, carcinogenic and mutagenic to animals as well as humans and is associated with decreased plant growth and changes in plant morphology.

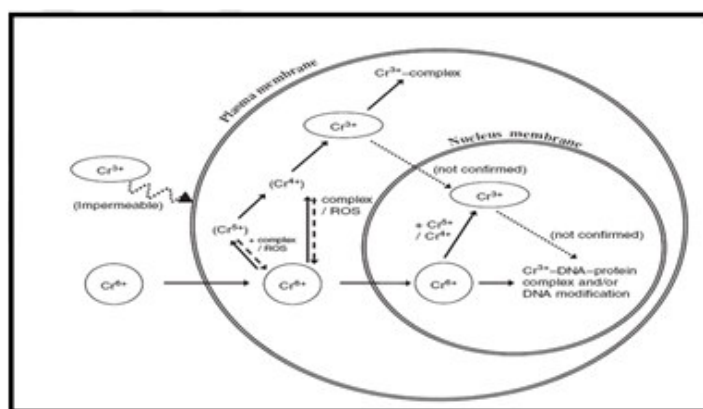
## SOURCES

Hexavalent chromium Cr (VI) is discharged into the environment as a result of the wide use of chromium compounds in industries such as leather tanning, electroplating, pigment production and thermonuclear weapons manufacture.<sup>7</sup> The effluent from electroplating industries contains heavy metals such as chromium, cadmium, lead, nickel, copper as well as acids, alkali and cyanide. Chromium enters into the environment through the anthropogenic activities, it is used in stainless steel plant, preparation of alloys, chrome plating, leather tanning, production of refractories, dye industry, industrial water cooling, paper pulp production, petroleum refining, wood preservation and nuclear power.<sup>8</sup> Chromium is vastly used in all fields so its presence in effluents originated from the different activities represents a serious pollutant of sediments, soil, water and air. Cr (VI) is introduced in the environment mainly as a consequence of its industrial use, while chromium in its trivalent form, Cr (III), naturally predominates in the environment. Cr (VI) is highly dispersed in sediments and surface waters and it is characterized by a much greater solubility, mobility and bioavailability than Cr (III) and all the forms of chromium. Cr (VI) has high water solubility and elevated mobility which lead to its easily diffusion away from the native site of contamination. Moreover, the increase in soil pH increases the leachability of Cr (VI). In contrast, Cr (III) shows a low mobility and is relatively inert and easily absorbable on mineral surfaces and solid-phase organic ligands, thus resulting less bioavailable in the environment. Mobility of Cr (III) decreases with absorption of clays and oxide minerals below pH 5. Binding of Cr (III) by iron oxides can be considered an example of these mechanisms, as this feature can decrease the solubility of this form of chromium.<sup>9</sup> In anaerobic environments, under reducing conditions, in

the presence of reducing agent as sulfides, ferrous iron and organic matter that are several of the organic and inorganic constituents, Cr (VI) may rapidly convert to Cr (III). Again, bacterially mediated reduction of Cr (VI) has also been considered in the chromium biogeochemical cycle. Cr (III) is stable in aquatic environments and its oxidation to Cr (VI) is improbable, even in the presence of dissolved oxygen. Different factors affect Cr (III) oxidation to Cr (VI) depending on the presence and mineralogy of Mn(III, IV) hydroxides, pH and the form and solubility of Cr (III). Oxidation of Cr(III) is improbable to occur in aquatic environments because aged waste materials containing Cr(III) are typically less soluble and more inert to oxidation and Cr(OH)<sub>3</sub> precipitates may form on surfaces of Mn (III, IV) hydroxide.

## TOXICITY

The biological effect of chromium is highly dependent on its oxidation state. Cr (VI) compounds can be toxic for biological systems. These compounds have been considered to be a group 'A' human carcinogen. The structural similarity of chromate to SO<sub>4</sub><sup>2-</sup> allows for the uptake of Cr (VI) into bacteria through the sulfate transport system. Chromium toxicity is related to the process of transformation of Cr (VI) to lower oxidation states, not necessarily to Cr (III) in which free radicals are generated. Transformation of Cr (VI) to Cr (III) has been reported in many biological systems; transient formation of Cr (V) is the most likely mechanism involved in Chromium toxicity. Cr (V) complexes are formed from Cr (VI) by physiological reducing agents such as NAD(P)H, FADH<sub>2</sub>, several pentoses and glutathione to generate the short-lived intermediates Cr(V) and/or Cr(IV) free radicals and the end-product Cr(III). Cr (V) undergoes a one-electron redox cycle to generate Cr (VI) and transferring the electron oxygen. The process produces a reactive oxygen species (ROS) that easily combines with DNA-protein complexes. Cr (IV) would bind to cellular materials and deter their normal physiological functions. Figure 1 summarizes the various pathways followed by Cr (VI) that gets into cells.<sup>10</sup>



**Figure 1**  
**Schematic diagram of toxicity and mutagenicity of Cr (VI)**

the intracellular Cr(VI) reductants naturally available are frequently obligatory one electron reducers, which

generate Cr(V) and a large amount of reactive oxygen species (ROS) that causes the deleterious effects of

Cr(VI). Modification of the DNA polymerase and other enzyme activities may be caused by the displacement of magnesium ions by Cr (III) ions. In aqueous system, chromium exists as oxyanions ( $\text{CrO}_4^{2-}$ ), chromate ( $\text{CrO}_4^{2-}$ ) and dichromate ( $\text{Cr}_2\text{O}_7^{2-}$ ), analogous in structure to sulphate and phosphate ions, which can readily permeate through bacterial and eukaryotic cells, their intracellular reduction results in chromate-induced toxicity. Chromate and dichromate are in equilibrium which is sensitive to pH changes, where lower pH pushes the equilibrium towards the dichromate ion.  $\text{H}_2\text{CrO}_4$  belongs to the strong acids and at  $\text{pH} > 1$ , its deprotonated forms are prevailing while above pH 7 only  $\text{CrO}_4^{2-}$  ions exist in solution throughout the concentration

range. In the pH between 1 and 6,  $\text{HCrO}_4^-$  is the predominant form up to the Cr(VI) concentration  $10^{-2}$  M when it starts to condense yielding the orange-red  $\text{Cr}_2\text{O}_7^{2-}$ . Within the normal pH range in natural waters, the  $\text{CrO}_4^{2-}$ ,  $\text{HCrO}_4^-$  and  $\text{Cr}_2\text{O}_7^{2-}$  ions are the forms expected. They constitute a lot of Cr (VI) compounds which are quite soluble and thus mobile in the environment. However, Cr (VI) oxyanions are readily reduced to trivalent forms by electron donors such as organic matter or reduced inorganic species, which are ubiquitous in soil, water and atmospheric system. Table 1 summarizes the speciation of chromium in the environment.

**Table 1**  
**Summarizes the speciation of chromium in the environment<sup>11</sup>**

Chemical species	Oxidation State	Examples	Remarks
Elemental Cr	Cr(0)	—	Does not occur naturally.
Divalent Cr	Cr(II)	$\text{CrBr}_2$ , $\text{CrCl}_2$ , $\text{CrF}_2$ , $\text{CrSe}$ , $\text{Cr}_2\text{Si}$	Relatively unstable and is readily oxidized to the trivalent state.
Trivalent Cr	Cr(III)	$\text{CrB}$ , $\text{CrB}_2$ , $\text{CrBr}_3$ , $\text{CrCl}_3$ , $6\text{H}_2\text{O}$ , $\text{CrCl}_3$ , $\text{CrF}_3$ , $\text{CrN}$	Forms unstable compounds and occurs in nature in ores, such as ferrochromite ( $\text{FeCr}_2\text{O}_4$ ).
Tetravalent Cr	Cr(IV)	$\text{CrO}_2$ , $\text{CrF}_4$	Does not occur naturally. The Cr (VI) ion and its compounds are not very stable and because of short half-lives, defy detection as reaction intermediates between Cr(VI) and Cr(III).
Pentavalent Cr	Cr(V)	$\text{CrO}_2$ , $\text{CrF}_4$	Does not occur naturally. Cr (V) species are derived from the anion $\text{CrO}_4^{3-}$ and are long-lived enough to be observed directly. However, there are relatively few stable compounds containing Cr (V).
Hexavalent Cr	Cr(VI)	$(\text{NH}_4)_2\text{CrO}_4$ , $\text{BaCrO}_4$ , $\text{CaCrO}_4$ , $\text{K}_2\text{CrO}_4$ , $\text{K}_2\text{Cr}_2\text{O}_7$	The second most stable state of Cr. However, Cr (VI) rarely occurs naturally, but is produced from anthropogenic sources. It occurs naturally in the rare mineral crocoites ( $\text{PbCrO}_4$ ).

*The genotoxic effect of Cr ion however cannot be fully explained by the sole action of ROS. Intracellular cationic Cr (III) complexes can interact electrostatically with negatively charged phosphate groups of DNA which could affect replication, transcription and cause mutagenesis.*

## MODE OF ENTRY

There are three different routes of entry of chromium into the human body. The gastro-intestinal route is the most important physiological condition, while in occupational exposure the airways are more important routes of entry and uptake. The valence state of chromium, water solubility, acidity of gastric juice and the passage time through the tract are the factors which control the uptake of chromium in the gastro-intestinal tract, while uptake in the airways is influenced by the particle size distribution and also on factors which govern the clearance time from the lungs. The third route is through the epidermis and this is very significant in pathological conditions. Almost every regulatory agency has listed Cr(VI) as a priority toxic chemical for control, with the maximum allowable level in drinking water of  $50\text{-}100 \mu\text{g L}^{-1}$ . In contrast to most metals, chromium is usually soluble under oxidising conditions and only limited removal can be achieved by conventional precipitation methods.<sup>12</sup> The search for new and innovative technology has drawn the attention in biotransformation of metals by microbes.

## BIOLOGICAL DEGRADATION OF CHROMIUM

Unlike organic contaminants, the metals cannot be ruined, but can be converted to a stable form or removed. The process of using microorganisms to degrade or remove hazardous pollutants from the environment is known as biodegradation or biological degradation. Even though Cr (VI) can be reduced by algae or plants, in soil microorganism has been confirmed to be most effective. The biological processes are highly specific with culture requirements and at time are difficult to extrapolate the results from lab to field. It also often takes longer time than other treatment such as excavation and removal of soil. There are numerous factors affecting the method of bioremediation such as depletion of preferential substrates, lack of nutrients, toxicity and solubility of contaminants, chemical reaction or reduction potential and microbial interaction. Mechanisms by which microorganisms act on heavy metals include bio sorption (metal sorption to cell surface by physicochemical mechanisms), bioleaching (heavy metal mobilization through the excretion of organic acids or methylation reactions), bio-mineralization (heavy metal immobilization through the formation of insoluble sulfides, etc), accumulation inside cell and enzyme-catalyzed reactions (redox reactions). The major microbial processes that influence the degradation of metals are summarized in Figure 2.<sup>13</sup>

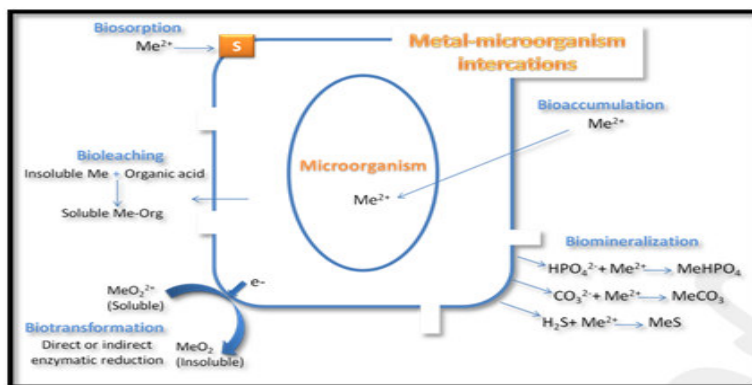


Figure 2  
Microbial processes used in bioremediation technologies<sup>13</sup>

**DETOXIFICATION OF HEXAVALENT CHROMIUM**

There are various technologies which are available for the removal of chromium from wastewaters.

**CONVENTIONAL TECHNIQUES**

The conventional techniques available for the bioreduction of hexavalent chromium includes chemical precipitation, lime coagulation, ion exchange, chemical

oxidation, electro dialysis, ultra filtration and solvent extraction<sup>14</sup> but these conventional methods have several disadvantages, such as high operating costs, the necessity of preliminary treatment steps, the difficulty of treating the solid waste subsequent generated and the requirement of large quantities of chemical adsorbent. Table 2 summarises the advantages and disadvantages of different conventional methods.

Table 2  
Summarises the advantages and disadvantages of different conventional methods

Some conventional methods			
Treatment method	Advantages	Disadvantages	References
Ion exchange	Metal selective, High regeneration of materials	High initial capital and maintenance cost	O'Connell <i>et al.</i> , (2008)
Chemical precipitation	Low capital cost, simple operation	Sludge generation, extra operational cost for sludge disposal	Kurniawan <i>et al.</i> , (2006)
Adsorption with new adsorbents	Low-cost, easy operating conditions, having wide pH range, high metal binding capacities	Low selectivity, production of waste products	Babel and Kurniawan (2003); Akil <i>et al.</i> , (2004)
Membrane filtration	Small space requirement, low pressure, high separation selectivity	High operational cost due to membrane fouling	Kurniawan <i>et al.</i> , (2006)
Electro dialysis	High separation selectivity	High operational cost due to membrane fouling and energy consumption	Mohammadi <i>et al.</i> , (2005)
Photo catalysis	Removal of metals and organic pollutant simultaneously, less harmful by-products	Long duration time limited applications	Barakat <i>et al.</i> , (2004); Kajitvichyanukula <i>et al.</i> , (2005)

Increasing the pH of the effluent is the simplest and cheapest method of removing most heavy metals from solution, thus converting the soluble metal into an insoluble form (i.e. its hydroxide) but this is not a selective process. Any iron (ferric ion) present in the liquid effluent will be precipitated first, followed by other heavy metals (Cu, Pb, Zn, Cd). Consequently, precipitation by alkali addition (usually lime) produces large quantities of solid sludge for disposal. The most commonly used conventional processes to remove Cr (VI) are: (a) reduction to Cr (III) followed by precipitation as chromium hydroxide, (b) removal by ion exchange and (c) removal by adsorption. These methods are costly due to operational, treatment and sludge disposal costs.<sup>15</sup>

**BIOLOGICAL TECHNIQUES**

Environment friendly processes need to be developed to clean up the contaminated environment without creating harmful waste products and to reduce metal content in wastewater or discharge to acceptable levels at affordable cost. Research for new and innovative technologies has centered on the biological treatment methods.<sup>16</sup> Fundamental to these biotreatment processes are the activities of living organisms, upon which transformation and detoxification of heavy metal pollutant depends. The biotreatment processes can be classified into two principal categories: the metabolically active (biomineralization, biotransformation, bioprecipitation and bioaccumulation) and metabolically passive (biosorptive) processes. Of the different biological methods, bioaccumulation and biosorption have been demonstrated to possess good potential to replace conventional methods for the removal of metals. Bioremediation is the use of microorganisms to break

down toxic and hazardous compounds in the environment. It generally utilizes microbes (bacteria, fungi, yeast and algae) although higher plants are used in some applications. The two main biological treatment processes under investigation are: the adsorption of Cr(VI) onto microbial cells (i.e. biosorption) and the reduction of Cr(VI) to Cr(III) by enzymatic reaction or indirectly by reducing compounds produced by microorganisms (i.e. biotransformation). New tools and techniques for use of bioremediation in situ, in biofilters and in bioreactors are contributing to the rapid growth of this field. The chromate presence in the environment inhibits most of the microorganisms, but it also promotes the selection of resistant bacteria. The physiological effects of chromate stress on bacteria are often observed through a decreased growth rate with increasing chromate concentration or longer lag phases before growth is observed. Uncoupling of energy expenditure and growth in sulphate reducing bacteria during chromate-stress, comparable to that observed in bacteria due to oxidative stress was showed. Chromate frequently causes morphological changes and observed as filamentous growth as is often seen with bacterial-stress responses. Microbial survival in polluted soil depends on intrinsic biochemical and structural properties, physiological and/or genetic adaptation including morphological changes of cell as well as environmental modification of metal speciation. Microbes apply various type of resistance mechanism in

response to heavy metals. Chromate reducing bacteria have been isolated and characterized mostly from chromium contaminated soil, wastewater and industrial effluent.<sup>17</sup>

## BACTERIA

Among microorganisms, bacteria are better candidates for heavy metal removal as these are easy to culture, easy to handle and have very simple nutritional requirements. Bacteria are the most abundant and versatile of microorganisms and constitute a significant fraction of the entire living terrestrial biomass of  $\sim 10^{18}$  g. Bacteria which are ubiquitous in nature play an important role in the mobility of metals and nucleotides. Small size of bacteria is very important because size affects a number of cell biological properties. Small size of bacteria ensures rapid metabolic processes. The smallest bacteria are about 0.3  $\mu\text{m}$ . *Escherichia coli* are about 1.1 to 1.5  $\mu\text{m}$  wide by 2.0 to 6.0  $\mu\text{m}$  long and cyanobacterium is about 7  $\mu\text{m}$  in diameter. The main advantages of using bacterial Cr(VI) reduction are that it does not require high energy input.<sup>18</sup> Moreover their cell wall surface contains many functional groups of carboxyl, hydroxyl, sulphhydryl, aminoacids, phosphate groups of lipids, proteins and polysaccharides having ability to bind metal ions. Table 3 shows the list of some bacteria which deals with the adsorption of chromium.

**Table 3**  
**Chromium adsorption capacity by different bacteria**

Bacteria	Adsorption capacity (mg/g)	References
<i>Bacillus amyloliquefaciens</i>	-	19
<i>Trichoderma gamsii</i>	44.8	20
<i>Trichosporon cutaneum</i>	-	21
<i>Enterococcus casseliformis</i>	512	22
<i>Lentinus edodes</i>	21.5	23

Pure cultures of Cr (VI) reducing bacteria have been tested and applied for detoxification of Cr (VI) contaminated waste water. Bacterial species such as *Bacillus*, *Pseudomonas*, *Streptomyces*, *Escherichia*, *Micrococcus* etc have been tested for uptake metals or organics. Bacteria may either possess the capacity for uptake of many elements or alternatively, depending on species, may be element specific. The identification of chromium resistant bacteria and assessment of their chromium detoxification capabilities are primary steps in developing process for biotransformation.<sup>24</sup> Biotransformation of hexavalent to trivalent chromium using bacteria is the most pragmatic approach with a well-established feasibility in biotransformation. Biological reduction of Cr (VI) using indigenous microorganisms offers a new cost-effective and environmentally compatible technology. The mechanisms by which these microorganisms reduce Cr (VI) are variable and are species-dependent. Several species use Cr (VI) as a terminal electron acceptor in their respiratory chains. At the extracellular level, Cr (VI) is highly toxic to most bacteria, whereas Cr (III) is relatively innocuous because of its insolubility and subsequent inability to traverse cell membranes. In the cytoplasm, chromium toxicity is mainly related to the

process of transformation of Cr (VI) to lower oxidation states i.e. Cr (V) and Cr (III) in which free radicals may be formed. Microorganisms especially bacteria which have been exposed to toxic compounds anthropogenically result in having a well-developed resistance mechanisms for metals. Bacteria may protect themselves from toxic substances in the environment by transforming toxic compounds through oxidation, reduction or methylation into more volatile, less toxic or readily precipitating form. Reduced trivalent form of chromium forms insoluble chromium hydroxide at neutral pH. These chromium compounds are very stable and they are unavailable to living organisms. Microbial transformation of toxic hexavalent chromium to relatively non-toxic trivalent form Cr (III) has been identified as a cost effective strategy for detoxification and removal of Cr(VI). A variety of chromate-resistant bacterial isolates have been reported and the mechanisms of resistance to this compound may be encoded either by plasmids or by chromosomal genes. A number of chromium-resistant microorganisms were subsequently isolated, such as *B. cereus*, *B. subtilis*, *Pseudomonas aeruginosa*, *Pseudomonas ambigua*, *Pseudomonas fluorescens*, *E. coli*, *Achromobacter Eurydice*, *Micrococcus roseus*, *Enterobacter cloacae*,

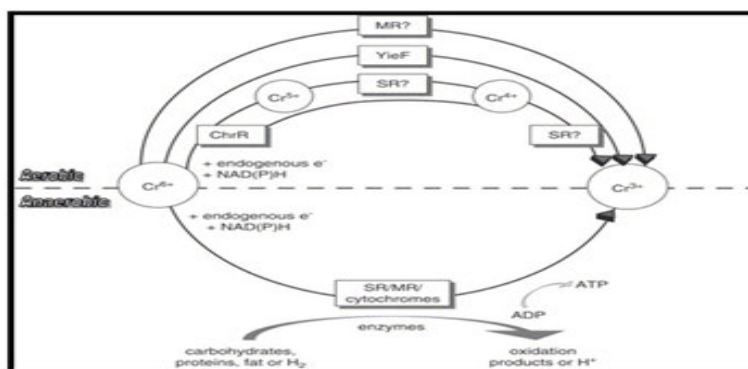
*desulfovibrio desulfuricans* and *D. vulgaris*.<sup>25</sup> Several species of bacteria such as *P. aeruginosa* and *Shewanella Oneidensis* contains membrane transporters responsible for mediating efflux of chromate ions from cell cytoplasm. Some species utilise a soluble enzyme that catalyse the transformation of Cr (VI) into the much less toxic and less mobile Cr (III), leading to decreased uptake of chromium such as *E.coli* and *Acidiphilium Cryptum*<sup>26</sup> even could transform Cr (VI) by periplasmic c-type cytochrome in the way of obtaining electrons from the respiratory chain and divert them to Cr(VI). In addition, *Shewanella oneidensis* has also use DNA repair system to resist toxicity caused by chromium compounds. The identification of chromium resistant bacteria and assessment of their chromium detoxification capabilities are primary steps in developing process for biotransformation. But the availability of efficient bacterial strains is the main bottleneck in developing a biotransformation process. There is a need to isolate bacteria from metal contaminated sites and to study their interactions with metals. Number of studies has shown that natural microorganisms, isolated from heavy metal contaminated habitats or from other extreme environments as well as some laboratory strains effectively interact with toxic metals by direct and indirect mechanisms. These interactions between microbes and metal can cause mobilization or immobilization of the metals. They encompass biotransformation such as oxidation or reduction of metals, biosorption by cell surface polymers, uptake of metals into the cells, induction of metal precipitation and generation of minerals or alteration of metal speciation caused by microbial induced redox changes in the environment. Hence microbial reduction strongly influences the fate of toxic metals in the environment. Bacteria have the potential to transform Cr (VI) to both soluble Cr (III) and insoluble Cr (III). Many researchers have explored the potential of microorganisms such as *Ochrobactrum anthropic* from tannery effluent. *Corynebacterium paurometabolum* from chromite mine quarries of Orissa, *Bacillus sp.* Ev3 from metal contaminated wastewater, *Stenotrophomonas maltophilia* T7D7, *Staphylococcus gallinarum* NT-S, *Pantoea sp.* 520b and *Aeromonas sp.* B27<sup>27</sup> for detoxification of hexavalent chromium. The soluble Cr (III) is probably complexed with organic ligands by enzymatic and chemical reductions in the presence of cellular organic compounds to form stable and soluble organo- Cr (III) complexes. Formation of organo-Cr (III) complexes from chromate reduction, with the formation of stable DNA- Cr (III) adducts, has been extensively studied in mammalian cells. Microbial treatments, based on biotransformation of Cr (VI) to Cr (III) which is easily mobilized as Cr (OH)<sub>3</sub>, are still efficient with low Cr (VI) amounts. These methods offer an economical as well as eco-friendly option for chromate detoxification and bioremediation. In aqueous systems, Cr (VI) exists as oxyanions (CrO<sub>4</sub><sup>2-</sup>) which are structurally analogous to sulphate and phosphate ions hence can easily be taken up by anionic transport systems of bacterial cells.<sup>28</sup> Their cell membrane is nearly impermeable to Cr (III) and thus Cr (III) has one thousandth of the toxicity of Cr (VI). Because of their insolubility, Cr (III) facilitates its precipitation and removal and hence the

biotransformation of Cr (VI) to Cr (III) has been considered as an alternative process for treating Cr (VI) contaminated wastes. The reduction of chromate proceeds aerobically, anaerobically or both. In aerobic systems most of the chromate reductases reported till date are soluble in cytosol and reduce Cr<sup>6+</sup> to Cr<sup>3+</sup> inside the cell or outside the plasma membrane while under anaerobic conditions chromate is reduced in the membrane. The constraints of chromate toxicity can be circumvented by using resistant organisms that could be effectively used for the biotransformation of chromate-contaminated wastewaters and soils. Cr (VI) removal capacity of microorganisms is also affected from some external factors such as salt ions, pH and temperature of waste water. Especially high salt concentrations in waste water treatment systems are most significant factor reducing microbial activity. The efficiency of bacterial strains may be increased by the optimisation of these factors. The influence of parameters: pH, temperature and initial chromium ion concentration on the chromium removal efficiency from synthetic effluents using *Sargassum sp.* as a biosorbent was observed. Cr (VI) transformation by microorganisms often results in consumption of large amounts of proton as reducing equivalents which results in the elevation of the background pH. The increased pH facilitates the precipitation of the transformed chromium as chromium hydroxide, Cr (OH)<sub>3</sub>. Microbial Cr (VI) reduction was first reported in the late 1970s when Cr (VI) reduction capability in *Pseudomonas sp.* grown under anaerobic conditions was observed. After that, several researchers have isolated new microorganisms that catalyse Cr (VI) reduction under different conditions. In *Pseudomonas aeruginosa*, Cr (VI) resistance is attributed to the decreased uptake and/or enhanced efflux of Cr (VI) by the cell membrane. A similar mechanism of resistance has been reported for *Alcaligenes eutrophus* CH34. On the other hand, investigation with *Pseudomonas fluorescens* shows that Cr (VI) resistance does not depend upon the capacity of the organism to reduce the metal. Other *Pseudomonas sp.* capable of reducing Cr (VI) includes a close relative of *Pseudomonas synxantha* and an unidentified species. A number of bacteria in other genera namely *Bacillus sp.*, *E. coli* ATCC33456, *Shewanella alga* BrY-MT and a few unidentified strains have also been shown to reduce Cr (VI). In *Pseudomonas maltophilia* 0-2 and *Bacillus megatarium* TKW3 Cr (VI) reduction was associated with the membrane cell fractions. The bacterial cell wall is the first component that comes into contact with metal ions, where the solutes can be deposited on the surface or within the cell wall structure. Due to nature of the cellular components, several functional groups are present on the bacterial cell wall, including carboxyl phosphonate, amine and hydroxyl groups. As they are negatively charged and abundantly available, carboxyl groups actively participate in the binding of metal cations. The solution chemistry affects not only bacterial surface chemistry but the metal speciation as well. Metal ions in the solution undergo hydrolysis as the pH increases. The extent of which differs at different pH values and with each metal but the usual sequence of hydrolysis is the formation of hydroxylated monomeric species, followed by the formation of polymeric species

and then the formation of crystalline oxide precipitates after ageing.

## MECHANISMS OF CR (VI) TRANSFORMATION BY BACTERIA

Bacteria can reduce Cr (VI) under aerobic or anaerobic conditions through electron-transport systems containing cytochromes. The mechanism of enzymatic Cr<sup>6+</sup> reduction under aerobic (upper) and anaerobic (lower) conditions is shown in Figure 3.



**Figure 3**  
**Possible mechanisms of enzymatic Cr(VI) transformation under aerobic (upper) and anaerobic (lower) conditions**<sup>29</sup>

Under aerobic conditions as shown in Figure 3 (upper), the transformation of Cr<sup>6+</sup> to the stable end product, Cr (III) occurs as two- to three-steps with Cr (V) and/or Cr (IV) as short-lived intermediates. The reduction of Cr (V) to Cr (IV) and Cr (IV) to Cr (III) is not known to be either spontaneous or enzyme-mediated. NADH, NADPH and electron from the endogenous reserve are implicated as electron donor in the Cr (VI) reduction process. The Cr (VI) reductase ChrR transiently reduces Cr (VI) with a one-electron shuttle to form Cr (V), followed by a two-electron transfer to generate Cr (III). Although a proportion of the Cr (V) intermediate is spontaneously reoxidized to generate reactive oxygen species. YieF enzyme from *E.coli* chromosome was found to have similar homology to chrR and showed maximum reduction of Cr (VI) at 35°C.<sup>30</sup> Enzyme YieF is unique in that it catalyzes the direct reduction of Cr (VI) to Cr (III) through a four-electron transfer, in which three-electrons are consumed in reducing Cr (VI) and the other is transferred to oxygen. Under anaerobic conditions, natural metabolites of anaerobes such as H<sub>2</sub>S produced by sulfate-reducing bacteria (SRB) are effective chemical Cr (VI) reductants under anoxic environment. In the absence of oxygen, Cr (VI) can serve as a terminal electron acceptor in the respiratory chain for a large array of electron donors, including carbohydrates, proteins, fats, hydrogen, NAD(P)H and endogenous electron reserves. The typical anaerobic Cr (VI) reduction is shown in Figure 3 (lower). SR and MR represent soluble and membrane-associated reductase, respectively.

## YEAST

The principal reason that yeasts are resistant to chromium relates more to their limited ion uptake rather than to biological reduction of Cr(VI) to Cr(III); such decreased uptake means decreased absorption and bioaccumulation in yeast cells. In chromate-resistant strains of *Candida maltosa*, NAD-dependent chromate-

reducing activity was discovered to take place in the soluble protein fraction, with the membrane fraction being less active. Recently, it has been discovered that Cr(VI) detoxification occurs via extracellular reducing substances that are secreted by the yeast cells such as sulfate and riboflavin.<sup>31</sup> Indeed, many yeast strains are known to biotransform Cr(VI) to the less toxic Cr(III); examples include *S. cerevisiae*, *Rhodotorula pilimanae*, *Yarrowia polytica* and *Hansenula polymorpha*, *Pichia guilliermondii* and *Rhodotorula mucilaginosa*.<sup>32</sup> Cr(VI) removal by *P. anomala* initially involves adsorption on functional groups (e.g., carboxyl group, amide I, amide II, amide III, polysaccharides and sulfonate) of cell surfaces, followed by intracellular accumulation and reduction of Cr(VI) to Cr(III). Three yeasts (viz., *Cyberlindnera fabianii*, *Wickerhamomyces anomalus* and *Candida tropicalis*) could be used for effectively remove Cr(VI) via adsorption from contaminated sites. Cr(VI) gains entrance into yeast cells in an oxy-anionic form in bacteria, i.e., via sulfate-specific transport systems. The genes involved in sulfate and chromate transport have been identified. Microbial cells are often impermeable to Cr(III), possibly because they form complexes that have low solubility. The mechanism of such transport is unknown and it is unclear if the known metal transport systems are responsible for the accumulation of Cr(III) in the cells. It is also unclear as to whether there is a specific system to transport this cation in yeasts. Yeasts were identified as convenient organisms to study bioremediation, because some strains are capable of growing in matrices that have high concentrations of chromium compounds and adsorb or accumulate significant quantities into cells and transform them via chelation to less toxic forms. Yeasts are known for playing an important role in the removal of heavy metals which are toxic. There is much information on biosorption of heavy metals by yeasts. In the case of hexavalent chromium Cr(VI) we found that *P. anomala* is able to remove Cr(VI). The capability of yeast to reduce hexavalent chromium was considered. The in



vitro decrease of hexavalent chromium using Crude Chromate Reductase (CChr) of *Pichia jadinii* M9 and *Pichia anomala* M10, isolated from a textile-dye factory effluent<sup>33</sup>.

## FUNGUS

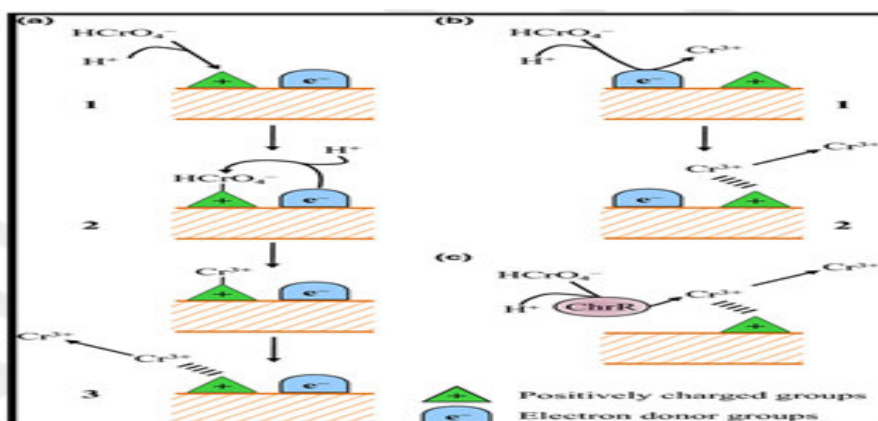
The common filamentous fungi can sorb heavy metals (Zn, Cd, Pb, Fe, Ni, Ag, Th, Ra and U) from aqueous solutions to varying extents. Fungal biosorption largely depends on parameters such as pH, metal ion and

biomass concentration, physical or chemical pretreatment of biomass and presence of various legends in solution. When compared with commercial ion exchange resins, carbons and metal oxides, fungal derivatives generally perform well. Biomass can be regenerated by using various elutants. The availability of variety of biomass and their metal binding potential makes it an economical and sustainable option for developing effluent treatment process for removal and recovery of heavy metals. Table 4 represent list of some fungi having adsorption capacity for chromium

**Table 4**  
**Chromium adsorption capacity by different fungus**

Fungi	Adsorption capacity (mg/g)	References
<i>Arthroobacter viscous</i>	12.6	34
<i>Aspergillus niger</i>	117.33	35
<i>Aspergillus parasiticus</i>	0.587	36
<i>Candida lipolytica</i>	10	37
<i>Mucor hiemalis</i>	30.5	38

Fungus acts as bioabsorptive material to remove hexavalent chromium. Biosorption mechanism is done by two methods- metabolism dependent and non-metabolism dependent. The chemicals get bound to the functional groups on the surface and get absorbed. Figure 4 illustrate the mechanisms of extracellular chromate reduction in fungi in a schematic manner<sup>39</sup>.



**Figure 4**  
**Mechanisms of extracellular chromate reduction in fungi**

(a) Mechanism I: 1 chromate binding by anionic absorption, 2 reduction by adjacent electron donor, and 3 release by electronic repulsion. (b) Mechanism II: 1 reduction by contacting with electron donor and 2 release by electronic repulsion. (c) Mechanism III: reduction by a chromate reductase enzymatic activity and release by electronic repulsion<sup>40</sup> Some of the fungi that remove chromium from tannery wastes are *Aspergillus*, *Nostoc* and *Cyanobacteria* etc. Any fungi can tolerate high concentration of potentially toxic metals and with other microbes; this may be correlated with decreased intracellular uptake or impermeability. A close relation between toxicity intracellular uptakes has been shown for Cu, Cd, Co<sup>2+</sup> and Zn<sup>2+</sup> in yeast *Saccharomyces cerevisiae*. Filamentous fungi can be profitably used in processes for heavy metals removal from wastewater due to their low cost and to the high ion exchange capacity of their cell walls. This property arises from the large density of functional groups present in the cell wall (carboxyl, hydroxyl, amine, phosphoryl, sulfhydryl), creating a negatively charged surface. The tolerance and accumulation of hexavalent

chromium by marine seaweed associated strains of *Aspergillus flavus* and *Aspergillus niger* was tested. They revealed that both the isolates accumulated more than 25% of the chromium supplied. *Aspergillus flavus* invariably exhibited higher accumulation potential. The chromium was bioremoved from tannery industries effluent by *Aspergillus oryzae*. *Aspergillus oryzae* can grow in different concentration of chromium 120-1080 mg/L. The maximum biomass growth and chromium removal rate is at pH 3.3. Hexavalent chromium was removed by a *Trichoderma inhamatum* fungal strain isolated from tannery effluent and the fungus exhibited a remarkable capacity to tolerate and completely reduced hexavalent chromium concentrations up to 2.43 mM. They indicated that the *Trichoderma inhamatum* fungal strain may have potential applications in bioremediation of hexavalent chromium contaminated wastewaters.

## ALGAE

Algae are photosynthetic organisms. Both growing and non-living algal cells are capable of removing Cr(VI).

The first step involved in the binding of Cr(VI) ions to algal species is binding to the cell surface. This process occurs rapidly and is independent of cellular metabolism. The second step of intracellular accumulation of a metal results from a simultaneous

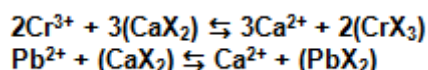
growth and surface biosorption effects. This step requires cell metabolic energy and is a much slower process. Table 5: summarised the list of some algae which help in adsorption of chromium in an effective manner

**Table 5**  
**Chromium adsorption capacity by different Algae**

Algae	Adsorption capacity (mg/g)	References
<i>Dunaliella</i> sp.	58	41
<i>Kappaphycus alvarezii</i>	0.86	42
<i>Spirulina platensis</i>	-	43
<i>Spirulina</i> sp.	90.91	44
<i>Pachymeniopsis</i> sp.	225	45

For *Chlorella miniata* and the green algae *Cladophora albida*, biosorption of Cr(VI) occurred first, followed by bioreduction of Cr(VI) and biosorption of Cr(III) onto the algal biomass. Among different types of biological material, algae have several advantages, i.e. they can be economically regenerated, the metal can potentially be recovered, less biological sludge is generated, algal material works at high efficiency in dilute effluents and has a large surface area to volume ratio. The main mechanism of heavy-metal cation sorption in algae biomass is the ion-exchange between the solution and the thallus. On the basis of the conducted studies, it was

found that the algae cell wall is built not only of organic compounds, but it also consists of, eg, Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup> ions. During cobalt biosorption by the alga *Ascophyllum nodosum*, the release of Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup> ions from the alga cells to the solution was observed. During sorption of Cr<sup>3+</sup> and Pb<sup>2+</sup> by the alga, an increase of calcium contamination in the solution, in which they were immersed, was observed. The process of ion exchange of heavy metals with Ca<sup>2+</sup> (CaX<sub>2</sub>) ions was described by the authors, by means of corresponding stoichiometric equations.

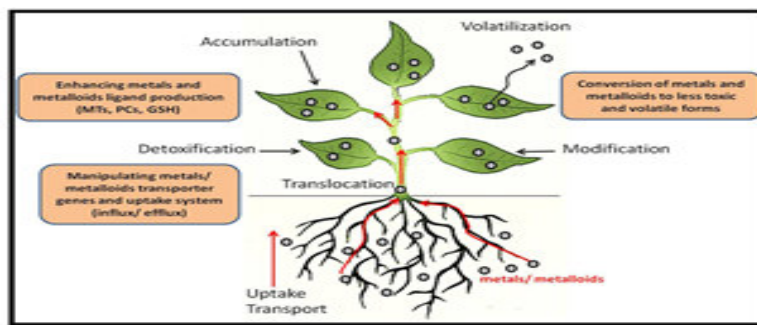


The process of ion exchange is a basic mechanism during heavy-metal sorption in the algae-solution system.<sup>46</sup>

## PLANTS

Cr is a toxic, nonessential element to plants; hence, they do not possess specific mechanisms for its uptake. Therefore the uptake of this heavy metal is through carriers used for the uptake of essential metals for plant metabolism. The pathway of Cr (VI) transport is an active mechanism involving carriers of essential anions such as sulphate. Fe, S and P are also known to compete with Cr for carrier binding. The first interaction Cr has with a plant is during its uptake process. Cr (VI) can easily cross biological membranes and has high oxidizing capacity, generating reactive oxygen species (ROS) which might induce oxidative stress. Cr may affect plant photosynthesis leading to decrease in productivity and ultimately to death. Exposure to Cr (VI) induced a reduction of both chloroplast auto fluorescence and volume in pea plants. Moreover, both Cr (III) and Cr (VI) can cause ultrastructural changes in the chloroplasts leading to inhibition of photosynthesis. Cr accumulates mainly in roots and shoots; however roots accumulate the major part, being usually only a small part translocated to the shoots.<sup>47</sup> In pea plants exposed to Cr there was an increase in concentration of

Cr in different parts of the plant with the increase of Cr supply. Accumulation of Cr in the different parts of the plant was in the following order roots > stem > leaves > seed. *Amaranthus viridis* L. were hydroponically grown and studied under different concentrations of Cr (VI) and it was found that Cr was accumulated primarily in roots. *Lolium perenne* grown in the presence of 500µM of Cr (VI) showed that roots accumulated 10 times more Cr than leaves. Spinach (*Spinacia oleracea* L. cv. "Banarasi") grown in the presence of Cr(VI) showed more accumulation of Cr in the roots than in leaves and stem showed the least accumulation. Chromium is toxic for agronomic plants at about 0.5 to 5.0 mg/l in nutrient solution and 5 to 100 mgkg<sup>-1</sup> of available Cr in soil. The species found to accumulate Cr are largely exotic. The plant species, *Lepto spermum scoparium* (Myrtaceae) is an accumulator of Cr it showed up to 20,000 mgCrkg<sup>-1</sup> in the foliage ash when grown on serpentine soils. Few Cr hyper accumulator species have been identified to date. *Brassica juncea* has been found to be an excellent accumulator plant for Cr in soils. Other metals accumulated by it are Cd, Ni, Zn and Cu In phytoremediation, hyperaccumulator plants are used to extract and transform toxic metals as Cr, into nontoxic and immobile compounds. Figure 5 summarised the potential biotechnological strategies for phytoremediation.<sup>48</sup>



**Figure 5**  
**Potential biotechnological strategies for phytoremediation**

Toxic elements can be mobilized and transported (influx) into roots through plasma membrane transporters. They can then be transported (efflux) out of the roots into the xylem and translocated into the shoots. At this stage, plant tolerance to toxic elements may be enhanced through manipulation of influx/efflux transporters or by increasing the level so ligands/chelators. Volatilization of the toxic elements can be achieved through enzymes that modify these toxic elements. Chelators or efflux transporters can also be used to export the toxic elements out of the cytosol and into vacuoles or the cell wall. Cr hyper accumulator plants can accumulate  $>1,000 \text{ mg Cr kg}^{-1}$  (DW), in plant leaves. These plants can tolerate metals through chelation with appropriate high-affinity ligands, biotransformation with reductants and compartmentalization in the cytoplasm or in the vacuole. The ability to uptake Cr from the soil by different organs of *Populus alba* and *Morus alba*. Leaves accumulated higher levels of Cr than stems or roots. However, neither *P. alba* nor *M. alba* showed potential of Cr phytostabilization, since presented translocation factor (TF)  $> 1$  and root bioconcentration factor (BCF)  $< 1$ ; also these plants are not suitable for phytoextraction as they presented a BCF  $< 1$ . *Ipomoea aquatica* is a chromium hyper accumulator that shows no toxicity symptoms when exposed to high levels of Cr (VI). Up to  $28 \text{ mg L}^{-1} \text{ Cr (VI)}$ , *I. aquatica* exhibits uniform absorption characteristics showing over 75% removal of added Cr (VI). Over 90% Cr (VI) is accumulated in stems and leaves i.e. aerial regions. The activity of antioxidant enzymes, namely, peroxidase, catalase (CAT), glucose-6-phosphate dehydrogenase and superoxide dismutase (SOD) increased in case of Cr-sensitive of mungbean exposed to different Cr concentrations. However, the level of antioxidant enzymes decreased in Cr-tolerant cultivars. SOD and CAT activities decreased in *T. aestivum* L. grown in the presence of  $\text{K}_2\text{Cr}_2\text{O}_7$  in roots and shoots. CAT activity also decreased in *A. viridis* L. exposed to Cr(VI) but an increase in SOD and guaiacol peroxidase (POX) activity was observed with increase of Cr(VI) concentration. Under chromium treatment, a decrease in both  $\alpha$  and  $\beta$ -amylase have been reported, which are one of the important factors for germination inhibition in many plants in view of the impaired supply of sugar to developing embryo axis. Chromium can affect roots of plants causing wilting and plasmolysis in root cells. Chromium can also inhibit the Hill reaction affecting both the dark and light reaction. Like other heavy metals, chromium can induce degradation of carotenoids in

plants. Chromium can degrade proteins and can result in the inhibition of nitrate reductase (NR) activity. Chromium is toxic to plants and does not play any role in plant metabolism. Accumulation of chromium by plants can reduce growth, induce chlorosis in young leaves, reduce pigment content, alter enzymatic function, damage root cells and cause ultra structural modifications of the chloroplast and cell membrane. Chromium toxicity can reduce seed germination and radical growth in plants. Cr (VI) is more phytotoxic than Cr (III) and retards growth, reduces the number of palisade and spongy parenchyma cells in leaves and increases the number of vacuoles and electron dense material along the walls of xylem and phloem. As a consequence of wide range of abiotic stresses including heavy metals, toxic reactive oxygen species (ROS) like  $\text{H}_2\text{O}_2$ ,  $\text{O}_2^-$ ,  $\text{OH}^-$  etc. are produced. The Cr (VI) form is toxic even in small amounts. It diffuses through the epidermis and is readily reduced to Cr (III) by gastric fluids, extracellular and intra-cellular low molecular weight molecules and proteins. The Cr (III) thus formed interacts with nuclear enzymes, protein nucleotide and DNA. This constitutes for the mutagenic and carcinogenic activity of Cr (VI). Through the food chains, chromium can be deposited in a living body part which at a certain size can cause toxicity.<sup>49</sup>

## GENETICALLY ENGINEERED MICROORGANISMS (GEM)

There are numerous approaches for increasing the efficiency of bacterial bioremediation. The first method is to promote bacterial growth by providing nutrients that favor a specific species, which allows it to compete with the natural bacteria present in the environment. This approach is called biostimulation. The second method is to introduce specific competent strains or consortia of microorganisms. This approach is named bioaugmentation. Another approach is to genetically engineer microorganisms to enhance their removal abilities. Genetic and protein engineering of suitable enzymes can improve bacterial bioremediation. ChrR were described as a dimeric flavoprotein that catalyzes the reduction of Cr(VI) optimally at  $70^\circ \text{C}$ . An open reading frame, yieF, on the *E. coli* chromosome with no assigned function was found to have a high homology to chrR. This gene was cloned and the encoded protein, YieF, showed maximum reduction of Cr(VI) at  $35^\circ \text{C}$ . Engineered bacteria to produce trehalose and found that they then reduced  $1 \text{ mM Cr(VI)}$  to Cr(III), whereas wild

type cells were only able to reduce half that amount. They concluded that by providing bacteria with a biochemical defense against the side-effects of chromate, reduction may be a new approach for cleaning up sites that are contaminated with high levels of chromate.<sup>50</sup> Transgenic approaches enhanced the heavy metal specificity and binding capacity of microalgae for efficient heavy metal, phytoremediation of contaminated wastewaters and sediments. The transgenic strategies include over expression of enzymes whose metabolic products ameliorate the effects of heavy metal-induced stress and the expression of high-affinity, heavy-metal binding proteins on the surface and in the cytoplasm of transgenic cells. Genetic engineering may also be utilized for more comprehension of the genetic basis of Cr(VI) resistance and its reduction. Using plasmid transfer and curing studies, both chromate resistance and reduction were plasmid mediated and *Bacillus brevis* harbored a stable 18 kb plasmid DNA.<sup>51</sup> GEMs may have higher activity in transforming metals. However, there is considerable controversy surrounding the release of such GEMs into the environment. Therefore, field testing of these organisms must be delayed until the human and environmental acceptability can be assured. Although this issue has been addressed by many regulatory agencies and scientists, no single set of guidelines with universal acceptance is presently available.

## CONCLUSION

Chromium (Cr), especially Cr (VI) is of particular environmental concern owing to its high solubility,

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## CONFLICT OF INTEREST

Conflict of interest declared none.

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