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BIOREMEDIATION OF CHROMIUM CONTAMINATION- A REVIEW

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Abstract :

Chromium (Cr) is a hard steel gray compound with oxidation state ranging from +2 to +6Cr but commonly exist as trivalent chromium Cr(III) and hexavalent chromium Cr(VI). Chromium is widely used in tannery, metallurgical and chemical industry. Cr (III) is an essential dietary nutrient and Cr (VI) is the toxic form of Cr, approximately 10 to 100 times more toxic than Cr(III) . The United States Environmental Protection Agency has identified $Cr6^+$ as one of the 17 chemicals posing the greatest threat to humans. It is considered to be one of the top 20 contaminants that need to be treated. According to CPCB the permissible limit of Cr (VI) in water to be discharged is 2 mg/L. But due to anthropological activity, large amount of chromium is discharged into soil and water resources which make it unfit for further usage. In India, 2,000 – 3,000 tons of elemental chromium escapes to the environment. This chromium released into the environment is treated using physical and chemical methods like adsorption, capping, soil washing, chemical precipitation etc. But this causes secondary pollution. This problem can be overcome by Bioremediation process which uses plants like hyperaccumulators, microbes for the treatment of contaminants. Though, it is time consuming this helps in complete restoration. This review focuses on the bioremediation of chromium by plants and microbes like bacteria, and fungus.

Keywords : *Bioremediation, hexavalent chromium, hyperaccumulators, microbes*

1. INTODUCTION:

The environment we live is composed of biotic and abiotic factors. These factors are polluted and contaminated by industrialization and large scale extraction of natural resource for human use. The pollutants that are emitted in to the environment are of two types: organic and inorganic. The inorganic pollutant that leads to threat to environment are heavy metal contamination. Environmental pollution from hazardous metals and minerals can arise from natural as well as anthropogenic sources. Natural sources are: seepage from rocks into water, volcanic activity, forest fires etc. In anthropogenic activity the pollution occurs both at the level of industrial production as well as end use of the products and run-off[1]. From the research conducted by Blacksmith Institute, it estimates that close to 125 million people are at risk from industrial pollution worldwide [2]. Based on data available for 976 National Priorities List (NPL) the contaminated sites in the year 1982–2003 given by US EPA is given in Fig 1.1[3].The major hazardous metals of concern for India in terms of their environmental load and health effects are lead, mercury, chromium, cadmium, copper and aluminium. These metals exist in soil leads to severe threat and needs to be treated. Data of CPCB show that Gujarat, Maharashtra and Andhra Pradesh contribute to 80% of hazardous waste in India [4].

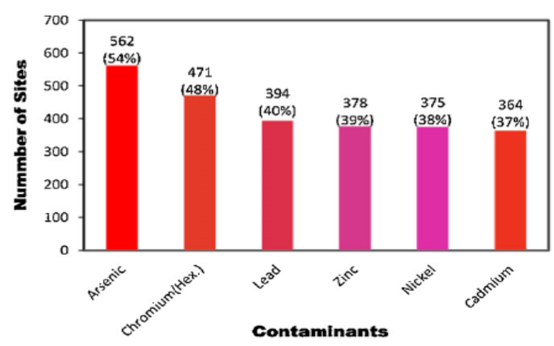

Fig 1.1: Frequencies of common contaminated sites at NPL sites

One of the heavy metal that causes potent threats to the environment is Chromium. Chromium is a hard steel-gray metal that is highly resistant to oxidation, even at high temperatures. It is the sixth most abundant element in the earth's crust, where it is combined with iron and oxygen in the form of chromite ore. Chromium

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is widely distributed in the earth's crust. It is a transition element located in the group VI-B and is the first element of the periodic table with a ground-state electronic configuration of Cr 3d54s1 [5]. Chromium has a wide application in applications in metallurgy, staining glass, anodizing aluminium, organic synthesis, leather tanning and wood preserving industries [6]. It can exist in oxidation states of +2 to +6Cr but exists primarily in two different oxidation states, hexavalent (Cr VI) and trivalent (Cr III). Cr(VI) is often found as chromate ($CrO_4{}^{2-}$), although it is typically in pH-dependent equilibrium with other forms including dichromate, and it is considered more soluble and more mobile than Cr(III). Cr(VI) is far more mobile than Cr(III) and more difficult to remove from water. The EPA classifies Cr(VI) as a known human carcinogen via inhalation, but classify Cr(III) as not known to cause cancer. The United States Environmental Protection Agency (US EPA) has identified Cr6+ as one of the 17 chemicals posing the greatest threat to humans[7]. The chromium (VI) can be made less toxic by converting it to Cr(III). This paper focuses the process and methods of bioremediation that were carried out to remediate the chromium contaminated soils, sludge, water and wastewater in detail.

2.CHROMIUM IN THE ENVIRONMENT:

Chromium is found in all phases of the environment, including air, water and soil. Naturally occurring in soil, Cr ranges from 10 to 50 mg/ kg depending on the parental material. In fresh water, Cr concentrations generally range from 0.1 to 117 µg /L, whereas values for seawater range from 0.2 to 50 µg/ L. Cr concentration varies widely in the atmosphere, from background concentrations of 5.0×10^{-6}– 1.2×10^{-3}µg/m^3 in air samples. Cr (III) in the air does not undergo any reaction. Cr (VI) in the air eventually reacts with dust particles or other pollutants to form Cr (III). However, the exact nature of such atmospheric reactions has not been studied extensively. The general population is exposed to chromium by eating food or food supplements, drinking water, and inhaling air that contain chromium. The mean daily dietary intake of chromium from air, water, and food is estimated to be <0.2-0.4, 2.0, and 60 micrograms, respectively. [8].

2.1 Standards for Chromium

According to US EPA, 0.1 milligrams per liter (mg/L) or 100 parts per billion (ppb) for total chromium, which includes all forms of chromium including Cr(VI) is permissible. The current federal drinking water standard for total chromium is 0.1 mg/L or 100 ppb. Cr(VI) and Cr(III) are covered under the total chromium drinking water standard because these forms of chromium can convert back and forth in water and in the human body, depending on environmental conditions. The concentration of Cr(VI) and total Cr prescribed under Indian Standards Specification for Drinking water quality is 0.05 mg/L. The Cr concentration in industries ranges from 2 to 5 g/L in the effluents. This imparts yellow color to the water making it unfit for drinking and other applications.

2.2 Chromium as an Environmental Contaminant

Chromium (Cr) is the chief heavy metal contaminant found in the tannery effluent accounting for 40% of the total industrial use. Cr used by the leather industry to tan hides is not taken up completely by leather and relatively large amounts escapes into the effluent [9]. Chromite ore processing industries and sewage treatment plants from industrial and residential sources discharge substantial amounts of Cr. Hexavalent chromium compounds that are used in industry activities have led to the widespread contamination in the environment and have increased its bioavailability and immobility. In India, about 2000–32,000 tons of elemental Cr annually escapes into the environment from tanning industries. Tamil Nadu is a leading finished leather producer in India. Over 250 tanneries had been functioning in the past decade and were actively involved in chrome tanning processes. Presently there are 6000 tanneries out of which a sizable percentage is actively involved in the chrome tanning process. Significant concentration of total and hexavalent chromium is observed in many wells located in the close vicinity of some of the industries in the industrial area of Ranipet, in Tamil Nadu which was considered as one of the “Critically polluted area” and an in-situ bioremediation (biotransformation) option was recommended by NEERI for implementation of bio-remediation of contaminated ground water [10].

2.3. Remediation of Chromium contaminated sites

Cr (VI) compounds are strong oxidants and can be quite readily reduced to Cr(III) forms in the presence of electron donors like organic matter and inorganic. Various physical, chemical and biological methods are used for remediation. Chemical process includes chemical like gaseous sulphurdioxide, sodium sulfite, sodium metabisulphite, ferrous sulphate, barium sulphite, lime and limestone for reduction of Cr (VI) to Cr(III)[11, 12]. The physical method involves treatment of contaminated system using physicochemical properties of the substances used for remediation. This involves techniques like Adsorption, capping, Electrokinetic method,Membrane Filtration, Granular Activated Carbon, photocatalysis and soil washing for remediation [13-16].Bioremediation is carried out by the action of microbes and plants which helps to transfer Cr(VI) to Cr(III) and makes it stable. Physical and chemical process have disadvantages like high operational cost, produces secondary contamination, high energy consumption which needs a cost effective approach for remediating such polluted environment. Bioremediation is a promising technology for remediation of such area and helps in complete restoration of land.

3. PRINCIPLE IN CHROMIUM REDUCTION:

Chromate is actively transported across biological membranes in both prokaryotes [11] and eukaryotes. Once inside the microbial cells, Cr(VI) is reduced to Cr(III) probably via the unstable Cr(V) and Cr(IV) states. This is done by two reduction process namely direct and indirect process. In plants, the metals get accumulated on the parts of plants by process of phytoaccumulation. Micro organisms both aerobic and anaerobic involves in conversion of Cr(VI) to harmless form. In the presence of oxygen, bacterial Cr^{6+} reduction commonly occurs as a two- or three-step process with Cr^{6+} initially reduced to the short-lived intermediates Cr^{5+} and/or Cr^{4+} which is further reduced to Cr^{3+} was spontaneous or enzyme mediated. NADH, NADPH and electron from the endogenous reserve are implicated as electron donors in the Cr^{6+} reduction process which is reduced in the presence of Cr^{6+} reductase ChrR.[17].In the absence of oxygen, Cr^{6+} 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. The cytochrome families (e.g., cytochrome b and cytochrome c) were frequently shown to be involved in the enzymatic anaerobic Cr^{6+} reduction. The widespread occurrence of anaerobes possessing Cr^{6+}-reducing activities offers great potential for in situ bioremediation of Cr^{6+}-contaminated sites [18]. Hyper accumulators are plants that are able to extract wide range of metals and to concentrate in the upper part of the plant [19-21]. These plants produce certain enzyme that helps in the conversion of hexavalent chromium to trivalent form and stores it in the vacuoles. Therefore, plants and microbes can be used in effective way to treat the chromium contaminated sites.

4. BIOREMEDIATION OF CHROMIUM:

4.1 Using Bacteria:

Hexavalent Chromium is toxic to bacteria that are present in the contaminated in soil or waste water. The bacterial species are able to grow in the toxic conditions and are generally assumed to be tolerant/ resistant to chromium [22]. *Pseudomonas sp.* was the first hexavalent chromium to be indentified from waste water [23]. Resistance is defined as the ability of a microorganism to survive toxic effects of metal exposure by means of a detoxification mechanism produced in direct response to the metal species concerned. Tolerance is defined as "the ability of a microorganism to survive metal toxicity by means of intrinsic properties and or environmental modification of toxicity [24]. The reduction of Cr (VI) can be identified by using Di Phenyl Carbazide and read at 560nm. Bacterial strain that are isolated from electroplating industry showed higher reduction rate compared with the procured one [25]. *Bacillus coagulans* isolated from electroplating industry was capable of reducing Cr(VI) by using soluble enzyme and utilizing malate as external electron donor. The biological reduction of Cr (VI) was found similar to Sulphate reduction process. The ability of sulphate-reducing bacterial bioflims [26] to reduce hexavalent chromium (Cr(VI)) to insoluble Cr(III) using lactose as carbon source was investigated. Almost 88% of 500µmol/L was removed by the bacteria within 48 hours. 80% of Cr was precipitated by bioflim. This study shows that the sulphate reducing bacteria have the ability to reduce chromium in the soil. Sulphate reducing bacteria capable of reducing Cr(VI) under anaerobic condition was isolated from metal contaminated marine sediments of Tokwawan, Hong Kong SAR [27]. The enrichment consortium almost completely (98.5%) reduced 0.6 mM Cr(VI) in 168h. *Bravibacterium* sp. CrT-13, CrT-11, CrT-12, CrT-14 was isolated from industrial wastewater had ability to reduce Cr(VI) at various concentration. They have wide pH (5 to 9) and temperature (24 to 42°C) growth range. They exhibited multiple metals (Ni, Zn, Mn, Cu, Co, Pb) and antibiotics like streptomycin, ampicillin, tetracycline, kanamycin, chloramphenicol resistances [28]. The bacteria isolated from highly contaminated of Tamil Nadu Chromates and Chemicals Limited (TCCL) premises, Ranipet, Tamil Nadu, India showed higher Cr (VI) remediation efficiency under aerobic and anaerobic action. The bacterial concentration of 15±1.0 mg/g of soil and 50 mg of molasses/g of soil as carbon source were required for the maximum Cr (VI) reduction. The bioreactor operated at these conditions could reduce entire 5.6 mg Cr(VI)/g of soil) in 20 days[29]. The bacteria *Providencia sp.* isolated from the contaminated site of chemical industry was found to reduce Cr (VI) to 100% at a concentration ranging from 100–300 mg/l and 99.31% at a concentration of 400 mg/l, pH 7 and temperature 37°C. It also showed cross metal resistance for Pb,Co, Hg,Zn,. This study showed that soluble fraction of bacteria was able to reduce Cr(VI) [30]. The strain identified from a steel-alloy factory in Hunan Province, China. *Pannonibacter phragmitetus* sp[31] showed that when sufficient nutrients were amended into the contaminated soils, total Cr (VI) concentration declined from the initial value of 462.8 to 10mg/kg at 10 days and the removal rate was 97.8%. Water soluble Cr (VI) decreased from the initial concentration of 383.8 to 1.7mg/ kg. Exchangeable Cr (VI) and carbonates-bound Cr (VI) were removed by 92.6% and 82.4%, respectively. Bacterial consortia also help in removal of Chromium from soil and wastewater. *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Saccharomyces cerevisiae* in consortia and in their immobilized forms reduces 770 mg/l of Cr(VI) before remediation, to 5.2 – 5.7 mg/L. The best activity was observed by *S. cerevisiae* - *P. aeruginosa* consortia, followed by immobilized beads of *S. cerevisiae* and *S. cerevisiae* - *B.subtilis* consortia [32]. The microbial ability to detoxify repeatedly and continuously in a non-modified medium were studied. Results showed that consortia of indigenous bacteria are resistant to greater than 200 mg/L Cr(VI). . The consortia was able to reduce Cr(VI) at various concentrations from 100 to 300 mg/L in a wide range of pH, using different electron donors like [33].

4.2 Bioremediation using fungus:

Fungus acts as Bioabsorptive material to remove hexavalent chroimium. 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.

Biosorption of the chromium ion Cr(VI) onto the cell surface of *Trichoderma* fungal[34] species in aerobic condition was investigated. The maximum efficiency of 97.39% was obtained at 5.5pH. The results of FT-IR analysis suggested that the chromium binding sites on the fungal cell surface were most likely carboxyl and amine groups. The absorption isotherm studied fit to Freundlich models. Acidic pH decreases the biosorption efficiency of fungus. The study performed using *Aspergillus* and *Penicillium* sp. showed removal efficiency of 80%. When pH decreases to pH 3, the removal efficiency decreases[34]. The Cr(VI) reduction process catalyzed by *Hypocrea tawa* was characterized in batch cultures conducted at initial Cr(VI) concentrations ranging from 0.59 to 4.13 mM. The fungus showed a remarkable capacity to completely reduce very high concentrations of Cr(VI) under aerobic conditions. Higher volumetric and specific rates as well as a greater capacity (77 mg Cr(VI)/g biomass) to reduce Cr(VI) were obtained with higher initial Cr(VI) concentrations, which suggests that the fungal strain could be potentially useful for detoxification of Cr(VI)-laden wastewaters [36]. Biosorbent matrix was developed using *Carica papaya* plant dry stem to colonize the fungal strain *Fusarium oxysporum* to facilitate bioabsorption process. Maximum efficiency of chromium removal by biosorption upto 90 per cent was achieved at the end of 5th day of incubation. FTIR spectroscopic analysis revealed that the main functional groups involved in the uptake of chromium by *Fusarium oxysporium* strain were carbonyl, carboxyl, amino and hydroxyl groups[37]. The ability of yeast to reduce hexavalent chromium was studied. The in vitro reduction of hexavalent chromium using Crude Chromate Reductase (CChR) of *Pichia jadinii*M9 and *Pichia anomala* M10, two yeasts isolated from a textile-dye factory effluent. CChRs were characterized based on optimal temperature, pH, use of electron donors, metal ions and initial Cr(VI) concentration in the reaction mixture [38].

4.3 Bioremediation using plants:

The translocation and accumulation of Cr inside the plant depends on the oxidation state of the supply, the concentration of Cr in the media, as well as on the plant species [39,40]. Several components like NAD(P)H, FADH2, several pentoses, glutathione, vitamins C and B12, cytochrome P-450, and the mitochondrial respiratory chain helps in conversion of Cr(VI) [41]. Callitriche cophocarpa (water-starwort) an aquatic widespread was found to be an excellent chromium accumulator. after the prolonged time of culture (up to ca.3 weeks) two-fold increase in Cr accumulation in these shoots accompanying two-fold increase in their biomass was found. In this case, the concentration of Cr in fresh shoots was up to 27 times greater than in medium. The accumulation of Cr might be performed via Cr(III) adsorption following Cr(VI) reduction. sulphate transporters carries Cr(VI) to the cell and converts it to Cr(III) [42]. High doses of Cr caused toxic effects in plants, as evident by a reduction in photosynthetic nitrate reductase activity and the contents of chlorophyll and soluble protein . Since plants lack a specific Cr-transport system, mineral nutrient contents also changed due to Cr toxicity [43]. Research shows that addition of chelating agents like EDTA, vermicompost have increased the uptake of chromium by the plants. This is because the chelating agents will have functional group that helps in absorption and conversion of chromium. In Sorghum plant, addition of vermicompost to the contaminated soil improves the biomass of the plants thus making room for more bioaccumulation [44]. *Pterocarpus indicus* and *Jatropha curcas* plants were able to remediate hexavalent chromium polluted soil of less than 90 mg/ Kg [45]. *Penisetum purpureum, Brachiaria decumbens* and *Phragmites australis* were grown hydroponically to determine chromium removal rate [46]. Studies shows that flowering crops like *Jasminum sambac*, *Jasminum grandiflorum*, *Polianthus tuberosa* and *Nerium oleander* have the ability to accumulate chromium of higher concentration[47]. *Pistia stratiotes* showed accumulation of chromium in its tissue when grown at contaminated water[48]. sunn hemp[49] and Zea mays [50] along with microbes like *Achromobacter* sp and *Streptomyces* shows higher accumulation of Chromium in its tissue. This shows that the microbes converts the Cr(VI) to less harmful form and makes the plants to accumulate higher amount in its parts.

4. CONCLUSION:

This study is focused on the chromium production, presence of chromium in the environment and hexavalent chromium contamination in various part of India and funding granted to remediate Chromium contaminated site in Ranipet, Tamilnadu. Though various physical and chemical process are available, bioremediation alone can only make the permanent solution for remediation. This study shows that the bioremediation process helps in full remediation of hexavalent chromium in the environment. The bacteria isolated from the native site can help in bioremediation by following aerobic and anaerobic degradation mechanism. Various fungus can be used as a natural biosorbents to absorb hexavalent chromium in the environment. The use of various hyperaccumulators, flowering plants, grasses, foddercrops and ornamental plants for remediation of chromium contaminated site have been discussed. Extensive research have to made in this field inorder to get biological products which helps in large scale remediation of land, water and wastewater.

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