Jr.of Industrial Polluction Control 37(10)(2021) pp 1-7
www.icontrolpollluction.com
Review Article

PHYTOREMEDIATION OF CHROMIUM THROUGH PLANT-SOIL-MICROBE INTERACTION: A REVIEW

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(Received 03 September, 2021; accepted 17 September, 2021)

Key words: Chromium, Phytoremediation, Plant, Microbe, Soil, Toxicity, Detoxification.

ABSTRACT

The use of chromium has been increased since last two-three decades due to its high demand in different industries. The anthropogenic activities and waste products from industries like steel, leather, tannery and dye production has released the chromium into soil and water bodies causes the environmental pollution. Chromium mostly occurs in two stables forms i.e. Cr^{6+} and Cr^{3+}. The hexavalent form of chromium is more toxic, mutagenic and carcinogenic as compared to its trivalent form which is less toxic and insoluble. The conversion of hexavalent chromium to trivalent is a basic process of detoxification. Behaviour of chromium in soil and its transfer/accumulation in plants and different plant parts vary with plant type, soil physio-chemical properties and its chemical form. Microbes present in the soil plays an important role in speciation and behaviour of Cr in soil. For the uptake of Chromium, there is no specific transporter used by plants and it is relay on the specific and non-specific transporter channels of essential ions. The accumulation of chromium is mostly seen in root tissues region of plants with a limited translocation to shoot parts. The chromium toxicity had adverse effects on plants physiological, morphological and biochemical processes. Apart from these effects, chromium also affects the plant growth, nutrient uptake and photosynthesis process, induces enhanced generation of reactive oxygen species and causes lipid peroxidation and alteration of antioxidant activities. Plants have various defence mechanisms against Cr toxicity via anti-oxidative enzymes such as complexation by organic ligands, compartmentation into the vacuole and scavenging ROS. The consumption of Cr-contaminated food can cause serious health hazards to humans by inducing severe clinical conditions. In order to achieve this, use of plants and microbes and their interaction through phytoremediation process has gained popularity in recent times. Microorganisms are omnipresent in the environment. Soil rhizosphere, a nutrient-rich bio-resource is the habitat of versatile microorganisms. Here the microbes plays an important role by their biological activities that are vital to retain soil fertility and plant growth by decomposition, assisting nitrogen fixation, mineralization and immobilization of different macro/micro nutrients. In addition, soil microbes play an important role by governing the biogeochemical behaviour of heavy metals in soil-plant system.

INTRODUCTION

The industrial development and increasing anthropogenic activity has increased the presence of heavy metals in the environment during past two- three decades. It is also raised the concern of countries worldwide. Heavy metal contamination is a serious threat to the environment and use of these metals has become a challenge for life on earth. Since the industrial revolution, the use of chromium has been rapidly increased. The most prevalent form of chromium is chromate Cr (VI) and it is present in solid/liquid waste due to anthropogenic activities. Chromium is extensively used in chromeplating resistant alloys formation (stainless steel), dye productions and leather tanneries. Chromium exists in different oxidation states due to its variation in valence cell electronic configuration ranging from -4 to +6, but hexavalent, Cr(VI) and trivalent, Cr(III) are the most stable and common forms available in soil (Fendorf, et al., 1995). Apart from its toxicity, Hexavalent chromium Cr(VI) is soluble in water with full pH range makes it mobile and biologically available to the ecosystem, while trivalent chromium Cr(III) is insoluble in slightly acidic and alkaline pH thus get adsorbed on the soil surface or precipitate as chromium hydroxide. Due to its carcinogenic nature, the U.S. Environmental Protection Agency (EPA) has classified chromium under Group 'A' human carcinogen and is one of the main pollutants.

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LITERATURE REVIEW

The possible mobile forms of chromium in soils are CrO_4^{2-} and $HCrO_4^-$ ions. Behaviour of chromium in soil and its transfer/accumulation in plants and different plant parts vary with plant type, soil physico-chemical properties and its chemical form. Chromium in soil can take up by plants with non-specific transporter/carriers and also it can easily leach out into the deeper soil layers leading to ground and surface water pollution. Hence, the removal /reduction of Cr(VI) in soil and industrial solid waste were important. There are various physico-chemical methods used for the treatment of this hazards contaminant such as adsorption, precipitation, reduction, ion exchange and electro-dialysis or land filling. These remediation methods are expensive as it is economically viable only at high or moderate concentrations and it has some disadvantages like high reagent and energy consumption, incomplete removal of metal and also causes ground water contamination with toxic sludge wastes disposal. To overcome these problems, bioremediation possibly have wider implications to remediate lower concentration of heavy metal. Now days, Researchers have great interest in bioremediation technology to remove, stabilize or recover heavy metals in contaminated soil and water. Phytoremediation technology has been reported to be more effective for the removal of soluble toxic forms of heavy metals from dilute solution and microbe-based technologies can provide better alternative in comparison to the conventional techniques of heavy metal removal/recovery. Several microorganisms had the capability of reducing hexavalent form of Cr(VI) to relatively less toxic trivalent form of Cr(III), plant-microbe interaction and phytoremediation gives an immense opportunity for technology development to detoxify Cr(VI)-contaminated soils as an alternative to the existing physico-chemical processes. In this review, we had highlighted about chromium and its toxic effects on plants, phytoremediation technology and its types, plant-microbe interaction, molecular mechanism (plant and microbes)and some of the important efforts to remediate potential Cr(VI) using plant-microbe interaction study for phytoremediation of soils/effluent sludge/mine and waste water.

Chromium and its Toxic Effects on Plants

Chromium (Cr) is a d-block element having an atomic number 24. The silver colour hard metal has a molecular weight of 51.1 u with density 7.19 g/cm^3. It is the 7th most abundant element and this metal has been ranked 7th among the top 20 hazardous substances by the agency for toxic substances and disease registry. Cr found in nature in several oxidation states between (-2 to +6) among which the trivalent and the hexavalent form are the prevalent one. Chromium exploration is usually performed through the open cast mining method and so there is a chance for the transformation of the comparatively lesser toxic form to the most toxic form i.e, trivalent to hexavalent form which is comparatively more stable and water soluble. Hence it is usually leaches out of the soil easily and contaminates the surrounding environment. The International Agency for Research on Cancer and the National Toxicology Program has categorized Chromium as no.1 carcinogen agent and that why, this metal requires in-depth monitoring as well as detailed understanding especially for soil-plant system in the environment.

Toxic effects of chromium on plants: The toxic nature of this metal is well-known for its harmful effects on plants and their development and growth. Chromium had adverse effects on biochemistry and physiological processes of plants and its exposure may induce toxic effects in biochemical processes like seed germination, root, shoot and leafs growth development.

Chromium stress on seed germination and root growth process is one of the first physiological effect. The germination of phaseolus vulgaris seed has inhibited by 90% at 0.5 mm conc. Cr (VI) reported by (Sharma, et al., 2016), in case of Avena sativa, Cr (VI) inhibits by 84% with 4000 mg/kg of Cr (VI) (Lopez-Luna, et al., 2009). It also inhibits Echinochloa colona by 25% at 200 mM of Cr (VI) (Rout, et al., 2000) and medicago sativa by 23% at 40 mg/kg of Cr (VI) (Peralta et al., 2001). Eruca sativa inhibition occurs at 500 ppm Cr (VI) while triticum aestivum at 100 ppm. Significant variations are observed in Cr toxicity in different plants. The Cr stress sensitivity and tolerance towards seed germination possible due to the suppression activities of α and β amylase. This enzyme plays an important role in hydrolysis starch and supply sugar to the developing embryo. Toxic nature of chromium reduces sugar availability to the developing embryo by decreasing amylase activity, thereby inhibiting seed germination.

Chromium stress also affects the growth and development of secondary roots and lateral roots. Cr (VI) treatment inhibits the growth of root length and root hairs number in case of zea mays. Cr (VI) also reduces the cell division/ extension of cell cycle by interfering the uptake of water and nutrient (Sundaramoorthy, et al., 2010). Cr stress also results in decreasing the mitotic index in root tip cells of Amaranthus viridis reported.

The leaf of a plant is act as a morphological bioindicator under various stress condition. Under Cr(III) stress, the leaf shows reduced growth with wilted chlorotic in comparison to the control plants. Long term chromium (VI) stress causes decrease in total leaf area, chlorosis and necrosis to the older leaves as well as permanently wilted the plants (Chatterjee, et al., 2000). Dube also reported that, Cr toxicity reduces cell division and cell number in the leaves of watermelon plant (Dube et al., 2003).

Heavy metal also affects the photosynthesis processes like photophosphorylation, CO_2 fixation, enzymatic activities and electron transport in plants which ultimately leads to decrease in chlorophyll (a, b, total) and carotenoid contents of plants (Sharma, et al., 2016). Treatment of chromium can changes in thylakoid arrangement in chloroplast, distortion in the chloroplastidic membrane and also inhibits the hill reaction (light and dark reactions). Chromium toxicity can redox the Fe and Cu carriers channel to inhibit electron transport or Cr can also binds to cytochrome (heme group) by blocking the electron transport. The high oxidative potential of Cr (VI) can produce ROS to reduce photosynthesis and serve as an alternative path for electrons. The metal-induced ROS production directly and indirectly interfering with photosynthesis process results slower plant growth and reduced pigment contents was experimentally demonstrated by Sharma. It is also reported that ROS altered the structure of pigment protein complex by three steps (a) protein degradation and destabilization of antenna complex, (b) substitution of Mg^{2+} with H^+ ions resulting in pheophytinization of the chlorophylls and (c) damage membranes of thylakoid.

The structural similarity of chromium to the essential ions needed for plant growth and development interfere plants nutrition uptake mechanism in a complex way. Previous studies shows the interference of chromium with essential ions/nutrients: S, P, Mn, Cu and Zn translocation in brassica oleracea, uptake of N, P and K in oryza sativa (Sundaramoorthy, et al., 2010), uptake of nutrients such as K, P, Fe, Mg, Ca and Mn in salsola kali and cocos nucifera and uptake of Fe, Cu, Zn and Mn ions by amaranthus viridis due to the competitive binding of chromium to other essential ion carriers, it decreases the uptake of essential nutrients needed for plant growth. Decrease in nutrient uptake can also decrease the H+ ATP case activity of plasma membrane induced by Cr (Shanker, et al., 2005). Long term exposure of plants to high conc. of Cr (VI) may alter the physiological binding sites of essential nutrients. According to vernay, there are some synergistic interactions in between chromium and essential nutrients like Cu, Ca, Mn, Mg (Vernay, et al., 2007).

The toxicity of chromium at genetic level has been reported in yeast and animals. The geno- toxicity studies shows changes in term of cross-linking in DNA strand (inter and intra), DNA- protein, breaking of DNA strand, changes in DNA transcription and replication function, changes genomic stability, altered the DNA repair mechanism and changing signalling pathways. Though there is critical significance of chromium toxicity, there is a lack of information and studies in plants in comparison to that in animals and human beings (Nickens, et al., 2010). Hexavalent form of chromium is highly considered as a carcinogenic and mutagenic pollutant. The toxic nature of chromium causes chromosomal disorders, improper cell cycle and cell division, reduces the efficiency of antioxidative enzymes and formation of micronuclei in plant cells.

The mutagenic, cytotoxic and genotoxic effects of Cr (VI) varies from organ to organ and plants to plants, so this gap need to be explored. This type of variations observed due to variation in intracellular contents of the particular chemical form (Shanker, et al., 2005), distribution pattern of Cr within cell compartments or by the differential ROS generation of cell organelles. Cr (VI) has higher mutagenic impact compared to Cr (III) in Bacillus subtilis cells reported.

According to shanker, the hexavalent chromium also affects the morphological changes in chromosome by enhancing chromosome stickiness frequency, chromosome and anaphase. Cr (VI) induction altered the ploidy level and dynamics of cell cycle in leaf cells and alteration in cell cycle was observed at G2/M phase along with polyploidy at both 2C and 4C levels in roots cells in Pisum sativum. Cr induction also increases the DNA damage in leaf and root cells was observed during comet assay and the degree of DNA damage also depends on the concentration of Cr and exposure time. It is also reported that Cr (VI) genotoxicity causes hypermethylation of DNA and increases DNA polymorphism in brassica napus. Kumari, et al. also reported the Cr genotoxicity using AFLP molecular marker in *arabidopsis thaliana*. Kumari et al. also reported that exposure of allium cepa to Cr (VI) enhances micronuclei formation and the mitotic index in root tips (Kumari, et al., 2016). Increased micronuclei formation is also an indication for clastogenicity. ROS generation can deregulate cell proliferation by interfering with Mitogenic-Activated Protein Kinase (MAPK).

The high concentration of chromium induced stress generates ROS and adversely affects the morphological and physiological process in plant. Enhanced production of ROS causes biochemical disorders by interacting with enzymes, proteins, lipids DNA resulting inactivation of enzymes and membrane leakage.

Cr Defence Mechanism of Plants

Plants have the ability to escape from different environmental stress by adopting various defence strategies against stress and tolerance towards different metals. The activation or suppression of antioxidant enzymes depends upon the ROS and plant type against the oxidative damage caused by metals. Plants developed different strategies against Cr toxicity includes: chelation of Cr with ligands with the help of phytochelatins, catalytic reduction of hexavalent Cr to trivalent Cr, sequestration of Cr in vacuoles and activation of antioxidant enzymes

(Shanker, et al., 2005). In order to defence the effect of oxidative stress induced by ROS, plants have developed an enzymatic complex mechanism with catalase (CAT), superoxide dismutase (SOD) and guaiacol peroxidase (POD) for scavenging of ROS.

There are different remediation technologies to remediate the heavy metals from the contaminated soil and water resources and it is shown in the Fig. 1.

In this review, we have mainly focused in the phytoremediation technology to remove the metal contaminants.

Phytoremediation

Phytoremediation is a plant-based remediation technology uses native or genetically modified plant species for restoration of contaminated soil and water resources. The primary reason for implementing this technology is a low-cost remediation process in comparison to the chemical and physical processes. Due to industrial emissions and anthropogenic activities the presence of heavy metals in urban areas, agricultural lands from fertilizers, pesticides, mining, sewage sludge, tannery effluents and electroplating industries are increased over the years (Wei, et al., 2010). The presence of heavy metals in the environment like arsenic, cadmium, chromium, iron, copper, etc., affects both the human health and the atmosphere (Alam, et al., 2013). The oxidative stress of heavy metals in living cells and biological macromolecules is mainly due to binding of metals to nuclear proteins and DNA. Fig. 2 Shows that the flowchart of various methods used in phytoremediation.

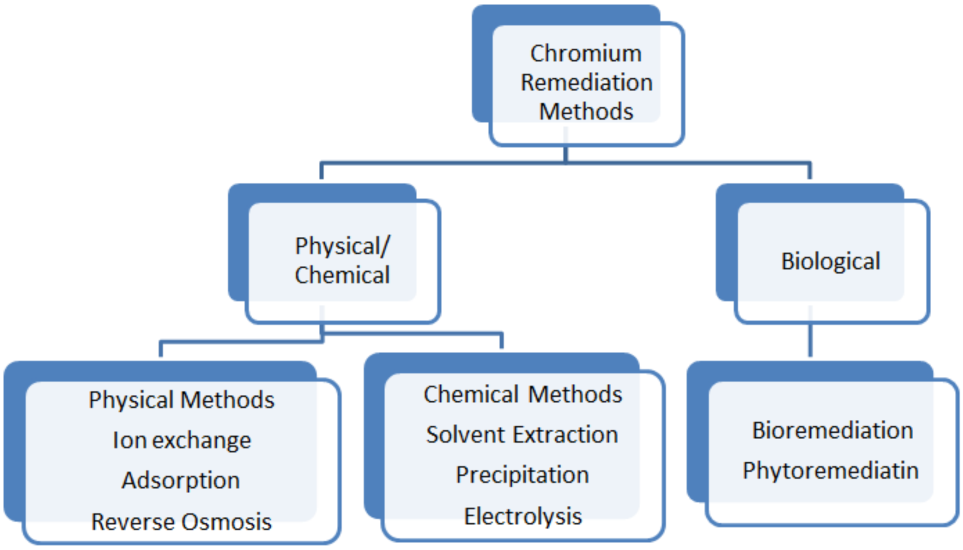

Fig. 1 Flowchart of various methods used in Cr remediation.

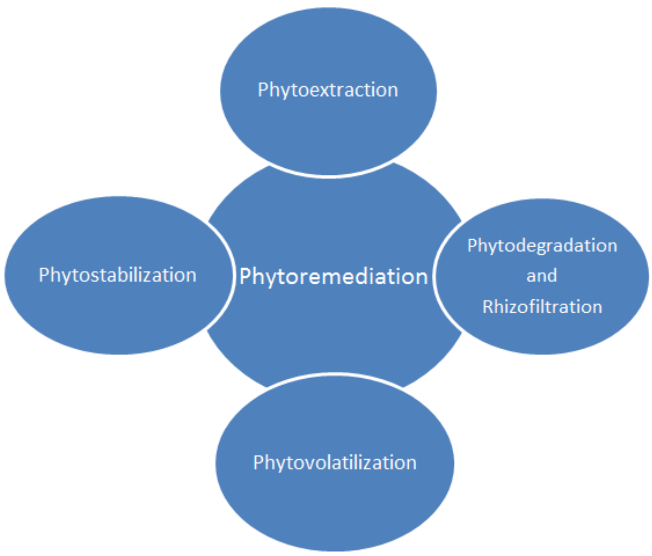

Fig. 2 Flowchart of various methods used in phytoremediation.

Phytoremediation has several advantages as compared to physico-chemical processes, as follows:

• It is an environmentally-friendly technology.

• It is a cost- effective technology (around 50% ~ 80% less costly than current methods or even less) and also has minimal engineering costs.

• It is useful for treating a wide range of environmental contaminants.

• There is minimal disruption of the environment and this can be applied both in situ and ex situ.

• There is the possibility of the recovery and re-use of valuable metals from post harvesting processes.

• It has also certain limitations. The limitations of this technology are:

• It is most effective only at the contamination sites with shallow contaminated soils and water (below 5 m depth).

• It is taking a long period of time and different climatic or seasonal conditions may interfere with metal accumulation and inhibit plant growth.

• Organic and inorganic contaminants may be toxic to plants and plants survival may be affected.

• The absorption of toxic contaminants by the plants may pose potential risks of transferring contaminants into the food chain.

Phytoaccumulation

This is a process in which plants absorb nutrients and water required for their growth along with the contaminants from contaminated sites. This method is widely used for remediation of metallic and radionuclide contaminants (Kamal, et al., 2004) as this technology required low initial investment and also has an opportunity to solve environmental problems, so there is a scope for commercialization of this technology. The researcher's experimental data shows various plant species have high accumulation potential for remediation purpose. The plants species having such type of accumulative potential are pistia stratiotes and spirodela polyrrhiza, mentha aquatic, ludwigina palustris and myriophyllum aquaticum (Harguinteguy, et al., 2013).

Phytostabilization

Phytostabilization is the process certain plant species were used to immobilize the contaminants at the site of contamination with the help of roots/root hairs, adsorption at root surface or precipitation in rhizosphere region of certain plant species. This process restricts the movement of the contaminants, prevents the entry of heavy metals into food chain and reduces the bioavailability. This method helps in re-vegetation at the contaminated sites having high metal concentrations (Regvar, et al., 2006). To restrict the movement of different contaminants from leaching into the groundwater by different carriers like wind, erosion and rain, the metal-tolerant plant species can be employed. The plant- microbe association enhance plant growth and increases the metal tolerance capacity of plants as well as minimize the metal uptake to shoot parts by reducing metal bioavailability in rhizospheric region. The microorganisms have different mechanism towards heavy metal resistance and these methods are as follows: (1) Prevent the entry non-essential metal by a permeability membrane or carriers molecules and kept the metal outside, (2) Attaching the toxic particles to polymers outside of the cell (3) Detoxification or chemical modification of more toxic metal into less toxic forms (Glick, et al., 2014).

Phytovolatilization

The uptake of contaminant by the plant, conversion into less toxic forms and releases them into the atmosphere by this process. Toxic metals like mercury and arsenic can be converted into volatile forms like mercuric oxide and dimethyl selenide respectively evaporated into the atmosphere. The volatilization of dimethyl selenide can be inhibited by the presence of sulphate and boron. This process is considered to be a permanent solution because the volatilized contaminant will not redeposit at the contaminated site. The remediated product cannot be used for other purposes like other remediation techniques because there is no trace of contaminant after volatization.

Typha latifolia L. an aquatic plant is used for phytovolatilization of selenium from the contaminated soil reported. Genetically modified *nicotiana tabacum* L. and *arabidopsis thaliana* L. have been used to volatize mercury with the help of mercuric reductase. The area with high population density and unusual weather pattern could facilitate the discharge of volatile substances, so this technique is not preferable for these areas.

Phytodegradation

In this method, the contaminants degraded into less toxic forms taken up by the plants and generally it occurs in two ways i.e. Plants metabolic process and enzymatic activity of the plants. The broken down of contaminants into simpler forms are used by the plant as nutrients for their growth. Pesticides, chemical solvents, organic and inorganic compounds can be degraded by this technique. Ethion present in the water hyacinth was reduced by ethion free culture solutions about 75–80% in root and 50–90% in stem. So, this plant can be used for the degradation of pollutants present in industrial wastewater as well as an economically efficient and alternative product for remediation. The phytodegradation process is affected by several factors such as uptake efficiency by the plan and concentration of pollutants in the soil. The efficiency of this process is mainly depends upon the phytochemical properties of the plants. The process can efficiently remove organic contaminants like benzene, ethyl benzene. Xylene toluene, chlorinated solvents and aliphatic hydrocarbons at shallow depths in soil(Arshad, et al., 2007).

Plant- Microbe Interaction

The microbes are omni present and in soil there are different types of microorganisms helps in plants growth, nitrogen fixation, nutrient uptake and useful activities. The plant growth promoting rhizobacteria are associated with plants which soil-borne bacteria are having the ability to enhance plant growth by different plant growth promoting mechanisms. The phytoremediation is mainly depends on several conditions such as plants rhizospheric activitity, metal tolerance and bioavailability. These factors greatly influence the efficiency of plants towards phytoremediation. The bioavailability of metal mainly depends on their chemical speciation. The PGPR strains can alter the nature of metal by producing various metabolic compounds like organic acids, biosurfactants, siderophores and they can change the nature by oxidation–reduction reactions and change their mobility by chelation process at rhizospheric region to enhance different phytoremediation efficiency of the plant. The microbial community at the rhizospheric region stimulating root proliferation to enhance plant growth, heavy metal tolerance and fitness of plant. PGPR can protect plants against pathogens to improve plant nutrient uptake as well as heavy metal uptake and their translocation. PGPR produces ACC deaminase to lower ethylene production to promote plant growth (Kärenlampi, et al., 2000). PGPR inoculated Plants with ACC deaminase shows increased biomass production and enhanced heavy metals uptake with increased phytoremediation efficiency (Muhammad, et al., 2017). PGPR also produce bacterial auxin (IAA) to stimulate root hair development by lateral root initiation. Arbuscular mycorrhizal fungi are important microbial community which are helping plants for phytoremediation. The presence of arbuscular mycorrhizal fungi increases the surface area of plant roots for absorption of water and nutrient and increases bioavailability of heavy metal by extensive hyphal network. AMF can also promote plant growth by producing phytohormones.

Future Perspective

The available techniques are need to improved and followed, implement and interpret the recent biotechnological advances in the field of bioremediation. The emphasis should be given on the cost-effectiveness, adoptive and sustainability of the techniques to mitigate the environmental change, contamination of food products and biological systems, impact of anthropogenic activities on the environment. The management and disposal of plants having phytoremediation potential with high contaminants are an important concern. Further research and knowledge is required to commercialize this technique on a large scale and will ensure the environmental security in a sustainable way and will make the planet Earth more beautiful place to live.

CONCLUSION

In this review, we have highlighted the remediation of chromium contamination from the environment with application of phytoremediation. As chromium is considered to be one of the most harmful heavy metals released to the environment, its remediation is inevitable. Many physical as well as chemical remediation methods were employed previously. However, the main drawback of these techniques is the requirement of high energy and toxic chemical reagents, with the possible production of secondary by-products. Phytoremediation is an emerging technology helping to clean the soil and water bodies from toxic pollutants. Phytoremediation can provide a low-cost and sustainable way to improve the economies of developing countries. Moreover, several metal chelating proteins involved in metal translocation and tolerance and the phytoremediation efficiency. This type of approach by adopting new technologies to study various stress factors and specific patterns of plants response towards phytoremediation.

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