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Review Article

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Detoxification of Hexavalent Chromium

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ABSTRACT

Chromium is a toxic metal that are abundant and persistent environmental pollutants introduced into environment through man-made sources. Signs of Cr toxicity in plants include progressive stages of chlorosis and leaf death. Conventional methods employed for removal of hexavalent chromium is expensive and lack specificity. Biosorption is an emerging technology uses biomass to remove pollutants from environment. Biosorption by bacteria and fungi as an substitute treatment for wastewater containing heavy metals. Fungi can tolerate high concentration of potentially toxic metals and with other microbes; this maybe correlated with decreased intracellular uptake or impermeability.

Keywords: Hexavalent chromium, detoxification, phytoremediation, microbial consortia, biosorption efficiency.

INTRODUCTION

The term ‘‘heavy metals’’ denotes any type of metallic element that has a reasonable high density of4 g/cm^3, or 5 times and is toxic or poisonous even at low concentration. Heavy metals are extensive environmental pollutants, and their harmfulness causes nutritive, natural and biological, dietary and ecological problems [1]. The pollutants that are emitted in to the environment are of two types: organic and inorganic. The inorganic pollutant which cause serious threat to environment are heavy metal contamination. Environmental pollution from detrimental metals and minerals can arise from usual as well as man- made sources. Natural causes include;, discharge from rocks into water, fires in forest, volcanic activity etc., In anthropogenic activity the contamination occurs both at the level of industrial construction as well as end use of the yields and run-off [2]. Ecological pollution is the occurrence of a pollutant in the environment air, water and soil, which may be lethal and will cause damage to living things. Heavy metals have leading availability in soil and aquatic ecosystems and to a relatively smaller quantity in atmosphere as particulate or vapours. Toxicity in plants differs with plant species, certain metal, concentration, chemical form and soil composition and pH, as numerous heavy metals are considered to be essential for development of plant. Particular metals Zinc and copper either serve as cofactor and activators of enzyme reactions e.g., informing enzymes/substrate metal complex [3] or to utilize a catalytic property such as prosthetic group in metalloproteins.

Chromium

Chromium(VI), one of Cr oxidation states is a suspected carcinogen and causes contaminants in soil, and groundwater contaminant. Extensive usage of Chromium (Cr) includes surface treatments, alloy and stainless steel production, leather processing, pigments, production of catalysts [4, 5, 6]. Tanneries are the chief source of chromium pollution and release Cr(VI) ranging from 40 – 25,000 mg/l of wastewater. The maximum tolerance limit of total Cr for public water supply has been fixed at 0.05 mg/l as per Indian standards[7, 8].The hexavalent form of chromium is more poisonous than trivalent chromium and is frequently present in wastewater as chromate (CrO_4^{2-}) and dichromate ($Cr_2O_7^{2-}$). This is of serious environmental concern as Cr(VI) persists indefinitely in the environment complicating its removal. The persistant nature makes it accumulate in the food chain which with time reaches damaging levels in living beings leading to serious health hazards such as cancer in digestive tract, stunted growth rates in plants and causes lethal damage to animals. Hence Cr(VI) must be removed from wastewater prior to its discharge into natural water systems, contiguous landmasses and sewer systems needs severe and instant attention

[9].The substantial issues impacting metals speciation in solution includes existence of metals and anions, pH, heavy metal absorption. Metals tend to precipitate, as hydroxide or carbonate forms. Elimination of metals from waste water is achieved principally by the application of several processes such as adsorption, sedimentation, electrochemical processes, ion exchange, coalescence property, filtration and membrane processes, chemical precipitation etc. Phytoremediation has arisen as the most appropriate technology which uses plants for removal of environmental pollutants or detoxification to make them harmless. There are several plants used in the phytoremediation has a significant capacity of metal absorption, its accumulation and decreasing the time of decontamination of an ecosystem.

Chromium toxicity in plants

Whereas Cr(VI) has been demonstrated to produce severe damage compared to Cr(III) because of it is less toxicity and extremely low solubility, which avoids its leaching into ground water. Serious problems were produced by Cr(III)in living tissues although at higher concentrations than Cr(VI) were also investigated in the preceeding experiments. 100 WM Cr(III) was incorporated in Barley, it showed 40% of growth inhibition whereas inhibition caused by the same Cr(VI) level reached up to 75% in shoots and 90% in roots [10]. Indications of Cr toxicity in plants include progressive stages of chlorosis and leaf death. Incorporation of 50 ppm Cr(VI) resulted in vital growth although with transformed appearance; further exposure to 100 ppm caused a stressed appearance subsequently 2 days, and later 7-10 days all barley plants have noticed in lethal condition. Cr(VI) have shown to produce more toxic symptoms than Cr(III), and occur earlier and at lower concentrations. A decline in protein content and in nitrate reductase activity analysed *in vitro* has been reported [11]. Cr also elicits the synthesis of polyamines in barley; Cr(VI) was a faster and more efficient inducer of putrescine synthesis than Cr(III). Subsequently, once putrescine has been induced the plants were observed for the presence of chlorosis, decline in growth, stimulation of leaf chitinase, reduction of shoot growth and lowered water content in leaves [12]. Chromosome aberrationsand micronuclei formation have been observed in *Viciafaba* and *Allium cepa* root tips exposed to heavy metals[13, 14]. Though micronuclei formation correlated with Cr levels detected in contaminated soils [15], the levels of other heavy metals existing in the soil samples were not distinguished, and hence the nuclear aberrations observed could be due to the presence of remaining heavy metals.

Biosorption Efficiency

Biosorption is a novel and emerging technology uses biomass to remove pollutants from wastewater, specifically those that are not easily biodegradable such as heavy metals and dyes [16, 17, 18, 19]. A diversity of biomaterials such as bacteria, fungi algae, and biowastes were designated to bind these pollutants [20, 21, 18, 22]. Several species of *Candida*, including *Candida utilis*, *Candida albicans*, *Candida tropicalis* and *Candida lipolytica* were found to be capable of proficiently accumulating heavy metals under a wide range of external conditions[23, 24, 25, 26]. These effective strains could either adsorb a range of metal ions or be firmly specific in respect of single metal ion. For example, intact and desiccated *C. utilis* cells have the capacity to remove Cr(VI) [23]. Moreover, responsiveness has been focused on increasing the biosorption ability of these microbial consortium. To initiate the activated approach the binding sites on the surface should be stimulised[27]. To improve the Cr(VI) resistance and biosorption efficiency in *C. utilis* protoplast mutagenesis technology was also employed [25]. Some easily available biomaterials have also been tested for the progress of low-cost biosorbents, in specific the sewage sludge from sewage treatment plants [28], bio-waste produced as a by-product of industry and agriculture [29], and other plant derived materials [30]. Meanwhile sewage sludge is the solid waste produced in abundant quantity. Similar experiments were conducted and stated that sewage sludge has the effective biosorption potential lying in its complex consortium of microbes, large surface capacity, external chemistry, permeability and excellent settling competency [31, 32]. Moreover, diminutive report is existing regarding the biosorption properties on the removal of heavy metals by the mixture of isolated *Candida* and sewage sludge.

Phytoremediation of Chromium

Literature survey indicates that very few workers have reported ameliorative measures for Cr toxicity in crop plants. It is due to the reason that most of the investigation has been focused on improving phytoaccumulation of Cr by plants and trees for its usage in phytoremediation. By the application of mycorrhizal inoculation decreased mineral nutrition due to Cr toxicity has been improved. Khan (2001) reported the potential of mycorrhizae in protecting tree species *Populuseuro americana* and *Dalbergi asisso* against the detrimental effects of heavy metal and phytoremediation of Cr contamination in tannery effluent-polluted soils[33]. Karagiannidis and Hadjisavva Zinoviadi (1998) studied the effect of the vesicular arbuscularmycorrhizal fungus (VAMF) *Glomusmosseae* on progress, and produced better yield of durum wheat and stated that VAMF enriched yield in wheat and simultaneously declined the Cr content in the herb [34]. The effect of Cr on the uptake and distribution of micronutrients (Fe, Mn, Cu and Zn) in mycorrhizal soybean and maize in sand culture, (Davies *et al*. 2001) found that VAMF enriched the ability of sunflower plants to tolerate Cr[35]; similarly, Davies *et al*. (2002) described that In chromium incorporated plants VAMF had a positive effect on tissue mineral concentration, gas exchange and development[36]. Free amino acids and glutathione are known to induce heavy-metal tolerance by antioxidant action and metal chelating activity, respectively [37]. Enhanced supply of sulphur caused an total intensification in total sulphur, sulfate and GSH in rhizomes and leaves of potato. The absorptions of the total free amino acid pools in leaves and tubers showed a two and threefold diminution [38]. Henceforth, it is possible that sulfate and iron supplementation can counter Cr toxicity in harvesting yields. The deprived translocation of Cr from roots to shoots is a main obstacle in using plants and trees for remediation purpose. Pulford *et al*. (2001) in a work with moderate trees confirmed that Cr was poorly taken up into the aerial tissues but was held primarily in the root[39]. These results mean that the trees can be used as phytoremediators on Cr-contaminated sites, their main assessment is to stabilise and monitor a site [40]. This has leaded to increase in Cr translocation by adding chemical and biological amendments to soil. Environmental risk can be declined by reducing the chromate to chromic oxide by chemical or organic methods, by recognising the solubility of chromic oxides in soil, restricted the formation of chromate[41]. Mycorrhizae and organic acids (citric and oxalic) have been stated to play an vital role in phytoremediation of Cr-contaminated soils by enhancing Cr uptake and increasing translocation to shoot [42, 34]. Nutrient culture studies displayed a marked development in uptake and translocation of chelated 51Cr in *P. vulgaris*. Cr chelation by DTPA was most efficientlytranslocated followed by 51Cr-EDTA and 51Cr-EDDHA [43]. Cr accumulation from Cr(III)-treated maize plants in the existence of increasing concentrations of organic acid have been observed [44]. Shahandeh and Hossner (2000b)described a high rise in Cr uptake assisted by organic acids[46]. Srivastava *et al*. (1999b) if the concentration of organic acids is high, it leads to increase in uptake of Cr without affecting the distribution in plant parts[45]. In wheat Source-to plant transfer coefficients of Cr tended to rise with increasing concentrations of organic acids. Chaney *et al*. (1997) depicted that phytostabilization [in situ conversion of Cr(VI) in soil to Cr(III)] appears to have strong promise with respect to chromium[47].

Chromium(VI) detoxification by bacteria

Microbial Cr(VI) reduction was first stated in the late 1970s, when Romanenkoand Koren'Kov (1977) observed a Cr(VI) reduction capability in *Pseudomona ssp.* grown under anaerobic conditions[48]. The bacterial strain, isolated from sewage sludge, classified as Pseudomonas dechromaticans was effective in this process. Several microorganisms were isolated that catalyze the reduction of Cr (VI) to Cr(III) under varying conditions. Initially, the attention was focused on facultative anaerobic bacteria such as *Aerococcus*, *Micrococcus* and *Aeromonas* [49], Cr(VI) can be reduced aerobically like *Thermuss cotoductus*[50] and anaerobically such us *Achromobacter sp*. [51]. Cr (VI) reduction depend strongly on the oxygen requirements of the bacterium. *Actinomycetes* have also been described to reduce Cr(VI). (Polti*et al*. 2007) identified 11 Cr(VI) resistant strains, ten from the genus *Streptomyces* and one from *Amycolatopsis*[52]. Recently, Sugiyama *et al*. (2012) isolated Flexivirgaalba with Cr(VI) reducing activity that is stimulated by molasses[53]. The bacteria capable of the capacity to reduce Cr(VI) levels are named chromium-reducing bacteria (CRB) [54]. CRB are commonly isolated from industrial effluents, particularly those from tanneries [55, 56], and textile [57] and electroplating manufacturing [58]. Similarly CRB are isolated from soil contaminated with effluents [59, 61].Cr (VI) bioremediation studies have been done by Monocultures of various bacterial strains [62, 63, 55]. Particular species occasionally survive in a complex environment. Therefore, using pure cultures under precise lab conditions may not match actual environmental conditions, particularly in highly polluted areas. Bacteria are more constant and survive better when they exist in mixed culture[64]. In addition, consortia of cultures are metabolically superior for eradicating metals andare more appropriate for field application, as the organisms are more competitive and are more possible to survive [65]. Therefore researchers have found that consortia cultures isolated from the environment are capable of Cr(VI) reduction [66, 67, 68]. Biological treatment of Cr(VI)-contaminated wastewater may be challenging because the metal's toxicity can kill the bacteria. Therefore, to protect the cells, cell immobilization techniques have been employed by several researchers [69, 70, 71], because (1) the biofilm-bound cells can withstand higher concentrations of Cr(VI) than planktonic cells, and (2) they allow easy separation of the treated liquid from the biomass [72]. Considering the lethal effect of certain physicochemical methods are essential to recognise alternative technologies for reducing/destroying chromium toxicity, researchers have recently focused on abatement of Cr(VI) toxicity by using plant-growth-promoting rhizobacteria (PGPR) [73].

PGPR are naturally occurring soil bacteria that destructively colonize plant roots and benefit plants by providing growth promotion [74]. The use of soil bacteria (often PGPB) as adjuncts in metal phytoremediation can significantly facilitate the growth of plants in the presence of high (and otherwise inhibitory) metal levels [75]. Efficiency can be increased by the application of plants along with selected microorganisms may be beneficial; such a technique is called rhizoremediation [76]. PGPR like *P. putida P18* and *P. aeruginosa P16* [77], N.T. Joutey *et al*[68]. P. corrugate 28 [78], *Bacillus sp. PSB10* (Wani and Khan 2010) are described to be capable of restoring chromium contaminated sites[79]. Tiwari*et al*. (2013) observed that when a consortium of *Bacillus endophyticus*, *Paenibacillus macerans*, and *Bacillus pumilus* was inoculated in the rhizospheric zone of *S. munja*, this has enriched metal uptake through mobilization and stimulated plant growth [80]. Bacterial action may change metal speciation to make metals water soluble and amenable to plant uptake.

Detoxification by fungi:
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. Biosorption of the chromium ion Cr(VI) onto the cell surface of *Trichoderma* fungal 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. The Biosorption productivity of fungus was decreased in acidic pH. The study performed using *Aspergillus* and *Penicillium* sp. states that it is efficient in degrading metals. When pH is set up in acidic condition, the elimination efficiency declines[81]. The Cr(VI) degradation was done using *Hypocreatawa* and characterized in batch cultures accompanied at initial Cr(VI) concentrations ranging from 0.59 to 4.13 mM. The fungus showed a significant capacity to entirely decrease very high concentrations of Cr(VI) under aerobic conditions. 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 possibly useful for detoxification of Cr(VI)-laden wastewaters [82]. 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. The results obtained by FTIR depicts that the main functional groups involved in the uptake of chromium by *Fusarium oxysporum* strain were carbonyl, carboxyl, amino and hydroxyl groups [83]. The ability of yeast to reduce hexavalent chromium was studied. The *in vitro* reduction of hexavalent chromium using Crude Chromate Reductase (CChR) of Yeast *Pichiajadinii*M9 and *Pichiaanomala*M10, 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 [84].

CONCLUSION

This review paper is focused to detoxification of the hexavalent chromium contamination in the environment. Biological process plays a major role in bioremediation and can make the permanent resolution for remediation. This study shows that the bioremediation process offers several benefits in remediation of hexavalent chromium in the surroundings. The bacteria isolated from the natural site can help in bioremediation by aerobic and anaerobic degradation mechanism. Various fungus can be used as a natural biosorbents to absorb hexavalent chromium in the environment. Extensive research has to be conducted in this field in order to get biological products which aids in large scale remediation of environmental and water contaminants.

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