P: ISSN NO.: 2394-0344 RNI No.UPBIL/2016/67980 VOL-3* ISSUE-1* April- 2018
E: ISSN NO.: 2455-0817 Remarking An Analisation

Phytoremediation of Chromium by Chickpea (Ciecer arietinum L.) and It's Toxic Effect

Abstract

The main objective of the study is to test the species of chickpea (*Ciecer arietinum L.*) plant in different concentration of Chromium as anhydrous Potassium dichromate ($K_2Cr_2O_7$) by germinating the seeds under normal environmental conditions. The present study includes effect of different conc. Of chromium on plant growth and its accumulation in the species. Standard growth parameters such as root Length, shoot length, chromium accumulation and remediation mechanism were tested as markers of chromium toxicity. The toxic effects were evaluated by measuring the change in plant height, pigment concentration. The plants are separately harvested after 10, 20 and 30 days after measurement root and shoot emergence. The accumulation of Chromium is also observed after the harvesting at different time interval.

Keywords: Chromium, Potassium dichromate, Chickpea, Phytoremediation, Root length, Shoot Length.

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Introduction

Chromium and its corresponding compounds have multifarious industrial uses. Basically they are used in leather processing and finishing, in the production of refractory steel, electroplating cleaning agents, catalytic manufacture and in the production of chromic acid and specialty chemicals. Hexavalent chromium compounds are used in industry for metal plating, cooling tower water treatment, hide tanning and,, wood preservation. Leather industry is the major cause for the high influx of chromium to the biosphere accounting for 40% of the total industrial use (J. Barnhart, 1997). Chromium (vi) is considered the most toxic form which usually occurs, associated with oxygen as chromate (CrO_4^{-2}) or dichromate ($Cr_2O_7^{-2}$) oxyanions. Chromium (III) is less toxic, less mobile and is mainly found bound to organic matter in soil and aquatic environments (T. Bacquer et al., 2003). It is also one of the important pollutants among other pollutants like cadmium, lead, mercury and aluminum. Until now, methods used for their remediation such as excavation & landfill, thermal treatment, acid leaching and electroclamation are not suitable for practical applications, because of their high cost, low efficiency, large destruction of soil structure and fertility. Thus the development of bioremediation strategies for heavy metal contamination is necessary. These anthropogenic activities have led to an increase in the level of chromium contamination in the biosphere.

Soil contamination with heavy metal is now days a worldwide problem leading to agricultural losses and hazardous health problems as metals enter in to food chain. Samantaray et al., (1998) reported that high concentrations of chromium exhibited severe chlorosis, necrosis and a host of other growth abnormalities and anatomical disorders including the regulation of the mineral metabolism, enzyme activity and other metabolic processes. Cr^{3+} is taken up by plants because of its mobile nature in soil

Chickpea [*Cicer arietinum* (L.)] Belongs to genus Cicer, tribe Cicereae, family Fabaceae, and subfamily Papilionaceae. It is known as gram or Bengal gram or chana. Chickpea is a herbaceous annual plant which branches from the base. It is almost a small bush with diffused, spreading branches. The plants grows 20-50 cm high and has small, feathery leaves on either side of the stem. Chickpeas are a type of pulse, with one seedpod containing two or three peas. It has white flowers with blue, violet, or pink veins.. Chickpeas are a nutrient dense food, providing rich content of protein, dietary fiber, and certain dietary minerals such as irons and phosphorus.

Aim of the Study

The main objective of the study is to collect the suitable varieties of chickpea (*Cicer arietinum L.*) Seeds and find out the effect of Cr(VI) on growth and development of plant species under natural conditions and also to study the root and shoot measurement assessment of different toxicity of treated plant species .The present study also has aim to evaluate theaccumulation potential from different concentration of Cr(VI) ions mg/g on the plant.

Review of Literture

Phytoremediation

Phytoremediation (from ancient Greek phyto- meaning "plant", and Latin remedian - meaning "restoring balances") refers to the technologies that use living plants to clean up soil, air, and water contaminated with hazardous chemicals. Phytoremediation is a cost – effective plant – based approach of remediation that takes advantage of the ability of plants to concentrate elements and compounds from the environment to mobilize various molecules in their tissues. It refers to the natural ability of certain plants called hyper accumulators to bio molecules degrade or render harmless contaminants in soil, water, and air. Toxic heavy metals and organic pollutants are the measure target for the Phytoremediation. Knowledge of physiological and molecular mechanisms of Phytoremediation began to emerge in recent years with biological and engineering strategies designed to optimize and improve phytoremediation in addition, several field trails confirmed the feasibility of using plants for environmental cleanup.

Plant Species for Phytoremediation

Several plants are having capacity to accumulate heavy metal from the environment**.** To identify plant population with the ability to accumulate heavy metals, 300 accessions of 30 plant species were tested by Ebbs et al. (1997) in hydroponic levels of Cu, Cd and Zn. The results indicate that many brassica spp. Such as B.juncea L., B.juncea L. czern, B.napus L., B. Rapa L. exhibited that moderately enhanced Zn and Cd accumulation. They were also found to be most effective in removing Zn from the contaminated soils. To date more than 400 plant species have been identified as metal hyperaccumulators representing less than 0.2% of all angiosperms (Brooks, 1998: décor etal., 2000). The plant species that have been identified for remediation of soil include either high bio mass plants such as willow (Landberg and Greger , 1996) or those that of have low biomass but high hyper accumulating characteristics such as *Thalaspi* and *Arabidopsis* species.

Hexavalent Chromium

Hexavalent chromium is a form of the metallic element chromium. Chromium is naturally occurring element found in rocks, animals, plants, soil, and volcanic dust and gases. It comes in several different forms, including trivalent chromium and hexavalent chromium. Trivalent chromium is often referred to as chromium (VI), is generally produced by industrial processes. Hexavalent chromium is not found naturally it is found in the environment due to manmade activities. The various source of hexavalent chromium get exposed to the environment are from airborne emission from Chemical plants, Cement Dust, contaminated landfill, effluents, tobacco, and from industry like glass making , leather tanning , Textile manufacturing , Welding of alloys or steel, Paints or pigments, Chrome electroplating etc.

Toxicity of Chromium (VI) on Plants

Since anthropogenic activities have lead to an increase in the level of chromium contamination in the biosphere that direct affects the plant growth and development. Chromium compounds are highly toxic to plants and are detrimental to their growth and development. Although some crops are not affected by low Cr concentration (3.8×10^{-4} µM) (Huffman and Allaway, 1937), Cr is toxic to higher plants at 100µM/kg dry weight (Davies et al., 2002). Barcelo et al.,(1985) described the inhibition of P and K translocation within the plant parts when bean plants were exposed to Cr in nutrient solutions. Continuous irrigation of agricultural land with industrial wastewater may cause heavy metal accumulation in the soil and vegetables (Sharma et al.,2007). Contamination of soil in cultivated fields by industrial effluents loaded with toxic heavy metals has emerged as a new threat to agriculture. Most of the effluents and wastes contain heavy metals in an amount sufficient enough to cause toxicity to crop plants (Khan et al., 2006). Root nodulation was suppressed and number of functional nodules appreciably decreased (Khan and Khan, 2010). Consequ- ently, it infuences cell size, leaf area and photo-synthetic activity (Kibe et al., 2006; Walley et al., 2005; Alam, Haider, 2006; Caliskan et al., 2008; Salvagiotti et al., 2008).

Chromium Uptake, Translocation and Accumulation

The pathway Cr (VI) transport is an active mechanism involving carries of essential anions such as sulphate (Cervates et al., 2001). Iron (Fe), Sulphur (S) and Phosphorus (P) are known also to compete with Cr for carrier binding (Wallace et al., 1976). Independent uptake mechanisms for Cr (VI) and Cr (III) have been reported in barley. The use of metabolic inhibitors diminished Cr(VI) uptake whereas it did not affect Cr(III) uptake, indicating that Cr(VI) uptake depends on metabolic energy and Cr(II)doesn't (Skeffington et al., 1976). In contrast , an active uptake of both Cr species, slightly higher for Cr(III) than for Cr(VI), was found in the same crop(Ramachandran et al.,1980).In 7 out of 10 crops analysed , more Cr accumulated when plants were grown with Cr(VI)than with Cr(III) (Zayed et al.,1998). Golovatyi et al. (1999) have shown that Cr distribution in crops had a stable character which did not depend on soil properties and concentration of this element ; the maximum quality of element contaminant was always contained in roots and a minimum in the vegetative and reproductive organs.

Effect of Chromium on Physiological Activity of Plant

Various physiological activity like, Root Growth, Stem Growth, Leaf Growth, Growth, Germination photosynthesis, Mineral Nutrition and also production yield of the plant is affected by the heavy metal pollution. The growth of root and shoot, dry matter were Adversely affected by heavy metals (Huma Naz et.al,2015). Growth is chiefly expressed as a function of genotype and environment, which consist of external growth factors and internal growth factors. Presence of Cr in the external environment leads to changes in the growth and development pattern of the plant. Germination of seed is the first physiological process is affected by Chromium and the tolerance capacity of seed determine by the medium containing chromium. High levels (500ppm) of Cr(VI) in soil reduced germination up to 48% in the bush *Phaseolus vulgaris* (Parr and Taylor, 1982). Peralta et al.(2001) found that 40ppm of Cr(VI) reduced by 23% the ability of seeds of *lucrene* (Medicago sativa cv. Malone) to germinate and grow in the contaminated medium. As the roots of plants are directly attached with soil, therefore, it is obvious that Cr greatly influenced on their growth (S. Medda, and N K Mondal, 2017) .Lead and chromium considerably affected germination of Chickpea (*C. Arietinum L.*) seeds (Sunayna Dasgupta et .al,2011).

Prasad et al.(2001)reported that the order of metal toxicity to new root primordial in *salix viminalis* is CD > Cr > Pb, whereas root length was more affected by Cr than other heavy metals studied. Anderson et al., (1972) observed 11%, 22% and 41% reduction in plant height, respectively, over control. Reduction in plant height due to Cr(VI) on *Curcumas sativus* . *Latuca sativa* and *Panicum miliaceum* was reported by joseph et al. (1995). Barton et al., (2002) observed that Cr(III) addition inhibited shoot growth in Lucerne cultures. Tripathi et al.(1999) found that leaf area and biomass of *Albizialebbek* seedlings was severely affected by a high concentration (200ppm) of Cr(VI). Jain et al. (2000) observed leaf chlorosis at 40ppm Cr that turned to necrosis at 80ppm Cr.

Most physiological and biochemical processes are severely affected by Cr and as a consequence, the yield and productivity of the crops are equally affected (Barcelo et al., 1993). Chromium stress is one of the important factors that affect photosynthesis in term of CO_2 fixation, electron transport, photophosphorylation and enzymes activities (Clijsters and Van Assche, 1978). Zeid (2001) observed in peas that Cr at the highest concentration tested (10^{-2}M) decreased photosynthesis drastically. Krupa and Baszynski (1995) explained some hypotheses concerning the possible mechanisms of heavy-metals toxicity on photosynthesis and presented a list of key enzymes of photosynthetic carbon reduction, which were inhibited in heavy-metal treated plants (mainly cereal and legume crops).It has been noticed that the 40% inhibition of whole plant photosynthesis in 52 days old plants at 0.1mM Cr(VI) was further enhanced to 65% and 95% after 76 and 89 days growth respectively(Bishnoi et al.,1993a).

Cr due to its structural similarity with some essential elements can affect mineral nutrition of plants in a complex way (Rafia Azmat* and Hira Akhter, 2010). Interaction of Cr with uptake and accumulation of other inorganic nutrients have received maximum attention by researches. Cr (III) and Cr (VI) are taken up by the plants by different mechanisms (zaccheo et al., 1985). khan et al. (2001)observed that threshold values of the concentrations of N, P, K in dry weight of rice plants showed significant decreases at 0.5ppm Cr, excess of Cr caused decreases in concentration of Fe and affected the translocation of P, S, Mn, Zn and Cu from roots to tops (Chatterjee and Chatterjee,2000) in cauliflower. Alternation in the production of pigments which are involved in the life substances of plants.(e.g chlorophyll and anthocyanin) (Boonyapookana et al.,2002).Increased production of metabolites as a direct response to Cr stress which may cause damage to the plants.

Material and Methods

Materials

Seeds of (*Cicer arietinum (*L.)) namely 'desi buta' were taken from the local market of Bhubaneswar, Odisha .Then the damaged and unhealthy seeds are removed by washing in tap water. The damaged seed were float on the water which can easily separate from the healthy seed.

Methods

The healthy seed were kept in a beaker with water for overnight. After 24 hours seeds were removed from the beaker and wrapped tightly with a cloth. Then the seed were kept in a dark place for about 24 hours. The chickpeas sprouted. Then the sprouted chickpeas planted in the pots contain soil collected from near the river bed of Dayanadi,Itipur, Dhauli, Bhubaneswar

Measurement of Growth

The growth of the shoot and root are measured in terms of length after 10, 20 and 30 days interval of time.

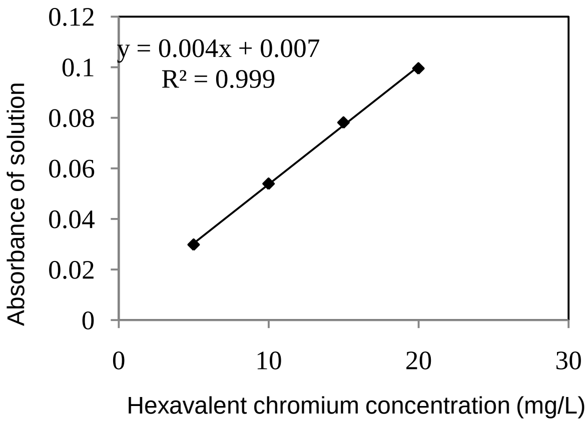
Standard Curve Preparation

Instrumentation calibration standards for transfer of 1ml, 2ml, 3ml, 4ml and 5ml. standard solution of Cr(VI) to 100ml volumetric flask add appropriate amount of matrix modifier and dilute to volume with distilled water. So the standard solution will be 5, 10, 15, 20 and 25 µg/L respectively. Details preparation procedure of Cr(VI) standard solution has been using manuals of spectrophotometer

Chromium Accumulation Analysis

Standard Curve Preparation

Figure:-1 A Calibration Curve of Cr(Vi) Standard Solution



After preparation of standard curve, chromium accumulation in shoot and root work was done by following methods: First two flasks were taken, in one flask is blank and other is sample content. In blank flask, there is 100ml of distilled water was added and then 0.25ml of $H_3 PO_4$ and 2ml of diphenyl carbazide solution was added. In sample content flask, 1st 15ml of distilled water was added then 5ml of leaf extract was added. This is collected as 100mg of leaf from different concentrations of each sample and then homogenized that leaf with acetone and volume was adjusted to 5ml, then 0.25ml of $H_3 PO_4$ and 2ml of diphenyl carbazide solution was added. After that, the two flasks were kept in a dark for 5-10 minutes for colour formation. At last absorbance was measured in spectrophotometer at 540nm. This same procedure was done in different concentration (control, 10ppm, 30ppm, 50ppm) of e chickpea. This procedure was repeated in 10th, 20th and 30th days.

Chlorophyll Content Analysis

Fresh shoots (100mg) were collected after 10th, 20th and 30th days from plants grown in natural condition which was treated with different concentration of Cr (VI). The samples were homogenized in 80% acetone and centrifuged at 5000rpm for about 10 minutes. The supernatant was used as the chlorophyll content was measured by colorimetric method. The absorbance measured in spectrophotometer at 645nm and 663nm.

Result

Effect of Chromium on Shoot and Root Growth

Effects of Cr (VI) on shoot length and root length of chickpea (*Cicer arietium* L.) at different concentrations and exposed times were presented in Table- 1 and 2 respectively. In chickpea treated with Cr (VI) solutions, the root and shoot lengths were significantly increased with respect to time and Temperature.

Table-1 Roots Length of Chickpea (*Cicer arietinum* (L.)

Conc. Of Cr (VI) (mg/L)		Time interval in Days		
		Day-10	Day-20	Day-30
	Control	8.6cm	12.5cm	14.8cm
	10	6.8cm	7.9cm	9cm
	30	6cm	6.7cm	7.3cm
	50	4.9cm	5.7cm	6.5cm

Table-2 Shoots Length of Chickpea (*Cicer arietinum(* L.)

		Time interval in Days		
Conc. Of Cr (VI) (mg/L)	**Cr(VI) conc-mg/l**	**Day-10**	**Day-20**	**Day-30**
	Control-0	19cm	21.4cm	23.1cm
	10	17.6cm	18.4cm	19.1cm
	30	14.7cm	15.6cm	16.4cm
	50	11.8cm	12.5cm	13.2cm

Translocation of Cr (VI) ions and accumulation:

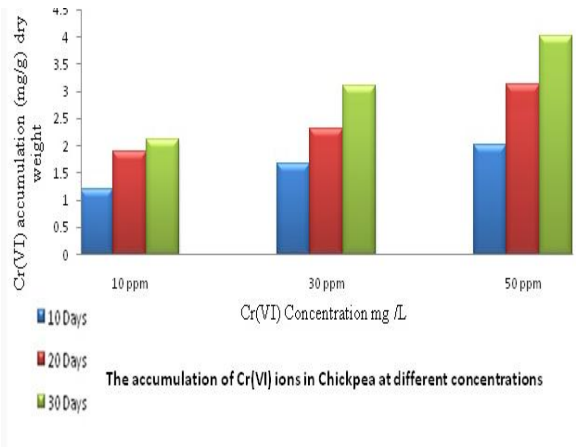
Phytoremediation methods using terrestrial plants to absorbed metal ions from their soil are highly efficient. Bio removal process using plant species contains two uptake processes.

1. Biosorption, which is an initial fast reversible and metal binding process.
2. Bioaccumulation, which is a slow irreversible and ions sequestration step(Arun k Shankar et al., 2005).In the present study the accumulation of hexavalent chromium ions by chickpea (*Cicer arietinum* L.) at ambient temperature with different concentrations and exposures of times was analyzed and were presented in table3. And figure 2

Table-3 Accumulation of Cr(VI)

	Time interval in Days		
Conc. of Cr(VI) mg/L	**10days**	**20days**	**30days**
10	1.210	1.890	2.121
30	1.670	2.320	3.110
50	2.010	3.120	4.010

Figure:2 Accumulation of Cr(VI) Ion in chickpea Plants Treated with Different Cr(VI) Concentration



Chlorophyll Analysis

Chlorophyll analysis was done by colorimetric method. The result shows that the chlorophyll content is found to be more in case of 10ppm, which was shown in Table-4.

Table-4 Changes in Chlorophyll Pigments (Chl-a and Chl-b) of Plants due to Cr(VI) Treatment.

Cr(VI) conc.mg/L	Chl-a	Chl-b
Control(0)	8.754mg	3.432mg
10	7.655mg	2.857mg
30	6.374mg	1.035mg
50	5.843mg	0.952mg

Discussion

Effect on Growth of shoot and root

After 10days the highest root and shoot length was obtained 6.8 cm and 17.6 cm respectively which were treated with 10ppm Cr(VI) solution and presented in Table-1. and 2 .After 20days the highest root and shoot length was obtained 7.9 cm and 18.4 cm respectively which were treated with 10ppm Cr (VI) solution and presented in Table-1 and 2. After 30days the highest root and shoot length was obtained 9 cm and 19.1cm respectively and presented in Table-1 and Table-2.

Similarly after 30days the highest root and shoot length was obtained 6.5 cm and 13.2 cm respectively which were treated with 50ppm cr(VI) solution and presented in Table-1 and Table-2 .The increasing Cr(VI) concentration and days , the shoot and root length of chickpea cultivars gradually increases in length. It may be due to the presence of suitable cations and anions that have been influencing due to effect of the nutrient uptake, which helps in plants physiological metabolic activity can grow easily.

Accumulation of Chromium (VI)

Chickpea species treated with 50mg/l of Cr(VI) after 30 days accumulated the highest level of metal ions 4.01mg/g dry weight. Shown in Figure-2. In the presence of excessive oxygen Cr(III) oxidizes into Cr(VI) which is highly toxic and more soluble in water than the other forms. Cr(VI) can easily cross the cell membrane whereas the phosphate- sulphate carrier also transports the chromites anions. Metals ions penetrate plants by passive process mostly by exchange of cations which occurred in the cell wall. It can be proposed that the root cells reached saturation during the period and there exist some mechanism in roots that could detoxify heavy metals or transport to aerial parts. The toxic properties of Cr(VI) originate from the action of this from which has an oxidizing agent as well as from the formation of free radicals during the reduction of Cr(VI) to Cr(III) inside the cell. Induction and activation of superoxide dismutase (SOD) and of antioxidant catalase are some major metal detoxification mechanism in plants.

Chlorophyll content

The increases in Cr(VI) concentrations and days, the chlorophyll content decreases gradually due to reaction of Cr(VI). But due to uptake of nutrient solution the chlorophyll content not decreased markedly.

Conclusion

The metal/metalloid pollution is a great concuss these hazardous pollutants are accumulated in living organism were responsible for many metabolic disorders. The phytoremediation techniques are relatively new methods for abetment of hazardous ions from water, soil and air. From the above table and figures, it is clear that the species of Cr are toxic at different degrees at different stages of plant growth and development and also that the toxicity is concentration and medium dependent. The toxic properties of Cr(VI) originate from the action of this from itself as an oxidizing agent, as well as from the formation of free radicals during the reduction of Cr(VI) to Cr(III) occurring inside the cell. Cr(III) occurring inside the cell .Cr(III), on the other hand, apart from generating reactive oxygen species, if present in high concentrations, can cause toxic effects due to its ability to corrdinate various organic compounds resulting in inhibition of some metallo-enzyme systems.in chickpea, the maximum value of accumulation of Cr(VI) is 4.01mg/g at 50ppm and the minimum is 1.21mg/g at 10ppm.

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