

EFFECT OF CHROMIUM ON SEED GERMINATION, PLANT GROWTH AND METABOLISM.

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INTRODUCTION

Alarming rate of increase in human population, consequently rapid increase in urbanization, increase in anthropogenic perturbations' of the biosphere manifest in a broad array of global phenomena, including accelerated industrialization, intensive agricultural activities, extensive mining etc. This has in turn caused widespread contamination of soil and water bodies with different heavy metals. Chromium was first discovered in Siberian red ore (crocoites) in 1798 by the French Scientist Vauquelin .

Chromium is the 17th most abundant element in the earth's mantle (Arredainayagam *et al.*, 2003), 7th most abundant element in the earth's crust (Katz and salem, 1994) and the 6th most abundant transition metal (Mohan and Pittman, 2006; Panda and Chowdhury, 2005) and its molecular weight is 51.1g. It has gained lesser attention from the plant scientists because of its complex inorganic chemistry (Shankar *et al.*, 2005). It occurs naturally as chromites (FeCr_2O_4) in ultramafic and serpentine rocks or complexed with other metals as crocoites (PbCrO_4), bentorite $\text{Ca}_6(\text{CrAl})_2(\text{SO}_4)_3$ and tarapacite (K_2CrO_4), vauquelinite ($\text{CuPb}_2\text{CrO}_4\text{PO}_4\text{OH}$) among others (Babula *et al.*, 2008). Chromium can be detected in various oxidation states i.e. from -2 to +6 ($\text{Cr}^{-2}, \text{Cr}^{-1}, \text{Cr}^0, \text{Cr}^{1+}, \text{Cr}^{2+}, \text{Cr}^{3+}, \text{Cr}^{4+}, \text{Cr}^{5+}, \text{Cr}^{6+}$). Chromium among all oxidation states, Cr^{3+} and Cr^{6+} are the most stable and common in terrestrial and aquatic environment (Augustynowicz *et al.*, 2010, Santos *et al.*, 2009, Kimbrough 1999, Zayed *et al.*, 1998) where as other oxidation states are unstable and short lived in biological system. The hexavalent form of the metal Cr (VI) is reported to be the more toxic and highly soluble in water than the relatively less reactive and less mobile. Cr (III) is mainly found in organic matter in soil and aquatic environment (Becquer *et al.*, 2003). Hexavalent Chromium compounds (mainly chromate, CrO_4^{2-} and dichromate, $\text{Cr}_2\text{O}_7^{2-}$) are extensively used in diverse fields of industry leading to environmental pollution and as a result growth and developments of plants are affected.

Cr salts are used in many industrial processes such as leather tanning ,electroplating ,steel production ,metal finishing ,catalyst application ,pigment manufacturing and metal corrosion inhibitors (Zayed and Terry,2003;erry,2003;Nath *et al.* ,2005;Venkateswaran *et. al.* ,2007).A high concentration of r has been found to be harmful to vegetation .As the Chromium concentration in plants increases ,it adversely affects several biological parameter .Ultimately there is loss of vegetation and land sometimes become barren .Symptoms of Chromium toxicity include inhibition of seed germination or of early seedling development ,reduction of root growth ,shoot growth ,leaf chlorosis ,reduction of rate of photosynthesis ,changes in water relation ,oxidative imbalances and in turn reduction in total biomass and dry matter production .There are many studies on Chromium toxicity in plants. Chromium significantly affects the metabolism of plants such as barley (*Hordeum vulgare*),citulus ,cauliflower ,vegetable crops ,wheat (*Triticum aestivum* Hd2204),Onion (*Alium cepa*),maize(*Zea mays*),Paddy(*Oryza sativa*,L),Tea (*Camellia sinensis* L).Increase in Chromium concentration in the environment of plant growth causes an increase in the plant tissues, but exposure to high concentration of Chromium impaired in some physiological processes and ultimately reduce the growth of plants and lead to toxic symptoms.

LITERATURE REVIEW

1.International level:

Heavy metals are globally considered as environmental pollutants which excessively causes serious risks for different plants in agricultural soil (Stambulska *et. al.* ,2018).Due to rapid industrialization and urbanization ,the metropolitan cities as well as in the developing countries pollution in air, water ,soil become increases day by day (Ademoroti, 1986; Tumi et al., 1990; Berthelsen *et. al.*,1995; Xiong, 1998) .The sources of heavy metals may be both anthropogenic like activities include sewage discharges, mining operations, runoffs from metal-refining Industries as well as natural environmental sources (Foy et al., 1978; Wheeler and Rolfe, 1979; Lepp, 1981 ; Cimino and Ziino, 1983; Moore and Ramamoorthy 1984; Adriano, 1986; Ho and Sachs, 1989; Alloway, 1990; Friedland, 1990; Filipinski and Grupe, 1990; Steffens, 1990; Seaward and Richardson, 1990).

Usually heavy metals are the group of metals having atomic density greater than $4g\text{ cm}^{-3}$ or 5 times more or greater than water (Nriagu and Pacyna ,1988;Hawkes,1977).Cadmium,chromium,Arsenic,cobalt,copper,Lead,mercury,nickel,uranium,vanadium,lead,mercury,nickel are some of the important heavy metals found in our environment disrupts natural ecosystems and microbial populations. Some of them are mobile and others are immobile and persistent. They ultimately concentrate on soils and sediments.

1.1 SOURCES AND UPTAKE OF CHROMIUM :

Several workers have been investigated the effects of heavy metals on the growth of plants and microbes till now (Coppola *et. al.*, 1988, Lorenz *et. al.*, 1992). Metal toxicity, occurrence, defensive strategies of plants have been significantly and widely reviewed quite a few of authors (Foy *et. al.*, 1978; Hamer, 1986; Baker, 1987; Roy *et. al.*, 1988; Barceló and Poschenrieder, 1990; Rauser, 1990; Steffens, 1990). Phytotoxicity of the metals is the main factor in the limitation of plant growth (Foy, 1988). Among the heavy metals Chromium is considered as an environmental hazard which is highly toxic for organisms'. Chromium is present ubiquitously in all phases of environment including air, water and soil. Chromium is listed as a hazardous environmental pollutant by Environmental Protection Agency (ATSDR, 1998). The average global Cr-emissions from natural sources have been estimated to be 43,000 tons/year. (Nriagu, 1988). Depending upon the parental material, Cr ranges from 10 to 50 mg kg⁻¹ naturally in soil. In ultramafic soil (serpentine), it can reach up to 125g kg⁻¹ (Adriano, 1986). Most of the countries harvest 50 Mg L⁻¹ (ppb) Cr (VI) as the maximum contaminant level (MCL) in the drinking water, where as MCL of 100 µg L⁻¹ (ppb) has been fixed in drinking water in USA (ATSDR, 1998). Cr concentration generally range from 0.1 to 117 µg L⁻¹ in fresh water, where as its value ranges from 0.2 to 50 µg L⁻¹ in saline water (Pawlisz et al 1997). Global discharge of Cr is 142,30 and 896 (x1000 metric tons/year) in water and soil respectively (Mohan and Pittman 2006). Cr concentration varies widely in the atmosphere, from background concentrations of 5.0 x 10⁻⁶ - 1.2 x 10⁻³ µg m⁻³ in air samples from remote areas such as Antarctica and Greenland 0.015 to 0.03 µg m⁻³ in air samples collected over urban areas (Nriagu 1988).

Heavy metal absorption in plants depend on many factors such as temperature, pH, aeration, conductivity, competition between different species, plant size, plant type, availability of root system element, type of leaf and soil and plant moisture content also (Yamamoto and Kozlowski, 1987). An immense level of literature related to uptake of metal ions and level of accumulation by plants which shows the differences between species and genotypes and between metals in the field of laboratory which ranges from nutrient to toxic heavy metals. (Lepp, 1981; Cseh, 2002). Some heavy metals are totally absent for plants due to their nature of insolubility and interactions among the soil particles. For example we can take Pb although it is present in large quantity but it is almost absent for plants due to its strong interactions with the soil particles and low in solubility nature. (Nriagu, 1988). The uptake and absorption varies for different heavy metals and also the different factors determine the uptake translocation and accumulation of it. There is no specific mechanism for uptake of chromium as it is not an essential element for plants. Thus the uptake and distribution mechanism of Cr in vegetative and reproductive parts of the plant are not fully understood. Therefore, uptake of this heavy metal is through carriers used for uptake of essential metals for plant metabolism. The mechanism of Cr uptake and translocation in plants differs with lapse of time. Both active and passive transports seem to be involved in the uptake mechanism. Active transport occurs at low concentration where as

passive transport occurs at toxic levels. The pathway of Cr (VI) transport is an active mechanism involving carriers of essential anions such as sulfate (Cervantes *et. al.*, 2001).

1.2 TOXICITY OF CHROMIUM :

Chromium is not an essential element for plants (Huffman and Allway ,1973),yet its solubility ,particularly of Cr (VI),in water is a threat to biota (Neiboer and Richardson,1980) .After the amount of Cr attains its threshold level the ability of the plant to hold the Cr metal expires and at that time Cr exerts its adverse effects on the seed of the plant if the metal accumulated in large amount (Bradshaw *et. al.*, 1965) . Although there is no exclusive evidence of essentiality of Cr in plant metabolism, some studies has shown that small additions of Cr have stimulating effects on growth and plant productivity (Zayed and Terry, 2003).In ecotoxicology, Cr found to be one of the important heavy metal. The adverse effects of Cr toxic level in *Cannabis sativa* L., lentil, , fenugreek, soya beans, wheat in different, date palm, pea ,number of vegetables was reported by a number of workers (Davies *et. al.*, 2002 ; Citterio *et. al.*, 2003). Jianmin, 2012 investigated effects of Cr stress on some physiological factors like growth, photosynthesis, enzymatic activities, proline, malondialdehyde, cell membrane permeability and accumulation of those in *Camellia sinensis* through pot cultivation technique results in adverse effects of Cr on tea plant and production. Transport of Cr falls under active mechanism carriers of essential ions such as sulphate (Curvantes et al.2001).Fe ,S ,P competed with Cr for binding of carrier to it.(Wallace et al,1976).In bean plants Cr accumulated in seed only for 0.1% and 98% was accumulated on the roots.

1.2.1 EFFECT ON GERMINATION:

Growth and development are the essential life processes of plant and for the propagation of the species. The first physiological process affected by Cr is seed germination; the level of tolerance of Cr is the ability of seed to germinate in a medium containing chromium (Peralta *et. al.*, 2001). There was a reduction in the germination of seed of the weed *Echinochola colonum* by 25% at 200 μ M Cr (Rout *et. al.*, 2000). Germination of bush bean, *Phaseolus vulgaris* was reduced by 48% in the soil containing high level of (500ppm) Cr (VI) (Parr and Taylor, 1982). It was observed by Peralta et al., the ability of seeds of lucrene is reduced by 23% when the seed germinated and grown in a Cr 40ppm contaminated medium. Reduction in germination of seed under stress of Cr has a depressive effect on the on the enzyme such as amylase and as a result there is a reduction in the transport of sugar to the axis of embryo. On the other hand activity of proteases increases with the increase in uptake of Cr which could also contribute to the reduction in percentage of germination of seed treated with Cr (Zeid, 2001). Some scientists suggested that Cr (VI) treatment may not affect seed germination, but instead inhibit radicals' growth when they emerge and contact Cr solution. For developing embryo sugar is essential which can be obtained by plant on hydrolysis of starch by amylase. Plant treated with Cr decreases the activity of amylase and as a result sugar becomes unavailable to the developing embryo and seed germination is inhibited (Oliveira, 2012). The effect of heavy

metals on seed germination relies on their ability to reach embryo tissues across physiological barriers predominantly the seed coat which directly depends on the seed coat structure which varies in diverse plant species and on the physical and chemical properties of metal ion themselves (Seregin and Kozhevnikova 2005). Barley seed germinated and the seedlings grew well at Cr (VI) levels up to 100mg/kg in soil but were always slower in development due to Cr inhibition, which is responsible for delayed mobilization the reserve starch necessary for initial growth (Zayed and Terry 2003). The reduction in seedling growth under chromium stress might be due to poor growth which will decrease transportation of water and nutrients to the shoot. In addition to this, Cr transport to the aerial part of the plant can have direct impact on its cellular metabolism contributing to the reduction in seedling growth.

1.2.2 EFFECT ON ROOT, SHOOT AND LEAF GROWTH :

Heavy metals also affect the growth of root beside germination of seed. Decrease in root growth is a well documented effect of Cr in trees and crops as they are the primary target for metal anions than aerial parts (Breckle, 1991; goldbold and Kettner, 1991; Tang *et. al.*, 2001). Peralta and coworkers in 2001 showed that 5mg/l of Cr (VI) increased root growth comparatively to the control, and at higher doses (20 and 40 mg/l) there was a dose inhibition effect. When mungbean is exposed to Cr (VI), growth of root decreases (Rout *et. al.*, 1997).

Cr also has adverse effects on height of plant and shoots growth (Rout *et. al.*, 1997). It was observed by Anderson *et al.*, in 1972 that plant height is reduced by 11%, 22% and 41%, respectively, over control. Plant height, due to Cr (VI), was reduced in *Curcumas sativus*, *Lactuca sativa*, and *Panicum miliaeceum* (Joseph *et. al.*, 1995). Barton *et al.*, observed that Cr (III) addition inhibited shoot growth in Lucerne cultures.

Reduction in certain root parameters, such as diameter, surface area, root hairs are observed on exposure to chromium and have also been determined as the cause of wilting and plasmolysis in root cells (Ali *et al.*, 2011; Moral *et al.*, 1995). Cr has also been shown to be toxic to bean plants causing changes in morphology compared to control plants (Azmat and Khanum , 2005) , including reduced root length with increase in Cr concentration (0, 5, 10, 50, 100, 150, 200, 250 mg/kg) . Canopy size, however, was less affected. Morphological changes of leaves of corn after one day of exposure to 300 mg /l of $K_2Cr_2O_7$ have also been observed, where young leaves suffer epinasty after 6 hour of exposure, and significant wilting was observed after 12 hour, possibly due to water stressed caused by Cr (Wang *et al.*, 2013). Toxicity symptoms of Cr (III) were also observed in *Ocimum basilicum* plants exposed to $CrCl_3.6H_2O$ concentrations of 0, 2, 4, 6 and 8 mg/l. Changes in organization of cytoplasm, ultra structure of chloroplast underdeveloped lamellar structure with widely spaced thylakoids and less amount of grannae (Bishekolaei *et al.*, 2011). Such changes in morphology can severely affect photosynthetic pigments and photosynthesis (Bishekolaei *et.al.*, 2011). Primary toxic effects of chromium (VI) has been demonstrated which is due to membrane damage

(Vazquez et al. 1987; Appenorth *et al.*, 2003). As a result of interference with essential mineral elements, adverse effect of Cr (VI) is observed in the aerial parts of plants which are also considered as secondary effects (Vazquez *et al.*, 1987).

1.2.3 EFFECT ON PRODUCTIVITY:

Yield of a plant depend on growth of leaf, area occupied by leaf, number of leaves etc. Productivity and yield are also affected by accumulation of chromium in plants as chromium affects most of the physiological and biochemical processes of plants. Cr (VI) in irrigation water decreased significantly grain weight and yield (kg/ha) of paddy up to 80% under 200mg/l of chromium (Oliveira, 2012) .As physiological and biochemical processes are adversely affected by chromium, yield and productivity are also affected (Barceló *et al.*, 1993). Golovatyj *et al.*, (1999) reported less production of barley and maize in pot with soil having concentration of chromium 100 to 300 mg/kg. In carrot, yield at harvest was not at all obtained on application Cr at 270 or 810 kg /ha. Reduction in yield of spring season *Hordeum vulgare* grown in soil contaminated with 100 to 150 mg / kg Cr(VI) was observed whereas the grain weight and yield of *Z. mays* was increased in the same soil contamination (Wyszkowski and Radziemska , 2010).

1.2.4 EFFECT ON ANATOMY :

Structural and ultra structural anomalies were also observed in plants grown in Cr contaminated soil. It was observed and reported by Han et al., in 2004, Cr (VI) reduced the palisade and spongy parenchyma cells in leaves, induced clotted deposition in the vascular bundles of stems and roots, and enhanced the number of vacuoles and electron dense materials along the walls of xylem and phloem elements in *B. juncea*. Complete destruction of cortical tissue in roots of *T. aestivum* seedlings by Cr (III, VI) was reported by Hansain and Sabri in 1997. Certain anatomical aberration was observed in plants by Bianchi et al., in 1998 on exposure to Cr (VI) (20 and 40 mg /l) such as , browning of roots with black tips, absence of statolytes on root cap , disorganization of cells in the central cylinder , heavy thickening of tangential walls , and development of lateral roots in the root hair zone . A loss of organization in tissue , change in palisade layer of cells , increase in intercellular spaces and reduction in mesophyll layer , and decrease in development of starch granules and chloroplast when *M. aquatic* plants are treated with Cr (VI) (Bianchi *et al.* , 1998).

1.2.4 EFFECT ON PHYSIOLOGY AND BIOCHEMISTRY:

Heavy metals alters the chlorophyll content and in turn photosynthesis. Photosynthesis inhibition during stress caused by metals is one of the main consequences in plants, since these elements invariably, directly or indirectly; affect the photosynthetic apparatus (Sytar *et al.*, 2013). Metals alter the functions of the chloroplast membrane and components of the electron transport chain in mitochondria (Ventralla *et al.*, 2011) and, thus, inhibit part of the energy transfer from one level to another (Sytar *et al.* , 2013). Metals can also affect the activity of photosystem I and the photosystem II located in the thylakoid membrane.

Photosystem I is less susceptible to toxic metals than photosystem II (Sytar *et. al.*, 2013). It has also been demonstrated that Cr interferes with exchange of gases parameters consisting of CO₂ assimilation, transpiration, stomatal conductance, and internal carbon (Rodriguez *et al.*, 2012). In this context , Cr (III) causes water imbalance in plants and affects stomatal opening leading to changes in stomatal conductance (Barbosa *et. al.* , 2007) . At high chromium concentrations production of enzymes are inhibited (Vazques *et. al.*, 1987) which ultimately leads to decrease in the photosynthetic yield (Nagajyoti *et. al.*, 2010). On comparison, it has been found that Cr (III) can increase assimilation of carbon in *E. crassipes* whereas Cr (VI) reduces carbon assimilation, content of chlorophyll a, and fluorescent parameter and this change in photosynthetic capacity is due to anomalous organization of the ultra structure of chloroplast (Van Assche and Clijsters, 1983). The decrease in amount of photosynthetic pigment due to metals occurs by inhibition in the activity of enzyme involved in the biosynthesis of chlorophyll, as well as by changing the position of Mg ion of the chlorophyll molecule by the metal, impairing the reception of light by the molecule and leading to reduction in photosynthetic rate (Kupper *et al.*, 2002; Starzalka 2011). In *Phaseolus vulgaris*, on exposure to 10⁻⁶, 10⁻⁴, and 10⁻² mol l⁻¹ of CrCl₆H₂O, it was observed that amount of pigment increased at lower and moderate concentration of Cr (III) , whereas the plants irrigated at highest Cr (III) concentration , chlorophyll a , b and carotenoid content is significantly reduced (Zeid, 2001) . In tomato plants, rate of photosynthesis is reduced on exposure for two weeks at 0, 10, 20, 30, 40, 50 µmol L⁻¹ Cr (III). The decrease in the yield of photosynthesis due to Cr (III) was gradual and slow whereas the reduction due to Cr (VI) is much more significant (Henriques, 2010).

If transpiration is eliminated without stopping photosynthesis, injury from drought would occur and crop plants will not thrive in large areas that are semi desert. Wilting of various crops and plant species due to Cr toxicity has been reported (Turner and Rust, 1971), but little information is available on the exact effect on Cr on water relation of higher plants. Barceló *et. al.*, (1985) observed a decrease in water potential of leaf in Chromium treated bean plants. When bush bean plants are exposed to Cr, turgor pressure and plasmolysis in epidermal and cortical cells were decreased (Vazques *et. al.*, 1987). Toxic levels of Cr in bean were found to decrease tracheary vessel diameter thereby reducing longitudinal water movement (Vazques *et. al.*, 1987). The presence of metal blocks water transport from roots to the above ground parts leading to severe dehydration of shoots (Haag- kerwer *et. al.*, 1999). Inspired spatial distribution and reduced root surface of Cr stressed plants can lower the capacity of plants to explore the surface for water. The significantly higher toxic effect of Cr (VI) in declining the stomata conductance could be due to high oxidative potential of Cr (VI), which in turn may be instrumental in damaging the cells and membrane of stomatal guard cells.

Chromium due to its structural resemblance with some essential elements can affect mineral nutrition of plants in a byzantine way. Interactions of chromium with uptake and accumulation of other inorganic nutrients have received maximum attention by researcher. Cr (III) and Cr (VI) are taken up by the plants by different mechanisms (Zaccheo *et. al.*, 1985). It is suggested that Cr stress interferes with the functions of mineral nutrients in rice plants, thus causing a serious inhibition of plant growth (Adriano 1986). The toxic effect of Cr may particularly be linked to the interactions of chromium with essential nutrient element due to changeable valences (Turner and Rust, 1971). Many investigations were done and determined (2010 a). Chromium accumulation and tolerance varies with varied genotypes within a crop (Zeng *et al.*, 2008 a). In soil grown rye grass, the influence of chromium on mineral nutrition was highly variable and depended on the source of Cr and soil properties (Ottabbong , 1989 a); it was further found that differences in soluble Mn fractions , interactions with P and critical effects on the uptake of Mn ,Cu , Zn , Fe and Al were influenced by Cr in rye grass (Ottabbong , 1989 b) . Cr induced chlorosis was also observed, whereas there was no clear correlation between Fe levels in leaf and chlorosis (Ottabbong, 1989 c). In non calcareous soil with Cr (III), the translocation of Fe, Zn and Mo to bean plants was decreased (Wallace *et al.*, 1976). It is important to obtain additional information on the possible mechanism of Cr tolerance by studying Cr stress on the sub cellular distribution and chemical form of mineral nutrients among the different genotypes. The results show that Cr concentration was significantly and negatively correlated with dry weight of all plant organs. In rice roots, Cr concentration has a significantly positive correlation with Mg concentration, but significantly negative correlation with Ca, Zn, and Fe concentrations. In rice stems, significant and positive correlations between Cr and Ca / Mg concentrations but negative correlations between Cr and Zn / Fe concentrations were found. In rice leaves , there was a significant and positive correlation between Cr and Ca concentrations , negative correlation between Mg / Zn concentrations and no relation with Fe concentrations (Zeng *et. al.* , 2010 b).

Cr stress can induce alteration in the activity of enzymes. Chlorosis endangered by heavy metals has been usually correlated with low Fe content in plant. Under Fe deficient condition, dicotyledonous plants elevated root Fe (III) reductase activity, thus increasing the ability to reduce Fe (III) to Fe (II), the form in which roots absorb (Alcantra *et. al.*, 1994). Cr applied to Fe deficient *Plantago lanceolata* roots increased the activity of root associated Fe (III) reductase. This effect was evident only with acceptor of the turbo reductase and was not observed in Fe sufficient plants (Wolfgang 1996). In split root experiments , which allows only a part of the root system to receive Cr while the other portion was grown in Fe free medium , roots obnoxious to either treatment showed an intermediate Fe – EDTA reductase activity with respect to non split control plants (Wolfgang 1996).

ATPase plays a significant role in the adaptation to heavy metal conditions and it is regulated to the molecular and biochemical level (Dietz *et. al.*, 2001). Pillay (1994) suggested that ATPase activity increased at higher treatment concentrations in a study on the effects of soil with treatment of Cr on different

metabolites and certain enzymes of *Hyptis suaveolens* and *Helinthus annuus* leaves. The inhibition of ATPase activity causes a decrease in proton extrusion. This in turn could cause a decrease in the transport activities of the root plasma membrane, thus reducing the uptake of most of the nutrient elements. It is also possible that Cr interfered with the mechanism controlling the intracellular pH which is supported by the fact that cellular Cr could be reduced in the cells thereby utilizing the protons (Zaccheo *et. al.*, 1985).

Some strategies have developed in plants to cope up with the worst conditions and the negative effects (Liu and Yao, 2007), and the process of development in plant also changes due to initiation of a variety of metabolic alterations as a result of excess of chromium and other metals (Hayat *et. al.*, 2012). Redox metals, such as, Cr can directly generate oxidative injury via Haber – Weiss and Fenton reactions, which in turn lead to the production of reactive oxygen species (ROS) in plants (Flora, 2009). Though the generation of reactive oxygen species (ROS) such as superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH^\cdot) and oxygen singlets (1O_2) are produced in less amount in aerobic organism but under stressed conditions, their generation dramatically increases (Miller *et. al.*, 2010). As a result of maximum ROS generation in plants can result in disruption of homeostasis in cells, rupturing of DNA strand, defragmentation of proteins, or cell membrane and damage of photosynthetic pigments, which may ultimately lead to cell death (Flora, 2009).

Plants need to effectively eliminate the toxic reactive oxygen species (ROS) which are generated as a result of environmental stresses. Plants have developed a complex antioxidant system which scavenges these ROS particles thereby providing protective shield against the oxidative attack (Vranova *et. al.*, 2002).

With the help of antioxidant compounds, ROS are eliminated from the site of their production (Hossain *et. al.*, 2012). The main antioxidant enzymes studied in plants include catalase (CAT, E. C. 1.11.1.6), guaiacol peroxidase (E.C.1.11.1.7), ascorbate peroxidase (E.C. 1.11.1.11), glutathione reductase (E.C. 1.8.1.7), superoxide dismutase (SOD, E.C. 1.15.1.1) (Gill and Tuteja, 2010; Panda and Choudhury, 2005). Beside enzymes increase in other compounds have also been reported in response to metal induced stress such as polyamines (Hussain *et. al.*, 2011), proline (Kishor *et. al.*, 2005), nitric oxide (Delledonne, 2005; Liu and Yao 2007) and metallothioneins (Teixeira *et. al.*, 2013). Though ROS have harmful effects, it has been noticed that H_2O_2 being a ROS can act in signaling mechanisms in response to stress (Mittler *et. al.*, 2004; Sharma and Deitz, 2009), and has proposed as a key molecule to elicit signal transduction for metal tolerance in plants, since it is immediately produced under stress by metals (Seth *et. al.*, 2012).

Though many studies have been conducted on the stress caused by chromium, the precise molecular mechanisms related to both the effects of chromium phytotoxicity, the defensive mechanism of plants against exposure to chromium as well as translocation and accumulation in plants in general remain poorly understood (Dubey *et. al.*, 2010). With the advancement technology, and field of “omics”, far more precise and greater number of variables linked to physiological response to Cr stress has been developed. This field

has maximum scope to understand the toxic effect of heavy metal pollutant and in consequence identification of new biomarkers of effect (Dowling and Sheehan; Lopez- Barea and Gomez-Briza, 2006).

2. National Level:

SOURCES AND UPTAKE OF CHROMIUM :

Most of the water sources of the whole world receive millions of liters of sewage, domestic wastes, agricultural and industrial and agricultural effluent. Effluent of Cr is highly toxic for plants for their growth and development. Effect of Cr on the plant *Hibiscus esculentum* was experimentally observed (Hira *et. al.*, 2013) on the seed germination, seedling growth, seedling vigor index, chlorophyll content and tolerance indices. The effects on plants Cr. Phytotoxic in their chlorophyll content increases with increase in the amount of effect of chromium on the germination, seedling growth of some wheat (*Triticum aestivum* L.) cultivars was studied by Datta (2011) where he found the phytotoxic percentage increases with increase in Cr concentration. India alone releases about 2000-3200 of Chromium in elemental form in the environmental form in the environment annually from tanning industry with Chromium concentration ranging between 2000 to 5000 mgL⁻¹ in the effluent ,compared to the recommended permissible limit of 2µgL⁻¹.Cr(VI) is a strong oxidant with a high redox potential in the range of 1.33-1.38eV accounting for a rapid and high generation of ROS and its resultant toxicity(Shanker *et. al.*,2004).The phytotoxic effects of Cr absorption and distribution dependent on the speciation of the metal which determine its uptake ,translocation and accumulation (Shanker *et. al.*,2005).Due to the environmental stresses it effects on plants by the responses by which the structural and functional integrity is affected .In the study of toxic effects of heavy metals on plant growth and metal accumulation in maize by Ghani,2010 found that Cr was found to be the least but it is considerably phytotoxic metal as compared to the other metals added to the soil. In the moist soil conditions ,low level of phytotoxicity has been recognized to its insoluble nature.(Smith ,1992).Cr (III) did not affect the plant until and unless the concentrations given to it are very large (Sharma,1996).In sand culture process of Maize plants under the green house conditions brought about considerable reduction in chlorophyll content, biomass ,activities of catalase and peroxidase activities (Kalayanaraman,1993).The heavy metals are accumulated in soil are occurred in parts of the soil where the roots are concentrated which is easily accessible for the plants.

2.2 TOXIC EFFECTS OF CHROMIUM

2.2.1 EFFECT ON GERMINATION:

Growth and development are continuous processes and mainly depend on external resources present in soil and air (Shankar *et. al.*, 2005). Sugarcane bud germination was reduced by 32.57% were observed on

exposure to 20 and 80 ppm Cr respectively (Jain *et al.*, 2000). The maximum in pigeon pea germination (100%) was recorded in control and 20 ppm Cr level. With increasing level of Cr after 20 ppm decreased the germination by 4, 5, and 7% in 40, 80 and 100 ppm Cr levels, respectively at 72 hours (Dotaniya *et al.*, 2014). Early growth stages of seedling are very important indicator in determining toxicity impacts of heavy metals like chromium in plants (Pandey and Pandey 2008).

2.2.2 EFFECT ON ROOT, SHOOT AND LEAF GROWTH:

Growth of paddy is decreased in concentration of Cr (VI) up to 200 mg /l in *Oryza sativa* L. (Sundarmurthi *et al.*, 2010). In mungbean root do not elongate on exposure to high concentration of Cr (VI) but in low concentrations root elongation is similar to the control (Samantary , 2002). Cr exposure also affects the development of lateral roots and root numbers. It was reported that toxicity of metal is in the order Cd > Cr > Pb in root primordia of *Salix viminalis*, where as root length was more affected by Cr than other heavy metals (Prasad *et al.*, 2001). Panda and Patra (2000) observed that 1 µM of Cr increased the root length growing under nitrogen nutrition level; higher Cr concentration decreased root length in all the nitrogen treatments .

An adverse effect is observed in the growth of shoot also on exposure to chromium. Sharma and Sharma in 1993 reported that after 32 and 96 days, plant height reduced significantly in wheat cv. UP 2003 in a glass house trial when sown in sand with 0.5 µM sodium dichromate.

Brown coloration of roots, stunted growth of roots, malformation of root hair, and increase in lateral growth of root is reported due to accumulation of Cr (VI) (Samantaray *et al.*, 1996; Mallick *et al.*, 2010). Aduini *et al.*, (2006) reported that formation of light blue deposit on roots of *M .sinensis* under 50 to 200 mg /l of Cr (III) . Cr (III) has been found to cause necrosis in root apices of *Genipa americana* plants contaminated water containing 30 mg /l (Barbosa *et al.*, 2007).

2.2.3 EFFECT ON PRODUCTIVITY :

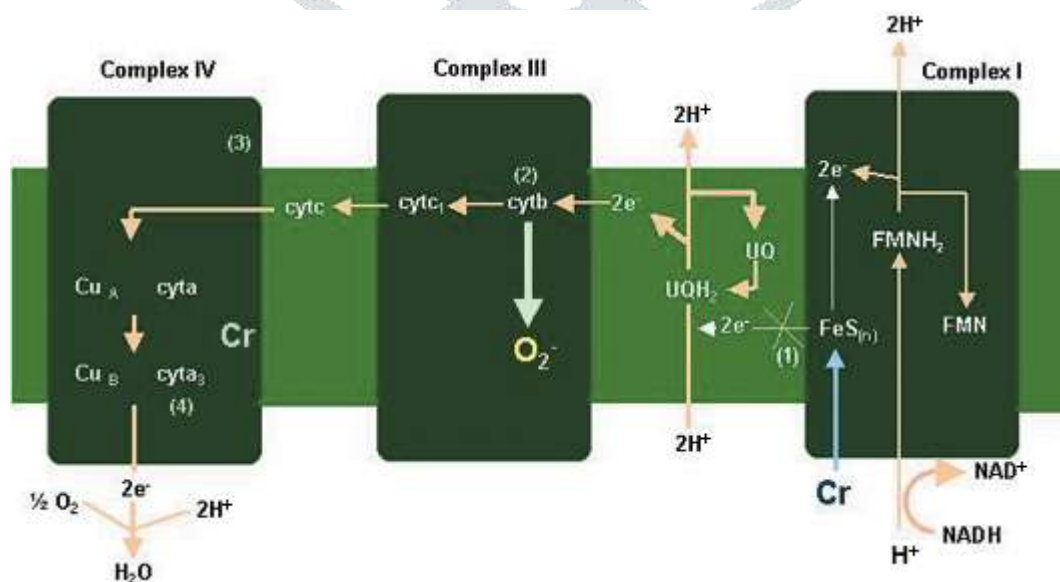
Grain yield of crop plants are significantly reduced when the soil is contaminated with chromium. It was reported by Bishnoi *et al .*, (1993) absorption of Cr (VI) by *P. sativum* reduces the number of flower and number of pods per pea plant and under Cr (VI) stress seeds were formed in a few pods and the number of seeds per pod was also significantly reduced. No drastic change was observed in total yield of *L. esculentum* though the no of fruits were reduced when grown in a nutrient solution containing Cr (III) 50 to 100 mg /l. It was reported by Sundarmoorthy *et al.*, in 2010 that weight of grain and yield of *O. sativa* was greatly reduced when Cr (VI) was there in irrigation water.

2.2.4 EFFECT ON ANATOMY :

When *Phyllanthus amarus* are stressed under Cr (VI), wax deposition is reduced in leaves and stomata are widely opened with enlarged subsidiary cells were observed by Rai and Merhotra in 2008 .reduction in the number of palisade and spongy parenchyma cells were observed by Su *et. al.*, in 2005 in *P. vittata* when grown in Cr (VI) 500 mg /kg and Cr (III) 1000 mg/ kg.

2.2.4 EFFECT ON PHYSIOLOGY AND BIOCHEMISTRY :

Due to increase in concentration of heavy metals in plants, photosynthetic pigments are significantly degraded. According to Dixit *et. al.*, (2002), the change in redox reactions of Cu and Fe carriers as shown in figure allows, for Cr (VI) to be transferred via cytochrome in mitochondria and allow this element to bind to cytochrome a_3 as well cytochrome IV of cytochrome oxidase (E. C. 1.9.3.1), thus causing severe inhibition of the activity of this enzyme (Dixit *et. al.*, 2002). When *Brassica juncea* was exposed to 200 and 400 mmol L^{-1} Cr (VI) , greater activity of Photosystem II was observed as compared to control plants (Gupta *et. al.* , 2009) . The Photosystem I activity in thylakoids of seedlings exposed to 200 $\mu mol L^{-1}$ Cr (VI) was similar to controls , whereas the PSI activity in plants exposed to over 200 mmol L^{-1} Cr (VI) concentrations were lower compared to control seedlings (Gupta *et. al.*, 2009) . In research conducted with chloroplast isolated from *Beta vulgaris* L. submitted to Cr (VI) exposure, a significant inhibition of electron transport activity in both PSI and PSII was observed. Within the PSII, the pheophytin and plastoquinone regions were more affected (Pandey *et. al.*, 2013). Metals at higher levels are more toxic than at lower levels. The decrease in growth due to metal treatment might be attributed to a loss in photosynthetic pigments (Sharma and Sharma, 2003) which ultimately lead to reduction in rate of photosynthesis. The Cr (VI) has more significant in photosynthesis.



Involvement of chromium in inhibition of electron transport system in plants (Panda & Chaudhury, 2005)

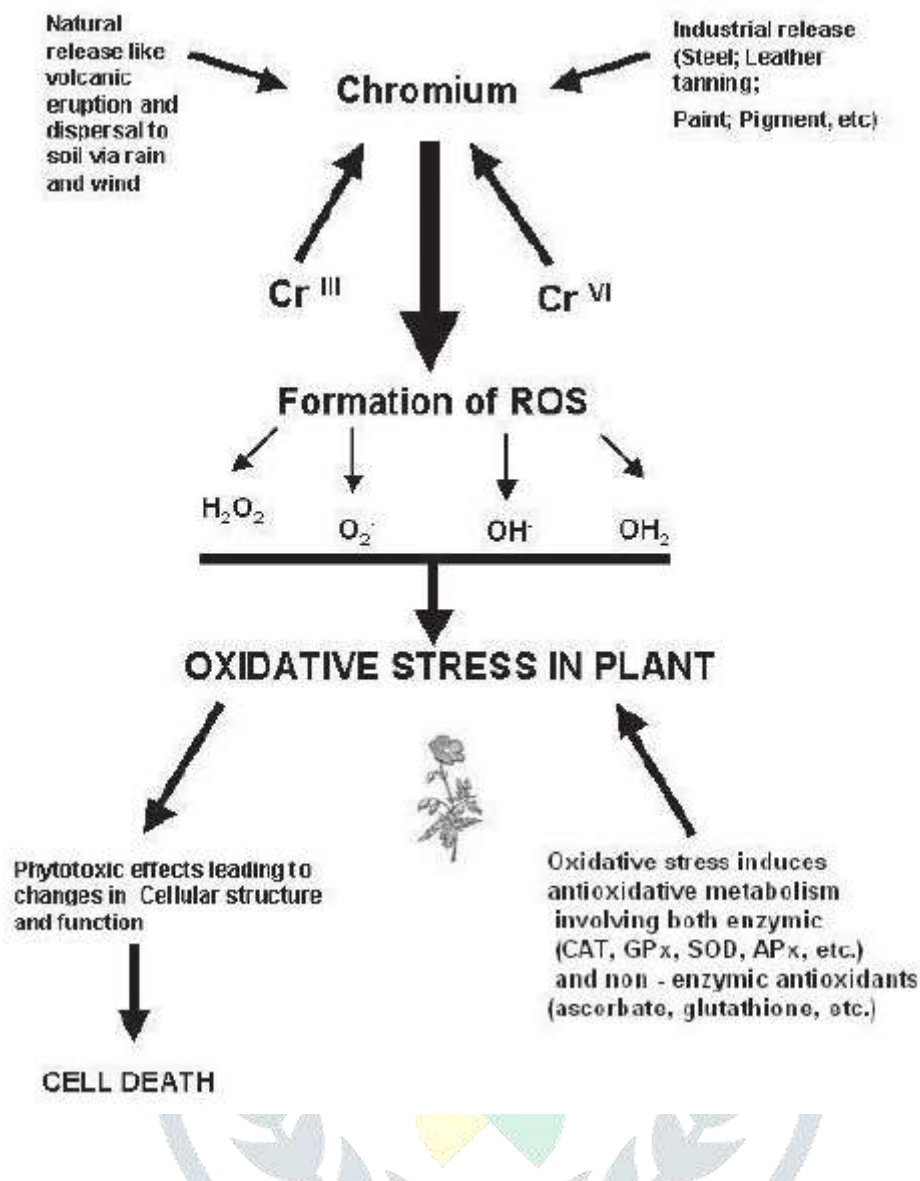
The chromium induced toxicities and abnormalities can cause defects both in dark and light reactions of photosynthesis. Bioaccumulation of Cr and its toxicity to photosynthetic organelle and pigments in various crops and trees is well documented (Shanker *et al.*, 2005; Sinha *et al.*, 2006).

Excess of heavy metals decreased the water potential too in plants. Presence of Cr decreased the water potential and rate of transpiration and increased diffusive resistance and relative water content in leaves of cauliflower (Chatterjee and Chatterjee, 2000). Gopal *et al.*, (2009) demonstrated that Cr (VI) reduces the physiological availability of water as indicated by a decrease in leaf water potential and increase in diffusive resistance in *S. oleracea* leaves, thereby suggesting development of water stress.

One of the reasons for the decreased uptake of most of the nutrient in Cr stressed plants could have been because of the inhibition of the activity of plasma membrane H⁺ ATPase (Shanker, 2003). Cr treatment also markedly inhibited the incorporation of P, K, Ca, Mg, Fe, Zn, and Cu in different cellular constituents in 1 year old West Coast Tall coconut plants growing in pots (Biddappa and Bopaiah, 1989). The decrease in N, K, P and other elements could be due to reduction in growth of root and anomalous penetration of roots into the soil due to toxic effect of chromium. Khan *et al.* (2001) noticed that threshold values of the concentration of N, P, and K in dry weight of rice plants showed significant reduction at 0.5 ppm Cr. When Cr is in excess (0.5 mM) caused a decrease in the concentration of Fe and affected the translocation of P, S, Mn, Zn, and Cu from roots to tops in cauliflower (Chatterjee and Chatterjee). Total P in sunflower hull was the highest with Cr (0.5ppm) 30 days after flowering (Gupta *et al.*, 2000), whereas Sharma and Sharma in 1996 observed that concentration of P in leaf is reduced with 0.5 mM Cr in wheat cv. UP 2003. Cr (VI) is actively taken up and is a metabolically driven process in contrast to Cr (III) which is passively taken up and retained by cation exchanges sites of the cell wall (Shankar *et al.*, 2004). In addition, it is known that P and Cr are competitive for surface sites and Fe, S and Mn are also known to compete with these elements to gain rapid entry into the plant system.

Heavy metals can change in enzyme content of a plant also which are basically the biocatalyst and in turn affects the metabolism of plants. The activity of nitrate reductase in leaves was considerably increased over that of the control and negatively complemented with root and shoot length, leaf area, and biomass of the plants, exhibiting stress due to Cr (VI) in *Albizia lebbek* (Tripathi *et al.*, 1999). Seedlings treated with 1 mM Cr resulted in the increased activity of nitrate reductase, whereas higher concentrations were toxic and reduces the activity of enzyme in wheat (Panda and Patra, 2000a). Cr is observed to affect Fe uptake in dicots either by averting reduction of Fe (III) to Fe (II) or by competing with Fe (II) at the site of absorption (Shanker 2004). One of the membrane bound enzyme whose activity is changed in Cr stressed plants seems to be H⁺ ATPase (Pandey *et al.*, 2009 a). This is the only proton pump operating in the plasma membrane, playing a crucial role in the regulation of ion homeostasis.

Reactive oxygen species (ROS) are generated in plants when exposed to Cr, and has been shown to result in oxidative stress leading to DNA, protein and pigment damage, as well as the initiation of lipid peroxidation (Choudhury and Panda, 2005; Panda, 2003). Absorption of chromium is facilitated by a carrier membrane; thereby ROS generation and their impact on plasma membrane are very important (Maiti *et al.*, 2012). Cr reactivity can be considered from its interaction with glutathione, NADH and H₂O₂, forming OH⁻ radicals in cell free systems. Production of H₂O₂, OH⁻, and O₂⁻ under stress of Cr has been demonstrated in plants, generating oxidative stress leading to damage of DNA, proteins and pigments as well as peroxidation of lipids (Bagchi *et al.*, 2000; Panda *et al.*, 2003). Cr at both toxic and mild concentrations can inhibit uncoupled electron transport (Dixit *et al.*, 2001), indicating the electron transport chain to be a common site of Cr binding in plants. Inhibition of electron transport by Cr may be a consequence of redox change in the Cu and Fe carriers, where Cr may be transferred by cytochrome in the mitochondria to reduce heme group of cytochrome may act as a site for Cr binding, blocking electron transport (Dixit *et al.*, 2001). The severe inhibition of cytochrome oxidase activity may be due to binding of Cr to complex IV where Cr may also bind to cytochrome a₃ (Dixit *et al.*, 2001). In pea plants, the treatment of Cr at different concentrations showed that O²⁻ is generated in the cytochrome b region complex (III) of root mitochondria and high at this site is high (Dixit *et al.*, 2001; Panda and Chaudhury, 2005). The antioxidant response of plants to metal induced oxidative stress is variable and depends on the type of plants and metals involved. It has not been reported much about antioxidative property of Cr unlike other heavy metals such as Cd, Zn and Fe. It has been observed that antioxidative property of *S. nigrum* is higher than that of *H. annuus* (Vijayalakshmi *et al.*, 2010). The increase in antioxidant enzyme activity observed might have been in direct response to the generation of superoxide radical by Cr-induced blockage of electron transport chain in the mitochondria.



Involvement of Chromium in inhibition of electron transport system in plants (Panda and Chaudhury , 2005)

3. Regional level

In regional level the effect of Cr in plants regarding its morphological and physiological changes are not worked in adequate. The metal chromium has no any biological functions in plants .The various effects on plants processes during its early growth stages. R.P.Kakati and NayanPathak,2014 investigated on the effect of Chromium induced in morphological and biochemical changes in seedling of Black gram(*Phaseolus mungo*).An another study has been done by karak and Paul,2014 ,the effect of Chromium in soil and tea (*Camellia sinensis L.*) infusion. Here they found that only $2.5\mu\text{gL}^{-1}$ to $4.8\mu\text{gL}^{-1}$ Cr was present in tea infusion when it was received from different doses of municipal solid waste compost. Choudhury, 2005 examined toxic effects of Pb and Cr, their oxidative stress and ultra structural changes in moss *Taxithelium*

nepalense (SCHWAEGR.) broth .After the application of both the metals the dry mass and total chlorophyll content decreased. At high concentration of metals, moss cells could be induced oxidative stress.

Cr interferes with several metabolic processes, causing toxicity to plants as exhibited by reduced germination or early seedling development (Sharma et al., 1995)

Sharma et al. in 1995 reported that less than 1 mM Cr (VI) stress, grain yield was severely affected in *Triticum aestivum* and no grain formation occurred.

CONCLUDING REMARKS:

After reviewing the literature about chromium toxicity in plants, it becomes easy to understand contamination of Cr is, increasingly, a major threat to the biota, provides new insight to the chromium toxicity to the plants and emerging as a serious health hazard to the living world. Cr is a non essential element for plants but is a toxic heavy metal which induces toxicity in plants. Cr is obtained in different oxidation states. The compounds of hexavalent chromium are much more toxic than that of trivalent chromium. The reason for such toxicity is due to its rapid permeability through biological and subsequent interactions with intercellular proteins and nucleic acids. Cr is capable of inducing several toxic effects on plants, including changes in the germination process and in the growth of roots, stems and leaves as well as harmful effects on morphological and physiological processes such as photosynthesis, water relation to plants, mineral nutrition, change in synthesis enzyme etc. From a molecular point of view Cr is also capable of inducing oxidative stress in plant cells , disrupting redox equilibrium .Certain plant species can tolerate high levels of Cr and even accumulate Cr in their tissues , thus highlighting their potential of Cr pollute sites. Mycorrhizal fungi help in translocation, accumulation and thus phytoremediation of Cr by plants. Moreover, salicylic acid can be used to mitigate Cr toxicity in plants.

From the overview of literature , it can be concluded that studies in this regard are still very less as chromium has a very complex chemistry and thus the mechanism are poorly understood though several defense mechanism are employed by plants .

Plant health can be improved and environmental stresses can be reduced by arbuscular mycorrhizal (AM) fungi which can enhance uptake of water, increasing resistance to disease. (Pfleger and Linderman 1994; Gother and Paszkowski 2006). AM fungi enhance plant tolerance to heavy metals with compounds secreted by AM fungi or absorption of heavy metals in fungal cell walls, chelation inside the fungi, or precipitation of metals in polyphosphate granules in the soil (Gother and Paszkowski 2006).

It has been observed that application of salicylic acid can alleviate toxicity of Cr in clusterbean plants. Exogenous salicylic can enhance activities of nitrogen metabolism pathways of enzymes and alleviates

disordered caused by Cr stress. It is considered as a useful compound for suppressing uptake of Cr in plants under Cr stressed soil. (Punesh Sangwan and U. N. Joshi)

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