

**Effect of Biochar, Poultry manure & Sewage sludge  
on Phytoremediation of Chromium Contaminated Soil  
by using Rice (*Oryza sativa* L.)**

काशी हिन्दू  
विश्वविद्यालय



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UNIVERSITY

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THE REQUIREMENTS FOR THE DEGREE OF  
**Master of Science (Agriculture)**  
in  
**Soil Science and Agricultural Chemistry**

Supervisor  
***Prof. P.K. Sharma***

Submitted by  
***Jiten Kumar Behera***

DEPARTMENT OF SOIL SCIENCE & AGRICULTURAL CHEMISTRY  
INSTITUTE OF AGRICULTURAL SCIENCES  
BANARAS HINDU UNIVERSITY  
VARANASI - 221 005  
INDIA

ID. No. 16412SAC023

2018

Enrolment No.389360



**Dr. P.K Sharma**  
Professor

Department of Soil Science & Agricultural Chemistry  
Institute of Agricultural Sciences  
Banaras Hindu University  
Varanasi – 221005

Ref. No. ....

Date .....

### **CERTIFICATE**

To,  
The Registrar (Academic)  
Banaras Hindu University,  
Varanasi-221005 (India)

**Through:** The Head,  
Department of Soil Science and Agricultural Chemistry, Institute of  
Agricultural Sciences, Banaras Hindu University,  
Varanasi-221005 (India)

Dear Sir,

I have great pleasure in forwarding the thesis "**Effect of Biochar, Poultry Manure & Sewage sludge on Phytoremediation of Chromium contaminated Soil by using Rice (*Oryza sativa* L.)**" submitted by **Mr. Jiten Kumar Behera**, (I.D. No. 16412SAC023), in partial fulfillment of the requirements for the degree of **Master of Science (Agriculture) in Soil Science and Agricultural Chemistry**, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi.

I certify that the entire scheme of investigation presented herein was planned and carried out solely by the candidate under my guidance and supervision. The data presented in the thesis, to the best of my knowledge and belief, are genuine and original. No part of the work has been submitted for any other degree or distinction.

Thanking you,

**Forwarded by**

**(Head)**

**(Prof. P.K Sharma)**

Supervisor

# Effect of Biochar, Poultry Manure & Sewage sludge on Phytoremediation in Chromium Contaminated Soil by using Rice (*Oryza sativa* L.)”

by

*Jiten Kumar Behera*

Thesis submitted in partial fulfilment of the requirements for the degree of

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**IN**

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Department of Soil Science and Agricultural Chemistry

Institute of Agricultural Sciences

Banaras Hindu University

Varanasi- 221005, Uttar Pradesh

India

## Approved by Members of the Advisory Committee

- Chairman and Supervisor** : **Dr. P.K. Sharma**  
Professor  
Department of Soil Science and Agricultural chemistry  
Institute of Agricultural Sciences, BHU, Varanasi
- Member** : **Dr. S. K. Singh**  
Professor  
Department of Soil Science and Agricultural chemistry  
Institute of Agricultural Sciences, BHU, Varanasi
- Member** : **Dr. R. Meena**  
Assistant Professor  
Department of Soil Science and Agricultural Chemistry,  
Institute of Agricultural Sciences, BHU, Varanasi
- Member** : **Dr. J.K Singh**  
Professor  
Department of Agronomy,  
Institute of Agricultural Sciences, BHU, Varanasi
- External Examiner** :

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## CHAPTER I

# INTRODUCTION

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Environmental pollution by heavy metals has become a serious problem in the world. The mobilization of heavy metals through extraction from ores and subsequent processing for different applications has led to the release of these elements into the environment. The problem of heavy metals' pollution is becoming more and more serious with increasing industrialization and disturbance of natural biogeochemical cycles. Unlike organic substances, heavy metals are essentially nonbiodegradable and therefore accumulate in the environment. Soil contamination is majorly due to accumulation of heavy metals and metalloids through emissions from the rapidly expanding industrial areas, disposal of high metal wastes, land application of fertilizers, wastewater irrigation, animal manures, pesticides, sewage sludge, mine tailings, coal combustion residues, leaded gasoline and paints, spillage of petrochemicals, and atmospheric deposition. The accumulation of heavy metals in soils and waters poses a risk to the environmental and human health.

Heavy metals are generally defined as metals with relatively high density, atomic weight or atomic number. It includes elements (both metal and metalloids) with an atomic density greater than  $6\text{ g cm}^{-3}$  (with an exception of Arsenic (As), Boron (B) and Selenium (Se)) or the metals which are having a density of five times higher than that of water. They are regarded to be cytotoxic, mutagenic and carcinogenic. Chromium is one of the top sixteen toxic pollutants.

Chromium (Cr) with atomic number 24, molecular weight 51.1 and density  $7.19\text{ g/cm}^3$  is a silver color hard metal. Chromium is the 7th most abundant element (Nriagu, 1988), and 21st most abundant metal (Sinha et al., 2005; Economou-Eliopoulos et al., 2013) of the Earth's crust. Chromium is also categorized as no.1 carcinogen according to the International Agency for Research on Cancer (IARC, 1987) and the National Toxicology Program. Chromium has a complex electronic and valence shell chemistry

owing to its high potential to easily convert from one oxidation state to another (Prado et al., 2016a). Chromium has several oxidation states (2 to 6), but hexavalent chromate [Cr (VI)] and trivalent chromite [Cr (III)] forms are the most common and stable in the natural environment (Ashraf et al., 2017).

The main human activities that increase chromium(VI) concentrations are chemical, leather and textile manufacturing, electro painting and other chromium(VI) applications in the industry. These applications will mainly increase concentrations of chromium in water. Through coal combustion chromium will also end up in air and through waste disposal chromium will end up in soils.

Most of the chromium in air will eventually settle and end up in waters or soils. Chromium in soils strongly attaches to soil particles and as a result it will not move towards groundwater. In water chromium will adsorb on sediment and become immobile. Only a small part of the chromium that ends up in water will eventually dissolve. Chromium(III) is an essential element for organisms that can disrupt the sugar metabolism and cause heart conditions, when the daily dose is too low. Chromium(VI) is mainly toxic to organisms. It can alter genetic materials and cause cancer.

Crops contain systems that arrange the chromium-uptake to be low enough not to cause any harm. But when the amount of chromium in the soil rises, this can still lead to higher concentrations in crops. Acidification of soil can also influence chromium uptake by crops. Plants usually absorb only chromium(III). This may be the essential kind of chromium, but when concentrations exceed a certain value, negative effects can still occur.

Chromium is not known to accumulate in the bodies of fish, but high concentrations of chromium, due to the disposal of metal products in surface waters, can damage the gills of fish that swim near the point of disposal. In animals chromium can cause respiratory problems, a lower ability to fight disease, birth defects, infertility and tumor formation.

Cr and its compounds have multifarious industrial uses. They are extensively employed in leather processing and finishing (Nriagu, 1988), in the production of refractory steel, drilling muds, 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, until recently, wood preservation. These anthropogenic activities have led to the widespread contamination that Cr shows in the environment and have increased its bioavailability and biomobility. The leather industry is the major cause for the high influx of Cr to the biosphere, accounting for 40% of the total industrial use (Barnhart, 1997). In India, about 2000–32,000 tons of elemental Cr annually escape into the environment from tanning industries. Even if the recommended limit for Cr concentration in water are set differently for Cr(III) (8  $\mu\text{g L}^{-1}$ ) and Cr(VI) (1  $\mu\text{g L}^{-1}$ ), it ranges from 2 to 5  $\text{g/L}$  in the effluents of these industries (Chandra et al., 1997). In the United States, 14.6  $\mu\text{g L}^{-1}$  in ground water and 25.9  $\text{gd kg}^{-1}$  in soil have been found in the vicinity of chrome production sites.

Remediation of heavy metals polluted soil could be carried out using physicochemicals processes such as ion exchange, precipitation, reverse osmosis, evaporation and chemical reduction. However, the measures require external man-made resources and therefore are very costly (Mangkoedihardjo and Surahmida, 2008).

Plant-based bioremediation technologies have received recent attention in order to clean-up contaminated soil and water. These strategies have collectively termed as phytoremediation which refers to the use of green plants and their associated micro biota for the in-situ treatment of contaminated soil, sediment and as well as ground water. Biological based remediation strategies, which includes phytoremediation, have been estimated to be four to 1000 times cheaper, on volume basis, than current non-biological technologies. For a country like India, phytoremediation is best suited as it requires low investment, and relies on plants natural capability to take up metal ions from soil (Ghosh and Singh, 2005).

Phytoremediation is an in situ remediation innovation that uses the inborn capacities of living plants. The proficiency and instruments of phytoremediation rely upon the sort of contaminant, bioavailability and soil properties. Highlights of plants in phytoremediation are leveling pH, radiating allelochemicals, exudation of restricting mixes like phenolics, quinols, peptides, enzymatic change and authoritative of contaminations. The uptake, translocation, and form of Cr in the plant are dependent on the form and concentration of the supplied Cr. Oxidation state (+3) of chromium was

found predominant, regardless of the Cr source supplied to the plant. Although at high Cr (VI) treatment concentrations, Cr (VI) and Cr (V) were also observed. At low Cr (VI) concentrations, the plant effectively reduced toxic Cr (VI) to less toxic Cr (III), which was observed both as a Cr (III) hydroxide phase in the roots and a Cr (III)– organic complex in the roots and shoots. At low Cr (VI) treatment concentrations, Cr in the leaves was observed predominately around the leaf margins, whereas at higher concentrations Cr accumulated in the leaf veins. Cr toxicity is thought to be caused by reaction of the Cr with reducing agents, such as NADPH, which in turn react with hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) to generate damaging hydroxide (OH) radicals, in addition to Cr reacting with the carboxyl and sulfhydryl groups of enzymes, thereby inhibiting their activity (Cervantes et al., 2001). The ability of a plant to minimize these effects and thereby withstand greater concentrations indicates a plants tolerance to the metal.

Rice paddy-based cropping systems cover more than 162.3 million hectares of land around the world. Rice plants belong to the family Poaceae, members of which are characterized by a fibrous adventitious root system that helps to pump water more efficiently from the soil, an important characteristic for meeting the primary requirement of a metal accumulator plant. Fibrous adventitious roots of rice plants also help to cope with nutrient deficiency. Extensive fibrous root systems firmly cling to soil particles, preventing paddy soil erosion. Thus the morphological features of roots of rice plants help to prevent outflow of TEs from the contaminated soil through soil erosion. The rice plant poses promising phenological characteristics of metal accumulation. Acclimation to dry as well as wetland cultivation and adaptability to grow under varying environmental conditions such as anoxia make this plant ideal for phytoremediation. In addition, assisted metal accumulation strategies can be easily incorporated in rice paddies. Rice cultivation also offers a vast amount of biomass that can be utilized for bioremediation. Rice industry by-products of commercial importance include straw, husk, bran, germ, and broken rice. These materials can be utilized to produce bioremediation agents such as biosorbents and substrates for microbe-assisted metal removal. Cultivation of various floating aquatic plants during wetland cultivation increases biomass yield from rice paddies and helps in sustainable bioremediation. Thus rice paddy-based bioremediation approaches radically contribute to economic gain.

Addition of organic matter amendments, such as compost, fertilizers and wastes, is a common practice for immobilization of heavy metals and soil amelioration of

contaminated soils (Clemente et al. 2005). Organic amendments, obtained mainly from plants, are characterized by great variation in the content of biogenic elements such as nitrogen, phosphorus, and potassium. They contain large amounts of carbon and other elements, which are part of the main organic substances. The quality of plant substrate for the production of compost is influenced by the content of organic material and fertilizers and the ratio of carbon to nitrogen. The effect of organic matter amendments on heavy metal bioavailability depends on the nature of the organic matter, their microbial degradability, salt content and effects on soil pH and redox potential, as well as on the particular soil type and metals concerned (Walker et al. 2003, 2004). The present study has an objective to broaden and increase the knowledge of the effect of organic amendments like biochar, poultry manure, sewage sludge on the phytoremediation of chromium contaminated soil by using rice plant.

Biochar application into agricultural soil has recently attracted attention due to its potential agronomic, economic, and environmental benefits. Biochar is a carbon (C) rich material produced by pyrolyzing waste biomass between 250 and 900 °C under continuous flow of nitrogen or anoxic conditions. Examples of waste biomass used for biochar include hard woods, rice straw, rice husks, bamboo, wheat straw, peanut hulls, poultry litter, corn stover, bamboo and animal manures.. Amending the soil with biochar has increasingly attracted widespread attention for two reasons. First, because of its chemical stability, its suitability for sequestering C in soil. Secondly, it can be used as a soil conditioner because its application rapidly increases the soil fertility and plant growth by supplying and retaining nutrients while simultaneously improving the physical and biological properties of the soil]. In addition, it reduces the Phyto availability of heavy metals, minimize the bioavailability of organic contaminants to earthworms, microbes, and plants, due to its high porosity, large surface area, and associated high sorptive capacity

For these reasons, application of biochar is done in degraded land to promote restoration process. Biochar often has an increased, negatively charged surface area compared to soil and can be enriched in nutrients. Thus, upon amendment to soil biochar may increase the cation exchange capacity, pH, water holding capacity, and decrease bulk density Biochar has been reported to have considerable agronomic values for enhancing crop production.

Poultry population is raising every year leaving large amount of poultry refuse. The poultry population in India is 489 million and the manure availability is estimated to be 12.1 million tonnes (Livestock Census, 2003). It has been become popular consequent to the rapid growth of poultry industry. It is used for extensive cropping such as rice, sugarcane and chillies. The percent nutrient composition of poultry manure is 2.2-3% N, 1.8% P and 0.5-1% K. poultry manure can be used as an organic amendemnet for phytoremediation of cr because it helps in biotransformation of Cr from its higher to lower oxidative state.

Sewage refers to the used-up water from towns and cities collected though a drainage system. It consists of solid and liquid excreta and liquid wastes from kitchen and bath rooms. It also contains animal vegetable and mineral matter in suspension, solution and colloidal state. It is the mineral matter that makes the purification difficult while sludge is the water drained from the kitchens, bathrooms and drainage water of the streets (open canal). The use of sewage sludge in reclamation processes contributes to the possibility of valuable elements recovery, for example, nitrogen, phosphorus, and other nutrients which are important for plant growth . The contents of individual components in sewage sludge result from the processes of wastewater treatment and the composition of effluent.

The use of sewage sludge for remediation has limitation due to the presence of hazardous substances, microorganisms in sewage sludge, and pathogenic micropollutants and undesirable odour. Despite such limitations, sewage sludge improves soil structure by the generation of large amounts of humus. This is the reason why sewage sludge plays an important role in the phytoremediation. In the heavy metal binding process, both inorganic substances (sulfides, phosphates, hydroxides and oxides of noncrystalline iron and aluminum, and manganese) and organic substances (living microorganisms, organic and mineral remains of dead organic compounds) are involved. Important mechanism for binding sewage sludge by heavy metals are ion exchange, precipitation and co-precipitation reactions, and adsorption of contaminants on the outer and inner surface of minerals. Sometimes the heavy metal present in sewage sludge has a positive effect on the growth of the microbial biomass and microorganisms present in the soil . sewage sludge used for the restoration of degraded land are involved in the processes chemophytostabilization, immobilization phytoremediation (phytoextraction and Phyto stabilization, bioaugmentation and biostimulation. Composts, containing

sewage sludge from municipal wastewater sources, may contain excessive amounts of heavy metals, such as Sn, Zn, Cd, Pb, Mn, Fe, Co, and Si, which in high A. Grobelak concentrations can be toxic. Manurial value of sewage sludge is 3.5% N- 1.0% P<sub>2</sub>O<sub>5</sub> - 0.5 to 1.0 % K<sub>2</sub>O.

The in-situ immobilization of Cr using these composts or biological waste materials to remediate contaminated soil is a viable and cheapest option. Large quantities of compost or organic matter can be added to contaminated soil with the aim of immobilizing Cr as stable complexes with organic colloids. During the organic matter decomposition, compounds such as citric acid or gallic acid are formed which have the potential for chelating Cr (III) or reducing Cr (VI), and thereby reducing the toxicity of Cr. The accumulation of Cr in soil is of major concern because of its possible phytotoxicity or increased movement of metals into the food chain and its potential for surface and groundwater contamination. Chromium contamination in soil and water has drastically reduced the crop yields (25 to 40%) over the years and decreased significantly

The aim of this research is to study following objectives:

**OBJECTIVES:**

1. To study the effect of organic amendments like biochar, poultry manure & sewage sludge on growth and yield and quality of rice in chromium contaminated soil.
2. To study the effect of organic amendments like biochar, poultry manure & sewage sludge on N, P, K, content and uptake by rice in chromium contaminated soil.
3. To study the Cr uptake ability of roots, shoots, and grain of rice in different Cr-levels contaminated soil amended with and without organic manure (biochar, poultry manure & sewage sludge)
4. To determine enrichment and translocation factor of chromium in rice.
5. To study the effect of organic amendments like biochar, poultry manure & sewage sludge on soil chemical properties, available NPK content and chromium content in the soil.

## REVIEW OF LITERATURE

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Heavy metals toxicity and their danger in bioaccumulation in the food chain which represents one of the major environmental and health issues of our modern society. Many heavy metals are existing which are carcinogenic in nature, out of which we are focusing here on chromium.

There are studies conducted by several scientists to know the behaviour of chromium in the plants and soil to find ways to minimize health hazards. But the information pertaining to effect chromium on the growth of cereals like rice plant, its physiological disorder and their behavior in relation to organic amendments is scanty, therefore, it becomes necessary to incorporate few other relevant findings. In this chapter, the work conducted by several scientists in India and abroad pertinent to the present studies has been reviewed and are presented as follows.

### 2.1 Sources of chromium in soils

#### 2.1.1 Geogenic sources

#### 2.1.2 Anthropogenic sources

### 2.2 Effect of chromium on crops.

#### 2.2.1 Growth, yield attributes, and chlorophyll content.

#### 2.2.2 Uptake of macronutrients and micronutrients.

#### 2.2.3 Critical toxic concentrations of chromium in plants.

### 2.3 Phytoremediation potential of rice in chromium contaminated soils.

### 2.4 Effect of organic amendment like biochar, poultry manure & sewage sludge on growth parameters and yield of plant

### 2.5 Effect of organic amendment (biochar, poultry manure & sewage sludge) & chromium on soil properties & postharvest macronutrient & micro nutrient content.

2.6 Effect of organic amendment like biochar, poultry manure & sewage sludge on chromium, macro & micronutrient uptake by rice in chromium contaminated soils.

## 2.1 Sources of chromium in soils

There are many potential sources of chromium present in the soil. There are mainly two types of sources, they are namely geogenic and anthropogenic.

### 2.1.1 Geogenic sources of chromium

**Babula *et al.*** (2008) reported that chromium naturally occurs as highly insoluble chromite ( $\text{FeCr}_2\text{O}_4$ ) in serpentine rocks and ultramafic rocks and they may also form complexes with other metals like crocoite ( $\text{PbCrO}_4$ ), tarapacaite, bentorite, vauquelinite and others.

**Dill *et al.*** (2012) reported that the average Cr content recorded as 34 mg/kg for sands, 77mg/kg for silts and 90mg/kg for clays in the Basara Bian siliciclastic rocks which are native to Eastern Carpathians Foreland Basin. Chromium content in soils can be up to 1000 mg/kg if soils are formed on basic rocks (Lacatusu, 2008).

**Becquer *et al.*** (2003) said that chromite ( $\text{FeOCr}_2\text{O}_3$ ) is the most stable trivalent form of chromium which is present in the environment, while chromate ( $\text{CrO}_4^{2-}$ ) or dichromate ( $\text{Cr}_2\text{O}_7^{2-}$ ) are hexavalent forms of chromium which are formed by making associations with oxygen.

**Oze *et al.*** (2004) gave Cr (VI) sources which are geogenic in origin are ultramafic rocks and serpentines of ophiolites, where they possess concentration of chromium as high as 60000mg/kg; they also observed that generation of Cr (VI) was found in a suspension of chromite and Mn oxide particles which were approximately 6 times faster at pH 5.0 i.e., they are found more available at acidic condition than at pH 6.7 and not detectable at pH 8.0 i.e., alkaline condition. Thus, acid-producing processes in soils contain both geogenic Cr (III) as well as Mn oxides could have a substantial effect on Cr(VI) production.

**Chung *et al.*** (2001) shown that Cr (VI) is generated in situ from naturally occurring minerals by incubating drill core material from the Sacramento Valley in the laboratory. Baize (1997) reported that total chromium levels in igneous and sedimentary

rocks are usually in the range of about 100 $\mu\text{g/g}$  and also said chromium concentration for most of the soils generally ranges from 15 to 100 $\mu\text{g/g}$  and the Cr content increases with increase in proportion of clay.

0.1 to 250 ppm Cr is the range of Cr found naturally, and in certain specific areas, soil content may reach as high as 400 ppm Cr reported by (Langard and Norseth, 1979).

Cr ranges from 10 to 50  $\text{mg kg}^{-1}$  found naturally occurring in soil, depending on the parental material. In soils formed from ultramafic rocks (serpentine), Cr content may reach up to 125 $\text{mg kg}^{-1}$  reported by (Adriano, 1986).

### **2.1.2 Anthropogenic sources of chromium**

Metal working industries, cooling tower water, and for preservation wood etc., all these activities have contributed to a significant increase in Cr concentration in the environment reported by (**Shanker *et al.*, 2005**)

Spillage of contaminated sludge coming from the leather working industries produces 2000 to 32,000 tons of elementary Cr per year which enters the environment given by (**Zayed and Terry, 2003**).

Global industrial-age cumulative Cr production was found in 2000, has been estimated at 105.4 million tons which are significantly increased since the 1950s given by (Han *et al.*, 2002). Metalliferous soils may possess Cr concentrations as high as a few thousand  $\text{mg kg}^{-1}$  (**Reeves & Baker, 2000**).

The leather industry is one of the major causes for a high influx of Cr into the biosphere, which accounts for 40% of total industrial use. The industries namely leather and tannery, in particular, are highly responsible for the release of a stream of chromium metal into the biosphere (Barnhart, 1997).

Different industries namely electroplating, production of paints, pigments, metallurgical tanning, wood preservation, chemicals production and pulp and paper production uses Chromium in huge amount. Wastes generated from such industries (e.g., fly ash, sludge, slag, etc.) are used as a filling material at numerous locations in order to reclaim marshlands, for tank dikes, and for backfill at sites following demolition which lead Cr entry into soil given by (**Salunkhe *et al.*, 1998**)

**Nriagu** (1988) reported that Chromium (Cr) used widely in industrial production such as leather finishing and processing which became a severe threat to crop production and food safety. Leather tanning, electroplating, and stainless steel industries are important for the production of Cr given by (**Riley and Zachara, 1991**).

Annually, tanning industries release 2000 to 3200 Mg of elemental Cr and with Cr concentration varying from 2000 to 5000 mg L<sup>-1</sup> of effluents into the environment in India (**Chandra, Sinha, and Rai, 1997**).

## **2.2 Effect of chromium on crops**

### **2.2.1 Growth, yield attributes, and chlorophyll content**

**Nagarajan (2014)** has reported that Cr altered growth significantly and reduced dry weights of shoot i.e., (7-58%) and roots (7-73%) of rice plants in different treatments. The impact of Cr was remarkably higher on stomatal conductance (2166%), photosynthetic rate (21-62%) and transpiration rate (5-59%). The pigments like chlorophyll a and b and carotenoid contents also reduced heavily in Cr-treatment plants by 17-47%, 12-43%, 31-50%, respectively.

**Ahmad *et al.* (2011)** have reported the stunted growth and less number of tillers/hill and leaves was observed in Cr-treated plants compared to control. Leaf senescence, slower rate of synthesis of carbohydrates, in which a number of green leaves remained alive for a longer period of time have also observed in Crcontaminated plants. Reduced fresh weights of shoot and roots along with corresponding reduced dry biomass of shoots and roots of paddy were also found upon harvest.

**Nagarajan & Ganesh (2015)** has found that there is a reduction of morphophysiological parameters of the plant of paddy seedlings if concentrations of chromium were more than 100 mg/L. Chromium content in roots and shoots of plant seedlings was uptaken more in Cr treated pots .He also reported that accumulation of chromium in the roots was much higher than the shoots of the paddy seedlings under treatment.

Root growth decreased in the contaminated soil it may be due to root surface damage, causing leakage of cell content and collapse of root hairs and of epidermal cells, it was reported by (**Castro et al., 2007**). Inhibitory effect of plant growth is especially

due to Cr phytotoxicity and stunted growth of shoots may occur in the excess amount of Cr found within the plant (**Faisal and Hasnain, 2005**).

**Chen *et al.* (2001)** gave results by conducting an experiment that root length and total root weight of wheat was affected by 20mg Cr (VI) /kg soil of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>. Panda and Patra (1997) found that chromium of 1 µM cons has increased the root length of seedlings growing under nitrogen (N) nutrition levels; higher Cr concentrations decreased root length in all the N treatments.

**Dube *et al.* (2003)** reported that a high concentration of chromium found harmful to vegetation, chromium will enter the food chain through consumption of plant material. The chromium concentration in plants increases, which adversely affects several biological parameters ultimately there is the loss of vegetation and land sometimes become barren. Relative to the control, a slight increase in root length occurred when exposed to 20 µm Cr, while maximum root length decreased by 18% upon exposure to 200 µm Cr (**Dixit *et al.*, 2002**).

Cr supra affects the root length when compared to any other heavy metal, Cr if accumulated more in the roots makes them a metal excluder reported by (**Raskin *et al.*, 1994**) they also reported that metal excluder will restrict metal from being translocated to aerial parts of the plants or which maintains lower levels over a wide range of its concentrations in the soil. Thus, higher Cr concentrations have decreased root length in all the treatments.

Roots affected by Cr showed increased growth of root hairs and increased the relative proportion of pith and cortical tissue layers observed by scanning electron microscope reported by (**Suseela *et al.*, 2002**). Excess Cr decreased the transpiration rate, water potential, increased diffusive resistance, and relative water content in leaves of cauliflower (**Chatterjee and Chatterjee, 2000**).

**Barcelo *et al.* (1986)** reported that root growth decreased this is due to Cr toxicity which could also be to inhibition of root cell division/root elongation or to the extension of the cell cycle in the roots. Under high concentrations of both the Cr species (trivalent and hexavalent) combination, the reduction in root growth could be due to the direct contact of seedlings roots with Cr in the medium causing a collapse and subsequent inability of the roots to absorb water from the medium.

Cr stress is an important factor which affects photosynthesis in terms of CO<sub>2</sub> fixation, electron transport, photophosphorylation, and enzyme activities (**Clijsters and Van Assche, 1985**). Toxic concentrations of Cr considerably reduced dry matter production in different plants reported by (**Sharma and Sharma, 1993**).

**Sharma and Sharma (1993)** reported that plant height reduced significantly after 32 and 96 days, in wheat cv, UP 2003 in a glasshouse trail which was sown in the sand with 0.5µM sodium dichromate. The reduction in plant height might be due to the reduced root length and consequent lesser nutrients and water transport to the aerial part of the plant can have a direct impact on the cellular metabolism of shoots contributing to the reduction in plant height.

**Kocik and illsky (1994)** studied the effect of chromium on quantity and quality of biomass in sunflower and maize and observed that dry matter production was not markedly effected by 200mg /kg Cr (VI), but the uptake of chromium in plant tissue was positively correlated with their contents in the soil.

### **2.2.3 Uptake of macronutrients**

**Nagarajan (2014)** reported that highly pronounced reductions were recorded in nitrogen (23-82%), potassium (6-42%) and phosphorous (4-37%) content of Cr treated plant leaves. Cr accumulation was extremely higher in shoots (3575- 19150%), followed by roots (1023-5869%), and seeds (21-249%) of treated plants compared with control.

**Mallick *et al.* (2010)** found uptake of both macronutrients (eg. N P K) as well as micronutrients decreased with increase in Cr (VI) in irrigation of paddy and on chromium exposure decreased copper absorption in *Zea mays* L. roots, while leaves were not affected and they also decreased uptake of the micronutrients Mn, Fe, Cu, and Zn was detected by Liu *et al.* in *A. Viridis* L. exposed to Cr (VI).

**Ali (2010)** reported a reduction in nutrients P, K, Mg, S, Fe, Zn and Mn contents in roots at pH 6.5 and P, K, Ca, Mg, S, Zn and Mn contents at pH 4.0 during chromium stress. Translocation was inhibited of all nutrients from roots to upper parts of plants except Ca at pH 6.5 upon Cr addition. Lower contents of all nutrients were observed at pH 4.0 as compared to pH 6.5 in barley. The reduction in nitrogen compounds, K and P, could be due to reduced root growth and impaired penetration of the roots in the soil due to Cr toxicity (Azmat and Khanum, 2005).

NPK and other elements reduction could be due to reduced root growth and impaired penetration of roots into soil due to chromium. It is assumed that when plants are exposed to Cr stress, Mg uptake is inhibited and more  $Mg^{2+}$  will be transported to the leaves from roots in order to maintain the normal function of leaves (**Zeng *et al.*, 2010a**).

**Khan *et al.* (2001)** observed that thresholds values of concentrations of NPK in dry weight of rice plants showed significantly decrease at 0.5 ppm Cr. Chromium, due to its structural resemblance with some essential elements, which can affect the mineral nutrition of plants in a by zantine way. Cr-induced changes in ion concentrations were associated with a significant reduction in plant biomass. 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**).

Cr (VI) transport is an active mechanism involving carriers of essential anions such as sulfate (**Cervantes *et al.*, 2001**) Fe, S and P are known also to compete with Cr for carrier binding. **Sharma & Mehrotra (1993)** reported that the overall adverse effect of Cr on growth and development of plants could be serious impairment of uptake of mineral nutrients and water leading to a deficiency in the shoot. Leaf nitrogen contents were reduced by 23-82%. Phosphorous is regarded as the energy currency of the cell, which was reduced (ranged from 4-37%) due to different Cr-treatments in rice plants while potassium that is needed in various metabolic activities was also reduced (ranged from 6-42%) in rice treated with various Cr levels.

The mineral elements such as nitrogen, phosphorus, potassium, calcium, magnesium, manganese, iron, copper and zinc content of paddy are gradually decreased with the increase in chromium concentrations. It may be due to the competition of chromium ions with potassium, which in turn exercised a regulatory control on potassium uptake (**Lanoreaux and Chaney, 1978**).

**Sharma and Sharma (2003)** reported that leaf p concentration decreased with 0.25 mM chromium in wheat cv.UP2003 .chromium, being structurally similar to other elements, may effect plant mineral nutrition.

#### **2.2.4 Uptake of Micronutrients**

A significant and positive correlations between occurred with Cr and Ca/Mg concentrations, but negative correlations between Cr and Zn/Fe concentrations were

found in rice stems. There was a significant and positive correlation between Cr and Ca concentration, negative correlation between Cr and Mg/Zn concentrations, and no relationship with Fe concentration found in rice leaves, (**Zeng *et al.*, 2010b**). Furthermore, Cr showed a significant and negative correlation with the accumulation of Ca, Mg, Zn, and Fe.

High Cr level reduced Zn concentration in each subcellular fraction of stems and leaves, except for the mitochondrial fraction in stems and the soluble fraction in leaves. Thus, it may be assumed that the marked decrease of Zn concentration in the cell wall, chloroplast, nucleus, and mitochondria of rice leaves under Cr stress may deteriorate photosynthesis (**Zeng *et al.*, 2010a**).

**Skeffington *et al.* (1976)** reported that chromium mainly moved in the xylem of the plants. He also suggested that both Cr (III) and Cr (VI), can interfere the uptake of several other ionically similar elements like Fe and S. Excess chromium interfered with the uptake of Fe, Mo, P and N (Adriano, 1986).

Unambiguous effect of stress on the concentration and Fe/Zn forms may be attributed to the activated root exudation (**Zeng *et al.*, 2008b**). **Turner and Rust (1971)** said that studies have determined the negative effect of Cr on the uptake of nutrients, such as Zn, Fe, Ca, Mg, Mn, and Cu.

**Ottabong (1989)** found the differences in soluble Mn fractions, interactions with P and critical effects on the uptake of Mn, Cu, Zn, Fe and Al were influenced by chromium in rye grass.

**Sharma and Pant (1994)** reported the effects of Cr and Fe concentration varied with plant organ and chromium level. Mn and Cu concentrations generally decreased with increasing Cr level, while Zn concentration decreased in leaves and flowers but increased in stem and roots of maize.

### **2.2.5 Critical toxic concentration of chromium in plants.**

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 (**Huffman and Allaway, 1973**).

**Zayed et al. (1998)** tested Cr (III) and Cr (VI) translocation in several crops and found that translocation of both Cr forms from roots to shoots was very low and accumulation of Cr by roots was 100-fold higher than in shoots, despite the Cr species.

### **2.3 Phytoremediation potential of rice of different chromium concentration level**

**Payus et al. (2015)** carried out a study on heavy metals contamination in paddy field using *Oryza sativa*. Rice roots accumulated the relatively large amount of lead, cadmium, chromium, and copper, while the stem accumulated a large amount of zinc. Enrichment factor (EF) showed that lead, cadmium, and copper were concentrated in the root of *Oryza sativa*, while chromium and zinc metals were more concentrated in the shoot part. In addition, the translocation factor (TF) showed that *Oryza sativa* was able to transfer zinc and chromium from root to the shoot part of *Oryza sativa*.

**Halim et al. (2015)** has estimated the heavy metal contamination in paddy soil and subsoil and uptake by rice plants which was collected from Barapukuria coal mine area of Bangladesh. The mean contents of As, Cr, Cu, Mn, Ni, Pb, and Zn in paddy soil and subsoil exceed the world averages, and the observed soils are moderate to extremely polluted with inputs from mining activities. Regression model and Correlation analyses suggest that pH and TOC have a distinct effect on the availability of observed metals in soils. Sequential extraction of paddy soil and subsoil samples demonstrated that the mobility of heavy metals increased in the order of Cu > Zn > Pb > Fe > Cr > Ni > Mn > As. The uptake of metals in rice root was much higher than those in straw and rice grains. Arsenic, Cr, and Pb uptake in rice grains are 6.87, 1.58, and 5.26 fold higher than the maximum permissible concentration which shows a tendency of transformation of these elements from contaminated soil to rice plants.

**Nagarajan (2014)** has found that rice plants that accumulate Cr was extremely higher in shoots (3575- 19150%), roots (1023-5869%), and seeds (21-249%) of treated plants compared with control.

**Mohanty et al. (2011)** conducted a study on chromium bioaccumulation in rice plants (*Oryza sativa* L. cv. Khandagiri) irrigated with Cr+6 contaminated mine wastewater was analyzed along with its attenuation from mine wastewater. Accumulation of Cr was significantly high in surface soils (0-20 cm) with a mean value of 11,170 mg kg<sup>-1</sup>, but it significantly decreased after the crop harvest. About 70% to 90% reduction of

Cr<sup>+6</sup> levels was observed in irrigated mine wastewater when passed through successive rice plots. High bio-concentration of Cr in leaves with values ranging from 125-498 mg kg<sup>-1</sup> as compared to stem (25-400 mg kg<sup>-1</sup>) and grain (5-23 mg kg<sup>-1</sup>) was noticed. Plant age, high biomass and area of water passage depend upon the reduction of Cr<sup>+6</sup> levels.

**Lai et al. (2009)** have reported that rice would possibly accumulate cadmium (Cd) from Cd-contaminated soils, but the Cd uptake was quite different between different rice species and in different soil situations. Similarly, a survey investigated As concentration in brown rice harvested from contaminated soils with different levels of total arsenic concentrations found that As was accumulated by rice roots more than shoots.

**Shahandeh and Hossner (2000)** have reported a high increase in Cr uptake aided by organic acids. **Srivastava et al. (1999b)** found that increasing concentrations of organic acids resulted in increased uptake of Cr without affecting the distribution in plant parts. Organic acids (citric and oxalic) and mycorrhizae and has been reported to play an important role in phytoremediation of Cr-contaminated soils by enhancing Cr uptake and increasing translocation to shoots (**Davies et al., 2001**)

#### **2.4 Effect of organic amendment like biochar, poultry manure & sewage sludge on growth parameters, yield on normal & chromium contaminated soil**

**Budhar et al. (1991)** observed higher **grain yields** of rice by incorporation of farm wastes and green manures, with the highest yield by poultry manure was obtained under lowland conditions indicating the superiority of poultry manure.

**Savithri et al (1991)** reported that application of coir pith based poultry litter at 6.35 t ha<sup>-1</sup> along with recommended levels of NPK registered highest yield of sorghum.

**Das et al. (1991b)** recorded highest **grain yield** of maize by application 5t of poultry manure + 28 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> as single super phosphate.

**Giardini et al. (1992)** reported an increased yield of onion bulbs due to poultry manure, which produced yields of more than 35 t ha<sup>-1</sup>. They have also reported that the highest yield of tomato and marketable yield of tomato due to combined application of poultry manure and mineral fertilizers. In ferralitic soils of Nigeria,

**Oikeh and Asiegbu (1993)** obtained highest tomato yields (10 t ha<sup>-1</sup>) out of poultry and swine manures.

**Nakamoto et al. (1994)** reported that application of 25% recommended commercial fertilizer with 75% biodigested poultry manure slurry was superior and

concluded that poultry manure has potential for supplementing or replacing commercial fertilizer in **sweet corn** production.

**Jayanthi (1995)** reported that application of recycled and composted poultry manure resulted in higher **grain yield** of rice. In a degraded soil in Southern Nigeria, Obi and Ebo (1995) found that average maize grain yield was significantly improved due to 100% poultry manure application and also in 50% poultry manure + 50% inorganic fertilizer application.

**Abdel-Magid et al. (1995)** reported that grain and straw yield of wheat increased with increased rate of chicken manure in Saudi Arabia and obtained the greatest economic return at 8.25 t ha<sup>-1</sup>.

In Nigeria,

**Ugbaja (1996)** found that castor seed yields were the highest with poultry manure or swine manure applied at the rate of 10 t ha<sup>-1</sup>.

**Opara and Asiegbu (1996)** observed an increase in the fruit yield of eggplant with increased rates of poultry manure upto 15 t ha<sup>-1</sup>.

**Akande et al. (2005)** reported that complementary application of rock phosphates with poultry manure increased maize grain yield by 33%, while cowpea yield was increased by 25%.

**Yamur et al. (2005)** showed that when lentil (*Lens culinaris*) was grown on soil with different doses of sewage sludge treatment the plant seed yield increased significantly. Some important plant characters such as plant height, first pod height, number of pod per plant, number of seed per plant, seed weight per plant, and 1000-seed weight and one of the yield characters showed an increase in the amended soils.

**Khan et al. (2007)** showed the variations in wheat yield with different doses of sewage sludge @ 10, 20, 40 and 80 Mg ha<sup>-1</sup>, keeping 120:60:60 NPK as a standard application rate (control). The results indicated that the sewage sludge @ 80 Mg ha<sup>-1</sup> recorded significantly higher yield when compared with control. Sewage sludge increased the levels of Zn and Cd, within the permissible limits, in wheat grain; whereas no significant increase was observed in Cu and Pb. The recommendable rate of 40 Mg ha<sup>-1</sup> was found to be better than 80 Mg ha<sup>-1</sup> though the yield was to some extent low. At higher doses sludge application, there are chances of risk of metal contamination while the possible risks of metal uptake and accumulation in the soil are very less with lower dose of sewage sludge.

**Nagy (2008)** studied the effect of mine tailing was assessed by using composted sewage sludge in different ratios, which can buffer the toxic effects of metals to some extent. Often applied indicator plant species, such as cabbage (*Brassica oleracea L. convar. Capitata provar. Alba*) and lettuce (*Lactuca sativa*) were applied to examine the phytotoxicological properties of mine tailing – compost mixtures. Based on the results of growth inhibition and seed germination test 5 V/V% compost is recommended to use for phytoremediation due to its buffering effect.

**Zoubi et al. (2008)** found that there was a significant increase in production of maize as well as vetch in sludge-fertilized treatments as compared to the control and the best results were obtained when they were fertilized with double the amount of sewage. However, no increase in the productivity of wheat was noted due to sludge application. Sludge application was found to increase the fresh weight of spinach (Ngole , 2010) by up to 31% and carrots by up to 10%, these increases also varied with type of soil on which vegetable was grown. Spinach accumulated more Zn than carrots. Carrots and spinach grown on this sludge amended soils had the highest mean concentration of Zn with values of 131.6 and 86.3 mg kg<sup>-1</sup>, respectively.

**Hossain et al. (2010)** investigated and quantified the effects of wastewater sludge biochar on soil quality, growth, yield and bioavailability of metals in cherry tomatoes, pot experiments were carried out in a temperature controlled environment and under four different treatments consisting of control soil, soil with biochar; soil with biochar and fertiliser, and soil with fertiliser only. The results revealed significant effect of biochar on the height of the plant, increase in fruits size & grain yield of the plant.

**Cui et al. (2012)** extended a field study involving wheat production in order to study the effect of biochar (BC) amendment in paddy soil that had long-term contamination of Cd. The BC was used as an amendment in Cd-contaminated soil for its special property. BC was amended at rates of 10 to 40 t ha<sup>-1</sup> during the rice season before rice transplantation in 2009. BC amendments increased grain yield of wheat as compared to control where BC was not applied.

**Houben et al. (2013)** tested phytoremediation of soils contaminated by heavy metals by liming (CaCO<sub>3</sub>) or adding biochar (1%, 5% and 10%, mass fraction) and by growing rapeseed (*Brassica napus L.*), a common bioenergy crop. Twelve weeks after sowing, all plants cultivated on the untreated soil and on the soil amended by biochar at 1% had died, while the plants grew normally on the soil that had the other treatments. Compared to liming, treatment with 10% biochar proved equally efficient in reducing

metal concentrations in shoots but the biomass production tripled as a result of the soil fertility improvement. Results showed a significant increase in the rapeseed biomass production when biochar was applied. By alleviating the metal phytoavailability, the biochar-5% and biochar-10% and the lime application enabled the plants to survive and grow without presenting any toxicity symptoms during the entire cultivation period (except a slight yellowing in shoots from the biochar-5% treatment)..

**Khan *et al.* (2013)** used rice plant pot experiments and investigated the influence of Sewage sludge biochar (SSBC) upon biomass yield, bioaccumulation of nutrients, and metal(loid)s, and greenhouse gas (GHG) emissions. Regarding rice plant properties, SSBC amendments significantly increased shoot biomass, tillers, height of the plant & grain yield.

**Bian *et al.* (2014)** conducted a field experiment in a contaminated rice paddy in southern China to determine if bioavailability of soil Cd and Pb could be reduced while grain yield was sustained over 3 years after a single soil amendment of wheat straw biochar. They observed a significant increase in rice yield in the third year by 16.6% and 18.3% under C20 and C40 treatments, respectively.

**Puga *et al.* (2015)** applied sugar cane straw-derived biochar (BC), produced at 700 °C, in their experiment to a heavy metal contaminated mine soil at 1.5%, 3.0% and 5.0% (w/w). Jack bean (*Canavalia ensiformis*) and *Mucuna aterrima* were grown in pots containing soil and biochar mixtures, and control pots without biochar. Pore water was sampled from each pot to confirm the effects of biochar on metal solubility, whilst soils were analyzed by DTPA extraction to confirm available metal concentrations. In their pot experiment, with increasing doses of BC in the soil there was an increase in dry mass of shoots of beans, and roots of both plants. This may be due to the decreased availability of heavy metals (Cd, Pb and Zn) in the soil and increasing concentration of P added by the biochar.

**Choppala *et al.* (2015)** studied, two carbon materials [chicken manure biochar (CMB) and black carbon (BC)] for investigating their effects on the reduction of hexavalent chromium [Cr(VI)] in two spiked [600 mg Cr(VI) kg<sup>-1</sup>] and one tannery waste contaminated [454 mg Cr(VI) kg<sup>-1</sup>] soils. The addition of carbon materials to the tannery soil was also effective in decreasing the phytotoxicity of Cr(VI) in mustard (*Brassica juncea* L.) plants. Plant growth decreased with Cr(VI) addition. In calcic red sandy loam soil that was spiked with 250 mg Cr(VI) kg<sup>-1</sup>, addition of 50 g BC and CMB kg<sup>-1</sup> increased the dry matter yield by 63 and 65 %, respectively. In tannery waste

contaminated soils, the amendments had a significant effect on growth and dry matter yields which increased by 83 and 81 % with the addition of 50 g BC and CMB kg<sup>-1</sup> soil, respectively.

**Jatav et al. (2016)** conducted greenhouse experiment in the net house of the Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (U.P.) during *kharif*, 2013 to find out the effect of biochar on availability of heavy metals in soil amended with sewage sludge. There were nine treatments, consisting of six different doses of biochar as 2.5, 5.0, 7.5, 10, 15, 20 t ha<sup>-1</sup> along with 100% RDF and sewage sludge @ 30 t ha<sup>-1</sup> applied in each biochar amended pot. In order to recover plant from initial stress, 50% recommended dose of nitrogen (RDN) was applied in each pot at 45 DAT. Results of this study showed a significant increase in grain yield with application of graded level of biochar along with sewage sludge. Application of biochar @ 20 t ha<sup>-1</sup> along with sewage sludge (30 t ha<sup>-1</sup>) was found to increase grain yield to 2.5 times over control (T<sub>1</sub>) and 8.5 % over 100 % RDF.

**Younis et al. (2016)** evaluated the role of Biochar (BC) (cotton stick at a rate of 0, 3, and 5 %) on Cd uptake and the photosynthetic, physiological and biochemical responses of spinach (*Spinacia oleracea*) grown in Cd-spiked soil (0, 25, 50, 75, and 100 mg Cd kg<sup>-1</sup> soil). The results showed that Cd toxicity decreased growth, photosynthetic pigments, gas exchange characteristics, and amino acid and protein contents in 52-day-old spinach seedlings. The application of BC ameliorated the harmful effects of Cd in spinach plants. Application of Cd in the growth medium significantly affected the chlorophyll a, b, total chlorophyll, carotenoid, lycopene and anthocyanin concentrations in the leaves of spinach. Cadmium treatments significantly decreased the chlorophyll a, b, and total chlorophyll contents as compared to the control. The BC application increased the chlorophyll a, b, total chlorophyll, and carotenoid concentrations as compared to the respective Cd-only treatments. Cadmium stress decreased the plant fresh and dry biomass. The application of BC showed a positive effect on the biomass of the plants under Cd stress, and maximum biomass was recorded at 5 % BC application in control plants.

**Ramzani et al. (2016)** investigated the potential role of biochar (BC) and gravel sludge (GS), alone and in combination, for in situ immobilization of Ni in an industrially Ni contaminated soil at original and sulfur-amended altered soil pH. Dry matter yield significantly increased by BC and GS in the treatments of unaltered pH soil and the pH-

amended soil. a significantly highest grain yield [pH 6.5 (27.2 g pot<sup>-1</sup>)] was observed in the treatment receiving GS, BC, and S together (T<sub>10</sub>) which was 56 % higher when compared to control (T<sub>1</sub>). It also observed that application of BC and GS alone or their mixture significantly increased the shoot dry weight per pot when compared to control

**Rehman *et al.* (2016)** conducted a greenhouse experiment to evaluate the effects of biochar (BC) and other organic amendments like farm manure (FM) from cattle farm and compost (Cmp) on physiological and biochemical characteristics of maize (*Zea mays* L.) under Ni stress. Maize was grown in Ni spiked soil without and with two rates of the amendments (equivalent to 1% and 2% organic carbon, OC) applied separately to the soil. After harvest, plant height, root length, dry weight, chlorophyll contents, gas exchange characteristics and trace elements in plants were determined. Nickel toxicity significantly decreased total chlorophyll content. The highest significant increase in chlorophyll content was recorded in the leaves treated with FM at 2% OC as compared to the control. Compared to the lower rate, application of amendments at higher rate significantly increased total chlorophyll content. all of the amendments increased plant height, root length, shoot and root dry weight with the maximum increase in all parameters by FM (2% OC) treatment.

**Abbas *et al.* (2017)** studied the effect of rice straw biochar (BC) on Cd immobilization in soil and uptake by wheat in an agricultural contaminated-soil was investigated. Different levels of rice straw BC (0%, 1.5%, 3.0% and 5% w/w) were incorporated into the soil and incubated for two weeks. After this, wheat plants were grown in the amended soil until maturity. The results show significantly increased in chlorophyll content, shoot and root dry mass, plant height in the BC amended soil.

**Rehman *et al.* (2017a)** studied to investigate the effect of organic and inorganic amendments on wheat growth and reducing Pb concentration in the plant. A greenhouse experiment was conducted on Pb spiked soil (500 mg kg<sup>-1</sup> of soil) with the application of farmyard manure, poultry manure, gypsum and di-ammonium phosphate (DAP). Stomatal conductance, chlorophyll content, and photosynthetic rate decreased with increasing Pb concentration in wheat shoots. Amendments significantly increased the chlorophyll content (Chl a + Chl b) in leaves & grain yield as compared to the control.

**Rehman *et al.* (2017b)** investigated the effects of limestone, lignite, and biochar on growth, physiology and Cd uptake in wheat and rice under rotation irrigated with raw effluents. Initially, each treatment was applied alone at 0.1% and combined at 0.05% each

and wheat was grown in the field and then, after wheat harvesting, rice was grown in the same field without additional application of amendments. Results showed that the amendments applied in the soil increased the total chlorophyll content & grain yield as compared to the control.

**Arshad *et al.* (2017)** investigate chromium ( $50 \text{ mg kg}^{-1}$ ) induced phytotoxicity in wheat (*Triticum aestivum* L.) and to reduce its phytoavailability by amending the contaminated soil with chromium reducing bacteria (CRB) and 1% or 5% biochar. For the phytotoxicity assay, wheat was grown at different concentrations of chromium (10, 20, 30, 40 and  $50 \text{ mg L}^{-1}$ ). After 3 weeks results showed that soil amendment with biochar and bacteria showed a significant improvement in root and shoot length, fresh and dry biomass, percentage germination, total chlorophyll, and carbohydrates as compared to soil without amendment.

**Zhang *et al.* (2017)** screened out the cost-efficient Cr(VI)-contaminated soil in situ amended materials, the effects of ordinary zero-valent iron (ZVI), nanoscale zero-valent iron (nZVI), biochar (B), biochar/zero-valent iron (BZVI), and biochar/nanoscale zero-valent iron (BnZVI) on the immobilization of Cr(VI) in spiked soil. The experimental groups consisted of unpolluted soil (BK), Cr(VI)-spiked soil (CK), biochar treated soil (B), ZVI-treated soil (ZVI), nZVI-treated soil (nZVI), biochar/ZVI-treated soil (BZVI), and biochar/nZVI-treated soil (BnZVI). The results showed that the combination of biochar on cabbage mustard with nZVI was capable to partly offset the adverse effects brought from remediation process, which became apparent from the higher seed germination rate, higher biomass than that of nZVI. Observed results for the length of harvest plants of cabbage mustard showed that BK plants were longer than other two groups (both roots and shoots), and root length of BnZVI (3.8 cm) was significantly longer than nZVI (2.2 cm).

### **2.5 Effect of organic amendment like biochar, poultry manure & sewage sludge on soil properties & postharvest macronutrient & micro nutrient & chromium content of soil**

**Hossain *et al.* (2010)** investigated and quantified the effects of wastewater sludge biochar on soil quality, growth, yield and bioavailability of metals in cherry tomatoes, pot experiments were carried out in a temperature controlled environment and under four different treatments consisting of control soil, soil with biochar; soil with biochar and

fertilizer, and soil with fertilizer only. The results showed that the application of biochar was found to significantly increase the phosphorus and nitrogen contents in soil.

**Cui *et al.* (2012)** extended a field study involving wheat production in order to study the effect of biochar (BC) amendment in paddy soil that had long-term contamination of Cd. The BC was used as an amendment in Cd-contaminated soil for its special property. BC was amended at rates of 10 to 40 t ha<sup>-1</sup> during the rice season before rice transplantation in 2009. BC amendments increased soil pH by 0.11 to 0.24 and by 0.09 to 0.24 units, respectively. Compared to the unamended soil, the pH of the soil with the BC amendment was significantly higher at a mean rate by 0.01 units per ton of BC amended consistently over two years. Compared to the unamended soil, the organic carbon of the soil with the BC amendment was significantly higher consistently over two years i.e. 2010 and 2011.

**Nigussie *et al.* (2012)** investigated the effect of biochar application on the selected properties of chromium polluted soils and uptake of lettuces grown in polluted soils. Biochar produced from maize stalk was applied at the rates of 0, 5 and 10t/ha on soils artificially polluted with Cr at the levels of 0, 10 and 20ppm. The study showed a significant increase in pH due to application of biochar. Biochar application significantly increases the pH values of chromium polluted and unpolluted soils. The study showed a significant increase in total nitrogen, available phosphorous and exchangeable bases like Ca, Mg, K and Na due to application of biochar on chromium polluted and unpolluted soils. biochar application also increases the EC of soil.

**Usman *et al*** reported that application of poultry manure resulted in considerable increase in soil pH, DOC, CO<sub>2</sub>-C efflx, net nitrogen mineralization in amended soil as compared to unamended soil. He also found that application of synthetic chelator with poultry manure increases availability of As.

**Rathore** investigated the effect of biochar application on the selected properties of chromium polluted soils and uptake of lettuces grown in polluted soils. Biochar produced from maize stalk was applied at the rates of 0, 5 and 10t/ha on soils artificially polluted with Cr at the levels of 0, 10 and 20 ppm. The study showed that the uptake of nitrogen, phosphorous and potassium were significantly increased by addition of biochar.

**Rangasamy *et al*** studied about reduction of the hexavalent chromium into trivalent chromium from chromium contaminated (300 µg g<sup>-1</sup>) soil. In the presence of organic amendments (poultry manure) and microbial inoculants (*Pseudomonas fluorescens* and *Trichoderma viride*) the chromium (VI) reduction was observed in

maximum in the poultry manure treatment (91%) whereas poultry manure with *Pseudomonas fluorescens* reduced the hexavalent chromium up to 90 %.

**Sabir *et al.* (2013)** investigated the effect of activated carbon, poultry manure and press mud on immobilization of nickel, manganese, copper, zinc, iron and lead in the contaminated soil, plant growth and metal concentrations in maize shoots. The amendments were applied to the soil at the rate of 4% on dry weight basis. All the amendments increased AB-DTPA extractable Ni in the soil with the exception of AC that decreased Ni by 33% compared to the control.

**Houben *et al.* (2013b)** investigated the effect of biochar application on the fate of Cd, Zn and Pb in a contaminated soil amended with three different rates of biochar (1%, 5% and 10%; w/w). In an incubation experiment, the 0.01 M CaCl<sub>2</sub>-extractability of metals after 1 h of incubation significantly decreased with increasing rate of biochar application. This effect was mostly attributed to the raise in soil pH. Compared to the control, the soil pH was significantly increased by the addition of biochar throughout the incubation experiment. Significant increases in available nutrients (P, K, Ca and Mg) were observed and raised with the amount of biochar added. Potassium was the nutrient which increased the most.

**Khan *et al.* (2013)** Used rice plant pot experiments and investigated the influence of Sewage sludge biochar (SSBC) upon biomass yield, bioaccumulation of nutrients, and metal(loid)s, and greenhouse gas (GHG) emissions. SSBC amendments increased soil pH, increased total nitrogen and available nutrient decreased bioavailable As, Cr, Co, Ni, and Pb (but not Cd, Cu, and Zn) in soil.

**Puga *et al.* (2015)** applied sugar cane straw-derived biochar (BC), produced at 700 °C, in their experiment to a heavy metal contaminated mine soil at 1.5%, 3.0% and 5.0% (w/w). Jack bean (*Canavalia ensiformis*) and *Mucuna aterrima* were grown in pots containing soil and biochar mixtures, and control pots without biochar. The P concentration in soil had an average increase of 78% with BC application. K, Ca and Mg concentration also increased in post-harvest soil by BC application. Biochar application also increased the N, P and S content in jack beans and *Mucuna aterrima*.

**Jatav *et al.* (2016)** conducted greenhouse experiment in the net house of the Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (U.P.) during *khariif*, 2013 to find out the effect of biochar on availability of heavy metals in soil amended with sewage sludge. There were nine treatments, consisting of six different doses of biochar as 2.5, 5.0, 7.5

10, 15, 20 t ha<sup>-1</sup> along with 100% RDF and sewage sludge @ 30 t ha<sup>-1</sup> applied in each biochar amended pot. In order to recover plant from initial stress, 50% recommended dose of nitrogen (RDN) was applied in each pot at 45 DAT. Soil pH and electrical conductivity did not show any significant change with application of sewage sludge but with increasing levels of biochar pH of soil showed a significant increase.

**Abbas *et al.* (2017)** studied the effect of rice straw BC on Cd immobilization in soil and uptake by wheat in an agricultural contaminated-soil was investigated. Different levels of rice straw BC (0%, 1.5%, 3.0% and 5% w/w) were incorporated into the soil and incubated for two weeks. After this, wheat plants were grown in the amended soil until maturity. The results show that soil pH linearly increased with increasing the doses of BC amendment in the soil as compared to the control. The results show that the EC of the soil increased when BC levels in soil increased up to a maximum value of 4.81 dS m<sup>-1</sup>, which was approximately 60% higher compared to the control.

**Bashir *et al.* (2017)** investigated the potential of sugarcane bagasse biochar in reducing the bioavailability of soil cadmium (Cd) and chromium (Cr) and assessing the impact of biochar application on soil microbial activities and plant growth in metal-contaminated soil. The addition of biochar (15 g kg<sup>-1</sup>) to metal-contaminated soil showed a slight increment in the soil pH by 0.34 units compared to the control sample without biochar addition (non-amended contaminated soil). All treatments with biochar application exhibited a higher TOC than the treatments without biochar application. The biochar application increased the MBC in Cr-contaminated and Cd-contaminated soil, respectively, compared with Cr- and Cd-contaminated soil without biochar.

**Lebrun *et al.* (2017)** conducted greenhouse experiment with contaminated technosols amended with biochar and garden soil, single or combined, revegetated with the 3 willow species. The aims of study were (i) to evaluate the effect of a pinewood biochar on the physicochemical properties of a former mine contaminated technosol, (ii) to assess the mobility and phytoavailability of As and Pb and (iii) to investigate the remediation potential of three willow species (*Salix alba*, *Salix viminalis* and *Salix purpurea*). Plant growth, *Salix* organ dry weight and metal(loid) uptake were determined in order to evaluate the phytoremediation potential of the three *Salix* species studied. Results indicated that biochar increased the pH of soil pore water (SPW).

**Ramzani *et al.* (2016)** investigated the potential role of biochar (BC) and gravel sludge (GS), alone and in combination, for in situ immobilization of Ni in an industrially

Ni contaminated soil at original and sulfur-amended altered soil pH. Application of the mixture of both amendments reduced soil DTPA-extractable Ni.

## **2.6 Effect of organic amendment like biochar, poultry manure & sewage sludge on chromium, macro & micronutrient uptake by rice in chromium contaminated soils**

**Iyengar et al. (1984)** reported that poultry manure resulted in significantly higher build up of P concentration in leaf sample of banana five months after planting.

**Prasad et al. (1984)** reported that addition of poultry manure alone or in combination with N, P, K, Zn and Fe increased the uptake of Zn and Fe by wheat and rice.

**More and Ghonsikar (1988)** reported that the P content in wheat was significantly higher due to the application of poultry manure with super phosphate.

**Faiyard et al. (1991)** recorded an increase in N, P, K, Fe, Mn and Cu contents in faba beans due to the application of poultry manure in comparison with FYM

**Yassen et al. (2007)** carried out pot experiment in the greenhouse to study the effect of some organic residues (farmyard manure, peanut residue and potassium humate) on heavy metal (Cd, Pb and Zn) toxicity of spinach plants grown on a polluted soil compared with non-polluted one. Data revealed that NPK content and uptake decreased in spinach plant in soil contaminated with heavy metals whereas the application of organic residues increased NPK content and uptake.

**Khan et al. (2013)** Used rice plant pot experiments and investigated the influence of Sewage sludge biochar (SSBC) upon biomass yield, bioaccumulation of nutrients, and metal(loid)s, and greenhouse gas (GHG) emissions. Amendments of SSBC significantly reduced the bioaccumulation of As, Cr, Co, Cu, Ni, and Pb, but increased that of Cd and Zn, though not above limits set by Chinese regulations.

**Choppala et al. (2015)** studied, two carbon materials biochar (CMB) and black carbon (BC) for investigating their effects on the reduction of hexavalent chromium [Cr(VI)] in two spiked [600 mg Cr(VI) kg<sup>-1</sup>] and one tannery waste contaminated [454 mg Cr(VI) kg<sup>-1</sup>] soils. The addition of carbon materials to the tannery soil was also effective in decreasing the phytotoxicity of Cr(VI) in mustard (*Brassica juncea* L.) plants. The metal (loid) transfer coefficient, as defined by the ratio between plant tissue concentration, and soil concentration decreased with the addition of carbon compounds.

**Ali et al. (2015)** investigated the effects of FA application on alleviating Cr phytotoxicity in wheat plants in a pot experiment conducted in sand- and soil-grown plants. Three Cr (0, 0.25, and 0.50 mM) treatments in the form of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> were applied

in both soils with or without foliar application of  $1.5 \text{ mg L}^{-1}$  FA. Plants were harvested after 4 months of treatments and data regarding growth characteristics, biomass, photosynthetic pigments and antioxidant enzymes were recorded. Chromium concentration, without FA, significantly increased in the wheat grains, leaves, stem, and roots with increasing Cr levels in both sand and soil. Chromium concentration in root was larger indicating that the root is the main part for Cr accumulation in a wheat plant.

**Abbas *et al.* (2017)** studied the effect of rice straw BC on Cd immobilization in soil and uptake by wheat in an agricultural contaminated-soil was investigated. Different levels of rice straw BC (0%, 1.5%, 3.0% and 5% w/w) were incorporated into the soil and incubated for two weeks. After this, wheat plants were grown in the amended soil until maturity. The results show that the BC treatments significantly increased Zn and Mn concentrations in shoots, roots and grains of wheat compared to the control.

**Shuhe *et al*** reported that the average Cd concentrations in root, stem, leaf, inflorescence and shoots of poultry manured *B. tripartite* were significantly decreased ( $p < 0.05$ ) by 35.5%, 34.4%, 31.0%, 46.5% and 22.6%, respectively, as compared to that of without the addition of poultry manure due to the decrease of extractable Cd in soil. Cd extraction capacities ( $\lg \text{ pot1}$ ) in shoot of *B. tripartite* were significantly increased ( $p < 0.05$ ) due to more than 4-fold increase in shoot biomass. Thus, poultry manure application lowered extractable Cd in soil thereby significantly decreased its uptake, however increased plant biomass and enhanced the Cd phytoextracting efficiency.

## **MATERIALS AND METHODS**

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An experiment has been conducted to study the “**Effect of Biochar, Poultry Manure & Sewage Sludge on phytoremediation in chromium contaminated soil by using rice (*Oryza sativa* L.)**” in Net house of the Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, respectively. The pot experiment was conducted in Factorial Completely Randomized Design (FCRD) taking rice as a test crop in 2015-16 during kharif season.

### **3.1 Experimental site**

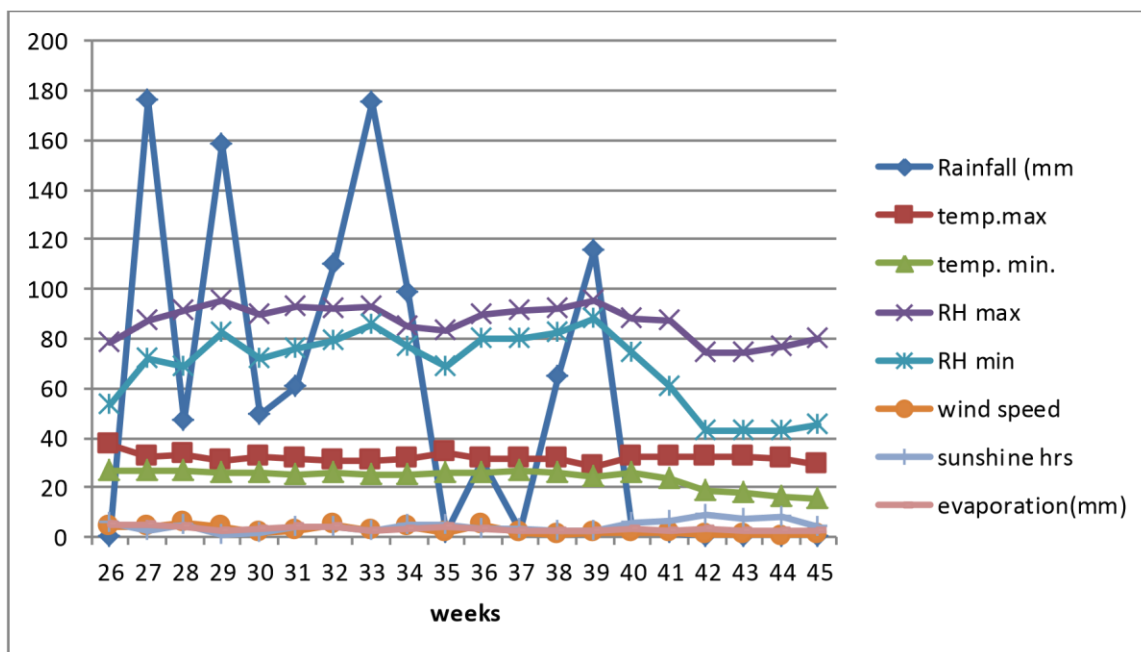
Varanasi is situated at an altitude of 78.71 meters above mean sea level and located between 25<sup>0</sup>18” North latitude and 80<sup>0</sup>36” East longitude.

### **3.2 Climate and weather**

Varanasi falls in the belt of semi-arid to sub-humid climate receiving a mean annual rainfall of 1100 mm and potential evapo-transpiration of about 1525 mm. Thus, there is a moisture deficit of 425 mm. The normal period for the onset of monsoon in this region is the third week of June and which lasts up to the end of September or sometimes extends to the first week of November. However, occasional showers are also common during winters. The winter months are cool whereas summers are hot and dry. Standard week wise data on meteorological parameters were obtained from the meteorological observatory, located at Agricultural Research Farm of Banaras Hindu University, Varanasi. A standard week-wise data on weather parameters are presented in Table 3.1 and depicted in Fig. 3.1.

**Table 3.1 Mean standard week-wise meteorological parameters during crop season (kharif), 2016.**

Week No.	Month & Date	Rainfall (mm)	Temperature (°C)		R.H. (%)		Wind Speed (km hr <sup>-1</sup> )	Sunshi ne hours	Evapor ation (mm)
			MAX	MIN	Morn.	Even.			
26	24-30	0.0	37.1	26.9	78	53	4.4	6.1	5.0
27	July 02-08	176.2	32.3	26.4	87	72	4.2	2.7	4.9
28	09-15	46.6	33.4	26.7	91	69	5.4	5.3	4.5
29	16-22	158.4	31.1	26.1	95	82	4.2	0.5	2.7
30	23-29	49.2	32.4	26.0	90	72	1.9	1.9	3.5
31	30-05	60.4	31.2	24.9	93	76	2.8	4.4	4.2
32	Aug 06-12	110	31.0	25.8	92	79	4.7	4.5	4.0
33	13-19	175.6	30.4	24.7	93	86	2.3	2.4	2.8
34	20-26	98.6	31.5	24.8	85	77	4.3	4.7	3.7
35	27-02	1.4	34.0	26.0	83	69	1.8	5.1	4.0
36	Sep 03-09	31.1	31.6	26.2	90	80	4.7	3.1	3.4
37	10-16	2.6	31.4	26.4	91	80	1.5	2.9	2.6
38	17-23	64.8	31.2	25.6	92	82	1.2	2.7	2.6
39	24-30	115.8	28.6	24.0	95	88	2.0	2.3	2.6
40	Oct 01-07	2.6	32.4	26.3	88	74	1.3	5.8	2.9
41	08-14	1.5	32.0	23.4	87	61	1.7	6.5	2.4
42	15-21	0.0	32.4	18.4	74	43	0.8	8.7	3.2
43	22-28	0.0	32.4	17.9	74	43	1.2	7.7	2.8
44	29-04	0.0	31.4	16.6	77	43	0.2	8.0	2.2
45	Nov 04-10	0.0	29.2	15.3	80	45	1.1	3.9	2.1



**Fig 3.1: Mean standard week-wise meteorological parameters during crop season (kharif), 2016.**

### 3.3 Experimental soil

The alluvial soils of Indo-Gangetic plains in general are deep, flat, well drained with low available nitrogen and medium in available phosphorus and potassium. To conduct the pot experiment, bulk surface (0-15 cm) soil was collected from the Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. The soil samples were analyzed for chemical properties and the results are presented in below Table 3.2.

**Table 3.2 Chemical characteristics of the initial experimental soil**

Properties	Values of initial soil	Method	Reference
<b>Chemical analysis</b>			
pH (1:2.5) soil: water suspension	7.9	Glass electrode digital pH meter	Jackson (1973)

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Electrical conductivity (dSm <sup>-1</sup> at 25 °C)	0.15	Electrical conductivity meter	Jackson (1973)
Organic carbon (%)	0.39	Wet digestion	Walkley and Black's (1934)
Available N (kg ha <sup>-1</sup> )	191.03	Alkaline permanganate method	Subbiah and Asija (1956)
Available P <sub>2</sub> O <sub>5</sub> (kg ha <sup>-1</sup> )	18.7.7	0.5M NaHCO <sub>3</sub> Extractable	(Olsen <i>et al.</i> , 1954) method
Available K <sub>2</sub> O (kg ha <sup>-1</sup> )	213.32	Flame photometer	Jackson (1973)
Available chromium (ppm)	0.08	AAS	Lindsay and Norwell(1978)
Available Fe(ppm)	18.8 ppm		
Available Cu(ppm)	1.75 ppm		
Available Mn(ppm)	6.05 ppm		
Available Zn(ppm)	1.04 ppm		

The data pertaining to physico-chemical properties of initial soil collected from Agricultural Research Farm had pH -7.9, EC-0.15 dSm<sup>-1</sup> and organic carbon 0.39 percent. The initial soil was low in available N (191 kg ha<sup>-1</sup>) while available P (18.7 kg ha<sup>-1</sup>) and K (213.3 kg ha<sup>-1</sup>) were in medium range .Available micronutrients were found very less in the initial soil.

### 3.4 Experimental details

- Year of experimentation : Kharif, 2016
- Recommended dose of fertilizers : N: P<sub>2</sub>O<sub>5</sub>: K<sub>2</sub>O @ 150:60:60 kg ha<sup>-1</sup>
  - No. of treatments : 15
  - No. of replication : 3
  - Total no. of pots : 36 (12 × 3)
  - Amount of soil : 10 kg pot<sup>-1</sup>
  - Experimental design : F.C.R.D.
  - Crop : Rice
  - Variety : HUR-105

The classified description of the treatments with corresponding symbol in order to facilitate their reference in the text is given in the below Table 3.3

**Table 3.3 Treatment details**

<b>Treatments</b>	<b>Abbreviations</b>
T1:Chromium 0 ppm	Cr0
T2:Chromium 25 ppm	Cr25
T3:Chromium 50 ppm	Cr50
T4:Chromium 0 ppm + Biochar	Cr0 + B
T5:Chromium 25ppm + Biochar	Cr25 + B
T6:Chromium 50 ppm + Biochar	Cr50 + B
T7:Chromium 0 ppm + Poultry manure	Cr0 + PM

T8:Chromium 25 ppm+ Poultry manure	Cr25 + PM
T9:Chromium 50 ppm + Poultry manure	Cr50 + PM
T10:Chromium 0 ppm + Sewage Sludge	Cr0 + SS
T11:Chromium 25 ppm + Sewage Sludge	Cr25 + SS
T12:Chromium 50 ppm + Sewage Sludge	Cr50 + SS

NPK recommended dose (150,60, 60kg hac<sup>-1</sup>), FYM @ (10t/hac) ,VC@ (5t/ hac)

### 3.5 Selection of variety

Malaviya Sugandh-105 (HUR-105)

It is used as a test crop that was developed at Banaras Hindu University, Varanasi and released in 2009. It is a semi dwarf, medium maturity scented rice variety suitable for cultivation in irrigated areas of U.P. Its maturity duration is 130135 days; average yield potential is up to 50 q ha<sup>-1</sup>. Plant grows up to height of 100110 cm. It is tolerant to stem borer, leaf and neck blast and brown spot under field condition.

### 3.6 Sources of nutrients

#### 3.6.1 Inorganic sources

##### 1. Urea

Urea is most widely used N fertilizer which is produced by heating ammonia with CO<sub>2</sub> under high temperature (160-170 °C). Urea contains the highest percentage of nitrogen (46.0%) among solid fertilizers. It contains nitrogen in the amide form.

This organic fertilizer is cheaper than any other solid nitrogenous fertilizer in India.

## 2. DAP

Di-ammonium phosphate is the most widely used phosphatic fertilizer and is produced by reacting calcium nitrate with mono-calcium phosphate and finally to di Ammonium phosphate and it contains 18% N and 46% P<sub>2</sub>O<sub>5</sub>.

## 3. MOP

Muriate of potash or potassium chloride (KCl) is most common and cheapest fertilizer among the potassic fertilizes. It contains 60% K<sub>2</sub>O.

### 3.6.2 Organic sources

The biochar, poultry manure & sewage sludge was collected from Agricultural Research Farm, IAS, BHU. Varanasi .

**1. Biochar:** About 1.08 kg of biochar is collected & 30g of biochar is applied per 10kg pot.

**2. Poultry manure:** 180g of poultry manure is collected & 5g of poultry manure is applied per 10kg pot

**3. Sewage & Sludge:** 1.8 kg of sewage sludge is collected & 50g of sewage sludge is applied per 10kg pot

## 3.7 Procedure

### Step1: Filling of pots

Processed 10 kg soil was filled in each polythene lined pots. The pots were irrigated up to field capacity and moisture level is maintained.

### Step 2: Application of chromium:

The ten kg of soil was treated with required amount of Cr through potassium dichromate ( $K_2Cr_2O_7$ ) i.e. Cr (VI), with five different levels like (0,25,50 mg/kg soil) and maintained contamination for 15days .

### **Step 3: Application of organic amendments**

After 15 days of application of chromium, the organic amendments like Biochar i.e., as 25gm/10kg soil Sewage sludge (50g/10kg soil ) & poultry manure ( 5g/10Kg) applied to the soil and thoroughly mixed well .

### **Step 4: Transplanting of rice seedlings in to contaminated pots**

Pots were incubated for 15 days with organic amendments and watered at field capacity four week old-seedlings were transplanted on august 1st and each pot transplanted with 5 seedlings  $pot^{-1}$ .

### **Step 5: Application of fertilizers**

The sources of nitrogen, phosphorus and potassium were urea, DAP and MOP, respectively. The recommended dose of N, P and K were 120, 60 and 60 kg  $ha^{-1}$ . One third N and full dose of P and K has been applied at the time of sowing and remaining dose of N has been applied in two equal splits after 30 and 60 days of transplanting in each pot. All the pots received uniform dose of NPK and irrigation had supplied to maintain field capacity. The crop was grown up to maturity.

### **Step 6: Harvesting and collection of samples**

Plants were harvested and plant parts like grain, straw, and root samples were collected separately from each pot and washed successively with tap water and thereafter with single and double distilled water, oven dried at 65°C and grinded in Willy mill to pass through 0.5 mm screen .

### **3.8 Biometric Observation**

For recording biometric observations at a regular interval of 30<sup>th</sup>, 60<sup>th</sup>, 90<sup>th</sup> days after sowing (DAS) and at harvest, five plants in each pot were taken into consideration. Yield attributes and yield were studied before and after harvesting as per investigation required.

#### **3.8.1 Growth Attributes**

##### **3.8.1.1 Plant height (cm)**

The height of plants was measured at 30, 60, 90 days transplanting and at harvest from the base of the plant to the tip of the upper most fully opened leaf. After panicle emergence, the height was measured up to the tip of the panicle.

##### **3.8.1.2 Number of the tillers hill<sup>-1</sup>**

Tillers were counted from each pot at 30, 60, 90 days after transplanting and at harvest total tillers/hill and productive tillers hill<sup>-1</sup> from each pot were computed.

##### **3.8.1.3 Dry matter accumulation hill<sup>-1</sup> (g)**

The plants were cut from ground level and sun dried thereafter the collected samples were oven dried at 70°C for 48 hours to constant weight. The weight thus obtained was recorded as average dry weight hill<sup>-1</sup> (g) after dividing the total weight of five plants by the total number of plants in a hill (05).

#### **3.8.2 Yield attributes and yeild**

The following observations on yield attributes and yield were recorded during the experimentation.

##### **3.8.2.1 Panicle length (cm)**

5 panicles were randomly selected from plants in each pot and the length was measured from the neck node to the tip of the upper most spikelet and average length was recorded.

**3.8.2.2 Filled grains panicle<sup>-1</sup>**

Grains of 5 panicles of randomly selected from each pot were separated and counted and their mean value expressed as average grains per panicle.

**3.8.2.3 Panicle weight (g)**

Five panicles were sampled from the plants in each pot and their weight was measured. The mean panicle weight was computed and expressed in g.

**3.8.2.4 1000 grains weight or test weight (g):**

Grain samples were taken from the threshed and cleaned produce of each pot and 1000 grains were counted and weighed.

**3.8.2.5 Grain yield (g pot<sup>-1</sup>)**

The mature harvested produce from the pot was sun dried and threshed to obtain grain yield (g pot<sup>-1</sup>).

**3.8.2.6 Straw yield (g pot<sup>-1</sup>)**

The straw yield was worked out by subtracting the grain yield from total biological yield and finally it was computed to g pot<sup>-1</sup>.

**3.8.2.7 Harvest Index (%)**

It is the economic yield expressed as percentage of biological yield and calculated as follows. It is expressed in percentage.

$$\text{Harvest index (\%)} = \frac{\text{Grain yield (kg ha}^{-1}\text{)}}{\text{Biological yield (kg ha}^{-1}\text{)}} \times 100$$

Biological yield= Grain yield + Straw yield

### **3.8.3 Chemical analyses of Soil Samples**

#### **3.8.3.1 Soil pH**

A soil-water suspension was prepared in the ratio of 1:2.5 (10 g soil with 25 mL of distilled water) and pH was measured with the help of pH meter.

#### **3.8.3.2 Electrical conductivity**

The soil water suspension prepared for determination of pH was used to determine the electrical conductivity of the soil. Soil suspension was allowed to settle till supernatant become clear. Electrical conductivity was measured with the help of EC meter and expressed as  $\text{dSm}^{-1}$ .

#### **3.8.3.3 Organic Carbon**

Organic Carbon in the soil was determined using Walkley and Black method (Walkley and Black, 1934). One gram of soil was taken in a 500 mL of conical flask. Ten mL of 1 N  $\text{K}_2\text{Cr}_2\text{O}_7$  solution was added and mixed. Then 20 mL of concentrated  $\text{H}_2\text{SO}_4$  was added, the flask was swirled 2-3 times and allowed to stand for 30 minutes in dark. The suspension was diluted with 200 mL of distilled water. Ten mL of 85%  $\text{H}_3\text{PO}_4$  and 1 mL of diphenyl indicator were added and titrated against the solution of 0.5 N Ferrous Ammonium Sulphate till color changed from violet to bright green. A blank titration was also carried out.

#### **3.8.3.4 Available nitrogen**

Available Nitrogen content in soil was determined using Kjeltec Semi-Auto Nitrogen Analyzer by alkaline Potassium permanganate method as proposed by Subbiah and Asija (1956). The method has been adopted widely in order to get a reliable index of Nitrogen availability in soil due to its rapidity and reproducibility. Five gram of soil sample was weighed and transferred in a distillation tube. Twenty five mL 0.32%  $\text{KMnO}_4$

was added to it and the distillation tube was set to the instrument. In a 250 mL conical flask, 20 mL of 2 % boric acid mixed indicator was taken and placed under the receiver tube. Twenty-five mL of 2.5% NaOH was sucked and added to the distillation tube. Then it was put on distillation for 9 min. During this process the N released in the form of ammonia is trapped in the boric acid, which develops green color. The flask containing the distillate was removed. The distillate was then titrated against 0.02 N H<sub>2</sub>SO<sub>4</sub> until pink color developed.

### **3.8.3.5 Available phosphorous**

Available phosphorus content of soil was determined by Olsen's method (Olsen *et al.*, 1954). Firstly reagent A was prepared by using ammonium molybdate, antimony potassium tartarate and H<sub>2</sub>SO<sub>4</sub>. Then reagent B was prepared with the help of reagent A. 2.5 gram of soil was taken in a 100 mL conical flask, a pinch of Darco G-60 and 50 mL of Olsen's reagent (0.5 M NaHCO<sub>3</sub>) was added to it. It was then shaken for 30 minute on mechanical shaker and the suspension was filtered through Whatman No. 1 filter paper. Five mL of filtrate was transferred in a 25 mL volumetric flask and was acidified with 2.5 M H<sub>2</sub>SO<sub>4</sub> to pH 5.0 and 20 mL distilled water was added followed by 4 mL of reagent B. After waiting for 10 min the intensity of blue colour was measured on spectrophotometer at 660 nm. Simultaneously a blank was also run. First standard reading was taken followed by sample reading.

### **3.8.3.5 Available Potassium**

Flame Photometer is used to determine available potassium in soil by using (1 N ammonium acetate extract) by using a method Jackson (1973). Five gram soil was transferred in a 100 mL conical flask and 25 mL of 1 N ammonium acetate solution was added and it was shaken for 5 minutes. The suspension was then filtered through Whatman No. 1 filter paper and potassium concentration in the filtrate was measured using flame photometer. First standard reading was taken followed by sample reading.

### **3.8.3.6 DTPA extractable micronutrient and chromium**

Available Zn, Mn, Cu and Fe in soil samples were determined by the method of (Lindsay and Norvell, 1978). In this method, 10 g of soil was extracted with 20 mL DTPA

extracting solution by shaking for 2 hours on a shaker. The suspension was then filtered and trace elements were determined by atomic absorption spectrophotometer (UNICAM-969) using respective cathode lamps.

### 3.8.4 Nutrient content and uptake studies

At harvest, the plant and grain samples were collected from each pot for chemical estimation and the samples were dried in an oven at 70°C for 48 hours, the plant material thus obtained was ground with the help of grinder and passed through 40 mesh sieve and preserved separately for determination of N, P and K content. The nutrient content was then estimated as per following methods as given in the Table 3.8 and nutrient uptake were also calculated by following formulae.

$$\text{Nutrient uptake (kg ha}^{-1}\text{)} = \frac{\text{Nutrient content (\%)} \times \text{yield (kg ha}^{-1}\text{)}}{100}$$

#### 3.8.4.1 Nitrogen content (%) and its uptake

Nitrogen content (%) was estimated both in grain and in straw by modified Kjeldahl method as described by Jackson (1973). Total nitrogen content in grain and straw was multiplied by the respective dry matter yield to get the total nitrogen uptake (kg ha<sup>-1</sup>) by plants.

#### 3.8.4.2 Phosphorus and its uptake

Phosphorus content (%) in grain and straw was determined by Vanomolybdo phosphoric acid yellow color method as suggested by Jackson (1973). Total phosphorus content of grain and straw was multiplied by the respective dry matter yield to get the total phosphorus uptake (kg ha<sup>-1</sup>) by plants.

#### 3.8.4.3 Potassium and its uptake

Potassium content (%) was estimated with the help of Flame photometer as described by Jackson (1973). Potassium per cent in grain and straw were multiplied by

their respective dry matter yield to obtain total potassium uptake ( $\text{kg ha}^{-1}$ ) by plants. The uptake of the entire major nutrient in  $\text{kg ha}^{-1}$  was calculated by using the formula given by Black (1967).

#### 3.8.4.4 Chromium

Chromium content (%) was estimated with the help of AAS as described by Lindsay and Norwell (1978). Chromium per cent in grain and straw were multiplied by their respective dry matter yield to obtain total chromium uptake ( $\text{kg ha}^{-1}$ ) by plants.

**Table 3.4 Chemical analyses of plant and grain:**

Analysis	Method	Reference
Total N	Micro Kjeldahl method	Jackson (1973)
Total P	Vandomolybdo phosphoric acid yellow colour method	Jackson (1973)
Total K	Flame photometer method	Jackson (1973)
Total Cr	AAS	Lindsay and Norwell(1978)

#### 3.9 Enrichment factor (EF) and transformation factor (TF)

Quantification of enrichment factor and transformation factor are shown in the equation (1) from (Lorestani *et al.*, 2011). The value of enrichment factors indicated the mechanism of heavy metal absorption from soil to *Oryza sativa*, however the translocation factor is to examine the transport or transfer of heavy metals from roots to shoots of *Oryza Sativa*. The EF and TF values that were examined in this study are in  $\text{mg kg}^{-1}$ .

$$\text{Enrichment factor (EF)} = C_p/C_s$$

Where

C<sub>p</sub>= heavy metal concentration in plant

C<sub>s</sub>=heavy metal concentration in soil

Translocation factor (TF)=C<sub>s</sub>/C<sub>r</sub>

Where

C<sub>s</sub>=heavy metal concentration in plant's shoot

C<sub>r</sub>=heavy metal concentration in plant's root

### 3.10 Statistical analysis

The observations recorded during the course of investigation were tabulated and analyzed statistically to draw a valid conclusion. The data were analyzed as per the standard procedure for "Analysis of Variance" (ANOVA) as described by Gomez and Gomez (1984). The significance of treatments was tested by „F“ test (Variance ratio). Standard error of mean was computed in all cases. The difference in the treatment mean were tested by using Critical Difference (CD) at 5% level of probability where „F“ test showed significant differences among means by the following formula:

$$SEm \pm = \frac{\sqrt{ErrorMSS}}{N}$$

Where ,

$$N = \frac{\text{Number of experimental unit}}{\text{Number of factor involved}}$$

$$CD = \sqrt{\frac{2 \times \text{error sum of square}}{N}} \times t (\text{error d.f. } 5\%)$$

### ANOVA

S.V.	d.f.	SS	MSS	Fcal	Ftab 5%

<b>Factor A</b>					
<b>Factor B</b>					
<b>A*B</b>					
<b>Error</b>					
<b>Total</b>					

The results have been presented in the tabular form and depicted in figure wherever necessary.

### Overall view of pots in the net house



## RESULTS AND DISCUSSION

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The present experiment was conducted to study the “Effect of biochar, poultry manure & sewage sludge on phytoremediation in chromium contaminated soil by using rice (*Oryza sativa L.*)” in the net house environment, Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh.

In this experiment data has been recorded regarding effect of biochar, poultry manure & sewage sludge on growth, yield and quality, nutrient content and uptake of chromium and nutrients of rice grown in chromium contaminated soil and regarding potential of rice to uptake chromium. The data recorded were analyzed statistically drawing the valid conclusions. Differential responses exhibited by varying experimental variables were discussed in this chapter as follows.

4.1 Effect of biochar, poultry manure & sewage sludge on plant parameters of rice plants in chromium contaminated soil

4.1.1 Growth (plant height)

4.1.2 Total tillers/hill and productive tillers/hill

4.2 Physiological characteristics

4.2.1 Chlorophyll

4.3 Yield attributes of rice plants

4.3.1 Panicle length

4.3.2 Numbers of grains panicle-1 and unfilled grain panicle-1

4.3.4 1000grains weight (g) or test weight

4.4 Yield of rice plants

4.4.1 Grain yield and straw yield of rice

#### 4.4.2 Harvest index (%)

#### 4.5 Nutrient content and uptake of rice straw and grain

##### 4.5.1 Nitrogen content in grain and straw (%)

##### 4.5.2 Phosphorus content in grain and straw (%)

##### 4.5.3 Potassium content in grain and straw (%)

#### 4.6 Effect of FYM and Vermicompost on soil properties in chromium contaminated soil

##### 4.6.1 pH

##### 4.6.2 EC

##### 4.6.3 Organic carbon

##### 4.6.4 Available Nitrogen

##### 4.6.5 Available Phosphorus

##### 4.6.6 Available potassium

#### 4.7 Effect of FYM and Vermicompost on Cr content

##### 4.7.1 In grain and straw of rice

##### 4.7.2 In post-harvest soil

##### 4.7.3 Accumulation of chromium in shoots and roots

##### 4.7.4 Translocation and enrichment factor

### **4.1 Effect of biochar, poultry manure & sewage sludge on plant parameters of rice plants in chromium contaminated soil**

#### **4.1.1 Plant height**

The data of total plant height of rice was recorded at 30 DAT, 60 DAT, 90 DAT. The height of rice plant in Cr-contaminated soil and that plant height influenced by organic amendments are presented in Table 4.1 and depicted in Fig 4.1. plant height increases upto 90DAT and thereafter decreases as shown in the data observed. Plant height was significantly affected by different levels of chromium at all growth stages. With increase

in the chromium concentration (0,25,50)ppm the plant height has decreased. Similar type of results reported by Nagarajan (2014).

There is significant increase in plant height (30 DAT) on addition of Organic amendments like biochar, poultry manure & sewage sludge as compared to control pots. The highest plant height (54.67) was found in treatment Cr0 +biochar followed by the treatment Cr0 + PM i.e., (54.33). On the other hand, the lowest values of these parameters were obtained from control pot with treatment Cr50 (47.33). The similar trend was noticed with plant height at 60 DAT and 90 DAT except a slight increase in plant height and there after a decline in height observed. Interaction effect of chromium with organic amendments shown on –significant on plant height.

Due to an adequate supply of nutrients by fertilizers & organic amendment there was increase in plant height. The availability of nutrients increased by improving important soil properties given by Ganai and Singh (1988), Singarum (1994). In the absence of any organic amendments, the rice growth was decreased due to the toxicity of Cr VI.

#### 4.1.2 Number of tillers hill-1

The data of total tillers per hill and productive tillers per hill was recorded at different observation days presented in Table 4.2 and Fig 4.2. A perusal of data presented that the number of tillers per hill increased slowly from 30 to 60 DAT and there after a gradual decline was observed. Different levels of chromium generally affect the tiller no. As the chromium concentration increases there was a decrease in number of total tiller and productive tiller per hill as shown in the table 4.2. Similar results like Cr-treated rice plants showed stunted growth and produced less number of tillers and leaves compared to counterparts grown in control has reported by Ahmad et al. (2011).

The results of the pot experiments showed that a number of tillers per hill of rice and productive tillers per hill were significantly affected due to the application of organic amendments. The highest total tillers hill-1 (13.67) and productive tillers per hill (12.33) was observed in all control pots of Cr0+ biochar followed by Cr0+ SS in total (12.667) and Cr0 + PM in productive (11)tillers per hill. The application of biochar, poultry manure & sewage sludge resulted in a significantly greater number of tillers per hill and productive tillers/hill of rice. Generally because of supply of better micronutrient by organic sources resulted in better tillering. Application of sewage sludge biochar(SSBC) results in increase in no of tillers as reported by Khan et al in 2013.

The interaction effect of Cr and amendments indicated that the adverse effect of Cr on tillers hill-1 and productive tillers hill-1 of rice could be alleviated to some extent by amendment application, especially with biochar application. Significantly, the highest number of tillers hill-1 and productive tillers hill-1 was recorded at treatment Cr0+ biochar ; while the maximum tillers reduction was noted by 25% per cent over control at a Cr50 level without amendment (Table 4.2). The interaction effect between chromium concentration levels and amendments on tillers per hill was found not- significant in all growth stages.

All these growth parameters have reduced with an increase in chromium concentrations this is seen because when metal content of soil becomes high, the plant will lose its role, possibly because of the lethal exploit by the metal, and the uptake especially increases. Further, because of this improved uptake, metals will interrelate with various cellular mechanisms and they disturb the normal metabolic reactions of plants, producing cellular damage and the death of the plants in severe cases. The decrease in plant growth could be due to the reduction of photosynthetic pigments (Wani et al., 2006 and Sheoran et al.,1990).

Organic amendments increases soils fertility, enhancing plant growth and decreasing Phyto-available fractions of metals, where similar results reported by Walker et al. (2004). The variation in the above-mentioned parameters recorded might be attributed due to the availability of nutrients. Nutrient availability from organic sources is due to microbial action which is slow and steady and further through improved physical conditions of the soils reported by Amitava et al. (2008) and Mirza et al. (2010).

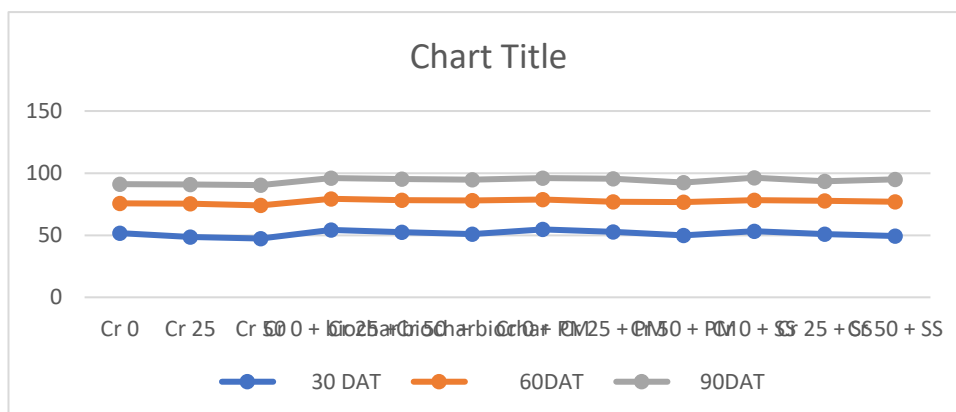
Biochar shows better affect as compared to other organic amendment because of its chemical stability as it widens the possibility of sequestering carbon besides it increases soil fertility and available nutrient for plant uptake as reported by Houben et al. it is also free from presence of other heavy metal as in sewage sludge.

The results indicated that Cr affects all growth parameters of rice even though it was reported as an accumulator and tolerant for heavy metals, So upon addition of these organic amendments will reduce the toxicity caused by Cr and significantly increase the growth rate of plants.

#### **4.1 Effect of Biochar, Poultry Manure & Sewage sludge on plant height of rice at 30,60,90 DAT in chromium contaminated soil**

Treatments	30 DAT	60DAT	90DAT
Cr 0	51.667	75.667	91.067
Cr 25	48.667	75.333	90.867
Cr 50	47.333	74	90.333
Cr 0 + biochar	54.667	79.333	96.067
Cr 25 + biochar	52.333	78.333	95.3
Cr 50 + biochar	51	78	94.767
Cr 0 + PM	54.33	78.667	96.3
Cr 25 + PM	52.667	77	95.5
Cr 50 + PM	50	76.667	92.4
Cr 0 + SS	53.333	78.333	96.3
Cr 25 + SS	51	77.667	93.333
Cr 50 + SS	49.333	77	95.1
CD	3.629	N/A	N/A
SE(m)	1.236	1.255	2.176
SE(d)	1.748	1.774	3.078
CV	4.168	2.816	4.014

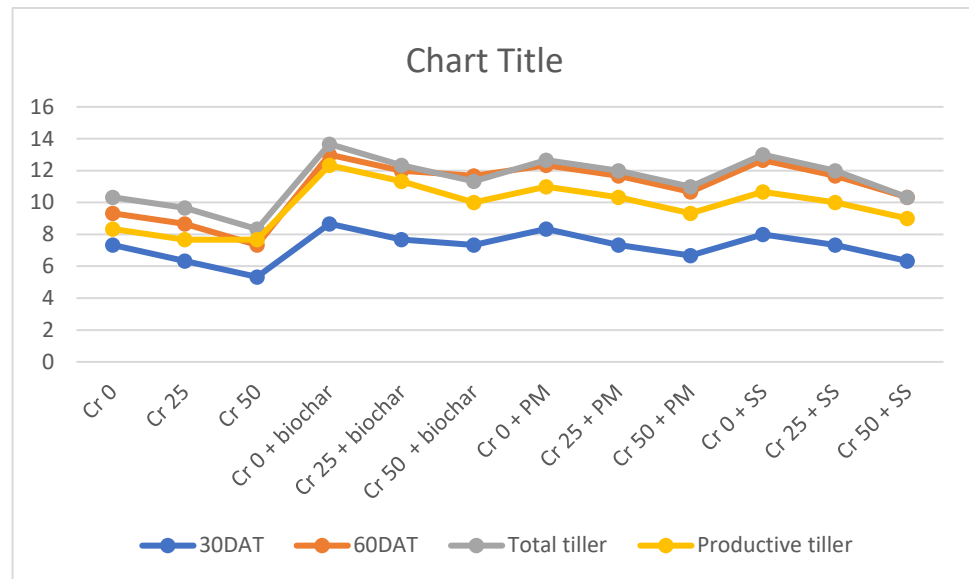
**4.1 fig Effect of Biochar, Poultry Manure & Sewage sludge on plant height of rice at 30,60,90 DAT in chromium contaminated soil**



**4.2 Effect of Biochar, Poultry Manure & Sewage sludge on number of tiller**

treatment	30DAT	60DAT	Total tiller	Productive tiller
Cr 0	7.333	9.333	10.333	8.333
Cr 25	6.333	8.667	9.667	7.667
Cr 50	5.333	7.333	8.333	7.667
Cr 0 + biochar	8.667	13	13.667	12.333
Cr 25 + biochar	7.667	12	12.333	11.333
Cr 50 + biochar	7.333	11.667	11.333	10
Cr 0 + PM	8.333	12.333	12.667	11
Cr 25 + PM	7.333	11.667	12	10.333
Cr 50 + PM	6.667	10.667	11	9.333
Cr 0 + SS	8	12.667	13	10.667
Cr 25 + SS	7.333	11.667	12	10
Cr 50 + SS	6.333	10.333	10.333	9
CD	0.937	1.232	1.165	1.355
SE(m)	0.319	0.419	0.397	0.461
SE(d)	0.451	0.593	0.561	0.653
CV	7.654	6.638	6.034	8.152

#### 4.2 fig Effect of Biochar, Poultry Manure & Sewage sludge on number of tiller



#### 4.2 Physiological Characteristics of Plants:

##### 4.2.1 Chlorophyll

The data of chlorophyll was recorded by SPAD at different observation days presented in Table 4.3 and depicted in Fig 4.3. A perusal of data presented that chlorophyll content increased slowly from 30 to 60 DAT and there after a gradual decline was observed at 90 DAT and maturity.

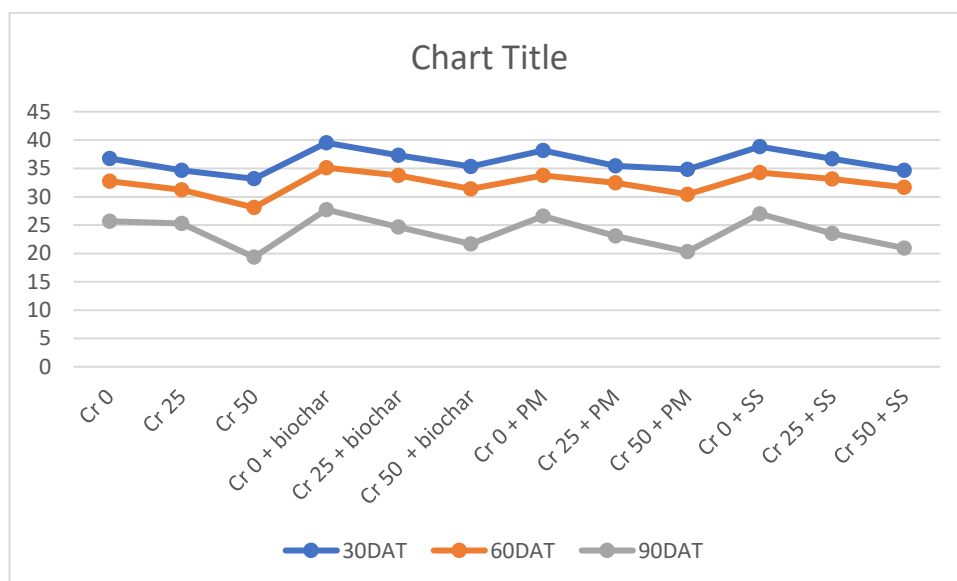
It is apparent from the data that chlorophyll content was significantly affected by different chromium levels at all growth stages. Data shows that as the level of chromium increases from 0 to 50 the amount of chlorophyll content decreases respectively. Oxidative stress is induced by more concentration of chromium, which involves induction of lipid peroxidation in plants that cause severe damage to cell membranes. The oxidative stress is induced by chromium initiates the degradation of photosynthetic pigments which causes a decline in growth of the plant. High chromium concentration can disturb the chloroplast ultra structure there by disturbing the photosynthetic process. Cr stress is one of the important factors that affect photosynthesis in terms of CO<sub>2</sub> fixation, electron transport, photophosphorylation, and enzyme activities said by Clijsters and Van Assche (1985).

The Chromium toxicity in plants occurs by inhibiting the growth more or less, showing chlorosis. High chromium concentration inhibits photosynthesis and seriously inhibits the root growth reported by Dheeba et al .(2012).

The results of the pot experiments showed that chlorophyll content rice was significantly affected due to the application of organic amendments. The highest chlorophyll content (39.51) was observed with treatment Cr0+ biochar followed by treatment Cr0+ SS(38.83).Plants grown on soil without any amendments at Cr50 had shown yellowing of leaves. The application of Biochar, Poultry Manure & Sewage sludge resulted in a significantly highest chlorophyll content of rice. A similar trend followed in 60DAT but during 90 DAT and maturity gradual decline in chlorophyll content due to toxicity effect of Cr VI. The interaction effect between chromium concentration levels and amendments on chlorophyll content was found non-significant in all growth stages. The Biochar application increased the chlorophyll a, b, total chlorophyll, and carotenoid concentrations as compared to control treated with heavy metals without any amendment as reported by Younis et al (2016)

**Table 4.3 Effect of Biochar, Poultry Manure & Sewage sludge on chlorophyll content of rice plant in chromium contaminated soil**

treatment	30DAT	60DAT	90DAT
Cr 0	36.727	32.73	25.67
Cr 25	34.667	31.19	25.273
Cr 50	33.167	28.1	19.33
Cr 0 + biochar	39.513	35.13	27.73
Cr 25 + biochar	37.333	33.73	24.67
Cr 50 + biochar	35.333	31.4	21.667
Cr 0 + PM	38.18	33.73	26.59
Cr 25 + PM	35.44	32.47	23.1
Cr 50 + PM	34.84	30.44	20.29
Cr 0 + SS	38.833	34.29	27
Cr 25 + SS	36.667	33.13	23.54
Cr 50 + SS	34.667	31.667	20.96
CD	1.479	1.012	2.751
SE(m)	0.504	0.345	0.937
SE(d)	0.712	0.488	1.325
CV	2.404	1.847	6.812

**Fig. 4.3 Effect of Biochar, Poultry Manure & Sewage sludge on chlorophyll content of rice plant in chromium contaminated soil**

### **4.3 Effect of Biochar, Poultry Manure & Sewage sludge on yield attributes of rice in chromium contaminated soil**

#### 4.3.1 Panicle length (cm):

The length of the panicle is generally influenced by different chromium concentration and addition of various organic amendment. Data was collected regarding length of panicle which is represented in Table 4.4. The results showed that plant has slight variations and can withstand with an increase in the levels of chromium up to concentration Cr25 in control and with use of amendments rice can withstand up to treatment Cr 50.

It is evident from the results that panicle length varied from (21.96 to 18.2cm). Among organic amendments, maximum panicle length (21.967cm) was found associated with the treatment Cr0+biochar which was significantly higher than the application of other amendment and control. The interaction effect between chromium levels and organic sources in respect to the length of panicle and weight of panicle was non-significant.

#### 4.3.2 Number of grains panicle-1(g hill-1)

There was a significant effect on a number of grains per panicle with a different application of different levels of chromium. A number of grains per panicle varied from (109 to 102g hill-1) in case of different chromium levels in control. The highest no. of grains /panicle was seen control pots i.e., Cr0 followed by Cr25 and Cr50 respectively. This result show that there was decrease in grains per panicle as the concentration of chromium increases.

It is evident from data that no.of grains /panicle varied in organic amended pots from (130.33 to 109.67g hill-1). These organic amendments significantly influenced a number of grains panicle-1of rice. A maximum number of grains panicle-1 (130.33g hill-1) was recorded with Cr0+biochar followed by Cr0+PM (125) & Cr0+SS (124) respectively. The addition of these organic amendments has significantly increased the number of grains panicle-1. The interaction effect between chromium levels and organic amendments in respect to a number of grains panicle-1 was found not-significant.

#### 4.3.3 1000 grains weight (g)

Various chromium levels and organic amendments influence grain weight of the plant at various growth stages of the crop has been represented in Table 4.4. Data revealed that 1000 grains weight varies from (22.26g) in Cr0 to (18.8g) in Cr50. This data shows that increase in chromium content decreases 1000 grain weight of plant.

Different organic amendments significantly influenced 1000 grains weight of rice. Maximum 1000 grains weight (24.77g) was recorded with treatment Cr0+biochar followed by Cr0+FYM (23.233g) & Cr0+SS (22.8) respectively. The addition of these organic amendments has significantly increased the 1000 grains weight. The interaction effect between chromium levels and organic amendments in respect to 1000 grains weight was found not-significant.

All these yield attributes like panicle number, grains panicle-1, 1000 grain weight, increased with the addition of biochar & other organic amendment as compared to control. SSBC amendments significantly increased grain yield in case of rice as reported by Khan et al (2013). Similarly Bian et al reported that grain yield was sustained over 3 years after a single soil amendment of wheat straw biochar. The improved growth coupled with the transport of photosynthates towards reproductive structure might have increased the yield attributes and yield due to organic addition reported by (Manivannan and Sriramachandrasekharan, 2009).

The results indicated that Cr affects the yield attributes of rice even though it was reported as a hyper accumulator and tolerant for heavy metals. Cr adversely affects the yield attributes of rice and the fertilizer amendments will alleviate the toxicity caused by Cr and significantly increase the yield attributes of plants.

**Table 4.4 Effect of Biochar, Poultry Manure & Sewage sludge on yield attributes of rice in chromium contaminated soil**

treatment	Panicle length (cm)	No of panicles per m <sup>2</sup>	Grains per panicle(g hill-1)	Unfilled grains/panicle (g hill-1)	Test weight in g
Cr 0	19.167	241.333	109	7	22.267
Cr 25	18.867	231.333	105	8	19.6
Cr 50	18.2	227.333	102	10	18.8
Cr 0 + biochar	21.967	273.667	130.333	4	24.777
Cr 25 + biochar	21.667	265.667	122.333	5.667	22.733
Cr 50 + biochar	20.9	254.333	115	6.667	21.133
Cr 0 + PM	21.1	267	125	5	23.233
Cr 25 + PM	20.7	259.667	117.333	7	22.433
Cr 50 + PM	20.133	249.333	112	7.667	20.8
Cr 0 + SS	20.967	265	124	6	22.8
Cr 25 + SS	20.733	259.667	116	6.667	21.733
Cr 50 + SS	20.133	244	109.667	7.667	20.267
CD	0.32	3.727	2.364	1.874	0.168
SE(m)	0.109	1.269	0.805	0.638	0.057
SE(d)	0.154	1.795	1.139	0.903	0.081
CV	0.925	0.868	1.206	16.311	0.457

#### **4.4 Effect of Biochar, Poultry Manure & Sewage sludge on the yield of rice in chromium contaminated soil:**

##### **4.4.1 Grain yield and straw yield**

The yield of grain and straw has been depicted in the Table 4.5 and 4.6 respectively. An adverse direct effect on rice grain yield was shown by application of Cr. The lowest value (23.37 g pot<sup>-1</sup>) found in the treatment at Cr50 with a reduction in the yield by 13 per cent over control (26.7 g pot<sup>-1</sup>). Similar results were also reported in case of the straw yield of rice due to the direct effect of Cr (Table 4.6). The highest reduction

in rice straw yield due to direct effect was observed at Cr50 (28.20g pot<sup>-1</sup>). The beneficial effect of Cr was seen at lower level upto Cr20 on rice which could be attributed to increased absorption of nutrients like K, Ca and Mg, while at very higher Cr concentration it might have adversely affected due to phytotoxicity. Similar results was observed by Parmar and Patel (2015). The Cr is inhibitory to metabolism and acts on a contributory factor in phytotoxicity of wheat (Sharma et al. 1995).

Grain yield of rice is enhanced due to the direct effect of all organic amendments over control which is shown in Table 4.5. Similar results were also observed in straw yield of rice due to organic amendments application reported in Table 4.6. The improvement in yield of rice could be attributed to the addition of amendment like biochar, poultry manure & sewage sludge to the soil, which increased the availability of nutrients due to enhancement in important soil properties reported by Ganai & Singh (1988) and Singarum (1994).

The interaction effect between Cr and amendments like biochar, poultry manure & sewage sludge had shown significance. The adverse effect of Cr on the yield of rice could be alleviated to some extent by amendment application, especially with biochar addition. Significantly, the highest rice grain yield (31.730g) was recorded at treatment Cr0 +biochar; while the minimum yield was observed at Cr50 (23.80) without amendment (Table 4.5). This beneficial effect of amendments like biochar, poultry manure & sewage sludge also ascribed to increased microbial activity which might have helped in reduction of the toxic form (Cr<sup>6+</sup>) to non-toxic form (Cr<sup>3+</sup>) by microbial activity, by acting as electron donors, and O<sub>2</sub> level of the soil is lowered thereby creating reducing conditions. The almost similar trend was noticed for a straw yield of rice as an influence by the direct effect of Cr as well as interaction effect of Cr and amendments (Table 4.6). Similar results have also been reported by Bolan et al. (2003) Yuji et al. (2004)

Role of organic amendments is to reduce the toxicity of Cr. This improvement in yield could be mainly attributed due to the reduction in the bioavailability of Cr and thus reduce toxic effects of Cr in the soil. Bioavailability of metal in the soil environment and soil particles is said to be the fraction of the total metal in the interstitial pore water that is available to the receptor organism. More specifically, it refers to the biologically available fraction (or pool) that can be taken up by an organism and can react with its

metabolic machinery. By immobilization of metals, the bioavailability of metal can be reduced. This could be achieved by the addition of organic amendments where these amendments increase the immobilization of metal through adsorption reactions, the formation of organo-chromic complexes or reduction of toxic in soil or by chelation.

The grain and straw yield has been dropped after applying heavy metals which have been accredited to the toxic effects of metals on the propagation of roots and shoots. The characters of rice vary under various treatments of chromium. High organic matter and macro and micronutrients enhance both the soil physical, chemical and biological properties and the plant yield. Decrease in biomass productivity might be attributed to a disruption in nitrogen metabolism of seedlings under chromium stress also (Chatterjee and Chatterjee, 2000). Bian *et al.* (2014) reported significant increase in rice yield with application of wheat straw biochar.

**Table 4.5 Effect of biochar, poultry manure & sewage sludge on grain yield of cr contaminated soil**

	<b>B1 control</b>	<b>B2 Biochar</b>	<b>B3 Poultry manure</b>	<b>B4 Sewage sludge</b>	<b>Mean cr</b>
A1 Cr0	26.700	31.730	30.107	29.810	29.587
A2 Cr25	25.190	30.310	29.210	29.113	28.456
A3 Cr50	23.800	28.197	27.520	26.577	26.523
<b>Mean B</b>	25.230	30.079	28.946	28.500	

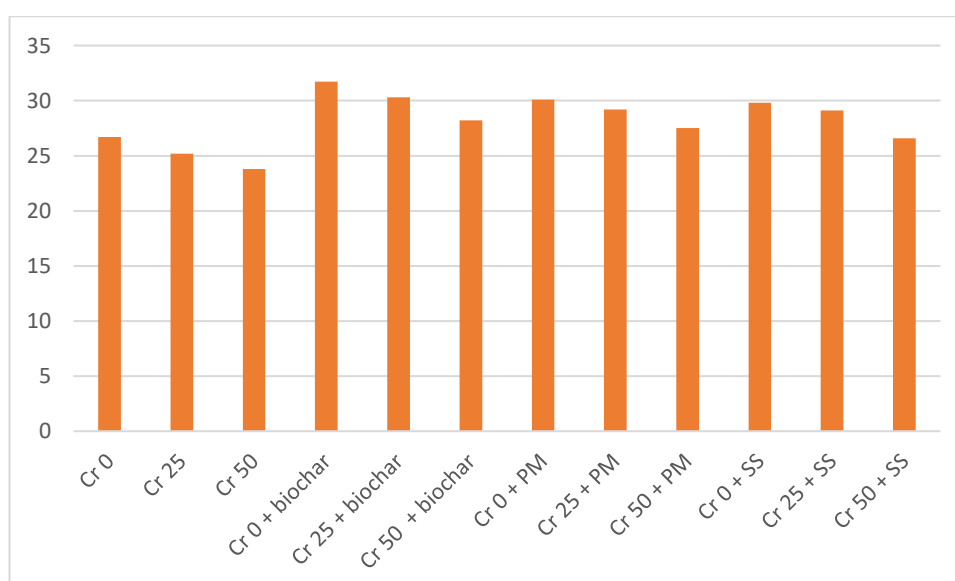
<b>Factors</b>	<b>C.D.</b>	<b>SE(d)</b>	<b>SE(m)</b>	
Factor(A)		0.076	0.054	significant
Factor(B)	0.183	0.088	0.062	significant
Factor(A X B)	0.317	0.153	0.108	significant

**Table 4.6 Effect of biochar, poultry manure & sewage sludge on straw yield of cr contaminated soil**

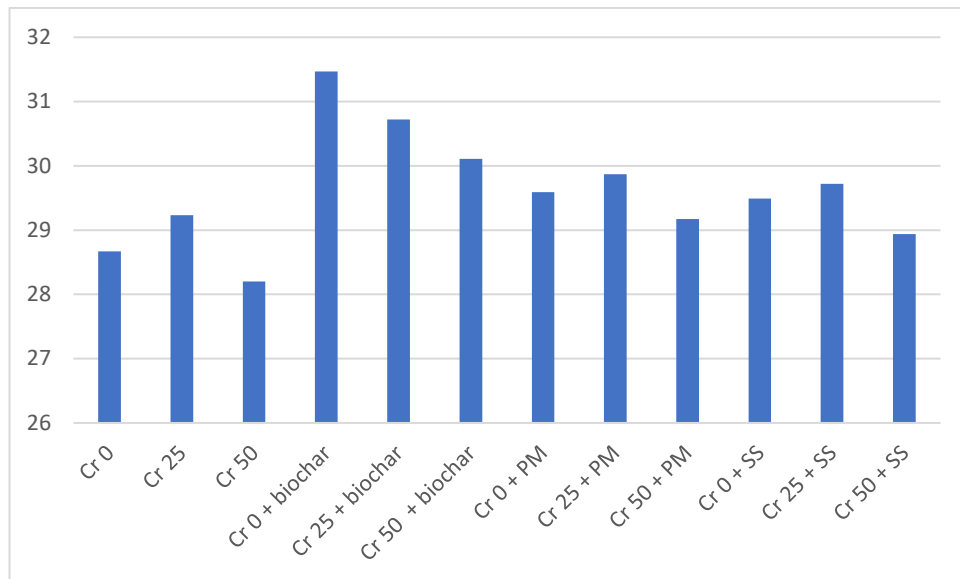
	<b>B1 control</b>	<b>B2 Biochar</b>	<b>B3 Poultry manure</b>	<b>B4 Sewage sludge</b>	<b>Mean cr</b>
A1 Cr0	28.670	31.470	29.590	29.493	29.806
A2 Cr25	29.230	30.720	29.867	29.720	29.884
A3 Cr50	28.200	30.110	29.170	28.937	29.104
<b>Mean amendment</b>	28.700	30.767	29.542	29.383	

<b>Factors</b>	<b>C.D.</b>	<b>SE(d)</b>	<b>SE(m)</b>	
Factor(A)	0.037	0.018	0.012	significant
Factor(B)	0.042	0.020	0.014	significant
Factor(A X B)	0.073	0.035	0.025	significant

**Fig 4.4 Effect of biochar, poultry manure & sewage sludge on grain yield of cr contaminated soil**



**Fig 4.5 Effect of biochar, poultry manure & sewage sludge on straw yield of cr contaminated soil**



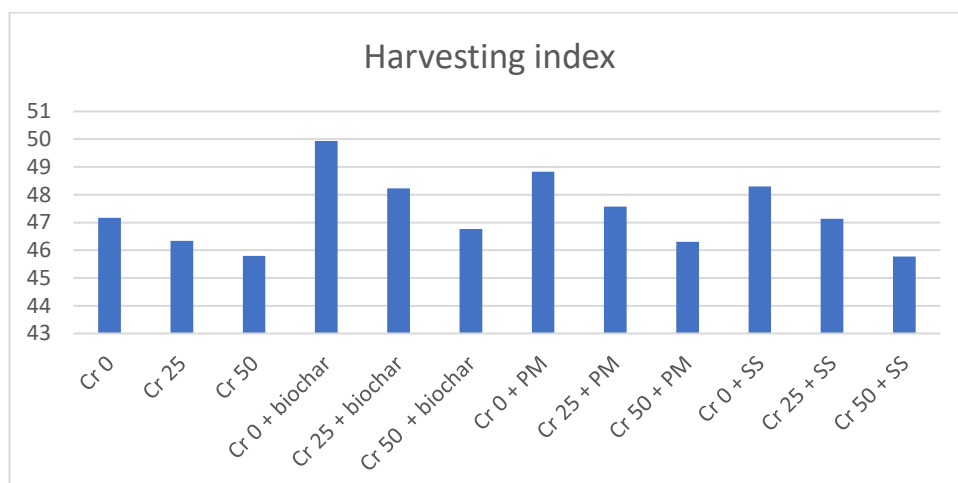
#### 4.4.2 Harvest index (%)

Harvest index of rice affected by chromium levels and organic amendments has been compiled in Table 4.7 and depicted graphically in Fig 4.6. Data revealed that harvest index varies from (47.16 %) in control to (45.8%) in Cr50. However, highest harvest index seen in treatment Cr0 is highest followed by the treatment Cr 25 & Cr50 respectively.

Different organic amendments significantly influenced harvest index of rice. Maximum harvest index was recorded with treatment Cr0+biochar(49.93%) followed by Cr0+PM (48.83%). The addition of these organic amendments has significantly increased the harvest index. There was a significant difference in the extent of Cr (VI) reduction among the soils treated with organic amendments (Bolan and Thiyagarajan, 2001 and Bolan et al. 2003). Interaction effect between chromium levels and organic amendments in respect to harvest index was found non-significant.

**Table 4.7 Effect of biochar, poultry manure & sewage sludge on harvesting index of the rice grown of cr contaminated soil**

treatment	Harvesting index
Cr 0	47.167
Cr 25	46.333
Cr 50	45.8
Cr 0 + biochar	49.933
Cr 25 + biochar	48.233
Cr 50 + biochar	46.767
Cr 0 + PM	48.833
Cr 25 + PM	47.567
Cr 50 + PM	46.3
Cr 0 + SS	48.3
Cr 25 + SS	47.133
Cr 50 + SS	45.767
CD	0.534
SE(m)	0.182
SE(d)	0.257
CV	0.665

**Fig 4.6 Effect of biochar, poultry manure & sewage sludge on harvesting index of the rice grown of cr contaminated soil**

4.5 Effect of chromium levels and organic amendments on nutrient content and uptake of rice grain and straw:

Data pertaining to analyses of N, P and K contents in grain and straw and their uptake as effected by chromium levels and organics sources have been presented in Table 4.8

#### 4.5.1 Nitrogen content in grain and straw (%)

The nitrogen content in grain and straw has been depicted in Table 4.8 and Fig 4.7. Data revealed that nitrogen content found highest in grain (1.077) compared to straw (0.42). Grain nitrogen content decreased with increase in the levels of chromium. The maximum reduction was seen at treatment Cr50. However, the treatment of Cr0 found statistically at par with treatment Cr25. Increasing chromium levels resulted in significant increase in nitrogen content of the rice straw up to the treatment Cr 20 tested in the experiment. There was the highest reduction in nitrogen content compared to P and K when interacted with chromium.

The nitrogen content in grain and straw of rice was found significantly higher under use of organic sources i.e. biochar, poultry manure, sewage sludge over control plant which contained minimum nitrogen content. The interaction effect between chromium and organic sources regarding nitrogen content in grain and straw failed to reach the level of significance

#### 4.5.2 Phosphorus content in grain and straw (%)

Phosphorus content in grain and straw as affected by chromium concentrations and organic sources have been compiled in Table 4.8 and depicted in Fig 4.8. Data revealed that minimum phosphorus content in grain and straw was found under treatment Cr50. Phosphorus content found highest in grain compared to straw. The reduction in the nutrient content may be due to the inhibition of enzymes involved in the synthetic process (Balashouri and Devi, 1994).

As regards the organic sources, the treatment Cr0+PM recorded highest phosphorus content in grain and straw though proved significantly higher over the treatment. However, The interaction effect between chromium levels and organic sources in respect to nitrogen content in grain and straw was recorded non-significant. The

addition of organic materials increased the intractable fraction of phosphorus and Microbial activity and thus biochemical transformation reported by Nziguheba (1998).

#### **4.5.3 Potassium content in grain and straw (%)**

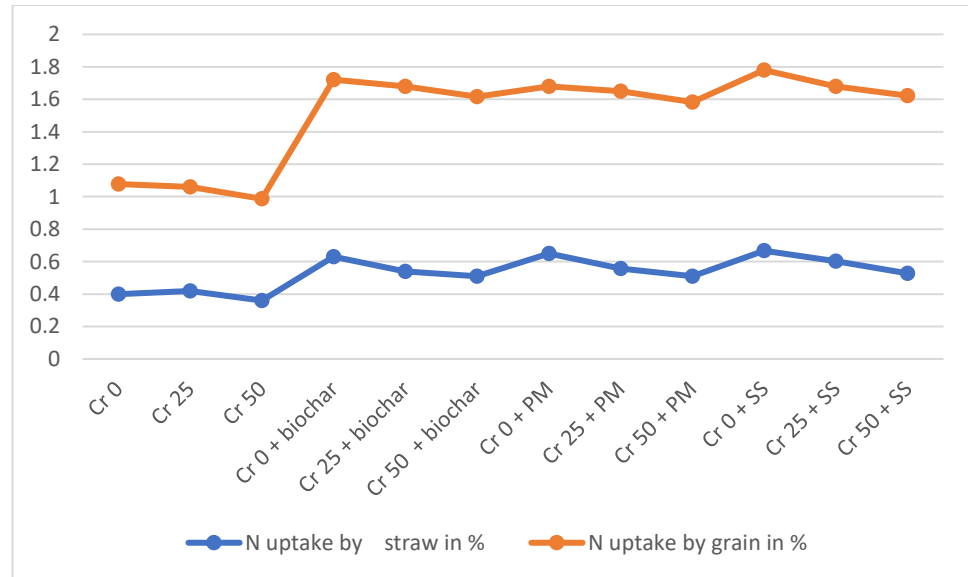
Potassium content in grain and straw as affected by chromium concentrations and organic sources had been compiled in Table 4.8 and depicted in Fig 4.8. Data revealed that minimum potassium content in grain and straw was found under treatment Cr50 which exhibited significantly lowest potassium content over rest of treatments.

As regards the organic sources, the treatment Cr0+PM recorded the highest potassium, content in grain and straw though proved significantly higher over the treatment Cr0 alone. Potassium content found highest in straw compared to grain. The interaction effect between chromium levels and organic sources in respect to potassium content in grain and straw was recorded non-significant.

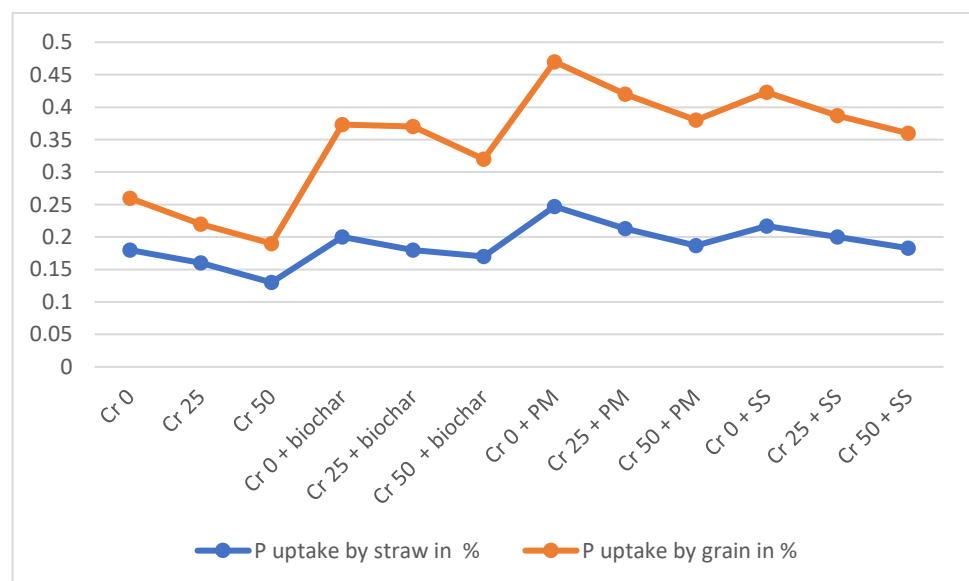
**Table no 4.8 Effect of biochar, poultry manure & sewage sludge on N,P,K uptake by grain and straw of chromium contaminated soil**

treatment	N uptake by straw in %	N uptake by grain in %	P uptake by straw in %	P uptake by grain in %	K uptake by straw in %	K uptake by grain in %
Cr 0	0.4	1.077	0.18	0.26	1.3	0.37
Cr 25	0.43	1.06	0.16	0.22	1.223	0.33
Cr 50	0.36	0.987	0.13	0.19	1.16	0.29
Cr 0 + biochar	0.63	1.72	0.2	0.373	1.63	0.39
Cr 25 + biochar	0.54	1.68	0.18	0.37	1.58	0.347
Cr 50 + biochar	0.51	1.617	0.17	0.32	1.487	0.313
Cr 0 + PM	0.65	1.68	0.247	0.47	1.75	0.48
Cr 25 + PM	0.557	1.65	0.213	0.42	1.65	0.417
Cr 50 + PM	0.51	1.583	0.187	0.38	1.567	0.37
Cr 0 + SS	0.667	1.78	0.217	0.423	1.693	0.437
Cr 25 + SS	0.603	1.68	0.2	0.387	1.63	0.382
Cr 50 + SS	0.527	1.623	0.183	0.36	1.537	0.357
CD	0.034	0.036	0.022	0.021	0.039	0.017
SE(m)	0.012	0.012	0.007	0.007	0.013	0.006
SE(d)	0.016	0.017	0.011	0.01	0.019	0.008
CV	3.789	1.399	6.863	3.618	1.533	2.76

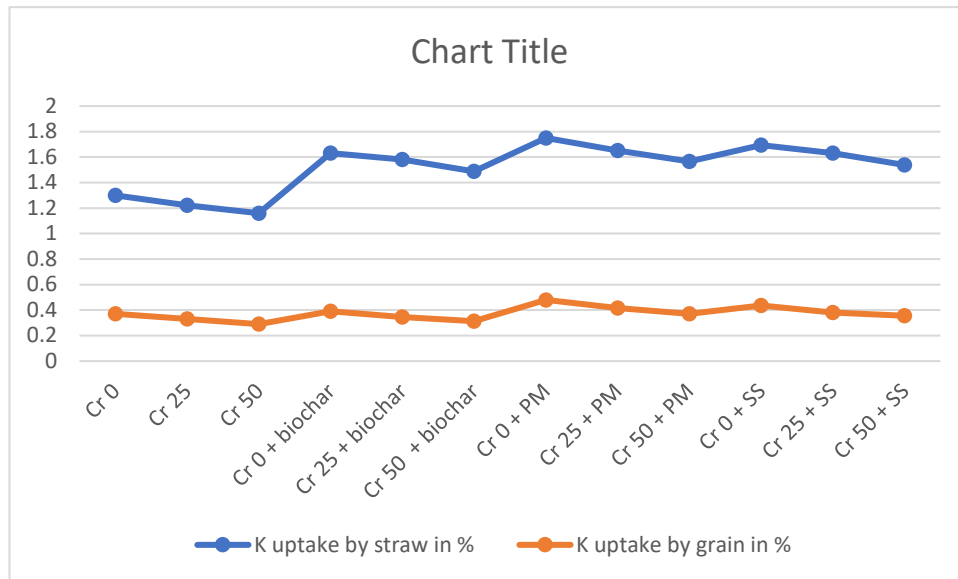
**Fig 4.7 Effect of biochar, poultry manure & sewage sludge on N uptake by grain and straw of chromium contaminated soil**



**Fig no 4.8 Effect of biochar, poultry manure & sewage sludge on P uptake by grain and straw of chromium contaminated soil**



**Fig 4.9 Effect of biochar, poultry manure & sewage sludge on K uptake by grain and straw of chromium contaminated soil**



#### 4.6 Effect of FYM and Vermicompost on chemical properties of post-harvest soil:

##### 4.6.1 Soil pH

The results indicated that the initial pH of soil was 7.9. The data pertaining to pH of the post harvest soil (Table 4.9 and Fig 4.11) showed that pH varied from 8.15 to 8.7. Maximum pH(8.7) was observed with treatment Cr0+biochar. minimum was observed with the treatment Cr25(8.15). Application of organic amendments in chromium contaminated pots showed a slight change in pH of the soil. Soil pH is the most important factor responsible for affecting metal availability. High pH under biochar & PM amended soil increased adsorption sites by increasing negative charges on the soil surface and reducing competing for metal cations similar results reported by Alamgir et al. (2011). Availability of all metals was significantly higher in non - amended soil due to lower soil pH. With the increase in soil pH, the bioavailability of Cd decreased given by Kim et al. (2009). In the present study, initial soil samples did not show a significant difference in pH (Table 4.12), but at the time of harvest, pH was significantly higher in biochar & PM amended the soil as compared to the control soil. Soil pH did not show any significant change with application of sewage sludge but with increasing levels of biochar pH of soil showed a significant increase as reported by Jatav et al. with application of poultry manure there was also increase in pH because of alkalinity of organic manure, releasing

of ammonia during nitrogen mineralization as reported by Usman et al. Interaction of organic manure with heavy metals causes the changes in soil pH which determine immobilization or mobilization of metals. The pH levels were expected to rise with the addition of the organic manure due to release of ammonia from the decomposing organic manures. The environment around the completing site will affect metal complexation by soil organic matter, particularly pH and the metal species taking part in the process (Ross, 1994).

#### **4.6.2 EC**

Data pertaining to EC of soil has been presented in Table 4.9 and depicted in Fig 4.12. It is evident from that the EC of the soil ranged between 0.3 to 0.473 dSm<sup>-1</sup>. Highest EC was found in biochar amended soil. The EC of the soil increased significantly with the application of organic amendments. The interaction effect of chromium and organic amendments was non significant. Metal content in the soil is directly associated with plant uptake, which is indirectly associated with physicochemical characteristics of soil and plant species similar results reported by Tariq and Rashid (2013). the EC of the soil increased when BC levels in soil increased up to a maximum value of 4.81 dS m<sup>-1</sup>, which was approximately 60% higher compared to the control as reported by Abbas et al. EC of soil does not show any significant change with application of sewage sludge but with increasing levels of biochar pH of soil showed a significant increase as reported by Jatav et al.

#### **4.6.3 Organic Carbon**

Highest Organic Carbon was recorded with the treatment Cr0+biochar (0.34%) followed by Cr0+PM (0.329%) ( Table 4.9). At the time of harvest, organic C increased by 25% in biochar and 22% in PM amended the soil when compared to the control Cr0. With the increase in chromium concentration, the organic carbon decreased. The minimum organic carbon found at treatment Cr50. Upon addition of organic amendments used as fertilizers will stimulate the soil biological activity, consequently increases the organic C content in the soil. So we have increased organic carbon upon addition of amendments compared to control. The application of organic amendments resulted in Cr (VI) reduction either from an enhancement of the microbial population and microbial activity. In addition, it enhanced the abiotic process of Cr (VI) reduction by supplying organic carbon supported by Losi et al. (1994).

#### 4.6.4 Available N

Highest available N were recorded with the treatment Cr25+SS (211.33kg ha<sup>-1</sup>) followed by Cr25+PM(208.67 kg ha<sup>-1</sup>), Cr25+biochar (200kg ha<sup>-1</sup>) respectively. At the time of harvest, available N increased due to application of organic amendment like biochar, poultry manure & sewage sludge. Interaction effect of organic amendments and chromium was found non-significant

The available nitrogen in the soil was generally found lower in range. Upon increasing chromium concentration at first N content increase but upon the higher concentration of chromium, the nitrogen content further decreased. So in order to make nitrogen available in the soil we are adding organic amendments. These organic amendments will supply all macronutrients. But upon an increase in chromium concentration even in the amended soil, we find a decrease in available Nitrogen. The interaction between chromium and organic amendments found non-significant.

#### 4.6.5 Available P

A critical perusal data presented under table (Table 4.10 and Fig 4.13) showed that available P in soil was in medium range. A significant increase was recorded with the application of organic amendment like biochar, poultry manure & sewage sludge. The available P ranged from 20.9 to 19.17kg ha<sup>-1</sup>. Lower in control higher in organic amended pots.

The connections with the soil mechanism to increase phosphorus uptake by the plants are most important given by Whalen (2001). The exchangeable cations (Na<sup>+</sup>, K<sup>+</sup>, and Ca<sup>2+</sup>) were highest for vermicompost followed by FYM amended soil.

#### 4.6.6 Available K

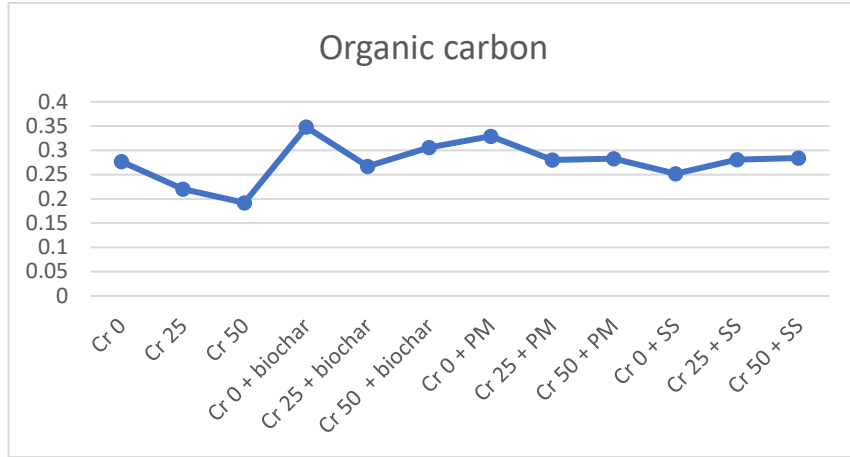
Highest available K was recorded with the treatment Cr0+ PM (211.33 kg ha<sup>-1</sup>) because poultry manure contains more amount of available K as compared to biochar and sewage & sludge. (Tables 4.10). Interaction effect of organic amendments and chromium was found non-significant.

Generally, initial soils are medium in available K content in the soil. Upon addition of organic amendments, there was an increase in soil available K. But with an increase in chromium concentration, the available K decreased in both amended and non-amended soil.

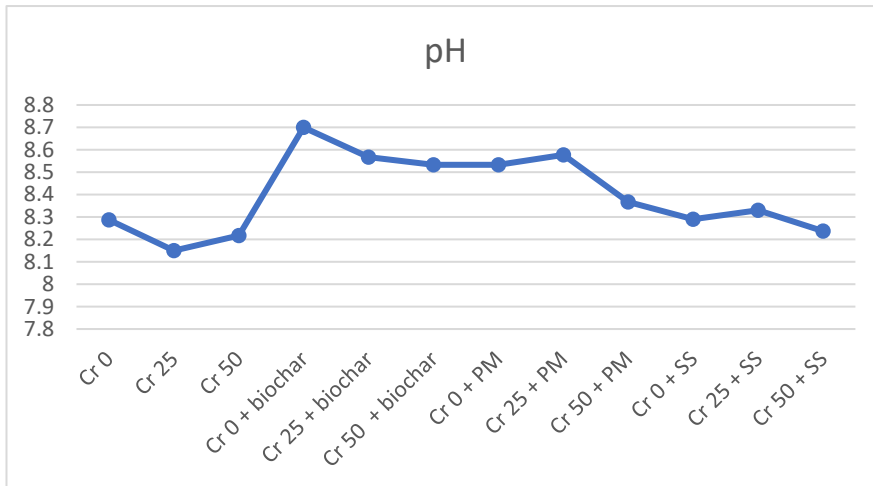
**Table 4.9 Effect of Biochar, Poultry Manure & Sewage sludge on pH, EC & organic carbon of soil**

treatment	Organic carbon	pH	EC
Cr 0	0.227	8.287	0.3
Cr 25	0.22	8.15	0.312
Cr 50	0.192	8.217	0.351
Cr 0 + biochar	0.348	8.7	0.427
Cr 25 + biochar	0.267	8.567	0.437
Cr 50 + biochar	0.306	8.533	0.473
Cr 0 + PM	0.329	8.533	0.377
Cr 25 + PM	0.28	8.577	0.403
Cr 50 + PM	0.283	8.367	0.42
Cr 0 + SS	0.281	8.29	0.355
Cr 25 + SS	0.251	8.33	0.38
Cr 50 + SS	0.244	8.237	0.385
CD	0.048	0.329	0.054
SE(m)	0.016	0.112	0.018
SE(d)	0.023	0.159	0.026
CV	10.205	2.313	8.212

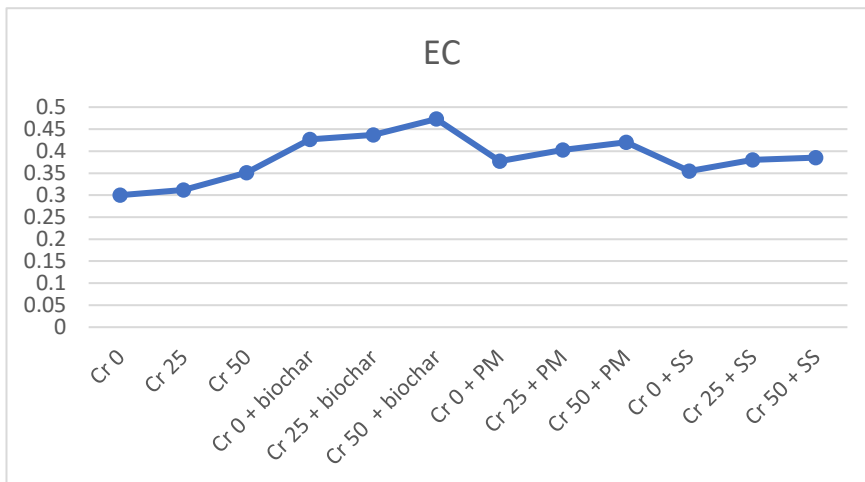
**Fig 4.10 Effect of Biochar, Poultry Manure & Sewage sludge on organic carbon of soil**



**Fig 4.11 Effect of Biochar, Poultry Manure & Sewage sludge on pH**



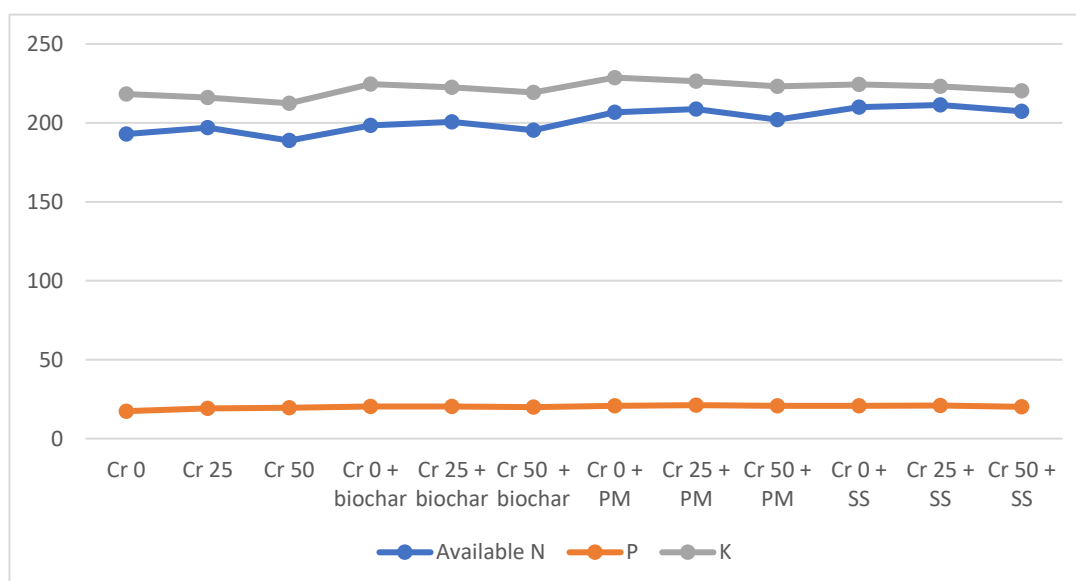
**Fig 4.12 Effect of Biochar, Poultry Manure & Sewage sludge on**



**Table 4.10 Effect of Biochar, Poultry Manure & Sewage sludge on NPK(Kg ha<sup>-1</sup>) content in post-harvest chromium contaminated soil**

treatment	Available N	P	K
Cr 0	193	17.4	218.2
Cr 25	197	19.17	216.1
Cr 50	188.8	19.49	212.4
Cr 0 + biochar	198.333	20.25	224.5
Cr 25 + biochar	200.667	20.37	222.6
Cr 50 + biochar	195.333	19.95	219.2
Cr 0 + PM	206.667	20.717	228.7
Cr 25 + PM	208.667	21.2	226.3
Cr 50 + PM	202	20.7	223.2
Cr 0 + SS	210	20.85	224.4
Cr 25 + SS	211.333	20.9	223.2
Cr 50 + SS	207.333	20.1	220.3
CD	1.854	0.485	0.333
SE(m)	0.632	0.165	0.113
SE(d)	0.893	0.234	0.16
CV	0.543	1.425	0.089

**Fig 4.13 Effect of Biochar, Poultry Manure & Sewage sludge on NPK(Kg ha<sup>-1</sup>) content in post harvest chromium contaminated soil**



## 4.7 Effect of biochar, poultry manure & sewage sludge on Cr content

### 4.7.1 Cr content in grain and straw of rice

Data regarding Cr content in rice grain and straw was given in Table 4. and 4. and Fig 4. and 4..Cr content in rice grain and straw increased significantly with increase in Cr concentrations (0,25,50 ppm). The highest Cr content of grain and straw of rice were observed in the treatment of Cr50 which was higher than control. The main reason is due to the higher addition of Cr in the soil, which in turn increased DTPA-extractable Cr in the soil. A similar effect was also noted by Bolan et al. (2003).

The application of organic amendments like biochar, poultry manure & sewage sludge significantly reduced the adverse effect of toxicity of Cr content in different parts of rice. Thus the effect of biochar, poultry manure & sewage sludge significantly decreased Cr content in grain and straw of rice over control. Biochar is found to be more effective in decreasing chromium concentration in grain & straw as compared to poultry manure & sewage sludge. Decrease in Cr toxicity in by biochar, poultry manure & sewage sludge is found to 33%,29%,26% respectively while for straw it is 31%,30%,26% respectively. This might be due to application of plant root exudates and organic matter which contained various organic acids like fulvic acid, humic acid, etc. These organic acids will reduce and immobilize Cr and might form organic compounds originated from a turnover of bacterial activities to make the reduction of Cr reported by Deng & Stone (1996).

With the increase in chromium concentrations, the Cr content in rice grain and straw was significantly increased in soils with and without amendment. The application of organic amendments in chromium contaminated soils, the Cr content decreased in grain in the corresponding level of Cr. These results might be ascribed to organic matter which expected to enhance the reduction of chromate in soil. Similarly, the use of organic materials could reduce the desorption of metals in soil due to formation of various insoluble compounds (Ram & Verloo 1985, Mengel & Kirkby 1987, Narwal et al. 1992).

**Table 4.11 Cr content in rice grain****Two Way Mean Table**

	<b>B1 control</b>	<b>B2 Biochar</b>	<b>B3 PM</b>	<b>B4 SS</b>	<b>Mean A</b>
A1 Cr0	0.847	1.360	1.237	1.177	1.155
A2 Cr25	1.787	1.503	1.697	1.737	1.681
A3 Cr50	4.600	1.970	2.133	2.320	2.756
<b>Mean B</b>	2.411	1.611	1.689	1.744	

**TABLE OF SEM,SED AND C.D.**

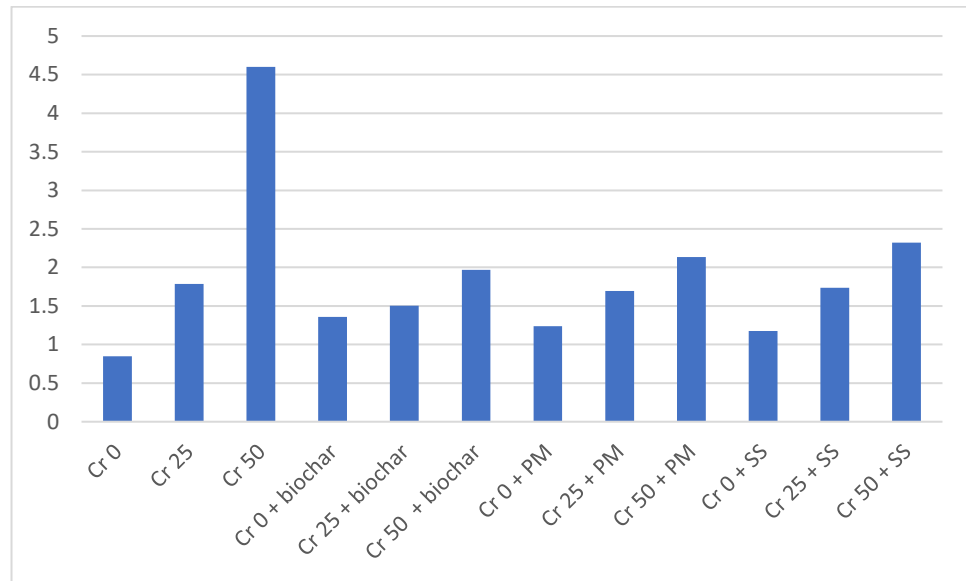
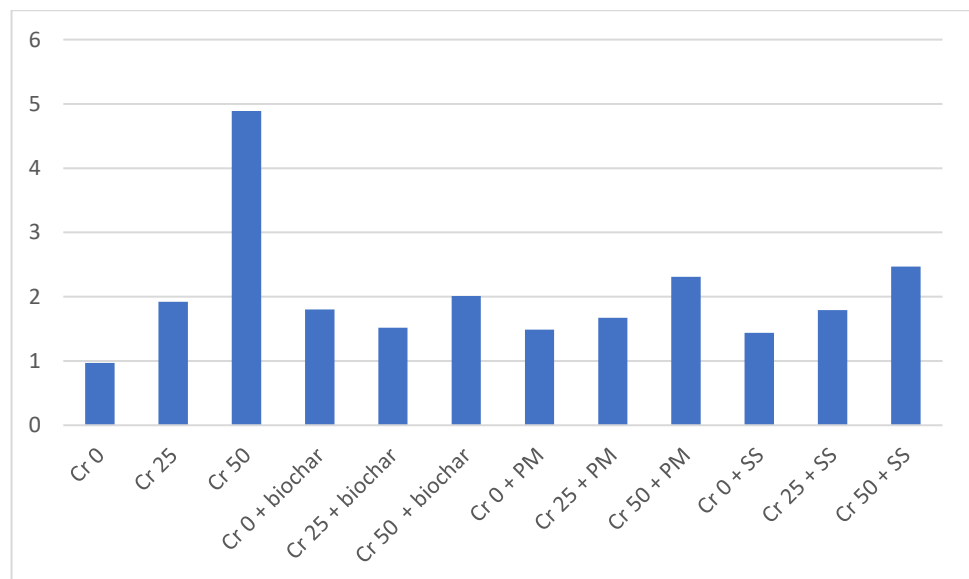
<b>Factors</b>	<b>C.D.</b>	<b>SE(d)</b>	<b>SE(m)</b>	
Factor(A)	0.026	0.012	0.009	significant
Factor(B)	0.030	0.014	0.010	significant
Factor(A X B)	0.052	0.025	0.018	significant

**Table 4.12 Cr content in straw of rice****Two Way Mean Table**

	<b>B1 control</b>	<b>B2 Biochar</b>	<b>B3 Poultry manure</b>	<b>B4 Sewage sludge</b>	<b>Mean cr</b>
A1 Cr0	0.970	1.800	1.490	1.437	1.424
A2 Cr25	1.920	1.517	1.670	1.790	1.724
A3 Cr50	4.890	2.010	2.310	2.470	2.920
<b>Mean B</b>	2.593	1.776	1.823	1.899	

**TABLE OF SEM,SED AND C.D.**

<b>Factors</b>	<b>C.D.</b>	<b>SE(d)</b>	<b>SE(m)</b>	
Factor(A)	0.023	0.011	0.008	significant
Factor(B)	0.026	0.013	0.009	significant
Factor(A X B)	0.045	0.022	0.015	significant

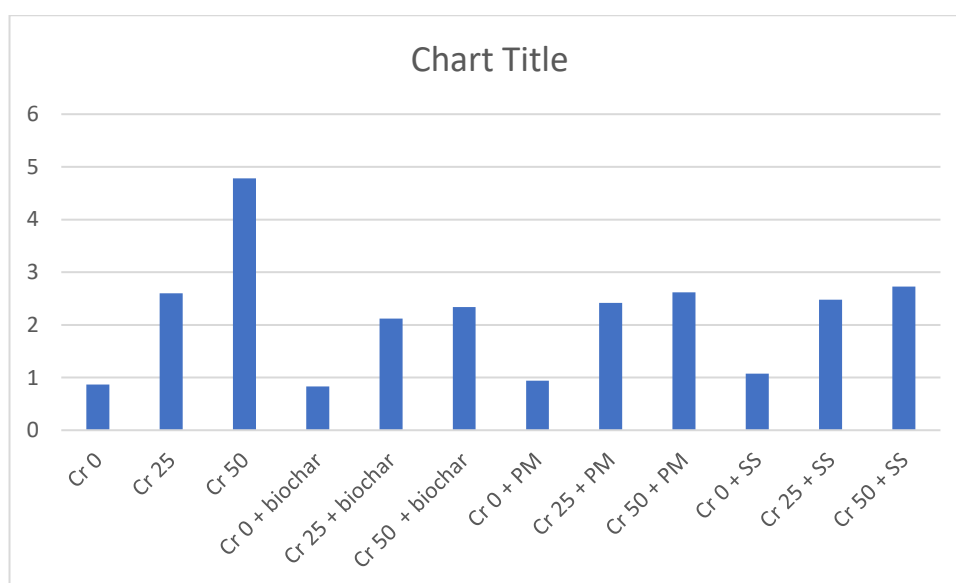
**Fig 4.14 Cr content in rice grain****Fig 4.15 Cr content in straw of rice**

**Table 4.13 Cr content in roots of rice**

	<b>B1 control</b>	<b>B2 Biochar</b>	<b>B3 PM</b>	<b>B4 SS</b>	<b>Mean A</b>
A1 Cr0	0.867	0.830	0.943	1.073	0.928
A2 Cr25	2.600	2.120	2.417	2.480	2.404
A3 Cr50	4.780	2.340	2.620	2.727	3.117
<b>Mean B</b>	2.749	1.763	1.993	2.093	

**TABLE OF SEM,SED AND C.D.**

<b>Factors</b>	<b>C.D.</b>	<b>SE(d)</b>	<b>SE(m)</b>	
Factor(A)	0.024	0.012	0.008	significant
Factor(B)	0.028	0.013	0.009	significant
Factor(A X B)	0.048	0.023	0.016	significant

**Fig 4.16 Cr content in roots of rice**

#### 4.7.2 Effect of biochar, poultry manure & sewage sludge on Cr content in post-harvest chromium contaminated soil

Result of data given in (Table 4. and Fig 4.). Indicated that DTPA extractable Cr increased with increase in Cr concentrations. The highest Cr content ( $5.6\mu\text{g g}^{-1}$ ) was observed in the treatment Cr50. The Cr content in postharvest soil was lowered in

respective levels of chromium i.e., the mean value of control pots is (3.23  $\mu\text{g g}^{-1}$ ). This may indicate that the applied Cr was heavily up taken by rice where similar results reported by Chandra et al. (1997). The remaining Cr was distributed among different fractions of Cr (Bolan et al. 2003).

The DTPA-extractable Cr decreased significantly over control according to their mean values. This is majorly due to direct effect of biochar, poultry manure & sewage sludge application to rice. The results reported that DTPA extractable -Cr content will change in soil after rice harvest. There is a significant decrease in DTPA extractable -Cr which is due to desorption of heavy metals in soil due to formation of various insoluble compounds. DTPA-extractable Cr was decreased with increasing rate of organic matter application where similar results reported by Ram & Verloo (1985), Mengel & Kirkby (1987) and Narwal et al. (1992).

The interaction effect between Cr and amendment was found significant which revealed that highest level of Cr i.e., with the treatment (Cr50) applied alone showed the highest content of DTPA-extractable -Cr (5.6mg  $\text{kg}^{-1}$ ), while lowest Cr status of 0.28 mg  $\text{kg}^{-1}$  was noted with absolute control Cr0 . Results have also shown that with application of Cr alone caused higher value of DTPA-extractable -Cr over other organic amendment in respective level of Cr. The addition of amendments combined with Cr levels decreased DTPA-Cr as compared to Cr application alone.

Finally, the results indicated that with increase in the concentration of Cr, DTPA extractable -Cr content of soil increased over corresponding control. However, application of organic amendment ,the DTPA-Cr status could be decreased more than that was noticed due to Cr application alone. Root exudates containing organic acids form complexes with Cr, making them available for plant uptake.

#### **4.7.3 Accumulation of Cr in rice tissues**

The accumulations of Cr in shoots and root tissues of rice with different Cr concentration of (0,25,50 ppm) in soil with and without organic manure was observed. The concentration of Cr was highest in root (2.74  $\mu\text{g g}^{-1}$ ) compared to straw (2.59  $\mu\text{g g}^{-1}$  shown in Table (4.12 and 4.13 and Fig 4.15 and 4.16). The results showed that, Cr accumulation was higher in root tissue and increased with the increase in Cr concentration. Cr accumulation in rice roots was higher than in leaves because of the high CEC of roots that can absorb Cr when in direct contact with contaminated water of Cr and then transmit Cr to the leaves reported by Kadlec (1996).

Cr mobility was lower from root to shoot this is because, there are barriers or lack of a transport mechanism suitable for Cr transport from roots to shoots reported by Kleiman (1998). No significant toxicity symptoms were detected in rice grown in soil contaminated with Cr.

Application of organic amendment enhanced the Cr accumulation in roots (3.42  $\mu\text{g g}^{-1}$ ) compared to shoot (2.29  $\mu\text{g g}^{-1}$ ). Thus these organic amendments help in retention of metal in the roots hence reduces photo availability of Cr.

#### 4.7.4 Enrichment factor and translocation factor:

Mechanism of absorption of heavy metal chromium by *Oryza sativa* has been analyzed by Enrichment factor and translocation factor analysis. The Enrichment factor from soil to other parts of *Oryza sativa* shown in the table 4.19 revealed that enrichment factor was greater than 1 which indicates that rice was a hyper accumulator with high potential to absorb metals from soil (Lorestani et al., 2011). The values of translocation factor given in (table 4.18) were greater than 1 which indicates that *O. Sativa* can hyper-accumulate from roots to shoots.

**Table 4.15 Effect of biochar, sewage sludge & poultry manure Cr content in post-harvest soil**

**Two Way Mean Table**

	<b>B1 control</b>	<b>B2 Biochar</b>	<b>B3 PM</b>	<b>B4 SS</b>	<b>Mean A</b>
A1 Cr0	0.280	0.260	0.270	0.333	0.286
A2 Cr25	4.020	2.070	2.750	3.067	2.977
A3Cr50	5.600	4.087	4.433	4.600	4.680
<b>Mean B</b>	3.300	2.139	2.484	2.667	

**TABLE OF SEM, SED AND C.D.**

<b>Factors</b>	<b>C.D.</b>	<b>SE(d)</b>	<b>SE(m)</b>	
Factor(A)	0.088	0.042	0.030	significant
Factor(B)	0.101	0.049	0.034	significant
Factor(A X B)	0.175	0.084	0.060	significant

**Table 4.15 Effect of biochar, sewage sludge & poultry manure on translocation factor****Two Way Mean Table**

	<b>B1 control</b>	<b>B2 Biochar</b>	<b>B3 PM</b>	<b>B4 SS</b>	<b>Mean A</b>
A1 Cr0	2.097	3.808	2.891	2.438	2.809
A2 Cr25	1.426	1.425	1.393	1.422	1.416
A3 Cr50	1.985	1.702	1.696	1.757	1.785
<b>Mean B</b>	1.836	2.312	1.993	1.872	

**TABLE OF SEM,SED AND C.D**

<b>Factors</b>	<b>C.D.</b>	<b>SE(d)</b>	<b>SE(m)</b>	
Factor(A)	0.041	0.020	0.014	significant
Factor(B)	0.048	0.023	0.016	significant
Factor(A X B)	0.083	0.040	0.028	significant

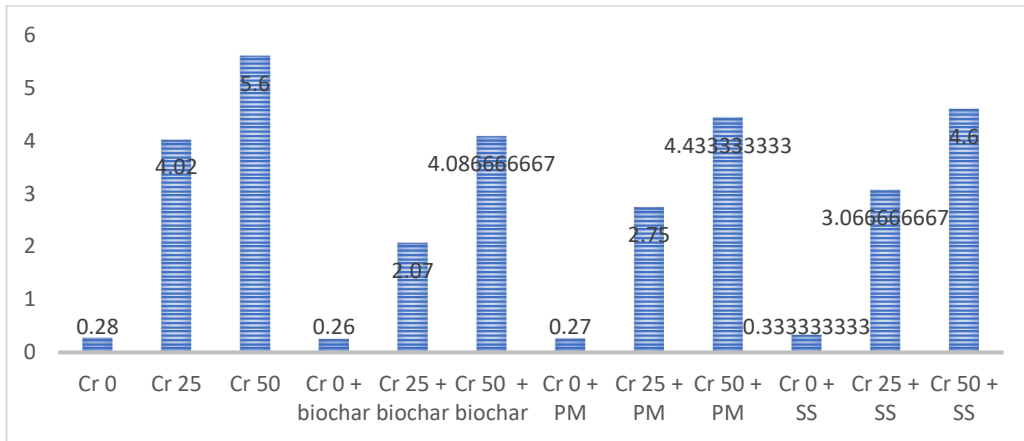
**Table 4.16 Effect of biochar, sewage sludge & poultry manure on enrichment factor****Two Way Mean Table**

	<b>B1 control</b>	<b>B2 Biochar</b>	<b>B3 PM</b>	<b>B4 SS</b>	<b>Mean A</b>
A1 Cr0	9.660	17.079	13.803	11.374	12.979
A2 Cr25	1.575	2.497	2.110	2.005	2.047
A3Cr50	2.585	1.532	1.609	1.640	1.842
<b>Mean B</b>	4.607	7.036	5.841	5.006	

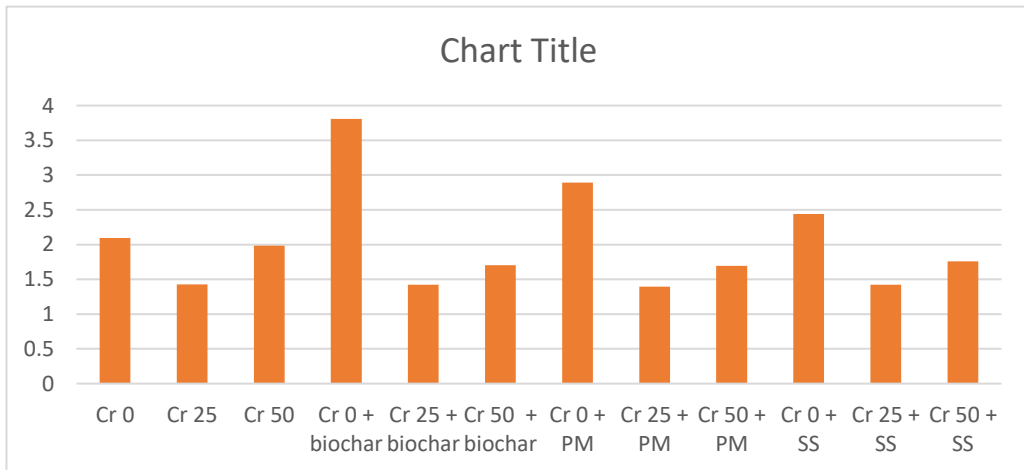
**TABLE OF SEM,SED AND C.D.**

<b>Factors</b>	<b>C.D.</b>	<b>SE(d)</b>	<b>SE(m)</b>	
Factor(A)	0.342	0.165	0.116	significant
Factor(B)	0.395	0.190	0.134	significant
Factor(A X B)	0.684	0.329	0.233	significant

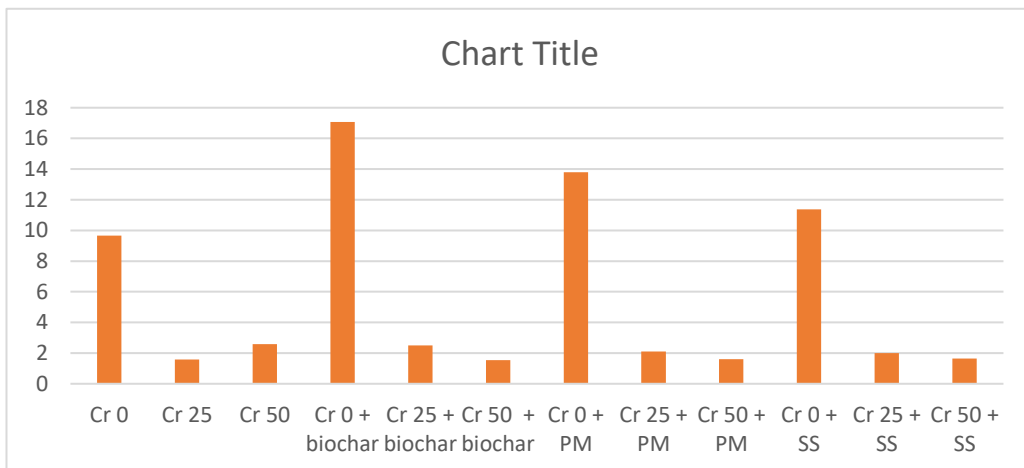
**Fig 4.17 Effect of biochar, sewage sludge & poultry manure Cr content in post-harvest soil**



**Fig 4.18 Effect of biochar, sewage sludge & poultry manure on translocation factor**



**Fig 4.19 Effect of biochar, sewage sludge & poultry manure on enrichment factor**



## CHAPTER V

**SUMMARY AND CONCLUSION**

Contamination of soil with chromium is an environmental concern. These accumulated metals have adverse effects on soil ecology, agricultural production, animal and human health as well as groundwater quality. The reduction of the mobility of heavy metals in soil involves Phyto stabilization. The addition of organic amendments, can decrease solubility of metals in soil and minimize leaching to groundwater.

The present research work entitled “Effect of biochar, poultry manure & sewage sludge on phytoremediation in chromium contaminated soil by using rice (*Oryza sativa* L.)” was carried out in net house of Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, during Kharif season, 2015-2016.

HUR-105 is the rice variety chosen for the experiment. A pot trail of 36 pots was conducted containing 12 treatments (Cr0, Cr25, Cr50, Cr0+biochar, Cr25+ biochar, Cr50+ biochar, Cr0+poultry manure, Cr25+ poultry manure, Cr50+ poultry manure, Cr0+sewage sludge, Cr25+ sewage sludge, Cr50+ sewage sludge) with 3 replications to study the effect of biochar, poultry manure, sewage sludge in association with different concentrations of chromium. Data regarding different growth parameters of rice like plant height, chlorophyll content, straw and grain yield etc were recorded. Harvested rice grain, straw & root samples were analysed for different macronutrient & Cr content using the standard procedure. Soil samples were collected after harvesting of crop, processed and stored in polythene bags for subsequent laboratory analysis. Data recorded in various aspects statistically analysed employing FCRD. The facts observed during the period of experimentation are summarized and concluded in this chapter.

- Growth parameters like plant height, no. of tillers/hill, productive tillers /hill decreased upon increasing doses of chromium i.e., (0,25,50ppm). The addition of organic amendments like biochar, poultry manure & sewage sludge has significantly increased the growth parameters of rice. The interaction found non –significant. Among treatments with organic amendments, biochar found to be more effective followed by poultry manure & sewage sludge. The results showed that growth parameters decreased in both organic amended and non-amended soils with increase in chromium concentrations.

- Results indicated that Cr has negative impact on the yield attributes like total tillers/hill, productive tillers /hill, grains/panicle, panicle length, panicle weight, of rice. Upon increasing concentration of chromium, the yield attributes decreased respectively. Among organic treatments like biochar, poultry manure & sewage sludge the highest values found in treatment Cr0+biochar treated pots followed by Cr0+PM & Cr0 + SS respectively in chromium contaminated soils. The application of organic amendments reduced Cr toxicity to some extent but not completely.
- Biomass of rice i.e., grain yield and straw yield dry weight decreased with increasing doses of chromium. The addition of organic amendments has significantly increased the biomass of rice. The interaction found significant. Among treatments with organic amendments, the highest values shown in the treatment Cr0+biochar followed by Cr0+PM & Cr0+SS respectively in chromium contaminated soils. The results showed that biomass of rice plant decreased in both organic amended and non-amended soils upon an increase in chromium concentration. The addition of these organic amendments significantly increased the biomass of rice
- Harvest index varied from (47.16 %) in Cr0 to (45.8%) in Cr50. However, more harvest index was seen in Cr0 followed by Cr 25 & Cr50 respectively shows decreasing in harvesting index with increasing the concentration of chromium. Different organic amendments significantly influenced harvest index of rice. Maximum harvest index was recorded with Cr0+biochar (49.93%) followed by Cr0+PM (48.83%) & Cr0+SS (48.3). The addition of these organic amendments has significantly increased the harvest index. The treatments of organic amendment are found at par with each other.
- NPK content of rice straw and grain decreased with increase in the levels of chromium i.e., maximum decrease was observed in treatment Cr50. However, the treatment of Cr25 was found statistically at par with treatment Cr0. Increasing chromium levels resulted in significant increase in NPK content of the rice straw up to the treatment Cr 25 tested in the experiment and then reduced with an increase in chromium concentration. NPK content in grain and straw was found significantly higher under use of organic sources. The addition of these organic amendments has significantly increased NPK content. The highest N content found in sewage sludge treated pots followed by PM & biochar treated pots while higher amount of P, K in grain & straw found in PM followed by Sewage sludge & biochar treated pots. N, P, K uptake by straw and grain was observed minimum with treatment Cr50. By addition of organic amendment NPK uptake increases. It is found that the nitrogen phosphorus uptake more in grain whereas potassium uptake more in the case of straw
- The pH of initial soil recorded as 7.9. The data pertaining to pH of the postharvest soil showed that pH varied from 8.15 to 8.7. Maximum pH (8.7) was observed with treatment

Cr0 +biochar. Addition of biochar & poultry manure increases the soil post-harvest soil pH as compared to control while pH of post-harvest soil by addition of sewage sludge is at par with the control. With the application of chromium along with amendments the pH of soil changed.

- EC of the soil didn't increase significantly with the application of sewage sludge while a slight increase is observed in case of biochar & poultry manure. The interaction effect of chromium and organic amendments was non-significant. This is evident that the EC of the soil ranged between 0.3 to 0.473 dSm<sup>-1</sup>. The minimum EC was found in treatment Cr0 (0.3 dSm<sup>-1</sup>). It is found that with increase in chromium concentration the EC of soil increases.
- Organic Carbon was recorded highest with the treatment Cr0+Biochar (0.34%) followed by Cr0+PM (0.32%), Cr0+SS(0.28) and then Cr0 (0.22%) i.e., not- amended the soil respectively. So we can conclude that with addition of organic amendment organic carbon of soil increases. With the increase in chromium concentration, the organic carbon decreased. The minimum organic carbon found at treatment Cr50.
- Available N was recorded highest with the treatment Cr25+SS (211kg ha<sup>-1</sup>) followed by Cr25+PM (206 kg ha<sup>-1</sup>), Cr25 + biochar (200 kg ha<sup>-1</sup>). Interaction effect of organic amendments and chromium was found non-significant for available nitrogen.
- Available phosphorus content increased in soil with an increase in chromium concentrations at low chromium concentration up to 25ppm. The treatment Cr25 +PM had the highest available phosphorus compared to rest treatments in control.
- Available K was recorded highest with the treatment Cr25+ PM (224 kg ha<sup>-1</sup>). Application of organic amendment increases the amount of available Interaction affect of organic amendment & various levels of Chromium is found not significant.
- Cr content in rice grain and straw increased significantly with increase in Cr concentrations. The highest Cr content of grain and straw of rice was observed with the treatment Cr50 which was higher than control. The use of organic amendment significantly decreased the adverse effect of toxicity of Cr content in different parts of rice; the direct application of Decrease in Cr toxicity in by biochar, poultry manure & sewage sludge is found to 33%,29%,26% respectively while for straw it is 31%,30%,26% respectively over control.
- The concentration of Cr (2.6 µg/g) found in root and (2.5µg/g) in straw was found in control pots. Upon application of organic amendments like FYM the Cr accumulation in roots was further enhanced compared to shoot. Thus, these organic amendments help in retention of metal in the roots, hence reduces Phyto availability of Cr.
- Enrichment factor was greater than 1 which indicates that *Oryza Sativa* was an hyper accumulator with high potential to absorb metals from soil. The mean values of

translocation factor was greater than 1 which indicates that rice can hyperaccumulate from roots to shoots. Upon application of organic amendments there was accumulation of Cr observed more in the roots. Hence uptake of Cr into grains is reduced

- The present study confirms rice is capable of accumulating chromium in root and shoots, roots accumulate more chromium than shoots. Exposure of rice to chromium reduced overall growth, yield, nutrient content and uptake. However, application of biochar, poultry manure & sewage sludge restored the damage caused by heavy metal chromium and showed increase nutrient content. Biochar, poultry manure & sewage sludge enhanced growth and yield parameters and accumulation of metal. It can be concluded that rice can be used as effective agent for phytoremediation in presence of organic amendments of soil polluted with chromium.

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