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Effect of biochar application on the chromium uptake of *Canna indica* L. from chromium spiked soil

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Abstract
To study the uptake of chromium from soil by the plant *Canna indica* L, a particular soil was chosen and screened for heavy metals. The soil was found to contain heavy metals other than chromium. This soil was spiked with chromium doses of 30 mg and 60 mg and the plant *Canna indica* L was chosen to phyto extract chromium from the spiked soil. The plant *Canna indica* L was selected as it is a sturdy, perennial, non edible plant with bright flowers and grows in adverse climatic conditions through out the year. The growth of the plant in different concentrations of chromium spiked soil was monitored. The uptake of chromium by the different plant parts were recorded on the 5th, 10th and 20th days. The experiments were repeated keeping all other conditions identical and adding 10% of coconut shell biochar to the soil. The growth of the plant and uptake of chromium by the plants were studied in the biochar amended soil. The plants phytostabilized chromium in the root region, not translocating it to the other parts and withstood the toxicity of chromium for 20 days.

Keywords: Chromium, *Canna indica* L, Biochar, Macropores, Phytoextraction, Phytostabilization

1. Introduction
The continuous influx of waste water effluents polluted with heavy metals into fresh water bodies results in severe contamination of water and soil. Of these heavy metals chromium (Cr) is one of the most hazardous pollutants that threaten the flora and fauna with long term effects. Tanning industries, dye industries, electroplating industries, aluminum and steel industries use and discharge Cr at different stages of their manufacture. Chromium exists in different oxidation states.

The most commonly occurring species are Cr(III) and Cr(VI). These two species of Cr prevail in the environment with different physicochemical properties and biochemical reactivities (Kotas and Stasicka, 2000) [1]. Cr(VI) is highly mobile, mutagenic, teratogenic and thermodynamically meta-stable in soil and exists as chromate and bi-chromate complexes in strongly oxidizing environments (Faybishenko *et al*., 2008) [2]. Due to the high solubility, mobility and toxicity of Cr(VI), its removal is a major challenge in water and soil remediation. The remediation of Cr(VI), in waste water usually involves the reduction of Cr(VI) to Cr(III), which is less toxic and less mobile.

Most of the methods used for heavy metal contaminated soil remediation are adapted from technologies originally developed in industrialized countries. Extraction, adsorption using activated carbon and microbial reduction are the ex-situ methods in use; whereas in-situ methods make use of stimulation of anaerobic and aerobic microbial activities. These methods need a large capital investment with high recurring expenses and human resources. Thus researchers are seeking ways to develop cheaper and faster alternatives to treat large areas of soils and wetlands. Under this scenario, phytoremediation has emerged as a very promising method for treatment of heavy metal pollution. The use of plants to remediate soil is an eco-friendly, cost-effective and hassle free method.

Phytoremediation consists of five major plant based technologies that include phytoextraction, phytofiltration, phytostabilization, phytovolatilization and phytodegradation. The following are the definitions of the plant based technologies (Paz-Ferreiro *et al*., 2014) [3].

- Phytoextraction: Plants accumulate contaminants in harvestable biomass.
- Phytofiltration: Sequestration of pollutants from contaminated water by plants.
- Phytostabilization: Limiting the mobility and bio availability of polluting sites by prevention of migration or immobilization.
- Phytovolatilization: Conversion of pollutants to volatile form followed by their release to the atmosphere.
- Phytodegradation: Degradation of organic xenobiotics by plant enzymes with in the plant tissues.

The plant, *Canna indica* L. has been used for the present study. It grows in damp edges of open sewage drains and water bodies. The roots of the plants are tuberous, the plants are perennial and highly tolerant to varied conditions. *Canna indica* L. produces a large biomass with robust rhizomes.

Advantages and limitations of phytoremediation techniques

Advantages

- Environmentally friendly and cost-effective.
- Metals absorbed by the plants may be recovered from harvested plant biomass.
- Prevents the leaching of contaminants into ground water.
- It is the least harmful method because it uses naturally occurring organisms and therefore totally eco-friendly.

Limitations

- Slow growth and low biomass hamper the process of phytoremediation.
- Leaching of contaminants into groundwater cannot be prevented completely by this method of remediation.
- The plant survival can be affected by the toxic metals depending on the Tolerance Limit (TL) of the plant.

To overcome the limiting factors which influence phytoremediation efficiency, the technique can be enhanced by the use of a soil amendment additive like biochar.

Biochar is the short form of bio-charcoal which is obtained through the pyrolysis of various low-cost biomass under a limited supply of oxygen. Biochar is found to have a rich porous structure, oxygen containing functional groups, high pH and cation exchange capacity. The porous structure which is a carbon matrix can hold on to air, water, metals and organic chemicals and provides a habitat for micro-organisms. The characteristics of biochar mainly depend on the type of feedstock and the pyrolysis temperature. Biochar has been found to have a positive impact on soil biology (Lehmann *et al*., 2011; Paz-Ferreiro *et al*., 2014) [4, 3], controlling diseases, improving the fixation of nitrogen, increasing crop biomass and reducing nutrient leaching loss. Biochar produced from a range of biomass can potentially be a relatively cost effective and beneficial tool for environmental remediation. Biochar (BC) from coconut shells (BC***cs***) is the additive used for soil amendment to enhance this phytoremediative study.

2. Highlights

The main objective of the study was to investigate the effect of biochar on the uptake of chromium by the plant *Canna Indica* L.

The broad objectives of this study were:

- To study the phytoremediation process for synthetic chromium containing soil, using BC***cs*** and Canna plant for fixed time intervals.
- To identify the functional groups present in BC***cs*** using FTIR and arrive at a possible mechanism for chromium absorption by the plant.
- To examine the surface and the size of pores of BC***cs*** by SEM analysis.

3. Materials and Methods

Materials

- All chemicals in the study were of analytical grade (Merck chemicals, Germany) which were used without any further purification. In all experiments, deionized water (Milli-Q Millipore 18.2MΩ/cm conductivity) was used for the preparation, dilution and analytical purposes. A stock Cr (VI) solution of 1000mg/L was prepared by dissolving 2.828g of anhydrous potassium dichromate ($K_2Cr_2O_7$) and appropriate dilutions were made using deionized water.
- *Canna indica* L plants were purchased from a nursery nearby. The plants were selected to be of the same average size.
- Chromium free nursery soil was also purchased from the local nursery.
- BC**cs** was made by crushing dried coconut shells and pyrolysing it in a muffle furnace with a reduced supply of oxygen for 3hrs at 500^{o}C.

Methods

3.1 Screening of heavy metals in the soil

A sample of nursery soil was cleaned, sieved and washed with water. After digestion with con HNO3 and con HCL for three hours, it was filtered through a Whatman filter paper 0.42 and made up to 50ml in the standard flask. The solution was screened for the following heavy metals by AAS; Cr, Zn, Fe, As, Cu, Mn, Co, Pb and B.

3.2 Potting Experiments

Estimation of chromium

The chromium free nursery soil was cleaned, sieved and dried. Appropriate doses of Cr were mixed with a known quantity of water and added to 1kg of soil and mixed thoroughly with a Hobart mixer. This was taken in a pot, labeled and the canna plants were measured (shoot and root size) and planted one in each. They were regularly watered at the same time of the day and were kept in sunlight. Three replicates were prepared.

The plants were harvested after 5, 10 and 20 days. The plant parts namely root, stem and leaf were segregated, washed with de-ionized water, measured, oven dried and weighed. The samples were subjected to acid digestion according to APHA method and Cr (VI) was estimated using diphenyl-carbazide method. A duplicate analysis was performed for each sample in the AAS. The standard deviation was calculated and the average values were presented.

BC***cs*** was weighed in the ratio of 1:10, mixed with 1kg of nursery soil and spiked with two different doses of Cr^{6+}. The plants were planted in this soil mixture in the pots and watered regularly. They were harvested on the 5th, 10th and 20th days as before. The measurements (shoot and root length) were made, the plant parts were segregated, dried and analyzed as before.

3.3 FT-IR of coconut shell biochar

FT-IR spectroscopy was analyzed on a Varian 670-IR (Agilent Technologies Inc., CA) using the pellet technique by mixing 1mg of dried biochar (BC*cs***)** with 300mg of pre-dried and pulverized spectroscopic-grade KBr (Merck).

3.4. SEM of Coconut shell biochar

The biochar derived from coconut shell pyrolysis was characterized by scanning electron microscope (Tescan Vega3 SBU-Czech Republic) with an acceleration voltage of 30kV at different magnification values to have a clear view on pore density and diameter.

4. Results and Discussion

The results of the various experiments are tabulated in the following manner.

4.1. Screening of heavy metals (Nursery Soil)

Table 1

Heavy Metals	Method	Unit	Results
Chromium as Cr	EPA 7190:1986	mg/l	BDL(DL:0.1)
Zinc as Zn	EPA7950:1986	mg/l	0.13
Iron as Fe	EPA 7380:1986	mg/l	0.89
Arsenic as As	EPA 7061A:1992(Rev - 1)	mg/l	0.03
Copper as Cu	EPA 7210:1986	mg/l	0.1
Manganese as Mn	EPA 7460:1986	mg/l	0.56
Cobalt as Co	EPA 7200:1986	mg/l	0.17
Lead as Pb	EPA 7420 : 1986	mg/l	BDL(DL:0.1)
Boron as B	4500 - B-B-ALPHA 22nd Ed.2012	mg/l	0.17

4.2. Potting Experiments

a. Soil amendment additive: nil

Plant: *Canna indica* L, No. of days: 5, 10 and 20

Table 2

ID	Nursery Soil	Dose of Cr^{6+} mg/kg	Plant Parts	5 D	10 D	20 D
Cr30	1	30	Leaf	0.042	2.67	0.283
			Stem	0.107	1.56	0.374
			Root	0.161	0.848	0.906
			Soil	0.162	0.037	0.048
Cr60	1	60	Leaf	0.257	1.780	0.380
			Stem	0.096	1.040	0.540
			Root	0.078	0.367	0.193
			Soil	0.111	0.065	0.430

The total Cr^{6+} in the root, stem and leaf of the various plants grown in different dosages of Cr^{6+} were recorded and tabulated. The concentration of Cr^{6+} left behind in the soil was also noted in each case.

b. Soil amendment additive: BCcs

Plant: *Canna indica* L

No. of days: 5, 10 and 20

Table 3

ID	Nursery Soil(Kg)	Dose of Cr^{6+}mg/kg	BC g	Plant Parts	5 D	10 D	20 D
$BC_{10} – Cr_{30}$	1	30	10	**Leaf**	0.031	0.045	0.058
				Stem	0.081	1.586	2.086
				Root	2.250	9.900	10.390
				Soil	0.950	0.420	0.344
$BC_{10} – Cr_{60}$	1	60	10	**Leaf**	0.152	0.210	0.263
				Stem	0.536	0.818	8.322
				Root	4.251	6.340	9.498
				Soil	0.972	0.943	0.357

The total Cr^{6+} in the root, stem and leaf of the various plants grown in different dosages of Cr^{6+}/BC were recorded and tabulated. The concentration of Cr^{6+} left behind in the soil was also noted in each case.

4.3. FT-IR of BC*cs*

The following results were obtained for the FT-IR analysis of coconut shell biochar (BC*cs*)

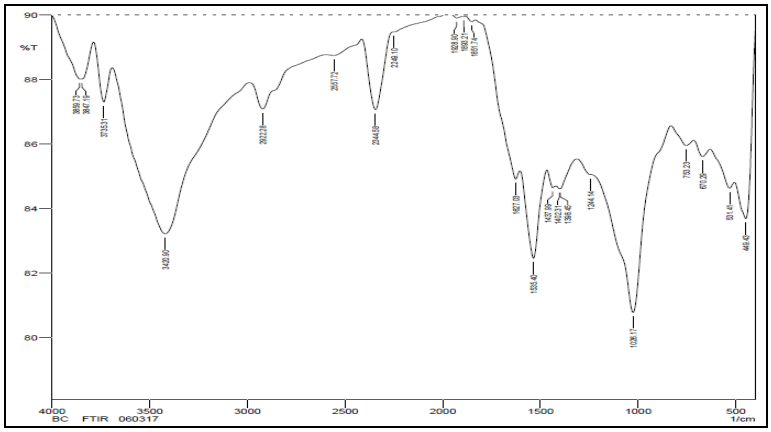

Fig 1: FT – IR of Coconut Shell Bio-Char

Table 4: FT-IR – BC*cs* – Result

Functional Group	Type of Vibration	Characteristic Absorptions (cm-1)	Intensity
O-H- free alcohol	Stretch	3735.31	Sharp
O-H- alcohol bonded	Stretch	3420.91	Strong, Sharp
C-H of alkane	Stretch	2922.28	Medium
O-H of acid	Stretch	2557.72	Weak
C=N	Stretch	2249.1	Weak
Aromatic compounds	Bending	1928.9, 1893.21, 1851.74	Weak
C=O	Stretch	1627.03	Weak
C=C	Stretch	1535.4, 1437.99, 1402.31, 1398.45	Medium, weak, multiple bands
C-O of Ether	Stretch	1244.14	Strong, Sharp
C-H of alkene	Bending	753.23	Weak
C-H	Bending	670.29	Weak

4.4. SEM Analysis of BC*cs*

The following image was obtained by SEM analysis of coconut shell biochar (BC*cs)*

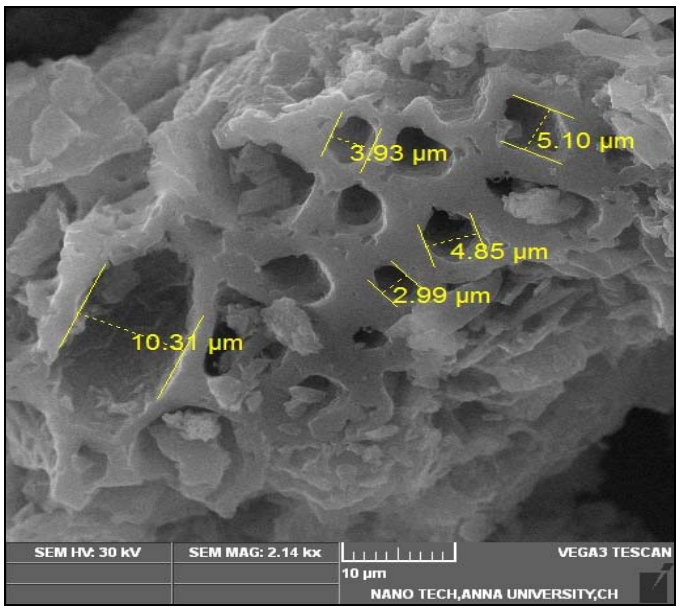

Fig 2: SEM Image of Coconut Shell Bio-Char

The SEM analysis of coconut shell bio-char revealed a rough surface and multiple sizes of pores with varying diameters as follows: 2.99 µm, 3.93 µm, 4.85 µm, 5.10 µm, 10.31 µm. These clear well developed pores are shown in the figure 2.

5. Discussion

5.1. Screening of heavy metals (Nursery Soil)

A complete screening of the nursery soil for the presence of heavy metals showed the following trend: Fe > Mn > B > Co > Zn > Cu > As > Cr > Pb. From the above result (Table 1) the concentration of chromium was found to be negligible in the soil chosen for the present study.

5.2. Potting Experiments

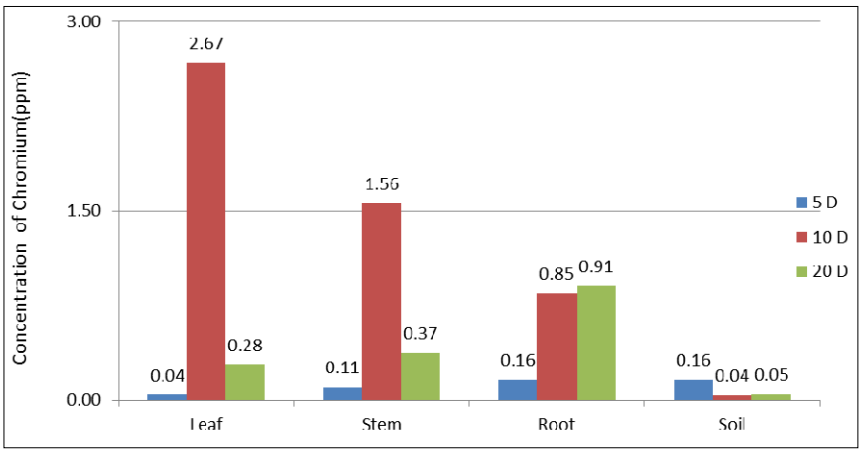

Fig 3: Cr-30

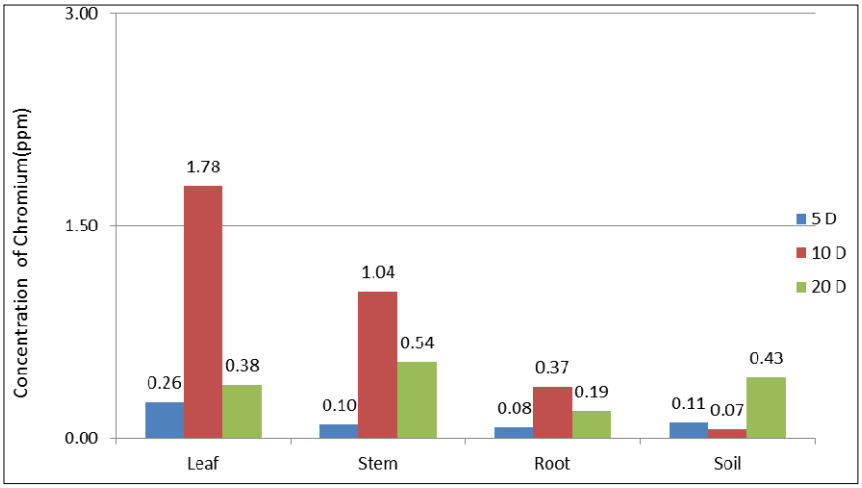

Fig 4: Cr-60

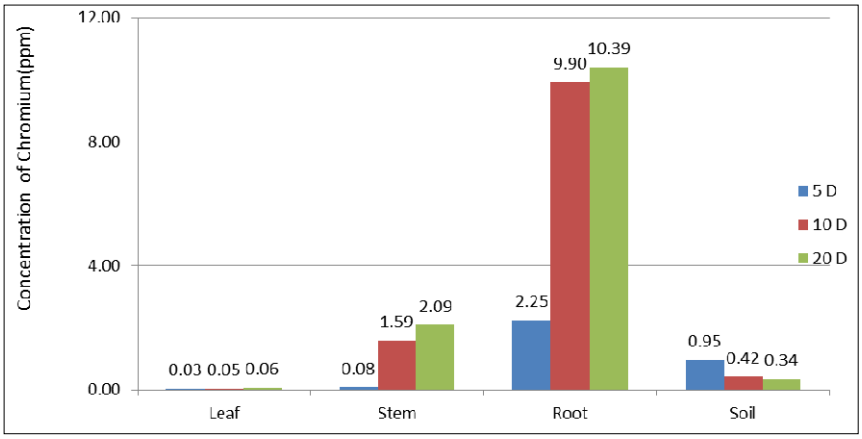

Fig 5: BC-10 Cr-30

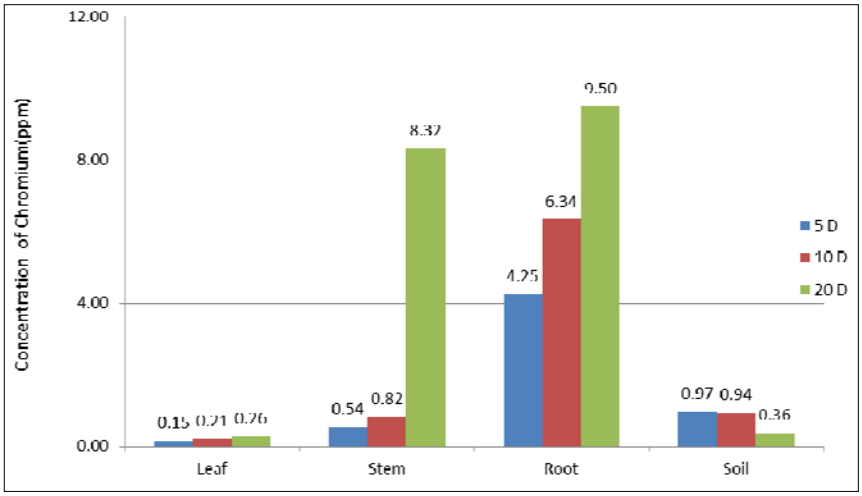

Fig 6: BC 10 Cr-60

From the above graphs, the following observations were made:

Fig 3 & Fig 4 represent the uptake of Cr(VI) by the plant *Canna indica* L. Cr(VI) has been trans-located to the above ground parts of the plant. It is accumulated to a maximum extent in the leaf. This is a classic example of phytoextraction. *Canna indica* L can thus be classified as a hyper accumulator. The maximum accumulation of chromium in the leaf was on the 10th day of planting. The plants could not with stand the toxicity of chromium beyond the 10th day.

Fig 5 & Fig 6 represent the uptake of Cr(VI) by the plant *Canna indica* L in the presence of a soil amendment *viz.*, coconut shell bio-char. Here the situation is altered. The roots are found to accumulate maximum amount of chromium. The process of phyto extraction has changed over to a process of phyto stabilization. The addition of coconut shell biochar to chromium spiked soil has brought about this change. The FT-IR of BC***cs*** **(**Fig 1) shows the presence of several oxygenated functional groups which would have contributed to the reduction of the highly mobile Cr (VI) ions to the less mobile

Cr (III) ions.

This is consistent with the finding of Losi *et al*., 1994 [5] and Bolan *et al*., 2003 [6], who reported substantial increase in the rate of reduction of Cr(VI) ions with the addition of organic amendments. This reduction may be attributed to the presence of several oxygen-containing acidic (carbonyl, lactonic, carboxylic, hydroxyl and phenol) and basic (chromone, ketone and pyrone) functional groups in biochar (Goldberg, 1985) [7]. Moreover, bio-char comprises of disordered polycyclic aromatic hydrocarbon sheets which are highly porous with high surface area. These sheets donate π electrons for the Cr(VI) to be reduced (Wang *et al*., 2010) [8]. The resultant Cr(III) either adsorbs or participates in the surface complexation with organic compounds (Hsu *et al*., 2009a) [9].

From the present study it is observed that maximum amount of chromium was accumulated in the roots over a period of 20 days. The Cr^{3+} ions are bigger in size than the Cr^{6+} ions and therefore they remain adsorbed on the roots of the plant. The Cr^{3+} is phytostabilized on the roots in the soil and it is not translocated to the stem and leaves of the plant.

5.3. FT-IR of coconut shell biochar

When freshly produced biochars are applied to soils, on exposure to the atmosphere, oxygenation of biochar surface occurs. Oxygen containing functional groups like carboxyl, hydroxyl, phenol and carbonyl groups are formed on the surface of the biochar (Liang *et al*., 2006, Lee *et al*., 2010, Uchimiya *et al*., 2010b) [10-12]. This is proved in the present study of FT-IR of BC***cs*** (Fig 1).

The FT-IR spectrum of biochar obtained from pyrolysis of coconut shell at the pyrolysis temperature of 500°C is shown in fig 1, Table 4.4. The O-H stretching vibrations at 3420cm^{-1} indicate the presence of phenols and alcohols. The sharp stretching vibration at 3735cm^{-1} shows the presence of free alcohol. The C-H stretching vibration at 2922cm^{-1} indicates the presence of alkanes. A weak stretching vibration at 2557cm^{-1} shows the presence of O-H of an acid. A weak stretching vibration at 2249cm^{-1} shows the presence of C≡N. The absorbance peaks at 1928cm^{-1}, 1893cm^{-1} and 1851cm^{-1} shows the presence of aromatic compounds. The stretching vibration at 1627cm^{-1} is due to C=O. The multiple bands at 1535cm^{-1}, 1437cm^{-1}, 1402cm^{-1} and 1398cm^{-1} show the stretching of an alkene group. A strong sharp peak at 1026cm^{-1} shows the presence of an ether. These results indicate the presence of many oxygenated functional groups on the surface of the coconut shell biochar. These functional groups are responsible for the reduction of Cr(VI) to Cr(III) in the soil.

Metal sorptions to biochar is an endothermic physical process (Kannan and Rengaswamy 2005, Leiu and Zhang 2009, Harvey *et al*., 2011) [13-15]. Aromatic structures of biochars having π electron clouds associated with C=O or C=C which can have an electrostatic interaction with a positively charged cation (Cao *et al*., 2009, Uchimiya *et al*., 2010b, Harvey *et al*., 2011) [16, 12, 13]. An increase in pyrolysis temperature of biochar increases the aromaticity and decreases the number of oxygenated functional groups (Harvey *et al*., 2010, Mc Beath *et al*., 2011) [17]. So increasing the pyrolysis temperature increase the cation vibration π interaction (i.e., electrostatic bonding).

In the present study the pyrolytic temperature being 500 °C, Cr^{6+} ion must have formed electrostatic bonds with the surface of biochar. These ions will be released to the soil, depending on the soil conditions, mainly pH. They become bio available to the plants along with other nutrients after Cr(VI) is reduced to Cr(III) by biochar, to be adsorbed by the roots.

5.4. SEM Analysis

The image shown in fig 2 represents the macroporus structure of coconut shell biochar. The size of the pores range from 2µm to 10µm. The honey-comb like structure is due to the presence of tubular structures originally emanating from the plant cells. As a result of these well developed pores, the coconut shell biochar will have a high surface area. This biochar produced at high temperature is analogous to activated carbon in environmental remediation. The macro porous structure of biochar is potentially important to water holding and adsorptive capacity of pollutant in soil and solution systems (Angin 2013, Gao *et al*., 2013) [18, 19]. Therefore in the present study also we find that the potted plants grew well in the biochar amended soil inspite of spiking it with chromium. The plants were able to withstand the toxicity of chromium beyond 20 days.

6. Conclusion

The phytoremediation of chromium spiked soil with *Canna indica* L. proved *to* be effective. The plant being a good hyper accumulator was able to withstand the toxicity of chromium. Initially chromium was translocated to the above ground parts within a short span of time. The maximum concentration was found in the leaves of *Canna indica* L. The accumulation of chromium in the leaves proved that *Canna indica.* L can phyto remediate the soil from chromium by the process of phytoextraction. As the leaves accumulate chromium, they can be cut off from the plant from time to time. The rhizome of the plant will once again start growing fresh leaves.

When a soil amendment like biochar was added to the soil, the roots started accumulating chromium and the translocation became minimal. From earlier studies it is known that biochar can reduce the mobility of heavy metals altering their redox state eg. Cr^{6+} to the less mobile Cr^{3+}. An increase in electrostatic attraction between positively charged heavy metals and biochar amended soil has been reported. Prior to the addition of biochar the highly mobile Cr^{6+} is absorbed by the roots and quickly translocated the leaves. The addition of coconut shell biochar converts Cr^{6+} to Cr^{3+}. The size and mobility of Cr^{6+} is less than that of Cr^{3+}. The Cr^{3+} ions thus formed are adsorbed by the roots and are phytostabilized. Therefore the chromium ions do not travel beyond the roots to the above-ground parts.

The plant *Canna indica* L is a phytoextractor of chromium from the soil in the absence of a soil amendment like biochar. In the presence of biochar it becomes a phytostabilizer of chromium in the soil. Thus the soil is phyto remediated by the same plant using two different remediation technologies.

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