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A comparative study of two biochars based on the temperature of pyrolysis for phytoremediation of chromium spiked soil by *Canna indica* L

Shyamala Lionel and Dr. R Joel Karunakaran

Abstract
The clean-up of chromium spiked soil combining two remediation techniques ie., biochar enhanced phytoremediation has been attempted. Two biochars viz coconut shell biochar (BC*cs*) and rice husk biochar (BC*rh*) have been used. The comparisons are made on the basis of the temperature at which they were pyrolysed. BC*cs* was pyrolysed at 400°C and BC*rh* at 500°C. *Canna indica* L. was chosen as the phytoremediating plant. Potting experiments were done spiking the soil with varying concentrations of hexa-valent chromium and biochars. The Canna plants were grown in these pots and their chromium uptake was recorded on the 5th, 10th and 20th days. Based on the accumulation of the metal in the plant parts, the impact of the biochars on the mechanism of chromium uptake was derived..

Keywords: Coconut Shell, Rice Husk, Pyrolysis, *Canna indica* L, Phytostabilization, Phytoextraction

1. Introduction
Plants are ideal agents for soil and water remediation because of their unique genetic, biochemical and physiological features (Parisa and Somaye, 2014) [1]. There is an abundance of reports in the literature about amendments being used to reduce the bio availability of heavy metals (Komarek *et al*. 2013) [2] and thus having the potential to be combined with phytoremediators (de Abreu *et al*. 2012) [3]. Phytoremediation consists of different plant-based technologies that include phytoimmobilization, phytostabilization, phytoextraction and phytovolatilization (Chaney, 1983) [4]. In principle, biochar prepared from any material would have the potential to increase plant yield and can thus be used in combination with phytoremediation. Phytoremediation and biochar are two sound environmental technologies which could be at the forefront to mitigate soil pollution (Paz-Ferreiro *et al*. 2014) [5].
Biochar is the solid produce of pyrolysis produced through heating of biomass at 300°C to 500°C in the absence of oxygen (Lehmann *et al*. 2002) [6]. Biochar has a high surface area, high porosity, variable charge and functional groups which can increase soil water-holding capacity, pH and cation exchange capacity (CEC), when added to soil (Glacer *et al*. 2002; Liang *et al*. 2006 and Tang *et al*. 2013) [7-9]. These properties vary with the pyrolysis temperature and properties of feedstock (Gundale and DeLuca, 2006) [10]. Recently the application of biochar to soil is drawing greater attention for sustainable soil quality improvement and carbon sequestration (Woolf *et al*. 2010; Sohi, 2012) [11, 12].
Phytoremediation is an eco-friendly modern technique for detoxifying pollutants, especially heavy metals from the soil. Since this technology is based on the capacity of plants to phytoextract the pollutants, a correct choice of plant is essential. *Canna indica* L is a sturdy plant with a fleshy rhizome and broad leaves. It is highly tolerant to diseases and can grow in adverse conditions producing a large biomass. It is not edible and therefore restricts the passage of contaminants into the food chain. This being a perennial plant, multiplies easily and spreads from the rhizome which grows underground, even if the leaves are trimmed. From earlier literature, this plant is a known hyper-accumulator and is therefore used in the present study. Phytostabilization also referred to as in-place inactivation, is primarily concerned with the use of certain plants to immobilize soil sediment and sludges (USEPA, 2000) [13]. Contaminants are absorbed and accumulated by roots, adsorbed on to the roots or precipitated in the rhizosphere. This reduces or even prevents the mobility of the contaminants preventing migration into the ground water or air and also reduces the bio availability of the contaminant thus preventing the spread through the food chain. Phytostabilization can occur through the process of sorption, precipitation, complexation or metal valent reduction. This technique is useful for the clean up of Pb, As, Cd, Cr, Cu and Zn (Jadia and Fulekar, 2009) [14].

2. Highlights

The main objective of the study was to investigate the effect of two 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 BCcs and BCrh with Canna plant, for fixed time intervals.
- To examine the surface and the size of pores of BCcs and BCrh by SEM analysis.
- To compare the functional groups present in BCcs and BCrh using FTIR.

3. Materials and Methods

Materials

- All chemicals in the study were of analytical grades (Merck chemicals, Germany) which were used without any further purification. In all experiments, deionized water (Milli-Q Millipore 18.2M/cm conductivity) was used for the preparation, dilution and analytical purposes. A stock Cr(VI) 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.

- **Coconut shells** were collected from the Madras Christian College canteen. It was cleaned, washed and dried in the sun. It was hand crushed to large size pieces and dried in air oven in batches at 105 °C for the removal of moisture. The dried coconut shell pieces are then sent to ball mill for grinding. The product is then ground to smaller size and the finer product is separated using a sieve.

- **Rice husk** was collected from a nearby rice mill and it was washed thoroughly with distilled water to remove adhering soil and clay. It was then dried in an air oven at 105 °C for 4 hours. The rice husk was milled and then passed through different sieves, to get the desired particle size.

Methods

3.1 Pyrolysis of biochar

It is a thermo chemical decomposition of organic material at elevated temperature in the absence of oxygen. It results in an irreversible phase and chemical composition. The products of pyrolysis are liquid fraction, non-condensable vapours, soil residue and char.

The present study makes use of two kinds of biochar:

Coconut shell biochar (BC*cs*)

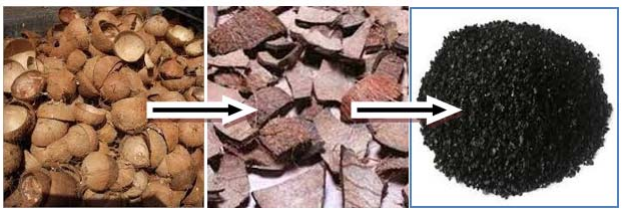

Fig 1: Conversion of Coconut Shell into Biochar (BC*cs*)

Rice husk biochar (BC*rh*)

Fig 2: Conversion of Rice Husk into Biochar (BC*rh*)

The pyrolysis of coconut shell and rice husk was done in ceramic crucibles, covered with a fitting lid and heated in a limited oxygen condition in a muffle furnace. Table 1 gives the heating conditions and time for the production of biochars.

Table 1: Pyrolysis of biochars

Production Conditions	Unit	BC*cs*	BC*rh*
Heating rate	°C/min	0.25-0.3	0.25-0.3
Initial temperature	°C	250	350
Peak temperature	°C	350 - 400	450-500
Residence time in peak temperature	min	30	30

The powdered biochars obtained from pyrolysis were stored in separate containers and labeled as BCcs and BCrh.

3.2 Characteristization of biochars

SEM Analysis

The char products derived from coconut shell pyrolysis and rice husk pyrolysis where characterized by scanning electron microscope (Model: Vega3 sbu – Czech Republic) with an acceleration voltage of 15kV at different magnification values to have a clear view on pore density and diameter. Large pores are found on the surface of cocnut shell biochar and smaller ones on rice husk biochar. Coconut shell has a higher volatile content which produces granulated carbon with even and large pore volume (Laine and Yunes, 1992) [15].

FTIR Analysis

Fourier Transform Infrared spectroscopy (FTIR) is an important analysis technique which detects various characteristic functional groups present in the char. On interaction of infrared light with the char the chemical bonds will stretch, contract and absorb infrared radiation in a specific wavelength range regardless of the structure of the rest of the molecule.

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

3.3 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. BC***cs*** and BC_{rh} were weighed in the ratio of 1:10 and mixed with the chromium spiked soil. These were taken in pots, 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.

4. Results and Discussion

4.1 Characterization of biochars

SEM Analysis of BC*cs* and BC*rh*

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

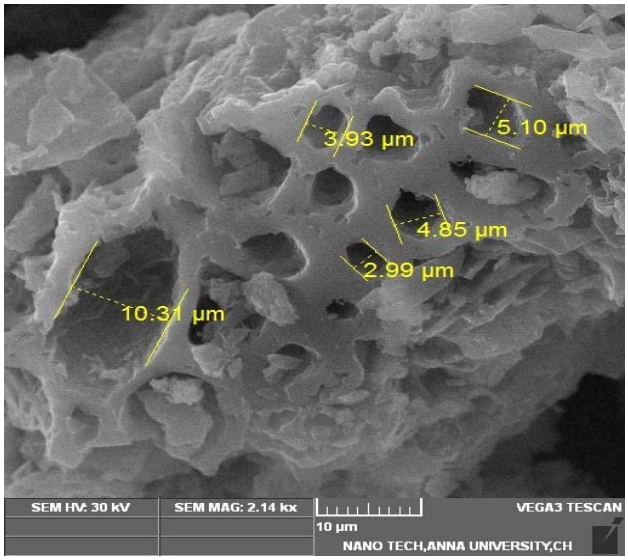

Fig 3: SEM Image of BC*cs* (Shyamala and Joel, 2017)[16]

The SEM analysis of coconut shell bio-char shows multiple sizes of pores on a rough surface 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 3.

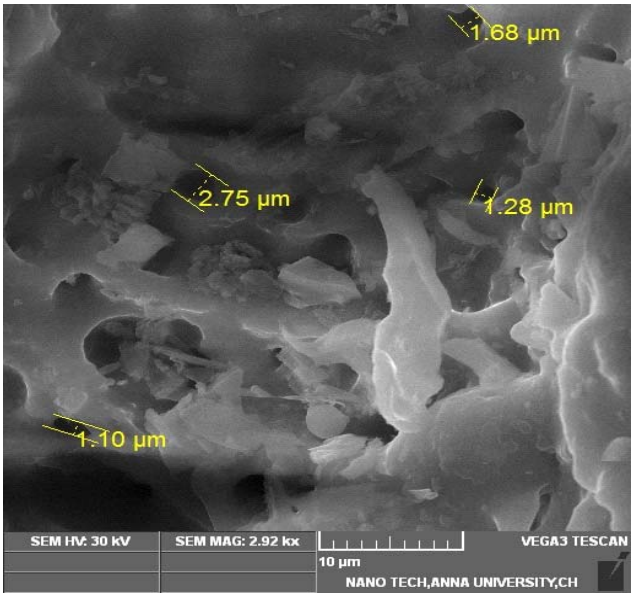

Fig 4: SEM Image of and BC*rh*

The SEM analysis of rice husk bio-char shows ill-defined pores and their sizes are as follows: 1.10 µm, 1.28 µm, 1.68 µm, 2.75 µm. as shown in figure 4.

FTIR of BC*cs* and BC*rh*

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

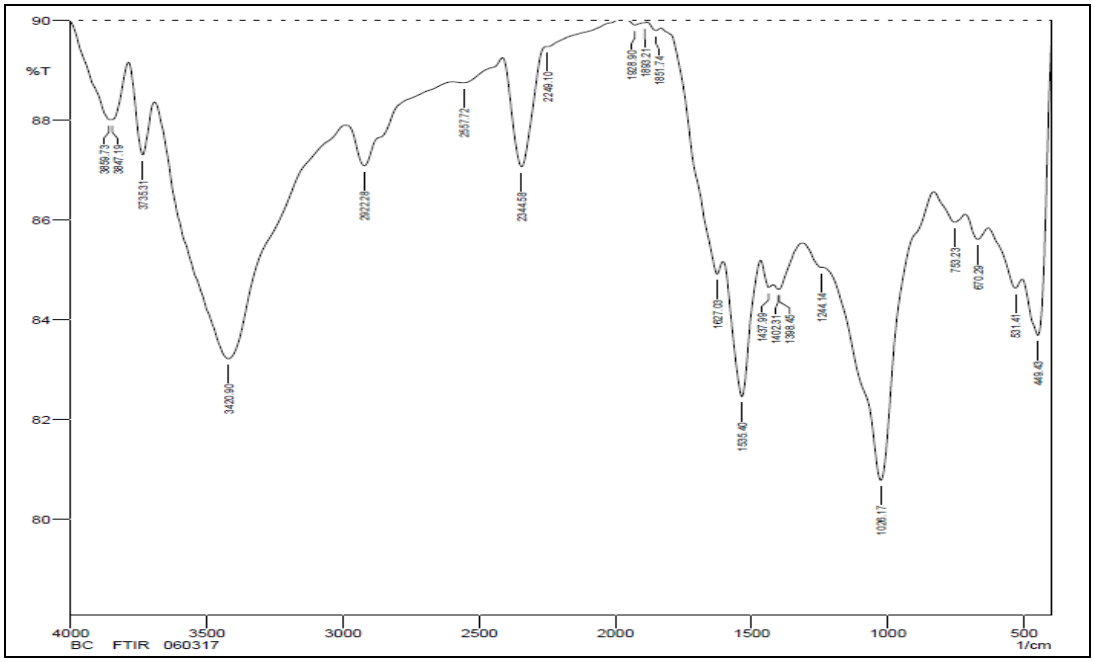

Fig 5: FTIR of BC*cs* (Shyamala and Joel, 2017) [16]

Coconut shell pyrolysed at a temperature of 400 °C (Fig.5) shows the presence of a number of oxygenated functional groups. These functional groups are responsible for the reduction of Cr(VI) to Cr(III).

The following results were obtained for the FTIR analysis of Rice Husk biochar (BC***rh***)

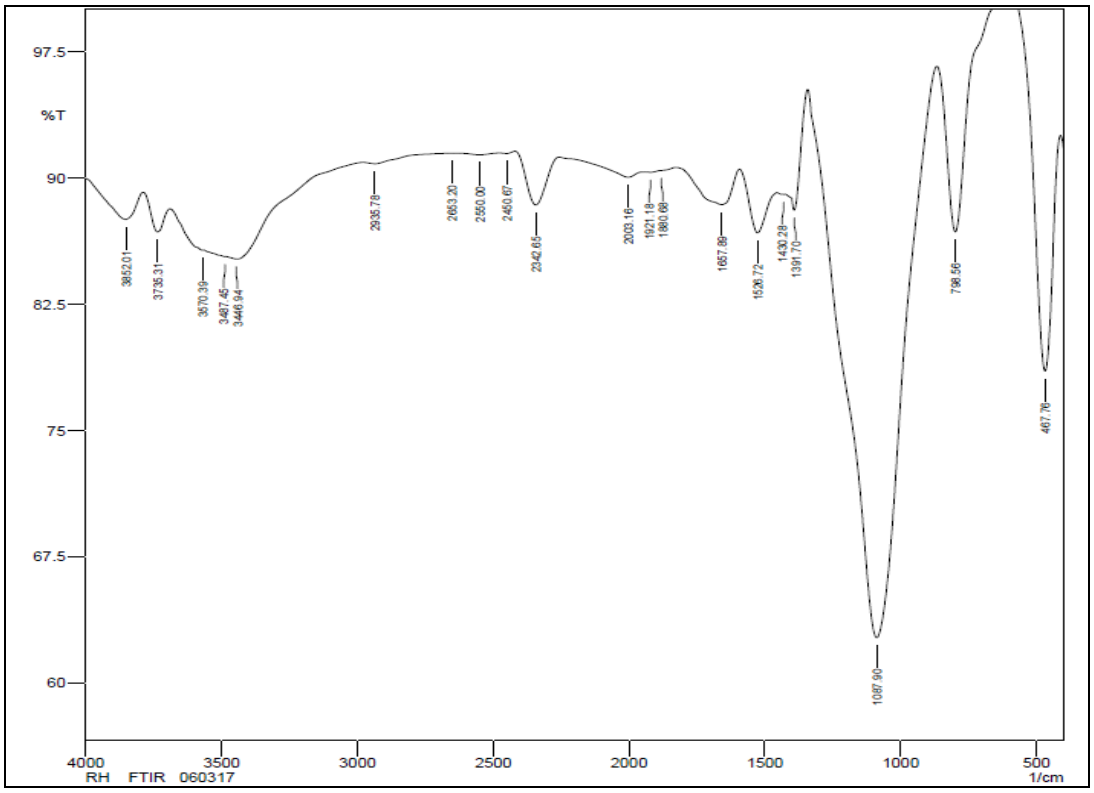

Fig 6: FTIR of and BC*rh*

Rice husk pyrolysed at 500 °C (Fig.6) shows a decrease in the number of oxygenated functional groups. This is in accordance with the previous studies (Harvey *et al*. 2012, Jindo *et al*., McBeth *et al*. 2013) [17-19]. There is also an increase in aromaticity which would increase the cation-interaction (electrostatic bonding). The chromium ion would have formed electrostatic bonds on the surface of the biochar. The results of the spectral analysis are recorded in Table 2 to make a comparison between BC*cs* and BC*rh*.

Table 2: Peaks and Functional Groups From FTIR Of Bc*cs* And Bc*rh*

Type of Vibration	Characteristic Absorptions (cm-1)		Functional Group
	BC*cs*	**BC*rh***	
O-H - free alcohol	3735.31	3570cm^{-1}	Alcohol
O-H - alcohol bonded	3420.91	3446.94, 3487.45	Phenol
C-H of alkane	2922.28	-	-
O-H of acid	2557.72	2550,2653,2935	-
C=N	2249.1	-	-
Cyclic Stretch	1928.9, 1893.21, 1851.74	-	-
C=O Stretch	1627.03	1087.9	Ketone/Carboxylic acid
C=C Stretch	1535.4, 1437.99, 1402.31, 1398.45	-	Aromatic
C-O Stretch	1244.14	-	Ether
C-H Bend	753.23	798.56	Aromatic
C-H Bend	670.29	-	Aromatic
Si-O	-	467.76	Si-O Stretching

4.2 Potting Experiments

a) Soil amendment additive: BC*cs*

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

Table 3: Chromium uptake by *Canna Indica* L (Shyamala and Joel, 2017)[16]

ID	Nursery Soil(Kg)	Dose of Cr^{6+}mg/kg	BC g	Plant Parts	5 D (ppm)	10 D (ppm)	20 D (ppm)
BCcs–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
BCcs–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

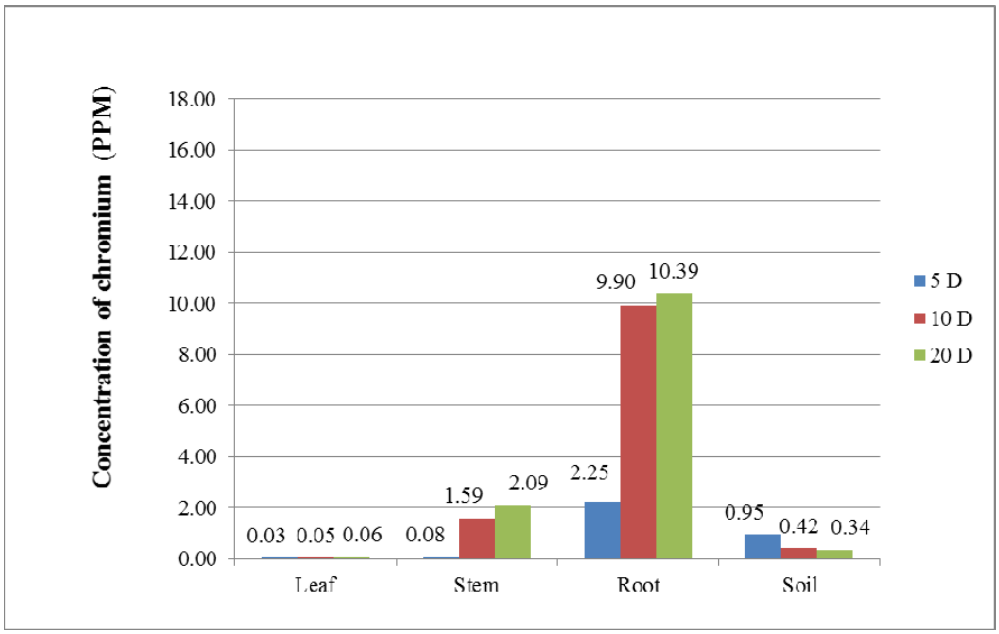

Fig 7: BCcs–10- Cr_{30} (Shyamala and Joel, 2017)[16]

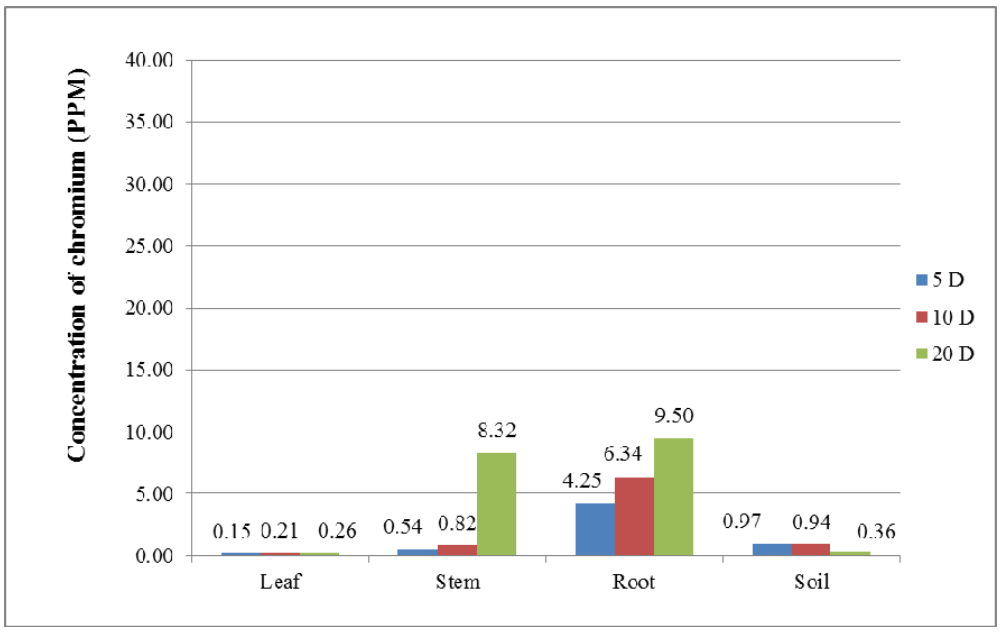

Fig 8: BCcs–10- Cr_{60} (Shyamala and Joel, 2017)[16]

The above graphs (Fig. 7 & 8) show that in the presence of BCcs, chromium has accumulated in the roots reaching a maximum concentration on the 20th day. Minimum amount of chromium has been translocated to the leaves. With increased concentration of chromium (Fig. 8), a considerable amount of chromium has been translocated to the stem as well, but this was not consistant. Quite a large concentration of chromium has been removed from the soil on the 20th day.

b) Soil amendment additive: BC*rh*

Plant: *Canna indica* L

No. of days: 5, 10 and 20

Table 4: Chromium uptake by *Canna Indica* L (Shyamala and Joel, 2017)[16]

ID	Nursery Soil (Kg)	Dose of Cr^{6+}mg/kg	BC g	Plant Parts	5 D (ppm)	10 D (ppm)	20 D (ppm)
BCrh–10- Cr_{30}	1	30	10	Leaf	1.31	1.90	1.45
				Stem	5.28	6.99	7.18
				Root	6.52	8.68	17.1
				Soil	2.23	1.61	0.83
BCrh–10- Cr_{60}	1	60	10	Leaf	5.64	9.57	7.50
				Stem	12.16	15.20	14.54
				Root	8.63	12.84	33.84
				Soil	2.53	1.29	0.98

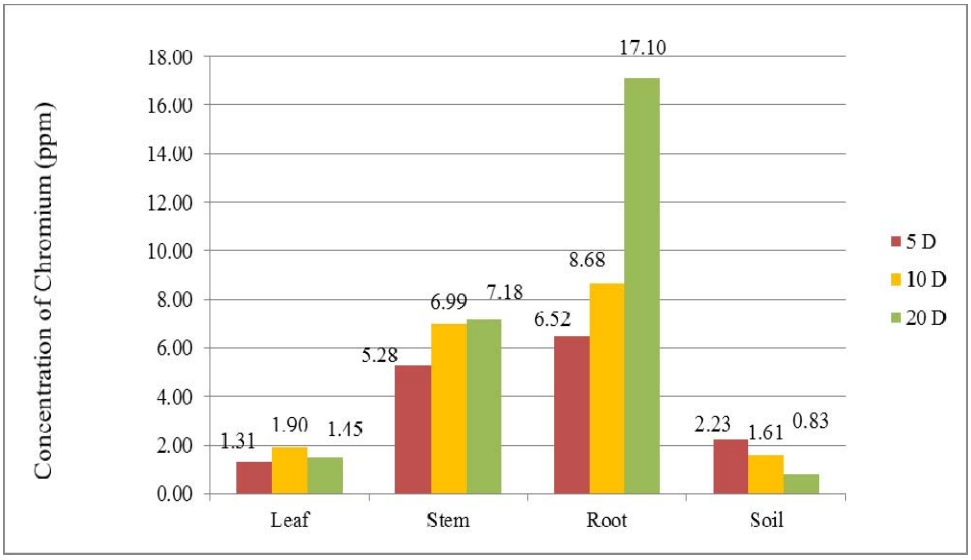

Fig 9: BCrh–10- Cr-30

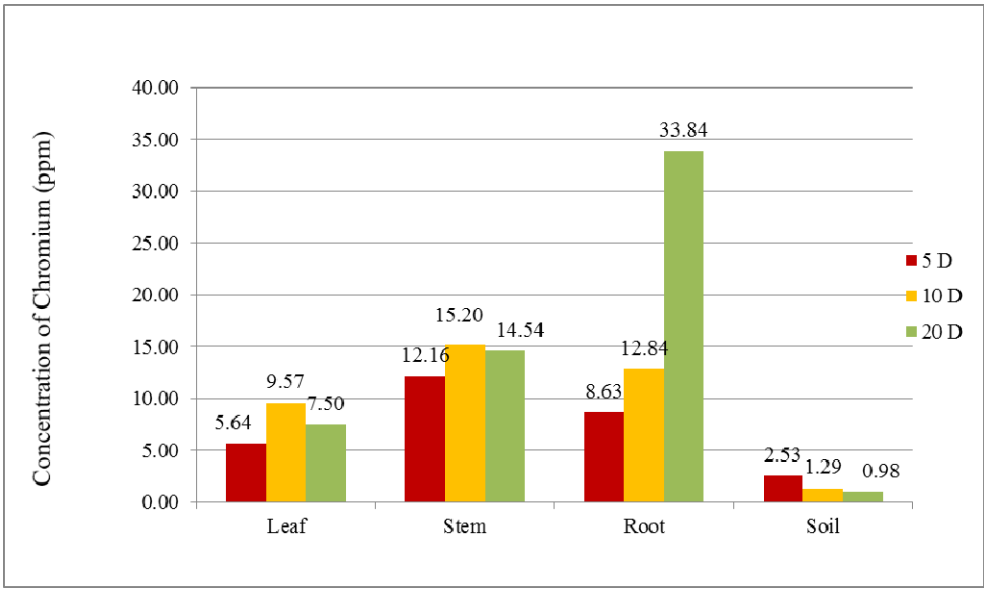

Fig 10: BCrh–10 Cr-60

The above graphs (Fig. 9&10) show that in the presence of BCrh, chromium has accumulated in the roots reaching a maximum concentration on the 20th day. It has also been translocated to the above ground parts viz, stem and leaf, to a greater extent than in the presence of BCcs. Chromium was not completely eliminated from the soil on the 20th day.

Discussion

SEM Analysis

The SEM analysis of BC*cs* and BC*rh* showed variations in the surface morphology. The image shown in fig 5 represents the macroporus structure of BC*cs.* BC*cs* which was pyrolysed at 400 °C had well defined pores and a regular structure. The size of the pores indicated that the volatile compounds had evaporated creating macro pores on the surface. 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) [20, 21]. 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.

BC_{rh} was formed at a temperature of 500°C and many volatile compounds would have escaped at this high temperature creating large voids as shown in figure 6. The surface of rice husk biochar shows a crumbled structure with micro pores all over. This must be due to the formation of ash that would have fallen into the pores. A close examination of the surface reveals ill-defined pores and micro pores. The ash in BC*rh* would have increased the Si content of the soil and increased water retention.

FTIR Analysis

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*. 2011b) [22-24]. This is proved in the present study of FTIR of BC***cs*** (Fig 5).

The FTIR spectrum of biochar obtained from pyrolysis of coconut shell at the pyrolysis temperature of 400 °C is shown in fig 5 table 2. 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 CN. 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. 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.

This is inline with the earlier studies which have shown that metal sorption to biochar is an endothermic physical process (Kannan and Rengaswamy 2005, Leiu and Zhang 2009) [25, 26]. Aromatic structures of biochars having electron clouds associated with C=O or C=C can have an electrostatic interaction with a positively charged cation (Cao *et al*. 2009, Uchimiya *et al*. 2011b, Harvey *et al*. 2012) [27, 24, 17]. An increase in pyrolysis temperature of biochar increases the aromaticity and decreases the number of oxygenated functional groups (Mc Beath *et al*. 2011) [19]. So increasing the pyrolysis temperature increase the cation vibration interaction (i.e., electrostatic bonding).

The FTIR spectrum of rice husk biochar (BC_{rh}) pyrolysed 500 °C is shown in fig 6 & table 2. The O–H stretching vibration at 3570cm^{-1} represents free alcohol and those at 3446.94 cm^{-1} and 3487.45 cm^{-1} show the presence of increased aromatic compounds, like phenols. The presences of O–H of aromatic acids are seen at 2550 cm^{-1}, 2653 cm^{-1} and 2935 cm^{-1}. The peak at 1087.9 cm^{-1} represents ketone. An aromatic C–H bend is seen at 798.56 cm^{-1} and Si–O stretching showing the presence of silicon is found at 467.76cm^{-1}. However, the numbers of functional groups have decreased drastically. Therefore, we find that BC_{rh} does not reduce Cr(VI) as effectively as BC_{cs}. Previous literature is inline with this observation whereby an increase in pyrolytic temperature decreased the number of functional groups on the surface of the biochar.

The removal of Cr (VI) decreased as the pyrolytic temperature increased. Cr (VI) removal was primarily attributed to the reduction of Cr(VI) to Cr(III). This was determined by the type and amount of oxygen containing functional groups of the material (Shen *et al*. 2012) [28]. In the present study the FTIR analysis reveals the presence of more number of oxygenated functional groups in BCcs than in BCrh. Accordingly reduction of Cr(VI) are found to be more in the soil amended with BCcs. The reduced Cr(III) ions are adsorbed on the root of *Canna indica* L by the adsorption process.

Mechanism of Chromium uptake

The adsorption process is divided into three parts as follows (Dong *et al*. 2013) [29]:

1. Negatively charged Cr(VI) species migrate to positively charged biochar (at low pH) with a help of electrostatic driving forces.
2. Cr(VI) is reduced to Cr(III) by the participation of hydrogen ions and the electron donors from biochar.
3. Part of the reduced Cr(III) is released to the aqueous solution and the other part of Cr(III) is complexed with the functional groups on biochar (Lu *et al*. 2012) [30].

In the present study, in case of BC_{cs}, apart from these three stages a part of Cr(III) ions is released in the soil. This is adsorbed on the roots of *Canna indica* L as we have combined biochar with phytoremediation. The root exudates which are organic acids decrease the pH around the root zone enabling the adsorption of Cr(III). The adsorbed Cr(III) is phytostabilized. This is clearly shown in the graphs, figures 7&8 that the maximum accumulation of chromium is on the roots. Translocation to the other parts of the plant is minimal.

Reduction of Cr(VI) to Cr(III) becomes less in case of BC_{rh} as there are less number of oxygenated functional groups on the surface of the biochar. Cr(VI) is absorbed as such by the plant as its solubility in a wide range of pH, enhances absorption by the plant. The plant *Canna indica* L shows its full potential as a Phytoextractor, being strengthened by the presence of BCrh. This is shown in the graphs, figures 9 & 10, where although the roots have accumulated the maximum amount of chromium a considerable amount has been translocated to the stem and leaf as well. Thus in this case BCrh has promoted the elimination of chromium from the soil by enhancing phytoextraction of Cr(VI).

5. Conclusion

Coconut shell biochar and rice husk biochar differed in their surface morphology in terms of pore size and number of functional groups. This difference is mainly attributed to the pyrolic temperature. Coconut shell biochar with well-defined macro pores and rice husk biochars with a crumbled, pore blocked, structure exhibited differences in the water holding capacity. Rice husk biochar, with more ash content filling up the pores, was more compact and was able to hold more water.

The most important observation was the difference in the number of oxygenated functional groups, on the surface of the two biochars, as shown by FTIR analysis. Rice husk which was pyrolysed at a higher temperature (500 °C) had few functional groups. Coconut shell biochar had more number of functional groups as the pyrolytic temperature was low (400 °C).

The reduction of Cr(VI) in the soil was found to increase in the case of coconut shell biochar as it had more number of oxygenated functional groups. Part of the Cr(VI) ions are reduced to Cr(III) and are held on to the surface of biochar. The remaining Cr(III) ions are released into the soil. These ions become available to the plant at a pH<4. The root exudates of the plant *Canna indica* L makes the alkaline soil acidic, near the root zone. This enables the Cr(III) ions to be adsorbed on the root system. The ions being larger than Cr(VI) do not cross over the cell membrane. Thus the chromium ions are immobilized on the roots ie., they are phytostabilized.

In the case of rice husk biochar the number of oxygenated functional groups are less. Therefore reduction of Cr(VI) decreases and less of Cr(III) ions are formed. The soil now contains more of the soluble, highly toxic, more mobile Cr(VI) which is directly absorbed by the plant *Canna indica* L. The presence of silicon in the rice husk biochar is a very

vital nutrient which enables a luxuriant growth of the plants. This spurt of growth with increased biomass helps the plant to absorb more of Cr(VI) from the soil and translocate to the stem and leaves. Therefore the presence of rice husk biochar pyrolysed at 500 °C makes the condition favorable for phytoextraction rather than phytostabilization. We infer that in both the cases the soil is remediated from hexa-valent chromium, by the plant *Canna indica* L, either by phytostabilization or by phytoextraction depending on the kind of biochar used. By altering the pyrolytic temperature, we can design the biochars and combine them with a hyper accumulating plant, to phytoremediate chromium contaminated soil effectively.

6. References

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