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EFFECT OF CADMIUM (CD) STRESS ON SPINACH (*Spinacea oleracea*) AND ITS RETENTION KINETICS IN SOIL IN RESPONSE TO ORGANIC AMENDMENTS

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Vegetables are irrigated with wastewater from industries in some parts of Pakistan. The wastewater from municipal and industries contains a variety of contaminants including metals. The aim of present study was to assess the effects of cadmium (Cd) stress from irrigation wastewater on spinach, its uptake by plant and subsequently development of a strategy to mitigate the stress. For this purpose, spinach was grown in pots irrigated with Cd contaminated wastewater and impacts were assessed using different physicochemical parameters. Two different organic amendments namely cow dung and wood charcoal at 20 and 30 t ha^{-1} were used to check the kinetics of Cd in soil and plants. Results revealed that maximum fresh biomass was recorded for treatment receiving 30 t ha^{-1} of charcoal (10 g per pot) followed by Cd alone treatment @ 9.5 g pot^{-1}. At the same rate of cow dung, the lowest uptake of Cd by spinach plant was recorded. Maximum Cd concentration was measured at treatment with metal alone control (0.91 mg kg^{-1}), while minimum of 0.016 mg kg^{-1} DM was at the highest level of cow dung in herbage. In soil, lowest Cd concentration (0.10 mg kg^{-1}) was noticed in the cow dung treatment i.e. T5. Organic carbon content was found highest (0.034 mg g^{-1}) in charcoal treatment at higher level. . The FTIR analysis of charcoal was conducted that showed peaks at 2250, 1000 and 2000 cm^{-1} showing C-C, C-O-C and C=C bonding, respectively. Overall, cow dung proved the best assimilator of Cd and the use of this bio-organic waste as a filter for wastewater treatment or soil conditioner is an innovative and emerging idea that deserves further attention and sorption studies.
Keywords: Cadmium, *Spinacea oleracea,* retention kinetics,organic amendments

INTRODUCTION

With an increase in uncontrolled industrialization since 1960, the need for freshwater resources for irrigation and drinking purposes has become greater than that in past. Pakistan is an agricultural country which contributes about 21% of GDP (Raza *et al.,* 2012). To meet the increasing demands of freshwater, different water conservation and remediation strategies are needed to be developed. With the industrial revolution, the wastewater production increased considerably, hence releasing contaminants due to use of metals in various industrial procedures (Bucker-Neto *et al.,* 2017). Different sources of water pollution including metallurgical operations, smelting, tanning and coal burning are contributed by anthropogenic activities (Qadir *et al.,* 2007). In several developing countries, urban and peri-urban agriculture depends on wastewater as a source of irrigation. Wastewater contaminated with heavy/trace elements like lead (Pb), zinc (Zn), copper (Cu), boron (B), chromium (Cr), cobalt (Co) molybdenum (Mo), arsenic (As) and manganese (Mn) are non-essential and toxic to plants, animals and human beings (Kanwar and Sandha, 2000; Mathur *et al.,* 2016). The leafy vegetables are reported as most affected (Chuan *et al.,* 2016) regarding food bio-security threats from metal contamination. Long-term application of untreated wastewater has resulted in a significant buildup of heavy metals in soil (Khan *et al.,* 2008; Ghosh *et al.,* 2012) and plants (Arshad *et al.,* 2015; Ahsan *et al*., 2018a,b).
Heavy metals are considered potent threat to flora and fauna in the environment and are also carcinogenic. Vegetables and plants have the enormous ability to accumulate metal in their tissues (Bigdeli and Seilsepour, 2008; Arshad *et al.,* 2016; Zhou *et al.,* 2016; Gul *et al., 2018*; Manzoor *et al.,* 2018) that later enter the food chain and affect whole cycle of producers, decomposers and omnivores.Heavy metals accumulation by plants depends on both plant species and soil properties (Arshad *et al.,* 2008). The uptake of heavy metals in vegetables and cerealsis likely to become higher that could lead to their deposition in human body. Among all heavy metals,Cd is widely used in industry like color pigments, nickel-cadmium battery, stabilizer and in plastics. Phosphate fertilizers are also one of the major sources of Cd in agricultural soils (Roberts, 2014). The permissible level of Cd in plants is 0.02 mg kg^{-1} recommended by World Health Organization (WHO, 1996) while in soil it is about 0.8 mg kg^{-1}. Generally, the use of Cd contaminated soil affects plant

by reducing biomass, yield, chlorophyll content, decreasing stomatal size and transpiration rate, affect the water and nutrient uptake and increase the production of reactive oxygen species (ROS). Once Cd is absorbed by the edible plants, it ultimately affects the human. High amount of Cd cause adverse effects including kidney, bone disorder, reproductive, cardiovascular and nervous system damage, also affect placenta and child growth, and could cause cancer (Ogundele *et al.,* 2015; Khalid *et al.,* 2017; Khan *et al.,* 2017). Taking suitable measures to prevent Cd accumulation in the environment is required as soils are not only source of essential nutrients for plant growth but also serve as the key sink for heavy metals.

To meet the upcoming food demands of increasing human population, cultivation of cereal crops is carried out at lands which don't have suitable irrigation water and soils for agriculture like in the vicinity of wastewater drains and other polluted places. Partial to address the water crisis, now-a-days irrigation in Pakistan is planned using large amount of wastewater discharged from the industries, household and agriculture sectors. Wastewater used for irrigation carries many contaminants, mainly organic and inorganic pollutants like heavy metals depending upon the origin (Pedrero *et al.,* 2010; Huibers and Van Lier, 2005)

In most of the underdeveloped countries, the discharge of wastewater from municipal, industrial and household areas is not pre-treated and also sometimes, if it is treated, the treatment process involves only primary physical processes like screening which generally, do not remove heavy metals from the water. Long term use of wastewater for irrigation can cause accumulation of heavy metals in soil which can later be translocated to plants and food crops, thereby enters the food chain (Gupta *et al.,* 2010; Singh and Agrawal, 2010).

It is desired to rehabilitate the soils from organic and inorganic contaminants. The most well-known and cost effective as well as economically feasible method is phytoremediation, sorption media, immobilization and phytoextraction of these contaminants (Arshad *et al.,* 2016; Gul *et al.,* 2018; Manzoor *et al.,* 2018). In most of the soils, some types of metals are less bio-available for plant uptake such as Pb, while Cd and Zn are readily bio-available for plants in some soils under certain conditions (Arshad *et al.,* 2008, 2011; Farid *et al.,* 2013; Gul *et al.,* 2018). To prevent theCd uptake by edible plants, there is a need to use some amendments which stabilized the Cd in soil and could reduce the bio-availability.

Organic amendments including animal manure, crop residues and biosolids has been widely used for the immobilization of Cd in contaminated soils through absorption reaction. Addition of organic amendments increase the surface charge therefore increase the retention of Cd in soils. Organic minerals, phosphate and aluminium compounds present in the organic amendments, retain Cd in soil. Moreover, the organic matter convert the soluble Cd into organic bond fraction, thus reducing the uptake (Khan *et al*., 2017).Organic manures like cow dung and charcoal/ biochar are carbon rich organic amendments that can be used to decrease absorption and uptake of Cd by plants. Keeping in view this background, specific objectives of the study are to evaluate the effect of Cd contaminated water on spinach vegetable, Cd uptake, and physicochemical parameters of plant and soil, and mitigate the Cd toxicity in spinach by charcoal and organic manure (cow dung) amendments.

MATERIALS AND METHODS

***Soil sampling and preparation*:** Soil samples were collected from the region near to canal in the town of Jalalpur Jattan, Punjab, Pakistan. Samples were air dried and then passed through a 2 mm sieve. Thick root material and left over of plants were removed by sieving process.

***Charcoal preparation and cow dung*:** Charcoal was prepared by burning of Kikar (*Vachellia karroo)* wood at 500°C. Particle size of grinded charcoal was 3 to 5mm. Cow dung was collected from dairy farm near the study area and air dried.

***Methods*:** Eighteen earthen pots were used for plantation of spinach vegetable/plants in soil in test study. Charcoal and cow dung at rates of 20 t ha^{-1} (80 mg kg^{-1}) to 30 t ha^{-1} (120 mg kg^{-1}) were used as amendments to soil (2 kg each pot) as treatments for metal reduced uptake by plants/spinach. About 7-8 seeds of spinach were sown in each pot which was later thinned to 5 plants per pot. Irrigation water used was collected from botanical garden and was applied to each pot on weight basis (Table 1) at 70% field capacity. Plants were allowed to grow on different treatments of organic origin for three weeks and then applied with Cd contaminated synthetic wastewater @ 20 mL per week of 100 µM of Cd metal solution.

Different treatments are shown below:

T1 = Control
T2 = Cadmium alone (100 µM Cd)
T3 = Charcoal 20 t ha^{-1} (100 µM Cd)
T4 = Charcoal 30 t ha^{-1} (100 µM Cd)
T5 = Cow dung 20 t ha^{-1} (100 µM Cd)
T6 = Cow dung 30 t ha^{-1} (100 µM Cd)

Table 1. Physicochemical characteristics of soil and water used in the study

S#	Parameter	Value
1.	Soil pH	8.32
2.	Soil texture	Clay loam
3.	Soil moisture content (%)	4.16
4.	Soil electrical conductivity (µS/cm)	245
5.	Total phosphorus (mg kg^{-1}) in soil	2177
6.	Available phosphorus (mg kg^{-1}) in soil	60
7.	pH of water	6.71
8.	Electrical conductivity (µS/cm) of water	555
9.	Turbidity (NTU) of water	1.3
10.	TDS (mg L^{-1}) of water	276

After six weeks (Fig. 1), plants were cut 5 cm above the top of soil. At harvest, soil was sampled along with roots and leaves. After harvesting the spinach, fresh weights were recorded and for dry weight, samples were oven dried at 65°C for two days. Samples were grinded using pestle and mortar. Roots were extracted from pots by flooding the soil. The organic Carbon content in soils (Table 3) in different treatments after harvest was determined by muffle furnace method at 600°C using method of Goldin (1987). According to the method, a weighed amount of oven dried soil (at 105°C; 24 hours) was placed in a porcelain crucible and kept in a muffle furnace (±5°C precision, 600°C) for combustion till 6 hours. The organic matter content was determined through the mass difference method in relation to the original soil sample (Pereira, 2006).

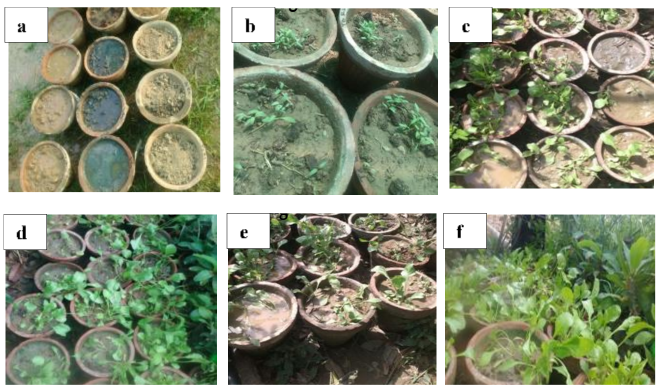

Figure 1. Different growth stages of pot trial conducted using various treatments; a = seed sowing and pot watering; b = first week after sowing; c = third week of sowing; d = fourth week of sowing; e = fifth week of sowing; f = sixth week of sowing.

***Digestion of biomass*:** The herbage and soil samples were digested with Aqua Regia di-acid mixture at hot plate and then diluted. Samples were then filtered by using Whatman filter paper No. 42 and stored in bottles and sent to Institute of Environmental Sciences, University of Agriculture, Faisalabad, Pakistan for further analysis by AAS (Atomic adsorption Spectroscopy - Hitachi Polarized Zeeman AAS, Z-8200, Japan) following the conditions described in AOAC (1990).

***Batch Cd sorption Study on Cow dung*:** Batch sorption study was conducted with different concentrations of Cd i.e. 5, 10, 20 and 30 mg/L (0.04, 0.08, 0.17, 0.26 µM) of Cadmium in triplicate @ 50 mL at sorbent dosage of 5000 mg (5 g) cow dung each in a set of glass conical flasks. The suspensions were agitated in horizontal shaker @ 150 rpm for about 5 hours at 20°C. The filtrate samples were collected after shaking and subjected to Cd analysis on AAS. Langmuir Adsorption Isotherm was drawn on the data (Fig. 6). The sorption data confirmed the retention affinity of cow dung for Cd.

***Statistical analysis*:** Analysis of variance (General linear model) and Tukey's test were used to find out the effect of charcoal and cow dung on plant growth and metal dynamics from synthetic Cd solution. The Minitab software was used for the statistical analysis.

RESULTS

***FTIR*:** The FTIR analysis of charcoal (Figure 2) was conducted that showed peaks at 2250, 1000 and 2000 cm^{-1} showing C-C, C-O-C (Wang *et al*., 2009) and C=C bonding, respectively.

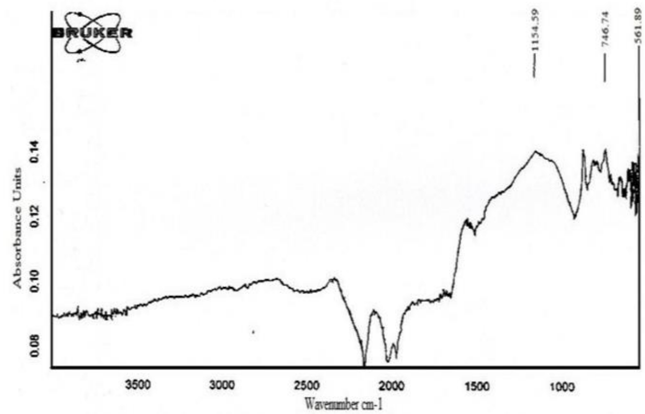

Figure 2. FTIR analysis of charcoal samples used in the study.

***Fresh shoot biomass of spinach*:** Fresh shoot biomass of spinach is presented in figure 3. Maximum fresh biomass (10 g) was observed upon 30 t ha^{-1} charcoal application in combination with 100 µM Cd. Minimum fresh biomass (4.3 g) was observed at 20 t ha^{-1} charcoal application along with 100 µM Cd. Fresh biomass of spinach was 9.1g and 8.9g, at 20 and 30 t ha^{-1}, respectively, cow dung application. No significant difference was observed in fresh biomass upon application of cow dung at 20 and 30 t ha^{-1} in combination with Cd. Fresh biomass of spinach was increased by 8.3%, 5.9 upon 20, 30 t ha^{-1}, respectively, cow dung application. However, 19% increase in fresh biomass was observed upon 30 t ha^{-1} Charcoal application as compared to control (no Cd added).

***Fresh root biomass*:** Figure 4 illustrates the fresh root biomass in Cd and organic amendment treated groups. The application of Cd alone affect the fresh root biomass. The fresh root biomass was decreased by 6.9% in Cd treated group as compared to control. The application of charcoal and cow dung increase the root fresh biomass except for 30 t ha^{-1} charcoal. Maximum fresh root biomass (0.51 g) was observed at T3 (charcoal 20 t ha^{-1} and 100 µM Cd); and the minimum fresh root biomass (0.03 g) was recorded at T4 (charcoal 30 t ha^{-1} and 100 µM Cd). The application of 20 and 30 t ha^{-1} cow dung increased the fresh root biomass by 48.1% and 10.3%, respectively as compared to control. The fresh root biomass was increased by 75.8% upon 20 t ha^{-1} charcoal application and 89.6% reduction in the fresh root biomass was observed upon 30 t ha^{-1} charcoal application as compared to control. The decrease in fresh biomass upon high level of charcoal applicationcould be due to the presence of aromatic form of carbon charcoal and less labile content thereby immobilizing the nutrients.

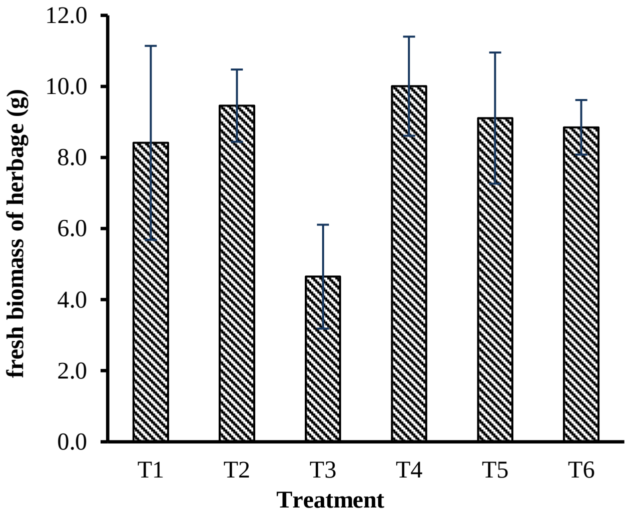

Figure 3. Fresh weight of Spinach Herbage under different treatments.

Where, T1 = Control, T2 = metal solution, T3= charcoal 20 t ha^{-1} and metal solution, T4 = charcoal 30 t ha^{-1} and metal solution T5 = cow dung 20 t ha^{-1} and metal solution, T6 = cow dung 30 t ha^{-1} and metal solution

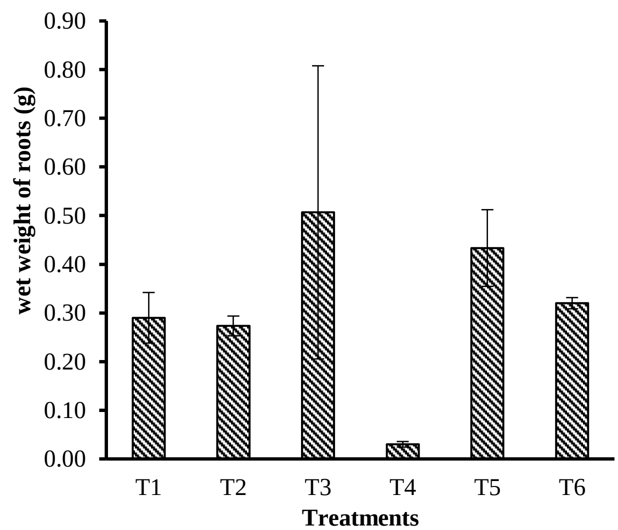

Figure 4. Fresh biomass of roots of Spinach under different treatments.

Where, T1=Control, T2=metal solution, T3=charcoal 20 t ha^{-1} and metal solution, T4= charcoal 30 t ha^{-1} and metal solution T5=cow dung 20 t ha^{-1} and metal solution, T6 =cow dung 30 t ha^{-1} and metal solution

Cd concentration in plant and soil**:** Concentration of Cd in spinach is presented in Table 2. The concnetration was increased by 32.7% in Cd treated group as compare to control. Maxiumum Cd concentration (0.911 mg kg^{-1}) was observed in Cd treated group. The application of organic amendments i.e. charcola and cow dung reduced the Cd concentration. Minimum Cd concentrtaion (0.016) was observed upon 30 t ha^{-1} cow dung application. Application of 20 and 30 t ha^{-1} charcoal and cow dung decreased the Cd concnetration by 96%, 97%; 97.9%, 98%, respectively, as compared to Cd alone.

Table 2. Cadmium concentration in spinach plant.

Treatments	Concentration in	
	mg g^{-1}	**mg kg^{-1}**
T1	0.000027	0.027
T2	0.000911	0.911
T3	0.000035	0.035
T4	0.000028	0.028
T5	0.000019	0.019
T6	0.000016	0.016

Where, T1=Control, T2=metal solution, T3=charcoal 20 t ha^{-1} and metal solution, T4= charcoal 30 t ha^{-1} and metal solution T5=cow dung 20 t ha^{-1} and metal solution, T6 =cow dung 30 t ha^{-1} and metal solution

The Cd concentration in soil is presented in Table 3. The highest Cd concentration (0.116 mg kg^{-1}) in soil was found in T4 treatment (30 t ha^{-1} Charcoal and 100 µM Cd) and lowest was in T5 (0.10 mg kg^{-1}) with cow dung at lower level.

Table 3. Concentration of cadimum in soil in different treatments.

Treat.	Concentration in mg g^{-1}	Mean mg kg^{-1}	Organic Carbon content (mg g^{-1})
T1	0.0000016	0.0096	0.0002
T2	0.000075	0.112	0.040
T3	0.000113	0.113	0.028
T4	0.000116	0.116	0.034
T5	0.000099	0.100	0.020
T6	0.000116	0.110	0.022

Where, T1=Control, T2=metal solution, T3=charcoal 20 t ha^{-1} and metal solution, T4= charcoal 30 t ha^{-1} and metal solution T5=cow dung 20 t ha^{-1} and metal solution, T6 =cow dung 30 t ha^{-1} and metal solution

In batch sorption study, the Langmuir Isotherm showed correlation value of 0.8629 (Fig. 5).The value of q_{max} was recorded 12.34 mg g^{-1} at the highest dose of 30 mg L^{-1} Cd influent concentration.

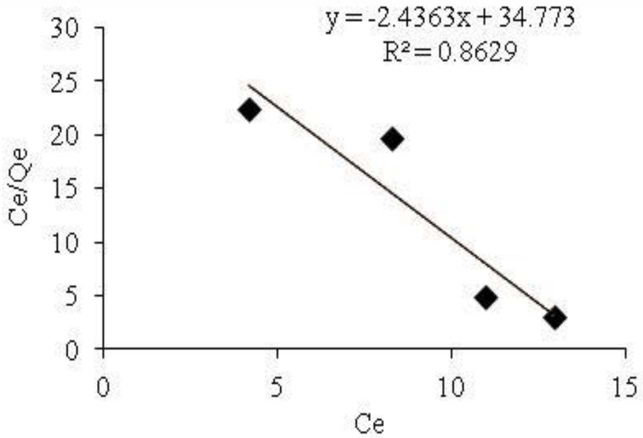

Figure 5. Langmuir Isotherm plot of Cd sorption data on cow dung.

DISCUSSION

The minimum fresh biomass (4.6 g) was observed in treatment 3 i.e. charcoal at rate of 20 t ha^{-1}, this decrease in biomass might be due to the fact that plant could not get enough available nutrients due to sorption or competition with Cd. Whereas, the highest value of herbage biomass was noted in treatment T4 (10 g) and T2 (9.5 g) that contained higher amount of charcoal at the rate of 30 t ha^{-1} and Cd solution alone, respectively. The statistical analysis at 95% Confidence Interval (0.05 p value) showed no significant difference among different treatments in biomass and Cd concentration data. The charcoal could be responsible for the nutrient binding and slow release of nitrogen to the plants. While the increase in biomass in response to Cd alone treatment could be due to the affinity of plant with the metal. It showed that spinach can tolerate Cd stress without showing symptoms at certain level and good for remediation of Cd contaminated soils.

Similar results are reported by (Arduini *et al.*, 2004) and (Tauqeer *et al.*, 2016) where effect of Cd was shown to relate to increased biomass of plant. Arduini *et al.* (2004) reported that Cd increased Miscanthus growth up to 0.50 mg L^{-1} and decreased it at 0.75 mg L^{-1}. In the present study, root biomass was not significantly affected by higher levels of Cadmium. Liu *et al.* (2008) found increase in biomass at the Cd level of 30 mg kg^{-1} of soil in ornamental plants i.e. *Calendula officinalis* and *Althaeea rosea*, while biomass and growth decreased with higher Cd levels. The positive trend of Cd on plant herbage biomass is suggested by two mechanisms according to (Kennedy and Gonsalves, 1987). Cadmium is known to induce genes, related to mammalian cell proliferation thereby leading to Cd caused carcinogenesis. Also low Cd levels hyperpolarize the plasma membranes at surface of root so increase in trans-membrane potential which is considered energy source for cation uptake.

In Table 2, the T2 treatment with metal alone solution showed more concentration of metal as compared to other treatments with amendments because the application of cow dung and charcoal immobilze the metal and make it less bio-available. Cow dung showed more promising results in reducing the bioavailability of Cd (Table 2) which is similar to results reported by Ojedokun and Bello (2016). They reported cow dung as important sorbent to remove metals like Zn, Cu, Pb, Cd and Ni from wastewaters and environment. In the present study, lowest Cd concentration (0.10 mg kg^{-1}) in soil was found upon 20 t ha^{-1} cow dung application.

Maximum herbage Cd concentration of 0.91 mg kg $^{-1}$ was recorded in treatment T2 while lowest (0.016 mg kg $^{-1}$) was in T6 with cow dung at higher level (Table 2). The correlation between soil and plant cadmium showed good relation with R^2 value of 0.99 (Fig. 6). Similar results were reported (Rafiq *et al.*, 2014) where Cd concentration in Pak Choi (*Brassica chinensis* L.) shoots were significantly correlated to total Cd content (R^2 = 0.95 to 0.99) and Mehlich-3 extractable Cd (R^2=0.97) in soils. The best relation of Pak Choi was established in Mollisols (R^2=0.99). The organic carbon content of soil (Table 3) showed the highest C (0.034 mg/g) in charcoal treatment at higher level. However, the correlation between plant Cd and soil organic C content was not promising and showed no significant relation between them. The Correlation value of R^2=0.86 was observed in Langmuir plot that confirmed the affinity of cow dung for Cd at higher levels (Fig. 5).

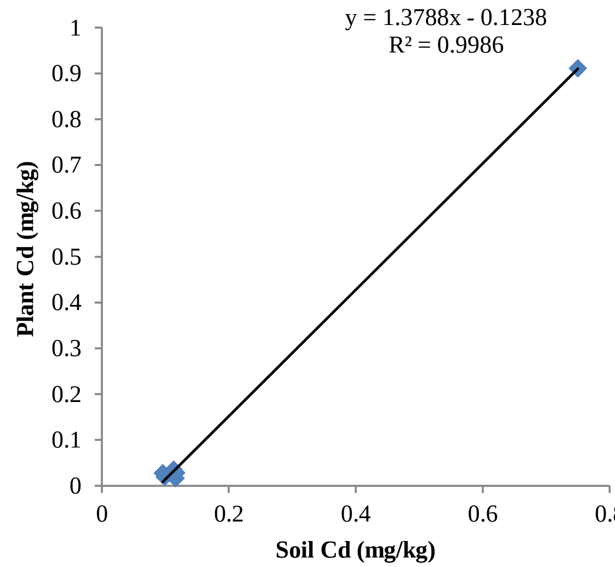

Figure 6.Regression analysis of soil and plant cadmium.

***Conclusion*:** Overall, the addition of Cd to spinach did not have any negative effect on yield/biomass of plant at 100 µM Cd concentration. Rather Cd addition alone to plant increased the biomass significantly than in the presence of other amendments. Cow dung proved to be more helpful as an amendment to stabilise Cd in soil and reducing its uptake significantly. However, charcoal was not found effective in retaining Cd in soil and Cd was more available in spinach biomass under this treatment. By increasing use of cow dung for agricultural activities, it could be effective organic manure and sorbent for contaminants' stabilization. It is cost effective and futher research is needed to find added value of this natural sorbent in industrial applications.

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