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Nutrient shifts modeling in Fenugreek (*Trigonella corniculata* L.) under biochar and cadmium treatments

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Abstract

Amelioration of cadmium (Cd) toxicity to plants through use of biochar (BC) is a promising technique. However, a little is known about interactive effect of biochar and Cd on nutrient shifts in plants. In present study, fenugreek (*Trigonella corniculata* L.) was grown in a soil-culture pots amended with three levels of BC (0%, 3% and 5%) and four levels of Cd (0, 25, 50 and 100 mg Cd kg^{-1} soil). After harvest, the modeling of nutrients shift under Cd and BC treatments was performed to check the uptake of various nutrients (N, P & K). For nutrients modeling an emerging technique vector analysis was used as it gives comparative results of relative nutrient concentration, contents and relative dry weight in a graphic format. The vector analysis explores the nutrients shifts by arrow movements in cadmium and biochar treatments. The relative concentration, contents of nitrogen, phosphorous, potassium and relative dry weight give treatment specific response as their increased or decreased is not related to specific treatments of cadmium and biochar but biochar showed positive influence on nitrogen and phosphorous concentrations and contents of *T. corniculata*. So, BC is useful in increasing uptake of nitrogen and phosphorous under high cadmium stress.

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Introduction

The status of nutrients in a plant is a very important determinant of plant growth and performance. The nutrient concentration is needed to be examined in agricultural field for better plant growth and more yields. The technique of vector analysis has been utilized from the former few spans to scrutinize responses of plants to various treatments. The basis for interpreting vector results lies in the association between plant nutrient concentrations and its growth (Larson and Isebrands 1972). Effect of any treatment will be clearly shown by nutrient concentrations and plant dry weights. As a handful tool researchers have been used this method to observe responses of plant to various treatments.

On the other hand, various other techniques (The Diagnosis, Recommendation integration system) are available to examine nutrient status of the plant but vector analysis is a technique which allows the comparison of growth, nutrient concentrations and nutrient content in a single graphic format (Hase and Rose, 1995). Similarly, vector analysis permits for a synchronized contrast of plant growth, nutrient-concentration, and nutrient-content in an integrated graphic format. The nutrients concentration and their uptake by the plants are greatly affected by heavy metals especially cadmium in soil.

As, cadmium (Cd) is a toxic heavy metal that has no defined biological role. It enters the food chain through use of contaminated irrigation water such as raw sewage water. In Pakistan, the practice is very common because of ready availability and low cost. After continuous use of such waters Cd accretion in numerous crops of Pakistan is one of foremost danger and scorching problem (Ahmed *et al.,* 1994). Though Cd is nonessential for plants but plants can uptake it through roots. In plants Cd affects the physiology and causes nutrients (K, Mn, Ca, Mg, and Fe) imbalances (Larbi *et al.,* 2002; Wallace *et al.,* 1992 (Dong *et al.,* 2006; Larbi *et al.,* 2002; Greger *et al.,* 1991).

The entry of Cd to crop plants can be avoided by use of various amendments. Use of biochar (BC) to remediate the Cd polluted soil has become a promising technique (Verheijen *et al.,* 2010). Biochar is a black-carbon biological complex (Verheijen *et al.,* 2010) that could be exploited as peat. It is porous and fine-grained substance that is well thought-out as a soil conditioner which can enhance the water holding capacity, CEC, pH of soil and remains in soil for a very long period than any other organic amendment (Downie *et al.,* 2011; Amonette and Joseph, 2009; Thies and Rillig, 2009; Gundale and DeLuca, 2007). It is prepared by pyrolysis of organic wastes in anaerobic conditions (Schnitzer *et al.,* 2007; Tagoe *et al.,* 2008). The prepared BC contains carbon, hydrogen, oxygen, calcium, nitrogen, potassium and ash. As a consequence the discharge of greenhouse gases in environment is diminished (Lehmann *et al.,* 2006). Biochar additions retain the nutrients in rhizosphere specially nitrogen that increase the vegetative growth and upsurge the efficacy of fertilizer practice (Steiner *et al.,* 2008). Phosphorous, calcium and potassium stored in biochar during pyrolysis boost up the plants growth via providing these elements in additional amount (Glaser *et al.,* 2002). Prendergast-Miller *et al.,* (2011) recommended that the vegetable which is grown in the presence of biochar had larger rhizosphere, where biochar elements becomes gathered in huge amount as paralleled to soil. These elements provide an easy uptake of nutrition like potassium, phosphorus and nitrogen. In addition biochar reduces the uptake of metals by the plants in contaminated conditions due to its structural modifications (Beesley *et al.,* 2010, Uchimiya *et al.,* 2010a).

Though many studies report reduced bioavailability of metals after application of BC, a little is known about interactive effect of BC and Cd on nutrient shifts in plants. The objective of present study was to interpret the impact of BC application on macronutrients (N, P, K) movement and accumulation in various parts of fenugreek (*Trigonella corniculata*) with respect to dry weights of plant. For this purpose the vector analysis technique was used as the display of three parameters in graphs may be difficult and puzzling, however, the graphing through vector analysis is a very simple depiction of drawing relative nutrient concentrations alongside relative nutrient content and relative dry weight. Each and every point on the display of vector diagram embodies the degree and directional move of every nutrient from the control treatment and their distances predict the receptiveness of the nutrient and treatment being analyzed. It is the first time to analyze nutrients shifts in plants in response to various treatments by vector analysis method. It was hypothesized that biochar decreases the toxic effects of Cd and increases the nutrient bioavailability to fenugreek and this shifts was study by vector analysis technique.

Materials and methods

Plant growth

In present study, Fenugreek (*Trigonella corniculata* L.) was grown in soil culture pots amended with three levels of biochar (0, 3, and 5% w/w) and four levels of cadmium (0, 25, 50 and 100 mg Cd kg^{-1} soil, using $CdCl_2$). The soil used in pots was claey loam. The calculated quantities of BC and Cd salt were mixed with 5 kg soil before pot filling and each treatment was replicated four times. The feedstock of BC was cotton stalks, treated at 450°C for 4 h in a specially designed double-walled stainless steel furnace. The seeds of fenugreek purchased from a native market were sown and at the seedling stage, 10 plants per pot were allowed to grow. The plants were irrigated regularly using tap water to maintain 50% of the water holding capacity.

Nutrient analysis

At maturity, the plants were harvested and fresh and dry biomasses were determined. The concentration of Cd in the plant samples were determined using di-acid digestion followed by measurement with atomic absorption spectrophotometry (Rashid, 1986). The nitrogen concentration in the plant samples was determined using kjeldahl distillation after digestion with H_2SO_4 (Schouwenberg and Walinge, 1973). For phosphorous and potassium determination, the plant samples were wet digested with HNO_3-$HClO_4$ (Chapman and Pratt, 1961). After digestion, phosphorous concentration was determined using colorimetric method (by Ammonium Vanadate-Ammonium Molybdate yellow color method), and the concentration of potassium was determined using flame photometer. The content of N, P, K and Cd were calculated by multiplying the respective concentration with dry matter.

Vector analysis

In vector analysis the nutrient content (x), nutrient concentration (y) and plant dry weight (z) satisfy the equation: $x=f(y,z)$. The values of nutrient concentration, content and plant dry weights are normalized in order to assist evaluation with a common point. The values of other treatments are than plotted and then arrows from the common or reference point are added for the clear representation of nutrients and dry weight shifting (Haase and Rose, 1995). The normalized values are known as relative values of nutrient concentration, content and dry weights.

Results

Effect of biochar and cadmium on nitrogen consumption and dry weight of plants

Nitrogen (N) shift modeling of fenugreek with respect to relative N concentration, content and relative dry weights of shoot and root under different application rates of cadmium and biochar is presented in Fig. 1. By analyzing arrow shifts in vector diagram it was observed that there was less effect of various Cd treatments (25, 50 & 100 mg Cd Kg^{-1} soil) on relative N contents, concentrations at different biochar applications rates (3% & 5%) which is noted by arrow shifting towards increased levels in case of root but at 5% BC application the T_2 (50 mg Cd Kg^{-1} soil) treatment showed some declined arrow shift in relative N concentrations and contents as compared to 3% BC. Whereas, in the case of shoot more concentration and contents of N with relative dry weights were noted at 0% BC, correspondingly, vector analysis interpretation suggested the dire shifting of arrows at T_2 and T_3 of 3% BC and T_1 (25 mg Cd Kg^{-1} soil) of 5% BC but this dropped shifts toward amplification at T_2 (50 mg Cd Kg^{-1} soil) treatment of

5% BC.

Effect of biochar and cadmium on phosphorous consumption and dry weight of plants

The analysis of vector with respect to relative phosphorous (P) concentration, contents and relative dry weights athwart numerous treatments of biochar and Cd in different parts (root & shoot) of fenugreek is presented in Fig. 2. Conferring to elucidation of vector analysis, without biochar amendment, there were more relative P contents, concentrations and relative dry weight in shoot. Although, the amassed application of biochar from 0% to 3% BC improved the relative dry weights of root with the decrease of P concentration and content as compared to some treatments of 0% biochar. Whereas, at 5% BC the vector analysis tells that the root behave treatment explicit with respect to relative P concentration, content and relative dry weights, as, the relative P concentration and contents of root increase with the increase of relative dry weight but opposite trend was true for shoot attributes. The more and more values of relative phosphorous concentration and contents of root were noted at T_2 (120.0 & 132.08) and T_3 (93.33 & 83.50) of 5% BC.

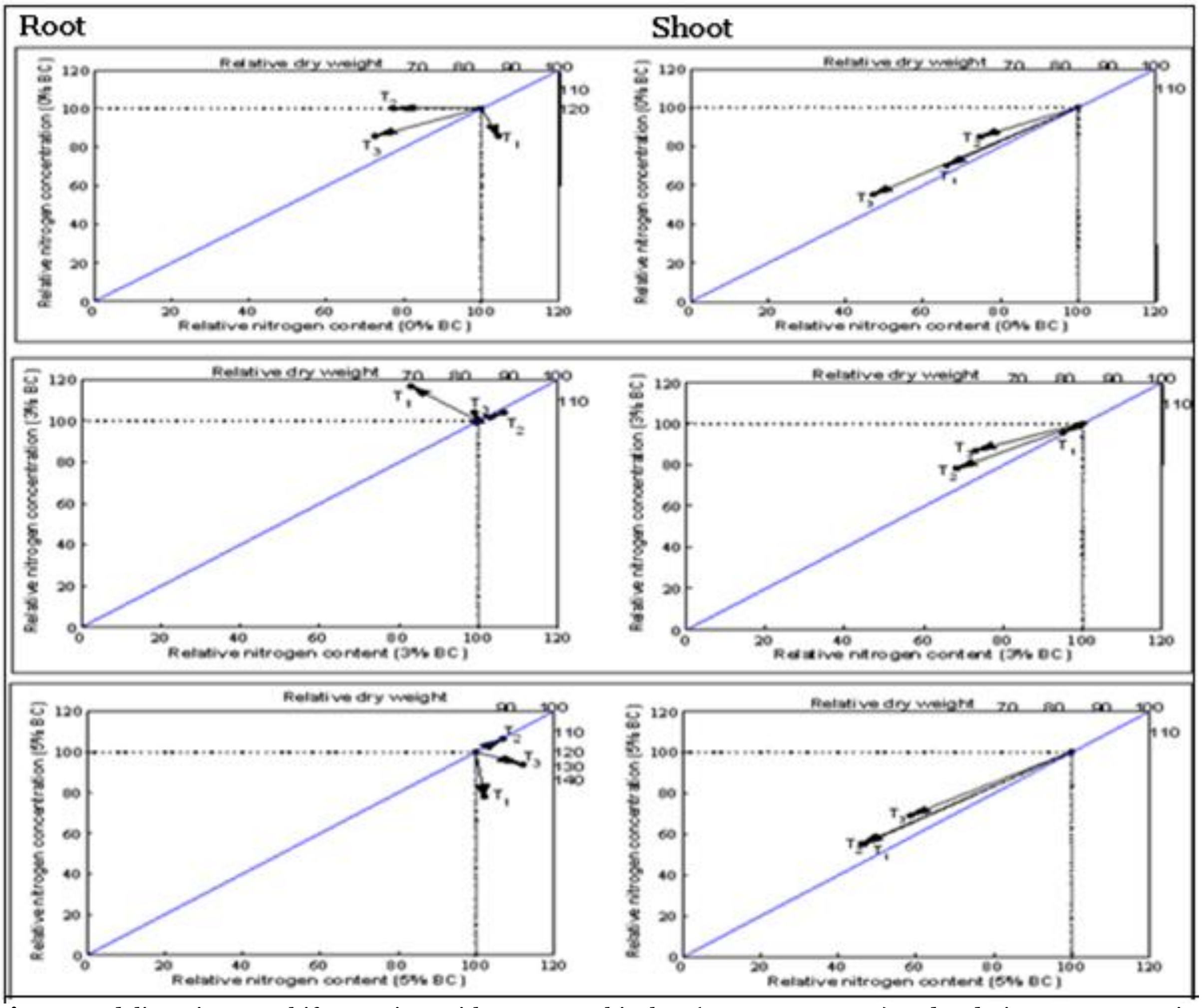

Fig. 1. Modeling nitrogen shift over time with respect to biochar (0%, 3% & 5% BC) and cadmium treatments in root and shoot of *Trigonella corniculata*. Note T_1 = 25 mg/kg Cd, T_2 = 50 mg/kg Cd & T_3 = 100 mg/kg Cd.

Effect of biochar and cadmium on potassium consumption and dry weight of plants

From the root/shoot vector analysis diagrams (Fig. 3) of fenugreek it was perceived that the relative potassium (K) concentrations and contents of shoot decreased with upturn of biochar application rate from 0% BC to 5% BC under various Cd treatments cadmium (0, 25, 50 & 100 mg Cd Kg^{-1} soil). The interpretation of arrow shifts in vector diagrams exhibited declined trend of relative K concentration and content at 3% and 5% BC in the shoot of *T. corniculata* than 0% BC the opposite was true for potassium contents of root at 5% BC. Although, the increased of biochar application from 0% BC to 3% BC caused an increase in the relative unit dry weights of root. Whereas, at 5% BC the vector analysis tells that the root perform treatment specific with respect to relative potassium content, as, this parameter of root increase with the increase of relative dry weight but opposite trend of potassium contents was true for shoot attributes.

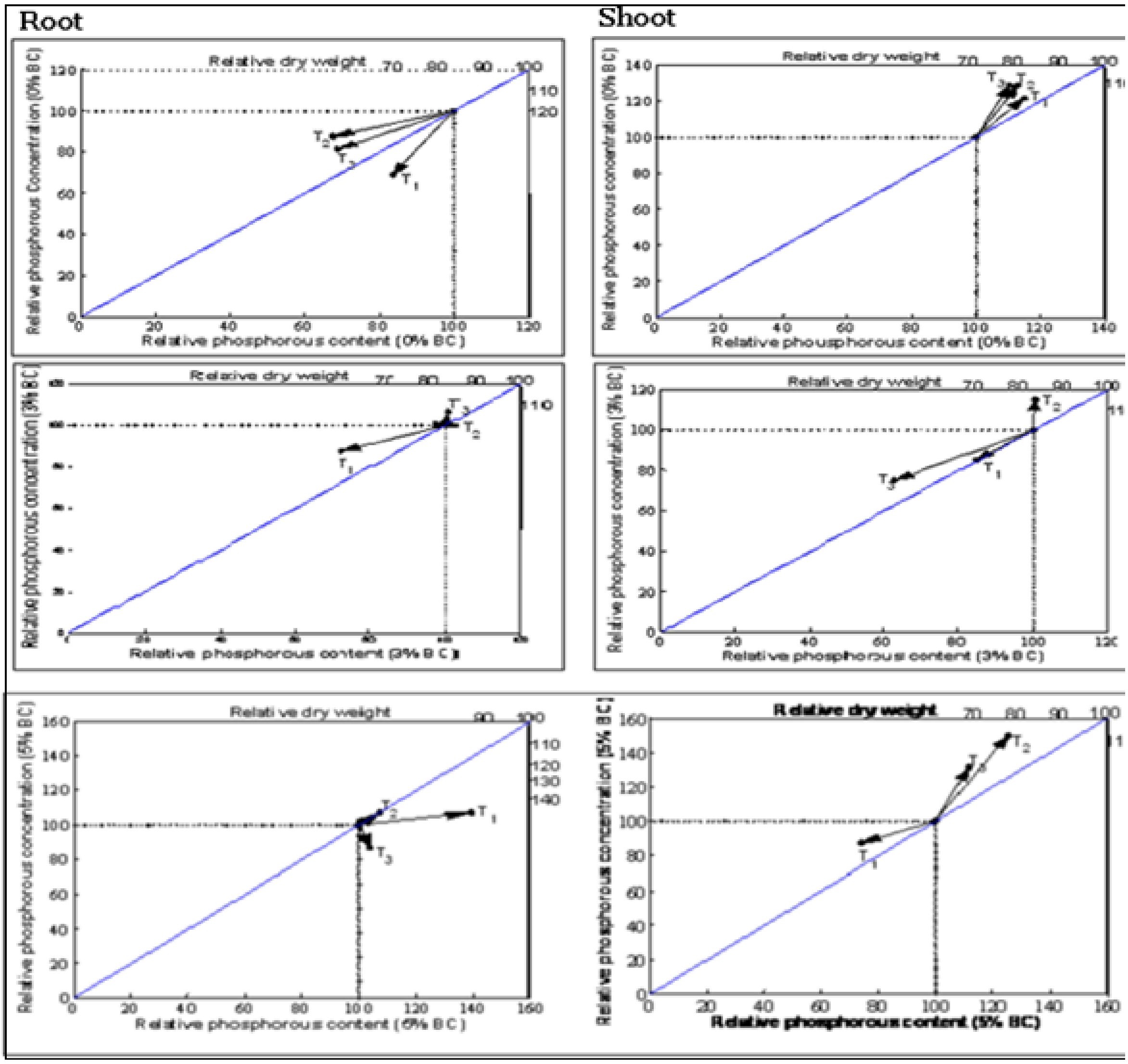

Fig. 2. Modeling Phosphorous shift over time with respect to biochar (0%, 3% & 5% BC) and cadmium treatments in root and shoot of *Trigonella corniculata*. Note T1 = 25 mg/Kg Cd, T2 = 50 mg/Kg Cd & T3 = 100 mg/Kg Cd.

Effect of biochar on cadmium consumption and dry weight of plants

The arrow interpretation of relative cadmium (Cd) content against relative Cd concentration of *fenugreek* across various treatments of Cd and biochar is given in Fig. 4. Rendering to vector analysis, there was spare of Cd concentration and content in plant samples at different treatments of cadmium (0, 25, 50 & 100 mg Cd Kg^{-1} soil) and 0% BC, but with biochar amendment at 5%, the drastic shifts in arrows was noted which describe the decrease of relative cadmium concentration and contents in the shoot of plant samples. This declined was clearly observed by vector diagrams of cadmium. Similarly, the cadmium concentrations in the root samples showed a positive declined at 5% BC.

Discussion

As hypothesized, use of biochar as soil amendment enhanced the nutrients bioavailability that is shown in Figures 1, 2, 3 & 4. The treatments where no biochar was applied, Cd caused negative effects on nutrient homeostasis as it was observed in decreased nutrient content and concentrations. In these treatments, Cd concentration was increased in the plant roots that might be due to interference of Cd with nutrient uptake by changing plasma membrane permeability (Li *et al.,* 2012; Dong *et al.,* 2006).

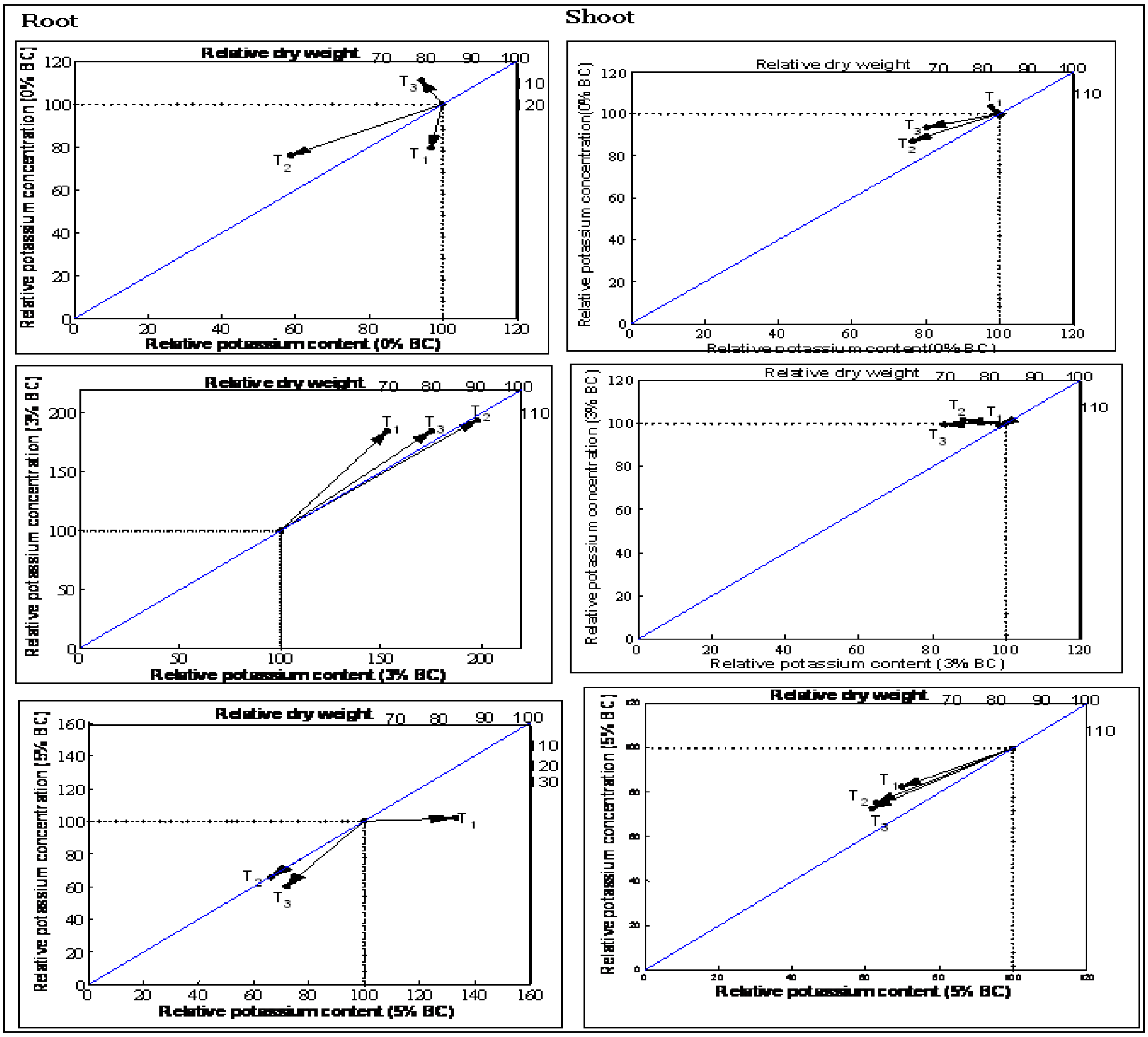

Fig. 3. Modeling Potassium shift over time with respect to biochar (0%, 3% & 5% BC) and cadmium treatments in root and shoot of *Trigonella corniculata*. Note T1 = 25 mg/Kg Cd, T2 = 50 mg/Kg Cd & T3 = 100 mg/Kg Cd.

In the present study, the uptake of N increased with increasing BC application from 0% to 3% at different concentrations of Cd, which specify that the influence of Cd was minor on N uptake by fenugreek merely in the existence of biochar. Biochar application may support in transformation of nitrogen, possibly refining its obtainability by plants. The vector analysis (Figure 1) clearly demonstrated that by the use of BC at 3%, the root N content increased very swiftly at different treatments of cadmium which is illustrated by arrow shifts in diagrams. There are two reasons for this, firstly; soil fauna is accountable for biotic N fixation and for N-mineralization and secondly is that biochar prepared from plants contains numerous N holding structures. On pyrolysis the compression of these structures from heterocyclic nitrogen compounds (Cao and Harris 2010; Novak *et al.,* 2010; Steiner *et al.,* 2008; Chan *et al.,* 2007; Koutcheiko *et al.,* 2006), thus demonstrating that biochar has ability to improve the efficiency of inorganic N fertilizer (Joseph *et al.,* 2010).

Ouzounidou *et al.,* (1995) reported that the uptake of heavy metals not only causes the chromosomal aberration but also division of cells become abnormal that significantly decreases the growth of plants. Abiotic stresses such as heavy metals induced stress can be demonstrated in term of dry biomass because dry biomass is the total outcome of different characters. In our study, BC application improved the dry biomass that indicates reduction in metals induced stress. Similar results were observed for root dry biomass of plants in our study.

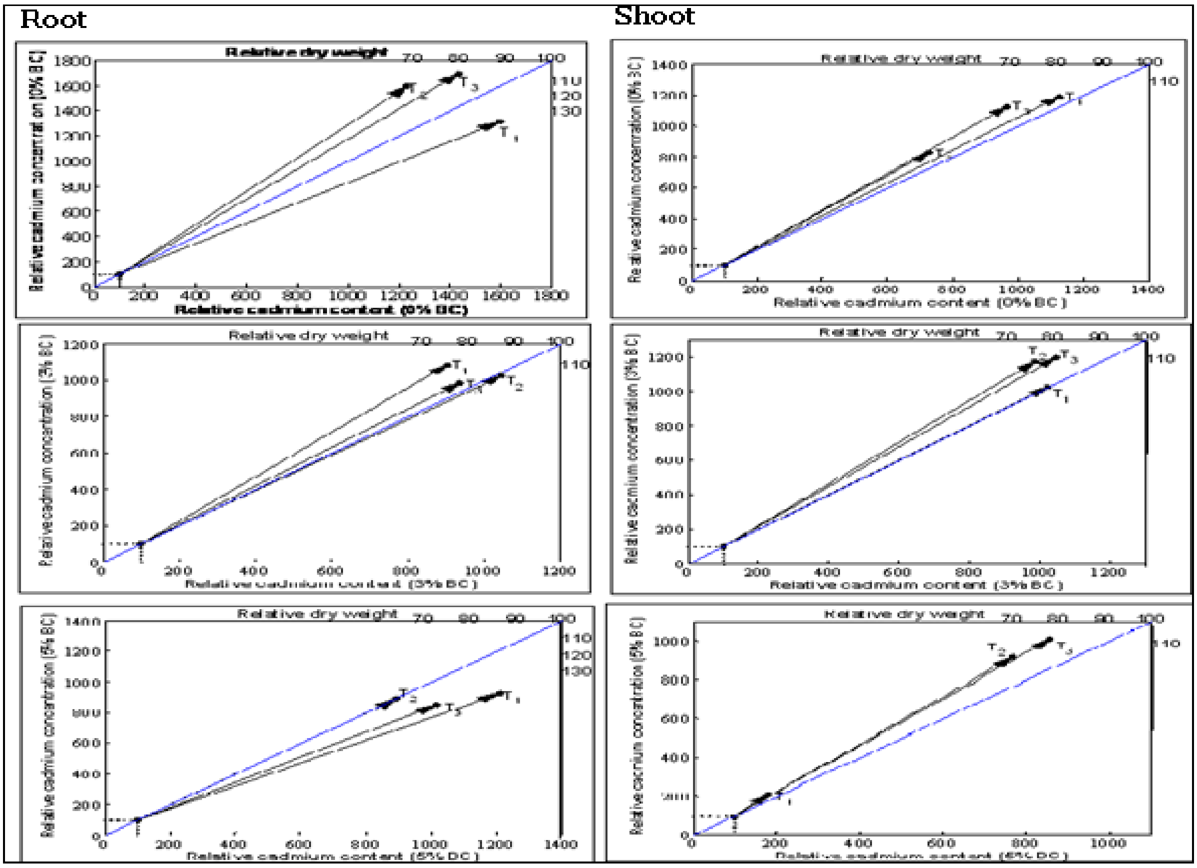

Fig. 4. Modeling cadmium shift over time with respect to biochar (0%, 3% & 5% BC) and cadmium treatments in root and shoot of *Trigonella corniculata*. Note T_1 = 25 mg/kg Cd, T_2 = 50 mg/kg Cd & T_3 = 100 mg/kg Cd.

The concentration and content of P were slightly increased in root of fenugreek and decreased in shoots due to the Cd contamination in the growth medium. However, after application of BC at increasing rates, it enhanced the concentrations and contents of root P. This increase was mainly due to fact that biochar is a direct source of P as it stores P at superficial sites by its anion exchange capacity. Moreover BC acts as pH modifier thus enhance phosphorous mineralization (DeLuca *et al.,* 2009). Bestowing to the Richardson *et al.,* (2009) the roots health depends on the P bioavailability. Similar trend of relative dry weights of root was observed in the present study. The highest value of relative root dry weight was noted at 25 mg Cd kg^{-1} soil amended with 3% BC. Park *et al.,* (2011) also observed similar trend of improvement in plants growth was and it was suggested that the addition of sludge's biochar in tomato gives 74% more yield as compared to the control. This upsurge in crop can be owed to enhanced photosynthesis by efficiently uptake of nutrients by plants (Lehmann *et al.,* 2006).

In the present study, shoot and root K concentration was decreased at 25 mg Cd kg^{-1} soil but increased at T_3 (50 mg Cd Kg^{-1} soil) in soils amended with 3% and 5% BC, whereas, at other treatments decreasing trend was observed. This increase in potassium at 100 mg Cd kg^{-1} soilwas due to the large concentrations of exchangeable K in biochar (Chan *et al.,* 2007) but the similar decrease in K of root and shoot was observed by Kevresan and Petrovic (2003) in pea and by Abdel-Latif, 2008 in wheat plants under Cd. Cadmium causes membrane depolarization and upward conductance of K that results in decreased concentration in roots (Li *et al.,* 2012).

According to Beesley and Marmiroli (2011) sorption is the mechanism through which metals can be immobilized in soils through biochar addition. Bilgic and Caliskan (2001) suggested that π-electrons play a vital role in the immobilization of metals when biochar is applied. These π-electrons are part of aromatic functional groups like –OH,–COOH and C=N. Uchimiya *et al.,* (2010b) suggested the semi sorption of surface by d-electrons in assistance with π-electrons that is major cause of heavy metals mobility reduction due to biochar addition. Similar trend of metals intake was observed by Park *et al.,* (2011) where they successfully immobilized the metals using sludge biochar as that in our experiment where cotton sticks biochar significantly decrease the Cd intake in *Trigonella corniculata* L.

Conclusions

It is concluded that in the case of *Trigonella corniculata* the BC plays a treatment specific role in modeling of nutrient shifts. Although, neither a similar trends (increased or decreased) in nutrients concentrations (nitrogen, phosphorous and potassium) were noted at various biochar and cadmium treatments. So, it is concluded that biochar amendment is useful at high cadmium concentration because increase of nutrients was noted at 100 mg Cd Kg^{-1} soil. Therefore, it is concluded that BC applications with high Cd stress proved to be useful in increasing nutrients uptake especially nitrogen and potassium by *T. corniculata*.

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