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# *Biochar Mitigates Cadmium Stress on Alfalfa Seeds During Germination*

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***Abstract* – Cadmium (Cd+2) is a ubiquitous toxic heavy metal (HMs) in the environment. Cadmium chloride was used in three concentrations (0.001, 1, and 5 mM) in presence and absence of Biochar (BC) to evaluate the efficiency of BC in remediation of the Cd toxicity on seed germination. Germination percentage, radicle length, fresh and dry weight gradually decreased with increasing Cd concentration and completely inhibited at 5 mM CdCl2. Seedling vigor index (SVI) and metal tolerance index (MTI) decreased, while phyto-toxicity (%) increased with increasing Cd concentration to become 100% at 5 mM CdCl2. Cd-stress increased malondialdehyde (MDA), hydrogen peroxide (H2O2) and proline content, whereas the total soluble sugars, total soluble proteins, DNA and RNA content decreased. Activity of catalase (CAT), peroxidase (POX) ascorbic acid oxidase (ASAO), polyphenol oxidase (PPO) increased under Cd-stress conditions. On the other hands, Cd negatively affected α-, and β-amylases and protease enzymes. Cd application also caused a great alteration in the permeability of membranes through increasing ion leakage and lipid peroxidation. Application of BC significantly alleviated the inhibitory effects of Cd and consequently, increased germination percentage, SVI and MTI. Phytotoxicity and inhibition value of radicle decreased by BC treatment to become 50% at 5 mM CdCl2. Increasing the germination percentage by BC treatment was associated with increasing the activity of the antioxidants and the hydrolytic enzymes under Cd stress.**

***Keywords* – Amylase; Antioxidant Enzymes; H2O2; MDA; Nucleic Acids; Protease.**

## *I. INTRODUCTION*

Cd is a highly toxic environmental pollutant which causes oxidative stress [1]. It is difficult to degrade and is readily taken up by plant roots and transported to aerial parts, thus entering into the food chain causing health problems in animals and humans [2]. The high concentration of Cd which observed in many agricultural fields is caused by long-term use of phosphate fertilizers, sewage sludge application, and wastewater irrigation [3]. After long-term exposure to Cd, roots became mucilaginous, brown, decomposed, decreasing the number of plumules and root elongation. Cd inhibits lateral root formation and causes the main root to become brown, rigid, and twisted [4], resulting in reduced growth and death of the plant [3]. Seed germination is an important stage of the plant growth cycle and highly sensitive to changes in the surrounding environment [5]. Reference [6] reported that barley, rice, and wheat subjected to HMs stress recorded a reduction in germination (%) and seedling length. Complete germination (from early imbibition to radicle protrusion) requires protection by antioxidants from ROS that are normally produced during these stages [7].

BC is a carbonaceous porous substance synthesized as a result of pyrolysis of organic feed stocks such as plant materials, organic manures and sludges [8]. BC has some specific physicochemical properties, i.e. large surface area for sorption of metals, alkaline nature, ash, carbon content, a microporous structure, O-containing functional groups, as well as a high pH value, cation exchange capacity (CEC) [9], [10]. BC had the ability to immobilize toxic HMs, which place it among good HMs remediators. Since having large surface area that available for sorption by forming heavy metals-BC complexes either by exchange of HM cations with other metal cations (i.e., Na, K, $Ca^{+2}$, $Mg^{+2}$) at functional groups that present in BC or by physical adsorption [11].

Alfalfa was chosen for this study because it is the oldest and the most important forage crop in the worldwide. It is one of the most important crops used for livestock feeding in Egypt, but there is a gap between the demand and the consumption of green forages. Thus, the present work aimed to evaluate the ability of alfalfa seeds (*Medicago sativa* L.) to germinate under Cd stress in absence and presence of BC for remediation purposes.

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## *II. Research Methods*

### 1. Plant Material:

Seeds of alfalfa (*Medicago sativa* L.) were kindly obtained from Legumes Crops Department; Field Crops Research Institute; Agricultural Research Centre, Giza, Egypt. BC was produced from rice husk stems. Rice husk stems were cut into pieces (10-20 cm) and after drying, the biomass was pyrolyzed at 300$^{o}$C for 2hrs, followed by quenching and subsequent drying in an oven at 105$^{o}$C. BC was then crushed in a 24-blade variable-speed rotator mill according to [12]. Finally, BC was grinded and sieved until obtain a uniform 50-75 µm particles size. The grinded and un-grinded BC samples were visualized using scanning electron microscope (SEM) (Fig. 1). $Cd^{+2}$ was applied as $CdCl_2$. $H_2O$.

### 2. Germination Experiment:

Seeds of alfalfa were surface sterilized with 2.5% sodium hypochlorite solution for three minutes, and thoroughly rinsed three times with distilled water. Cd was applied at three concentrations 0.001, 1 and 5 mM of $CdCl_2$. $H_2O$. The experiment was carried out in presence and absence of BC (0.5 %, w/v) for 4 days in the dark at 25$^{o}$C. Seeds of alfalfa were spread on a filter paper in Petri dishes (25 seeds). Distilled water was used as control. Each treatment had four replicates. The seeds were considered germinated once the radicle emerged to about 2 mm. Some parameters were measured on the 4$^{th}$ day of germination:

**2.1. Germination percentage, seedling fresh and dry weight.**

**2.2. Seedling vigor index (SVI)** was calculated according to [13] by multiplying the germination percentage with radicle length.

**2.3. Percentage of phytotoxicity and inhibition value of radicle** was calculated using the formula of [14]:

$$\text{Percentage of Phytotoxicity} = \frac{\text{Radicle length of control- Radicle length of test}}{\text{Radicle length of control}} \times 100$$

**2.4. Metal tolerance index (MTI)** was calculated using the formula of [15]:

$$\text{Metal Tolerance Index (MTI)} = \frac{\text{Radicle length of metal polluted seeds}}{\text{Radicle length of control seeds}} \times 100$$

### 3. Enzyme activity assays:

Alfalfa seeds were homogenized at a ratio of 1:2 (w/v) with 0.1 M potassium phosphate buffer, pH 7.0. Extracts were filtered through nylon cloth and centrifuged at 1000 xg, for 30 min at 4˚C. Enzymes activity was assayed by spectrophotometric methods during germination (4-d-old).

### 3.1. Hydrolases:

**3.1.1. α- Amylase (E.C. 3.2.1.1)** activity was assayed by using soluble starch in phosphate buffer (pH 7.0) as a substrate, and 3,5-dinitrosalicylic acid as a color reagent [16].

**3.1.2. β- Amylase (E.C. 3.2.1.2)** activity was assayed by using soluble starch in acetate buffer (pH 4.8) as a substrate, and 3, 5-dinitrosalicylic acid as a color reagent [16].

**3.1.3. Protease (EC 3.4.21.40)** activity was assayed according to [16] method. Casein in 0.1 M phosphate buffer (pH 7.5) was used as a substrate. The reaction was terminated by addition of tricarboxylic acid (TCA). The soluble peptides content of TCA- component was measured

after reaction with Folin-phenol reagent as described by [17].

**3.2. Antioxidant enzymes:**

**3.2.1. Catalase (CAT) (EC. 1.11.1.6)** was measured according the method of [18], the assaying mixture contained 1 ml of $H_2O_2$ (65 mM $H_2O_2$ in Na/K P pH 7.4) and 0.2 of enzyme extract. The decomposition of $H_2O_2$ was followed by the decline in absorbance at 405 nm.

**3.2.2. Peroxidase (POX) (EC. 1.11.1.7)** activity was measured by the guaicol oxidation method according to [19]. The reaction was initiated by adding 0.1 ml enzyme extract to 2.2 ml (pH 7) potassium phosphate buffer, 0.5 ml of 0.018 mM guaicol and 0.2 ml $H_2O_2$ 30 %. Changes in absorbance every 30 seconds up to 3 minutes were recorded at 470 nm.

**3.3. Oxidase enzymes:**

**3.3.1 Ascorbic acid oxidase (ASAO) (EC. 1.10.3.3)** activity was measured according to the method of [20] and [21]. To a clean quartz cuvette of a UV spectrophotometer 1 ml of 0.2 M phosphate buffer pH 6.2, 0.2 ml of 1mM ascorbic acid and 0.2 ml of enzyme extract were added. Distilled water was added to bring volume to 3.0 ml. The initial absorbance ($A_O$) was recorded and then the optical densities after 30 sec intervals up to 3 minutes to follow the rate of disappearance of ascorbate at 265 nm.

**3.3.2. Polyphenol oxidase (PPO) (EC. 1.10.3.1)** activity was assayed by the method described by [22]. To a clean cuvette of a UV spectrophotometer 3.0 ml buffered catechol solution (0.01M catechol, freshly prepared in 0.1 M phosphate buffer, pH 6.0) and 1ml of enzyme extract were added. Changes in absorbance every 30 seconds up to 5 minutes were recorded at 495 nm.

**4. Chemical Analysis:**

The germinating seeds (4-d-old) were extracted in 70% ethanol and diluted to volume for estimating the total soluble sugars and total soluble proteins content.

**4.1. Total soluble sugars** content was determined by using anthrone reagent (2 g $L^{-1}$ $H_2SO_4$) as described by [23].

**4.2. Total soluble proteins** content was estimated by using Folin-phenol reagent in alkaline medium containing copper sulphate according to [17].

**4.3. Proline content** was estimated according to [24]. Samples were extracted with 3% sulfosalicylic acid, and acid-ninhydrin was used as a color reagent.

**4.4. Nucleic acids** were extracted in Tris–EDTA buffer pH 8.5 according to [25]. DNA was measured according to [26] by using diphenylamine reagent (DPA). RNA was determined using the method adopted by [27] using orcinol reagent.

**4.5. Hydrogen peroxide ($H_2O_2$)** content was determined according to [28]. Samples (0.3 g) were homogenized with 4 ml of 0.1% (w/v) TCA in ice bath. The homogenate was centrifuged at 12,000 ×g at 4°C for 15 minutes and mixed with 0.5 ml of the supernatant with 0.5 ml of 10 mM potassium phosphate buffer (pH 7.0) and 1 ml KI was also added. Then, absorbance was read at 390 nm and $H_2O_2$ content was calculated by using standard curve.

**4.6. Lipid peroxidation**, malondialdehyde (MDA) in the germinated seeds was determined as 2-thiobarbituric acid (TBA) reactive substances using the method of [29]. For each assay, 10 primary root tips were homogenized with 10 ml of TBA reagent using a pestle and mortar (TBA reagent was prepared by mixing 18% TCA with 0.45% TBA in a 1:2 ratio). The extract was kept in a boiling water bath for 15 min, and filtrated. Absorbance of the filtrate was measured at 532 nm using Cecil CE. 1010 spectrophotometer. The values were corrected for non-specific turbidity by subtracting the absorbance at 600 nm. MDA equivalents were calculated by the method of [30]:

MDA equivalents ($nmol.cm^{-1}$) = 1000 [(Abs 532 - Abs 600nm)/155].

**4.7. Electrolyte leakage**: Total electrolyte leakage was estimated in the roots. Primary root tips were excised and washed briefly with deionized water to remove adhering electrolytes. The tissue sections were then immersed in test tubes containing 20 ml deionized water stirred continuously at 28 ˚C. After 5 h, conductivity meter estimated the electrolyte leakage. The samples were then boiled for 30 min. and the conductivity measured again. The percentage of leakage was calculated as: 100 (Conductivity before boiling / Conductivity after boiling) as described by [31].

**5. Scanning Electron Microscopy (SEM)**

BC samples (grinded and un-grinded) were coated firstly with gold using EMITECH K550X sputter coater (England). Samples were analyzed using SEM Model; Quanta 250 FEG (Field Emission Gun) attached with EDX Unit (Energy Dispersive X-ray Analyses), with accelerating voltage 30 K.V., magnification14x up to 1000000 and resolution for Gun.1n). FEI Company, Netherlands. At Central Laboratories Sector, the Egyptian Mineral Resources Authority, the Ministry of Petroleum.

**6. Statistical analysis:**

Statistical analysis was carried out according to the method of complete randomized blocks design (CCRBSE. Bas) using analysis of variance and the significance was determined using LSD values at p = 0.05 and 0.01 according to [32].

## *III. Result And Discussion*

Germination was negatively affected by the presence of Cd ions in the surrounding medium (Tab. 1). The results showed that increasing Cd concentration in the surrounding medium of alfalfa seeds led to decreasing germination percentage, fresh and dry weight and radicle length at 0.001 and 1 mM Cd, while severely inhibited at 5 mM $CdCl_2$. Phytotoxicity percentage and radicle inhibition were increased in comparison with control to become 100% at 5 mM $CdCl_2$ (Tab. 2). This was further corroborated by the reduction in vigor index (VI) and metal tolerance index (MTI) under Cd stress compared to control. This might be due to inhibition of the hydrolytic enzymes (α- and β-amylases, and protease) activity during seed germination (Tab. 3) which convert the stored food (e.g., starch and protein) into simple forms (e.g., sugars and amino acids) required for growth of the embryo axes, and the reduction of water uptake from the external medium. These results support [33] who reported that when activity of the hydrolytic enzymes decreases, the food does not reach to the embryo, thereby affects seed germination. Higher Cd concentration in the germination medium seems to prevent water uptake and water movement in the embryo axis [34], decrease the germination percentage, SVI and MTI, but increase phytotoxicity percentage [35]. At high Cd levels in the germination medium, Cd can interfere with trace and macro elements transport which is essential for seed germination [36]. Thus, some HMs-poisoning symptoms have been attributed to physiological and biochemical disturbances due to nutritional disorders.

The cellular content of total soluble sugars and protein decreased with increasing Cd concentration from 0.001 to 1 mM (Tab. 4). This might be due to inhibition of the hydrolytic enzymes α-, β-amylases and protease activity. These results are in harmony with [37] who reported that both α- and β-amylase activity decrease under Cr and Co stress on alfalfa germinated seeds. The decrease in amylase activity under Cd stress is assumed to decrease sugar availability to the developing embryo which may be one of the reasons behind germination inhibition.

DNA and RNA content of alfalfa germinating seeds progressively decreased with increasing Cd concentration in the external medium (Tab. 4). It has been suggested that high concentration of Cd inhibited cell division and elongation of the roots. This was in line with [38] who recorded a toxic effect in response to high concentrations of Cr and Co on cell division and induction of chromosomal aberrations in root tips of *Allium cepa*. Reference [39] recorded a decrease in RNA synthesis and increased activity of ribonuclease (RNase), leading to further decrease in RNA content under stress of Cu, Ni, Cd and Pb.

Proline content in the germinating seeds increased with increasing Cd concentration (Tab. 4). Proline has been considered as indicator for stress. Proline increases stress tolerance of plants through such mechanisms as osmoregulation, protection of enzymes against denaturation and stabilization of protein synthesis [40].

The total electrolyte leakage, lipid peroxidation (MDA content) and $H_2O_2$ content exhibited a marked increase with increasing Cd concentration (Tab. 5). The present results clarified that the Cd toxicity enhances the production of $H_2O_2$ as one of ROS that induces oxidative stress and attacks plasma membranes in the cells. Consequently, the plasma membrane becomes dysfunction due to peroxidation of its lipid content and high electrolyte leakage. Reference [41] reported that the major sources of ROS production (such as $H_2O_2$) in hydrated seeds during germination as well as the ROS targets can be attributed to mitochondrial activity, since the resumption of respiration in imbibed seeds can lead to electron ion leakage and increased production of ROS [42]. Seed germination reduction can be attributed to the changes of permeation properties of cell membrane [43]; the interference and alteration in the cell membrane permeation properties by $Cd^{+2}$, which resulted in reduction of water absorption and transport [44].

Activity of some antioxidant and oxidase enzymes such as CAT, POX, PPO and ASAO increased with increasing Cd concentration compared to control (Tab. 6). Increasing the activity of such enzymes was a response to the high production of ROS under Cd stress. The increased activity of the antioxidant enzymes may trigger the Cd-tolerance of alfalfa germinated seeds.

BC is an organic material produced via the pyrolysis of C-based organic wastes. The cations in the BC after pyrolysis transformed into oxides, hydroxides, and carbonates which are alkaline components act as a liming agent. So, alkalinity of BC is the main responsible for high pH values that result in HMs precipitation. The porous nature of BC could increase the water holding capacity, cation exchange capacity (CEC) and surface sorption

capacity. Thus, the high surface area of BC particles implies a high capacity for HMs adsorption on their surfaces.

In the present study, BC was produced from rice husk at 300$^{o}$C, grinded and sieved to obtain a uniform 50-75 µm particles size. This led to great increasing of the BC surface area. This was supported by scanning electron microscope (SEM) analysis of BC before and after grinding (Fig. 1). So, its capacity for cation adsorption greatly increased with increasing its surface area. So, when BC was added to Cd solutions (0.001, 1 and 5 mM), the Cd toxicity on germination had been significantly ameliorated. Similar results were obtained by [45].

BC may be enhanced alfalfa seed germination through excluding the Cd ions, consequently increasing the enzymes activity, metabolites, nucleic acids content, water uptake, and maintaining the membrane status. The membrane stability may be improved in presence of BC through increasing nutrients, cations exchange capacity and alleviating the membrane peroxidation that caused by Cd pollution. So, the electrolyte leakage was significantly decreased.

**TABLE 1**: Effect of different concentrations of Cd (0.001, 1 and 5 mM) on germination percentage (%), radicle length (cm), fresh and dry weight (g) of alfalfa germinated seeds after 4 days of germination, in presence and absence of biochar (0.5 %).Each value is a mean of four replicates.

| Biochar | Cd (mM) | Germination (%) | Radicle length (Cm) | Fresh weight (g) | Dry weight (g) |
|---|---|---|---|---|---|
| -- | 0.0 | 100 | 5.5** | 0.277** | 0.109** |
| | 0.001 | 83** | 5.0** | 0.214** | 0.087** |
| | 1 | 18** | 2.0** | 0.148** | 0.084** |
| | 5 | 0 | 0.0 | 0.0 | 0.0 |
| (0.5 %) | 0.001 | 100** | 6.0** | 0.336** | 0.136** |
| | 1 | 100** | 5.5** | 0.274** | 0.108** |
| | 5 | 36** | 2.7** | 0.213** | 0.080** |
| L.S.D. at 0.05* | | 2.843 | 0.394 | 0.005 | 0.007 |
| L.S.D. at 0.01** | | 3.885 | 0.537 | 0.0058 | 0.0087 |

**TABLE 2**: Effect of different concentrations of Cd (0.001, 1 and 5 mM) on seedling vigor index(SVI), phyto-toxicity and inhibition value of radicle (%), metal tolerance index (MTI) of alfalfa germinated seeds after 4 days of germination, in presence and absence of biochar (0.5 %).Each value is a mean of four replicates

| Biochar | Cd (mM) | SVI | Phyto-toxicity and Inhibition Value of radicle (%) | MTI |
|---|---|---|---|---|
| | 0.0 | 550.00** | 0 | 0 |
| -- | 0.001 | 376.40** | 15.23** | 84.76** |
| | 1 | 33.00** | 59.60** | 40.40** |
| | 5 | 0 | 100 | 0 |
| (0.5 %) | 0.001 | 603.30** | 0 | 100 ** |
| | 1 | 540.70** | 3.68** | 98.20** |
| | 5 | 95.13** | 51.10** | 48.90** |
| L.S.D. at 0.05* | | 31.417 | 5.546 | 6.710 |
| L.S.D. at0.01** | | 42.92 | 7.032 | 8.507 |

**TABLE 3**: Effect of different concentrations of Cd (0.001, 1 and 5 mM) on the activity of some hydrolytic enzymes (µg $g^{-1}$f.wt. $s^{-1}$) of alfalfa germinated seeds after 4 days of germination, in presence and absence of biochar (0.5 %). Each value is a mean of four replicates.

| Biochar | Cd (mM) | α-amylase | β-amylase | Protease |
|---|---|---|---|---|
| -- | 0.0 | 150.1** | 153.7** | 0.428** |
| | 0.001 | 138.0** | 143.5** | 0.395** |
| | 1 | 114.5** | 124.0** | 0.225** |
| | 5 | 0 | 0 | 0 |
| (0.5 %) | 0.001 | 155.0** | 157.8** | 0.429** |
| | 1 | 141.4** | 146.4** | 0.355** |
| | 5 | 81.4** | 58.5** | 0.141** |
| L.S.D. at 0.05* | | 2.160 | 0.634 | 0.010 |
| L.S.D. at 0.01** | | 3.317 | 0.867 | 0.014 |

**TABLE 4**: Effect of different concentrations of Cd (0.001, 1 and 5 mM) on total soluble sugars, total soluble proteins, proline and nucleic acids (mg $g^{-1}$ d.wt) content of alfalfa germinated seeds after 4 days of germination, in presence and absence of biochar (0.5 %). Each value is a mean of four replicates.

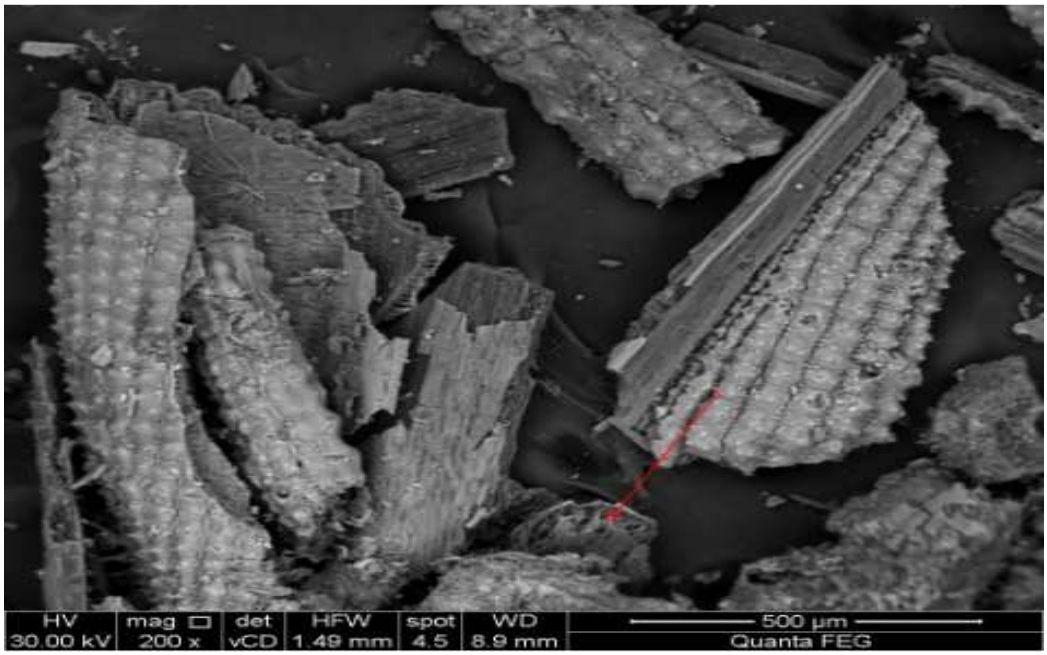
| Biochar | Cd (mM) | Total soluble sugars | Total soluble proteins | Proline | DNA | RNA |
|---|---|---|---|---|---|---|
| -- | 0.0 | 6.83** | 18.80** | 1.44** | 1.724** | 6.42** |
| | 0.001 | 5.97** | 17.30** | 1.55** | 1.383** | 3.40** |
| | 1 | 4.95** | 9.88** | 1.89** | 1.004** | 1.65** |
| | 5 | 0 | 0 | 0 | 0 | 0 |
| (0.5 %) | 0.001 | 6.95** | 18.84** | 1.40** | 1.719** | 6.45** |
| | 1 | 6.75** | 15.60** | 1.48** | 1.674** | 6.02** |
| | 5 | 3.70** | 6.19** | 0.50** | 0.983** | 4.37** |
| L.S.D. at 0.05* | | 0.007 | 0.254 | 0.075 | 0.0040 | 0.328 |
| L.S.D. at 0.01** | | 0.010 | 0.347 | 0.102 | 0.0058 | 0.447 |

**TABLE 5**: Effect of different concentrations of Cd (0.001, 1 and 5 mM) on the total electrolyte leakage (%), lipid peroxidation (MDA) content (µ mol $g^{-1}$f.wt) and hydrogen peroxide ($H_2O_2$) content of alfalfa germinated seeds after 4 days of germination, in presence and absence of biochar (0.5 %). Each value is a mean of four replicates.

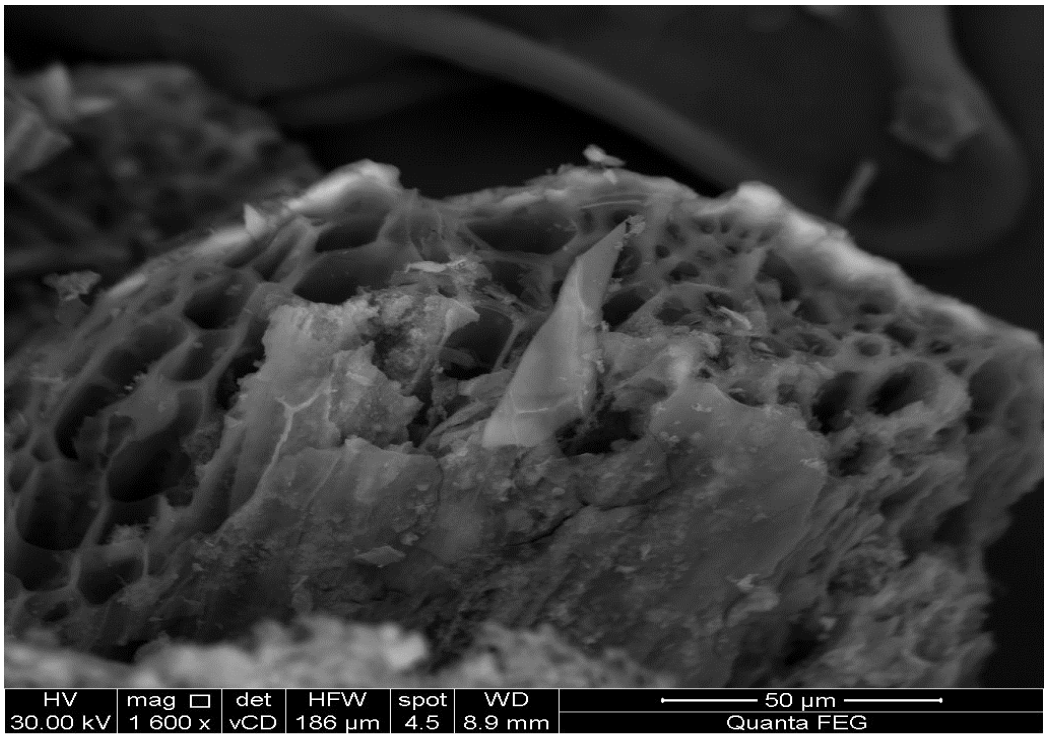
| Biochar | Cd (mM) | Ion leakage | Lipid peroxidation | $H_2O_2$ |
|---|---|---|---|---|
| -- | 0.0 | 9.0** | 1.00** | 0.274** |
| | 0.001 | 17.9** | 1.70** | 0.296** |
| | 1 | 35.3** | 2.04** | 0.540** |
| | 5 | 0 | 0 | 0 |
| (0.5 %) | 0.001 | 0.066** | 0.80** | 0.270** |
| | 1 | 0.089** | 0.81** | 0.287** |
| | 5 | 29.2** | 1.90** | 0.298** |
| L.S.D. at 0.05* | | 1.364 | 0.114 | 0.018 |
| L.S.D. at 0.01** | | 1.863 | 0.155 | 0.026 |

**TABLE 6:** Effect of different concentrations of Cd (0.001, 1 and 5 mM) on the activity of some antioxidants and oxidases enzymes ($\mu g\ g^{-1}$f.wt. $s^{-1}$) of alfalfa germinated seeds after 4 days of germination, in presence and absence of biochar (0.5 %). Each value is a mean of four replicates.

| Biochar | Cd (mM) | Catalase | Peroxidase | Ascorbic acid oxidase | Polyphenol oxidase |
|---|---|---|---|---|---|
| -- | 0.0 | 42.3** | 221 | 0.109** | 1.44** |
| | 0.001 | 48.4** | 231 | 0.160** | 1.55** |
| | 1 | 53.1** | 295 | 0.225** | 1.98** |
| | 5 | 0 | 0 | 0 | 0 |
| (0.5%) | 0.001 | 40.1** | 222 | 0.107** | 1.46** |
| | 1 | 42.8** | 233 | 0.115** | 1.48** |
| | 5 | 15.0** | 79 | 0.090** | 0.50** |
| L.S.D. at 0.05 | | 0.010 | N/A | 0.009 | 0.018 |
| L.S.D. at 0.01** | | 0.015 | N/A | 0.012 | 0.023 |




**(a)**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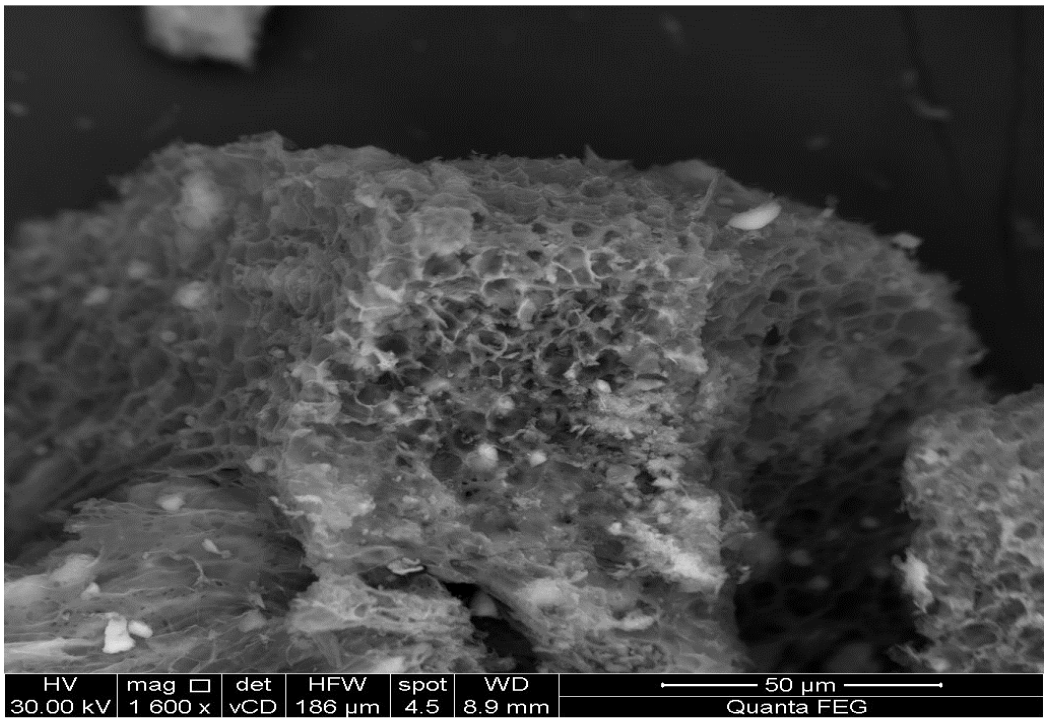
**(b)**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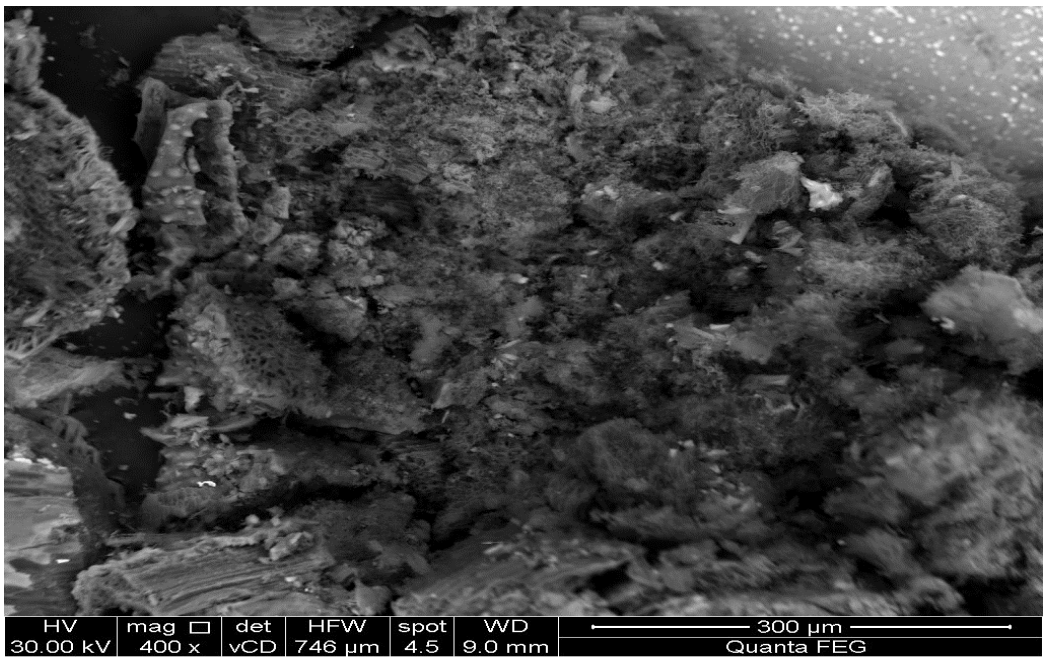


**(d)**

**FIGURE 1:** Scanning electron microscope (SEM) images of BC materials produced from rice husk at various phases; (a, b) un-grinded and (c, d) grinded forms. Pyrolysis was at 300$^{o}$ C for 2 hours,using SEM Model Quanta 250 FEG (Field Emission Gun) with accelerating voltage 30 K.V., magnification14x up to 1000000 and resolution for Gun.1n). FEI Company, Netherlands.

## *IV. CONCLUSION AND SUGGESTION*

### *A. Conclusion*

1. Increasing Cd concentration in the surrounding medium of alfalfa germinating seeds led to decreasing of germination percentage, consequently the phytotoxicity percentage and radicle inhibition increased.

2. Mixing the grinded BC with the Cd solution excluded the Cd ions from the germinating medium and alleviated the Cd toxicity on the germinating seeds through adsorption on the surface of BC particles. So, the germination percentage might be increased through improving the physiological activities such as increasing the activity of the hydrolytic enzymes and the antioxidant enzymes.

### *B. Suggestion*

1. Filtration of wastewater that polluted with HMs through BC as excellent adsorbent for removing HMs.

2. BC is easy to apply, cheap and safe on plant, soil and in environment. This would maintain soil fertility, should limit the contamination with HMs in environment and HMs accumulation in plants, and consequently maintains animal and human health.

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