34 *Egypt. J. Bot.*, **Vol. 56**, No. **3** (Part 1) pp. 573-594 (2016)

Alleviation of Cadmium Toxicity in *Triticum aestivum* Using the Coagulant Defatted *Moringa oleifera* and *Moringa peregrina* Seeds Powder.

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THE PRESENT study explores the unexploited sorption properties of *Moringa oleifera* Lam (MO)and *Moringa peregrina* Foresk (MP) for decontamination of Cd^{2+} at laboratory scale. *Triticum aestivum* plants were grown under Cd^{2+} stress. *Moringa* seed powder (3 g/ kg soil) of both species were mixed with the soil before wheat sowing. After ten days, the Cd-concentrations were applied to the treated pots (0.0, 0.5, 1.0 and 1.5 mM). Plant samples were collected at 5 weeks post sowing to assess the growth traits and certain physiological parameters. Growth traits, relative water content, photosynthetic pigments were decreased under Cd^{2+} stress. However, the application of *Moringa* seed powder can detoxify the toxic effect of cadmium acetate stress on the above parameters. The Cd^{2+} stress increased the Cd^{2+} contents in roots and shoots of wheat plants, but in the *Moringa* seed powder-treated plants under Cd^{2+} stress revealed a reverse situation. Both *Moringa* species can coagulate Cd^{2+} from the soil by the presence of proteins having coagulation properties (MWts 64.5, 51.4, 41.2, 38.3, 27.9 and 18.9 KDa). The effect of MO seed powder in mitigating the adverse effect of Cd^{2+} stress on wheat plant was much pronounced than that of MP seed powder.

Keywords: Biodegradable, Biosorption, *Moringa oleifera* seed powder, *Moringa peregrine* seed powder, Organic fertilizer, Seed-cake.

Abbreviation: MO: *Moringa oleifera*, MP: *Moringa pergrina*, MSP: *Moringa* seed powder, OSP: Oleifera seed powder, PSP: Peregrina seed powder.

Wheat is a major agricultural commodity and dietary components across the world, where it is the most widely grown crop species in the world (Rezzoug *et al*., 2008). Wheat is one of the most important cereals in view of nutritional value (Abd El-Baky, 2009). It serves as a rich source of carbohydrates, essential amino acids, fiber components, minerals and vitamins in the human diet (Fardet *et al*., 2008). In Egypt, wheat is considered as the first strategic food crop. It has maintained its position as the basic staple food in urban areas, and mixed with maize in rural areas for bread making. In addition, wheat straw can be considered as an important fodder (Amin *et al*., 2008).

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Heavy metal stress leads to loss in a regular productivity and induces hazardous health effects. Heavy metals have largest availability in soil and aquatic ecosystems and to a relatively smaller proportion in atmosphere as particular or vapors. Among the toxic heavy metals, Cd^{2+} is a non-essential heavy metal pollutant naturally present in the environment. Cadmium containing phosphate fertilizers, sewage sludge and industrial emissions are the major sources of Cd^{2+} (Howlader, 2014). Cadmium and certain Cd^{2+} compounds are listed by International Agency for Research on Cancer (IARC) as carcinogenic agent. Cd^{2+}- at low levels over prologed periods- causes high blood pressure, sterility among males, kidney damage and flu disorders (Nand *et al*., 2012). If plants exposed to high levels of Cd^{2+} , photosynthetic rate, water and nutrient uptake be will inhibited and finally death will occur (Agami and Mohamed, 2013).

Environmental pollution by toxic metals occurs globally through military, industrial and agricultural process and was disposal (Kumari *et al*., 2005), this has led to a need of means of purifying of water for various roles it plays in our lives. This can be achieved by the addition of chemical coagulants such as aluminum sulphate and synthetic electrolyte that may be toxic, to the raw water (Foidle *et al*., 2001). Also activated carbon is an absorbent that widely used in the treatment of contaminated water, however its use in large scale becomes costly (Babel and Kurniawan, 2003). Thus , the search for low cost and high efficiency adsorbents has led to study alternative natural absorbents such as waste agricultural and agro-industrial byproducts, which generally have the advantage of being produced in large quantities, and many of these materials have potential in sorption of metal ions and hence are known as biosorbants (Santos *et al*., 2011). One of these materials is the byproduct obtained from *Moringa* seeds which has the ability to join the heavy metals to form complex with ions in solutions (Pagnanelli *et al*., 2003). Due to this characteristic, *Moringa* becomes a very attractive option for water remediation. *Moringa* crushed seed powder yields water soluble proteins that can coagulate wastes from water (Foidle *et al*., 2001). in this respect, Ndabigengesere *et al*. (1995) reported that the active components in *Moringa oleifera* seeds are soluble cationic proteins and peptides with molecular weight ranging from 6 to 16 KDa. However, Himesh *et al*. (2007) reported the isolation of a high molecular mass protein (66 KDa) having coagulation activity. The percentage removal of heavy metals from waste water by *Moringa oleifera* seeds was recorded 80% for Pb and 60% for Cd (Nand *et al*., 2012). The present investigation was conducted as a trial to mitigate Cd toxicity on wheat plant using defatted seeds powder of *Moringa oleifera* and *Moringa peregrina* plants as coagulants.

Materials and Methods

Plant material preparation

The seeds of both mature *Moringa* species (MO and MP) were collected from El- Kanatter Horticultural Research Station (Agricultural Research Center, Kalubia Governorate, Egypt) and El- Orman Garden (Giza), respectively. The seeds were then air-dried and defatted from their oil content using n-hexane through soxhlet apparatus (Meara, 1955). The seed cake resulted from extraction was left in air to evaporate the residual solvent. The seed-cake was applied (about 3 g for each pot) to soil-cultivated with wheat to test the activity of both *Moringa sp*. to coagulate $Cd^{2+}$from the Cd-contaminated soil (Foidle *et al*., 2001). Also, the seeds of both species were analyzed, then their chemical constituents and protein pattern, were presented in (Table 1, 2 and Plate 1).

Growth conditions

Before sowing wheat grains, every Kg soil was mixed with three g of defatted *Moringa oleifera* or *peregrina* seeds powder except for controls (pots irrigated with H_2O, 0.5, 1.0 and 1.5 mM Cd), then seeds of wheat (*Triticum aestivum*) were sown in plastic pots (15 cm in diameter and 15 cm in depth). Each pot was filled with one kg soil (clay: sand; 2:1 w/w) and contained 5 plants. The study was conducted under natural conditions during winter. The average temperature was 21±2 ˚C, the seedlings were irrigated with tap water and raised at 70% water holding capacity. The plants were left to grow in the greenhouse and after ten days post sowing, Cd-acetate with different concentrations (0.5 mM, 1.0 mM and 1.5 mM) was added to the soil in treatments alone, or in combination with MO or in combination with MP in treatments. Each treatment was carried out in three replicates. Plant samples were collected at 5 weeks post sowing to assess the growth traits and certain physiological parameters (fresh leaves of plant samples were used to determine photosynthetic pigments, relative water content and Cd^{+2} content in leaves and roots. Protein banding pattern in fresh leaves was also determined.

Plant growth analyses

Wheat plants were removed from the pots along with the soil and were dipped in a beaker filled with water. Plants were moved smoothly to remove the adhering soil particles. Root and shoot lengths were measured by using a meter scale. The dry weights of shoots and roots were determined by placing the fresh materials in an oven run at 70 ˚C up to constant weight. These dried plants were weighed to record dry mass of shoots and roots (Howladar, 2014).

Hormonal analysis

The method of hormone extraction was essentially similar to that adopted by Wasfy *et al*. (1974). During the extraction method there are two fractions, the fraction of the ethanol extract (acidic fraction) containing the acidic hormones (IAA, GA3) and was determined with the method described by Shindy and Smith (1975) using HEWLETT PACKERED Gas Chromatography (5890) with a Flame Ionization Detector (FID). The aqueous fraction comprised cytokinins that were quantified using High Performance Liquid Chromatography (HPLC) according to the method adopted by Muller and Hilgenberg (1986).

Determination of relative water content

Relative water content (RWC) was determined in fresh leaf segments (1 cm x 3 mm). Segments were weighed quickly and immediately placed on double distilled water in Petri-dishes to saturate them with water for about 24 hr in dark. The leaf segments were reweighed to note the adhering water and turgid mass. Dry mass of the leaf segments was recorded after dehydrating them at 70 ˚C for 48 hr. The % RWC was calculated using the following formula giving by Howladar (2014)

$$RWC = \frac{\text{Fresh mass- dry mas}}{\text{Turgor mass- dry mass}} \times 100$$

Determination of photosynthetic pigments

The photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were determined according to spectrophotometric method adopted by Metzner *et al*. (1965). A sample of 0.5 g of 35-day old fresh leaves was homogenized in 85% aqueous acetone for 5 min. The homogenate was centrifuged. The optical densities of acetone extract were measured at 3 wave lengths of 663, 644 and 452.5 nm using Spectronic 601 Spectrophotometer.

Determination of proline contents

Free proline was estimated according to the method described by Bates *et al*. (1973). Intensity of color of toluene phase was red at 520 nm using Spectronic 601 Spectrophotometer.

Determination of carbohydrate contents

The carbohydrates were extracted according to Prud'homme *et al*. (1992). The total soluble fraction was determined using modification of the procedures of Yemm and Wilis (1954). The polysaccharides were determined using Thayermanavan and Sadasivam (1984). Total carbohydrates were calculated as the sum of the amount of soluble sugars and polysaccharides.

Determination of protein contents

The total proteins (soluble and insoluble proteins) were determined by using Folin-Ciocalteu reagent according to the method described by Daughaday *et al*. (1952) and the total proteins were calculated as the sum of the amount of soluble and insoluble proteins.

Determination of amino acids and total nitrogen contents

Amino acids extracted by using the method adopted by Hassanein (1977). Free amino acids were directly determined in the extract according to Muting and Kaiser (1963). Total-N was determined by the conventional micro-kjeldahl method.

Determination of ascorbate and glutathione contents

The method used for estimation of ascorbic acid (ASA) was described by Barakat *et al*. (1973). The reduced glutathione was determined by using the method adopted by Hissin and Hilf (1976).

Determination of total phenols and flavonoids

Total phenols were extracted by the method of Danial and George (1972) and measured at 725 nm using Spectronic, 601. Whereas, the flavonoids extracts were prepared using the modified method of Malkowski and piotrowska (2006). The intensity of pink color of flavonoid samples was measured at 510 nm using Spectoronic 601.

Determination of certain elements

Elements extracted according to Chapman and Pratt (1961). Potassium elements was estimated by the Flame Photometer Emission Technique as described by Ranganna (1977). Calcium, magnesium, iron, manganese, phosphorus and cadmium were determined simultaneously by inductively coupling plasma (ICP) spectroscopy according to the method of Saltanapour (1985) and calculated as mg/100 g DW

Determination of fibers content

Jenkins method (1930) was used to determine cellulose content. The method used for pectin content was that adopted by Nanji and Norman (1928).

Determination of oil content

Oils were extracted as described by Meara (1955). Extraction made with petroleum ether in a soxhlet apparatus and continued for 18 hours.

protein banding pattern

The method used in analyzing the protein banding pattern of soluble protein was carried out according to the method described by Hames and Ricwood (1981).

Statistical Analysis

Data were analyzed by a simple variance analysis (ANOVA) using program SPSS17.

Results

Biochemical composition of seeds of MO and MP

MO and MP seeds were analyzed and their chemical constituents were presented in (Table 1). As shown from the table, the seeds of the two *Moringa* species contained considerable amounts of phytohormones namely IAA, GA_3, zeatin, zeatin riboside and benzyl adenine. With regard to primary metabolites, namely total carbohydrates, total proteins and total lipids; they are higher in MO compared with MP. Concerning the mineral elements, both *Moringa* species contained higher levels of Ca, K, P and Mg, but minute amounts of Mn and Fe.

In addition, MO and MP seeds are rich in antioxidant compounds such as ascorbic acid, reduced glutathione, total flavonoids and total phenols.

TABLE 1. Biochemical composition of fully matured seeds of *Moringa oleifera* and *Moringa peregrina* plants.

Chemical Compounds		M. *oleifera* seed	M. *peregrina* seed
Phytohormones (µg/g DW)	Auxin (IAA)	91.16 ± 3.242	143.50 ± 4.912
	GA3	186.77 ± 6.110	247.17 ± 4.900
	Zeatin	6088.33 ±136.961	4070.20 ± 180.639
	Zeatin riboside	25.78 ± 4.595	63.10 ± 10.523
	Benzyl adinine	455.83 ± 25.750	344.20 ± 15.235
Carbohydrates (g/100g DW)			
	Total soluble Sugars (TSS)	3.77 ± 0.221	2.16 ± 0.208
	Poly saccarides	5.61 ± 1.013	2.26 ± 0.354
	Total carbohydrates	9.38 ± 1.276	4.42 ± 0.148
Nitrogenous compounds (g/100g DW)	Soluble proteins	11.25 ± 0.107	6.61± 0.023
	Insoluble proteins	3.42 ± 0.312	2.70 ± 0.033
	Total proteins	14.67 ± 0.219	9.3 ± 0.010
	Total nitrogen	29.14 ± 3.732	16.11 ± 1.609
	Amio nitrogen	0.17 ± 0.017	0.12 ± 0.008
	Proline	0.01 ± 0.001	0.01 ± 0.000
Total lipids (%)		32.71 ± 4.503	25.53 ± 1.861
Minerals (mg/100g DW)	Mn^{2+}	0.73 ± 0.085	0.49 ± 0.032
	Fe^{3+}	2.84 ± 0.268	5.38 ± 1.295
	Ca^{2+}	333.17 ± 7.281	171.17 ± 5.901
	K^{+}	733.51 ± 6.500	338.83 ± 6.001
	P^{3+}	415.91 ± 4.556	272.67 ± 11.504
	Mg^{2+}	90.84 ± 7.780	110.67 ± 11.676
Fibers (g/100 g DW)		49.82 ± 0.351	48.28 ± 0.300
Ascorbic acid (mg/100g DW)		100.60 ± 7.006	56.54 ± 0.676
Reduced glutathione (mg/100g DW)		2139.23 ± 194.45	3696.30 ± 583.35
Total flavonoids (mg/100g DW)		0.16 ± 0.013	0.33 ± 0.025
Total phenols (mg/100g DW)		1316.19 ± 39.942	403.21 ± 10.261

Protein banding pattern of seeds of MO or MP

Seeds of MO are characterized by the presence of 5 polypeptides, their molecular weights are 87.82, 73.0, 58.7, 46.23, and 23.3 KDa. However, seeds of MP are characterized by the presence of 4 protein bands, their molecular weights are 115.4, 97.0, 53.9 and 36.50 KDa (Table 2 and plate 1). The two *Moringa* species are characterized by the presence of 6 protein bands, their molecular weights are 64.5, 51.4, 41.2, 38.3, 27.9 and 18.9 KDa (Table 2 and plate 1).

TABLE 2. Protein banding pattern of *Moringa oleifera* and *Moringa peregrina* seeds (molecular weight; MWt and amounts).

MW	*Moringa oleifera* seeds		*Moringa peregrina* seeds		MW
KDa	MW	amount	MW	amount	MW
116	-----	-----	-----	-----	116
115.44	-----	-----	115.4	1.85	-----
100.40	-----	-----	-----	-----	-----
98.70	-----	-----	-----	-----	-----
97.0	-----	-----	97.0	1.94	97
94.7	-----	-----	-----	-----	-----
91.3	-----	-----	-----	-----	-----
87.82	87.82	3.87	-----	-----	-----
84.40	-----	-----	-----	-----	-----
79.8	-----	-----	-----	-----	-----
75.8	-----	-----	-----	-----	-----
73.0	73.0	2.75	-----	-----	-----
66.0	-----	-----	-----	-----	66
64.5	64.5	3.97	64.5	4.97	-----
58.7	58.7	2.13	-----	-----	-----
55.0	-----	-----	-----	-----	55
53.9	-----	-----	53.9	5.31	
51.4	51.4	7.55	51.4	9.46	-----
46.23	46.23	5.11	-----	-----	-----
45.0	-----	-----	-----	-----	45
41.20	41.20	5.08	41.20	3.92	-----
38.30	38.30	4.07	38.30	3.21	-----
36.50	-----	-----	36.50	4.59	-----
36	-----	-----	-----	-----	36
29.0	-----	-----	-----	-----	29
27.9	27.9	8.55	27.9	8.7	-----
26.0	-----	-----	-----	-----	-----
24.0	-----	-----	-----	-----	24
23.3	23.3	3.67	-----	-----	-----
18.9	18.9	4.10	18.9	4.25	-----
15.5	-----	-----	-----	-----	-----
14.2	-----	-----	-----	-----	14.2
12.2	-----	-----	-----	-----	-----
Number of bands	11	-----	10	-----	-----

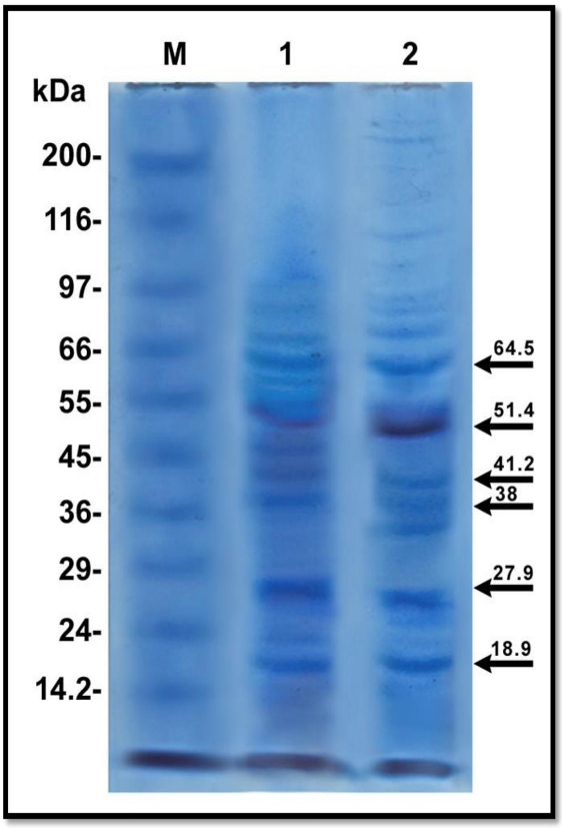


Plate 1. Electrograph of soluble protein pattern by one dimension SDS-PAGE showing the proteins presented in the seeds of *Moringa oleifera* and *Moringa peregrina*.
Lane(1): *Moringa oleifera.*
Lane(2): *Moringa peregrina.*

Effect of seed powder of MO or MP on wheat growth under Cd^{2+} stress

Cadmium application significantly reduced the growth traits (lengths of shoot and root, fresh and dry weights of root and shoot) of wheat plant, especially at the highest concentration (1.5 mM) compared to control plants. It had been found that soil application with *Moringa* seed powder of the two species separately, significantly increased the above mentioned growth traits higher than the control plants, especially in the case of MO. Application of *Moringa* seed powder of the two species in combination with different concentration of Cd^{2+} significantly increased the previous growth traits of wheat plants compared with their relative treated controls. Generally, the seed powder of both species (either separately or combined with Cd^{2+}) significantly increased both fresh and dry weights of root of wheat plant with the superiority of MO seed powder compared to that of MP seed powder (Table 3).

TABLE 3. Effect of soil application of defatted *Moringa oleifera* (OS) and *Moringa peregrina* (PS) seeds on growth parameter of 35 day old seedlings of wheat (*Triticum aestivum*) grown under Cd-acetate stress. Each value is the mean of three variables.

Parameters	Length of Shoot (cm)	Shoot FW	Shoot DW	Length of Root (cm)	Root FW	Root DW
Control	25.82 ±0.27 [b]	334.03 ± 9.35 [b]	30.15 ± 0.76 [c]	11.22 ± 0.22 [bc]	41.28 ± 1.26 [c]	8.14 ± 0.51 [cd]
MO	27.64 ± 0.21 [a]	350.14 ± 9.36 [a]	34.88 ± 1.80 [a]	12.44 ± 0.41 [a]	55.02 ± 1.81 [a]	9.39 ± 0.41 [a]
MP	26.29 ± 0.22 [b]	342.58 ± 3.13 [ab]	31.39 ± 0.86 [b]	11.79 ± 0.31 [b]	44.52 ± 1.62 [b]	8.95 ± 0.37 [ab]
Cd (0.5 mM)	24.64 ± 0.42 [c]	242.68 ± 1.71 [de]	26.92 ± 0.41 [e]	9.89 ± 0.61 [e]	33.95 ± 0.56 [fg]	7.21 ± 0.30 [ef]
Cd (1.0 mM)	21.92 ± 0.46 [g]	182.95 ± 5.37 [g]	21.72 ± 0.59 [h]	8.39 ± 0.54 [gh]	29.73 ± 0.73 [i]	6.18 ± 0.30 [g]
Cd (1.5 mM)	17.85 ± 0.35 [h]	139.25 ± 2.58 [h]	18.05 ± 0.30 [j]	6.81 ± 0.30 [i]	21.28 ± 0.80 [k]	5.09 ± 0.46 [h]
Cd (0.5mM)+MO	25.61 ± 0.43 [b]	303.27 ± 8.58 [c]	28.53 ± 0.20 [d]	10.75 ± 0.36 [cd]	37.42 ± 1.05 [d]	8.35 ± 0.15 [bc]
Cd (0.5 mM) +MP	24.28 ± 0.68 [cd]	246.62 ± 3.56 [d]	27.86 ± 0.30 [de]	10.28 ± 0.21 [de]	35.95 ± 0.66 [de]	7.58 ± 0.47 [de]
Cd (1.0 mM)+MO	23.64 ± 0.76 [de]	242.32 ± 3.10 [de]	24.61 ± 0.56 [f]	9.78 ± 0.31 [e]	34.56 ± 0.59 [ef]	7.42 ± 0.30 [ef]
Cd (1.0 mM) +MP	22.88 ± 0.20 [ef]	232.52 ± 2.40 [f]	23.61 ± 0.50 [fg]	9.05 ± 0.31 [f]	32.29 ± 1.16 [gh]	6.79 ± 0.56 [fg]
Cd (1.5 mM)+MO	23.08 ± 0.25 [ef]	234.62 ± 4.71 [ef]	22.88 ± 0.30 [gh]	8.89 ± 0.35 [fg]	31.58 ± 1.02 [h]	6.81 ± 0.36 [fg]
Cd (1.5 mM) +MP	22.31 ± 0.95 [fg]	178.95 ± 3.24 [g]	20.35 ± 0.51 [i]	8.11 ± 0.26 [h]	26.41 ± 0.51 [j]	6.45 ± 0.26 [g]
$LSD_{0.05}$	0.827	9.183	1.208	0.618	1.783	0.651

Effect of MO or MP on wheat relative water content under Cd^{2+} stress

Cadmium stress significantly reduced the percentage of relative water content (RWC) in leaves of wheat plants at different applied concentrations (0.5 and 1.5 mM) as compared to untreated control and other treated plants. The treatment of soil with *Moringa* seed powder separately, increased the percentage of relative water content (%RWC) in leaves of wheat plants in comparison with the untreated control plants. The soil application with the combined treatments MO + 0.5 mM Cd and MO + 1.5 mM Cd or MP + 0.5 mM Cd and MP + 1.5 mM Cd induced the high significant increases in %RWC compared to their corresponding controls. The application of MO seed powder reveals the most increase in the percentage of RWC compared to MP (either alone or in combination with Cd^{2+} stress) (Table 4).

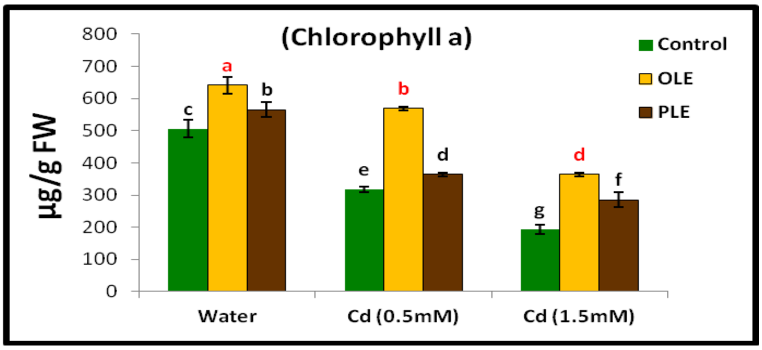
TABLE 4. Effect of soil application of defatted *Moringa oleifera* (MO) and *Moringa peregrina* (MP) seeds on percentage of relative water content (% RWC) of leaves of 35 day old seedlings of wheat (*Triticum aestivum*) grown under Cd- acetate stress. Each value is the mean of three variables.

Parameters	% of RWC
Control	81.5 ± 3.04 [b]
MO	93.7 ± 3.51 [a]
MP	90.27 ± 3.64 [a]
Cd (0.5 mM)	68.47 ± 5.39 [de]
Cd (1.5 mM)	44.7 ± 3.85 [f]
Cd (0.5 mM) + MO	79.05 ± 2.16 [bc]
Cd (0.5 mM) + MP	73.53 ± 2.34 [cd]
Cd (1.5 mM) + MO	74.53 ± 3.82 [cd]
Cd (1.5 mM) + MP	64.8 ± 1.7 [e]
$LSD_{0.05}$	2.81

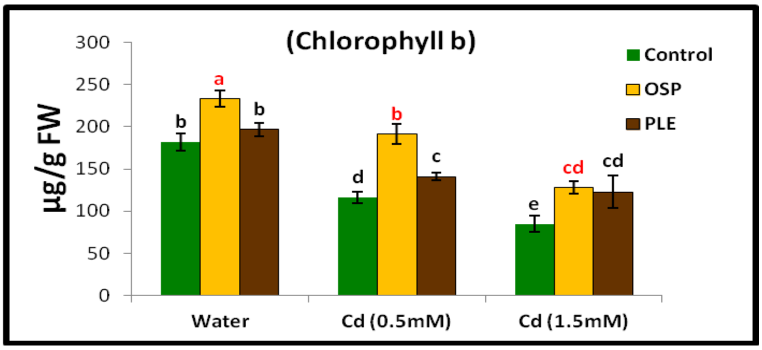
Effect of MO or MP seed powder on wheat photosynthetic pigments under Cd^{2+} stress

Wheat plants subjected to Cd stress show high significant decreases in leaf contents of chlorophylls (a & b) and carotenoids below those of untreated controls. The soil application with MO or MP improved the above parameters above those of untreated controls. The toxic effect of Cd^{2+} stress was significantly mitigated in case of combination between MO or MP and Cd combination, where the MO and MP can improve the photosynthetic pigments compared with their corresponding Cd-treated controls (Fig. 1).

(A) Chlorophyll a



(B) Chlorophyll b



(C) Carotenoids

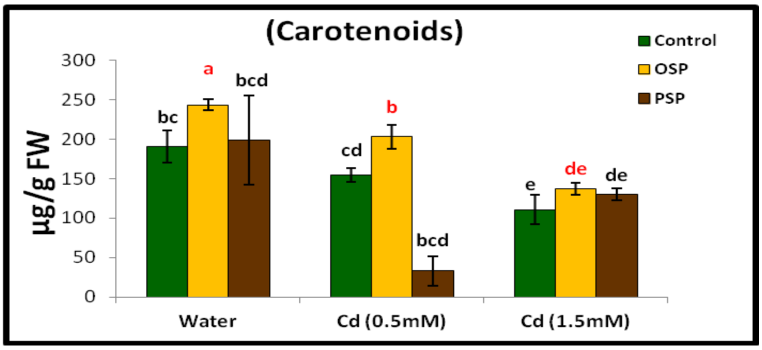
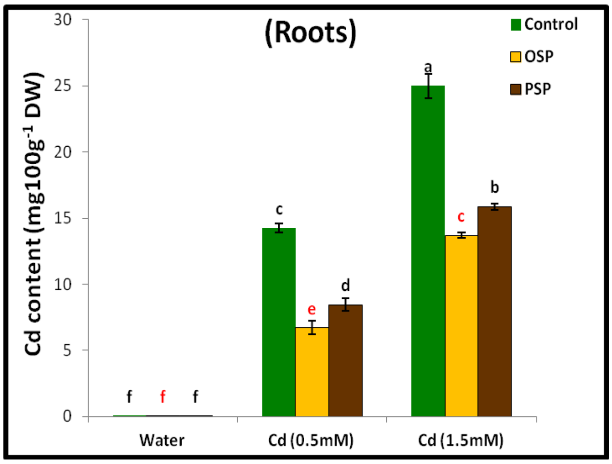


Fig. 1. Effect of soil application of defatted *Moringa oleifera* (MO) and *Moringa peregrina* (MP) seeds powder on photosynthetic pigments content of leaves (μg g^{-1} FW) of 30 day old seedlings of wheat (*Triticum aestivum*) grown under Cd- stress.

Effect of MO or MP on wheat content of Cd^{2+} under Cd^{2+} stress

The stress generated by Cd^{2+} led to high accumulation of Cd^{2+} content in the roots and shoots of wheat plants. Plant roots were shown to be more affected than shoots. The toxic effect of cadmium stress was improved by applying the MO or MP to the soil-cultivated wheat plants. Non-significant changes in Cd^{2+} contents were observed in roots and shoots of wheat plants- treated with MO or MP- as compared with those of their corresponding controls (Fig. 2).

(A) Cd^{2+} content in Roots



(B) Cd^{2+} content in Shoots

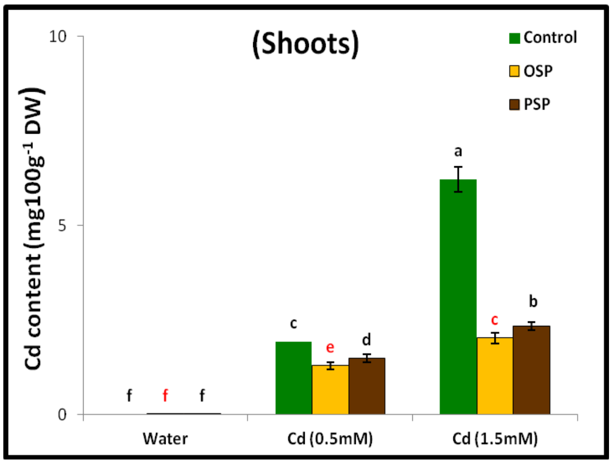


Fig. 2. Effect of soil treatment of defatted *Moringa oleifera* seed powder (MO) and *Moringa peregrina* seed powder (MP) on Cd^{2+} content of shoots and roots of 35 day old seedlings of wheat (*Triticum aestivum*) grown under Cd-stress.

Effect of MO or MP on protein banding pattern of wheat plant under Cd^{2+} stress

Cadmium induced changes in protein patterns of wheat plant Table 5 and plate 2, reveal that 15 protein bands of molecular weights: 182.24, 105.6, 100.6, 89.0, 63.41, 58.5, 55.0, 47.8, 42.2, 36.0, 32.73, 27.1, 25.0, 21.43 and 15.87 KDa were expressed in the leaves of untreated control wheat plant. The band of MW 182.24 KDa is a unique band while the others were polymorphic proteins. Treatment with 0.5 mM Cd induced the expression of two proteins of molecular weights 261.38 and 170.92 KDa, while 1.5 mM Cd induced the expression of 3 proteins of molecular weights 114.0, 97.0, 74.14 KDa in the leaves of treated wheat plants. In addition, the 2 concentrations of Cd induced the expression of 5 proteins, their molecular weights are 217.77, 135.4, 51.6, 45.0 and 38.2 KDa in the leaves of Cd treated wheat plants. The combined effect of MO defatted seed powder or MP defatted seed powder with 0.5 mM or 1.5 mM Cd on protein banding pattern of wheat leaves indicated the expression of 11 proteins, their molecular weights are 261.38, 217.77, 177.38, 135.4, 114.0, 97.0, 74.14, 51.6, 38.2, 24.0 and 13.5 KDa. Of the above eleven proteins, one might be expressed due MO or MP powder (177.38 KDa) and the others (13.5 and 24 KDa) were induced by MP seed powder.

Discussion

The industrial activities greatly polluted the environment and deteriorate the ecosystems due to the presence of contaminants in their effluent (Lenardão *et al*. 2003), from these activities contaminant toxic heavy metals that cause damage to ecosystems and human health due to their incorporation into the food chain (Bazrafshan *et al*., 2013). Cadmium is considered as the most poisonous heavy metals even at low doses (Yurtsever and Sengil 2009), the presence of excessive amount of Cd in soil causes many toxic symptoms in plants such as chlorosis, growth inhibition, browning of root tips and finally death (Guo *et al*., 2008). For the removal of heavy metals, there are many synthetic coagulants such as activated carbon which was used widely in the treatment of effluents (Wan Ngah and Hanafiah, 2008), also synthetic coagulants of aluminum (alum lime, aluminum sulphate, and polyaluminum silico sulphate), ferric salts (iron hydroxide and iron chloride) and soda ash are also widely used processes (Bazrafshan *et al*., 2013). But their use in large scale becomes costly and in some cases has limited application and also serious to the environment (Ahluwalia and Goyal, 2007). Thus, the search for an alternative, low cost, highly efficient adsorbents must be needed and widely used in the developing world (Choong *et al*., 2007). The alternative materials such as waste agricultural and agro-industrial byproducts, that are produced in large quantities, relatively inexpensive and many of these materials have ability in sorption of metal ions and hence are known as biosorbents (Santos *et al*. 2011) and also have advantages such as biodegradability, low sludge production and fewer risks to health and environment (Mahdavi *et al*., 2012).

TABLE 5. protein banding pattern of leaves of wheat plant (*Triticum aestivum*) treated with cadmium alone or in combination with defatted seed powder of *Moringa oleifera* (MO) or *Moringa peregrina* (MP), (Mwt & amount).

MW KDa	H2O	0.5 mM Cd	1.5mM Cd	MO	MP	MO+0.5 mM Cd	MP+0.5 mM Cd	MO+1.5 mM Cd	MP+1.5 mM Cd	MW KDa
261.38	-	3.66	-	-	-	-	3.35	2.83	3.5	
251.69	-	-	-	-	-	-	-	-	-	
225.85	-	-	-	2.64	3.08	-	-	-	-	
217.77	-	3.82	4.41	-	-	2.69	2.69	3.52	6.4	
206.46	-	-	-	-	-	-	-	-	-	-
200.00	-	-	-	-	-	-	-	-	-	-
190.31	-	-	-	3.12	5.09	-	-	-	-	-
182.24	3.78	-	-	-	-	-	-	-	-	-
177.38	-	-	-	-	-	3.24	2.95	3.90	4.15	
170.92	-	4.08	-	-	-	-	-	-	-	-
145.1	-	-	-	-	3.6	--	-	-	-	-
135.4	-	4.39	4.21	-	-	3.08	3.0	3.13	2.9	-
116.0	-	-	-	2.73	6.77	-	-	-	-	116.
114.0	-	-	3.77	-	-	2.81	3.65	3.0	3.3	
105.6	3.54	4.89	3.87	3.1	3.52	2.9	14.7	-	5.62	
100.6	2.58	-	-	2.37	3.26	-	-	2.88	-	
97.0	-	-	3.17	-	-	3.3	2.8	-	-	97.0
89.0	4.15	6.47	2.9	3.33	3.31	3.81	3.09	2.78	-	-
74.14	-	-	3.9	-	-	3.43	2.21	2.28	3.78	
68.3	-	-	-	4.41	2.79	-	-	-	-	-
66.0	-	-	-	-	-	-	-	-	-	66.0
63.41	4.35	4.64	-	3.91	3.16	3.46	3.12	3.46	3.57	
58.5	3.6	4.42	3.95	-	5.32	4.0	3.51	4.62	3.29	
55	3.66	-	-	3.42	4.5	-	3.26	-	3.59	55
51.6	-	4.8	4.04	-	-	4.34	3.32	5.12	7.25	
47.8	5.26	-	-	4.28	-	3.66	-	-	-	-
45.0	-	4.9	3.42	-	4.29	3.72	-	-	4.0	45.0
42.2	4.45	-	4.17	4.0	4.5	-	-	6.14	3.77	
38.2	-	5.54	4.24	-	-	3.86	4.53	-	-	
36.0	5.82	-	-	-	-	7.83	-	-	-	36.0
32.73	10.8	9.62	10.1	10.3	13.6	-	10.8	12.9	10.3	
29	-	-	-	3.08	-	4.1	-	3.53	4.33	29
27.1	4.13	4.34	3.87	4.29	-	-	3.99	-	-	
25.0	4.42	4.28	33.85	-	5.12	-	5.25	-	4.2	-
24	-	-	-	-	-	9.08	-	5.91	-	24
21.43	5.42	4.64	31.0	8.65	6.53	-	4.21	-	4.86	-
15.87	4.78	4.24	5.74	-	-	4.26	3.83	5.27	4.64	
14.2	-	-	-	-	4.45	-	-	-	-	14.2
13.5	-	-	-	-	-	4.14	-	-	-	
No. of bands	15	16	17	16	17	19	19	14	18	

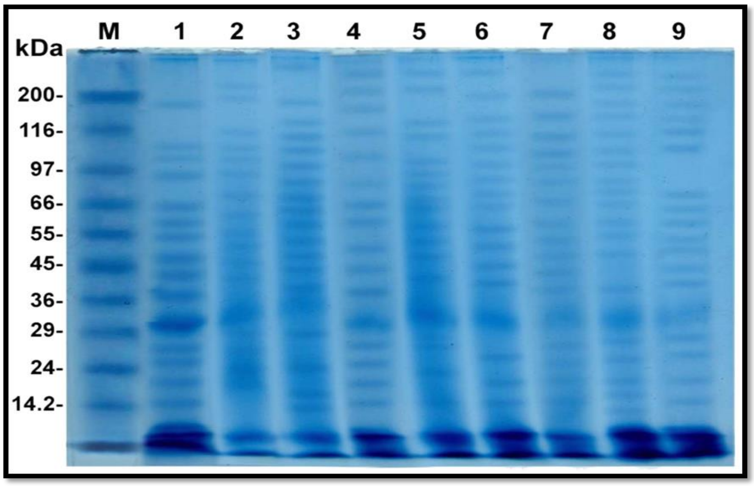


Plate 2. electrograph of soluble protein pattern by one-dimension SDS-PAGE showing the changes of protein bands in response to different concentrations of cadmium either alone or in combination with defatted seed powder of *Moringa oleifera* (MO) or *Moringa peregrina* (MP)

Lan(1): H_2O Lan(2): Cd (0.5 mM)
Lan(3): Cd (1.5 mM) Lan(4): MO
Lan(5): MP Lan(6): MO + Cd (0.5 mM)
Lan(7): MP + Cd (0.5 mM) Lan(8): MO + Cd (1.5mM)
Lan(9): MP + Cd (1.5mM)

In this study, the byproduct (seed cake originated after the oil extraction of seeds) coms from the seeds of *Moringa* species (*Moringa oleifera and Moringa peregrina*) which contained 6 protein bands, their molecular weights are 64.5, 51.4, 41.2, 38.3, 27.9 and 18.9 KDa. These proteins may have coagulant properties with the ability to bind the ions of heavy metals to form complexes (Bazrafshan *et al*., 2013). From our results presented in (Fig. 2) the application of MO and MP seed powders separately or in combination with Cd- treatments has the ability to coagulate the Cd^{2+} in the soil, the mechanisms by which the seed powder of both species can coagulate the cadmium in the soil must be understood. In this respect, Meneghel *et al*. (2013), suggested that the process of adsorption and coagulation of Cd^{2+} is a result of electrostatic interactions and formation of complexes between the metal ions and the functional groups present in the adsorbent biomass. These groups (O–H, N–H, C–H, and C–O) present in carbohydrates, proteins, lipids, and lignin. Also, Yongabi *et al*. (2011), suggested that the active coagulant components is a protein materials. Okuda *et al*. (2001), reported that there are two active coagulant components were extracted from *Moringa oleifera* seeds one of them extracted by using salt solution (MOC-SC) (Agrawal *et al*., 2007) and the other extracted by distilled water (MOC-DW), both of them have the ability to coagulate wastes from turbid water. MWt of the former MOC-SC was about 3 KDa, while that of the later (MOC-DW) was about 12-14 KDa (water soluble- cationic polypeptide) (Santos *et al*., 2012). The coagulation mechanism of MOC-DW was reported by Ndabigengesere *et al*. (1995) which include adsorption and neutralization of charges or adsorption and bridging of destabilized particles where this polypeptide carry a net positive charges so it proposed to bind to negatively charged particles (silt, clay, bacteria, etc. suspended in a colloidal form), that make raw waters turbid. The previous results that the coagulation component was proteins have been reported by Agrawal *et al.* (2007), where they extracted and purified a high molecular mass protein with MWt about 66 KDa on 15% SDS-PAGE. Finally, Agrawal *et al.* (2007) suggested that the flocculation coagulation activity and antimicrobial activity of seeds come from MO plant is a cumulative effect of various active components. The purified active components if used together for water treatment can be more effective in clarifying the water than the individual active components.

It is clearly shown from Table 3 that Cd-treatment of wheat plant inhibited all the growth parameters (shoot length, shoot FW & DW, root length and root FW & DW) and this inhibition increased with increasing Cd concentration. The reduction of growth by Cd toxicity could be mainly due to the role of Cd on inhibition of chlorophyll synthesis (Padmaja *et al*., 1990) and photosynthetic rate as well as detrimental effects on chloroplast replication and cell division (Baryla *et al*., 2001). Inhibition of root growth in Cd-treated plants may be due to the reduced water uptake, also, the reduction in root growth may be due to reductions in both new cell formation and cell elongation of the root (Raziuddin *et al*., 2011). In the present investigation, Table 4 shows that Cd^{+2} adversely affect the percentage of RWC in Cd treated-wheat plants. It was found from our results that using *Moringa* seed cake in soil for wheat plants improved the growth of seedling if compared to Cd-treated and non- treated control plants. This was confirmed by our results (Table 1) and those of Emmanuel *et al*. (2011), who reported that MO seed cake has high content of phytohormones, macro, micronutrient and antioxidant compounds such as ascorbic acid, reduced glutathione and total phenol, so increased the mineral content of the soil, and hence improved the growth of the crop plants as compared to the control ones. The decrease in leaf conductance and relative water content could be due to a direct Cd^{+2}-induced stomatal closure (Perfus-Barbeoch *et al.*, 2002), through disturbance of hormonal balance that may reduce water transport and reduced % RWC (Table 4).

Cadmium stress negatively affects the photosynthetic pigments (ch a, ch b and carotenoids) in stressed wheat plants (Fig. 1). This reduction in chlorophyll contents in Cd-treated plants may be due to inhibition of its biosynthesis (Padmaja *et al.*, 1990). Cadmium may substitute Mg ion in the chlorophyll molecules and this will lead to chlorophyll destruction (Baryla *et al.*, 2001). Also lowering the chlorophyll contents under Cd stress result from the activation of its enzymatic degradation by chlorophyllase (Vassilev and Yordanov, 1997).

As shown in (Fig. 2), wheat roots had much higher Cd^{2+} concentration than shoots, this result was acceptable in cereal crops because these parts are not generally utilized as food or feed (Zhang *et al.*, 2002).

The effects of Cd^{2+} ions on protein expression revealed differential expression of various proteins (Kranner and Colville, 2011), five protein bands of molecular weights 217.77, 135.4, 51.6, 45.0 and 38.2 KDa were expressed in Cd-treated wheat plants. They may be Cd stress proteins (Table 5, Plate 2). During Cd stress, the activity of proteins can be affected by reactive oxygen species (Romero-Puertas *et al.*, 2002). Where proteins can undergo oxidative modification through direct reaction with ROS, and also through interaction with the aldehyde products of lipid peroxidation (Sandalio *et al.*, 2001), these products reacts with free amino groups of proteins leading to protein carbonylation which is the common oxidative protein modification in which the side-chains of amino acids are converted to aldehyde or keto groups, leading to inactivation of proteins and cross linking. On the other hand, Cd-stress can promote expression of stress-responsive genes and subsequently proteins, thereby increasing the levels of RNA and proteins (Maheshwari and Dubey, 2008). These proteins may bind Cd^{2+} forming phytochelatins and metallothioneins which have been sequestered in the vacuole. Application of the seed powder of both *Moringa* species in the soil of wheat plants can lead to the expression of 11 new protein bands in leaves. These protein bands having molecular weights of 261.38, 217.77, 177.38, 135.4, 114.0, 97.0, 74.14, 51.6, 38.2, 24.0 and 13.5 KDa, that may have the ability to chelate the Cd^{2+} ions, thus decreasing their content in roots and shoots of wheat plants (Table 5 and Plate 2). In our opinion the improvement in growth of wheat seedlings in pots treated with both *Moringa* species separately or in combination with Cd^{2+} concentrations may be related to the ability of seed cake to chelate Cd^{2+} from the *Moringa*- treated soil, thus the plants can grow in normal conditions and revealed normal growth. Generally, we reported that application of *Moringa oleifera* seed cake was more effective in removing Cd^{2+} ions from the soil than *Moringa peregrina* (Fig. 2). There is no information recorded concerning this study.

Conclusion

Moringa seed powder being a good coagulant for Cd^{2+} metals from the soil under stress condition. MSP can enhance the tolerance of wheat plants to heavy metal stress. Also improved the tolerance of wheat plant by increasing growth of wheat plant under stress-free development under stress-free condition and stress conditions via phytohormones, antioxidant compounds, macro and micronutrient-rich *Moringa* seeds.

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(*Recrived* 27/12/2015;
accepted 7 /1 /2016)

تخفيف سمية عنصر الكادميوم على نبات القمح باستخدام مسحوق بذور البان (اللبان) أوليفيرا و بريجرينا منزوعة الدهون كمرسبات لعنصر الكادميوم.

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يتضمن هذا البحث دراسة الخصائص الغير متوقعة لنباتي، البان (اللبان) أوليفيرا وبريجرينا في امتصاص عنصر الكادميوم من التربة في نطاق المعمل. تم خلط ٣ جم من بودرة بذور نباتي البان (اللبان) على حدة مع كل كجم من التربة المستخدمة في زراعة نبات القمح (*Triticum aestivum*) قبل الزراعة مباشرة. بعد عشرة أيام تم ري التربة بتركيزات مختلفة من الكادميوم (٠,٥، ١,٠ و ١,٥ ميللي مولار). تم جمع عينات نبات القمح بعد خمس اسابيع من زراعته لكي تتم دراسة الصفات الظاهرية، وبعض المعايير الفسيولوجية. لوحظ ان المعاملة بعنصر الكادميوم تؤدي الى نقص في الصفات الظاهرية لنبات القمح المعامل بتركيزات من الكادميوم مقارنة بنباتات المقارنة، كما تؤدي الى نقص كل من المحتوى المائي النسبي و الأصباغ الضوئية لنباتات القمح المجهدة بعنصر الكادميوم اذا ماقورنت بنباتات المقارنة والنباتات المعاملة ببودرة نباتي البان. على النقيض، فقد وجد ان معاملة نباتات القمح ببذور نباتي البان تؤدي الى تقليل سمية عنصر الكادميوم (على الصفات المدروسة اعلاه) على نباتات القمح المعاملة بهذه البذور. كما يؤدي الإجهاد بعنصر الكادميوم الى زيادة محتوى هذا العنصر في كل من المجموع الجذري والمجموع الخضري لنبات القمح، ولكن يحدث العكس في النباتات المعاملة ببذور نباتي البان حيث تقلل محتوى عنصر الكادميوم في جذور وأوراق نبات القمح. وقد لوحظ ان بودرة بذور نباتي البان لها القدرة على امتصاص عنصر الكادميوم من التربة عن طريق بروتينات لها القدرة على جذب آيونات الكادميوم حولها و الوزن الجزيئي لهذه البروتينات (٦٤,٥، ٥١,٤، ٤١,٢، ٣٨,٣، ٢٧,٩ و١٨,٩ كيلو دالتون). من خلال هذا البحث، تم استنتاج ان استخدام بودرة بذور نبات البان أوليفيرا اكثر فاعلية من البان بريجرينا في تقليل سمية عنصر الكادميوم -على نباتات القمح المعاملة به- وامتصاصه من التربة.