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Influence of moringa extract application on growth and chemical constituents of *Alstonia scholaris* grown in sandy soil polluted by cadmium

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Abstract : Two pot experiments were carried out at green house of National Research Centre , Egypt, during 2014 and 2015 seasons. The purpose of this study is to investigate the influence of moringa extract (0 and 5cm/pot) on the growth and constituents of *Alstonia scholaris* grown in sandy soil polluted by cadmium (0, 10, 15 and 20 mg / kg soil). Results show that, moringa extract gave the highest values of plant height, leaves number, root length, stem diameter and fresh and dry weight of leaves and stems. The same behavior was noticed concerning nitrogen, phosphorus and potassium percentage as well as nitrogen, phosphorus and potassium uptake in leaves, stems and roots. in addition, plant height, leaves number, root length, stem diameter, fresh and dry weight of all plant organs decreased as cadmium concentrations increased. Data also observed that, all growth parameter increased by interaction treatments between moringa extract plus all cadmium concentrations. Cadmium at 15 mg/kg soil gave the greatest values of chlorophyll a, b and carotenoid. Whereas, the maximum values of Pb and Cd ppm were obtained by cadmium at 20 mg / kg soil in leaves, stems and roots. It could be recommended to use Cd concentration up to 10 mg/ kg soil to stimulate the growth parameters and chemical constituents and to treat plants in regions polluted with cadmium with moringa extract to overcome the dangerous and destructive of effect of the concentrations of cadmium.
Keywords : Pollution - *Alstonia scholaris* – Cadmium –Moringa- Phytoremediation.

Introduction

The plant *Alstonia scholaris* belongs to the family Apocynaceae that originates in India. It is an evergreen three that ranges in size from medium to large, it also has straight buttressed and fluted stem. The tree has dark – grey and shiny leaves coming out of world branches, whereas the bark itself is black ashtray and rough with crocks that are shallow. One whorl has 4-10 leaves of obviate and obtuse shape classed in a petiole. The tree's flowers are greenish-white and give follicular fruit, the plant is mostly used for its wood in pocking cases, boxes and writing boards, also gun powder is made from the wood charcoal. After proper and it is also good enough for match boxes manufacturing as well as splints. The bark is sometimes used as astringent, tonic and as medicine for diarrhea and dysentery. It can used in treating sores and ulcers by applying its milky juice to them. Fiber is yielded from the plant's bark and the flowers give an oil and alkaloid pyridine that is used as an anti-depressant [1,2].

Heavy metals are any metals of more than 5 g/cm3 in density. Only 53 metals, of the total 20 naturally occurring elements, are considered heavy metals[3] and a few of those has any biological significance. 17 heavy metals of those 53 mentioned above may be available to living cells based on their solubility under physiological conditions, they may also be important for both communities of plants and animals in different ecosystems [3]. Among those heavy metals are Zn, Ni, Cu, U, Co, W and Cr which are non- toxic elements at low concentrations. As, Hg, Ag, Sb, Cd, Pb and Al all do not have any known functions as nutrients, they also appear to be toxic to plants and microorganisms[4,5,6].

Some heavy metals have shown on inhibition in the growth and an decrease in the crops productivity at high concentrations[7]. One of those is cadmium which is quite known for its toxicity as an environmental element and its movement from soil to plants and further down the food chair[8]. Almost all organisms can accumulate and incorporate Cd in great sums. Cd interferes in physiological metabolism in plants such as photosynthesis, assimilation of nitrogen, transpiration and respiration[9,10,11].

Furthermore, Cd is a divalent heavy metals action (Cd^2) which results in photo toxicity as it is readily taken up. The excess cadmium in the environment reduces the content of chlorophyll[12] and growth as well[13]. In this regard, all the macro elements in herbage and roots are decreased by the increment in the concentration of cadmium. The same outcome was observed in microelements. However, the content of cadmium increased when the concentration of cadmium was increased[14].

Bio-extracts that contain beneficial micro and macro elements are known to improve the growth of the plant by supplying nutrients to plants that might help in sustaining the health of the environment and the productivity of the soil as well. Additionally, the use of breaking synthetic chemicals turned out to cost a lote and to cause contamination in the environment[15]. Plant hormones influence all phases of plant growth and development which is why they are used to increase the yield per unit area.

Traditionally, five groups of growth regulators are listed which are auxins abscise acid, cytokines and ethylene [16]. Cytokines improve the production of food. Seating is a very common form of cytokine that occurs naturally in side plants. Fresh *Moringa oleifera* leaves have been found to have a high content of zeatin. The plants leaves were taken from different parts of the world and studied, they were shown to have high concentrations of seating that range between 5 mcg and 200 mcg/g of leaves[17].

The current study focuses on testing the effect of moringa extract on the growth and chemical constituents of *Alstonia scholaris* grown in sandy soil polluted by cadmium.

Materials and Methods

Pot experimental trails were carried out during the two successive seasons of (2014 and 2015) at the green house of national research Centre, Cairo, Egypt. The effect of Moringa extract on vegetative growth and chemical constituents of *Alstonia scholaris* grown under cadmium pollution in sandy soil was studied in this experiment.

One year – old seedlings of *Alstonia scholaris* were obtained from nursery of forestry department Horticulture research Institute, Agriculture Research Centre. The seedlings were planted on 15th March in plastic pots 30cm (one plant/ pot, the average height of seedlings were 15.20cm) filled with 12kg soil. The physical and chemical characteristics of the used soil are determined according to method of [18] and presented in Tables (1 and 2).

Table(1): Some physical properties of used soil:

Soil sample	coarse sand %	Fine sand %	Silt%	Clay %
Sand	71.0	18.0	4.5	6.5

Table(2): Some chemical constituents of used soil:

Soil sample	EC. m.mohs/cm^3	pH	Sp%	Anion (meqL-1)			Cation (meqL-1)			
				HCO_3^-	Cl^-	SO_4^-	Ca^{++}	Mg^{++}	Na^{++}	K^+
Sand	2.1	7.1	23.0	2.0	23.0	-	4	3	15	5

Cadmium as cadmium sulphate was added at (0, 10, 15 and 20mg/1kg soil) after one month from planting. Moringa extract at (0 and5 cm / pot) was added once after a month and another time after two months of planting.

The experiment was set in completely Randomized in factorial experiment Design with four cadmium levels and two rates of moringa extract to give 8 treatments with replicates.

All plants were supplied by mixed by ammonium nitrate (33.5 N%), super phosphate (15.5% P_2O_5) and Potassium sulphate (48.5 K_2O) at the rate of 5gm/ pot in four doses. At the end of the experiment at November 15th, the following data was recorded : plant height (Cm), No. of leaves/ plant, root length (Cm), stem diameter (Cm), fresh and dry weight of leaves, stems and roots. All previous data were subjected to statistical analysis of variance according to the method described by[19] and the combined analysis of the two seasons was calculated according to the method of [20]. Chlorophyll (a), (b) and carotenoids content was determined in fresh leaves according to[21]. The following data were determined in leaves, stems and roots dry weight. Carbohydrates percentage was determined according to[22]. Microelements (Nitrogen, Phosphorus and Potassium) were determined according to the method described by[23]. Cadmium percentage was determined using the Atomic Absorption Spectrophotometer Zeiss FMD_3 according to [24].

Results and Discussion

Vegetative growth:

The results obtained in Table (3 and 4) showed that cadmium at high concentrations (15 and 20 mg/ Kg soil) decreased plant height, number of leaves/ plant, root length and stem diameter of *Alstonia scholaris* compared with the control and other treatments. The decrease was (8.96 and 11.88%) plant height, (2.56 and 23.08%) number of leaves / plant, (7.86 and 23.21%) root length and (3.24 and 5.38%) stem diameter respectively, compared with the control. In this respect, the lowest values of fresh and dry weight of all plant organs were obtained by (20mg/ Kg soil), fresh weight gave (56.19, 117.38 and 72.23 gm) leaves, stems and roots and dry weight gave (15.28, 35.10 and 24.34 gm).

Table (3): Effect of moringa extract on plant height (cm), No. of leaves /plant, Length of root (cm) and Stem diameter (cm) of *Alstonia scholaris* seedlings grown under cadmium pollutants.

Characters / Treatments	Plant height(cm)	No. of leaves	Length of root(cm)	Stem diameter(cm)
Control	80.0	39	28	1.86
Moringa extract	110.25	68.5	38.5	2.7
Cd 10 mg	104.75	60	34.5	2.2
Cd 15 mg	72.83	38	25.8	1.80
Cd 20 mg	70.50	30	21.5	1.76
Moringa+Cd 10 mg	101.72	58	32.0	2.1
Moringa+Cd 15 mg	99.75	52	31.5	1.95
Moringa+Cd20 mg	86.24	46	29.5	1.91
L.S.D at 5%	7.19	4.10	2.36	0.21

Table (4): Effect of moringa extract on fresh and dry weight of leaves, stems and roots (g) of *Alstonia scholaris* seedlings grown under cadmium pollutants.

Characters / Treatments	F.W of leaves	F.W of stems	F.W of roots	D.W of leaves	D.W of stems	D.W of roots
Control	63.41	143.27	83.12	17.56	44.13	28.43
Moringa extract	106.62	205.44	114.52	31.99	67.80	41.91
Cd 10 mg	95.49	185.73	147.67	28.27	60.73	54.64
Cd 15 mg	68.31	131.52	87.84	19.20	40.90	30.57
Cd 20 mg	56.19	117.38	72.23	15.28	35.10	24.34
Moringa+Cd 10 mg	78.46	174.90	112.90	22.75	55.79	40.87
Moringa+Cd 15 mg	89.29	177.11	99.97	26.07	57.03	35.79
Moringa+Cd 20 mg	73.85	161.42	92.85	21.05	50.85	32.78
L.S.D at 5%	6.98	13.14	9.88	2.23	4.87	3.22

The reduction could be due to the stopping that occurs in the rate of elongation growth of cells as cadmium performs an irreversible inhibition on proton pump that does the process [25,26]. The result in harmony with those of [14].

On the other hand, the highest values of plant height, number of leaves/ plant, root length, stem diameter, fresh and dry weight of leaves and stems were obtained by moringa extract 5cm/pot compared with the control and other treatment. The values were (110.25, 68.5, 38.5, 2.7, 106.62, 205.44, 31.99 and 67.80) respectively. This is may be due to Moringa extracts that contain high zeatin hormone which stimulates growth. These results were confirmed with those obtained by [27,28,29,30].

In addition, Interaction between moringa extract 5cm/pot plus cadmium in any concentrations (0, 10, 15 and 20mg/1kg soil) increased all growth characters (Plant height, number of leaves/ plant, root length, stem diameter, fresh and dry weight of leaves, stem and roots) compared with the untreated plant.

Total carbohydrates content:

Table (5): Effect of moringa extract on Carbohydrates% and Pigments content mg/gm f.W of *Alstonia scholaris* seedlings grown under cadmium pollutants .

Characters / Treatments	Carbohydrates%			Pigments content mg/gm f.W		
	leaves	Stems	Roots	Chlorophyll (a)	Chlorophyll (b)	Carotenoids
Control	26.2	26.0	46.4	2.379	0.933	2.804
Moringa	28.0	24.0	24.2	2.047	1.024	2.791
Cd 10 mg	27.2	27.6	25.4	2.421	1.151	2.832
Cd 15 mg	37.0	27.2	25.2	2.458	1.322	2.889
Cd 20 mg	25.4	24.0	23.4	2.453	1.210	2.860
Moringa +Cd10mg	25.4	25.8	28.2	2.169	0.707	2.718
Moringa+Cd15mg	25.4	23.8	28.8	2.359	0.914	2.814
Moringa+Cd20mc	28.4	24.0	28.8	2.162	0.804	2.784

The data obtained in Table (5) reported that in leaves carbohydrates percentage increased by Cd at 10 and 15 mg / 1kg soil as compared to the control plants. The increments were (3.82 and 41.22%) by Cd at 10 and 15mg/kg soil respectively. Similar results were obtained by moringa extract and Moringa extract plus Cd at 20 mg/Kg soil. Total carbohydrates percentage increased by moringa extract and Moringa extract plus Cd at 20mg/ Kg, were (6.87 and 8.40%) respectively compared with untreated plants. Whereas, Cd at 20 mg/Kg soil and Moring a extract + Cd at 10 or 15 mg/ Kg soil reduced total carbohydrates percentage compared with the control and other treatments. In stems, the highest percentage of total carbohydrates was obtained in plants

treated with Cd at 10 and 15 mg/ Kg soil. The increments were (6.15 and 4.62%) by Cd at 10 and 15 mg/ Kg soil respectively. The lowest value of carbohydrates % was obtained by Moringa extract + Cd at 15 gm/ Kg soil compared with other treatments. In roots, the greatest value of total carbohydrates % was achieved by control plants compared with other treatments. In this respect, total carbohydrates % decreased by all treatments compared with the untreated plants. This effect was previously found by [31] reported that the low export from mesophyll and higher starch accumulation harmed *Alstonia scholaris* might cause stronger resistance of photosynthetic apparatus of theirs and weak starch export from the mesophyll. Heavy metals bad effect on the metabolism of carbon results from their possible interaction with the center of ribulosebis phosphate carboxylase.

Pigments Content (mg/g F.W.) :

From the given data in Table (5) cleared that, cadmium at 15 mg/kg soil showed the highest content of chlorophyll a, b and carotenoids (2.458, 1.322 and 2.889 mg/g F.W.) followed by cadmium at 20 mg/Kg soil (2.453, 1.210 and 2.860 mg/g F.W.) in the leaves respectively. While, the lowest content of chlorophyll – a (2.047 mg/g F.W.) was determined in leaves of the plants treated with Moringa extract. But, the least values of chlorophyll – band carotenoids were recorded, when plants were treated by Moringa + Cd at 10mg/Kg Soil.

Nutrient Contents:

The results obtained in Table (6) showed that moringa extract increased all microelements in all plants orgasm except potassium in roots of *Alstonia scholars* plants. Plants applicator with moringa increased nitrogen percentage in leaves, stems and roots compared with the control. The increments were (125%, 128.2 and 64.71%) in leaves, stems and roots respectively compared with the control. The untreated plant gave the lowest value of nitrogen % in leaves, but, cadmium at 20 mg/kg soil gave the least values of nitrogen % in stems and roots. The same results were obtained in phosphorus % in all plant organs. The results indicated that the phosphorus percentage in leaves, stem and roots increased in plants applicator by moringa (0.44, 0.32 and 0.22%) respectively followed by plants treated with moringa + Ca at 10mg / Kg soil (0.32, 0.25 and 0.16%) respectively and then, plants applicator with Cd at 10 mg/ Kg soil (0.28m 0.22 and 0.13%) respectively. The lowest values of P% were recorded with untreated plants (0.18%) leaves, (0.08%) stem and (0.08%) roots were recorded with untreated plants compared with the other treatments. In this respect, plants applicator with moringa extract gave the greatest values of potassium % in leaves and stem (1.61 and 2.21%) respectively followed by plants treated with moringa extract + Cd at 10mg/ Kg soil (1.41 and 1.92%) respectively, compared with other treatments. In roots the highest value of potassium % was resulted in untreated plants (1.15%) followed by moringa extract or moringa extract + Cd at 10mg / Kg soil (1.10 and 1.10%) respectively.

Table (6): Effect of moringa extract on Nitrogen, Phosphorus and Potassium percentage of *Alstonia Scholaris* seedlings grown under cadmium pollutants.

Characters / Treatments	Nitrogen %			Phosphorus %			Potassium %		
	Leaves	Stem	Roots	Leaves	Stem	Roots	Leaves	Stem	Roots
Control	1.20	0.85	0.85	0.18	0.08	0.08	0.46	1.15	1.15
Moringa	2.70	1.94	1.40	0.44	0.32	0.22	1.61	2.21	1.10
Cd 10 mg	1.69	0.90	0.68	0.28	0.22	0.13	0.98	1.45	0.62
Cd 15 mg	1.52	0.82	0.59	0.21	0.17	0.11	0.89	1.22	0.51
Cd 20 mg	1.36	0.75	0.51	0.18	0.14	0.08	0.81	1.00	0.48
Moringa +Cd10mg	1.93	1.36	0.87	0.32	0.25	0.16	1.41	1.92	1.10
Moringa+Cd15mg	1.72	1.15	0.75	0.27	0.21	0.13	1.20	1.84	0.69
Moringa+Cd20mg	1.65	1.00	0.63	0.23	0.15	0.11	0.98	1.73	0.58

Table (7): Effect of moringa extract on lead and cadmium content of *Alstonia Scholaris* seedlings grown under cadmium pollutants.

Characters / Treatments	Pb ppm			Cd ppm		
	leaves	Stems	Roots	leaves	stems	Roots
Control	1.70	0.70	4.00	0.54	0.41	2.2
Moringa	11.00	7.00	18.00	0.77	0.62	5.1
Cd 10 mg	18.00	10.00	28.00	1.28	1.00	7.80
Cd 15 mg	20.00	13.00	36.00	1.50	1.15	8.30
Cd 20 mg	24.00	16.00	43.00	1.80	1.30	13.00
Moringa +Cd10mg	15.00	8.00	23.00	1.10	0.88	6.3
Moringa+Cd15mg	1.20	11.00	30.00	1.22	0.97	7.90
Moringa+Cd20mg	21.00	13.00	37.00	1.60	1.10	8.20

Additionally, It is obvious from the data in Table (7) that Pb and Cd ppm were markedly increased in all plant organs in all treatments compared with the untreated plants. The highest values of Pb were obtained in plants applicated with Cd at 20 mg/kg soil compared with other treatments. The values were (24.00, 16.00 and 43.00 ppm) in leaves, stems and roots respectively. The lowest values of Pb ppm resulted from the untreated plants and they were *1.70, 0.70 and 4.00 ppm) in leaves, stems and roots respectively. The same results were observed in Cd ppm. Cadmium ppm increased in all treatments. The greatest values of Cd were achieved in plants treated with Cd at 20 mg/Kg soil. The values were (1.80 ppm) in leaves, (1.30 ppm) in steams and (13.00ppm) in roots. Whereas, the least values of Cd ppm resulted from untreated plants. The values were (0.54, 0.41 and 2.2 ppm) in leaves, stems and roots respectively. Generally, cadmium content gradually increased by increasing Cd concentrations. The result is in harmony with those of [32,33,34,14].

Results in Table (8) indicated that the highest N uptake in leaves was obtained by plants applicated with moringa extra (864 mg) followed with Cd at 10 mg/Kg soil(478 mg) compared with other treatments and with the untreated plants. Similar results were obtained with N uptake in roots. The greatest values of N uptake were recorded by plants treated with moringa extract (586.20 mg) followed by Cd 10mg/Kg soil (371.55 mg) compared with other treatments and with the control. But the value of N uptake in stem resulted from plants applicated with moringa extract followed by moringa extract +Cd at 10 mg/Kg soil compared with control and with other treatments. In general, the least values of nitrogen uptake in leaves and steams were recorded with Cd at 20 mg/Kg soil and in roots was recorded in untreated plants.

Data presented in Table (8) revealed that moringa extract gave the greatest values of phosphorus uptake (140.7 mg) in leaves, (217 mg) in steams and (92.20 mg) in roots compared with the control and other treatments.

Table (8): Effect of moringa extract on Nitrogen, Phosphorus and Potassium uptake of *Alstonia scholaris* seedlings grown under cadmium pollutants.

Characters / Treatments	Nitrogen mg			Phosphorus mg			Potassium mg		
	Leaves	Stem	Roots	Leaves	Stem	Roots	Leaves	Stem	Roots
Control	210	375	56.68	31.6	35	8.53	81	507	34.12
Moringa	864	1315	586.20	140.7	217	92.20	515	1498	461.61
Cd 10 mg	478	547	371.55	79.2	133	71.00	277	880	338.8
Cd 15 mg	292	335	180.36	40.0	70	33.64	170	499	155.91
Cd 20 mg	208	263	124.13	27.5	49	19.47	124	351	116.83
Moringa +Cd10mg	439	758	355.57	72.8	139	65.25	320	1071	318.79
Moringa+Cd15mg	448	655	268.43	70	120	46.53	312	1049	246.95
Moringa+Cd20mg	347	513	206.51	48.4	76	36.06	206	880	190.12

The lowest values of P uptake in leaves were recorded in Cd at 20 mg/Kg soil, in steams and roots were recorded in untreated plants giving (27.5, 35.0 and 8.53 mg) respectively.

Data also reported that the highest values of potassium uptake in all plant organs (leaves, stem and roots) were observed in plants applicated with moringa extract (515.0, 1498.0 and 461.61 mg) respectively in comparison with the control. But the lowest values of potassium uptake resulted from untreated plants in leaves and roots giving (81.0 and 34.12 mg) respectively and in plants applied with Cd at 20 mg/Kg soil in stem gave (351.0 mg).

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