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REMOVAL OF SOME PLANT TOXIC HEAVY METALS FROM SOIL USING *MIMOSA PIGRA* L. PLANT AND EFFECT OF METHANOLIC EXTRACT OF *ACACIA NILOTICA L.* ON METAL REMOVING EFFICACY

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Abstract

The principal concern of our study, which is carried out in microcosmic conditions, is focused on removing some plant toxic heavy metals such as Ni, Se, Zn, Pb, Cd, Cr and Cu from soil by using *Mimosa pigra* plant, as well as the measuring the effect of these harmful elements on some morphological characters of seedlings such as length, number of leaves, fresh weigh, dry weigh and chlorophyll A and B. The investigation also evaluates the effect of *Acacia. nilotica* methanolic extract (ANME) on heavy metals removing process. Seedlings of *M. pigra* were subjected to two treatments; first by two different concentrations of heavy metals solutions, the second by adding of ANME. Atomic absorption analysis of seedlings exhibited that *M. pigra* seedlings had a good ability to remove Ni (20 ppm), Zn (80.01 ppm), Cr (8.82 ppm), Cd (17.57 ppm) and Cu (223.75), especially in case of seedlings treated with high concentrations of heavy metals, while accumulation of Se and Pb was not affected by increasing the concentration of elements. Addition of ANME enhanced the accumulation of Ni (35.1 ppm), Cr (11ppm), and Se (186.24 ppm). Accumulation of heavy metals resulted in reduction in length, number of leaves, fresh weight, dry weight and chlorophyll a and b content of *M. pigra* seedlings. Statistical data analysis was carried out using One-way analysis of variance (ANOVA).

Key words: *Mimosa pigra,* Toxic, Heavy metals, *Acacia nilotica*, Methanolic extract, Morphological features.

Introduction

Soil pollution with toxic heavy metals is considered the main environmental problem in many areas particularly the industrial countries (Grytsyuk *et al*., 2006). When plant roots take in nutrients and water from polluted source, most of these plants have the capability to remove harmful and toxic chemicals from soil and water. Phytoremediation is actually a generic term for many ways in which plants can be used to clean up contaminated water and soils. The most toxic heavy metals such as Lead (Pb), Mercury (Hg), Arsenic (As), Cadmium (Cd), Selenium (Sn), Chromium (Cr), Zinc (Zn) and Copper (Cu) (Gosh, 2010) come from industrial manufacturing processes, domestic refuse and waste materials (Yadav *et al*., 2009). Presence of toxic heavy metals in soil affect plant growth causing loss of the agricultural productivity of any planted crop (Ghani, 2010).

Mimosa pigra L. belongs to Fabaceae family, and Mimosoideae subfamily. It is a rare shrub restricted to the Nile banks and granite islands of Aswan, considered as one of the endangered species in Egypt (EL-Hadidi *et al*., 1992). The species is used in many herbal remedies and magic rites in Africa (Burkill, 1995). *Mimosa pigra* L. contains many phytochemical contents such as; alkaloids, amino acids, Anthraquinones, Flavonoids, Glycosides, Saponins (*Mbatchou et al.,* 2011).

Acacia nilotica is a native species distributed from Egypt to Mozambique, grows in tropical and subtropical countries. Phytochemical analysis of aerial parts of the plant resulted in the identification of a variety of phenolic constituents, among which catechin derivatives. *Acacia nilotica* has many compounds that have a wide range of biological activities, particularly as anticarcinogenic, antioxidant and anti-inflammatory activities (Maldinia *et al.,* 2011).

In this study, removing of some toxic heavy metals by using *M. pigra* was investigated. Moreover, the effects of the plant extract of *A. nilotica* - which grow side by side to *M. pigra* in the study area- on the removal efficiency, were evaluated.

Materials and Methods

Plants materials: Seeds *of M. pigra*, as well as *A. nilotica* fruits were collected from the first cataract island (Saluga and Ghazal) protected area at Aswan. All plant materials were identified according to Boulos (1999).

Phytochemical analysis of *Acacia nilotica* and *Mimosa pigra*: Protein and Carbohydrates were determined according to (Lowry *et al.,* 1951) and (Morris, 1948). Phenols, flavonoids and saponins were determined according to (Singelton *et al*., 1999; Zhishen *et al.*, 1999; Ebrahimzad & Niknam, 1998), respectively. Total antioxidant capacity (TAC) was determined by spectrophotometry using ascorbic acid standard method, at ʎ= 695 nm (Prieto *et al*., 1999).

Seeds treatment: *M. pigra* seeds were treated using concentrated sulfuric acid for 20 min, and then washed carefully with distilled water (Yoursheng & Sziklai, 1985).

Irrigation solutions: Irrigation solutions containing heavy metals were prepared in two concentrations of each element according to Aydinalp & Marinova,(2009); Ilhan *et al.*, (2004); John *et al.*, (2009); Malone *et al.*, (1974); Manios *et al.*, (2003) and Ali *et al.*, (2012).

Preparation of methanolic extract of *Acacia nilotica* fruits: 200 grams of the air-dried of *Mimosa pigra* and *Acacia nilotica* fruits were powdered separately and extracted with MeOH 80% by maceration until exhaustion. The alcoholic extract was concentrated under freeze dryer (Christ Alpha 2-4 LD) system and pulverized (Mahalel, 2015). Concentration of 1% of each extract was used in treatment of irrigation solutions.

Chemical analysis of *M. pigra* seedlings and soil: After 2 months, seedlings of each treatment were harvested separately and washed carefully with distilled water, weighted, then dried in the oven at 105°C. The dried samples were ground to powder, and then digested by nitric acid for heavy metals analysis using atomic absorption (Havlin & Soltanpour, 1980). Chlorophyll content of seedlings was determined according to Arnon, (1949) spectrophotometerically at ʎ = 645. Soil used in the experiment was also analyzed by using atomic adsorption.

Data analysis: One-way analysis of variance (ANOVA) through the statistical computer programme MINITAB was used to test the correlation and significance of quantitative data of the removing of the studied elements.

Results and Discussion

Chemical analysis of soil exhibited that selenium content was the highest among the tested elements; and lead content was the lowest (Table 1).

Total flavonoids, total phenols, saponins and total antioxidant capacity (TAC) of *A. nilotica* are shown in table 2. Secondary metabolites molecules in *A. nilotica* recorded high contents of flavonoids (80.50 mg/g) followed by saponins (60.05 mg/g), phenols (23.06 mg/g) and total antioxidant (6.25 mg/g). On the other hand, maim primary metabolites molecules such as carbohydrates and proteins recorded 5.93 mg/g and 23.31 mg/g, respectively.

Removing heavy metals bioassay: Tables 3 to 9 show the data of the effect of accumulation of studied elements; Ni, Zn, Cr, Cd, Cu, Se, and Pb as well as the effect of adding ANME on the investigated morphological features and chlorophyll a,b of *Mimosa pigra* seedlings.

Nickel (Ni): The data exhibited that *M. pigra* seedlings had the ability to remove nickel from the soil and accumulate it in their shoots and roots, about 20 ppm of Ni were accumulated in case of seedlings treated with high concentration (16 ppm) (Fig. 1). Adding of ANME enhanced significantly the removal process of Ni ($p< 0.5$), in case of concentration of 16 ppm about 35.7 ppm of Ni were accumulated. A remarkable decrease was observed on plant length, number of leaves, chlorophyll A and chlorophyll B (Table 3). Our data were in agreement with Netty *et al*., (2013) who considered *M. pigra* as Ni tolerance species and it could accumulate more amount of Ni in their roots and shoots. Like *M. pigra* many plants such as *Brassica juncea* and *Brassica napus* are considered as *hyper - accumulate* of nickel from soil and water (Brown, 1995). The growth reduction in *M. pigra* seedlings may happen as a result of changes in physiological and biochemical processes in plants growing on heavy metal polluted soils (Oancea *et al.*, 2005).

Zinc (Zn): Figure 1 shows that *M. pigra* seedlings have the ability to remediate Zinc in all treated seedlings Significant variations were observed ($p<0.05$). 15.53 ppm of Zn (2.2 times of amount accumulated by the control) was accumulated in seedlings treated with low concentration (100 ppm). Where the amount accumulated in the high concentration (800 pm) was 11.9 times as amount accumulated by control (83.82 ppm). Seedlings treated with low concentration of Zn and increasing in their length, number of leaves, fresh weight and dry weight was observed, where no noticeable effect was recorded in chlorophyll a or b. Otherwise, in seedlings treated with high concentration of Zn, a remarkable decrease in all parameters was noticed (Table 4). The same was found *with Mimosa pudica* indicating that it was a good accumulator of Zn in their roots and shoots (Ashraf *et al*., 2011). Treatment of seedlings with ANME enhanced Zn remediation in case of low concentrations of element (100 ppm), which may refer to its high content of saponins (60.05mg/gm). Saponins as secondary metabolite molecules could affect on the uptake of zinc which was recorded in Italian ryegrass (Zhu et al., 2015). Changes in morphological feature of seedlings resulted from the direct toxic effects of high concentrations of heavy metals such as inhibition of cytoplasmic enzymes and damaging of cell structures because of its oxidative stress (Van Assche & Clijsters, 1990). Although Zn in excessive amount is toxic for the plant, but its presence in small quantity may help the plant because it's importance as cofactors for many enzymes and essential for both functions of chloroplast and mitochondria (Clemens, 2001).

Table 1. Chemical analysis of soil and water used in the experiment.

	Ni	Se	Zn	Pb	Cr	Cd	Cu
Soil (ppm)	0.0413	0.1115	0.0523	0.0096	0.0594	N.D.	0.0102
Water (ppm)	0.003	N.D.	0.541	N.D.	N.D.	0.0029	0.0299

Table 2. Phytochemical analysis of *Mimosa pigra* and *Acacia nilotica.*

	Carbohydrates (mg/g)	Proteins (mg/g)	Flavonoids (mg/g)	Phenols (mg/g)	Saponins (mg/g)	Total antioxidant capacity (TAC) (mg/g)
Mimosa pigra	14.87 ± 1.479	10.72 ± 1.21	41.80 ± 3.90	23.07 ± 0.47	28.10 ± 0.49	7.66 ± 1.18
Acacia nilotica	5.928 ± 0.602	23.31 ± 0.71	80.50 ± 4.49	23.06 ± 1.04	60.05 ± 1.42	6.25 ± 0.66

Table 3. Effect of Nickel accumulation and adding ANME on some morphological features and chlorophyll a,b of *Mimosa pigra* seedlings.

Treatment	Plant length (cm)	Number of leaves	Fresh weight (gm)	Dry weight (gm)	Chlorophyll A %	Chlorophyll B %
Control	13.13 ± 5.37	34.89 ± 11.63	20.13	6.03	28.23	29.96
5 ppm	15.83 ± 1.61	35.48 ± 2.83	14.02	4.32	32.98	37.57
16 ppm	11.50 ± 2.75	24.96 ± 7.01	15.88	5.44	15.58	15.42
5 ppm + ANME	14.81 ± 2.57	29.04 ± 5.33	15.89	4.78	N.D.	N.D.
16 ppm + ANME	8.28± 1 .26	11.56 ± 7.85	5.85	1.73	21.09	28.36

ANME = *A. nilotica* methanolic extract, ND = Not detected

Table 4. Effect of Zinc accumulation and adding ANME on some morphological features and chlorophyll a, b of *Mimosa pigra* seedlings.

Treatment	Plant length (cm)	Number of leaves	Fresh weight (gm)	Dry weight (gm)	Chlorophyll A %	Chlorophyll B %
Control	11.38 ± 0.00	22.25 ± 0.00	11.37675	2.89955	3.195076	3.458488
100ppm	13.25 ± 1.59	25 ± 4.24	20.534933	6.4637333	3.0277255	3.310929
800ppm	5.63 ± 0.26	6.38 ± 0.18	3.2866333	0.8139	N.D.	N.D.
100ppm + ANME	8.28 ± 0.57	19.38 ± 4.07	8.2436	2.3607333	1.748579	1.927726
800ppm + ANME	3.75 ± 0.79	1.25 ± 0.71	3.9885333	1.0172333	N.D.	N.D.

ANME = *A. nilotica* methanolic extract, ND = Not detected

Table 5. Effect of Chromium accumulation and adding ANME on some morphological features and chlorophyll a, b of *Mimosa pigra* seedlings.

Treatment	Plant length (cm)	Number of leaves	Fresh weight (gm)	Dry weight (gm)	Chlorophyll A %	Chlorophyll B %
Control	17.04 ± 1.26	16.93 ± 3.13	12.18	3.21	3.13	4.13
9.2ppm	15.39 ± 6.31	12.50 ± 4.55	10.65	2.21	1.75	3.06
12ppm	2.95 ± 0.25	4.71 ± 1.49	1.91	0.45	0.99	2.59
9.2ppm + ANME	3.07 ± 0.61	4.93 ± 0.10	2.18	0.44	N.D.	N.D.
12ppm + ANME	4.43 ± 0.40	5.50 ± 2.93	5.12	1.48	2.27	2.59

ANME = *A. nilotica* methanolic extract, ND = Not detected

Chromium (Cr): *M. pigra* seedlings could remediate Cr even if they are present in low concentration in the soil. About 7.09 and 8.82 ppm of the element were accumulated in plant seedling treated with low and high concentration of the element, respectively (Fig. 1). Toxic element removal efficacy of Mimosa seedling when treated with low concentration of Cr (9.2 ppm) was increased by adding ANME reached to 11 ppm. Morphological features of *M. pigra* seedlings were affected by Cr accumulation. Table 5 showed that generally, accumulation of Cr caused stress symptoms on *M. pigra* seedlings; including reducing of plant length number of leaf, fresh and dry weight. More decrease in plant length, number of leaves, chlorophyll a, b, fresh and dry weight was observed in seedlings treated with low concentration of in presence of ANME as a result of high accumulation of Cr. This data was considerably close to previous findings of Gardea-Torresdey *et al.*, (2004), who found that *Prosopis laevigata* was a potential hyper-accumulator of chromium from the environment and promising candidate for phytoremediation purposes. Umadevi & Avudainayagam (2013) reported the same results with *Leucaena leucocephala* that a decline in total dry matter production and enzyme activities was observed after treatment with Cr. Chromium toxic effects causes harmful effects on physiological processes of plant like mineral nutrition and photosynthesis by oxidative stress resulting from produce reactive oxygen effecting the morphological features such as root and shoot growth as well as dry weight production and yield (Shanker *et al.*, 2005).

Cadmium (Cd): *M. pigra* seedlings displayed high ability to accumulate Cd (Fig. 1). Seedlings of *M. pigra* treated with high concentration of the element (50 ppm) accumulated about 17.57 ppm of Cd, which represented about 2 times in respect to control. Many plants do like that, Wahab *et al.*, (2014) found that *Neptunia oleracea* could remediate more amount of Cd after 10 days of treatment. No noticeable effect of adding of ANME on remediation of Cd was observed. Morphological features of *M. pigra* seedlings were affected by accumulation of Cd in their tissues (Table 6). The plant length, number of leaves, fresh and dry weight were affected negatively in case of high concentration of Cd, as well as in case of presence of ANME. Generally, growth parameters of plant such as leaf area and dry weight are reduced with the accumulation of Cd (Ibrahim *et al.*, 2017).

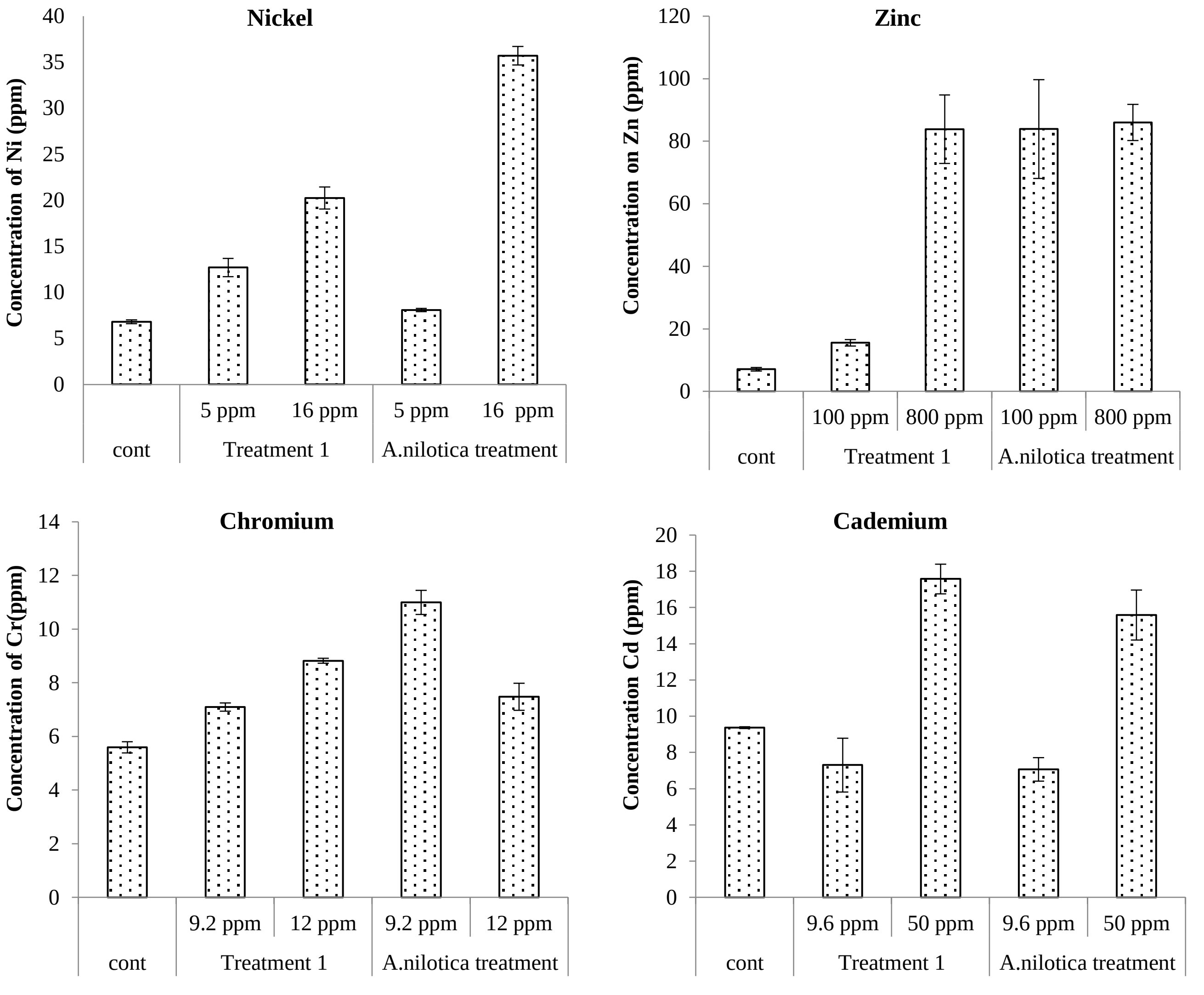

Fig. 1. Removal of Ni, Zn, Cr and Cd by *M.pigra* plant and effect of *A. nilotica methanolic extract on* removing efficacy.

Table 6. Effect of Cadmium accumulation and adding ANME on some morphological features and chlorophyll a, b of *Mimosa pigra* seedlings.

Treatment	Plant length (cm)	Number of leaves	Fresh weight (gm)	Dry weight (gm)	Chlorophyll A %	Chlorophyll B %
Control	21.42 ± 0.00	29.67 ± 0.00	7.37	2.19	2.25	2.01
9.6ppm	17.27 ± 1.17	31.50 ± 0.24	11	3.66	1.25	1.55
50ppm	5.58 ± 2.58	10.50 ± 3.88	2.26	0.47	N.D.	N.D.
9.6ppm +ANME	7.69 ± 2.54	16.72 ± 2.87	5.18	1.25	2.14	1.93
50ppm + ANME	2.91 ± 0.37	6.33 ± 1.33	0.89	0.27	N.D.	N.D.

ANME = *A. nilotica* methanolic extract, ND = Not detected

Table 7. Effect of Copper accumulation and adding ANME on some morphological features and chlorophyll a, b of *Mimosa pigra* seedlings.

Treatment	Plant length (cm)	Number of leaves	Fresh weight (gm)	Dry weight (gm)	Chlorophyll A %	Chlorophyll B %
Control	5.90 ± 0.00	10.20 ± 0.00	1.55	0.3	0.03	0.13
120ppm	6.30 ± 2.12	13.10 ± 6.08	1.98	0.33	0.01	0
3100ppm	3.40 ± 0.70	7.90 ± 1.56	1.03	0.27	N.D.	N.D.
120ppm + ANME	1.6 ± 0.00	2 ± 1.06	1.06	0.24	N.D.	N.D.
3100ppm +ANME	2.22 ± 1.24	7.70 ± 0.99	0.61	0.16	N.D.	N.D.

ANME = *A. nilotica* methanolic extract, ND= Not detected

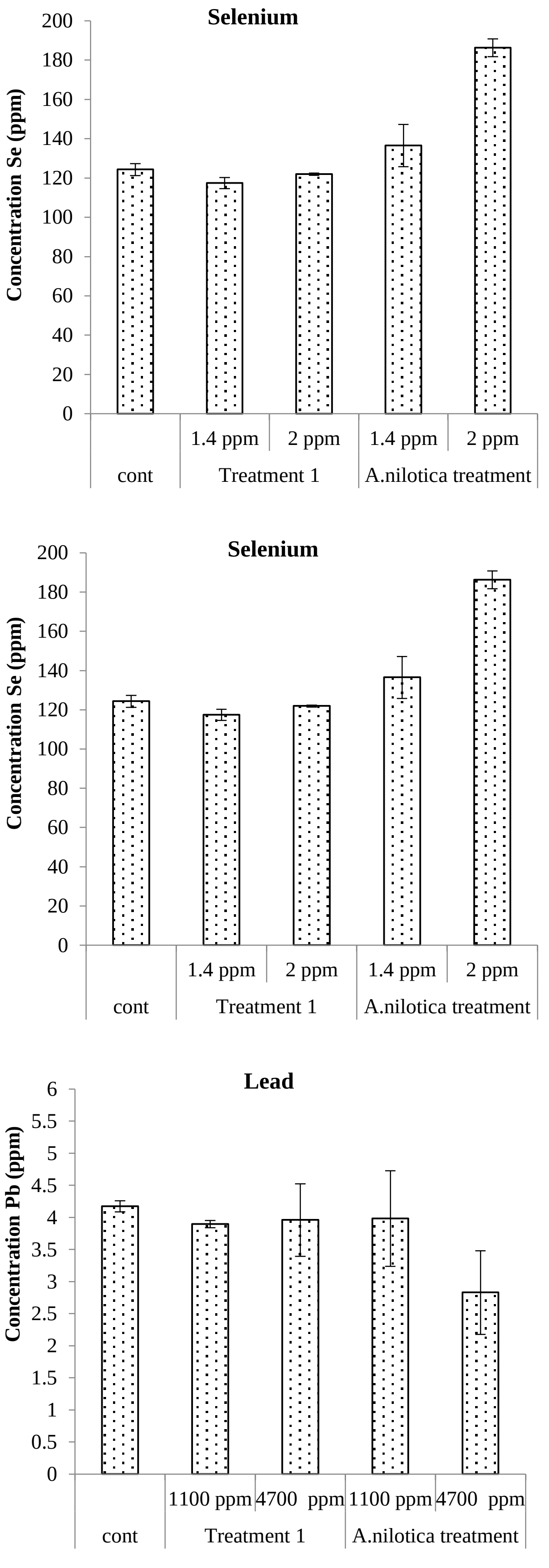

Fig. 2. Phytoremediation of Copper, Selenium and Lead by *M.pigra* and the effect of adding of *A. nilotica* and *M. pigra* extracts.

Copper (Cu): Data in (Fig. 2) reveals that *M. pigra* has the ability to remove copper from the soil and accumulate it in the tissues. 223.75 ppm of Cu was accumulated in seedlings treated with high concentration of cu (3100 ppm). Seedlings treated with low concentration (120 ppm) accumulated about 20.7 ppm of the element and this concentration was considerably enhanced (36 ppm) by adding of ANME. Many plants such as *Mimosa bimucronata, Cynodon dactylon, Piptochaetium montevidense* and *Baccharis dracunculifolia* have the ability to remediate Cu from soils, (Zocche, 1989). Accumulation of Cu in *M. pigra* seedlings resulted in noticeable decrease in plant length, number of leaves, fresh weight and dry weight and chlorophyll a and b contents (Table 7). Presence of Cu in the plant in higher amount causes harmful and toxic effects on the plant tissues (Clemens, 2001). The data was in accordance with Ibrahim *et al*., (2017) who found that the accumulation of copper affected many morphological features of the copper plant accumulator.

Selenium (Se): Slight differences in accumulation of Selenium in all treated seedlings were detected in comparison with control except seedlings treated with high concentration of the element in presence of ANME (186.24 ppm) (Fig. 2). The effect of adding of ANME treatment was statistically significant (p<0.05). Morphological features of seedlings such as plant length, number of leaves, fresh weight and dry weight as well as chlorophyll a and b content were affected positively by accumulation of Se in the tissues (Table 8). According to Chibuike & Obiora (2014) some heavy metals, beneficial to plants, could actually improve plant growth and development, although, at higher concentrations of these metals, reductions in plant growth could happen. Schiavon *et al*., (2017) reported that selenium in plant tissues enhanced production of sulphate-organic compounds which were very important for plant to resist abiotic and biotic stress conditions and stimulate some metabolites enzymes. Whereas, high amount causes decreasing in Ni-compound level which can trigger negatively on the plant metabolites.

Lead (Pb): Lead is the most toxic among heavy metals because of its non-degradable properties (Yongpisanphop *et al*, 2017). The obtained data revealed that no significant differences were noticed in accumulation of lead among all treated seedling except with the high concentration (4700 ppm) in presence of ANME (Fig. 2). The accumulated amounts of Pb were much closer to each other and ranged from 3.62 to 4.17 ppm. Reduction in length, number of leaves, fresh weight and dry weight as well as chlorophyll a and b contents of all treated seedling was noticed compared to control (Table 9). The harmful effect of Pb on the morphological feature of the plant may be related to its physicochemical characters. Ribeiro *et al*., (2012) found that *Mimosa caesalpiniaefolia* was the most tolerant species to Pb toxicity among the studied species in germination assay, accompanying with reduction in shoot biomass and leaf area. Alves *et al*., (2008) reported that increasing in Pb levels caused significant reductions of dry mass of the shoot, root of *Vetiveria zizanioides, Desmanthus virgatus* and *Prosopis juliflora.*

Table 8. Effect of Selenium accumulation and adding ANME on some morphological features and chlorophyll a, b of *Mimosa pigra* seedlings.

Treatment	Plant length (cm)	Number of leaves	Fresh weight (gm)	Dry weight (gm)	Chlorophyll A %	Chlorophyll B %
Control	4.94 ± 0.00	16.22 ± 0.00	7.06	1.84	1.03	0.51
1.4ppm	8.50 ± 3.46	20.44 ± 8.64	11.8	5.08	1.13	0.77
2ppm	6.67 ± 1.26	12.56 ± 3.30	3.94	1.11	N.D.	N.D.
1.4ppm + ANME	5.92 ± 1.53	15.28 ± 4.01	13.37	8.1	1.53	1
2ppm +ANME	16.19 ± 0.04	26.67 ± 5.66	13.16	4.66	2.51	1.91

ANME = *A. nilotica* methanolic extract, ND= Not detected

Table 9. Effect of Lead accumulation and adding ANME on some morphological features and chlorophyll a, b of *Mimosa pigra* seedlings.

Treatment	Plant length (cm)	Number of leaves	Fresh weight (gm)	Dry weight (gm)	Chlorophyll A %	Chlorophyll B %
Control	16.71 ± 0.00	28.57 ± 0.00	11.53	3.32	2.48	2.29
1100ppm	15.21 ± 0.97	27.07 ± 3.13	9.53	2.83	2	1.43
4700ppm	3.14 ± 1.07	5.71 ± 1.32	2.32	0.44	N.D.	N.D.
1100ppm + ANME	3.36 ± 0.50	2.86 ± 0.81	4.49	1.19	0.83	0.52
4700ppm + ANME	3.69 ± 0.36	7.95 ± 1.63	2.38	0.55	N.D.	N.D.

ANME = *A. nilotica* methanolic extract, ND= Not detected

Conclusion

This investigation has a promising applied data about the efficacy of *M. pigra* to remove considerable quantity of toxic heavy metals from soil and accumulate it in the plant tissues. Seedlings of *M. pigra* were able to remediate Ni, Zn, Cr, Cd, Se, Pb and Cu in variable degrees. Total metal amounts that remediated by *M pigra* seedlings were as the following sequence; Pb< Cr< Cd< Ni< Zn< Se< Cu. It is concluded that *M pigra* plants could be considered as a good removal for toxic heavy metals from the soil, this may have an important application in pre- purify of soil from that toxic metals. Moreover, the presence of *A. nilotica* trees near *M. pigra* along the Nile bank in the study area could improve the removal efficacy of *M. pigra* for toxic heavy metals, especially that there are berth of floating hotels close to this area.

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