Phytoremediation of Polluted Soil Using Some Ornamental Trees

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ABSTRACT: This experiment was conducted in the Nursery of the Department of Floriculture, Ornamental Horticulture and landscape Gardening - Faculty of Agriculture Shatby - University of Alexandria from August 1st to February 1st in two successive seasons (2014-2015 and 2015-2016), to study the biological treatment of contaminated soil with the use of some ornamental trees, A tree with a type of inoculation obtained from the nursery of the Department of Forestry and Wood Technology - Abyss - Alexandria University: *Acacia saligna: Rhizobium* sp., (which used for inoculation of *A. saligna* root). The treatments of the three elements (cadmium - zinc - lead) were: Control - 250 mg/kg - 500 mg/kg and 1000 mg/kg soil. The first part is inoculated seedlings and the second part uninoculated seedlings. **The most important results obtained can be summarized as follows:**

- The application of the high level of heavy metals (500 and 1000 mg/kg) led to decrease the growth parameters (growth rate, total dry weight and nodule/root ratio). The negative effect of the pollutants was more significant in uninoculated seedlings with *Rhizobium*, i.e., the inoculated ones had displayed growth parameters significantly higher than those of uninoculated ones.
- Both of Cd and Pb had harmful impact on plant growth than what Zn did.
- Total chlorophyll of the phyllodes was decreased as a result of application of heavy metals. Both of Pb and Zn had detrimental impact on chlorophyll content relative to Cd.
- Analyzing the Rhizosphere of the seedlings treated with the heavy metals, it has been found that lead was the most metals fixed in the soil as it compared with Zn and Cd, particularly under high levels of such metals amended.

It is recommended, however, to use *A. saligna* seedlings as a phytoremediator for heavy metals polluted soil, since it is regarded to be tolerant to such stress, in addition to potential of the *Rhizobium* bacterial, the symbiotic partner of the plant, for increasing the efficiency of the phytoremediation.

Keywords: Phytoremediation, *Rhizobium*, Heavy metals, *Acacia saligna,* contaminated soil.

INTRODUCTION

Acacia saligna is a multipurpose, fast growing tree species (MPTS), which belongs to family *Fabaceae*. It is a dense and multi-stemmed, thornless, spreading shrub or single stemmed small tree (Maslin, 1974). It occurs naturally in Southwest and Western Australia and has been introduced to the Mediterranean coast in Egypt for many different purposes, including re-vegetation, tanning, fodder, protein-rich seeds and fruits, firewood, agroforestry, windbreak, control of soil erosion, enhancement of bio-productivity and overcoming salt stress problems, which is reported to be salt tolerant (5 to 10 dS/m), because these plants enrich the soil with nitrogen in symbiotic association with *Rhizobium* and form associations with *Arbuscular mycorrhizal* fungi (AMF) (Hobbs *et al.*, 2006, Swelim *et al.,* 2010 and Amira *et al.*, 2012).

Rhizobium is a genus of Gram-negative soil bacteria that can fix the atmospheric nitrogen. *Rhizobium* species form an endosymbiotic nitrogen-fixing association with roots of legumes and Parasponia. The bacteria colonize plant cells within root nodules, where they convert atmospheric nitrogen into ammonia and then provide organic nitrogenous compounds such as glutamine or ureides to the plant. The plant, in turn, provides the bacteria with organic compounds made by photosynthesis. This mutually beneficial relationship is true of all of the rhizobia, of which the genus Rhizobium is a typical example (Sawada *et al.*, 2003). The term heavy metals (HM) refers to any element whose density is more than 5 or 6 g/cm^3 (Lozet and Mathieu 1991, Adriano 2001, Davies 1987 and Thornton, 1995).

Heavy metals are a group of pollutants that are currently of much environmental concern due to their toxicity to a wide range of organisms. The high concentration of heavy metals in soil is not only caused by natural processes as a result of weathering of rock minerals, but also by anthropogenic activities, such as mining, metals ores smelting, gas exhaust, energy/fuel production, electroplating, industrial emission, and the use of fertilizers and insecticides (Baker, 1987, Alloway, 1994 and Garbisu and Alkorta, 2003). In recent decades, the annual global release of heavy metals reached 22,000, 783,000, 939,000, and 1,350,000 metric tons for cadmium (Cd), lead (Pb), copper (Cu), and zinc (Zn), respectively (Singh *et al.*, 2003). There is an interest towards the ecological threats of heavy metals which led to in-depth research on new economical remediation technologies based on plants. Conventional methods employed for remediation of contaminated soils, including chemical, physical, and microbiological methods, are expensive and time-consuming, they can also release secondary wastes and put workers at risk (Wenzel *et al.*, 1999, Lombi *et al.*, 2000, Fitz and Wenzel, 2002 and Danh *et al.*, 2009).

Phytoremediation of contaminated soils is achievable by various processes such as rhizofiltration, phytostabilization or phytoimmobilization, phytodegradation, phytotransformation, phytovolatilization, phytostimulation, and phytoextraction (Schwitzguebel, 2000 and Ali *et al.,* 2013). Among these strategies, phytoextraction (phytoaccumulation), which is a plant's capability to absorb inorganic (mainly metals) contaminants from soil, is the most prevalent remediation technology (McCutcheon and Schnoor, 2003). Phytostabilization is defined as the ability of certain plants to reduce the availability of toxic metals in soil (Gwozdz and Kopyra, 2003). Such a process could reduce the danger of the pollutant, but it could not remove toxic metals from the soil (Li *et al.*, 2000 and Reza *et al.*, 2018). An encouraging and eco-friendly approach is phytoremediation technology wherein plants are utilized to extract, sequester, and/or detoxify contaminants to prevent their dissipation by groundwater or run-off or impede their accumulation via food webs (McGrath *et al.*, 2002, Suresh and Ravishanker, 2004, Arthur *et al.*, 2005). It has been reported that phytoremediation is an efficient, nonintrusive, cheap, and esthetically pleasant technology for remediation of contaminated natural environments (Garbisu and Alkorta 2003, Weber *et al.,* 2001and Reza *et al.*, 2018). The legumes are one of the most important family, including 15 species (Del Rio *et al.*, 2002). For this reason we focused in the use of *Rhizobium*– legume symbiosis as an alternative procedure for bioremediation of polluted soils. There is an advantage of using microorganisms as major agents affecting metals solubility, bioavailability and mobility, and plant legumes as a bioremediation system. Furthermore it is also capable of providing N-compounds to the soil from a source of non-combined N (Vance and Lamb, 2001 and Jose´ *et al.,* 2005).

The aim of the present study is the soil remediation from pollutants, the heavy metals in terms of phytoextration of some heavy metals pollutants, using *Acacia saligna* seedlings either uninoculated or inoculated with *Rhizobium* bacteria.

MATERIAL AND METHODS

The experiment was conducted using seedlings of *Acasia saligna*. It started on 1st August to 1st February in two successive seasons (2014-2015 and 2015-2016) at The Experimental Station of Forestry and Wood Tech. Dept., Abies, Alexandria and the Department of the Floriculture, Ornamental Horticulture and Landscape Gardening, Faculty of Agriculture, El -Shatby, Alexandria University. Data were analyzed in the Flowers and Ornamental Plants Research Gardens and Laboratory as well as the Unit of Analysis and Scientific Services, Soil and Water Activity of Information Services and Training (SWUIST).

1. Materials

1. Plant material

Seedlings of *Acacia saligna* were obtained from the nursery of the Forestry and Wood Tech. Dept., Abies, Faculty of Agriculture - Alexandria University.

2. Soil

The soil used was a mixture of clay and sand (1:3, v: v). the chemical and physical characteristics of the soil are presented in Table (1).

Table (1). Some physical and chemical characteristics of the soil used in the study

		Particle Size Distribution			
pH	E.C. (dS/m)	Sand %	Silt %	Clay %	Soil Texture
7.82	3.45	94	3	4	Sandy
		Soluble Cations (meq/l)			
Na^{+}	K^{+}	Ca^{++}	Mg^{++}		
20.70	0.50	7.40	10.80		
		Soluble anions (meq/l)			
CO_3^{-}	HCO_3^{-}	Cl^{-}	SO_4^{--}		
—	3.60	21.00	14.80		
		SAR			
		6.90			

3. Symbiotic agents:

A group of micro-organisms located on the roots of plants during plant growth. Size of those micro organisms didn't exceed few milligrams.Nodules used were:

- *Rhizobium* sp.: **Family:** Rhizobiaceae, which was used for inoculation of *A. saligna* root.

2. Methods:

Certified seeds of *A. saligna* obtained from the experimental station of Forestry and Wood Tech. Dept. were obtained as a source of plant material used in this work. *Acacia saligna* seeds were pretreated to breakdown seed coat dormancy by soaking in boiled water, kept overnight, then sowing in polyethelyne bags, 3 seeds/pot then they thinned to only one strong seedling. The resultant seedlings were transplanted after 2 months of sowing in 30 cm plastic pot (one plant/pot), 4.9 kg capacity. Soil medium was autoclaved previously to assure the absence of any microorganisms.
Sowing of the seeds of both species, however was conducted on 1^{st} August, 2014.

1. Artificial inoculation of seedlings with the inocula of the symbiotic agents:

Crushed nodule of certified strains of both symbiotic agents, *Rhizobium*, ASAAS was used for the inoculation by mixing the crushed nodules with sterilized peat and farmyard material to assure stability of symbiotic agent spores and for keeping it tightly adhised in inoculum media. The inocula were homogenized using sterilized water as slurry. As the plant reached 3 months old, the seedlings were inoculated with *Rhizobium* for *A. saligna*, using about 1.0 g of slurry, fresh weight and amended in a depth of 3 cm at rhizosphere of the seedlings.

2. Heavy metals treatment:

After one month of transplanting used as an antibiotic was tetracycline amended to assure the absence of any symbiotic agent, especially N_2-fixer ones. The heavy metals, Cadmium (Cd), zinc (Zn) and lead (Pb), were administered in irrigation water at 3 concentrations 250, 500 and 1000 mg for every kilogram of potting soil.

3. Growth parameter's determination:

1. Growth rate (GR)

The GR of seedlings was determined as follows:

$$\text{Growth rate (cm/ month)} = \frac{SH_2 - SH_1}{\text{The number of months between measured } SH_1, SH_2}$$

SH_1: Shoot height (cm) at the beginning of the treatment
SH_2: Shoot height (cm) just before harvesting
Months: Growth rate during the trial period (monthly)

2.Total dry weight (TDW)

Total dry weight was calculated by sum of all parts, i.e., phyllodes, stem and roots with nodule, based on dry weight in gram.

3.Nodule/root ratio (NRR)

Nodule/root ratio was calculated according to the following formula:

$$\text{Nodule/root ratio} = \frac{\text{Nodule dry weight}}{\text{Root dry weight}}$$

4. Chemical analysis:

1. Total chlorophyll contents of phyllodes (mg/g)

Total leaf chlorophyll was determined according to (Moran, 1982) as follows:

Half gram of fresh phyllodes were extracted by 10 ml. of N,N,Dimethyl formamide for 24 hours, then kept in dark place, at 4°C until all pigments were extracted. One ml. was taken from this mixture then completed with N,N,Dimethyl formamide to 10 ml. The absorbance of the extracts was measured at a wave length of 664 nm for chlorophyll A and 647 nm for chlorophyll B using spectrophotometer. The total chlorophyll content was calculated as mg/g fresh weight. The equations for the determination of total chlorophyll content were:

$$\textbf{Total chlorophyll} = 7.04\, A_{664} + 20.27\, A_{647} = \mu g/ml$$

$$\mu g/ml = ((\mu g \times 10^{ml})/ml \times 1000 \times 0.1g) = mg/g$$

The samples of treatments were collected separately from each replicate and the average of the three replicates was calculated.

2. Heavy metals content in phyllodes and roots (mg/kg)

Total nutrients in the plant samples were obtained by dry ashing of 1.00 g of biomaterial at 500 °C for 6 hrs and the ash was extracted by adding 10 mL of aqua regia (HNO_3 : HCl, 1:3). After cooling to room temperature, the digested solution was filtered using filter paper Whatman No. 1 into a 100-mL volumetric flask and brought to the volume with de-ionized water. Total concentrations of Cd, Zn and Pb were measured in this solution by Atomic Absorption Spectrophotometer, Varian, SpectrAA-220.

5. Experimental layout and statistical analysis:

The experimental design used in this work was randomized complete block design (RCBD) using 3 replicates. The obtained data were analyzed using split split plot system, where the main plot was for inoculation (two treatments) and subplot for type of pollutant (3 treatments, Zn, Cd and Pb) and sub subplot was for concentration of soil heavy metals pollutants (4 treatments, 0.00, 250, 500 and 1000 mg/kg). Data of the present study were statistically analyzed using computer program according to the used experimental design as illustrated by (Sendecor and Cochran, 1967) and using the statistical analysis system (SAS Institute, 1988). Statistical analysis of the collected data was carried out and means were compared by L.S.D according to (Sendecor and Cochran, 1967).

RESULTS

1. Plant growth:

1. Growth rate (GR) (cm/month)

The inoculated seedlings of *A. saligna* had displayed GR (4.96 and 4.51 cm/month) significantly higher than that obtained in those uninoculated ones (3.62 and 3.30 cm/month) in both seasons, respectively (Table 2).

Concerning the impact of heavy metals, however Zn and Pb-amended seedlings had displayed GR (4.69 and 4.31 cm/month) significantly higher than those Cd and Pb (4.12 and 3.63 cm/month) and Pb and Cd-amended ones (4.06 and 3.77 cm/month) and both the later were different significantly between each other in both seasons, respectively (Table 2). As for the impact of the concentration of amended heavy metals soil, however, the addition of 250 mg/kg has brought about the highest GR (4.86 and 4.47 cm/month), while the lowest GR (3.71 and 3.33 cm/month) was detected in those applied with 1000 mg/kg in both seasons, respectively (Table 2).

The significant interaction between the effect of inoculation and concentrations of heavy metals has revealed that the inoculated and uninoculated seedlings applied with 250 and 1000 mg/kg displayed the highest and the lowest GR (5.70, 5.22 and 2.47, 2.06 cm/month, respectively) in both seasons, respectively (Table 3). As for the significant interaction between the type of heavy metals and its concentration, the treatment with 250 mg/kg of Zn has induced the highest GR (5.56 and 5.22 cm/month), while the lowest GR (2.96 and 2.47 cm/month) was detected in those applied with 1000 mg/kg Zn in both seasons, respectively (Table 3).

Based on the significant triple interaction among all the studied factors, the highest GR (7.29 and 6.80 cm/month) was obtained in the inoculated seedlings of *A. saligna* treated with 250 mg/kg Zn, the lowest GR (2.40 and 1.92 cm/month) was detected in the uninoculated ones treated with 1000 mg/kg Cd and Zn in both seasons, respectively (Table 4).

2. Total dry weight (TDW) (g)

The inoculated seedlings of *A. saligna* had displayed TDW (70.03 g) significantly higher than that obtained in those uninoculated ones (53.03 g) in the first season (Table 2).

Concerning the impact of heavy metals, however, Zn-amended seedlings had displayed TDW (79.63 g) significantly higher than those Pb (77.22 g) and Cd (64.71 g) and both the later were different significantly between each other in the second season (Table 2). As for the impact of the concentration of amended heavy metals, however the addition of 0.00 mg/kg has brought about the highest TDW (106.10 and 117.82 g), while the lowest (32.76 and 53.16 g) was detected in those applied with 1000 mg/kg in both seasons, respectively (Table 2).

Regarding the significant interaction between the impact of the inoculation with *Rhizobium* and heavy metals type, the inoculated seedlings of *A. saligna* contaminated with Zn and Pb had displayed the highest TDW (75.72 and 87.69 g), while the lowest (41.86 and 62.57 g) was obtained in those uninoculated ones that applied with Pb and Cd in both seasons, respectively (Table 3).

The significant interaction between the effect of inoculation and concentrations of heavy metals has revealed that the uninoculated seedlings applied with 0.00 and 1000 mg/kg displayed the highest and the lowest TDW (126.06 and 19.01 g, respectively). It is worth pointing also that the detrimental impacts concentrations of 250, 500 and 1000 mg/kg applied for inoculated seedlings with *Rhizobium* were less extent as it compared with their matul uninoculated ones. While the second season, had revealed that the inoculated and uninoculated seedlings applied with 0.00, 250 mg/kg displayed the highest and the lowest TDW (120.37 and 49.79g, respectively) (Table 3).

As for the significant interaction between the type of heavy metals and its concentration, the treatment with 0.00 mg/kg of Cd and Pb has induced the highest TDW (121.72 and 123.01 g), while the lowest (21.81 and 42.91 g) was detected in those applied with 1000 mg/kg Cd in both seasons, respectively (Table 3). Based on the significant triple interaction among all the studied factors, the highest TDW (166.19 and 139.36 g) was obtained in the uninoculated and inoculated seedlings of *A. saligna* treated with 0.00 mg/kg Cd and Pb, while the lowest TDW (14.27 and 34.72 g) was detected in the uninoculated ones treated with 1000 mg/kg Pb and Cd in both seasons, respectively (Table 4).

Table (2). Average of Growth rate (cm/month) and Total dry weight (g) of *Acacia saligna* as affected by inoculation with *Rhizobium*, application of heavy metal and its concentration

Treatment	Growth rate (cm/month)		Total dry weight (g)	
	2014-2015	2015-2016	2014-2015	2015-2016
Inoculation with *Rhizobium* (A)				
Uninoculated	3.62b	3.30b	53.03b	67.61
Inoculated	4.96a	4.51a	70.03a	80.11
LSD 0.05	0.44	0.32	3.60	N.S
Heavy metal type (B)				
(Cd)	4.12b	3.77b	60.04	64.71b
(Zn)	4.69a	4.31a	65.44	79.63a
(Pb)	4.06b	3.63b	59.12	77.22a
LSD 0.05	0.17	0.22	N.S	5.68
Heavy metal Con. (C)				
0	4.58a	4.22a	106.10a	117.82a
250 mg/kg	4.86a	4.47a	43.88c	60.63b
500 mg/kg	4.01b	3.59b	63.37b	63.80b
1000 mg/kg	3.71c	3.33b	32.76d	53.16c
LSD 0.05	0.29	0.37	6.42	3.48

Table (3). Average of interaction of Growth rate (cm/month) and Total dry weight (g) of *Acacia saligna* as affected by inoculation with *Rhizobium*, application of heavy metal and its concentration

Treatment		Growth rate (cm/month)		Total dry weight (g)	
		2014-2015	2015-2016	2014-2015	2015-2016
Inoculation with *Rhizobium* X Heavy metal type					
Uninoculated	(Cd)	3.46	3.24	62.08b	62.57c
	(Zn)	3.96	3.70	55.14b	73.50b
	(Pb)	3.44	2.96	41.86c	66.75bc
Inoculated	(Cd)	4.78	4.30	57.98b	66.85bc
	(Zn)	5.42	4.93	75.72a	85.76a
	(Pb)	4.69	4.29	76.38a	87.69a
LSD 0.05		N.S	N.S	9.77	8.03
Inoculation with *Rhizobium* X Heavy metal Con.					
Uninoculated	0	4.30c	4.08c	126.06a	115.27b
	250 mg/kg	4.03cd	3.72c	30.64e	49.79d
	500 mg/kg	3.70d	3.34d	36.41e	52.45d
	1000 mg/kg	2.47e	2.06e	19.01f	52.91d
Inoculated	0	4.85b	4.37bc	86.15b	120.37a
	250 mg/kg	5.70a	5.22a	57.11c	71.47c
	500 mg/kg	4.33c	3.84c	90.33b	75.16c
	1000 mg/kg	4.96b	4.60b	46.52d	53.40d
LSD 0.05		0.42	0.52	9.09	4.93
Heavy metal type X Heavy metal Con.					
(Cd)	0	3.18d	2.84cd	121.72a	114.94b
	250 mg/kg	5.22ab	4.88ab	26.58e	52.81e
	500 mg/kg	3.62cd	3.24c	70.01bc	48.17ef
	1000 mg/kg	4.47b	4.10b	21.81e	42.91f
(Zn)	0	5.29ab	5.03ab	116.8a	115.50b
	250 mg/kg	5.56a	5.20a	52.16cd	66.89d
	500 mg/kg	4.95b	4.55b	63.81c	82.03c
	1000 mg/kg	2.96d	2.47d	28.97e	54.09e
(Pb)	0	5.26ab	4.80ab	79.80b	123.01a
	250 mg/kg	3.81c	3.33c	52.88cd	62.19d
	500 mg/kg	3.47cd	2.98cd	56.29cd	61.21d
	1000 mg/kg	3.71c	3.41c	47.50d	62.47d
LSD 0.05		0.51	0.64	11.13	6.04

Table (4). Average of triple interaction of Growth rate (cm/month) and Total dry weight (g) of *Acacia saligna* as affected by inoculation with *Rhizobium*, application of heavy metal and its concentration

Treatment			Growth rate (cm/month)		Total dry weight (g)	
			2014-2015	2015-2016	2014-2015	2015-2016
Inoculation with *Rhizobium* X Heavy metal type X Heavy metal Con.						
Uninoculated	(Cd)	0	2.76fg	2.56de	166.19a	124.57b
		250 mg/kg	5.04cd	4.84bc	15.89g	50h
		500 mg/kg	3.66ef	3.38cd	43.18f	40.99i
		1000 mg/kg	2.40g	2.16e	23.07g	34.72i
	(Zn)	0	5.16cd	5.13b	120.67b	114.58c
		250 mg/kg	3.83ef	3.59cd	44.89ef	51.4h
		500 mg/kg	4.45de	4.13c	35.35fg	65.75fg
		1000 mg/kg	2.42g	1.92e	19.68g	62.27g
	(Pb)	0	4.91cd	4.54bc	91.33cd	106.67cd
		250 mg/kg	3.22f	2.73de	31.15fg	47.98hi
		500 mg/kg	2.98fg	2.49de	30.69fg	50.61h
		1000 mg/kg	2.59fg	2.10e	14.27g	61.74g
Inoculated	(Cd)	0	3.60ef	3.12d	77.25d	105.31d
		250 mg/kg	5.41c	4.92bc	37.28fg	55.63gh
		500 mg/kg	3.58ef	3.09d	96.83c	55.36gh
		1000 mg/kg	6.54b	6.05a	20.56g	51.11h
	(Zn)	0	5.42c	4.93bc	112.93b	116.43bc
		250 mg/kg	7.29a	6.80a	59.44e	82.39e
		500 mg/kg	5.45c	4.97bc	92.27cd	98.31d
		1000 mg/kg	3.51ef	3.02de	38.26fg	45.91hi
	(Pb)	0	5.54c	5.05b	68.27de	139.36a
		250 mg/kg	4.41de	3.92cd	74.62de	76.4ef
		500 mg/kg	3.95e	3.47cd	81.89cd	71.82f
		1000 mg/kg	4.84d	4.73bc	80.74d	63.19g
LSD 0.05			0.72	0.90	15.74	8.54

3. Nodules/ root ratio (NRR)

It has been found that the seedlings of *A.saligna* which applied with Cd had exhibited the highest NRR (0.59 and 0.51), which was significantly higher than those applied with Zn and Pb (0.25 and 0.31) in both seasons, respectively (Table 5). Concerning, the effect of heavy metals concentration, it has been found that the highest value (0.59 and 0.59) was obtained in the seedlings of *A. saligna* applied with 250 and 1000 mg/kg, while the lowest value (0.26 and 0.15) was detected in those applied with 500 mg/kg in both seasons, respectively (Table 5). Thanks to the significant difference interaction among the all the studied factors in terms of NRR of *A. saligna* recorded, the highest value (1.17 and 1.04) was in the control plant and 1000 mg/kg with Pb and Cd, while the lowest (0.12 and 0.08) was detected in those applied with 500 mg/kg Zn in both seasons, respectively (Table 5).

Table (5). Averages of Nodules/ root ratio of *Acacia saligna* seedlings as affected by inoculation with *Rhizobium*, application of heavy metals and its concentration

Heavy metals type (A)	Heavy metals Con. (B)									
	2014-2015					2015-2016				
	0	250 mg/kg	500 mg/kg	1000 mg/kg	Average	0	250 mg/kg	500 mg/kg	1000 mg/kg	Average
(Cd)	0.53b	1.17a	0.24bc	0.410bc	0.588a	0.420bc	0.373bc	0.200c	1.04a	0.509a
(Zn)	0.32bc	0.12c	0.22c	0.340bc	0.251b	0.446bc	0.286c	0.083c	0.436bc	0.313b
(Pb)	0.91a	0.48bc	0.32bc	0.143c	0.461a	0.663b	0.123c	0.173c	0.283c	0.310b
Mean	0.59a	0.59a	0.26b	0.297b		0.510a	0.261b	0.152b	0.587a	
LSD 0.05 (Heavy metals type)					0.158					0.176
LSD 0.05 (Heavy metals Con.)					0.182					0.203
LSD 0.05 (Heavy metals type x Heavy metals Con.)					0.316					0.352

2. Chemical analysis

1. Total chlorophyll content in phyllodes (mg/g)

Concerning the impact of heavy metals type, however, Cd-amended seedlings showed the highest Total chlorophyll (1.51 and 1.65 mg/g), while the lowest (0.84 and 1.20 mg/g) was detected in those applied in Pb-amended ones in both seasons, respectively (Table 6).

As for the impact of the concentration of amended heavy metals, however the control plants displayed the highest Total chlorophyll (1.66 and 1.97 mg/g), while the lowest concentration (0.87 and 1.07 mg/g) was detected in those applied with 500 and 1000 mg/kg in both seasons, respectively (Table 6). According to the significant interaction between the effect of inoculation and concentration of heavy metals, the uninoculated seedlings applied with 0.00 and 500 mg/kg displayed the highest and the lowest Total chlorophyll (1.75 and 0.79 mg/g, respectively) in the first season, while the second season, theuninoculated seedlings applied with 0.00 and 1000 mg/kg displayed the highest and the lowest Total chlorophyll (1.99 and 1.03 mg/g, respectively) (Table 7).

As for the significant interaction between the type of heavy metals and its concentration, concentration of 0.00 mg/kg Cd has brought about the highest Total chlorophyll (2.02 and 2.10 mg/g), while the lowest concentration (0.45 and 0.86 mg/g) was detected in those applied with 1000 mg/kg Pb and Zn in both seasons, respectively (Table 7). Based on the significant triple interaction among all the studied factors, the highest Total chlorophyll (2.04 and 2.11 mg/g) was obtained in the uninoculated seedlings of *A. saligna* treated with 0.00 mg/kg Cd, while the lowest concentration (0.25 and 0.859 mg/g) was detected in the inoculated and uninoculated ones, treated with 1000 mg/kg Pb and Zn in both seasons, respectively (Table 8).

2.Cadmium content (Cd) in phyllodes (mg/kg)

The phyllodes of inoculated seedlings of *A. saligna* had displayed Cd content (0.325 mg/kg) significantly higher than that obtained in those uninoculated ones (0.144 mg/kg) in the second season (Table 6). Concerning the impact of heavy metals type, however Cd, Zn-amended seedlings had displayed Cd content (0.015 and 0.303mg/kg) significantly higher than that of Zn, Pb and Pb-amended ones (0.005 and 0.137 mg/kg) and both the later were different significantly between each other in both seasons, respectively (Table 6).

As for the impact of the concentration of amended heavy metals, however the seedlings applied with 1000 mg/kg displayed the highest Cd content (0.012 and 0.399 mg/kg), while the lowest content (0.007 and 0.025 mg/kg) was detected in those applied with 0.00 mg/kg in both seasons, respectively (Table 6). Upon the significant interaction between the impact of the inoculation with *Rhizobium* and heavy metals type, the inoculated seedlings of *A. saligna* contaminated with Zn has displayed the highest Cd content (0.481 mg/kg), while the lowest (0.099 mg/kg) was obtained in those uninoculated ones that applied with pb in the second season (Table 7).

The significant interaction between the effect of inoculation and concentrations of heavy metals has revealed that the inoculated seedlings applied with 1000 and 0.00 mg/kg displayed the highest and the lowest Cd content (0.019 and 0.005 mg/kg, respectively) in the first season, while the second season, it is clear that the phyllode of inoculated seedling contained heavy metal concentrations significantly higher than its 1000 mg/kg matual of uninoculated ones of control plants, which proved that the presence of nodules may induce more obsorption of heavy metal by plants (Table 7).

As for the significant interaction between the type of heavy metals and its concentration, the highest Cd content (0.024 and 0.704 mg/kg) was found in the phyllodes of *Acacia saligna* seedlings applied with 1000 mg/kg Cd, while the lowest content (0.005 and 0.016 mg/kg) was detected in those applied with 0.00 mg/kg Pb and Zn in both seasons, respectively (Table 7).

Based on the significant triple interaction among all the studied factors, the highest Cd content (0.043 and 1.30 mg/kg) was obtained in the inoculated seedlings of *A. saligna* treated with 1000 mg/kg Cd, whereas the lowest content (0.005 and 0.004 mg/kg) was detected in the uninoculated ones treated with 0.00 mg/kg Pb and Zn in both seasons, respectively (Table 8).

3. Zinc content (Zn) in phyllodes (mg/kg)

The phyllodes of uninoculated and inoculated seedlings of *A. saligna* had displayed Zn (0.753 and 0.911 mg/kg) significantly higher than that obtained in those inoculated and uninoculated ones (0.678 and 0.856 mg/kg) in both seasons, respectively (Table 6).

Concerning the impact of heavy metals type, however Zn-amended seedlings had displayed Zn content (0.942 and 1.16 mg/kg) significantly higher than those Pb- (0.688 and 0.820 mg/kg) and Cd-amended ones (0.517 and 0.665 mg/kg) and both the later were different significantly between each other in both seasons, respectively (Table 6).

As for the impact of the concentration of amended heavy metals, however the addition of 500 mg/kg has brought about the highest Zn content (1.19 and 1.46 mg/kg), while the lowest content (0.050 and 0.066 mg/kg) was detected in those applied with 0.00 mg/kg in both seasons, respectively (Table 6).Upon the significant interaction between the impact of the inoculation with *Rhizobium* and heavy metals type, the inoculated seedlings of *A. saligna* contaminated with Zn had displayed the highest Zn content (0.942 and 1.17 mg/kg), while the lowest (0.485 and 0.541 mg/kg) was obtained in those uninoculated ones that applied with Cd in both seasons, respectively (Table 7).

The significant interaction between the effect of inoculation and concentrations of heavy metals has revealed that the inoculated seedlings applied with 500 and 0.00 mg/kg had displayed the highest and the lowest Zn content (1.20 and 0.046 mg/kg, respectively) in the first season, while the second season, had applied with 500 and 0.00 mg/kg displayed the highest and the lowest Zn content (1.50 and 0.056 mg/kg, respectively) (Table 7).

As for the significant interaction between the type of heavy metals and its concentration, the treatment with 500 mg/kg of Zn has induced the highest Zn content (1.82 and 2.06 mg/kg), while the lowest content (0.033 and 0.050 mg/kg) was detected in those applied with 0.00 mg/kg Zn and Cd in both seasons, respectively (Table 7).

Based on the significant triple interaction among all the studied factors, the highest Zn content (2.01 mg/kg) was obtained in the inoculated seedlings of *A. saligna* treated with 500 mg/kg Zn, the lowest content (0.022 mg/kg) was detected in the uninoculated ones treated with 0.00 mg/kg Zn in the first season, while the second season, the highest Zn content (2.30 mg/kg) was obtained in the inoculated seedlings of *A. saligna* treated with 500 mg/kg Zn, whereas the lowest content (0.031 mg/kg) was detected in the inoculated ones treated with 0.00 mg/kg cd (Table 8).

4. Lead content (Pb) in phyllodes (mg/kg)

The phyllodes of inoculated seedlings of *A. saligna* had displayed Pb content (0.600 and 0.990 mg/kg) significantly higher than that obtained in those uninoculated ones (0.540 and 0.655 mg/kg) in both seasons, respectively (Table 6).

Concerning the impact of heavy metals type, however Pb-amended seedlings had displayed Pb content (0.737 mg/kg) significantly higher than those Zn-(0.529 mg/kg) and Cd-amended ones (0.457 mg/kg) and both the later were different significantly between each other in the first season, while the second season, had applied pb-amended seedlings had displayed pb content (1.01 mg/kg) significantly higher than those Cd (0.785 mg/kg) and Zn-amended ones (0.667 mg/kg) and both the later were different significantly between each other (Table 6).

As for the impact of the concentration of amended heavy metals, however the addition of 250 mg/kg has brought about the highest Pb content (0.942 and 1.13 mg/kg), while the lowest content (0.055 and 0.061 mg/kg) was detected in those applied with 0.00 mg/kg in both seasons, respectively (Table 6). Upon the significant interaction between the impact of the inoculation with *Rhizobium* and heavy metals type, the inoculated seedlings of *A. saligna* contaminated with Pb had displayed the highest Pb content (0.940 and 1.13 mg/kg), while the lowest (0.413 and 0.599 mg/kg) was obtained in those inoculated and uninoculated ones that applied with Cd in both seasons, respectively (Table 7).

The significant interaction between the effect of inoculation and concentrations of heavy metals has revealed that the inoculated seedlings applied with 1000 and 0.00 mg/kg displayed the highest and the lowest Pb content (1.18 and 0.042 mg/kg, respectively) in the first season, while the second season, had revealed that the inoculated and uninoculated seedlings applied with 250, 0.00 mg/kg displayed the highest and the lowest pb content (1.61 and 0.054mg/kg, respectively) (Table 7).

As for the significant interaction between the type of heavy metals and its concentration, the treatment with 1000 mg/kg of Cd has induced the highest Pb content (1.50 mg/kg), while the lowest content (0.036 mg/kg) was detected in those applied with 0.00 mg/kg Pb in the first season, while the second season, the treatment with 250 mg/kg of pb has induced the highest pb content (1.59 mg/kg), while the lowest content (0.048 mg/kg) was detected in those applied with 0.00 mg/kg Cd (Table 7).

Based on the significant triple interaction among all the studied factors, the highest Pb content (2.35 mg/kg) was obtained in the uninoculated seedlings of *A. saligna* treated with 1000 mg/kg Cd, the lowest content (0.021 mg/kg) was detected in the uninoculated ones treated with 0.00 mg/kg Zn in the first season, while the second season, the highest pb content (2.74 mg/kg) was obtained in the inoculated seedlings of *A. saligna* treated with 250 mg/kg pb, the lowest content (0.030 mg/kg) was detected in the uninoculated ones treated with 0.00 mg/kg Cd (Table 8).

Table (6). Average of total chlorophyll content (mg/g), cadmium content (mg/kg), zinc content (mg/kg) and lead content (mg/kg) of phyllodes of *Acacia saligna* as affected by inoculation with *Rhizobium*, application of heavy metal and its concentration

Treatment	Total chlorophyll content (mg/g)		Cadmium content (mg/kg)		Zinc content (mg/kg)		Lead content (mg/kg)	
	2014-2015	2015-2016	2014-2015	2015-2016	2014-2015	2015-2016	2014-2015	2015-2016
Inoculation with *Rhizobium* (A)								
Uninoculated	1.17	1.42	0.008	0.144b	0.753a	0.856b	0.54b	0.655b
Inoculated	1.10	1.47	0.009	0.325a	0.678b	0.911a	0.60a	0.990a
LSD 0.05	N.S	N.S	N.S	0.0024	0.0033	0.0041	0.0075	0.002
Heavy metal type (B)								
(Cd)	1.51a	1.65a	0.015a	0.264b	0.517c	0.665d	0.457c	0.785b
(Zn)	1.06b	1.49b	0.005b	0.303a	0.942a	1.166a	0.529b	0.667c
(Pb)	0.84c	1.20c	0.005b	0.137c	0.688b	0.820b	0.737a	1.01a
LSD 0.05	0.87	0.92	0.0023	0.001	0.0072	0.0028	0.0022	0.0026
Heavy metal Con. (C)								
0	1.66a	1.97a	0.007b	0.025d	0.050d	0.066d	0.055d	0.061d
250 mg/kg	1.11b	1.42b	0.008b	0.239c	0.742c	0.876c	0.942a	1.13a
500 mg/kg	0.87c	1.32c	0.007b	0.274b	1.19a	1.46a	0.618c	1.03c
1000 mg/kg	0.90c	1.07d	0.012a	0.399a	0.874b	1.12b	0.684b	1.06b
LSD 0.05	0.82	0.93	0.0024	0.001	0.0085	0.0047	0.0039	0.005

Table (7). Average of interaction of Total chlorophyll content (mg/g), cadmium content (mg/kg), Zinc content (mg/kg) and lead content (mg/kg) of phyllodes of *Acacia saligna* as affected by inoculation with *Rhizobium*, application of heavy metal and its concentration

Treatment		Total chlorophyll content (mg/g)		cadmium content (mg/kg)		Zinc content (mg/kg)		Lead content (mg/kg)	
		2014-2015	2015-2016	2014-2015	2015-2016	2014-2015	2015-2016	2014-2015	2015-2016
Inoculation with *Rhizobium* X Heavy metal type									
Uninoculated	(Cd)	1.23	1.51	0.014	0.210c	0.550d	0.541f	0.500d	0.599f
	(Zn)	1.12	1.45	0.005	0.125e	0.941a	1.16b	0.527c	0.648e
	(Pb)	1.17	1.30	0.006	0.099f	0.769b	0.863c	0.590b	0.719c
Inoculated	(Cd)	1.20	1.58	0.016	0.318b	0.485e	0.788d	0.413f	0971b
	(Zn)	1.01	1.41	0.0062	0.481a	0.942a	1.17a	0.462e	0.687d
	(Pb)	1.09	1.41	0.005	0.175d	0.607c	0.778e	0.940a	1.31a
LSD 0.05		N.S	N.S	N.S	0.0014	0.0102	0.0040	0.0031	0.0036
Inoculation with *Rhizobium* X Heavy metal Con.									
Uninoculated	0	1.75a	1.99a	0.010b	0.019h	0.053g	0.056h	0.042g	0.054g
	250 mg/kg	1.17c	1.37bc	0.009b	0.170e	0.811d	0.779f	0.695c	0.663e
	500 mg/kg	0.79f	1.29c	0.009b	0.243d	1.18b	1.42b	0.652d	0.950d
	1000 mg/kg	0.99e	1.03d	0.006c	0.144f	0.960c	1.16c	1.18a	0.953d
Inoculated	0	1.58b	1.94a	0.005c	0.030g	0.046g	0.076g	0.068f	0.067f
	250 mg/kg	1.06de	1.46b	0.007bc	0.309b	0.673f	0.974e	0.521e	1.61a
	500 mg/kg	0.95e	1.35bc	0.005c	0.306c	1.20a	1.50a	0.715b	1.11c
	1000 mg/kg	0.80f	1.11d	0.019a	0.655a	0.789e	1.09d	0.715b	1.16b
LSD 0.05		1.15	1.31	0.0033	0.0014	0.0119	0.0066	0.0054	0.0070
Heavy metal type X Heavy metal Con.									
(Cd)	0	2.02a	2.11a	0.0121bc	0.041h	0.045j	0.060l	0.073i	0.048k
	250 mg/kg	1.78b	1.39d	0.015b	0.297d	0.725f	0.816g	0.782k	0.968g
	500 mg/kg	1.09e	1.49cd	0.01c	0.401b	0.818e	1.20c	0.667e	1.05d
	1000 mg/kg	0.79fg	1.19e	0.024a	0.704a	0.483h	0.574i	0.702d	1.33b
(Zn)	0	1.67b	2.04a	0.005d	0.016i	0.033k	0.050k	0.036k	0.065j
	250 mg/kg	1.27d	1.56c	0.005d	0.246e	0.599g	0.740h	0.707d	0.848h
	500 mg/kg	0.67g	1.27de	0.005d	0.246e	1.82a	2.06a	0.807b	1.04e
	1000 mg/kg	0.64g	0.86f	0.007cd	0.318c	1.31b	1.80b	0.567g	0.708i
(Pb)	0	1.44c	1.76b	0.005d	0.017i	0.0713i	0.089j	0.056j	0.069j
	250 mg/kg	0.95ef	1.30de	0.005d	0.176g	0.902d	1.07e	0.612f	1.59a
	500 mg/kg	0.85f	1.20e	0.006d	0.177fg	0.947c	1.11d	0.380h	0.986f
	1000 mg/kg	0.45h	1.16e	0.006d	0.177f	0.831e	1.00f	1.50a	1.13c
LSD 0.05		1.41	1.61	0.0040	0.0017	0.0146	0.008	0.0067	0.0086

Table (8). Average of triple interaction of Total chlorophyll content (mg/g), cadmium content (mg/kg), zinc content (mg/kg) and lead content (mg/kg) of phyllodes of *Acacia saligna* as affected by inoculation with *Rhizobium*, application of heavy metal and its concentration

Treatment			Total chlorophyll content (mg/g)		cadmium content (mg/kg)		Zinc content (mg/kg)		Lead content (mg/kg)	
			2014-2015	2015-2016	2014-2015	2015-2016	2014-2015	2015-2016	2014-2015	2015-2016
Inoculation with *Rhizobium* X Heavy metal type X Heavy metal Con.										
Uninoculated	(Cd)	0	2.04a	2.11a	0.019b	0.043m	0.040pq	0.031t	0.064o	0.030t
		250 mg/kg	1.20d	1.34cd	0.018b	0.190i	0.653k	0.444p	0.662i	0.453o
		500 mg/kg	0.95e	1.35cd	0.015bc	0.406c	1.03f	1.42d	0.531m	0.922j
		1000 mg/kg	0.84ef	1.24cd	0.006d	0.201g	0.475n	0.265q	0.843e	1.45b
	(Zn)	0	2.03a	2.10a	0.005d	0.004r	0.022p	0.048s	0.021r	0.051s
		250 mg/kg	1.37cd	1.54bc	0.005d	0.196h	0.586m	0.577o	0.863c	0.854k
		500 mg/kg	1.21d	1.33cd	0.005d	0.196h	1.63b	1.82c	0.933b	1.12h
		1000 mg/kg	0.80ef	0.83e	0.005d	0.104l	1.49c	2.20b	0.572j	0.563n
	(Pb)	0	1.65bc	1.76b	0.005d	0.0126q	0.075o	0.089r	0.041q	0.066r
		250 mg/kg	0.94e	1.24de	0.005d	0.126k	1.19d	1.31e	0.562k	0.683m
		500 mg/kg	0.89ef	1.19d	0.007d	0.129j	0.896h	1.01i	0.682h	0.803l
		1000 mg/kg	0.52g	1.01de	0.007d	0.129j	0.909h	1.03h	2.35a	0.843k
Inoculated	(Cd)	0	1.80b	2.10a	0.005d	0.039n	0.049p	0.089r	0.082n	0.094p
		250 mg/kg	1.52c	1.43c	0.012c	0.405c	0.797i	1.18g	0.722g	1.25d
		500 mg/kg	1.23d	1.63bc	0.005d	0.396d	0.603lm	0.994j	0.803f	1.19f
		1000 mg/kg	0.74f	1.14d	0.043a	1.30a	0.491n	0.882m	0.552l	1.21e
	(Zn)	0	1.68bc	1.97ab	0.005d	0.029o	0.045q	0.051s	0.051p	0.079q
		250 mg/kg	1.18d	1.58bc	0.005d	0.296e	0.613l	0.904l	0.552l	0.843k
		500 mg/kg	0.82ef	1.22de	0.005d	0.296e	2.01a	2.30a	0.682h	0.973i
		1000 mg/kg	0.49g	0.89e	0.01cd	0.434b	1.12e	1.41d	0.562k	0.853k
	(Pb)	0	1.36cd	1.76b	0.005d	0.022p	0.066op	0.089r	0.071o	0.045s
		250 mg/kg	0.97e	1.37cd	0.005d	0.226f	0.611l	0.832n	0.662i	2.74a
		500 mg/kg	0.80ef	1.20d	0.005d	0.226f	0.997g	1.21f	0.078no	1.16g
		1000 mg/kg	0.25h	0.72f	0.005d	0.226f	0.752j	0.973k	0.853d	1.43c
LSD 0.05			1.99	2.28	0.0057	0.0024	0.0207	0.011	0.0094	0.0121

5. Cadmium content (Cd) in roots (mg/kg)

Concerning the impact of heavy metals type, however Cd-amended seedlings had displayed Cd content (0.357 mg/kg) significantly higher than those Zn- (0.271 mg/kg) and Pb-amended ones (0.138 mg/kg) and both the later were different significantly between each other in the second season (Table 9).

As for the impact of the concentration of amended heavy metals, however the addition of 1000 and 250 mg/kg has brought about the highest cd content (0.338 and 0.363 mg/kg), while the lowest (0.006 and 0.036 mg/kg) was detected in those applied with 0.00 mg/kg in both seasons, respectively (Table 9).

Upon the significant interaction between the impact of the inoculation with *Rhizobium* and heavy metals type, the uninoculated seedlings of *A. saligna* contaminated with Cd had displayed the highest Cd content (0.284 and 0.357 mg/kg), while the lowest (0.138 and 0.176 mg/kg) was obtained in those inoculated ones that applied with Cd and Pb in both seasons, respectively

(Table 10). The significant interaction between the effect of inoculation and concentrations of heavy metals has revealed that the uninoculated seedlings applied with 250 and 0.00 mg/kg displayed the highest and the lowest Cd content (0.343 and 0.006 mg/kg, respectively) in the first season, while the second season, had revealed that the uninoculated seedlings applied with 250 and 0.00 mg/kg displayed the highest and the lowest Cd content (0.385 and 0.031 mg/kg, respectively) (Table 10).

As for the significant interaction between the type of heavy metals and its concentration, the treatment with 500 mg/kg of Cd has induced the highest Cd content (0.593 mg/kg), while the lowest content (0.017 mg/kg) was detected in those applied with 0.00 mg/kg Pb in the second season (Table 10). Based on the significant triple interaction among all the studied factors, the highest Cd content (0.529 mg/kg) was obtained in the uninoculated seedlings of *A. saligna* treated with 250 mg/kg Cd, while the lowest content (0.006 mg/kg) was detected in all the studied factors had treated with 0.00 mg/kg in the first season, while the second season, thehighest Cd content (0.753 mg/kg) was obtained in the uninoculated seedlings of *A. saligna* treated with 500 mg/kg Cd, whereas the lowest concentration (0.012 mg/kg) was detected in the uninoculated ones treated with 0.00 mg/kg Pb (Table 11).

6. Zinc content (Zn) in roots (mg/kg)

The inoculated seedlings of *A. saligna* had displayed Zn content (0.626 and 0.924 mg/kg) in *A. saligna* roots the significantly higher than that obtained in those uninoculated ones (0.396 and 0.615 mg/kg) in both season, respectively (Table 9).

Concerning the impact of heavy metals type, however Pb-amended seedlings had displayed Zn content (0.586 mg/kg) significantly higher than those Cd (0.542 mg/kg) and Zn-amended ones (0.405 mg/kg) and both the later were different significantly between each other in the first season, however, the second season had displayed Pb-amended seedlings showed the highest Zn content (0.789 mg/kg), while the lowest content (0.613 mg/kg) was detected in those applied with Zn (Table 9).

As for the impact of the concentration of amended heavy metals, however the addition of 1000 and 500 mg/kg has brought about the highest Zn content (0.775and 1.06 mg/kg), while the lowest (0.049 and 0.073 mg/kg) was detected in those applied with 0.00 mg/kg in both season, respectively (Table 9).

According to the significant interaction between the impact of the inoculation with *Rhizobium* and heavy metals type, the inoculated seedlings of *A. saligna* contaminated with Cd had displayed the highest Zn content (0.743 and 1.10 mg/kg), while the lowest (0.262 and 0.381 mg/kg) was obtained in those uninoculated ones that applied with Zn in their roots in both season, respectively (Table 10). The significant interaction between the effect of inoculation and concentrations of heavy metals has revealed that the inoculated seedlings applied with 1000 and 0.00 mg/kg displayed the highest and the lowest Zn content (1.09, 0.046 and 1.48, 0.062 mg/kg, respectively) in their roots in both season, respectively (Table 10).

As for the significant interaction between the type of heavy metals and its concentration, concentration 1000 mg/kg of Zn has inducted the highest Zn content (0.964 mg/kg), while the lowest content (0.040 mg/kg) was detected in those applied with 0.00 mg/kg Zn in the first season, while the second season, the roots of *A. saligna* seedling applied with 500 mg/kg Cd has induced the highest Zn content (1.38 mg/kg), while the lowest content (0.063 mg/kg) was detected in those applied with 0.00 mg/kg Cd (Table 10).

Based on the significant triple interaction among all the studied factors, the highest Zn content (1.50 mg/kg) was obtained in the inoculated seedlings of *A. saligna* treated with 1000 mg/kg Zn, the lowest content (0.022 mg/kg) was detected in the inoculated ones treated with 0.00 mg/kg Zn in the first season, while the second season, the highest Zn content (1.93 mg/kg) was obtained in the inoculated seedlings of *A. saligna* treated with 500 mg/kg Cd, while the lowest concentration (0.038 mg/kg) was detected in the inoculated ones, treated with 0.00 mg/kg Cd (Table 11).

7. Lead content (Pb) in roots (mg/kg)

The uninoculated seedlings of *A. saligna* had displayed Pb content (1.16 mg/kg) significantly higher than that obtained in those inoculated ones (0.750 mg/kg) in the first season (Table 9). Concerning the impact of heavy metals type, however Pb-amended seedlings had displayed Pb content (1.30 and 1.60 mg/kg) significantly higher than those Cd-(0.854 and 0.935 mg/kg) and Zn-amended ones (0.716 and 0.861 mg/kg) and both the later were different significantly between each other in both season, respectively (Table 9).

As for the impact of the concentration of amended heavy metals, however the addition of 1000 and 500 mg/kg has brought about the highest Pb content (1.39 and 1.71 mg/kg), while the lowest (0.113 and 0.059 mg/kg) was detected in those applied with 0.00 mg/kg in both season, respectively (Table 9).Upon the significant interaction between the impact of the inoculation with *Rhizobium* and heavy metals type, the uninoculated seedlings of *A. saligna* contaminated with Pb had displayed the highest Pb content (1.54 mg/kg), while the lowest (0.503 mg/kg) was obtained in those inoculated ones that applied with Cd in the first season, while the second season, the inoculated seedlings of *A. saligna* contaminated with Pb had displayed the highest Pb content (1.63 mg/kg), while the lowest content (0.698 mg/kg) was obtained in those inoculated ones that applied with Cd (Table 10).

The significant interaction between the effect of inoculation and concentrations of heavy metals has revealed that the uninoculated and inoculated seedlings applied with 1000 and 0.00 mg/kg had displayed the highest and the lowest Pb content (1.85 and 0.066 mg/kg, respectively) in their roots in the first season, while the second season, had revealed that the uninoculated and inoculation seedling applied with 500 and 0.00 mg/kg had displayed the highest and the lowest Pb content (1.79 and 0.051 mg/kg, respectively) (Table 10).

As for the significant interaction between the type of heavy metals and its concentration, the treatment with 500 mg/kg of Pb has induced the highest Pb content (1.87 and 2.48 mg/kg), while the lowest content (0.068 and 0.035 mg/kg) was detected in those applied with 0.00 mg/kg Cd and Zn in both season, respectively (Table 10).

Based on the significant triple interaction among all the studied factors, the highest Pb content (2.71 mg/kg) was obtained in the uninoculated seedlings of *A. saligna* treated with 1000 mg/kg Cd, while the lowest content (0.039 mg/kg) was detected in the roots of inoculated ones treated with 0.00 mg/kg Pb in the first season, while the second season, the highest Pb content (2.60 mg/kg) was obtained in the inoculated seedlings of *A. saligna* treated with 500 mg/kg Pb, the lowest content (0.012 mg/kg) was detected in the uninoculated ones treated with 0.00 mg/kg Zn (Table 11).

Table (9). Average of cadmium content (mg/kg), zinc content (mg/kg) and lead content (mg/kg) of roots of *Acacia saligna* as affected by inoculation with *Rhizobium*, application of heavy metal and its concentration

Treatment	Cadmium content (mg/kg)		Zinc content (mg/kg)		Lead content (mg/kg)	
	2014-2015	2015-2016	2014-2015	2015-2016	2014-2015	2015-2016
Inoculation with *Rhizobium* (A)						
Uninoculated	0.237	0.256	0.396b	0.516b	1.164a	1.20
Inoculated	0.208	0.254	0.626a	0.924a	0.750b	1.08
LSD 0.05	N.S	N.S	0.005	0.010	0.109	N.S
Heavy metal type (B)						
(Cd)	0.212	0.357a	0.542b	0.758b	0.854b	0.953b
(Zn)	0.218	0.271b	0.405c	0.613c	0.716c	0.861c
(Pb)	0.239	0.138c	0.586a	0.789a	1.301a	1.605a
LSD 0.05	N.S	0.012	0.004	0.007	0.071	0.078
Heavy metal Con. (C)						
0	0.006b	0.036d	0.049d	0.073d	0.113d	0.059d
250 mg/kg	0.281b	0.363a	0.520c	0.746c	1.016c	1.16c
500 mg/kg	0.266b	0.340b	0.700b	1.06a	1.306b	1.72a
1000 mg/kg	0.338a	0.282c	0.775a	1.00b	1.393a	1.62b
LSD 0.05	0.046	0.016	0.019	0.020	0.084	0.084

Table (10). Average of interaction of cadmium content (mg/kg), Zinc content (mg/kg) and lead content (mg/kg) of roots of *Acacia saligna* as affected by inoculation with *Rhizobium*, application of heavy metal and its concentration

Treatment		Cadmium content (mg/kg)		Zinc content (mg/kg)		Lead content (mg/kg)	
		2014-2015	2015-2016	2014-2015	2015-2016	2014-2015	2015-2016
Inoculation with *Rhizobium* X Heavy metal type							
Uninoculated	(Cd)	0.284a	0.357a	0.341d	0.416e	1.20b	1.20b
	(Zn)	0.182a	0.311b	0.262e	0.381f	0.740d	0.815c
	(Pb)	0.245a	0.099e	0.585b	0.750d	1.54a	1.57a
Inoculated	(Cd)	0.138b	0.355a	0.743a	1.100a	0.503e	0.698d
	(Zn)	0.254a	0.231c	0.547c	0.844b	0.692d	0.906c
	(Pb)	0.232a	0.176d	0.586b	0.827c	1.05c	1.63a
LSD 0.05		0.112	0.017	0.006	0.010	0.099	0.112
Inoculation with *Rhizobium* X Heavy metal Con.							
Uninoculated	0	0.006c	0.031e	0.051f	0.083g	0.159f	0.065e
	250 mg/kg	0.343a	0.385a	0.644c	0.703e	1.08c	1.05d
	500 mg/kg	0.264b	0.359b	0.433d	0.759d	1.55b	1.79a
	1000 mg/kg	0.335a	0.247d	0.456d	0.517f	1.85a	1.88a
Inoculated	0	0.006c	0.040e	0.046f	0.062g	0.066g	0.051e
	250 mg/kg	0.218b	0.340bc	0.394e	0.788c	0.947de	1.26c
	500 mg/kg	0.267b	0.319c	0.967b	1.35b	1.05d	1.64b
	1000 mg/kg	0.341a	0.317c	1.09a	1.48a	0.932e	1.35c
LSD 0.05		0.065	0.022	0.027	0.028	0.118	0.118
Heavy metal type X Heavy metal Con.							
(Cd)	0	0.006	0.054h	0.050i	0.063i	0.068g	0.057g
	250 mg/kg	0.315	0.407c	0.578f	0.761f	0.681f	0.606f
	500 mg/kg	0.253	0.593a	0.904b	1.38a	1.08d	1.48d
	1000 mg/kg	0.273	0.371d	0.636e	0.82e	1.57bc	1.66c
(Zn)	0	0.007	0.035hi	0.040i	0.074i	0.084g	0.035g
	250 mg/kg	0.264	0.503b	0.264h	0.497h	0.927e	1.07e
	500 mg/kg	0.264	0.248f	0.350g	0.682g	0.948e	1.18e
	1000 mg/kg	0.339	0.298e	0.964a	1.19b	0.904e	1.14e
(Pb)	0	0.006	0.017i	0.054i	0.080i	0.185g	0.083g
	250 mg/kg	0.264	0.178g	0.716d	0.978d	1.43c	1.79c
	500 mg/kg	0.283	0.178g	0.847c	1.109c	1.87a	2.48a
	1000 mg/kg	0.403	0.177g	0.724d	0.987d	1.69b	2.05b
LSD 0.05		N.S	0.028	0.033	0.035	0.145	0.145

Table (11). Average of triple interaction of cadmium content (mg/kg), Zinc content (mg/kg) and lead content (mg/kg) of roots of *Acacia saligna* as affected by inoculation with *Rhizobium*, application of heavy metal and its concentration

Treatment			Cadmium content (mg/kg)		Zinc content (mg/kg)		Lead content (mg/kg)	
			2014-2015	2015-2016	2014-2015	2015-2016	2014-2015	2015-2016
Inoculation with *Rhizobium* X Heavy metal type X Heavy metal Con.								
Uninoculated	(Cd)	0	0.006d	0.039i	0.041m	0.089o	0.052j	0.091h
		250 mg/kg	0.529a	0.321e	0.640g	0.524l	0.667h	0.459g
		500 mg/kg	0.362b	0.753a	0.355j	0.838i	1.381ef	1.77d
		1000 mg/kg	0.240c	0.316e	0.329jk	0.213n	2.71a	2.50ab
	(Zn)	0	0.006d	0.041hi	0.059m	0.088o	0.093j	0.012h
		250 mg/kg	0.279bc	0.706b	0.308k	0.390m	0.888gh	0.887f
		500 mg/kg	0.106d	0.198f	0.257l	0.539l	1.05fg	1.24e
		1000 mg/kg	0.339bc	0.298e	0.425i	0.508l	0.928g	1.12e
	(Pb)	0	0.006d	0.012i	0.052m	0.073op	0.331i	0.092h
		250 mg/kg	0.221c	0.128g	0.985cd	1.19e	1.69d	1.82d
		500 mg/kg	0.326bc	0.128g	0.688f	0.900h	2.24b	2.36b
		1000 mg/kg	0.426ab	0.127g	0.615g	0.829i	1.91c	2.03c
Inoculated	(Cd)	0	0.006d	0.069h	0.059m	0.038p	0.083j	0.022h
		250 mg/kg	0.100d	0.492c	0.515h	0.999g	0.696h	0.754f
		500 mg/kg	0.142cd	0.433d	1.453b	1.93a	0.797gh	1.19e
		1000 mg/kg	0.305bc	0.425d	0.944d	1.42c	0.436i	0.828f
	(Zn)	0	0.008d	0.03i	0.022m	0.060op	0.076j	0.059h
		250 mg/kg	0.248bc	0.299e	0.220l	0.605k	0.967g	1.25e
		500 mg/kg	0.421ab	0.297e	0.442i	0.824i	0.846gh	1.13e
		1000 mg/kg	0.339bc	0.298e	1.504a	1.88b	0.880gh	1.17e
	(Pb)	0	0.006d	0.022i	0.057m	0.088o	0.039j	0.074h
		250 mg/kg	0.306bc	0.228f	0.447i	0.759j	1.18f	1.77d
		500 mg/kg	0.239c	0.228f	1.005c	1.31d	1.51de	2.60a
		1000 mg/kg	0.378b	0.227f	0.834e	1.14f	1.48e	2.07c
LSD 0.05			0.114	0.039	0.047	0.049	0.205	0.205

DISCUSSION

To a great extent, the seedlings of *Acasia saligna* were significantly affected by all the studied factors, the inoculation with *Rhizobium*, heavy metals type and its concentration in terms growth parameters and heavy metals concentration in plant.

Upon the findings of this study, it has been revealed that the inoculation of *Acasia saligna* seedlings with *Rhizobium* has brought about the highest growth parameters either under heavy metals pollutants or in normal condition. So it is hypothesized that the inoculation with *Rhizobium* may act as remediator in terms of the soil pollutants. Kim *et al.* (2014) highlighted the role of *Rhizobium*, which is widely recognized in plant growth promotion.

Plant growth promotion by *Rhizobia* through nitrogen fixation, Nitrogen (N) is required for synthesis of nucleic acids, enzymes, proteins and chlorophyll and hence it is a vital element for plant growth. Although 78 %of the atmospheric air is N, this gaseous form is unavailable for direct assimilation by plants. Currently, a variety of industrial N fertilizers is used for enhancing agricultural productivity. However, economic, environmental and renewable energy concerns dictate the use of biological alternatives (Gopalakrishnan *et al.*, 2014).

Bloemberg and Lugtenberg (2001) studied a host-specific symbiosis with leguminous plants. The symbiosis is initiated by the formation of root or stem nodules in response to the presence of the bacterium. Lipooligosaccharide signal molecules that are secreted by the bacterium play a crucial role in this process. The bacteria penetrate the cortex, induce root nodules, multiply and subsequently differentiate into bacteroids, which produce the nitrogenase enzyme complex. Within the root nodules, the plant creates a low oxygen concentration, which allows bacterial nitrogenase to convert atmospheric nitrogen into ammonia. In turn, the plant supplies the bacteria with a carbon source.

The concentration of 1000 mg/kg Pb and Cd had brought about the lowest growth of plants, however, the higher the concentration of heavy elements the lower the growth of plants in case of uninoculated seedlings. The harmful effect of higher level of either Cd or Pb may be due to the inhibitory effect on plant metabolism, and toxic the effects of these metals on systematic enzyme (Rivetta *et al.*, 1997 and Kabata- Pendias and Pendias, 2001). It has been found detrimental impact of high levels of heavy metals on the plant, in terms of chlorophyll content. Pietrini *et al.* (2009) found the same effect of Cd, Zn and Pb in the poplar plant.

The results obtained indicated that the highest concentration of heavy metals was detected in the soil of *A. saligna,* which proved that the presence of nodules may induce more absorption and may phytostablization of heavy metals by plants. Plants were able to absorb of heavy metals (Labrecque *et al.*, 1995), in additions, (Landberg *et al.*, 1996) found that the *Salix* clones from the polluted area had higher accumulation of heavy metals in their roots and lower translocation of it to the shoots than clones from the unpolluted area**.**

Amira (2015) found significant differences between selected species in their absorption of Pb and Cd, either on the bases of roots or shoots. It is clear also that the tolerant species such as *A. saligna* has the ability to keep the heavy metals in their root system and do not move them to the aboveground parts. These results support those obtained by (Gratao *et al.*, 2005), who found a positive relationship between the accumulation of Pb or Cd and the ability of plants to tolerate these metals. Therefore, some species have a mechanism to tolerate heavy metals toxicity through prevention of the uptaking of Pb and Cd from roots to shoots. Therefore, microbes were either sufficiently tolerant or selected for survival in this environment with less bio-available metals (Smolders *et al.*, 2003 and Smolders *et al.*, 2004). This is the main reason why most soil microbial processes were still found even in highly contaminated field soils (Renella *et al.*, 2002 and Smolders *et al.*, 2003).

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الملخص العربي

العلاج الحيوي للتربة الملوثة باستخدام بعض أشجار الزينة

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أجريت التجربة في مشتل قسم الزهور ونباتات الزينة وتنسيق الحدائق بكلية الزراعة الشاطبي بالأسكندرية وذلك من أول أغسطس إلى أول فبراير ٢٠ لموسميين متتاليين (٢٠١٤- ٢٠١٥ / ٢٠١٥- ٢٠١٦)، لدراسة العلاج الحيوى للتربة الملوثة بأستخدام بعض أشجار الزينة وهي نبات الاكاسيا سالجينا ملقحة ببكتريا الريزبيوم التي تم الحصول عليها من مشتل قسم الغابات وتكنولوجيا الأخشاب بأبيس بالإسكندرية، وكذلك تقييم صفات التربة بعد الزراعة لمعرفة مدي قدرتها علي تنقية التربة من العناصر الثقيلة بوجود الكائنات الدقيقة – المعاملات المستخدمة من العناصر الثقيلة (كادميوم – زنك – رصاص) كانت: ٠،٠٠ مجم/ كجم تربة (كنترول) – ٢٥٠ مجم/ كجم تربة – ٥٠٠ مجم/ كجم تربة و ١٠٠٠ مجم/ كجم تربة وذلك علي كل النباتات غير الملقحة والملقحة بالريزوبيوم.

<u>وفيما يلي أهم النتائج المتحصل عليها:</u>

- التركيزات العالية من العناصر الثقيلة (٥٠٠ – ١٠٠٠ مجم/ كجم تربة) قللت لحد ما من صفات النمو (معدل النمو النبات – الوزن الكلي الجاف – نسبة عقد الريزوبيوم / الجذر) وظهر التأثير السلبي بوضوح في النباتات غير الملقحة بالريزبيوم أي أن النباتات الملقحة بالريزوبيوم كانت أعلي فى صفات النمو من النباتات غير الملقحة.
- أكثر العناصر الثقيلة أثرا علي النمو كانت الكادميوم والرصاص أما تأثير الزنك كان أقل ضررا.
- تأثر محتوي الكلوروفيل الكلي في أوراق النبات بأضافة العناصر الثقيلة وكانت التركيزات العالية من الزنك والرصاص هي الأكثر أثرا بالسلب علي محتوي الكلوروفيل الكلي.
- وجد أن عنصر الرصاص هو أكثر المعادن الثقيلة ثباتا في النبات مقارنة بكل من الكادميوم والزنك وذلك عند إضافته بالمستوي الأعلي (١٠٠٠مجم/ كجم تربة).

التوصـية

يوصي باستخدام نباتات الأكاسيا سالجينا كنبات معالج (Phytoremediator) للتربة الملوثة نظرا لتحمله المستويات العالية من الملوثات فضلا عن أن البكتريا المثبتة للنيتروجين والمتكافلة علي جذوره لها دور فعال في علاج التربة الملوثة بالعناصر الثقيلة.