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Spirulina platensis Alleviating Effect To Rocket Plant (*Eruca Vesicaria*) Grown On A Pb-Polluted Soil

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Abstract

Lead (Pb) is a hazardous heavy metal causing environmental pollution in various sources. A 3-factor pot experiment was conducted to asses the use of *Spirulina platensis* for alleviating Pb pollution on rocket plant (*Eruca vesicaria*) grown in pots (10-kg pot^{-1}). Factors and treatments were (1) Pollution (L): non-polluted (L_0) and polluted soil with 400 mg Pb kg^{-1} (L_1) as $PbCl_2$ (2) Priming (P):none (P_0), priming (soaking) of rocket seeds in 40 mgL^{-1}(P_1) and 80 mgL^{-1} (P_2) (3) .Spirulina foliar spray (F) : with distilled water (F_0), 40 mg L^{-1} (F_1) and 80 mgL^{-1} (F_2). *Spirulina* alleviated contamination and increased growth and photosynthetic pigments and activity as well as increased activity of carboxylating enzymes (Phosphoenol pyruvate carboxylase (PEPC, EC 4.1.1.31) and ribulose1,5-bisphosphate carboxylase (RuBPC, EC 4.1.1.39) and NPK Highest positive effect was by the the combination of foliar and soaking at 80mg/l of *Spirulina platensis* solution.

Keywords:

Introduction

Lead (Pb) is malleable, heavy and non-corrosive metal and is used in paints, plumbing , batteries, ammunition and petrol (Csuros, 1994; Alloway and Ayres, 1997). It is toxic to plant (Sharma and Dubey 2005; Han *et al.*, 2008; Liu *et al.* 2008; Zhou *et al.*, 2010) disturbing its morphology, growth and photosynthesis as well as enzyme activities, water imbalance, membrane permeability and nutrition (Patra *et al.*, 2004; Dogan *et al.*, 2009; Ling and Hong, 2009; Maestri *et al.*, 2010 and Nas and Ali 2018). It can damage cells generating reactive oxygen species 'ROS' (Fargasova, 1994; Dixit *et al.*, 2001; Erdei *et al.*, 2002; Islam *et al.*, 2011). ROS such as superoxide radical ($O_2^{\cdot -}$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH.) are produced as a result of oxidative stress and detrimental effects to plant cells (Islam *et al.*, 2008).

Photosynthesis is most sensitive to such pollution which decreases chlorophyll contents "probably reduction of δ-aminolevulinic acid dehydratase activity" (Sharma and Dubey, 2005, Cargnelutti *et al.*, 2006; Gonçalves *et al.*, 2009; Rossato *et al.*, 2012) , enhances chlorophyll degradation due to high chlorophyllase activity (Drazkiewicz, 1994, Rashid *et al.*, 1994; Gajic *et al.*, 2009) and decreases carotenoids which protects lipids of membranes (Haider *et al*., 2006; Li *et al.*, 2012). Heavy meyals decrease photosynthetic pigments (Haider *et al.*, 2006; Mishra *et al.*, 2006; Li *et al.*, 2012).

Spirulina platensis algae (blue green alga) can be a biofertilizer and a protein, with vitamins, essential amino acids and fatty acids (Leduy and Thorein, 1977; Ciferri and Tibani, 1985; Vonshak and Richmond, 1985 , Vonshak, 1986 and Ahmed *et al*., 2011). Jagannath *et al*. (2002) found that *Spirulina* increased growth, protein, carbohydrates and grain yield of chick peas (***Cicer arietinum***). Liu-ShiMing and Liang-ShiZhong (1998) noted that cyanobacteria increased roots, epicotyl and hypocotyl growth, and weight of mung beans (***Vigna radiate***).

Cyanobacteria as biofertilizer increased seed germination, growth parameters and nitrogenous compounds, of wheat (***Triticum aestivum***), sorghum(***Sorghum vulgare***), maize (***Zea mays***), lentil (***Lens culinaris***) and sugar beet (***Beta vulgaris***) (Adam, 1999; Aly *et al.*, 2008 and Indira and Biswajit, 2012).Spirulina application as foliar spray on radish (***Raphanus sativus***) increase yield and chlorophyll (Godlewska et al. 2019)

Rocket (***Eruca vesicaria subsp. sativa***) can grow throughout the year and its fresh green leaves are consumed by the Egyptians for their spicy hot taste. Leaves are rich in nutrients and vitamins and seeds contain medicinal oil ; with an acerage of more than 2200 ha cultivated in Egypt during 2006 (Moussa, 2006).

The present study aims at assessing the effect of treating rocket grown on Pb polluted soil with *Spirulina platensis* in alleviating such pollution.

Materials and Methods

A pot experiment (PVC pots 40-cm high and 35-cm diameter) filled with 10 kg clay soil was conducted . Table 1 shows main properties of the soil (analysis according to methods cited in Black et al (1965). The experiment was kept in a growth chamber to assess the efficiency of spirulina in decontaminating a Pb-polluted soil and alleviating the negative effect on rocket (*Eruca vesicaria* subsp. *sativa*).Seeds were surface sterilized in 0.1% (w/v) sodium dodecyl sulphate solution then rinsed with sterile deionized water. The soil was non-saline non-sodic heavy clay. Twenty seeds per pot were seeded and immediately after germination reseeding for non-germinated seeds was done. All pots were supplied with 60, 20 and 40

mg of N, P and K respectively per kg soil as urea (640 g Nkg^{-1}) , super phosphate (68 g P kg^{-1}) and potassium sulphate (40 g K kg^{-1}) . The design of the experiment was a Randomized Complete Block , factorial (3 factors) in 3 replicates. **Factor 1** is pollution (L): non-polluted (L_0) and polluted with 400 mg Pb kg^{-1} (L_1) as $PbCl_2$ **Factor 2** spirulina seed priming (P) ; none (P_0), priming in 40 mgL^{-1}(P_1) and in 80 mgL^{-1} (P_2) .Priming lasted overnight. Non-primed seeds were soaked in distilled water. **Factor 3** spirulina foliar spray (F) with none (F_0) spray with distilled water , spray with 40 mgL^{-1} (F_1) and with 80 mgL^{-1} (F_2) Thus the number of treatment combinations = 18 (2 "L" x 3 "P" x 3 "F"). Watering was done up to about 85% of water holding capacity.

Photosynthetic pigments Chlorophyll (Chl *a*, and Chl *b*) and carotenoids were determined according to Metzner *et al*. (1965); Photosynthetic activity ($^{14}CO_2$-fixation) was assayed as described by Moussa (2006). Activities of carboxylating enzymes (Phosphoenol pyruvate carboxylase "PEPC, EC 4.1.1.31") were measured using a method described by Cánovas and Kornberg (1969) .Ribulose-1,5-bisphosphate carboxylase/oxygenase activity (RuBPC, EC 4.1.1.39) was measured using a method described by Robinson and Portis (1988). N and P were determined using an auto-analyzer (QuikChem, Series 8000, Lachat Instruments Inc., USA), while K and Pb were measured using atomic absorption spectrophotometer (Perkin-Elmer 5100 PC).

Table 1. Main properties of the soil used in the experiment

Property	**Value**	**Available nutrient**	
pH (1:2.5 H_2O)	7.55	N	4.0
EC, dS m^{-1} (Paste extract)(	0.86	P	4.1
ESP	0.52	K	100
Organic Matter (g kg^{-1})	20.1	Fe	9.9
Sand %	31.5	Mn	2.7
Silt %	11.8	Pb	0.01
Clay,%	56.7	Zn	0.7
Texture*	Heavy Clay	Extracts: KCl(N); NaCHO3 (P); NH4Ac(K); DTPA (Fe,Mn,Zn Cu and Pb)	
WHC (w/w) %	38.6		
Bulk Density (Mg m^{-3})	1.33		

Results and Discussion

Fresh weight of above soil plant growth :

Shoots fresh weight was affected negatively by Pb contamination and positively by spirulina application (Table 2). The lowest of 210 g pot^{-1} occurred upon application of Pb with no spirulina ($L_1P_0F_0$) while the highest was given by non Pb-contaminated treatment receiving the high dose of both priming and spraying of spirulina ($L_oP_2F_2$) which surpassed the lowest by 552% which demonstrates a considerable alleviation of the negative effect caused by Pb. The main effect of Pb contamination was a decrease of 30.8 %. The decrease was particularly high – being 46.3%- where no spirulina was given. In presence of spirulina at its combined high dose of spray+priming the negative effect less approaching 25.6% .The alleviating effect of spraying spirulina surpassed its priming counterpart particularly where the high dose was used. Priming caused increases averaging 26.7 and 66.3 % at the low and high doses respectively compared with 43.7 and 114% respectively using the spray method.

Roots fresh weight followed a pattern resembling that of the shoots (Table 2). The lowest of **86** g pot^{-1} occurred with Pb without spirulina ($L_1P_0F_0$) and the highest was given by no Pb treatment receiving the high dose of spirulina priming +spraying ($L_oP_2F_2$) which surpassed the lowest by 631% indicating an extremely high alleviation effect of growth retardation caused by Pb. The main effect of Pb contamination was a decrease of 33.5 %. The decrease was particularly high amounting to 23.2%, where no spirulina was given. In presence of spirulina at its combined high dose of "spray+priming" the negative effect was a decrease of 50.1%. The alleviating effect of spraying spirulina surpassed its priming counterpart particularly where the high dose was used. The priming effect averaged 41.3 and 94.5 % at the low and high doses respectively compared with 58.5 and 138% respectively using the spray method.

Contents of carotenoids in shoots:

Response of carotenoids (Table 2) followed a pattern resembling those of the plant growth . Contents were affected negatively by Pb and positively by spirulina application. The lowest content of 0.50 $mgkg^{-1}$ f.w. occurred due to Pb contamination with no spirulina treatment ($L_1P_0F_0$).The highest existed in the non-contaminated high spirulina ($L_0P_2F_2$) plants surpassing the lowest by an extremely high 584%. Under no spirulina the decease caused by Pb was 38.3% .The main effect of Pb contamination was an average decrease of 16.0 % . Under the combined high doses of both spirulina methods the decrease by Pb was 15.2% . The spray method of spirulina was more efficient than the priming method. Increases averaged 38.4 and 112% by the low and high spray respectively compared with respective averages of 23.2 and 58.3% by the priming method.

Table 2. Fresh weight ($gpot^{-1}$) of rocket plants and carotenoids in shoots as a affected by contaminating the soil with Pb and alleviation with *Spirulina platensis* (blue-green algae)

Pb Pollution (L)	Seed primin (P)	Foliar spray (F)											
		F_0	F_1	F_2	Mean	F_0	F_1	F_2	Mean	F_0	F_1	F_2	Mean
		Shoots ($g\ pot^{-1}$)				Roots ($g\ pot^{-1}$)				Shoots Carotenoids ($mg\ kg^{-1}$)			
L_0	P_0	391	613	1150	**718**	112	285	400	**266**	0.81	1.85	2.87	**1.84**
	P_1	552	871	1275	**899**	208	352	528	**363**	1.40	2.08	3.16	**2.21**
	P_2	924	993	1369	**1095**	367	498	629	**498**	2.41	2.31	3.42	**2.71**
Mean		**622**	**826**	**1264**	**904**	229	378	519	**376**	1.54	2.08	3.15	**2.25**
L_1	P_0	210	408	729	**449**	86	112	314	**171**	0.50	1.31	2.45	**1.42**
	P_1	313	526	903	**581**	100	180	482	**254**	1.09	1.60	2.74	**1.81**
	P_2	617	909	1018	**848**	227	315	508	**350**	2.08	2.35	2.90	**2.44**
Mean		**380**	**614**	**883**	**626**	**138**	**202**	**435**	**250**	**1.22**	**1.75**	**2.70**	**1.89**
G. Mean		**501**	**720**	**1074**		**183**	**290**	**477**		**1.38**	**1.91**	**2.92**	**2.07**
		Means of P											
P_0		301	511	940	**584**	99	199	357	**218**	0.65	1.58	2.66	**1.63**
P_1		432	699	1089	**740**	154	266	505	**308**	1.24	1.84	2.95	**2.01**
P_2		770	951	1193	**971**	297	406	569	**424**	2.24	2.33	3.16	**2.58**
LSD 0.05		L:2.1 P:2.7 F:2.2 LP:3.8 LF:3.8 PF:1.6 LPF:2.3				L:0.1 P:0.2 F:0.2 LP:0.2 LF:0.2 PF:0.3 LPF:0.4				L:0.09 P:0.001 F:0.001 LP:0.06 LF:0.01 PF:0.001 LPF:0.001			

Notes: L_1 and L_2 are Pb pollution of 0, and 400 $mgkg^{-1}$ respectively (source Pb Cl_2) ; P_0,P_1 and P_2 are seed soaking (priming) in spirulina suspension (SS) of 0, 40 and 80 mgL^{-1} ; F_0,F_1 and F_2 are foliar spray with SS of 0, 40 and 80 mgL^{-1} respectively.

Contents of Chlorophyll$_a$:

Contents of chlorophyll$_a$ were affected negatively by Pb contamination and positively by spirulina application (Table 3). The lowest content of chlorophyll$_a$ of 0.76 $mgkg^{-1}$ f.w. occurred upon application of Pb with no spirulina ($L_1P_0F_0$).The highest contents existed in the non Pb-contaminated treatment receiving the high dose of both priming and spraying of spirulina ($L_oP_2F_2$) which surpassed the lowest by 345% indicating a considerable alleviating effect caused by the combined effect of spirulina seed priming and foliar spray . The main effect of Pb was an average decrease of 17.1 %. The decrease was 40.6% where no spirulina was given and 12.7% where spirulina was given at the high dose of the two methods combined. Effectiveness of spirulina spray surpassed its priming counterpart. Spray caused average increases of 37.4 and 101% by the low and high doses respectively compared with respective 11.8 and 34.9.% by the priming method.

Contents of chlorophyll$_b$:

Table 3 shows that results of chlorophyll$_b$ resemble those of chlorophyll$_a$. The lowest chlorophyll$_b$ of 0.25 $mgkg^{-1}$ f.w. was given by the Pb-contaminated non-spirulina treated ($L_1P_0F_0$) plants. The highest were given by non-Pb polluted plants which received combined high doses of spirulina which surpassed the lowest by as much as 864% demonstrating an extremely alleviating effect against Pb toxicity . The main effect of Pb was an average decrease of 37.7%. The decrease was 38.5% where no spirulina was given and 36.9% where spirulina was given at the high dose of the two methods combined. Effectiveness of spirulina spray was more than its priming. For the spray method ,increases averaged 75.3 and 137% for the low and high spray dose respectively compared with respective averages of 27.4 and 62.1%.

Contents of chlorophyll$_{a+b}$:

Table 3 shows that the pattern of response to chlorophyll$_{a+b}$ was very similar to each of chlorophyll$_a$ and chlorophyll$_b$ .Pb caused a negative effect while spirulina caused a positive one. The lowest chlorophyll$_{a+b}$ of 1.01 $mgkg^{-1}$ f.w. occurred upon applying Pb with no spirulina ($L_1P_0F_0$) while the highest existed in the non Pb-contaminated which of the high combined dose of both priming and spraying spirulina ($L_oP_2F_2$) which surpassed the lowest by 422% , reflecting considerable alleviating effect against Pb contamination .

Main effect of Pb was a decrease of 25.2 % and the decrease was particularly high of 45.1% where no spirulina was given but with combined high doses of spirulina , the decrease was lowered to 20.1%. Spirulina was more effective when sprayed than when primed. Spraying caused increases averaging 44.0 and 105% due to the low and high dose respectively as compared with 10.9 and 32.3 % for the priming methods.

Photosynthetic activity

The pattern of response regarding photosynthetic activity (Table 4) was in line with those of the chlorophyll pigments. Photosynthetic activity was

affected negatively by Pb contamination and positively by spirulina application.

Table 3. Chlorophyll contents (mg kg^{-1} fresh wt.) in shoots of rocket grown on a Pb-contaminated soil as affected with *Spirulina platensis* treatment

Soil Pb Poll. (L)	Seed prime (P)	Foliar spray (F)											
		F_0	F_1	F_2	Mean	F_0	F_1	F_2	Mean	F_0	F_1	F_2	Mean
		Chlorophyll$_a$ '				Chlorophyll$_b$				Chlorophyll$_{a+b}$			
L_0	P_0	1.28	2.08	2.92	**2.09**	0.85	1.33	1.87	**1.26**	1.84	3.40	4.79	**3.34**
	P_1	1.53	2.21	3.18	**2.30**	0.89	1.50	2.08	**1.41**	2.41	3.65	4.97	**3.68**
	P_2	2.11	2.45	3.38	**2.65**	1.31	1.87	2.41	**1.87**	3.42	3.81	5.27	**4.17**
Mean		**1.64**	**2.25**	**3.16**	**2.35**	**0.93**	**1.57**	**2.12**	**1.54**	2.55	3.62	5.01	**3.73**
L_1	P_0	0.76	1.58	2.55	**1.63**	0.25	0.78	1.15	**0.72**	1.01	2.36	3.71	**2.36**
	P_1	1.08	1.71	2.78	**1.85**	0.51	0.92	1.34	**0.92**	1.57	2.56	3.83	**2.65**
	P_2	2.03	2.14	2.95	**2.37**	0.85	1.29	1.52	**1.22**	2.83	3.08	4.21	**3.37**
Mean		**1.29**	**1.81**	**2.76**	**1.95**	**0.54**	**1.00**	**1.33**	**0.96**	**1.30**	**2.66**	**3.91**	**2.79**
G. Mean		**1.47**	**2.02**	**2.96**		**0.73**	**1.28**	**1.73**		**2.18**	**3.14**	**4.46**	**3.26**
		Means of F											
P_0		1.02	1.83	2.73	**1.86**	0.41	1.05	1.51	**0.95**	1.42	2.88	4.25	**2.85**
P_1		1.30	1.96	2.98	**2.08**	0.70	1.21	1.71	**1.21**	1.99	3.10	4.40	**3.16**
P_2		2.07	2.30	3.16	**2.51**	1.08	1.58	1.97	**1.54**	3.13	3.45	4.74	**3.77**
		L:0.001 P: 0.22 F:0.001 LP:0.05 LF:0.01 PF0.001 LPF: 0.001				L 0.001 P 0.50 F 0.001 LP0.06 LF:0.06 PF0.001 LPF 0.001				L 0.09 P0.001 P 0.001 LP 0.06 LF 0.01 LFP 0.001			

See notes of Table 2 for designation of treatments.

The lowest activity of 9.63 kBq mg-1 fw occurred in the Pb-contaminated treatment under conditions of no spirulina ($L_1P_0F_0$) whereas the highest of 43.13 was given by the non Pb-contaminated with high dose of spirulina priming and spraying ($L_oP_2F_2$) which surpassed the lowest by 348%. This demonstrate considerable alleviating effect caused by spirulina seed priming and foliar spray against the negative Pb effect . The main effect of Pb contamination was a decrease of 23.6 %. The decrease was particularly high(-41.4%) where no spirulina was applied. %. Where spirulina was given at the high dose (of both methods combined), the negative effect of Pb was 20.8 Effectiveness of spirulina spray surpassed its priming counterpart ; Spray caused 24.3 and 80.2% increases at the low and high dose respectively compared with 19.3 and 35.4% respectively by the priming method.

Assessment of plant growth, chlorophyll ,carotenoids and photosynthetic activity:

The considerable decrease in plant growth, chlorophylls and carotenoids as a result of Pb contamination is a demonstration of retardation and toxicity caused by this element . Pollution of soil with Pb inhibits growth and proper functions of systems in many plant species (**Sharma and Dubey 2005; Han et al., 2008; Liu et al. 2008; Zhou et al., 2010**). Retardation of photosynthetic processes is an outcome of Pb toxicity (**Patra *et al.*, 2004; Dogan *et al.*, 2009; Ling and Hong, 2009; Maestri *et al.*, 2010**). Lead has a profound negative effect on photosynthesis (**Sharma and Dubey, 2005**) and causes degradation to plant chlorophyll (**Drazkiewicz, 1994**). Oxidative cell damage by generating reactive oxygen species 'ROS' is caused by Pb toxicity (Fargasova, 1994, Dixit *et al.,* 2001, Erdei *et al.,* 2002 and Islam *et al.,* 2011).

On the other hand the positive effects of spirulina on plant growth which are shown in the present study were demonstrated in alleviating Pb toxicity as well as increasing plant growth. This was evident through spraying as well as seed priming. Toxicity of Pb decreased pigment contents drastically in plants not treated with spirulina and less in plants treated with spirulina. Spirulina can form associations with plants and produce growth-promoting substances (**Nanjappan-Karthikeyan *et al.*, 2007**) which enable plant to resist stress conditions (**Malik *et al.*, 2001; Song *et al.*, 2005 and Indira and Biswajit, 2012**).

A number of research work attributed the decline in photosynthetic rate caused by Pb to a number of reasons which include the four followings: A- Blocked electron transport (**Qadir *et al.*, 2004 and Gajic *et al.*, 2009**), B- Inhibiting the activities of the 5 Calvin cycle enzymes of RuBP carboxy lase, **fructose** 1,6-bisphosphatase "FBPase" , sedoheptulose 1,7-bisphosphatase "SBPase", Ru5P kinase and NADP-glyceraldehyde 3-phosphate "GAP" dehydrogenase (**Sharma and Dubey, 2005**; **Mishra *et al.*, 2006 and Liu *et al.*, 2008**), C- Distorting the chloroplast ultrastructure (**Sharma and Dubey, 2005; Islam *et al.*, 2008**), especially through disorganizing the thylakoid arrangement (**Basile *et al.*, 2012) .** Inhibition of chlorophyll biosynthesis was attributed in part to reduction of δ-aminolevulinic acid dehydratase

activity (**Cargnelutti *et al.*, 2006; Gonçalves *et al.*, 2009; Rossato *et al.*, 2012).** Enhancement of cytokinins to plant physiologic activities was stated by **Thomas (1996**). upon application of algae extracts .The extracts contain auxins which increase vitamins , hormones producing gibberellic acids GA_3, and GA_7 and vitamin all of which increase photosynthetic activity (**O'Dell, 2003**). **Kowalski *et al.* (1999**) noted positive effects of algae extracts on growth and yield of potato plant (*Solanum tuberosum*). Foliar application of spirulina to sesame plant (*Sesamum indicum*) increased its growth parameters . Other positive effects were noted on other plants (**Aly and Esawy 2008; Bhowmik et al., 2010;amal et al., 2010; Chojnacka et al., 2012 , Piyanast et al., 2014 and Hicham et al., 2016).** Algal extracts contain gibberellic acids (GAs) particularly GA_3, and GA_7 both of which contribute to proper plant growth (**Drazkiewicz, 1994**).

Decreased carotenoids in plants is another negative effect of Pb toxicity noted by **Haider *et al.* (2006)** on mungo beans (*Phaseolus mungo*) and black lentils (*Lens culinaris*) and noted also by **Li *et al.* (2012)** on rice (*Orsa sativa*). Photosynthetic electron transport in plant was inhibited by Pb (**Rashid *et al.*, 1994 and Gajic *et al.*, 2009 and Qadir *et al.*, 2004**).

Table 4. photosynthetic enzymes activity , Ribulose-1,5-bisphosphate-carboxylase/oxygenase, RuBPCase. Phosphoenol-pyruvate carboxylase enzymes(PEPCase) and Pb (mg kg^{-1} dw) in shoots

Soil Pb Poll. (L)	Seed prime (P)	Foliar spray (F)											
		F_0	F_1	F_2	Mean	F_0	F_1	F_2	Mean	F_0	F_1	F_2	Mean
		Photosynthetic activity (kBq mg^{-1} fw)				carboxylase enzymes (µmol CO_2 min^{-1} mg^{-1} protein)				Pb (mg kg^{-1} dw)			
L_0	P_0	16.8	18.8	23.1	19.5	53.8	69.0	78.0	66.8	0.41	0.07	0.08	0.18
	P_1	17.9	21.6	26.8	22.1	61.4	73.2	80.6	71.8	0.05	0.05	0.07	0.05
	P_2	20.0	22.4	28.1	23.5	66.0	80.2	91.0	79.0	0.04	0.06	0.08	0.06
Mean		18.2	21.0	26.0	21.7	0.16	0.06	0.08	0.10	0.16	0.06	0.08	0.10
L_1	P_0	11.3	15.2	17.9	14.8	11.3	15.2	17.9	14.8	7.80	4.31	3.01	5.00
	P_1	13.7	16.8	18.2	16.2	13.7	16.8	18.2	16.2	5.00	3.11	2.40	3.50
	P_2	17.7	18.0	20.0	17.5	17.7	18.0	20.0	17.5	3.20	2.50	2.01	2.60
Mean		14.2	16.6	18.7	16.2	5.30	5.30	2.40	3.70	5.30	5.30	2.40	3.70
G. Mean		16.0	19.0	22.3	19.1	2.80	1.70	1.20	1.90	2.80	1.70	1.20	1.90
		Means of F											
P_0		14.0	17.0	20.5	17.2	4.10	2.21	1.60	2.60	4.10	2.21	1.60	2.60
P_1		15.8	19.2	22.5	19.1	2.50	1.60	1.20	1.80	2.50	1.60	1.20	1.80
P_2		17.3	20.2	24.0	20.5	1.60	1.20	1.00	1.30	1.60	1.20	1.00	1.30
		L: 0.0001 P: 0.001 F:0.001 LP :0.002 LF: 0.001 PF: 0.004 LFP: 0.0001				L: 0.00 9 P: 0.0007 F:0.0001 LP :0.03 LF: 0.0001 PF:0.001 LFP: 0.0001				L: 0.001 P: 0.001 F:0.0001 LP :0.02 LF: 0.001 PF: 0.001 LFP: 0.001			

See notes of Table 2 for designation of treatments.

Phosphosynthetic enzymes activity.

Table 4 shows that the activity of photosynthesis enzymes (in Ribulose-1,5-bisphosphate-carboxylase/oxygenase,RuBPCase). Activities are of carboxylating enzymes (Phosphoenol pyruvate carboxylase **"**PEPC, EC 4.1.1.31") and ribulose1,5-bisphosphate carboxylase (RuBPC, EC 4.1.1.39**).** Values ranged between 11.3 and 28.1 kBq mg (kilo benzoquinone per mg fw). The lowest was given by the Pb non- primed non-sprayed ($L_1P_0F_0$) while the highest was caused by the non-polluted highly primed and sprayed ($L_0P_2F_2$) treatment ; an increase of 149%.This indicates a very high alleviating effect caused by spirulina seed priming combined with foliar spray against the negative effect of Pb on plant in photosynthetic activity. The main effect of Pb contamination was a decrease of 25.3 %. Where no spirulina was given, the decrease was particularly high , being -32.7%, but where spirulina was given at the high dose (of the two methods combined) , the negative effect of Pb was -28.8%. Effectiveness of spirulina foliar spray surpassed its priming counterpart; the foliar spray increased the activity by averages of 18.7 and 39.4% at the low and high dose respectively compared with 11.0 and 19.1% at the low and high priming methods respectively.

Phosphoenol-pyruvate carboxylase enzymes (CPEPase)

Table 5 shows **CPEPase** activity as affected by Pb contamination and spirulina treatment. Values (**µmol CO_2 min^{-1} mg^{-1} protein)** ranged from 41.7 to 91.0 µmol CO_2 min^{-1} mg^{-1} protein .Results show patterns very much similar to those of the photosynthetic activity. The lowest was given by the Pb none-primed non-sprayed ($L_1P_0F_0$) while the highest was caused by

the non-polluted highly primed and sprayed ($L_0P_2F_2$) .The high value surpassed the low one by 118% indicating a considerable increase in enzyme activity as a result of subjecting the contaminated plant to a high dose of combined spirulina seed priming and foliar spraying. The main effect of Pb contamination was a decrease of 26.9 %. Where no spirulina was given the decrease was 22.5%. Under conditions of spirulina at the high dose of combined priming+spraying the negative effect of Pb was a decrease of 25.4%. Foliar spray surpassed its priming counterpart .Foliar spray increased the activity by averages of 18.2 and 38.5% at the low and high dose respectively compared with respective 7.6 and 19.1%.

Contents of Pb in plant:

Contamination with Pb caused a drastic increase in plant Pb. Content in shoots under no Pb application was 0.41 $mgkg^{-1}$ dw . Content in plant subjected to contamination increased by as much as 18 times (Table 4) .Spirulina decreased Pb in plant. The lowest content of 0.04 $mgkg^{-1}$ dw occurred in the non-contaminated treatment where seeds were primed at the high spirulina dose and no spray was done ($L_0P_2F_0$).The highest existed in the Pb contaminated non-spirulina treated plants ($L_1P_0F_0$) where Pb content were 19 times those in the lowest. The main effect of Pb contamination was an average increase of 36 times. Spirulina suppressed contents of Pb in plant. Plants receiving spirulina at the high combined dose and growing on non-Pb contaminated soil showed as much as 99% decrease in Pb as compared with those grown on Pb-contaminated non-spirulina treated ones.

The spray method of spirulina was more effective than the priming method. Decreases caused by spraying averaged 39.3 and 57.1 % at the low and high dose respectively compared with 30.8 and 50.0% at the low and high dose of priming respectively.

Assessment of Phosphosynthetic and PEPCase activities and Pb in plant

The retarding and toxic effects of Pb was manifested in decreased phosphoenol pyruvate carboxylase activity in plants grown on the Pb contaminates soil. In a study by **van Assche and Clijsters (1990**) where rocket was grown on a soil contaminated with 350 mg Pb kg^{-1} , a considerable decrease occurred in phosphoenol pyruvate carboxylase (PEPC, EC 4.1.1.31) and ribulose1,5-bisphosphate carboxylase (RuBPC, EC 4.1.1.39) enzymes (superoxide dismutase activity, EC1.15.1.1; catalase activity, EC 1.11.1.6 and peroxidase activity, EC 1.11.1.7. Lead inhibits activities of enzymes of the reductive pentose phosphate pathway (**Hampp et al., 1973**). The activity of ribulose-bis-phosphate carboxylase/oxygenase was inhibited in spinach (***Spinacia oleracea***) grown in a nutrient solution containing 1555 ug Pb L^{-1} as nitrate (**Vallee and Ulmer, 1972**)..Spirulina increases plant growth and enables plants to withstand adverse effect (**Liu-ShiMing and Liang-ShiZhong ,1998**).. It was reported that Increased contents of Pb in plants grown on Pb-contaminated soils occurred in presence of chelating substances in soil (Huang et al 1997). Spirolina increases plant growth, protein and carbohydrates (Jagannath et al. 2002)

Contents of N, P and K in plant leaves:

N contents

Contents of N in leaves were affected negatively by Pb contamination and positively by spirulina application (Table 5). The lowest of 20.1 mgg^{-1} dw occurred upon application of Pb where no spirulina was given ($L_1P_0F_0$) and the highest was given by non Pb-treatment receiving the high dose of both priming and spraying of spirulina ($L_oP_2F_2$) which surpassed the lowest by 200% indicating a marked alleviation of the suppression caused by Pb.

The main effect of Pb was a decrease of 27.9 %. The decrease was particularly high (38.7%) where no spirulina was applied. In presence of spirulina at its combined high dose of spray+priming , the decrease was less (36.4%) .The alleviating effect of spraying spirulina surpassed the priming effect particularly where the high dose was used. Average increases due to priming were 12.1 and 35.1 % at the low and high doses respectively compared with 31.0 and 48.7% respectively using the spray method.

P contents

Results resembled those of the N contents. Plants growing on Pb contaminated soils were negatively affected by Pb and contained lower contents of P (Table 5). On the other hand spirulina affected plants positively .The lowest P of 11.0 **m**gg^{-1} dw was given upon application of Pb with no spirulina applied ($L_1P_0F_0$) and the highest was given by the non Pb-treatment receiving the high combined dose of primed and sprayed spirulina ($L_oP_2F_2$) which surpassed the lowest by 331% indicating a marked alleviation of the suppression caused by Pb. The main effect of Pb was a decrease of 21.8 %. The decrease was particularly high, being 44.4% where no spirulina was applied. In presence of spirulina at its combined high dose of spray+priming , the negative effect was less (24.6%) .The alleviating effect of spraying spirulina surpassed that of the priming particularly where the high dose was used. Priming caused increases averaging 18.8 and 41.6 % at the low and high doses respectively compared with 28.1 and 65.6% respectively using the spray method.

Table 5. NPK (mgg^{-1} dw of rocket leaves grown on Pbl contaminated soil as affected with *Spirulina platensis* treatment

Soil Pb Poll. (L)	Seed prime (P)	Foliar spray (F)											
		F_0	F_1	F_2	Mean	F_0	F_1	F_2	Mean	F_0	F_1	F_2	Mean
		N				P				K			
L_0	P_0	32.8	38.4	46.3	39.1	19.8	28.2	37.9	28.7	10.8	15.0	21.6	15.8
	P_1	34.8	41.8	55.1	43.9	24.9	29.8	41.8	32.1	13.9	17.6	25.1	18.9
	P_2	41.7	58.7	60.4	53.6	32.7	35.2	47.4	38.4	20.7	19.8	28.2	22.9
Mean		36.4	46.3	54.0	45.6	25.8	31.0	42.3	33.0	15.1	17.5	25.0	19.2
L_1	P_0	20.1	28.9	36.9	28.7	11.0	22.8	27.1	20.3	4.9	11.2	17.3	11.1
	P_1	25.2	33.8	37.2	32.1	21.8	25.6	30.6	26.0	8.8	13.2	19.8	13.9
	P_2	31.3	41.1	41.7	38.0	24.6	30.2	37.8	30.9	14.2	16.2	23.0	17.8
Mean		25.6	34.8	38.4	32.9	19.1	26.2	31.9	25.8	9.3	13.6	20.0	14.3
G. Mean		31.0	40.6	46.1	39.2	22.4	28.7	37.1	29.4	12.2	15.5	22.5	16.7
		Means of F											
P_0		26.4	33.7	41.6	33.9	15.4	25.6	32.5	24.5	7.8	13.1	19.4	13.5
P_1		30.0	37.8	46.1	38.0	23.3	27.7	36.2	29.1	11.3	15.4	22.4	16.4
P_2		36.5	50.2	50.8	45.8	28.6	32.7	42.6	34.7	17.4	18.0	25.7	20.3
		L: 0.0001 P: 0.001 F:0.001 LP :0.002 LF: 0.001 PF: 0.004 LFP: 0.0001				L: 0.00 9 P: 0.0007 F:0.0001 LP :0.03 LF: 0.0001 PF:0.001 LFP: 0.0001				L: 0.001 P: 0.001 F:0.0001 LP :0.02 LF: 0.001 PF: 0.001 LFP: 0.001			

See notes of Table 2 for designation of treatments.

K contents

The pattern of response to contents of K in plan was rather similar to those of the contents of N and P. Plants growing on Pb contaminated soils contained lower K than those non-contaminated (Table 5) and spirulina showed positive effect alleviating the retarding effect of Pb .The lowest K of 4.9 **m**gg^{-1} dw was given upon application of Pb with no spirulina applied ($L_1P_0F_0$) and the highest was given by the non Pb-treatment receiving the high combined dose of primed and sprayed spirulina ($L_oP_2F_2$) which surpassed the lowest by 476% indicating an extremely high alleviation of the suppression caused by Pb. The main effect of Pb was an average decrease of 37.6 % , and the decrease was particularly high 45.6% where no spirulina was applied. In presence of spirulina at its combined high dose of spray+priming , the negative effect of Pb was less (18.4%) .The alleviating effect of spraying spirulina surpassed that of the priming particularly where the high dose was used. Priming caused increases averaging 21.5 and 50.4 % at the low and high doses respectively compared with 27.0 and 84.4% respectively using the spray method.

Assessment of NPK contents in plant as affected by Pb and spirulina:

The retarding effect of Pb contamination was inflicted on plants and their contents of plant (**Sharma and Dubey 2005; Han et al., 2008; Liu et al. 2008; Zhou et al., 2010**). Cell damage is caused by Pb through many mechanisms including generating reactive oxygen species 'ROS' (Fargasova, 1994, Dixit *et al.,* 2001, Erdei *et al.,* 2002 and Islam *et al.,* 2011). Was shown to cause imbalances in the uptake of plant nutrients of K, Ca, Mg, Mn, Zn, Cu, Fe within the tissues through blocking their absorption access via plant roots (Pallavi and Rama, 2005).

On the other hand the positive effect of *Spirulina platensis* is most certainly due to its being a biofertilizer containing plant nutrients besides its ameliorating properties (Misra and Kasushik, 1989, Singh and Trehan, 1973 and venkataraman and Neelakantan, 1969).. A number of workers observed an increase in rice seed germination, root and shoot growth, weight of rice grains and their N content with use of cyanobacteria (Misra and Kasushik, 1989, Singh and Trehan, 1973 and venkataraman and Neelakantan, 1969). Analysis of Spirulina dry biomass revealed that it contains nitrogen, phosphorus and potassium, respectively (Aly and Esawy, 2008).

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الأثر التخفيفي للسبيرولينا Spirulina Platensis على نبات الجرجير النامي في تربة ملوثة بالرصاص

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[1] قسم الأراضي والمياه كلية الزراعة بمشتهر جامعة بنها .. [2] قسم الأراضي والمياه مركز البحوث النووية هيئة الطاقة الذرية

الرصاص هو عنصر سام يشكل تلوثا للبيئة في اشكال مختلفة. أقيمت التجربة الاصصية (الاصيص سعة 10 كجم تربة طينية) علي نبات الجرجير المزروع في تربة ملوثة بالرصاص لتقييم الأثر المخفف الذي تسببه االسبيرولينا للنبات الذي ينمو في تربة ملوثة بالرصاص . التصميم الاحصائي هو قطاعات عشوائية كاملة ..عاملية 3 عوامل هي 1- التلوث : معاملتان هما تربةغير ملوثة وتربة ملوثة ..2- نقع البذور 3 معاملات هي نقع في ماء و نقع في محلول سبيرولينا 40 مجم/لتر و محلول 80 مجم/لتر..3- رش علي النبات 3 معاملات هب رش بالماء ورش بمحلول 40 مجم سبيرولينا /لتر و رش بمحلول 80 مجم/لتر. حدث تخفيف للتأثير السام للرصاص نتيجة المعاملات بالسبيرولينا كما حدثت زيادة في نمو النبات وكذا محتوياته من الكلوروفيل و النشاطات الانزيمية نتيجة المعاملة بالسبيرولينا.