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Effectiveness of Algae on Growth of Parsley (*Petroselinum crispum)* and Spinach *(Spinacia oleracea)* Plants under the Stress of some Heavy Metals

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ABSTRACT

Soil contamination with heavy metals has become an environmental crisis due to their long-term stability and adverse biological effects. Therefore, bioremediation is an eco-friendly technology to remediate contaminated soil. This study was conducted in two pot experiments at the greenhouse of the National Research Center. Experiments were carried out to study the influence of algal extract on growth of spinach and parsley plants under different levels of polluted soil. The algal species used in this study were *Chlorella vulgaris* and *Anabaena sphaerica* which used in a mixture with concentration (2%). Increasing the level of pollution decreased growth of spinach and parsley plants in presence of lead or cadmium. Adding the mixed algal extract reduces the impact of pollution on the growth. In general addition of heavy metal decreased all macronutrients (N, P and K) compared with control treatment either in presence of algae or not. The uptake of nitrogen, phosphorous and potassium by spinach and parsley plants increases when treated with algae. Application of algae reduced the concentration of Pb or Cd at the same treatments for spinach and parsley plants. Data also, show a great variation in value of Cu content in spinach or parsley as it increases with increasing levels of soil pollution under all treatments.
KEY WORDS: heavy metal, microorganisms, bioremediation, algae,polluted soil, parsley, spinach.

INTRODUCTION

Environmental pollution with heavy metals is a dangerous crisis, and the different uses of these elements have enhanced in the world due to industrial progress **(Dhal et al., 2013).** These pollutants are toxic and pose a severe threat to human and environmental health due to their accumulation in the food chain **(Song et al., 2017, Tao et al., 2017).**

Heavy metal toxicity to various environmental niches is a great concern for environmentalists. Because these metals are difficult to be eliminated from the environment and, unlike many other pollutants, cannot be degraded chemically or biologically and are eventually indestructible. Hence, their toxic effects last longer **(Ahemad, 2012).**

Increasing the use of chemical fertilizer led to high costs in vegetable production. It affected soil fertility and a series of negative environmental consequences **(Tilman et al., 2001)** because some fertilizers contain heavy metals (e.g. cadmium, and chromium) and high concentrations of radionuclides. As the global food demand is expected to double by 2050 **(Singh, 2016),** it has become essential to find alternative untraditional methods to increase crop production. Several methods have been utilized for remediation of metal contaminated soils **(Liu et al. 2018).** The remediation of metal-contaminated soils consequently becomes imperative because such soils generally cover large areas that are rendered inappropriate for sustainable agriculture. One of them is the bioremediation method. The bioremediation methods are gaining increasing prominence because their cost-effective, environment friendly **(Shah &Daverey 2020),** simple to implement **(Liu et al. 2018),** without disturbing the soil fertility and biodiversity **(Ahmad et al. 2016, Xiao et al. 2019).**

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Phytoremediation has emerged as the most desirable technology which uses plants for removal of environmental pollutants or detoxification to make them harmless **(Cunningham and Berti, 1993).** Many living organisms can accumulate certain toxicants to body concentrations much higher than present in their environments **(Nyangababo et al, 2005; Igwe et al, 2008 and Kord et al, 2010).** Thus, the use of plants for the decontamination of heavy metals has attracted growing attention because of several problems associated with pollutant removal using conventional methods.

Recently, the strong interest in crop production has been focused on using microorganisms, including cyanobacteria and green algae, as eco-friendly biofertilizers, which can be an alternative to chemical fertilization and offer economic and ecological benefits to farmers. Microalgae only need sunlight, simple nutrients, including nitrogen, sulfur, phosphorous, and carbon dioxide (Pignolet et al., 2013), and complete an entire growth cycle within hours. Their inexpensive growth requirements as well as their advantage of being utilized simultaneously for multiple technologies (e.g., biofuel production, antimicrobial compounds **(Abd El-Aty et al., 2014)** and bioremediation of heavy metals **(Abd El-Aty et al., 2013**) and organic contaminants **(Abd El-Aty et al, 2015)** have made microalgae more popular for various biotechnological applications **(Suresh Kumar et al., 2015).**

Previous studies proved that cyanobacteria could be developed as a bioremediating agent for plant growth promotion and salt-affected soil remediation through the most effective mechanisms, such as nitrogen fixation, production of extracellular polysaccharides, and growing the organic carbon contents **(Li et al., 2019).** Therefore, cyanobacteria inoculation can be considered a novel technique for remediation of contaminated soil **(Biglari Quchan Atigh et al., 2020).**

The genus *Chlorella* includes single-celled, spherical green microalgae of about 2–10 μm in diameter. *Chlorella* is currently the most cultivated microalga worldwide, mainly due to its rapid growth rate, high photosynthetic efficiency and high nutritional value **(Masojídek and Torzillo, 2008).** *Chlorella* cells can contain up to 70% of protein (in dry weight), making the biomass valuable to the food industry **(Liu & Hu, 2013).** *Chlorella vulgaris* is one of the most commonly reported Chlorella species for heavy metal removal. Algal extract foliar application was recommended for increasing the growth parameters of tomato **(Nour et al., 2010),** green gram **(Pramanick et al., 2013),** garlic plants **(Shalaby and El-Ramady, 2014),** Parsely **(Kandil et al., 2020)** and Spinach **(Mahmood et al., 2019).**

Petroselinum crispum (Mill), popularly known as parsley, belongs to the family Apiaceae **(Borges *et al.,* 2016).** It is native to Europe and the Mediterranean region **(Sayilikan *et al.,* 2011).** Parsely is an evergreen biennial or shortlived perennial herb **(Midrad, 2011).** It has strong aromatic compound leaves inflorescences in the shape of terminal umbels over the leaves, with small yellow-greenish flowers **(Borges et al., 2016).** Parsley is widely cultivated commercially for its strong aromatic edible leaves, fleshy roots **(Kmiecik and Lisiewska, 1999)** and essential oils **(Mylavarapu and Zinati, 2009).** The vitamin C rich leaves are used fresh, dried or frozen as a garnish or spice to add flavour to food **(Mirdad, 2011).**

Parsley is a good source of carotene (pro-vitamin A), vitamins B1, B2 and C, and iron and other minerals **(Osman and Abd El-Wahab, 2009).** The plant has many medicinal uses that include antispasmodic carminative, diuretic; since it contains essential oil of 0.3% in leaf and 2-7% in the fruit **(Midrad, 2011).** The oil contains pinene, myrcene, phellandrene, cymene, methatriene, elemene, myristicin and apiole **(Petropoulos et al., 2008).** It is used in the food industry or as a fragrance in manufacturing perfumes **(Diaz-Maroto et al., 2002).**

Spinach *(Spinacia oleracea* L.*)* is one of the most important and commonly consumed leafy vegetables. It is commercially known as spinach, which possesses therapeutic properties and is a rich source of flavonoids and phenolic compounds besides its economical and ease of availability **(Metha and Belemkar, 2014).** Spinach has low calorific value with an ample supply of vitamins, micro-and macronutrients, and other phytochemicals, including polyphenols and fibre **(Llorach et al., 2008).**

This study was conducted to assess the ability of of mixed extract of two different algal speceies namely *Anabaena sphaerica* and *Chlorella vulgaris* as biofertilizers to improve the growth and chemical composition of parsely and spinach cultivated in contaminated soil with some heavy metals.

MATERIALS AND METHODS

Soil description and characterization

The soil was collected from the Agricultural Experimental Station of National Research Centre, Nobaria sector, Behara governorate, Egypt. This soil was sandy in texture and represented the deserted light-textured soils of Egypt. The sample was gathered from 0 to 30 cm of soil depth, air-dried at room temperature, ground, sieved (2 mm mesh). The physic-chemical characteristics of the studied soil were determined according to **Klute, (1986),** contents of organic matter and $CaCO_3$ as well as EC and pH were evaluated according to **Black *et al.,* (1982).** Total N and available P, K, total Cu, Pb, Cd and available Cu, Pb and Cd were also determined according to **Jackson (1973)** and summarized in Table (1).

Table (1): Some physical and chemical properties of El-Nobaria soils.

% Sand	% Silt	% Clay	Soil texture	pH 1:2.5	E.C dS/m	% $CaCO_3$	% O.M	%			Available heavy metals (ppm)		
								N	P	K	Pb	Cd	Cu
93.47	5.5	1.03	Sand	7.68	0.62	0.7	0.3	0.016	0.013	0.011	0.44	0.009	0.32

Soil pre-treatment

The sampled soils were subdivided, each soil sample weighed 5kg was put into a plastic pots. A Lead (Pb) and cadmium (Cd) solution was added separately into the plastic pots to simulate soil contamination. The concentration of Pb and Cd in soil was (0, 200 and 300 ppm).

Algal strains and growth condition

The two microalgal species used in this study were *Anabaena sphaerica*, N_2-fixing *Cyanobacterial* species and *Chlorella vulgaris*, green alga isolated from the phytoplankton community of the River Nile, purified and recultivated in a fresh algal nutrient medium BG11 **(Carmichael, 1986).** *Chlorella vulgaris* isolated in 100 % $NaNO_3$ (1.5 g/l), where $NaNO_3$ was entirely excluded from *Anabaena sphaerica* media **(Abd El- Aty, et al., 2015).** The cultures were incubated under continuous white fluorescent illumination (33.3 E/m2/s) and temperature of 24 ± 2°C for *Chlorella vulgaris* and 30 ± 2°C for *Anabaena sphaerica* growth. Cultures were shaken once daily to prevent clumping of algal cells. Cultivation was carried out in sterilized conical flasks. The cultivation time differed from one strain to another depending on the optimum growth rate till reaching the stationary phase, which always ranged between (10-15) days.

Preparation of algal biomass and extract

At maximum growth of each species, the algal species were harvested by centrifugation at 3000 rpm for 15 min. Then, after removing clear liquid, the algae pellet was washed several times by distilled water till the effluents became almost transparent. The washed biomass was then dried in an oven at 40°C until a constant weight was reached. The dried biomass was then ground into fractions. The algae were stored in an airtight container in a dry place. The preparation of algal extracts and the chemical composition of algal strain were previously described by Mahmoud et al.*,* 2019.

Seedling experiments

Pots experiment were carried out in the National Research Centre (NRC) greenhouse, Dokki, Giza, Egypt. Mixed algal extract concentration (2%) was used. Ten seeds of the two studied plants, namely parsley *(Petroselinum crispum)* and spinach (*Spinacia oleracea*), were planted in 5kg capacity of air-dried soil plastic pots by pressing them into contaminated soil to a depth of 0.5 cm and the following treatments were applied: 1- Control, 2- 200 ppm Cd, 3- 300 ppm Cd, 4- 200 ppm Pb, 5- 300 ppm Pb, 6- Control with algae, 7- 200 ppm Cd with algae, 8- 300 ppm Cd with algae, 9- 200 ppm Pb with algae and 10- 300 ppm Pb with algae. The pots were watered daily to 70% of the water holding capacity, thinned out to 5 seedlings per pot after 10 days, and then treated with algae and allowed to grow for 75 days. The necessary mineral fertilizers were applied (20 kg fed^{-1} ammonium sulphate, 100 kg fed^{-1} superphosphate and 50 kg fed^{-1} potassium sulphate) to each pot. At harvesting, plants were carefully removed, washed with tap water (to remove any attached particles), rinsed twice with distilled water. Fresh and dry weights were recorded. Nitrogen, phosphorus, potassium and trace elements (Cd ,Pband Cu) in the plant were analyzed. All the analyses were determined using the standard methods of **Jackson (1973), Lindsay and Norvell (1978).**

Statistical analysis of data
All the analytic determinations were carried out in triplicates. Statistical analyses were performed as described by **Sendecor and Cochran (1982)** and the treatments were compared by using the L.S.D. test at 0.05 level of probability.

RESULTS AND DISCUSSIONS

algae play an important role in improving growth of many plants when applied as biofertilizers. This evidence was clearly appeared in growth criteria of spinach plant which grown in soil polluted by Pb and Cd in presence or absence of algal addation are presented in Table (2) and Fig (1).

The growth of spinach plants which grown in polluted soil in terms of fresh (FW) and dry weight (DW) was increased by algal application when compared with the same treatments in absence of algal addition. Exposing of spinach plants to various concentrations of lead or cadmium resulted in a significant reduction of fresh and dry biomass. In general, increasing the level of pollution with lead or cadmium led to decreases in growth while adding mixed algal extract reduce the impact of this pollution on the growth of spinach. This results was confirmed by **Mahmoud et al.(2019)**.

Table (2): Growth criteria of spinach plants cultivated in soil polluted with cadmium and lead in absence and presence of algae

Treatments	rate	FW (g)	DW (g)	N (%)	P (%)	K (%)
Without Algae						
Control	0	52.7	12.8	2.04	0.10	2.1
Cd	200	72.5	16.6	2.41	0.14	2.5
	300	45.4	13.1	2.56	0.12	3.0
Pb	200	81.6	18.5	3.55	0.16	3.2
	300	47.8	13.7	1.44	0.11	3.0
With Algae						
Control	0	60.7	14.0	1.70	0.05	2.5
Cd	200	116.2	18.7	2.18	0.17	3.4
	300	99.0	17.7	2.80	0.16	4.5
Pb	200	70.5	16.9	2.80	0.18	2.8
	300	64.0	16.2	1.86	0.15	3.7
LSD at 0.05%		1.11	0.24	0.06	0.005	0.02

The obvious impact of alga concentrate might be credited with its impact in expanding cell layer penetrability and advancing plant productivity in the retention of supplements . In addition, green growth concentrate may assume a part through its substance of cytokinins in postponing the maturing of leaves by decreasing the debasement of chlorophyll, (Enan et.al 2016) .The highest fresh and dry weight of spinach plant was recorded at 200 ppm Cd after adding the of mixter algae (116.2, 18.7) for fresh and dry weight respectively. Treatment with mixed algal extract led to an increase in spinach growth by (60.28%, 25.5%) for fresh and dry weight compared with control, respectively, when treated with 200 ppm cadmium. Blue green algal extract excretes a great number of substances that influence plant growth and development **(Ordog, 2004).** may be due to algae extract contains cytokines which induce the physiological activities and increase total chlorophyll in plants which, reflects on the activity of photosynthesis and the synthesized materials which will positively reflects on the growth characteristics (Ghalab and Salem, 2001) and (Enan et al.,2016).

These results are in line with **(Chekroun and Baghour, 2013)** who found that the accumulation of heavy metals by micro and macroalgae provides an advantage for phytoremediation over other methods which are more costly and not eco-friendly.

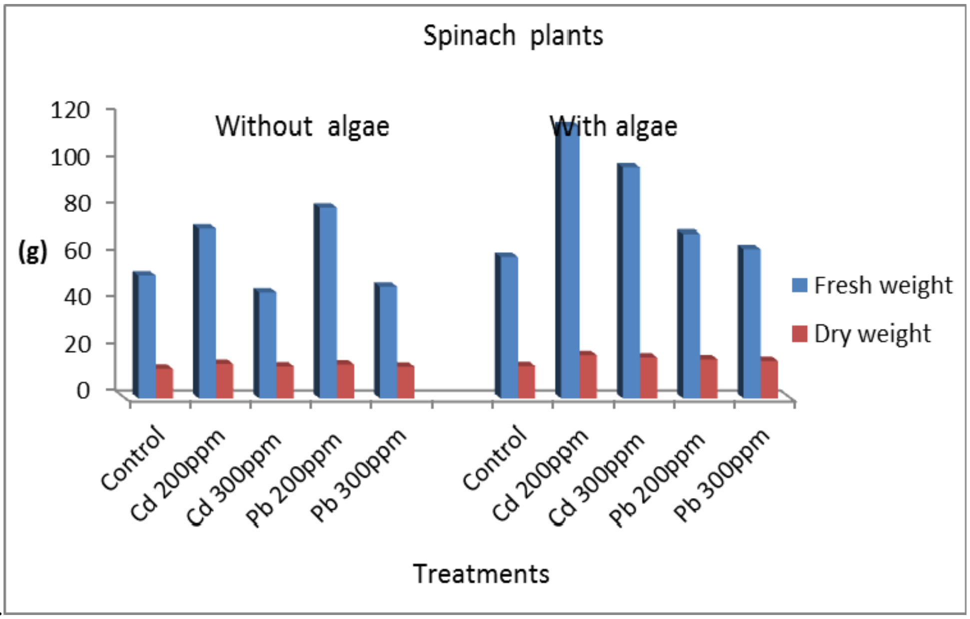

Fig. (1): Effect of Cadmium and lead in absence and presence of algae on fresh (FW) and dry weight (DW) of spinach plants.

The concentration of N and K in spinach plants significantly increased in presence of different concentrations of heavy metals either cadmium or lead when compared with control except at the concentration of 300 ppm Pb. Also, the phosphorous was increased when the concentration of 200 ppm of Cd or Pb was added ,however as the concentration of heavy metal was increase to 300ppm, the phosphorous content of the spinach plant was decreased. This results have the same trend in absence and presence of algae (Table 2 and Fig 2).

These results were in accordance with those obtained by **Ordog (2004)** who documented that the suspension of cyanobacterial and microalgal extract contains a special set of biologically active compounds including plant growth regulators, which can be used for treatment to decrease senescence, transpiration as well as to increase leaf, chlorophyll, protein content and root development, also**, Ghallab and Salem (2001)** found that the two biofertilizers increased nutrients content in wheat plant. A Similar trend was observed by **Adam (1999)** who showed an improvement in the nitrogen contents of the seed and related processes of wheat, sorghum, maize and lentil under application of cyanobacteria as biofertilizers.

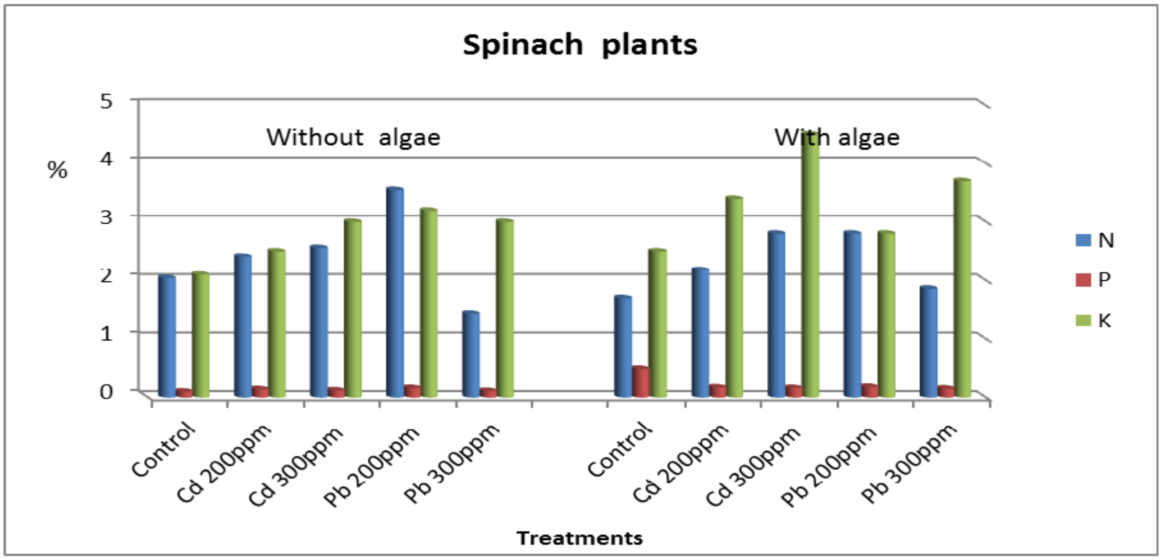

Fig. (2): Effect of Cadmium and lead in absence and presence of algae on nitrogen (N), phosphrous (P) and potassium (K) contentes of spinach plants.

Data recorded in Table (3) and Fig (3) show the effect of different levels of heavy metals namely Cd and Pb in absence and presense of algae on the growth criteria of parsley plants which represented by fresh weigt,dry weight ,N, P and K . It is noticed a significant decrease in the fresh and dry weight with an increase in the concentration levels of heavy metals in absence of algae. Meanwile there is an increase in fresh and dry weight after treatments with algal extract under all levels of heavy metals treatments. These results in harmony with those obtained by **Kandil et al., (2020)** who reported that the application of algae caused positive effect on parsley fresh and dry weight.

Table (3): Growth criteria of parsley plants cultivated in soil polluted with cadmium and lead in absence and presence of algae

Treatments	rate	FW (g)	DW (g)	N (%)	P (%)	K (%)
Without Algae						
Control	0	26.0	14.8	0.70	0.19	1.65
Cd	200	23.2	12.3	0.68	0.32	1.58
	300	24.7	12.4	0.44	0.25	1.48
Pb	200	22.0	12.2	0.52	0.28	1.64
	300	18.0	14.5	0.45	0.22	1.10
With Algae						
Control	0	30.5	15.5	0.70	0.30	1.50
Cd	200	42.2	23.1	0.69	0.40	1.11
	300	30.8	17.5	0.48	0.34	1.60
Pb	200	37.6	21.0	0.66	0.42	1.42
	300	35.9	17.5	0.46	0.38	1.60
LSD at 0.05%		0.96	0.31	0.004	0.002	0.05

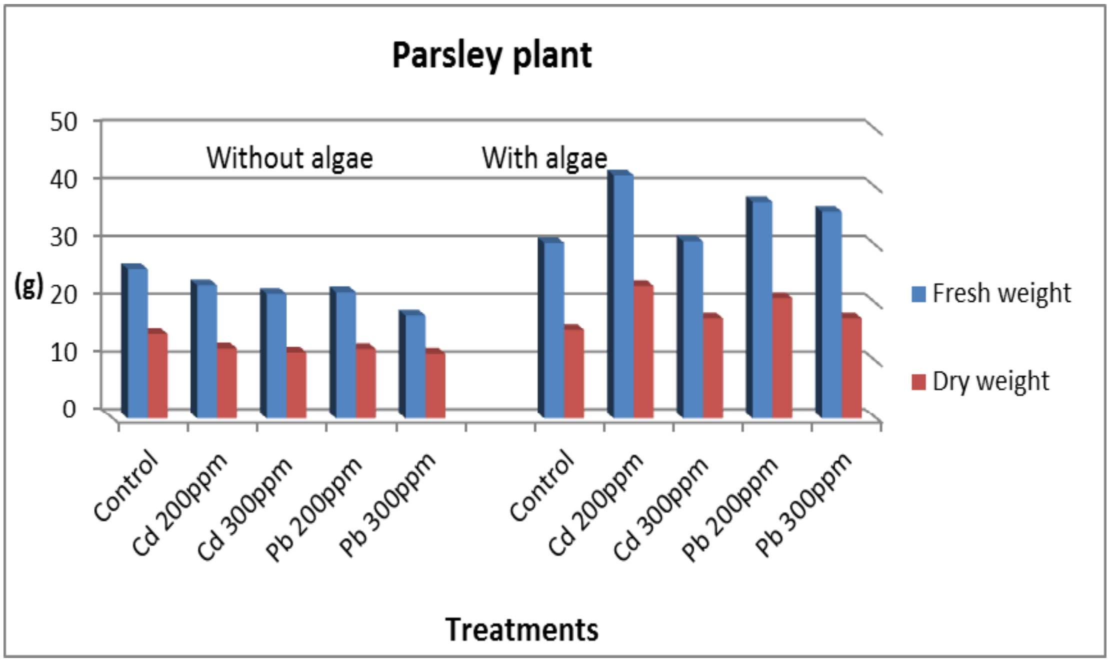

Fig.(3): Effect of Cadmium and lead in absence and presence of algae on fresh (FW) and dry weight (DW) of parsley plants.

The highest fresh and dry weight was obtained at 200 ppm Cd in presence of algae (42.2, 23.1 g) for fresh and dry weight of parsley respectively. This result is comfied with **Kublnovskaya et al., (2019),** who stated that using *Anabaena sphaerica* as the foliar application biofertilizer gave highest fresh and dry weight.

Data presented in Table (3) and Fig (3) cleary show that the application of different concentrations of heavy metal decreased all macronutrients (N, P and K) as compared with control either in presense or absence of algal application. Despite that, the macronutrient uptake by plants increase as a result of the increase in the dry weight of parsley plants.

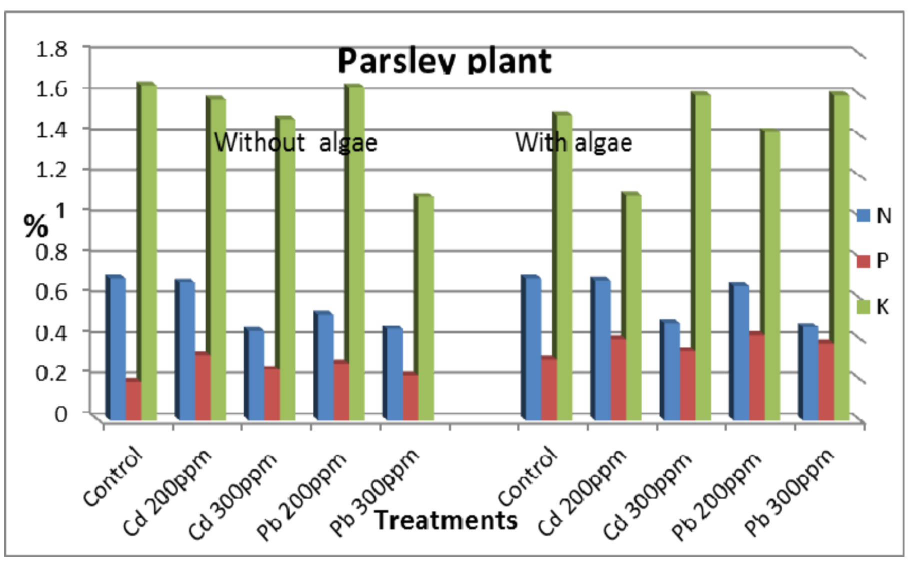

Fig.(4): Effect of Cadmium and lead in absence and presence of algae on nitrogen (N), phosphrous (P) and potassium (K) contentes of parsley plants

These results are in harmony with those obtained by **Brahmbhatt and Kalasariya (2015)** who reported that addition of algal extracts can enhance plant growth of radical. **Ghallab and Salem (2011)** found that the two biofertilizers increased nutrients content in wheat plants. Also, **Mahmoud et al. (2019)** reported that *Anabaena sphaerica* strain gave better results for spinach growth and macronutrients.

Data presented in Table (4) and Figs (5 and 6) showed that the effect of bioremediation with algae on spinach and parsley plants grown in soil polluted with different levels of heavy metals (Cd and Pb). The concentration of Cd in spinach and parsley plants which grown in diferent levels (Cd and Pb) significantly increased by Cd addition either in presence or absence of algae . The highest values of Cd concentrations in spinach and parsley plants were recorded (6.8 and 21.5 ppm) at 300 ppm Cd in absence of algal treatments for spinach and parsley respectively. However, the application of mixed algal extract reduced polluted Cd to (5.3 and 13.4 ppm) at the same treatments for spinach and parsley plants recorded.

Table (4): Effect of Cadmium and lead in absence and presence of algae on on heavy metals contentes (Cd, Pb and Cu) of spinach and parsley plants

Treatments	rate	spinach plants			parsley plants		
		Cd (ppm)	Pb (ppm)	Cu (ppm)	Cd (ppm)	Pb (ppm)	Cu (ppm)
Without Algae							
Control	0	3.0	5.50	8.9	3.5	5.6	14.4
Cd	200	5.5	5.20	8.4	15.5	5.8	15.1
	300	6.8	5.20	9.0	21.5	6.1	21.5
pb	200	3.3	15.90	10.9	3.6	15.0	15.4
	300	3.5	19.00	11.7	3.7	17.0	15.9
With Algae							
Control	0	2.5	4.50	9.5	2.5	4.5	15.5
Cd	200	4.4	5.50	8.8	9.0	3.0	13.8
	300	5.3	6.50	9.7	13.4	5.5	17.2
pb	200	1.4	11.30	9.5	1.0	12.0	12.5
	300	1.8	15.50	10.0	1.4	15.4	13.0
LSD at 0.05%		0.03	0.25	0.04	0.04	0.23	0.05

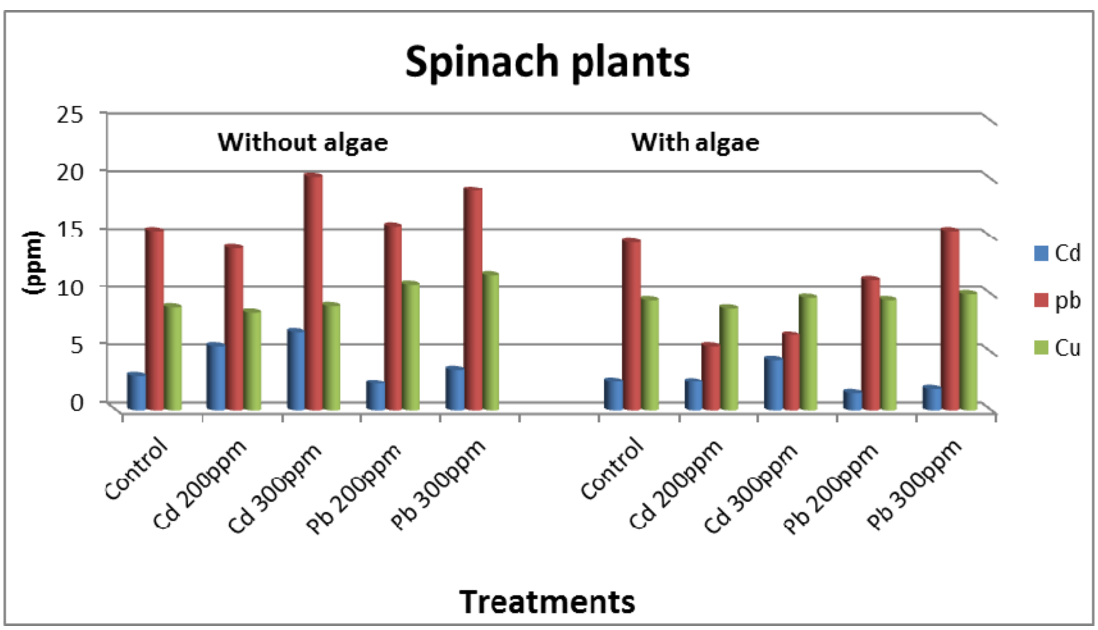

Fig.(5): Effect of Cadmium and lead in absence and presence of algae on heavy metals contentes (Cd, Pb and Cu) of spinach plants.

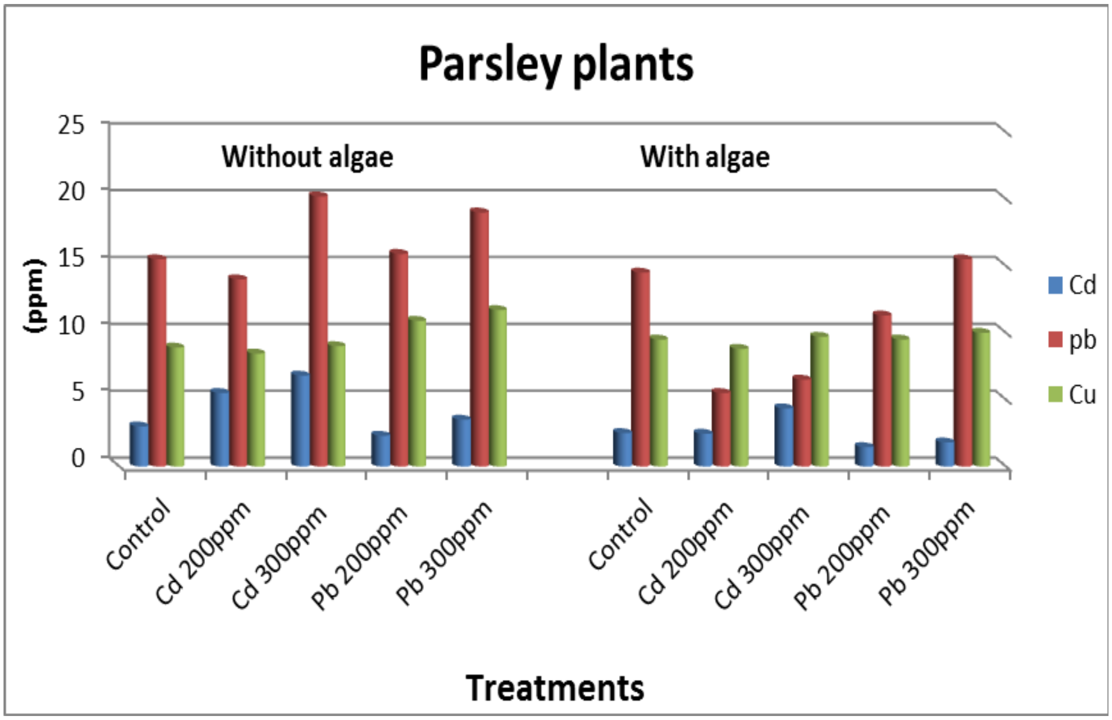

Fig. (6): Effect of Cadmium and lead in absence and presence of algae on heavy metals contentes (Cd, Pb and Cu) of parsley plants.

Lead concentration in spinach and parsley plants grown on different levels of (Cd and Pb) significantly increased by increasing lead concentration either in presence or absence of agae.

The highest values of Pb concentrations in spinach and parsley plants were recorded (19 and 17 ppm) at 300 ppm Cd without algal treatments for spinach and parsley respectively. Also, application of algae reduced polluted Pb to (15.5 and 15.4 ppm) at the same treatments for spinach and parsley plants recorded.

These results agree with **Ali *et al.* (2009) and Mahmoud *et al.*(2019)** they found that application algae reduced of heavy metals concentration in the plant. **Kaoutar and Mourad, (2013)** found that using algae or aquatic plants to remove pollutants from the environment**.** Also, **Mahmoud *et al.* (2019)** and **Kandil *et al.*, (2020)** reported that all biofertilizers treatment reduced heavy metals contents in spinach and parsley plants.

Recently, the use of aquatic plants especially micro and macro algae has received much attention due to their ability to absorption of metals and take up toxic elements from the environment or rendering them less harmful **(Matagi *et al*., 1998).**

Several studies (**Shariatmadari et al., 2011; Koliai et al., 2012; Sokhangoy et al., 2012)** have been reported that biological fertilizers are able to change the basic nutrients lead to better seed germination, plant growth and yield. Application of biofertilizer to Cd2+ - contaminated nutrient medium resulted in an improvement of plasma membrane integrity in which increased the maintenance of *Zea* plants to absorb considerable water (increase of succulent) and macronutrients K, Ca, Mg and P, and hence increased the tolerance index.

Data show a great variation in value of Cu content in spinach or parsley as it increases with increasing levels of soil pollution under all treatments. The Cu contents in spinach decrease with algae treatment compared without algae under the same pollution level, whether with Pb or Cd, while with parsley it does not give the same effect. **Kandil *et al.*, (2020)** revealed that all biofertilizers treatment reduced Cu contents in parsley plants.

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