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Use of Cyanobacteria and Organic Fertilizer Mixture as Soil Bioremediation

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Abstract: Soil pollution with heavy metals is spreading worldwide along with industrial progress. The exocellular polysaccharides of cyanobacteria had been suggested to alleviate the heavy metal toxicity by bio-sorption. This work aimed to study the effect of cyanobacteria suspension, *Nostoc minutum* and *Anabaena spiroides*, either alone or in addition to chemical or organic fertilizer on broad beans cultivated in contaminated soil with heavy metals. The obtained results showed that treatment with mixtures of cyanobacteria and organic fertilizer significantly increased dry weight of broad bean more than full chemical and organic fertilizers doses by 41% and 103%, respectively. All algal treatments caused an increase in nitrogen content in seeds more than full dose of both chemical and organic fertilizers. Mixtures of cyanobacteria and organic fertilizer reduced Pb, Cd and Ni contents in both shoot and seed more than other treatments. Soil pH, electric conductivity, Pb, Cd and Ni were highly significantly decreased by inoculation with cyanobacteria. The study recommends the use of mixture of cyanobacteria and organic fertilizers to improve the contaminated soil.

Key words: Heavy Metals · Bioremediation · Cyanobacteria · *Nosto Cminutum* · *Anabaena Spiroides*.

INTRODUCTION

Environment pollution with heavy metals is getting worse and spreading worldwide along with industrial progress. They are non-biodegradable and tend to accumulate in living organisms throughout the food chains, causing significant problems to both ecosystems and human health [1-3]. The low cost, high removal efficiency and good performance of bio-remediation in low-heavy metal concentrations has been paid a great deal of attention [3-6]. Microalgae and other microorganisms play an important role in the transformation of heavy metal ions in the environment [7,8]. Cyanobacteria are microalgae suggested having some added advantages over other microorganisms because of their larger surface area, great mucilage volume with high adherence resemblance and simple nutrient requirements [9]. Early, [10], it was suggested that cyanobacteria could be used to reclaim alkaline soils because they form a thick stratum on the surface of the soil during the rainy season and the winter months. The exo-cellular polysaccarides of *Nostoc* had been suggested to modify the heavy metal toxicity by biosorption [11, 12]. The algal material incorporated in the soil conserves organic C, organic N and organic P as well as moisture and converts Na^+ clay to Ca^{2+} clay. Organic matter and N added by cyanobacteria bind the soil particles, causing improvement of soil permeability and aeration [10]. Kaushik and Subhashini [13] and Kaushik [14] documented improvement of soil aggregation by lowering the pH and electrical conductivity and by increasing the hydraulic conductivity of saline and alkali soils by cyanobacteria. Moreover, the use of cyanobacteria as biofertilizers can improve plant growth and crop yield since they raise organic matter in soil [15, 16], thus improving soil structure [17-22]. Inoculation of soil with a suspension of *Nostoc entophytum* and *Oscillatoria angustissima* or a combination of the two species significantly increased the germination percentage and enhanced the other detected growth parameters and photosynthetic pigment fractions of pea [23]. The use of living micro algal biomass offers an efficient, simple and cost-effective method. Soil inoculation with cyanobacteria with these attributes may therefore represent a simple and low-cost method for improving the productivity of degraded lands in

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developing countries, where very little or no inorganic fertilizers are usually applied. So far, little research has been undertaken with regard to the possibility of using N_2- fixing cyanobacteria in Egyptian soils. This work aimed to study the effect of cyanobacteria suspension, *Nostoc minutum* and *Anabaena spiroides*, either alone or in addition to chemical or organic fertilizer on broad beans cultivated in contaminated soil with heavy metals.

MATERIALS AND METHODS

The soil samples collected from a contaminated area, Nubaria-Egypt, (N 30° 47', E 29° 46') was wetted with sterilized distilled water in Petri dishes and then incubated at 30° C ±1 in a culture chamber with continuous fluorescent light intensity of 45 µmolphoton $m^{-2}s^{-1}$. A green mat of algae appeared on the surface of dishes after 3-4 weeks and small patches represented colonies of algal growth. A colony of each species was picked up by a platinum wire and examined under the binocular microscope for identification. The identified species were then transferred to Petri dishes containing fresh solid medium and were subcultured several times. For purification of cyanobacterial species, algal growth mats were picked up separately by a platinum wire, suspended in sterilized liquid medium and then centrifuged at 1,000×g for 1 min. The supernatant (contained bacteria) was decanted, whereas the pellet was resuspended in a fresh sterilized liquid medium and centrifuged again under the same conditions. This process was repeated for at least ten times. Finally, cells were spread on a solid medium with inoculating needle. cyanophytes were identified according to Prescott [24, 25]. Cyanobacteria were cultured in 2l Erlenmeyer flasks. Each flask was inoculated with 100 ml of exponentially growing cyanobacterial liquid culture and incubated at 30°C under fluorescent light with light intensity of 45 µmolphoton $m^{-2}s^{-1}$. Cultures were aerated with air mixed with 3% CO_2 to accelerate algal growth. Each cyanobacterium was harvested during exponential growth phase by centrifuging at 2,000×g for 10 min. Cells pellet was rinsed four times and re-suspended in sterilized distilled water to eliminate traces of growth medium [26]. Fresh weights of both identified algal biomass, *Nostoc minutum* and *Anabaena spiroides*, (1, 1.5 and 2 gm-representing 50, 75 and 100%), taken from cyanobacterial culture having optical density (0.95) at wavelength 700 nm, was added to soil (7 kg soil per pot), 15 days before cultivation. Pure identified broad bean (*Vicia faba*) seeds obtained from obtained from Agronomy Department, Agriculture Research Center, Giza, Egypt, were surfaces sterilized by 0.01% $HgCl_2$ for 1 min and washed completely with distilled water. Cultivation took place under normal environmental conditions of light and temperature in pots containing equal amounts. All treatments were replicated five times. Fifteen Broad bean seeds were sown in each pot. Treatments were as follow: T1: 100% chemical fertilizers (The recommended dose of the chemical fertilizers by the Ministry of Agriculture, super phosphate 200 kg/ fadden and ammonium sulfate 200 kg/fadden), T2: 100% Organic compound, T3: 100% algae, T4: 75% algae +25% chemical fertilizers, T5: 75% algae+25% organic compound, T6: 50% algae +50%chemical fertilizers and T7: 50% algae +50% organic compound. Dry weight of shoots (drying in oven at 40°C for three days) were measured. Total N (gram per 100 g) and total P (gram per 100 g) were estimated for seeds and shoots of broad bean as reported by Allen *et al*. [27] and analyzed using GBC.932 (AAS),At the end of the cultivation season, all fruited plants were harvested. Roots, shoots and fruits were separated and stored in paper bags. Some yield parameters as shoot dry weight, number and weight of seeds per legume were detected. Available P, K, Cd, Ni and Pb in soil were extracted using ammonium bicarbonate AB-DTPA according to Soltanpaur, [28] and determined by (ICP-Plasma JY.), except k which was determined using the flame photometer. Soil available nitrogen was extracted using KCl (2M) and determined by using the Kjeldahl method according to Chapman and Pratt [29]. Dried plant materials (shoot and seed of *vicia faba* plants) were digested by using a mixture of concentrated sulphuric-perchloric acids according to the procedure of Chapman and Pratt [29] and determined by the inductively Coupled Plasma (ICP). Total nitrogen was determined by Kjledahel technique and Potassium was determined by the flame photometer [30].

Statistical Analyses: Results are presented as mean of five replicates ± standard error. The results obtained were subjected to one way analysis of variance to determine the significant difference of treatments, at P>0.05 using SPSS software v.15.0.

RESULTS AND DISCUSSION

The most pronounced growth parameters stimulation was recorded by treatment T7, followed by T1. Table (1) showed that treatment with mixtures of cyanobacteria and organic fertilizer (T7) increased dry weight,

Table 1: Effect of different treatments on vegetative growth of broad bean plant

	Vegetative growth			
Treatments	Dry weight/plant(g)	Shoot height/plant(cm)	Seed weight/plant(g)	Seed number/plant
T1	13.2 ±1.39	59.33 ±5.49	2.9 ±0.23	5.0 ±0.58
T2	9.2 ±1.01	47.67 ±4.33	1.7 ±0.07	4.0 ±0.58
T3	9.0 ±0.30	55.67 ±3.84	1.8 ±0.29	4.0 ±0.58
T4	9.5 ±0.31	28.97 ±1.03	1.7 ±0.29	4.7 ±0.67
T5	8.9 ±0.29	72.33 ±1.45	1.9 ±0.22	4.3 ±0.33
T6	7.8 ±0.61	22.17 ±0.83	1.2 ±0.21	3.7 ±0.33
T7	18.7 ±1.44	73.33 ±4.41	2.8 ±0.73	4.3 ±1.45
L.S.D (p=0.05)	1.176	5.627	0.440	1.087

Table 2: Effect of different treatments on some soil chemical properties cultivated by broad bean plant before and after different treatments. Values are means of five replicates ±S.D.

	Chemical Properties of Soil							
Treatments	N (mg/kg)	P (mg/kg)	K (mg/kg)	PH	EC (dS m^{-1})	Pb (mg/kg)	Cd (mg/kg)	Ni (mg/kg)
Before Treatment	304.58±25.30	25.49±0.80	578.40±61.50	8.12±0.10	6.82±0.10	0.884±0.02	0.022±0.01	0.67±0.01
T1	222.85±41.97	17.36±0.73	207.8±21.360	7.6±0.000	5.0±0.116	0.61±0.030	0.019±0.001	0.30±0.023
T2	308.70±12.730	13.33±0.403	222.3±36.320	7.7±0.000	2.9±0.088	0.52±0.001	0.001±0.001	0.138±0.017
T3	305.10±6.370	12.64±0.005	200.7±10.570	7.6±0.058	1.7±0.000	0.51±0.001	0.005±0.006	0.224±0.001
T4	264.60±25.460	18.30±0.179	258.0±0.000	7.57±0.033	2.5±0.176	0.42±0.001	0.008±0.001	0.222±0.001
T5	308.70±4.240	14.87±0.070	244.9±7.560	7.2±0.058	1.5±0.058	0.41±0.001	0.005±0.001	0.23±0.0151
T6	171.50±16.070	17.72±0.4770	231.8±0.003	7.6±0.033	1.7±0.058	0.50±0.004	.007±0.001	0.175±0.016
T7	132.30±25.810	15.53±0.694	208.7±28.460	7.6±0.058	1.3±0.000	0.45±0.014	0.004±0.001	0.191±0.011
L.S.D (p=0.05)	32.705	0.630	25.188	0.063	0.114	0.057	0.001	0.020

highly significantly, more than full chemical, full organic fertilizer and only cyanobacteria by 41%,103% and 107% respectively, also recorded the highest shoot length with an increase by 23.5%, 53.6% and 31.7%, respectively. No significantly difference in seed weight obtained by T7 with the highest value recorded by full chemical fertilizer (T1). The increase in growth parameters of broad bean could be due to the action of one or more of the growth promoting chemicals secreted by cyanobacteria especiall yauxins [31], cytokinins [32] and gibberellins [31]. The increase of dry weights and shoot length can be also due to increase of nutrients added by cyanobacteria and uptake of these nutrients by pea seeds.

Soil NPK content decreased significantly after cultivations in all treatments, this shortfall because of depletion of these elements by broad bean plants (Table 2). All algal treatments caused an increase in nitrogen content in seeds more than full dose of both chemical and organic fertilizers. (T3) recorded the highest nitrogen content in both shoot and seed, while treatment T6 (75% algae + 25% organic compound)caused the highest phosphorous content in both seed, as well as shoot and the highest Kcontent in seed. The increase in the total N content can be a result of nitrogen fixation and nitrate reductase activities of cyanobacteria or to uptake of the amino acids and peptides produced by cyanobacteria [33, 34]. The increases in the P content with the used fertilization treatments can be attributed to the production of a variety of organic acids by the tested cyanobacteria, with decrease in the soil pH leading to conversion of the non-available P into the available P. Halder *et al*.[35] concluded that soil inoculation with cyanobacteria increase soil microorganisms, which can also make P available to plant by producing chelating substances, which lead to solubilization of phosphates. In addition, cyanobacteria may be a reservoir of P with P release due to cell death and decadence [36, 37]. Soil pH and electric conductivity (EC) were highly significantly reduced by inoculation with cyanobacteria (Table 2). The highest reduction (80%) in soil salinity was recorded by T7, while the lowest reduction was showed by T1. Soil salinity decreased due to cyanobacterial exudates, which remove Na^+ from the aqueous medium due to bio sorption, thus the osmotic as well as ionic effect of Na^+, which otherwise have an

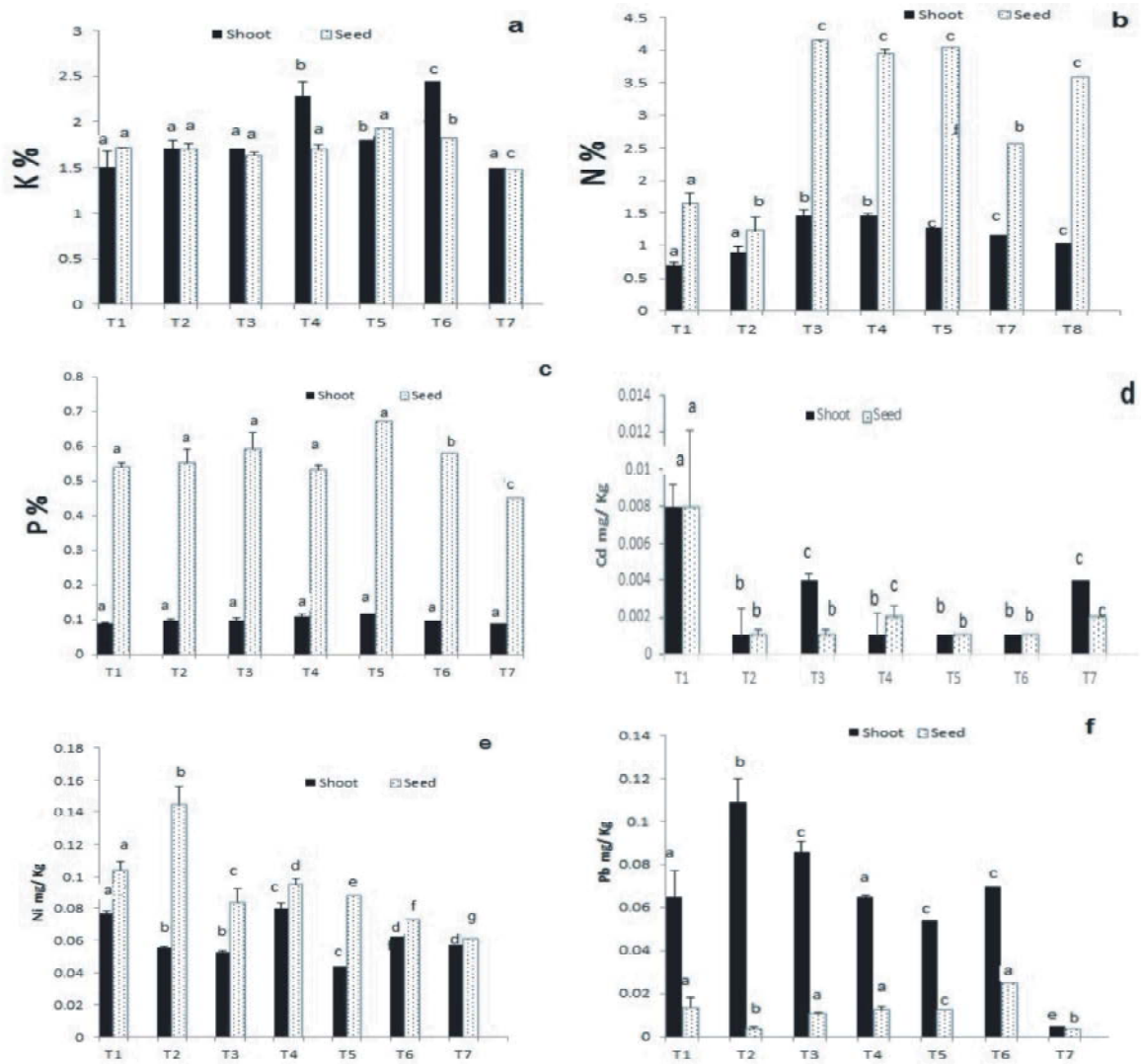

Fig. 1: Effect of different treatments on K (a), N (b), P (c), Cd (e), Ni (e) and Pb (f) contents in shoot and seed of broad bean. Error bars represent one standard deviation of five replicates and superscripts (a, b, c) indicate statistically significant differences in the values ($p<0.05$).

inhibitory effect on growth [38] get a big drop since after betting bound to the cyanobacterial secretion, Na^+ ions are no more available as free ions in the medium. Similar stimulation of crop growth has earlier been reported by Misra and Kaushik [39] and Arora *et al*. [40]. Kaushik and Murti (13) recoded that the application of a mixture of cyanobacteria promoted the leaching of sodium, decreased pH from 9.5 to 7.6 and increased the exchangeable calcium by about 25% in an alkali soil in Northern India where plant growth was poor. Mixtures of cyanobacteria and organic fertilizer reduced Pb, Cd and Ni contents in both shoot and seed more than other treatments (Fig. 1). On the other hand treatment by 100% chemical fertilizer (T1) showed the highest content of these heavy metals in both shoot and seed. It has been proposed that polysaccharides secreted by cyanobacteria, which are especially differentiate by their anionic nature, play an important role in the sequestering or immobilization of metal ions, which are respectively essential or harmful to bacterial life [41]. Pereira *et al*. [42] reported that biofertilization with a mixture of N fixing cyanobacteria (*Nostoc commune*, *Nostoclinckia*, *Nostoc* sp and *Anabaena iyengarii* var. *tenuis*) reduced the use of N fertilizer by 50%, to obtain the same grain yield and quality of rice compared with the full dose of chemical fertilizer.

CONCLUSIONS

The study strongly recommends inoculation of soil contaminated with heavy metals with suspensions of species *Nostoc minutum* and *Anabaena spiroides*, as bio fertilizers, in combination with different doses of organic fertilizers to improve soil characters and increase the growth of broad bean. In conclusion, the two tested species can be used for bioremediation of metal-polluted soils. In order to bring this potential to the applicable stage on a commercial basis, more information on metal detoxification efficiency upon exposure of micro algal biomass to various metal-contaminated effluents is required.

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