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ASSESSMENT OF HEAVY METAL ACCUMULATION AND PERFORMANCE OF SOME PHYSIOLOGICAL PARAMETERS IN *ZEA MAYS* L. AND *VICIA FABA* L. GROWN ON SOIL AMENDED BY SEWAGE SLUDGE RESULTING FROM SEWAGE WATER TREATMENT IN THE STATE OF QATAR

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تقييم تراكم العناصر الثقيلة وأداء بعض العمليات الفسيولوجية في نباتات الذرة الشامية والفول المنماة على تربة مسمدة بالرواسب الصلبة الناتجة من معالجة مياه الصرف في دولة قطر

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أجريت تجربة على نباتات الذرة الشامية والفول المنماة في إصيصات تحت ظروف النمو في البيت الزجاجي وذلك لدراسة مدى تراكم العناصر الثقيلة في هذه النباتات ومدى تأثر بعض العمليات الفسيولوجية بهذا التراكم إن وجد . وقد أظهرت الدراسة أن محتوى العناصر الثقيلة كان أكبر بعدة مرات من نظيره في التربة الزراعية العادية ، كما أن النباتات المنماة على التربة المعاملة ركزت في أنسجتها هذه العناصر الثقيلة بصورة أكبر من تلك التي نميت على التربة الغير معاملة . وقد تأثرت العمليات الفسيولوجية المدروسة بهذا التجميع للعناصر الثقيلة ، فبينما تأثرت في نبات الذرة إيجابيا فقد تأثرت نظيراتها في نبات الفول سلبيا . وقد لوحظت آثار تسمم أو نقص عنصري على نباتات الفول وليس الذرة .

كما أن المحتوى الكلوروفيللي والنشاط الانزيمي لإنزيمي نايتريت ريداكتيز وبيب كربوكسيليز والمحتوى الكلي للبروتينات الذائبة قد انخفض في نبات الفول وارتفع قليلاً في نبات الذرة . كذلك قد اختفت العقد البكتيرية في نباتات الفول المعاملة وقد انعكست هذه الآثار على النمو فبينما ينشط قليلا في الذرة قد انخفض في الفول . وقد نوقشت نتائج هذا البحث من وجهة نظر مدى عملية وفائدة استخدام نواتج معالجة مياه الصرف الصحي في الزراعة بدولة قطر .

Key words : Accumulation, Agriculture, Heavy metal, Phytotoxicity, Sewage sludge.

ABSTRACT

Pot experiment was conducted to explore extent of concerns regarding agricultural use of sewage sludge for crop production in the state of Qatar. Extent of heavy metal accumulation and its subsequent impact on physiological performance in *Zea mays* and *Vicia faba* plants grown on sludge - amended soils was explored. Analysis revealed that all tested heavy metals were several times higher in pure sludge compared to pure garden soil. Plants grown in sludge - mixed soil accumulated large amounts of all the tested heavy metals, except Mn. Tissue content of all metals exhibited continuous increase as the ratio of sludge in soil mixtures increased. Compared to other metals, Zn and Cd were strongly accumulated. Magnitude of accumulation was greater in *Vicia faba* than in *Zea mays*. Growth, in response to sludge treatment, was slightly promoted in *Zea mays* and strongly reduced in *Vicia faba*. Toxicity or deficiency symptoms were noticed only on *Vicia faba*. Nodulation in *Vicia faba* was inhibited by sludge treatment. Chlorophyll synthesis was slightly promoted in *Zea mays* and retarded in *Vicia faba* plants in response to growth on sludge mixed soil. Nitrate reductase activity and content of total soluble proteins in leaves, was generally, promoted in *Zea mays* and reduced in *Vicia faba*. PEP carboxylase activity in *Zea mays* and in *Vicia faba* was reduced. Results, generally, indicate that physiological performance was better in *Zea mays* than in *Vicia faba*. Results are discussed from the point of view of practicality of using sewage sludge in agriculture in the State of Qatar.

INTRODUCTION

Over the past few decades, urban water supplies have improved considerably in developing countries and with this advance has come an increasing problem of waste water disposal.

In the state of Qatar, reuse of water effluent and sludge, resulting fom sewage treatment, in agriculture seems attractive as it offers a solution of other problems beside the problem of disposal, such as the scarcity of conventional water resources and the poor soil physical and chemical properties. Application of the sewage sludge on agricultural land for enhancing crop productivity and imporving soil physical properties has been a common practice for many years [3, 7].

During the waste water treatment process, heavy metals are concentrated in the primary and secondary sludges [19, 20]. Since less than 1% of the total flow of sewage to a treatment works is produced as sludge and this typically contains between 50 and 80% of the total quantity of Cd, Cu and Pb entering the works [13], these and other metals are concentrated in sewage sludge to a significant degree. The heavy metal content of sewage sludge is about 0.5 - 2% on a dry weight basis. In some cases, extremely high concentration (up to 4% w/w) of chromium, copper, lead and zinc have been recorded [22]. With this in mind, if sewage sludge is to be used for agriculture, both public health and agronomic effects must be considered. The potential health hazards associated with land disposal of sludge are metal uptake by plants and the subsequent accumulation of metals in the food chain [22]. Among the agronomic concerns is the effects of these heavy elements of sludge on the yield of crops as a result of the phytotoxicity they may induce in plants [5].

The objective of this study was, therefore, to explore the extent of these two concerns, if sludge is decided to the used on agricultural land for crop production in state of Qatar. In other words, uptake of some heavy metals, growth and response of some metabolic parameters was studied in corn and faba bean plants as a consequence of growth on sewage sludge produced by sewage treatment plant at Abu Hamour, Doha, State of Qatar.

MATERIALS AND METHODS

A pot experiment was conducted, in which corn (*Zea mays* L.) and faba been (*Vicia faba* L.) were grown on graden soil mixed with dry sewage sludge. Dry sewage sludge, resulting from sewage treatment at Abu Hamour treatment plant, was brought to experiment site at the green house of the College of Sciences, University of Qatar, Doha, Qatar. Dry sludge was mixed with garden soil to give three mixtures with sludge representing 0, 50%, and 100% of the mixture.

Seeds of test plants used in this study were purchased from the local market. Ten seeds were germinated on the soil mixtures in plastic pots and only 4 healthy seedlings were chosen to continue growth. For either corn or faba, 3 treatments (0%, 50%, and 100%), and 3 replica for each treatment were used. The soil mixture with ratio 0% served as the control treatment. Each pot contained two kilograms of that soil. During germination and growth, plants were being watered with tap water. Whenever needed, all pots were receiving equal volume of water. Plants were being supplied with a half strength Hoagland nutrient solution once every 4 days. Plants were left to grow under green house conditions (temperature, 25°C ± 2; relative humidity, 65 ± 5%; light intensity~1300 μ Em^{-2} s^{-1}; and 12 hours light period) and after 4 weeks, plants were harvested for analysis.

For heavy metal analysis in plant meterial, one whole plant was harvested from each pot, and fresh weight was determined. Plants were then washed thoroughly with distilled deionized water and oven dried at 80°C for 72 hours and dry weight was determined for each plant. The dried plants were then finely ground and 1 gm powder of this material, was prepared for atomic absorption flame spectrophotometric analysis of Cu, Zn, Pb, Cd and Hg following the method employed by Heckman et al. [10]. Chlorophll content in fresh leaves was estimated essentially according to the method described by Arnon [2]. Total soluble proteins were determined by the Bradford procedure [4]. Leaf nitrate reductase activity was assayed according to Jaworski [12], where leaf segment of 0.1 cm^2 incubated in 5 ml of reaction mixture containing 0.1 M phosphate buffer PH 7.5, 1 M KN03 and 1% (v/v) n-propanol for 60 minutes at room temperature (25°C) in the dark. The reaction was terminated and the color was developed by adding 1% sulphanilamide in 3 N HC1 and 0.02% N-1-naphthyl ethylene diamine dihydrochloride. The enzyme activity is expressed as µmol NO2 formed per gram fresh weight per hour. Leaf PEP carboxylase was assayed following the method previously employed by Foster et al. [8]. Sludge or graden soil analysis for heavy metal was carried out according the analytical methods taken from Standard Methods [1].

RESULTS

Analysis for heavy metal content in graden soil and sludge revealed that all tested heavy metals were several times higher in pure sludge compared to pure garden soil (Table 1). In pure sludge, for example, Cu was 2.7 times, Zn was 3.3 times, Pb was at least 12 times, Cd was at least 5 times, Ni was around 2 times and Mn was around 5.2 times higher than their counterparts in garden soil.

Plants of both *Zea* and *Vicia* grown on sludge-amended soils accumulated large amounts of all the tested heavy metals, except Mn especially in case of *Vicia faba* (Table 1). The rate of sludge application strongly influenced the metal content of the two plants. Plant tissue concentrations of all metals exhibited continuous increase as the ratio of sludge in soil mixtures increased, except for Mn, where its contents were lower in plants grown on sludge-amended soils, especially in *Vicia faba.* Compared to other micro elements, Zn and Cd were strongly accumulated by the two plants. Zn content increased from 30 mg Kg^{-1} to 134 mg Kg^{-1} in *Zea mays* and from 26 mg Kg^{-1} to 169 mg Kg^{-1} in *Vicia faba*, while Cd content rised from 0.05 mg Kg^{-1} to 0.22 mg Kg^{-1} and from 0.04 mg Kg^{-1} to 0.26 mg Kg^{-1} in *Zea mays* and **Vicia faba,** respectively. Generally, the magnitude of accumulation of heavy metals was greater in *Vicia faba* compared to *Zea mays.*

Table 1 . Micro elemental content in pure sludge, pure graden soil, and in *Zea mays* and *Vicia faba* plants grown on sludge-mixed soil .

Sample	Sludge Ratio (%) (in Soil mixture)	Cu	Zn	Pb	Cd	Ni	Mn
		mg Kg^{-1}					
Sludge		187	707	12	5.3	59	350
Garden soil		69	218	ND	ND	33	67
Zea	0 %	6.8	30	ND	0.05	3.2	49
	50 "	7.7	80	"	0.12	4.3	89
	100 "	8.4	134	"	0.22	5.4	44
Vicia	0 "	6.2	26	"	0.04	2.6	52
	50 "	8.1	94	"	0.11	3.8	34
	100 "	7.9	169	"	0.26	6.2	32

Values are the mean of three replicates.

Growth of plants was differently influenced by sewage sludge where it was slightly promoted in *Zea mays* and significantly reduced in *Vicia faba* (Table 2). With regard to external appearance, *Zea mays* plants grown on sludge were not different from those grown on garden soil. No toxicity or deficiency symptoms were observed. On the other hand, *Vicia* plants grown on sludge looked chlorotic and stunted compared to those grown on garden soil. Nodulation in *Vicia* plants, grown on sludge-containing soils was inhibited compared to those grown on garden soil (Table 2). Inhibition in nodulation was dependent on the rate of sludge in soil.

Table 2 . Influence of sludge application to soil on growth of *Zea mays* and on growth and nodulation in *Vicia faba* plants grown on sludge-mixed soil

Plant	Sludge Ratio (%) (in Soil mixture)	Fresh Biomass	Dry Biomass	Nodulation
		Gram / plant		
Zea	0 %	29.6	5.4	--
	50 "	29.1	7.8	--
	100 "	31.7	7.4	--
Vicia	0 "	22.7	4.7	0.17
	50 "	20.3	4.1	0.07
	100 "	17.5	2.8	0.00

Values are the mean of three replicates.

Figure 1 shows the response of chlorophyll contents in *Zea* and *Vicia* plants to growth on sludge. Sludge application to growth soil influenced chlorophyll content in leaves of the test plants and the extent of effect was dependent on the ratio of sludge in soil. Chlorophyll synthesis in *Zea* plants grown on sludge was very slightly promoted or at least unaffected (Fig. 1). In *Vicia* plants, on the other hand, it was slightly retarded.

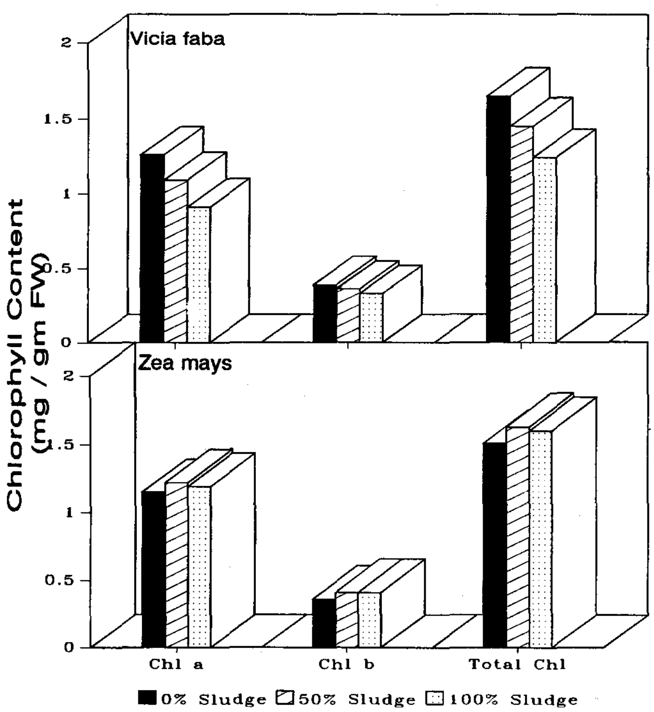

Figure 1 : Chlorophyll content in *Zea mays* and *Vicia faba* in response to growth on sludge-mixed soil.

In response to growth on sludge-containing soil, nitrate reductase activity in leaves, was generally promoted in *Zea mays* and reduced in *Vicia faba* (Fig. 2). In *Zea mays*, the highest activity was recorded for plants grown on soil with 50% sludge and not in case of pure sludge. In *Vicia faba*, in contrary to *Zea*, the depression increased with increase of sludge content in soil.

Phosphoenol pyruvic acid carboxylase (PEPCase) activity in *Zea* mays and *Vicia faba* was reduced by growth on sludge-amended soils or on pure sludge (Fig. 2). Activity reduction was the highest in plants grown on pure sludge soil.

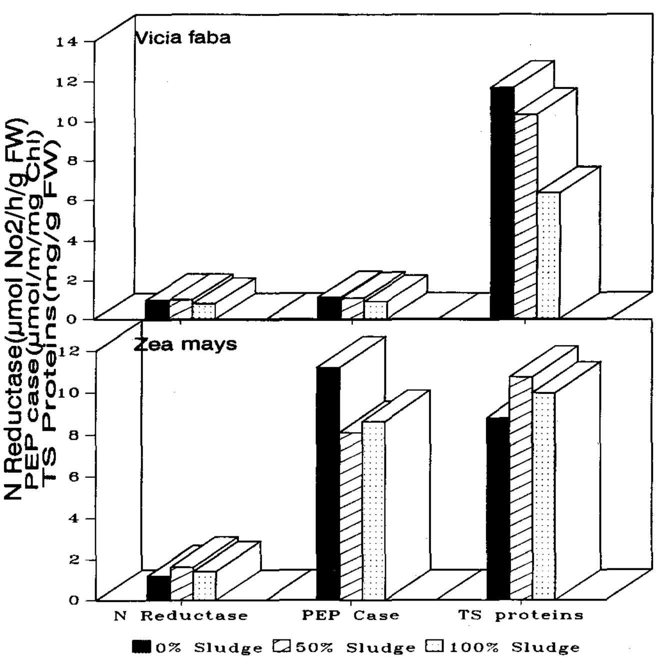

Figure 2 : Response of PEP carboxylase activity, nitrate reductase activity, and total soluble protein contents to growth of *Zea mays* and *Vicia faba* on sludge-mixed soil.

Pattern of response of total soluble proteins in the two test plants exactly followed that of nitrate reductase, where their content increased in *Zea mays* , with the highest content was reached by plants grown on soil with 50% sludge (Fig. 2). Content of total soluble proteins in *Vicia faba* plants, on the other hand, decreased continuously as sludge content increased in soil.

DISUSSION

The present study has shown accumulation of heavy metals in tissues of plants grown on sludge-amended soils. The bioaccumulation ability has been shown to be species dependent. Previous studies have shown the accumulation of metals by different plants and tissues after their growth on sludge-amended soils [10, 15] Based on results from this and previous studies, using sludge in soil amendment seems to result in unavoidable uptake of heavy metals by plants growing on those soils. Other strategies should then be considered to deal with the concern of heavy metal introduction and concentration in the food chain through using sludge in agriculture. One possible strategy is to alleviate rather than to totally prevent the effect. Of the factors that strongly influence metal uptake from soil is soil pH [13]. Heckmen et al. [10] found that low pH increased uptake and that raising pH by liming decreased uptake significantly. Other strategies are:

1) Incorporating sludge on the land which is used for the production of non-food chain crops. Sewage sludge, for example, had been used in forest lands [6].

2) following sludge treatment technologies to remove or reduce heavy metals in sewage sludge.

3) Considering the Long term effects of sludge applications on crops. In this context, it was found by Hinsely et al. [11] that uptake by *Zea mays* dropped strongly strongly after four years of sludge application to soil. Study of the different conditions for optimization is necessary, if it is decided to use sewage sludge in agriculture in state of Qatar.

With respect to phytotoxicity, results of the present study have shown visual signs of phytotoxicity on *Vicia faba* but not on *Zea mays*. Observed phytotoxicity in *Vicia faba* may be due to additive toxic effects from different metals. According to Chaney et al. [5] crops may suffer yield reductions even when metal phytotoxicity is not visually indicated. This may not apply to *Zea mays* plants in this case. Performance of studied physiological parameters in *Zea mays* was generally promoted or at least negatively unaffected contrary to the case in *Vicia faba* under the same soil conditions of sludge treatment. The physiological performance was reflected into enhanced dry matter yield in *Zea mays* and retarded one in *Vicia faba.* Previous stuudies indicated that the application of sewage sludge has produced varied growth responses in different plants with positive growth responses to older crops [23].

It is deceptive to evaluate application of sludge for crop production on the basis of physiological performance in a plant irrespective of extent of ability of this plant to accumulate heavy metals. A plant may be able to accumulate high levels of toxic metals and its physiology is endurant to these levels of metals and ends up with good yield. Under such conditions of sludge use for crop production, plants with least metal accumulation ability and good growth and yield should be the ones to be cultivated.

Heavy metal accumulation, due to growth on sludge-amended soil, caused reduction of chlorophyll synthesis in *Vicia faba.* This may be explained by the heavy metal interference with protochlorophyllide reductase complex responsible for chlorophyll formation [17], by their damaging effect on chloroplast structure [16], or by inhibition of photosynthetic enzyme activity [21]. The metabolic systems in Zea mays seem to be less sensitiive than those in *Vicia faba,* under these conditions.

Nodulation in *Vicia faba* was inhibited by growth on sludge-amended soil. Nodulation reduction in this case may be attributed to the reasones mentioned in similar studies, namely mineralization of sludge N. [9] and reduction in rhizobial population in sludge-amended soil, due to metal toxicity, [18].

In *Zea mays*, nitrate reductase activity and total soluble proteins increased. This is expected, because used sludge was fresh and hence rich in nitrogen. This has not been the case with *Vicia faba.* The reduced activity of nitrate reductase and hence total soluble proteins in *Vicia faba* may be attributed to metal toxicity of that enzyme. Another possibility may be the inhibition through toxification of photosynthetic machinery.

Photosynthetic NAD (P) H can be a limiting factor for nitrate reductase activity . In support of the latter possibility, is the reduction in chlorophyll content and PEP carboxylase activity in response to accumulation of heavy metals.

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