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ORIGINAL PAPER

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Heavy Metal Accumulation and Physiological Consequences in Selected Food Plants Grown on Sewage Sludge Amended Soils

Abstract: Pot experiments were conducted to explore concerns regarding agricultural use of sewage sludge for crop production. The extent of heavy metal accumulation and its subsequent impact on the physiological performance of six food plants grown on sludge mixed soils was explored. The effect of liming of sludge amended soil on the reduction of metal uptake by the raised plants was also tested. The contents of tested heavy metals were several times higher in pure sludge than in garden soil. Heavy metals were accumulated by plants cultivated on sludge mixed soil (SMS) or limed sludge mixed soil (LSMS) to levels significantly higher than those in control plants grown on garden soil (GS). The magnitude of accumulation was dependent on plant species and was generally higher in roots than in the shoot system. Although contents of all metals in tissues of SMS were significantly higher than those of GS plants, they were reduced by liming. No toxicity or deficiency symptoms were observed on SMS plants but contrary to expectations, cultivation of plants on SMS was generally enhancing to all tested physiological criteria in all tested plants. For example, plant growth, the end product of physiological performance, was improved by 30%, 40%, 25%, and 25% in *Corchorus*, *Eruca*, *Raphanus* and *Spinacia* respectively. Similar effects were recorded for chlorophyll content, activity of leaf nitrate reductase and total soluble proteins. Results are discussed from the viewpoint of the practicality of using sewage sludge in agriculture in the State of Qatar.

Keywords: Food plants, Heavy metals, Liming, Nitrate reductase, Total soluble proteins, Sewage sludge.

تراكم العناصر الثقيلة والتبعات الفسيولوجية المترتبة على ذلك في بعض نباتات الغذاء المختاره المنماه على تربات مسمدة بالرواسب الصلبة للصرف الصحي

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المستخلص: يستهدف هذا البحث إستطلاع حدود المخاوف من إستخدام نواتج معالجة مياة الصرف الصحى فى الزراعة بدولة قطر، ومن هذه المخاوف إمتصاص وتركز العناصر المعدنية الثقيلة فى أنسجة النباتات، وما يترتب على ذلك من دخول هذه العناصر الثقيلة الى السلسلة الغذائية للإنسان، ومن تأثر العمليات الفسيولوجية فى النباتات نفسها، بما ينعكس سلباً على إنتاجها المحصولى. أجريت التجارب على 6 من النباتات التى يأكلها الإنسان، وهى الملوخية، الجزر، الجرجير، الخس، الفجل والسبانخ. وتم فى هذه التجارب اختبار أهمية اضافة الجير للتربة المعالجة بالنواتج الصلبة لمعالجة مياه الصرف الصحى على تخفيض مستوى امتصاص النباتات للعناصر المعدنية الثقيلة من التربة المعالجة بالمخلفات الصلبة للصرف الصحى. وأسفرت نتائج هذه الدراسة عن أن محتوى انسجة النباتات على تربة الحديقة المخلوطة 50% من وزنها بالمخلفات الصلبة لنواتج الصرف الصحى، كان أعلى بصورة جوهرية من تلك التى بالنباتات فى تربة غير معالجة. وأن مقدار الزيادة يختلف من نبات لآخر وأن محتوى المجموع الجذرى من هذة العناصر اعلى من المحتوى فى المجموع الخضرى للنبات الواحد. كذلك بينت النتائج أن اضافة الجير قد خفض امتصاص كل العناصر الثقيلة فى كل النباتات. لم تظهر فى هذه الدراسة أية أعراض تسمم على النباتات الترب المعالجة بالمخلفات الصلبة للصرف الصحى، بل على العكس من ذلك، كانت مظاهر النمو لها أفضل من مثيلاتها المزروعة على ترب غير معالجة. كما ان معدل سيرالعمليات الفسيولوجية مثل بناءالكلوروفيل ومعدل نشاط انزيم مختزل النترات *Nitrate Reductase* والمحتوى الكلى للبروتينات الذائبة*Total Soluble Proteins* كان أفضل أيضاً وقد انعكس ذلك على وزن المادة الجافة فمثلاً ارتفع وزن المادة الجافة، بمقدار30%، 40% ، 25% و 25% فى نباتات الملوخية والجرجير والفجل والسبانخ على الترتيب. نوقشت نتائج هذا البحث من وجهة نظر معقولية إستخدام نواتج معالجة مياه الصرف الصحى فى الزراعة بدولة قطر.

كلمات مدخلية: نباتات غذاء، معادن ثقيلة، تركين، مختزل النترات، البروتينات الذائبة، الصرف الصحي.

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Introduction

The sludge that results from municipal wastewater treatment processes contains organic matter and nutrients that, when properly treated and applied to farmland, can improve the physical properties and agricultural productivity of soils (Peles, *et al.* 1996). Sewage sludge application to land is the principal way of deriving a beneficial use for sludge by recycling plant nutrients and organic matter for crop production (Bouldin, *et al.* 1985 and Epstein, *et al.* 1976). Agricultural use of sludge provides an alternative to disposal options, such as incineration or land filling. It is becoming ever more important as other options for disposal are diminishing. Agricultural application also provides a cost effective method of sludge disposal, but it is essential that sludge recycling in agriculture is regulated to minimize potential environmental problems. Agricultural application of municipal wastewater and sludge has been practiced since the advent of modern wastewater management about 150 years ago. (Bouldin, *et al.* 1985; Brown *et al.* 1998; Cobb *et al.* 2000; Epstein, *et al.* 1976)

In the State of Qatar, reuse of water effluent and sludge in agriculture seems attractive as it offers a solution for other problems beside the problem of waste disposal, such as the scarcity of conventional water resources and the poor physical and chemical properties of soil (Mazen, 1995). On the other side of the picture, farmers and the food industry have expressed their concerns that agricultural use of sludge may affect the safety of food products and the sustainability of agricultural land, and may carry potential economic and liability risks (Brown, *et al.*, 1998; Cobb, *et al.*, 2000; Dudka and Miller, 1999; Grant, *et al.*, 1998).

While sludge is generally recognized as a fertilizer resource, the potential for excessive uptake of heavy metals continues to be an area of concern (Brown, *et al.* 1998; Cobb, *et al.* 2000; Dudka, and Miller, 1999; Grant, *et al.* 1998).). During the wastewater treatment process, heavy metals are concentrated in the primary and secondary sludges (Stephenson and Lester, 1987; Sterrit and Lester, 1984). 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 (Lester, *et al.* 1983), 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 (Tyagi and Couillard, 1989) 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 include metal uptake by plants and the subsequent accumulation of metals in the food chain (Tyagi and Couillard, 1989; Logan, *et al.* 1997; Dudka, *et al.* 1999). Among the agronomic concerns are the effects of these heavy elements of sludge on the yield of crops as a result of the phytotoxicity they may induce in plants (Chaney, *et al.* 1978; Mazen 1995).

The present work was a continuation to the work the author adopted to assess the health and agronomic concerns raised because of using sludge on agricultural land for crop production in State of Qatar (Mazen, 1995). In other words, uptake of some heavy metals, growth and the consequences on performance of some vital metabolic processes was studied in ten common food plants grown on soils mixed with sludge produced by a sewage treatment plant at Abu Hamour, Doha, State of Qatar.

Materials and Methods

A pot comparative experiment was conducted in which six food plants (*Corchorus olitorious*, *Raphanus sativa*, *Eruca sativa*, *Daucus carota*, *Spinacia oleracea*, and *Lactuca sativa*) were grown on garden soil and garden soil mixed with dry sewage sludge. Dry sewage sludge, resulting from sewage treatment at the Abu Hamour treatment plant, was brought to experiment site at the greenhouse of the College of Sciences, University of Qatar, Doha, Qatar. Dry sludge was mixed with garden soil (GS) to produce a sludge mixed soil (SMS) with sludge representing 50% of the mixture by weight. Part of the resulting SMS was limed by addition of burned limestone (10% w/w). Plants were then planted in triplicates on these three types of soils, with garden soil (GS) serving control.

Seeds of test plants used in this study were purchased from the local market. Ten seeds were germinated on the soil-sludge mixtures in plastic pots and only 4 healthy seedlings were chosen to continue growth. Each pot contained two kilograms of soil. During germination and growth, plants were watered with tap water. Whenever needed, all pots were receiving equal volumes of water. Plants were 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 $\mu Em^{-2} S^{-1}$; and 12 hours light photo period) and after 4 weeks, plants were harvested for analysis.

For heavy metal analysis in plant material, 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. Then, the 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, and Cd following the method employed by Heckman, *et al.* (1987). Analysis of heavy metals in sludge and garden soil was carried out according to Jinadasa, *et al.* (1997).

Chlorophyll content in fresh leaves was estimated according to the method described by Arnon, (1949). Total soluble proteins were determined by the Bradford procedure (Bradford, 1976). Leaf nitrate reductase activity was assayed following the methods described by Jaworski (1971) and Abdel Wahab and Abd-Alla (1995). Leaf segments of 0.1 cm^2 were incubated in 5 ml of reaction mixture containing 0.1 M phosphate buffer pH 7.5, 1 M KNO_3 and 1% (v/v) n-propanol for 60 minutes at room temperature (25°C) in the dark. The reaction was terminated by boiling for 1 minute and the color was developed by adding 1% sulphanilamide in 3 N HCl and 0.02% N-1-naphthyl ethylene diamine dihydroehloride.

Data were statistically analyzed using the IBM computer program "*Epistat*" (written by Tracy L. Gustafson, MD, USA). One-way analysis of variance (*ANOVA*) was performed on results of each experiment to test significance of difference between data means of treatments and controls.

Results

Heavy metal-analysis revealed that content of all tested metals were several times higher in pure sludge than in garden soil (see Table 1). For example, Cu was 4.3 times, Zn was 3.8 times, Pb was at least 6 times, and Cd was at least 7 times higher than their counterparts in garden soil.

Mixing sludge to GS was significantly effective on heavy metal uptake by cultivated plants and generally, all tested plants grown on SMS or LSMS accumulated all the tested heavy metals to levels several times higher than those in counterpart plants grown on GS. Accumulation magnitude of elements was dependent on the plant species and it was generally higher in the root than in the shoot system in all tested plants. For example, increase of Cu and Zn levels ranged from 2 to 5 times in shoots and 3-9 times in roots. Similarly, levels of Cd and Pb which were not detectable in tissues of plants grown on GS, increased significantly in the counterpart tissues of plants cultivated on SMS. In a general sense, liming reduced contents of the four metals in both shoot and root systems of LSMS plants to levels lower than those of SMS and reduction in some cases was significant. Similar to the situation with SMS plants, metal contents of LSMS plants were generally significantly higher than those of control plants grown on GS.

Generally, the dry biomass of all experimental plants (see Fig. 1) was significantly promoted by cultivation of these plants on SMS, except for *Daucus* and *Lactuca* where the effect on these two plants was not clear. Dry biomass improved by 30%, 40% & 25%, 25% in *Corchorus*, *Eruca*, *Raphanus* and *Spinasia* respectively. With regard to external appearance, there were no clear differences between plants of all tested plants grown on both soils. No toxicity or deficiency symptoms were observed but contrary to expectations SMS-plants looked more healthy.

Chlorophyll synthesis responded positively to cultivation on SMS for all tested plants except *Raphanus* (see Fig. 2). In *Raphanus*, chlorophyll synthesis in plants grown on SMS was slightly promoted or at least unaffected. The extent of effect on chlorophyll content was dependent on the plant species. Chlorophyll content increased by 40%, 25%, 45%, 40%, 20% and 30% in *Corchorus*, *Daucus*, *Eruca*, *Lactuca*, *Raphanus* and *Spinasia* respectively over those in the counterpart plants grown on GS.

For all tested plants, cultivation on sludge mixed-soil significantly promoted the enzymatic activity of leaf nitrate reductase (see Fig. 3). Enzyme activity increased in SMS plants by around 60%, 40%, 50%, 45%, 30% and 100% in *Corchorus*, *Daucus*, *Eruca*, *Lactuca*, *Raphanus* and *Spinasia* respectively over the contents in their counterpart plants grown on GS.

The pattern of response of total soluble proteins (see Fig. 4) in all tested plants almost followed that of nitrate reductase, except for *Daucus* where the increase was insignificant. Cultivation on SMS lead to 30%, 20%, 25%, 40%, 45% and 50% more total soluble proteins in *Chorcorus*, *Daucus*, *Erukka*, *Lactuca*, *Raphanus* and *Spinasia* respectively over their counterpart plants grown on GS.

Table 1: Content of four heavy metals (Cu, Zn, Pb and Cd) in garden soil (GS), sludge mixed soil (SMS), limed sludge mixed soil (LSMS) and in the shoot (S) and the root (R) systems of six food plants grown on them.

Sample			Cu	Zn	Pb	Cd
				mg Kg^{-1} Dry Weight		
Soil		Garden Soil	52 ±11	187 ± 45	2.52	0.95
		Pure Sludge	224 ±76	718 ±134	17 ± 2.1	7 ±
Corchorus	S	GS	3.3 ± 1.0	13.3 ± 2.3	ND	ND
		SMS	13.0 ± 4.2	49 ± 8.2	0.73 ± 0.012	31 ± 0.0
		LSMS	9.4 ± 4.2	31 ± 5.7	0.53 ± 0.051	0.21 ± 0.06
	R	GS	6.4 ± 2.2	21.4 ± 7.2	ND	0.04 ±
		SMS	43.7 ± 3.6	153 ± 11.2	1.84 ± 0.022	0.67 ± 0.04
		LSMS	35.1 ± 2.3	113 ± 12.3	0.99 ± 0.17	0.45 ± 0.0
Daucus	S	GS	4.0 ± 1.5	14.7 ± 4.1	ND	ND
		SMS	11.2 ± 2.8	49.9 ± 5.5	0.04 ± 0.00 0	21 ± 0.07
		LSMS	7.7 ± 3.4[B]	29.6 ± 6.7	0.03 ± 0.012[B]	0.19 ± 0.08[B]
	R	GS	4.3 ± 1.7	19.0 ± 1.1	ND	ND
		SMS	37 ± 6.3	134 ± 13.2	2.46 ± 0.22	0.45 ± 0.11
		LSMS	31 ± 4.5[B]	86 ± 26.4	1.83 ± 0.19	0.33 ± 0.06[B]
Eruca	S	GS	6.3 ± 1.9	26.3 ± 9.0	ND	ND
		SMS	19.3 ± 3.9	76.0 ± 7.4	0.04 ± 0.0	0.21 ± 0.05
		LSMS	11.1 ± 2.6	55 ± 3.0	0.03 ± 0.0[B]	0.19 ± 0.03[B]
	R	GS	4.1 ± 1.3	23.1 ± 7.9	ND	ND
		SMS	31.7 ± 5.2	166 ± 7.9	3.65 ± 1.10	0.53 ± 0.13
		LSMS	17.6 ± 4.7	128 ± 12.3	2.97 ± 0.45[B]	0.44 ± 0.05[B]
Lactuca	S	GS	4.3 ± 1.4	14.5 ± 7.3	ND	ND
		SMS	19.5 ± 3.9	48.8 ± 6.9	0.06 ± 0.02	0.18 ± 0.02
		LSMS	16.2 ± 5.6[B]	37.0 ± 3.6	0.04 ± 0.56[B]	0.14 ± 0.7[B]
	R	GS	6.2 ± 2.1	21.9 ± 7.2	ND	ND
		SMS	47.3 ±11.6	187.3 ± 9.2	2.62 ± 0.74	0.33 ± 0.03
		LSMS	41.5 ± 7.8[B]	136 ± 11.7	2.1 ± 0.94[B]	0.29 ± 0.05[B]
Raphanus	S	GS	3.2 ± 1.0	9.9 ± 5.9	ND	0.02 ± 0.0
		SMS	11.3 ± 4.3	33.12± 6.8	1.34 ± 0.23	0.35 ± 0.4
		LSMS	9.7 ± 3.1[B]	26.1 ± 13.2	1.12 ± 0.85[B]	0.27 ± 0.07[B]
	R	GS	5.2 ± 1.1	22.5 ± 1.6	ND	0.04 ± 0.01
		SMS	44.6 ±13.5	171.0 ± 17.2	3.12 ± 0.0	0.54 ± 0.16
		LSMS	33.8 ±11.2[B]	118 ± 13.7	3.0 ± 0.93[B]	0.47 ± 0.13[B]
Spinacia	S	GS	5.6 ± 1.8	11.1 ± 0.9	ND	ND
		SMS	22.3 ± 5.6	44.9 ± 7.5	0.84 ± 0.12	0.07 ± 0.0
		LSMS	9.3 ± 3.6	24.3 ± 4.7	0.74 ± 0.11[B]	0.06 ± 0.02[B]
	R	GS	7.7 ± 2.4	16.2 ±	ND	ND
		SMS	46.1 ± 5.2	122.5 ± 7.2	2.23 ± 0.32	0.37 ± 0.02
		LSMS	29.7 ± 6.3	67.1 ± 11.3	1.96 ± 0.76[B]	0.33 ± 0.04[B]

Each value is the mean±SD of three replicates. All mean values in case of pure sludge are significantly different compared with those in garden soil (*P<0.05*). Mean value in case of SMS or LSMS is significantly different compared with value in GS (*P<0.05*) except where denoted by ([A]). Mean value in cases where LSMS is significantly different compared with value in case of SMS (*P<0.05*) is followed by ([B]). ND = not detected

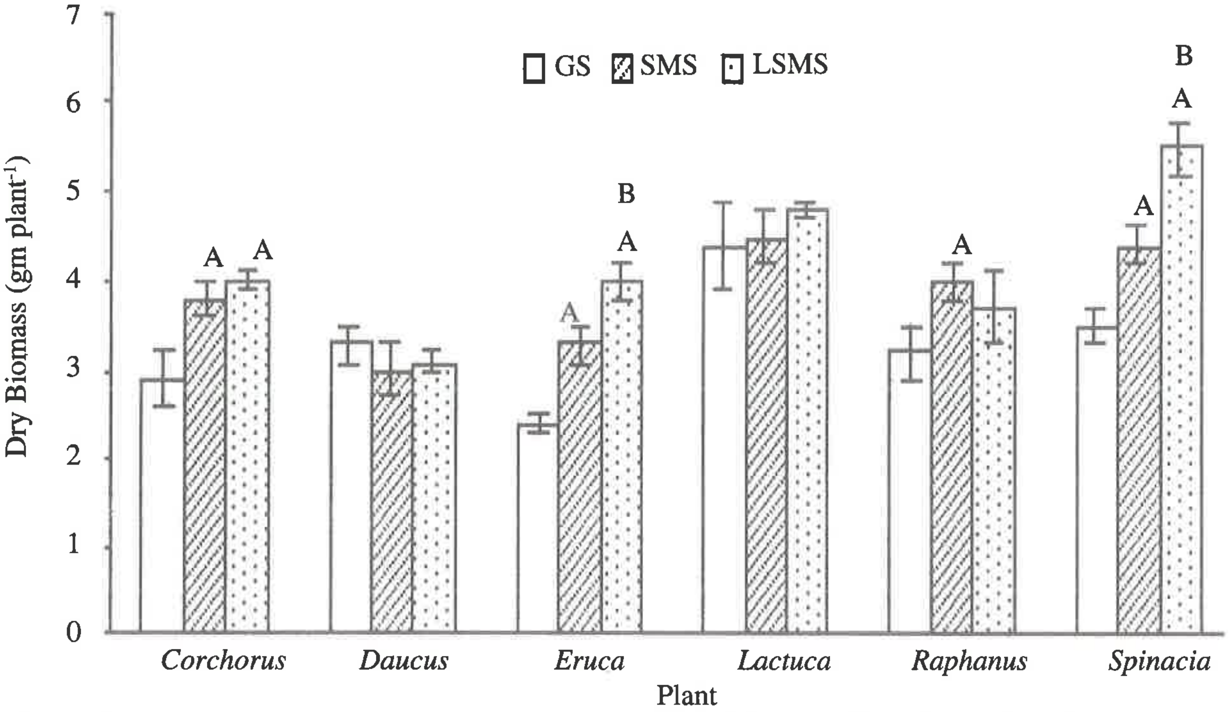

Figure 1: Growth of food plants represented by dry weight/plant as affected by cultivation on garden soil (GS), sludge mixed garden soil (SMS), or limed sludge mixed garden soil (LSMS). Each data point represents the mean of three different determinations and error bars represent standard deviations from the means. The mean of each data point in case of SMS or LSMS is significantly different (*P<0.05*) from its counterpart in case of GS whenever marked by (A). Also, the mean of each data point in case of LSMS is significantly different (*P<0.05*) from its counterpart in case of SMS whenever marked by (B).

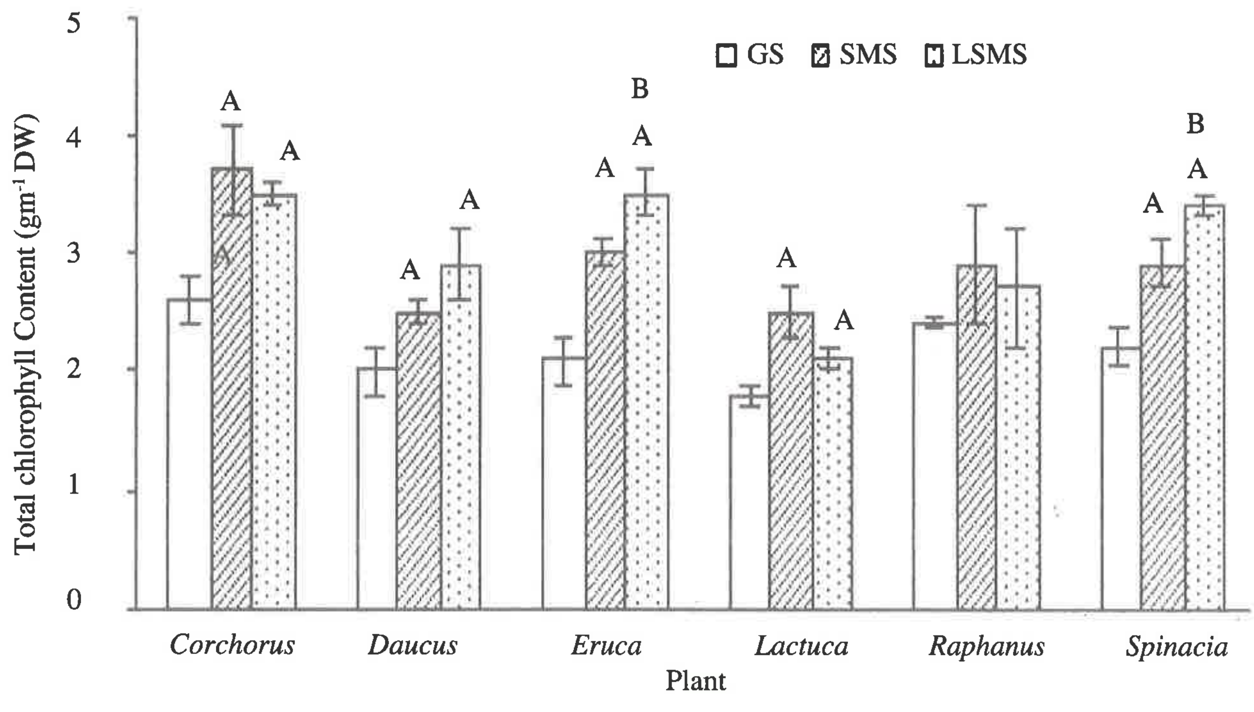

Figure 2: Total chlorophyll content of food plants as affected by cultivation on garden soil (GS), sludge mixed garden soil (SMS), or limed sludge mixed garden soil (LSMS). Each data point represents the mean of three different determinations and error bars represent standard deviations from the means. The mean of each data point in case of SMS or LSMS is significantly different (*P<0.05*) from its counterpart in case of GS whenever marked by (A). Also, the mean of each data point in case of LSMS is significantly different (*P<0.05*) from its counterpart in case of SMS whenever marked by (B).

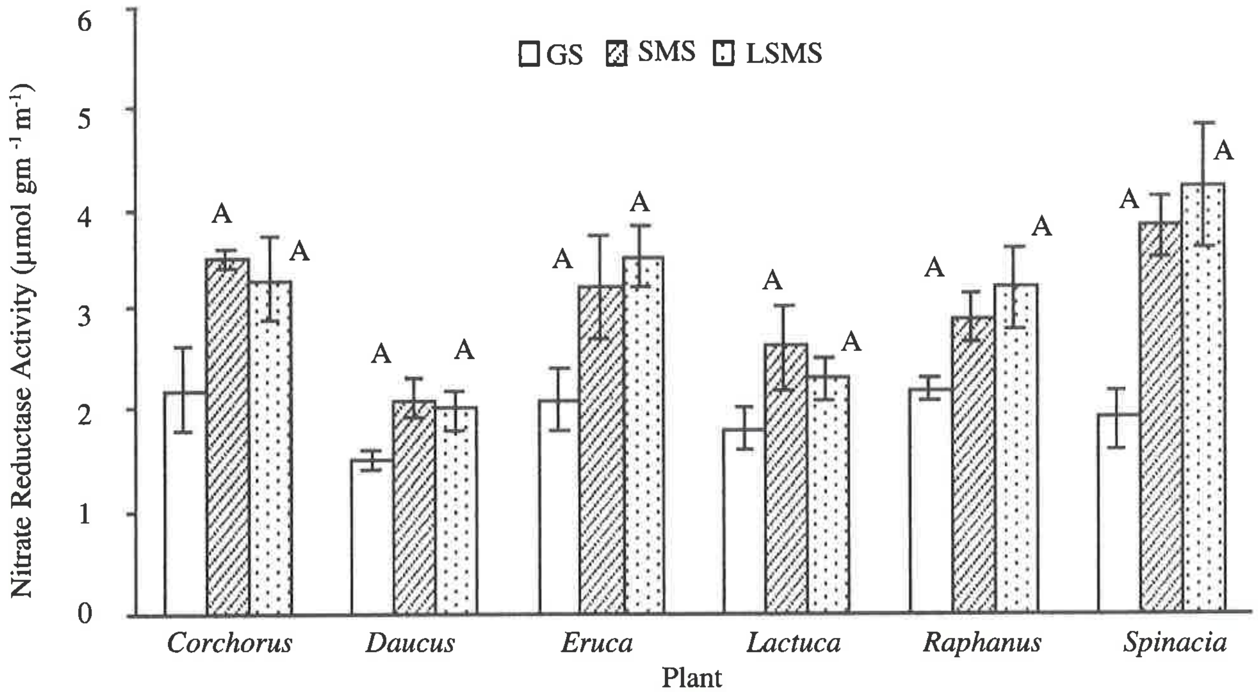

Figure 3: Activity of nitrate reductase in leaf tissues of food plants as affected by cultivation on garden soil (GS), sludge mixed garden soil (SMS), or limed sludge mixed garden soil (LSMS). Each data point represents the mean of three different determinations and error bars represent standard deviations from the means. The mean of each data point in case of SMS or LSMS is significantly different ($P<0.05$) from its counterpart in case of GS whenever marked by (A). Also, the mean of each data point in case of LSMS is significantly different ($P<0.05$) from its counterpart in case of SMS whenever marked by (B).

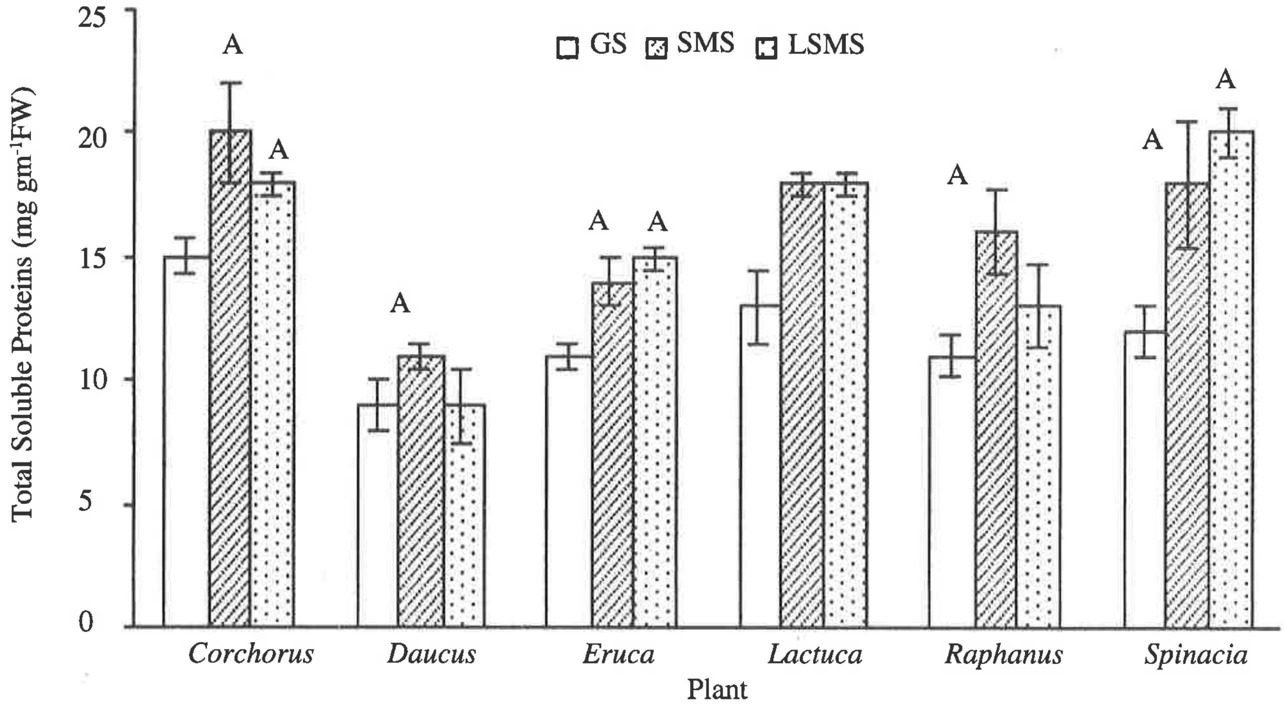

Figure 4: Content of total soluble proteins in leaf tissues of food plants as affected by cultivation on garden soil (GS), sludge mixed garden soil (SMS), or limed sludge mixed garden soil (LSMS). Each data point represents the mean of three different determinations and error bars represent standard deviations from the means. The mean of each data point in case of SMS or LSMS is significantly different ($P<0.05$) from its counterpart in case of GS whenever marked by (A). Also, the mean of each data point in case of LSMS is significantly different ($P<0.05$) from its counterpart in case of SMS whenever marked by (B).

Comparing tested criteria (Figs. 1-4) of plants grown on LSMS to those of plants grown on SMS, liming of SMS seemed to have no clear significant effect on any of them except in *Eruca* and *Spinasia* where liming enhanced these criteria significantly. As was the case with SMS plants, values of these criteria of LSMS plants were generally significantly higher than those of control plants grown on GS.

Discussion

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

Uptake and concentration of heavy metals by plants due to cultivation on sludge amended soils seems to be an inevitable process. Previous studies have reported accumulation of toxic metals in different plants and tissues after their growth on sludge-amended soils (Heckman, *et al.* 1987; Lubben and Sauerbeck, 1991; Mazen 1995). In the present study, we confirm this fact again on six widely consumed food plants. The bioaccumulation efficiency has been shown to be species dependent. Accordingly, I believe that I have to support concerns that have been raised concerning heavy metal introduction and concentration in the food chain through using sludge in agriculture. Based on this, I have the tendency to recommend that sludge be incorporated on the land which is used for the production of non-food chain crops if it has to be disposed of on farm lands. Sewage sludge, for example, has been used in forest lands (Davis, 1987). Alternatively, other strategies should then be considered to deal with this situation. 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 (Lester, *et al.* 1983). Low soil pH increases metal ion uptake and according to Heckman *et al.* (1987), raising it by soil liming leads to significant uptake reduction. Results of this research support this view where liming of sludge mixed soil to raise pH reduced metal uptake by plants cultivated on it. Another possible strategy I suggest here for consideration is to consider the long term effects of sludge applications on crops. In this context, it was found by Hinsely *et al.* (1979) that uptake by *Zea mays* dropped strongly after four years of sludge application to soil. One more suggestion to consider is to follow other sewage treatment technologies to remove or minimize heavy metals in sludge. Serious study of the different conditions for optimization is necessary if it is decided to use sewage sludge in agriculture in State of Qatar.

Previous studies indicated that the application of sewage sludge has produced varied growth responses in different plants with positive growth responses in older crops (Zasoski, *et al.* 1983). Results of the present study have shown no signs of phytotoxicity on any of the tested plants raised on SMS but on the contrary , they were even healthier than control plants. According to Chaney, *et al.* (1978) crops may suffer yield reductions even when metal phytotoxicity is not visually indicated. In this research, the performance of studied physiological parameters in plants tested was generally improved or at least negatively unaffected. The physiological performance was reflected in enhancing dry matter yield in these plants. Based on these results, the concerns regarding negative effects of agricultural use of sludge on crop yield seem to be of little importance at least in this case of Qatari sludge and plants tested. Low levels of metals in sludge may be the reason. Periodical monitoring and case by case evaluation studies are recommended if sludge or treated sewage water are to be used for agricultural purposes in state of Qatar.

Conclusions

Results presented here support raised concerns about heavy metal introduction and concentration in the food chain through using sludge in agriculture since uptake and concentration of heavy metals by plants due to cultivation on sludge amended soils seems to be an inevitable process. In the present study, this fact is confirmed again on six widely consumed food plants although levels of metals taken up were still within the safe limits.

Liming of sludge mixed soil to raise pH proved to be an effective means to minimize the risk of toxic metal uptake by plants cultivated on sludge amended soils.

Results of the present study have shown no signs of phytotoxicity on any of the tested plants raised on sludge mixed soil, but on the contrary, they were even healthier than control plants.

Based on results of this research, the concerns regarding negative effects of the agricultural use of sludge on crop yields seem to be of little importance at least in this case of Qatari sludge and plants tested. Low levels of metals in sludge may be the reason.

Periodical monitoring and case by case evaluation studies are recommended if sludge or treated sewage water are to be used for agricultural purposes in the State of Qatar.

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