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Effects of scrubber by-product-stabilized dairy lagoon sludge on growth and physiological responses of sunflower (*Helianthus annuus* L.)

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Abstract

Brick manufacturing industries are challenged to comply with clean air mandates. Dry air scrubbers have been used to remove acid gases from the exhaust air from brick manufacturing plants. The use of dry air scrubbers results in the production of large quantities of an alkaline powder by-product. A greenhouse experiment was conducted to evaluate the potential of using dairy lagoon sludge stabilized with the scrubber by-product as a soil amendment. Lagoon sludge was stabilized with scrubber by-product at an application rate of $20\ g\ l^{-1}$. The sludge-scrubber by-product mixture was applied to a sandy loam soil to provide amendments ranging between 28 and 168 kg of plant available nitrogen (PAN)/ha for the growth of *Helianthus annuus* (sunflower). Use of the sludge-scrubber by-product mixture as a nitrogen fertilizer did not adversely affect sunflower seedling emergence; however, significantly higher ($p < 0.05$) plant volume indices, leaf area, dry shoot and root masses, and seed yields were obtained for mature plants grown in sludge-treated soil relative to the control or fertilizer treatment. The sludge amendment did not severely impact gas exchange or chlorophyll *a* fluorescence of the plants and nutrient content of the sunflower tissues was generally within a sufficient range. The increased growth and yield of sunflower plants indicated the potential of the sludge-scrubber by-product mixture as a soil amendment in agricultural crop production.

Keywords: Sunflower; Scrubber by-product; Sludge; Gas exchange; Photosynthesis; Dry lime scrubber

1. Introduction

Land application of animal waste is a common practice which when conducted judiciously can provide a cost-effective utilization strategy for recycling organic matter and essential plant nutrients, as well as assist in solid waste disposal. The production benefits garnered from animal sludge has been extensively documented (Adegbidi and Briggs, 2003; Yang et al., 2004; Hiltbrunner et al., 2005; Zhou et al., 2005). There are concerns regarding the potential hazards associated with the constituents of animal waste, particularly volatilization of ammonia, leaching of nitrate, phosphorus, and metals into surface water and groundwater, as well as accumulation of metals in plant tissues (Wong, 1985; Sommer, 1997; Veeken and Hamelers, 2002). The introduction of pathogenic organisms during the application of animal waste is also a concern. The challenge in land application of animal or sewage sludge, therefore, is to strategically utilize the potential benefits in an environmentally sustainable manner.

A key issue that the brick manufacturing industry must face is the reduction of emissions to meet the Environmental Protection Agency's (EPA) regulatory standards (US

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EPA, 2003a,b). One solution to this problem is the use of by-products from dry lime scrubber systems that neutralize acid gases produced from brick kilns. The scrubbing process, however, produces large quantities of neutralizing reagent. Landfills are the current method of disposal of this dry powder scrubber by-product. However, the initial scrubbing agent (lime) has been traditionally used as a chemical to control the environment required for pathogen activity in biosolids to meet the EPA regulations. Other studies have indicated that this material has the potential to be an effective chemical agent in the stabilization of animal lagoon sludge because it enhances settling and metal precipitation, as well as elevating sludge pH for pathogen control (Omoike and Vanloon, 1999; Chastain et al., 2001; Duan and Gregory, 2003). By combining two independently potential waste products (animal sludge) and (brick emission neutralizing reagent), we set out to determine if a constructive alternative use could be found.

The objectives of the present study were:

(i) To determine the effects of different treatments of the sludge-scrubber by-product mixture on emergence, growth, and nutrient accumulation of seedlings of sunflower (*Helianthus annuus* L. cv. Blazer).
(ii) Assess the effects of different treatments of the sludge-scrubber by-product mixture on volume indices, chlorophyll *a* fluorescence, gas exchange, and nutrient accumulation of sunflower plants over the entire sunflower plant life cycle.

2. Materials and methods

2.1. Sludge preparation

Air scrubber by-product was obtained from Boral Bricks manufacturing plant (Augusta, GA). The sludge used in this study was collected from the primary lagoon at the Clemson University LaMaster Dairy Cattle Center, weighed, and mixed to produce a concentration equivalent to 20 g of scrubber by-product per l of sludge. This concentration was selected based on the results of previous studies, which indicated that treatment of lagoon sludge with scrubber by-product at this concentration provided a pH sufficient for pathogen control and enhanced nutrient removal and settling (Chastain et al., 2001; Thomas, 2005). The scrubber by-product-sludge mixture was allowed to settle for 24 h, the supernatant was removed, and the settled material was retained for use in the study as a source of major and minor plant nutrients.

2.2. Plant species

Sunflower was selected based on its agricultural, horticultural, and heavy metal phytotoxic indicator importance (Goodman and Ennos, 1998; Ruiz et al., 2003; Turgut et al., 2004). The American Standards and Testing Materials lists it as one of the plants suitable for phytotoxicity testing (ASTM, 1998).

2.3. Seedling emergence studies (experiment 1)

Treatment levels for the application of the settled solids as a soil amendment were calculated based on the recommended plant available nitrogen (PAN) application rate of 112 kg N/ha for sunflower for a total plant yield of 2240 kg/ha. The plant available nitrogen of the sludge was estimated as

$$\text{PAN} = (0.9 \times \text{TAN}) + (0.6 \times \text{Org-N}) + \text{NO}_3\text{-N}, \qquad (1)$$

where PAN is the plant available nitrogen; TAN, the total ammoniacal nitrogen; Org-N, the organic nitrogen, and NO_3-N is the nitrate nitrogen.

This equation accounts for loss of TAN during incorporation of the sludge. The treatment levels selected for the study represented PAN application rates at, as well as above, and below the recommended rate: 28, 56, 84, 112, and 168 kg PAN/ha. For positive control purposes (treatment control), an additional treatment comprised of a 10:10:10 NPK inorganic commercial fertilizer formulation at the recommended N application rate of 112 kg PAN/ha was included. The sludge slurry was applied to the surface of 40 cm diameter pots that contained a sandy loam soil from the Clemson University Edisto Research and Education Center (Blackville, SC). The sludge was immediately incorporated to a depth of approximately 5 cm using a small hand rake. Pots were completely randomized in a controlled environment glasshouse (25–28 °C ambient temperature; 53–57% relative humidity) and left to stabilize for three d. Sunflower seeds ($n = 16$) obtained from Seeds 2000 Inc. (Breckenridge, MN) were sown in each pot. All experimental treatments were done in triplicate (6 treatments × 3 replicates × 2 harvests). The pots were watered daily and the number of seedlings emerging from each treatment was recorded. Plants were harvested 21 d after planting and again on day 53. Upon harvesting, leaf area was measured with a LiCor 3100 leaf area meter (LiCor Inc., Lincoln, NE). Whole plants were then oven-dried to a constant weight at 70 °C and the dry-weights were recorded. Nutrient contents of the plant tissues at both harvests were determined by the Clemson University Agricultural Service Laboratory. Analytical procedures included dry ash for B and Al and wet ash with HNO_3/H_2O_2 for mineral nutrients followed by inductively coupled plasma spectroscopy (ICP).

2.4. Seedling growth and nutrient accumulation

Relative growth rates (RGR) and net assimilation rates (NAR) of the sunflower seedlings were calculated for each treatment according to Evans (1972) and Hunt (1978), as later modified by Hunt et al. (2002). The relative growth rate and NAR across the two harvest intervals were calculated from the following formula:

$$\mathrm{RGR} = \frac{(\mathrm{Ln}\,W_2 - \mathrm{Ln}\,W_1)}{(t_2 - t_1)}, \quad \text{and} \tag{2}$$

$$\mathrm{NAR} = \frac{(W_2 - W_1)(\mathrm{Ln}\,L_{\mathrm{A}2} - \mathrm{Ln}\,L_{\mathrm{A}1})}{(L_{\mathrm{A}2} - L_{\mathrm{A}1})(t_2 - t_1)}, \tag{3}$$

where W_1 is the dry mass (g) of plant tissue at first harvest; W_2, the dry mass (g) of plant tissue at second harvest; L_{A1}, the cumulative leaf area per plant at first harvest; L_{A2}, the cumulative leaf area per plant at second harvest; t_1, the time of first harvest (d), and t_2 is the time of second harvest (d).

Relative accumulation rates of nutrients (RNuAR) were computed similar to relative growth rates (Hocking and Steer, 1983a,b):

$$\mathrm{RNuAR} = \frac{(\mathrm{Ln}\,\mathrm{Nu}_2 - \mathrm{Ln}\,\mathrm{Nu}_1)}{(t_2 - t_1)}, \tag{4}$$

where Nu_1 and Nu_2 represent masses of a given nutrient at times t_1 and t_2, respectively.

2.5. Growth and ecophysiological determinations (experiment 2)

Physiological responses of sunflowers were studied over the entire life cycle of the plants, where the treatments were prepared as described for the seedling emergence study and comprised a 56, 112, and 168 kg N/ha sludge treatment, as well as a commercial fertilizer treatment at 112 kg N/ha and an unfertilized control. All treatments were done in replicates of six. Three sunflower seeds were sown per pot and thinned to one seedling per pot upon emergence. Weekly measurements of shoot height and diameter, gas exchange, leaf chlorophyll *a* fluorescence, and estimates of chlorophyll concentration were taken. Volume indices were calculated from shoot height and diameter at 10 cm above the soil surface.

2.5.1. Gas exchange measurements

Leaf gas exchange measurements were made in situ with a portable CO_2/H_2O infrared gas exchange system equipped with an integral cuvette air supply unit and an automatic leaf cuvette (CIRAS-1, PP Systems, Inc., Amesbury, MA). Upon foliage development, measurements were made randomly on the first fully expanded leaf on each of three plants per treatment between 09:00 and 16:00 h.

Measures of CO_2 assimilation rate (A) in relation to calculated internal leaf CO_2 concentrations (C_i) were made at a photosynthetic photon flux density (PPFD) of 1200 μmol m^{-2} s^{-1} and leaf temperature of 25 °C. Leaf cuvette CO_2 concentration (C_a) was initially set at 370 μmol mol^{-1} (ambient) and decreased incrementally to 175, 150, 100, and 50 μmol mol^{-1}, followed by a repeat of C_a at 370 μmol mol^{-1} and then sequentially increased to 600, 800, 1000, and 1200 μmol mol^{-1}. A final measurement was taken at zero PPFD and ambient C_a. From the A/C_i measurements, photosynthetic parameters of A_{max} (maximum photosynthetic rate), A_{net} (net photosynthetic rate), V_{cmax} (maximum rate of carboxylation by Rubisco), J_{max} (photosynthetically active radiation-saturated electron transport rate), R_d (dark respiration rate), Γ^* (CO_2 compensation point), and g_s (stomatal conductance) were deduced according to the biochemical models of Farquahar et al. (1980) and Bernacchi et al. (2001). Parameters were estimated using non-linear regression (SAS Institute Inc., Cary, NC; version 9.1, 2002–2003) as described in Geber and Dawson (1997) with modifications by Bauerle et al. (2003a).

Photosynthetic light–response curves were constructed from gas exchange measurements on the leaves that were used to generate A/C_i data. The temperature inside the leaf cuvette was set at 25 °C and C_a was set to 370 μmol mol^{-1}. Measurements were recorded sequentially at PPFDs of 1200, 900, 600, 425, 300, 200, 100, 50, and 0 μmol m^{-2}s^{-1}. The photosynthesis-light data were fitted to the model described by Küppers and Schulze (1985), which allowed parameterization of Q_{app} (apparent quantum yield):

$$A_{\mathrm{net}} = A_{\mathrm{max}}[1 - \mathrm{e}^{-\phi(I - I_{\mathrm{c}})}], \tag{5}$$

where I is photosynthetic photon flux density, I_c is the light compensation point, A_{max} is the maximum rate of CO_2 assimilation, and ϕ is derived from the measured net photosynthesis rate (A_{net}) and absorbed irradiance. The model was implemented using the non-linear function of SPSS (SPSS Inc., Chicago, IL; version 11.5, 1989–2002). The Q_{app} was calculated as the slope of the first four points of each light response curve.

2.5.2. Leaf chlorophyll a fluorescence determination

Dark-adapted leaf chlorophyll fluorescence was measured using a modulated fluorometer (model OS5-FL, Opti-Sciences, Tyngsboro, MA). The nomenclature for the fluorescence parameters follows Maxwell and Johnson (2000). Determinations were made on the same leaves that were used for gas exchange measurements. Leaves were dark adapted for 30 min. Following dark adaptation, measurements of initial fluorescence (F_0), maximal fluorescence (F_m), and quantum efficiency of photosystem II (F_v/F_m), where F_v is the variable fluorescence were collected.

2.5.3. SPAD chlorophyll meter measurements

Leaf greenness was estimated in situ using a hand-held Minolta SPAD 502 chlorophyll meter (Minolta Camera Corp., Ramsey, NJ). An average of five SPAD readings was recorded for each selected leaf. Apparent quantum yield was adjusted to quantum yield (Q) following Bauerle et al. (2004).

2.5.4. Leaf area, flower head diameter, and plant tissue analysis

Sunflower plants were harvested after 10 weeks. Cumulative leaf area for each plant was measured using a LiCor 3100 leaf area meter immediately following the destructive harvest. Diameters of the flower heads were measured with a rigid metric ruler. Plants were oven dried at 70 °C for 4 d and dry weights were determined. Plant tissue elemental concentrations were determined as described in the seedling emergence studies.

2.6. Statistical analysis

Plant response data were analyzed using SAS (SAS Institute, Cary, NC, 2003). One-way analysis of variance (ANOVA) was used to analyze the significance of main effects (*F*-test at $p < 0.05$) and differences between means were analyzed by least significant difference (LSD) at $p < 0.05$ (Little and Hills, 1978). Pearson's correlation was used to assess correlation between variables.

3. Results and discussion

3.1. Seedling growth and nutrient accumulation

Characteristics of the sludge-scrubber by-product mix used in this study were presented in (Table 1). When compared to the positive control, the scrubber by-product stabilized dairy lagoon sludge did not adversely impact sunflower seedling emergence percentages, with the exception of the 56 kg PAN/ha treatment, which had significantly lower ($p < 0.05$) percentage emergence than all the other treatments (Table 2). Relative growth rates and NARs of plants growing in sludge amended soil were significantly higher for plants that grew in commercial fertilizer. The RGRs in all the treatments are consistent with values within the range 0.08–0.146 reported by Hocking and Steer (1983a) for sunflower with similar ontogeny.

Of the plant macronutrients analyzed, the highest RNuAR were obtained for Ca followed by Mg, and NO_3-N, particularly in plants growing in sludge amended soil (Table 3). The comparatively higher RNuARs are indicative of the high concentrations of these constituents in the scrubber by-product. Significantly higher total N accumulation rates were obtained in all sludge treatments when compared to the fertilizer treatment and attained a rate of 0.043 d^{-1} for the 168 kg PAN/ha treatment (an approximate 2-fold difference when compared with the fertilizer rates). Similarly, RNuARs for Ca in sludge treatments were significantly higher than in fertilizer treatments, with the highest rates (0.082 d^{-1}) obtained in the 84 and 112 kg PAN/ha treatments. The RNuARs for NO_3-N were higher in the sludge treatments when compared to the fertilizer treatments and generally increased with increasing application rates. The highest rate (0.098 d^{-1}) was obtained for plants from the 168 kg PAN/ha, which is 6-fold higher than the fertilizer treatment. The RNuARs for the micronutrients were also influenced by treatments. The highest rate of Zn accumulation (0.091 d^{-1}) was obtained for the 84 kg PAN/ha treatment, which was in fact significantly different from the rates obtained for all the other treatments. The highest RNuARs of Cu were obtained for plants from sludge treatments above 56 kg PAN/ha. Likewise, the highest Mn RNuARs were obtained for the 112 and 168 kg PAN/ha sludge treatments. The highest RNuAR for Fe, however, was obtained for plants from the fertilizer treatment (0.132 d^{-1}) and generally decreased with increasing sludge application rates, with the exception of the 84 kg PAN/ha treatment for which the RNuAR was the highest among the sludge treatments (0.082 d^{-1}). The reduction in Fe accumulation rates is indicative of reduced bioavailability as sludge treatment application increases. Boron RNuAR was higher in plants from the fertilizer treatment (0.076 d^{-1}), but not significantly different from rates in plants from sludge treatments, with the exception of the 56 kg PAN/ha treatment which had the lowest rate of 0.067 d^{-1} and is statistically lower than that of all the other treatments. The RNuARs for Na and Al were not statistically different among treatments.

Table 1
Selected properties of dairy lagoon sludge-scrubber by-product mix (mix), sludge, and by-product used in this study

Constituent	Mix	Sludge	By-product
TAN (mg/l)	260 (3.02)	186.67 (43.34)	NM
Org-N (mg/l)	1113 (11.93)	926.67 (38.44)	NM
NO_3-N (mg/l)	0	1833.33 (1833.33)	4380.8 (263.04)
P (mg/l)	893 (7.81)	760.33 (18.27)	300 (0)
K (mg/l)	576 (5.47)	403.67 (21.88)	1450 (101.67)
Ca (mg/l)	9591 (7.96)	794.33 (35.32)	393000 (10816.48)
Mg (mg/l)	314 (13.98)	184.67 (4.81)	4033.3 (134.98)
S (mg/l)	738 (4.95)	297.67 (6.39)	12785 (3165.49)
Zn (mg/l)	21.02 (1.85)	16.7 (0.42)	25.5 (19.28)
Cu (mg/l)	18.34 (1.24)	8.74 (1.05)	1.5 (0.29)
Mn (mg/l)	21.90 (1.85)	14.17 (0.22)	184.3 (9.58)
Fe (mg/l)	446.12 (7.01)	377.4 (66.79)	2458 (173.96)
B (mg/l)	5.49 (0.59)	0.58 (0.02)	6.8 (0.23)
Al (mg/l)	1232.00 (12.25)	652.4 (136.98)	22.8 (1.5)
Na (mg/l)	124.45 (1.85)	83.32 (2.96)	1556.8 (75.29)
Cl (mg/l)	1200 (8.15)	5.97 (1.09)	17183.3 (1428.02)
pH	11.9 (0.05)	NM	NM

Unmeasured constituents are denoted NM. Means are presented with standard errors in parentheses.

Table 2
Seedling emergence percentages, relative growth rates (RGR), and net assimilation rates (NAR) of sunflower seedlings

Treatment (kg PAN/ha)	% Seedling emergence	RGR (d^{-1})	NAR ($g\ m^{-2}\ d^{-1}$)
Fertilizer (112 kg PAN/ha)	85.4 ± 8.3 ab	0.081 ± 0.002 b	9.87 ± 0.45 c
28	97.9 ± 2.0 a	0.090 ± 0.002 a	14.92 ± 0.83 a
56	83.3 ± 2.0 b	0.088 ± 0.001 a	13.00 ± 0.25 b
84	95.8 ± 2.1 ab	0.090 ± 0.001 a	14.68 ± 0.05 a
112	87.5 ± 3.6 ab	0.093 ± 0.000 a	13.26 ± 0.04 b
168	89.6 ± 4.2 ab	0.092 ± 0.002 a	14.67 ± 0.16 a

Values are means ± standard errors. Values followed by the same letter within the same column are not significantly different from each other ($p \leqslant 0.05$).

Table 3
Relative nutrient accumulation rates (RNuAR) of sunflower seedlings

Treatment	RNuAR (d^{-1})					
	Fertilizer	28 kg PAN/ha	56 kg PAN/ha	84 kg PAN/ha	112 kg PAN/ha	168 kg PAN/ha
N	0.027 d (0.0008)	0.040 ab (0.0015)	0.033 c (0.002)	0.037 bc (0.002)	0.040 b (0.0003)	0.043 a (0.001)
P	0.049 ab (0.003)	0.043 b (0.004)	0.050 ab (0.0009)	0.050 ab (0.002)	0.052 ab (0.0004)	0.049 ab (0.003)
K	0.052 b (0.002)	0.060 ab (0.003)	0.061 ab (0.004)	0.062 a (0.001)	0.057 ab (0.003)	0.063 a (0.003)
Ca	0.062 b (0.002)	0.080 a (0.003)	0.075 a (0.004)	0.082 a (0.004)	0.082 a (0.0005)	0.080 a (0.006)
Mg	0.070 b (0.002)	0.076 ab (0.0006)	0.078 ab (0.005)	0.083 a (0.002)	0.080 ab (0.001)	0.077 ab (0.006)
S	0.052 ab (0.001)	0.056 ab (0.009)	0.046 ab (0.007)	0.044 b (0.0001)	0.062 ab (0.009)	0.066 a (0.005)
NO_3-N	0.016 c (0.002)	0.081 ab (0.003)	0.063 b (0.007)	0.090 a (0.003)	0.093 a (0.011)	0.098 a (0.008)
Zn	0.061 b (0.001)	0.052 b (0.008)	0.053 b (0.001)	0.091 a (0.002)	0.061 b (0.002)	0.061 b (0.005)
Cu	0.060 dc (0.002)	0.065 bc (0.002)	0.054 d (0.002)	0.070 abc (0.003)	0.073 a (0.002)	0.070 abc (0.004)
Mn	0.060 ab (0.003)	0.067 ab (0.011)	0.040 ab (0.004)	0.032 b (0.006)	0.074 a (0.015)	0.077 a (0.018)
Fe	0.132 a (0.003)	0.070 c (0.003)	0.059 d (0.004)	0.082 b (0.002)	0.063 cd (0.002)	0.055 d (0.009)
Na	0.060 (0.002)	0.050 (0.010)	0.063 (0.011)	0.060 (0.007)	0.050 (0.003)	0.044 (0.007)
B	0.076 a (0.002)	0.071 ab (0.003)	0.067 b (0.004)	0.072 ab (0.001)	0.072 ab (0.002)	0.070 ab (0.004)
Al	0.058 (0.0002)	0.070 (0.014)	0.055 (0.009)	0.075 (0.002)	0.081 (0.006)	0.055 (0.007)

Values are means with standard error in parentheses. Values followed by the same letter within the same column are not significantly different from each other ($p \leqslant 0.05$).

In general, the RNuARs of selected nutrients in this study are within the levels reported for sunflower seedlings by Hocking and Steer (1983a), with the exception of P, which was lower. The RNuARs were compared with the RGRs of sunflower seedlings to elucidate any synchronicities between the accumulation of dry matter and the accumulation of particular nutrients.

There was an apparent correlation of RNuARs with RGRs for some of the nutrients: the RNuAR of NO_3-N, Ca, N, Mg, and K increased with increasing plant dry matter. A negative relationship between RNuAR for Fe and RGR was obtained, which indicates reduced accumulation rates of Fe as the plant accumulates dry matter over time. These relationships were significant at $p = 0.01$.

3.2. Volume index

Fig. 1 illustrates changes in volume indices from weeks 3 to 7. The sludge-scrubber by-product treatments of 56 and 112 kg PAN/ha resulted in plants at weeks 3 and 4 with significantly higher volume indices (20.84 and 26.70 cm^3, respectively, for week 3; 51.60 and 62.96 cm^3, respectively, at week 4). As plant growth ensued over the next three weeks, significantly higher volume indices were obtained for all the plants growing in the sludge mixture compared with the fertilizer treatment, the volume index of the latter being statistically similar to that of the control. At the seventh week, plants growing in the 168 kg PAN/ha treatment had the highest volume index (267.98 g), which were 2- and 1.5-fold higher than that of the control and fertilizer treatments, respectively. The apparent higher volume indices obtained for plants growing in the sludge-scrubber by-product mixtures may be construed as the consequence of essential nutrients supplied by the mixture. Whereas, the fertilizer treatment, being an inorganic formulation of N, P and K, supplied these respective nutrients and the sludge mixtures provide macro- and micronutrients that promote plant growth.

3.3. Chlorophyll a fluorescence

The sludge-scrubber by-product mixtures did not influence the Q in Photosystem II (PS II) (F_v/F_m), initial fluorescence (F_0), or maximal fluorescence (F_m) as evidenced by the statistically similar values for these three parameters when pooled across the 10-week period. Mean values of F_v/F_m were 0.831, 0.838, 0.834, 0.810, and 0.830 for the control, fertilizer, 56, 112, and 168 kg PAN/ha, respectively. These values are not indicative of plant stress due to the sludge-by-product mixture since measures were close to 0.84, the optimum reference value for healthy plants (Björkman and Demming, 1987; Johnson et al., 1993;

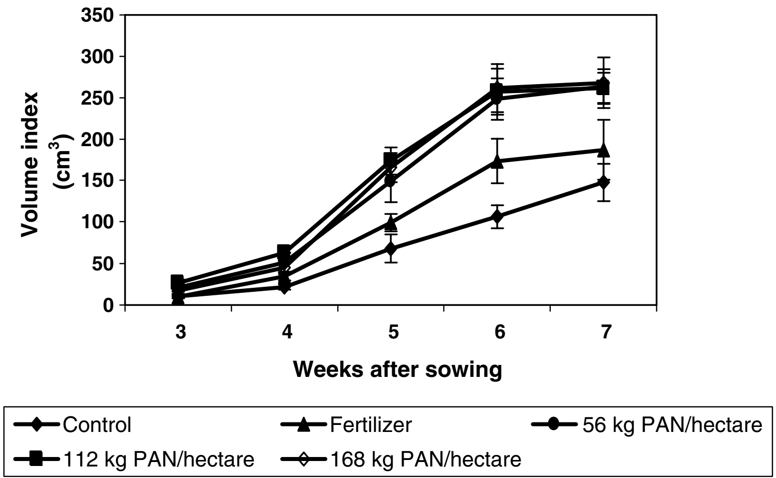

Fig. 1. Change in volume indices for sunflower plants (*H. annuus* cv. Blazer) growing in untreated soil, control (◆), commercial fertilizer treatment (▲) and dairy sludge-scrubber by-product treatments of 56 kg PAN/ha (●), 112 kg PAN/ha (■), and 168 kg PAN/ha (◇). Volume index is calculated as the product of shoot height and square of shoot diameter 10 cm above the soil surface. Vertical bars represent standard errors.

Bauerle et al., 2003b). The values for F_v/F_m obtained in this study are also consistent with values reported for unstressed sunflower plants (Ciompi et al., 1996). There is extensive documentation explicating the influence of environmental factors on the normal functioning of PS II (e.g. Smillie and Nott, 1982; Flagella et al., 1994; Basu et al., 1998; Bauerle et al., 2003b; Mohammadian et al., 2003). Measures of chlorophyll *a* fluorescence are useful in the quantitative characterization of stress on the photosynthetic apparatus. In particular, F_0 is known to be sensitive to any stress that interferes with the structural integrity of the PS II pigment level, with slight increases being indicative of photoinhibition, while F_v is sensitive to changes in the rates of electron transfer and membrane ultrastructure (Mohammadian et al., 2003).

3.4. Gas exchange measurements

Table 4 shows the light response gas exchange parameters pooled across a 7-week growth period. Initially, the gas exchange parameters for sunflower plants growing in each treatment were not statistically different ($p > 0.05$). During week 4, however, A_{max} for control plants exceeded that of other treatments by approximately 20%. This changed by week 6, where A_{max} was highest for plants growing in the 168 kg PAN/ha treatment and lowest in the 112 kg PAN/ha treatment. Changes in A_{max} thereafter were not significantly different among treatments. A similar trend was observed for changes in A_{net} with time. The value for A_{net} was approximately 20% higher for control plants during weeks 3 and 4. The sludge-scrubber by-product treatments did not significantly impact A_{net} over the next six weeks since differences between treatments were not statistically significant ($p > 0.05$).

Stomatal conductance of plants in each treatment fluctuated over the growth period of the sunflower plants with no apparent trend. Whenever g_s differed significantly among treatments (weeks 6 and 7), values for the control and fertilizer treatments lagged behind those for the sludge-scrubber by-product treatments. This indicates an apparent influence of the sludge-scrubber by-product mixture on g_s. Dark respiration rates were not statistically different among treatments except at week 5 when R_d for plants growing in the commercial fertilizer and the 168 kg PAN/ha treatment was >200% of the mean value for control plants. Values for the 56 and 112 kg PAN/ha treatments were also higher than the controls.

A comparison of the values obtained for V_{cmax} for sunflower plants growing in sludge-scrubber by-product mixes with values for fertilizer or control plants revealed that significant differences among treatments occurred only during weeks 3 and 8. The value for V_{cmax} was significantly higher in plants in the 168 kg PAN/ha treatment than the other treatments during week 3, while plants of the 112 kg

Table 4
Light response gas exchange parameters pooled across a 7-week growth period for sunflower plants (*H. annuus* cv. Blazer) in different treatments

Treatment (kg PAN/ha)	A_{max} (μmol m^{-2} s^{-1})	$Q \times 10^{-2}$ (μmol m^{-2} s^{-1})	I_c (μmol m^{-2} s^{-1})
Control (untreated soil)	34.07 ± 7.83	1.52 ± 0.25	50.27 ± 19.79
Fertilizer (112 kg PAN/ha)	32.99 ± 6.46	1.47 ± 0.11	33.74 ± 8.84
56	28.37 ± 4.00	1.57 ± 0.18	36.40 ± 10.90
112	26.99 ± 5.70	1.81 ± 0.36	19.00 ± 1.46
168	25.30 ± 3.40	1.51 ± 0.12	29.82 ± 6.33

Means are presented ± standard errors. A_{max} = maximum photosynthetic rate at ambient CO_2 and saturated PPFD; Q = quantum yield; I_c = light compensation point.

PAN/ha treatment had significantly lower V_{cmax} values than plants of all other treatments. Similarly, values of J_{max} were constant among treatments over the growth period except at weeks 6 and 7 when values obtained for the 168 kg PAN/ha treatments were significantly higher than the control plants. The CO_2 compensation point differed significantly among treatments only at weeks 4, 9, and 10. The scenario at weeks 4 and 10 were similar where the Γ^* values for plants from the 112 kg PAN/ha treatment were statistically higher than that for the 56 kg PAN/ha treatment. At other time points, values did not differ significantly among treatments; at week 9, Γ^* for control plants was significantly higher than that for the 112 kg PAN/ha treatment but still was not different from that of other treatments.

Values for A_{max} (at ambient CO_2 and saturated PPFD), Q, and I_c were pooled across the growth period. Mean values for A_{max} for the treatments were in the order: Control > Fertilizer > 56 kg PAN/ha > 112 kg PAN/ha > 168 kg PAN/ha. These values were, however, not statistically different from each other at $p = 0.05$. The standard error in the measurements did not indicate a biologically significant change, where A_{max} variation among treatments could be attributed to the precision in the instrumentation. Similarly, mean values for I_c were lower for plants growing in the sludge-scrubber by-product treatments, although these values were not significantly different either. In fact, lower I_c values indicate a lower energy requirement for photosystem II excitation and hence a positive attribute in activating the photosynthetic apparatus (Wang and Bauerle, in press). Lastly, values for Q were not statistically different among treatments (Table 4).

Several studies have indicated a high dependency of photosynthesis on adequate nitrogen supply (Shangguan et al., 2000; DaMatta et al., 2002; Cechin and Fumis, 2004; Chen et al., 2005). Ciompi et al. (1996) reported a decline in A_{net} and rise in stomatal conductance of sunflower plants under nitrogen deficient conditions and attributed it to reduced mesophyllic activity. The results obtained in the present study suggest that the photosynthetic characteristics of the sunflower plants were largely unaffected by the sludge-scrubber by-product applications. This may be explained by the exceptional photosynthetic ability of sunflower plants conferred by its high Rubisco enzyme activity, efficient electron transport (Ranty and Cavaliè, 1982) and high g_s (Connor and Sadras, 1992; Connor and Hall, 1997). Taken together, these characteristics could explain the relative invariance in the photosynthetic parameters among treatments, particularly during the latter stage of growth. Connor et al. (1993) also reported that Q_{app} in sunflower plants did not change with N treatments.

3.5. Biomass and leaf area determinations

Dry root masses for the sludge treatments were significantly higher than for the control treatment (Fig. 2). Root masses for the 56 and 168 kg PAN/ha treatments (5.12 and 5.36 g/plant, respectively) were both significantly higher than that of the control and fertilizer plants (2.92 and 3.65 g/plant, respectively), however, dry root mass for the 112 kg PAN/ha treatment (4.70 g/plant) was not significantly different from that of the fertilizer treatment. While shoot masses were not significantly different among the sludge and fertilizer treatments, masses for the sludge treatments were all significantly different (approximately 20% higher) from that of control plants. Mean seed yields for the treatments (relative to the surface area of the pots) were in the order: 168 kg PAN/ha > Fertilizer > 112 kg PAN/ha > 56 kg PAN/ha > Control. Values for plants from the 112 and 168 kg PAN/ha sludge-scrubber by-product treatments as well as for the fertilizer treatment were significantly higher than that of the control and 56 kg PAN/ha treatments. Flower head diameters for the sludge treatments and fertilizer treatment were significantly higher than that of the controls but did not differ among each other. The highest leaf area was recorded for plants from the 56 kg PAN/ha treatment (2265.81 cm^2/plant), which

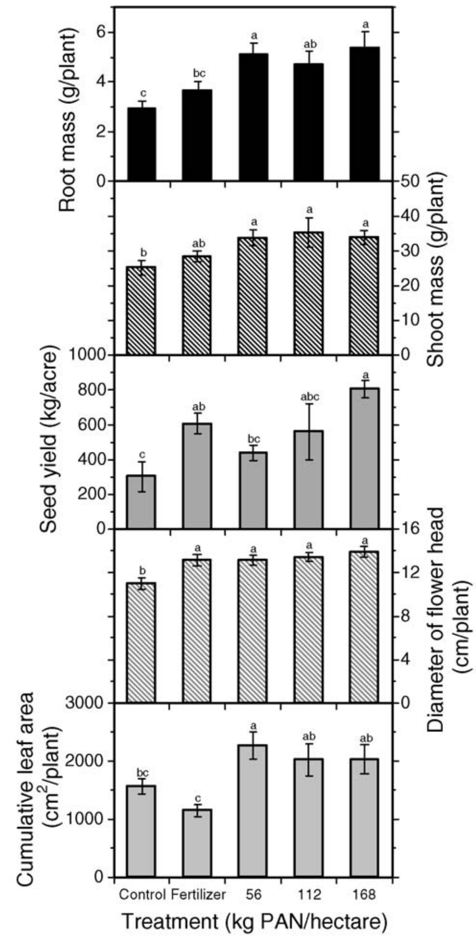

Fig. 2. Root mass, shoot mass, seed yield, flower head diameter, and cumulative leaf area for sunflower (*H. annuus* L. cv. Blazer) growing in untreated soil (control), soil with commercial fertilizer, and dairy sludge-scrubber by-product mixtures supplying 56, 112, and 168 kg PAN/ha. Vertical bars represent standard errors. Means with the same letter are not statistically different from each other at $p = 0.05$.

was 2- and 1.5-fold higher than the fertilizer and control treatments, respectively. Leaf areas were, however, not different among the sludge treatments and values for the 112 and 168 kg PAN/ha treatments were statistically similar to that of the control. There is a widely accepted view that leaf expansion for sunflower is the most important adaptive mechanism to stress (Hocking and Steer, 1983a,b; Connor and Sadras, 1992). Also, the primary impact of nitrogen fertilization is leaf area growth (Garcia et al., 1988).

3.6. Plant tissue analysis

The analysis of plant tissues is an important tool in elucidating the nutritional status of plants. For each nutrient there are critical threshold concentrations indicating deficiency or toxicity (Ulrich and Hills, 1973), the disparity between the two being different for each nutrient. The levels of each nutrient in plant tissues is useful in deducing whether the plant has an adequate supply of the nutrient, is deficient, or is experiencing toxic stress due to an excess of the nutrient. Shoot tissue concentrations of all the nutrients were within the sufficient ranges reported for sunflower by Hocking and Steer (1983a,b) and Blamey et al. (1997). Hocking and Steer (1983a,b) reported the following concentrations of nutrients in sunflower roots (mg/kg): 680 P, 6470 S, 1790 K, 1230 Mg, 5220 Ca, 1980 Cl. A comparison of these values with those of the present study indicates that the concentrations of these nutrients were generally higher across all the treatments in this study for K, Mg, Ca, and Cl (data not shown). The concentrations for K, Ca, S, Zn, Fe, Mn, and Cu were generally higher than values reported by Blamey et al. (1997). Conversely, however, Seiler (1986) reported the following concentrations in sunflower seeds (mg kg^{-1}): 30000 N, 6900 P, 8200 K, 3500 Ca, and 1800 Mg; while values reported by Hocking and Steer (1983a,b) were (mg/kg): 4300 P, 7600 K, 1200 Ca, 3000 Mg, and 4700 S. The ranges of the reported values for each nutrient in these studies encompass the nutrient values obtained in this study (data not shown). This suggests adequate sunflower seed nutrient composition.

4. Conclusions

The results of this study indicate that relative growth rates and net assimilation rates of sunflower seedlings were higher in plants growing in sludge-scrubber by-product treatments than in controls or soil treated with commercial fertilizer. Also, the relative nutrient accumulation rates of selected nutrients by the seedlings varied among treatments for each nutrient and increased concurrently with plant dry matter for some nutrients. The study also showed that sludge treatments did not significantly impact the photosynthetic apparatus of sunflower plants, as indicated by similar measures of quantum yield of photochemistry among treatments. Furthermore, the results of A/C_i and light–response analyses suggest that gas exchange parameters were not adversely impacted by the sludge applications. The tissue concentrations of selected elements were also within the ranges reported by other researchers. The morphological responses to the sludge applications were more pronounced than the physiological responses, as indicated by higher volume indices, cumulative leaf area, and plant yields obtained from sludge treated plants compared with control or fertilizer treatments.

The study, therefore, indicates that the sludge mix when applied at rates of 28–168 kg PAN/ha has the potential to be used as a soil additive in crop production without detrimental affects on plant physiological response or growth.

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