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ABSTRACT

Studies were conducted to evaluate if sludge amendment improves the characteristics of soil and supports plant growth. Spinach (*Spinacea oleracea*) var. All Green and cowpea (*Vigna unguiculata*) var. Pusa Komal plants were raised in pots containing nutrient deficient soil supplemented with different proportions (25% and 50%) of sludge. Application of sludge has been shown to improve the physico-chemical properties of the soils. Sludge increases the organic content, water holding capacity, cation exchange capacity (CEC) and enriches soil with macronutrients such as nitrogen (N), phosphorus (P), potassium (K) and sulphur (S). Increased nutrient availability promotes growth of plants as evidenced through biochemical estimations. The growth was significantly higher in soil amended with sludge in comparison to control. The present findings suggest that sludge can serve as a good soil supplement as it has capacity to improve soil properties. It can be used for improvement of nutrient depleted and degraded soils.

INTRODUCTION

Chemical supplements such as fertilizers have been used since decades in improving the soil nutrient status. Long term use of agrochemicals deteriorates soil health and pollutes the environment. Efforts are made to search for alternate such as organic/biomanures to reduce environmental contamination. Earlier studies suggest that sludge, a waste generated after wastewater treatment can be supplemented in soil of wastelands/degraded lands, agriculture lands since it is a rich source of organic matter and macronutrients such as nitrogen, phosphorus (Garcı´a *et al.*, 2000; Garcı´a-Gil *et al.*, 2004; Jamil *et al.*, 2006; Ferna´ndez *et al.*, 2009; Ahmed *et al.*, 2010). Sludge supplementation is known to improve the physical, chemical and biological properties of the soil. According to literature reports sludge has improved the properties of soil especially the nutrient content hence supporting the growth of plants. Increased growth response has been noted in crop plants such as *Zea mays*, *Corchorus*, *Educe*, *Raphanus*, *Spinacia, Hordeum vulgare* (Antolin *et al*., 2005; Ozyazıcı, 2013).

Studies have been undertaken to explore the utility of sludge as a partial substitute fertilizer. The interest in the replacement of synthetic agrochemicals with organic amendments has prompted the need for the present study (Al Zoubi *et al.,* 2008). The major objectives of the present study include (a) assessment of sludge as a soil supplement to improve the properties of nutrient deficient/degraded soil, (b) assessment of its effect on plant growth and productivity.

MATERIAL AND METHODS

Sludge and soil analysis

Air-dried sludge generated after wastewater treatment was collected from Common Effluent treatment Plant (CETP) located at Mayapuri, New Delhi, India. The sludge samples were air dried, homogenized, passed through a 2-mm sieve and analyzed for physico-chemical properties such as pH, water holding capacity (WHC), electrical conductivity (EC), total dissolved solutes (TDS), organic matter (OM), available potassium, nitrogen, phosphorus (Dhir and Srivastava, 2013). EC and pH values were measured from water extract (1:5 w/v) of samples using an electrical conductivity probe and a pH electrode using multiparameter meter (Orion star, Bench top meter, Thermo Fischer Scientific).

Soil organic matter (%) was determined with method of Kalra and Maynard (1991). The total organic carbon (TOC) was measured according to Walkley-Black wet oxidation method. Available nitrogen was estimated by Kjeldahl method. Potassium was measured following the protocol of Black *et al.* (1965). Heavy metals were determined in acid digested samples (reduce interferences by organic matter and to convert metal associated with particulates to a free form) using atomic absorption spectrophotometer (AA-6300, Shimadzu Corporation, Kyoto, Japan) (Lindsay and Norvel, 1978).

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Raising of plants

The sludge was mixed with soil to get 25% and 50% concentrations. The soil without any supplement was referred as the control (C). Crop plants viz. Spinach (*Spinacea oleracea*) var. All Green and cowpea (*Vigna unguiculata*) var. Pusa Komal were raised in pots containing soil amended with sludge in the concentrations of 25% and 50%. The plants were raised in outdoor natural conditions for 45 days.

Plant analysis

The plants were harvested and biochemical estimations were carried out in leaves.

Biochemical studies

Leaf samples were used for determination of biochemical attributes such as photosynthetic pigments, protein and sugar levels.

Photosynthetic pigments**:** Fresh leaves (1g) were suspended in DMSO. The suspension was incubated at 80°C for 2h and the pigment content was estimated spectrophotometrically at 645, 663 and 750 nm respectively. The amount of chlorophyll a, b and total chlorophyll were calculated and expressed as mg per g fresh weight of leaf (Hiscox and Israelstam, 1979).

Total soluble protein**:** Leaves homogenized in Tris-NaOH buffer (pH 7.0) were centrifuged at 12,000 g for 10 min. The supernatant was mixed with Bradford reagent and optical density was measured at 595 nm (Bradford, 1976).

Total soluble sugars**:** Soluble sugars were quantified following the phenolsulfuric acid method. Five hundred milligram of leaves was extracted in 80% (v/v) methanol heated to 70°C in water bath. The extract was then centrifuged at 5,000 × g for 10 min. The supernatant was used for the estimation of soluble sugar concentrations. The mixture consisted of 5% phenol and 98% sulphuric acid. The extract was cooled and the absorbance was determined at 490 nm using D-glucose as standard (Dubois *et al*., 1956).

Physiological analysis

The net photosynthetic rate was measured using Pulse Amplitude Modulator fluorometer (mini-PAM, Walz, Effeltrich, Germany) (Dhir and Srivastava, 2013). The instrument is equipped with a halogen lamp to provide actinic light and a leaf-clip holder with a light sensor to monitor incident photosynthetically active radiation (PAR). Actinic irradiance (PAR intensities between 0 and 900 μmol photons $m^{-2}s^{-1}$) provided the changes in chlorophyll *a* fluorescence signals. A saturation pulse (0.8s at 6.000 μmol m^{-2} s^{-1}) is applied for measurements. The parameters such as efficiency of photosystem II (Fv/Fm), photochemical (qp), non-photochemical (qN) quenching, photochemical efficiency of PSII (Y), the rate of electron transport (ETR) are recorded.

Statistical analysis

The data obtained as mean values from three independent experiments each with three replicates was subjected to Duncan's Multiple Range tests to check the level of significance differences between control and treated samples.

RESULTS AND DISCUSSION

Sludge showed the presence of heavy metals such as Cr, Zn, Ni, Pb, Cu in trace amounts. The nutrients such as N, P, K and S were present in high amounts. It showed features such as high organic carbon content and good water holding capacity (Table 1). The sludge amendment in soil altered the properties of soil (Table 2). Parameters such as water holding capacity, cation exchange capacity CEC), nutrient content (N, P, K, S) and organic carbon noted an enhancement in sludge amended soil (Table 2). Soil was enriched with nutrients and the improvement in soil properties after sludge amendment is in accordance with several previous reports (Mtshali *et al.,* 2014).

Table 1 Composition of sludge.

Parameters	Values
pH	6.3 ± 0.8
EC (mS/cm)	5.6 ±0.4
TDS (mgL^{-1})	2179 ± 23
Water holding capacity (%)	104 ± 11
Cation exchange capacity (meq/100g)	38.1 ± 6
Organic Carbon (%)	5.49 ± 0.9
Nitrogen (mg g^{-1})	0.39 ± 0.07
Phosphorus (mg g^{-1})	0.21 ± 0.07
Potassium (mg g^{-1})	0.40 ± 0.1
Sulphur (mg g^{-1})	1.71 ± 0.8
Metals (mg g^{-1})	
Cr	0.72 ± 0.08
Cd	0
Zn	0.22 ± 0.015
Ni	0.01 ± 0.003
Pb	0.30 ± 0.05
Co	0
Cu	0.11 ± 0.07

Table 2 Changes in the physico-chemical characteristics of soil after sludge amendment.

Parameters	Treatments		
	Soil	Soil with 25% sludge supplementation	Soil with 50% sludge supplementation
pH	6.5± 0.6[a]	6.8± 0.8[a]	6.8± 0.9[a]
EC (mS/cm)	0.071± 0.01[c]	1.33± 0.4[b]	3.8± 0.5[c]
TDS (mgL^{-1})	37± 3[a]	256± 10[b]	1133± 31[c]
Water holding capacity (%)	42.1± 4[a]	56 ± 3[ab]	106 ± 11[b]
Cation exchange capacity (meq/100g)	13.6 ± 2[a]	26.2 ±3[ab]	41.2 ± 3[b]
Organic Carbon (%)	0.15 ± 0.1[c]	0.88 ± 0.3[b]	2.18 ± 0.5[a]
Nitrogen (mg g^{-1})	0.04 ± 0.01[a]	0.12± 0.01[b]	0.22 ± 0.05[c]
Phosphorus (mg g^{-1})	0.007± 0.001[a]	0.188± 0.09[b]	0.257 ± 0.05[c]
Potassium (mg g^{-1})	0.08 ± 0.07[a]	0.315± 0.1[b]	0.500 ± 0.10[c]
Sulphur (mg g^{-1})	0.03 ± 0.01[a]	0.09± 0.03[ab]	0.15± 0.06[b]

Each value represents mean ± SE
Data followed by different letters in rows are significantly different at $p \leq 0.05$

The soil enrichment positively affected the plant growth. Plant height noted an increase in plants raised in sludge supplemented soil in comparison to control. The chlorophyll content showed an increase in sludge supplemented soil in both the plant species. Total soluble protein and sugar levels noted an increase in plants raised in soils amended with sludge in comparison to control. Among the physiological parameters, Fv/Fm, photochemical efficiency of chlorophyll a, showed no significant changes in any of the treatments with respect to control (Table 3, 4). Electron transport rate (ETR) and quantum yield of photosystem II [Y(II)] did not show any significant change in all the treatments. Photosystem II activity was not affected hence inducing no alteration in photosynthesis. The present results are in agreement with earlier findings in *Solanum, Lycopersicon* (Dhir and Rajam, 2015; Dhir 2016). The present findings are in agreement with earlier responses of plant growth improvement noted in several plant species including *Zea mays*, *Vigna radiata*, *Helianthus annus*, *Abelmoschus esculentus*, *Triticum*, *Vigna*, *Daucus*, *Lactuca*, *Raphanus*, *Spinacia* (Al Zoubi *et al*,. 2008; Tamrabet *et al.*, 2009; Singh and Agrawal, 2010; Roy *et al*,. 2010; Hbaiz *et al.*, 2014).

Table 3 Physiological and biochemical parameters measured in spinach.

Parameters	Treatments		
	Control	25%	50%
Plant height (cm)	23.5± 3[a]	24.6± 3[a]	25.3± 4[a]
Total chlorophyll (mg g^{-1} fresh wt.)	2.23 ± 0.5[a]	2.46 ± 0.6[a]	3.20 ± 0.4[b]
Total soluble protein (mg g^{-1} fresh wt.)	4.34 ± 0.8[a]	4.97 ± 0.7[a]	5.64 ±0.7[b]
Total soluble sugars (mg g^{-1} fresh wt.)	2.50 ± 0.8[a]	2.83 ± 0.8[a]	3.14 ± 0.5[b]
Fv/Fm	0.765 ± 0.08[a]	0.788 ± 0.08[a]	0.750 ± 0.09[a]
Y(II)	0.610 ± 0.05[a]	0.660 ± 0.07[a]	0.627 ± 0.09[a]
ETR	48 ± 3[a]	52 ± 4[a]	49 ± 4[a]

Each value represents mean ± SE
Data followed by different letters in rows are significantly different at p≤0.05
ETR-represents electron transport rate
Fv/Fm- Efficiency of photosystem II
Y(II)–Quantum yield of photosystem II

Table 4 Physiological and biochemical parameters measured in cowpea.

Parameters	Treatments		
	Control	50%	50%
Plant height (cm)	32 ± 2[a]	33.4± 3[a]	34.1± 3[a]
Total chlorophyll (mg g^{-1} fresh wt.)	1.02 ± 0.3[a]	1.88 ± 0.3[a]	2.11 ± 0.4[b]
Total soluble protein (mg g^{-1} fresh wt.)	2.14 ± 0.8[a]	2.88 ±0.8[a]	2.80 ±0.73[a]
Total soluble sugars (mg g^{-1} fresh wt.)	1.59 ± 0.7[a]	1.89 ± 0.8[a]	2.14 ± 0.9[b]
Fv/Fm	0.737 ± 0.08[a]	0.751 ± 0.08[a]	0.739 ± 0.1[a]
Y(II)	0.561 ± 0.1[a]	0.589 ± 0.08[a]	0.554 ± 0.1[a]
ETR	44 ± 3[a]	47 ± 3[a]	44 ±4[a]

Each value represents mean ± SE
Data followed by different letters in rows are significantly different at p≤0.05
ETR-represents electron transport rate
Fv/Fm- Efficiency of photosystem II
Y(II)–Quantum yield of photosystem II

CONCLUSION

The present study demonstrates that sludge supplementation till 50% improves soil nutritional quality. The nutrients as well as organic matter present in sludge improve soil fertility, which supports plant growth. It can be suggested that sludge could partly substitute for fertilizer if applied in the right amounts to soil as it can replenish nutrients in the soils depleted of macro-nutrients like N, P, K and S. Further studies for optimization of dose and frequency of sludge supplementation in soil are required. The composition (presence of toxic contaminants such as microbes, metals) of sludge is crucial for deciding response of the plants. Therefore reuse of sludge as soil supplement can prove to be a sustainable approach for its management.

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