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Soil Amendment with Municipal Sludge Does not Alter the Physiological Status of *Solanum melongena*

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Abstract

Investigations were carried to evaluate the effect of municipal sludge amendment in soil on physiological status of crop plant- *Solanum melongena* var. Pusa Hybrid 6. Preliminary studies proved sludge to be non-phytotoxic. The germination index (GI), the radicle and hypocotyl length did not exhibit differences between control and sludge treated samples hence reflecting the non-toxic nature. Soil supplementation with sludge in different proportions does alter the physiological status of plants. The parameters such as efficiency of photosystem II (Fv/Fm), electron transport rate (ETR), quantum yields of photosystem II (Y) did not show much differences within the treatments. Chlorophyll content and sugar levels also did not showed much differences in plants rose in sludge supplemented soil and control. The present studies demonstrated that sludge can be utilized as a soil supplement as it improves soil properties without altering the physiological status of plants.

Keywords: Photosynthesis; Phytotoxicity; *Solanum;* Sludge; Toxicity

Introduction

Sludge is a by-product (insoluble residue) generated from wastewater treatment after aerobic or anaerobic digestion processes or industrial practices. Sludge comprises of organic matter, macronutrients such as nitrogen, phosphorus, potassium, calcium, magnesium and non-essential trace elements, and microorganisms. The quantity of sludge generated around the world is estimated to be around 10 million tons (dry solids) and its disposal is a issue of major concern because of pathogenicity and toxicity. Sludge may contain many toxic substances such as heavy metals, pesticides, organics, hormone disruptors, detergents and various salts that might cause risk to human and animal health [1].

Most of the sludge is disposed in landfills (both surface and subsurface). Alternatively it is used for land reclamation, horticulture, landscaping, and energy recovery. According to an estimate, about 40% of the total sludge generated is put to agricultural use in many developing countries around the world. Its use is completely banned in developed countries because of stringent policies. Land application of sewage sludge has been tried as an effective disposal method and practised throughout Canada, the United States and Europe from last few years. Soil supplementation and in agricultural lands has been demonstrated in earlier studies [2]. The macro and micronutrients present in the sludge enrich the soil, organic matter cause enhancement in the soil microbial activity bringing the positive effect on plant growth and productivity [3].

India produces around 450-500 tonnes of sludge every year and its disposal is also an issue of major concern. Soil supplementation can be a viable and eco-friendly option for sludge disposal in developing countries like India. Therefore, the present studies aimed at assessing the utility of sludge as a soil supplement and test its effect on soil properties and the physiological status of plants.

Material and Methods

Physico-chemical characteristics of sludge

Air-dried sludge was collected from Common Effluent treatment Plant (CETP) located at Mayapuri, New Delhi, India. The physicochemical characterization was done. The parameters studied include pH, electrical conductivity (EC), total dissolved solutes (TDS), metal content, organic matter, carbon, nitrogen, phosphorus, potassium content.

Phytotoxicity assay

1 g of air dried sludge was dissolved in 10 ml sterilized water. Five ml of the extract was used for moistening Whatman No. 3 filter paper placed in a petri dish. Ten seeds of *Solanum melongena* (brinjal) var. Pusa Hybrid 6 were placed on it. Each dish was covered and placed in the germination chamber at 22 ± 2°C for 120 hours in darkness [4]. Radicles and hypocotyls emerging from seeds were measured with a metric ruler after the germination period. Germination index (GI), and vitality index (VI) were calculated for each treatment using the following equation: GI =$\sum$ Gt/Dt, where Gt is the number of germinated seeds at time t, and Dt represents the corresponding day of germination [5].

$$\text{Seed germination (\%)} = \frac{\text{Number of seeds germinated x 100}}{\text{Total number of seeds}}$$

VI = S•GI, where S is the length of seedlings.

Experimental set up

Clay loam garden soil was used for the experiment. The sludge was air-dried, crushed to pass a 2 mm sieve, and dry sludge was mixed with soil to have four sludges soil mixtures representing the ratios 1:10 (S1), 1:5 (S2) and 1:2 (S3) of the mixtures. The soil mixture with ratio 0% served as the control. Ten seeds of brinjal were place in each pot containing two kilograms of soil-sludge mixtures (25 cm in diameter and 30 cm in depth). The plants were watered with tap water every other day.

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Soil analysis

Soil samples taken from the surface at a depth of 10 cm were air dried, homogenized, passed through a 2-mm sieve and analyzed for physico-chemical properties as described earlier [6]. The physical parameters such as pH, water holding capacity (WHC), electrical conductivity (EC), total dissolved solutes (TDS), organic matter (OM), available potassium, nitrogen, and phosphorus were evaluated. The heavy metals were measured with Atomic Absorption Spectrophotometer.

Plant analysis

Total chlorophyll content: One gram (fresh weight) leaves were homogenized in 10 ml of 80% acetone and the homogenate was centrifuged at 10,000 g for 10 min. The optical density of the supernatant was measured spectrophotometrically at 645, 663, and 750 nm (Smart Spec Plus, Bio-Rad, USA) as described by Arnon [7].

Photosynthetic rate: The photosynthetic rate was measured using mini-portable Pulse Amplitude Modulator (PAM) (mini-PAM, Walz, Effeltrich, Germany). 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). With increasing actinic irradiance (PAR intensities between 0 and 900 µmol photons $m^{-2}s^{-1}$), the changes in chlorophyll *a* fluorescence signals are measured. A saturation pulse (0.8s at 6.000 µmol m^{-2} s^{-1}) is applied and the parameters including the 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 [6].

Statistical Analysis

Mean values from three independent experiments each with three replicates were subjected to Duncan's Multiple range test to measure level of significance of differences observed between control and treated samples.

Results and Discussion

Phototoxicity analysis

Sludge can prove dangerous for crops/vegetation, humans, animals and the environment hence it becomes mandatory to assess the phytotoxicity [8,9]. The phytotoxicity or ecological risks is assessed using plant growth test based bioassays [10,11]. According to literature, the parameters such as seed germination are sensitive to any toxicity or environmental stress. In the present investigation, seed germination (%) was not affected in sludge treated samples. Other parameters such as germination index, length of radical, and hypocotyl also did not exhibit any significant differences from control suggesting the non-phytotoxic nature of sludge (Table 1).

Sludge and soil analysis

Sludge characterization revealed the presence of high organic carbon, nutrients such as nitrogen, phosphorus, potassium. The heavy metals were not present in traceable levels sludge (Table 2). Soil amendment with sludge improved soil properties including water holding capacity, cation exchange capacity (CEC), organic carbon, electrical conductivity (EC) organic matter (%) and nutrients such as nitrogen, phosphorus, potassium. The soil pH however, did not show much change (Table 2). The increase in the nutritional status of the soil is in accordance with previous studies where high organic matter and macro and micronutrients present in the sludge have modified/improved the physico-chemical properties of soil [12,13]. The macronutrients in the sludge increase nutritional status, while organic constituents provide beneficial soil conditioning properties [1]. These modifications in soil have benefited agricultural soils through increased availability of nutrients hence causing increased plant productivity [14]. Sludge amendment at lower rates has been shown to improve growth in measured in term of shoot and root lengths, number and area of leaves in *Zea mays, Vigna radiata, Helianthus annus, Abelmoschus esculentus* [15] and the major reason for growth improvement in these plants was higher availability of nutrients in soil.

Photosynthetic rate

Studies showed no change in F0 and Fv (Table 3). This indicated no PSII inactivation and change in non-photochemical quenching process or close to the reaction center. The Fv/Fm ratio, which characterizes the maximal quantum yield of the primary photochemical reactions in dark adapted leaves also did not changed significantly in plants grown in sludge supplemented soils. The proportion of energy driven to the photochemical (qP), non-photochemical quenching (qN) and quantum yield of electron transport Y showed no change at pre-flowering stage. However, the non-photochemical process showed a decline at flowering stage. Electron transport rate (ETR) showed increment in plants raised in sludge supplemented soils. Since there was no decrease in this parameter hence photosystem II activity was not affected apparently indicating no down-regulation of photosynthesis.

Pigment and sugar level: The pigment contents of the plants showed slight increase in plants raised in sludge amended soils. Increased pigments levels are also correlative of increase in photosynthetic rates. Total soluble sugars of plants raised in sludge amended soils were positively affected. These observations are in accordance with earlier studies in wheat, maize, mungbean, *Daucus*, *Lactuca*, *Raphanus, Spinacia* [16,17]. Since photosynthesis was not affected hence the level of leaf total soluble sugars was maintained in plants grown in sludge supplemented soils.

The results of this study suggests that amendment of sludge in soil at application rate of below 50% may be a good option for use as a soil fertilizer positive effects on plants physiological status, though number of environmental, physiological and genetically reasons also decide the threshold concentration of sludge use in agriculture for different plants.

Parameters	Treatments	
	Sludge	Control
Germination index	5 ± 0.98 ^{a}	5 ± 1.02^{a}
Vitality index	21 ± 4^{a}	20.5 ± 3.8^{a}
Length of radicle (cm)	3.2 ± 0.6^{a}	3 ± 0.5^{a}
Length of hypocotyl (cm)	1.8 ± 0.3^{a}	1.5 ± 0.3^{a}

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

Table 1: Germination index and vitality index of plants under different treatments.

Parameters	Physiochemical characteristics				
	Sludge	Control soil	S1 soil	S2 soil	S3 soil
pH	6.8	6.5	6.8	6.8	6.8
EC (mS/cm)	3.6	0.067	0.27	0.34	0.48
TDS (mgL^{-1})	1769	34	128	165	189
Water holding capacity (%)	114.27	40.12	42.14	44.04	47
Cation exchange capacity (meq/100 g)	46.8	16.6	19.9	23.3	26.2
Organic Carbon (%)	5.52	0.16	0.98	1.15	1.98
Nitrogen (Kg/ha)	289	56	67	79	89
Phosphorus (Kg/ha)	335.06	6.25	69	158	255
Potassium (Kg/ha)	664	174	288	408	479

S1- 1/10 of soil supplemented with sludge
S2- 1/5 of the soil supplemented with sludge
S3- ½ of the soil supplemented with sludge

Table 2: Physico-chemical characters of sludge and soil before and after sludge supplementation.

Growth stage	Parameters	Treatments			
		control	S1	S2	S3
Pre-flowering					
	Chlorophyll (µg g^{-1} fresh wt.)	383 ± 78[a]	423 ± 88[b]	390 ± 78[a]	378 ± 69[a]
	Sugars (µg g^{-1} fresh wt.)	933 ± 89[a]	976 ± 87[a]	989 ± 99[a]	1003 ± 89[a]
	Fv/Fm	0.764 ± 0.1[a]	0.773 ± 0.1[a]	0.785 ± 0.1[a]	0.781 ± 0.1[a]
	ETR	47 ± 6[a]	46 ± 7[a]	50.2 ± 8[a]	48 ± 6[a]
	qp	0.798 ± 0.1[a]	0.776 ± 0.1[a]	0.784 ± 0.1[a]	0.814 ± 0.1[a]
	qN	0.130 ± 0.07[a]	0.144 ± 0.09[a]	0.124 ± 0.05[a]	0.133 ± 0.04[a]
	Y (II)	0.588 ± 0.1[a]	0.577 ± 0.1[a]	0.646 ± 0.1[b]	0.606 ± 0.09[a]
Flowering					
	Chlorophyll (µg g^{-1} fresh wt.)	1136 ± 123[a]	1188 ± 118[a]	1201 ± 122[a]	1098 ± 198[a]
	Sugars (µg g^{-1})	1633 ± 89[a]	1676 ± 88[a]	1685 ± 79[a]	1603 ± 89[a]
	Fv/Fm	0.780 ± 0.1[a]	0.780 ± 0.1[a]	0.773 ± 0.1[a]	0.776 ± 0.1[a]
	ETR	45 ± 5a	48 ± 6[a]	43.9 ± 4[a]	43.2 ± 4[a]
	qp	0.793 ± 0.1 [a]	0.795 ± 0.1[a]	0.865 ± 0.2[b]	0.751 ± 0.1[a]
	qN	0.210 ± 0.07 [a]	0.139 ± 0.08 [ab]	0.089 ± 0.01[b]	0.178 ± 0.5[ab]
	Y(II)	0.573 ± 0.09[a]	0.602 ± 0.1[ab]	0.657 ± 0.1[b]	0.549 ± 0.08[a]

S1- 1/10, S2- 1/5, S3- 1/2
Each value represents mean ± SE
Data followed by different letters in columns are significantly different at $p \leq 0.05$
Efficiency of photosystem II (Fv/Fm), Photochemical quenching (qP), non-photochemical quenching (qN), electron transport rate (ETR), quantum yield of electron transport (Y (II))

Table 3: Alterations in parameters after different treatments measured during different growth stages of plant.

Conclusions

Preliminary results suggested that sludge could partly substitute for fertilizer in crop production. The macro and micro nutrients as well as organic matter present in sludge help in improving soil condition and plant growth. Optimization of dose and frequency of sludge supplementation in soil is required to implement the use of sludge supplementation in soil. Long term investigations and field studies are required to validate the findings.

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