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Potential of sewage sludge towards germination, growth and chlorophyll content in *Vigna radiata* L. at Prayagraj, Uttar Pradesh, India

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ABSTRACT

The use of sewage sludge in agriculture is an alternative strategy to reduce waste production. The goal of this study was to evaluate the impact of sewage sludge (SS) from various sewage treatment plants (STPs) on seed germination, growth metrics, and chlorophyll content in *Vigna radiata* L. var. Samrat. The experiment was first carried out under controlled conditions, then in the Zaid season at the nursery of forestry Department, SHUATS, Prayagraj, a field experiment with seven treatments and three replications was conducted. Sludge's initial physico-chemical examination had revealed greater EC, lower pH, and more macro- and micronutrients as well as heavy metals over control. According to the results of the controlled experiment, there was less seed germination, shoot length, and seedling vigour index compared to the control. At lower concentrations, data in a field trial indicated good results, but at 45 and 60 days after sowing, substantial reductions in several growth metrics and chlorophyll content were seen at greater concentrations with various sludge applications as compared to the control.

Key words: *Vigna radiata L., Germination percent, Sewage sludge, Heavy metals, Waste management*

Introduction

The management and disposal of SS provide particularly challenging environmental issues in the current era of modernisation or urbanisation. Additionally, although sewage sludge (SS) is a helpful fertiliser, it may also include heavy metals that harm the ecosystem and reduce production. The typical techniques for getting rid of SS include soil filling, land application, and incineration. The most common and preferred disposal option among these is landfilling. Industrial and home sewage has also been employed in a number of agricultural production techniques since it contains significant amounts of macro and micronutrients as well as organic components (Warman *et al*., 2005).

According to several researches, using SS for agricultural areas has both beneficial and detrimental effects (Bazai *et al*., 2006). The sludge from sewage can also be used as manure to grow plants, however frequent usage of sludge in large dosages can cause environmental issues such the building of heavy metals and harmful chemicals in soils, which then affect plants, animals, and water bodies. The physiological and metabolic processes of the several crops tested by Singh and Agrawal were significantly impacted by the accumulation of heavy metals in the soil brought on by the addition of sewage sludge.

At various rates of sewage sludge amendments in the soil, favourable impacts on dwarf bean yield components and flax growth and productivity have been recorded by Theodorates *et al.* (2000) and Tsakou *et al.* (2002) respectively.

The most common pulse is the gram, which accounts for about 40% of all Indian pulse production. *Vigna radiate* L. contributes 8–10% of all gram production. In order to assess the impact of STP sewage sludge treatedon agricultural fields on *Vigna radiata* L. var. Samrat in the Prayagraj district of Uttar Pradesh, no such data are available for the various places.

Keeping this in view, this study was designed to evaluate the potentian of various STP's sewage sludge towards germination, growth and chlorophyll content of *Vigna radiata* L. at Prayagraj, Uttar Pradesh.

Materials and Methods

Study areas and sample collection

The field experiment was conducted at the nursery of Forestry department, SHUATS, Prayagraj, Uttar Pradesh. Mean ambient temperature varied from 20 ^{o}C to 43 ^{o}C during the experimentation period from March to June, 2018. Sewage sludge was collected from several STPs in Prayagraj, U.P., including the pikh patti area near the ancient bridge (STP A1), Rajapur (STP A2), and Kodra, Pritam Nagar (STP A3).

Experimental design

In order to assess the effect on seed germination, shoot length, and seedling vigour index, the experiment was first conducted under carefully controlled laboratory conditions using a petri dish filled with three areas of STP of sludge. The seeds were sterilized for 10 minutes with sodium hypochlorite (2%) and then washed with distilled water (DW). For seed germination investigations, 20 healthy seedlings were placed in a sterile petri dish along with wet (DW) tissue paper that had been filled with 5 gramme of soil (control) and sludge.

Experiment second was set up in randomized block design with seven treatments and three replications, (twenty-one plots of 2 m × 2 m size) for guiding sewage sludge application at different levels collected from different STPs. Collected sewage sludge was used according to their treatment combinations (mixed before sowing at @ of 10t ha^{-1} and 15t ha^{-1}, respectively in each plot). Treatment was designed T_0 (Control), T_1 (A_1+10t ha^{-1}SS), T_2 (A_1+15t ha^{-1}SS), T_3 (A_2+10t ha^{-1}SS), T_4 (A_2+15t ha^{-1}SS), T_5 (A_3+10t ha^{-1} SS) and T_6 (A_3+15t ha^{-1}SS). The tested variety of *Vigna radiata* L. var. Samrat was used for experiments then after maintaining identical moisture levels in plots, seeds were sown manually with spacing row to row and plant to plant was (30x10 cm). Irrigation and manual weeding were done when required.

Soil and sewage sludge analyses

Analyze the initial physiochemical characteristics of the soil and the three locations where sewage sludge from STPs is present. After being crushed and put through a sieve with a mesh size of 2 mm, the samples were air dried and stored separately for subsequent examination. The pH of samples was measured in the suspension of 1:2(w/v) with the help of a pH meter and electrical conductivity (EC) by conductivity meter. Organic C (%) in the soil and sewage sludge were analysed from wet oxidation methods Walkley and Black (1947). A spectrophotometer analyzer (ModelKB 8S, Germany), respectively, $NaHCO_3$ extraction method was used for available phosphorous (P) analysis by Olsen et al. (1954) and potassium (K) was determined by Toth and Prince (1949). For analyses of micronutrients and heavy metals,the acid digestion method is as follows (HNO_3:H_2SO_4:$HClO_4$, 3:2:1) Allen *et al.* (1986). Concentrations of micronutrients (Fe, Cu, Mn and Zn) and heavy metals (Cr, Cd, Pb and Ni) in various sludge were analyzed by using a Flame Atomic Absorption Spectrophotometer (Perkin Elmer, A. Analyst 400) on a dry weight basis.

Seed germination and seedling vigor index

Three different STPs sewage sludge were taken for determination of seed germination, shoot length and seedling vigor index (*Vigna radiata* L.). Note the number of germinated seeds in each petri dish to calculate the germination percentage by using formula (a), for shoot length of seedlings measured by scale (cm) and seedling vigor index calculated respectively by using the formula (b) given by Abdul and Anderson (1973).

$$\text{(a) Seed germination percent} = \frac{\text{Number of seeds germination}}{\text{Total number of seeds sown}} \times 100$$

(b) Seedling vigor index = Germination (%) × Total seedlings length (cm)

Analysis of growth parameters

Plants were sampled randomly from each treatment plot to take average data of all replications at the time interval of 15 DAS. Plants height and root length were measured at 30, 45 and 60 DAS intervals, respectively in (cm plant^{-1}). The fresh and dry weights were recorded at different times interval, washed to removed soil particles and oven-dried at 80 ^{o}C till a constant weight was achieved. The number of nodules and number of branches was measured at 45 DAS. Chlorophyll content was determined by SPAD-502 (Minolta, Tokyo, Japan) at 30 & 60 DAS.

Statistical analysis

The data were subjected to one-way analysis of variance (ANOVA) using the computer software package SPSS with Duncan's multiple range tests were performed as post hoc to test the significance of the difference between the treatments.

Results and Discussion

The initial analysis of the physicochemical characteristics of the soil and several STPs' sewage sludge areas (pH, EC, OC, accessible N, P, K, Zn, Cu, Fe, Mn, Cr, Pb Cd, Ni) was provided in (Table 1). Different STPs parameters' physico chemical characteristics differ significantly. However, pH was recorded as the lowest and EC was increased in all STP samples compared to control. The different SS exhibited somewhat acidic pH (ranging from 5.9 to 6.4) with higher EC (ranging from 1.08 mS cm^{-1} to 1.78 mS cm^{-1}). Similar results have been reported by numerous other researchers as well. Mazeed *et al.* (2020) discovered that low soil pH at various concentrations of sewage sludge may be responsible for the release of humic acid owing to the biodegradation of organic carbon-rich sewage sludge. The results of the current investigation demonstrated that all STP sludge had higher amounts of organic C percent, accessible macronutrients (N, P, K), micronutrients (Fe, Cu, Mn, Zn) and heavy metals (Cr, Cd, Pb, Ni) than the control. According to Sharma *et al.* (2006) and Kesherwani *et al*. (2020), the pH and EC concentrations were affected by the high levels of heavy metal concentrations present in sludge. Reduced soil pH in the receptor soil increased the availability of the heavy metals at higher concentrations of sewage sludge. Thus, in the Naini (A1) and Rajapur (A2) STPs samples, only Cd was above the allowable level. Similar to this, Latare *et al.* (2014) showed that excessive (20 t ha) or greater levels of

Table 1. Physio-chemical parameters of soil and different areas (STPs) of sewage sludge.

Parameters	Control Soil	Naini STP (A_1)	Rajapur STP (A_2)	Pritamnagar STP (A_3)
pH (1:2)	7.2^{d}	5.9^{b}	6.0^{b}	6.4^{c}
EC (mS cm^{-1})	0.34^{a}	1.19^{b}	1.78^{c}	1.08^{b}
Organic C (%)	0.40^{a}	8.50^{c}	12.41^{d}	6.68^{b}
Avail. N(mg kg^{-1})	23.98^{a}	486.02^{c}	591.54^{d}	460.64^{b}
Avail. P (mg kg^{-1})	5.51^{a}	32.88^{c}	30.51^{b}	47.44^{d}
Avail. K (mg kg^{-1})	71.66^{a}	348.00^{b}	340.66^{b}	373.36^{b}
Fe (g kg^{-1})	5.59^{a}	13.26^{d}	8.20^{b}	14.03^{e}
Cu (mg kg^{-1})	8.74^{a}	226.00^{e}	118.33^{d}	29.26^{b}
Mn (mg kg^{-1})	89.33^{a}	155.66^{b}	88.88^{a}	266.00^{b}
Zn (mg kg^{-1})	23.26^{a}	114.33^{c}	25.60^{a}	37.79^{b}
Cr (mg kg^{-1})	2.40^{a}	96.44^{d}	25.74^{b}	36.18^{c}
Cd(mg kg^{-1})	0.07^{a}	222.66^{c}	121.33^{b}	0.32^{a}
Pb (mg kg^{-1})	1.16^{a}	25.13^{e}	13.53^{d}	3.46^{b}
Ni (mg kg^{-1})	9.27^{a}	21.00^{d}	13.13^{b}	13.13^{b}

Control soil, A_1: (Naini STP), A_2: (Rajapur STP), A_3: (Pritamnagar STP), DAS: (Days after showing). Different letters in each group show significant differences at $p<0.05$.

sludge application increased the heavy metals contents in soil and plants as well as in sludge disposal land, and the Cd content in rice grain was beyond the Indian safe limit.

The germination % and seedlings lengths were observed significant reduction (Table 2) by 76% and 5.16 cm, 88% and 4.50 cm, 93% and 5.26 cm, respectively recorded in various STPs sludge applications as compared to control. In seedling vigour index also reduced from 396 to 464 as compared to control. The same research, which was previously reported by Indra and Sivaji (2006), found that the excess amounts of micronutrients, heavy metals and hazardous compounds present in SS decreased seed germination, shoot length, and seedling vigour index of *Vigna radiata* L. A similar finding also reported that high salinity might cause physiological stress on the seedlings (Neelam and Sahai, 1998).

In growth parameters, shoot length increased at a lower concentration of SS (10t ha^{-1}) at all intervals of DAS compare to a higher concentration (15t ha^{-1}). Shoot length, at the initial stage (30 DAS) no signifi-

Table 2. Effect of different areas (STPs) of sewage sludge on seed germination (%), shoot length and seedling vigor index of *Vigna radiata* L.

Treatments	Seed germination percentage	Shoot length (cm)	Seedling vigor index
Control soil	100.00^{e}	13.40^{c}	1273.33^{c}
Naini STP (A_1)	76.66^{b}	05.16^{b}	396.53^{b}
Rajapur STP (A_2)	88.33^{c}	4.50^{b}	420.33^{b}
Pritamnagar (A_3)	93.33^{d}	5.26^{b}	464.30^{b}

Control soil, A_1: (Naini STP), A_2: (Rajapur STP), A_3: (Pritamnagar STP). Different letters in each group show significant differences at $p<0.05$.

Table 3. Effect of different areas (STPs) of sewage sludge on growth parameters (shoot length, root length, total fresh and dry weight) of *Vigna radiata* L.

Days after sowing	Treatments	Shoot length (cm plant^{-1})	Root length (cm plant^{-1})	Total fresh weight (g plant^{-1})	Total dry weight (g plant^{-1})
30 DAS	T_0 Control	28.00^{a}	09.30^{a}	5.29^{bc}	0.99^{a}
	T_1 (A_1+10t ha-^{1}SS)	27.62^{a}	11.27^{c}	5.05^{bc}	1.14^{b}
	T_2 (A_1+15t ha-^{1}SS)	27.03^{a}	09.36^{a}	3.96^{a}	1.05^{b}
	T_3 (A_2+10t ha-^{1}SS)	26.40^{a}	10.46^{b}	5.83^{c}	1.18^{b}
	T_4 (A_2+15t ha-^{1}SS)	27.63^{a}	11.01^{bc}	4.05^{a}	1.16^{b}
	T_5 (A_3+10t ha-^{1}SS)	27.40^{a}	15.20^{d}	8.19^{d}	1.48^{c}
	T_6 (A_3+15t ha-^{1}SS)	27.13^{a}	09.30^{a}	4.61^{ab}	0.83^{a}
45DAS	T_0 Control	55.14^{b}	12.90^{ab}	25.20^{c}	5.80^{c}
	T_1 (A_1+10t ha-^{1}SS)	43.60^{a}	12.10^{a}	14.23^{a}	4.45^{ab}
	T_2 (A_1+15t ha-^{1}SS)	39.80^{a}	13.71^{ab}	12.60^{a}	3.76^{a}
	T_3 (A_2+10t ha-^{1}SS)	44.90^{a}	14.00^{ab}	15.73^{a}	4.43^{ab}
	T_4 (A_2+15t ha-^{1}SS)	44.70^{a}	12.11^{a}	14.40^{a}	4.33^{ab}
	T_5 (A_3+10t ha-^{1}SS)	46.36^{ab}	15.36^{b}	20.50^{b}	5.26^{bc}
	T_6 (A_3+15t ha-^{1}SS)	42.45^{a}	12.50^{ab}	15.53^{a}	3.96^{a}
60 DAS	T_0 Control	67.33^{a}	18.13^{a}	42.06^{e}	11.33^{d}
	T_1 (A_1+10t ha-^{1}SS)	65.06^{a}	18.53^{a}	25.30^{cd}	5.93^{b}
	T_2 (A_1+15t ha-^{1}SS)	52.93^{a}	17.66^{a}	17.13^{a}	5.06^{a}
	T_3 (A_2+10t ha-^{1}SS)	60.93^{a}	18.46^{a}	20.70^{ab}	8.46^{c}
	T_4 (A_2+15t ha-^{1}SS)	59.20^{a}	17.26^{a}	19.33^{ab}	5.96^{b}
	T_5 (A_3+10t ha-^{1}SS)	67.73^{a}	18.85^{a}	28.63^{d}	9.06^{c}
	T_6 (A_3+15t ha-^{1}SS)	56.13^{a}	17.53^{a}	22.70^{bc}	6.20^{b}

T_0: (control soil), A_1: (Naini STP), A_2: (Rajapur STP), A_3: (Pritamnagar STP), SS: (sewage sludge), DAS: (Days after showing). Different letters in each group show a significant difference and similar letters in each group show not very significant differences at $p<0.05$.

cant differences were noted (Table 3), however at 45 and 60 DAS shoot length recorded a slightly significant reduction, Overall the decrease was recorded in shoot length of *Vigna radiata* at an amendment rate of SS (15t ha^{-1}) at 60 DAS as compared to a lower concentration. A similar finding was also studied by Sinha *et al.* (2008) that significant increments in growth parameters of two varieties of *Vigna radiata* at lower tannery sludge but reduced at a higher ratio. The present study also shows the reductions in growth parameters at the highest amendment rate of SS (15t ha^{-1}). It was evident that higher rates of sludge with long day application decreased shoot length in various crops revealed by Latare *et al.* (2014).

Root length of *Vigna radiata* recorded increment in both concentrations (10t ha^{-1} and 15t ha^{-1}) at different time intervals of 15 days. Root length at initial stage 30 DAS showed a significant difference with increment at lower concentration of SS in T_1 and T_5 was increased by 17.48% and 38.81% respectively as compared to control. At 45 DAS root length was significantly increased by 16.02% in T_5 compared to T_0 and at 60 DAS 3.82% increase was observed in T_5 compared to control (Table 3) the reduction in root length might beas the decomposition and release of nutrients from sludge was take time thus its effect was more visible after 45 and 60 days. Singh and Sinha (2004) reported that significant increment in root length of mustard at lower tannery sludge amendment ratios, but very low incensement was observed athigher ratio with a long period, others also reported similar results of SS on different crops (Singh and Agrawal, 2010).

Fresh weight of *Vigna radiata* showed significant differences in treatments as well as in all time intervals of (30, 45 and 60 days) compared to control T_0. It was founded that the maximum increment was recorded by 35.45% in T_5 compared to T_0 at early stage 30 DAS but the result also observed that in treatment T_1 and T_5 with a lower concentration of SS observed increment by 21.6 % and 43.7% respectively compared to T_2 and T^6 at higher concentration of SSat 30 DAS. Overall reduction was observed at 45 and 60 DAS compared to control but T_1, T_3 and T_5 showed increment as lower concentration as compared to T_2,T_4 and T_6 at 45 DAS. However, T_1 increased by 32.3 % followed by T_5 (20.7%) as compared to T_2 and T_6, respectively at 60 DAS similar studies also reported by Eid *et al*. (2017).

In this study, the dry weight of *Vigna radiata* showed significant differences intreatments at various days after sowing at 30, 45 and 60 as compared to control. At the primary stage, (30 DAS) dry weight production was higher in treatment T_5 which was observed maximum increased by 33.10% except decrease was recorded in T_6 but after 45 & 60 days dry weight showed a significant reduction in other treatments as compared to T_0 still T_5 showed increased at lower concentration of SS application at 45 and 60 days. Similar results were also reported by Smith (1992) that higher availability of heavy metals in the soil at higher SS amendment rate reduces the magnitude of increment in biomass at different rates at the early application but others also revealed that SS application increased morphometric parameters and biomass but with higher concentration, decreased the cucumber shoot height and total biomass after long days application Eid *et al*. (2017).

In *Vigna radiata*, number of root nodules and

Table 4. Effect of different areas (STPs) sewage sludge on growth parameters (number of root nodules and number of branches) of *Vigna radiata* L.

Days after showing	Treatments	Number of root nodules plant $^{-1}$	Number of branches plant $^{-1}$
45 DAS	T_0 Control	53.76^{e}	7.00^{b}
	T_1 (A_1+10t ha-^{1}SS)	35.50^{cd}	5.33^{a}
	T_2 (A_1+15t ha-^{1}SS)	28.33^{b}	5.66^{a}
	T_3 (A_2+10t ha-^{1}SS)	33.33^{bc}	5.66^{a}
	T_4 (A_2+15t ha-^{1}SS)	21.33^{a}	5.00^{a}
	T_5 (A_3+10t ha-^{1}SS)	41.83^{d}	6.00^{ab}
	T_6 (A_3+15t ha-^{1}SS)	36.33^{cd}	4.66^{a}

T_0:(control soil), A_1:(Naini STP), A_2:(Rajapur STP), A_3:(Pritamnagar STP),SS:(sewage sludge), DAS:(Days after showing). Different letters in each group show a significant difference and similar letters in each group show not a very significant difference at $p<0.05$.

number of branches were counted at 45 DAS, showed in (Table 4). The results have shown a significant reduction in treatments as compared to control but an increase was recorded in T_5 at lower concentration of SS as compared to other treatments. Overall, it was observed that increasing the levels of sludge application decreased the root nodulations. The reduction in nodulation may be due to the availability of soluble salts and heavy metals in sewage sludge that was toxic to Rhizobia. A similar reduction was noticed by several researchers Giller *et al.* (1989) and Madariaga *et al*. (1992).

In this study chlorophyll content of *Vigna radiata* showed significant differences in treatments at 30 and 60 DAS (Table 5). The top, middle and lower leaves of the crop at 30 DAS showed higher chlorophyll content however at 60 days chlorophyll content was decreased but in middle and lower leavesin T_1, T_3 and T_5 with a lower concentration of SS showed increment compared to T_2, T_4 and T_6 in which higher SS concentration was applied. The maximum increase was observed in T_5 at lower concentrations in the top, middle and lower leaf by 11.43%, 7.48% and 7.98%, respectively at 30 DAS and also increase was recorded by 31.30%, 30.17% and 26.84%, respectively in T_5 at 60 DAS in chlorophyll content as compared to control with lower application of SS. Similar findings were also observed by several researchers Singh *et al*. (2004 b); found that in the varieties, of *Vigna radiata* increase in photosynthetic pigments in the lower amendments (up to 35% TS) in the early stages of growth could be attributed to the presence of essential metal ions in tannery sludge required for chlorophyll biosynthesis. However, reduction in chlorophyll content at higher sludge amendments may be attributed to the interference of heavy metals in the formation of chlorophyll through direct inhibition of an enzymatic step Sinha and Gupta (2007)

In conclusion, the present investigation revealed the application of a higher concentration of SS for (*Vigna radiata* L.) var. Samrat, had harmful effects on seed germination (%), shoot length and seedling vigor index however lower concentration of SS at the initial stage promoted growth parameters and chlorophyll content. Treatment T_5 showed the best result as compared to other areasof sewage sludge. Overall, it was concluded that the lower level of SS application might be useful for growth parameters and chlorophyll content of *Vigna radiata*. Sewage sludge contains a sufficient amount of organic matter, N, P, K and micronutrients which will be a good option to use as fertilizers in agricultural productivity but it is recommended that the application of sewage sludge will be used for the short term with lower concentration to reduce their hazardous effects and increase sustainability.

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Table 5. Effect of different area (STPs) sewage sludge on Chlorophyll Content (SPAD Unit) of *Vigna radiata* L.

Days after showing	Treatments	Chlorophyll content SPAD unit (Top leaves)	Chlorophyll content SPAD unit (Mid leaves)	Chlorophyll content SPAD unit (Low leaves)
30 DAS	T_0 Control	49.10^e	47.47^d	45.73^b
	T_1 (A_1+10t ha-^{1}SS)	48.74^d	44.32^c	46.36^c
	T_2 (A_1+15t ha-^{1}SS)	48.06^c	40.43^b	43.60^a
	T_3 (A_2+10t ha-^{1}SS)	52.27^f	48.59^d	49.30^f
	T_4 (A_2+15t ha-^{1}SS)	45.36^b	44.40^c	48.30^d
	T_5 (A_3+10t ha-^{1}SS)	55.44^g	51.31^e	49.70^e
	T_6 (A_3+15t ha-^{1}SS)	43.16^a	36.33^a	49.16^e
60 DAS	T_0 Control	32.63^c	32.33^c	29.40^c
	T_1 (A_1+10t ha-^{1}SS)	37.23^d	34.40^d	37.10^f
	T_2 (A_1+15t ha-^{1}SS)	26.30^a	26.50^a	32.50^e
	T_3 (A_2+10t ha-^{1}SS)	40.46^f	37.60^e	26.40^b
	T_4(A_2+15t ha-^{1}SS)	39.20^e	34.53^d	25.46^a
	T_5(A_3+10t ha-^{1}SS)	47.50^g	46.30^f	39.66^f
	T_6 (A_3+15t ha-^{1}SS)	31.46^b	29.10^b	30.66^d

T_0: (control soil), A_1: (Naini STP), A_2: (Rajapur STP), A_3: (Pritamnagar STP), SS: (sewage sludge), DAS: (Days after showing). Different letters in each group show significant differences at $p<0.05$.

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Conflict of Interest

The authors did not have any conflicts of interest.

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