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Potential influence of lime treated sewage sludge application on soil micro fauna and their impact on growth of Radish

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Abstract— *Lime-treated sewage sludge (LTSS) is a potential soil amendment with both benefits and drawbacks. This study aims to investigate the influence of LTSS application on soil microfauna and their subsequent impact on the growth of radish (Raphanus sativus). Soil microfauna, comprising nematodes, protozoa, and other small invertebrates, play a crucial role in nutrient cycling. LTSS application can alter soil properties like pH, nutrient availability, and organic matter content. These changes can influence the abundance and diversity of microfauna populations. LTSS can be a source of essential nutrients for radish growth, potentially leading to increased biomass and yield. Additionally, some microfauna, like certain nematodes, can promote plant growth through root-fungus interactions. High application rates of LTSS could disrupt the microfauna community, potentially reducing beneficial populations and hindering nutrient cycling. Furthermore, LTSS may contain residual heavy metals or pathogens that could negatively impact radish growth. This study hypothesizes that LTSS application will have a biphasic effect on radish growth. At moderate application rates, LTSS may enhance radish growth by stimulating beneficial microfauna populations and improving nutrient availability. However, at high application rates, negative effects on microfauna and potential heavy metal contamination might hinder radish growth*

Keywords— *Sewage sludge, soil, radish and plant growth*

I. INTRODUCTION

The radish (*Raphanus sativus L*) is a versatile root vegetable enjoyed globally. Originally hailing from Europe and Asia, it belongs to the Brassicaceae family (Gill, 1993). In India, radishes thrive year-round in various regions. This popular vegetable offers more than just its crisp, edible root. Radishes are typically eaten raw in salads or cooked as a side dish. But that's not all! The leafy greens are packed with vitamins A and C and can also be enjoyed cooked. Additionally, young radish pods, known as "mongree," are a culinary delight, eaten raw or cooked on their own or mixed with other vegetables. Radishes boast significant medicinal value. They are often recommended for individuals suffering from piles, liver problems, and jaundice (Khan et al., 2008).

As mentioned, radish performance heavily depends on these factors. Different varieties have specific soil type and climate preferences for optimal growth .This is a crucial factor influencing radish growth and yield. However, the specific nutrient needs vary depending on: Different soil types hold nutrients differently, impacting availability for the radish plant. Richer soils may require less fertilizer amendments compared to less fertile ones. Factors like temperature and rainfall can influence nutrient uptake and utilization (Rahman et al., 2016).

Municipal wastewater treatment generates sewage sludge, a heterogeneous mixture of solid, semi-solid, and liquid residues (National Research Council, 2002). The industry increasingly utilizes the term bio solids to designate treated sludge intended for beneficial reuse (U.S. EPA, 1999). This distinction highlights the processing steps that transform raw sludge into a potential soil amendment. Historically, wastewater sludge disposal posed a significant challenge for urban environments. Traditional methods like incineration and landfilling are becoming less favourable due to limited landfill capacity, rising disposal costs, and environmental concerns . Ocean disposal, once practiced, is no longer considered an acceptable option. Bio solids offer a potential solution, as they contain valuable nutrients like nitrogen, phosphorus, and micronutrients, along with organic matter (U.S. EPA, 2000). Land application represents a sustainable approach to nutrient recycling and organic matter replenishment in soil. However, this strategy necessitates a risk-benefit analysis (Nadeem & Wajid, 2018). Bio solids can harbor metals such as Lead, Cadmium, Chromium, Copper, Zinc, Mercury, etc., and pathogenic microorganisms, posing threats to human health, agricultural productivity, and ecological integrity. Heavy metal mobilization within the soil system is a key concern. These contaminants can be absorbed by plants or leached into drainage water, potentially contaminating associated water resources. Industrial activities significantly contribute to heavy metal concentrations in bio solids. These activities includes the sources such as surface treatment processes employing elements like Cu, Zn, Ni, and Cr, along with the industrial waste improperly disposed of through drainage systems.

II. MATERIALS AND METHODS

The glassware utilized in the present study includes conical flasks, measuring cylinders, pipettes, funnels, Petri plates, test tubes, volumetric flasks, and beakers. Additionally, miscellaneous materials such as inoculating needles, scalpels, spirit lamps, corks borers, and rubber bands were employed. To prepare the glassware for experimentation, they were first immersed in tap water overnight and subsequently washed with detergent powder under running tap water. After sun drying, the cleaned glassware was individually wrapped in clean paper and subjected to sterilization in a hot air oven at a temperature of 150°C for two to four hours. Petri dishes and pipettes were similarly wrapped and sterilized at 160°C for 2 hours. Solid and liquid media were sterilized by autoclaving at a pressure of 1.1 kg/cm² (121.6°C) for 20 minutes during all laboratory studies. All cultural experiments were conducted under aseptic conditions within a laminar airflow hood. Finally, the tips of inoculation needles, forceps, and cork borers were sterilized using an open flame.

2.1 Nutrient Agar

Nutrient Agar is a basic culture medium commonly used for the cultivation of non-fastidious microorganisms specially bacteria.

Materials used:

- Nutrient agar powder
- Distilled water
- Autoclave or pressure cooker
- Conical flask
- Stirring rod
- pH meter or pH paper

2.2 Potato Dextrose Agar (PDA)

Potato Dextrose Agar (PDA) is a widely employed microbiological growth medium that serves as a foundation for cultivating and identifying yeasts and molds. It is prepared by infusing potatoes with dextrose, which provides a source of energy for fungal growth. PDA is particularly useful for the isolation and cultivation of various fungi, including those associated with plant pathogens.

Materials used:

- Potatoes
- Dextrose (glucose)
- Agar powder
- Distilled water
- Autoclave or pressure cooker
- Conical flask
- Sterilized petri dishes

Fig. 2.1 Potato dextrose agar media

2.3 Experimental site

The research study was conducted during two consecutive Zaid seasons in 2017 and 2018 at the Research Farm of the Department of Environmental Science & Natural Resource Management (NRM), College of Forestry, Sam Higginbottom University of Agriculture, Technology, and Sciences (SHUATS), located in Prayagraj, Uttar Pradesh, India. The university campus spans approximately 1020 acres and is situated on the right bank of the Yamuna River in the southern region of Allahabad city. The geographical coordinates of the area are latitude 25°24'42" N and longitude 81°50'56" E, with an altitude of 98 meters above mean sea level.

2.4 Percent pore space

Porosity was calculated from the particle density and bulk density of the soil using formula given by Muthuval et al., (1992)

(1) % pore space = $(1 - \frac{Bulk\ Density}{Partial\ Density}) \times 100\%$

(2) Soil pH
A soil-water suspension was meticulously prepared using a 1:2.5 ratio (10 g of soil mixed with 25 mL of distilled water). Subsequently, the pH value was determined using a pH meter (Jackson 1958).

(3) Electrical conductivity (dS m-1)
The soil water suspension prepared for measurement of pH was utilized to analyze the electrical conductivity of soil. Soil suspension was allowed to settle till supernatant become clear. Electrical conductivity was measured with the help of EC meter and expressed as dS m- 1 (Wilcox 1950).

(4) Organic carbon (%) (Walkley and Black, 1934)
Procedure: One g of soil was taken in a 500 ml of conical flask. Ten ml of 1 N $K_2Cr_2O_7$ solution was added and stirred. Then 20 ml of Conc. H_2SO_4 was added, the flask was swirled 2-3 times and allowed to stand for 30 minutes on an asbestos sheet for the reaction to occur. The suspension was diluted with 200 cc of distilled water. Ten ml of 85 percent H_3PO_4 and 1 ml of diphenylamine indicator were added and titrated against the solution of 0.5 N Ferrous Ammonium Sulphate till color changed from violet to bright green. A blank titration was also carried out.

(5) Electrical conductivity (dS m-1)
The soil water suspension prepared for measurement of pH was utilized to analyze the electrical conductivity of soil. Soil suspension was allowed to settle till supernatant become clear. Electrical conductivity was measured with the help of EC meter and expressed as dS m- 1 (Wilcox 1950).

III. RESULT AND DISCUSSION

3.1 Mechanical analysis of the soil

Table 3.1 Mechanical analysis of the soil of experimental field during 2017 and 2018

Physical analysis of soil	Value (unit)		Method (references)
	2017	2018	
Sand	57.58 (%)	58.12 (%)	Bouyoucos hydrometer method (Bouyoucos, 1927)
Silt	24.78 (%)	24.62 (%)	
Clay	15.24 (%)	15.26(%)	
Textural class	Sandy loam	Sandy loam	Triangular method (Piper, 1950)
Bulk density	1.35 (mg m^{-3})	1.39 (mg m^{-3})	Muthuvel, *et al.*, 1992
Particle density	2.65 (mg m^{-3})	2.61(mg m^{-3})	
Pore space%	48.47(%)	49.76(%)	
Solid space %	51.53 (%)	50.24 (%)	

Table 3.2 Total bacteria from raw sewage sludge

S. No.	Total number of colonies	Type of colonies	Texture of colony
1.	300	1 type	smooth
2.	300	1 type	smooth or whitish
3	290	1 type	smooth

Fig. 3.1 Isolation of bacteria colonies on Nutrient Agar

Table 3.3 Total bacteria from raw lime

S. No.	Total number of colonies	Type of colonies	Texture of colony
1.	5	1 type	smooth
2.	20	1 type	smooth
3	21	1 type	smooth

Fungi Isolation

A total of 6 fungal strains were successfully isolated from the lime treated sewage sludge. The isolated fungal strains displayed diverse morphological features, including hyphal structure, spore formation, and colony pigmentation (Table 3.2 & Fig. 3.1). Initial identification based on colony morphology suggested the presence of various fungal genera, including *Fusariumaspergillus niger*, *Aspergillus tirius*, *Mucur Penicillium*, *Aspergillus flavus*, and *Aspergillus fumigatus* (Table 3.5). Overall, the isolation results indicate the presence of a diverse microbial community in lime treated sewage sludge, comprising both bacteria and fungi with potential implications for bioremediation and nutrient cycling processe (Raouf MS & Raheim ARM, 2016).

Table 3.4 Isolation of total fungi from sewage sludge

S. No.	Total number of colonies	Type of colony	Texture of colony
1.	13	3 types (black yellow and green)	Rough cottony (Smooth)
2.	12	4 types (black yellowish, greenish whitish	rough, smoothy, cottony (smoothy or cottony)
3	15	2 types (black and whitish)	rough, colony
4	13	3 types (black, white, yellowish and greenish	rough cottony smoothy
5	1	1types black color	black rough
6	1	types (brownish)	brownish rough

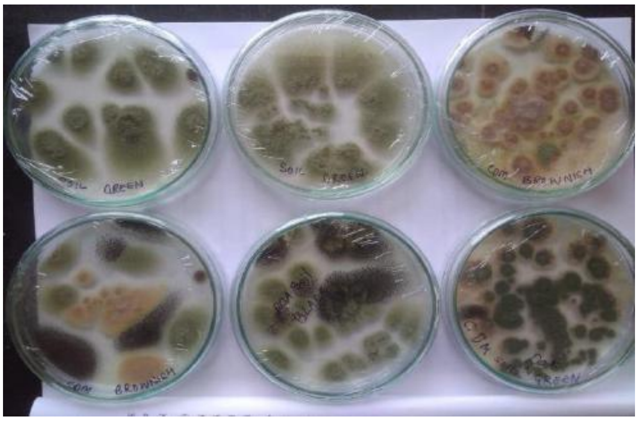

Fig. 3.2 Isolation of total fungi from sewage sludge

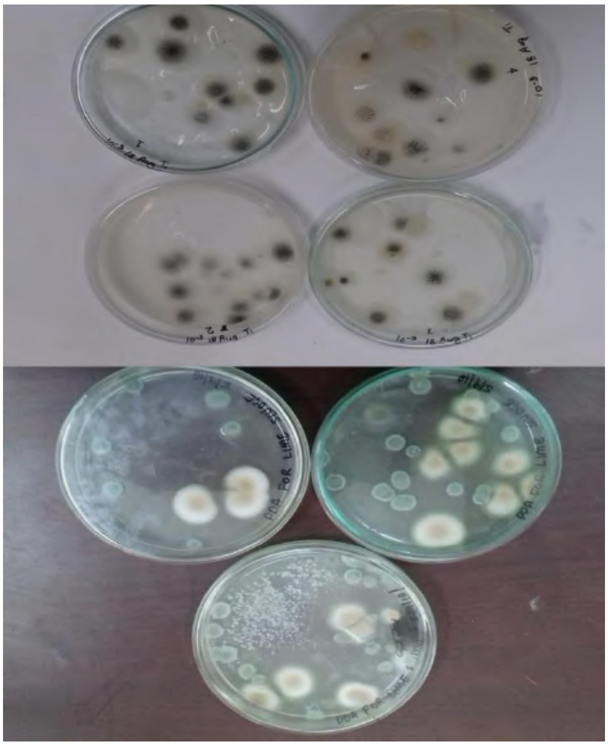

Fig 3.3 Identification of Fungi.

Table 3.5 Identification of fungi

S. No.	Fungi	Colour
1.	Aspergillus niger	black colony
2.	Aspergillus tirius	brown whitish colony
3.	Mucur	Pure white
4.	Penicillium	green colonies
5.	Aspergillus flavus	
6.	Aspergillus fumigatus	

Table 3.6 Effects of Sewage sludge and soil management practices on plant height of Radish

Treatments	Treatment explanation	Plant height (cm)		
		2016-2017	2017-2018	Pooled
T_1	Control	28.01	30.64	29.33
T_2	RSS 100% (Raw Sewage Sludge)	31.38	33.54	32.46
T_3	LTSS 100% (Lime Treated Sewage Sludge)	32.90	34.53	33.71
T_4	CDM 100% (Cow Dung Manure)	33.43	35.36	34.40
T_5	RSS 50% + CDM 50%	35.96	36.50	36.23
T_6	LTSS 50% + CDM 50%	36.25	37.12	36.68
	F-Test	S	S	S
	C.D at 0.5%	2.372	0.512	1.143
	S.Ed	1.065	0.230	0.513

Table 3.7 Effects of Sewage sludge and soil management practices on number of leaves per plant

		Number of leaves per plant		
Treatments	Treatment explanation	2016-2017	2017-2018	Pooled
T_1	Control	9.56	10.31	9.94
T_2	RSS 100% (Raw Sewage Sludge)	10.66	11.56	11.11
T_3	LTSS 100% (Lime Treated Sewage Sludge)	11.05	12.09	11.57
T_4	CDM 100% (Cow Dung Manure)	11.51	13.64	12.58
T_5	RSS 50% + CDM 50%	12.42	14.50	13.46
T_6	LTSS 50% + CDM 50%	13.64	15.27	14.46
	F-Test	S	S	S
	C.D at 0.5%	0.671	0.650	0.445
	S.Ed	0.301	0.292	0.200

Table 3.8 Effects of Sewage sludge and soil management practices on root length (cm)

		Root length (cm)		
Treatments	Treatment explanation	2016-2017	2017-2018	Pooled
T_1	Control	10.65	16.54	13.60
T_2	RSS 100% (Raw Sewage Sludge)	15.37	17.60	16.49
T_3	LTSS 100% (Lime Treated Sewage Sludge)	16.08	18.55	17.31
T_4	CDM 100% (Cow Dung Manure)	17.51	19.66	18.59
T_5	RSS 50% + CDM 50%	18.60	20.40	19.50
T_6	LTSS 50% + CDM 50%	19.75	21.71	20.73
	F-Test	S	S	S
	C.D at 0.5%	1.296	0.518	0.800
	S.Ed	0.582	0.233	0.359

Table 3.9 Effects of Sewage sludge and soil management practices on Root weight (gm)

		Root weight (gm)		
Treatments	Treatment explanation	2016-2017	2017-2018	Pooled
T_1	Control	102.62	101.99	102.31
T_2	RSS 100% (Raw Sewage Sludge)	105.29	110.94	108.12
T_3	LTSS 100% (Lime Treated Sewage Sludge)	110.42	117.44	113.93
T_4	CDM 100% (Cow Dung Manure)	116.12	121.20	118.66
T_5	RSS 50% + CDM 50%	119.93	127.46	123.69
T_6	LTSS 50% + CDM 50%	131.94	135.40	133.67
	F-Test	S	S	S
	C.D at 0.5%	6.777	5.202	2.820
	S.Ed	3.401	2.335	1.266

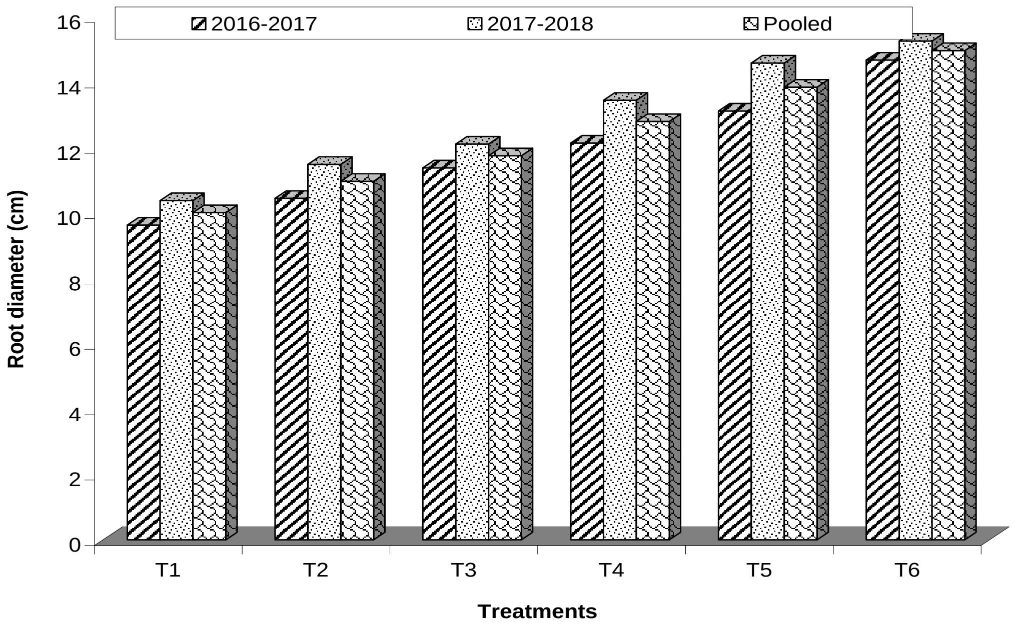

Fig. 3.4 Effects of Sewage sludge and soil management practices on Root diameter (cm)

IV. CONCLUSION

The maximum plant height (cm) (36.25, 37.12 and 36.68) was recorded for the treatment T_6 LTSS 50% + CDM 50%. Whereas the minimum plant height (28.01, 30.60 and 29.33) was found in treatment T_1 Control during 2016-17 and 2017-18 with pooled data respectively.

The maximum number of leaves per plant (13.64, 15.27 and 14.46) was recorded for the treatment T_6 LTSS 50% + CDM 50%. Whereas the minimum number of leaves per plant (9.56, 10.31 and 9.94) was found in treatment T_1 Control during 2016-17 and 2017-18 with pooled data respectively.

The maximum root length (cm) (19.75, 21.71 and 20.73) was recorded for the treatment T_6 LTSS 50% + CDM 50%. Whereas the minimum root length (cm) (10.65, 16.54 and 13.60) was found in treatment T_1 Control during 2016-17 and 2017-18 with pooled data respectively.

The maximum root weight (gm) (131.94, 135.40 and 133.67) was recorded for the treatment T_6 LTSS 50% + CDM 50%. Whereas the minimum root weight (gm) (10.65, 16.54 and 13.60) was found in treatment T_1 Control during 2016-17 and 2017-18 with pooled data respectively.

The maximum root diameter (cm) (14.68, 15.26 and 14.97) was recorded for the treatment T_6 LTSS 50% +

CDM 50%. Whereas the minimum root diameter (cm) (9.63, 10.38 and 10.01) was found in treatment T_1 Control during 2016-17 and 2017-18 with pooled data respectively.

The maximum fresh weight of plant (gm) (229.22, 233.60 and 231.41) was recorded for the treatment T_6 LTSS 50% + CDM 50%. Whereas the minimum fresh weight of plant (g) (201.82, 204.48nd 203.15) was found in treatment T_1 Control during 2016-17 and 2017-18 with pooled data respectively.

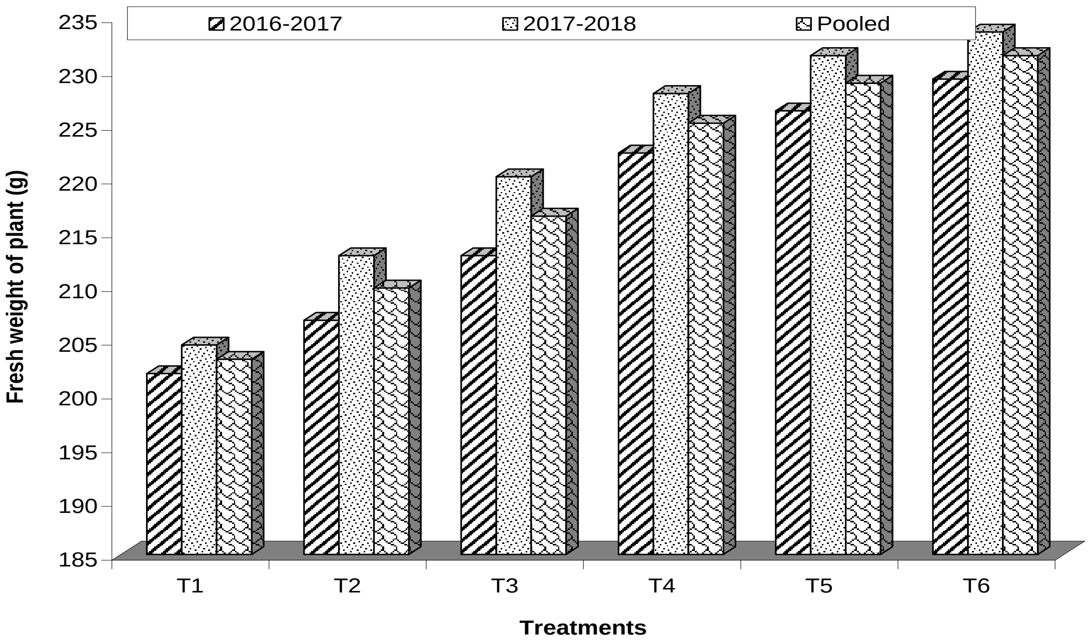

Fig. 3.5 Effects of Sewage sludge and soil management practices on Fresh weight of plant (gm)

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