CUMULATIVE EFFECT OF SEWAGE SLUDGE AND FERTILIZERS APPLICATION ON ENHANCING SOIL MICROBIAL POPULATION UNDER RICE - WHEAT CROPPING SYSTEM

H. S. Jatav1*, S. K. Singh2, J. S. Yadav3

1Research Scholar, Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University Varanasi, Uttar Pradesh, India -221 005.  
2Professor, Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University Varanasi, Uttar Pradesh, India -221 005.  
3Department of Botany, Institute of Science, Banaras Hindu University Varanasi, Uttar Pradesh, India -221 005

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ABSTRACT

A field experiment in randomised block design was conducted at Agriculture Research Farm, Banaras Hindu University, Varanasi, U.P. (India) to find out the effect of conjoint application of sewage sludge and fertilizers on soil microbial population of rice-wheat cropping system (RWCS). Total ten treatments consisting of sewage sludge and fertilizer was formulated. The study demonstrated that increase in bacterial population ($10^6$ cfu g$^{-1}$) in treatment containing 30 t ha$^{-1}$ sewage sludge was applied along with 100% RDF followed by treatments 20 t ha$^{-1}$ sewage sludge was applied along with 100% RDF where, 30 ton ha$^{-1}$ sewage sludge was applied with 100% RDF which was followed by treatments 20 t ha$^{-1}$ sewage sludge was applied along with 100% RDF where, 20 ton ha$^{-1}$ sewage sludge was applied along with 100% RDF. The bacterial population subsequently decreased in remaining three crops after taking 1$^{st}$ rice. In case of Fungi ($10^4$ cfu g$^{-1}$) the maximum population was in 30 t ha$^{-1}$ sewage sludge applied with 100% RDF followed by treatments 20 t ha$^{-1}$ sewage sludge was applied along with 100% RDF where as Actinomycetes was maximum in 30 t ha$^{-1}$ sewage sludge applied with 100% RDF. There was depletion of microbial population in subsequent three crops after first rice crop in which sewage sludge was added. The minimum microbial population was found in treatment without fertilizer and sewage sludge in rice wheat cropping system.

* Corresponding author  
E-mail: hanumanjatav@gmail.com (H. S. Jatav)  

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1 Introduction

The world’s population is increasing and concentrating in urban centres. This trend is particularly intense in developing countries, where an additional 2.1 billion people are expected to be living in cities by 2030 (United Nations, 2014; United Nations, 2015). The fact itself says that population is burgeoning with an alarming rate coupled with the problems of soil health, climate change and food security, and this compels us for the adoption of intensive farming system to supply the food requirements and address the challenges we will face by 2050. The living body soil is a non-renewable resource because its formation takes thousands of years. It may be considered that the native inherent capacity of this versatile and worthy natural resource may get exhausted in order to fulfil our daily food demands. In the coming time we have to adopt new technological interventions or cropping systems to supply our increasing food demands, out of which intensive cultivation is one of them. The intensive cultivation will accelerate the nutrient depletion rate from soil that will lead to depletion of native fertility status which will emerge in the form of deficiency or lack of SOC (soil organic carbon) (Lal et al., 2007; Lal, 2016). In many countries the deficiency of macro or micronutrients is emerging in soil at an alarming level like India and Africa. To overcome from this problem, researcher and scientific communities need to pay more attention not only on managing soil fertility but also on recycling of the wastes generated in cities. Depletion of organic matter in soil discourages activity of soil micro flora responsible for decomposition of organic matter to enrich soil fertility (Sharma & Subehila, 2014). The high nutrients and organic matter contents of sewage sludge make it an excellent fertilizer to enhance soil fertility and crop production. However, presence of heavy metals may be a problem for utilization of sewage in agriculture sector, but it’s utilisation in a proper manner makes it suitable to fulfil the nutritional requirement of the crops (Smith, 2009; Cieślik et al., 2015). The management and disposal of sewage sludge in an economically and environmentally acceptable manner, is one of the society’s most persistent problem. The previous study suggested that if the dose of sewage sludge exceed from 45 ton ha\(^{-1}\), there could be chance to build-up of heavy metals (Latare et al., 2014). So, the dose of sewage sludge in present study was reduced to 20 to 30 ton ha\(^{-1}\). Soil organism acts as primary driving agents of nutrient cycling, regulating the dynamics of soil organic matter, soil carbon sequestration greenhouse gas emission, modifying soil structure and water regimes, enhancing the amount of nutrient acquisition by vegetation, conferring stress tolerance, resisting pathogens and improving plant health (Magdoff & Van Es, 2009).

2 Materials and methods

The field experiment was conducted in randomised block design on alluvial soil representing an Inceptisol (Typic Ustochrept) during July-2015 to April-2017, at Agriculture Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (U.P.) using Rice (cv. Arize-6444) and Wheat (cv. Pusa Gautmi, HD-3086) as test crop. The previously rice and wheat cropping system was followed subsequently. The Varanasi, India is located between 25\(^{0}\)19’ North latitude and 83\(^{0}\) 10’ East longitudes. The initial properties of experimental soil and sewage sludge are presented in Table 1. The sewage sludge (Figure 1) was collected from Bhagwanpur sewage treatments plant (STPs).

| Properties                  | Initial Soil | Sewage sludge |
|-----------------------------|--------------|---------------|
| pH (Soil:water, 1:2.5)      | 8.24±0.5     | 6.58±0.62     |
| EC (dS m\(^{-1}\))          | 0.15±0.01    | 2.49±0.02     |
| Organic C (%)               | 0.46±0.04    | 8.19±0.59     |
| Available N (mg kg\(^{-1}\))| 63.27±2.24   | 155.43±6.23   |
| Available P (mg kg\(^{-1}\))| 7.78±0.12    | 68.56±3.15    |
| Available K (mg kg\(^{-1}\))| 59.26±2.26   | 174.19±5.85   |
| Bacteria 10\(^{6}\) cfu g\(^{-1}\) soil | 14.50±0.29 | 38.65±2.54 |
| Fungi 10\(^{7}\) cfu g\(^{-1}\) soil | 7.20±0.21 | 24.55±3.65 |
| Actinomycetes 10\(^{3}\) cfu g\(^{-1}\) soil | 17.25±0.35 | 39.20±2.84 |

Figure 1 Experimental material sewage sludge collected from Bhagwanpur Sewage Treatment Plants (STPs) after dry
sewage sludge was applied at initial stage before transplanting of first rice crop and there after only graded level of recommended dose of fertilizer was applied in remaining three crops. The post harvest soil samples of each crop were stored at low temperature in a freezer (Temperature 10-15 °C) and used for measurement of different soil biological properties. The population of bacteria, fungi and actinomycetes in post harvest soil was determined by serial dilution of soil and plating technique using Asparagine-Mannitol agar medium (Thornton, 1922); Rose Bengal streptomycin agar medium (Martin, 1950) and Ken Knight and Munair’s medium by pour plate method (Chhonkar et al., 2002), respectively. The initial soil samples (0-15 cm) were subjected to analyzed for pH and EC (dS m⁻¹) in 1:2.5 (soils: water suspension) (Sparks, 1996); organic carbon by Walkley & Black (1934) method; available N by alkaline potassium permanganate method (Subbiah & Asija, 1956), available P by sodium bicarbonate (Olsen et al., 1954) with spectrophotometer, available K by ammonium acetate extraction (Jackson, 1973) using flame photometer. Sewage sludge was also digested in di-acid and analyzed for P, K and for the determination of total N sludge was digested in concentrated H₂SO₄. The data were subjected to one way analysis of variance (ANOVA P=0.05) using SPSS version 22 software. Duncan’s Multiple Range Test (Duncan, 1955) was performed to test the significance of difference between the treatments.

3 Results and discussion

3.1 Bacterial Population:

The data presented in table 2 explained that effect of conjoint application of sewage sludge (SS) and fertilizers on bacterial population (10⁶ cfu g⁻¹ soil) in post harvest soils of RWCS. Application of sewage sludge in soil increased the population of bacteria which ranged from 14.33 (10⁶ cfu g⁻¹ soil) to 34.97 (10⁶ cfu g⁻¹ soil) in post harvest soil I rice crop. A significant increase in bacterial (10⁶ cfu g⁻¹ soil) population was observed over RDF to the treatments in which 20 t and 30 ton ha⁻¹ sewage sludge was applied. The minimum bacterial population was observed in treatment without fertilizer which was 7.43 (10⁶ cfu g⁻¹ soil) in post harvest soil (PHS) OF II wheat crop. The maximum bacterial population was found in treatment containing 30 ton ha⁻¹ SS along with 100% RDF which was 34.72 (10⁶ cfu g⁻¹ soil), this population reduced to 24.04 in II wheat crop. Rest of the treatments are showing either similar or at par the first rice crop. One time application of sewage sludge before first rice crop leads to a sharp increase in population of bacteria but a decline in its population was observed with the subsequent cropping. It was reported that sewage sludge is rich source of organic carbon as well as macro and micro nutrients which release nutrients slowly as it decompose slowly (Late and Singh 2013; Latere et al., 2014; Jatav et al., 2016). The enhancement in bacterial population was found due to application of sewage sludge as sewage sludge is

| Treatments* | 2015-2016 | 2016-2017 |
|-------------|-----------|-----------|
|             | I-Rice    | I-Wheat   | II-Rice | II-Wheat |
| Without fertilizer | 14.33±0.58b | 11.92±0.79a | 8.42±0.74a | 7.43±0.27a |
| RDF (100%)  | 23.72±1.20b | 22.16±0.76b | 21.26±1.19b | 18.47±0.60b |
| 20 t ha⁻¹ SS + RDF (100%) | 32.97±1.35b | 30.72±1.59b | 26.65±0.61b | 23.18±0.82d |
| 30 t ha⁻¹ SS + RDF (100%) | 34.72±1.19b | 31.73±1.15b | 27.38±0.90b | 24.04±0.32d |
| 20 t ha⁻¹ SS + RDF (50%) | 26.91±0.83b | 24.93±0.82b | 21.43±0.82b | 19.11±0.34c |
| 20 t ha⁻¹ SS + RDF (60%) | 28.57±0.98b | 26.90±0.65d | 23.40±0.65b | 20.19±0.65b |
| 20 t ha⁻¹ SS + RDF (70%) | 29.81±0.35d | 26.99±0.90d | 23.70±0.71b | 20.49±0.71b |
| 30 t ha⁻¹ SS + RDF (50%) | 29.43±1.97d | 27.12±1.91d | 23.62±1.91b | 20.56±2.04b |
| 30 t ha⁻¹ SS + RDF (60%) | 29.59±2.07d | 27.32±1.8d | 24.64±2.68b | 21.82±2.16b |
| 30 t ha⁻¹ SS + RDF (50%) | 30.26±0.53d | 28.21±0.57c | 24.91±0.41b | 22.93±0.36d |

Value given in table is mean of three replicates; Mean ± Standard error. Values with same letter differ non-significantly (P>0.05). Different letters for each parameter show significant difference at p < 0.05; RDF-Recommended dose of fertilizer, SS-Sewage sludge, Rice 100% RDF 150:60:60 kg N:P₂O₅:K₂O ha⁻¹; Wheat 100% RDF 120:60:60 kg N:P₂O₅:K₂O ha⁻¹)
rich source of organic carbon and addition of organic matter in soil provide more carbon substrate for growth of microbes. The incensement in microbial population by addition of organic matter has also reported by Kamlesh et al. (1991) and Rajshree & Piliai (2002). Singh et al. (2014) also reported that application of organic and inorganic sources of nutrients significantly affected the population of fungi, bacteria and actinomycetes.

3.2 Fungal Population

The data on fungal population ($10^4$ cfu g$^{-1}$ soil) is presented in Table 3 and Figure 2. The fungal population ranged from 8.17 to 20.38 ($10^4$ cfu g$^{-1}$ soil) in post harvest soil of being the minimum in control where no treatment was applied. The study showed a significant enhancement of microbial population with application of sewage sludge along with recommended dose of fertilizers. The treatments containing 20 or 30 t ha$^{-1}$ SS and 50-70% RDF have similar fungal population whereas the treatment containing 20 t ha$^{-1}$ SS+100% RDF and 30 t ha$^{-1}$ SS+100% RDF show significantly higher fungal population over the RDF. The decrease in fungal population was recorded with subsequent cropping due to depletion of organic matter as sewage sludge was only applied only in 1st rice crop only. Sewage sludge enhanced the microbial population in soil because it is not only rich source of organic carbon but also provide the essential nutrients for growth and development of microbes.

Table 3 Effect of conjoint application of sewage sludge and fertilizers on fungal population ($10^4$ cfu g-1 soil) in post harvest soils of rice-wheat cropping system (RWCS)

| Treatments* | I-Rice 2015-2016 | I-Wheat 2015-2016 | II-Rice 2016-2017 | II-Wheat 2016-2017 |
|-------------|------------------|--------------------|-------------------|-------------------|
| Without fertilizer | 8.17±0.60$^a$ | 6.98±0.24$^a$ | 5.18±1.06$^a$ | 4.95±1.24$^a$ |
| RDF (100%) | 12.85±1.11$^b$ | 11.58±0.35$^b$ | 10.30±0.63$^b$ | 10.25±0.46$^b$ |
| 20 t ha$^{-1}$ SS + RDF (100%) | 18.63±0.75$^{ef}$ | 17.71±0.39$^{ef}$ | 16.71±0.75$^{de}$ | 15.80±0.21$^e$ |
| 30 t ha$^{-1}$ SS + RDF (50%) | 13.64±0.29$^{bc}$ | 13.20±0.57$^{bc}$ | 11.27±0.58$^{bc}$ | 11.01±0.70$^{bc}$ |
| 20 t ha$^{-1}$ SS + RDF (60%) | 14.23±0.32$^{bed}$ | 13.51±0.54$^{bc}$ | 12.31±0.38$^{bc}$ | 12.28±0.23$^{cd}$ |
| 20 t ha$^{-1}$ SS + RDF (70%) | 15.48±0.99$^{bcd}$ | 14.46±0.89$^{bcd}$ | 13.55±0.95$^{cd}$ | 12.79±0.13$^{cd}$ |
| 30 t ha$^{-1}$ SS + RDF (50%) | 15.10±1.34$^{bd}$ | 14.41±1.53$^{bd}$ | 13.17±1.35$^{bc}$ | 12.76±1.01$^{cd}$ |
| 30 t ha$^{-1}$ SS + RDF (60%) | 16.26±1.20$^{de}$ | 15.25±1.57$^{de}$ | 13.33±1.55$^{bc}$ | 12.73±0.66$^{cd}$ |
| 30 t ha$^{-1}$ SS + RDF (50%) | 17.28±1.42$^{de}$ | 16.93±1.65$^{de}$ | 15.01±1.72$^{de}$ | 13.78±0.53$^{de}$ |

Value given in table is mean of three replicates; Mean ± Standard error. Values with same letter differ non-significantly (P>0.05). Different letters for each parameter show significant difference at p < 0.05; RDF—Recommended dose of fertilizer, SS—Sewage sludge, Rice 100% RDF 150:60:60 kg N:P:K ha$^{-1}$; Wheat 100% RDF 120:60:60 kg N:P:K ha$^{-1}$

Figure 2 Fungal population ($10^4$ cfu g-1 soil) in post harvest soils of First Rice crop in treatment compared with Sewage sludge and 100% RDF.
Result of the study revealed that the application of sewage sludge enhanced fungal population which provide or solubilise the essential enzyme and nutrients for growth and development of plant. Sewage sludge was more effective in causing significant increase in microbial population. This might to be explained in the light of the heterotrophic nutritional behaviour of micro organism in soil. The significant and positive relationship of organic carbon with microorganism indicates that the increase in fungal population of soil may be due to high organic matter build up with the regular addition of sewage sludge (Mahajan et al., 2007).

### 3.3 Actinomycetes population

The data showing the Actinomycetes population (10^5 cfu g⁻¹ soil) is presented in Table 4. The population of actinomycetes ranged from 17.20 to 38.55 (10^5 cfu g⁻¹ soil). The observation showed that the enhancement in actinomycetes population in soil was found due to application of sewage sludge. The maximum population of actinomycetes was significant with the application of 30 ton⁻¹ hectare sewage sludge and this was followed by 20 ton⁻¹ hectare along with 100% RDF in all four crops of rice and wheat taken during two cropping cycle. The treatments containing 20 or 30 t ha⁻¹ SS along with 50-70% RDF have similar or at par actinomycetes population in PHS of Iᵣ while as in case of Iᵣ wheat the treatment 30 and 20 ton⁻¹ hectare sewage sludge along with RDF are superior and rest treatments are not significantly different to each other. The depletion of actinomycetes population was observed as in the last crop (IIᵣᵣ wheat). The application of fertilizer (RDF) also enhanced the population of actinomycetes might be due to supply of nutrient. Finding of Bharadwaj & Omanwar (1992) also show the enhancement of microbial population with application of fertilizer. Among the soil under different treatment combination the maximum population of actinomycetes registered when chemical fertilizer was integrated with 30 ton⁻¹ hectare sewage sludge followed by 20 ton⁻¹ sewage sludge. The increment in the population of actinomycetes may be due to application of sewage sludge and release of nutrients (Selvi et al., 2005; Singh et al., 2014).

### Conclusions

The result of the study revealed a significant increase in the population of microorganism that is bacteria, fungi and actinomycetes with application of sewage sludge along with chemical fertilizers. A significant increase in population of micro-organism and during the initial years which subsequently declined with time because of sewage sludge was applied only in Iᵣᵣ rice crop.

### Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this research paper.

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**Table 4 Effect of conjoint application of sewage sludge and fertilizers on Actinomycetes population (10⁵ cfu g⁻¹ soil) in post harvest soils of rice-wheat cropping system (RWCS)**

| Treatments*          | 2015-2016 | 2016-2017 |
|----------------------|-----------|-----------|
|                      | I-Rice    | I-Wheat   | II-Rice   | II-Wheat  |
| Without fertilizer   | 17.20±1.10a | 12.92±0.45b | 10.18±0.53c | 9.03±0.50d |
| RDF (100%)           | 24.29±1.42b | 20.49±1.03b | 18.84±1.02b | 17.33±0.65b |
| 20 t ha⁻¹ SS + RDF (100%) | 36.55±0.90f | 27.68±0.80f | 25.12±0.89d | 24.34±0.78d |
| 30 t ha⁻¹ SS + RDF (100%) | 38.53±0.73d | 32.69±1.42d | 29.86±1.46d | 27.17±0.38f |
| 20 t ha⁻¹ SS + RDF (50%) | 27.32±0.58f | 21.06±0.39b | 19.09±0.22bc | 17.53±0.27b |
| 20 t ha⁻¹ SS + RDF (60%) | 28.47±0.65cd | 21.35±0.49b | 19.28±0.13bc | 17.60±0.92b |
| 20 t ha⁻¹ SS + RDF (70%) | 28.88±0.36cd | 23.60±1.18b | 21.04±1.18bc | 19.50±1.18b |
| 30 t ha⁻¹ SS + RDF (50%) | 29.15±1.97cd | 22.65±2.01b | 20.09±2.01bc | 18.55±2.01b |
| 30 t ha⁻¹ SS + RDF (60%) | 31.22±0.82bc | 24.40±1.80bc | 22.40±1.72cd | 20.37±1.08bc |
| 30 t ha⁻¹ SS + RDF (50%) | 34.28±1.50cd | 27.70±1.75bc | 24.41±0.91bc | 22.87±0.91cd |

Value given in table is mean of three replicates; Mean ± Standard error. Values with same letter differ non-significantly (P>0.05). Different letters for each parameter show significant difference at p < 0.05; RDF-Recommended dose of fertilizer, SS-Sewage sludge, Rice 100% RDF 150:60:60 kg N:P₂O₅:K₂O ha⁻¹; Wheat 100% RDF 120:60:60 kg N:P₂O₅:K₂O ha⁻¹

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