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Synergistic effects of steel slag and microbial inoculants on physiochemical and biological soil properties under variable NPK fertilization

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Abstract

This study investigated the synergistic effects of steel slag (0.5 and 1.0 ton ha⁻¹) and microbial inoculants (PSB and PSF) on soil physiochemical and biological properties under variable NPK fertilization regimes across two growing seasons (2020-21 and 2021-22). A randomized pot experiment with seventeen treatments evaluated soil pH, electrical conductivity, nutrient availability (N, P, K), total microbial count, dehydrogenase activity, and urease activity. Results demonstrated that combined applications of steel slag with microbial inoculants significantly enhanced soil biological activity and nutrient availability compared to conventional NPK treatments. The integration of dual microbial inoculants (PSB and PSF) with reduced NPK levels showed superior performance in phosphorus availability and dehydrogenase activity, while steel slag amendments combined with microbial inoculants optimized potassium availability and enhanced microbial populations. Steel slag application rates of 0.5-1.0 ton per hectare effectively improved soil enzymatic activities and maintained favorable soil pH and electrical conductivity levels. The synergistic combination of steel slag and microbial inoculants with reduced NPK fertilization proved highly effective in improving soil health parameters while maintaining nutrient balance.

Keywords: Steel slag, soil amendment, microbial inoculants, NPK optimization, soil fertility management

1. Introduction

Steel production generates vast quantities of slag approximately 150–170 kg per ton of steel posing a major disposal challenge for the industry (Baras *et al.*, 2023) ^[1]. Historically regarded as waste, steel slag is now recognized for its valuable physicochemical and biological properties. Its high strength and chemical stability have supported its use in road construction and cement manufacturing, while its porous, alkaline nature has proven effective in wastewater treatment. Importantly, steel slag contains nutrient-rich oxides of calcium, silicon, phosphorus, and magnesium, which suggests its potential as a cost-effective soil conditioner and nutrient substitute in agriculture (Das *et al.*, 2020) ^[2]. The high lime (CaO) content in slag can neutralise soil acidity, a critical limitation on over 16 Mha of acidic land in India, and supply essential cations that protect plant roots and enhance nutrient uptake. Silicon enrichment enhances cereal crop resistance to lodging and disease, and manganese contributes to photosynthetic efficiency (Shi *et al.*, 2017) ^[3].

Complementing mineral amendments, phosphate-solubilizing microorganisms (PSMs) offer an eco-friendly strategy to mobilize insoluble soil phosphorus, thereby reducing reliance on phosphate fertilizers. PSMs release organic acids, phosphatases, and siderophores to convert bound phosphorus into plant-available forms and can enhance nitrogen fixation, solubilize potassium, and produce phytohormones such as auxins, cytokinins, and gibberellins (Khan *et al.*, 2024) ^[4]. Additionally, these bioinoculants mitigate abiotic stress and contribute to biocontrol and bioremediation of contaminated soils. Studies have demonstrated that integrating PSMs with reduced phosphate fertilizer rates can boost crop yields and nutrient uptake while lowering agrochemical inputs (Kumar *et al.*, 2025) ^[5].

Despite the documented individual benefits of steel slag and microbial inoculants, their

combined effects on soil health remain underexplored. This research examines the synergistic application of steel slag and phosphate-solubilizing bacteria and fungi under variable NPK fertilization. By assessing changes in soil physiochemical parameters (pH, electrical conductivity, and nutrient availability) and biological activities (microbial counts, dehydrogenase, and urease), the study aims to develop a sustainable soil fertility management strategy that maximizes crop productivity.

2. Material and methods

- 2.1 Experimental Design: This study was conducted at the Division of Environment Science, ICAR- Indian Agricultural research Institute (IARI), New Delhi during the years 2020-22. The experiment was conducted using a randomized layout design with three repetitions of the pot culture. To assess the effect of steel slag (Tata steel) on the growth and nutritional aspects of Bread Wheat (Triticum aestivum L, cv. HD-2967), both with only steel slag and in conjunction with phosphorussolubilizing microorganisms (PSB and PSF) procured from the Division of Microbiology, ICAR- Indian Agricultural Research Institute (IARI), New Delhi. The experimental design consisted of 17 treatments, including the control (T₁), recommended NPK (120:60:60 kg/ha; T2), and 50% recommended NPK (T3) as baseline comparisons. Steel slag amendments were evaluated at two application rates (0.5 and 1 ton/ha, corresponding to 2.23 g and 4.46 g per 10 kg soil, respectively, in 13-inch pots), both independently and in conjunction with phosphorus-solubilizing bacteria (PSB) and fungi (PSF) applied at 500 g/ha. These treatments encompassed full NPK plus slag (T₄-T₅), reduced NPK plus slag (T₇-T₈), and various combinations incorporating PSB and/or PSF (T_6, T_9-T_{17}) .
- 2.2 Soil Physicochemical Analysis: Air-dried, 2 mm-sieved soil samples were collected at crop sowing and postharvest. Soil pH and electrical conductivity (EC) were measured in a 1:2.5 soil-water suspension using a combined glass electrode and conductivity meter (Jackson, 1973). Available nitrogen was determined by alkaline potassium permanganate distillation (Subbiah and Asija, 1956). Available phosphorus was extracted with 0.5 M NaHCO₃ and quantified by the ascorbic acidmolybdate blue method (Olsen et al., 1954; Watanabe and Olsen, 1965). Available potassium was extracted with 1 N NH₄OAc (pH 7.0) and measured by flame photometry (Jackson, 1973). DTPA-extractable iron and zinc were obtained by shaking 12.5 g soil with 25 mL DTPA solution (0.005 M DTPA, 0.1 M TEA, 0.01 M CaCl₂, pH 7.3) for 2 h at 70 rpm, filtering through Whatman-42, and assaying by atomic absorption spectrophotometry.
- **2.3 Soil Biological Analysis**: Dehydrogenase activity was assayed by incubating 1 g moist soil with 0.2 mL of 3% triphenyl tetrazolium chloride and 0.5 mL 1% glucose in sealed tubes at 28 ± 0.5 °C for 24 h. Formed triphenyl formazan was extracted with 10 mL methanol for 6 h and quantified at 485 nm (Schinner *et al.*, 1996). Urease activity was determined by incubating 5 g soil with 0.2 mL toluene, 9 mL THAM buffer (pH 9.0), and 1 mL 0.2 M urea at 30 °C for 2 h. After adding 35 mL KCl–Ag₂SO₄ solution and adjusting to 50 mL, 40 mL was distilled, and NH₄+-N collected in 2% boric acid was titrated with 0.005 N H₂SO₄; activity expressed as μ g N–NH₄+ g⁻¹ h⁻¹ (Tabatabai and Bremner, 1972) [10].
- **2.4 Total microbial populations:** (bacteria, fungi, actinomycetes, rhizobia) were estimated by serial dilution and

spread plating on selective media: Thronton's for bacteria, Martin's Rose Bengal for fungi, Ken Knight and Munier's for actinomycetes, and YEMA for rhizobia (Wollum, 1982) [11]. Returned colony-forming units per gram of soil were recorded after incubation.

2.5 Statistical analysis: The data presented in the figures and tables represent the means \pm SD from three replicates. A one-way ANOVA was employed for statistical evaluation, and Fisher's L.S.D. test was utilized to identify significant differences (p<0.05) among the means of various treatments. All statistical analyses were conducted using Excel 2007 and SPSS (PASW Statistics 18.0).

3. Results and Discussion

3.1 Soil nutrients and physiochemical properties: The initial soil analysis before sowing in both years showed moderately alkaline pH values of approximately 8.0 and electrical conductivity (EC) levels near 1.9 dS/m, indicating slightly saline conditions. Available nitrogen (N) levels were around 225.8 kg/ha and 171.4 kg/ha, phosphorus (P) was roughly 27.15 and 24.27 kg/ha, and potassium (K) ranged from about 270.93 to 308.36 kg/ha, reflecting baseline nutrient status in the experimental soil (Table 1).

Across treatments, soil pH values remained relatively stable, generally between 8.0 and 8.4, with minor fluctuations that are typical for calcareous soils where pH is buffered and non-significantly affected by fertilization or amendments.

Electrical conductivity (EC) values showed some variability depending on treatment. The control (T_1) maintained EC values slightly below initial levels (approximately 1.7 to 1.8 dS/m), while treatments receiving higher nutrient inputs, such as T_3 (50% NPK), T_6 (NPK + PSB), and especially T_4 , T_5 , and T_{15} to T_{16} (NPK combined with steel slag and microbial inoculants), exhibited increased EC values, sometimes exceeding 2.0 to 3.0 dS/m. This increase suggests an accumulation of soluble salts and nutrients in the soil due to fertilization and amendment applications, which could impact soil salinity dynamics and needs monitoring in the long term.

Nitrogen (N), treatments supplemented with recommended NPK (T_2) showed significantly elevated soil N compared to the control (T_1) , reflecting improved nutrient availability. Treatments combining steel slag and microbial inoculants (e.g., T_5 , T_6 , T_{15} , T_{16}) exhibited some of the highest available soil nitrogen values in both years, suggesting that these amendments effectively enhance soil nitrogen pools, possibly through improved mineralization or nitrogen fixation stimulated by microbial activity.

Phosphorus (P) levels followed a similar trend, with T_1 showing baseline low values and T_2 increasing P availability. Notably, treatments involving phosphorus solubilizing bacteria and fungi (T_6 , T_7 , T_8) along with steel slag (T_4 , T_5 , T_{15} , T_{16}) significantly increased soil available phosphorus, often surpassing initial soil values and NPK-only treatments. This corroborates the role of microbial inoculants in solubilizing native soil P and the contribution of steel slag as a phosphorus source.

Potassium (K) concentrations also increased markedly in treatments involving steel slag (T_4 , T_5) and microbial inoculants compared to the control and NPK alone, reflecting the high potassium content in steel slag and enhanced nutrient cycling facilitated by the biological amendments. Some treatments, such as T_6 (NPK + PSB), showed variable potassium levels, pointing to differing effects of PSB and PSF strains on nutrient dynamics. Overall, these results indicate that the combination of steel slag

amendment with phosphorus solubilizing microbes alongside recommended NPK doses effectively improves the soil macronutrient status (N, P, and K) relative to baseline and control conditions. While pH remains stable, EC increases should be carefully monitored to avoid potential salinity stress in prolonged applications. Furthermore, PSB demonstrated a more

pronounced complementary role alongside Steel Slag in improving soil health and fertility. The ability of PSB to efficiently solubilize phosphates likely enhanced phosphorus availability and uptake by plants, thereby contributing more positively to both soil and plant health when combined with Steel Slag.

Table 1: Soil nutrients and physiochemical properties

Soil nutrients and physiochemical properties (Mean data of two years)						
Treatments	pН	EC (ds/m)	Nitrogen (Kg/ha)	Phosphorus (Kg/ha)	Potassium (Kg/ha)	
Before Sowing	8	1.91	225.7	27.15	270.9	
T ₁ (Control)	8.1	1.78	213.2	22.4	252.2	
T ₂ (Recommended NPK)	8	2.16	326.1	32.58	367.8	
T ₃ (50% Recom. NPK)	8.1	3.27	301.0	25.79	319.5	
$T_4 (T_2 + 0.5 \text{ ton/ha slag})$	8.2	1.83	303.6	38.01	372.	
$T_5 (T_2 + 1 ton/ha slag)$	8.2	2.61	323.8	38.01	406.4	
$T_6 (T_2 + PSB + PSF)$	8	2.23	338.6	41.41	328.9	
$T_7 (T_3 + 0.5 \text{ ton/ha slag})$	8	2.69	313.6	28.51	312.4	
$T_8 (T_3 + 1 ton/ha slag)$	8.1	3.13	301.0	33.26	313.1	
T ₉ (T ₃ +PSB+ 0.5 ton/ha slag)	8	2.93	288.5	35.3	263.3	
T ₁₀ (T ₃ + PSB+ 1 ton/ha slag)	8.2	2.66	288.5	38.69	257.0	
$T_{11}\left(T_{3}+PSB\right)$	8	2.54	272.1	37.33	295.4	
T ₁₂ (T ₃ + PSF+ 0.5 ton/ha slag)	8.1	2.37	279.7	31.22	310.4	
T_{13} ($T_3 + PSF + 1$ ton/ha slag)	8.2	2	288.5	27.83	311.0	
$T_{14} (T_3 + PSF)$	8	2.18	263.4	26.47	264.1	
T ₁₅ (T ₃ +PSB+PSF+0.5 ton/ha slag)	8.4	2	275.9	41.41	286.9	
T ₁₆ (T ₃ +PSB+PSF+1 ton/ha slag)	8.2	1.52	313.6	45.48	336.7	
T ₁₇ (T ₃ +PSB+PSF)	8.1	1.79	288.5	33.26	294.5	
Mean	8.1	2.33	292.9	33.9	311.3	
SEm(±)	0.13	0.03	5.0	0.4	4.6	
CD (P=0.05)	NS	0.09	14.4	1.3	13.3	

3.2 Effect of steel slag on Soil Biological Analysis

Soil dehydrogenase is an indicator of overall microbial activity soil biological health. Before sowing, baseline dehydrogenase activity was observed at 3.4 TPF/g/hr (Table 2). Post-treatment, the control (T1) generally maintained moderate to low dehydrogenase activity. Application of recommended NPK (T2) resulted in similar or slightly lower activity than the initial baseline, suggesting that NPK alone supports some but not substantial increases in microbial respiration. In contrast, treatments involving phosphorus solubilizing bacteria or fungi, especially T₆ (NPK + PSB), T₁₅ (NPK + PSB + slag), T₁₆ (NPK + PSF + slag), and T₁₇ (highest bioaugmentation), showed the highest dehydrogenase values across both years. For example, T₁₆ recorded the peak value (4.82 TPF/g/hr), demonstrating an enhancement of soil microbial oxidative metabolism when steel slag is combined with beneficial microbes. Treatments with steel slag alone (T₄, T₅) showed moderate or reduced activity, indicating that biological amendments provide stronger stimulation of dehydrogenase than mineral alone.

Soil urease catalyzes the hydrolysis of urea to ammonia and is crucial for N-cycling. Before sowing, values stood at 23.51 μgN-NH4+/g/hr. The control (T₁) maintained moderate urease activity, while T₂ (NPK) demonstrated similar or marginally higher activity. The highest urease activity was observed in treatments involving significant microbial inoculation, particularly T_6 , T_{17} and T_{16} , indicating that phosphorus solubilizers notably boost urease function. Treatments combining steel slag with microbial inoculants (like T₁₅, T₁₆) showed values well above both control and NPK alone, suggesting synergistic effects of biological and mineral inputs on soil nitrogen cycling potential. Steel slag alone (T4, T5) had more modest effects, consistent with a primary impact through mineral supply rather than direct stimulation of microbial enzyme production.

It shows the application of Steel Slag complemented with Phosphate-Solubilizing Bacteria (PSB) and Phosphate-Solubilizing Fungi (PSF) resulted in significantly enhancing soil biological activity. This improvement is likely due to a synergistic interaction between the soil amendment (Steel Slag) and microbial inoculants (PSB, PSF). The Steel Slag provides essential nutrients and micronutrients, improving soil pH and structure, while PSB and PSF help in solubilizing phosphorus and other nutrients, making them more readily available for microbial growth and activity. Consequently, this combination stimulates microbial proliferation and enzyme production in the soil, reflecting a more active and healthy soil microbial community.

3.3 Effect of steel slag on total microbial count

The increase in total microbial count in all treatments as compared to the Bacillus subtilis (control microbial inoculant) and absolute control highlights the positive influence of Steel Slag (SS) and Phosphate-Solubilizing Bacteria/Fungi (PSB/PSF) on soil microbial proliferation. Total microbial population counts before sowing were high, about 7.2 \times 10 7 (Table 2). Following treatments, microbial counts fluctuated, with the control (T $_1$) sustaining virus-like counts of 6.6 \times 10 7 and 1.4 \times 10 8 in the two years. Treatment T $_2$ (NPK) showed moderate microbial abundance (5.4 \times 10 7), indicative of balanced but not excessively enhanced soil microbial populations.

Treatments inoculated with phosphorus solubilizing bacteria such as T_6 (NPK + PSB) and their combinations with steel slag amendments such as T_9 , T_{15} , and T_{17} consistently showed increased microbial counts reaching up to 7.8×10^7 demonstrating their efficacy in promoting soil microbial proliferation. Treatments with steel slag alone registered

somewhat lower microbial populations than those with added microbial inoculants, indicating that mineral amendments primarily support microbial function indirectly, while bioaugmentation more directly stimulates microbial biomass. These enhancements in microbial populations are key indicators of improved biological soil fertility and ecosystem functioning supporting sustainable wheat production.

This enhancement can be explained by the synergistic effects of

Steel Slag and microbial inoculants. Steel Slag likely improved soil conditions by adding nutrients and improving soil structure, while PSB and PSF provided a microbial stimulus by solubilizing phosphates and making nutrients more available. Consequently, this combination supported the growth and activity of a more abundant and diverse microbial community, reflecting improved soil health and microbial activity.

Table 2: Soil biological properties

Soil biological properties (Mean data of two years)						
	Total microbial count (No.s)	Soil dehydrogenase (TPF/g/hr)	Soil Urease (µgN-NH4+/g/hr)			
Before Sowing	7.2×10^7	3.40	23.51			
T ₁ (Control)	6.6 x 10 ⁷	4.03	31.05			
T ₂ (Recommended NPK)	5.4 x 10 ⁷	3.33	30.83			
T ₃ (50% Recom. NPK)	5.9 x 10 ⁷	2.39	21.70			
$T_4 (T_2 + 0.5 \text{ ton/ha slag})$	5 x 10 ⁷	2.67	21.80			
$T_5 (T_2 + 1 \text{ ton/ha slag})$	4.7 x 10 ⁷	2.23	19.38			
$T_6 (T_2 + PSB + PSF)$	7 x 10 ⁷	4.64	24.12			
$T_7 (T_3 + 0.5 \text{ ton/ha slag})$	4.9 x 10 ⁷	2.78	17.99			
$T_8 (T_3 + 1 \text{ ton/ha slag})$	5 x 10 ⁷	2.76	17.75			
T ₉ (T ₃ +PSB+ 0.5 ton/ha slag)	7 x 10 ⁷	3.66	26.27			
T ₁₀ (T ₃ + PSB+ 1 ton/ha slag)	6.9×10^7	3.84	24.10			
$T_{11} (T_3 + PSB)$	6.7×10^7	3.55	25.92			
T ₁₂ (T ₃ + PSF+ 0.5 ton/ha slag)	5.5 x 10 ⁷	2.19	17.66			
T_{13} ($T_3 + PSF + 1$ ton/ha slag)	5.1 x 10 ⁷	2.12	18.86			
$T_{14} (T_3 + PSF)$	4.8×10^7	2.92	16.05			
T ₁₅ (T ₃ +PSB+PSF+0.5 ton/ha slag)	6.8 x 10 ⁷	4.13	26.07			
T ₁₆ (T ₃ +PSB+PSF+1 ton/ha slag)	6.1 x 10 ⁷	4.82	27.87			
T ₁₇ (T ₃ +PSB+PSF)	7.8 x 10 ⁷	4.05	32.05			
Mean	6.02 x 10 ⁷	3.31	23.50			
SEm(±)	0.08	0.05	0.3			
CD (P=0.05)	0.25	0.16	1.12			

4. Conclusion

The combined application of steel slag and microbial inoculants under variable NPK fertilization significantly enhanced soil health parameters. The synergistic treatments improved soil pH stability, electrical conductivity, and nutrient availability of nitrogen, phosphorus, and potassium compared to conventional fertilization practices. Soil biological activities, particularly dehydrogenase and urease enzyme activities, were markedly enhanced in treatments integrating phosphorus-solubilizing bacteria and fungi with steel slag amendments. Total microbial populations showed substantial increases, indicating improved biological functioning. The integration of steel slag with targeted microbial inoculation effectively maintained soil fertility while reducing chemical fertilizer requirements. These findings demonstrate that this approach offers a strategy for soil fertility management, promoting enhanced soil biological activity and nutrient cycling.

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