Ameliorative Response of Humic Acid and Plant Growth Promoting Rhizobacteria on Soil Microorganism Population in the Northern Region of India

Pooja Bhatt[1*], V. K. Singh[2], Sanjeev Kumar[3] and Parul Chaudhary[1]

[1]School of Agriculture, Graphic Era Hill University, Dehradun, Uttarakhand, India-248002
[2]College of Agriculture, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand-263145
[3]Department of Civil Engineering, National Institute of Technology Hamirpur, Himachal Pradesh-177005

*Corresponding author: bhattpooja1991@gmail.com

Received: 28 August 2024 / Accepted: 04 April 2025

Abstract. Soil microorganisms play a vital role in improving soil fertility. The addition of organic amendments increases the microbial population in the soil. The present study aimed to investigate and compare the impact of organic amendments (HA-Humic acid and PGPR -Plant growth-promoting bacteria) and recommended dose of fertilizer (RDF) solely and in combination on the soil's microbial population (fungi, actinomycetes, and bacteria). The two years of study were investigated at GB Pant University of Agriculture and Technology during winter. The experiment was designed using a randomized block design (RBD), and it consisted of ten treatments and three replications. HA (2.5 kg/ha- soil application, 20 g/kg-seed treatment), PGPR (20 g/kg seeds), and RDF (150 kg/ha) are all used individually or in combination. The result shows that the T_{10} (RDF + HA-2.5 kg/ha (soil application) + PGPR -20 g/kg seed (seed treatment) significantly increases the population of fungi, bacteria, and actinomycetes 42.54, 35.57, & 41.87% and 21.14, 41.11 & 49.38% over T_5 (RDF) at 70 days after sowing in the first and second year, respectively. Also, soil application of HA was found to be more effective than seed treatment in increasing microbial population. The study concluded that applying HA and PGPR enhances the biological properties of soil and can minimize the fertilizer dose, which can be a better approach for sustainable agriculture.

Keywords: humic acid, Plant Growth-Promoting Rhizobacteria (PGPR), chick pea cultivation, rhizosphere microbiology, soil health enhancement.

1. Introduction

Food security is a major concern facing agricultural researchers today, particularly as the global population expands. This challenge is exacerbated by the decline in agricultural land due to urbanization. To meet the food demands of the current generation and conserve resources for future generations, intensive agriculture has become the primary approach. However, this intensification often involves the continuous exposure of soil to chemicals (Zahedi, 2016). Field crops and food production are frequently affected by insect pests, resulting in significant yield losses. Consequently, the use of pesticides has become indispensable in modern agriculture (Singh, 2015). The long-term use of inorganic fertilizers and pesticides has been shown to have detrimental effects on soil fertility over time. These practices can lead to soil degradation, reduced microbial activity, and imbalanced soil nutrient levels (Saffari and Saffari, 2021). As a result, it is crucial to adopt sustainable practices that nourish the soil and help maintain ecological balance (Singh and Kumar, 2024). Sustainable practices such as organic farming, agroforestry, and integrated pest management (IPM) can play a vital role. Organic farming relies on natural processes and inputs, such as compost, manure, bio-fertilizer, green manure, and some organic compounds (humic, humin, and fulvic acid), to improve soil fertility and structure (Meena et al., 2015). Soil fertility and productivity are influenced by many factors, including soil structure, porosity, water-holding capacity, pH, cation exchange capacity, nutrient availability, organic matter content, and microbe activity (Moradkhani et al., 2021). These three aspects of soil—biological, physical, and chemical—are intricately interconnected. The microbes-rich soil exhibits favorable physical characteristics, such as desirable structure, optimal water retention capacity, and suitable bulk density. Additionally, nutrient availability is higher in microbial-active soils, as microorganisms are crucial in nutrient cycling and mineralization processes (Abbaslou and Bakhtiari, 2017). This article focused on humic acid and PGPR because their distinguished characteristics are essential in improving soil properties.

Plant growth-promoting rhizobacteria (PGPR) are a group of microorganisms or free-living bacteria in the rhizosphere. A wide range of microbes in soil, including bacteria, fungi, actinomycetes, and enzymes, play crucial roles in maintaining soil health. These microorganisms are not only abundant but also highly beneficial. They contribute to the ecosystem by enhancing nutrient availability through decomposition, solubilization, and fixation, ensuring a balanced

nutrient profile for plant growth (Prasad et al., 2015). Furthermore, these microbes help to regulate soil temperature, increase soil porosity, and aid in the biodegradation of heavy metals, thus promoting a healthier and more sustainable soil environment. PGPR can play a significant role in sustainable farming by enhancing plant growth and health (Niu et al., 2024). They typically function in three main ways: 1. Synthesizing Compounds: PGPR can produce various compounds such as phytohormones (e.g., auxins, cytokinin, and gibberellins) and enzymes [e.g., 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase] that benefit plants. Phytohormones stimulate plant growth and development, while ACC deaminase decreases the levels of stress ethylene in plants, enhancing their stress tolerance (Vejan et al., 2016). 2. Nutrient Uptake: PGPR can solubilize and mobilize nutrients like phosphorus, iron, and zinc in the soil, making them more available to plants. They can also fix atmospheric nitrogen into an inorganic form that mostly plants can use, reducing the need for synthetic nitrogen fertilizers. 3. Disease Suppression: PGPR can act as a bio-control agent, suppressing the growth of plant pathogens and competing for nutrients and space, antibiosis (production of antibiotics), and inducing systemic resistance in plants (Hayat et al., 2010).

Humic acid (HA) is a dark brown, fully decomposed organic substance resistant to further breakdown. The primary source of HA is the leonardite layer. These are sedimentary layers found under the soil and formed after an extended period, although coal and peat are the other sources (Hong et al., 2020; Bhatt and Singh, 2022). HA has a complex chemical composition and contains various functional groups: aromatic carboxylic, phenolic, and aliphatic carboxylic acids. It contains 51 to 57% C, 4 to 6% P, and 0.2 to 1% other micronutrients. HA indeed plays a crucial role in soil enhancement and plant growth. Here's a more detailed explanation of how HA can impact various soil properties and agronomic factors: 1. Soil Physical Properties: HA can improve soil structure by promoting aggregation, which enhances soil aeration and root penetration (Suh et al., 2014). This, in turn, improves the overall physical condition of the soil, making it easier for plants to access nutrients and water. 2. Soil Chemical Properties: HA can increase the cation exchange capacity (CEC) of the soil, which is the soil's ability to hold positively charged ions like Ca, Mg, and K. It helps nutrient retention and plant availability (Y. Li et al., 2019). HA can also help balance soil pH, making it more suitable for plant growth (D. Duan et al., *2020).* 3. Soil Biological Properties: HA can stimulate microbial activity in the soil, promoting the decomposition of organic matter and the cycling of nutrients. It enhances the soil's fertility and

improves nutrient availability to plants. 4. Water-Holding Capacity: HA can improve the soil's water-holding capacity (WHC) by increasing its ability to retain moisture. It is especially beneficial in arid and semi-arid regions with limited water availability (Lian et al., 2020). Incorporating products based on humic acid into crop production can improve soil health, enhance plant growth, and increase agricultural output, making it an attractive option for sustainable agriculture practices (Nunes et al., 2019).

Farmyard manure (FYM) and compost are widely utilized in crop production on a large scale. However, their application often requires large quantities due to their relatively low content of major nutrients (Bouajila and Sanaa, 2011). To address this challenge, biofertilizers, humic acid, and humic acid have emerged as promising alternatives to traditional manure and compost. Researchers have conducted several studies to understand the physiochemical properties of HA and its role in improving soil quality (Yu et al., 2024; Sherafi et al., 2024). Most experiments were performed in pots or under laboratory conditions, but there has been little emphasis on its influence on soil microbes in field conditions. The knowledge gap prompted our focus on investigating the impact of HA on soil microbes in a field experiment. After reviewing past research, we found that HA has an important role in improving soil characteristics, but it doesn't compete effectively with inorganic fertilizer (Amerian et al., 2024). Since PGPR is directly linked to pulses and boosts microbial activity, we believe it would be a good combination with HA. For this reason, we headed out to investigate the effects of HA and PGPR on soil microbes separately and in combination. Besides the many benefits of HA and PGPR, they require a lower application rate than FYM and compost. Furthermore, they offer an array of advantages over conventional fertilizers. Farmers may benefit by adopting these alternatives into their farming methods. This modification promotes the well-being and efficiency of agricultural systems while providing a more environmentally friendly method of managing soil fertility.

2. Material and Methods

The present study was conducted at the crop research center of GBPUAT, Pantnagar, during the winter of 2020 and 2021. The experimental site comes under an irrigated area. Two irrigation systems are provided: pre-sowing irrigation and another 45 days after sowing (DAS).

The climatic condition of the region can be described as humid subtropical, with cold winters and dry hot summers. The soil texture was silty clay loam. The soil's initial properties (before sowing) are given in Table 2. Ten treatments with three replications were allocated as per randomized block design in 30 plots. Treatment details are presented in Table 1. Each plot size was 3.6 × 5 m. Humic acid was procured from Shahi Krishi Seva Kendra, Udham Singh Nagar. The powder form of HA extract (85% minimum) was used in the experiment. HA was applied as a soil application (2.5 kg/ha) and seed treatment (20g/kg of seed) at the time of sowing only. The PGPR (*Bacillus cereus* NE-10, NCBI Acc No. KR868766) was obtained in solid formulation from AICRP on Pulses, Department of Soil Science, GBPUAT, Pantnagar. PGPR is only used as seed treatment (20 g/kg) at sowing time. The Chickpea variety (PG-186) was used in both years and developed by crossing ILC613 × Pant chana 114**.** This variety is suitable for normal and late-sown conditions.

Table 1: Overview of different treatments used in the study

S. No	**Treatment Descriptions**
1	Control (RDF only)
2	HA - 2.5 kg/ha (applied to soil)
3	HA - 20 g/kg (seed treatment)
4	PGPR - 20 g/kg (seed treatment)
5	Recommended Dose of Fertilizer (RDF)-150 kg/ha of NPK mixture
6	RDF + HA - 2.5 kg/ha (applied to soil)
7	RDF + HA - 20 g/kg (seed treatment)
8	RDF + PGPR - 20 g/kg (seed treatment)
9	HA - 2.5 kg/ha (applied to soil) + PGPR - 20 g/kg (seed treatment)
10	RDF + HA - 2.5 kg/ha (applied to soil) + PGPR - 20 g/kg (seed treatment)

Soil samples

Soil samples were taken from each plot at 70 and 140 days after sowing (DAS) in both studies, at 0–15 cm depth. In order to facilitate subsequent biological examination, soil samples were stored in a deep freezer at a low temperature of 40 degrees Celsius.

Table 2. Initial soil properties of the trial site

S.No	Soil properties parameter	Before sowing
1	Bulk density (g/cm^3)	1.39
2	Particle density (g/cm^3)	2.50
3	Porosity (%)	44.40
4	Water holding capacity (%)	24.13
5	Soil pH	6.29
6	Electrical conductivity (ds/m)	0.22
7	Organic carbon (%)	0.65
8	Available nitrogen (kg/ha)	175.5
9	Available phosphorus (kg/ha)	14.2
10	Available potassium (kg/ha)	133.2

Microbial count

The soil microorganism count was determined using the serial dilution pour plate method with a Laminar flow chamber (Wollum, 1982). Initially, 10 grams of soil were diluted in 90 mL sterile blanks in a conical flask and mixed thoroughly using a vortex shaker. Subsequently, 1 mL of the soil suspension was transferred to a 9 mL sterile blank to achieve a 10^{-2} dilution, and this process was repeated for the desired dilutions. For the next step, 0.5 mL of the suspension (Table 2) was placed onto sterilized petri dishes. Then, 20-25 mL of selective media (Plate count Agar, Martin's Rose Bengal, and Ken night's agar for bacteria, fungi, and actinomycetes, respectively) were poured into the plates containing the soil suspension of the respective microorganisms. Different stages of Microbial count have been mentioned in Fig. 1.

The plates were gently rotated 10-15 times clockwise and anticlockwise to ensure homogeneous mixing. The plates were inverted and incubated in a BOD incubator at 28±2°C. Once the colonies were visible, they were enumerated. The microbial population in the soil was expressed as colony-forming units (CFU) per gram of soil (Eq.1). The microbial count of actinomycetes, bacteria, and fungi was done after standard durations.

$$\textbf{No. of microorganism}\left(\frac{cfu}{g}\right) = \frac{\text{No. of colonies} \times \text{Dilution factor}}{\text{The volume of soil suspension}} \qquad \textbf{(Eq. 1)}$$

Statistical analysis

A one-way analysis of variance (ANOVA) was performed with SPSS, version 16.0 software, to determine the difference in the microbial population. Duncan's Multiple Range Test (DMRT) was used to determine whether there were significant differences between the means at a significance level of $P<0.05$. The data presented in the tables are expressed as the means of three replicates ± the standard deviation.

Fig. 1. Pictorial representation of the microbial count of actinomycetes, bacteria, and fungi in a laboratory at 70 DAS during the first year of the experiment.

3. Results and Discussion

Effect of HA and PGPR on the population of fungi

The fungi population was found to be significantly influenced by different treatments. The fungi population was higher at 70 days after sowing (DAS) than at harvesting time due to more microbial activity and root extension at this time. (Table 3) Overall, 57.1% more fungal population was obtained in the second year than in the first year. All the treatments were significantly superior to T_1 (control) for the fungi population except T_3 (HA-seed treatment) at 70 DAS in the second year. Among all, T_{10} (RDF+ HA-soil application+ PGPR) recorded the maximum colony of fungi at both stages of observation during the experiment. T_{10} (RDF+HA-soil application +PGPR)

recorded 236.5, 73.8, and 70.8, 26.9% higher number of colonies of fungus in 1 g of soil over T_1 (control) and T_5 (RDF) at 70 DAS in the first and second year, respectively. It might be due to the cumulative effect of HA, PGPR, and RDF. Integrated application of inorganic and organic fertilizer may contribute to a balanced proportion of nutrients in the soil as the soil is an inhabitant of multiple microorganisms that require nutrition to survive (Kumar and Kumar, 2023). Humic acid, a rich carbon source (51-57%), increased carbon availability in soil and provided a food source for fungi. Also, HA plays a crucial role in improving soil properties, and facilitating nutrient availability (Fontaine et al., 2007).

Table 3. Fungi population as influenced by different treatments at 70 and 140 DAS during both the years of investigation.

Treatment	**Fungi (× 10^3 CFU g^{-1} soil)**			
	I year		**II year**	
	70 DAS	**140 DAS**	**70 DAS**	**140 DAS**
Absolute control	9.33±1.52 ^{a}	6.33±0.57 ^{a}	24.00±2.00 ^{a}	9.33±1.52^{a}
HA (soil application)	14.33±2.30 ^{bc}	11.33±1.52 ^{bc}	30.00±2.00 ^{abc}	12.66±2.00^{b}
HA (Seed treatment)	12.66±1.52 ^{b}	9.00±1.00 ^{b}	29.00±8.18 ^{ab}	13.33±0.57^{b}
PGPR (seed treatment)	17.00±1.73 ^{cd}	14.66±1.52 ^{de}	34.33±2.08 ^{bcde}	15.00±1.00^{b}
RDF	18.00±2.00 ^{d}	12.66±1.15 ^{cd}	32.33±2.51^{bcd}	14.00±1.00^{b}
RDF+ HA (soil application)	22.66±0.57 ^{e}	15.33±0.57 ^{e}	37.00±2.64^{cde}	21.00±1.00^{c}
RDF+ (Seed treatment)	19.00±1.00 ^{d}	13.00±1.00 ^{cde}	36.33±3.78^{cde}	14.33±0.57^{bc}
RDF+ PGPR (seed treatment)	25.00±2.64 ^{e}	18.00±2.64 ^{f}	38.66±3.05^{de}	23.33±1.52^{d}
HA (soil application) + PGPR (seed treatment)	18.33±1.52 ^{d}	14.33±1.52 ^{de}	31.66±4.04^{bcd}	22.66±1.52^{cd}

HA (soil application) + PGPR (seed treatment) + RDF	31.33±2.30 ^{f}	20.33±1.15 ^{f}	41.00±2.00^{e}	26.00±2.00^{e}

Values in each column followed by the same letter were not significantly different (P<0.05)

Inoculation of seed with PGPRs (T_4 and T_8) recorded a more significant number of fungi than that of control (T_1)**.** It might be due to the inoculation of PGPR, which provided a congenial environment to the rhizosphere and enhanced the soil health by releasing some growth hormones, viz. auxin and gibberellin acid, and facilitating the solubilization of phosphorus in soil. Most organisms use phosphorus as phosphate (PO_4) (Alnasra et al., 2024). Phosphorus plays a crucial role in energy metabolism, the storage and transmission of biological information, and the integrity of an organism's membranes (Gupta et al., 2020).

Effect of HA and PGPR on the population of bacteria

The different treatments during both years also significantly influenced the bacterial population. 24.81% more bacterial colonies were counted in the second year over the first year. Unlike fungi, the bacterial population increased during harvesting during both years as most bacteria survive under starvation conditions. There is a proper water supply and nutrients during flowering and fruiting, but it is depleted during harvesting (Bhardwaj et al., 2018).

All the treatments recorded significantly more bacteria than T_1 (control). T_{10} (RDF+ PGPR+ HA-soil application) recorded the maximum colonies of bacteria at each observation during both years of study, followed by T_8 (RDF+ PGPR). T_{10} (RDF+HA-soil application +PGPR) recorded 153, 55.2, and 169.8, 80.9% higher number of bacterial colonies in 1 g of soil over T_1 (control) and T_5 (RDF) at 70 DAS in the first and second year, respectively (Table 4). All sole HA, PGPR, and RDF applications recorded significantly more bacteria activity than the control.

Table 4. Effect of different combinations of HA and PGPR treatments on the bacteria population in two years of study.

Treatment	Bacteria (× 10^6 CFU g^{-1} soil)			
	I year		II year	
	70 DAS	140 DAS	**70 DAS**	140 DAS
Absolute control	10.0±1.00^{a}	11.6±0.57^{a}	5.6±1.52^{a}	9.0±1.00^{a}
HA (soil application)	15.0±1.00^{bc}	15.0±1.00^{ab}	10.0±1.00^{b}	11.3±1.52^{b}
HA (Seed treatment)	12.6±1.15^{b}	12.0±0.57^{a}	9. 6±0.57^{b}	11.6±0.57^{b}
PGPR (seed treatment)	18.0±2.00^{de}	18.6±1.52^{bc}	13.3±1.52^{cd}	14.6±1.52^{c}
RDF	16.3±1.52^{cd}	17.3±1.15^{b}	10.6±1.15^{b}	16.0±1.52^{c}
RDF+ HA (soil application)	21.0±1.00^{f}	23.6±0.57^{d}	13.3±0.57^{cd}	20.0±1.00^{de}
RDF+ (Seed treatment)	17.3±1.52^{cde}	22.0±1.00^{cd}	11.0±1.00^{b}	18.6±1.52^{de}
RDF+ PGPR (seed treatment)	21.3±1.52^{f}	29.3±0.00^{e}	15.0±0.00^{d}	20.0±1.00^{d}
HA (soil application) + PGPR (seed treatment)	19.6±1.52^{ef}	22.0±1.52^{cd}	11.6±1.52^{bc}	21.3±1.15^{ef}
HA (soil application) + PGPR (seed treatment) + RDF	25.3±1.52^{g}	31.3±1.73^{e}	18.0±1.73^{e}	22.6±0.57^{f}

Values in each column followed by the same letter were not significantly different (P<0.05)

Bacteria need a favorable environment to live in soil. Generally, bacteria regulate all primary nutrient cycles like nitrogen, phosphorus & sulfur. HA has various properties that improve the soil condition by increasing the water-holding capacity and porosity in soil, as moisture and aeration are prerequisites for most bacteria. On the other hand, T_8 (HA- soil application + PGPR) recorded more bacterial colonies than T_5 (RDF) during both years. It may have happened due to the cumulative effect of both HA and PGPR, as both are organic substances and facilitate nutrient availability in soil. In addition, PGPR helps colonize the roots, which provides more rhizospheric area for rhizobacteria by releasing growth hormones. Also, PGPR plays a crucial role in various biochemical reactions in soil, viz. ammonification, nitrification, mineralization, etc. All these processes are responsible for the availability of nutrients in the soil, which regulate the population of bacteria in the soil (Chen et al., 2017).

Effect of HA and PGPR on the population of Actinomycetes

Table 5 depicts the range of actinomycetes colonies varied from 4.3 to 32 and 13.0 to 39.0 × 10^5 CFU g^{-1} soil in the first and second years, respectively. Overall, 29.53% more population was recorded in the second year than in the first year. All the treatments were significantly superior to T_1 (control) concerning the actinomycetes population.

Among all the treatments, T_{10} (RDF+HA- soil application +PGPR) recorded the maximum number of actinomycetes colonies at each observation during both years, followed by T_8 (RDF+ PGPR). T_{10} (RDF+HA-soil application +PGPR) recorded 220, 72, and 154.9, 72.6% more number of colonies of actinomycetes in 1 g of soil over T_1 (control) and T_5 (RDF) at 60 DAS in first and second year, respectively. T_8 (RDF+ PGPR) was found to be on par with T_6 (RDF+ HA-soil application) and T_9 (HA-soil application +PGPR).

Table 5. The population of actinomycetes as influenced by various treatments of HA and PGPR, solely or in different combinations with RDF.

Treatment	Actinomycetes (× 10^5 CFU g^{-1} soil)			
	I year		II year	
	70 DAS	140 DAS	70 DAS	140 DAS
Absolute control	10.0±1.00^{a}	15.3±1.52^{a}	4.3±1.52^{a}	13.0±1.00^{a}
HA (soil application)	17.3±1.15^{c}	16.3±1.52^{ab}	10.0±1.00^{b}	17.0±1.73^{bc}
HA (Seed treatment)	14.0±2.00^{b}	18.3±2.51^{abc}	8.3±0.57^{b}	16.0±1.72^{b}
PGPR (seed treatment)	21.0±1.00^{d}	20.6±1.15^{bc}	15.0±1.73^{de}	19.3±3.21^{cd}
RDF	18.6±1.52^{cd}	22.6±0.57^{c}	12.3±0.57^{c}	20.6±1.15^{de}
RDF+ HA (soil application)	28.3±2.08^{f}	31.6±4.72^{de}	17.0±1.00^{f}	22.6±1.15^{ef}
RDF+ (Seed treatment)	24.6±1.52^{e}	29.3±4.16^{d}	13.6±0.57^{cd}	24.0±1.00^{fg}
RDF+ PGPR (seed treatment)	29.6±1.52^{fg}	35.0±5.00^{ef}	19.0±1.00^{g}	26.0±1.00^{gh}

HA (soil application) + PGPR (seed treatment)	28.3±2.51^{f}	33.3±1.52^{de}	15.6±1.15^{ef}	22.6±2.51^{ef}
HA (soil application) + PGPR (seed treatment) + RDF	32.0±2.00^{g}	39.0±1.73^{f}	24.3±1.15^{h}	28.0±1.00^{h}

Values in each column followed by the same letter were not significantly different (P<0.05)

Seed inoculation with PGPR (T_4 and T_8) counted significantly more actinomycetes than T_2 and T at all observations during both years. This might be due to the characteristics of PGPR, which provide a favorable environment for the growth of rhizospheres' microbes (Bhatt et al., 2018). Root-associated rhizobacteria produce an incredible variety of biomolecules, which is remarkable—incorporating these biomolecules into the soil results in an additional improvement to the soil's overall health. In addition, they affect the byproducts of plants, leading to the decomposition and mineralization of many organic compounds (Mehmood et al., 2018).

4. Conclusion

The study explains the combined effects of humic acid (HA) and plant growth-promoting rhizobacteria (PGPR) in conjunction with the recommended dose of fertilizers (RDF) on soil microbial dynamics in chickpea cultivation. The findings indicate that soil application of HA yields a more pronounced enhancement in microbial populations, including bacteria, fungi, and actinomycetes, compared to seed treatment. This phenomenon is likely attributable to HA's role in improving soil physicochemical properties, such as moisture retention, porosity, and nutrient bioavailability, thereby fostering a conducive environment for microbial proliferation. Moreover, PGPRs contribute to microbial activity by projecting phytohormones and catalyzing phosphorus solubilization, further augmenting soil fertility and microbial resilience.

Integrating HA and PGPR with RDF presents a holistic approach to nutrient management, facilitating a more efficient nutrient cycling process, bolstering microbial diversity, and ultimately reinforcing soil health. This equilibrium between organic and inorganic amendments sustains soil microbial communities and enhances plant physiological responses and crop productivity, underscoring the necessity of adopting integrated soil fertility management practices. Despite the promising implications of this study, further investigations are needed to delineate optimal application rates across diverse edaphic conditions and cropping systems. Additionally, long-term field trials are essential to elucidate HA and PGPR's persistence and cumulative effects on soil

microbiota and agroecosystem stability. The outcomes of this research provide a foundational framework for advancing sustainable soil fertility management strategies, emphasizing the critical role of microbial interventions in achieving long-term agricultural sustainability.

Acknowledgement

The authors acknowledge the laboratory facilities provided by the soil science department and research material provided by the agronomy department of GBPUAT, Pant Nagar.

Conflict Of Interest

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

Funding

This research has not received any funding or support.

ORCID

Pooja Bhatt https://orcid.org/0000-0002-3040-5813

References

Abbaslou H. & Bakhtiari S., 2017, Phytoremediation potential of heavy metals by two native pasture plants (Eucalyptus grandis and Ailanthus altissima) assisted with AMF and fibrous minerals in contaminated mining regions. *Pollution* 3(3): 471-486. doi: 10.7508/pj.2017.03.012

Alnasra O.A., Khalili F.I. & Alhnafat F.A., 2024, Enhanced removal of Pb(II), Zn(II) and Cd(II) ions by insolubilized humic acid: Characterization and sorption behaviors. *Desalination and Water Treatment* 100604–4.

Amerian M., Palangi A., Gohari G. & Ntatsi G., 2024, Humic acid and grafting as sustainable agronomic practices for increased growth and secondary metabolism in cucumber subjected to salt stress. *Scientific Reports* 14(1).

Badeplam, 2006, Unidad de información para la biodiversidad (UNIBIO). Instituto de Biología, Universidad Nacional Autónoma de México. Retrieved from http://unibio.unam.mx/html/proyectos/badeplam.htm.

Barot S.F.S., Barré P., Bdioui N., Mar B. & Rumpel C., 2007, Stability of organic carbon in deep soil layers controlled by fresh carbon supply. *Nature* 450(7167): 277–280.

Bhardwaj A., Kumar S. & Singh D., 2023, Tannery effluent treatment and its environmental impact: a review of current practices and emerging technologies. *Water Quality Research Journal* 58(2): 128–152. doi: 10.2166/wqrj.2023.002

Bhatt P., Singh V.S. & Kumar S., 2025, Optimizing soil health: comparative effects of humic acid, PGPR, and RDF on soil properties and fertility. *Ecological Questions* 36(1): 1–17. https://doi.org/10.12775/EQ.2025.007

Bhatt P. & Singh V.K., 2022, Effect of humic acid on soil properties and crop production– A review. *The Indian Journal of Agricultural Sciences* 92(12).

Blancas J., Casas A., Pérez-Salicrup D., Caballero J. & Vega E., 2013, Ecological and socio-cultural factors influencing plant management in Náhuatl communities of the Tehuacán Valley, Mexico. *Journal of Ethnobiology and Ethnomedicine* 9: 1-23. https://doi.org/10.1186/1746-4269-9-39

Bouajila K. & Sanaa M., 2011, Effects of organic amendments on soil physico-chemical and biological properties. *Environmental Science* 2(S1): 485–490.

Chen X., Kou M., Tang Z., Zhang A., Li H. & Wei M., 2017, Responses of root physiological characteristics and yield of sweet potato to humic acid urea fertilizer. *PLoS One* 12(12): e0189715.

Domiciano N.R.A., Nogueira A.P.A., Canellas S. et al., 2019, Evaluation of the effects of humic acids on maize root architecture by label-free proteomics analysis. *Scientific Reports* 9(1).

Duan D., Tong J., Xu Q., Dai L., Ye J. & Wu H. et al., 2020, Regulation mechanisms of humic acid on Pb stress in tea plant (Camellia sinensis L.). *Environmental Pollution* 267: 115546–6.

Gupta S.C., Trivedi B.K. & Singh P., 2020, Effect of diverse nutrient application on symbiotic traits, yield attributes, nutrient uptake, microbial population, dehydrogenase activity and productivity of chickpea (Cicer arietinum L.) in black soils. *Legume Research-An International Journal* 43(6): 844–849.

H.N R., Hong-ying Z.H., Lin Y. & Y.T. Z., 2024, Variations in structure and adsorption characteristics of humic acid during pressure oxidation process. *Transactions of Nonferrous Metals Society of China* 34(5): 1694–1709. doi: 10.1016/S1003-6326(24)66500-3

Kumar M.R., Kumar S.R., Pal S.N., Kumari M.S. & Meena S., 2015, Isolation of low temperature surviving plant growth–promoting rhizobacteria (PGPR) from pea (Pisum sativum L.) and documentation of their plant growth promoting traits. *Biocatalysis and Agricultural Biotechnology* 4(4): 806–811.

Kumar P.R., Kumar M. & Varma A., 2014, Role of PGPR in Soil Fertility and Plant Health. *Soil Biology* 247–260.

Kumar S. & Kumar D., 2023, Biofiltration of volatile organic compounds using chir pine cone nuts inoculated with Pseudomonas putida. *Ecological Questions* 35(2): 1–16. doi: 10.12775/EQ.2024.013

Lian X., Liao S., Yang Y., Zhang X. & Wang Y., 2020, Effect of pH or metal ions on the oil/water interfacial behavior of humic acid-based surfactant. *Langmuir* 36(36): 10838–45.

Li Y., Fang F., Wei J., Wu X., Cui R. & Li G. et al., 2019, Humic Acid Fertilizer Improved Soil Properties and Soil Microbial Diversity of Continuous Cropping Peanut: A Three-Year Experiment. *Scientific Reports* 9(1): 12014.

Man-hong Y., Lei Z., Sheng-tao X., McLaughlin N.B. & Jing-hui L., 2020, Effect of water-soluble humic acid applied to potato foliage on plant growth, photosynthesis characteristics and fresh tuber yield under different water deficits. *Scientific Reports* 10(1).

Mehmood U., Muhammad I., Saeed M., Altaf A., Azam F. & Hayat S., 2023, A Brief Review on Plant Growth Promoting Rhizobacteria (PGPR): A Key Role in Plant Growth Promotion. *Plant Protection* 2(2): 77–82.

Moradkhani P., Oustan S., Reyhanitabar A. & Alidokht L., 2021, Efficiency of humic acid from various organic sources for reducing hexavalent chromium in aqueous solutions. *Pollution* 7(2): 321-331. doi: 10.22059/poll.2021.308924.880

Saffari V.R. & Saffari M., 2021, Improving phytoremediation efficiency of copper-spiked calcareous soils by humic acid applications. *Pollution* 7(4): 871-884. doi: 10.22059/poll.2021.324145.1095

Sharafi R., Gholamreza S.J., Karimi E., Hosein G. & Mojegan K., 2024, Integrating bioprocess and metagenomics studies to enhance humic acid production from rice straw. *World Journal of Microbiology and Biotechnology* 40(6).

Shailendra S.G.G., 2015, Plant Growth Promoting Rhizobacteria (PGPR): Current and Future Prospects for Development of Sustainable Agriculture. *Journal of Microbial & Biochemical Technology* 7(2).

Singh D. & Kumar S., 2023, Challenges of Municipal Solid Waste Management in Jalandhar, Punjab (India): A Case Study. *Lecture Notes in Civil Engineering* 207–214. doi: 10.1007/978-981-99-4045-5_18

Suh H.Y., Yoo K.S. & Suh S.G., 2014, Tuber growth and quality of potato (Solanum tuberosum L.) as affected by foliar or soil application of fulvic and humic acids. *Horticulture Environment Biotechnology* 55: 183–189.

Vejan P., Khadiran A.R., Ismail T. & Nasrulhaq Boyce, 2016, Role of plant growth promoting rhizobacteria in agricultural sustainability—A review. *Molecules* 21(5): 573.

Wollum A.G., 1982, Cultural Methods for Soil Microorganisms. *Agronomy* 781–802.

Zahedi H., 2016, Growth-Promoting Effect of Potassium-Solubilizing Microorganisms on Some Crop Species. *Agronomy* 31–42.