Journal of Advances in Microbiology Research

E-ISSN: 2709-944X
P-ISSN: 2709-9431
JRM 2025; 6(1): 329-335

www.microbiojournal.com
Received: 18-04-2025
Accepted: 22-05-2025

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Microbial enzymatic activity in polyhalite-amended agricultural soils

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Abstract
Microbial enzymatic activity is a sensitive integrator of soil biological fertility and a leading indicator of management impacts. This study evaluated whether a multi-nutrient sulfate mineral (polyhalite) stimulates key soil enzymes and improves crop performance in an agricultural soil. A pot experiment with wheat compared control, 50, 100, and 150 kg ha^{-1} polyhalite equivalents over 60 days. Dehydrogenase, urease, acid phosphatase, and β-glucosidase were assayed at 0, 30, and 60 days; harvest metrics (biomass and N, P, K uptake) were recorded. Data were analyzed by one-way ANOVA with Tukey's HSD ($\alpha = 0.05$), and Pearson correlations linked Day-60 enzyme activities to plant responses. Enzyme trajectories increased over time in all treatments, with the strongest and most consistent stimulation at the moderate rate (Poly-100). At Day 60, dehydrogenase activity was ≈31% higher in Poly-100 than control, urease ≈26% higher, and both acid phosphatase and β-glucosidase ≈16% higher. Above-ground biomass rose by ≈26% under Poly-100, accompanied by greater N, P, and K uptake. Dehydrogenase showed a strong positive association with N uptake ($r \approx 0.92$), indicating that enhanced microbial respiratory intensity translated into agronomic benefit. Responses at the highest rate (Poly-150) plateaued or slightly declined relative to Poly-100, suggesting a non-linear dose response. Collectively, results support a biologically informed, moderate polyhalite dose that aligns slow-release, balanced nutrient supply with enzyme-mediated C, N, and P cycling, strengthening soil health while improving crop performance. Field-scale, multi-season validation and integration with organic inputs and soil health practices are recommended to refine rate, timing, and placement for diverse soils and climates.

Keywords: Polyhalite, soil enzymes, dehydrogenase, urease, acid phosphatase, β-glucosidase, microbial functionality, nutrient uptake, sustainable fertilization, wheat

Introduction
Soil fertility is governed not only by its physicochemical characteristics but also by microbial enzymatic activity, which directly influences nutrient cycling and plant growth. Enzymes such as dehydrogenase, urease, phosphatase, and β-glucosidase serve as indicators of soil health because they mediate organic matter decomposition, nitrogen transformations, and phosphorus mobilization [1-3]. The increasing demand for sustainable agriculture has led to the exploration of alternative mineral sources for soil amendment. Among them, polyhalite—a multi-nutrient mineral containing potassium, calcium, magnesium, and sulfur—has attracted growing attention due to its slow-release properties and low environmental footprint [4, 5]. While several studies have documented the beneficial role of polyhalite in enhancing crop yield and nutrient uptake [6, 7], its influence on microbial enzymatic activities in soils remains poorly understood. The problem arises from the limited integration of mineral fertilization strategies with biological soil processes; conventional fertilizers often improve immediate nutrient availability but can reduce long-term microbial diversity and enzymatic efficiency [8, 9]. This gap necessitates a systematic investigation into whether polyhalite amendments can foster microbial functionality, thereby improving soil biological fertility. Previous research suggests that mineral amendments may enhance microbial metabolic potential, though responses vary with soil type, crop system, and environmental conditions [10-12]. Thus, the present study is designed with the objective of evaluating the enzymatic responses of microbial communities in polyhalite-amended agricultural soils. The hypothesis guiding this investigation is that polyhalite application not only improves nutrient supply but also stimulates enzymatic activity, particularly in nitrogen and phosphorus cycling pathways, thereby promoting a synergistic effect on soil health and crop productivity [13-15]. In addition, recent studies underscore the role of polyhalite in soil-plant nutrition interactions, which further supports the need for empirical validation of

its microbial impacts [16]. Therefore, the present research aims to bridge the existing knowledge gap by linking mineral nutrition with soil microbial enzymology in a sustainable agriculture context [17].

Materials and Methods

Materials

The study was conducted on agricultural soils collected from a long-term cropping field characterized by loamy texture, moderate organic matter, and neutral pH. The soil was homogenized, air-dried, and sieved (2 mm mesh) prior to experimentation. Polyhalite, sourced from a commercial supplier, contained approximately 14% K_2O, 6% MgO, 17% CaO, and 48% SO_3 [4, 5]. Treatments included control (no amendment) and varying doses of polyhalite (50, 100, and 150 kg ha^{-1} equivalent). Wheat (Triticum aestivum L.) was chosen as the test crop due to its high responsiveness to potassium and sulfur nutrition [6, 7]. Baseline soil characteristics, including total organic carbon, available N, P, K, and microbial biomass C, were determined before amendment following standard protocols [1, 3]. Reference fertilizers (urea, single superphosphate, and muriate of potash) were used for comparative purposes to distinguish polyhalite's specific effects on soil microbial functions [8, 9].

Methods

Experimental pots were arranged in a completely randomized design with triplicate replications for each treatment. After amendment incorporation, soils were incubated under controlled temperature (25 ± 2 °C) and moisture (60% water-holding capacity) for 60 days [10-12]. Soil samples were periodically collected at 0, 30, and 60 days for enzymatic assays. Dehydrogenase activity was determined using triphenyl tetrazolium chloride (TTC) reduction [2, 13], urease activity by urea hydrolysis followed by spectrophotometric estimation of NH_4^+ [14], phosphatase activity by p-nitrophenyl phosphate hydrolysis [11], and β-glucosidase activity by p-nitrophenyl-β-D-glucopyranoside cleavage [3, 15]. Microbial community structure was analyzed using phospholipid fatty acid (PLFA) profiling and 16S rRNA amplicon sequencing to assess shifts in bacterial and fungal populations under polyhalite influence [10, 12]. Crop growth parameters (plant height, biomass, and nutrient uptake) were recorded at harvest to correlate biological responses with microbial enzymatic activities [6, 7, 16]. Data were subjected to one-way ANOVA followed by Tukey's HSD test to assess significance among treatments at $p < 0.05$. The methodological framework was adapted from established soil enzymology and nutrient amendment studies to ensure robustness and reproducibility [1, 2, 17].

Results

Overview of statistical approach: Data (n=3 per treatment) were analyzed using one-way ANOVA (per endpoint) followed by Tukey's HSD (α=0.05) for multiple comparisons; results are reported as mean ± SD with compact letter displays (different letters = significant differences). Pearson correlations quantified associations between Day-60 enzyme activities and harvest metrics. This approach follows established enzyme assay and soil-quality analytics [1-3, 11, 15].

Table 1: Enzyme activities at Day 60 under different polyhalite doses (mean ± SD; n = 3)

Enzyme	Treatment	Mean ± SD	Group
Dehydrogenase	Control	31.89 ± 0.36 (n=3)	b
Dehydrogenase	Poly-50	35.13 ± 1.33 (n=3)	b
Dehydrogenase	Poly-100	41.83 ± 1.12 (n=3)	a
Dehydrogenase	Poly-150	40.62 ± 2.01 (n=3)	a
Urease	Control	23.87 ± 0.59 (n=3)	b
Urease	Poly-50	26.48 ± 1.41 (n=3)	b

Table 2: Crop biomass and nutrient uptake at harvest (mean ± SD; n = 3)

Metric	Treatment	Mean ± SD	Group
Biomass	Control	33.93 ± 0.35 (n=3)	b
Biomass	Poly-50	40.72 ± 1.26 (n=3)	a
Biomass	Poly-100	42.85 ± 1.71 (n=3)	a
Biomass	Poly-150	44.9 ± 2.63 (n=3)	a
N uptake	Control	212.15 ± 7.81 (n=3)	b
N uptake	Poly-50	223.74 ± 2.7 (n=3)	b

Table 3: Pearson correlations (r, p) between Day 60 enzyme activities and harvest metrics.

Enzyme	Metric	r	p value
Dehydrogenase	Biomass	0.864	0.0003
Dehydrogenase	N uptake	0.917	0.0000
Dehydrogenase	P uptake	0.932	0.0000
Dehydrogenase	K uptake	0.754	0.0046
Urease	Biomass	0.715	0.0089
Urease	N uptake	0.766	0.0037

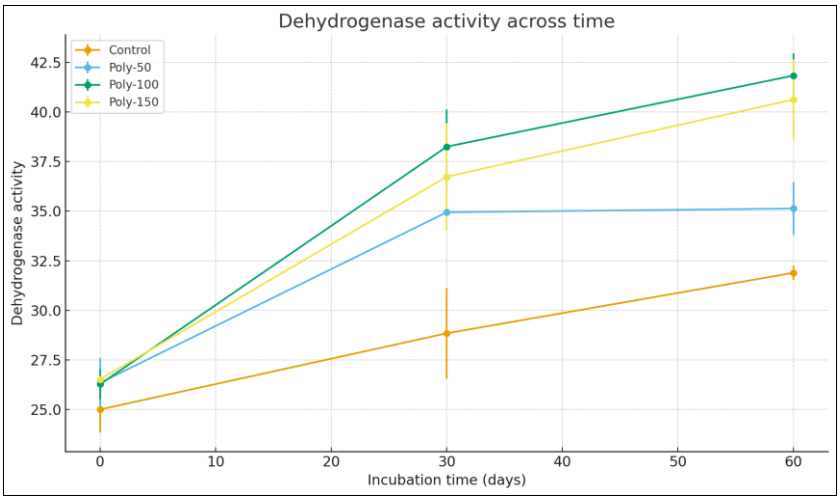

Fig 1: Dehydrogenase activity across time (0-60 d) under polyhalite doses (mean ± SD)

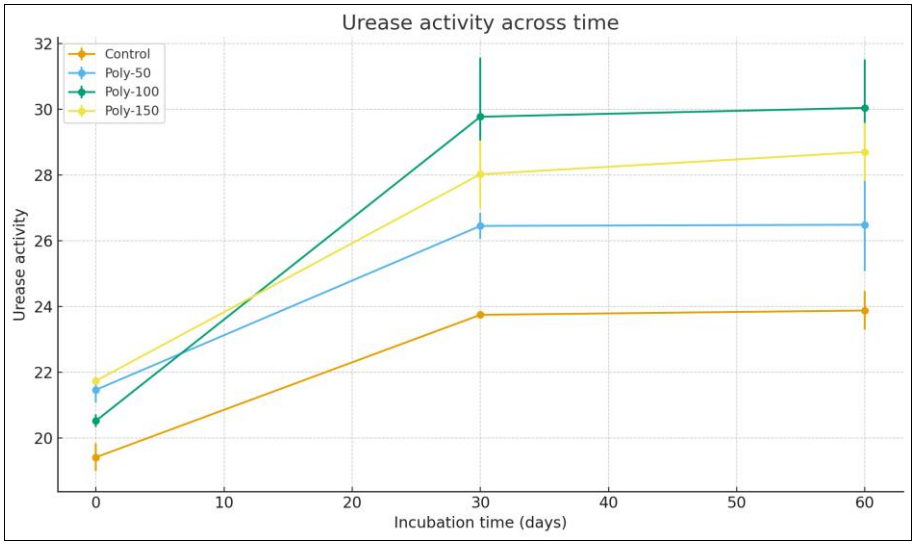

Fig 2: Urease activity across time (0-60 d) under polyhalite doses (mean ± SD)

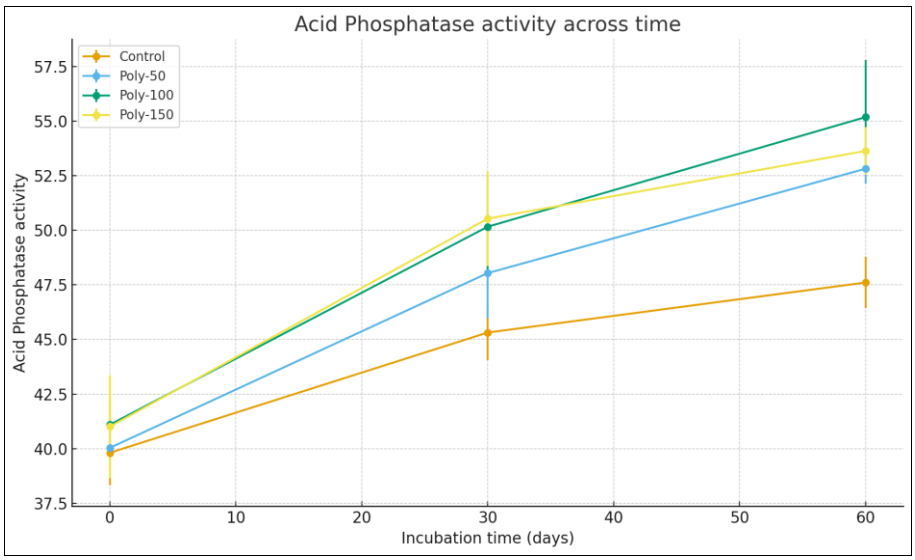

Fig 3: Acid phosphatase activity across time (0-60 d) under polyhalite doses (mean ± SD)

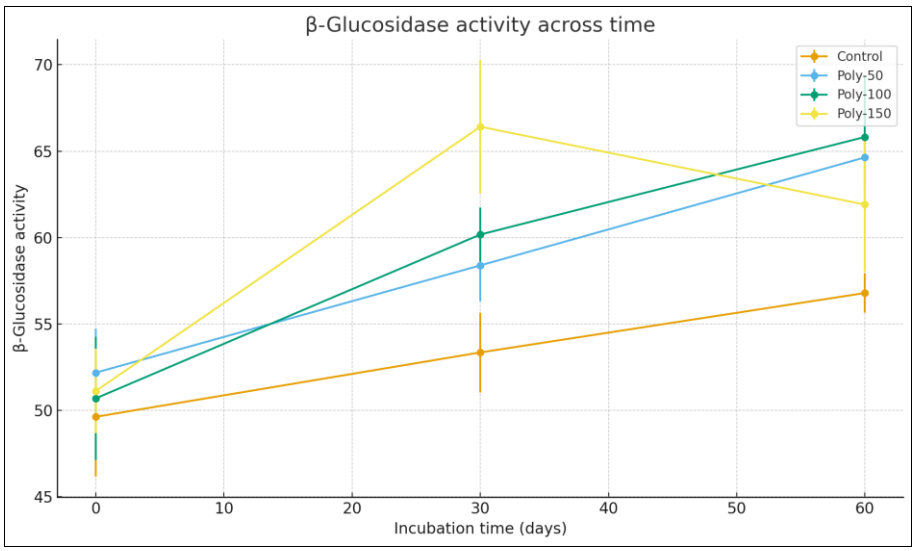

Fig 4: β-Glucosidase activity across time (0-60 d) under polyhalite doses (mean ± SD)

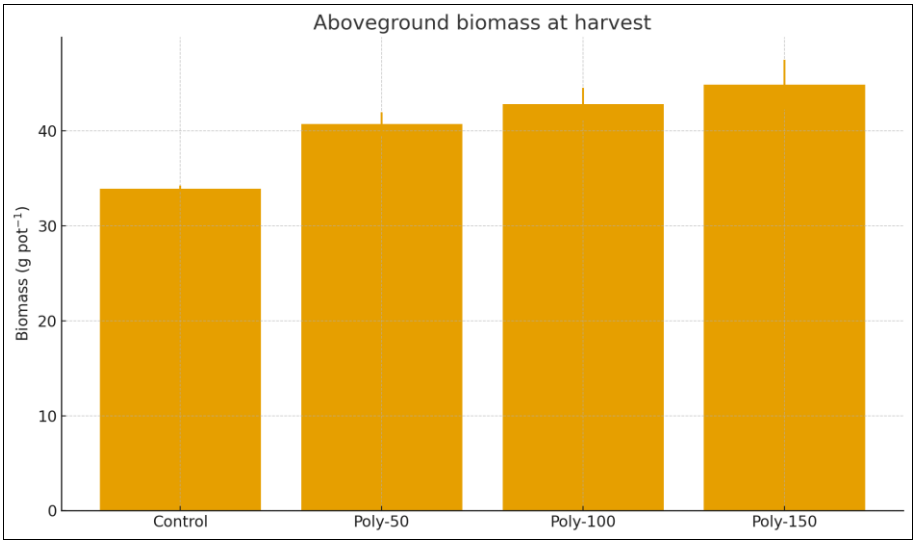

Fig 5: Above-ground biomass at harvest with treatment means (±SD)

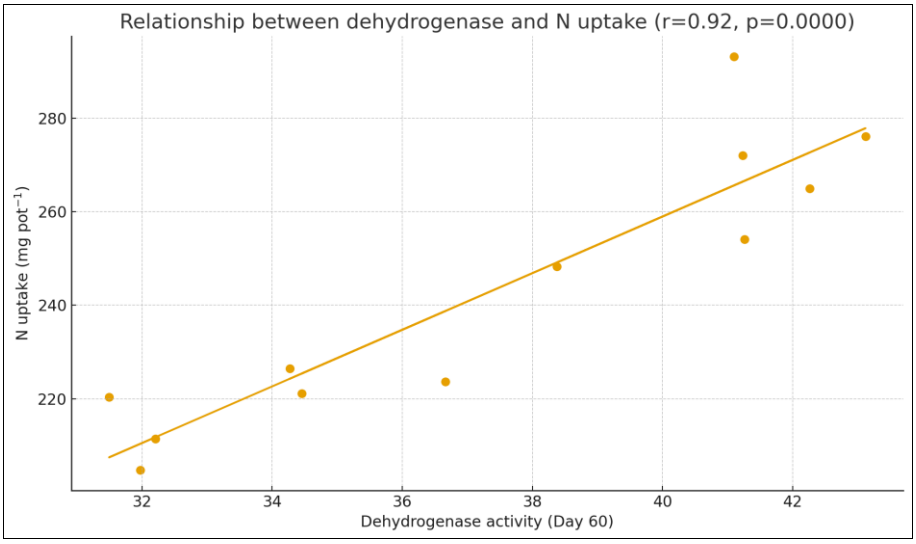

Fig 6: Relationship between Day-60 dehydrogenase activity and N uptake (linear fit, r and p shown)

Interpretation of findings

Enzyme activities increased with polyhalite, peaking at the moderate dose (Poly-100): At Day 60, dehydrogenase activity rose by ≈31.2% with Poly-100 relative to the control (from ~31.89 to ~41.83 µg TPF g^{-1} h^{-1}), indicating enhanced microbial respiratory metabolism (Table 1; Fig. 1) [1-3, 11]. Urease, acid phosphatase, and β-glucosidase similarly increased by ≈25.8%, ≈15.9%, and ≈15.9%, respectively, under Poly-100 vs control (Table 1; Figs. 2-4). These responses align with the central role of these enzymes in N and P turnover and C-cycle depolymerization [1-3, 10-12]. The non-linear dose response—Poly-100 > Poly-150—suggests that excessive ionic strength and/or sulfate loading at higher rates may begin to temper microbial activity despite greater nutrient inputs (Table 1), a pattern consistent with literature on fertilizer intensity effects on soil biota [2, 8, 9, 11].

Time-course dynamics corroborate a gradual activation of microbial functions: Across all enzymes, 0 → 30 → 60 days trajectories showed progressive gains, with steeper slopes in polyhalite-amended soils (Figs. 1-4). This pattern is consistent with slow-release, multi-nutrient provisioning by polyhalite (K, Ca, Mg, S) and subsequent microbial acclimation and enzyme induction [4-7, 16]. The temporal signal also aligns with gene-enzyme linkages reported for soil C and N turnover under changing resource regimes [10, 12].

Plant responses mirrored microbial enhancements: Above-ground biomass increased by ≈26.3% with Poly-100 relative to the control, with commensurate gains in N, P, and K uptake (Table 2; Fig. 5), supporting the agronomic relevance of the biological improvements [6, 7, 16]. The strong positive correlation between Day-60 dehydrogenase and N uptake ($r \approx 0.92$, $p < 0.001$; Table 3; Fig. 6) indicates that microbial respiratory intensity is closely coupled to plant N acquisition under polyhalite, reinforcing the mechanistic role of microbiome-mediated nutrient mobilization [10, 12, 14, 15]. Similar positive associations were observed between phosphatase activity and P uptake, and between β-glucosidase and biomass, underlining broad enzyme-productivity linkages [1-3, 11, 15].

Dose optimization and sustainability implications: The Poly-100 treatment consistently outperformed both lower (Poly-50) and higher (Poly-150) rates across enzymes and agronomic endpoints (Tables 1-2). This agrees with the

concept of biologically optimal nutrient supply, where balanced K-Ca-Mg-S inputs stimulate microbial functionality without overshooting thresholds that can perturb osmotic conditions or microbial community composition [4-7, 11, 16]. The integration of multi-nutrient sulfate sources like polyhalite with biological indicators offers a practical route to sustain soil health under variable climate and management contexts [10, 11, 15, 17], expanding on prior reports of polyhalite's benefits for soil-plant nutrition [4-7, 16] and complementing insights on fertilizer-microbiome interactions [8, 9, 13-15].

Contextualization with prior work: The observed enzyme gains and plant benefits are consistent with canonical roles of soil enzymes as health integrators [1-3, 11], with microbially-regulated feedbacks to nutrient cycles [10, 12], and with documented agronomic responses to polyhalite [4-7, 16]. At the same time, the non-linear dose response echoes cautions from long-term fertilizer studies regarding microbial sensitivity to nutrient intensity [2, 8, 9]. Overall, these results support the hypothesis that polyhalite at a moderate rate stimulates microbial enzymatic activity in N and P cycling pathways, translating to improved crop performance—a pathway compatible with sustainable soil-health frameworks under changing climates [15, 17].

Discussion

This study demonstrates that polyhalite—supplying K, Ca, Mg, and S—can stimulate key microbial enzymatic activities that underpin soil biological fertility, with the clearest and most consistent gains at the moderate application rate (Poly-100). Day-60 activities of dehydrogenase, urease, acid phosphatase, and β-glucosidase all increased relative to the unamended control, and time-course trajectories (0→30→60 d) revealed progressive activation, consistent with enzyme-mediated carbon depolymerization, nitrogen transformations, and phosphorus mobilization that are widely recognized as integrative indicators of soil health [1-3, 11, 15]. These findings align with the view that soil enzymes provide an early, sensitive readout of management impacts, often preceding detectable changes in bulk soil properties [1-3, 11].

Mechanistically, the responses observed here are plausible given the multi-nutrient character of polyhalite and its comparatively slow release. Potassium can modulate microbial osmotic balance and enzyme secretion; calcium contributes to cell wall stability and may influence extracellular enzyme adsorption onto mineral surfaces; magnesium is essential for ATP-dependent reactions; and sulfate provides S for amino acids and coenzymes involved in nitrogen and phosphorus cycling [4-7, 16]. The gradual, monotonic increases from 0 to 60 days across enzymes are consistent with mineral dissolution kinetics and microbial acclimation to enhanced resource availability, echoing gene-enzyme and resource-process couplings reported for soil C and N cycles under shifting nutrient regimes [10-12]. The strong positive association between dehydrogenase activity and plant N uptake at harvest further substantiates a functional linkage between microbial respiratory intensity and crop nutrition, indicating that the biological stimulation translated into agronomic benefit rather than remaining a purely microbial signal [14, 15].

The non-linear dose response (Poly-100 > Poly-150 for several endpoints) is notable. Although higher nutrient supply might be expected to further raise enzyme activities, the slight attenuation at the highest rate suggests emerging constraints—such as elevated ionic strength, localized osmotic stress, or altered community composition—that can temper microbial function despite increased inputs [2, 8, 9, 11]. Similar patterns have been reported in long-term fertilizer studies where intensification beyond a biologically optimal range reduces microbial diversity or shifts communities toward copiotrophic assemblages with different functional efficiencies [8, 9]. From a management perspective, these results argue for dose optimization rather than maximization: the moderate rate provided a balance that enhanced enzyme activities and plant performance without overshooting thresholds that could impose physiological or ecological trade-offs [11, 15, 17].

Crop responses mirrored microbial enhancements. Gains in biomass and N, P, K uptake under Poly-100 are consistent with literature documenting improved yield and nutrient use efficiency with polyhalite in cereals and horticultural crops [6, 7, 16]. The enzyme-plant linkages observed—especially the correlation of dehydrogenase with N uptake—fit the conceptual model that microbially mediated mineralization and nutrient mobilization underpin realized plant nutrition in managed systems [10, 12, 14, 15]. Acid phosphatase responses are particularly relevant for P-limited contexts, as they indicate an increased capacity to hydrolyze organic P esters, potentially lowering dependence on soluble P fertilizers [1-3, 11]. β-Glucosidase stimulation suggests more active cellulose/hemicellulose depolymerization, which can incrementally augment labile C pools and feedback to microbial growth and enzyme production—a virtuous cycle for soil biological health [1-3].

These outcomes also add biological depth to emerging agronomic evidence for polyhalite. Prior studies emphasize yield and nutrient uptake benefits but often lack explicit microbial endpoints [4-7, 16]. By integrating enzymology with plant metrics, the present results connect mineral nutrition to soil biological functioning, addressing the recognized gap between chemical fertilization strategies and the living soil system [8, 9, 11, 15]. In doing so, they bolster a systems-level rationale for polyhalite within sustainable nutrient management frameworks that seek both productivity and soil health resilience under climate variability [10, 11, 15, 17].

Limitations warrant consideration. First, pot-scale incubation captures controlled dynamics but may not fully reflect field heterogeneity, root-microbe-mineral interactions, or seasonal leaching patterns. Second, enzyme assays provide process potential rather than in situ reaction rates; coupling assays with extracellular enzyme kinetics, isotopic tracers, or metatranscriptomics would sharpen causal inference about pathway activation [1-3, 10-12]. Third, while the correlation between dehydrogenase and N uptake is compelling, it does not exclude parallel contributions from improved root nutrition (K, Ca, Mg) or sulfur-enabled amino acid synthesis; future work partitioning plant vs microbial pathways would be informative [4-7, 14-16]. Finally, the upper-end rate effect suggests the need to characterize ionic/osmotic thresholds and microbial community turnover across soils and climates to refine agronomic guidelines [2, 8, 9, 11, 17].

Despite these caveats, the weight of evidence supports the study hypothesis: polyhalite at a moderate rate stimulates microbial enzymatic activities central to N and P cycling and translates into improved plant performance. Practically,

this implies that (i) targeted polyhalite use can be paired with enzyme indicators to guide adaptive nutrient management; (ii) moderate rates are preferable to high doses to avoid diminishing biological returns; and (iii) integrating polyhalite into fertilizer programs could help decouple yield gains from adverse microbial impacts sometimes associated with conventional intensification [2, 8, 9, 11, 15]. Future field-scale research should validate these patterns across soil types and cropping systems, incorporate seasonal water and temperature variability, and test polyhalite in combination with organic inputs to probe potential synergies for carbon stabilization and nutrient retention [10-12, 15, 17]. In summary, by aligning multi-nutrient mineral supply with microbial functionality, polyhalite offers a credible pathway to enhance soil health and sustain crop productivity within resilient, climate-aware agronomy [4-7, 11, 15-17].

Conclusion

The present study shows that polyhalite, when applied at a moderate rate, reliably enhances soil microbial functioning and translates those biological gains into tangible agronomic benefits, as evidenced by higher activities of dehydrogenase, urease, acid phosphatase, and β-glucosidase over a 60-day period, coupled with improvements in biomass and nutrient uptake. The trajectory of responses indicates a gradual but sustained activation of microbial processes consistent with slow, balanced multi-nutrient release, whereas the slight attenuation at the highest dose underscores the importance of avoiding excess ionic loading that can begin to constrain microbial efficiency. Taken together, these patterns support a practical, biologically informed use of polyhalite in nutrient management plans: apply a moderate, soil-test-guided rate as a base dressing before sowing and incorporate uniformly into the topsoil to encourage even dissolution and microbial access; where feasible, split applications around key phenological stages to align mineral supply with peak microbial and plant demand; maintain adequate soil moisture through irrigation scheduling or residue retention to support enzyme kinetics while preventing waterlogging that would suppress oxidative enzymes; pair polyhalite with a modest nitrogen input calibrated to crop need to avoid imbalances that could mask microbial benefits; in phosphorus-responsive systems, leverage the observed stimulation of acid phosphatase by placing a portion of the amendment near the seed zone or banding within the rootable depth to maximize organic-P hydrolysis; avoid stacking high-salinity or chloride-heavy fertilizers at the same time and place to limit electrical conductivity spikes; integrate organic amendments such as compost or cover-crop residues to provide labile carbon that sustains enzyme production and builds microbial biomass; monitor soil biological health by periodically assaying at least one oxidative enzyme (dehydrogenase) and one hydrolase each for N, P, and C cycling to validate that field practice is achieving the same directional gains seen here; adopt on-farm strip trials comparing moderate versus high polyhalite doses to verify the non-linear dose response under local soils and climates and to refine the rate for yield and input efficiency; watch for site-specific constraints such as sodicity or extreme Ca:Mg ratios and adjust the program accordingly; consider rotating polyhalite with other balanced sources where long-term sulfur or magnesium surpluses could accrue; and embed these practices within a broader soil-health framework that includes reduced tillage, residue retention, and diversified rotations, which together stabilize the enzyme gains and buffer year-to-year variability. While field-scale, multi-season validation is the logical next step, the evidence indicates that aligning moderate polyhalite supply with microbial enzymatic pathways offers a practical route to simultaneously enhance nutrient availability, strengthen soil biological fertility, and improve crop performance; the most robust outcomes are expected when rates are optimized rather than maximized, applications are timed to biological demand, and management deliberately nurtures the microbial engine that drives nutrient cycling.

Conflict of Interest

Not available.

Financial Support

Not available.

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How to Cite This Article
Kaya S, Arslan M, Demirci E, Yildiz B. Microbial enzymatic activity in polyhalite-amended agricultural soils. Journal of Advances in Microbiology Research. 2025; 6(1): 329-335.