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Eco-enzyme-assisted restoration of soil fertility and microbial activity in nickel mining-contaminated soils

Hendra Jultrisno Rusman[1], Fahruddin[2*], Nurlina Kasim[3], Baharuddin[4], Eymal Bashar Demmallino[1], Muh. Jayadi[5]

[1] Environmental Science Study Program, The Graduate School, Hasanuddin University, Makassar, Indonesia
[2] Department of Biology, Faculty of Mathematics and Natural Science, Hasanuddin University, Makassar, Indonesia
[3] Department of Agrotechnology, Faculty of Agriculture, Hasanuddin University, Makassar, Indonesia
[4] Department of Pest and Plant Disease, Faculty of Agriculture, Hasanuddin University, Makassar, Indonesia
[5] Industrial Engineering Study Program, Faculty of Engineering, Muhammadiyah University of Luwuk, Indonesia
* Corresponding author's e-mail: fahruddin_science@unhas.ac.id

ABSTRACT

Nickel mining activities often cause severe soil degradation, reducing fertility as well as microbial activity, and hindering ecological recovery. This study evaluated the effectiveness of eco-enzyme application in improving the physicochemical and biological properties of nickel mining-contaminated soils. A laboratory incubation experiment was conducted using two soil types treated with eco-enzyme concentrations ranging from 0 to 10% and incubated for up to 30 days. Soil pH, soil organic carbon, total nitrogen, available phosphorus, cation exchange capacity, base saturation, aggregate stability, and microbial populations were analysed. The results showed that the eco-enzyme significantly improved soil quality, with the best performance achieved at 10% concentration after 30 days of incubation. Under this treatment, soil pH, nutrient availability, cation exchange capacity, aggregate stability, and microbial populations increased substantially in both soil types. These findings indicate that eco-enzyme is a low-cost and environmentally friendly amendment with strong potential for sustainable rehabilitation of nickel mining-degraded soils.

Keywords: eco-enzyme, nickel mining, soil fertility restoration, microbial activity, soil rehabilitation, contaminated soils.

INTRODUCTION

Nickel mining activities have become one of the major drivers of land degradation in tropical regions, particularly in the areas dominated by open-pit laterite mining systems (Tambara et al., 2025; Atika et al., 2026; Giljum et al., 2025). Intensive excavation, vegetation removal, and topsoil stripping alter soil structure, accelerate erosion, and reduce soil fertility, thereby affecting the ecological stability of surrounding environments. In many mining areas, the exposure of sulphide-bearing materials also promotes soil acidification and increases the mobility of potentially toxic elements, resulting in long-term deterioration of soil quality and agricultural productivity (Fahruddin et al., 2024a; Fahruddin et al., 2024b; Rashid et al., 2023; Akpa et al., 2025). Contaminated runoff and sediment transport from mining sites may further affect nearby agricultural land, particularly paddy soils located downstream of mining operations (Wang et al., 2024; Rashmi et al., 2022). Previous studies demonstrated that the mining-affected soils commonly exhibit low

pH, reduced nutrient availability, poor aggregate stability, and severe depletion of soil organic matter, all of which limit ecosystem recovery and sustainable land use (Punia and Bharti, 2023; Rajput et al., 2025; Sánchez-Castro et al., 2023).

Soil degradation associated with nickel mining also strongly affects soil biological processes. Acidic conditions and heavy metal stress suppress microbial growth, inhibit enzymatic activity, and reduce organic matter decomposition, thereby disrupting nutrient cycling and soil biochemical functioning (Naz et al., 2022; Xiao et al., 2023). As a consequence, mining-contaminated soils generally contain low levels of soil organic carbon, nitrogen, phosphorus, and exchangeable base cations required for plant growth and microbial metabolism (Rashmi et al., 2020; Rashmi et al., 2024). Low soil pH additionally increases the solubility and mobility of heavy metals, further aggravating soil toxicity and reducing natural rehabilitation capacity (Alsafran et al., 2023; Wang et al., 2022). These conditions indicate that restoration of mining-degraded soils requires the remediation approaches capable of simultaneously improving soil chemical fertility and supporting biological recovery.

Various remediation technologies have been developed to improve the quality of mining-contaminated soils. Biological and organic-based remediation approaches have received increasing attention because they are considered more environmentally compatible and sustainable than conventional chemical remediation methods (Chaudhary et al., 2023; Haroun et al., 2023; Rajput et al., 2025). Previous studies reported that organic amendments, compost, biochar, and microbial inoculants can improve soil pH, nutrient retention, cation exchange capacity, and microbial activity in degraded soils (Mensah et al., 2022; Rahman et al., 2020; Wang et al., 2022). Sulphate-reducing bacteria (SRB) were also reported to contribute to remediation processes through sulphate reduction and acidity neutralisation, thereby improving soil biochemical conditions in contaminated environments (Ayangbenro et al., 2018; Hu et al., 2024; Mafane et al., 2025). However, most previous remediation studies primarily focused on single remediation agents and emphasised either contaminant stabilisation or soil fertility improvement separately, while integrated approaches addressing multiple aspects of soil recovery remain limited.

Mining activities have become a major environmental concern in many nickel-producing regions, particularly in tropical countries such as Indonesia. Large-scale open-pit nickel mining often results in the removal of topsoil, destruction of vegetation cover, soil compaction, nutrient depletion, and disruption of soil microbial communities. In several mining areas of Sulawesi and other nickel-producing regions, extensive land degradation has reduced soil productivity and hindered natural ecosystem recovery. Furthermore, the accumulation of potentially toxic metals and the decline of organic matter content have created unfavourable conditions for plant establishment and long-term land rehabilitation. These challenges have intensified the need for sustainable and cost-effective remediation technologies capable of restoring both soil fertility and biological functions. Therefore, developing environmentally friendly approaches that can simultaneously improve soil physicochemical properties and microbial activity has become an important priority for post-mining land management.

Eco-enzyme has recently emerged as a potential organic amendment for environmental management and soil restoration. Eco-enzyme is generally produced through fermentation of organic waste materials with sugar and water, generating organic acids, soluble nutrients, and diverse microbial communities during the fermentation process (Indraloka et al., 2023; Benny et al., 2023). Previous studies demonstrated that eco-enzyme applications can improve organic matter decomposition, stimulate microbial activity, and enhance nutrient availability in environmental remediation systems (Salvi et al., 2024; Madan et al., 2025). Organic compounds produced during fermentation may also contribute indirectly to soil recovery through nutrient solubilisation, cation retention, and stabilisation of soil biochemical processes (Ren et al., 2023; Vega et al., 2022). Nevertheless, the studies evaluating eco-enzyme application for rehabilitation of nickel mining-contaminated soils are still very limited, particularly regarding their effects on soil fertility restoration and microbial recovery in post-mining and contaminated agricultural soils.

In addition, many previous studies on eco-enzyme applications focused mainly on wastewater treatment or organic waste utilisation, while comprehensive evaluations of soil physicochemical and biological recovery following eco-enzyme application remain scarce (Benny et al., 2023;

Salvi et al., 2024). The information regarding the response of soil fertility indicators, such as soil organic carbon, total nitrogen, phosphorus availability, exchangeable base cations, cation exchange capacity, and microbial abundance under eco-enzyme treatment in mining-degraded soils is also still limited. Therefore, further studies are needed to evaluate the potential of eco-enzymes as a sustainable remediation strategy for restoring soil quality in mining-affected ecosystems.

In the study area, nickel mining activities have caused substantial soil degradation, resulting in highly acidic conditions and poor soil fertility. Initial soil characterisation showed that post-mining soils were strongly acidic (pH 3.82) and contained very low levels of soil organic carbon (0.82%) and total nitrogen (0.07%), indicating severe nutrient depletion and limited biological activity. Such conditions restrict vegetation establishment, reduce ecosystem resilience, and hinder the success of post-mining land rehabilitation programs. Although various soil amendments have been investigated, the application of eco-enzyme as a sustainable remediation material for nickel mining-contaminated soils remains insufficiently explored. Therefore, evaluating its effectiveness under local soil conditions is important for developing environmentally friendly rehabilitation strategies in mining-affected areas.

On the basis of these conditions, this study evaluated the effectiveness of eco-enzyme application in restoring the quality of nickel mining-contaminated soils. The study focused on post-mining soil and contaminated paddy soil subjected to different eco-enzyme concentrations and incubation periods under controlled laboratory conditions. The effectiveness of the eco-enzyme was assessed through changes in soil physicochemical and biological properties to determine its potential as a sustainable soil remediation amendment. The findings are expected to contribute to the development of environmentally friendly and low-cost rehabilitation strategies for mining-degraded lands and contaminated agricultural soils.

MATERIAL AND METHODS

Study area and research design

This study was conducted from November to December 2025 at the Biochemistry Laboratory, Faculty of Mathematics and Natural Sciences, Hasanuddin University, using soil samples collected from a nickel mining area and adjacent agricultural land in Siuna Village. Two soil types were evaluated, namely post-mining soil (TT) collected from a nickel mining site and contaminated paddy soil (TS) collected from agricultural land located near the mining area. The study area is characterised by intensive open-pit nickel mining activities associated with soil acidification and land degradation.

Soil sampling was conducted using a stainless-steel hand auger to minimise contamination during collection. For each land-use type, soil samples were collected from a depth of 0–20 cm, representing the topsoil layer most affected by mining activities and soil management practices. Five sampling points were established at each site using a purposive sampling approach. At each sampling point, three subsamples were collected within a radius of approximately 5 m and homogenised to form one composite sample. The distance between sampling points was approximately 20–30 m to ensure representative spatial coverage of the study area. Composite samples were air-dried, sieved through a 2-mm mesh, and stored for subsequent laboratory analyses.

Although the study area is located within an active nickel mining region and exhibits clear symptoms of mining-induced soil degradation, direct measurements of nickel and other heavy metal concentrations were not conducted in the present study. The soil contamination status was inferred from their proximity to mining activities and their degraded physicochemical characteristics. Future studies should incorporate detailed heavy metal analyses to better quantify contamination levels and evaluate the effectiveness of eco-enzyme application in reducing metal mobility and bioavailability.

A laboratory-scale experiment was conducted to evaluate the effects of eco-enzyme application on soil fertility restoration and microbial recovery. The experiment used different eco-enzyme concentrations (0%, 2.5%, 5.0%, 7.5%, and 10%) combined with incubation periods of 0, 10, 20, and 30 days under controlled laboratory conditions. Soil quality recovery was assessed using physicochemical and biological parameters, including soil pH, soil organic carbon, total nitrogen, available phosphorus, exchangeable base cations, cation exchange capacity, base saturation, soil aggregate stability, and microbial populations. The location of the study area and soil sampling sites

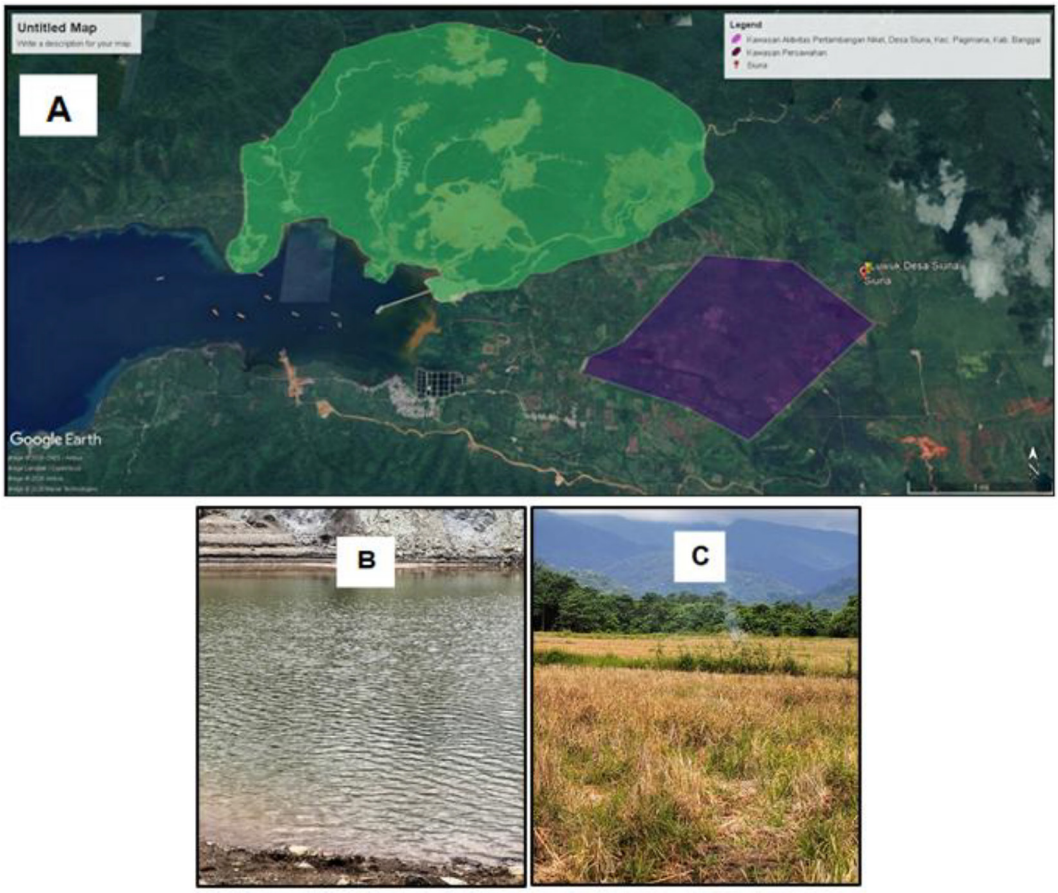

Figure 1. Location of soil sampling sites in nickel mining-affected areas showing the geographical position of the study area and sampling points

is presented in Figure 1. The sampling locations were selected to represent both post-mining land and agricultural land potentially affected by mining activities in Siuna Village, Central Sulawesi, Indonesia.

Preparation of eco-enzyme and soil incubation

The eco-enzyme was prepared through anaerobic fermentation of organic waste materials, brown sugar, and water at a ratio of 1:3:10 (w/v/v), following the eco-enzyme production approach commonly reported in previous studies (Indraloka et al., 2023; Benny et al., 2023; Madan et al., 2025). The formulation was further enriched with sulphate-reducing bacteria (SRB) and indigenous bacteria isolated from nickel mining soil to enhance microbial activity and support the remediation of contaminated soils, considering the important role of SRB in heavy metal immobilisation and mine soil rehabilitation (Ayangbenro et al., 2018; Hu et al., 2024; Mafane et al., 2025). Fermentation was carried out in closed plastic containers under anaerobic conditions at room temperature for three months until a stable dark-brown liquid with a characteristic acidic odour was obtained. The composition of the eco-enzyme formulation is presented in Table 1.

The eco-enzyme formulation was developed based on the standard fermentation ratio of organic substrates, sugar, and water (1:3:10) reported in previous eco-enzyme studies (Indraloka et al., 2023; Benny et al., 2023), with

Table 1. Composition of the integrated eco-enzyme formulation used in this study

Component	Composition/Source
Organic substrates	Mixed fruit and vegetable waste
Brown sugar	1 part (w/v)
Water	10 parts (v/v)
Sulphate-reducing bacteria (SRB)	Enriched culture isolated and propagated in the laboratory
Indigenous mining soil bacteria	Isolated from nickel mining-contaminated soil

additional enrichment using sulphate-reducing bacteria and indigenous mining soil bacteria to enhance the remediation potential for mining-contaminated soils (Ayangbenro et al., 2018; Hu et al., 2024).

Soil incubation experiments were conducted using 5 L plastic bioreactors under laboratory conditions. Each treatment consisted of 300 g of soil mixed with 100 mL of synthetic acid mine drainage solution and eco-enzyme according to the treatment concentration. Five eco-enzyme concentrations were evaluated, namely 0% (control), 2.5%, 5.0%, 7.5%, and 10% (v/w), as presented in Table 2. Based on the preliminary concentration test, the optimum concentration (10%) was subsequently incubated for 0, 10, 20, and 30 days to evaluate temporal changes in soil quality and microbial recovery. All treatments were maintained at room temperature under identical laboratory conditions throughout the incubation period. Figure 2 presents the laboratory incubation process applied to post-mining soil and contaminated agricultural soil, showing the experimental setup used to evaluate the effects of eco-enzyme application on soil restoration and microbial recovery.

Microbiological and chemical characterisation of eco-enzyme

Prior to soil application, the eco-enzyme was chemically and microbiologically characteriaed to evaluate its properties and ensure the reproducibility of the remediation experiment. The eco-enzyme was produced through anaerobic fermentation of mixed fruit waste (citrus, pineapple, and papaya residues), brown sugar, and distilled water at a ratio of 3:1:10 (w/v/v) for 90 days at room temperature (28 ± 2 °C), following procedures commonly reported for eco-enzyme production (Indraloka et al., 2023; Benny et al., 2023). Chemical characterisation of the liquid eco-enzyme was conducted using methods specifically applicable to liquid organic amendments. The pH and electrical conductivity (EC) were measured using a calibrated digital pH/EC meter following standard procedures for liquid samples (APHA, 2017). Total organic carbon (TOC) was determined using the Walkley–Black wet oxidation method, while total nitrogen (TN) was analysed using the Kjeldahl digestion method (Bremner, 1996). Phosphate and potassium concentrations were measured according to standard analytical procedures for liquid organic fertilisers (APHA, 2017). Organic acids were identified and

Table 2. Experimental design for eco-enzyme concentration treatments

Treatment code	Treatment composition	Eco-enzyme concentration
C0	300 g soil + 100 mL acid mine drainage	0%
C1	7.5 mL eco-enzyme + 300 g soil + 100 mL acid mine drainage	2.5%
C2	15 mL eco-enzyme + 300 g soil + 100 mL acid mine drainage	5%
C3	22.5 mL eco-enzyme + 300 g soil + 100 mL acid mine drainage	7.5%
C4	30 mL eco-enzyme + 300 g soil + 100 mL acid mine drainage	10%

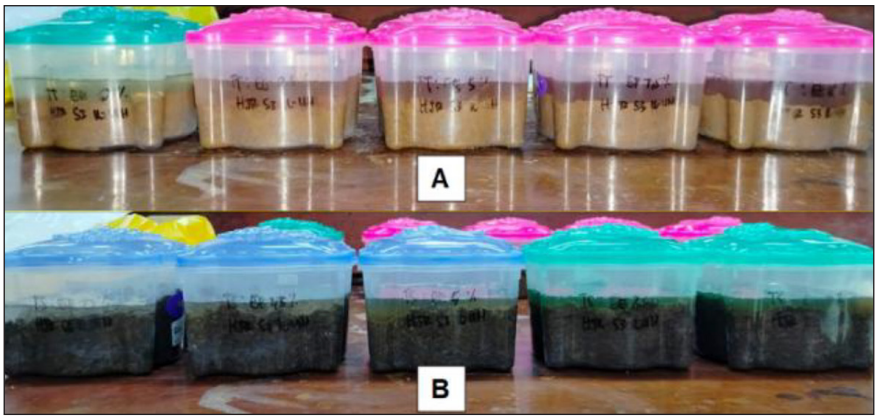

Figure 2. Laboratory incubation experiment showing eco-enzyme application in (A) post-mining soil and (B) contaminated agricultural soil (paddy field) collected from the study area

quantified using high-performance liquid chromatography (HPLC), following the procedure described by Indraloka et al. (2023). Hydrolytic enzyme activities were determined spectrophotometrically according to standard enzymatic assay protocols. These analytical procedures differ from those used for soil characterisation because the eco-enzyme is a liquid fermentation product requiring liquid-phase analytical methods.

Microbiological characterisation was conducted using serial dilution and spread plate techniques to determine culturable microbial populations in the eco-enzyme. Total bacterial populations were enumerated on nutrient agar, while sulphate-reducing bacteria (SRB) were isolated and cultivated using selective SRB growth media following standard microbiological procedures for anaerobic bacteria (Ayangbenro et al., 2018; Mafane et al., 2025). Dominant bacterial isolates were identified through 16S rRNA gene sequencing using molecular characterisation procedures widely applied for environmental microbial identification (Chaudhary et al., 2023). The eco-enzyme contained several dominant bacterial genera, including *Bacillus*, *Lactobacillus*, *Pseudomonas*, and *Desulfovibrio*. The presence of SRB was further confirmed by sulphide production under anaerobic incubation conditions, following established approaches for sulphate-reducing bacterial verification (Hu et al., 2024; Ayangbenro et al., 2018). The characterisation results demonstrated that the eco-enzyme contained active microbial populations, organic acids, hydrolytic enzymes, and nutrient compounds that potentially contributed to soil fertility improvement and microbial stimulation during soil incubation. The chemical and microbiological characteristics of the eco-enzyme are presented in Table 3.

Heavy metal analysis

Total concentrations of Ni, Pb, Fe, and Cr in soil samples were determined using Atomic Absorption Spectrophotometry (AAS) following acid digestion procedures modified from Mensah et al., (2022). Approximately 1 g of air-dried soil (<2 mm) was digested using concentrated HNO_3 under heating conditions until complete mineralisation was achieved. The digested solution was filtered and diluted to 50 mL with distilled water prior to analysis. Metal concentrations were measured using an Atomic Absorption Spectrophotometer (Buck Scientific 235 ATS) at wavelengths of 232.0 nm (Ni), 283.2 nm (Pb), 248.3 nm (Fe), and 357.9 nm (Cr). Heavy metal concentrations were expressed as mg kg^{-1} dry soil. All analyses were performed in triplicate, and quality control was maintained using reagent blanks and standard calibration solutions. Heavy metal concentrations were calculated based on the absorbance values

Table 3. Chemical and microbiological characteristics of eco-enzyme used in this study

Parameter	Value	Unit
pH	3.54 ± 0.08	-
Electrical conductivity	3.28 ± 0.14	mS cm^{-1}
Total organic carbon	18.42 ± 0.76	g L^{-1}
Dissolved organic carbon	12.85 ± 0.52	g L^{-1}
Total nitrogen	1.62 ± 0.05	g L^{-1}
Phosphate	0.84 ± 0.03	g L^{-1}
Potassium	2.36 ± 0.08	g L^{-1}
Acetic acid	4.15 ± 0.12	g L^{-1}
Citric acid	2.74 ± 0.10	g L^{-1}
Protease activity	18.52 ± 0.64	U mL^{-1}
Amylase activity	12.63 ± 0.58	U mL^{-1}
Cellulase activity	9.84 ± 0.42	U mL^{-1}
Total bacterial population	8.6×10^7	CFU mL^{-1}
Sulphate-reducing bacteria	3.4×10^6	CFU mL^{-1}
Dominant bacterial genera	Bacillus, Lactobacillus, Pseudomonas, Desulfovibrio	–

Note: Values represent mean ± standard deviation of triplicate measurements. The eco-enzyme contained active microbial populations, organic acids, hydrolytic enzymes, and nutrient compounds that potentially contributed to soil remediation and nutrient mobilization.

obtained from the calibration curve and corrected using the dilution factor and final digestion volume, following analytical procedures commonly applied in heavy metal assessment studies (Akpa et al., 2025; Wang et al., 2024). The concentration was determined using Equation 1:

$$[LB] = \frac{V_{final}}{V_{initial}} \times Concentration \times f_p \quad (1)$$

Soil pH measurement

Soil pH was measured using a soil-to-water suspension method. Briefly, 10 g of air-dried soil was mixed with 25 mL of distilled water (1:2.5, w/v), shaken for 30 min, and allowed to settle prior to measurement. The pH of the supernatant was determined using a calibrated digital pH meter following the method of Nurrisma, (2023). Measurements were performed in triplicate for all treatments. Soil pH was monitored before and after eco-enzyme application to evaluate changes in soil acidity during the remediation process.

Determination of soil organic carbon and nitrogen

Soil organic carbon (C-organic) was analysed using the Walkley–Black oxidation method, while total nitrogen (N-organic) was determined using the Kjeldahl digestion-distillation method following standard procedures commonly applied in soil fertility and contaminated soil assessment studies (Mensah et al., 2022; Haroun et al., 2023). The analyses of C-organic and N-organic were conducted because mining activities, particularly open-pit nickel mining, are known to reduce soil organic matter, alter soil structure, suppress microbial-mediated nutrient cycling, and decrease nutrient availability and soil fertility (Punia and Bharti, 2023; Giljum et al., 2025; Naz et al., 2022; Xiao et al., 2023). Previous studies have also reported that biological amendments, including biofertilisers, organic substrates, sulphate-reducing bacteria, and integrated bioremediation systems, can improve nutrient availability, stimulate microbial activity, and enhance heavy metal stabilisation in contaminated soils (Chaudhary et al., 2023; Hu et al., 2024; Haroun et al., 2023; Alsafran et al., 2023). Therefore, the evaluation of C-organic and N-organic contents in this study was intended to assess the effectiveness of integrated eco-enzyme application in restoring soil fertility and supporting sustainable post-mining land rehabilitation.

Determination of available phosphorus and exchangeable minerals

Available phosphorus (P) was determined using the Bray I extraction method for acidic soils, followed by spectrophotometric measurement of phosphate concentration. Exchangeable base cations (K, Ca, Mg, and Na) were extracted using 1 N ammonium acetate (NH_4OAc) at pH 7.0 and quantified using atomic absorption spectrophotometry (AAS). Cation exchange capacity (CEC) was calculated from the total exchangeable cations retained by the soil exchange complex, while base saturation (BS) was determined as the percentage of exchangeable base cations relative to the CEC. These parameters were evaluated to assess nutrient availability and soil fertility recovery following eco-enzyme application, as nutrient depletion and exchangeable cation losses are common consequences of mining-induced soil degradation (Punia and Bharti, 2023; Tambara et al., 2025; Rashid et al., 2023). Previous studies have shown that biological and organic amendments can improve nutrient retention, increase cation exchange capacity, and enhance soil fertility in degraded and contaminated soils (Mensah et al., 2022; Haroun et al., 2023; Chaudhary et al., 2023).

Determination of cation exchange capacity and base saturation

Cation exchange capacity (CEC) was determined using the ammonium acetate (NH_4OAc) extraction method at pH 7.0 following the standard procedures recommended by Soil Survey Staff (2022). Soil samples were extracted with a 1 N ammonium acetate solution to replace exchangeable cations adsorbed on soil colloids. The extracted cations were subsequently quantified, and the CEC value was calculated as the total amount of exchangeable cations retained by the soil exchange complex. CEC was used as an indicator of the soil's capacity to retain and supply essential nutrients during remediation. The CEC value was calculated using Equation 2.

$$CEC = (Sample\ titration - Blank) \times \frac{100}{0.1 \times 1000} \quad (2)$$

Base saturation (BS) was calculated as the percentage of exchange sites occupied by base cations (K, Mg, Ca, and Na) using Equation 3:

$$BS\ (\%) = \frac{K+Mg+Ca+Na}{CEC} \times 100 \quad (3)$$

All analyses were performed in triplicate to evaluate changes in soil fertility and nutrient retention following eco-enzyme treatment.

Soil texture analysis

Soil texture was analysed using the standard pipette method following the procedures recommended by Soil Survey Staff (2022). The soil samples collected from 0–30 cm were pretreated to remove organic matter and dispersed using a chemical dispersing agent before particle-size separation. Sand, silt, and clay fractions were determined based on sedimentation principles and Stokes' law. The percentages of each fraction were subsequently used to classify soil texture and evaluate soil physical characteristics. All analyses were conducted in triplicate.

Microbial population analysis

Soil microbial populations were determined using a serial dilution and plate count technique commonly applied in soil microbiology studies (Naz et al., 2022; Xiao et al., 2023). Briefly, 10 g of soil was suspended in sterile 0.9% NaCl solution and serially diluted up to 10^{-7}. Aliquots from appropriate dilutions were inoculated onto Nutrient Agar medium and incubated at 28–31 °C for 3–5 days. The number of colonies was counted and expressed as colony-forming units (CFU g^{-1} soil). All analyses were performed in triplicate to evaluate changes in microbial abundance following eco-enzyme application and incubation.

Data analysis

All data were expressed as mean ± standard deviation of triplicate measurements. Differences among treatments were evaluated using analysis of variance (ANOVA) followed by the appropriate post hoc test at a significance level of $p < 0.05$. Statistical analyses were performed to assess the effects of eco-enzyme concentration and incubation period on soil properties. The overall experimental workflow used in this study, including soil sampling, eco-enzyme preparation and characterisation, incubation treatments, as well as physicochemical and microbiological analyses, is presented in Figure 3.

The analytical procedures used in this study followed internationally recognised standard

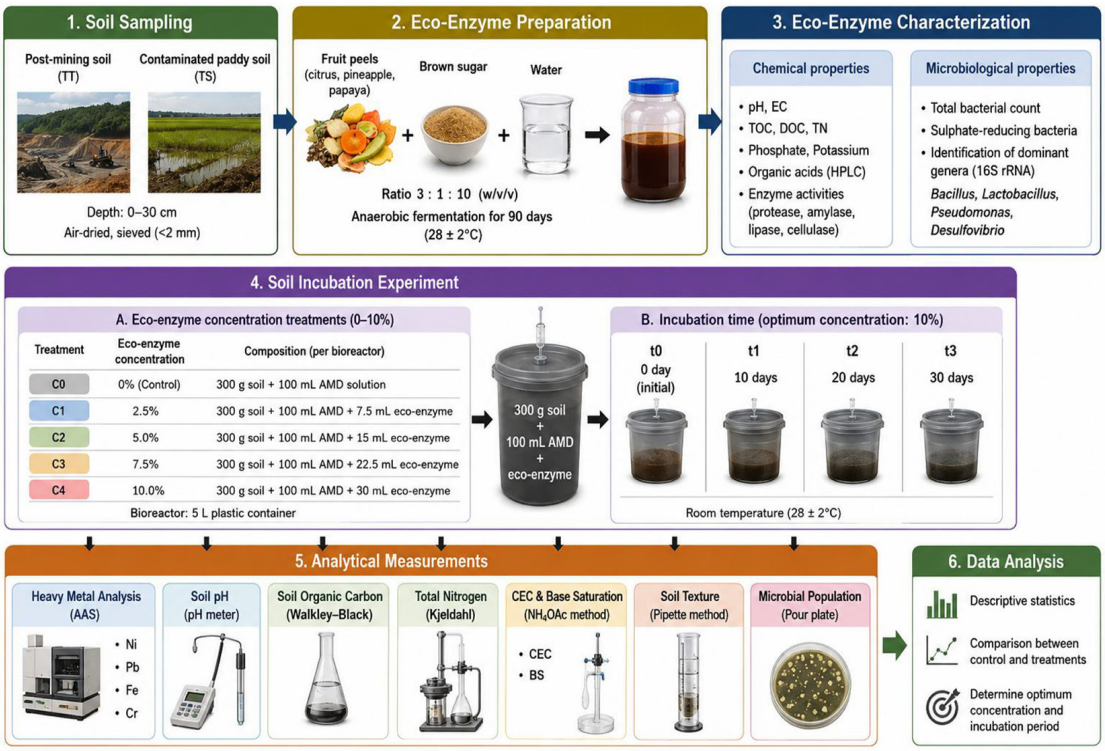

Figure 3. Schematic overview of the experimental design, incubation treatments, and physicochemical and microbiological analyses, including heavy metals, soil pH, soil organic carbon, total nitrogen, available phosphorus, exchangeable cations, cation exchange capacity (CEC), base saturation (BS), soil texture, and microbial populations

methods. Soil pH was determined using a glass electrode pH meter in a soil-water suspension. Soil organic carbon (SOC) was analysed using the Walkley–Black method, while TN was determined using the Kjeldahl digestion method. Available P was measured using the Bray I extraction method. Exchangeable bases (Ca, Mg, K, and Na) were determined by ammonium acetate extraction, and cation exchange capacity (CEC) was calculated based on the sum of exchangeable cations. Aggregate stability was assessed using the wet-sieving method, whereas microbial population was quantified using the total plate count (TPC) method. All analyses were conducted following standard soil analysis procedures recommended by the Soil Science Society of America (SSSA) and relevant laboratory protocols.

Experimental procedure

The experimental work was conducted through four main stages: soil sampling, eco-enzyme application, incubation, and laboratory analysis.

- First, soil samples were collected from post-mining land and contaminated paddy fields affected by nickel mining activities. The collected samples were air-dried, homogenised, and sieved to remove coarse materials before treatment application.
- Second, eco-enzyme solutions were prepared and applied at concentrations of 0%, 2.5%, 5.0%, 7.5%, and 10% (v/w) based on the dry weight of soil. Each treatment was thoroughly mixed to ensure uniform distribution of the eco-enzyme throughout the soil matrix.
- Third, the treated soils were incubated under controlled laboratory conditions for 0, 10, 20, and 30 days. Soil moisture was maintained throughout the incubation period to support microbial activity and biochemical processes associated with eco-enzyme decomposition.
- Finally, the soil samples from each treatment and incubation period were analysed to determine changes in physicochemical and biological properties, including soil pH, soil organic carbon, total nitrogen, available phosphorus, exchangeable bases, cation exchange capacity, base saturation, aggregate stability, and microbial population.

RESULTS AND DISCUSSION

Changes in soil pH following eco-enzyme application

Changes in soil pH under different eco-enzyme concentrations and incubation periods are presented in Table 4 and Figure 4. Initial soil conditions showed strongly acidic pH values in both TT and TS, indicating severe acidification associated with nickel mining activities and acid mine drainage. Soil pH increased progressively with eco-enzyme concentration and incubation time in both soil types. The highest pH values were observed under the C4–t3 treatment (10% eco-enzyme, 30 days incubation), reaching 5.86 in TT soil and 6.21 in TS soil. These results indicate that eco-enzyme application effectively reduced soil acidity and improved soil chemical conditions during the remediation process.

The increase in soil pH was likely associated with microbial activity and organic compounds produced during eco-enzyme fermentation. The

Table 4. Changes in soil pH under different eco-enzyme concentrations and incubation periods in post-mining soil (TT) and contaminated paddy soil (TS)

Treatment	Eco-enzyme concentration (%)	Incubation period (days)	Soil pH (TT)	Soil pH (TS)
C0-t0	0	0	3.82 ± 0.04	4.15 ± 0.03
C1-t1	2.5	10	4.21 ± 0.05	4.56 ± 0.04
C2-t1	5.0	10	4.48 ± 0.03	4.87 ± 0.05
C3-t1	7.5	10	4.79 ± 0.04	5.18 ± 0.03
C4-t1	10.0	10	5.03 ± 0.05	5.42 ± 0.04
C4-t2	10.0	20	5.41 ± 0.03	5.78 ± 0.05
C4-t3	10.0	30	5.86 ± 0.04	6.21 ± 0.03

Note: Values are presented as mean ± standard deviation. TT = post-mining soil; TS = contaminated paddy soil. Increasing eco-enzyme concentration and prolonged incubation significantly improved soil pH in both soil types, indicating progressive neutralization of soil acidity and enhanced remediation performance.

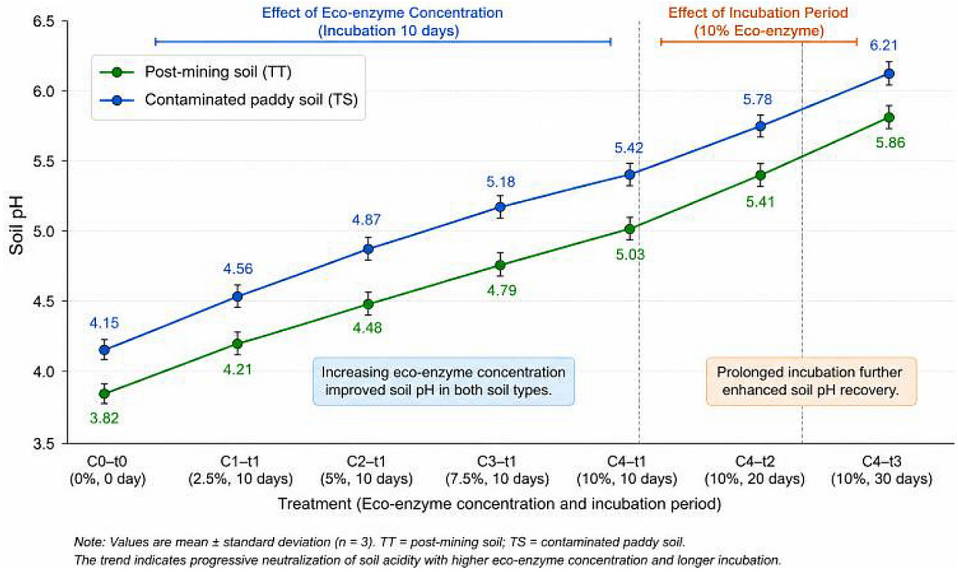

Figure 4. Trend soil pH changes under different eco-enzyme concentrations and incubation periods in post-mining soil (TT) and contaminated paddy soil (TS)

presence of sulphate-reducing bacteria (SRB) in the eco-enzyme formulation may have contributed to acidity neutralisation through sulphate reduction and alkalinity generation, resulting in lower metal solubility and improved soil stability (Ayangbenro et al., 2018; Hu et al., 2024; Mafane et al., 2025). In addition, longer incubation periods likely enhanced microbial adaptation and decomposition processes, leading to more stable pH recovery over time.

Both soil types exhibited a similar trend of pH increasing with eco-enzyme concentration and incubation period. The observed differences in final pH values were primarily associated with the initial soil pH conditions, where contaminated paddy soil had a higher baseline pH than post-mining soil. The results indicate that eco-enzyme application effectively ameliorated soil acidity in both soil types, suggesting a consistent remediation response across different soil conditions. These findings are consistent with previous studies reporting that biologically based remediation systems and organic amendments can improve soil pH and restore degraded soils through enhanced microbial activity and nutrient cycling (Chaudhary et al., 2023; Haroun et al., 2023; Sánchez-Castro et al., 2023). Overall, the findings demonstrate that integrated eco-enzyme application effectively improved soil pH and supported the recovery of acidic nickel mining-contaminated soils, particularly under higher eco-enzyme concentrations and longer incubation periods.

Changes in soil organic carbon and total nitrogen

Changes in SOC and total nitrogen after eco-enzyme application are presented in Table 5 and Figure 5. Both parameters increased progressively with increasing eco-enzyme concentration and incubation time in TT and TS. The highest values were observed in treatment C4–t3 (10% eco-enzyme, 30 days incubation), where SOC increased from 0.82% to 2.41% in TT soil and from 1.14% to 2.78% in TS soil. Total nitrogen also increased from 0.07% to 0.27% in TT soil and from 0.09% to 0.31% in TS soil. These results indicate that eco-enzyme application improved nutrient availability and accelerated soil fertility recovery in nickel mining-affected soils.

The increases in SOC and nitrogen were likely associated with the addition of organic substrates and microbial activity during eco-enzyme incubation. Organic compounds contained in the eco-enzyme may have contributed directly to carbon accumulation, while microbial decomposition enhanced nutrient mineralisation and nitrogen availability. The gradual increase observed from t1 to t3 also indicates that longer incubation promoted more stable decomposition and nutrient transformation processes.

Contaminated paddy soil consistently showed higher SOC and nitrogen values than post-mining soil. This difference may be related to the greater initial organic matter content and better buffering

Table 5. Changes in soil organic carbon (SOC) and total nitrogen under different eco-enzyme concentrations and incubation periods in post-mining soil (TT) and contaminated paddy soil (TS)

Treatment	Eco-enzyme concentration (%)	Incubation period (days)	SOC (%) TT	SOC (%) TS	Total N (%) TT	Total N (%) TS
C0–t0	0	0	0.82 ± 0.03	1.14 ± 0.04	0.07 ± 0.01	0.09 ± 0.01
C1–t1	2.5	10	1.05 ± 0.04	1.36 ± 0.05	0.09 ± 0.01	0.12 ± 0.01
C2–t1	5.0	10	1.28 ± 0.05	1.61 ± 0.04	0.12 ± 0.01	0.15 ± 0.01
C3–t1	7.5	10	1.54 ± 0.04	1.88 ± 0.05	0.15 ± 0.01	0.18 ± 0.02
C4–t1	10.0	10	1.79 ± 0.05	2.12 ± 0.04	0.18 ± 0.02	0.22 ± 0.01
C4–t2	10.0	20	2.08 ± 0.04	2.43 ± 0.05	0.22 ± 0.01	0.26 ± 0.02
C4–t3	10.0	30	2.41 ± 0.05	2.78 ± 0.04	0.27 ± 0.02	0.31 ± 0.01

Note: Values are presented as mean ± standard deviation. SOC = soil organic carbon; TT = post-mining soil; TS = contaminated paddy soil. Increasing eco-enzyme concentration and prolonged incubation significantly improved SOC and total nitrogen contents in both soil types, indicating enhanced nutrient cycling and progressive restoration of soil fertility.

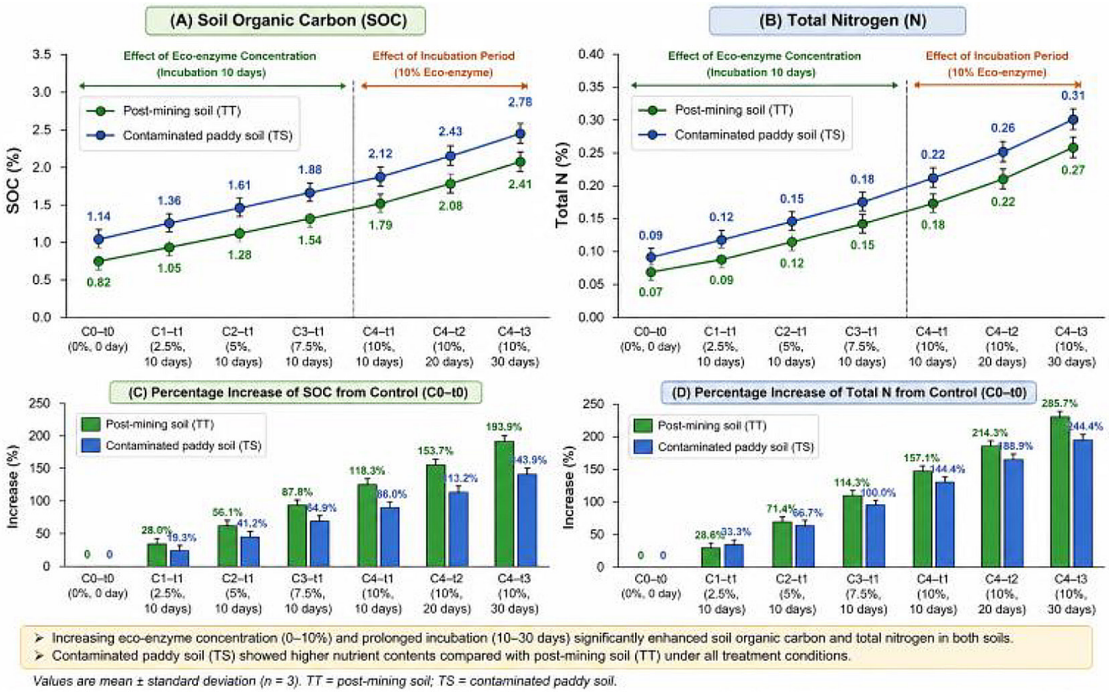

Figure 5. Trends of soil organic carbon (SOC) and total nitrogen (N) under different eco-enzyme concentrations and incubation periods

capacity of agricultural soil compared with severely degraded post-mining soil. Nevertheless, substantial improvements in TT soil demonstrate that eco-enzyme application was effective in restoring nutrient-deficient mining soil conditions.

Overall, the results suggest that integrated eco-enzyme treatment improved soil biochemical properties and supported soil fertility recovery in nickel mining-contaminated soils. These findings are consistent with previous studies reporting that organic-based bioremediation systems can enhance soil organic matter, nutrient availability, and microbial-mediated nutrient cycling in degraded soils (Chaudhary et al., 2023; Hu et al., 2024; Kumar et al., 2024).

The application of the eco-enzyme resulted in a progressive increase in both SOC and TN throughout the incubation period. The improvement was attributed to the addition of organic substrates, stimulation of microbial activity, and enhanced nutrient cycling within the contaminated soils. Although the increases observed in this study indicate a positive response to eco-enzyme treatment, the final SOC and TN levels suggest that further improvement may still be required to achieve optimal soil fertility conditions,

particularly in post-mining soils where severe organic matter depletion is common. Previous studies have reported that mining activities significantly reduce soil organic matter and nutrient reserves, thereby limiting biological productivity and ecosystem recovery (Punia and Bharti, 2023; Rashmi et al., 2024; Tibbett, 2024). Therefore, the observed increases demonstrate the potential of eco-enzyme as a soil restoration amendment, while also highlighting the need for longer-term rehabilitation strategies to further enhance soil quality and ecosystem functionality.

Changes in available phosphorus and exchangeable cation

The effects of eco-enzyme application on available phosphorus and exchangeable mineral elements are presented in Table 6 and Figure 6. The results showed consistent increases in available P, K, Ca, Mg, and Na in both TT and TS following eco-enzyme treatment and incubation. The highest nutrient values were observed in treatment C4–t3 (10% eco-enzyme, 30 days incubation), indicating that eco-enzyme application improved nutrient availability and soil chemical fertility.

The increase in nutrient availability was likely associated with organic acids and microbial activity produced during eco-enzyme fermentation, which enhanced nutrient solubilisation and mineral release in contaminated soils. Calcium and magnesium showed the largest increases, indicating improved soil buffering capacity and reduced soil acidity after remediation treatment. Longer incubation periods also promoted more stable nutrient recovery, suggesting that microbial decomposition and nutrient transformation continued throughout the incubation process.

Contaminated paddy soil showed higher nutrient concentrations than post-mining soil across all treatments. This result may be related to the higher initial organic matter content and better nutrient retention capacity of agricultural soils compared with severely degraded post-mining soils. However, substantial nutrient improvement in TT soil indicates that eco-enzyme application effectively restored soil chemical properties even under highly degraded conditions.

The application of eco-enzyme significantly increased the availability of essential macronutrients, including P, K, Ca, and Mg, indicating improved nutrient availability and soil fertility. These improvements were likely associated with the decomposition of organic substrates, enhanced microbial activity, and increased nutrient mineralisation during the incubation process. In addition to these beneficial nutrients, exchangeable sodium (Na) also showed a gradual increase following eco-enzyme application. Although elevated Na concentrations may potentially contribute to soil solicits, structural degradation, and reduced plant growth when present at excessive levels, the Na values observed in this study remained within a relatively low range and did not indicate a risk of solicits development. Therefore, the increase in Na should be interpreted as a secondary effect of organic matter decomposition and nutrient release rather than a negative consequence of eco-enzyme application. Nevertheless, long-term monitoring of exchangeable Na is recommended if the eco-enzyme is applied continuously in large quantities to ensure that soil quality

Table 6. Changes in available phosphorus and exchangeable mineral elements in post-mining soil (TT) and contaminated paddy soil (TS) following eco-enzyme application and incubation treatments

Treatment	Available P (mg kg^{-1}) TT	Available P (mg kg^{-1}) TS	K (cmol(+) kg^{-1}) TT	K (cmol(+) kg^{-1}) TS	Ca (cmol(+) kg^{-1}) TT	Ca (cmol(+) kg^{-1}) TS	Mg (cmol(+) kg^{-1}) TT	Mg (cmol(+) kg^{-1}) TS	Na (cmol(+) kg^{-1}) TT	Na (cmol(+) kg^{-1}) TS
Initial soil	4.82	6.15	0.12	0.18	1.42	2.16	0.48	0.72	0.10	0.14
C0–t0	4.76	6.08	0.11	0.17	1.38	2.11	0.45	0.69	0.09	0.13
C1–t1	6.92	8.54	0.19	0.25	2.14	3.08	0.76	1.02	0.14	0.19
C2–t1	8.45	10.26	0.24	0.31	2.86	3.94	1.01	1.28	0.18	0.24
C3–t2	10.83	12.74	0.31	0.39	3.68	4.85	1.29	1.61	0.23	0.29
C4–t2	12.47	14.38	0.36	0.45	4.21	5.47	1.54	1.88	0.27	0.33
C4–t3	14.26	16.81	0.42	0.53	5.08	6.34	1.82	2.15	0.31	0.38

Note: TT = post-mining soil; TS = contaminated paddy soil; C0 = control (0% eco-enzyme); C1 = 2.5% eco-enzyme; C2 = 5% eco-enzyme; C3 = 7.5% eco-enzyme; C4 = 10% eco-enzyme; t1 = 10 days; t2 = 20 days; t3 = 30 days incubation period. Values represent mean concentrations obtained from laboratory analyses after eco-enzyme remediation treatments.

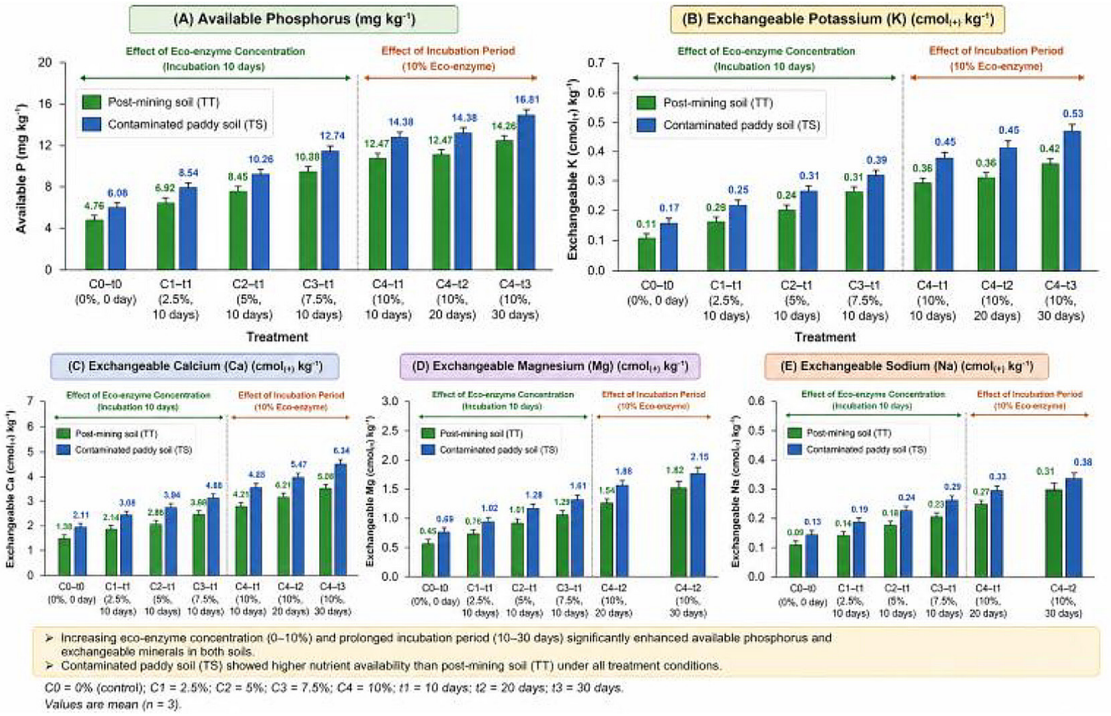

Figure 6. Effect of eco-enzyme application on available phosphorus and exchangeable cations in post-mining soil (TT) and contaminated paddy soil (TS)

remains suitable for sustainable agricultural production and post-mining land rehabilitation.

Overall, the findings demonstrate that integrated eco-enzyme treatment improved nutrient availability and exchangeable mineral status in nickel mining-contaminated soils. These results support previous studies showing that organic-based bioremediation systems can enhance nutrient cycling and promote recovery of soil fertility in degraded mining environments (Mensah et al., 2022; Haroun et al., 2023; Hu et al., 2024). This finding is consistent with previous studies reporting that organic amendments can increase the availability of both essential nutrients and soluble cations in soil, while the environmental significance of sodium accumulation depends on its concentration and the resulting exchangeable sodium percentage (ESP) in the soil system (Mensah et al., 2022; Wang et al., 2022; Haroun et al., 2023).

Changes in cation exchange capacity and base saturation

Changes in CEC and BS after eco-enzyme application are presented in Table 7 and Figure 7. Both parameters increased progressively with increasing eco-enzyme concentration and incubation time in TT and TS. The highest values were recorded in treatment C4–t3 (10% eco-enzyme, 30 days incubation), where CEC increased from 8.42 to 21.45 cmol(+) kg^{-1} in TT soil and from 11.38 to 26.17 cmol(+) kg^{-1} in TS soil. Base saturation also increased from 24.75% to 64.83% in TT soil and from 31.62% to 71.95% in TS soil. These results indicate substantial improvements in soil nutrient retention capacity and soil chemical fertility after remediation treatment.

The increase in CEC was likely associated with the accumulation of organic matter and microbial metabolites resulting from eco-enzyme decomposition, which promoted the formation of negatively charged functional groups capable of retaining nutrient cations. Organic amendments and biologically active materials are known to enhance soil colloidal surfaces and increase cation retention through improved soil organic carbon content and microbial-mediated nutrient transformations (Rahman et al., 2020; Mensah et al., 2022). Longer incubation periods further improved CEC, indicating the continued stabilisation of organic matter and the gradual development of nutrient exchange sites during the remediation process. Similar findings have been reported in the studies demonstrating that organic-based remediation strategies and microbial inoculants can significantly improve nutrient retention and overall soil fertility in degraded and

Table 7. Changes in cation exchange capacity (CEC) and base saturation (BS) in post-mining soil (TT) and contaminated paddy soil (TS) following eco-enzyme application and incubation treatments

Treatment	CEC (cmol(+) kg^{-1}) TT	CEC (cmol(+) kg^{-1}) TS	BS (%) TT	BS (%) TS
Initial soil	8.42	11.38	24.75	31.62
C0–t0	8.15	11.02	23.84	30.91
C1–t1	10.68	13.84	32.46	39.27
C2–t1	12.94	16.35	40.18	46.92
C3–t2	15.82	19.44	48.53	55.74
C4–t2	18.37	22.68	55.41	63.28
C4–t3	21.45	26.17	64.83	71.95

Note: TT = post-mining soil; TS = contaminated paddy soil; C0 = control (0% eco-enzyme); C1 = 2.5% eco-enzyme; C2 = 5% eco-enzyme; C3 = 7.5% eco-enzyme; C4 = 10% eco-enzyme; t1 = 10 days; t2 = 20 days; t3 = 30 days incubation period. Values represent mean laboratory measurements obtained after eco-enzyme remediation treatments.

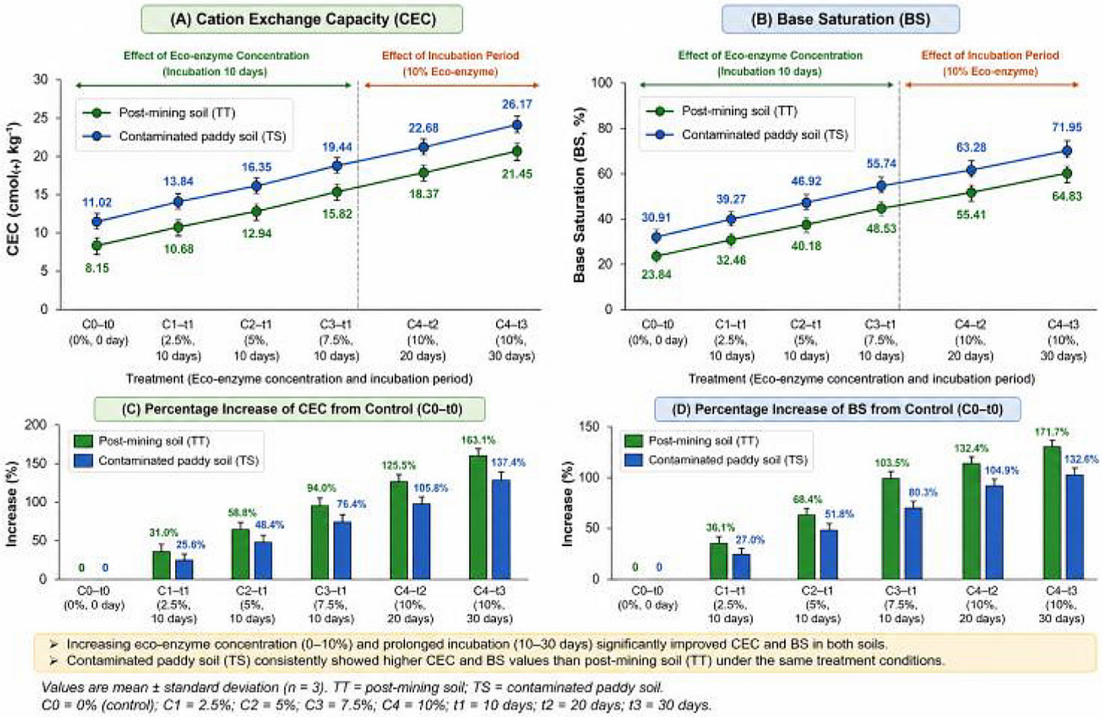

Figure 7. Changes in cation exchange capacity (CEC) and base saturation (BS) in post-mining soil (TT) and contaminated paddy soil (TS) under different eco-enzyme concentrations and incubation periods

contaminated soils (Haroun et al., 2023; Chaudhary et al., 2023).

The application of eco-enzyme substantially improved both CEC and BS in post-mining soil and contaminated paddy soil. The increase in CEC indicates an enhanced capacity of the soil to retain and exchange nutrient cations, which is essential for maintaining soil fertility and supporting plant growth. Similarly, the increase in BS reflects a greater proportion of exchange sites occupied by basic cations such as Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}, indicating reduced soil acidity and improved nutrient availability. According to commonly accepted soil fertility criteria, the soils with higher CEC and BS values generally exhibit better nutrient retention and greater resistance to nutrient losses through leaching. The results suggest that eco-enzyme application shifted the soil from poor fertility conditions toward more favourable fertility status, particularly under the 10% eco-enzyme treatment and 30-day incubation period. These improvements can be attributed to the addition of organic matter, microbial activity enhancement, and the gradual release of nutrient cations during decomposition (Rahman et al., 2020; Mensah et al., 2022; Haroun et al., 2023). Based on soil

fertility classification, the increase in CEC from approximately 8 cmol(+)/kg to 18 cmol(+)/kg indicates a transition from low to moderate fertility status. Likewise, BS increased from approximately 25% to 65%, indicating a shift from low base saturation to a level generally considered favourable for nutrient availability and plant growth.

Base saturation (BS) also increased consistently across treatments, reflecting greater occupancy of soil exchange sites by essential base cations, such as Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}. An increase in BS generally indicates a reduction in soil acidity and an improvement in nutrient availability because a larger proportion of the cation exchange complex is occupied by plant-essential nutrients rather than acidic ions such as H^{+} and Al^{3+}. Organic amendments and biologically active remediation agents have been reported to enhance BS through nutrient release, improved microbial-mediated nutrient cycling, and increased cation retention capacity (Rahman et al., 2020; Haroun et al., 2023; Mensah et al., 2022). According to commonly accepted soil fertility criteria, BS values below 20% are generally considered very low, 20–35% low, 35–50% moderate, 50–80% high, and values exceeding 80% very high. Higher BS values are beneficial for plant growth because they increase nutrient availability, improve root nutrient uptake, and reduce the toxic effects of soil acidity. Therefore, the increase in BS observed in this study indicates a substantial improvement in soil fertility status and suggests that eco-enzyme application enhanced the capacity of both post-mining and contaminated agricultural soils to support plant nutrient absorption and sustainable vegetation establishment. Contaminated paddy soil exhibited higher CEC and BS values than post-mining soil, which may be attributed to its greater organic matter content and stronger buffering capacity that facilitated nutrient retention and exchange processes (Punia & Bharti, 2023; Rashmi et al., 2024; Tibbett, 2024).

Overall, the combined increase in CEC and BS demonstrates that integrated eco-enzyme treatment effectively improved soil chemical quality and nutrient retention in nickel mining-contaminated soils. These findings support previous studies reporting that organic-based remediation systems can enhance soil fertility recovery and stabilise degraded mining soils (Rahman et al., 2020; Wang et al., 2022; Haroun et al., 2023).

Soil texture characteristics following eco-enzyme treatment

Changes in soil texture characteristics after eco-enzyme treatment are presented in Table 8 and Figure 8. The results showed slight decreases in sand fraction and gradual increases in silt and clay fractions in both TT and TS after 30 days of incubation. Although eco-enzyme application did not change the basic soil texture class,

Table 8. Soil texture fractions (%) of post-mining soil (TT) and contaminated paddy soil (TS) before and after eco-enzyme treatment

Soil type	Treatment	Sand (%)	Silt (%)	Clay (%)	Texture class	Aggregate stability observation
TT	Initial soil	68.4	24.1	7.5	Sandy loam	Loose and highly dispersive
	C0–t3	67.9	24.6	7.5		Low aggregate formation
	C1–t3	66.8	25.3	7.9		Slight aggregate improvement
	C2–t3	65.7	26.0	8.3		Moderate aggregation observed
	C3–t3	64.5	26.8	8.7		Stable aggregates began forming
	C4–t3	63.2	27.6	9.2		Strong aggregate stability
TS	Initial soil	55.3	32.7	12.0	Sandy clay loam	Moderate structural instability
	C0–t3	54.8	33.0	12.2		Slightly compacted structure
	C1–t3	53.7	33.6	12.7		Improved particle cohesion
	C2–t3	52.5	34.2	13.3		Moderate aggregate formation
	C3–t3	51.4	34.8	13.8		Stable aggregate development
	C4–t3	50.1	35.5	14.4		High aggregate stability and improved structure

Note: TT = post-mining soil; TS = contaminated paddy soil; C0 = control (0% eco-enzyme); C1 = 2.5% eco-enzyme; C2 = 5% eco-enzyme; C3 = 7.5% eco-enzyme; C4 = 10% eco-enzyme; t3 = 30 days incubation period. Data represent the final incubation stage used to evaluate changes in soil physical characteristics following eco-enzyme application.

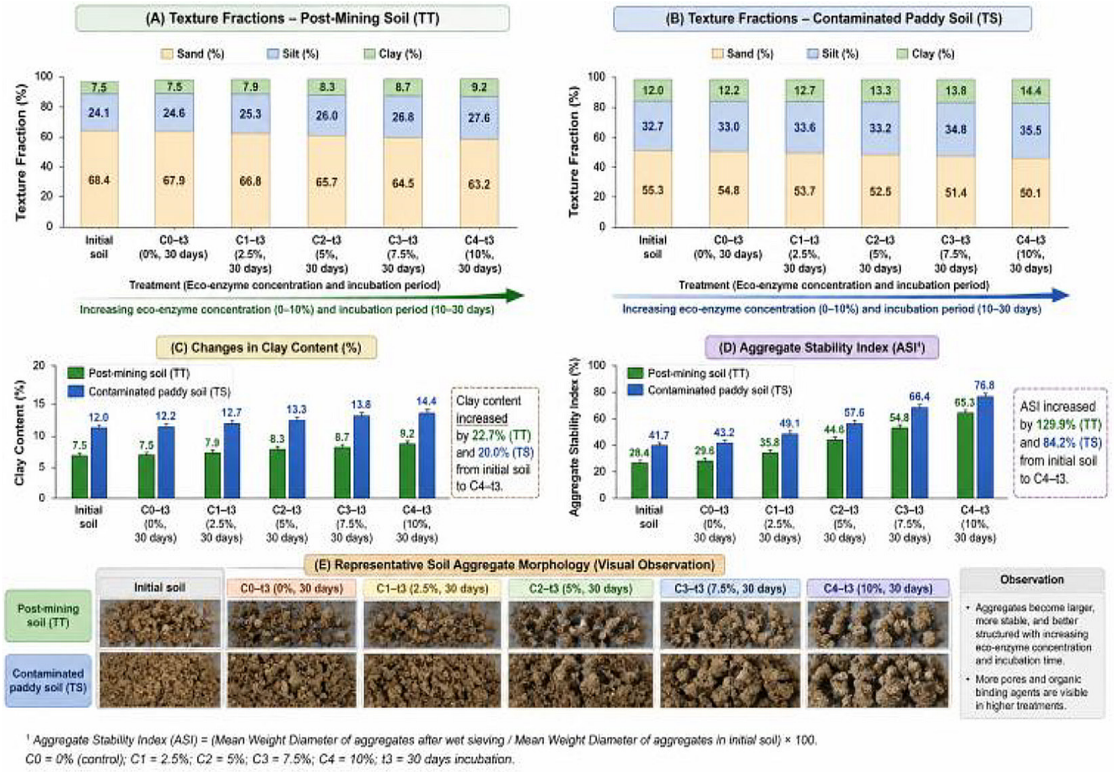

Figure 8. Soil texture characteristics and aggregate stability following eco-enzyme treatment in post-mining soil (TT) and contaminated paddy soil (TS)

improvements in aggregate stability and particle cohesion were observed, particularly at higher eco-enzyme concentrations (C3 and C4).

The improvement in aggregate stability was more evident in the treatments with higher eco-enzyme concentrations, suggesting that organic matter accumulation and microbial activity promoted stronger inter-particle binding during incubation. Post-mining soil initially exhibited loose, dispersive characteristics, whereas the treated soils developed more stable aggregates and improved structural cohesion after remediation.

Contaminated paddy soil exhibited better aggregate development than post-mining soil, likely due to higher initial clay and organic matter contents that supported aggregate formation. Improved soil aggregation may enhance water retention, nutrient retention, aeration, and resistance to erosion in degraded soils.

Eco-enzyme application improved soil aggregate stability in both post-mining soil and contaminated paddy soil, with the highest values observed under the 10% eco-enzyme treatment and 30-day incubation period. Improved aggregate stability indicates enhanced soil structural quality, which is important for water infiltration, root penetration, resistance to erosion, and long-term soil productivity. The positive effect may be attributed to the accumulation of organic compounds and microbial metabolites that acted as natural binding agents between soil particles. Organic amendments are known to promote the formation of stable soil aggregates through enhanced microbial activity and increased organic matter content, thereby improving soil physical resilience and ecosystem recovery in degraded lands (Rahman et al., 2020; Rashmi et al., 2024; Tibbett, 2024). Although minor changes in soil texture fractions were observed, the overall texture class remained relatively stable because soil texture is primarily controlled by the mineral composition of the soil and generally changes only over long-time scales. Therefore, the primary physical improvement associated with eco-enzyme application was reflected in enhanced aggregate stability rather than substantial changes in soil texture composition.

Overall, these results indicate that eco-enzyme application improved soil physical quality and structural stability in nickel mining-contaminated soils. Similar improvements in soil aggregation following organic-based remediation treatments have also been reported in previous studies (Rahman et al., 2020; Haroun et al., 2023; Tibbett, 2024).

Microbial population dynamics

Changes in soil microbial populations after eco-enzyme application are presented in Table 9 and Figure 9. Microbial populations increased consistently with increasing eco-enzyme concentration and incubation time in both TT and TS. The highest microbial abundance was observed in treatment C4–t3 (10% eco-enzyme, 30 days incubation), reaching 10.3 × 10^6 CFU g^{-1} soil in TT and 11.7 × 10^6 CFU g^{-1} soil in TS.

The increase in microbial populations indicates that eco-enzyme application improved soil biological conditions during remediation. Higher microbial abundance was likely associated with increased soil pH, greater organic carbon availability, and reduced heavy metal stress following incubation. Previous studies have demonstrated that improvements in soil pH and organic matter content create more favourable conditions for microbial growth, while reductions in heavy metal toxicity can stimulate microbial activity and nutrient cycling processes (Naz et al., 2022; Xiao et al., 2023). In addition, organic compounds, hydrolytic enzymes, and microbial metabolites produced during eco-enzyme fermentation may have provided additional carbon sources and growth-promoting substances that supported microbial proliferation and adaptation in contaminated soils (Haroun et al., 2023; Chaudhary et al., 2023; Hu et al., 2024).

Table 9. Microbial population dynamics (CFU g^{-1} soil) under different eco-enzyme concentrations and incubation periods in post-mining soil (TT) and contaminated paddy soil (TS)

Treatment	Eco-enzyme concentration (%)	Incubation period (days)	Microbial population (CFU ×10^6 g^{-1} soil) TT	Microbial population (CFU ×10^6 g^{-1} soil) TS
C0–t0	0	0	1.2 ± 0.10	1.6 ± 0.12
C1–t1	2.5	10	2.4 ± 0.15	3.1 ± 0.14
C2–t1	5.0	10	3.8 ± 0.18	4.6 ± 0.20
C3–t1	7.5	10	5.2 ± 0.22	6.3 ± 0.25
C4–t1	10.0	10	6.5 ± 0.25	7.8 ± 0.28
C4–t2	10.0	20	8.1 ± 0.30	9.4 ± 0.32
C4–t3	10.0	30	10.3 ± 0.35	11.7 ± 0.38

Note: Values represent microbial colony-forming units (CFU ×10^6 g^{-1} soil) in post-mining soil (TT) and contaminated paddy soil (TS) under different eco-enzyme concentrations and incubation periods. Microbial populations increased progressively with higher eco-enzyme dosage and longer incubation time, indicating improved microbial habitat conditions driven by increased organic carbon availability, pH normalization, and reduced heavy metal stress.

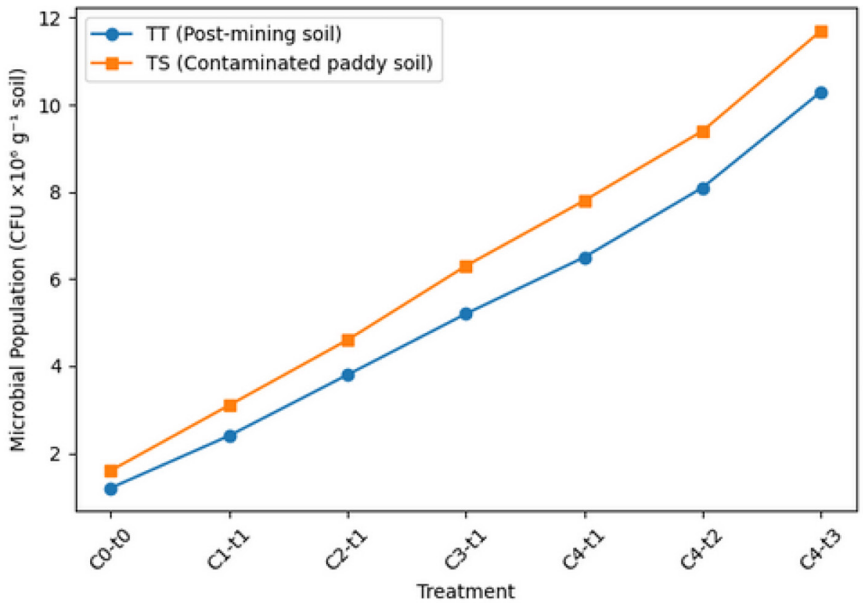

Figure 9. Microbial population dynamics under eco-enzyme treatment

Contaminated paddy soil consistently showed higher microbial populations than post-mining soil, suggesting that agricultural soils provided more favourable conditions for microbial recovery due to higher residual organic matter and nutrient availability. In contrast, microbial recovery in post-mining soil was slower because of severe degradation, topsoil removal, nutrient depletion, and lower initial fertility, which are common characteristics of mining-disturbed landscapes (Punia and Bharti, 2023; Rashmi et al., 2024; Tibbett, 2024). Nevertheless, the observed increase in microbial abundance across all treatments demonstrates the potential of eco-enzyme application as a biological amendment for restoring microbial functions and accelerating ecological recovery in mining-affected soils.

Overall, these findings demonstrate that eco-enzyme treatment supported microbial restoration and improved biological activity in nickel mining-contaminated soils. Similar responses have been reported in previous studies using organic-based and microbial remediation approaches in degraded mining environments (Haroun et al., 2023; Hu et al., 2024; Rajput et al., 2025).

Integrated remediation performance of eco-enzyme in nickel mining-contaminated soils

The overall remediation performance of eco-enzyme application is summarised in Table 10 and illustrated conceptually in Figure 10. The results showed that eco-enzyme application improved the physicochemical and biological properties of both TT and TS, with the highest remediation performance observed under the C4–t3 treatment (10% eco-enzyme; 30 days incubation). The improvement was reflected by increased soil pH, soil organic carbon, total nitrogen, available phosphorus, cation exchange capacity (CEC), base saturation (BS), and microbial population. These results indicate that eco-enzyme application simultaneously contributed to acidity reduction, nutrient recovery, and restoration of soil biological activity.

Compared with the initial condition, post-mining soil showed substantial increases in soil quality parameters after treatment. Soil pH increased from 3.82 to 5.86, SOC from 0.82% to 2.41%, total nitrogen from 0.07% to 0.27%, and microbial population from 1.20×10^6 to 10.30×10^6 CFU g^{-1} soil. Similar improvements were also observed in contaminated paddy soil, where pH increased from 4.15 to 6.21, and microbial population reached 11.70×10^6 CFU g^{-1} soil. The greater response observed in the TS soil was likely related to its higher initial organic matter content and better nutrient retention capacity compared with degraded post-mining soil.

The improvement in soil quality was associated with the combined effects of organic acids, microbial activity, and nutrient compounds contained in the eco-enzyme. Increased pH may have reduced heavy metal mobility and toxicity, thereby creating more favourable conditions for microbial growth and nutrient cycling. In addition, the increase in CEC and BS suggests improved nutrient retention and soil buffering capacity after remediation. Similar trends have been reported in previous studies using biologically based remediation approaches in mining-affected soils (Ayangbenro et al., 2018; Haroun et al., 2023; Hu et al., 2024).

Table 10. Integrated remediation performance of eco-enzyme application in post-mining soil (TT) and contaminated paddy soil (TS) under initial (C0–t0) and optimum (C4–t3) conditions

Parameter	TT initial	TT final (C4–t3)	TT improvement (%)	TS initial	TS final (C4–t3)	TS improvement (%)
pH	3.82	5.86	+53.4	4.15	6.21	+49.6
Soil organic carbon (%)	0.82	2.41	+193.9	1.14	2.78	+143.9
Total nitrogen (%)	0.07	0.27	+285.7	0.09	0.31	+244.4
Available phosphorus (mg kg^{-1})	4.82	14.26	+195.9	6.15	16.81	+173.3
CEC (cmol(+) kg^{-1})	8.42	21.45	+154.8	11.38	26.17	+130.1
Base saturation (%)	24.75	64.83	+162.0	31.62	71.95	+127.6
Microbial population (×10^6 CFU g^{-1})	1.20	10.30	+758.3	1.60	11.70	+631.3

Note: Values represent initial conditions (C0–t0) and optimum remediation performance (C4–t3). The percentage improvement was calculated based on relative increase from initial to final treatment. TT = post-mining soil; TS = contaminated paddy soil. The table demonstrates consistent improvements in chemical fertility, nutrient availability, and microbial recovery following eco-enzyme application.

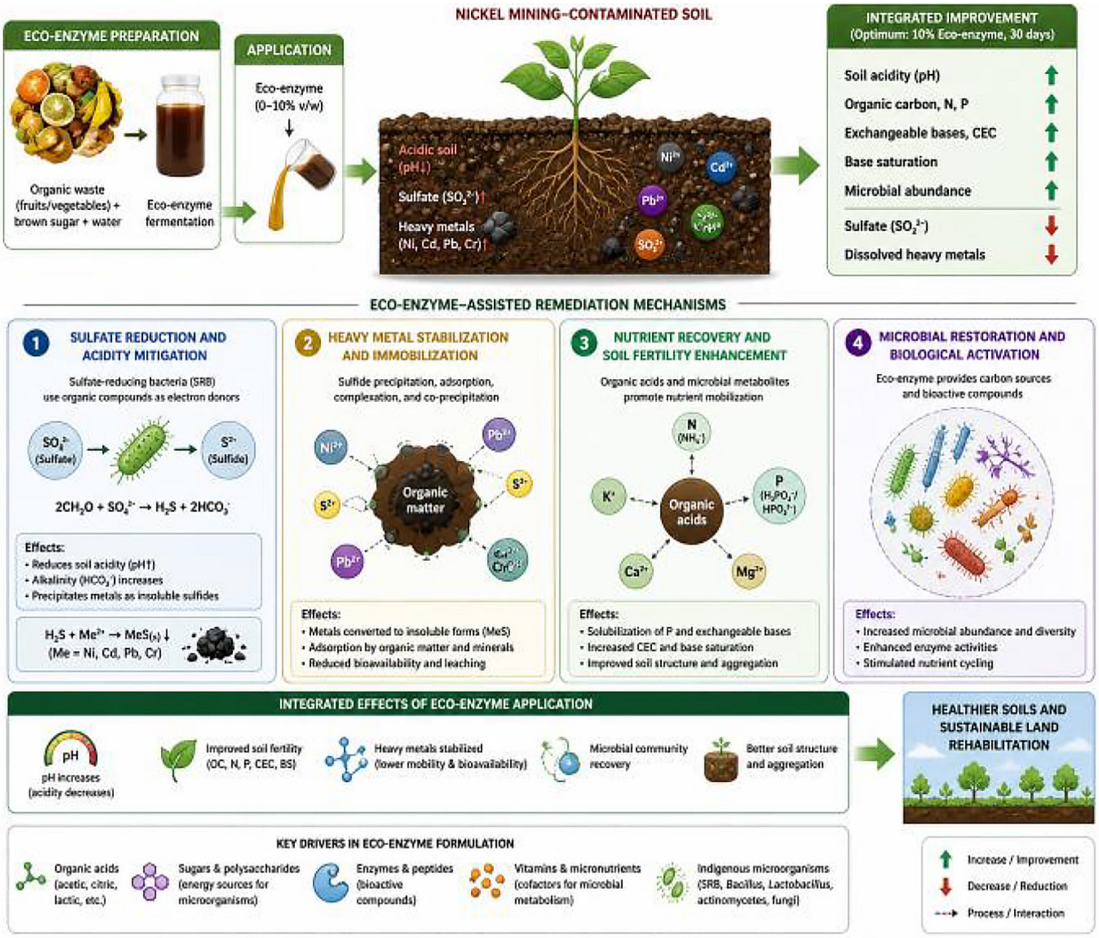

Figure 10. Conceptual mechanism of eco-enzyme-assisted remediation in nickel mining-contaminated soils

Overall, the results demonstrate that integrated eco-enzyme application effectively improved soil fertility and biological recovery in nickel mining-contaminated soils. The combined enhancement of chemical, physical, and biological properties indicates that eco-enzyme has strong potential as a sustainable remediation approach for rehabilitation of degraded mining land.

CONCLUSIONS

This study demonstrated that eco-enzyme application significantly improved the physicochemical and biological properties of nickel mining-contaminated soils. The optimum treatment (10% eco-enzyme with 30 days of incubation) produced the highest remediation performance in both TT and TS. In post-mining soil, pH increased from 3.82 to 5.86 (+53.4%), soil organic carbon increased from 0.82% to 2.41% (+193.9%), total nitrogen increased from 0.07% to 0.27% (+285.7%), available phosphorus increased from 4.82 to 14.26 mg kg^{-1} (+195.9%), cation exchange capacity increased from 8.42 to 21.45 cmol(+) kg^{-1} (+154.8%), base saturation increased from 24.75% to 64.83% (+162.0%), and microbial populations increased from 1.20×10^6 to 10.30×10^6 CFU g^{-1} soil (+758.3%). Similar improvements were observed in contaminated paddy soil, where pH increased from 4.15 to 6.21 (+49.6%), soil organic carbon increased by 143.9%, total nitrogen by 244.4%, available phosphorus by 173.3%, cation exchange capacity by 130.1%, base saturation by 127.6%, and microbial populations by 631.3%. These findings confirm that eco-enzyme is an effective and environmentally friendly amendment for improving soil fertility, nutrient retention, and microbial recovery in degraded mining environments. The novelty of this study lies in demonstrating the integrated effects of the eco-enzyme on chemical, physical, and biological soil properties simultaneously under different concentrations and incubation periods in nickel mining-contaminated soils. This study contributes new evidence supporting the use of eco-enzymes as a sustainable and low-cost remediation strategy for post-mining land rehabilitation and long-term ecosystem recovery.

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