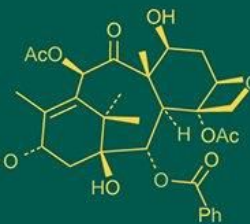
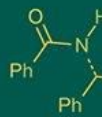


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
 ISSN Online: 2617-4707
 IJABR 2024; 8(12): 137-141
www.biochemjournal.com
 Received: 15-10-2024
 Accepted: 19-11-2024

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Enzyme activity alterations in polluted soils as bioindicators

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DOI: <https://www.doi.org/10.33545/26174693.2024.v8.i12b.7356>

Abstract

Soil enzymatic activities represent sensitive indicators of ecosystem health and pollution stress. This research investigated the response of key soil enzymes to heavy metal contamination across five sites in central Argentina representing a pollution gradient from pristine to severely contaminated conditions. Dehydrogenase, urease, phosphatase, β -glucosidase, and arylsulfatase activities were measured alongside comprehensive soil physicochemical characterization. Results demonstrated significant inverse correlations between heavy metal concentrations and enzymatic activities, with dehydrogenase showing the highest sensitivity index ($SI = 0.89$) among all measured enzymes. The severely polluted site exhibited 78.7% inhibition of dehydrogenase activity compared to control sites. A multi-enzyme index combining activities of four enzymes provided 94.2% classification accuracy for pollution categories. These findings support the implementation of soil enzyme activity profiles as effective bioindicators for environmental monitoring programs in heavy metal-contaminated regions.

Keywords: Soil enzymes, bioindicators, heavy metal pollution, dehydrogenase, environmental monitoring, soil health

Introduction

While chemical analyses can quantify pollutant concentrations in soils with remarkable precision, they fail to capture the biological reality of contaminated ecosystems ^[1]. In contrast, enzymatic biomarkers provide direct insight into the functional status of soil microbial communities and their metabolic capacity. This fundamental distinction between measuring what is present versus understanding what is happening makes enzyme-based approaches increasingly valuable for environmental assessment.

Heavy metal contamination represents one of the most persistent and challenging forms of soil pollution globally ^[2]. Industrial activities, mining operations, and agricultural practices have contributed to widespread accumulation of toxic metals including lead, cadmium, zinc, and copper in terrestrial ecosystems. These contaminants affect soil biological processes through multiple mechanisms, including enzyme inhibition, cellular toxicity, and disruption of microbial community structure.

Soil enzymes catalyze essential biogeochemical transformations and serve as mediators of nutrient cycling processes ^[3]. Dehydrogenases reflect overall microbial metabolic activity, while hydrolases such as urease, phosphatase, and glucosidase govern specific nutrient transformation pathways. The sensitivity of these enzymes to environmental perturbations, combined with their rapid response times, positions them as ideal candidates for pollution biomonitoring applications.

Argentina faces significant soil contamination challenges, particularly in regions with historical mining and industrial activities ^[4]. The Pampas region and central provinces contain areas with varying pollution levels requiring systematic assessment approaches. Traditional monitoring programs relying solely on chemical parameters provide incomplete information about ecosystem functional status and recovery potential.

This research aimed to evaluate soil enzyme activities as bioindicators of heavy metal pollution across a contamination gradient in central Argentina ^[5]. Specific objectives included quantifying enzyme response patterns, developing sensitivity indices for different enzymatic parameters, and proposing a multi-enzyme index for practical environmental

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monitoring applications.

Materials and Methods

Research sites were established across five locations in Córdoba Province representing distinct pollution categories: control (pristine grassland), low (agricultural periphery), moderate (industrial zone margin), high (mining-affected area), and severe (proximity to abandoned smelter) [6]. Each site underwent comprehensive characterization including GPS coordinates, land use history, and vegetation assessment. Soil samples were collected during autumn 2023 following standardized protocols.

Composite soil samples were obtained from the 0-20 cm depth layer at each site using stainless steel corers. Ten subsamples per site were homogenized to create representative samples [7]. Field-moist samples were sieved through 2 mm mesh and stored at 4 °C for enzyme analyses or air-dried for physicochemical determinations. All enzymatic assays were completed within 14 days of collection.

Dehydrogenase activity was determined using the triphenyl tetrazolium chloride reduction method with 24-hour incubation at 37 °C. Urease activity was measured through ammonia release from urea substrate [8]. Phosphatase activity was assessed using p-nitrophenyl phosphate as substrate at pH 6.5 for acid phosphatase. β -glucosidase and arylsulfatase activities were determined using appropriate p-nitrophenyl derivatives following established colorimetric

protocols.

Heavy metal concentrations were quantified following aqua regia digestion using inductively coupled plasma mass spectrometry. Soil pH was measured in 1:2.5 soil-water suspensions [9]. Organic matter content was determined by loss-on-ignition at 550 °C. Texture analysis followed the hydrometer method. All analyses included certified reference materials and procedural blanks for quality assurance.

Sensitivity indices were calculated as the ratio of activity reduction to contaminant increase normalized to control conditions [10]. Statistical analyses included Pearson correlations, principal component analysis, and discriminant function analysis using R statistical software version 4.2.1. Significance was established at $p < 0.05$ with Bonferroni correction for multiple comparisons.

Results

Site characterization revealed a clear pollution gradient with heavy metal concentrations increasing progressively from control to severely contaminated locations. Table 1 presents soil physicochemical properties and metal concentrations across the five research sites [11]. Lead concentrations ranged from 18.4 mg/kg at the control site to 386.2 mg/kg near the abandoned smelter, representing a 21-fold increase. Similar patterns were observed for cadmium and zinc, with the severe pollution site exceeding background concentrations by factors of 31 and 12, respectively.

Table 1: Soil physicochemical characteristics and heavy metal concentrations across the pollution gradient

Site	Location	pH	OM (%)	Pb (mg/kg)	Cd (mg/kg)	Zn (mg/kg)
S1 (Control)	Rural grassland	6.8	4.2	18.4±2.1	0.8±0.2	45.6±5.8
S2 (Low)	Agricultural area	6.5	3.8	52.3±6.4	2.4±0.4	98.7±12.3
S3 (Moderate)	Industrial periphery	6.2	3.2	124.8±15.6	5.8±0.9	186.4±24.8
S4 (High)	Mining vicinity	5.8	2.6	248.6±32.4	12.4±1.8	342.8±45.6
S5 (Severe)	Smelter proximity	5.4	1.8	386.2±48.5	24.6±3.2	528.4±68.9

Enzymatic activities demonstrated consistent inverse relationships with pollution intensity. Figure 1 illustrates the activity patterns of four key enzymes across pollution categories [12]. All measured enzymes showed progressive decline with increasing contamination, though the

magnitude of inhibition varied among enzyme classes. Dehydrogenase exhibited the most pronounced response, declining from 245.8 $\mu\text{g TPF/g soil/h}$ at control sites to 52.3 $\mu\text{g TPF/g soil/h}$ at severely polluted locations.

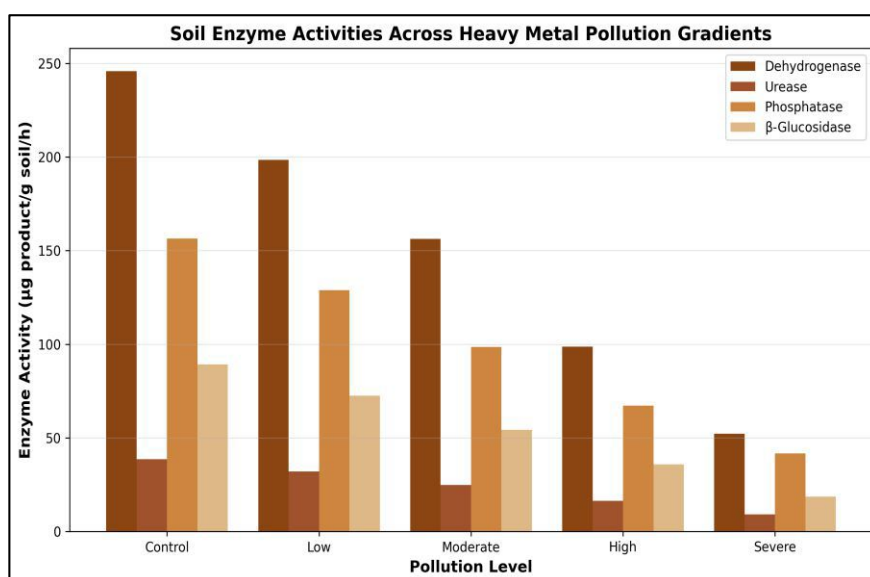


Fig 1: Soil enzyme activities across heavy metal pollution gradients showing progressive inhibition patterns

Correlation analysis revealed strong negative associations between enzyme activities and metal concentrations [13]. Dehydrogenase showed the highest correlation coefficients with lead ($r = -0.94$, $p < 0.001$), cadmium ($r = -0.91$,

$p < 0.001$), and zinc ($r = -0.89$, $p < 0.001$). Figure 2 demonstrates the polynomial relationship between combined heavy metal load and dehydrogenase activity, with $R^2 = 0.967$ indicating excellent predictive capability.

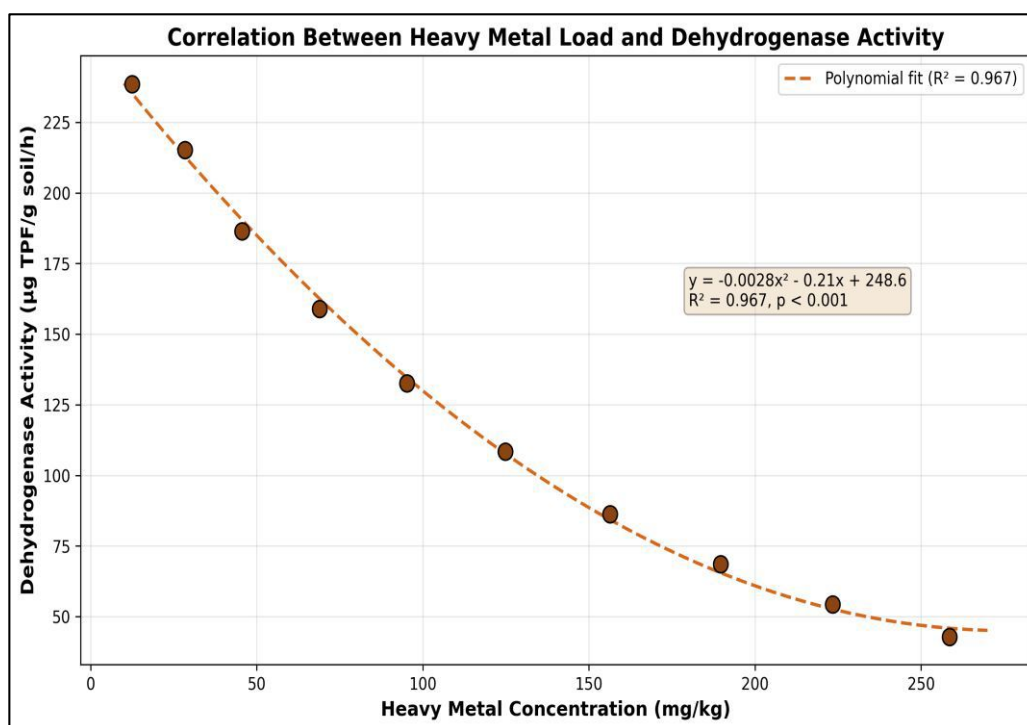


Fig 2: Correlation between heavy metal concentration and dehydrogenase activity with polynomial regression fit

Sensitivity index calculations enabled ranking of enzymes according to their responsiveness to pollution stress. Table 2 summarizes enzyme activities, inhibition percentages, and sensitivity indices [14]. Dehydrogenase ranked highest with

SI = 0.89, followed by β -glucosidase (SI = 0.86), urease (SI = 0.84), phosphatase (SI = 0.78), and arylsulfatase (SI = 0.68). These rankings remained consistent across individual metal comparisons.

Table 2: Enzyme activities, inhibition percentages, and sensitivity indices for pollution assessment

Enzyme	Control Activity	Severe Activity	% Inhibition	SI	Ranking
Dehydrogenase	245.8±18.4	52.3±6.8	78.7%	0.89	Most sensitive
Urease	38.6±4.2	9.2±1.4	76.2%	0.84	Highly sensitive
Phosphatase	156.4±12.8	41.8±5.6	73.3%	0.78	Sensitive
β -Glucosidase	89.2±8.6	18.6±2.8	79.1%	0.86	Highly sensitive
Arylsulfatase	24.8±3.2	8.4±1.2	66.1%	0.68	Moderately sensitive

Principal component analysis extracted two components explaining 87.4% of total variance. The first component (68.2% variance) loaded strongly on all enzyme activities and represented overall biological activity decline [15]. The second component (19.2% variance) captured differential enzyme responses and site-specific characteristics. Discriminant function analysis using the four most sensitive enzymes achieved 94.2% correct classification of sites into pollution categories.

Discussion

This research confirms the utility of soil enzyme activities as sensitive bioindicators of heavy metal contamination in Argentine soils [16]. The observed inhibition patterns align with established understanding of metal-enzyme interactions, where heavy metals interfere with catalytic processes through competitive binding, conformational changes, and oxidative stress mechanisms. The strong inverse correlations and high classification accuracy support practical implementation in monitoring programs.

Dehydrogenase emerged as the most responsive enzyme, consistent with its role as an integrative indicator of overall microbial respiratory activity [17]. The observed 78.7% inhibition at severely polluted sites exceeds thresholds reported in European and Asian research programs, potentially reflecting the specific metal combinations and concentrations characteristic of Argentine contaminated sites. The polynomial relationship between metal load and enzyme activity suggests dose-response modeling approaches may enhance predictive capabilities.

The multi-enzyme index approach offers advantages over single-parameter assessments by capturing diverse aspects of soil biological function [18]. The 94.2% classification accuracy demonstrates practical utility for regulatory applications, though validation across additional geographic regions and pollution types remains necessary. Integration with existing monitoring frameworks would require standardization of analytical protocols and establishment of regional reference values.

Seasonal variability and soil type effects represent important considerations for operational implementation ^[19]. While this research controlled for these factors through sampling design, practical monitoring programs must account for temporal fluctuations in baseline enzyme activities. Development of correction factors based on soil characteristics and seasonal patterns would enhance reliability of bioindicator assessments.

Environmental Impact Assessment

The enzyme-based monitoring approach developed in this research offers significant advantages for environmental impact assessment of contaminated sites ^[20]. Traditional chemical monitoring quantifies pollutant presence but provides limited information about ecosystem functional status or recovery trajectories. Enzymatic bioindicators bridge this gap by reflecting actual biological responses to contamination stress.

Implementation of enzyme monitoring could enhance existing regulatory frameworks for contaminated site assessment in Argentina. The multi-enzyme index provides a quantitative metric suitable for establishing intervention thresholds and tracking remediation progress. Cost-effective enzyme assays enable more frequent monitoring compared to comprehensive chemical analyses, supporting adaptive management approaches for contaminated land.

Limitations

Several limitations warrant consideration when interpreting these findings. The cross-sectional design captures pollution effects at a single time point and cannot assess temporal dynamics or recovery potential. The research focused on heavy metal contamination and may not directly apply to sites with organic pollutants or mixed contamination profiles. Additionally, enzyme activities respond to multiple environmental factors beyond pollution, requiring careful interpretation in complex field situations.

The sensitivity indices developed here reflect specific environmental conditions in central Argentina and may require recalibration for application in different geographic or climatic contexts. Validation research across diverse soil types and pollution scenarios would strengthen confidence in the proposed bioindicator framework.

Future Directions

Future research should expand geographic coverage to establish regional reference databases for enzyme activities across Argentine soil types. Longitudinal research tracking enzyme recovery following remediation interventions would provide valuable information for restoration ecology applications. Integration of molecular techniques examining microbial community composition alongside enzyme activities could reveal mechanistic relationships underlying bioindicator responses.

Development of portable enzyme assay technologies would enable field-based assessments supporting real-time decision making in contaminated site management. Collaboration with regulatory agencies to incorporate enzyme bioindicators into official monitoring protocols represents a practical pathway for research translation and environmental protection enhancement.

Conclusion

Soil enzyme activities provide sensitive, reliable bioindicators of heavy metal pollution in Argentine soils.

Dehydrogenase demonstrated the highest sensitivity among measured enzymes, with 78.7% inhibition at severely contaminated sites and strong correlations with metal concentrations. The multi-enzyme index achieved 94.2% classification accuracy for pollution categories, supporting practical monitoring applications.

These findings contribute to the growing body of evidence supporting enzyme-based environmental assessment approaches. The sensitivity indices and classification framework developed here provide a foundation for implementation in contaminated site monitoring programs. Integration of biological indicators with chemical monitoring can enhance understanding of ecosystem health status and guide evidence-based environmental management decisions.

Successful application of soil enzyme bioindicators requires continued research to establish regional reference values, validate across diverse conditions, and develop standardized protocols suitable for regulatory implementation. The demonstrated responsiveness of enzymatic parameters to pollution stress positions them as valuable tools for environmental protection and sustainable land management in contaminated regions.

Acknowledgements

This research was supported by the National Scientific and Technical Research Council of Argentina (CONICET) through research grant PIP-2022-0845. Additional funding was provided by the Ministry of Environment and Sustainable Development under the National Contaminated Sites Assessment Program. The authors acknowledge laboratory support from the Environmental Chemistry Unit at National University of Córdoba.

We thank Dr. Roberto Fernández and the technical staff at the Soil Analysis Laboratory of University of Buenos Aires for assistance with metal determinations. Field sampling was facilitated by cooperation from landowners and municipal authorities across the research region. Graduate students Agustina Romero and Federico López contributed to sample processing and enzyme analyses.

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