



ABSTRACT BOOK

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Metal-induced impairment of iNOS-mediated immune responses in earthworms from contaminated soils

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Abstract

Soil contamination by toxic metals represents a global environmental challenge, particularly in rapidly industrializing regions where systematic soil health monitoring is limited. The present study assessed metal-induced immunotoxic stress in the endogeic earthworm *Metaphire posthuma* inhabiting soils contaminated by cadmium, chromium, lead, and mercury from the Calcutta Leather Complex, India, with special emphasis on inducible nitric oxide synthase (iNOS) activity and Integrated Biomarker Response (IBR). Seasonal field sampling was conducted across three contaminated sites and a reference site over a two-year period. Metal concentrations were quantified in soil, whole earthworms, and extruded coelomocytes, revealing substantially higher bioaccumulation factors in coelomocytes, identifying them as primary targets of metal toxicity. Morphofunctional analyses demonstrated significant shifts in coelomocyte subpopulations, accompanied by extensive cellular damage, cytoplasmic vacuolation, membrane blebbing, and elevated frequencies of micronucleation and binucleation, indicating genotoxic and cytotoxic stress. A pronounced suppression of nitric oxide generation and significant downregulation of iNOS expression were detected in both amoebocyte and chloragocyte populations, suggesting impairment of innate immune signaling and microbicidal defense mechanisms. In parallel, marked inhibition of acetylcholinesterase activity indicated associated neurotoxic effects of accumulated metals. Principal component analysis revealed strong correlations between soil physicochemical deterioration, metal burden, and coelomocyte-level biomarker responses. Integration of multiple cellular, biochemical, and immunological endpoints using the IBR framework yielded consistently elevated stress indices in earthworms from contaminated sites, with maximum responses during high-precipitation seasons. IBR star plots identified iNOS expression, nitric oxide generation, nuclear aberrations, and cellular damage as dominant contributors to cumulative stress. Overall, the study demonstrates that suppression of the iNOS–NO axis is a key mechanistic feature of metal-induced immunotoxicity and establishes IBR as a robust tool for holistic soil health assessment using *M. posthuma* as a sentinel species.