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NICKEL IN TROPICAL LEGUMES:
Growth Responses, Impacts on Morphophysiology, and Sustainability Perspectives

Ilha Solteira - SP
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NICKEL IN TROPICAL LEGUMES:
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Thesis submitted to São Paulo State University (UNESP), Faculty of Engineering, Ilha Solteira, in fulfillment of the requirements for the degree of Doctor of Agronomy

Concentration Area: Production Systems

Advisor: Prof. Dr. Liliane Santos de Camargos

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POTENTIAL IMPACT OF THIS RESEARCH

The information presented in this thesis significantly contributes to basic science by investigating the effects of nickel, a toxic element, on species such as *Calopogonium mucunoides* and *Canavalia ensiformis*. Despite their important ecological roles, these species are not widely valued in a commercial context, which limits their inclusion in scientific studies, particularly in fields related to agriculture and large-scale production. The literature highlights that, although these legumes are essential for ecological processes such as biological nitrogen fixation and soil quality improvement, the focus on species of higher economic value, such as soybean and common bean, draws greater attention due to their relevance to food security and agricultural economics, resulting in the underrepresentation of other species in scientific research.

This research also expands knowledge on how nickel affects plant functioning in tropical ecosystems. By addressing the mechanisms of response and acclimation, the study contributes to developing new approaches to mitigate the effects of pollution by potentially toxic elements. The knowledge generated has the potential to be applied in the restoration of degraded soils, promoting ecosystem recovery and contributing to sustainable development.

IMPACTO POTENCIAL DESTA PESQUISA

As informações contidas nesta tese contribuem significativamente para a ciência básica ao investigar os efeitos do níquel, um elemento tóxico em espécies como *Calopogonium mucunoides* e *Canavalia ensiformis*, apesar de desempenharem um papel ecológico importante não são amplamente valorizadas no contexto comercial, o que limita sua inclusão em estudos científicos, especialmente em áreas relacionadas à agricultura e produção em larga escala. A literatura aponta que, embora essas leguminosas sejam fundamentais para processos ecológicos como a fixação biológica de nitrogênio e a melhoria da qualidade do solo,o foco em espécies de maior valor econômico, como a soja e o feijão, que recebem mais atenção devido à sua relevância para a segurança alimentar e a economia agrícola, o que resulta em sub-representarão de outras espécies em pesquisas científicas.

A pesquisa também amplia o conhecimento sobre como o níquel afeta o funcionamento das plantas em ecossistemas tropicais e ao abordar esses mecanismos de resposta e aclimatação, o estudo contribui para o desenvolvimento de novas abordagens para mitigar os efeitos da poluição por elementos potencialmente tóxicos. O conhecimento gerado tem potencial para ser utilizado na restauração de solos degradados, promovendo a recuperação de ecossistemas e contribuindo para o desenvolvimento sustentável.

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I respectfully dedicate this work to Olinda Diniz Gonçalves (*in memoriam*).

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Truly, I too learned to wait, but to wait for
myself. And above all, I learned to stand, to
walk, to run, to leap, to climb, and to dance.
(...)

This – is now my way – where is yours?
I replied to those who asked me, ‘the way.’ For
the way – that does not exist!"

Nietzsche, F. (1883–1885). Thus Spoke Zarathustra (p. 194).

ABSTRACT

This thesis investigates the physiological and biochemical responses of tropical legumes from the Fabaceae family to stress induced by nickel, a potentially toxic element present in soil at elevated concentrations. The study is structured around three chapters that explore different aspects of these species' reactions to nickel. The first chapter, concerning the scoping review, was conducted following the PRISMA protocol and aimed to map the Fabaceae species studied, the methodologies employed, and the physiological markers analyzed in peer-reviewed articles published between 2013 and 2022. The results highlight *Glycine max* as the most extensively studied species, while most research focuses on biotic stress, fertilization, and toxicity. However, physiological aspects such as nodules, biological nitrogen fixation, carbon metabolism, and gas exchange are underrepresented in the literature. The second chapter focuses on changes in biomass, nickel accumulation, and morphophysiological aspects of *C. mucunoides* and *C. ensiformis* in contaminated soils, revealing that *C. ensiformis* shows higher tolerance to nickel, with anatomical adaptations that allow greater accumulation of the element. The third chapter examines alterations in the leaf structure of these species under nickel stress, using morphological and biochemical analyses to understand how plants maintain cellular stability and physiological functioning under stress. The study showed that *C. mucunoides* exhibits higher sensitivity, with significant changes in water use efficiency and pigment activity, while *C. ensiformis* demonstrates greater resistance, maintaining gas exchange even under elevated stress conditions. The findings contribute to the understanding of the acclimatization strategies of tropical legumes to potentially toxic elements, with implications for the use of these species in phytoremediation programs and the restoration of degraded soils.

Keywords: nickel; tropical legumes; Fabaceae; abiotic stress; phytoremediation; morphophysiological acclimatization; biochemistry.

RESUMO

A presente tese investiga as respostas fisiológicas e bioquímicas de leguminosas tropicais da família Fabaceae ao estresse provocado pelo níquel, um elemento potencialmente tóxico presente no solo em concentrações elevadas. O estudo foi desenvolvido a partir de três capítulos que exploram diferentes aspectos das reações dessas espécies ao níquel. O primeiro capítulo, referente à revisão de escopo, foi conduzido seguindo o protocolo PRISMA e teve como objetivo mapear as espécies da família Fabaceae estudadas, as metodologias empregadas e os marcadores fisiológicos analisados em artigos revisados por pares publicados entre 2013 e 2022. Os resultados destacam *Glycine max* como a espécie mais investigada, enquanto a maioria dos estudos se concentra em estresses bióticos, fertilização e toxicidade. No entanto, aspectos fisiológicos como nódulos, fixação biológica de nitrogênio, metabolismo do carbono e trocas gasosas são sub-representados na literatura. O segundo capítulo foca nas modificações na biomassa, acumulação de níquel e aspectos morfofisiológicos de *C. mucunoides* e *C. ensiformis* em solos contaminados, revelando que *C. ensiformis* apresenta maior tolerância ao níquel, com adaptações anatômicas que permitem maior acúmulo do elemento. O terceiro capítulo examina as alterações na estrutura foliar dessas espécies sob estresse por níquel, utilizando análises morfológicas e bioquímicas para entender como as plantas mantêm a estabilidade celular e o funcionamento fisiológico frente ao estresse. O estudo mostrou que *C. mucunoides* apresenta maior sensibilidade, com alterações significativas na eficiência do uso de água e na atividade de pigmentos, enquanto *C. ensiformis* demonstra maior resistência, mantendo as trocas gasosas mesmo em condições de estresse elevado. As informações geradas contribuem para o conhecimento das estratégias de aclimatação das leguminosas tropicais a elementos potencialmente tóxicos, com implicações para o uso dessas espécies em programas de fitorremediação e recuperação de solos degradados.

Palavras-chave: níquel; leguminosas tropicais; Fabaceae; estresse abiótico; fitorremediação; aclimatação morfofisiológica; bioquímica.

SUMMARY

1 INTRODUCTION

To elucidate the correlation between nickel and legume physiology, it is important to highlight some of the key discoveries made over time.

In 1926 (Sumner, 1926), urease was presented in crystalline form for the first time. In 1975 (Dixon *et al*., 1975), it was established that urease is a metalloenzyme dependent on nickel for its activation. Both experiments were conducted using *Canavalia ensiformis*, a legume species. In 1976 (Polacco, 1976), in a study with soybean tissue culture, the hypothesis was raised that urea is produced from arginine, clarifying the intricate relationship between nitrogen metabolism and urease enzyme activity. Additionally, it was suggested that the presence of Ni^{2+} in soil microorganisms makes nickel an essential micronutrient in fields fertilized with urea (Polacco, 1976).

In 1984 (Eskew; Welch; Norvell, 1984), in a study using different Ni doses in soybean and cowpea, it was demonstrated that the role of Ni is of general importance due to the similarities in species' responses to Ni deficiency. However, it had not yet been established whether Ni was an essential element for all higher plants. By the late 1980s, the essentiality of Ni for higher plants was confirmed (Brown; Welch; Cary, 1987).

Ni nutrient management in agricultural systems has emerged as a promising and low-cost strategy to improve crop productivity and sustainability by enhancing nitrogen metabolism—through enzymes such as urease and NiFe-hydrogenase—optimizing calcium uptake, and increasing the efficiency of water and nutrient use by plants. Integrated application methods, particularly foliar spraying combined with soil or seed treatments, have shown greater effectiveness under varying environmental conditions, reducing the need for intensive agricultural inputs and minimizing resource waste (Rodak *et al*., 2024; Rabinovich *et al*., 2024)

Despite its agronomic benefits, nickel (Ni) also poses environmental risks, as its presence in soils—although influenced by geological factors—is significantly amplified by human activities such as mining, the use of agrochemicals, fossil fuel combustion, and improper disposal of industrial and agricultural waste, leading to contamination of soil, water, and air; furthermore, acid rain increases Ni mobility, exacerbating its environmental impact and compromising the safety and productivity of agricultural systems (Rizwan; Usman; Alsafran, 2024).

Furthermore, Ni poses risks to human health due to its widespread distribution in air, water, and food, with exposure occurring through ingestion, inhalation, or dermal contact, particularly among workers in Ni-related industries, and the contamination of agricultural soils by Ni compromises environmental and food safety, making the adoption of mitigating strategies essential, such as phytoremediation, which employs plants to absorb and accumulate heavy metals in their tissues (Genchi *et al*., 2020).

This thesis investigates the physiological and biochemical responses of tropical legumes from the Fabaceae family to stress induced by nickel , a potentially toxic element that contaminates soils. Given the widespread environmental distribution of nickel, through processes such as industrial activities, mining, and the improper disposal of waste, its presence in agricultural soils poses significant risks to both environmental and human health.

The importance of this research is underscored by the need to better understand the acclimation mechanisms of tropical legumes to nickel-induced stress. This understanding can potentially contribute to the development of more effective phytoremediation strategies, aiding in the recovery of contaminated soils. Additionally, the study aims to expand existing knowledge by focusing on underrepresented species such as *Calopogonium mucunoides* and *Canavalia ensiformis*, both of which are essential to tropical ecosystems but have received limited attention in previous studies. The growing relevance of this topic lies in the context of tropical ecosystems, where nickel pollution presents a pressing environmental concern that requires sustainable solutions.

This thesis is organized into three chapters, which include a comprehensive review of the responses of Fabaceae species to nickel, along with experimental analyses of biomass production, nickel accumulation, and morphological and biochemical changes in *C. mucunoides* and *C. ensiformis*. Despite the valuable insights this research offers, limitations exist, particularly in the variability of species' responses and the complexity of environmental interactions that modulate the effects of nickel stress.

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4 CONCLUDING REMARKS

This study synthesized the main conclusions from three distinct approaches to the interaction between legumes and Ni stress, highlighting the contributions of each study to advancing knowledge in the field.

In the first article, the scoping review provided an overview of how legumes have been investigated in recent years, with a focus on the potential role of nickel, the diversity of experimental variables, and plant physiology. It was evident that studies, predominantly conducted in controlled environments, prioritize the toxic impacts of nickel over its role as a nutrient or in the context of biotic stresses. Notably, soybean has received significant attention due to its economic relevance, whereas important gaps remain in both basic and applied research on other species within the Fabaceae family.

The second article revealed contrasting tolerance and responses to nickel stress between the species. *C. ensiformis* exhibited greater tolerance, with higher Ni accumulation and more pronounced anatomical and physiological changes. In contrast, *C. mucunoides* showed significant alterations in root proteins and amino acids, indicating a distinct strategy for coping with stress. Multivariate analysis reinforced the differences between species, highlighting specific anatomical and physiological strategies.

In the third article, morphological responses were investigated using scanning electron microscopy, while physiological responses were assessed through antioxidant activity, pigment composition, and gas exchange, focusing on the effects of Ni absorbed by the roots and translocated to the leaves. *C. mucunoides* exhibited a clear antioxidant response, with increased peroxidase (POX) activity and maintenance of photosynthesis, despite negative effects on growth and low adaptive plasticity. On the other hand, *C. ensiformis* displayed a greater acclimation capacity, with changes in stomatal density, increased superoxide dismutase (SOD) activity, and a positive correlation between gas exchange and antioxidant defense. However, both species showed growth impairment under high-stress conditions.

The distinct strategies observed in *C. mucunoides* and *C. ensiformis* in response to nickel stress provide valuable insights into potential phytoremediation approaches, especially in tropical ecosystems affected by nickel contamination. By revealing the varying tolerance levels and specific physiological and biochemical adaptations of these species, the research emphasizes the importance of selecting appropriate plant species for effective soil decontamination. This research supports the development of sustainable agricultural practices, offering an eco-friendly and cost-effective solution to mitigate soil pollution. Furthermore, the understanding gained from this study could be pivotal in advancing strategies for the restoration of degraded lands, promoting long-term environmental sustainability, and improving soil health, which is essential for food security in contaminated regions.

In addition to its environmental implications, this research carries significant social value, addressing critical concerns regarding public health and food safety. The contamination of agricultural soils with potentially toxic elements, such as nickel, poses substantial risks to human health, as these contaminants can enter the food chain through crop cultivation. By enhancing our understanding of how various legume species respond to nickel stress, this study contributes to identifying crops with potential for soil remediation and safer agricultural practices. Moreover, the findings highlight the importance of interdisciplinary research in addressing the challenges of environmental pollution, benefiting not only the scientific community but also policymakers, farmers, and local populations. Through the application of phytoremediation and other sustainable solutions, this research provides a foundation for creating safer, more resilient ecosystems, ultimately enhancing the well-being of communities affected by the contamination of potentially toxic elements.

Future research should focus on understanding the genetic and molecular mechanisms behind tropical legumes' tolerance and accumulation of potentially toxic elements like nickel, particularly identifying genes linked to stress responses such as antioxidant defense and metal sequestration. Expanding studies to underrepresented species and conducting long-term field experiments will be crucial to assess the practical effectiveness of phytoremediation under diverse environmental conditions. Additionally, exploring the combined effects of multiple stressors, such as drought, salinity, and temperature, alongside nickel exposure, will enhance our understanding of plant resilience under climate change. Lastly, evaluating the economic and environmental feasibility of large-scale phytoremediation compared to traditional methods is vital for ensuring its sustainability.