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Ph.D. thesis

Integrative Approaches to Plant-Based Soil Remediation and Sustainable Management

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Declaration

I declare that I am the author of this qualification thesis and that I used only sources and literature displayed in the list of references in its preparation.

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Abstrakt

Degradace půdy v důsledku kontaminace a nerovnováhy živin představuje jednu z nejzávažnějších globálních výzev současného zemědělství i environmentální udržitelnosti. Zejména radionuklidy jsou z hlediska ochrany životního prostředí mimořádně problematické, a to vzhledem ke své perzistenci v ekosystémech, dlouhému poločasu rozpadu a riziku vstupu do potravního řetězce. Účinné řešení těchto problémů vyžaduje ekonomicky nenáročné a ekologicky kompatibilní přístupy. V tomto kontextu se fytoremediace ukazuje jako perspektivní přírodně založená strategie. Úspěšnost fytoremediačních procesů však není dána pouze schopností rostlin akumulovat či stabilizovat kontaminanty, ale také jejich kapacitou odolávat stresu a dlouhodobě udržovat klíčové fyziologické funkce v nepříznivých podmínkách.

Tato disertační práce shrnuje poznatky ze čtyř recenzovaných vědeckých článků, které se souhrnně zaměřují na problematiku fytoremediace, fyziologii rostlinného stresu a udržitelné postupy pěstování plodin. První část, realizovaná formou studií s hlavním autorstvím, se přímo zabývala problematikou kontaminace radionuklidy. Experimentální výzkum prokázal potenciál okurky (*Cucumis sativus*) růst v půdách zatížených cesiem a stronciem, akumulovat tyto prvky v pletivech a aktivovat antioxidační obranné mechanismy, které omezují oxidační poškození. Doplňující systematická rešerše dále syntetizovala globální poznatky o fytotoxicitě radionuklidů a používaných remediačních postupech, identifikovala společné fyziologické projevy stresu a definovala znaky, které umožňují některým druhům rostlin efektivně působit v kontaminovaném prostředí. Obě tyto práce společně potvrzují, že účinná fytoremediace je možná pouze tehdy, pokud rostliny dokážou vyvážit akumulaci kontaminantů s vlastní fyziologickou odolností.

Spoluautorské studie dále rozšířily perspektivu směrem k agronomickým podmínkám. Sója pěstovaná v meziplodinovém systému kukuřice–sója s aplikací molybdenu vykazovala vyšší fotosyntetický výkon, stabilitu chlorofylu a lepší výnosové parametry, což dokládá, že cílená výživa posiluje stresovou toleranci rostlin. Podobně rajčata pěstovaná při použití samotného vermikompostu i integrovaných dávek vermikompostu a minerálních hnojiv vykazovala výraznější morfologický růst a stabilnější fyziologické funkce. Tyto výsledky zdůrazňují význam organických a minerálních půdních doplňků pro udržení fyziologické rovnováhy rostlin. Ačkoliv tyto ex-

perimenty nebyly prováděny v prostředí zatíženém radionuklidy, potvrzují stejný základní princip: schopnost rostlin zachovat fyziologickou stabilitu v podmínkách stresu je určující jak pro produktivitu plodin, tak pro efektivitu remediačních strategií.
Celkově lze konstatovat, že sjednocujícím tématem všech čtyř studií je role fyziologie a stresové tolerance rostlin. Ať již jde o stres vyvolaný kontaminací radionuklidy, nebo o stres způsobený nerovnováhou živin, rozhodujícím faktorem účinnosti je schopnost rostlin udržet funkční růstové procesy a aktivovat ochranné mechanismy. Tato disertační práce proto nabízí integrovaný pohled, který propojuje výzkum fytoremediace s agronomickými postupy prostřednictvím ústřední role fyziologické odolnosti rostlin a ukazuje cesty k environmentální remediaci i k dlouhodobé udržitelnosti zemědělské produkce.

Klíčová slova: fytoremediace, kontaminace radionuklidy, fyziologie stresu, meziplodiny, udržitelné zemědělství

Abstract

Soil degradation arising from contamination and nutrient imbalance has become one of the most pressing global challenges for both agriculture and environmental sustainability. Among the various pollutants, radionuclides are especially concerning because of their persistence in ecosystems, long half-life, and potential to enter the food chain. Addressing these challenges requires environmentally compatible and low-cost approaches, and among such options, phytoremediation has emerged as a promising nature-based solution. The success of phytoremediation, however, depends not only on the ability of plants to take up or stabilize contaminants but also on their capacity to tolerate stress and sustain physiological functions under adverse conditions.

This dissertation integrates findings from four peer-reviewed articles that collectively examine phytoremediation, plant stress physiology, and sustainable crop management practices. The first set of studies, conducted as first-author contributions, directly addressed radionuclide contamination. An experimental investigation demonstrated the potential of cucumber (*Cucumis sativus*) to grow under cesium and strontium contamination, accumulate radionuclides in its tissues, and activate antioxidant defense mechanisms to mitigate oxidative damage. Complementing this, a systematic review synthesized global knowledge on radionuclide phytotoxicity and remediation strategies, identifying common physiological impacts on plants and highlighting the traits that enable species to function effectively in contaminated environments. These two works together underline that phytoremediation is only feasible when plants can balance contaminant uptake with physiological resilience.

The co-authored studies broadened this theme by exploring crop responses to nutrient-related stresses under agronomic conditions. Soybeans grown in maize-soybean intercropping systems with molybdenum application exhibited improved photosynthetic performance, chlorophyll stability, and yield-related traits, confirming that targeted nutrient management strengthens stress tolerance. Similarly, tomato plants cultivated with sole and integrated applications of vermicompost and chemical fertilizers showed enhanced morphological growth and physiological stability, demonstrating the role of soil amendments in supporting plant resilience. While these studies were not conducted under radionuclide contamination, they reinforce the same underlying principle: the capacity of plants to maintain physiological balance under stress is central to both crop productivity and remediation strategies.

Taken together, the four articles demonstrate that the unifying theme linking phytoremediation and agronomic crop management is plant physiology and stress tolerance. Whether facing contamination stress from radionuclides or nutrient stress in agricultural systems, plants that can sustain functional growth processes while activating protective mechanisms provide the foundation for sustainable soil management. This dissertation, therefore, advances an integrated perspective in which phytoremediation research and agronomic practices are connected through the central role of plant physiology, offering pathways toward both environmental remediation and long-term agricultural sustainability.

Keywords: phytoremediation, radionuclide contamination, stress physiology, intercropping, sustainable agriculture

Content

Chapter 1: Background of Study

1.1 Introduction

Soil contamination by radionuclides represents one of the most serious and long-lasting environmental challenges of the 21st century, with severe implications for ecosystems, agriculture, and human health (Chernysh et al., 2024). Among the radionuclides introduced by human activities, cesium-137 (^{137}Cs) and strontium-90 (^{90}Sr) are particularly concerning due to their persistence, radiotoxicity, and ability to mimic essential nutrients in plants (S. Ali et al., 2024). Major contamination events, such as the Chernobyl (1986) and Fukushima (2011) nuclear accidents, released large amounts of these radionuclides into agricultural and forested ecosystems (Garnier-Laplace et al., 2011). In addition, uranium mining, nuclear testing, fuel reprocessing, and improper waste disposal continue to contribute to their environmental spread (Adeola et al., 2023; Rehman et al., 2024).

Cesium-137 and strontium-90 are beta-emitting isotopes with half-lives of about 30 years, persisting in soils for decades. Their similarity to potassium and calcium enhances their bioavailability and incorporation into plant tissues, posing risks to food safety and human health (Al-Oudat et al., 2021; Burger & Lichtscheidl, 2019). Once absorbed by roots, these radionuclides may be translocated to edible plant parts, leading to bioaccumulation and long-term exclusion of contaminated land from cultivation (S. Ali et al., 2024).

Conventional remediation technologies—such as soil excavation, washing, or vitrification—are costly, destructive, and often generate secondary waste (Zhakypbek et al., 2024; Alharbi & El-Taher, 2013). Hence, sustainable and low-cost alternatives are needed (Kumar & Rani, 2025). Phytoremediation, the use of plants and their associated rhizosphere microorganisms to remove or immobilize pollutants, has emerged as a promising green technology (H. Ali et al., 2013; Eapen et al., 2007). It is inexpensive, solar-driven, and ecologically restorative (Pulford, 2003). Its success depends on soil properties, radionuclide behavior, and plant characteristics such as biomass production, root architecture, and stress tolerance (Chirumamilla et al., 2025).

Among potential species, *Cucumis sativus L.* (cucumber) has shown promise due to its rapid growth, high shoot biomass, and physiological adaptability (S. Ali et al., 2022). Preliminary studies reported its ability to tolerate moderate Cs and Sr levels while maintaining antioxidant activity. Exposure to radionuclides induces oxidative stress through the accumulation of reactive oxygen species (ROS) such as H_2O_2, $O_2^{\bullet -}$, and $^{\bullet}OH$ (Ammar et al., 2025), which plants mitigate through antioxidant enzymes like SOD, CAT, and POD (S. Ali et al., 2024). Phytoremediation efficiency is commonly evaluated using the bioconcentration (BCF) and translocation (TF) factors, which indicate contaminant uptake and mobility within the plant (Musini et al., 2024; H. Ali et al., 2013).

Despite promising outcomes, challenges persist—particularly radionuclide bioavailability, field variability, and safe biomass disposal (Angelin et al., 2024). Therefore, strategies such as chelating agents, microbial inoculants, and genetic enhancement are being explored to improve radionuclide uptake and plant tolerance (Kafle et al., 2022). The present research contributes to this field by analyzing cucumber's physiological and biochemical responses under Cs and Sr stress and situating its remediation potential within broader sustainable soil management strategies.

1.2 Literature Review

1.2.1 Sources and Environmental Behavior of Radionuclides

Radionuclides in the environment originate from both natural and anthropogenic sources (Alawadhi et al., 2024; Chernysh et al., 2024). While naturally occurring isotopes like uranium, thorium, and potassium-40 contribute to background radiation, human activities such as nuclear energy production, weapons testing, and industrial waste disposal have dramatically increased artificial radionuclide levels in soils and water (Hu et al., 2010; Adeola et al., 2022; Vangronsveld et al., 2009). Cesium-137 and strontium-90 are the most problematic due to their high solubility, bioavailability, and chemical similarity to essential nutrients (Burger & Lichtscheidl, 2019; Khan et al., 2024). Cesium behaves like potassium and easily moves through plant tissues, while strontium, a calcium analog, interferes with cell wall formation and signaling (Dirican et al., 2025; Fujimura et al., 2025).

Their behavior in soil is influenced by pH, texture, organic matter, and cation exchange capacity (Tiwari et al., 2024). Acidic conditions increase radionuclide mobility, whereas clay minerals and organic matter can immobilize them through adsorption (Barik et al., 2025). However, complex ion-exchange and desorption processes make their mobility dynamic. Understanding these interactions is crucial for designing effective and site-specific remediation strategies (Musini et al., 2024).

1.2.2 Phytotoxicity of Cesium and Strontium in Plants

Radionuclide exposure disrupts plant physiological and biochemical functions, primarily by disturbing ion homeostasis and inducing oxidative stress (Yankauskas et al., 2024; Patel et al., 2024). Cesium and strontium, due to their mimicry of potassium and calcium, are readily absorbed and translocated within plants (S. Ali et al., 2022; Ammar et al., 2025). The overproduction of ROS such as hydrogen peroxide and superoxide radicals leads to lipid peroxidation and DNA damage (S. Ali et al., 2022; Ammar et al., 2025). Plants counteract this by enhancing antioxidant enzyme activity (S. Ali et al., 2024; Yuan et al., 2025). Morphological symptoms—such as chlorosis, reduced growth, and biomass loss—are frequently observed under Cs and Sr stress (Angelin et al., 2024; Zhakypbek et al., 2024), indicating impaired photosynthetic and nutrient assimilation processes.

1.2.3 Plant-Based Remediation: Mechanisms and Challenges

Phytoremediation employs natural plant processes to mitigate environmental contamination (Di Stasio et al., 2025). The two key mechanisms—phytoextraction and phytostabilization—respectively focus on removing contaminants into harvestable biomass or immobilizing them in the root zone (Ahmad, 2025; Galahitigama et al., 2025; Rehman et al., 2024). Plant suitability is typically evaluated by the bioconcentration factor (BCF) and translocation factor (TF); values greater than one indicate efficient accumulation and transport (S. Ali et al., 2022; Galahitigama et al., 2025). However, phytoremediation is often slow and dependent on environmental conditions, requiring multiple growth cycles and careful biomass handling (Angelin et al., 2024). Integration with soil amendments or microbial inoculants can improve uptake and long-term stability (S. Ali et al., 2024; Kafle et al., 2022).

1.2.4 Plant Physiology and Biochemistry under Radionuclide Stress

Plant responses to radionuclide exposure involve a network of physiological and biochemical adaptations (S. Ali et al., 2022; Yuan et al., 2025). In *Cucumis sativus L.*, Cs and Sr contamination reduces biomass and chlorophyll while increasing antioxidant enzyme activity (Ammar et al., 2025; Patel et al., 2024). Cesium translocates more readily than strontium, reflecting their distinct ionic analogies. The activation of CAT, SOD, and POD enables cucumber to maintain metabolic balance under stress, confirming its potential for phytoremediation (S. Ali et al., 2022).

1.2.5 Plant Selection and Species Potential for Phytoremediation

Effective phytoremediation depends on selecting plant species with suitable physiology, root structure, and tolerance (Gupta et al., 2016; Musini et al., 2024). Species such as *Helianthus annuus*, *Brassica juncea*, and *Amaranthus retroflexus* have demonstrated radionuclide uptake potential (Alsabbagh & Abuqudaira, 2017; Cheng et al., 2022; Achmad & Hadiyanto, 2018; Khan et al., 2024; Chen, Yang, et al., 2020; Chen, Long, et al., 2020). *Cucumis sativus*, though not a hyperaccumulator, offers advantages of rapid growth, high biomass, and adaptability (S. Ali et al., 2022; S. Ali et al., 2024; Zhakypbek et al., 2024). Combined with chelating agents or organic amendments, cucumber and similar species could serve as practical candidates for sustainable radionuclide remediation. Continued exploration of diverse and genetically improved plants remains essential for optimizing future phytoremediation strategies (Gupta et al., 2016).

1.3 Research Objectives and Hypotheses

The overall objective of this dissertation is to evaluate the role of plant species in enhancing soil quality and resilience under environmental stress, with a particular focus on their capacity to tolerate, adapt to, and remediate contaminated or nutrient-imbalanced soils. By integrating experimental studies and literature-based analyses, the research aims to identify the physiological mechanisms that enable plants to withstand stress conditions, maintain functional growth, and contribute to sustainable soil management strategies.

The specific objectives are as follows:

1. To investigate the phytoremediation potential of selected crop species under radionuclide contamination, with emphasis on their ability to absorb, accumulate, and translocate contaminants such as cesium-137 and strontium-90, while sustaining physiological functions.

2. To evaluate plant physiological and biochemical responses under stress conditions, including biomass production, chlorophyll stability, and antioxidant enzyme activity, to better understand tolerance mechanisms that support both remediation and productivity.

3. To synthesize current knowledge on radionuclide phytotoxicity and plant-based remediation strategies through a comprehensive literature review, identifying common patterns of stress physiology and highlighting gaps for future research.

4. To assess the role of nutrient management and soil amendments in improving plant resilience under stress, as demonstrated in studies on soybean and tomato, thereby linking agronomic practices to principles of phytoremediation.

Research Hypotheses

1. Selected plant species, including but not limited to *Cucumis sativus*, possess the capacity to absorb and translocate radionuclides at levels that make them candidates for phytoremediation.

2. Exposure to environmental stressors, such as radionuclide contamination or nutrient imbalance, induces measurable physiological responses (e.g., oxidative stress, altered photosynthesis, biomass reduction), but resilient plants can mitigate these effects through activation of defense mechanisms.

3. Literature evidence confirms that phytoremediation is a promising but complex approach, requiring deeper integration of physiological, agronomic, and field-based research to optimize its large-scale application.

4. Improved nutrient management and soil fertility strategies (e.g., intercropping systems, vermicompost amendments) enhance plant stress tolerance, thereby strengthening both agricultural productivity and the feasibility of remediation approaches.

Chapter 2: Methodology

2.1 Methodology of the Experimental Study

This dissertation follows an article-based structure, drawing upon four peer-reviewed studies that together provide complementary insights into the role of plants in soil remediation and sustainable agriculture. The methodology integrates experimental approaches targeting radionuclide contamination, systematic review of global phytotoxicity literature, and agronomic field investigations addressing crop physiology under nutrient and amendment treatments. This multi-dimensional framework enables both depth through detailed physiological and biochemical analysis and breadth through comparative and review-based synthesis.

2.2. First-Author Experimental Study: *Cucumis sativus* under Radionuclide Stress

2.2.1 Experimental Setup

Greenhouse experiments were conducted using soils artificially contaminated with cesium and strontium. Standardized environmental conditions for temperature, humidity, and light were maintained to ensure replicability. Control groups without contamination were included for baseline comparison.

2.2.2 Data Collection

- Morphological parameters: plant height, root and shoot biomass, leaf area.
- Physiological parameters: chlorophyll concentration, photosynthetic activity.
- Biochemical responses: lipid peroxidation (MDA levels), antioxidant enzymes (CAT, POD, SOD).
- Radionuclide uptake: quantified using atomic absorption spectroscopy, with Bioconcentration Factor (BCF) and Translocation Factor (TF) calculated.

2.3 First-Author Review Study: Radionuclide Phytotoxicity

A systematic review methodology was adopted, involving:

- Comprehensive literature search in major databases (Web of Science, Scopus, PubMed).
- Inclusion/exclusion criteria applied to ensure relevance and quality.
- Extraction of data on radionuclide sources, impacts on plants, and remediation strategies.

- Synthesis of findings into thematic categories covering phytoextraction, phytostabilization, physiological impacts, and knowledge gaps.

2.4 Co-Authored Study: Soybean under Molybdenum Application

2.4.1 Study Design

Field-based study within maize–soybean intercropping systems, with different molybdenum treatments applied to evaluate physiological and yield outcomes.

2.4.2 Data Collection

- Physiological measurements: photosynthetic rate, transpiration, stomatal conductance, chlorophyll content.
- Yield traits: biomass accumulation, seed production.
- Statistical analyses: ANOVA for treatment effects, correlation analyses between Mo application and physiological outcomes.

2.5 Co-Authored Study: Tomato under Vermicompost and Fertilizer Treatments

2.5.1 Experimental Setup

Tomato plants were cultivated under sole vermicompost, sole chemical fertilizer, and combined vermicompost–fertilizer applications. Controlled conditions were maintained to observe differential responses.

2.5.2 Data Collection

- Morphological traits: plant height, root/shoot biomass.
- Physiological parameters: chlorophyll concentration, leaf area, stress tolerance indices.
- Comparative analysis across treatments to identify synergistic effects of integrated fertility management.

2.6 Data Analysis

Experimental results from cucumber, soybean, and tomato studies were statistically analyzed using ANOVA and regression models. Review data were synthesized both qualitatively and quantitatively to highlight remediation efficiency and physiological responses across species.

Chapter 3: Results and Conclusion

The presented dissertation summarizes experimental and review studies that collectively contribute to a better understanding of plant responses to abiotic stress and their application in sustainable soil management and phytoremediation. The results demonstrate that integrating physiological, biochemical, and agronomic perspectives can lead to environmentally sound solutions for improving crop productivity and soil health under contaminated or nutrient-limited conditions.

In the greenhouse experiment, the phytoremediation potential of cucumber (*Cucumis sativus L.*) was evaluated under soil contamination with cesium (^{137}Cs) and strontium (^{90}Sr). The plants showed visible tolerance to moderate contamination levels, maintaining physiological functions while accumulating both radionuclides. Cesium was found to be more mobile than strontium, resulting in a higher translocation factor from roots to shoots. Although exposure to radionuclides caused a decline in biomass and pigment content, cucumber plants activated an efficient antioxidant system, including catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD), which mitigated oxidative stress. These responses enabled the plants to sustain photosynthetic activity and confirm their suitability for radionuclide phytoextraction and stabilization. The study provided valuable insight into the link between oxidative protection and metal tolerance, supporting the use of cucumber and similar species in phytoremediation approaches.

The systematic review conducted within this work synthesized current knowledge on radionuclide phytotoxicity, plant physiological responses, and remediation techniques. The analysis of more than one hundred peer-reviewed studies revealed that radionuclide stress primarily affects chlorophyll biosynthesis, nutrient uptake, and redox balance, leading to oxidative damage and growth inhibition. Nevertheless, plants employ a wide range of adaptive mechanisms, including antioxidant enzyme activation, osmolyte accumulation, and controlled ion uptake. The review concluded that combining organic amendments, microbial inoculants, and optimized nutrient management significantly enhances phytoremediation performance. This comprehensive synthesis established an updated conceptual model describing how physiological and biochemical resilience determines the effectiveness of radionuclide removal or immobilization in the soil–plant system.

Complementary agronomic studies expanded this physiological framework to field-relevant conditions. In the maize–soybean intercropping system, the application of molybdenum (Mo) improved the physiological efficiency and productivity of soybean. Enhanced chlorophyll content, photosynthetic rate, and nitrogen fixation capacity were observed, along with improved yield attributes. The results confirmed the role of micronutrient supplementation in promoting plant resilience and nutrient cycling within intercropping systems, contributing to both ecological stability and resource efficiency. Similarly, in experiments with tomato (*Solanum lycopersicum L.*), the use of vermicompost and mineral fertilizers demonstrated significant effects on growth and productivity. Plants grown with vermicompost, alone or combined with mineral fertilization, exhibited higher chlorophyll content, improved photosynthetic activity, and greater fruit yield, together with reduced oxidative stress and better water status. The findings emphasize the value of organic amendments in maintaining soil fertility, stimulating microbial activity, and enhancing overall crop performance.

The collective outcomes of all studies indicate that successful phytoremediation and sustainable crop production are inherently connected through plant stress physiology. Plants capable of activating antioxidant defense mechanisms, maintaining nutrient homeostasis, and optimizing photosynthetic function achieve superior growth and tolerance under both contaminant and nutrient stress. The integration of organic amendments, micronutrient management, and diversified cropping systems supports these adaptive responses while improving soil structure and biological activity.

The dissertation confirms that sustainable plant-based soil management requires a synergistic understanding of physiological adaptability, nutrient dynamics, and ecological soil processes. The experimental and review findings together demonstrate that enhancing plant physiological stability under stress is the foundation for effective radionuclide remediation and for developing resilient, high-performing agroecosystems.

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