学 位 論 文 内 容 の 要 旨

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学 位 論 文 題 名

The impact of lead pollution and *Azospirillum brasilense* Sp7 inoculation in lemongrass cultivation on soil nitrogen cycling and microbial communities

（鉛汚染及び *Azospirillum brasilense* Sp7 株の接種を伴う
レモングラスの植栽が土壌中の窒素循環と微生物群集に及ぼす影響）

Soil contamination by heavy metals is a significant environmental concern worldwide. It results from activities such as mining, industrial waste disposal, exhaust gas emissions, and the overuse of chemical fertilizers and pesticides. Heavy metals like lead (Pb) are non-biodegradable, lack biological function, and exhibit high toxicity. Such contamination not only diminishes ecosystem functions and vegetation cover but also poses human health risks through the dispersal of polluted topsoil. Therefore, effective remediation must simultaneously remove heavy metals and restore soil fertility.

The nitrogen cycle is a key indicator of soil fertility and is particularly sensitive to environmental disturbances such as heavy metal contamination. Despite this, only a limited number of studies have examined both the levels of inorganic nitrogen compounds and the abundance of nitrogen cycle-related functional genes under heavy metal stress. Many studies have focused on isolated steps or genes, leaving uncertainty about the overall impact on the nitrogen cycle.

In recent years, strategies combining plant growth-promoting microorganisms (PGPMs) with heavy metal-tolerant plants have attracted attention as low-cost and environmentally friendly solutions. However, differences in microbial species and inoculation methods across studies complicate efforts to identify practical approaches tailored to specific plants. Furthermore, most studies emphasize plant growth, with fewer evaluating impacts on soil functions. Lemongrass has recently emerged as a promising candidate due to its heavy metal tolerance and economic potential, but research on its interaction with PGPMs under contamination is still limited.

This study aims to address these gaps by: (1) conducting a comprehensive assessment of the nitrogen cycle under Pb contamination by measuring both inorganic nitrogen levels and the abundance of functional genes; (2) evaluating the effects of microbial inoculation and lemongrass cultivation on nitrogen cycling in Pb-contaminated soil; and (3) comparing the effectiveness of different inoculation methods on nitrogen cycling and plant growth.

In the first experiment, a soil incubation study assessed changes in denitrification activity and microbial communities in response to Pb exposure. Four Pb concentrations (0, 200, 400, 1600 mg/kg soil) were tested, with or without added nitrate. Results showed that 1600 mg/kg Pb significantly inhibited NO_3^- and NO_2^- reduction, while increasing N_2O emissions. The narG gene, responsible for NO_3^- reduction, decreased markedly under high Pb levels, whereas nirS showed no clear trend. 16S rRNA gene abundance declined, while Shannon diversity increased. Proteobacteria abundance dropped rapidly, and *Azotobacter* populations fell to less than 50% of control levels at Pb concentrations of 400 mg/kg or more.

In the second experiment, an eight-week pot study was conducted to assess the effects of *Azospirillum brasilense* Sp7 inoculation on lemongrass under Pb contamination. Three inoculation strategies—seed, root, and combined—were compared. Combined inoculation resulted in the highest leaf and root biomass, while root-only inoculation had a suppressive effect. By the fourth week, combined inoculation also led to the highest abundance of nitrogen cycling genes (nxrB, amoA, nirK, nirS, norB, nosZ) and 16S rRNA, with several genes showing significantly higher expression than in the control.

Together, the results of both experiments demonstrate that Pb contamination at 400–1600 mg/kg impairs denitrification processes, reduces the abundance of functional genes and total microbial biomass, and alters microbial community structure. In contrast, inoculating lemongrass with *Azospirillum brasilense* Sp7—particularly using both seed and root methods—enhances plant growth and promotes the recovery of microbial activity and nitrogen cycling functions in Pb-contaminated soils.

These findings underscore the potential of microbial inoculation and phytoremediation with lemongrass to improve nutrient cycling and ecosystem function under heavy metal stress. The study also provides insights into how denitrification activity may influence nitrogen use efficiency in plants growing in contaminated environments. However, further research is needed to address limitations such as the lack of transcriptomic analysis, quantification of Pb in soils and plants, and validation under long-term field conditions. Future studies should include gene expression profiling, Pb accumulation measurements, and field-scale trials to better understand the long-term effectiveness of microbial-assisted phytoremediation strategies.