ABSTRACT

In response to growing concerns about the impact of environmental pollutants, including antibiotics, on ecosystems, this doctoral dissertation focuses on investigating the effects of tetracycline on plants and their microbiomes. The work comprises five publications and additional unpublished material aimed at exploring molecular biomarkers of damage and regeneration in plants exposed to tetracycline, the antibiotic's impact on the photosynthetic apparatus, optimization of DNA isolation methods from the phyllosphere, and the analysis of soil and rhizosphere microbiome composition in contaminated environments.

The primary goal of this research was to identify molecular biomarkers of plant damage and recovery in response to tetracycline exposure. A literature review revealed significant gaps in current knowledge, particularly regarding tetracycline's effects on chlorophyll biosynthesis and the activity of key photosynthetic enzymes. To address these gaps, a series of experiments evaluated the impact of tetracycline on plant chlorophyll content. It was determined that tetracycline exposure leads to a significant reduction in chlorophyll concentration, demonstrating the antibiotic’s toxic effects on the photosynthetic system.

Further experiments involved monitoring the growth of *Lemna minor* over seven days in the presence of various tetracycline concentrations (1 mM, 2.5 mM, and 10 mM). Results showed a pronounced decline in chlorophyll content, with a reduction of up to 64% at the highest concentration. Additionally, studies with pea plants revealed that even low tetracycline concentrations in soil (5 mg/kg) reduced chlorophyll levels by 17%, while concentrations of 500 mg/kg caused a 28% decline. Negative effects on chlorophyll biosynthesis pathways were identified, including decreased activity of the key enzyme δ-aminolevulinic acid dehydratase (ALAD).

Research on pea plants also examined the impact of tetracycline on ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), a critical enzyme in photosynthesis. Findings showed that tetracycline reduces the abundance of RuBisCO's large subunit and its activity, posing a significant threat to photosynthetic efficiency. These changes correlated with both tetracycline concentration and exposure duration.

Another focus of the research was cellular damage mechanisms. In *Lemna minor,* tetracycline exposure increased free radical accumulation, leading to membrane and mitochondrial damage. Markers such as elevated malondialdehyde (MDA) levels indicated enhanced lipid peroxidation, while mitochondrial damage was confirmed using the WST-1 assay.

In addition to studying damage mechanisms, the research also investigated plant regeneration capacities. High tetracycline concentrations (1 mM, 2.5 mM, and 10 mM) revealed that plants could recover growth capacity after tetracycline removal from the medium. Biomarkers such as HSP70 and antioxidant enzyme activity indicated activation of regeneration mechanisms in response to stress.

A crucial objective of the dissertation involved optimizing DNA isolation methods from the plant phyllosphere. Fifty different protocols were analyzed, leading to the identification of optimal parameters for DNA isolation, including the amount of starting material, cell lysis methods, and bacterial cell collection techniques. Results showed that a combination of

mechanical-chemical lysis with sonication and membrane filtration yielded high-quality DNA, critical for microbiome studies.

Microbiome analyses demonstrated that tetracycline significantly alters the composition of soil and rhizosphere microbial communities in lettuce. Taxonomic analysis before and after tetracycline application revealed that Proteobacteria dominated, including families capable of soil remediation. Results highlighted substantial shifts in microbial composition in response to tetracycline, with potential implications for soil quality and crop health.

These studies provide essential insights into the effects of tetracycline on plants and their microbiomes. The identified damage and regeneration biomarkers can be used to assess environmental ecotoxicity and the phytoremediation potential of plants. Microbiome studies offer new perspectives on plant-environment interactions, which are crucial for future strategies in environmental protection and sustainable agriculture.

The findings also have practical applications for monitoring pollution in agricultural ecosystems, contributing to the development of methods for assessing soil and crop quality as well as the impacts of antibiotic use in agriculture. Future research could focus on the long-term effects of tetracycline exposure and methods for mitigating its adverse environmental impacts