Effects of selenium uptake on plant proteins for phytoremediation

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Statement of authorship

CERTIFICATE OF ORIGINAL AUTHORSHIP

I, Divya Vinod, declare that this thesis is submitted in fulfillment of the requirements for the award of Ph.D. in the School of life sciences in the science faculty at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise referenced or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This document has not been submitted for qualifications at any other academic institution.

This research is supported by the Australian Government Research Training Program.

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Abstract

Selenium (Se) is a naturally occurring element first identified as a contaminant in the 1970s in agricultural drain water acting as a breeding ground for wetland wildlife. In addition to coal ash slurry waste, Se released from fossil fuel combustion, garbage, paper, and tire incineration, forest fires, soil erosion, volcanism, agriculture, coal combustion, insecticide production, oil refining, photovoltaic, and glass manufacturing among others, and can easily evolve into a significant environmental concern, despite also being essential for animal and plant tissue function. Se bioavailability, uptake, distribution, metabolism, and volatilization within living organisms are influenced by the type of vegetation, the chemical form of Se in soil, pH, moisture content, and soil composition.

Australia is well known for its rich coal exports, but coal is also consumed domestically for electricity generation by power plants around the country. One of the by-products of coal use for electricity production is a coal ash slurry. Coal ash slurry is dumped in unlined ponds near power plants and leaches into the surrounding soils and eventually into waterways. While there are several methods of seepage prevention, power plant companies are not obliged by government regulations to line historical coal ash slurry dump sites. They are also not required to remediate the land before or after power plant closure leaving behind a legacy of coal ash slurry waste contamination in soil. The resulting impact of the coal ash slurry seepage is an elevated level of carcinogenic and toxic compounds in the ground, including cadmium, arsenic, mercury, and selenium (Se). There have been numerous studies to show how plants absorb these contaminants from soil. Still, there are no studies to date demonstrating the phytoremediation of Se from the earth using plants as a potential solution in Australia.

The plants selected for this study were *Brassica* 'vitamin green', *Helianthus annuus*, *Neptunia amplexicaulis,* and *Solanum tuberosum* species used for phytoremediation on contaminated sites through tissue engineering. The specific aims of the current research project were:

1. To determine sublethal selenite and selenate concentrations in the selected plants
2. To measure the effects of sublethal selenite and selenate concentrations on reactive oxygen species enzyme activity under Se stress
3. To establish and compare patterns of Se uptake on a whole plant and proteomic scale
4. To study the effects of Se stress on plant protein regulation

The experiments described in this thesis attempt to quantify the uptake patterns in the leaves, stems, and roots of three plants, *Brassica species, Helianthus annuus and Neptunia amplexicaulis*. These plants are known for varying levels of selenium absorption. Understanding the location of Se accumulation in the plants will determine their suitability *in situ* phytoremediation, where the efficacy of currently used species is constrained by slow growth rates and low biomass production. Se is also an essential micronutrient for human function so an added benefit of using edible crops for phytoremediation is that if plants are storing Se in the edible parts of the plant such as the leaves or seeds, there is the potential for adding these plants to livestock feedstock. The addition of the Se enriched plants to livestock feedback may then be used to supplement to the human diet.

Sublethal Se concentrations were determined by subjecting the test plants to increasing concentrations of two selenium salts over 21 days, with sublethal concentrations determined by measurements of the shoot and root length, fresh and dry weights, and the number of leaves present as the selenium concentrations increased. Sublethal concentrations ranged between 15 μM for *Solanum tuberosum* to 500 μM for *Neptunia amplexicaulis*.

The reactive oxygen species were measured in the leaves, stems and roots based on the above exposure concentrations. The results indicated that exposure to elevated levels of selenite and selenate in plants results in higher reactive oxygen species (ROS) enzyme activity than seen in the controls, confirming that plants exposed to selenite and selenate were experiencing oxidative stress.

A radioactive isotope of selenium (Se^{75}) was used to quantify the Se concentration in each plant and translocation of Se in each plant part. The study indicated higher uptake in the shoots than the roots for selenite and the opposite for selenate uptake in the *Brassica* species and *Helianthus* annuus. At the same time, *Neptunia amplexicaulis* showed no significant differences in Se translocation between the roots and shoots.

Given that the results from the autoradiographic study were more reliable in *Helianthus annuus* and *Neptunia amplexicualis,* these two plants were the focus for subsequent proteomic analysis to identify whether selenoproteins could be detected in selenium-exposed plants. If selenoproteins were not detected then what effect selenite and selenate exposure have on protein expression in the selected plants. The final component of the research project indicated

that proteins associated with reactive oxygen species were elevated in the selenium-treated plants compared to controls; however, proteomic methods did not conclusively identify specific selenoproteins.

This research identified the need to further optimize the use of radioisotopes for plant autoradiography and proteomic techniques for plant studies. It also highlighted the potential of using *Neptunia amplexicualis* as a tool for the phytoremediation of Se contaminated soils. However, further research is needed.