

Effects of selenium uptake on plant proteins for phytoremediation

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9 November 2021

Table of Contents

Statement of authorship	7
Acknowledgments	8
Abstract	10
Chapter 1. General introduction	13
1.1 Australian coal production	13
1.2 Impacts of coal production	13
1.3 Regulation of Coal ash waste management	14
1.4 An introduction to Se	15
1.5 Naturally occurring sources of Se	15
1.6 Se as a contaminant	17
1.7 Impact of Se soil contamination on humans	17
1.8 Phytoremediation as a solution for Se soil contamination	17
1.9 Research gaps	21
1.10 Research aims	22
Chapter 2. Sublethal selenium concentration selection	24
Abstract	24
2.1 Introduction	25
2.1.1 Plant selection for potential phytoremediation use	25
2.1.2 Selenium accumulating plant groups	26
2.1.3 <i>Helianthus annuus</i> for potential Se phytoremediation	26
2.1.4 <i>Brassica rapa</i> for potential Se phytoremediation	27
2.1.5 <i>Neptunia amplexicaulis</i> for potential Se phytoremediation	27
2.1.6 Aim	28
Methods	28
2.1.7 Se solution stock preparation	28
2.1.8 Tissue culture preparation	28

2.1.9	Seed preparation	29
2.1.10	Data collection	29
2.1.11	Statistical analyses	30
2.2	Results	30
2.2.5	Selenite and selenate sublethal concentrations in <i>Brassica rapa</i>	31
2.2.6	Selenite and selenate sublethal concentrations in <i>Helianthus annuus</i>	32
2.2.7	Selenite and selenate sublethal concentrations in <i>Neptunia amplexicaulis</i>	35
2.2.8	Selenite and selenate sublethal concentrations in <i>Solanum tuberosum</i>	37
2.3	Discussion	39
2.3.5	<i>Brassica rapa</i> sublethal concentrations	39
2.3.6	<i>Helianthus annuus</i> sublethal concentrations	40
2.3.7	<i>Neptunia amplexicaulis</i> sublethal concentration	41
2.3.8	<i>Solanum tuberosum</i> sublethal concentration	42
2.3.9	Comparison to soil Se levels	42
2.3.10	Limitation of methodology	
2.4	Conclusion and Future Direction	44
Chapter 3. Effects of Selenium species on reactive oxygen species enzyme activity		45
Abstract		45
3.1	Introduction	46
3.1.1	Antioxidant pathways	46
3.1.2	Selenium as a stressor: superoxide dismutase (SOD)	46
3.1.3	Selenium as a stressor: catalase	48
3.1.4	Selenium as a stressor: Ascorbic acid peroxidase (APX)	49
3.1.5	Selenium as a stressor: Guaiacol Peroxidase (GPX)	50
3.1.6	Selenium as a stressor: Lipoxygenase (LOX)	50
3.1.7	Selenium as a stressor: Glutathione Reductase (GR)	51
3.1.8	Proteases	52

3.2	Method	54
3.2.1	Sample preparation for enzymes	54
3.2.2	Total protein	54
3.2.3	Super oxide dismutase (SOD) (1.15.1.1)	55
3.2.4	Catalase (CAT) (1.11.1.6)	55
3.2.5	Ascorbic Acid Peroxidase (APX) (1.11.1.11)	56
3.2.6	Guaiacol Peroxidase (GPX) (1.11)	56
3.2.7	Lipoxygenase (LOX) (1.13.11.12)	56
3.2.8	Glutathione Reductase (GR) (1.8.1.7)	56
3.2.9	Acid Protease (AP) and Neutral Protease (NP)	57
3.2.10.	Replications and statistics	57
3.3	Results	58
3.3.1	Selenite and selenate SOD and CAT concentrations in <i>Brassica rapa</i>	58
3.3.2	Selenite and selenate APX and LOX concentrations in <i>Brassica rapa</i>	59
3.3.3	Selenite and selenate GPX and GR concentration in <i>Brassica rapa</i>	60
3.3.4	Selenite and selenate AP and NP concentration in <i>Brassica rapa</i>	60
3.3.5	Selenite and selenate SOD and CAT concentration in <i>Helianthus annuus</i>	61
3.3.6	Selenite and selenate APX and LOX concentration in <i>Helianthus annuus</i>	62
3.3.7	Selenite and selenate GPX and GR concentration in <i>Helianthus annuus</i>	63
3.3.8	Selenite and selenate AP and NP concentration in <i>Helianthus annuus</i>	64
3.3.9	Selenite and selenate SOD and CAT concentration in <i>Neptunia amplexicualis</i>	65
3.3.10	Selenite and selenate APX and LOX concentration in <i>Neptunia amplexicualis</i>	66
3.3.11	Selenite and selenate GPX and GR concentration in <i>Neptunia amplexicualis</i>	67
3.3.12	Selenite and selenate AP and NP concentration in <i>Neptunia amplexicualis</i>	68
3.4	Discussion	69
3.4.1	Effect of selenate and selenite on SOD concentration	69
3.4.2	Effect of selenite and selenate on CAT concentration	70

3.4.3	Effect of selenite and selenate on GPX concentration	71
3.4.4	Effect of selenite and selenate on GR concentration	72
3.4.5	Effect of selenite and selenate on APX concentration	72
3.4.6	Effect of selenite and selenate on protease concentration	72
3.5	Conclusion and future direction	73
Chapter 4. Selenium distribution in plants		75
Abstract		75
4.1	Introduction	76
4.1.1	Selenium species	76
4.1.2	Selenium distribution	76
4.1.2	Selenium distribution methods	77
4.2	Methods	80
4.2.1	Culture Preparation.	80
4.2.2	Seed preparation.	80
4.2.3	Radiotracer stock preparation.	81
4.2.4	Autoradiography	81
4.3	Results	82
4.4	Discussion	84
4.4.1	Effect of selenite and selenate exposure on Se uptake in <i>Brassica rapa</i>	84
4.4.2	Effect of selenite and selenate exposure on Se uptake in <i>Helianthus annuus</i>	85
4.4.3	Effect of selenite and selenate exposure on Se uptake in <i>Neptunia amplexicaulis</i>	86
4.4.4	Autoradiography technique used for Se detection in plants	87
4.5	Conclusion and Future Direction	87
Chapter 5 Effect of selenite and selenate exposure on seed storage protein selenium uptake		88
Abstract		88
5	Introduction	89

5.5.1	Seed storage proteins (SSPs)	89
Aims		93
5.6	Method	94
5.6.1	Culture Preparation.	94
5.6.2	Seed preparation.	94
5.2.3	Radiotracer stock preparation.	95
5.2.4	Seed storage protein fractionation.	95
5.3	Results	96
5.4	Discussion	97
5.4.1	Selenite and selenate distribution in <i>Brassica rapa</i>	98
5.4.2	Selenite and selenate distribution in <i>Helianthus annuus</i>	99
5.4.3	Selenite and selenate distribution in <i>Neptunia annuus</i>	100
5.4.4	Method optimization	100
5.5	Conclusion and Future direction	100
5.5.1	Significance of seed storage proteins for Se accumulation	100
Chapter 6-	Effects of selenium on protein regulation in plants	102
Abstract		102
6.1	Introduction	104
6.1.1	Selenoproteins	104
6.2	Methods	105
6.2.1	Sample preparation.	105
6.2.2	Protein extraction.	106
6.2.3	Protein Pellet Rehydration and Solubilisation.	107
6.2.4	Alkylation and reduction reaction.	107
6.2.5	Protein assay gel preparation.	107
6.2.6	Staining technique	108
6.2.7	Trypsin digestion	108

6.2.8	Modified 6-minute Trypsin Digest	109
6.2.9	Loading for MS preparation	109
6.2.10	Protein analysis	109
6.2.11	Protein identification	110
6.3	Results	111
6.4	Discussion	113
6.5	Conclusion and Future Direction	122
Chapter 7	General discussion, the significance of findings and conclusions	123
Chapter 8	Conclusion and Future directions	132
References		134

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.

Signature: Production Note:
Signature removed prior to publication.

Date: 9 November 2021

Acknowledgments

This thesis results from almost 5 years of late and sometimes sleepless nights, missed family events, and the support of family, friends, and university staff at UTS. Acknowledgments are below.

I would like to thank AINSE for providing me partial funding to research ANSTO. I would like to thank UTS for the use of facilities and staff.

I would like to acknowledge and thank Dr. Lou Defillipis who introduced me to this research project.

An acknowledgment to Dr. Tom Cresswell, Dr. Paul Callaghan, Dr. Nicholas Howell for their guidance and support both personal and professional during my time at the Lucas Heights facilities.

I would like to thank Dr. Fraser Torpy for kindly stepping in to assist after the unexpected departure of Dr. Lou Defillipis during the last stages of my Ph.D., for his edits and encouragement through the writing process.

Dr. Matt Padula and Nasim Shah are to be thanked for their support in the proteomics component of this work and for always making time for my questions in the early morning.

A huge thank you goes to the people at UTS particularly within the science faculty who made this journey enjoyable, shared their passion, stories, and experiences, including all the guys in the science IT support team who spent a large amount of their time fixing my computer. The kindness and patience of the technical staff, Gemma, Susan, Lucia in particular who were never too busy to assist me.

I would like to thank all the friends I've made along the way who have shared this journey with me and supported me especially when experiments haven't worked, people who have challenged me and navigated me towards opportunities.

To my dear husband, Rahul, who joined me on this Ph.D. rollercoaster ride for the last 3 years, thank you for your patience, your understanding, accepting that I keep falling asleep all the time and for all the support while I wrote this thesis.

My younger brother Michael, who is also completing his Ph.D., who always made time for me, for his wisdom, kindness, and get-the-job-done attitude had fuelled me throughout this experience and I still have a lot to learn from him.

Above all, I would like to thank my family, my parents Edward and Harathi, who have also completed this Ph.D. with me, sharing in the late nights, the late drop-off and pick-ups from the lab, for the hot meals at the end of a tiring day and for keeping me going in what has been at times an overwhelmingly difficult Ph.D. journey. No words can ever express the gratitude and love I have for my family.

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 feedstock 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 amplexicaulis*, 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.