FANTASTIC METALS & WHERE TO PHYT THEM

Assessing the Potential of Metal Accumulation in Edible Garden Plants

Annaclaire Gwendolyn McDonald

Thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

Under the supervision of Megan L. Murray & Brad R. Murray

University of Technology Sydney

May 2021

DECLARATION

CERTIFICATE OF ORIGINAL AUTHORSHIP

I, Annie McDonald, declare that this thesis is submitted in fulfilment of the requirements for the award of Doctor of Philosophy in the School of Life Science, Faculty of Science at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise reference 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.

Production Note:
Signature: Signature removed prior to publication.

Date: 21/05/2021

ABSTRACT

Land contamination is a major threat to global food security. Heavy metals are ubiquitous contaminants contributing to agricultural land degradation across the globe. Their potential to cause serious harm to ecosystems and human health has led to the development of innovative remediation technologies. Harnessing the natural uptake ability of plants, phytoremediation offers an environmentally friendly, and cost-efficient method of remediating heavy metals from soils. Among known phytoremediators, many are plants with edible tissues, which can be deployed on degraded sites to promote decontamination.

However, plants that accumulate high quantities of heavy metals into their edible tissues are a risk to food safety. With urban gardens in Sydney and Melbourne exceeding Australia's Health Investigation Guideline level for heavy metals in residential soils, this thesis investigates the potential of edible plants in remediating legacy soil contamination in Australian environments and evaluates the associated risks of these species to food safety.

A database analysis of edible phytoremediator plants from the literature identified a research gap of edible species tested under Australian environmental conditions. This study was followed by a germination experiment investigating single and multi-metal contaminant effects on the germination of eight commercially important crop species. Carrots were the only species able to germinate under complex multi-metal conditions inferring a greater risk to food safety if they continue to grow and accumulate metals *in situ*. In a controlled glasshouse experiment, mature root vegetable plants (i.e., carrots and radishes) posed the greatest risk to food safety, while common beans were found to accumulate appreciable concentrations of lead into brown leaf tissues compared to green leaves. These results present a possible avenue for the application of common beans as phytoindicators in lead-contaminated environments. Leafy herbs presented greater risk in a real-world investigation of homegrown produce from residences in North Sydney, NSW. In addition, a landmark field garden trailed on the heritage-listed White Bay Power Station, NSW, showed potential for phytoremediation using edible plants as a non-invasive, long-term strategy for contaminated industrial sites.

The work presented in this thesis advances knowledge of the potential for edible plant phytoremediators to be used in Australian contexts with consideration of the associated risks to food safety. This research identifies crop species that pose lower or greater health risks based on edible tissue accumulation patterns. These findings inform species selection for the mitigation of risks to urban gardeners as well as providing candidates for future applications of phytoremediation in Australia.

ACKNOWLEDGEMENTS

Wait, who said never meet your heroes? I didn’t just meet mine, I had the incredible fortune to be taken under her wing as her student. Megan’s passion for science and teaching is infectious and I’m awed by her generosity, optimism, and spunk. Unlike other celebrities, I’ve got your autograph (on all the forms you’ve had to sign). Seriously though, you are an incredible supervisor. I am forever grateful to you for your mentorship, friendship and support of this work which went above and beyond. It has opened doors and made this achievement possible. I have learned so much from you over the years, both professionally and personally. I’m so stoked to be privy to your wisdom. Thank you from the bottom of my heart for taking me on, guiding me, and helping me grow as a scientist and a human.

I owe a lot of gratitude to co-superstar Brad for his invaluable feedback, contributions, and discussions on nitty gritty aspects of my research. It was through your guidance that I developed my statistical skills and pushed myself technically to think deeply about my data. Plus, a side thank you for the gentle introduction to R and graciously letting me bug you every single semester to fill out the same stipend reference.

Very special thanks to Daniel Krix for your wizardry statistical and chart contributions to parts of this research.

I wish to acknowledge the School of Life Sciences, Faculty of Science for financial support for my research including travel funds to present at the ESA19 conference.

To the incredibly hardworking and talented technical staff Sue Fenech, Gemma Armstrong, Helen Price and Rachel Keeys, thank you so much for your help; often dropping what you were doing to assist me. It also made my day chatting about your artistic talents.

A special thank you to analytical magician Helen Price who spent countless hours schooling me in ICP-MS and MP-AES analysis and sample prep. Helen is *price*less for good banter and has a characteristic tough love approach "Glass is evil. Put it on a T-shirt" – so I did. I thank you not just for your gobsmacking scientific expertise but also your patience in teaching me, as well as introducing me to the wonderful sport of Bouldering. Helen is a machine whisperer and I've unconfirmed suspicions that she herself may be a machine…

HUGE thanks to my brother Angus for our frequent coffee chats at the Startup Hub (you shouted way more than your fair share 谢谢弟弟), and older brother David for regularly asking how Uni was going but once admitting that he wasn't really interested – also fair. Love ya both.

3 borks for my best companion and confidant, Copper. Despite being a dog, Cop is tertiary qualified by proxy after supporting her human not just through high school, but a Bachelors degree and now a PhD. Most insistent on sitting within a 5m radius, under my desk, or first class (my lap), Cop has learned that Copper is essential to human health. She knows we're not just talking about the heavy metal.


Copper the Dog

Thanks to my lab buddy Kieran Young who made fumbling through countless mistakes that much easier. "You just do it ay? You just drink lots of water and do it."

Shoutout to my very good friend Philippa Alvarez for research chats, zooming through the pandemic, and sharing artistic and philosophical interests. "A seed scientist and a comedian walk into a silent meditation retreat? Just when 2020 couldn't get any weirder."

– Megan. Likewise, Ichia Chen for your friendship and chats about PhD puddles together. Pip and Ichia's magnificent scientific achievements inspired me a great deal.

Speaking of badass lady academics, thanks to Katherine Whitworth for PhD pep talks and what she called 'unsolicited advice' as well as providing valuable feedback on parts of my thesis. Thanks to Alex Pulkownik for lending me her thesis. A hearty thanks to the numerous research volunteers who helped me process a mountain of samples and/or made an appearance on my podcast 'HDR *what*'; Julia Fyvie-Neill, Liv Schmidt, Lisa Danzey, Miranda Crossley, Nicola Huber-Smith, Elisabeth Morley, Jagmeet Brar, Rebecca Cohen, Amelia Pezzano, and Jules Kidstan-Lattari. Y'all are 'chop & chat' superstars.

Cheers to my ukulele for accompanying me in research presentations including its 'instrumental' role in winning the 2019 International Visualise Your Thesis competition.

Thanks to UTS Football WAAB team (the best team ever and innovators of Bruise Day Tuesday), the Sydney University Dramatic Society (SUDS) 2020 KCD cast, and What She Said Comedy for warmly welcoming me into your shenanigans to keep me grooving outside of research. Credit to my friends June, Kien, Dakota and Kieran H. for supporting me through tough times with frank chats and banter. I loved that you repeatedly asked, "How's Uni going?" or "How long do you have left?" – My answer was always "Yes."

Finally, I'm blessed with absolutely extraordinary parents who have cheered me on all the way through my education. I can't thank you both enough for your unwavering love and encouragement. Your support has meant so much to me and I dedicate this thesis to you.

PREFACE

All research presented here was completed for my PhD thesis.

A version of Chapter 3 has been published in the *Australian Journal of Crop Science*.

A version of Chapter 3 was also presented as a poster at the 2019 Ecological Society of Australia's annual conference in Launceston.

TABLE OF CONTENTS

Declaration i

Abstract ii

Acknowledgements iv

Preface vii

Table of Contents viii

List of Figures xii

List of Tables xvi

CHAPTER 1: SETTING THE SEED 1

1.1 Introduction 1

1.2 Plants as Conduits for Contaminants 2

1.3 Food Safety 7

1.4 Heavy Metal Contamination 9

1.5 Toxicology Profiles of Selected Metals in Australia 11

1.6 Comparing Australian Soil and Food Safety Standards with International Limits 15

1.7 Research Aims 18

1.8 Thesis Structure 19

CHAPTER 2: DATASET OF EDIBLE PHYTOREMEDIATORS 21

2.1 Introduction 21

2.2 Methods 22

2.2.1 *Dataset Compilation* 22

2.2.3 *Statistical Analyses* 23

2.3 Results 23

2.4 Discussion 28

2.4.1 Conclusion 31

CHAPTER 3: THE EFFECTS OF COPPER, ZINC AND LEAD CONTAMINATION ON THE GERMINATION OF EDIBLE CROP SPECIES 32

3.1 Introduction..32

3.2 Materials and Methods..36

3.2.1 *Study Species* 36

3.2.2 *Experimental Design and Procedure*...37

3.2.3 *Statistical Analyses* 39

3.3 Results 40

3.3.1 *Germination inhibition*..40

3.3.2 *Germination onset* 40

3.3.3 *Germination duration* 41

3.3.4 *Total germination* 41

3.4 Discussion..45

3.4.1 Conclusion 48

CHAPTER 4: LEAD ACCUMULATION IN THE TISSUES OF EDIBLE CROP SPECIES 49

4.1 What Pb us here? 49

4.2 Materials and Methods..51

4.2.1 *Glasshouse Setup*...51

4.2.2 *Plants and Soils - Laboratory Processing* 53

4.2.3 *Statistical Analyses* 55

4.2.4 *Phytoremediation Index Calculation* 56

4.3 Results 57

4.3.1 *Species and plant tissues* 57

4.3.2 *Tissue age and location on plant*...59

4.3.3 Phytoremediation Indices 62
4.4 Discussion 63
4.4.1 Conclusion 66

CHAPTER 5: HEAVY METAL ACCUMULATION IN HOMEGROWN GARDEN PLANTS IN NORTH SYDNEY, NSW 67

5.1 Introduction 67
5.2 Materials and Methods 70
5.2.1 *Study Location* 70
5.2.2 *Promoting Citizen Participation* 71
5.2.3 *Donated Plants and Soils - Laboratory Processing* 71
5.2.4 *Statistical Analyses* 72
5.3 Results 73
5.3.1 *Study Species* 73
5.3.2 *Heavy Metals in North Sydney Garden Soils* 75
5.3.3 *Soil and Plant Tissue Relationships* 75
5.4 Discussion 80
5.4.1 *Food Safety* 80
5.4.2 *Soil Concentrations and Site-Specific Features* 81
5.4.3 *In the Context of Heavy Metal Guidelines* 84
5.4.4 Conclusion 85

CHAPTER 6: 'POWER PLANTS'– PHYTOREMEDIATION OF WHITE BAY POWER STATION, ROZELLE NSW 86

6.1 Introduction 86
6.1.1 *White Bay Power Station: Site History* 87
6.1.2 *Phytoremediation of White Bay Power Station* 90

6.2 Materials and Methods 92

6.2.1 *Study Location* 92

6.2.2 *Project stakeholders* 92

6.2.3 *Phytoremediation Garden & Sampling Design* 93

6.2.4 *Laboratory Processing* 95

6.2.5 *Statistical Analyses* 96

6.3 Results 97

6.3.1 *Soils* 97

6.3.2 *Edible Tissue and Soil Relationships* 100

6.3.3 *Plant Compartmentalisation – Above vs. Below-ground Tissues* 105

6.3.4 *Garden Growth* 108

6.4 Discussion 109

6.4.1 Conclusion 113

CHAPTER 7: SYNTHESIS 115

7.1 Were the aims of this thesis met? 115

7.2 Safe Gardening 120

7.3 Edible Outlooks and the Ongoing Search for Fantastic Plants 121

7.4 Final Conclusions 123

APPENDICES 124

REFERENCES 152

LIST OF FIGURES

Figure 1.1. Summary of phytoremediation processes for inorganic heavy metal pollutants…4

Figure 1.2. Sources of heavy metals in the environment..10

Figure 2.1. Percentage of species by family where contaminants in edible tissues were within or exceeded Australian and New Zealand Food Standards (FSANZ 2016). Chart shows data for the 9 most specious families of 25 recorded in the database.................................24

Figure 2.2. Percentage of species by culinary plant type and whether edible tissues recorded contaminants within or over FSANZ (2016) guideline levels. Due to expected values recording < 5, categories 'Within Guidelines' and 'No Guidelines or Untested' were pooled for χ^2 contingency test analysis. 25

Figure 2.3. Percentage of species commonly grown in domestic gardens and association with edible tissues exceeding FSANZ (2016) guidelines...26

Figure 2.4. Number of phytoremediation papers on edible species by location of study......27

Figure 2.5. Percentage of papers from the top 8 contributors (of a total of 28 countries) of studies by country and proportion of *in situ* and *ex situ* study designs................................27

Figure 2.6. Percentage of species categorised as having low, moderate, and high potential for phytoremediation and the application of additional phytoremediation enhancing substances...28

Figure 3.1. Germination onset across the eight study species in relation to the heavy metal treatments (mean + SE). An asterisk shows those treatments that differed significantly (P < 0.05) from the uncontaminated control within each species...42

Figure 3.2. Germination duration across the eight study species in relation to the heavy metal treatments (mean + SE). An asterisk shows those treatments that differed significantly (P < 0.05) from the uncontaminated control within each species..43

Figure 3.3. Total germination across the eight study species in relation to the heavy metal treatments (mean + SE). An asterisk shows those treatments that differed significantly ($P < 0.05$) from the uncontaminated control within each species. 44

Figure 4.1. Mean lead concentration (ppm) in a) edible tissues, b) roots, c) stems and d) leaves of edible species. Red letters indicate statistically different groups within a single plot. Grey boxes represent the 95% confidence interval of the model's estimated mean indicated with a black bar. 58

Figure 4.2. Proportion of edible tissues by species exceeding the FSANZ (2016) guideline level for lead in food (0.1 ppm Pb). 59

Figure 4.3. Mean lead concentration (ppm) in chilli pepper and tomato plant tissues. Red letters indicate statistically different groups. Grey boxes represent the 95% confidence interval of the model's estimated mean, indicated with a black bar. 60

Figure 4.4. Lead concentration (ppm) in upper (red) and lower (blue) tissues of fruits, leaves and stems of chilli pepper and tomato species. 61

Figure 4.5. Mean lead concentration (ppm) in brown vs. green leaves of beans. 62

Figure 5.1. Study location map of suburbs Northbridge and Castlecrag in North Sydney, Australia. 70

Figure 5.2. Plots of mean heavy metal concentrations in soils (top right; log transformed data). Pearson's correlation value and significance values are indicated at the top of each plot. Blue points indicate significant associations while red indicate non-significant associations. The strip plot (bottom left) shows untransformed mean soil metal values across sites, ranked on the x-axis by their median indicated with a black bar. Red letters below the x-axis labels indicate groups that were significantly different. 76

Figure 5.3. Strip plot of relationships between soils and paired tissue type for heavy metal concentrations. Red dotted lines indicate the median for soil concentration. Blue strips indicate plant tissues while red strips indicate soils. 77

Figure 5.4. Plot of robust linear regressions between soils and paired tissue type for heavy metal concentrations, performed on log transformed data. Red lines show the line of best fit for a given relationship, with significance values shown at the top of each plot. Blue plots indicate significant correlations while red plots are non-significant. Fruits/edible tissues were omitted due to low replication. 78

Figure 5.5. Geospatial map of maximum soil concentration (ppm) of lead (a), arsenic (b), cadmium (c) and copper (d). 79

Figure 6.1. White Bay Power Station, Rozelle NSW. 87

Figure 6.2. Features from the northern aspect of White Bay Power Station. 88

Figure 6.3. Study location of White Bay Power Station and phytoremediation garden, Robert Street, Rozelle, NSW. 92

Figure 6.4. White Bay Power Station from a northern aspect of the garden prior to planting. 93

Figure 6.5. Phytoremediation garden design (From Ware *et al.* 2018) 94

Figure 6.6. (Above) The first garden plot (monoculture circles) from the south facing north. 95

Figure 6.7. (Right) Evidence of rusting pipe material in the 7th garden plot (monoculture circle of *Cucurbita pepo*) facing north. 95

Figure 6.8. Boxplot of mean heavy metal concentrations in soil samples collected at White Bay Power Station from original condition to January 2019, analysed with MP-AES. 98

Figure 6.9. Boxplot of mean heavy metal concentrations in soil samples collected at White Bay Power Station from February 2019 to May 2019, analysed with ICP-MS. 98

Figure 6.10. Plots of mean heavy metal concentrations in soils (log transformed data). Pearson's correlation value and significance values are shown at the top of each plot. Blue points indicate significant associations while red indicate non-significant associations. 99

Figure 6.11. Relationship of edible or reproductive tissue concentration of arsenic (ppm) and paired soil concentrations (ppm), showing movement of the metalloid from the soil matrix into plant tissues. Tissues below the detection limit of 0.5 ppb are indicated as 0 ppm. 101

Figure 6.12. Relationship of edible or reproductive tissue concentration of chromium (ppm) and paired soil concentration (ppm). Tissues below detection limits are indicated as 0 ppm. 102

Figure 6.13. Relationship of edible or reproductive tissue concentration of copper (ppm) and paired soil concentration (ppm). Tissues below detection limits are indicated as 0 ppm....103

Figure 6.14. Relationship of edible or reproductive tissue concentration of lead (ppm) and paired soil concentration (ppm). Tissues below detection limits are indicated as 0 ppm....104

Figure 6.15. Relationship of edible or reproductive tissue concentration of nickel (ppm) and paired soil concentration (ppm). Tissues below detection limits are indicated as 0 ppm....105

Figure 6.16. Heavy metal concentrations in below-ground and above-ground tissues (ppm) of plants harvested in May 2019. Individuals of the same species harvested from different garden plots are indicated by mean ± SEM. 106

Figure 6.17. Heavy metal concentrations in below-ground and above-ground tissues (ppm) of plants harvested in April 2019. 107

Figure 6.18. Heavy metal concentrations in below-ground and above-ground tissues (ppm) of plants harvested in October 2018. 108

Figure 6.19. White Bay Power Station with phytoremediation garden in the foreground. .108

LIST OF TABLES

Table 1.1. Guidelines for heavy metals in soils and food. 17

Table 3.1. Study species used in the experiments to assess seed germination responses to copper, zinc and lead contamination. *Solanum lycopersicum* has been grouped with vegetables commonly grown in domestic gardens because many domestic growers consider it a vegetable crop based on its culinary function (Bergougnoux 2014). 37

Table 4.1. Study species, harvest dates, and number of replicates by species′ family........... 52

Table 4.2. Leaf and soil bioconcentration factors (BCF_{leaf} and BCF_{root}), and fruit translocation factor (TF_{fruit}) of lead in crop species (mean ± SD). Values > 1 are marked in bold. 62

Table 5.1. List of donated species by taxonomic family.. 73

Table 6.1. Descriptive statistics for heavy metal concentrations in soils at White Bay Power Station. 97

CHAPTER 1

SETTING THE SEED

1.1 Introduction

The World is a Dirty Place

Human-led industrial advancement has positively contributed to the quality of life among human communities. Regrettably, positive progress has imposed negative outcomes for global environments. With prominent issues like climate change, resource sustainability, species extinctions, and land contamination more frequently evoking anxiety, or sentiments of concern, environmentalism is gaining wider traction in the wake of escalating ecosystem degradation (Wright & Nyberg 2012; Clayton 2020). Industrial growth has facilitated an unprecedented increase in environmental contamination events spanning a wide range of contaminant types (Munton 2002; Laidlaw *et al*. 2011; Mackay *et al.* 2013; Kennen & Kirkwood 2015). According to Anjum *et al.* (2013), over 16% of the world's total land area hold soils that have been subjected to some degree of contamination.

Remediation technologies are required to remedy this broad assortment of organic and inorganic contaminant classes; from petroleum hydrocarbons, chlorinated solvents, heavy metals and metalloids, pesticides, pharmaceuticals, explosives, propellants, radionuclides, polycyclic aromatic hydrocarbons (PAH), to fire retardants perfluoroalkyl and polyfluoroalkyl substances (PFAS) (Kennen & Kirkwood 2015). Adding to the challenge of remediation are varied chemical characteristics of these contaminants that influence their movement, speciation, and toxicity in local environments (Shahid *et al.* 2014).

With growing populations and greater dependence on arable lands, the movement of contaminants into food webs is of high concern worldwide (Shahid *et al.* 2014; Sarwar *et al.* 2017; Xiao *et al.* 2017; Eisazadeh *et al.* 2018). Food security can be limited by space where pollution sources like mining practices can overlap with zones of agriculture (Xiao *et al.* 2017). Questions of food safety extend to local contaminant sources in domestic gardens particularly in cities where brownfield spaces are increasingly being reclaimed to create urban gardens (Leake *et al.* 2009; Säumel *et al.* 2012).

The natural world contains a plethora of remarkable uses, remedies, and ecosystem services. Plants have been found to inhibit haemorrhaging from snake venom (de Moura *et al.* 2015), Mycelia fungi reduce viruses in European Honey Bee populations (Stamets *et al.* 2018), and humans who interact with green spaces experience profound benefits in mental and physical health including microbiome related immune function (Leake *et al.* 2009; Flies *et al.* 2017). Fittingly, plants provide an environmentally friendly option to be used as tools in remediation where nature may hold its own antidote to the poison of anthropogenic pollution. It is the distinction between deliberate application of edible plants in remediation and incidental uptake of contaminants into edible tissues that provides context to the risk to food safety.

1.2 Plants as Conduits for Contaminants

Plants in a Sucky Business

'Phytotechnology' is a hypernym for the rapidly emerging collection of environmentally friendly, plant-based solutions that can address complex contamination issues. It pivots on harnessing natural properties of plants including their physical attributes, absorbent propensity, and symbiosis with bacteria or mycorrhizal fungi (i.e., plant-microbe interactions) (Thijs *et al.* 2017). Examples of applied phytotechnologies include green roofs and walls for air-filtering particulate matter, highway vegetation buffers for noise and pollution mitigation, and bioswales or constructed wetlands for stormwater treatment (Kennen & Kirkwood 2015). Pre-emptive planting of gardens with the intent of future-proofing a site from contamination is also considered a phytotechnology.

Phytoremediation is a phytotechnology used to clean-up contaminated soils, air or groundwater. It is a cost-efficient (Mosa *et al.* 2016), non-invasive (Dietz & Schnoor 2001), longer-term biotechnology that can be applied *in situ* to decontaminate sites where contaminants are within reach of plant roots. Traditional methods of contaminated site remediation include fast removal via 'dig and dump' where contaminated soils are excavated and exported offsite to landfills. These strategies damage soil microbial ecology as native soils are replaced with imported fill that can be infertile, consequently restricting future use (Kennen & Kirkwood 2015). Without treating the contamination present in the soil, these strategies have been referred to as a 'band aid fixes' (Ware *et al.* 2018), equally referring to the unsustainability of landfills.

Phytoremediation involves several mechanisms engaged by plants, sometimes concurrently depending on contaminant types present, species, and soil chemistry. For inorganic pollutants, mechanisms include phytoextraction, phytostabilisation[1] and phytovolatilization (Fig. 1.1). Non-land based phytohydraulics are used in the redirection,

---

[1] Phytotechnology and phytoremediation are sometimes used interchangeably with the latter preferred in scientific contexts. Strictly, the term phytoremediation is when the objective is for remediation of land or groundwater contaminants via degradation or removal (Kennen & Kirkwood 2015). Phytostabilization is excluded under this definition because contaminants are not degraded or removed but stabilised in the soil.

evapotranspiration, or containment of groundwater contaminant plumes if in reach of plant roots (Martino *et al.* 2019). Phytoaccumulator plant species can be used as bioindicators of pollution levels (Calzoni *et al.* 2007).

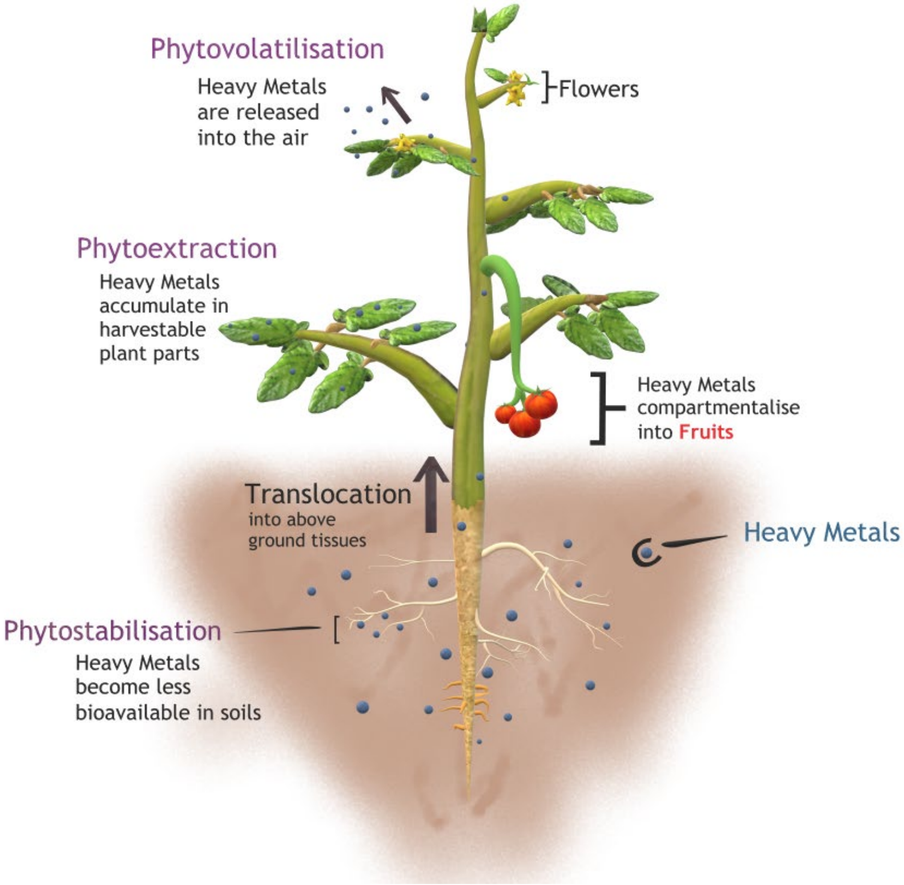


Figure 1.1. Summary of phytoremediation processes for inorganic heavy metal pollutants.

Inorganic pollutants like heavy metals can attach themselves to surfaces of matter in the soil either physically or chemically, or they can bind to matter based on opposing positive (cation) and negative (anion) ionic charges (Kennen & Kirkwood 2015). Soil pH plays a large role in the bioavailability of inorganic contaminants to plant roots where acidic soils (lower pH) containing more H+ ions render cation pollutants more bioavailable while attracting and binding to anion pollutants. Conversely, alkaline soils (higher pH) contain more OH- ions conducive for attracting positively charged cations and metaphorically ‘locking’ them to the soil (referred to as phytostabilisation; Fig. 1.1). Therefore, the cation exchange capacity (CEC), soil temperature, texture type (i.e., clay content), oxygen content, organic matter and plant species are factors that influence contaminant bioavailability to a plant’s roots (Kennen & Kirkwood 2015).

Phytoextraction

Phytoextraction is when contaminants are extracted from the soil and compartmentalised into upper tissues of a plant. Usually this occurs incidentally with natural uptake of nutrients or water (via phytohydraulics). Organic contaminants may be degraded within the plant’s biomass (called phytodegradation) effectively removing them altogether. Conversely, inorganic contaminants remain in plant tissues where they must be harvested to remove them from a site. Harvested plant sections can be reused as pulp, fuel, or hardwoods, or incinerated before being transported to landfill (Kennen & Kirkwood 2015).

Hyperaccumulator species are plants that uptake disproportionally high concentrations of contaminants into their above-ground biomass (Brooks 1998; Koleli *et al.* 2015). While uniform definitions of phytoremediation indices have yet to be decided (Buscaroli 2017), typically a ratio of metal content in a plant’s tissue (originally leaves) divided by the concentration in the soil > 1 is used to identify hyperaccumulators. This ratio is frequently referred to as the bioconcentration or bioaccumulation factor among at least 20 other synonymous terms in the literature depending on author preference, which plant part is being compared, and whether it is compared to soils or other plant parts (Buscaroli 2017). Identifying hyperaccumulator species can be useful because they can be characteristically tolerant to contaminants making them better candidates for application on heavily polluted sites where other plants may be limited in their ability to grow or establish.

The application of hyperaccumulator species on contaminant rich soils for the purposes of harvesting the contaminants and converting them into a commercially viable ‘bio-ore’ product is called phytomining (Nkrumah *et al.* 2016). The first phytoextraction patent was lodged in Japan in 1980 for phytomining cadmium (Utsunomiya 1980). Phytomining of nickel or cobalt using *Alyssum* genus (from the Brassicaceae family) with adjustments to soil pH has proven successful and the technique has been patented in the U.S. (Rufus *et al.* 2007; Nkrumah *et al.* 2016). Ongoing identification of hyperaccumulator species is useful in adding to a bank of remediation tools, in addition to informing associated food safety risks of their application on degraded sites.

Phytoremediation in Australia

In Australia, phytoremediation projects have largely focussed on urban or industrial wastewater treatment, landfill leachate treatment, road vegetation design, and the rehabilitation of mines, often first trialled in pot experiments. Examples include remediation of tailings dams at mine sites (e.g. Cannington Mine, Queensland; Lottermoser *et al.* 2009) or rehabilitating former industrial sites into parklands that entail filtering of contaminated leachate through wetland designs (Robinson & Anderson 2007). Wetland examples include Sydney Olympic Park (Ying *et al.* 2009), White’s Creek wetland in Annandale (Murray 2019), and Werribee’s sewage filtering wetland in Melbourne (Robinson & Anderson 2007).

Australian native flora have been useful as bioindicators in new mine exploration. For example, *Eucalyptus* tree leaves were found to contain deposits of gold (Au) particles in the Yilgarn Craton, 40km North of Kalgoorlie, Western Australia (Lintern *et al.* 2013). *Eucalyptus* trees have long root systems that can tap into deep gold deposits and translocate the particles into upper plant tissues where they are discarded (via dropping leaves) to reduce metal stress (Lintern *et al.* 2013).

To be effective, phytoremediation technology is required to be tailored to site-specific features. Australia boasts its own unique ecology, climate, and soil structure owing to its tectonic stability, geographic isolation, and age (Orians & Milewski 2007). Soils used for agriculture and mining are commonly acidic in Australia which can increase the mobility and bioavailability of inorganic contaminants (de Caritat *et al.* 2011; Abraham *et al.* 2018).

Phytoremediating species that are tested internationally inform selection for Australian sites but suitability is limited by Australia's sensitive biodiversity, characteristically long droughts, and nutrient poor soils (Orians & Milewski 2007). For the technology to be leveraged successfully to remediate Australia's degraded sites, it is important that plants are considered within the context of Australia's ecological and climatic conditions.

1.3 Food Safety

More Than You Bargained from Your Garden

Food security is a global priority in supporting human populations. Patterns of heavy metal contamination of foodstuffs differ between world regions. In Poland, for example, higher lead exposure was found to be derived from eating meats, vegetables and cereals, whereas in Finland, it was more likely to originate from diary and beverage products (Yale University

2018), where processing practices can also introduce contaminants. Overuse of pesticides or the application of wastewater for irrigation are known pathways of heavy metal contaminants into agricultural crops (Anwar *et al.* 2016; Edelstein & Ben-Hur 2018). Additionally, as demand for resources rises, competition for land space between agricultural and mining industries amplifies (Langkamp 1985). It could be conjectured that fertile land for growing crops coincides with mineral rich sites attractive for mining. Lechner *et al.* (2016) note that conflict between agricultural and coal industries are prominent in China and Australia. Examples of regions in Australia where coal industries operate near agriculture include the Hunter Valley NSW, the Darling Downs QLD, and Liverpool Plains NSW (Langkamp 1985). Nearby industrial practices can contaminate land used for agriculture where subsequent accumulation of contaminants into crops exposes people to health risks via dietary intake (Xiao *et al.* 2017).

Voutsa *et al.* (1996) studied the impact of industry derived atmospheric pollution causing heavy metal contamination in vegetables grown in Greece. The results of the study indicated significantly higher levels of metal accumulation in leafy vegetables (brassicas) as compared with root vegetables. A trend in brassicas accumulating higher levels of heavy metals is supported throughout the literature (Kachenko & Singh 2006; Anjula & Sangeeta 2011; Ning *et al.* 2015). The European Union (EU) and Food Standards Australia and New Zealand's (FSANZ) lead (Pb) contamination investigation trigger guideline for brassicas is 0.3 ppm as opposed to 0.1 ppm allowable lead in other vegetables (EU 2006; FSANZ 2016). These standards are important for commercial compliance to food safety, however urban gardeners are not subject to such standards, nor do they have opportunity for their vegetables to be tested.

In cities, food safety concerns arise from deposition of heavy metals within urban household soils and guerrilla gardens via vectors of home pesticide use, traffic fumes, historical use of leaded petrol and chipping leaded house paint (Rouillon *et al.* 2017). Through phytoremediation mechanisms, these heavy metals can accumulate into edible tissues of vegetables and fruits (Finster *et al.* 2004; Kachenko & Singh 2006; Antisari *et al.* 2015). In a community project by Rouillon *et al.* (2017), soil from 1200 Sydney, Australia home gardens were analysed revealing 40% of homes contained lead concentrations over the national Health Investigation Level for residential soils (HIL-A) of 300 ppm (NEPM 2013). 15% of homes had lead concentrations over 1000 ppm, with an overall correlation found between lead contamination and houses located closer to the city CBD. A similar study in Melbourne found 8% of the 13 community gardens tested and 21% of 136 residential gardens contained soil lead over the HIL-A guideline (Laidlaw *et al.* 2018). With many Australian homes well above the lead investigation trigger thresholds in their soils, understanding relationships between soil contaminant levels and food toxicity is critical for domestic food safety.

1.4 Heavy Metal Contamination

Heavy Metal – A Popular Genre

Heavy metals and metalloids are one of the most globally widespread and persistent classes of contaminants (Rubio *et al.* 2000; Anjula & Sangeeta 2011; Jennings 2013; Shahid *et al.* 2014; Tóth *et al.* 2016). While natural processes such as weathering contribute to heavy metal deposition in the environment, most sources are anthropogenic (Fig. 1.2).

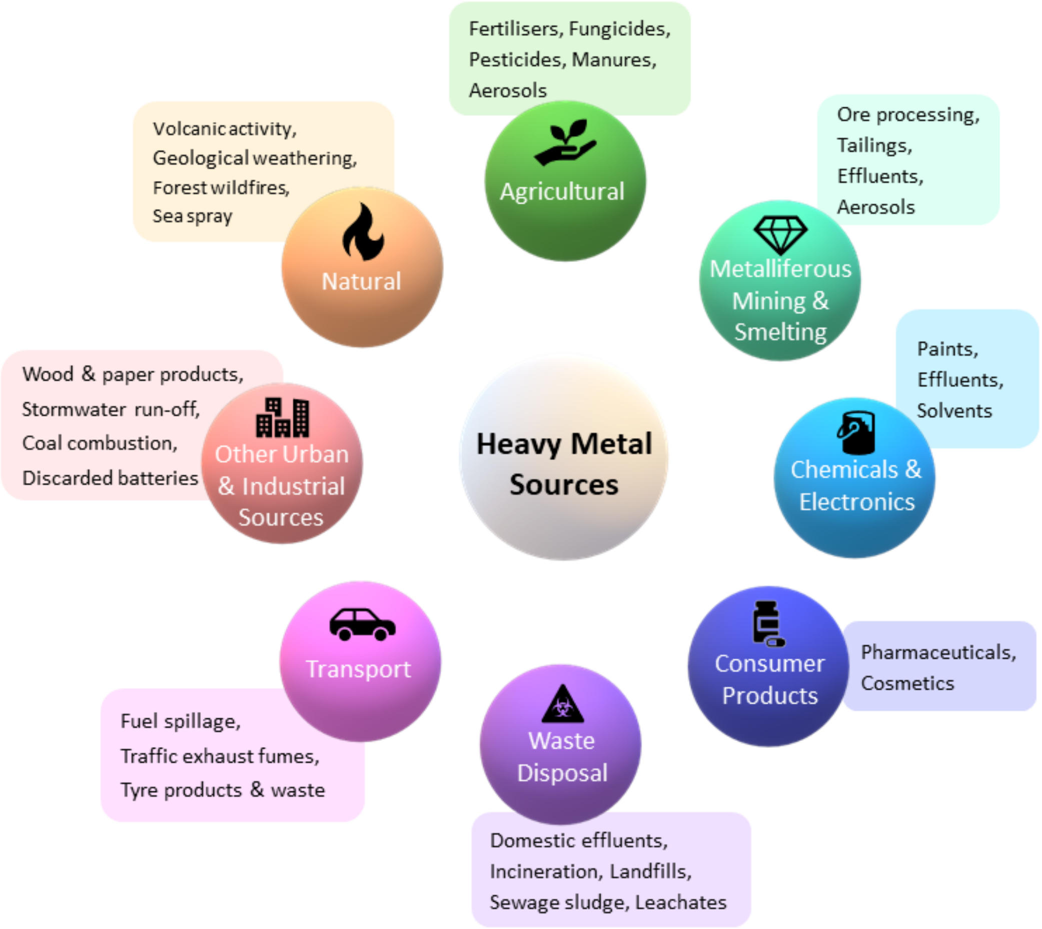


Figure 1.2. Sources of heavy metals in the environment with examples (Pulkownik 2000; Borowska *et al.* 2015; Edelstein & Ben-Hur 2018).

Heavy metals are grouped with metalloids as elements that have an atomic density > 4 g/cm^3 (Edelstein & Ben-Hur 2018). Some are essential micronutrients for the health of plants, humans and animals (e.g. Cu, Cr, Fe, Mn, Ni, Se, Zn), while others serve no biological function (e.g. As, Cd, Hg, Pb) (Edelstein & Ben-Hur 2018). Nearly all have the potential to be toxic depending on their dosage. Arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), and mercury (Hg) are flagged among the most concerning for public health because of their carcinogenicity and potential to cause harm even in low doses (WHO 2007; Tchounwou *et* *al.* 2012). Unlike long-chained organic compounds, heavy metals are inorganic contaminants and do not readily break down in the environment or the body. Predicting the fate and mobility of trace elements in environments is further complicated by natural conditions like soil type, temperature (Johnston *et al.* 2016) or the presence of adsorption sites like bacteria (Moon & Peacock 2011). Heavy metals can be converted into biologically unavailable forms thereby reducing their risk of toxicity, or they can be extracted from the environment altogether.

1.5 Toxicology Profiles of Selected Metals in Australia

Metal Mugshots

Arsenic

Arsenic occurs naturally in low abundances in most environments, usually ranging from 1 to 40 ppm (Tchounwou *et al.* 2012). High doses in plants inhibits growth of shoots, discolours roots and causes death of cells (necrosis) in leaves (Edelstein & Ben-Hur 2018). Diet is the most likely form of human exposure to arsenic however workers in industries like wood preservation, vineyards, smelting, waste disposal, glassmaking, mining or pesticide use are at greater risk (Kennen & Kirkwood 2015; Rai *et al.* 2019). Arsenic is used in human and veterinary medications including in treatment for acute promyelocytic leukemia (Kian *et al.* 2020). This anticancer property is interesting given that the metal is also strongly linked to numerous cancers with reportedly higher mortality rates (Tchounwou *et al.* 2012). Arsenic can interfere with nearly all body organ systems and is associated with neurological disorders, cardiovascular disease and disruption to DNA (Khan *et al.* 2020). There are over 100 compounds of arsenic where toxicity is dependent on compound type and oxidation state (Reid *et al.* 2020). It is understood that the extent of harm depends on dose and duration of exposure however the precise mechanisms of arsenic's toxicity remain uncertain (Tchounwou *et al.* 2012).

Cadmium

In contaminated environments, cadmium often co-occurs with elevated levels of zinc (Zn) (Kennen & Kirkwood 2015; Abraham *et al.* 2018). It is a non-essential element that can cause serious disruption to human biological functions including mutagenic and carcinogenic illnesses, endocrine disruption, renal failure and anemia (Ali *et al.* 2013). In plants, cadmium causes oxidative stress by production of reactive oxygen species (ROS). This can lead to chlorosis (an insufficient production of chlorophyll), necrosis, growth inhibition and metabolic disruption where essential nutrients like calcium must compete for uptake resulting in nutrient deficiencies (Charfeddine *et al.* 2017).

Chromium

Chromium (Cr) is contained in fresh foods typically in concentrations of < 0.01 and 1.3 mg/kg (Tchounwou *et al.* 2012). Trivalent chromium Cr(III) plays an essential role in protein and fat metabolism by assisting in the activation of insulin (Tchounwou *et al.* 2012; Vincent & Lukaski 2018). Chromium has several oxidation states ranging from Cr(II) to Cr(VI) where Cr(III) is considered relatively stable and is the state typically found in nature. Hexavalent chromium Cr(VI) is a toxin that can more readily pass through cell membranes where it can cause mutagenic and carcinogenic effects (Vincent & Lukaski 2018). In plants, chromium disrupts electron transport, carbon dioxide fixation and enzyme activity that overall impair photosynthesis (Edelstein & Ben-Hur 2018).

Copper and Zinc

Copper (Cu) and zinc (Zn) naturally occur in the environment with trace concentrations essential to human, animal, and plant health (Bost *et al.* 2016). An average adult weighing 70 kg contains approximately 1500-3000 mg zinc making it the most abundant trace metal in the body compared to copper at 100 mg (WHO 2004; Bost *et al.* 2016). Globally, copper and zinc rank third and fourth respectively as the most widely used elements, only exceeded by iron (Fe) and aluminium (Al) (WHO 2004).

Elevated zinc in a human body causes copper deprivation in the bowels as zinc ions outcompete copper ions to a limited number of protein binding sites (WHO 2004). Copper cycles between Cu (II) and Cu(I) (oxidised state to reduced state) making it valuable for enzymes that use it in oxidative stress-related functions (Tchounwou *et al.* 2012). This beneficial feature is also what makes copper toxic in higher concentrations as reactive oxygen species (ROS) like hydroxyl radicals and superoxides can form (Edelstein & Ben-Hur 2018). Excess copper can cause sickness with increased risk of mortality (Duruibe *et al.* 2007). Acute symptoms of copper poisoning by ingestion include vomiting, hypotension, coma, jaundice, and gastrointestinal distress (Klaasen *et al*. 1995). In plants, high concentrations of copper can impair plant growth and function, cause iron deficiency leading to chlorosis, and has been known to alter root structures (Lequeux *et al.* 2010, Feigl *et al.* 2013, Nair & Chung 2015).

Lead

In trace amounts, lead (Pb) is naturally present within the earth's crust but does not serve any biological function in organisms. Acute lead poisoning can cause brain damage, gastrointestinal diseases and kidney damage in humans. Similar to cadmium, lead interferes with nutrient uptake by taking the place of calcium (Tchounwou *et al.* 2012). Aside from nutrient deficiency, the consequences for plants exposed to elevated lead include impaired photosynthesis, water imbalances, inhibition of enzyme activity, oxidative stress and abnormal morphology (Mohamed 2011; Edelstein & Ben-Hur 2018).

The status of lead contamination is apparent by European Union (EU) and World Health Organisation (WHO) efforts to reduce our reliance on lead-based products (e.g. surface paints, industrial parts, motor fuel, children's toys) and track global lead pollution (WHO 2018). Through these organisations, a lead-paint targeted alliance known as the 'Global Alliance to Eliminate Lead Paint' was established in 2011 (United Nations Environment Programme 2013). According to recent WHO data, 66% of countries do not have legislation on lead-paint use, production, and distribution (WHO 2018). This is concerning given firm evidence linking lead exposure to neurotoxic effects particularly in children, including developmental delay, reduced Intelligence Quotient (IQ) and behavioural problems (Finster *et al.* 2004; Laidlaw & Taylor 2011; Mackay *et al.* 2013). Interestingly, child lead exposure has also been linked to criminality occurring 22 years later into adulthood observed across six American cities (Mielke & Zahran 2012). Furthermore, a pattern in the reduction of violent crime is reportedly consistent with the rates these states phased out leaded petrol (Mielke & Zahran 2012; ABC 2015).

1.6 Comparing Australian Soil and Food Safety Standards with International Limits

Choose Your Own Adventure

Presented side by side, standards from well-regarded organisations like the World Health Organisation (WHO) and the Food and Agriculture Organisation of the United Nations (FAO) illustrate how heavy metal content in different soils and vegetables can be categorised as 'contaminated' under one standard and 'safe' under another.

Comparing soil and food standards in Australia to countries like Canada that have updated their lead exposure allowances helps contextualise locally-permissible levels of contamination. Australia has a large mining and industrial history which has implications for higher average levels of heavy metals in the soils where these activities are current (e.g. mining towns like Mount Isa; Mackay *et al.* 2013) or where former industry has left legacy contamination (e.g. inner Sydney; Rouillon *et al.* 2017). Soil lead values in residential soils in Australia are flagged at 300 ppm (NEPM 2013), a threshold more than double the threshold in Canada (140 ppm; CCME 2018).

Australia and New Zealand differentiate in soil heavy metal guidelines but share guidelines for food stipulated by Food Standards Australia and New Zealand (FSANZ 2016; Table 1.1). These mirror European Union food safety standards for metals (EU 2006). Both organisations do not define standards for many metals focussing on known problem metals like arsenic, cadmium, lead, mercury, and tin as a priority due to their greater health concerns, including their potential to bioaccumulate. FSANZ divides heavy metal standards into food groups fruits, brassicas, and non-brassica vegetables in relation to home-grown produce (Table 1.1). While the reason is unclear, brassicas may have different guidelines because they are known to accumulate high levels of heavy metals, or their growth form may promote greater collection of airborne contaminants due to a large leaf surface area. In terms of soil management and urban gardening, international and local guidelines contextualise risks of exposure to heavy metal contaminated foods and soils. Furthermore, the investigation of heavy metal content in edible plant species can inform baseline standards of heavy metals that do not yet have recommended guideline levels.

Table 1.1. Guidelines for heavy metals in soils and food. Adapted from Rouillon *et al.* (2017).

Governing Body	**Region**	**Soil or Food Type**	**Arsenic** (ppm)	**Cadmium** (ppm)	**Chromium VI** (ppm)	**Cobalt** (ppm)	**Copper** (ppm)	**Lead** (ppm)	**Manganese** (ppm)	**Nickel** (ppm)	**Selenium** (ppm)	**Vanadium** (ppm)
NEPM (2013)	Australia	Residential soil with home garden produce	100	20	100	100	6,000	300	3,800	400	200	-
CCME (2018)	Canada	Residential soil with home garden produce	12	10	64	50	63	140	-	45	1	130
MFE (2013)	New Zealand	Residential soil with home garden produce	20	3	460	100	No Limit[b]	210	1,500	50	100	250
FSANZ (2016)	Australia & New Zealand	Vegetables (except Brassicas[a])	1	0.1	-	-	-	0.1	-	-	-	-
		Brassicas[a]	1	0.1	-	-	-	0.3	-	-	-	-
		Fruit	-	-	-	-	-	0.1	-	-	-	-
European Union (2006)[c]	EU	Other Vegetables & Fruit	-	0.05	-	-	-	0.1	-	-	-	-
		Stem & Root Vegetables	-	0.1	-	-	-	0.1	-	-	-	-
		Leafy Vegetables	-	0.2	-	-	-	0.3	-	-	-	-
		Berries & Small Fruits	-	0.05	-	-	-	0.2	-	-	-	-

[a] Leafy vegetables (e.g. cabbages, spinach, kale, brussels sprouts, broccoli).

[b] No limit because the derived standard exceeded 10,000 ppm which was deemed unlikely in practice (MFE 2013).

[c] EU values are based on wet weight while other values are based on dry weight.

- No threshold found.

1.7 Research Aims

From Root to Fruit

Food safety is paramount in fostering safe communities and viable enterprises for growers of edible produce at any scale. Given the extensive legacy of heavy metal contamination in Australian soils, and the potential for plants to absorb and accumulate metals, it is critical to investigate food safety risks of edible species growing under a range of conditions in Australia. Focussing on species typically grown in urban gardens, this new research examines contamination of homegrown foodstuffs in a unique, multi-species and multi-contaminant approach reflecting real-world environmental scenarios in Australia. It aims to explore food safety of urban gardens while documenting the potential for select edible species to be used as tools in phytoremediation of Australia's heavy metal contamination legacies. The research presented in this thesis is summarised by the following aims:

Aim 1. Investigate the application of edible crop species in heavy metal phytoremediation projects in Australia to inform suitable species selection.

Aim 2. Determine the extent of heavy metal translocation, and sites of accumulation, of lead in aerial and edible tissues of common groups of garden plants grown under a controlled glasshouse experiment.

Aim 3. Explore the risk of existing heavy metal contamination in homegrown produce of urban Sydney gardens and reflect on current recommended safety levels, background contamination sources, and levels found in the glasshouse experiment.

Aim 4. Explore the effects of single and multi-metal contamination on the germination of edible plant species seeds.

1.8 Thesis Structure

The Garden Path

To address these research aims, a combination of desktop, laboratory, field and glasshouse studies were conducted to investigate both real world and controlled phytoremediation capabilities of edible plants. These studies are structured by the following chapters:

Chapter 2: *Database Compilation and Analyses*

Desktop searches of peer-reviewed phytoremediation literature were compiled into a unique *Database of Edible Phytoremediators* to analyse for trends in edible species and associated phytoremediation potential. The database also indicates if a species' edible tissues contained contaminants over Australian guideline levels.

Chapter 3: *Seed Survival and Germination Response in Contaminated Media*

Seed germination response is investigated in eight commercially important crop species grown in media contaminated with copper, zinc, and lead.

Chapter 4: *Edible Crops in Spiked Pots*

Accumulation, tolerance and remediation of lead at the Australian residential guideline level (300 ppm) by common crop species is presented from a controlled glasshouse experiment.

Chapter 5: *In situ Metal Analysis of Home Gardens in North Sydney*

Assessment of contaminant risk to homegrown produce of home gardens was investigated in a local scale *in situ* analysis of heavy metals in soils and edibles grown in residential gardens of North Sydney, New South Wales.

Chapter 6: *White Bay 'Power Plants' Phytoremediation*

Heavy metal plant tissue compartmentalisation and real-world decontamination assessment of a phytoremediation garden at a heritage listed, former industrial site, White Bay Power Station, New South Wales.

Chapter 7: *Synthesis and Discussion*

A synthesis of findings from Chapters 2-6 are discussed followed by recommendations for future research directions.

CHAPTER 2

DATASET OF EDIBLE PHYTOREMEDIATORS

Recipe Book of Decontaminating Delicacies

2.1 Introduction

A major pathway of heavy metal exposure in humans is through food web accumulation via plants (Kachenko & Singh 2006; Liu *et al.* 2013; Rai *et al.* 2019). Common agricultural crop species and domestic garden plants are listed among known hyperaccumulators (Chaturvedi *et al.* 2019), but not all plants translocate and compartmentalise contaminants in the same way. Some edible plants may be useful in phytostabilising contaminants within their rhizosphere, with edible tissues remaining safe for consumption (Madejón *et al.* 2018). With negligible effect to plant health and yield, identification of phytoexcluder crops can be valuable for cultivation in agricultural areas where soils are degraded.

Unfortunately, there is no clear pattern that distinguishes metal tolerant plants from non-tolerant plants (Pulkownik 2000) making it imperative that potential phytoremediators are tested under an array of conditions, contaminant types, and concentrations. With a vast variety of investigative methods for testing potential phytoremediators, it can be difficult to identify edibles that may or may not pose risks to food safety.

In this study, a large dataset of phytoremediation literature specific to edible plants was collated and assessed to provide an overview of current knowledge in crop phytoremediation and associated food risks. During this scientific literature evaluation, the following research questions were asked:

(i) Are different types of edible tissues (e.g. fruits, herbs, vegetables) more likely to exceed guideline levels and risk food safety?

(ii) Is there an association between crops commonly found in domestic gardens and edible tissues exceeding contaminant guideline levels?

(iii) Is there a difference in proportion of *in situ* and *ex situ* experimental tests of edible phytoremediators and country the study was conducted in?

(iv) Is there a relationship between application of phytoremediation assistance (e.g. treatments of chelating agents, biochar, nutrient additions) and food safety?

2.2 Methods

2.2.1 *Dataset Compilation*

Phytoremediation literature was found using search terms (phytoremediation OR phytoremediator OR hyperaccumulation) OR (scientific name + (phytoremediation OR phytoremediator OR hyperaccumulation)) OR (common name + (phytoremediation OR phytoremediator OR hyperaccumulation)) within the Web of Science Core Collection database, Google Scholar and UTS Library Online Catalogue.

The dataset was categorised by species. Numerous categories were populated including genus, family, common name, culinary plant type (e.g. fruit, herb, vegetable), contaminants and their concentrations tested, contaminant category, whether the plant received additives to assist in phytoremediation, scale of phytoremediation potential (accumulation into tissues; low, moderate, high), commonality in domestic gardens, if contaminants in edible tissues exceeded Australian and New Zealand Food Standards (FSANZ 2016) (where applicable), *in situ* or *ex situ* design, timeline of experiment, country where the study was conducted, reference, and year of publication.

The scale of phytoremediation potential was categorised based on concentrations accumulated into the plant's tissues regardless of contaminant concentrations tested in the soil as these were highly variable. High accumulation in root tissues were considered 'high' because these plants could be candidates for phytostabilisation. 'Low' category plants typically had concentrations in ppb range or below FSANZ (2016) guidelines.

2.2.3 *Statistical Analyses*

Pearson's χ^2 tests for association were conducted to detect differences in the proportions between categorical data. Fisher's Exact Test is reported where < 20% of expected values recorded < 5 (Field 2009). Analyses were performed in IBM SPSS Statistics v. 26 (IBM Corp. 2019) with a statistical significance level of $\alpha = 0.05$.

2.3 Results

79 unique papers published between 2001 and 2020 were collated from research conducted in 28 countries. Of these publications, 70 edible species spanning 25 taxonomic families were reported upon. A summary table of the dataset is presented in Appendix 2.1. Phytoremediation of contaminants from 5 categories were identified including metals and metalloids (n = 55), organic pollutants (n = 9), petroleum hydrocarbons (n = 4), explosives (n = 1), and pharmaceuticals (n = 1). The 6 most speciose families were *Brassicaceae*, *Fabaceae*, *Poaceae*, *Apiaceae* and *Lamiaceae* (Fig. 2.1). Analysis of family data were prevented due to expected values < 5 in categories, violating assumptions of the χ^2 test for association.

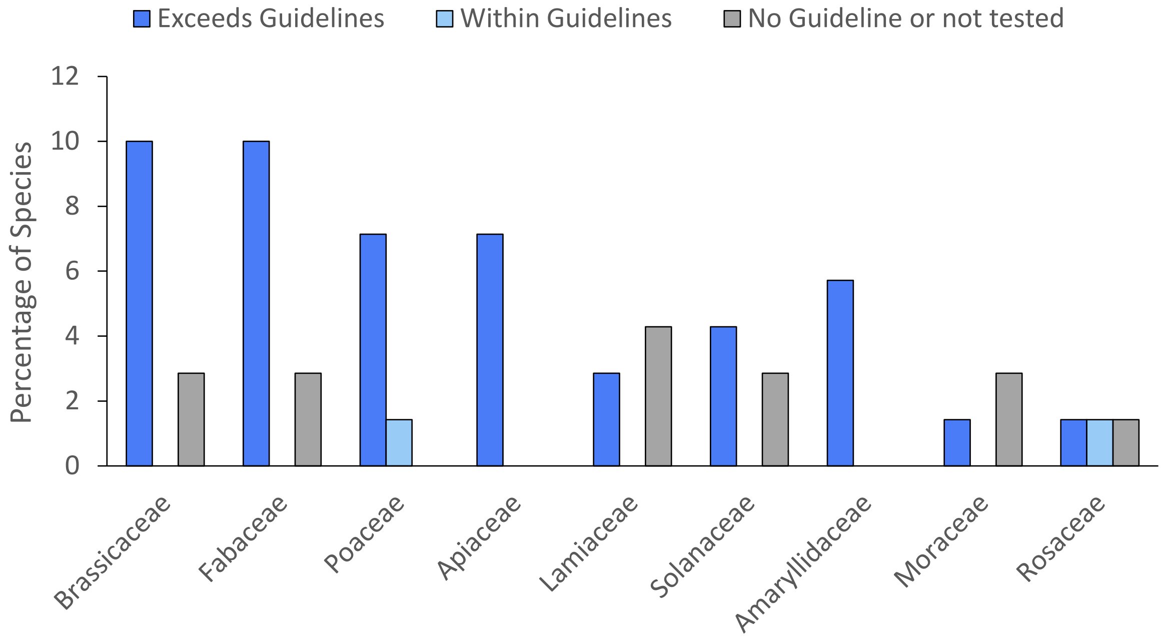


Figure 2.1. Percentage of species by family where contaminants in edible tissues were within or exceeded Australian and New Zealand Food Standards (FSANZ 2016). Chart shows data for the 9 most specious families of 25 recorded in the database.

64% of plants recorded contaminants (predominately heavy metals) in their edible tissues over Australian and New Zealand food standards (FSANZ 2016). 7% did not exceed guidelines and the remaining 29% of species were recorded from studies that did not test edible tissues or tested contaminants that have no reported guideline level. A χ^2 test for association showed a significant difference between the culinary type of edible tissue (i.e., fruit, herb, vegetable and a pooled category of grains, nuts and legumes) and if contaminants were found to exceed guidelines in these tissues ($\chi^2 = 7.778$, 3 d.f., $P = 0.047$, Fisher's Exact Test; Fig. 2.2). However, standardised residuals from these tests were not significant (i.e., < ± 1.96) indicating no particular group was driving this association (Field 2009).

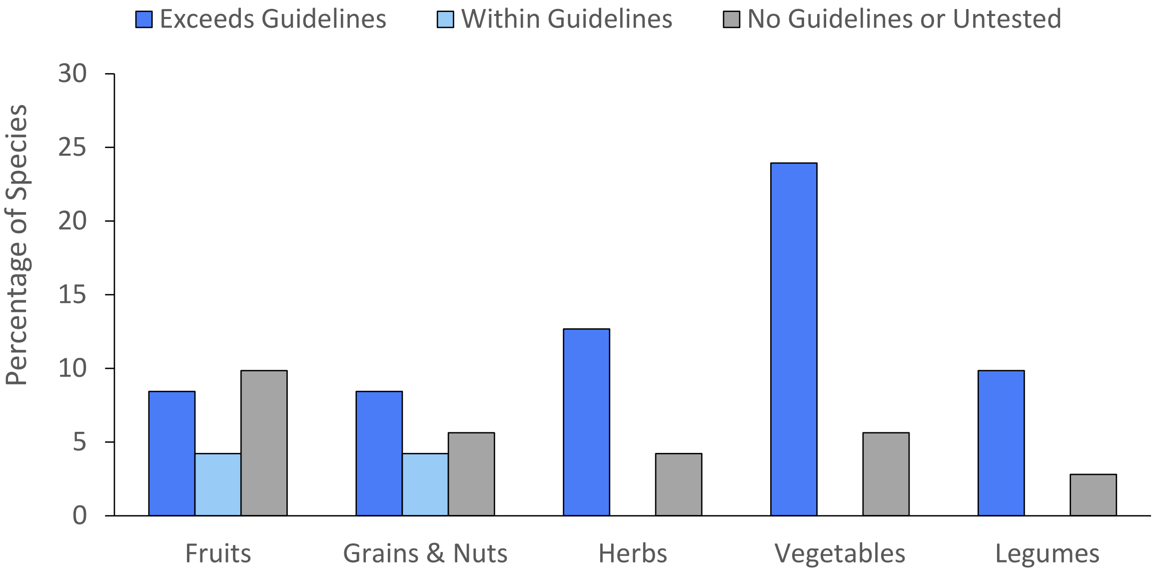


Figure 2.2. Percentage of species by culinary plant type and whether edible tissues recorded contaminants within or over FSANZ (2016) guideline levels. Due to expected values recording < 5, categories 'Within Guidelines' and 'No Guidelines or Untested' were pooled for χ^2 contingency test analysis.

Pearson's χ^2 test found no association between species commonly found in domestic gardens and the presence of contaminants in edible tissues over guidelines ($\chi^2 = 0.259$, 1 d.f., $P = 0.799$; Fig. 2.3).

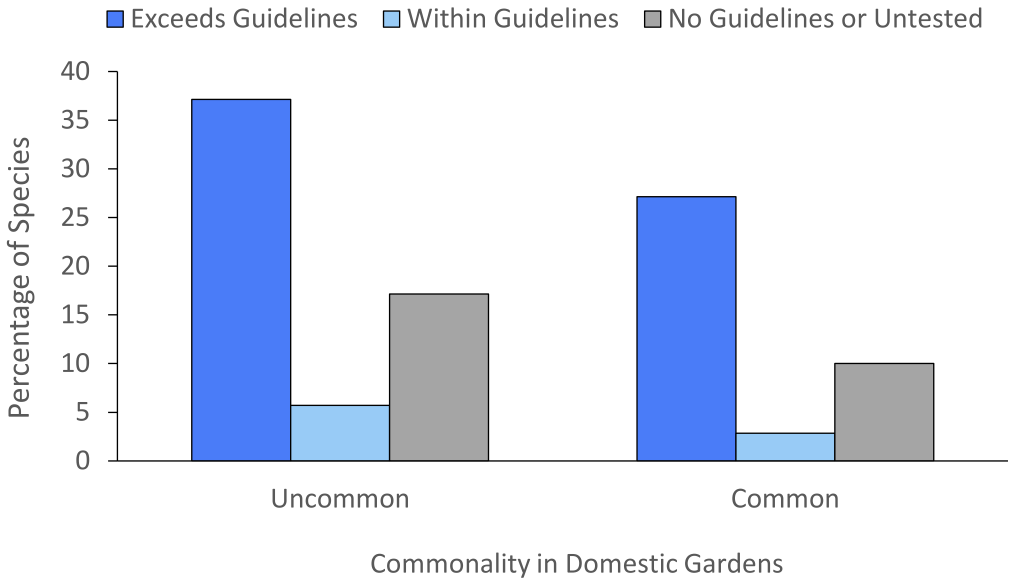


Figure 2.3. Percentage of species commonly grown in domestic gardens and association with edible tissues exceeding FSANZ (2016) guidelines.

A greater proportion of studies were conducted in China and India (*n* = 11 each) followed by America, Pakistan and Spain (*n* = 6 each; Fig. 2.4). China also had the greatest proportion of *in situ* research (Fig. 2.4; 2.5).

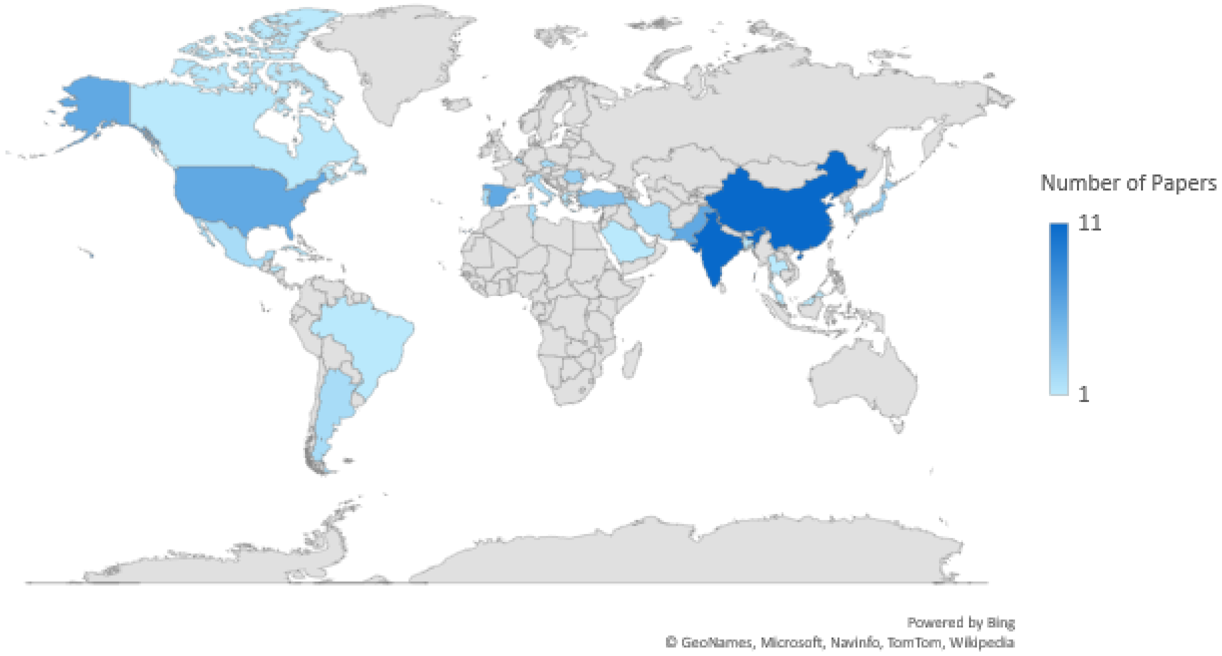


Figure 2.4. Number of phytoremediation papers on edible species by location of study.

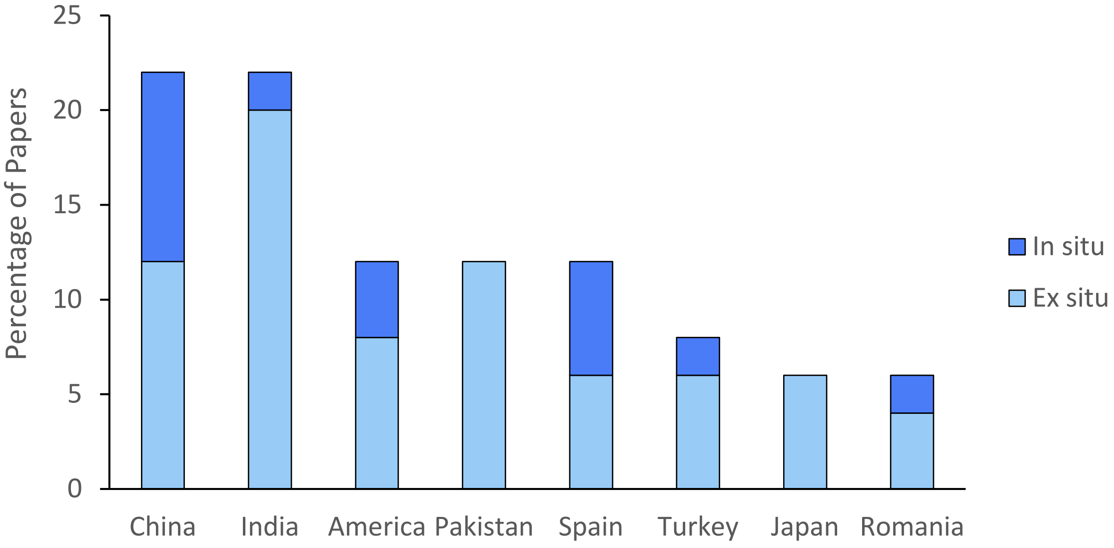


Figure 2.5. Percentage of papers from the top 8 contributors (of a total of 28 countries) of studies by country and proportion of *in situ* and *ex situ* study designs.

No association was found between plants that received treatments aiding phytoremediation (e.g. chelating agents, biochar, or soil amendments) and the scale of contaminant uptake ($\chi^2 = 1.565$, 2 d.f. $P = 0.503$; Fig. 2.6).

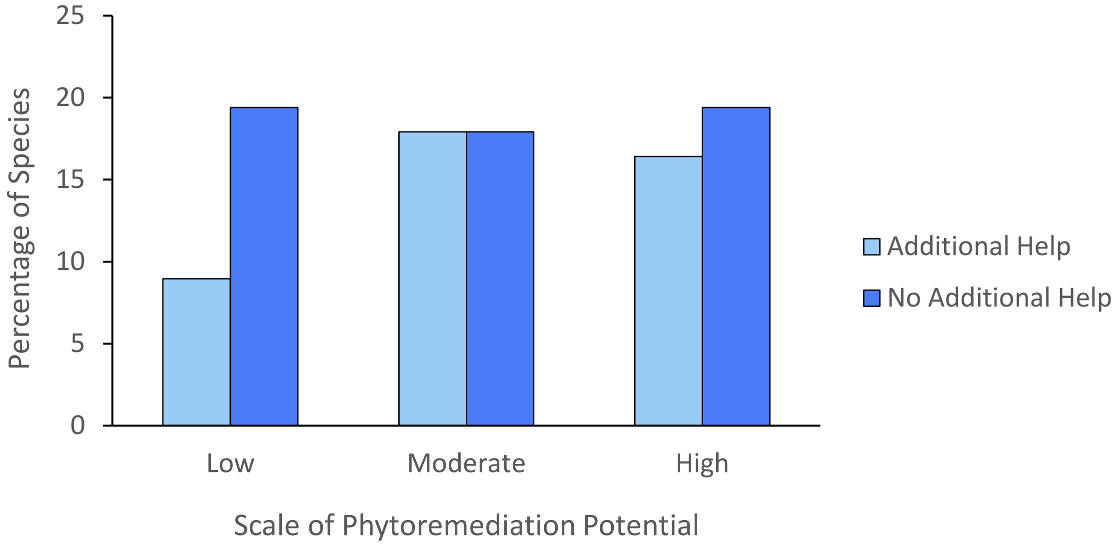


Figure 2.6. Percentage of species categorised as having low, moderate, and high potential for phytoremediation and the application of additional phytoremediation enhancing substances.

2.4 Discussion

A relationship was detected between the type of edible tissue and whether contaminant concentrations exceeded FSANZ (2016) guidelines for foods ($\chi^2 = 7.778$, 3 d.f., $P = 0.047$, Fisher's Exact Test; Fig. 2.2). It should be noted that over a quarter (28.6%) of species reported on could not be categorised due to the absence of guidelines for specific contaminants in Australia, as well as globally. For example, studies focussing on phytoremediation of heavy metals chromium, copper, cobalt, manganese, nickel, thallium, or zinc did not have safe food guidelines recorded in Australia (FSANZ 2016). The European

Union (2006) safety guidelines for contaminants in foods similarly only focus on cadmium, lead, and tin in edible plant products.

The culinary type of edible tissue (i.e., fruit, herb, vegetable) and relationship with contaminant uptake could be inferred from previous studies, identifying leafy vegetables in particular as potential hyperaccumulators (Anwar *et al.* 2016). In their analysis of 268 vegetables from Zhejiang province, Southeast China, Liu *et al.* (2013) found leafy vegetables accumulated greater portions of heavy metals (As, Cd, Cr, Hg, Pb) than root vegetables followed by solanaceous vegetables, and legumes. Chaturvedi *et al.* (2019) also noted that of over 500 known hyperaccumulators, 18% are from the Brassicaceae family. Leafy edibles like Brassicas can grow successfully on agricultural lands where wastewater irrigation is used (Edelstein & Ben-Hur 2018). In these scenarios where contaminants like organic pollutants or heavy metals are in high abundance, they can be accumulated into edible leaves (Edelstein & Ben-Hur 2018) which may decrease their likelihood of being safe for consumption.

These agricultural practices may reflect the greater proportion of studies conducted in China and India where contamination of arable land is cause for ongoing concern for farms and consumer communities in these regions (Liu *et al.* 2013; Kooner *et al.* 2014). The dataset revealed *ex situ* studies, including those that take quantities of field soils to test plants in offsite locations, represented most experimental designs reported in literature (72% *ex situ* vs 28% *in situ*). This is unsurprising given *in situ* experiments are logistically more difficult and costly to set up, monitor, and control.

There were no research papers conducted in Australia or the Oceania region (Fig. 2.4). However, this may be due to search terms limiting the scope of papers to phytoremediation and contaminant accumulation, rather than broader research on contamination and plant ecotoxicity. Tests of domestic chicken eggs and vegetables grown in home gardens near metal smelters in Australia have been conducted previously, revealing elevated levels of contamination in both the plants and eggs, but did not assess of the phytoremediation capacity of the plants (Kachenko & Singh 2006; Grace & Macfarlane 2016).

Species classified as commonly found growing in urban gardens were not associated with contaminant uptake into their edible tissues (Fig. 2.3). Previous phytoremediation databases have focussed on species selection for landscape architecture (Famulari & Witz 2015). Incorporating a food safety warning when applying phytoremediators is an important consideration for practitioners given patterns of accumulation are largely still under investigation. Guidelines for application are complicated where local growing conditions will affect phytoremediation performance. Even without interactions like organic matter composition, temperature, and pH interferences related to soils, there can be high variation in accumulation patterns found in plants from the same species. For example, Soudek *et al.* (2009) found variations of heavy metal accumulation between cultivars of the same species from the *Allium* (Garlic) genus grown under hydroponic conditions possibly due to differing phenolic content.

The dataset found a relatively even proportion of low, moderate, and high phytoremediation potential scaled plants (Fig. 2.6). In terms of research within the field, this is notable as it could indicate that studies where relatively low phytoremediation occurs are still published thereby reducing a publishing bias towards only high performing species.

Furthermore, low accumulating species could be key for application on degraded agricultural land as phytoexcluders that can support food security.

Future studies can address limitations of this study where some food types had to be grouped together for statistical power. For example, a larger sample of vegetable plants could allow for analysis of a greater number of food categories including separate categories for brassicas, root vegetables, herbs, and fruiting vegetables. These would be beneficial in forming recommendations on food safety for specific edible plant types.

2.4.1 Conclusion

This study categorises trends from the literature on phytoremediation potential of edible plants and their associated risk to food safety. A dataset of 70 culinary species spanning 25 taxonomic families was collated and analysed. An association was detected between the culinary type of edible tissue and its corresponding concentration of contaminant in its edible tissue exceeding Australian guideline levels for foods. A research gap of edible plants tested in Australian environmental conditions was identified.

CHAPTER 3

THE EFFECTS OF COPPER, ZINC AND LEAD CONTAMINATION ON THE GERMINATION OF EDIBLE CROP SPECIES

Seeds taken to the brink; that's what the coppers were lead to zinc.

This chapter has been published as: McDonald A. G., Murray B. R., Krix D. W. & Murray M. L. (2021) Complex soil contamination severely impacts seed-sown crop viability in Australia. *Australian Journal of Crop Science* **15,** 531-537.

3.1 Introduction

Land contamination is an ongoing threat to food security around the globe (Wang *et al.* 2005; European Union 2006; Rickson *et al.* 2015; Rojas *et al.* 2016). Heavy metals such as copper, zinc, and lead are of great concern with respect to their worldwide distribution as soil pollutants, detrimental impacts on crop species and subsequent health risks to humans and other organisms exposed to these contaminant pathways (Duruibe *et al.* 2007; Jennings 2013; Kooner *et al.* 2014; Tóth *et al.* 2016). In many instances some of the most arable lands for growing crops for human consumption are those that have also been highly suitable for mining of non-renewable resources (Langkamp 1985; Lechner *et al.* 2016). The contaminant legacies of these mining practices can potentially pose a serious risk to human health if these lands are used for growing edible crops (Alam *et al.* 2003; Roy & McDonald 2015). Unfortunately, farmers in developing nations may have little choice but to use contaminated landscapes and risk food contamination given the climatic, spatial, and socio-economic limitations to landscapes where key food crops can be produced (Xiao *et al.* 2017).

Similar safety concerns are relevant for domestic food gardens, especially in urban cities where edible plants, including crop species, may be grown in positions exposed to soil and airborne pollutants from industrial processes, road traffic, historical use of leaded petrol, and corrosion of building materials (Finster *et al.* 2004; Clark *et al.* 2006; Antisari *et al.* 2015; Rouillon *et al.* 2017). For example, 88% of urban garden soils tested by Clark *et al.* (2006) in Massachusetts, USA, contained lead levels over the reported US EPA threshold of 400 µg g^{-1}. Similarly, in Kano, Nigeria, urban crop species were discovered to contain levels of zinc in vegetables well above the WHO/FAO guideline at that time (Nafiu *et al.* 2011). Recent investigations of the soil lead content of domestic gardens in Australia revealed 40% of participating Sydney homes exceeded the health investigation guideline level for residential soils (Rouillon *et al.* 2017; NEPM 2013). This is important to note as urban communities are increasingly embracing guerrilla gardening and home gardening trends (Iveson 2013; Antisari *et al.* 2015).

Seed germination is a critical life-history stage in plant survival and establishment that can affect later growth, function and plant health (Phillips & Murray 2012; Márquez-García *et al.* 2013; Sánchez-Rendón *et al.* 2017). Understanding the germination requirements of edible crop species in relation to contaminants such as heavy metals in particular is of vital importance considering the widespread problem of complex soil contamination (Sethy & Ghosh 2013; Sánchez-Rendón *et al.* 2017). This is especially important from an agricultural management perspective where specific crop species may be restricted to cultivation in suboptimal landscapes that have a history of heavy metal contamination (García-Gómez *et al.* 2018). In the case of urban gardens, gardeners who direct-seed crops into contaminated soils could unknowingly be experiencing contaminant related inhibition of germination (Xiong 1998). Furthermore, from an ecological perspective, an understanding of the effects of heavy metal pollution on seed germination can help to inform broader knowledge of plant population dynamics, seed dispersal, frugivory and a range of life-history and ecological features (Robertson *et al.* 2006).

The present study investigated the effects of the heavy metals copper, zinc and lead on the germination of seeds of eight edible fruit and vegetable species (Table 3.1). The species span a wide variety of plant genera from six taxonomic families and range from common crop species used in domestic gardens (*Phaseolus vulgaris*, *Raphanus sativus*, *Lactuca sativa*, *Daucus carota*, *Solanum lycopersicum*) to exotic fruit species (*Morus nigra*, *Morus rubra*, *Morus alba* var. *tatarica*). Previous studies investigating germination effects of heavy metals lead and cadmium on common bean (*P. vulgaris*) show that the species is capable of germinating in concentrations up to 500 ppm at the cost of average root length, moisture content and germination rate compared to lower level contaminant exposure (Glasgow 2018). Very low thresholds (0 to 1024 µM) of lead, cadmium, nickel and copper have been found to have no significant effect on germination of radish (*R. sativus*), lettuce (*L. sativa*), carrot (*D. carota*) and tomato (*S. lycopersicum*) (Di Salvatore *et al.* 2008). Limited knowledge exists for seed germination responses of Moraceae species to heavy metal contaminants. With this in mind, the germination responses of these eight species were compared to the presence of copper, zinc and lead using concentrations of these heavy metals reported at degraded sites and current National Environment Protection Measure (NEPM) thresholds for domestic soils (i.e., copper 6,000 ppm, zinc 4,700 ppm, lead 300 ppm).

Copper thresholds of 6,000 ppm have been found in soils at Dolfrwynog Bog, a former copper mine in North Wales, UK (Brewin *et al.* 2007). Putting Australian guidelines into perspective, this figure coincides with the health investigation level (HIL) for residential soils containing homegrown produce in Australia (NEPM 2013). Soils in proximity to areas of car battery salvaging in Denmark were found to contain 6,600 ppm of copper while 4,700 ppm of zinc was associated with activities of scrap metal cutting (Jensen *et al.* 2000). Zinc concentrations of 4,700 ppm have also been found in dredged sediments of historic smelting regions of France (Panfili *et al.* 2005) and were discovered in multi-elemental dust deposition on residential shelves and public building entrances as a result of coke oven use in County Durham, England (Davenport 1953). Recently, lead concentrations over 300 ppm have been detected in 21% of tested Melbourne home vegetable gardens, Australia (Laidlaw *et al.* 2018). Lead at 300 ppm is the current health investigation level for residential soils in Australia (NEPM 2013). Thus, these heavy metal thresholds were chosen for this study because they are high compared to international agricultural and domestic guidelines (Jennings 2013; Rouillon *et al.* 2017) and therefore germination observed in these conditions informs likely success in lower thresholds globally.

Because contamination events are often characterized by a combination of heavy metals within the soil profile (da Rosa *et al.* 2018), the effect of each heavy metal was not only examined on its own, but also compared with germination responses to a combination of all three metals. Combinations of heavy metals have the potential to increase phytotoxicity as plants are subjected to multiple stress responses and higher concentrations of contaminants overall. Important to the predictions tested below, previous work has shown that alteration to plant function and chlorosis effects are more pronounced in species exposed to excess copper compared with zinc (Ivanova *et al.* 2010). In addition, while copper and zinc are both essential elements in trace concentrations, lead has no biological function (Alvarado-López *et al.* 2019). From knowledge to date, germination success of mulberry species in heavy metals copper, lead and zinc has yet to be investigated. Furthermore, seed germination responses to Australian guideline levels of lead have yet to be tested in these crop species. Three predictions were tested: (1) seed germination will be inhibited by the presence of each heavy metal; (2) inhibition of seed germination will be greatest to least in the following order of the three metals: copper > lead > zinc > no contaminant; and (3) a combination of all three metals in soils will have the greatest overall effect on seed germination.

3.2 Materials and Methods

3.2.1 *Study Species*

The 8 species selected for the study (Table 3.1) are all readily accessible crop species and affordable for domestic garden growers. Vegetable species were further selected based on their common occurrence in domestic and urban vegetable gardens. Mulberries (*Morus* spp.) are included in particular because they have demonstrated heavy metal robustness in previous work (Rafati *et al.* 2011) but have yet to be tested at Australian residential guideline levels. Fresh seeds of the fruit species *M. nigra* were purchased from the Australian seed seller OleLantana and Fair Dinkum Seeds, located in Toowomba and Gin Gin, Queensland. Seeds of the other two fruit species (*M. rubra* and *M. alba* var*. tatarica*) were purchased from the USA (TreeSeeds.com). Seeds of the five vegetable species (Table 3.1) were purchased from a local nursery Honeysuckle Garden in Mosman, Sydney.

Table 3.1. Study species used in the experiments to assess seed germination responses to copper, zinc and lead contamination. *Solanum lycopersicum* has been grouped with vegetables commonly grown in domestic gardens because many domestic growers consider it a vegetable crop based on its culinary function (Bergougnoux 2014).

Taxonomic Family	Common name	Scientific Name	Classification	Seed Origin
Apiaceae	Carrot	*Daucus carota* L.	Vegetable	Australia
Asteraceae	Lettuce	*Lactuca sativa* L.	Vegetable	Australia
Brassicaceae	Radish	*Raphanus sativus* L.	Vegetable	Australia
Fabaceae	Common Bean	*Phaseolus vulgaris* L.	Vegetable	Australia
Moraceae	Russian Mulberry	*Morus alba* var. *tatarica* L.	Fruit	USA
Moraceae	Black Mulberry	*Morus nigra* L.	Fruit	Australia
Moraceae	Red Mulberry	*Morus rubra* L.	Fruit	USA
Solanaceae	Tomato	*Solanum lycopersicum* L.	Vegetable	Australia

3.2.2 *Experimental Design and Procedure*

To determine the impacts of heavy metal contamination on seed germination experiments targeted each heavy metal on its own as well as including a treatment that represented a combination of all three heavy metals. Experimental treatments for seed germination consisted of agar media in standard Petri dishes spiked with either (i) copper sulphate ($CuSO_4$) (Anhydrous form, LR grade, 98% purity, Chem-Supply Pty Ltd, Australia), (ii) zinc nitrate ($Zn(NO_3)_2.6H_2O$) (Hexahydrate form, AR grade, 99% purity, Chem-Supply Pty Ltd, Australia), (iii) lead nitrate ($Pb(NO_3)_2$) (AR grade, 99% purity, Chem-Supply Pty Ltd, Australia) or (iv) a combination of all three heavy metals. In addition, an uncontaminated agar control set of Petri dishes was included. There were three replicate Petri dishes for each experimental treatment per species, with 10 seeds placed in each dish. Final agar concentrations of copper (6,000 mg/kg), zinc (4,700 mg/kg), and lead (300 mg/kg) were derived from levels previously detected at degraded sites and current Australian residential soil guidelines based on total mass (NEPM 2013). The combination treatment was spiked with copper 6,000 ppm, zinc 4,700 ppm, and lead 300 ppm.

Agar was made in 500 ml batches using a temperature-controlled heat pad and thermocouple (VELP AREC.X, Italy) and 5 g of bacteriological grade (LR) agar powder (Chem-Supply Pty Ltd, Australia) measured using analytical scales (Sartorius A-120S analytic, Australia). Contaminant masses were determined by calculating the relative atomic mass of each compound's constituents to find the proportion of the target contaminant. The desired concentration was divided by this proportion to obtain the amount required in grams. These equated to 10.6925 g of zinc nitrate, 7.5350 g of copper sulphate and 0.2398 g of lead nitrate per 500 ml of agar. The heat pad was maintained below boiling point at 90°C.

Seeds were vacuum cleaned in a vacuum filtering flask with a sidearm hose and loosely fitted cork prior to germination experiments. A single drop of detergent and 1 ml of bleach were added to 200 ml of milli-Q water in the flask. Species groups of seeds were vacuum cleaned for 15 to 20 minutes. The seeds were drained into a pre-autoclaved tea strainer and rinsed lightly with ethanol (undenatured AR grade, 99.5% purity, Chem-Supply Pty Ltd, Australia). Petri dishes were made up in a class II biological safety cabinet (Gelaire Pty Ltd, Australia, SFT 212 DTT model) to reduce contamination from laboratory air. Dishes were double bagged in plastic ziplocks to prevent contamination and moisture loss and then positioned on benches in the glasshouse at The University of Technology Sydney where they received natural light and dark cycles for the duration of the experiment. Cycling of light and dark conditions has previously proved beneficial for seed response compared to single light level exposure (Aamlid & Arntsen 1998).

Observations of germination were recorded twice a week for nine weeks. Germination was defined as the point where the radicle visibly emerged from the seed coating. Seeds that had not germinated by completion of the experiment were monitored until a state of decomposition was reached where there was no further possibility of germination (Phillips & Murray 2012).

3.2.3 *Statistical Analyses*

An ANOVA approach via the implementation of separate generalised linear models (Crawley 2012) was used to determine the impacts of heavy metal contamination on seed germination. The response variable in each model was either germination onset (the time taken for seeds to begin to germinate), germination duration (the period of time over which seeds germinated) or total germination (the total proportion of seeds that germinated). There were two categorical explanatory variables in all models which included heavy metal treatment and species. Treatment had five levels including each of the three individual contaminants, the combined treatment with all contaminants and the uncontaminated control group. Species had eight levels with one for each species. The treatment x species interaction was included in all models, with a significant interaction in models demonstrating variation among species in the type of effects of the heavy metals. Mean values of the three replicates per species were used in the models. Because the data were in the form of counts, a Poisson error structure with a logit link was used for germination onset and germination duration generalised linear models. A quasibinomial model was used to account for overdispersion in the total germination model. Significant ANOVA results were followed by post-hoc pairwise tests (with P value adjustment for multiple testing; Benjamini & Hochberg 1995) to determine significant contrasts between heavy metal treatments and the uncontaminated control. All statistical analyses were performed using R statistical software (R core team 2019) through R Studio (Version 3.5.1) and the 'emmeans' package post-hoc tests (Lenth 2019).

3.3 Results

3.3.1 *Germination inhibition*

Seed germination was completely inhibited by the combined heavy metal treatment in seven of the eight study species including bean, lettuce, the three mulberry species, radish and tomato (Fig. 3.3). Complete germination inhibition was also found in lettuce in response to the copper and zinc treatments, no germination in bean and black mulberry in response to copper and no germination in red mulberry to zinc (Fig. 3.3).

3.3.2 *Germination onset*

There was a significant treatment x species interaction for germination onset (χ^2 = 39.30, 16 d.f, P = 0.001) with significant treatment (χ^2 = 272.40, 4 d.f., P < 0.0001) and species (χ^2 = 435.06, 7 d.f., P < 0.0001) effects. Results found carrot germination onset was most heavily impacted by heavy metal contamination, with significant delays in germination in response to copper, zinc, and the combined heavy metal treatment (Fig. 3.1). In addition, the effect of zinc was to significantly delay germination in both black mulberry and Russian mulberry, while copper significantly delayed germination in Russian mulberry and radish (Fig. 3.1).

3.3.3 *Germination duration*

A significant treatment x species interaction emerged for germination duration (χ^2 = 128.60, 16 d.f., $P < 0.0001$) with significant treatment (χ^2 = 17.35, 4 d.f., $P < 0.0001$) and species (χ^2 = 200.27, 7 d.f., $P < 0.0001$) effects. Germination duration was significantly reduced in red mulberry by copper and lead and in Russian mulberry by copper and zinc (Fig. 3.2). In contrast, germination duration was significantly extended in carrot by zinc and lead (Fig. 3.2).

3.3.4 *Total germination*

There was a significant treatment x species interaction for total germination (χ^2 = 114.19, 28 d.f., $P < 0.0001$) with significant treatment (χ^2 = 513.94, 4 d.f., $P < 0.0001$) and species (χ^2 = 334.82, 7 d.f., $P < 0.0001$) effects. Total germination was significantly reduced in carrot by each of the copper, zinc and lead treatments as well as the combined treatment (Fig. 3.3). The zinc treatment significantly reduced the number of seeds germinating in bean, Russian mulberry, radish and tomato (Fig. 3.3). The copper treatment significantly reduced seed germination in Russian mulberry and radish (Fig. 3.3).

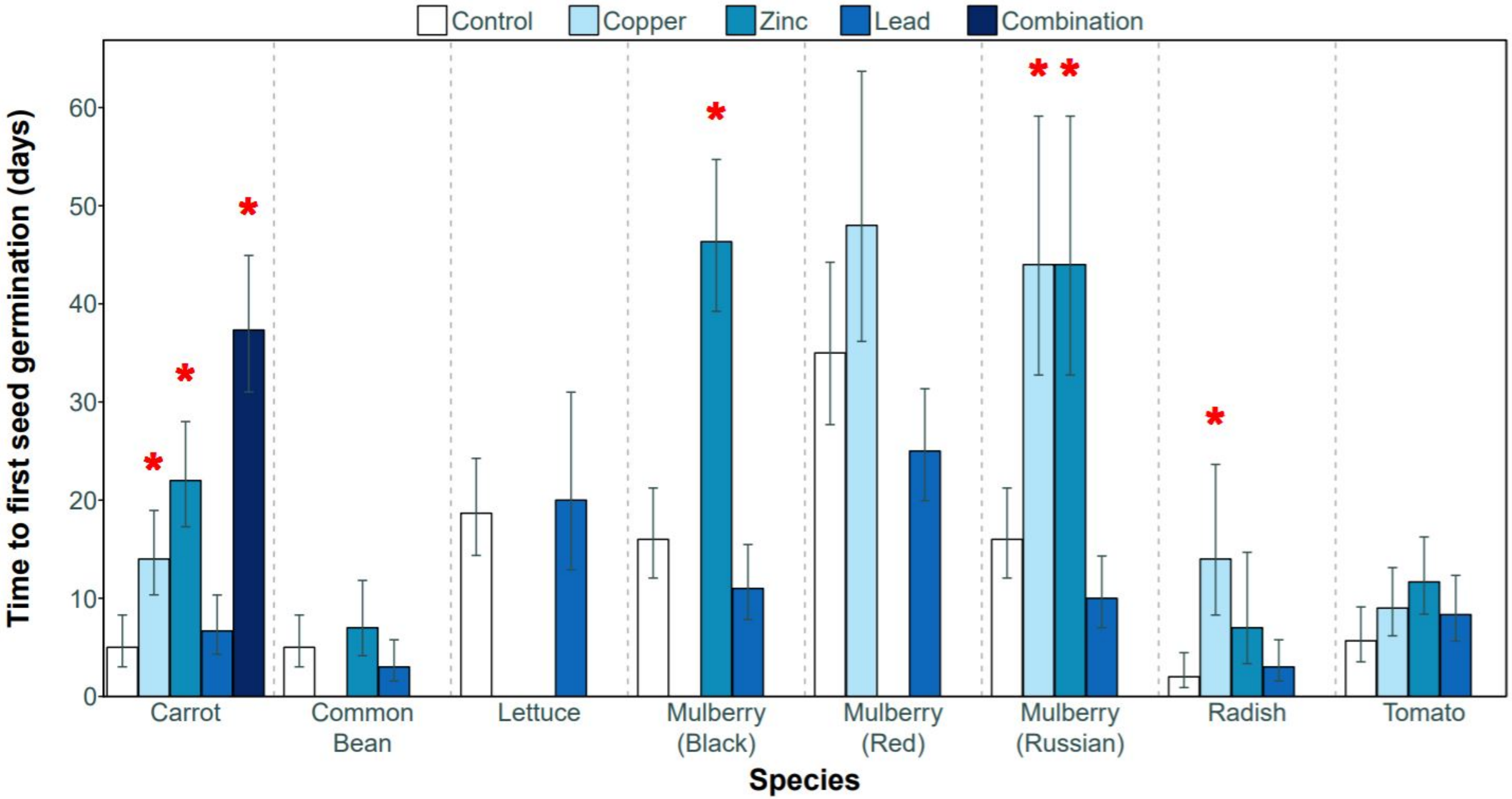


Figure 3.1. Germination onset across the eight study species in relation to the heavy metal treatments (mean + SE). An asterisk shows those treatments that differed significantly ($P < 0.05$) from the uncontaminated control within each species.

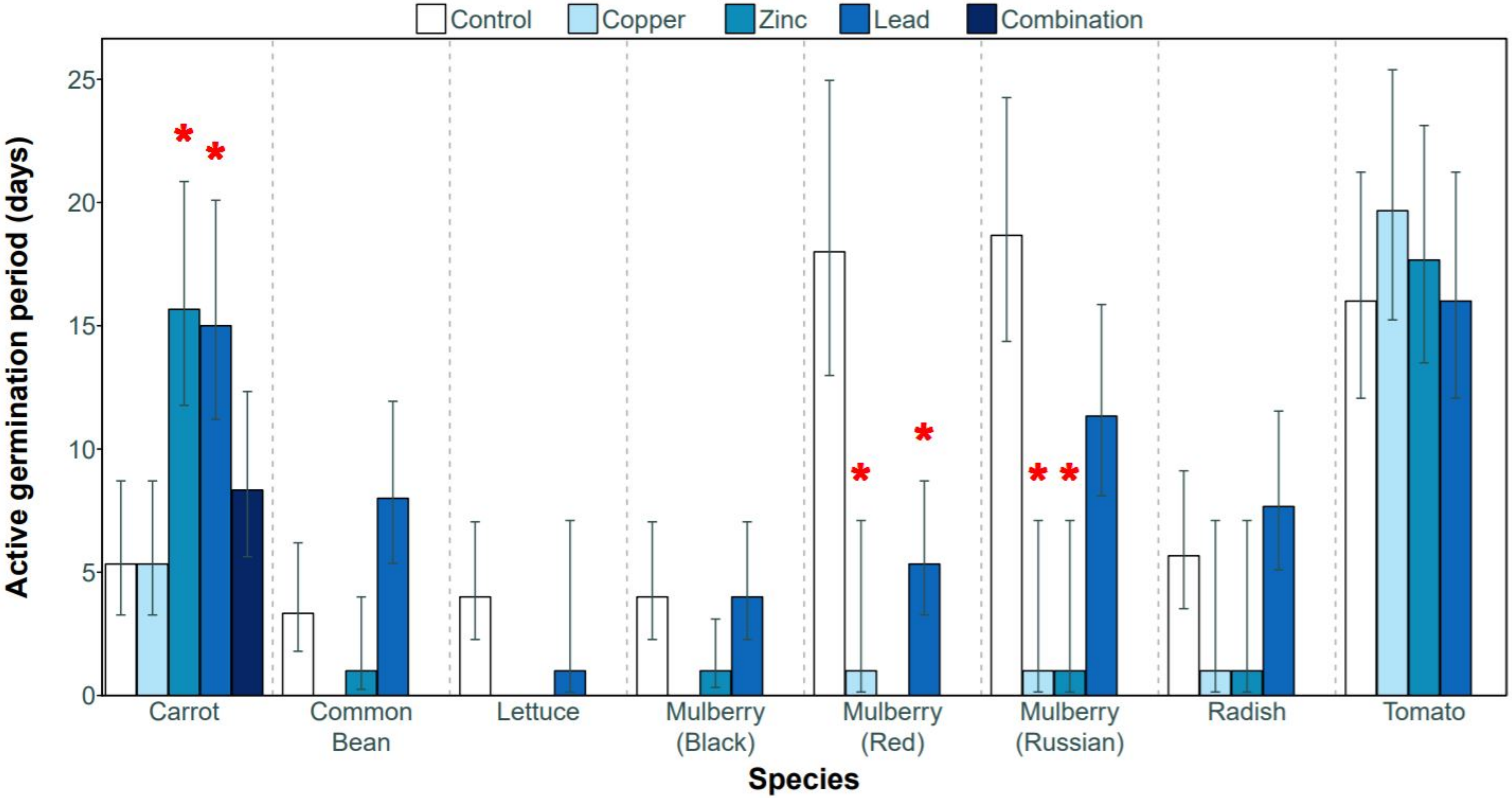


Figure 3.2. Germination duration across the eight study species in relation to the heavy metal treatments (mean + SE). An asterisk shows those treatments that differed significantly ($P < 0.05$) from the uncontaminated control within each species.

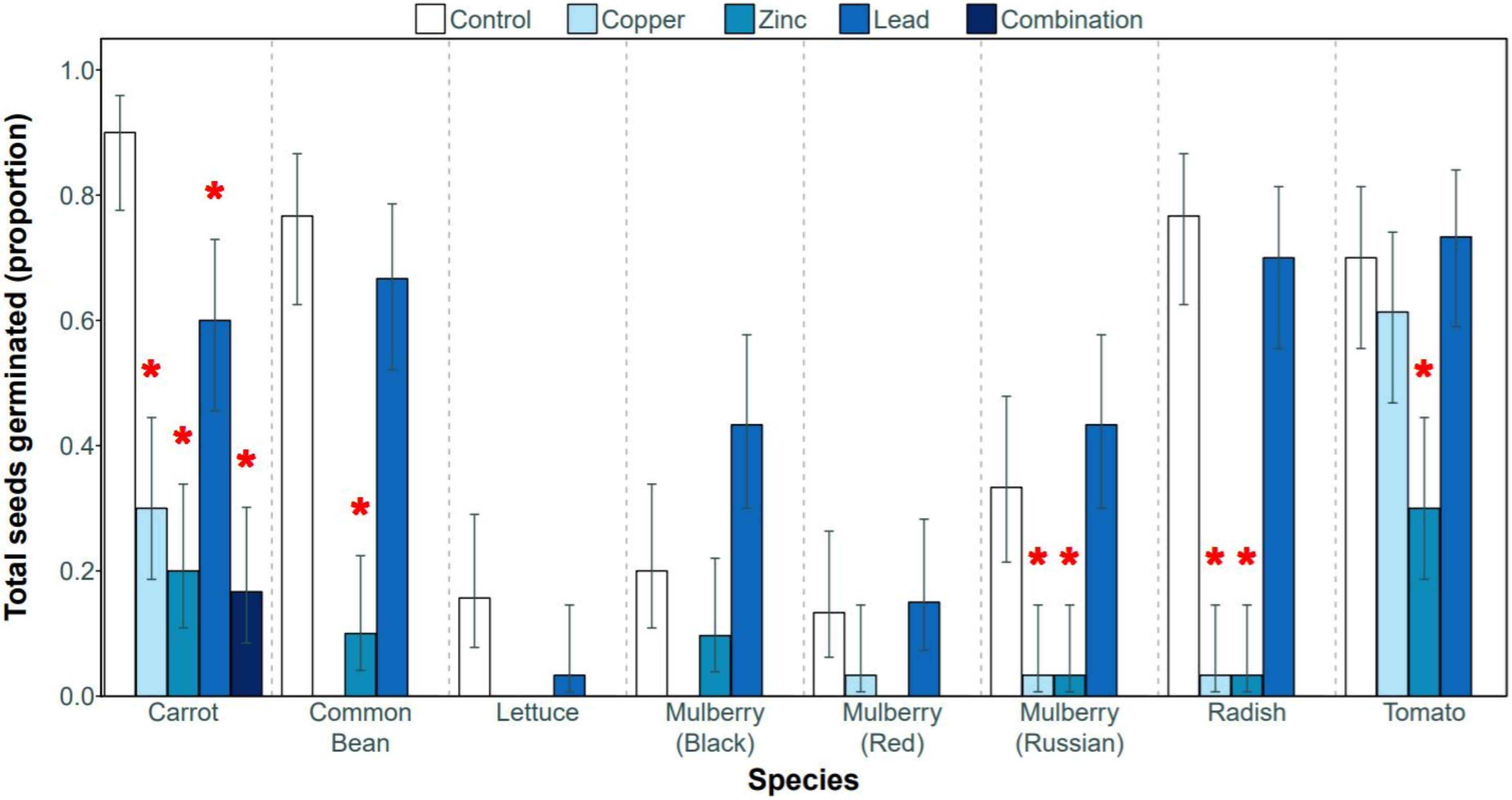


Figure 3.3. Total germination across the eight study species in relation to the heavy metal treatments (mean + SE). An asterisk shows those treatments that differed significantly (P < 0.05) from the uncontaminated control within each species.

3.4 Discussion

Germination inhibition from heavy metal salts can manifest in a variety of physiological and biochemical stressors including instability to membranes, genomic structure, enzyme activity, oxidative stress, as well as metabolism and nutrient loss (Sethy & Ghosh 2013). It follows that the greatest occurrence of germination inhibition arose in the combination treatment where seeds were exposed to the highest toxicity potential. This treatment also had a higher concentration of both sulphate and nitrate salts that can induce salinity related osmotic or ionic stressors contributing to overall toxicity (Manzoor *et al.* 2017). However, enrichment with nitrate salts has also previously been linked to promoting germination success (Hendricks & Taylorson 1974). Relevant to the context of this study, instances of heavy metal contamination events are related to increased soil salinity and associated adverse effects on plants (Byeong *et al.* 2011; Sethy & Ghosh 2013).

This study found that individual crop species had statistically different germination responses to each heavy metal treatment with overall patterns not consistently predictable by treatment or species groups. Smaller group patterns indicate germination inhibition from heavy metals with potential for further studies to investigate specific thresholds of inhibition for these species using greater seed quantities. For example, the three species from taxonomic family Moraceae recorded a delay in onset of germination in copper (Russian mulberry and red mulberry) and zinc treatments (Russian mulberry and black mulberry). This taxonomic family took approximately twice as long as the other species to germinate in copper and zinc treatments with very little overall germination recorded in these treatments if at all (Fig. 3.1; Fig. 3.3). Comparing these results to control dishes could suggest a sensitivity of these species to copper and zinc heavy metal pollution however a greater sample would be required to confirm this.

Mulberries (*Morus* spp.) have a wide range of uses including a pivotal role in supporting economically and culturally significant silk trades across Asia (Maji *et al.* 2005; Sánchez-Rendón *et al.* 2017). The species boasts antioxidant rich fruits and numerous traditional Chinese remedies from improving eyesight to treating premature grey hair (Zhang *et al.* 2018). Aside from its acclaimed health benefits it has also demonstrated robustness to heavy metal contamination and in some cases phytoremediation abilities (Olson & Fletcher 1999; Rezek *et al.* 2009). Recent research has reported that a mulberry plant's sex can play a role in its tolerance to multi-contaminant effects. It was found that lead induced stress on white mulberry (*M. alba*) saplings is reduced in the presence of zinc with this reduction observed further in female saplings compared to males (Qin *et al.* 2018). This multi-contaminant effect has yet to be investigated on germination of mulberry seeds and could be a further line of enquiry for much lower heavy metal concentrations than those used in this study.

Carrot and tomato had the most success across treatments with carrot demonstrating to be the only species able to germinate under combination treatments (Fig. 3.3). As a root vegetable capable of both assisted (Alvarado-López *et al.* 2019) and non-assisted (Roy & McDonald 2015) lead accumulation, this has implications for crop growers and domestic food safety as carrots will tolerate germinating in contaminated soils where metals can compartmentalise into their edible tissues.

Radishes and common beans germinated within days in control dishes but were stunted in copper and combination treatments in line with earlier predictions. It is well documented that excess copper induces stress related growth inhibition in plants (Lequeux *et al.* 2010; Feigl *et al.* 2013; Nair & Chung 2015). Copper related germination suppression was experienced across all species except for tomato where copper treatments did not differ from control treatments. In their investigation of vegetable seed germination and root elongation, Di Salvatore *et al.* (2008) tested seeds in comparatively low concentrations (0 to 1024 µM) of Cd, Pb, Ni and Cu finding that there was no significant effect of metals on germination. However, their results for root elongation tests noted that tomatoes were more sensitive to copper than radishes, despite no significant effect to germination success. The sensitivity of lettuce was replicated in this experiment, but Di Salvatore *et al.* (2008) found that a low dose of copper (2 µM) increased lettuce root growth where this amount is likely to be beneficial as an essential nutrient. Finally, the authors discuss the resistance of radishes in their root elongation tests to lead treatments. Radishes may exhibit greater resistance to low lead and copper exposure but transition to mechanisms of tolerance in higher concentrations (Gadd 1993; McLaughlin *et al.* 2000). Future studies could explore this comparison further with the inclusion of root elongation tests of radishes in higher doses of lead contamination.

Australian residential guideline levels of copper and lead were tested in this experiment as a conservative approach to evaluating germination inhibition in heavy metal contaminated soils. However, it is important to note that these levels are based on total dry weight concentrations (mg/kg) (NEPM 2013). The levels include leeway for non-bioavailable quantities of each metal that are inevitably present in soils as some metal constituents are immobilised into safer forms when they bind to matter present in the soil (Gadd 1993). Furthermore, agar media suspends seeds in concentrations where entire seed coats are subjected to the target concentration contributing to higher surface area potential for absorption and therefore toxicity than what would occur in soils (Di Salvatore *et al.* 2008). Testing seeds for final heavy metal concentrations post-experiment would assist in confirming exact concentrations species were subjected to. Additionally, future work could further investigate the effects of anions by comparing nitrates and sulphates in control treatments. The germination patterns from the edible species tested in this study are noteworthy as they indicate that different groups of species respond with their own mechanisms to concentrated levels of heavy metal pollution in their environments.

3.4.1 Conclusion

This study investigated seed response of eight edible crop species to five levels of heavy metal treatments. Species and treatment groups did not consistently predict rates of germination across all species. However, a combination of copper, zinc and lead greatly inhibited germination with only one crop (i.e., carrot) able to germinate under these conditions. It is suggested that carrots pose the highest risk to crop growers who direct-seed this species into complex, multi-metal contaminated sites.

CHAPTER 4

LEAD ACCUMULATION IN THE TISSUES OF EDIBLE CROP SPECIES

*Lead*ible Crops in Spiked Pots

4.1 What Pb us here?

Australian studies of heavy metal content in soils routinely highlight lead as a problem pollutant due to its toxicity and abundance. Rouillon *et al.* (2017) discuss that content of lead in Sydney home vegetable garden soils is linked to house age, dripline location and distance from the CBD. A similar study of Melbourne community garden soils found 8% of community gardens tested and 21% of residential gardens tested contained lead over 300 ppm (Laidlaw *et al.* 2018). Furthermore, Kandic *et al.* (2019) found that lead content in vegetable garden soils of greater Melbourne were directly related to house characteristics and distance to arterial roads.

In their review of lead contamination of inner-city soils, Laidlaw & Taylor (2011) discuss sources and history of lead pollution in inner cities of the United States and Australia. They note that lead in urban inner cities of Australia is highly bioavailable and that this bioavailability is likely correlated with higher total concentrations (Murray *et al.* 2009). Where heavy metals are bioavailable, there is a greater likelihood for uptake into plants (Kennen & Kirkwood 2015). The growing body of evidence of heavy metal contamination in urban garden soils is raising concerns of food safety for urban growers (Izquierdo *et al.* 2015).

Unlike other heavy metals, lead has no biological function in plants, animals, and humans. In spiked concentrations, it is known to cause chronic illnesses like liver and kidney damage, as well as serious neurotoxic effects including developmental delay in children who are most at risk (Laidlaw & Taylor 2011). In plants, lead can disrupt membrane function, respiration, transpiration, inhibit enzymes, and cause chlorosis (Pulkownik 2000; Edelstein & Ben-Hur 2018).

Previous studies have investigated phytoremediation and plant tissue allocation of lead (de Souza *et al.* 2012) however distinction between young and old tissues in addition to upper and lower plant biomass is limited. Rafati *et al.* (2011) found that fallen leaves compared to green leaves of white poplar *(Populus alba)* and white mulberry *(Morus alba)* had higher concentrations of cadmium and chromium, and chromium and nickel, respectively. Elevated concentrations in fallen leaves can indicate a metal-stress coping mechanism in plants that strategically accumulate heavy metals into extremity tissues where there could be a lower chance of biochemical damage to occur (Lintern *et al.* 2013). Upper, middle, and lower leaves in tobacco *(Nicotiana tabacum)* were found to have statistically different accumulation of cadmium, lead, and zinc where concentrations were ordered by upper leaves < middle leaves < lower leaves (Yang *et al.* 2019). A difference in old, new, upper and lower plant leaf tissue accumulation patterns of heavy metals may extend to other tissues like edible fruits. Testing fruit accumulation in relation to biomass position or time of harvest could inform risks to food safety of homegrown plants cultivated in contaminated urban soils. Conversely, crop species that reduce metal load in edible portions by preferentially translocating to non-edible leaf or stem tissues provides potential for concurrent soil remediation and food sources.

The present study investigates lead uptake and plant tissue compartmentalisation in five common crop species; carrots *(Daucus carota)*, tomatoes *(Solanum lycopersicum),* radishes *(Raphanus sativus)*, common beans *(Phaseolus vulgaris)* and chilli peppers (*Capsicum annuum*). My aims were to (1) investigate edible tissue risks to food safety in common crop species grown in the Australian residential soil guideline level for lead, 300 ppm (NEPM 2013), (2) identify lead accumulation patterns in upper and lower leaf, stem and fruit biomass of tomato and chilli pepper plants, and (3) compare accumulation of lead in young, fallen leaf tissues and mature leaf tissues of common beans.

4.2 Materials and Methods

4.2.1 *Glasshouse Setup*

Five crop species were selected based on their common occurrence in domestic and urban gardens (Table 4.1). Fresh seeds of carrots *(D. carota)*, tomatoes *(S. lycopersicum),* radishes *(R. sativus)*, common beans *(P. vulgaris)* and chilli peppers (*C. annuum*) were purchased from Honeysuckle Garden in Mosman, Sydney. Potting mix (Osmocote Professional Premium Plus) was purchased from Bunnings Warehouse, Chatswood. A total of 98 plants were cultivated in individual pots in the glasshouse at The University of Technology, Sydney where species replicates were positioned randomly across glasshouse benches. Plants were established over a 5-week period to allow for fruit growth from the 18th of June 2019 before individual pots were spiked to a soil concentration of 300 ppm using a lead nitrate solution on the 12th of September 2019. The target contamination level was derived from the current Australian soil residential guideline level outlined by the National Environment Protection Measure (NEPM 2013).

Table 4.1. Study species, harvest dates, and number of replicates by species' family.

Family	**Scientific name**	**Common name**	**No. of Spiked Plants**	**No. of Control Plants**	**Date Harvested**
Apiaceae	*Daucus carota*	Carrot	13	5	07/11/2019
Brassicaceae	*Raphanus sativus*	Radish	13	5	10/10/2019
Fabaceae	*Phaseolus vulgaris*	Common Bean	16	4	30/09/2019
Solanaceae	*Capsicum annuum*	Chilli Pepper	15	6	11/11/2019
Solanaceae	*Solanum lycopersicum*	Tomato	15	6	06/11/2019 *Early Fruits:* 17/10/2019

As the potting mix contained large bark components that had the potential to adsorb heavy metals and reduce target concentrations, the soil was sieved using a large 5 mm plastic sieve prior to potting. Soil volume was filled to a height of ~ 10.5 cm in the pots where air filled porosity for fine potting mix was calculated as ~ 15% following potting mix comparisons made by Passioura (2006). This placed soil aeration levels safely over the hypoxia risk threshold of 10% seen in pot experiments using finer soils where root structures can be altered by air related abiotic stress (da Silva *et al.* 1994; Passioura 2006). Similarly, uniform, red coloured pots were selected to reduce temperature fluctuations typical of darker pots in glasshouses and associated changes to root growth and soil organism compositions (Passioura 2006). Soil density was calculated as 515.3 g/L by taking the average density of 3 pots filled to a uniform height.

The lead nitrate solution was made gravimetrically using 24.7143g of $(Pb(NO_3)_2$ salt (AR grade, 99% purity, Chem-Supply Pty Ltd, Australia) and milli-Q water in a 1L volumetric flask. Individual pots were spiked on the 12th of September 2019 by carefully dispensing

10mls of lead nitrate solution evenly across the surface of the soil. Control plants of each species remained uncontaminated.

4.2.2 *Plants and Soils - Laboratory Processing*

Plants were separated into 666 paper bags containing tissue sections (i.e., roots, stems, leaves, edible tissues/fruits, flowers) or soils, and all were oven dried in the laboratory at The University of Technology Sydney at 80℃ for at least 48 hours. Below-ground tissue sections of root vegetables were separated into both stringy roots and the edible portion of the plant.

Due to successful fruiting and a greater rate of senescence, common beans were harvested first on the 30th of September 2019. Where sufficient biomass was available, leaf tissues of common beans were further separated into brown leaves (i.e., leaves that were near to or had been dropped by the plant) and green leaves. Radish species were harvested on the 10th of October 2019 after going to seed. This species was also harvested earlier to mitigate plant health effects from an establishing infestation of mites and aphids.

Chilli pepper and tomato plant leaves, stems and fruit tissues were separated into upper plant and lower plant where upper tissues were harvested from the upper half of the above-ground biomass. Fruits of tomato species were further separated into ‘new’ and ‘old’ fruits where ‘old’ fruits were harvested approximately one month earlier on the 17th of October 2019 and ‘new’ fruits were harvested with the rest of the plant on the 6th of September 2019.

Soil samples were sieved using a 2mm sieve and thicker plant tissue samples were chopped with stainless steel secateurs into smaller digestible chunks. Analytical scales (Sartorius A-120S analytic, Australia) were used to record the total dry weight and 1-gram sub-samples were weighed into 50ml polypropylene digestion tubes (Enviro Express, USA). Samples were hot block digested in nitric acid (70% HNO_3 AR grade, Rowe Scientific Pty Ltd, Australia) and hydrogen peroxide (30% H_2O_2 AR grade, Rowe Scientific Pty Ltd) as per EPA method 3050B (U.S. EPA 1996). Where only 0.5 grams of dried plant sample was available, a half digestion was performed. Samples were filtered through a 45 µm luer lock syringe filter (Minisart®, Sartorius AG, Germany) with milli-Q water into 15ml tubes with a final acid concentration of 1%. All digested, filtered, and diluted samples were finally analysed using Microwave Plasma Atomic Emission Spectroscopy (MP-AES 4210, Agilent Technologies, USA) for lead (Pb) (i.e., MP-AES wavelength 405.781 nm; correlation coefficient across analyses ranged between $R^2 = 0.99992$ and $R^2 = 0.99998$). Values were reported as the mean of 4 replicate readings. Data were collected in ppb and back-calculated using recorded subsample and filtering masses to obtain the original sample concentration in ppm.

Quality controls (QC) for each matrix type (i.e., tissue type) were measured by spiking a known concentration of lead in samples and measuring percentage recovery. QCs were within acceptable limits measuring 100% ± 3% for most tissue types and within 100 ± 10% for stem tissues. The MP-AES was calibrated every 35 samples with standards made using single elemental lead standard (ACROS Organics, Belgium) prepared to the same acid concentration as samples (1% v/v HNO_3). Certified reference materials (CRMs Pine needles for plants, and Loam Soil Level D, Choice Analytical, Australia), check standards (used top and tail of every recalibration), procedural blanks, duplicate machine readings across analyses, and intermittent duplicate subsampling of plant sections were used throughout analyses to determine percentage metal recovery, assess procedural contamination, and ensure the accuracy of all experimental data.

4.2.3 *Statistical Analyses*

To test for differences in accumulation among species and plant tissues, a general linear model (LM) was fitted with terms for species (fixed categorical factor with five levels), tissue type (fixed categorical factor with four levels: edible tissue, root, stem or leaf), and a species x tissue type interaction term. The inclusion of the interaction term was used to test whether patterns of accumulation within tissue types were similar across species. Following this, two further models were fitted to explore the degree to which species might accumulate metals in edible tissues. The first (LM) using the values for accumulation of lead, and the second a binomial model where samples were given a value of one in cases where they exceeded Australian Food Standards for lead in non-brassica vegetables (> 0.1 ppm; FSANZ 2016) and zero where they did not. Both models used species as the sole predictor (fixed categorical factor). Radishes and carrots were excluded from the binomial model as all samples exceeded Australian Food Standards for lead in vegetables thereby generating a complete separation (where the model is unable to estimate the parameter value or its standard error).

For the two species where tissue samples were taken from different locations on the plant (chilli pepper and tomato) a linear mixed model (LMM) was employed to test for differences between the species (fixed categorical term with two levels), if any effect of tissue location on the plant was detectable (fixed categorical term with two levels: upper and lower), and variation associated with tissue type (fixed categorical term with three levels: stem, leaf and fruit). Two-way interaction terms for species x tissue location, species x tissue type, tissue type x tissue location, and the three-way species x tissue location x tissue type were included to test the consistency of patterns among levels of the three predictors. As multiple measures were taken from individual plants, a random effect term was included using the plant identification number, to account for any intraindividual variation in lead accumulation of individual plants. A final model (LMM), comparing the lead accumulation of brown and green leaves, was fitted to data collected from the bean plants, using lead concentration as the response, and leaf colour as the predictor (fixed categorical factor with two levels: brown and green), with a random effect of replicate plant.

For all models, ANOVA tables were generated to assess the significance of the effects included in the models. Full results for all models can be found in Appendix 4.1. Where significant effects for factors with more than two levels, or significant interactions were found, post-hoc tests were performed to clarify which factor levels generated the significant results. Data for the first three models (i.e., where tissue location, or leaf colour was not considered) used mean values calculated for each replicate plant species and tissue type. Mean values for tissue type within tissue location were calculated for chilli pepper and tomato for all replicate plants. Similarly, mean values for green and brown leaves on common bean plants were used where this distinction between leaf colour was available during sample collection. Prior to analysis, lead concentration values were 5th root transformed, and values below detection limits (< 0.001 ppm) replaced with zeros. Analysis was conducted in R statistical software (R core team 2019), using the packages ‘car’ (Fox & Weisberg 2019), ‘emmeans’ (Lenth 2020), ‘lme4’ (Bates *et al.* 2015), ‘lmerTest’ (Kuznetsova *et al.* 2017) and ‘beeswarm’ (Eklund 2016).

4.2.4 *Phytoremediation Index Calculation*

A bioconcentration factor (BCF_{leaf}) was calculated to assess the extent of lead translocation from soils into leaf tissues where a value > 1 is indicative of hyperaccumulation (Buscaroli 2017). This was calculated as:

$$BCF_{leaf} = C_{leaf} / C_{soil}$$

where C_{leaf} represents the total metal concentration in leaves (ppm) and C_{soil} represents the total metal concentration in the soil (ppm).

A second bioconcentration factor (BCF_{root}) was calculated to assess lead accumulation into roots where a value > 1 indicates phytostabilisation potential. This was calculated as:

$$BCF_{root} = C_{root} / C_{soil}$$

where C_{root} represents the total metal concentration in roots (ppm) and C_{soil} represents the total metal concentration in the soil (ppm).

An adapted translocation factor (TF_{fruit}) was calculated to estimate the ability of a plant to translocate metals from roots to edible tissues. TF was calculated as:

$$TF_{fruit} = C_{fruit} / C_{root}$$

where C_{fruit} represents the total metal concentration in fruits (ppm) and C_{root} represents the total metal concentration in the root (ppm).

4.3 Results

4.3.1 *Species and plant tissues*

There was a significant tissue x species interaction for lead concentration ($F_{12,268} = 19.97$, $P < 0.0001$; Fig. 4.1) generated by species specific effects in all tissue types. The edible tissues of radish showed far greater accumulation than those of all other plants, with carrot the next highest, differing significantly from bean, chilli pepper and tomato tissues (Fig. 4.1a). Bean plants showed statistically higher lead accumulation in roots compared to all other species, followed by chilli pepper and tomato relative to radish and carrot (Fig. 4.1b). The stems of carrot and beans showed significantly higher lead compared to chilli peppers, radishes and tomatoes (Fig. 4.1c). Bean leaves accumulated significantly more lead than all other species' leaves (Fig. 4.1d), while lead was greater in both carrot and radish compared to chilli pepper and tomato leaves.

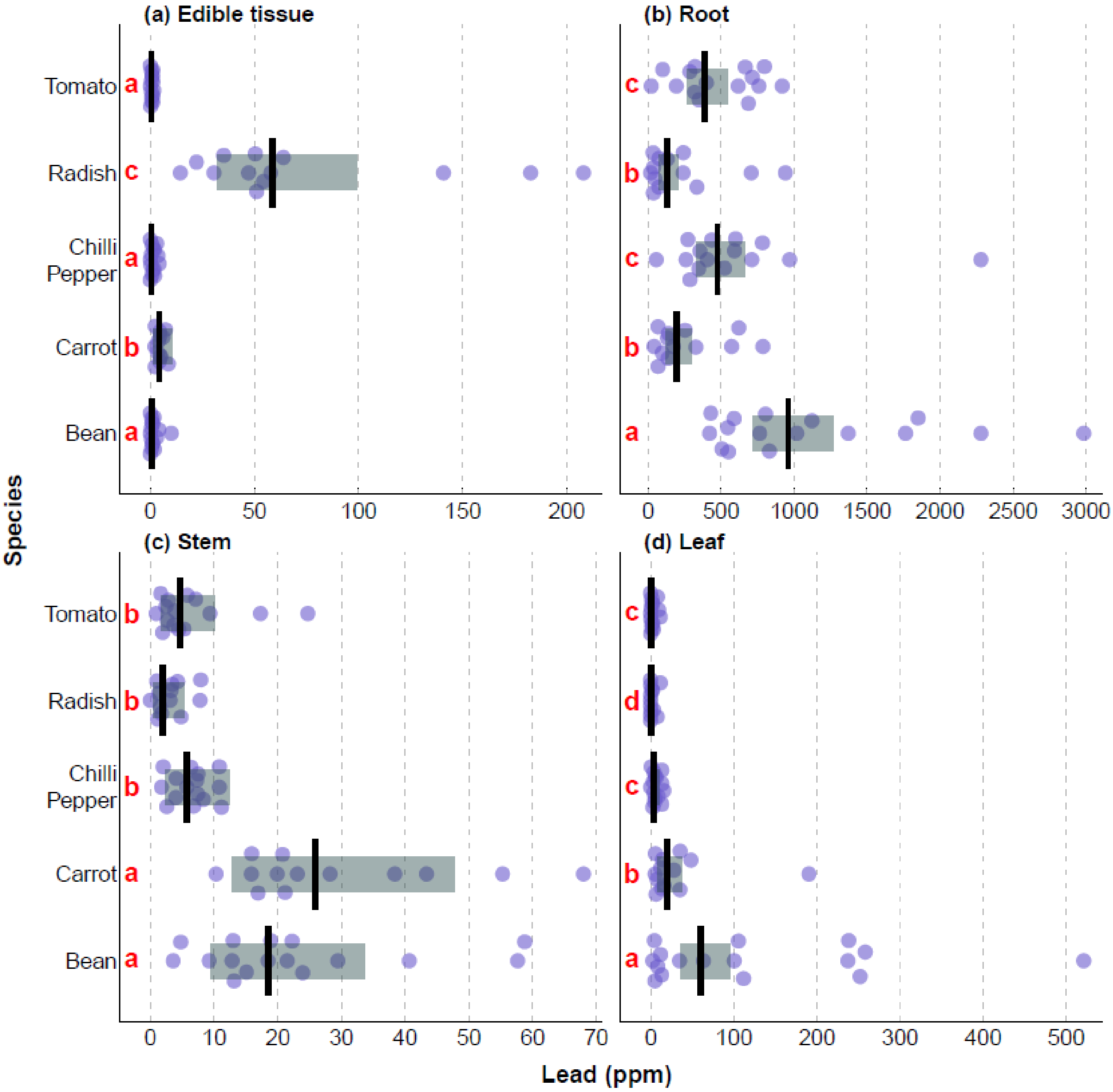


Figure 4.1. Mean lead concentration (ppm) in a) edible tissues, b) roots, c) stems and d) leaves of edible species. Red letters indicate statistically different groups within a single plot. Grey boxes represent the 95% confidence interval of the model's estimated mean indicated with a black bar.

In lead-treated soil, all radish and carrot edible tissues recorded lead over the Australian food standard of 0.1 ppm (FSANZ 2016; Fig 4.2). Of the species that did not exceed this level, no significant difference was found in the proportion of samples with potentially unsafe levels of lead content ($\chi^2 = 0.255$, 2 d.f., $P = 0.9$; Fig 4.2).

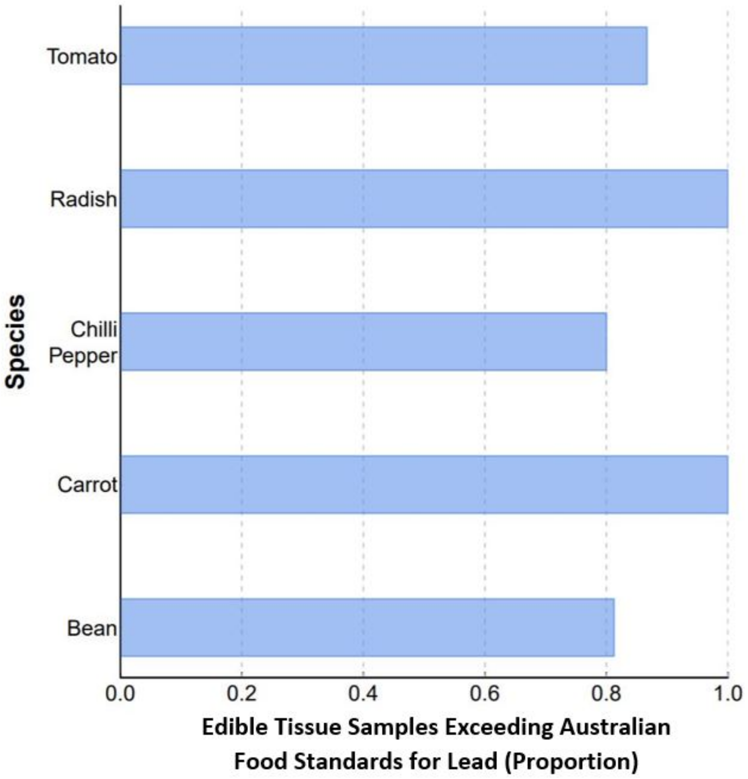


Figure 4.2. Proportion of edible tissues by species exceeding the FSANZ (2016) guideline level for lead in food (0.1 ppm Pb).

4.3.2 *Tissue age and location on plant*

Where upper and lower tissues were sampled (i.e., chilli pepper and tomato), chilli pepper accumulated significantly more lead than tomato across all tissue types ($\chi^2 = 23.36$, 1 d.f., $P < 0.0001$; Fig. 4.3). Significant differences were also found among tissue types ($\chi^2 = 62.36$, 2 d.f., $P < 0.0001$), with stem tissue showing significantly higher lead accumulation compared to leaves and fruits, where leaves were significantly greater compared to fruits. No significant difference was found in lead concentrations in tissues from upper and lower locations of the plant's biomass ($\chi^2 = 3.52$, 1 d.f., $P = 0.06$; Fig. 4.4), with consistent patterns of accumulation in upper and lower tissues across tissue types and species (all higher order interactions non-significant; Appendix 4.1).

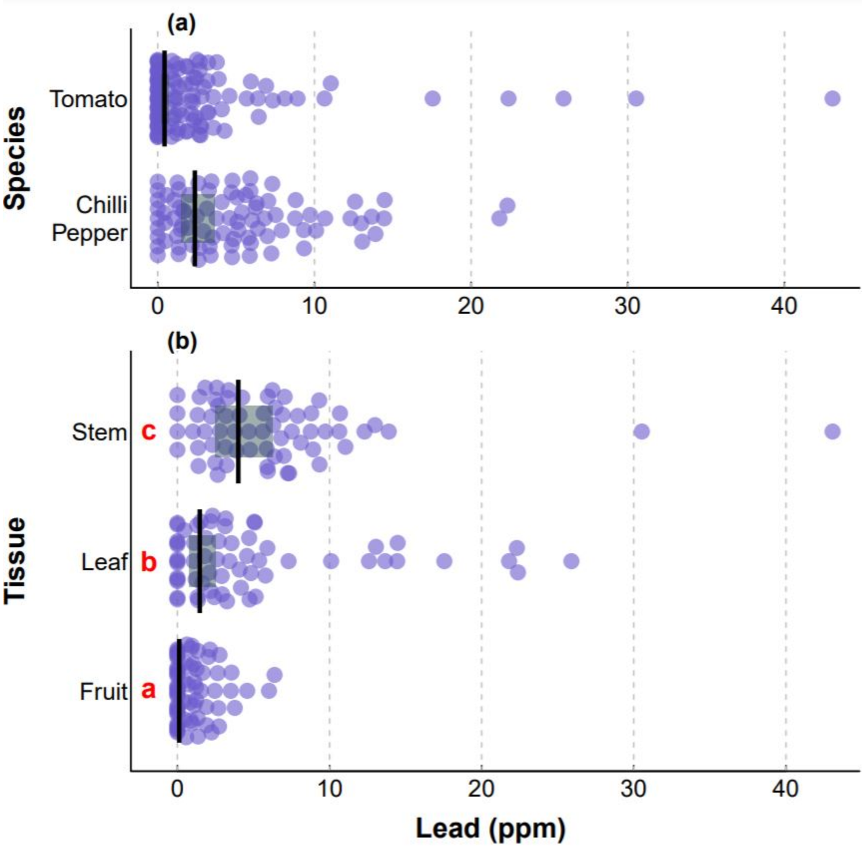


Figure 4.3. Mean lead concentration (ppm) in chilli pepper and tomato plant tissues. Red letters indicate statistically different groups. Grey boxes represent the 95% confidence interval of the model's estimated mean, indicated with a black bar.

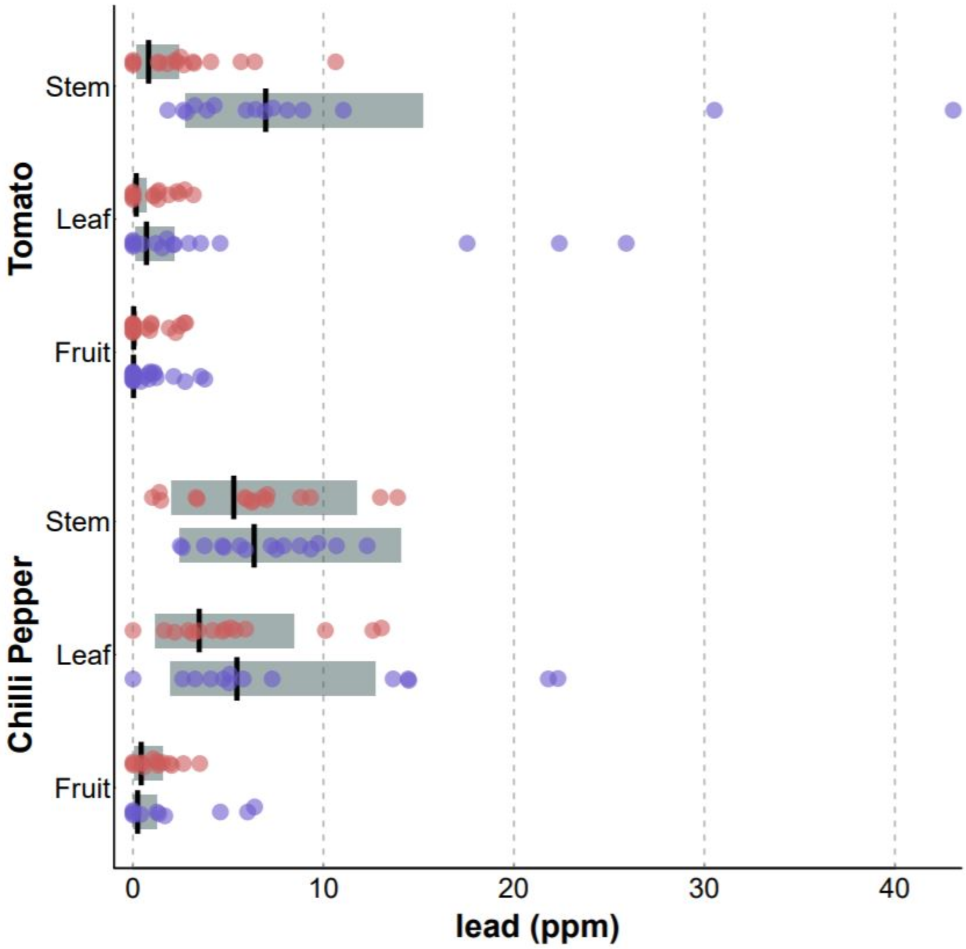


Figure 4.4. Lead concentration (ppm) in upper (red) and lower (blue) tissues of fruits, leaves and stems of chilli pepper and tomato species.

Within bean species, brown leaves accumulated significantly more lead compared to green leaves (χ^2 = 59.80, 1 d.f., P < 0.0001; Fig. 4.5).

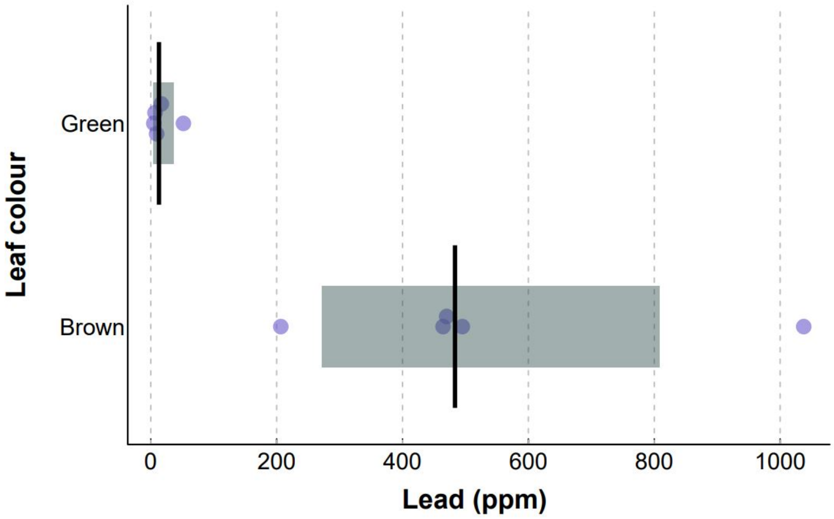


Figure 4.5. Mean lead concentration (ppm) in brown vs. green leaves of beans.

4.3.3 Phytoremediation Indices

Mean bioconcentration factors > 1 were recorded for BCF_{root} ratios in beans, chilli pepper and tomato. Translocation factor ratios of lead from roots to fruits (TF_{fruit}) were low except for radishes ($\bar{x}$ = 0.98 ± 1.42 SD), and control plants of chilli pepper and tomato (Table 4.2). All indices showed large variability.

Table 4.2. Leaf and soil bioconcentration factors (BCF_{leaf} and BCF_{root}), and fruit translocation factor (TF_{fruit}) of lead in crop species (mean ± SD). Values > 1 are marked in bold.

	Treatment (ppm Pb)	Bean	Carrot	Chilli Pepper	Radish	Tomato
BCF_{leaf}	0	0	0	0.56 ± 1.34	0	0
	300	0.42 ± 0.78	0.06 ± 0.07	0.02 ± 0.02	0.005 ± 0.006	0.01 ± 0.01
BCF_{root}	0	0	0	0.55 ± 1.35	0	0
	300	**3.31 ± 2.87**	0.57 ± 0.52	**2.10 ± 3.91**	0.61 ± 0.82	**1.14 ± 0.82**
TF_{fruit}	0	0	0	0.84 ± 0.29	0	0.55 ± 0.79
	300	0.002 ± 0.004	0.03 ± 0.03	0.01 ± 0.01	0.98 ± 1.42	0.01 ± 0.02

4.4 Discussion

The present study investigated lead accumulation of five common crop species cultivated in soils contaminated at the Australian residential guideline level for lead, 300 ppm (NEPM 2013). Results found significant species-specific effects in all tissue types. Radishes posed the greatest risk to food safety showing far greater accumulation of lead in edible tissues compared to other plants in the study, ranging between 14 and 208 ppm (Fig. 4.1a). The second highest risk was found in the edible tissues of carrots. Field tests of homegrown produce in Chicago, USA, have detected lead in the edible tissues of root vegetables, carrots, onions and radishes as 10, 21 and 12 ppm Pb respectively (Finster *et al.* 2004). Similar to our findings, Murray *et al.* (2009) found that while soil lead limits were well below Canadian guideline levels at the time (70 ppm Pb), carrots and radishes recorded at, or exceeded consumption limits defined by the Codex Alimentarius Commission (1 ppm Pb). The average accumulation into radish edible tissues in this study was 73 ppm, while carrots averaged 5 ppm Pb. Roy & McDonald (2015) similarly found greater concentrations of lead in radishes (77 ppm) compared to carrots (24 ppm). Under multi-contaminated treatments (Cd, Cu, Pb and Zn), Murray *et al.* (2009) found greater accumulation of lead into carrots (6.5 ppm), followed by radishes (3.9 ppm).

Australia's residential soil guideline level of 300 ppm used in this study is contextualised by Canada's current guideline of 140 ppm, and New Zealand's guideline of 210 ppm Pb (see Table 1.1). All replicate edible tissues of root vegetables carrots and radishes in lead treatments recorded lead over Australian and New Zealand food standards (FSANZ 2016; Fig 4.2) and were exceeded in 81.2% of beans, 80.0% of chilli peppers, and 86.7% of tomato fruits (Fig. 4.2). These proportions suggest that food safety of edible plants, particularly root vegetables, is an additional risk to the 40% of Sydney home gardeners and the 21% of Melbourne home gardeners with lead content exceeding 300 ppm detected by Rouillon *et al.* (2017) and Laidlaw *et al.* (2018).

In terms of all tissue types, the greatest proportion of lead was accumulated into roots in line with other studies (Li *et al.* 2017; Roy & McDonald 2015). Potential for phytostabilisation was indicated by root-to-soil bioconcentration factors > 1 in bean, chilli pepper, and tomato (Table 4.2). However, values were highly variable and possibly not a reliable indication of a plant's ability to immobilise contaminants in the rhizosphere.

Visual signs of metal stress such as chlorosis and wilting were observed in bean species which were harvested first due to a greater rate of senescence. Plant toxicity thresholds of lead have been reported for concentrations between 30 – 300 mg/kg dry weight in stem tissues (de Souza *et al.* 2012). Average stem concentrations of lead in beans were below 30 ppm (Fig. 4.1c), however appreciable concentrations in leaves indicate that stem tissues are more likely a translocation pathway of lead into leaves. Başar *et al.* (2009) reported that heavy metal accumulation in leaves does not consistently indicate whether a plant is safe for consumption suggesting that in addition to leaves, edible portions should be tested. Results from our study confirm that within leaf types, and depending on the species, high variability in metal content can arise. Brown leaves, including those that had fallen off bean plants ranged between 207 – 1037 ppm Pb, while green leaves ranged between 5 – 52 ppm Pb (Fig. 4.5). Lintern *et al.* (2013) discuss the dropping of leaves as a possible metal stress alleviation mechanism of *Eucalyptus* trees where gold (Au) is translocated from roots to extremity tissues like leaves where they can be discarded to reduce plant metal load and lower risk of biochemical damage.

Of the crop species tested, bean plants demonstrated lower concentrations of lead in their edible tissues along with chilli peppers and tomatoes. This result is important as highly toxic levels of lead are taken up into beans but directed into leaf tissues, away from edible portions of the plant. In instances where soil lead levels reflect the residential guideline level of 300 ppm, domestic gardeners who plant common beans may be incidentally remediating their soils with lower risk of food contamination compared to planting root vegetables like carrots and radishes. From a remediation perspective, the application of beans in lead contaminated soils would be more efficient in short-term phytoremediation projects to match their short life cycle, low metal tolerance, and requirement for ongoing collection of fallen leaves so that the metals contained in foliage do not return to the soil from composting. These characteristics also lend themselves to the application of beans as relatively fast, non-invasive bioindicators of lead contamination in urban gardens, especially given they are able to establish from seed under lead concentrations of 300 ppm (see Chapter 3, Fig. 3.3).

Measured concentrations in lead spiked soils varied greatly and included plants with an increased concentration of lead from target concentrations of 300 ppm, compared to below detection limits in control plants (excluding one chilli pepper control plant with 0.9 ppm Pb). Recent findings from a pot experiment report that plants do not phytoremediate heavy metals from soils in a homogenous way, but rather from sections of soils (He *et al.* 2020). The authors note that increases in metal concentrations over time could be attributed to these plant soil interactions affecting metal movement in the rhizosphere. Uncertainty in target concentrations may be mitigated in future studies with soil tests conducted immediately after spiking, and at the end of harvest. Future directions of this work could investigate the efficiency and viability of common beans as bioindicators of lead contamination in site assessments.

4.4.1 Conclusion

This study investigated lead compartmentalisation of five common crop species in a pot experiment where plants were treated with lead at the Australian residential investigation level of 300 ppm (NEPM 2013). Radishes posed the greatest risk to food safety showing far greater accumulation of lead than all other species, followed by carrots. 100% of carrot and radish edible tissues recorded lead over Australian and New Zealand food standards (FSANZ 2016) and were exceeded in 81.2% of beans, 80.0% of chilli peppers, and 86.7% of tomato fruits. These proportions suggest that food safety of edible plants, particularly root vegetables, is a risk to the 40% of Sydney home gardeners and the 21% of Melbourne home gardeners with lead content exceeding 300 ppm detected by Rouillon *et al.* (2017) and Laidlaw *et al.* (2018).

Statistical differences were found between species and tissue type (i.e., edible tissues, roots, stems and leaves). No differences were found in accumulation between upper and lower tissue location on plants, however fallen, brown leaves of common beans contained hyperaccumulated concentrations of lead compared to green leaves. These findings present an opportunity for the application of common bean species as bioindicators of lead contamination in urban gardens.

CHAPTER 5

HEAVY METAL ACCUMULATION IN HOMEGROWN GARDEN PLANTS IN NORTH SYDNEY, NSW

Science to the Streets!

5.1 Introduction

Popularity in home and urban gardening has grown markedly in cities around the world as community groups engage with the recreational, economic, and aesthetic benefits of green spaces that produce edible plants (Leake *et al.* 2009; Säumel *et al.* 2012; Grace & Macfarlane 2016). There is a direct financial advantage for individuals and communities to grow their own crops, with indirect contributions to improving local food security (Temple 2008) as well as decreasing the carbon footprint of meals in a small but positive way (Vávra *et al.* 2018). Urban gardens also promote regular physical and social activity benefitting community mental and physical health (Coventry *et al.* 2019), as well as providing air-filtering services that have flow-on potential to lower costs in health care (Leake *et al.* 2009; Turner *et al.* 2011). Moreover, the importance of gardens and bush corridors in urban areas is paramount to sustain the health of already-fragmented urban ecosystems that rely on these areas for biodiversity preservation (Antisari *et al.* 2015; Lepczyk *et al.* 2017).

Inner city gardeners can be restricted by space where occasionally the only positioning option for their garden is near a road or railway line (Turner *et al.* 2011; Antisari *et al.* 2015). One step further is the growing movement of ‘guerrilla gardening’ where unused plots of land, from small roadside corridors to roundabouts, are being converted into gardens without the permission of the landowners (Adams & Hardman 2014). There are dedicated guerrilla gardening community groups all around the world with their own branding and objectives. A group in Brussels launched an International Day of Guerrilla Gardening Sunflowers (coincidently a hallmark phytoremediator species) on the 1st of May (Reynolds 2012). In his TED talk, Finley (2013) described his transformation of vacant plots of South Los Angeles into vital crop resources for a community that suffered disproportionately from diet-related illnesses and limited access to fresh produce. He suggested gardening can translate into community empowerment, "growing your own food is like printing your own money. (…) Gardening is the most therapeutic and defiant act you can do." Reclaiming public land, cultivating community spirit and investing in local food security at their core are well-intentioned, but trespassing and gardening on private land belonging to others is illegal in most countries and offenders can face legal actions. In this regard, the practice could amusingly be considered 'community-organised crime'. Others see it as an opportunity for peaceful protest such as planting flowers in road potholes to bring attention to council neglect of road maintenance (Reynolds 2012).

With all its positive aspects, an increase in guerrilla gardening has also increased concerns of home produce safety; urban areas are more likely to contain greater concentrations of contaminants than rural areas (Leake *et al.* 2009; Säumel *et al.* 2012). Heavy metal contamination sources in urban areas include exhaust fumes from heavy traffic, overabundant pesticide use, chipping leaded house paint, and remnant contaminants from sites formerly used for industrial practices (Davis & Birch 2011; Laidlaw & Taylor 2011; Grace & Macfarlane 2016; Rouillon *et al.* 2017). Many home gardeners are unaware of the potential risks of exposure to heavy metals when they choose plots to grow their vegetable gardens. This includes risks associated with bioaccumulation and compartmentalisation of heavy metals into edible plant tissues. Elevated concentrations of heavy metals are often dangerous to humans and animals causing serious and ongoing health complications. For example, lead is a neurotoxin that has been linked to developmental delay and behavioural problems (Laidlaw & Taylor 2011). Similarly, the risks extend to elements that are essential to human health, such as copper and zinc.

Understanding how edible plant species respond to contaminants in their environment is imperative for developing guidelines for safe gardening practices (Antisari *et al.* 2015). In Australia, there have been multiple studies investigating sources and concentrations of heavy metal contaminants in residential properties. For example, Davis and Birch (2011) found population density and traffic volume correlated to particulate matter deposition of heavy metals copper, zinc and lead in Sydney's metropolitan area. Furthermore, their modelling forecasts that 40% of the total airborne concentrations of these heavy metals accumulates in residential properties. Similar results were replicated by Rouillon *et al.* (2017) who found that generally, lead soil contamination in Sydney residential homes was greatest in the inner city and decreased with distance from the CBD. Recently, Laidlaw *et al.* (2018) tested domestic and community garden soils in Melbourne, Victoria for heavy metals (As, Cr, Mn, Ni, Cu, Zn, Pb) revealing 21% of residential soils exceeded the current Australian guideline level for lead (300 ppm Pb) outlined by the National Environment Protection Measure (NEPM 2013).

Australian-based studies have focussed on heavy metal content of domestic Sydney and Melbourne soils with emphasis on the concern of high lead concentrations (Grace & Macfarlane 2016; Rouillon *et al.* 2017; Laidlaw *et al.* 2018; Kandic *et al.* 2019). However, implications for lead and other heavy metal concentrations in the edible tissues of plants growing in these types of soil profiles has yet to be investigated within an Australian context. Laidlaw & Taylor (2011) discuss high bioavailability of lead in Sydney urban soils. This could facilitate a greater likelihood of lead uptake into plants. In this study, an exploratory screening for public health risk of domestic garden plants and soils was conducted using a local scale, *in situ* citizen science design. This research aims to (1) investigate *in situ* concentrations of an array of heavy metals and plant species in existing domestic gardens, (2) compare detected soil and edible tissue concentrations with Australian and International guidelines, and (3) assess the risk to home growers of North Sydney who consume their produce.

5.2 Materials and Methods

5.2.1 *Study Location*

Figure 5.1. Study location map of suburbs Northbridge and Castlecrag in North Sydney, Australia.

Situated 6.5 km from Sydney's CBD, Northbridge and Castlecrag are neighbouring peri-urban suburbs located within the Willoughby City Council of NSW, Australia (Fig. 5.1). The Willoughby council area was included in a home soil testing study by Rouillon *et al.* (2017). Of the vegetable garden soils tested in the council area, lead soil concentrations ranged from less than 99 ppm Pb to 1000-1999 ppm Pb. These data points came from residences within surrounding suburbs of Northbridge and Castlecrag.

5.2.2 *Promoting Citizen Participation*

Using a citizen science approach, appeals for plant donations were advertised using social media, an article run in the community's newsletter, and word-of-mouth. Participating residents had the option to sample their own gardens using a sampling guide, or request researcher assistance, with all but two participants opting for the latter. Features such as garden location, rough proximity to roads or driveways, and whether the soil was original or imported were noted, where possible. Where available, soil samples were collected with each of the corresponding plants at a site during daylight hours between September and December 2018.

5.2.3 *Donated Plants and Soils - Laboratory Processing*

Within 48 hours of collection, plants were separated into paper bags containing tissue sections (i.e., roots, stems, leaves, fruits, flowers) and soils, and all were oven dried in the laboratory at The University of Technology Sydney at 80℃ for at least 48 hours. Soil and plant samples were processed, and acid digested as per methods outlined in Chapter 4 (EPA method 3050B; U.S. EPA 1996). All digested, filtered, and diluted samples were analysed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS, 2900 Agilent Technologies, USA) for arsenic (As), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), lead (Pb), manganese (Mn), nickel (Ni), selenium (Se), vanadium (V) and zinc (Zn).

Due to complex matrices, stems, roots and soil samples were filtered with a 50% dilution factor to mitigate ionic interferences. Quality controls for each matrix type (i.e., tissue type) were measured with spike recoveries that came within 12% of the expected recovery, except for zinc which was subsequently omitted. The ICP-MS was tuned using an internal standard and multi-element calibration curve (flowers, fruits and leaves: R^2 = 0.9998 (V, Cu) and 0.9999 (all other analytes); stems, roots and soils: R^2 = 0.9992 (Cu), 0.9995 (Mn), 0.9998 (Pb), 0.9999 (Se), and 1.000 (all other analytes). Certified reference materials (CRMs Pine needles for plants, Sandy Soil Level C, and Loam Soil Level D, Choice Analytical, Australia), procedural blanks, triplicate machine readings, and intermittent duplicate subsampling of plant sections were used throughout analyses to determine percentage metal recovery, assess procedural contamination, and ensure the accuracy of all experimental data.

5.2.4 *Statistical Analyses*

For each residence, the means of soil metal concentrations were calculated from the available number of soil samples taken at each site. Prior to analysis, values below detection limits (< 0.001 ppm) were replaced with zeros. To test for associations between concentrations of the metals across the sample sites, Pearson correlation tests were performed between all combinations of metal (values log transformed prior to analysis after addition of 0.01 to all values). Following this, differences among individual metal concentrations across sites were tested using non-parametric ANOVA (Kruskal-Wallis test) followed by pair-wise comparisons (one-sample Wilcoxon test), both using the untransformed data. Relationships between heavy metal concentrations in plant tissues (leaves, roots and stems, omitting fruits due to low replication) and soils were then modelled using linear mixed models where for each model the fixed predictor was the soil concentration of the relevant metal (log transformed data with addition of 0.01 to all values), including a random effect for site to account for site specific effects. Statistical analysis was performed in R statistical software (R core team 2019), using the packages ‘lme4’ (Bates *et al.* 2015) and ‘lmerTest’ (Kuznetsova *et al.* 2017), with a statistical significance level of α = 0.05.

5.3 Results

5.3.1 *Study Species*

A total of 139 samples from 28 species spanning 16 families were donated from 9 residential sites and 1 childcare centre (Table 5.1). Heavy metal concentrations in each tissue and soil in relation to Australian and international guideline values are presented in the thesis appendix for each site (Appendices 5.1 – 5.9).

Table 5.1. List of donated species by taxonomic family.

Family	Scientific name	Common name	No. of Plants Donated	Site Reference
Amaryllidaceae	*Allium ampeloprasum*	Leek	1	N2
Amaryllidaceae	*Allium schoenoprasum*	Chives	1	N1
Apiaceae	*Coriandrum sativum*	Coriander	1	N6
Apiaceae	*Petroselinum crispum*	Parsley	2	N6, N9
Asparagaceae	*Asparagus aethiopicus*	Asparagus Fern	1	N1
Asphodelaceae	*Aloe vera*	Aloe vera	1	N9

Asteraceae	*Sonchus oleraceus*	Sow Thistle	1	N1
Asteraceae	*Lactuca sativa*	Lettuce	1	N6
Brassicaceae	*Eruca vesicaria ssp. sativa*	Rocket	1	N4
Bromeliaceae	*Ananas comosus*	Pineapple	1	N9
Fabaceae	*Vicia faba*	Broad Bean	1	N2
Lamiaceae	*Mentha*	Mint	6	N1, N3, C5
Lamiaceae	*Mentha x piperita f. citrata*	Mint (“Chocolate” cultivar)	1	N9
Lamiaceae	*Origanum vulgare*	Oregano	1	N8
Lamiaceae	*Rosmarinus officinalis*	Rosemary	3	N3, C5, N9
Lamiaceae	*Thymus vulgaris*	Thyme	1	N4
Moraceae	*Morus nigra*	Black Mulberry	1	N1
Ochnaceae	*Ochna serrulata*	Mickey Mouse Bush	1	N9
Passifloraceae	*Passiflora edulis*	Passionfruit	1	N9
Rosaceae	*Fragaria × ananassa*	Strawberry	2	N2, N9
Rutaceae	*Citrus × aurantiifolia*	Lime	1	N4
Rutaceae	*Citrus × limon*	Lemon	3	N4, N9, N8
Rutaceae	*Citrus × paradisi*	Grapefruit	1	N4
Rutaceae	*Murraya paniculata*	Orange Jessamine (Traffic barrier plant)	1	N9
Solanaceae	*Capsicum annuum*	Capsicum (Bell pepper)	1	N9
Solanaceae	*Capsicum annuum (cultivar Cayenne)*	Chilli Pepper (Cayenne)	1	N4
Solanaceae	*Solanum lycopersicum*	Tomato	1	N8
Zingiberaceae	*Alpinia caerulea*	Australian Native Ginger	1	N9

5.3.2 *Heavy Metals in North Sydney Garden Soils*

Significant positive relationships were found in mean soil concentrations of heavy metals across sites, except for arsenic and cobalt ($r = 0.39$, $P = 0.07$; Fig. 5.2). This is further supported by patterns in geospatial data (Fig. 5.5 and Appendices 5.10 – 5.12). Mean soil metal concentrations varied significantly across sites ($\chi^2 = 194.28$, 6 d.f., $P < 0.0001$) with significant variation among all individual metals except for copper and lead ($P = 1$; Fig. 5.2).

5.3.3 *Soil and Plant Tissue Relationships*

The distribution of accumulation between plant tissues and soils varied for each heavy metal. Lead was the only contaminant that exceeded Australian Health Investigation Levels for residential soils (inclusive of childcare centres; Fig. 5.3). Significant positive associations between soil and root tissue metals were found for all metals except chromium (Fig. 5.4). Arsenic and lead showed positive relationships between soil concentrations and leaf tissues, and lead only between soil and stem tissue concentrations, while no significant soil-tissue associations were found for any other metals.

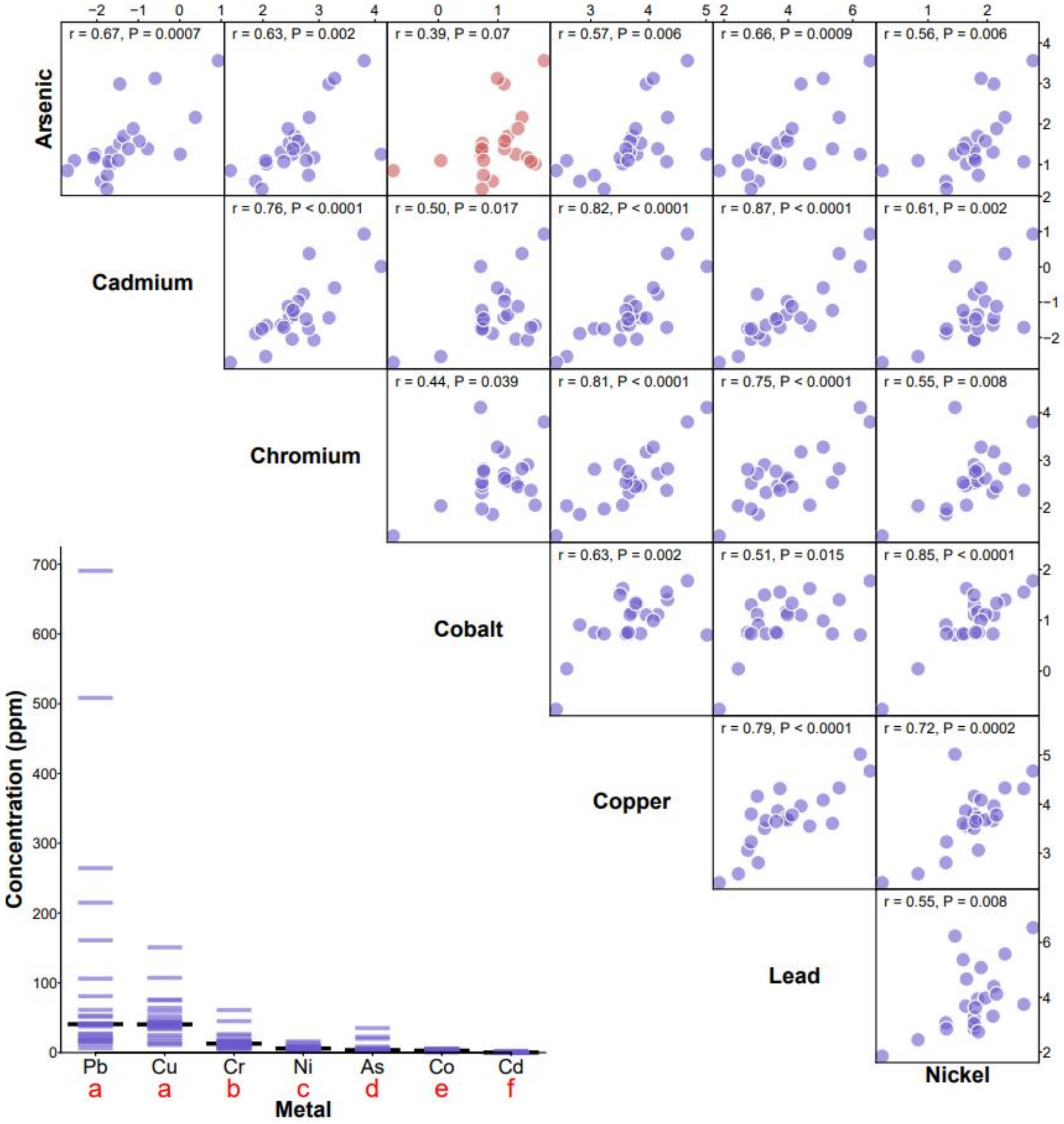


Figure 5.2. Plots of mean heavy metal concentrations in soils (top right; log transformed data). Pearson's correlation value and significance values are indicated at the top of each plot. Blue points indicate significant associations while red indicate non-significant associations. The strip plot (bottom left) shows untransformed mean soil metal values across sites, ranked on the x-axis by their median indicated with a black bar. Red letters below the x-axis labels indicate groups that were significantly different.

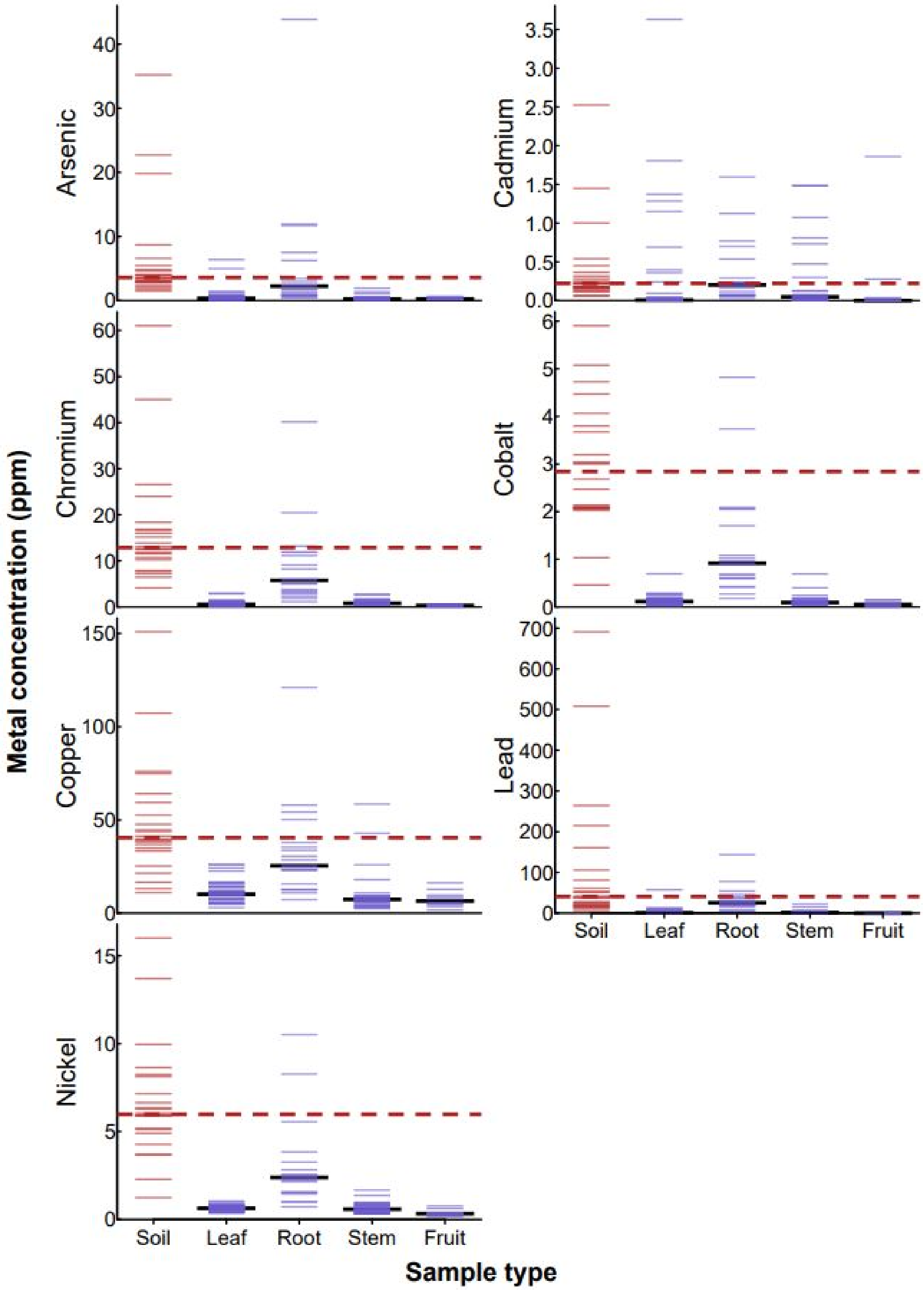


Figure 5.3. Strip plot of relationships between soils and paired tissue type for heavy metal concentrations. Red dotted lines indicate the median for soil concentration. Blue strips indicate plant tissues while red strips indicate soils.

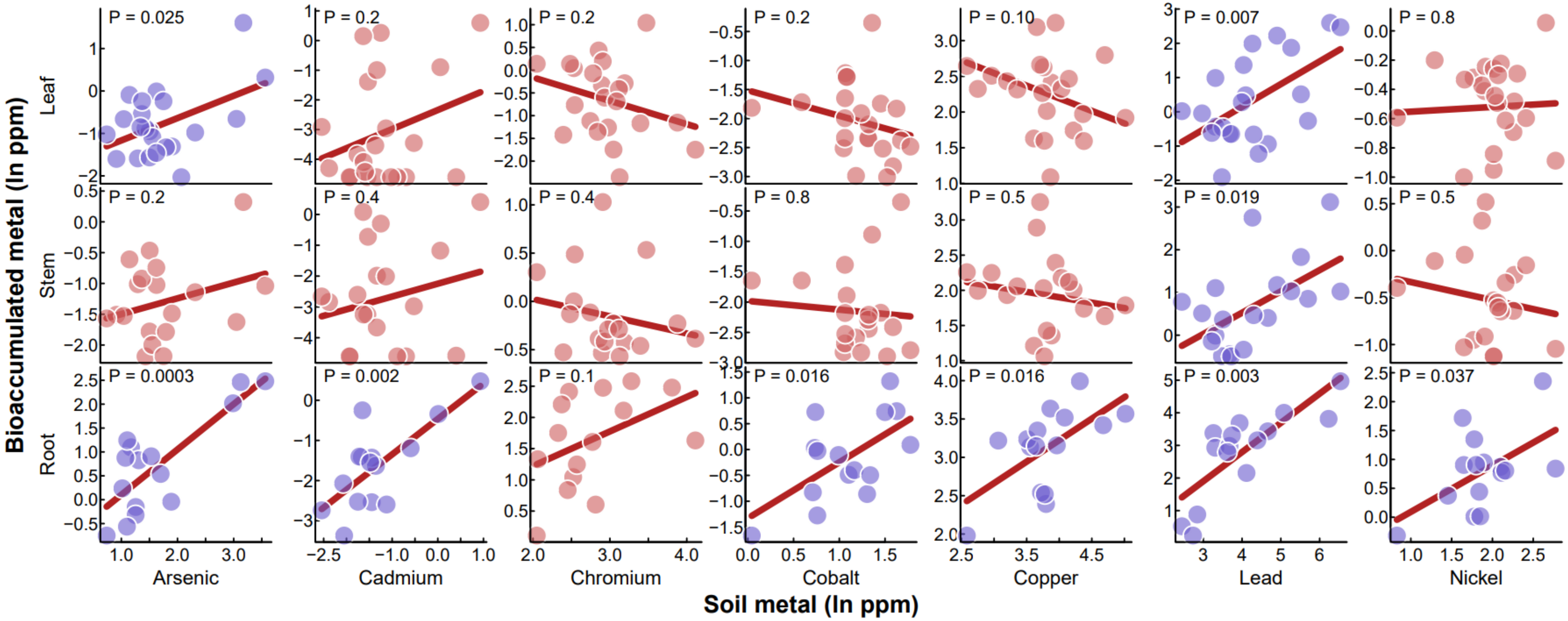


Figure 5.4. Plot of robust linear regressions between soils and paired tissue type for heavy metal concentrations, performed on log transformed data. Red lines show the line of best fit for a given relationship, with significance values shown at the top of each plot. Blue plots indicate significant correlations while red plots are non-significant. Fruits/edible tissues were omitted due to low replication.

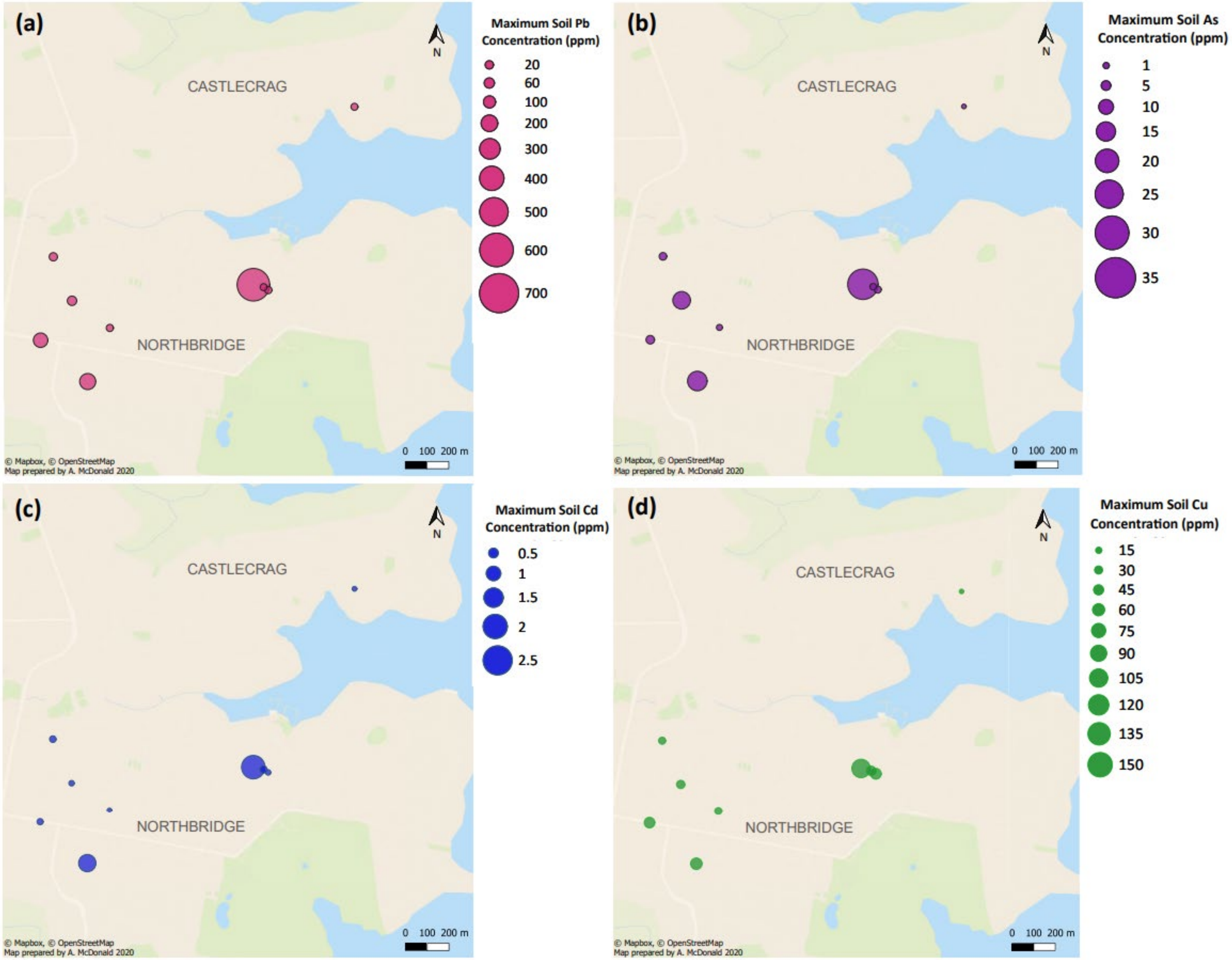


Figure 5.5. Geospatial map of maximum soil concentration (ppm) of lead (a), arsenic (b), cadmium (c) and copper (d).

5.4 Discussion

5.4.1 *Food Safety*

Several species were identified with heavy metal concentrations over safe limits in their plant tissues. As noted in previous chapters, of the contaminants tested, only arsenic, cadmium and lead have existing Australian guidelines for safe concentrations in food. Outside Australia, the European Union similarly specifies safe thresholds for cadmium and lead only in foodstuffs.

Lead exceeded Australian limits in 23 tissue sections edible for humans from aloe vera, capsicum, chives, leek, lime, mint, mulberry, parsley, rosemary and strawberry (see Appendices 5.1 – 5.9). Every site that donated mint plants contained lead over safe limits in their edible leaves. Mint is a popular herb that has demonstrated potential to rhizostabilize heavy metals (Prasad *et al.* 2010; Hasanpour *et al.* 2019) and accumulate lead and cadmium into leaves (Anwar *et al.* 2016; Kachenko & Singh 2006).

Lead was exceeded in 48 other plant tissue sections more likely to be eaten by herbivores or pollinators. Cadmium concentrations exceeded food guidelines for edible leaf sections of coriander, lettuce, mint and rocket. Arsenic was exceeded with lead in oregano and thyme leaves. These species patterns suggest that leafy herbs generally present a greater risk of heavy metal accumulation in line with former findings (Finster *et al.* 2004; Anjula & Sangeeta 2011; Liu *et al.* 2013). Greater leaf surface area may contribute to higher atmospheric deposition of heavy metals onto surfaces of herb and brassica tissues where it is possible for heavy metal particulates to be absorbed through the cuticle via the stomata (Shahid *et al.* 2017). Finster *et al.* (2004) found no significant difference between washing leafy vegetables in water with or without detergent and the resulting differences in concentrations of lead in fruiting vegetables tested under the same conditions. The authors recommend urban growers wash herbs with detergent regardless to remove any adhering contaminated soil remnants. This would mitigate deposition factors including soils that are deposited when disturbed during harvesting. In practice, it may be an unlikely behavioural change for domestic growers to use detergent on their crops before eating.

5.4.2 *Soil Concentrations and Site-Specific Features*

While not eaten directly, contaminated soils pose a great risk to children and pets that are more likely to ingest dirt carried on toys and hands (Laidlaw & Taylor 2011). In this study, soils that exceeded Australia's health investigation levels only did so for lead (> 300 ppm Pb). These soils were planted with asparagus fern (508 ppm Pb), sow thistle (690 ppm Pb) and one of the duplicate samples of lemon soil (326 ppm Pb). The first two species were sampled at residence 1; a 1920s built house where the soil was historic to the site and in ground-level garden beds close to either the driveway or house, respectively. Chipped leaded house paint is a common source of lead in household gardens (Rouillon *et al.* 2017; Kandic *et al.* 2019) and a likely cause of contamination at this site given flaking paint was observed during collection. Similarly, the asparagus fern was located on the edge of a driveway where the cadmium found in the berries could be attributed to vehicle exhaust fumes (Davis & Birch 2011; Kandic *et al.* 2019).

The soil at the base of the lemon tree at residence 8 had been sampled from the backyard garden where the owner suspected a former domestic incinerator had been located. Incineration of wastes are associated with emissions of heavy metals like As, Cd, Cr, Ni, Pb, V, and Zn that can settle in soils and enter the food chain via plant uptake (Edelstein & Ben-Hur 2018). This fits with results finding positive correlations between metal concentrations suggesting that where higher contamination occurs in one metal, the presence of other metals in these types of gardens is likely (Fig. 5.2). The lemon fruit at this site has safe concentrations of heavy metal content (FSANZ 2016), potentially indicating that lemon trees (*Citrus × limon*) may be phytoexcluders of these heavy metals. Soil contamination posed greater risk for the oregano and young tomato plant sampled at this same site. Tomato leaves contained cadmium over Canadian soil guidelines (4 ppm Cd), as well as elevated levels of arsenic (44 ppm As) and copper (121 ppm Cu) held in its root tissues. The edible oregano leaf tissues likewise were over guideline levels for arsenic (5 ppm As) and lead (6 ppm Pb). For both analytes, the oregano soil was over Canadian guidelines and the compartmentalisation pattern of all heavy metals except cadmium and selenium into the plant suggests that contaminants that were not bound by the root tissues, once absorbed, were more likely to translocate past the middle stems into the upper edible leaves (i.e., As, Cr, Co, Cu, Pb, Mn, Ni and V accumulated in the order of soils > roots > leaves > stems). A general link between mean soil concentrations across sites and paired root concentrations was found for As, Cd, Co, Cu, Ni, and Pb analytes (Fig. 5.4). Lead concentrations in soil were also positively correlated with concentrations detected in leaves and stems, while leaf tissues were correlated for arsenic. Average lead and copper heavy metal concentrations were not different across sites but were statistically greater than Cr < Ni < As < Co < Cd (Fig. 5.2).

The childcare centre included in this study is located in close proximity to a main road with high volume traffic density (approximately 17,443 private vehicles and 2079 buses per day with an average speed of 21.5 km/hr, averaged over a 5 day work week; Compass IOT Pty Ltd 2020). Centres that are positioned near major roads with greater exposure to exhaust fumes have been connected to development of childhood asthma (Dantzer & Keet 2015). Heavy metal results for the childcare centre in this study indicate successful implementation of protective barrier plants as an effective way of mitigating possible heavy metal deposition in their gardens (Säumel *et al.* 2012). The tall, roadside hedge species Orange Jessamine and Mickey Mouse Bush (a weed that grew within the hedge) contained smaller than expected concentrations of heavy metal load in their plant tissues perhaps because speeds of traffic are quite low where fumes may not travel as far. The paired soil section was historic to the site explaining its elevated lead content (215 ppm Pb). Risks associated with exposure to this soil sample area are very low as the soil was collected from behind a brick wall with a thick hedge on one side, and fence on the other making it inaccessible to children. All other soils at the site were store bought and planted in raised garden beds with an added protective mulch layer.

Small amounts of arsenic were detected that may originate from leachate of wooden play equipment treated with arsenate (Ursitti *et al.* 2004). Concentrations where well within naturally occurring ambient levels of environmental arsenic (1 to 40 ppm As; Tchounwou *et al.* 2012). Interestingly, Native Ginger (*A. caerulea*) appeared to be a hyperaccumulator of manganese and to a lesser extent, copper. Like many of the residential sites, some edible sections of the childcare centre’s plants were marginally over Food Standards Australia and New Zealand (FSANZ 2016) for lead. To deduce the extent of exposure risks from Tolerable Daily Intake (TDI) models underlying FSANZ standards, plant consumption rates would need to be recorded because they are highly variable depending on dietary behaviour, crop types, growing season, and the number of plants available for eating at any one time. This is a possible future direction of this work.

5.4.3 *In the Context of Heavy Metal Guidelines*

International guidelines help contextualise contamination risks as standards are derived from alternative methods. Australian soil guidelines (NEPM 2013) are conservative when compared to Canada (CCME 2018) or New Zealand (MFE 2013) because they incorporate leeway for contaminant bioavailability (Kim *et al.* 2015) and possibly reflect background concentrations in the context of Australia's mining and industrial history. Some results that were not flagged under Australian guidelines were exceeded by Canadian soil standards particularly for selenium (> 1 ppm), followed by copper (> 63 ppm) and arsenic (> 12 ppm). Selenium is an essential nutrient for the health of humans and plants making it low risk at concentrations of 1-3 ppm in the context of these sites. Selenium may even be beneficial at these levels as it has potential to alleviate oxidative stress in plants (Mozafariyan *et al.* 2015). Furthermore, selenium enrichment experiments have been trialled to reduce the accumulation of other heavy metals such as arsenic, cadmium, copper, lead, manganese and mercury into plants, including crops (Hu *et al.* 2014).

Measuring species grown under common suburban conditions is beneficial in exploring real-world data and informing application of knowledge from *ex situ* studies. A citizen science design creates a positive approach for education and raising awareness in communities but also presents limitations. Uprooting crops is undesirable for growers and unfortunately no root vegetables were donated to the study. An array of species commonly found in domestic gardens could be tested in exchange for lower replication and ad-hoc soil collection. Important future directions of this work could investigate relationships between socio-economic status and levels of contamination as an environmental justice issue.

5.4.4 Conclusion

This study aimed to explore real world heavy metal concentrations in urban garden plants and soils using a small-scale citizen science approach. Comparing edible tissue concentrations to Food Standards Australia and New Zealand (FSANZ 2016), the overall risk of heavy metal exposure to home growers in North Sydney was considered low. Two residences had moderate risk from soils containing lead over current National Environment Protection Measure values (NEPM 2013) (maximum values of 690 ppm and 326 ppm Pb). Contamination at these sites is thought to originate from various sources, including a former domestic incinerator, vehicle exhaust fumes, and historic soil contaminated with leaded house paint. All heavy metal analytes were positively correlated, except for arsenic and cobalt, indicating that where heavy metal contamination occurs in domestic gardens, it is more likely be multi-metal. Lead was the only heavy metal contaminant where soil concentrations positively correlated with leaf, stem, and root tissue concentrations. In general, leafy herbs presented greater risk of heavy metal accumulation. Every residence that donated mint had edible leaf sections containing lead over Food Standards Australia and New Zealand (FSANZ 2016) guidelines.

In situ investigation of domestic plants and soils is valuable for community food security and adding to knowledge of various species responses to heavy metal contamination. Following this, knowledge of how edible crops compartmentalise heavy metals in highly contaminated soils can inform species selection in phytoremediation projects coupled with assessment of risks to humans, herbivores and pollinators that interact with these gardens.

CHAPTER 6

POWER PLANTS – PHYTOREMEDIATION OF WHITE BAY POWER STATION, ROZELLE NSW

Who you gonna coal? Plant Musters!

The research presented in this chapter was part of a funded project collaboration between:

- The University of Newcastle, (UoN) School of Architecture and Built Environment
- The University of Technology Sydney, (UTS) School of Life Sciences and School of Architecture
- The University of New South Wales, (UNSW) School of Built Environment and Art and Design
- Landcom
- UrbanGrowth NSW

6.1 Introduction

Innovation in power generation from the early to mid-20th century revolutionised way of life for human populations. Progress in coal-fired power generation enabled unprecedented economic progress in transport and manufacturing sectors and remains a dominant source of power in developing nations today (Gohlke *et al.* 2011; Du & Mao 2015). Fossil fuel power generation has had profound impact on the environment with leading contributions to global CO2 emissions and mining-related land degradation as a non-renewable resource (Atilgan & Azapagic 2015). Power plants pollute local environments with aerosols and heavy metals up to a 5 km radius (Mandzhieva *et al.* 2016). Cohen *et al.* (2012) estimated that 30-50% of all sulphate emissions detected in the greater Sydney region are sourced from coal-fired power stations. As market demand fluctuates with a competitive gas industry, and environmental laws on emissions tighten, outdated factories are being decommissioned at an increasing rate. According to a *Nature* summary news article (2014), 85 smaller coal-fired power stations were shut down in 2012 alone. Once industrial sites are decommissioned, active pollution from emissions cease but the legacy of local soil and building contamination remain.

Brownfields are pockets of disused land often neglected due to complex and costly contamination and often feature comparable safety challenges to those present at former industrial sites (Martinat *et al.* 2018). Land value of brownfields can be significant where plots of land are waterfront or close to the city making their remediation attractive for site developers (Su-Lin 2016). In cases where industrial sites hold significant cultural heritage, a delicate balance must be navigated between preserving the site's heritage value and remediation for future use.

6.1.1 *White Bay Power Station: Site History*

Figure 6.1. White Bay Power Station, Rozelle NSW.

White Bay Power Station is an iconic landmark of Sydney's foreshore boasting a rich heritage as the last standing metropolitan station of its kind in Australia (State of NSW and Office of the Environment and Heritage 2013). It is located 3km from Sydney's CBD in Rozelle and is a key feature of the Bays Precinct; an urban renewal project of Sydney's industrial foreshore currently led by Infrastructure NSW (formerly UrbanGrowth NSW) (Infrastructure NSW 2018).

White Bay Power Station was the longest serving coal-fired power station in Sydney (State of NSW and Office of the Environment and Heritage 2013). It was constructed in 3 phases between 1912 and 1948 by the Railways Commissioners Department originally to service tram and rail networks (Design 5 Architects Pty Ltd 2011). Ownership changed in 1953 to the Electricity Commission of NSW (State of NSW and Office of the Environment and Heritage 2013).

As general electricity use in the city increased, factory additions such as a second boiler house were constructed to meet demand. The first boiler house was demolished after World War II and later reconstructed (Design 5 Architects Pty Ltd 2011). It is the only boiler

Figure 6.2. Features from the northern aspect of White Bay Power Station.

house remaining of the three that were constructed, and one of the 4 heritage listed buildings in the complex. The coal handling unit, turbine house and emblematic steel stacks are among the other official heritage listings (State of NSW and Office of the Environment and Heritage 2013).

Around the 1970s, power generation was superseded by newer power stations constructed nearer coal mines, rendering inner city power stations unviable (Design 5 Architects Pty Ltd 2011). On December 25th, 1983, White Bay Power Station was decommissioned and subsequently used as a substation only (Design 5 Architects Pty Ltd 2011). In 1995, Pacific Power (formerly named Electricity Commission of NSW) instigated a management plan for the site including extensive removal of hazardous asbestos. The Sydney Harbour Foreshore Authority bought the property 5 years later in 2000 and in 2010 also acquired the former site of the White Bay Hotel which had been demolished by fire in 2008. The White Bay Hotel held cultural significance as the onsite pub frequented by workers at the power station (Design 5 Architects Pty Ltd 2011).

The site’s heritage value is derived from rich oral and written archives as well as physical preservation of original machinery demonstrating distinguished technical and creative innovation for electricity generation in the early to mid-20th century (State of NSW and Office of the Environment and Heritage 2013). White Bay Power Station has also showcased in high profile fashion shoots and films including The Great Gatsby, Mad Max Fury Road and The Matrix Reloaded (Ware *et al.* 2018).

There is consensus between community, government, and other stakeholders that the site be adapted for new use while respecting its valuable attributes and heritage (Design 5 Architects Pty Ltd 2011; State of NSW and Office of the Environment and Heritage 2013; UrbanGrowth NSW 2015). Repurposing the site has proved difficult given the scale and cost of remediation required to address hazards. The original proposal from UrbanGrowth NSW (2015), aimed to transform White Bay Power Station with immediate priority into a community technological hub and “recognise its history in an authentic way.” The task went out for tender and 13 development proposals were received in 2015, including a joint proposal by Google and Lendlease (Su-Lin 2016).

None of the proposals were accepted. Risk mitigation and responsibility for the property remained in ownership of UrbanGrowth NSW. The initial momentum and vision for White Bay Power Station has since idled making way for other infrastructure priorities like the Rozelle Rail Yards and WestConnex freeway where part of the land near White Bay was used as a temporary truck marshalling area (Infrastructure NSW 2018).

6.1.2 *Phytoremediation of White Bay Power Station*

While the complexities of its future are considered, ongoing maintenance and management of the site continues providing opportunity for longer-term remediation strategies like phytoremediation. Phytoremediation is an ideal decontamination strategy for heritage listed brownfields because it is non-intrusive and inexpensive. Constructing a garden has added artistic quality and aesthetic architectural design to complement historic features (Ware *et al.* 2018). At the Sydneysiders Summit event held by UrbanGrowth NSW in 2015, community gardens were proposed during brainstorming for The Bays Precinct urban development to aid food security and community building (UrbanGrowth NSW 2015, p.54). While gardens create an aesthetic environment for communities and support biodiversity, edibles planted at former industrial sites like White Bay Power Station are at risk of contamination.

This study aims to investigate compartmentalisation patterns of heavy metal contaminants into separate tissue sections of plants applied *in situ* at White Bay Power Station's former coal yard. The project aims to decontaminate soils while assessing the potential risks to edible portions of plants growing onsite. Existing studies of applied bioremediation on Australian power plant sites are limited, with most phytotechnology focussing on filtering wastewater. For example, live macroalgae bioremediation of heavy metal contaminated effluent proved feasible in a trial conducted on wastewater of Tarong coal-fired power station, Queensland (Roberts *et al.* 2015).

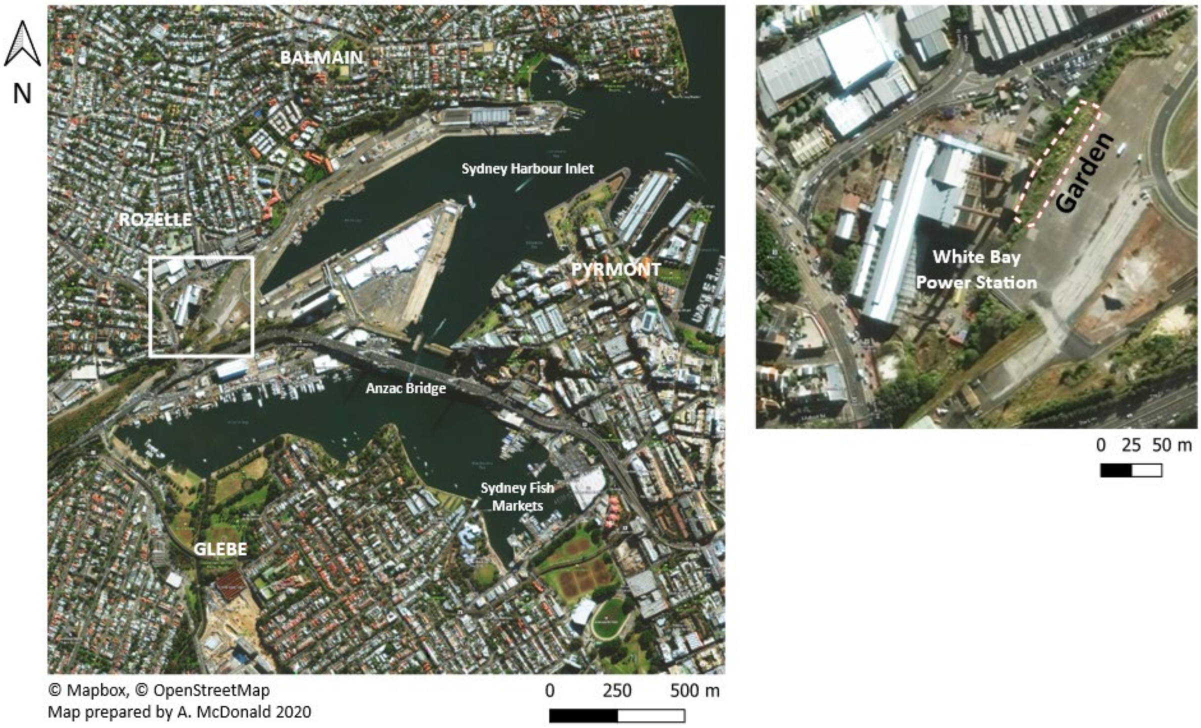
Internationally, the application of phytoremediation at coal-fired power stations has been successfully employed on fly ash dumps in India. For example, a prominent fruit tree, *Ziziphus mauritiana*, was found to accumulate an array of heavy metals including As, Cd, Co, Cu, Cr, Ni, and Pb into plant parts from fly ash dumps at the Panki Thermal Power Station in Uttar Pradesh (Pandey & Mishra 2018). Analysis of edible fruits from this study highlighted that *Z. mauritiana* should be used with caution due to concentrations exceeding local food safety limits for some heavy metals. Other international studies have examined heavy metal accumulation in pre-existing vegetation growing in areas surrounding power stations for the purpose of evaluating their potential for local phytoremediation. For example, mangroves were found to be protecting coastal ecosystems by remediating Pb in their environment near the Masinloc Power Plant in Zambales, Philippines (Paz-Alberto *et al.* 2014). These studies can be used to inform local environmental management. Site-specific investigations that account for local climate, soil chemistry and ecology are required to assess the viability of similar remediation projects in Australia.

This study, named *Power Plants*, marks Australia's first active *in situ* terrestrial phytoremediation project on a heritage listed site of national significance.

6.2 Materials and Methods

6.2.1 *Study Location*

White Bay Power Station is located on the foreshore of the Bays Precinct of Sydney in the suburb of Rozelle, NSW (GPS coordinates 33' 51S, 151' 11E). Since its closure, other possible contamination sources near the site include heavy traffic on nearby Anzac Bridge and local marine industries.



© Mapbox, © OpenStreetMap
Map prepared by A. McDonald 2020

Figure 6.3. Study location of White Bay Power Station and phytoremediation garden, Robert Street, Rozelle, NSW.

6.2.2 *Project stakeholders*

Power Plants is a collaborative project partnered with Landcom, UrbanGrowth NSW, The University of Newcastle (UoN), The University of New South Wales (UNSW), and The University of Technology Sydney (UTS). Documentation of the project in film and media was created to facilitate public education and engagement with the project given the site remained closed to the public due to safety concerns. The scientific team at UTS were responsible for sampling and analysing plants used in the design to measure the ongoing effectiveness of the garden in decontamination. The results from scientific analyses are presented here.

6.2.3 *Phytoremediation Garden & Sampling Design*

The phytoremediation garden is positioned on the eastern side of the White Bay Power Station complex on top of the former coal yard, spanning approximately 1000 m^2 (Fig. 6.3; Fig. 6.4). As the first trial garden was designed to span one year, fast growing annual species were selected based on architectural design with many commonly used in agricultural sectors (Ware *et al.* 2018). These species were further nominated on their previous phytoremediation performance in similar contaminant classes known to be present on the site. These include heavy metals, polycyclic aromatic hydrocarbon (PAHs), polychlorinated biphenyls (PCBs) and total petroleum hydrocarbons (TPHs) (JBS&G Australia Pty Ltd 2015). The garden design was split into 10 segments (approximately 10 m^2 each) alternating between monoculture and mixed meadow plots (Fig. 6.5).

Figure 6.4. White Bay Power Station from a northern aspect of the garden prior to planting.

Figure 6.5. Phytoremediation garden design (From Ware *et al.* 2018)

Original soil and plant samples were collected in August 2018. After the area was cleared and the garden planted, sampling occurred monthly from October 2018 to May 2019. Plants were sampled from each of the 10 plots with species randomly selected in consideration of reducing damage to the garden. In later months, thickness of the garden only allowed for sampling near garden edges with ticks and snakes causing safety hazards.

Figure 6.6. (Above) The first garden plot (monoculture circles) from the south facing north.

Figure 6.7. (Right) Evidence of rusting pipe material in the 7th garden plot (monoculture circle of *Cucurbita pepo*) facing north.

6.2.4 *Laboratory Processing*

On the same day of collection, plants were separated into paper bags containing either below-ground tissues (i.e., roots or root vegetables) or above-ground tissues (i.e., leaves, stems, fruits, flowers) and soils and processed in the laboratory at UTS following the method outlined in Chapter 4 (EPA method 3050B; U.S. EPA 1996). Digested and filtered samples from August 2018 to January 2019 were analysed using MP-AES (MP-AES 4210, Agilent Technologies, USA). Opportunity to analyse subsequent monthly samples (February 2019 to

May 2019) with ICP-MS instrumentation (ICP-MS 2900, Agilent Technologies, USA) enabled the scope of heavy metal data to include additional As, Cd, Co, Cu, Mn, Ni, Se and V analytes.

Check standards and additional recalibration for MP-AES were performed every 30 samples to check for machine drift. Quality controls for each matrix type (i.e., tissue type) were measured with majority recording within ± 12% of the expected recovery. One root matrix recorded as low as 70.29% for copper recovery (MP-AES) however this was considered within acceptable limits (U.S. EPA 1994). QC recoveries for zinc were not within acceptable limits so the data were omitted. Certified Reference Materials (Choice Analytical, Australia), procedural blanks, duplicate sampling, and inclusion of CRMs from previous instrument analyses were used to ensure the data were accurate.

6.2.5 *Statistical Analyses*

Prior to analysis, values below detection limits (< 0.001 ppm) were replaced with zeros. After adding 0.01 to all values (to allow statistical analysis), the data were log transformed and Pearson’s correlation were performed to test for associations between metal concentrations. Following this, a non-parametric ANOVA (Kruskal-Wallis test) was used to test for differences among mean individual metal concentrations in soils across harvest months followed by pair-wise comparisons (with Bonferroni correction), using the untransformed data. Statistical analyses were performed in IBM SPSS Statistics v. 26 (IBM Corp. 2019) with a significance level of $\alpha = 0.05$.

6.3 Results

6.3.1 *Soils*

A statistical difference between average heavy metal concentrations within soils across months was observed for As, Cr, Co, Cu, Mn, Pb, Se, while the distribution of Ni, Cd, and V were not statistically different (Appendix 6.1). Pairwise comparisons indicated that differences were largely driven by a peak recorded in soils collected at the first garden harvest in October 2018 (Fig. 6.8), or distributions from the 5th harvest in February 2019 for analytes only analysed in the latter 4 months (Fig. 6.9). Excluding one outlier, lead concentrations remained within Australian soil guideline levels for domestic soils (300 ppm) and well within industrial soil guidelines (Table 6.1; NEPM 2013). A Jonckheere-Terpstra test showed a trend in soil arsenic decontamination measured over the latter 4 months from February 2019 to May 2019 ($\chi^2 = 83.00$, 3 d.f., $P < 0.0001$; Fig. 6.9), however Bonferroni adjusted pairwise comparisons were insignificant ($P = 1$).

Table 6.1. Descriptive statistics for heavy metal concentrations in soils at White Bay Power Station.

Metal	**Soil Concentration Mean ± SE (ppm)**	**Range (ppm)**	**NEPM (2013) Residential Guideline (ppm)**	**NEPM (2013) Industrial Guideline (ppm)**
Arsenic	70.6 ± 6.7	6.7 – 332.9	100	3000
Cadmium	0.2 ± 0.03	0 – 1.0	20	900
Chromium	13.2 ± 0.8	4.7 – 88.2	100	3600
Cobalt	7.4 ± 0.6	2.3 – 20.1	100	4000
Copper	24.5 ± 1.0	5.1 – 65.8	6000	240,000
Lead	70.6 ± 6.7	6.7 – 332.9	300	1500
Manganese	127.3 ± 8.1	34.7 – 255.7	3800	60,000
Nickel	12.6 ± 1.1	3.5 – 46.4	400	6000
Selenium	1.04 ± 0.1	0.4 – 2.2	200	10,000
Vanadium	14.1 ± 0.5	7.3 – 22.3	No Guideline	No Guideline

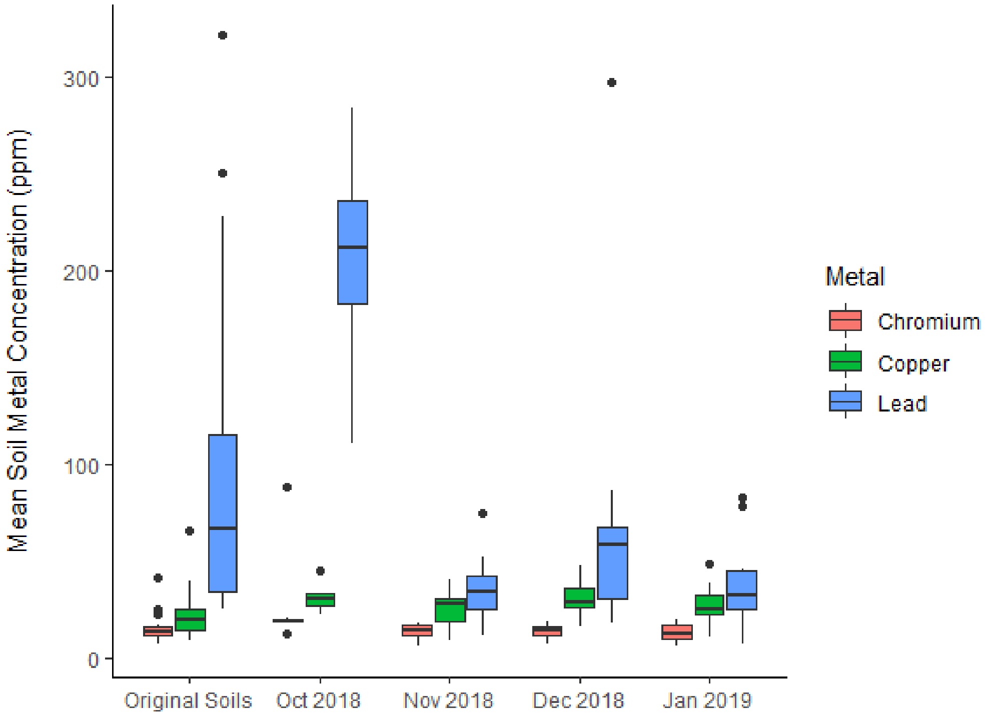


Figure 6.8. Boxplot of mean heavy metal concentrations in soil samples collected at White Bay Power Station from original condition to January 2019, analysed with MP-AES.

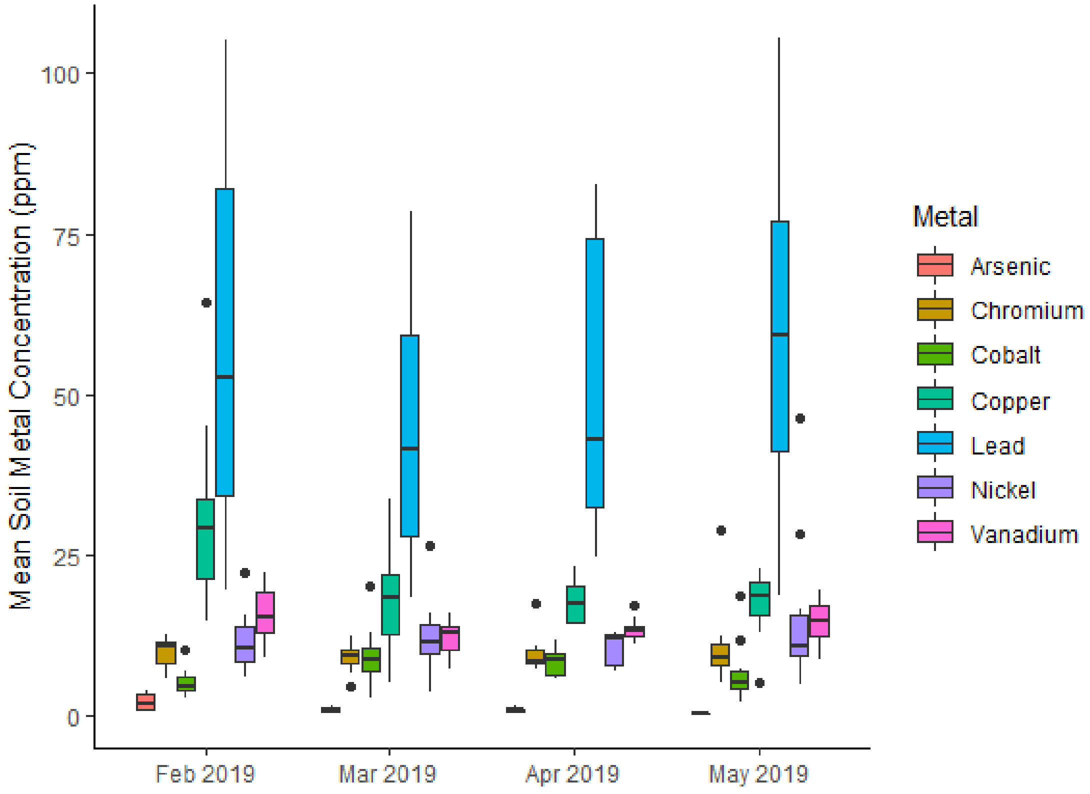


Figure 6.9. Boxplot of mean heavy metal concentrations in soil samples collected at White Bay Power Station from February 2019 to May 2019, analysed with ICP-MS.

Approximately half of relationships between heavy metal analytes As, Cd, Cr, Co, Cu, Pb and Ni were positively correlated for mean soil concentrations across all sampling months (Fig. 6.10).

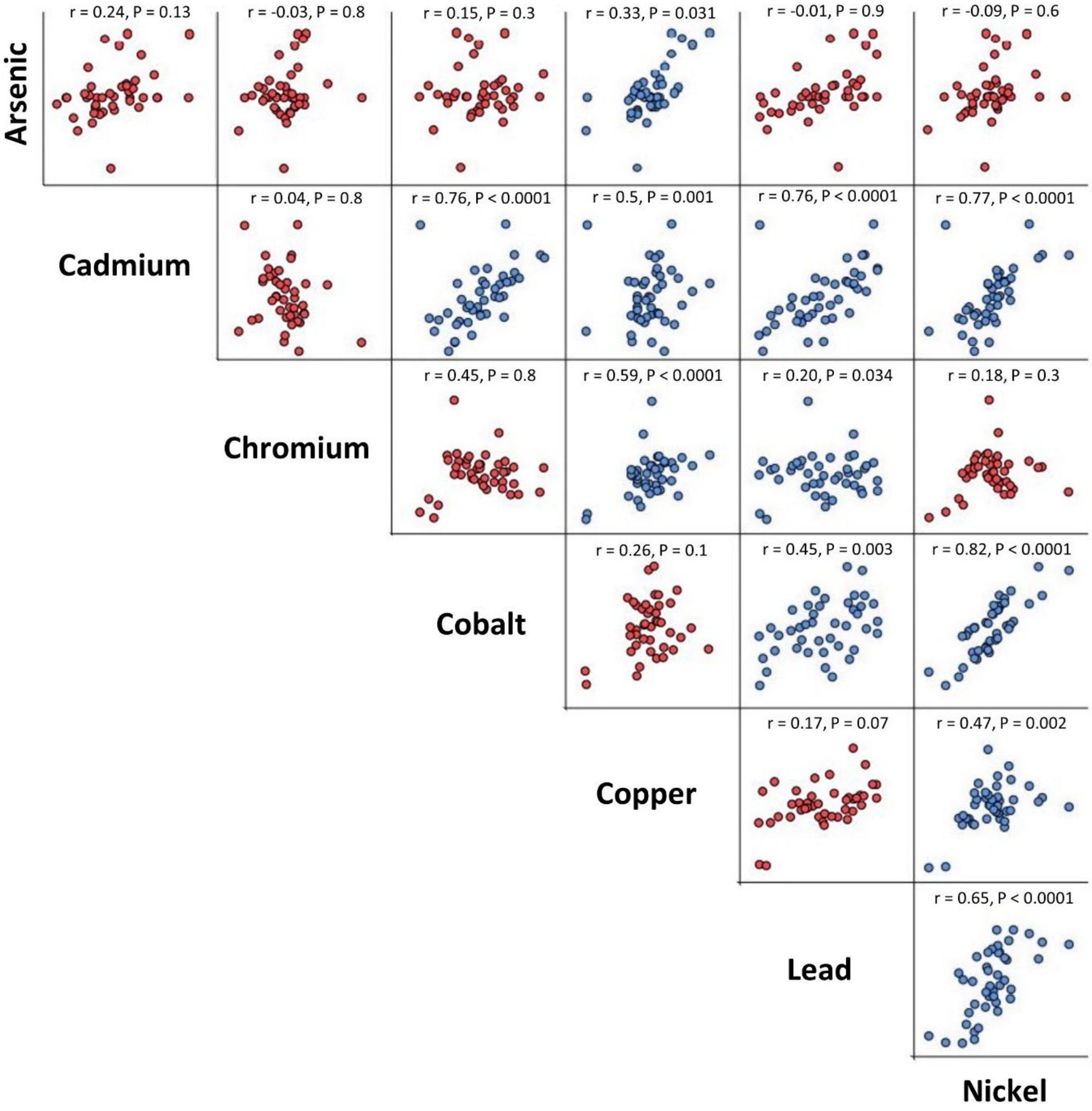


Figure 6.10. Plots of mean heavy metal concentrations in soils (log transformed data). Pearson's correlation value and significance values are shown at the top of each plot. Blue points indicate significant associations while red indicate non-significant associations.

6.3.2 *Edible Tissue and Soil Relationships*

Relationships between soil heavy metal content and edible tissues were highly variable for species and heavy metals. Arsenic observed a marked movement from soils to edible portions (excluding vetiver grass) where plants sampled in February 2019 had concentrations ranging 1-5 ppm in soils and below detection limits (< 0.5 ppb) in their corresponding edible portions. Between March 2019 to May 2019, arsenic gradually shifted to greater portions recorded in edible or reproductive tissues (0-4.3 ppm) and below detection limits in paired soils (Fig. 6.11).

Over 8 months, chromium concentrations in soils were reduced in a general trend where earlier months had a greater number of species recorded between 15-20 ppm compared to more species between 5-10 ppm from March to May 2019 (Fig. 6.12). Edible tissue concentrations remained within 0 and 5 ppm except for carrots and moss which recorded considerable levels for all metals. For example, above-ground tissues of moss contained 347 ppm of lead compared with soil concentration of 99 ppm (Fig. 6.14). Moss was not a selected garden species and was sampled opportunistically in February 2019 only. The results suggest the potential for further investigation of hyperaccumulation abilities and a notable tolerance for this plant species.

Copper concentrations in soils were reduced with earlier months recording a greater number of species containing between 20-40 ppm while latter months showed a greater number ranging 10-30 ppm (Fig. 6.13). From December 2018, copper concentrations in edible portions of alfalfa, carrot, clover, and marigold marginally trend downwards with soil concentrations.

Excluding moss, nickel concentrations in edible portions were < 10 ppm with no significant reduction in soil concentrations over the latter 4 month period (Fig. 6.15).

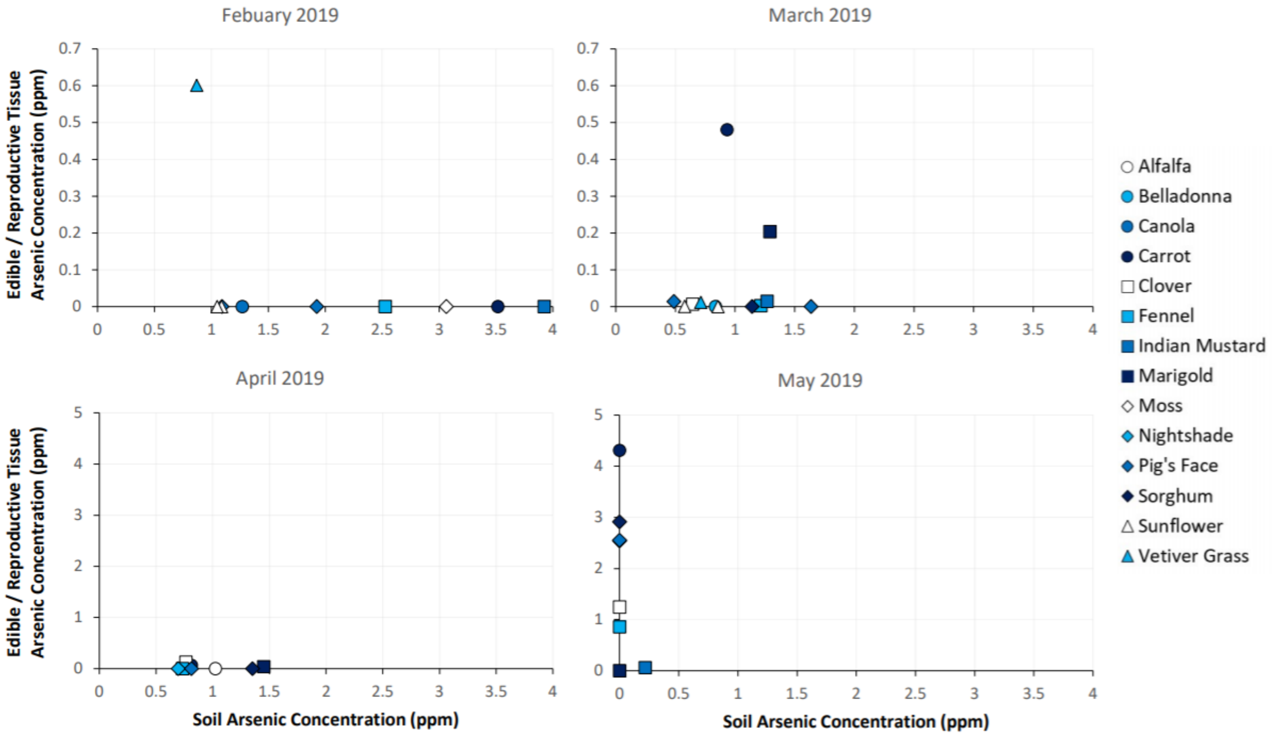


Figure 6.11. Relationship of edible or reproductive tissue concentration of arsenic (ppm) and paired soil concentrations (ppm), showing movement of the metalloid from the soil matrix into plant tissues. Tissues below the detection limit of 0.5 ppb are indicated as 0 ppm.

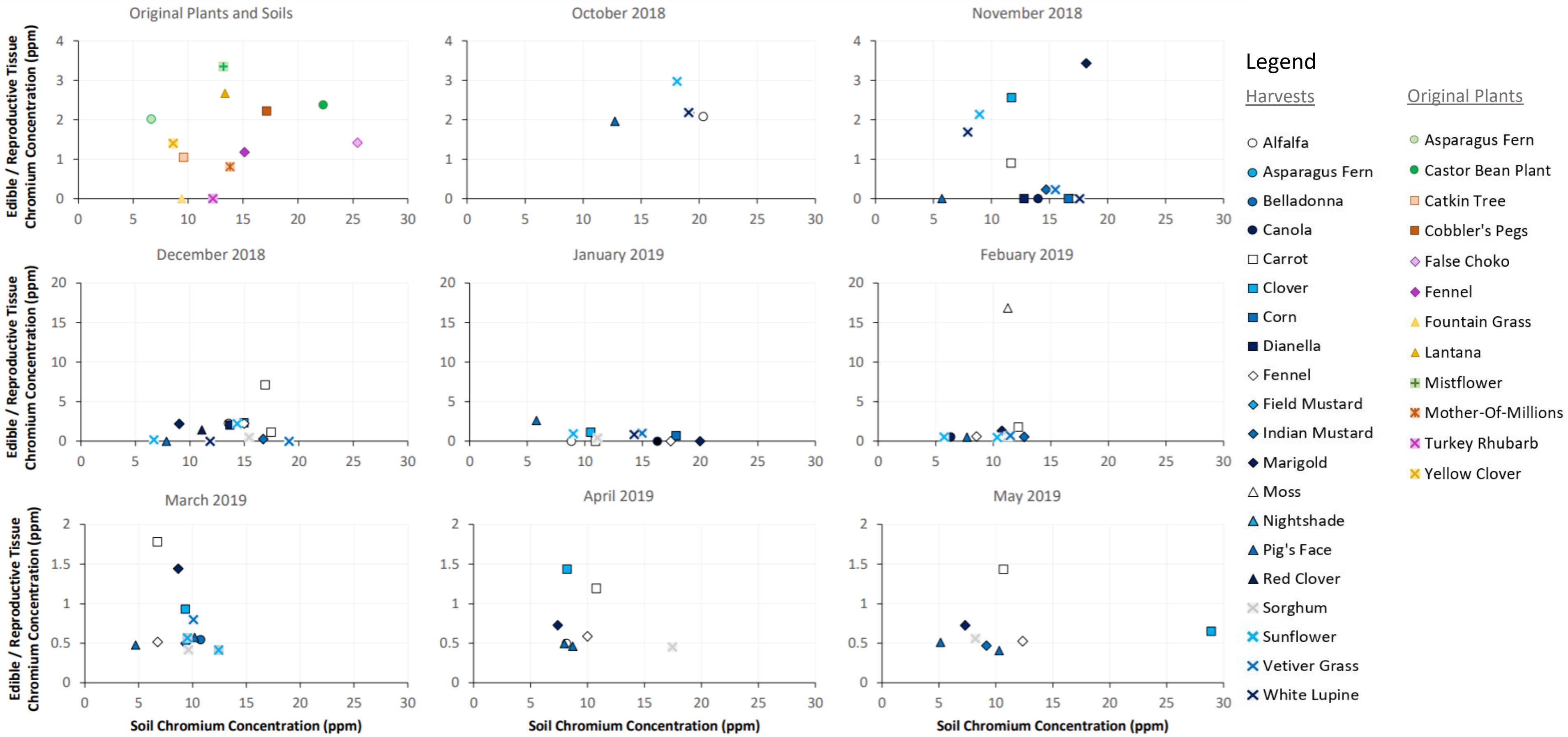


Figure 6.12. Relationship of edible or reproductive tissue concentration of chromium (ppm) and paired soil concentration (ppm). Tissues below detection limits are indicated as 0 ppm.

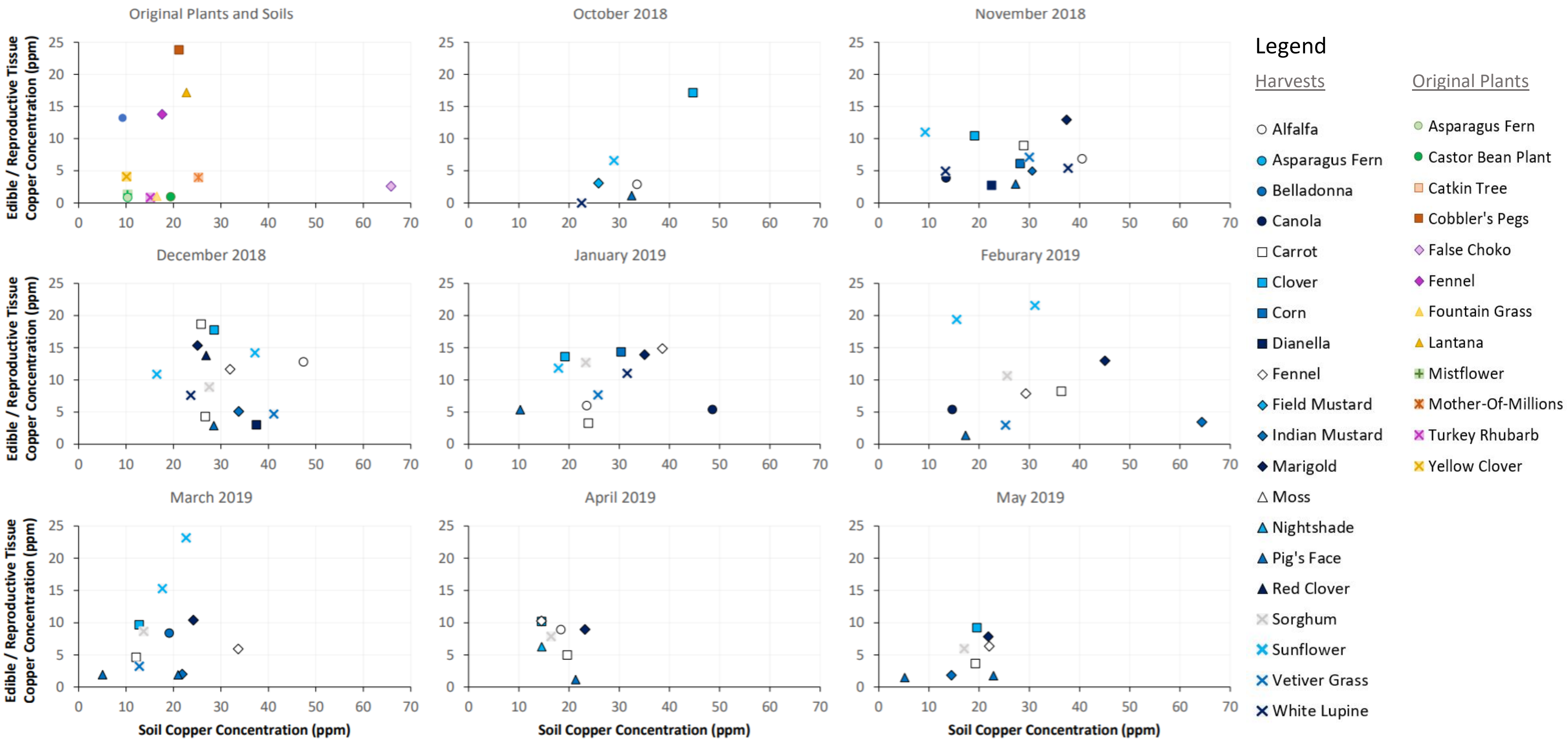


Figure 6.13. Relationship of edible or reproductive tissue concentration of copper (ppm) and paired soil concentration (ppm). Tissues below detection limits are indicated as 0 ppm.

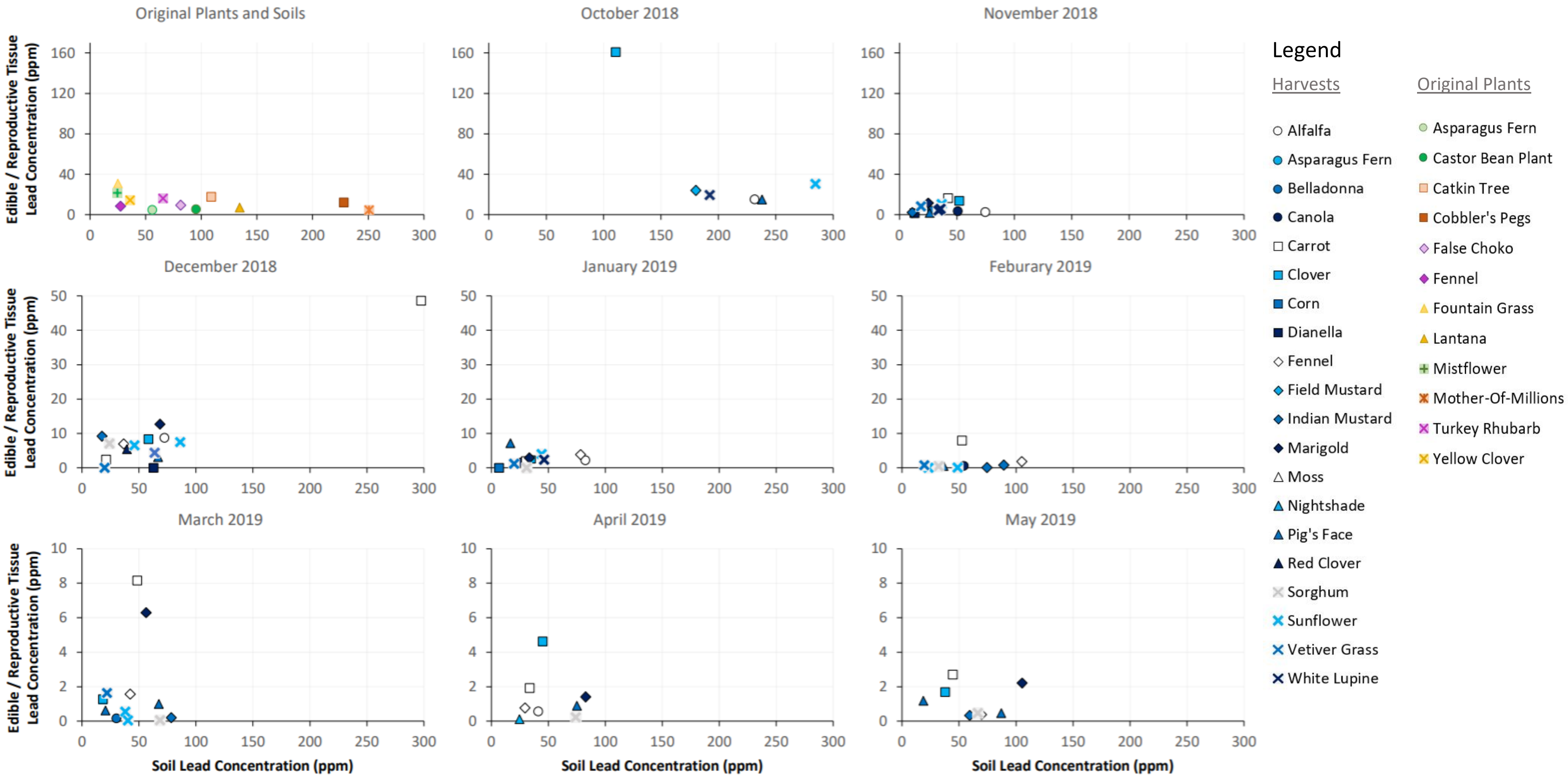


Figure 6.14. Relationship of edible or reproductive tissue concentration of lead (ppm) and paired soil concentration (ppm). Tissues below detection limits are indicated as 0 ppm.

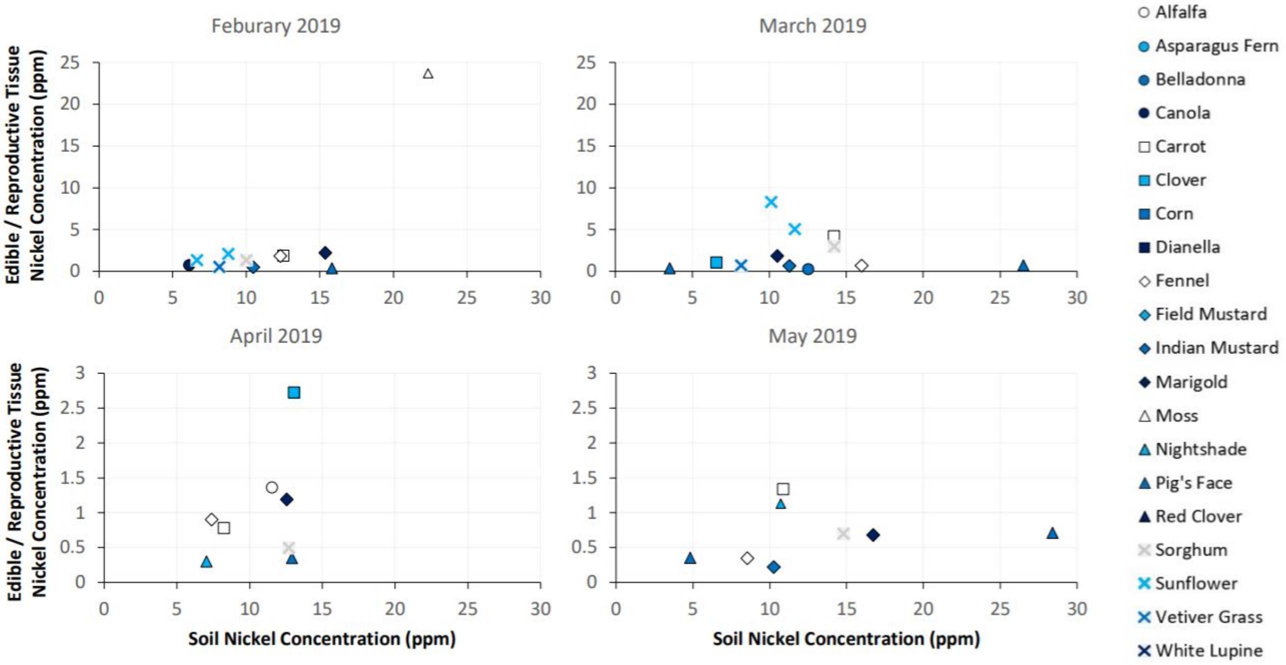


Figure 6.15. Relationship of edible or reproductive tissue concentration of nickel (ppm) and paired soil concentration (ppm). Tissues below detection limits are indicated as 0 ppm.

6.3.3 *Plant Compartmentalisation – Above vs. Below-ground Tissues*

Below-ground plant tissues (i.e., roots and carrot fruits) generally contained greater portions of heavy metals compared to the above-ground tissues. Phytostabilisation of heavy metals, particularly copper and lead, is observed in marigold followed by Indian mustard and sorghum (Fig. 6.16 – 6.17 and Appendices 6.3 – 6.7). In addition to these species, alfalfa, carrot, clover, corn, fennel, and sunflower were more likely to accumulate copper, and to a lesser extent lead, into their above-ground tissues (Fig. 6.16 – 6.18 and Appendices 6.3 – 6.7). All below-ground carrot fruits recorded over Australian guidelines for lead in edible vegetables (> 0.1 ppm; FSANZ 2016).

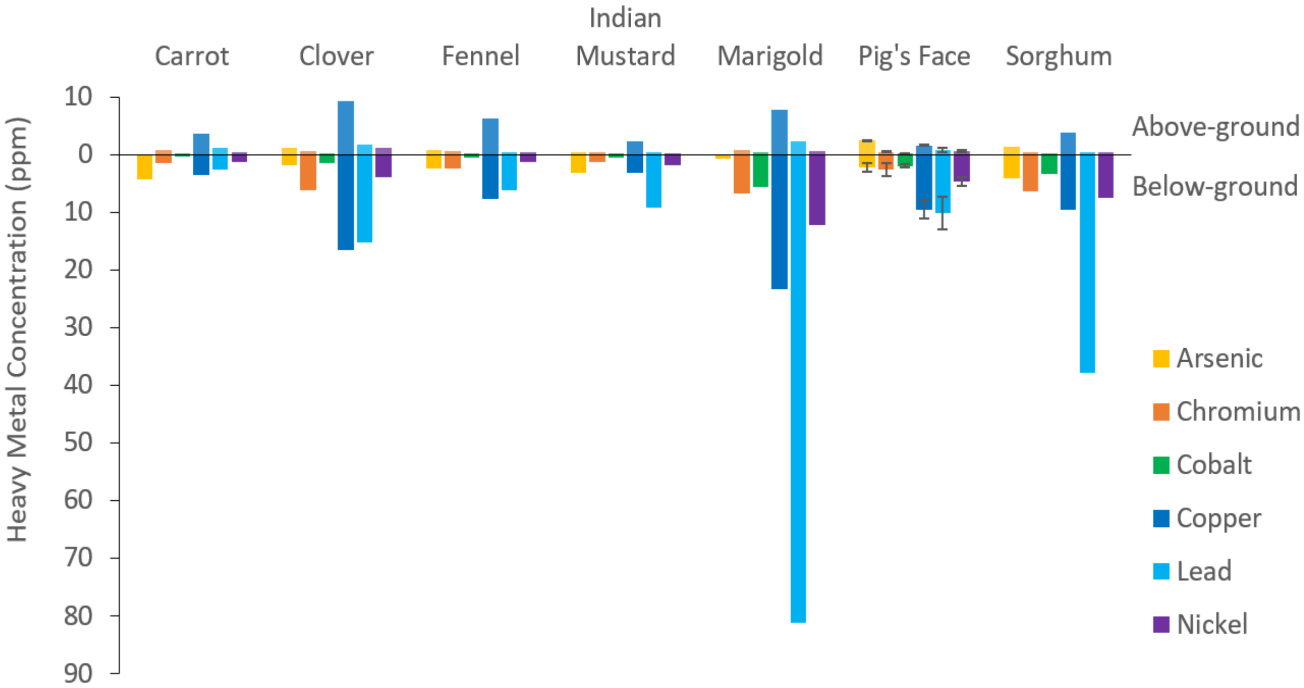


Figure 6.16. Heavy metal concentrations in below-ground and above-ground tissues (ppm) of plants harvested in May 2019. Individuals of the same species harvested from different garden plots are indicated by mean ± SEM.

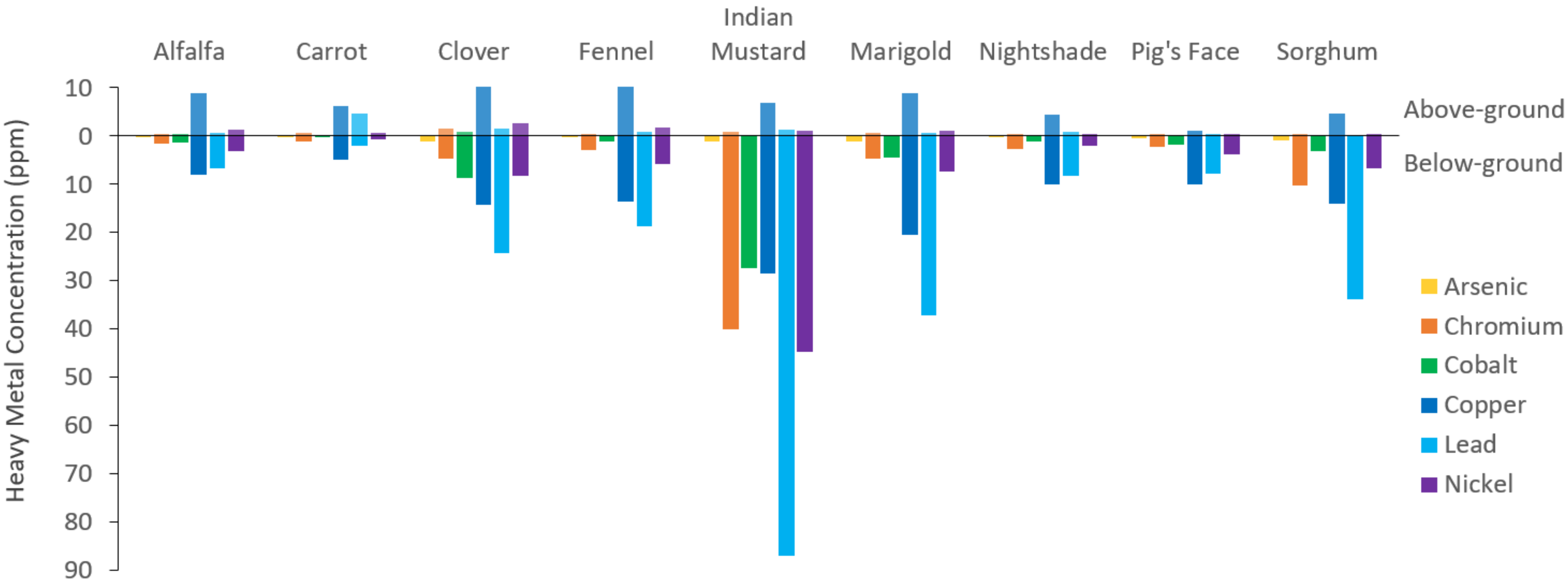


Figure 6.17. Heavy metal concentrations in below-ground and above-ground tissues (ppm) of plants harvested in April 2019.

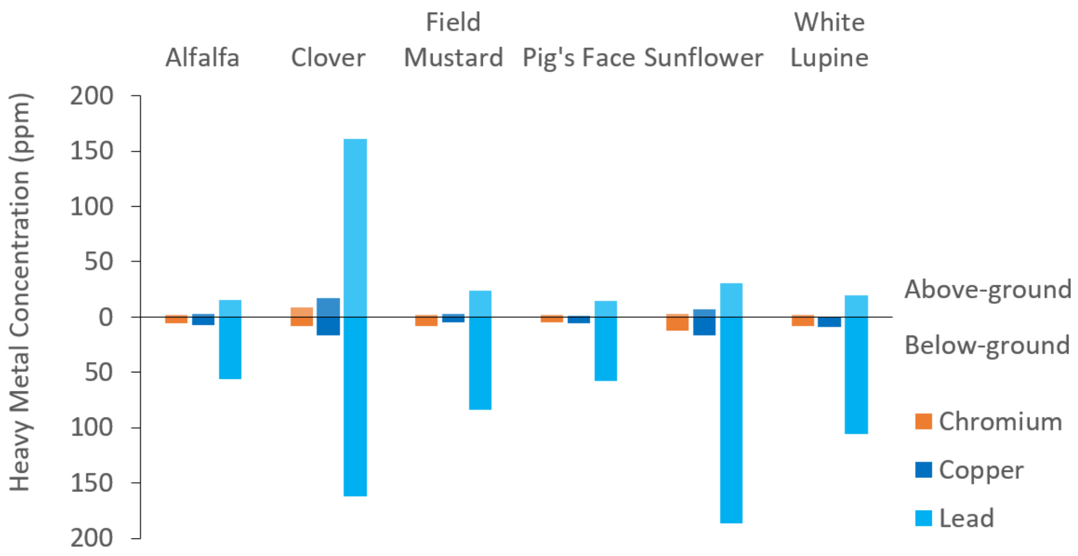


Figure 6.18. Heavy metal concentrations in below-ground and above-ground tissues (ppm) of plants harvested in October 2018.

6.3.4 *Garden Growth*

Over the course of the experimental trial, the garden became thick with several aggressive invasive plant species that had established themselves possibly from the existing seed bank of the site as well as wind vectors. Some garden plots were more prone to water logging or conversely exhibited tightly bound clay surfaces that made some sections more difficult for plants to establish. Ecological signs of garden health were evident with observations of birds and insects including bees, butterflies, moths, lady beetles, slugs, spiders, and worms.

Figure 6.19. White Bay Power Station with phytoremediation garden in the foreground**.**

6.4 Discussion

Phytoremediation can take up to 5 years or more for full results to be achieved (Kennen & Kirkwood 2015). The performance of *Power Plants* over its 9-month garden life is promising for future application of phytoremediation in industrial brownfields containing heavy metal contaminated soils. Plants were able to decontaminate arsenic from soils over this relatively short timeframe. Moreover, plants compartmentalised accumulated arsenic into their edible or reproductive tissues (Fig. 6.11). Arsenic concentrations in these tissues exceeded Australian food guideline levels (FSANZ 2016) for carrot, clover, pig's face, and sorghum in the final month. Chemically, arsenate resembles similarities to phosphate (Pigna *et al.* 2009) and mobility of arsenic into plants from soils may be attributed to phosphates replacing arsenic at soil binding sites (Cao *et al.* 2003). This provides important context for future avenues of investigation into the potential of arsenic phytoremediation on agricultural or urban garden soils where phosphorus is applied as fertiliser.

Soil decontamination of other heavy metals may have required a longer timeframe as average soil concentrations did not decrease significantly over time. Relationships between individual heavy metal concentrations varied with approximately only half of correlations between As, Cd, Cr, Co, Cu, Pb and Ni recording statistical significance (Fig. 6.10). This suggests that the distribution of metals was not homogenous across the site and the presence of one metal did not reliably predict presence of any other metal. Mean concentrations of lead were unexpectedly higher (by approximately 150 ppm) in soils sampled in the first harvest of October 2018. It is possible that point sources of lead were present within the soils of clover and sunflower species sampled during this month (Fig. 6.18). Recent findings from a pot experiment by He *et al.* (2020) suggest that the evolution of soil metal concentration over time is not as indicative of decontamination as calculating metal uptake in plant tissues. These conclusions could be important in the context of field experiments where the potential area of contaminant movement is greater than in contained pot experiments. The marginal downward trend of copper in the system, for example (Fig. 6.13), could be due to leachate possibly caused by heavy irrigation on some garden beds.

Despite limited overall soil decontamination indicated by varying soil results, plant tissue accumulation shows that plants were interacting with all heavy metals via mechanisms of phytostabilisation or phytoaccumulation. Above-ground accumulation was greatest for copper, followed by lead, and to a lesser extent, chromium, nickel, and cobalt for most species. Lead has no known biological function and is considered less mobile in plants (Pulkownik 2000; Forte & Mutiti 2017) while copper may be more mobile due its role as an essential nutrient in plant function and health (Feigl *et al.* 2013).

Marigold (*Tagetes tenuifolia*) accumulated and translocated heavy metals into above-ground tissues in the order of soil > roots > leaves > flowers. In the final harvest (May 2019), marigold recorded a large proportion of lead in its roots (81 ppm) suggesting phytostabilisation processes (Fig. 6.16). Meeinkuirt *et al.* (2019) found that marigold cultivars were similarly very effective phytostabilisers of cadmium in soils. Accelerated accumulation of cadmium, chromium, lead, and nickel by marigolds is also observed when plants are assisted with application of EDTA (ethylenediaminetetraacetic acid) or bacterial inoculum that convert metal ions into bioavailable forms for uptake (Yousra *et al.* 2020). Chelating agents like EDTA could be trialled to enhance heavy metal uptake by plants but increasing the mobility of heavy metals risks leachate movement into nearby Sydney Harbour (Ebrahimi 2016).

According to Peer *et al.* (2003) about 25% of known hyperaccumulators belong to the Brassicaceae family and studies affirm their ability to phytoremediate heavy metals and pharmaceutical drugs (Rahman *et al.* 2013; Gahlawat & Gauba 2016). Gisbert *et al.* (2006) found accumulated levels of cadmium and lead in above-ground tissues of cabbage (*B. oleracea*) posed health risks to humans and livestock. This study found greater concentrations in soils. A multi-elemental hotspot was discovered within the roots of Indian mustard harvested in April 2019 with limited above-ground accumulation (Fig 6.17). A limiting effect on heavy metal accumulation can be due to metal ion interactions in multi-contaminated soils (Israr *et al.* 2011) or when concentrations exceed a plant's exclusion tolerance (Gisbert *et al.* 2006). This may clarify low-level above-ground accumulation in our results for Indian mustard (*B. juncea*) and field mustard (*B. rapa*).

Characteristically tolerant in contaminated media (from results presented in Chapter 3), carrots (*Daucus carota*) established with great success providing an opportunity to harvest most months without overharvesting. All carrot fruits contained lead in concentrations over Australian food standards (> 0.1 ppm; FSANZ 2016). In lower soil concentrations of 30 - 33 ppm (January 2019 and April 2019), fruits contained 1.9 ppm of lead. December 2018 soil concentrations of 297 ppm Pb corresponded to 49 ppm in the carrot fruit (Fig. 6.14). While not a residential site, this result is interesting in the context of Australian residential soil guidelines where 297 ppm of lead is closely within acceptable limits (NEPM 2013) but poses a significant risk to carrot food safety. From a remediation viewpoint, the results highlight that carrots are an easily harvestable species effective in phytostabilising lead from contaminated soils.

The hyperaccumulating abilities of sunflowers (*Helianthus annuus*) are well documented (Rahman *et al.* 2013; Forte & Mutiti 2017). Sunflowers are excellent phytoremediation candidates because they are tolerant in a range of heavy metals (Rizwan *et al.* 2016), have large biomass for contaminant extraction (Alaboudi *et al.* 2018), and can secrete organic acid through roots thereby lowering soil pH which mobilises contaminants (Rahman *et al.* 2013). In our study, sunflowers became a hallmark feature of the garden from an architectural and artistic standpoint (Fig. 6.19; Appendix 6.2), however did not accumulate substantial amounts as expected. In contrast to results from Rahman *et al.* (2013), we found a greater portion of contaminants were held in roots rather than translocated to above-ground tissues.

Unexpectedly, soil heavy metal concentrations were within Australian guidelines for residential soils which were well below values for industrial soils (Table 6.1; NEPM 2013). Considering the garden was not accessible to the public, there are low risks to humans from heavy metals accumulated in plants. Some edible tissues and flowers recorded values of cadmium and lead over guidelines for foods (FSANZ 2016). These above-ground tissues are more likely to pose bioaccumulation risks to herbivores and pollinators (Devkota *et al.* 2000).

‘Real-world’ application of remediation solutions like phytoremediation are important for progressing and testing viability of these technologies despite an unavoidable range of experimental variables. Striking a balance between overharvesting and collection of plants for improved replication is one of the challenges of field trials where multiple species are applied. While it was valuable to sample a large range of species, low replication across species is a limitation of this study. Other limitations include the change in analytical equipment and restricted accessibility to collect replicates from the middle of garden beds during later months. This may overrepresent edge effects in the data.

Predictably, soils at White Bay Power Station had an uneven distribution of contaminant and plant growth, however most species thrived in the multi-contaminated brownfield environment. In addition to heavy metals, plants may have been tolerating, accumulating, or excluding other toxic contaminant types present onsite including polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB), and total petroleum hydrocarbons (TPH) (JBS&G Australia Pty Ltd 2015). Assessing the extent and effect of plants that remediate other contaminant types in tandem with heavy metals is an area for future research for phytoremediation of multi-contaminated brownfields. While average soil heavy metal concentrations at White Bay Power Station were within Australian residential guideline levels, it is recommended the site remain closed to the public given the presence of these other hazards.

6.4.1 Conclusion

Power Plants was a unique opportunity for exploratory analysis of *in situ* levels of heavy metals on an Australian urban brownfield site of heritage significance. Plants decontaminated soils of low-level arsenic and compartmentalised it into plant tissues, including their edible or reproductive tissues. Human risks of exposure to edible portions of plants are low however some concentrations of arsenic and lead in these tissues exceeded Australian food standards (FSANZ 2016), posing new questions of bioaccumulation risks for herbivores and pollinators.

Extraction of heavy metals by plants at White Bay Power Station signals the future for brownfield phytoremediation projects in Australia, particularly for heritage-listed sites where traditional methods of soil decontamination (e.g., excavation, soil washing) are not suitable. Future directions could incorporate chelating agents to increase metal bioavailability and uptake into plants, a focus on native Australian species that are adapted to local climates, and a longer experimental timeframe where perennial species may be applied and monitored.

CHAPTER 7

SYNTHESIS

Experimental Stir Fry

7.1 Were the aims of this thesis met?

This thesis took an exploratory approach to understanding phytoremediation potential of edible plant species within an Australian context. Broadly, research was conducted with a two-way outcome in mind for edible phytoremediators. The first, as incidental accumulators of existing heavy metals in urban and agricultural gardens where they may pose risks to food safety, and the second, as potential candidates in remediating heavy metal contaminants from degraded sites. This thesis met its primary objective of investigating accumulation patterns of heavy metals into edible garden plants via the following specific research aims:

1. Investigate the application of edible crop species in heavy metal phytoremediation projects in Australia to inform suitable species selection.

In Chapter 2, compilation and analysis of a phytoremediation dataset of 70 culinary species from 25 taxonomic families identified a research gap of edible plants tested in Australian environments for the purposes of phytoremediation. To address this research gap, a field study was conducted on edible phytoremediators applied on a heritage-listed site of national significance, discussed in Chapter 6. This was the first major phytoremediation trial of this type and scale in Australia. Edible species applied *in situ* on the ground of the White

Bay Power Station in Rozelle, NSW, were found to accumulate a range of heavy metal contaminants with relative success given the short-term 9-month timeframe. Opportunistic sampling provided an opportunity to test a range of species with lower replication to reduce overharvesting of the garden. Nevertheless, suitable species for further investigation were identified. Accumulation in root tissues of marigold, Indian mustard and sorghum indicated phytostabilisation potential for these species. Notably, arsenic concentrations decreased in soils and increased in the edible tissues of plants over a 4-month timeframe exceeding Australian food standards in the edible tissues of carrots, clover, pig’s face and sorghum (> 1 ppm; FSANZ 2016). All below-ground carrot tissues exceeded Australian and European Union standards for lead in edible vegetables highlighting carrots as possible food hazards in contaminated soils but noteworthy accumulators for remediation purposes. Results from Chapter 6 have set a precedent for future phytoremediation projects in Australia including a follow-on phytoremediation vegetable garden planned to be implemented on a residential site in an industrial area of Newcastle, NSW (Murray *pers. Comm.* 6 July 2020).

2. Determine the extent of heavy metal translocation, and sites of accumulation, of lead in above-ground and edible tissues of common groups of garden plants grown under a controlled glasshouse experiment.

Chapter 4 presents findings from a glasshouse experiment, where five commonly cultivated crop species were compared for their accumulation of lead in concentrations derived from the residential guideline level for soils in Australia. The concentration of lead was chosen in consideration of the knowledge gap identified in Chapter 2 where no phytoremediation research had been conducted on edible species in an Australian context. Novelty of this work was demonstrated by comparing lead accumulation in upper and lower positioned stem, leaf, and fruit tissues of chilli pepper and tomato plants, as well as fallen brown leaves and green leaves in common bean species. Meeting the requirements of this research aim, significant differences in metal accumulation patterns were detected between species tissue types (i.e., leaves, stems, edible tissues, roots), however no difference was found between upper and lower tissue locations. Radishes posed the greatest risk to food safety showing far greater accumulation of lead than all other study species, followed by carrots. All edible tissues in carrots and radishes recorded lead levels over Australian and New Zealand food standards (FSANZ 2016) and lead levels were also exceeded in 81.2% of beans, 80.0% of chilli peppers, and 86.7% of tomato fruits grown in these conditions. These proportions suggest that edible plants, particularly root vegetables, may be a food safety risk to the 40% of Sydney home gardeners and the 21% of Melbourne home gardeners with lead content exceeding 300 ppm detected by Rouillon *et al.* (2017) and Laidlaw *et al.* (2018), respectively.

Differences in root vegetable accumulation could relate to an association found in the literature synthesis dataset of Chapter 2, where the culinary type of edible tissue was linked to its corresponding concentration of contaminant within its edible portions, exceeding Australian safe standards for foods. There is opportunity to investigate this relationship further with a greater range of root vegetables and culinary types for their phytoremediation performance and advise accordingly for remediation or edible garden settings.

Not only were specific sites of accumulation identified in this thesis, but a noteworthy accumulation pattern was found in common bean species where brown leaves contained significantly elevated concentrations of lead compared to green leaves. This discovery is important as risks to food safety were lowered by beans directing metals away from edible tissues, and into discardable leaf tissues. Chapter 3 results found that common beans were capable of germinating under lead contaminated environments. Under this context, common beans could present a cost efficient, easily applicable method of site assessment where beans sown from seed can be used as a relatively fast bioindication of lead contamination in urban gardens from heavy metal analysis of brown leaf tissue accumulation. Future research could target this possibility and carry it forward to other contaminant types, species and field conditions.

3. Explore the risk of existing heavy metal contamination in homegrown produce of urban Sydney gardens and reflect on current recommended safety levels, background contamination sources, and levels found in the glasshouse experiment.

An exploratory screening of heavy metal concentrations in the plants and soils of home gardens were presented in Chapter 5. Of the 9 home garden sites tested, 2 properties contained lead concentrations in soils over Australian health investigation levels. Lead contamination is highlighted as the most likely risk to food safety with positive correlations of soil to leaf, stem, and root tissues. Residences, including the childcare centre, that used raised garden beds with imported soil and barrier plants posed low risk of heavy metal contamination in fresh produce. In the absence of root vegetable donations from participants, leafy herbs presented greater risk of heavy metal accumulation. Every residence that donated mint had edible leaf sections containing lead over Australian standards for food. Positive correlations in mean soil concentrations between heavy metal contaminants emphasises that where heavy metal contamination occurs in home garden soils, it is likely to be multi-metal contaminated. Examined in Chapter 3, this has implications for plant establishment and germination response for urban growers who direct seed their crops.

Reflecting on current guidelines for heavy metals in soils and food, the results from this work have exposed additional questions regarding the suitability of Australia’s soil guidelines. There was a high frequency of unsafe concentrations, particularly lead, in edible plant sections found in domestic gardens and the controlled glasshouse experiment. While guidelines outlined in the NEPM (2013) incorporate bioavailability and site-specific features, these results uncover a need for further evaluation of the lack of overlap between soil guidelines and food safety. Referring to international guidelines for heavy metals in soils may assist in updating Australia’s permissible limits given the noteworthy discrepancy between the two. Australia and New Zealand’s food standards for arsenic, cadmium, lead, mercury, and tin are in line with international standards but it is unclear why guidelines have not been established for all other heavy metals. Given elements like copper, nickel and zinc can occur at elevated concentrations in the environment and in edible phytoremediator plants, it is an important future direction to derive safe levels for these in foods.

4. Explore the effects of single and multi-metal contamination on the germination of edible plant species seeds.

This final aim was met in Chapter 3 by comparing seed survival and germination rates under single and multi-metal contamination of copper, lead and zinc. Species and heavy metal treatments did not consistently predict germination success across all 8 species tested. Further exploration of the effects of multiple contaminants and toxicity from their anions on germination in field environments is an important future direction of this work. While onset and duration were affected in all treatments for carrots, these species were the only crop able to germinate under multi-contaminant effects. Given results from Chapters 4 and 6 highlight carrots as accumulators of lead into edible tissues, this raises food safety concerns for carrot crops that are able to establish in multi-metal contaminated soils.

7.2 Safe Gardening

Don't Pardon Your Garden

This research encourages safe gardening practices as a critical step in supporting local biodiversity, contributing to food security, and rehabilitating the loss of connection to nature that arguably enabled environmental degradation in the first instance. While the focus of this research was on phytoremediation of edible plants rather than gardening practices specifically, aspects of this work add to recommendations made by other authors for the mitigation of heavy metal deposition in soils. The soil used in Chapter 4 was selected because it is widely commercially available and a convenient option for home gardeners. Results from control plants grown in this experiment confirm that replacing residing soil with store bought soil (Rouillon *et al.* 2017) is a safe alternative for gardeners who engage in guerrilla gardening or live-in areas affected by heavy metal contamination. Furthermore, tests of home gardens in Chapter 5 reinforce recommendations from Rouillon *et al.* (2017) that raised garden beds, positioning gardens away from driveways and busy roads, or using barrier hedge plants and mulch layers are effective strategies in mitigating heavy metal deposition in domestic gardens.

7.3 Edible Outlooks and the Ongoing Search for Fantastic Plants

Room to Grow

Ecological restoration and human health are intertwined (Breed *et al.* 2020), and economically viable environmental remediation strategies are paramount to creating a healthier, more ecologically connected future. There is no one silver bullet for ecological rehabilitation but phytoremediation has the potential to provide a unique, environmentally friendly and cost-efficient part of a wider solution to Australia's land contamination.

While not a new science, the application of phytoremediation in Australia is still in its infancy and the search for suitable phytoremediating plants remains ongoing. Important future directions of this work can entail investigation of safe soil to edible tissue thresholds for different edible types (e.g., root vegetables, fruiting vegetables, legumes) and contaminant classes outside heavy metals and metalloids. Some authors incorporate average daily intake risk assessments (e.g., Roy & McDonald 2015) but because home gardens are varied in crop yield and species type (as demonstrated in Chapter 5), a targeted approach for daily intake of specific types of edibles will provide a more meaningful snapshot of contaminant dose ratios. Targeted daily intake could be added in a survey design like those used by Rouillon *et al.* (2017) who captured information about house age and garden location in their analysis of home garden soils. Furthermore, in larger studies there is opportunity to assess relationships between socio-economic status and edible plant contamination to encompass broader issues of environmental justice.

Some limitations are to be considered in the interpretation of results from this research. Firstly, follow-up studies assessing the effect of increased anion related stress on germination would better substantiate germination responses of edible species to heavy metal anions (Chapter 3). This study used a mix of nitrate and sulphate anions for heavy metal salt additions that resulted in an additional experimental variable which should be explored. This could be evaluated in larger laboratory experiments with greater seed quantities and applied in field studies where salinity is naturally high due to contamination.

The work in this thesis found accumulation differences of lead in young and old leaf tissues of bean species which provides an exciting opportunity for further exploration of other species with similar abilities and evaluation of their efficacy in site assessments as cost effective bioindicators. Future glasshouse experiments are required to understand the underlying mechanisms employed by common beans and whether leaves could be mined for other heavy metals other than lead. This experiment may have been limited by the glasshouse environment where micro-climates could have affected biomass of individual plants. Considering this, a greater number of climate variables could be controlled in pot and germination studies by using growth cabinet technologies in future.

With greater replication and deeper exploration of soil-chemistry, mechanisms employed by plants in arsenic movements from soil to edible plant tissues at White Bay Power Station are needed to understand the underlying potential of these findings. Latter months of sampling were limited by overgrowth of the garden where results may overrepresent edge effects. Greater consistency in irrigation, species, and equal edge to centre garden plot harvesting would be recommended for similar field trials.

In exchange for a technology that is low in cost and more environmentally sustainable than traditional methods, a major drawback of phytoremediation is that it can take years for full site remediation results to be accomplished. Phytoremediation is a long-term strategy and time is a major factor in the outcome of accumulation patterns in edible species. Longer-term field trials in Australia will help to gain a deeper understanding of the role of time and its influence on outcomes to food safety.

7.4 Final Conclusions

The work presented in this thesis contributes to the ongoing search for crop species that are either efficient phytoremediators of heavy metal contaminants or in agricultural contexts, pose a low risk of accumulation into edible tissues. Edible species are among known effective phytoremediators and their application could be part of the solution to rehabilitating contaminated environments in Australia with added aesthetic and biodiversity benefits. Using a combination of field, glasshouse, laboratory and desktop studies, this thesis identifies edible crop species that pose health risks to urban garden growers where heavy metal contamination reflects Australian guideline values. In addition, species with potential in becoming part of the solution to heavy metal contaminated environments were identified where plant accumulation patterns indicated low risk to food safety. I believe this thesis provides a meaningful contribution to public food safety and the emerging field of phytoremediation in Australia.

APPENDICES

Appendix 2.1. Summary table of the dataset of edible phytoremediators.

Genus	**Species**	**Family**	**Common Name**	**Edible Type (colloquial)**	**Contaminants tested (multiple lines for more than one reference)**	**Contaminant Concentrations**	**Scale of Phyto Potential**	**Exceeds Food Standards in Edible Tissue?**	**In situ or Ex situ?**	**Scientific Reference/s**
Allium	*cepa*	Amaryllidaceae	Onion	Root Vegetable	Cd, Co, Cu, Ni, Pb and Zn As, Cd, Pb	0.05 and 0.25 mM As, Cd, Pb at background levels	High	Yes	Both	Soudek et al. (2009); Islam et al. (2016)
Allium	*porrum*	Amaryllidaceae	Leek	Vegetable	Cd, Co, Cu, Ni, Pb and Zn	0.05 and 0.25 mM	High	Yes	Ex situ	Soudek et al. (2009)
Allium	*sativum*	Amaryllidaceae	Garlic	Root Vegetable	Cd, Co, Cu, Ni, Pb and Zn	0.05 and 0.25 mM	High	Yes	Ex situ	Soudek et al. (2009)
Allium	*schoenoprasum*	Amaryllidaceae	Chives	Herb	Cd, Co, Cu, Ni, Pb and Zn Cd	0.05 and 0.25 mM Cd (0, 15, 30, 60, and 120 mg/kg soil)	High	Yes	Ex situ	Soudek et al. (2009); Eisazadeh et al. (2018)
Ananas	*comosus*	Bromeliaceae	Pineapple	Fruit	Textile Dye Basic Blue 3 & Congo Red	20 ml of dye and 0.03 g of ground pineapple stem	N/A	No	Ex situ	Chan et al. (2016)
Apium	*graveolens*	Apiaceae	Celery	Vegetable	Cd with help from fungi and EDTA	Cd (0, 5, 10, and 20 mg/kg soil)	Moderate	Yes	Ex situ	Anju et al. (2015)
Arbutus	*unedo*	Ericaceae	Strawberry tree	Fruit	As, Cd, Cu, W, Zn, Al, Fe and Pb	As (158-7,790 mg/kg), Cd (0.6-79 mg/kg), Cu (51-4,080 mg/kg), W (19-1,450 mg/kg) and Zn (142-12,300 mg/kg)	Low	Partly	In situ	Abreu et al. (2014)
Avena	*sativa*	Poaceae	Oat	Grain	Cd, Cr, Ni and Pb	Clay soil: Cd 6.81, Cr 137.3, Ni 104.2, Pb 126 Silt soil: Cd 7.81, Cr 331.8, Ni 148.5, Pb 270 (mg/kg)	Moderate	Yes	Ex situ	Mahmood-ul-Hassan et al. (2017)
Beta	*vulgaris*	Amaranthaceae	Beetroot	Root Vegetable	Cd	Cd (2.82 to 3.17 mg/kg)	Mid-high	Yes	In situ	Song et al. (2012)
Brassica	*oleracea*	Brassicaceae	Cabbage	Leafy Vegetable	Methyl bromide Se Tl Cd, Pb	Se (0, 5, 10 and 15 mg/kg) Tl (0.56 mg/kg) (Thallium removed 101-192 mg/kg mostly in the leaves)	Low & High	No guidelines	Ex situ	McCutcheon & Schnoor (1997); Esringü & Turan (2012); Ning et al. (2015)
Brassica	*carinata*	Brassicaceae	Ethiopian Rape or Mustard	Leafy Vegetable	Cu, Fe, Mn, Pb, Zn	Area contaminated by decades of industrial activity in Valencia (soil metal concentrations up to: Cu 5500 mg/kg, Cd 64 mg/kg, Ni 1200 mg/kg, Pb 13,000 mg/kg, and Zn 11,500 mg/kg).	Low	Yes	Ex situ	Gisbert et al. (2006)

Brassica	*juncea*	Brassicaceae	Indian Mustard	Leafy Vegetable	Cd, Pb Aspirin and tetracycline Zn Cu Cd, Hg, and Zn Cr	The concentrations of aspirin and tetracycline varied from 0.5% to 7% calculated as percentage by volume. Total concentrations of copper removal ranged from 21.8 - 87.7 ug/kg in fresh soil and 21.2 - 69.3 ug/kg in aged soil. Mean tissue concentrations of toxins in plant biomass: Cd 1.28 (±0.01) mg/kg, Hg 0.33 (±0.01), Zn 143.5 (±11) Cr (0.15 and 0.3 mM K2CrO4, 5 days) alone and in combination with GABA (125 µM) 50 µM Pb(NO3)2 and 25 µM CdSO4	Moderate	Yes	Both	Chigbo & Batty (2013); Adediran et al. (2015); Gahlawat & Gauba (2016); Dalyan et al. (2017); Guarino & Sciarrillo (2017); Mahmud et al. (2017)
Brassica	*napus*	Brassicaceae	Canola	Leafy Vegetable	Cd, Cu, Ni, Pb, Zn and to a lesser extent As	Several metals ranging between 100 and 2000 mg/kg in soil.	High	Yes	Ex situ	Dhiman et al. (2016)
Brassica	*nigra*	Brassicaceae	Black Mustard	Leafy Vegetable	Cu - much stronger in roots	Cu 50, 100, 200, and 500 µM	Low	No guidelines	Ex situ	Cevher-Keskin et al. (2019)
Brassica	*rapa* subsp. *Narinosa* (Or var. *rosularis*)	Brassicaceae	Field Mustard; Tatsoi, Chinese flat cabbage; Turnip	Leafy Vegetable	Cu, Cd with bioaccumulation assistance from Methyl bromide	Cu 0, 40, 80, 120, 160 mg/kg & Cd 0, 3, 6, 9, 12 mg/kg	Moderate	Yes	Ex situ	McCutcheon & Schnoor (1997); Nakagawa et al. (2010)
Cannabis	*sativa*	Cannabaceae	Cannabis	Herb	Cd Chrysene and benzo[a]pyrene Zn, Cu, Cd, Pb, Ni Pb	Cd accumulation ranged from 230 to 3338 µg/g with different fertilizer mixes in the soil with 100 mg cadmium acetate/kg 50, 100, and 200 µg/g of Chrysene and Benzo were used with adult plants	High	Yes	Both	Campbell et al. (2002); Meers et al. (2005); Ahmad et al. (2015); Chandra et al. (2017)
Capsicum	*annuum*	Solanaceae	Chili Pepper	Fruit	Cu	0, 2, 4 and 8 mM CuSO4	Moderate	No guidelines	Ex situ	Ruscitti et al. (2017)
Celtis	*australis*	Cannabaceae	European Nettle Tree / Honeyberry	Fruit	As, Cd, Cu, Pb, Zn	As 202, Cd 4.4, Cu 119, Pb 471, and Zn 381 mg/kg (averages).	Low	Yes	In situ	Madejón et al. (2018)
Ceratonia	*siliqua*	Fabaceae	Carob tree	Legume	As, Cd, Cu, Pb, Zn	As 202, Cd 4.4, Cu 119, Pb 471, and Zn 381 mg/kg (averages).	Low	Yes	In situ	Madejón et al. (2018)
Cocos	*nucifera*	Arecaceae	Coconut	Fruit (nut)	Cr Metal ions with various modifications: Cd2+ Ag+ Hg2+ Cr3+	It started with 8.25 ppm Cr suspended in sea water, there were 5 different husk products used for filtration ranging from 15.49% to 54.93% after 24 hours. The reduction was greater after 48 hours ranging from 20.34% to 76.63% and ranging from 33.16% to 98.30% after 96 hours.	N/A	N/A	Ex situ	Parimala et al. (2004); de Sousa et al. (2010)

					Pb2+ Cu2+ Ni2+					
Coriandrum	*sativum*	Apiaceae	Coriander	Herb	As, Pb	As and Pb in control and in tailing soil was 0.27, 0.141, 1.77, and 0.35 ppm	Mid-high	Yes	Ex situ	Gaur et al. (2017)
Cucurbita	*pepo*	Cucurbitaceae	Zucchini (var. cylindrica) [Field Pumpkin]	Fruit	DDT	1000 g of dry DDT contaminated soil equating to 63.5–101.3 ng/g of DDT and 381.4–455.3 ng/g of DDE	High	No guidelines	Ex situ	Mitton et al. (2018)
Daucus	*carota*	Apiaceae	Wild Carrot	Root Vegetable	As, Cd, Pb Pb	Background concentrations Pb: 200 mg/l	Moderate	Yes	Both	Islam et al. (2016); Alvarado-López et al. (2019)
Elaeagnus	*angustifolia*	Elaeagnaceae	Russian Olive	Fruit	Total Petroleum Hydrocarbons	TPH 0, 5, 10, 20 g/kg	Moderate	No guidelines	Ex situ	Zhang et al. (2013)
Eruca	*vesicaria ssp. sativa*	Brassicaceae	Rocket	Leafy Vegetable	As, Pb	As 191 and Pb 1040 mg/kg	Moderate	Yes	Ex situ	Tang et al. (2018)
Ficus	*pumila*	Moraceae	Creeping Fig	Fruit	Atmospheric nitrogen dioxide (NO2)	Irradiated with ion beam (C-12(5+), C-12(6+), or He-4(2+))	Low	N/A	Ex situ	Takahashi et al. (2012)
Foeniculum	*vulgare*	Apiaceae	Fennel	Herb	Pb, & antimony (Sb). Dramatically high concentrations were also found for As, Cd, Cu, Mn, Ni, Sn and Zn	Wastes contained 139532 ± 9601 mg/kg (≈14%) Pb and 3645 ± 194 mg/kg (≈0.4%) Sb	High	Yes	In situ	Mykolenko et al. (2018)
Glycine	*max*	Fabaceae	Soybean	Legume	Cd	Cd 50.35 ± 2.87 mg/kg total, and 9.18 ± 0.54 mg/kg bioavailable CdCl2 (0, 25, 50 and 100 µM)	Mid-high	Yes	Ex situ	Rojjanateeranaj et al. (2017); Yang et al. (2017)
Hyssopus	*officinalis*	Lamiaceae	Hyssop	Herb	Polycyclic Aromatic Hydrocarbons (PAHs)	Total PAH range 66.42–88.13 mg/kg	Low	N/A	In situ	Ling (2013)
Hirschfeldia	*incana*	Brassicaceae	Shortpod Mustard	Leafy Vegetable	Cd, Cu, Ni, Pb, Zn Cu	Cd 64 mg/kg, Cu 5500 mg/kg, Ni 1200 mg/kg, Pb 13,000 mg/kg and Zn 11,500 mg/kg 355 mg/kg Cu in dry shoot matter	Low	Yes	Ex situ	Poschenrieder et al. (2001); Gisbert et al. (2006)
Hordeum	*vulgare*	Poaceae	Barley	Grain	Hg Pb	22.9 mg/kg Hg Hg accumulated in the shoots at a concentration of 0.76 mg/kg (from Almadén contaminated soil containing 32.16mg/kg of Hg) and 2.70 mg/kg (HgCl2 spiked Almadén soil containing 33.56 mg/kg of Hg)	Low	No for humans, yes for fodder	In situ	He et al. (2015)
Lactuca	*sativa*	Asteraceae	Red leaf lettuce	Leafy Vegetable	Cd, Cu, Cr, Mn, Ni, Pb, Zn Ibuprofen (phytotoxicity tested) As and Pb	Many metals in high concentrations including Pb ranging 345 - 1017 mg/kg Environmental representative concentration of ibuprofen (3 ng/g) Lettuce in waste water accumulated in the order of Cu > Cr > Ni > Zn > Pb > Mn > Cd	High	Yes	Both	Malandrino et al. (2011); Rede et al. (2016)

Lathyrus	*sativus*	Fabaceae	Grass Pea	Legume	Cd, Cu, Pb, Zn (Cd and Pb accumulator when grown with resistant bacteria)	Soil1: Cd <0.27, Cu 5.1, Pb 11, Zn 26 mg/kg Soil2: Cd 3.8, Cu 23, Pb 761, Zn 88 mg/kg Soil3: Cd 5, Cu 42, Pb 816, Zn 225 mg/kg Soil4: Cd 17, Cu 30, Pb 3044, Zn 250 mg/kg	High	Yes	In situ	Abdelkrim et al. (2019)
Lolium	*multiflorum*	Poaceae	Italian ryegrass, alfalfa	Fodder	Trichlorophenol Pb, Cd Terbuthylazine (TBA) in aqueous solution	TCP: 3 concentrations of 2, 4, 6 - trichlorophenol (1, 10, 100 mg/kg) were tested along with the control. Cd and Pb (0, 100, 200, 400, and 600 mg/g) 0.5, 1.0 and 2.0 mg/L TBA	High	Yes	Ex situ	Parrish et al. (2004); Ding et al. (2011); Salama et al. (2016)
Malus	*domestica*	Rosaceae	Apple (Golden Delicious and Jonathan)	Fruit	Tebuconazole fungicide	Active ingredients were sprayed in a mist, at a normal dose (ND) and double dose (DD), then tested over 2, 5, 7.5, 10, and 15 days	Low	No	In situ	Coman-Babusanu et al. (2019)
Medicago	*sativa*	Fabaceae	Alfalfa	Legume	Cd, Zn with oxytetracycline antibiotic (OTC) as an enabler for metal uptake Cd, Cu, Zn	OTC concentrations (0, 1, 5, and 25 mg/kg dry weight soil), and low dosage daily input of OTC 0.28 mg/kg soil. Background concentrations (Cd 3.73 ± 0.11, Cu 80.6 ± 0.9, and Zn 316 ± 2 mg/kg)	Moderate	Yes	Ex situ	Gao et al. (2013); Ma et al. (2016)
Melilotus	*officinalis*	Fabaceae	Yellow sweet clover	Legume	PAHs: Phenanathrene Benz[a]anthracene Chrysene Fluroanthene Pyrene Benzo[b]fluoranthene Benzo[k]fluoranthene Benzo[a]pyrene Benzo[g,h,i]perylene Indeno[1,2,3-cd]pyrene	Soil used from a former gold mine site in Bedford, IN. Total PAHs % removal over the course of 12 months was 9.1% compared to the 5% for the controls.	Low	N/A	Ex situ	Parrish et al. (2004)
Mentha	*arvensis*	Lamiaceae	Wild mint	Herb	Cd, Pb	30 and 60 mg/kg soil Cd & Pb. Original farm soil taken from field contained 0.022 mg/kg Cd, and 0.521 Pb	Moderate	N/A	Ex situ	Prasad et al. (2010)
Mentha	*× piperita*	Lamiaceae	Peppermint	Herb	Cd, Pb	30 and 60 mg/kg soil Cd & Pb. Original farm soil taken from field contained 0.022 mg/kg Cd, and 0.521 Pb	Moderate	N/A	Ex situ	Prasad et al. (2010)
Morus	*alba*	Moraceae	White Mulberry	Fruit	Cd, Cr, and Ni	Cd (40, 80, and 160 mg/kg), Cr (60, 120, and 240 mg/kg) and Ni (120, 240, and 480 mg/kg)	Moderate	Yes	Ex situ	Rafati et al. (2011)
Morus	*rubra*	Moraceae	Red Mulberry	Fruit	Fluoranthene and pyrene	103.5 and 83.3 mg/kg, respectively. Decreased to 28.0 and 18.0 mg/kg	High	N/A	Ex situ	Rezek et al. (2009)
Nicotiana	*tabacum*	Solanaceae	Tobacco (cultivar K326)	Herb	Cd, especially in its leaves. Cu, Pb, Zn, were also tested	Initial concentration in the mixture of tobacco leaves before extraction was Cd 18.2, Cu 16.8, Pb 7.0 and Zn 203 mg/kg.	Very High	Yes	In situ	Yang et al. (2019)
Ocimum	*basilicum*	Lamiaceae	Basil	Herb	Translocation of Cd nanoparticles	Compared CdSSe nanoparticles and ionic Cd solution at 25 mg/kg soil & 50 mg/kg soil	High	Yes	Ex situ	Alamo-Nole & Su (2017); Dinu et al. (2020)

					Cd, Co, Cu, Cr, Ni, Pb, Zn	Cd and Ni 5 mg/L, Co and Cr 10 mg/L, Pb 350 mg/L, and Zn 100 mg/L				
Olea	*europaea*	Oleaceae	Olive Tree	Fruit	As, Cd, Cu, Pb, Zn	As 202, Cd 4.4, Cu 119, Pb 471, and Zn 381 mg/kg (averages).	Low	No	In situ	Madejón et al. (2018)
Origanum	*vulgare*	Lamiaceae	Oregano (Greek ssp. hirtum)	Herb	Cr (VI)	Cr (50, 100, 150, 200 spiked field soil mg/kg)	Very High	Yes	Ex situ	Levizou et al. (2019)
Petroselinum	*crispum*	Apiaceae	Parsley	Herb	Pb	Pb (0, 200, 600, 1,000 and 1,200 mg/kg soil)	High	Yes	Ex situ	Saeed et al. (2015)
Phaseolus	*acutifolius*	Fabaceae	Tepary bean	Legume	Cs, Sr	Hazard Waste Management Plant soil concentrations of Cr 137 and Sr 90 mg/kg	Low	N/A	In situ	Fuhrmann et al. (2002)
Phaseolus	*vulgaris*	Fabaceae	Common Bean	Legume	As (excluder and has the potential for phytostabilization)	20 and 50 mg/kg of As (III), As(V) or DMA (dimethylarsinic acid)	Moderate	Yes	Ex situ	Saha & Pal (2018)
Pinus	*pinea*	Pinaceae	Stone Pine	Fruit (nut)	As, Cd, Cu, Pb, Zn	As 202, Cd 4.4, Cu 119, Pb 471, and Zn 381 mg/kg (averages).	Moderate	No	In situ	Madejón et al. (2018)
Pistia - Aquatic	*stratiotes*	Araceae	Water cabbage, water lettuce	Leafy Vegetable	Uranium (U) Cd Al, Ca, Fe, K, Mg, Mn, Na, Zn, Cd, Co, Cr, Cu, Ni, Pb Chemical oxygen demand (COD), Ammonium, Nitrates, Phosphates	U concentration range of abandoned mine pit water was 0.4mg/L - 0.6mg/L. The mean concentrations (dry weight) in roots were 1,015.40 mg/kg. Cd (5, 10, 15 and 20 mg/L) Stormwater detention ponds: Al 16 - 55 g/ha, Ca 357 - 546 g/ha, Fe 29 - 57 g/ha, K 344 - 853 g/ha, Mg 70 - 134 g/ha, Mn 5.3 g/ha, Na 138 - 370 g/ha, Zn 1.2 - 1.3 g/ha, Cd 4 - 11 g/ha, Co 4.9 - 10 g/ha, Cr 92 - 189 g/ha, Cu 107 - 336 g/ha, Ni 31 - 52 g/ha, Pb 51 - 100 g/ha. Phytofiltration lagoon: COD was in the range of 47.82 +/- 39.3% to 88.00 +/- 15.0%. Ammonium N 76.78 +/- 21% to 98.79 +/- 0.9%. Nitrates were removed in the range of 16.92 +/- 64%. to 97.14 +/- 4.5%. And Phosphates were removed very effectively, from 73.72 +/- 18.5% to 92.89 +/- 4.3%.	High	Yes	Both	Lu et al. (2011); Das et al. (2014); Nie et al. (2015); Olguin et al. (2017)
Pisum	*sativum*	Fabaceae	Pea	Legume	As, Cd, Cu, Pb, Zn	As 5.3 ± 0.064, Cd < 0.0048 (i.e., not detected), Cu 37 ± 0.85, Pb 35 ± 0.28, and Zn 300 ± 5.2 mg/kg	High	Yes	Ex situ	Hur & Jho (2017)
Prunus	*avium*	Rosaceae	Cherry, sweet cherry	Fruit	Methyl bromide Cu, Cr, Fe, Mn, Ni, Co, Pb, Zn	Cu 4.10 - 9.93 mg/kg, Cr 0.00 - 5.94, Fe 97.2 - 153.1, Mn 45.64 - 74.20, Ni 3.10 - 9.03, Co 0.00 - 3.89 and Pb 3.02 - 12.96, Zn 13.15 - 21.86.	High	Yes	In situ	McCutcheon & Schnoor (1997); Başar et al. (2009)
Prunus	*dulcis*	Rosaceae	Almond	Fruit (nut)	It wasn't shown to phytoremediate Cd. Toxicity was tested	CdCl2 (0, 25, 50, 100 and 150 µM)	N/A	N/A	Ex situ	Elloumi et al. (2014)

Quercus	*ilex*	Fagaceae	Holly Oak	Fodder	As, Cd, Cu, Pb, Zn	As 202, Cd 4.4, Cu 119, Pb 471, and Zn 381 mg/kg (averages).	Low	No	In situ	Madejón et al. (2018)
Raphanus	*sativus*	Brassicaceae	Radish	Root Vegetable	Ni Pb, Zn	Ni (0, 50, 100, 150 mg/kg soil) Pb and Zn (25, 50 and 100 mg/kg)	High	Yes	Ex situ	Akhtar et al. (2018); Chaturvedi et al. (2019)
Sedum	*plumbizincicola*	Crassulaceae	Stonecrop	Succulent herb	Cd, Zn with oxytetracycline antibiotic (OTC) as an enabler for metal uptake	OTC concentrations (0, 1, 5, and 25 mg/kg dry weight soil), and low dosage daily input of OTC 0.28 mg/kg soil.	High	Yes	Ex situ	Ma et al. (2016)
Solanum	*lycopersicum - (cultivars)*	Solanaceae	Pusa ruby & Arka vikas (Tomato)	Fruit	As, Pb with glutathione (GSH) and citric acid (CA) DDT	As 10 µM, Pb 10 µM, Pb 10 µM + GSH 250 µM, As 10 µM + GSH 250 µM, Pb 10 µM + CA 250 µM and As 10 µM + CA 250 µM for 7 days 1000 g of dry DDT contaminated soil equating to 63.5–101.3 ng/g dry weight DDT and 381.4–455.3 ng/g dry weight DDE	Low	N/A	Ex situ	Kumar et al. (2017); Mitton et al. (2018)
Solanum	*Nigrum*	Solanaceae	Black nightshade	Fruit	Cu, Cr, Fe, Mn, Ni, Pb, Zn	Sludge concentration and water extractable leachate respectively: Cd (0.255, 0.02), Cu (2.15, 0.09), Cr (2.30, 0.11), Fe (67.53, 1.05), Mn (11.0, 0.07), Ni (3.30, 0.19) and Pb (1.05), Zn (13.9, 0.27) mg/l	Moderate	Yes	In situ	Chandra et al. (2017)
Solanum	*tuberosum*	Solanaceae	Potato	Root Vegetable	As, Cd, Pb	Background field levels	Moderate	Yes	In situ	Islam et al. (2016)
Sorghum	*bicolor*	Poaceae	Sorghum	Grain	Cd, Cr, and Pb	Clay soil: Cd 6.81, Cr 137.3, Ni 104.2, Pb 126 Silt soil: Cd 7.81, Cr 331.8, Ni 148.5, Pb 270 (mg/kg)	High	Yes	Ex situ	Mahmood-ul-Hassan et al. (2017)
Silene	*vulgaris*	Caryophyllaceae	Bladder campion	Leafy Vegetable	Mercury (Hg)	Hg (0.6 and 5.5 mg/kg soil)	Low	N/A	Ex situ	Araceli et al. (2012)
Spinacia	*oleracea*	Amaranthaceae	Spinach	Leafy Vegetable	Lindane (organochlorine pesticide (OCPs) found in insecticide) Methyl bromide	Lindane (5, 10, 15 and 20 mg/kg)	Moderate	N/A	Ex situ	McCutcheon & Schnoor (1997); Dubey et al. (2014)
Stellaria	*media*	Caryophyllaceae	Common Chickenweed	Leafy Vegetable	Cd	Spiked as CdCl2·2.5H2O, final Cd concentrations were 10 mg/kg	Moderate	Yes	Ex situ	Lu et al. (2017)
Tagetes	*erecta*	Asteraceae	Marigold	Edible Flower	Cd, Cr, Ni, Pb	Cd 162, Cr 101, Ni 168, and Pb 15 mg/kg	Moderate	N/A	Ex situ	Yousra et al. (2020)
Theobroma	*cacao*	Malvaceae	Cacao (powdered for chocolate)	Fruit (nut)	Cd into cacao beans but mostly depends on cultivar	*in situ* levels ranged between 0.72 & 0.837 ± 0.2 mg/kg total Cd in soils	High	Yes	In situ	Engbersen et al. (2019)
Triticum	*aestivum*	Poaceae	Common wheat	Grain	Cu, Cr, Fe, Mn, Ni, Pb, Zn Ba, Cu, Pb, Zn and a small amount of explosive octahydro-1,3,5,7-	Sludge concentration and water extractable leachate respectively: Cd (0.255, 0.02), Cu (2.15, 0.09), Cr (2.30, 0.11), Fe (67.53, 1.05), Mn (11.0, 0.07), Ni (3.30, 0.19) and Pb (1.05), Zn (13.9, 0.27) mg/l	Moderate	Yes	In situ	Chandra et al. (2017); Groom et al. (2002)

					tetranitro-1,3,5,7-tetrazocine (HMX)	Soil at the Firing range contained Ba (100−120 ppm), Cu (790−1000 ppm), Pb (85−96 ppm), and Zn (100−120 ppm)				
Vaccinium	*Sp.*	Ericaceae	Blueberry	Fruit	Ba, Cu, Pb, Zn and a small amount of explosive octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)	Soil at the Firing range contained Ba (100−120 ppm), Cu (790−1000 ppm), Pb (85−96 ppm), and Zn (100−120 ppm)	Low	N/A	In situ	Groom et al. (2002)
Vicia	*faba*	Fabaceae	Fava bean	Legume	Cd, Pb	2 field sites: Cd in seeds was 0.12 mg/kg & 0.14; Pb 0.2 mg/kg & 0.39 (mean values)	Low	Yes	In situ	Tang et al. (2019)
Zea	*mays*	Poaceae	Corn	Grain	Methyl bromide, Pb Cd, Cu, Ni, Pb, Zn As	Pb: 800 mg $Pb(NO_3)_2$ Zn: 64 - 1800 mg/kg dry weight As ($Na_2HAsO_4·7H_2O$) at 0, 40, 80, and 120 mg/kg dry soil	Moderate	Yes	Ex situ	McCutcheon & Schnoor (1997); Meers et al. (2005); Hadi et al. (2010); Gheju et al. (2013); Mehmood et al. (2017)
Ziziphus	*mauritiana*	Rhamnaceae	Indian jujube / Chinese date	Fruit	As, Cd, Cu, Co, Cr, Fe, Mn, Mo, Ni, Pb, Se, Zn	Field concentrations. Fe followed by Mn > Se > Zn > Mo > Cu > Cr > Pb > Cd >Ni > As > Co in rhizospheric substrate on fly ash dump of a power station	Low	Yes	In situ	Pandey & Mishra (2018)

Appendix 4.1. Results from Chapter 4 modelling. All models using lead as a response were 5th root transformed prior to analysis.

Response	Model	Terms	SS	DF	F/χ^2	P
Lead ppm	General linear model	Species	15.239	4	17.979	< 0.0001
		Tissue type	197.116	3	310.087	< 0.0001
		Species x Tissue type	50.784	12	19.972	< 0.0001
		Residuals	56.787	268		
Lead ppm	General linear model	Species	60.197	4	59.011	< 0.0001
		Residuals	17.087	67		
Dichotomous (lead > 0.1 ppm)	Binomial	Species		2	0.255	0.9
Lead ppm	Linear mixed model	Species		1	23.357	< 0.0001
		Tissue type		2	62.365	< 0.0001
		Tissue location		1	3.521	0.06
		Species x Tissue type		2	3.493	0.2
		Species x Tissue location		1	2.2	0.1
		Tissue type x Tissue location		2	4.777	0.09
		Species x Tissue type x Tissue location		2	1.545	0.5
Lead ppm	Linear mixed model	Leaf colour		1	59.804	< 0.0001

Appendix 5.1. Residence 1, Northbridge: Edible plants and metals (FL = Flower, F = Fruit, L = Leaf, S = Stem, R = Root; **Bold red** = Exceeds Australian Health Investigation Levels (NEPM 2013), [a]Exceeds Canadian guidelines (CCME 2018), [b]Exceeds New Zealand guidelines (MFE 2013), [c]Exceeds EU Food guidelines (EU 2006)).

Donated Sample	**Location Collected**	**As (ppm)**	**Cd (ppm)**	**Cr (ppm)**	**Co (ppm)**	**Cu (ppm)**	**Pb (ppm)**	**Mn (ppm)**	**Ni (ppm)**	**Se (ppm)**	**V (ppm)**
Asparagus Fern plant	Front yard near driveway	F: 0.67 L: 0.38 S: 0.62 R: 0.85	**F: 0.28** L: 0.4 S: 0.3 R: 0.7	F: 0.53 L: 0.16 S: 0.67 R: 5.1	F: 0.05 L: 0.07 S: 0.05 R: 0.43	F: 9.66 L: 6.8 S: 5.96 R: 35.43	F: 0.08 L: 13.4 S: 22.57 R: 45.34	F: 23.34 L: 38.84 S: 5.64 R: 45.94	F: 0.35 L: 0.72 S: 0.38 R: 1.44	F: 0.89 L: 0.2 S: 0.87 R: 0.66	F: 0.08 L: 0.13 S: 0.13 R: 1.57
Asparagus Fern soil	Front yard near driveway	3.5	1	61.03	2.03	**150.83**[a]	**508.3**	138.59	4.27	**1.39**[a]	11.57
Chives plant	Backyard Pot	L: < 0.5 ppb	L: 0.09	L: 0.47	L: 0.12	L: 5.41	**L: 57.42**	L: 50.77	L: 0.76	L: 0.33	L: 0.22
Mint 1 plant	Backyard Pot 1	L: 0.24 S: 0.29	L: < 0.5 ppb S: 0.02	L: 0.55 S: 0.99	L: 0.06 S: 0.05	L: 19.56 S: 9.48	**L: 1.74** S: 2.82	L: 59.18 S: 39.83	L: 0.7 S: 0.82	L: 0.1 S: 0.25	L: 0.16 S: 0.15
Mint 1 soil	Backyard Pot 1	2.18	0.17	7.92	3.94	27.12	**187.72**[a]	383.99	3.96	**1.38**[a]	8.25
Mint 2 plant	Backyard Pot 2	S: 0.41	S: 0.03	S: 2.24	S: 1.34	S: 26.5	S: 3.63	S: 83.34	S: 2.52	S: 0.52	S: 5.03
Mint 2 soil	Backyard Pot 2	3.21	0.21	9.96	5.63	52.9	70.18	376.58	7.18	**1.93**[a]	17.61
Mint 3 plant	Backyard Pot 3	L: 0.15 R: 1.26	L: < 0.5 ppb R: 0.23	L: 0.36 R: 3.77	L: 0.1 R: 2.09	L: 28.93 R: 23.01	**L: 16.71** R: 30.94	L: 84.38 R: 172.44	L: 0.85 R: 2.46	L: 0.21 R: 1.09	L: 0.27 R: 8.59
Mint 3 soil	Backyard Pot 3	2.89	0.16	5.67	5.66	24.65	60.27	461.65	4.44	**1.97**[a]	12.62

Mulberry plant	Large tree hanging over backyard fence	F: 0.23 L: 0.23 S: 0.23	F: < 0.5 ppb L: < 0.5 ppb S: < 0.5 ppb	F: 0.59 L: 0.55 S: 1.05	F: 0.06 L: 0.04 S: 0.04	F: 12.76 L: 11.89 S: 7.07	**F: 1.78** L: 1.09 S: 2.55	F: 51.79 L: 54.16 S: 28.3	F: 0.77 L: 0.89 S: 0.82	F: 0.16 L: 0.15 S: 0.3	F: 0.13 L: 0.06 S: 0.05
Sow Thistle plant	Backyard original soil bed next to house	FL: 0.23 L: 1.36 S: 0.34 R: 11.94	FL: 0.58 L: 1.81 S: 1.48 R: 1.6	FL: 0.46 L: 0.31 S: 0.79 R: 11.91	FL: 0.04 L: 0.07 S: 0.05 R: 1.08	FL: 19.28 L: 16.39 S: 5.1 R: 30.52	FL: 0.16 L: 11.79 S: 2.77 R: 143.79	FL: 19.66 L: 22.2 S: 11.48 R: 27.76	FL: 0.75 L: 0.4 S: 0.34 R: 2.31	FL: 0.2 L: 0.47 S: 0.24 R: 0.64	FL: 0.01 L: 0.16 S: 0.04 R: 2.63
Sow Thistle soil	Backyard original soil bed next to house	**35.22**[ab]	2.53	45.03	5.91	**107.26**[a]	**690.44**	173.15	16	**1.36**[a]	10.8

Appendix 5.2. Residence 2, Northbridge: Edible plants and metals (F = Fruit, L = Leaf, S = Stem, R = Root, rep = Replicate)

Donated Sample	Location Collected	As (ppm)	Cd (ppm)	Cr (ppm)	Co (ppm)	Cu (ppm)	Pb (ppm)	Mn (ppm)	Ni (ppm)	Se (ppm)	V (ppm)
Broad Bean plant	Median Strip, imported soil	F: 0.18 L: 0.20 S: 0.16 R: 0.72	F: < 0.5 ppb L: < 0.5 ppb S: < 0.5 ppb R: 0.02	F: 0.42 L: 0.71 S: 0.58 R: 2.83	F: 0.14 L: 0.17 S: 0.10 R: 0.41	F: 16.21 L: 11.14 S: 3.88 R: 10.92	F: < 0.5 ppb L: 0.64 S: 0.98 R: 2.40	F: 27.25 L: 66.08 S: 11.26 R: 37.30	F: 0.37 L: 0.76 S: 0.32 R: 1.00	F: 0.07 L: 0.07 S: 0.25 R: 0.23	F: < 0.5 ppb L: 0.30 S: 0.07 R: 1.97
Broad Bean mulch	Median Strip, imported soil	1.16	0.05	3.19	0.78	14.90	7.19	122.77	1.91	0.76	3.31
Broad Bean & Leek soil	Median Strip, imported soil	3.50	0.12	12.44	3.67	44.59	17.21	320.20	5.91	**2.10**[a]	16.84
Leek plant	Median Strip, imported soil	L: 0.11 S: 0.12 R: 6.25	L: < 0.5 ppb S: < 0.5 ppb R: 0.16	L: 1.15 S: 0.83 R: 20.44	L: 0.02 S: 0.09 R: 4.82	L: 9.81 S: 2.48 R: 57.95	**L: 0.41** **S: 0.50** R: 25.31	L: 40.43 S: 11.90 R: 418.77	L: 0.99 S: 0.38 R: 8.27	L: 0.13 S: 0.18 R: 2.44	L: < 0.5 ppb S: 0.10 R: 28.99
Strawberry plant	Median Strip, imported soil	F: 0.24 L: 0.40 S: 0.10 S rep: 0.20 R: 2.98	F: < 0.5 ppb L: < 0.5 ppb S: < 0.5 ppb S rep: < 0.5 ppb R: 0.12	F: 0.43 L: 0.74 S: 0.64 S rep: 0.62 R: 11.87	F: 0.02 L: 0.05 S: 0.08 S rep: 0.07 R: 2.05	F: 5.49 L: 5.09 S: 3.35 S rep: 4.76 R: 25.47	F: < 0.5 ppb L: 0.51 S: 0.63 S rep: 0.51 R: 29.33	F: 13.34 L: 23.46 S: 5.22 S rep: 14.52 R: 171.50	F: 0.31 L: 0.38 S: 0.31 S rep: 0.30 R: 3.84	F: 0.03 L: 0.07 S: 0.22 S rep: 0.12 R: 1.22	F: < 0.5 ppb L: 0.27 S: 0.14 S rep: 0.18 R: 16.10
Strawberry soil	Median Strip, imported soil	3.22	0.12	18.35	4.47	33.35	26.09	303.61	5.90	**1.65**[a]	22.78

Appendix 5.3. Residence 3, Northbridge: Edible plants and metals (L = Leaf, S = Stem, L+S = Pooled Leaf and Stem)

Donated Sample	**Location Collected**	**As (ppm)**	**Cd (ppm)**	**Cr (ppm)**	**Co (ppm)**	**Cu (ppm)**	**Pb (ppm)**	**Mn (ppm)**	**Ni (ppm)**	**Se (ppm)**	**V (ppm)**
Mint plant	Backyard, near house dripline	L+S: 0.78	L+S: < 0.5 ppb	L+S: 0.73	L+S: 0.08	L+S: 14.51	**L+S: 0.46**	L+S: 50.03	L+S: 0.56	L+S: 0.07	L+S: 0.20
Mint soil	Backyard, near house dripline	4.83	0.22	11.94	3.46	**77.09**[a]	30.12	181.80	6.28	**2.06**[a]	18.33
Rosemary plant	Backyard garden	L: 0.32 S: 0.38	L: < 0.5 ppb S: 0.05	L: 0.65 S: 0.98	L: 0.03 S: 0.12	L: 8.35 S: 7.34	**L: 0.26** S: 0.50	L: 7.39 S: 2.59	L: 0.41 S: 0.35	L: 0.08 S: 0.66	L: 0.12 S: 0.14

Appendix 5.4. Residence 4, Northbridge: Edible plants and metals (F = Fruit, L = Leaf, S = Stem, L+S = Pooled Leaf and Stem)

Donated Sample	**Location Collected**	**As (ppm)**	**Cd (ppm)**	**Cr (ppm)**	**Co (ppm)**	**Cu (ppm)**	**Pb (ppm)**	**Mn (ppm)**	**Ni (ppm)**	**Se (ppm)**	**V (ppm)**
Chilli Pepper plant	Backyard garden	F: 0.30	F: 0.03	F: 0.11	F: 0.06	F: 11.28	F: 0.05	F: 12.69	F: 0.19	F: 0.05	F: < 0.5 ppb
Grapefruit plant	Backyard garden	F: 0.33 L: 0.98 S: 0.35	F: < 0.5 ppb L: < 0.5 ppb S: < 0.5 ppb	F: 0.09 L: 0.16 S: 0.78	F: 0.05 L: 0.09 S: 0.08	F: 8.22 L: 5.70 S: 7.45	F: 0.05 L: 0.14 S: 0.60	F: 11.13 L: 11.97 S: 3.92	F: 0.30 L: 0.42 S: 0.58	F: 0.06 L: 0.05 S: 0.38	F: < 0.5 ppb L: 0.03 S: 0.05

Grapefruit soil	Backyard garden	4.01	0.45	15.19	3.03	**64.10**[a]	20.80	267.90	5.91	**1.73**[a]	14.58
Lemon plant	Backyard garden	F: 0.28 L: 0.49 S: 0.39	F: < 0.5 ppb L: < 0.5 ppb S: < 0.5 ppb	F: 0.38 L: 0.21 S: 0.61	F: 0.00 L: 0.09 S: 0.08	F: 3.62 L: 10.21 S: 3.88	F: < 0.5 ppb L: 0.40 S: 0.82	F: 3.25 L: 8.76 S: 3.15	F: 0.36 L: 0.58 S: 0.63	F: 0.05 L: 0.07 S: 0.33	F: < 0.5 ppb L: 0.08 S: 0.08
Lime plant	Backyard garden	F: 0.32 S: 0.21	F: < 0.5 ppb S: < 0.5 ppb	F: 0.14 S: 0.55	F: 0.07 S: 0.07	F: 7.87 S: 6.87	**F: 0.28** S: 0.85	F: 13.62 S: 3.48	F: 0.47 S: 0.51	F: 0.06 S: 0.18	F: 0.02 S: 0.03
Rocket plant	Backyard garden	L+S: 0.32	**L+S: 0.22**	L+S: 0.15	L+S: 0.10	L+S: 7.74	L+S: 0.23	L+S: 13.78	L+S: 0.17	L+S: 0.05	L+S: < 0.5 ppb
Thyme plant	Backyard garden	**L: 1.38** S: 1.08	L: 0.01 S: 0.08	L: 0.46 S: 1.09	L: 0.08 S: 0.11	L: 22.54 S: 58.53	**L: 0.58** S: 1.14	L: 68.24 S: 43.84	L: 0.58 S: 0.60	L: 0.01 S: 0.19	L: 0.18 S: 0.44

Appendix 5.5. Residence 5, Castlecrag: Edible plants and metals (L = Leaf, S = Stem, R = Root)

Donated Sample	**Location Collected**	**As (ppm)**	**Cd (ppm)**	**Cr (ppm)**	**Co (ppm)**	**Cu (ppm)**	**Pb (ppm)**	**Mn (ppm)**	**Ni (ppm)**	**Se (ppm)**	**V (ppm)**
Mint plant	Driveway	L: 0.29 R: 1.68	**L: 0.69** R: 0.54	L: 0.75 R: 6.03	L: 0.19 R: 1.71	L: 15.94 R: 50.29	**L: 3.14** R: 37.39	L: 33.78 R: 96.38	L: 0.61 R: 3.26	L: 0.37 R: 1.64	L: 0.77 R: 6.7
Rosemary plant	Driveway	L: 0.19 S: 0.21	L: 0.01 S: 0.06	L: 0.23 S: 0.58	L: 0.04 S: 0.07	L: 12.27 S: 9.45	**L: 0.62** S: 1.44	L: 8.92 S: 7.12	L: 0.36 S: 0.35	L: 0.08 S: 0.28	L: 0.24 S: 0.32
Rosemary soil	Driveway	1.8	0.14	6.47	2.47	16.58	21.46	120.02	3.66	**1.8**[a]	10.94

Appendix 5.6. Residence 6, Northbridge: Edible plants and metals (F = Fruit, L = Leaf, S = Stem, R = Root, rep = Replicate)

Donated Sample	**Location Collected**	**As (ppm)**	**Cd (ppm)**	**Cr (ppm)**	**Co (ppm)**	**Cu (ppm)**	**Pb (ppm)**	**Mn (ppm)**	**Ni (ppm)**	**Se (ppm)**	**V (ppm)**
Coriander plant	Backyard garden, imported soil	L: 0.33 S: 0.13 S rep: 0.13 R: 2.27	**L: 0.24** S: 0.47 S rep: 0.73 R: 0.77	L: 0.32 S: 0.88 S rep: 0.70 R: 5.75	L: 0.09 S: 0.06 S rep: 0.07 R:1.02	L: 13.82 S: 7.62 S rep: 8.87 R: 28.49	**L: 0.51** S: 0.60 S rep: 1.38 R: 18.70	L: 44.90 S: 14.05 S rep: 14.57 R: 69.57	L: 0.49 S: 0.52 S rep: 0.42 R: 2.38	L: 0.07 S: 0.10 S rep: 0.26 R: 0.69	L: 0.10 S: 0.02 S rep: 0.04 R: 4.10
Coriander soil	Backyard garden, imported soil	3.69	0.18	10.23	2.07	39.08	27.35	112.83	8.13	**1.54**[a]	11.17
Lettuce plant	Backyard garden, imported soil	L: 0.78 S: 0.10 R: 2.47	**L: 0.36** S: 0.13 R: 0.23	L: 1.53 S: 0.67 R: 11.14	L: 0.29 S: 0.07 R: 2.06	L: 25.74 S: 10.91 R: 37.92	**L: 3.91** S: 0.70 R: 19.72	L: 38.24 S: 11.14 R: 78.22	L: 0.65 S: 0.39 R: 5.56	L: 0.17 S: 0.22 R: 0.81	L: 1.08 S: 0.04 R: 6.27
Lettuce soil	Backyard garden, imported soil	4.62	0.23	11.80	2.08	47.62	39.48	128.14	5.12	**1.45**[a]	11.39
Parsley plant	Backyard garden, imported soil	L: 0.51 S: 0.19 S rep: 0.24 R: 7.52	L: < 0.5 ppb S: 0.02 S rep: < 0.5 ppb R: 0.07	L: 0.30 S: 0.62 S rep: 0.58 R: 8.24	L: 0.09 S: 0.09 S rep: 0.09 R: 0.60	L: 10.23 S: 8.79 S rep: 9.10 R: 23.47	**L: 0.38** S: 1.49 S rep: 1.30 R: 23.46	L: 20.82 S: 12.46 S rep: 13.05 R: 34.21	L: 0.61 S: 0.76 S rep: 0.70 R: 2.15	L: 0.07 S: 0.17 S rep: 0.14 R: 0.57	L: 0.03 S: 0.04 S rep: 0.03 R: 2.96
Parsley soil	Backyard garden, imported soil	**19.83**[a]	0.23	23.98	3.00	52.62	80.92	134.36	8.24	**1.74**[a]	14.86

Appendix 5.7. Residence 7, Northbridge: Edible plants and metals (F = Fruit, L = Leaf, S = Stem, R = Root)

Donated Sample	**Location Collected**	**As (ppm)**	**Cd (ppm)**	**Cr (ppm)**	**Co (ppm)**	**Cu (ppm)**	**Pb (ppm)**	**Mn (ppm)**	**Ni (ppm)**	**Se (ppm)**	**V (ppm)**
Capsicum plant	Backyard garden	F: 0.24 L: 0.26 S: 0.22 R: 1.70	F: 0.04 L: 1.29 S: 0.73 R: 0.19	F: 0.12 L: 0.54 S: 0.65 R: 3.47	F: 0.09 L: 0.69 S: 0.40 R: 0.66	F: 4.95 L: 7.49 S: 4.15 R: 12.68	**F: 0.21** L: 7.30 S: 15.66 R: 39.83	F: 8.61 L: 75.80 S: 49.00 R: 53.97	F: 0.19 L: 0.60 S: 0.56 R: 1.54	F: 0.02 L: 0.14 S: 0.15 R: 0.45	F: < 0.5 ppb L: 0.57 S: 0.13 R: 4.08
Capsicum soil	Backyard garden	5.47	0.25	13.07	3.20	41.23	51.26	285.45	6.27	**1.89**[a]	16.46
Lemon plant	Backyard garden	F: 0.16 F: 0.19 L: 0.26 S: 0.16	F: < 0.5 ppb F: < 0.5 ppb L: < 0.5 ppb S: < 0.5 ppb	F: 0.15 F: 0.14 L: 0.27 S: 0.74	F: 0.04 F: 0.04 L: 0.11 S: 0.10	F: 1.63 F: 1.95 L: 4.93 S: 2.88	F: < 0.5 ppb F: < 0.5 ppb L: 0.51 S: 1.59	F: 2.91 F: 3.36 L: 8.91 S: 6.87	F: 0.18 F: 0.19 L: 0.53 S: 0.70	F: 0.03 F: 0.03 L: 0.06 S: 0.26	F: < 0.5 ppb F: < 0.5 ppb L: 0.13 S: 0.04
Lemon soil	Backyard garden	4.85	0.37	13.83	3.03	39.63	53.12	175.50	7.15	**2.06**[a]	16.69

Appendix 5.8. Residence 8, Northbridge: Edible plants and metals (F = Fruit, L = Leaf, S = Stem, R = Root, rep = Replicate)

Donated Sample	**Location Collected**	**As (ppm)**	**Cd (ppm)**	**Cr (ppm)**	**Co (ppm)**	**Cu (ppm)**	**Pb (ppm)**	**Mn (ppm)**	**Ni (ppm)**	**Se (ppm)**	**V (ppm)**
Lemon plant	Backyard near suspected former domestic incinerator	F: 0.19 L: 0.37 S: 0.31	F: < 0.5 ppb L: < 0.5 ppb S: < 0.5 ppb	F: 0.04 L: 0.09 S: 0.56	F: 0.01 L: 0.04 S: 0.05	F: 4.62 L: 4.91 S: 5.68	F: 0.06 L: 0.76 S: 2.33	F: 4.91 L: 11.47 S: 8.00	F: 0.63 L: 0.54 S: 0.85	F: 0.02 L: 0.04 S: 0.19	F: < 0.5 ppb L: 0.05 S: 0.08

Lemon soil	Backyard near suspected former domestic incinerator	8.69 rep: 9.36	1.45 rep: 1.75	16.85 rep: 20.61	4.07 rep: 5.03	**76.00^{a}** **rep: 84.04^{a}**	**264.35^{ab}** **rep: 325.73**	296.69 rep: 356.69	9.96 rep: 10.90	**2.16^{a}** **rep: 2.76^{a}**	14.94 rep: 19.32
Oregano plant	Backyard garden	**L: 4.97** S: 1.37 R: 11.73	L: 0.02 S: 0.04 R: 0.29	L: 2.82 S: 1.69 R: 13.18	L: 0.14 S: 0.05 R: 0.89	L: 11.76 S: 8.31 R: 33.72	**L: 6.47** S: 2.78 R: 54.58	L: 16.09 S: 11.30 R: 67.68	L: 0.79 S: 0.51 R: 2.57	L: 0.09 S: 0.13 R: 0.77	L: 0.79 S: 0.19 R: 5.98
Oregano soil	Backyard garden	**22.73^{ab}**	0.54	26.55	2.69	59.36	**161.01^{a}**	180.56	6.64	**1.80^{a}**	14.34
Tomato plant	Backyard garden	L: 6.39 S: 1.95 **R: 43.86^{ab}**	**L: 3.63^{b}** S: 1.48 R: 1.13	L: 2.94 S: 2.55 R: 40.14	L: 0.15 S: 0.13 R: 0.69	L: 26.26 S: 8.69 **R: 121.06^{a}**	L: 2.67 S: 4.60 R: 77.97	L: 51.22 S: 28.32 R: 83.10	L: 0.69 S: 0.82 R: 2.83	L: 0.06 S: 0.12 R: 0.67	L: 0.50 S: 0.31 R: 5.15

Appendix 5.9. Childcare Centre: Edible plants and metals (FL: Flower, F = Fruit, L = Leaf, S = Stem, R = Root, rep = Replicate, A = Upper Sandpit, B = Lower Sandpit)

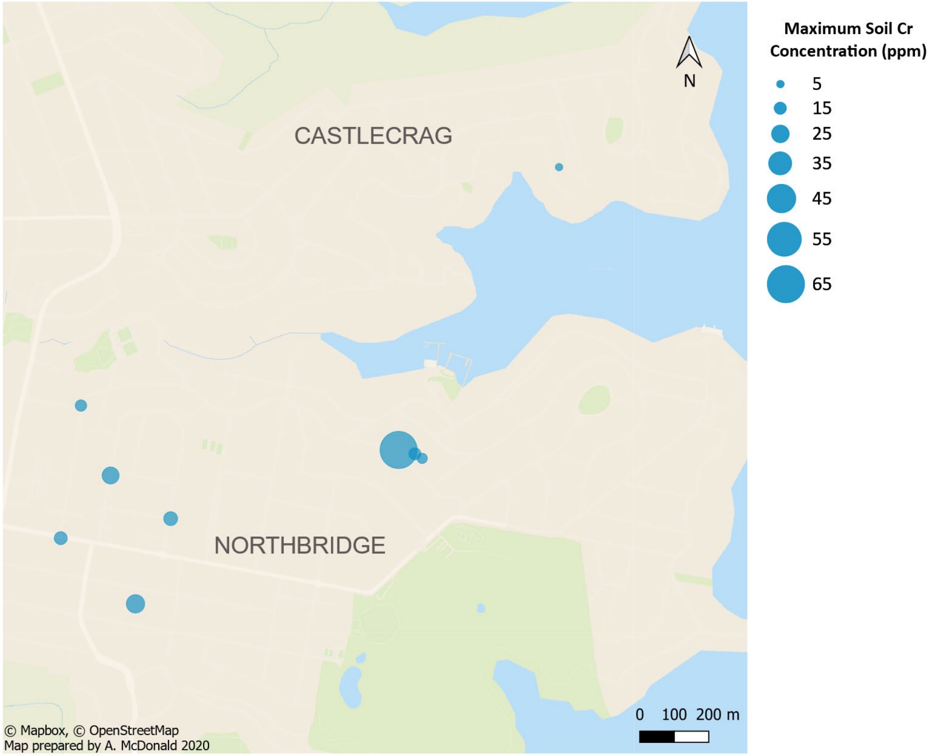
Donated Sample	**Location Collected**	**As (ppm)**	**Cd (ppm)**	**Cr (ppm)**	**Co (ppm)**	**Cu (ppm)**	**Pb (ppm)**	**Mn (ppm)**	**Ni (ppm)**	**Se (ppm)**	**V (ppm)**
Aloe vera plant	Garden-bed at back of centre, away from road	L: 0.12 R: 0.95	L: < 0.5 ppb R: 0.06	L: 0.25 R: 2.30	L: 0.07 R: 0.60	L: 2.96 R: 12.40	**L: 0.28** R: 8.61	L: 21.72 R: 33.99	L: 0.74 R: 2.23	L: 0.03 R: 0.40	L: 0.04 R: 1.84
Aloe vera soil	Garden-bed at back of	6.60	0.32	11.58	3.80	43.76	61.26	166.13	8.64	**1.85^{a}**	10.70

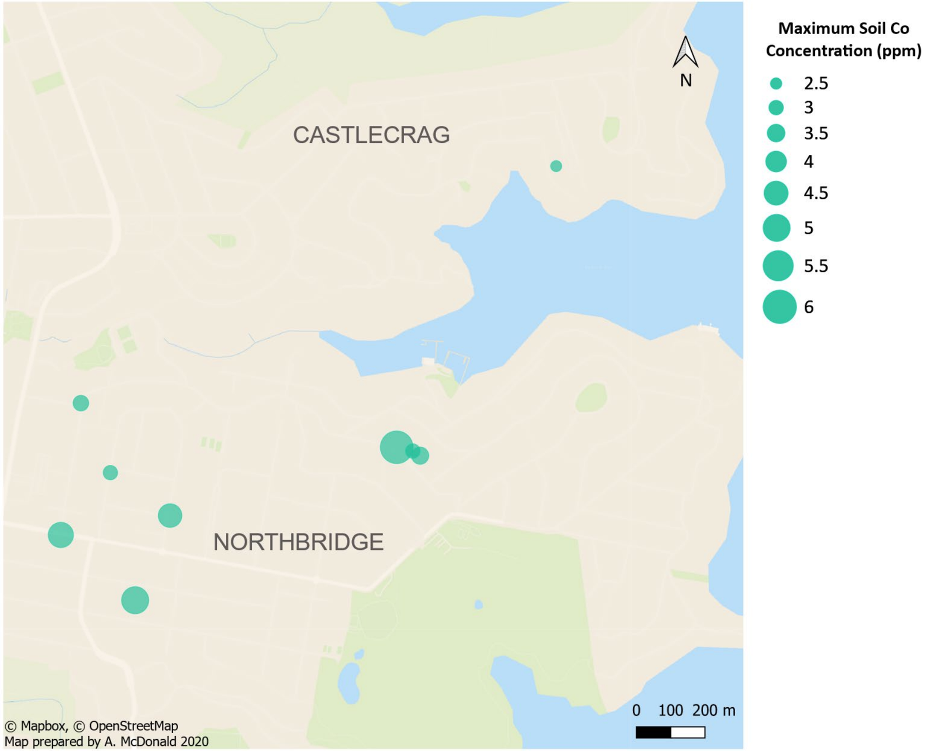
	centre, away from road										
Native Ginger plant	Back of centre, away from the road	L: 0.57 S: 0.39 R: 0.56	L: < 0.5 ppb S: 0.05 R: 0.05	L: 1.04 S: 0.99 R: 1.10	L: 0.17 S: 0.18 R: 0.18	L: 10.21 S: 7.32 R: 7.23	L: 0.94 S: 1.66 R: 1.67	L: 116.83 S: 24.20 R: 25.37	L: 0.81 S: 0.89 R: 0.72	L: 0.11 S: 0.22 R: 0.16	L: 0.56 S: 0.27 R: 0.47
Native Ginger soil	Back of centre, away from the road	2.99	0.07	7.75	1.03	13.21	11.57	54.65	2.28	**1.14**[a]	5.29
Mickey Mouse Bush plant	Road barrier hedge	FL: 0.35 F: 0.41 L: 0.47 S: 0.24	FL: 2.47 F: 1.86 L: 1.37 S: 0.81	FL: 0.89 F: 0.17 L: 1.13 S: 1.31	FL: 0.13 F: 0.05 L: 0.17 S: 0.16	FL: 16.11 F: 8.61 L: 16.74 S: 42.76	FL: 1.02 F: 0.21 L: 1.53 S: 6.03	FL: 16.18 F: 6.60 L: 43.18 S: 45.33	FL: 0.54 F: 0.30 L: 0.87 S: 0.96	FL: 0.06 F: 0.01 L: 0.25 S: 0.16	FL: 0.72 F: 0.05 L: 0.67 S: 0.65
Mint “Chocolate” plant	Behind a hedge barrier near the road	L: 0.18 R: 0.89	L: < 0.5 ppb R: 0.08	L: 0.40 R: 3.17	L: 0.23 R: 0.92	L: 15.30 R: 15.67	**L: 0.33** R: 3.85	L: 63.93 R: 93.22	L: 0.62 R: 1.55	L: 0.04 R: 0.53	L: 0.29 R: 3.35
Orange Jessamine plant	Road barrier hedge	FL: 0.23 L: 0.22 S: 0.46	FL: 0.01 L: 0.04 S: 0.12	FL: 0.64 L: 1.20 S: 2.79	FL: 0.12 L: 0.13 S: 0.24	FL: 13.95 L: 14.31 S: 25.91	FL: 0.43 L: 1.66 S: 6.22	FL: 24.22 L: 16.78 S: 16.44	FL: 0.58 L: 0.68 S: 1.37	FL: 0.03 L: 0.22 S: 0.22	FL: 0.30 L: 0.45 S: 1.30
Orange Jessamine soil	Road barrier hedge soil located behind a low brick wall	4.00	0.28	12.65	2.07	36.90	**214.88**[ab]	122.48	4.90	**1.82**[a]	17.18

Parsley plant	Behind a hedge barrier near the road	L: 0.51 S: 0.21 R: 0.46	L: 0.01 S: 0.03 R: 0.07	L: 0.65 S: 0.74 R: 1.82	L: 0.27 S: 0.14 R: 0.27	L: 11.35 S: 6.89 R: 24.97	**L: 0.53** S: 0.85 R: 1.25	L: 20.12 S: 12.30 R: 32.81	L: 0.73 S: 0.54 R: 1.01	L: 0.09 S: 0.15 R: 0.44	L: 0.48 S: 0.28 R: 0.84
Parsley soil	Behind a hedge barrier near the road	2.08	0.16	16.67	2.13	21.40	15.42	222.95	6.35	**1.62**[a]	16.82
Passionfruit plant	Vine within the front gate	L: 0.35 S: 0.20	L: 1.15 S: 1.07	L: 1.14 S: 0.87	L: 0.18 S: 0.10	L: 10.12 S: 7.80	L: 2.67 S: 3.00	L: 63.51 S: 18.60	L: 0.71 S: 0.95	L: 0.10 S: 0.23	L: 0.88 S: 0.15
Passionfruit soil	Vine within the front gate	1.48	0.16	7.25	2.07	25.31	17.17	148.45	3.70	**1.17**[a]	11.07
Pineapple plant	Back of centre, away from the road	L: 0.42 R: 2.39	L: < 0.5 ppb R: 0.24	L: 0.92 R: 9.09	L: 0.15 R: 3.74	L: 7.17 R: 54.18	L: 1.61 R: 27.32	L: 55.71 R: 143.79	L: 1.05 R: 10.51	L: 0.06 R: 0.88	L: 0.61 R: 9.10
Pineapple soil	Back of centre, away from the road	2.91	0.17	10.72	4.73	**74.91**[a]	42.04	203.02	13.70	**1.22**[a]	10.82
Rosemary plant	Behind a hedge barrier near the road	L: 0.90 S: 0.53	L: 0.04 S: 0.06	L: 1.14 S: 1.34	L: 0.15 S: 0.18	L: 14.07 S: 9.55	**L: 1.01** S: 2.17	L: 13.33 S: 9.36	L: 0.54 S: 0.66	L: 0.48 S: 0.08	L: 0.49 S: 0.67
Rosemary soil	Behind a hedge barrier near the road	2.34	0.06	4.12	0.46	11.02	6.37	25.19	1.24	0.51	2.77

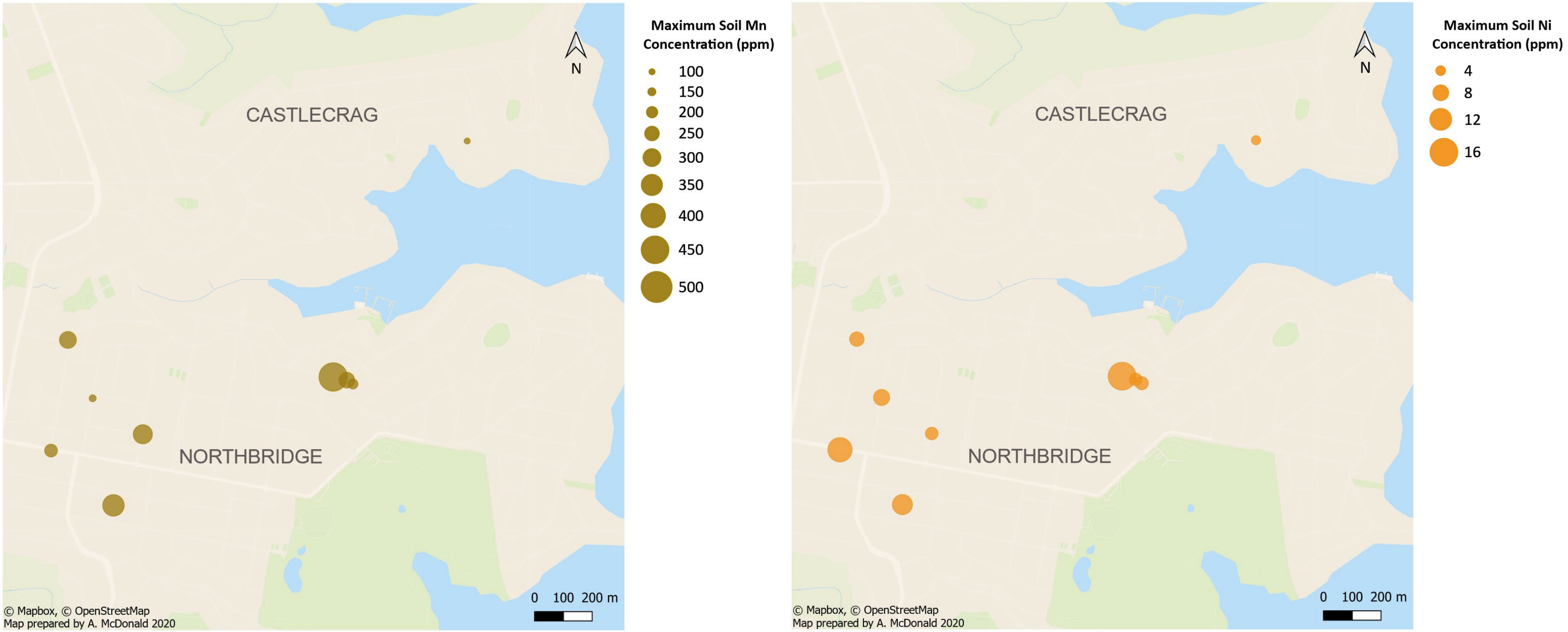
Strawberry plant	Front-middle section of centre	F: 0.44 L: 0.78 R: 3.46	F: 0.02 L: 0.02 R: 0.20	F: 0.21 L: 0.49 R: 4.98	F: 0.15 L: 0.27 R: 0.96	F: 7.60 L: 9.63 R: 23.21	**F: 0.21**[c] L: 1.31 R: 16.33	F: 187.89 L: 278.32 R: 118.05	F: 0.28 L: 0.63 R: 2.46	F: 0.06 L: 0.08 R: 0.53	F: 0.11 L: 0.36 R: 4.64
Strawberry soil	Front-middle section of centre	3.01	0.22	15.99	2.13	38.28	37.42	169.63	6.04	**1.39**[a]	15.45
Garden-bed soil	Near the middle-back section of the centre away from the road	2.68	0.17	12.38	1.87	56.78	14.35	212.39	3.86	**1.04**[a]	10.21
Sandpit	Middle of the centre with two sections: Upper and Lower	A: 1.51 B: 1.72	A: < 0.5 ppb B: < 0.5 ppb	A: 1.12 B: 1.23	A: 0.15 B: 0.16	A: 1.38 B: 0.66	A: 0.75 B: 0.82	A: 5.36 B: 5.65	A: 0.46 B: 0.47	A: 0.17 B: 0.19	A: 1.14 B: 1.37
Front herb garden box mulch	Behind a hedge barrier near the road	0.78	0.09	9.32	1.08	15.88	5.23	247.80	3.17	0.86	9.10
Front herb garden box soil	Behind a hedge barrier near the road	2.97	0.23	10.25	2.33	54.73	69.23	182.92	5.11	**1.46**[a]	11.01

Appendix 5.10. Geospatial map of maximum soil concentration (ppm) for chromium (left) and cobalt (right).

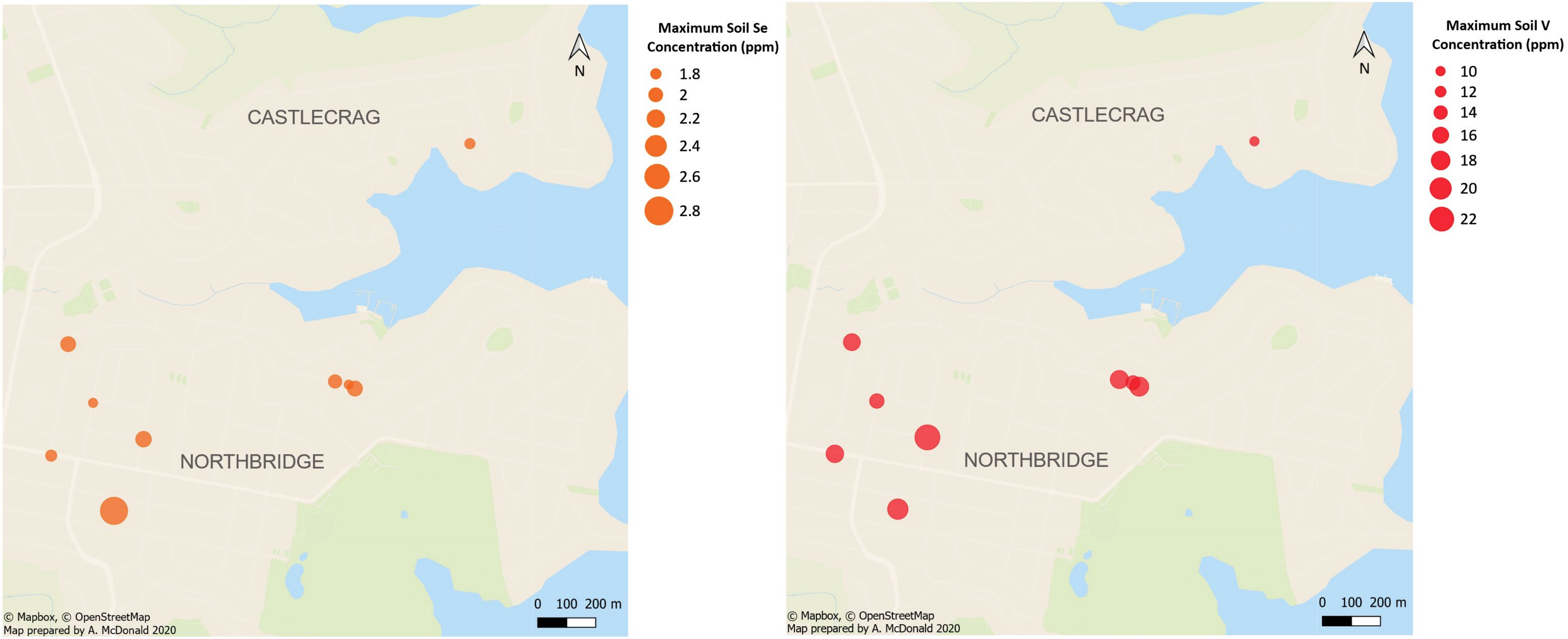




Appendix 5.11. Geospatial map of maximum soil concentration (ppm) for manganese (left) and nickel (right).



Appendix 5.12. Geospatial map of maximum soil concentration (ppm) for selenium (left) and vanadium (right).



© Mapbox, © OpenStreetMap
Map prepared by A. McDonald 2020

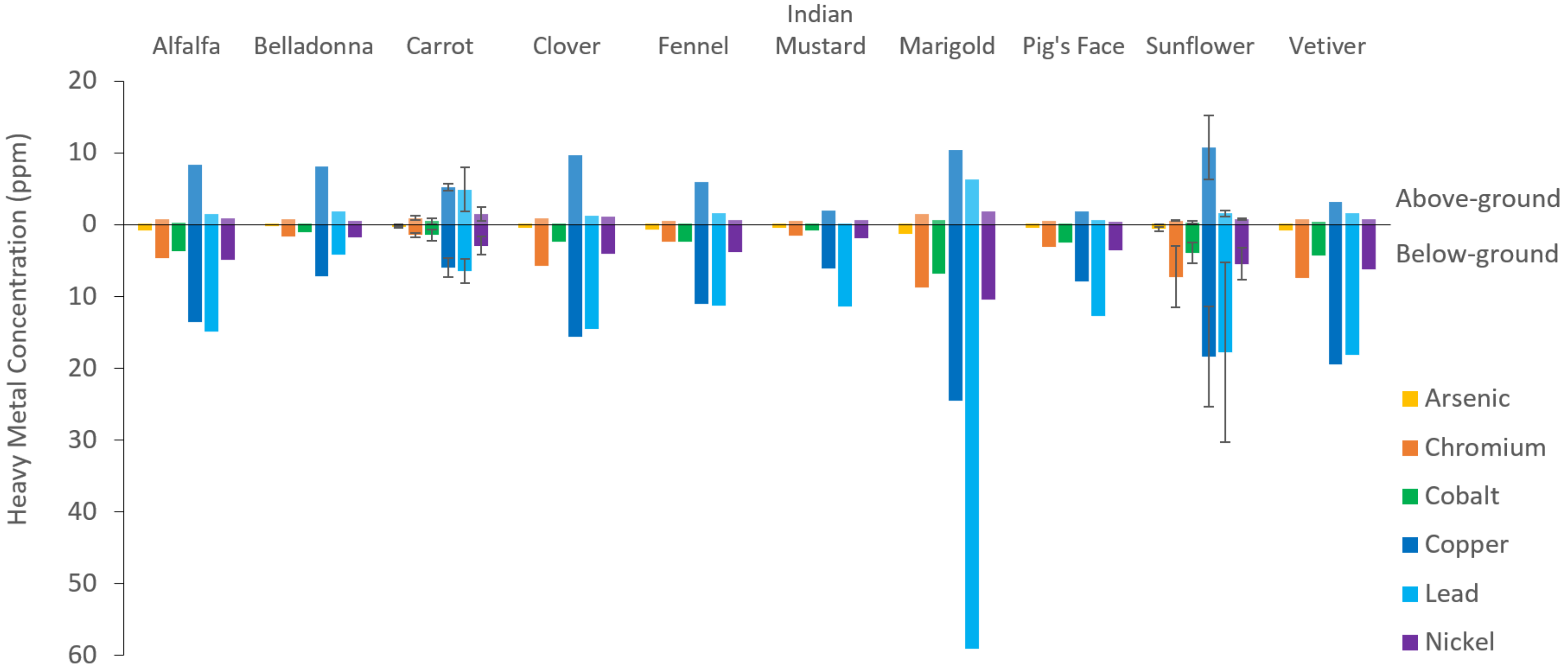
© Mapbox, © OpenStreetMap
Map prepared by A. McDonald 2020

Appendix 6.1. Results from Chapter 6 Kruskal-Wallis analyses for differences in distribution of mean individual heavy metal concentrations across harvest months.

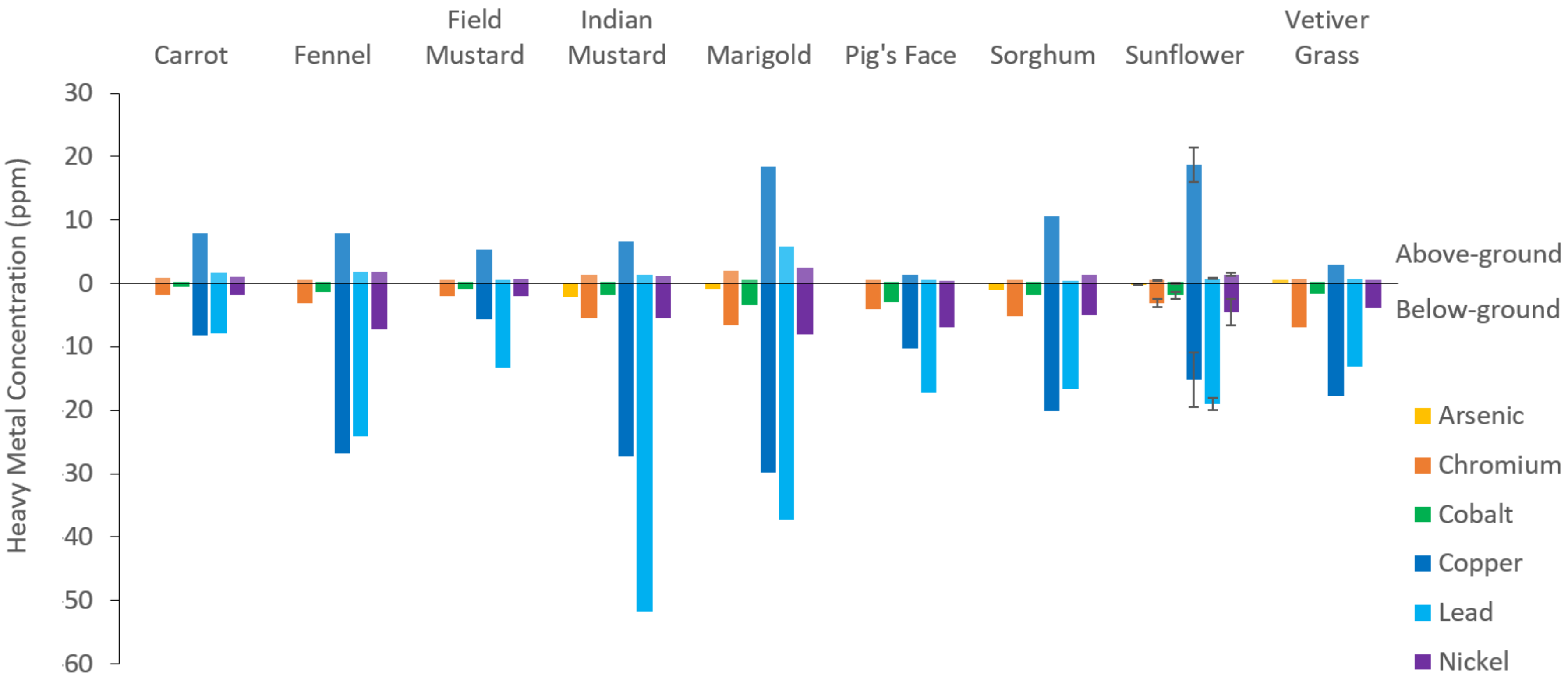
Heavy Metal	χ^2	DF	P value
Arsenic	29.464	3	< 0.0001
Cadmium	0.971	3	0.8
Chromium	35.039	8	< 0.0001
Cobalt	11.1	3	0.011
Copper	33.39	8	< 0.0001
Lead	33.76	8	< 0.0001
Manganese	13.78	3	0.003
Nickel	0.395	3	0.941
Selenium	32.397	3	< 0.0001
Vanadium	6.07	3	0.108

Appendix 6.2. Sunflowers at the White Bay Power Station Phytoremediation Garden

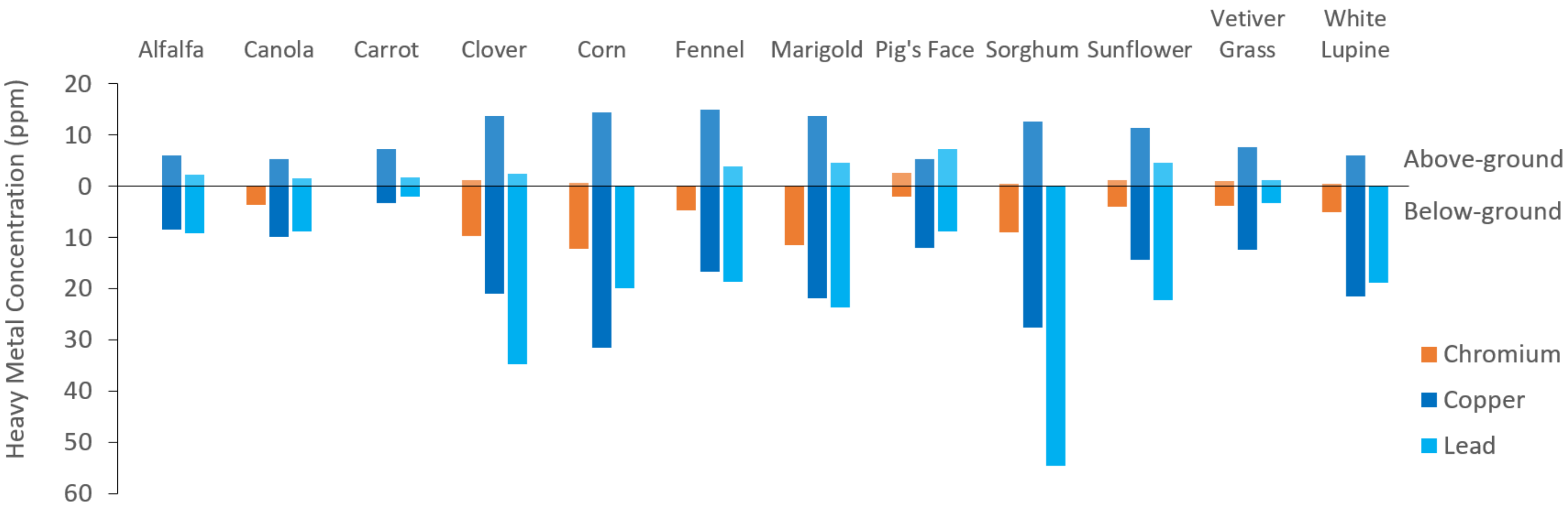
Appendix 6.3. Heavy metal concentrations in below-ground and above-ground tissues (ppm) of plants harvested in March 2019. Individuals of the same species harvested from different garden plots are indicated by mean ± SEM.



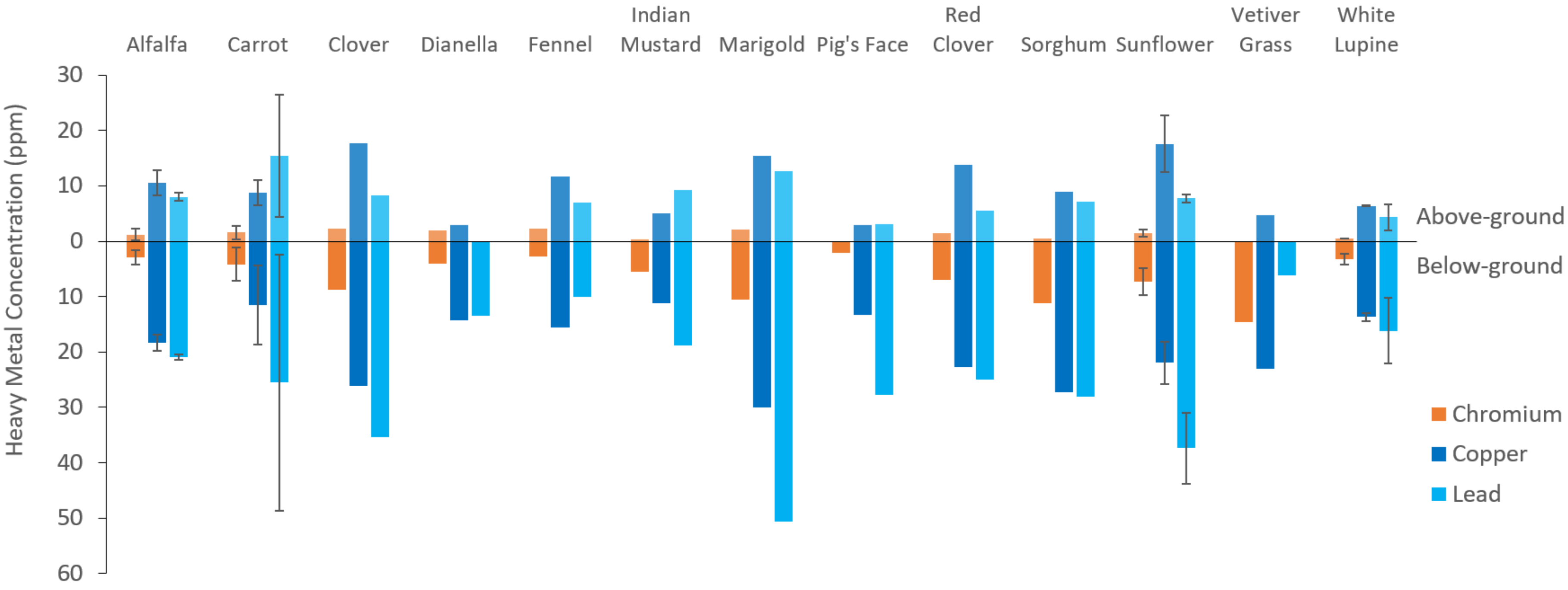
Appendix 6.4. Heavy metal concentrations in below-ground and above-ground tissues (ppm) of plants harvested in February 2019. Individuals of the same species harvested from different garden plots are indicated by mean ± SEM.



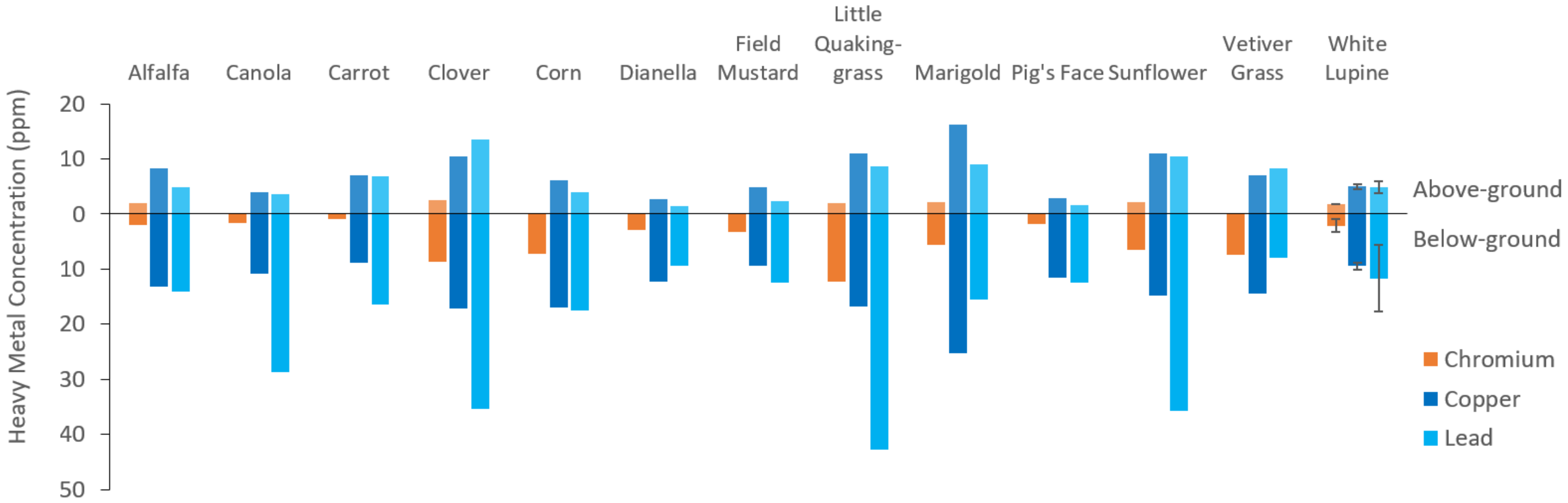
Appendix 6.5. Heavy metal concentrations in below-ground and above-ground tissues (ppm) of plants harvested in January 2019.



Appendix 6.6. Heavy metal concentrations in below-ground and above-ground tissues (ppm) of plants harvested in December 2018. Individuals of the same species harvested from different garden plots are indicated by mean ± SEM.



Appendix 6.7. Heavy metal concentrations in below-ground and above-ground tissues (ppm) of plants harvested in November 2018. Individuals of the same species harvested from different garden plots are indicated by mean ± SEM.



REFERENCES

Aamlid T. S. & Arntsen D. (1998) Effects of light and temperature on seed germination of *Poa pratensis* from high latitudes. *Acta Agriculturae Scandinavica, Section B — Soil & Plant Science* **48,** 239-47.

Abdelkrim S., Jebara S. H., Saadani O., Chiboub M., Abid G., Mannai K. & Jebara M. (2019) Heavy metal accumulation in *Lathyrus sativus* growing in contaminated soils and identification of symbiotic resistant bacteria. *Archives of Microbiology* **201,** 107-21.

Abraham J., Dowling K. & Florentine S. (2018) Assessment of potentially toxic metal contamination in the soils of a legacy mine site in Central Victoria, Australia. *Chemosphere* **192,** 122-32.

Abreu M. M., Godinho B. & Magalhães M. C. F. (2014) Risk assessment of *Arbutus unedo* L. fruits from plants growing on contaminated soils in the Panasqueira mine area, Portugal. *Journal of Soils and Sediments* **14,** 744-57.

Adams D. & Hardman M. (2014) Observing Guerrillas in the Wild: Reinterpreting Practices of Urban Guerrilla Gardening. *Urban Studies* **51,** 1103-19.

Adediran G. A., Ngwenya B. T., Mosselmans J. F. W., Heal K. V. & Harvie B. A. (2015) Mechanisms behind bacteria induced plant growth promotion and Zn accumulation in *Brassica juncea*. *Journal of Hazardous Materials* **283,** 490-499.

Ahmad A., Hadi F. & Ali N. (2015) Effective Phytoextraction of Cadmium (Cd) with Increasing Concentration of Total Phenolics and Free Proline in *Cannabis sativa* (L) Plant Under Various Treatments of Fertilizers, Plant Growth Regulators and Sodium Salt. *International Journal Of Phytoremediation* **17,** 56-65.

Akhtar M. J., Ullah S., Ahmad I. Rauf A., Nadeem S. M., Khan M. Y., Hussain S. & Bulgariu L. (2018) Nickel phytoextraction through bacterial inoculation in *Raphanus sativus*. *Chemosphere* **190,** 234-42.

Alaboudi K. A., Ahmed B. & Brodie G. (2018) Phytoremediation of Pb and Cd contaminated soils by using sunflower (*Helianthus annuus*) plant. *Annals of Agricultural Sciences* **63,** 123-7.

Alam M. G. M., Snow E. T. & Tanaka A. (2003) Arsenic and heavy metal contamination of vegetables grown in Samta village, Bangladesh. *Science of The Total Environment* **308,** 83-96.

Alamo-Nole L. & Su Y. (2017) Translocation of cadmium in *Ocimum basilicum* at low concentration of CdSSe nanoparticles. *Applied Materials Today* **9,** 314-8.

Ali H., Khan E. & Sajad M. A. (2013) Phytoremediation of heavy metals - Concepts and applications. *Chemosphere* **91,** 869-81.

Alvarado-López C. J., Dasgupta-Schubert N., Ambriz J. E., Arteaga-Velazquez J. & Villegas J. A. (2019) Lead uptake by the symbiotic *Daucus carota* L.–Glomus intraradices system and its effect on the morphology of extra- and intraradical fungal microstructures. *Environmental Science and Pollution Research* **26,** 381-91.

Anjula A. & Sangeeta L. (2011) A Comparative Analysis of Trace Metals in Vegetables. *Research Journal of Environmental Toxicology* **5,** 125-132.

Anjum N. A., Pereira M. E., Durate A. C., Ahmed I., Umar S. & Khan N. A. (2013) Phytotechnologies: Remediation of Environmental Contaminants. New York: CRC Press.

Antisari L. V., Orsini F., Marchetti L., Vianello G. & Gianquinto G. (2015) Heavy metal accumulation in vegetables grown in urban gardens. *Agronomy for Sustainable Development* **35,** 1139-47.

Anwar S., Nawaz M., Gul S., Rizwan M., Ali S. & Kareem A. (2016) Uptake and distribution of minerals and heavy metals in commonly grown leafy vegetable species irrigated with sewage water. *Environmental Monitoring and Assessment* **188**, 1-9.

Atilgan B. & Azapagic A. (2015) Life cycle environmental impacts of electricity from fossil fuels in Turkey. *Journal of Cleaner Production* **106,** 555-64.

Australian Broadcasting Corporation (ABC) (2015) *Catalyst: Lead Astray*. ABC, Sydney. Viewed online 13 June 2018. <http://www.abc.net.au/catalyst/stories/4174798.htm>

Başar H., Gürel S. & Katkat A. V. (2009) Metal Status of Soils and Plants Irrigated with Water from Lake İzincik, Turkey*. Communications in Soil Science and Plant Analysis.* **40,** 2545-61.

Bates D., Maechler M., Bolker B. & Walker S. (2015) Fitting Linear Mixed-Effects Models using lme4. *Journal of Statistical Software* **67**, 1-48.

Benjamini Y. & Hochberg Y. (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B (Statistical Methodology)* **57,** 289-300.

Bergougnoux V. (2014) The history of tomato: From domestication to biopharming. *Biotechnology Advances* **32,** 170-89.

Borowska S., Brzoska M. & Borowska S. (2015) Metals in cosmetics: implications for human health*.* *Journal of Applied Toxicology* **35,** 551-72.

Breed M. F., Cross A. T., Wallace K., Bradby K., Flies E., Goodwin N., Jones M., Orlando L., Skelly C., Weinstein P., Aronson J. & Breed M. F. (2020) Ecosystem Restoration: A Public Health Intervention. *EcoHealth*.

Brewin L. E., Mehra A., Lynch P. T. & Farago M. E. (2007) Bioavailability of copper within Dolfrwynog Bog soils, North Wales, UK. *Chemical Speciation & Bioavailability* **19,** 149-62.

Brooks R. R. (1998) Plants that hyperaccumulate heavy metals. CAB International, Wallingford, UK.

Buscaroli A. (2017) An overview of indexes to evaluate terrestrial plants for phytoremediation purposes (Review). *Ecological Indicators* **82,** 367-80.

Byeong Y. P., Jae K. L., Hee M. R. & Kim Y. H. (2011) Effects of Heavy Metal Contamination from an Abandoned Mine on Tomato Growth and Root-knot Nematode Development. *The Plant Pathology Journal* **27,** 266-71.

Calzoni G. L., Antognoni F., Pari E., Fonti P., Gnes A. & Speranza A. (2007) Active biomonitoring of heavy metal pollution using *Rosa rugosa* plants*. Environmental Pollution* **149,** 239-45.

Campbell S., Paquin D., Awaya J. D. & Li Q. X. (2002) Remediation of Benzo[a]pyrene and Chrysene-Contaminated Soil with Industrial Hemp (*Cannabis sativa*). *International Journal of Phytoremediation* **4,** 157-168.

Canadian Council of Ministers of the Environment (CCME) (2018) Canadian Environmental Quality Guidelines (CEQGs). Online Database. Viewed 22 June 2018. <http://st-ts.ccme.ca/en/index.html>

Cao X., Ma L. Q. & Shiralipour A. (2003) Effects of compost and phosphate amendments on arsenic mobility in soils and arsenic uptake by the hyperaccumulator, *Pteris vittata* L. *Environmental pollution* **126,** 157-67.

Cevher-Keskin B., Yıldızhan Y., Yüksel B., Dalyan E. & Memon A. R. (2019) Characterization of differentially expressed genes to Cu stress in *Brassica nigra* by Arabidopsis genome arrays. *Environmental Science and Pollution Research* **26,** 299-311.

Chan S., Tan Y. P., Abdullah A. H. & Ong S. (2016) Equilibrium, kinetic and thermodynamic studies of a new potential biosorbent for the removal of Basic Blue 3 and Congo Red dyes: Pineapple (*Ananas comosus*) plant stem. *Journal of the Taiwan Institute of Chemical Engineers* **61,** 306-15.

Chandra R., Yadav S. & Yadav S. (2017) Phytoextraction potential of heavy metals by native wetland plants growing on chlorolignin containing sludge of pulp and paper industry. *Ecological Engineering* **98,** 134-45.

Charfeddine M., Charfeddine S., Bouaziz D., Messaoud R. B. & Bouzid R. G. (2017) The effect of cadmium on transgenic potato (*Solanum tuberosum*) plants overexpressing the StDREB transcription factors. *Plant Cell, Tissue and Organ Culture (PCTOC)* **128,** 521-41.

Chatterjee J. & Chatterjee C. (2000) Phytotoxicity of cobalt, chromium and copper in cauliflower. *Environmental Pollution* **109,** 69-74.

Chaturvedi R., Favas P., Pratas J., Varun M. & Paul M. (2019) EDTA-Assisted Metal Uptake in *Raphanus sativus* L. and *Brassica oleracea* L.: Assessment of Toxicity and Food Safety. *Bulletin of Environmental Contamination and Toxicology* **103,** 490-5.

Chigbo C. & Batty L. (2013) Phytoremediation potential of *Brassica juncea* in Cu-pyrene co-contaminated soil: Comparing freshly spiked soil with aged soil. *Journal of Environmental Management* **129,** 18-24.

Clark H. F., Brabander D. J. & Erdil R. M. (2006) Sources, sinks, and exposure pathways of lead in urban garden soil*. Journal of Environmental Quality* **35,** 2066-74.

Clayton S. (2020) Climate anxiety: Psychological responses to climate change. *Journal of Anxiety Disorders* **74**.

Cohen D. D., Crawford J., Stelcer E. & Atanacio A. J. (2012) Application of positive matrix factorization, multi-linear engine and back trajectory techniques to the quantification of coal-fired power station pollution in metropolitan Sydney. *Atmospheric Environment* **61,** 204-11.

Coman-Babusanu A., Olariu R. I., Butnaru E. & Arsene C. (2019) Dissipation kinetics of tebuconazole on *Malus domestica* (Golden Delicious and Jonathan) in an apple orchard from north-eastern Romania. *Journal of Liquid Chromatography & Related Technologies* **42,** 582-92.

Compass IOT Pty Ltd. (2020) Traffic Analytics Data Console. Online Database. Viewed 21 March 2020. <https://www.compassiot.com.au/>

Coventry P. A., Neale C., Dyke A., Pateman R. & Cinderby S. (2019) The Mental Health Benefits of Purposeful Activities in Public Green Spaces in Urban and Semi-Urban Neighbourhoods: A Mixed-Methods Pilot and Proof of Concept Study. *International Journal of Environmental Research and Public Health* **16,** 2712.

Crawley M. J. (2012) The R Book, 2nd Ed. John Wiley and Sons Ltd.

da Rosa G. M., Gabriel M., Wastowski A. D. & da Rosa R. S. (2018) Behavior of copper and zinc metals in soil profile, submitted to different sources of fertilization. *Environmental Quality Management* **28,** 89-93.

da Silva A. P., Kay B. D., Perfect E. (1994) Characterization of the least limiting water range of soils. *Soil Science Society of America Journal* **58,** 1775–1781.

Dahbia Z., Mhamed M., Benchaban H. & Mohamed A. H. (2013) Phytoremediation of Contaminated Agricultural Soil by Lead from Traffic Pollution Using a Common Barley *Hordeum Vulgare*. *International Journal of Environment and Resource* **2,** 49-57.

Dalyan E., Yüzbaşıoğlu E., Keskin B. C., Yıldızhan Y., Memon A., Ünal M. & Yüksel B. (2017) The identification of genes associated with Pb and Cd response mechanism in *Brassica juncea* L. by using Arabidopsis expression array. *Environmental and Experimental Botany* **139,** 105-15.

Dantzer J. & Keet C. (2015) The Influence of Childhood Traffic-Related Air Pollution Exposure on Asthma, Allergy and Sensitization: A Systematic Review and a Meta-analysis of Birth Cohort Studies. *Pediatrics* **136,** 245.

Das S., Goswami S. & Talukdar A. D. (2014) A Study on Cadmium Phytoremediation Potential of Water Lettuce, *Pistia stratiotes* L. *Bulletin of Environmental Contamination and Toxicology* **92,** 169-174.

Davenport S J. (1953) *Review of Literature on Health Hazards of Metals: Copper, Volume 1.* U.S. Department of the Interior, Bureau of Mines, United States of America.

Davis B. S. & Birch G. F. (2011) Spatial Distribution of Bulk Atmospheric Deposition of Heavy Metals in Metropolitan Sydney, Australia*. Water, Air, & Soil Pollution* **214,** 147-62.

de Caritat P., Cooper M. & Wilford J. (2011) The pH of Australian soils: field results from a national survey. *Soil Research* **49,** 173-182.

de Moura V. M., Freitas de Sousa, Luciana A., Cristina Dos-Santos M., Raposo J. D. A., Lima A. E, de Oliveira R. B., da Silva M. N. & Mourão R. H. V. (2015) Plants used to treat snakebites in Santarém, western Pará, Brazil: An assessment of their effectiveness in inhibiting hemorrhagic activity induced by *Bothrops jararaca* venom. *Journal of Ethnopharmacology* **161,** 224-32.

de Sousa D. A., de Oliveira E., da Costa Nogueira M. & Espósito B. P. (2010) Development of a heavy metal sorption system through the PS functionalization of coconut (*Cocos nucifera*) fibers. *Bioresource Technology* **101,** 138-143.

de Souza S., Ribeiro C., de Andrade S., Lopez A., de Souza L. A. & Schiavinato M. A. (2012) Lead tolerance and phytoremediation potential of Brazilian leguminous tree species at the seedling stage. *Journal of Environmental Management* **110,** 299.

Design 5 Architects Pty Ltd (2011) White Bay Power Station Conservation Management Plan, Volume 1. Executive Summary, Final Report, Viewed 5 May 2020. <https://thebayssydney.nsw.gov.au/assets/Document-Library/White-Bay-Power-Station-resources-2004-2011/2011-WBPS-Conservation-Management-Plan.pdf>

Devkota B., Schmidt G. & Devkota B. (2000) Accumulation of heavy metals in food plants and grasshoppers from the Taigetos Mountains, Greece. *Agriculture, Ecosystem & Environment* **78,** 85-91.

Dhiman S. S., Selvaraj C., Li J., Singh R., Zhao X., Kim D., Kim J. Y., Kang Y. C. & Lee J. (2016) Phytoremediation of metal-contaminated soils by the hyperaccumulator canola (*Brassica napus* L.) and the use of its biomass for ethanol production. *Fuel* **183,** 107-14.

Di Salvatore M., Carafa A. M. & Carratù G. (2008) Assessment of heavy metals phytotoxicity using seed germination and root elongation tests: A comparison of two growth substrates. *Chemosphere* **73,** 1461-4.

Dietz A. C. & Schnoor J. L. (2001) Advances in phytoremediation. *Environmental Health Perspectives Supplements* **109,** 163.

Ding K. Q. & Liu T.-F. (2011) Effect of alfalfa (*Lolium multiflorum* L) on degradation of trichlorophenol in soil. *2011 Second International Conference on Mechanic Automation and Control Engineering*, 15-17 July 2011, 2107-2111.

Dinu C., Vasile G., Buleandra M., Popa D. E., Gheorghe S. & Ungureanu E. (2020) Translocation and accumulation of heavy metals in *Ocimum basilicum* L. plants grown in a mining-contaminated soil. *Journal of Soils and Sediments* **20,** 2141-54.

Du L. & Mao J. (2015) Estimating the environmental efficiency and marginal CO2 abatement cost of coal-fired power plants in China. *Energy Policy* **85,** 347-56.

Dubey R. K., Tripathi V., Singh N. & Abhilash P. C. (2014) Phytoextraction and dissipation of lindane by *Spinacia oleracea* L. *Ecotoxicology and Environmental Safety* **109,** 22-6.

Duruibe J. O., Ogwuegbu M. O. C. & Egwurugwu J. N. (2007) Heavy metal pollution and human biotoxic effects*. International Journal of Physical Sciences* **2,** 112-8.

Ebrahimi M. (2016) Enhanced Phytoremediation Capacity of *Chenopodium album* L. Grown on Pb-Contaminated Soils Using EDTA and Reduction of Leaching Risk. *Soil and Sediment Contamination: An International Journal* **25,** 652-67.

Edelstein M. & Ben-Hur M. (2018) Heavy metals and metalloids: Sources, risks and strategies to reduce their accumulation in horticultural crops. *Scientia Horticulturae* **234,** 431-44.

Eisazadeh S., Asadi Kapourchal S., Homaee M., Noorhosseini S. & Damalas C. (2018) Chive (*Allium schoenoprasum* L.) response as a phytoextraction plant in cadmium-contaminated soils. *Environmental Science Pollution Research* **26,** 152-60.

Eklund A. (2016) beeswarm: The Bee Swarm Plot, an Alternative to Stripchart. R package version 0.2.3.

Elloumi N., Zouari M., Chaari L., Jomni C., Rouina B. B. & Abdallah F. B. (2014) Ecophysiological responses of almond (*Prunus dulcis*) seedlings to cadmium stress. *Biologia* **69,** 604–609.

Engbersen N., Gramlich A., Lopez M., Schwarz G., Hattendorf,B., Gutierrez O. & Schulin R. (2019) Cadmium accumulation and allocation in different *cacao cultivars*. *Science of The Total Environment* **678,** 660-70.

Esringü A. & Turan M. (2012) The Roles of Diethylenetriamine Pentaacetate (DTPA) and Ethylenediamine Disuccinate (EDDS) in Remediation of Selenium from Contaminated Soil by Brussels Sprouts (*Brassica oleracea* var. *gemmifera*). *Water, Air, & Soil Pollution* **223,** 351-362.

European Union (EU) (2006) Commission regulation (EC) No. 1881/2006. Setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Union* **364**, 5–24, Viewed 16 May 2018 <http://data.europa.eu/eli/reg/2006/1881/oj>

Famulari S. & Witz K. (2015) A User-Friendly Phytoremediation Database: Creating the Searchable Database, the Users, and the Broader Implications. *International Journal of Phytoremediation* **17,** 737-44.

Feigl G., Kumar D., Lehotai N., Tugyi N., Molnar A., Oerdoeg A., Szepesi A., Gemes K., Laskay G., Erdei L. & Kolbert Z. (2013) Physiological and morphological responses of the root system of Indian mustard (*Brassica juncea* L. *Czern*.) and rapeseed (*Brassica napus* L.) to copper stress*. Ecotoxicology Environmental Safety* **94,** 179-89.

Field A. (2009) Discovering Statistics Using SPSS, 3rd Edition, SAGE Publications Ltd., UK.

Finley R. (2013) A Guerrilla Gardener in South Central LA. *TED2013* Conference Feb 2013, California, Accessed online 26 Mar 2020, <https://www.ted.com/talks/ron_finley_a_guerrilla_gardener_in_south_central_la?utm_campaign=tedspread&utm_medium=referral&utm_source=tedcomshare>

Finster M. E., Gray K. A. & Binns H. J. (2004) Lead levels of edibles grown in contaminated residential soils: a field survey*. Science of the Total Environment* **320,** 245-57.

Flies E. J., Skelly C., Negi S. S., Prabhakaran P., Liu Q., Liu K., Goldizen F. C., Lease C. & Weinstein P. (2017) Biodiverse green spaces: a prescription for global urban health. *Frontiers in Ecology and the Environment* **15,** 510-6.

Food Standards Australia and New Zealand (FSANZ) (2016) Schedule 19 – Maximum levels of contaminants and natural toxicants. Standards Code. Viewed 20 June 2018

<http://www.foodstandards.gov.au/code/Documents/Sched%2019%20Contaminant%20MLs%20v157.pdf>

Forte J. & Mutiti S. (2017) Phytoremediation Potential of *Helianthus annuus* and *Hydrangea paniculata* in Copper and Lead-Contaminated Soil. *Water, Air, & Soil Pollution* **228,** 1-11.

Fox J. & Weisberg S. (2019). An R Companion to Applied Regression, Third Edition. Thousand Oaks CA: Sage.

Fuhrmann M., Lasat M. M., Ebbs S. D., Kochian L. V. & Cornish J. (2002) Uptake of cesium-137 and strontium-90 from contaminated soil by three plant species: Application of phytoremediation. *Journal of Environmental Quality* **31,** 904-9.

Gadd G. M. (1993) Interactions of fungi with toxic heavy metals. *New Phytologist* **124,** 25-60.

Gahlawat S. & Gauba P. (2016) Phytoremediation of aspirin and tetracycline by *Brassica juncea*. *International Journal of Phytoremediation* **18,** 929-35.

Gao J., Liang L. & Chen Z. (2013) Phytoremediation of Heavy Metals Contamination in Sediments of Baihua Lake by Two Plants. *Guizhou Agricultural Sciences* **41,** 184-187.

García-Gómez C., Obrador A., González D., Babín M. & Fernández M. D. (2018) Comparative study of the phytotoxicity of ZnO nanoparticles and Zn accumulation in nine crops grown in a calcareous soil and an acidic soil*. Science of the Total Environment* **644,** 770-80.

Gaur N., Kukreja A., Yadav M. & Tiwari A. (2017) Assessment of phytoremediation ability of *Coriander sativum* for soil and water co-contaminated with lead and arsenic: a small-scale study. *3 Biotech* **7,** 196.

Gheju M. & Stelescu I. (2013) Chelant-assisted phytoextraction and accumulation of Zn by *Zea mays*. *Journal of Environmental Management* **128,** 631-636.

Gisbert C., Clemente R., Navarro-Aviñó J., Baixauli C., Ginér A., Serrano R., Walker D. J. & Bernal M. P (2006) Tolerance and accumulation of heavy metals by Brassicaceae species grown in contaminated soils from Mediterranean regions of Spain. *Environmental and Experimental Botany* **56,** 19-27.

Glasgow G (2018) Synergistic effect of cadmium (Cd) and lead (Pb) on *Phaseolus vulgaris* seed germination and physiology. ProQuest Dissertations and Theses. Michigan, USA.

Gohlke J. M., Thomas R., Woodward A., Campbell-Lendrum D., Prüss-Üstün A., Hales S. & Portier C. J. (2011) Estimating the global public health implications of electricity and coal consumption. *Environmental Health Perspectives* **119,** 821-6.

Grace E. & Macfarlane G. (2016) Assessment of the bioaccumulation of metals to chicken eggs from residential backyards. *Science of the Total Environment* **563-564,** 256-60.

Groom C. A., Halasz A., Paquet L., Morris N., Olivier L., Dubois C. & Hawari J. (2002) Accumulation of HMX (Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine) in Indigenous and Agricultural Plants Grown in HMX-Contaminated Anti-Tank Firing-Range Soil. *Environmental Science & Technology* **36,** 112-8.

Guarino C. & Sciarrillo R. (2017) The effectiveness and efficiency of phytoremediation of a multicontaminated industrial site: Porto Marghera (Venice Lagoon, Italy). *Chemosphere* **183,** 371-9.

Hadi F., Bano A. & Fuller M. P. (2010) The improved phytoextraction of lead (Pb) and the growth of maize (*Zea mays* L.): the role of plant growth regulators (GA3 and IAA) and EDTA alone and in combinations. *Chemosphere* **80,** 457-462.

Hasanpour R., Zaefarian F., Rezvani M. & Jalili B. (2019) Potential of *Mentha aquatica* L., *Eryngium caucasicum* Trautv. and *Froriepia subpinnata* Ledeb. for phytoremediation of Cd-contaminated soil. *Brazilian Journal of Botany* **42,** 399-406.

He F., Gao J., Pierce E., Strong P. J., Wang H. & Liang L. (2015) In situ remediation technologies for mercury-contaminated soil. *Environmental Science and Pollution Research* **22,** 8124-8147.

He W., Long A., Zhang C., Cao M., Luo J. & He W. (2020) Mass balance of metals during the phytoremediation process using *Noccaea caerulescens*: a pot study. *Environmental Science and Pollution Research*.

Hendricks S. B. & Taylorson R. B. (1974) Promotion of seed germination by nitrate, nitrite, hydroxylamine, and ammonium salts. *Plant Physiology* **54,** 304-9.

Hu Y., Norton G., Duan G., Huang Y. & Liu Y. (2014) Effect of selenium fertilization on the accumulation of cadmium and lead in rice plants. *Plant & Soil* **384,** 131-40.

Hur J. R. & Jho E. H. (2017) Effect of hemoglobin on the growth and Cd accumulation of pea plants (*Pisum sativum* L.). *Applied Biological Chemistry* **60,** 673-8.

IBM Corp. (2019). IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp

Infrastructure NSW (2018) Bays West Project Update. Accessed 14 May 2020. <http://www.infrastructure.nsw.gov.au/projects-nsw/bays-west/>

Islam M., Ahmed M., Habibullah-Al-Mamun M., Raknuzzaman M., Ali M. & Eaton D. (2016) Health risk assessment due to heavy metal exposure from commonly consumed fish and vegetables. *Environment Systems & Decisions* **36,** 253-65.

Israr M., Jewell A., Kumar D. & Sahi S. V. (2011) Interactive effects of lead, copper, nickel and zinc on growth, metal uptake and antioxidative metabolism of Sesbania drummondii. *Journal of Hazardous Materials* **186,** 1520-6.

Ivanova E. M., Kholodova V. P. & Kuznetsov V. V. (2010) Biological effects of high copper and zinc concentrations and their interaction in rapeseed plants. *Russian Journal of Plant Physiology* **57,** 806-14.

Iveson K. (2013) Cities within the City: Do-It-Yourself Urbanism and the Right to the City. *International Journal of Urban and Regional Research* **37,** 941-56.

Izquierdo M., de Miguel E., Ortega M. F. & Mingot J. (2015) Bioaccessibility of metals and human health risk assessment in community urban gardens. *Chemosphere* **135,** 312.

JBS&G Australia Pty Ltd (2015) Site Wide Remedial Concept Plan. The Bays Precinct Urban Transformation Area, Report for UrbanGrowth NSW. Accessed 15 May 2020. <https://thebayssydney.nsw.gov.au/assets/Reference-Group/Bays-Precinct-SiteWide-Remedial-Concept-Plan-WEB.pdf>

Jennings A. A. (2013) Analysis of worldwide regulatory guidance values for the most commonly regulated elemental surface soil contamination*. Journal of Environmental Management* **118,** 72-95.

Jensen D. L., Holm P. E. & Christensen T. H. (2000) Soil and groundwater contamination with heavy metals at two scrap iron and metal recycling facilities. *Waste Management & Research* **18,** 52-63.

Johnston S. G., Burton E. D., Moon E. M. & Johnston S. G. (2016) Arsenic Mobilization Is Enhanced by Thermal Transformation of Schwertmannite. *Environmental Science & Technology* **50,** 8010-9.

Kachenko A. G. & Singh B. (2006) Heavy Metals Contamination in Vegetables Grown in Urban and Metal Smelter Contaminated Sites in Australia*. Water, Air & Soil Pollution* **169,** 101-23.

Kandic S., Tepe S. J., Blanch E. W., De Silva S., Mikkonen H. G. & Reichman S. M. (2019) Quantifying factors related to urban metal contamination in vegetable garden soils of the west and north of Melbourne, Australia. *Environmental Pollution* **251**, 193-202.

Kennen & Kirkwood (2015) Phyto, Principles and resources for site remediation and landscape design. Routledge, London.

Khan K. M., Chakraborty R., Bundschuh J., Bhattacharya P. & Parvez F. (2020) Health effects of arsenic exposure in Latin America: An overview of the past eight years of research*. Science of the Total Environment* **710,** 136071.

Kian M. M., Haghi A., Salami M. Chahardouli B., Rostami S., Malekzadeh K., Foumani H. K., Mohammadi S. & Nikbakht M. (2020) Arsenic Trioxide and Thalidomide Combination Induces Autophagy Along with Apoptosis in Acute Myeloid Cell Lines*.* *Cell Journal* **22,** 193-202.

Kim R., Yoon J., Kim T., Yang J. E., Owens G. & Kim K. (2015) Bioavailability of heavy metals in soils: definitions and practical implementation--a critical review*. Environmental Geochemistry and Health* **37,** 1041-61.

Klaasen C. D., Casarett L. J. & Doull J. (1995) Casarett & Doull's Toxicology: The Basic Science of Poisons, Fifth Edition, Edited by Curtis D. Klassen, Ph.D., McGraw-Hill, New York. pp 715.

Koleli N., Demir A., Kantar C., Atag G. A., Kusvuran K. & Binzet R. (2015) Soil Remediation and Plants: Prospects and Challenges. *Chapter 22*, Elsevier Inc.

Kooner R., Mahajan B. V. C. & Dhillon W. S. (2014) Heavy Metal Contamination in Vegetables, Fruits, Soil and Water - A Critical Review. *International Journal of Agriculture, Environment and Biotechnology* **7,** 603-12.

Kumar A., Pal L. & Agrawal V. (2017) Glutathione and citric acid modulates lead- and arsenic-induced phytotoxicity and genotoxicity responses in two cultivars of *Solanum lycopersicum* L. *Acta Physiologiae Plantarum* **39,** 151.

Kuznetsova A., Brockhoff P. B. & Christensen R. H. B. (2017) lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software* **82,** 1-26.

Laidlaw M. A. S. & Taylor M. P. (2011) Potential for childhood lead poisoning in the inner cities of Australia due to exposure to lead in soil dust*. Environmental Pollution* **159,** 1-9.

Laidlaw M. A. S., Alankarage D. H., Reichman S. M., Taylor M. P. & Ball A. S. (2018) Assessment of soil metal concentrations in residential and community vegetable gardens in Melbourne, Australia. *Chemosphere* **199,** 303-11.

Langkamp P. J. (1985) Potential conflict between the coal and arable land resources in Australia: A case for corporate responsiveness*.* *Environmental Management* **9,** 49-60.

Leake J. R., Adam-Bradford A. & Rigby J. E. (2009) Health benefits of 'grow your own' food in urban areas: implications for contaminated land risk assessment and risk management? *Environmental Health* **8 Suppl 1,** S6.

Lechner A. M., Baumgartl T., Matthew P. & Glenn V. (2016) The Impact of Underground Longwall Mining on Prime Agricultural Land: A Review and Research Agenda*.* *Land Degradation & Development* **27,** 1650-63.

Lenth R. (2019) Emmeans: Estimated Marginal Means, Aka Least-squares Means. R Package Version 1.3.3.

Lenth R. (2020) Emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.5.0.

Lepczyk C. A., Aronson M. F. J., Evans K. L., Goddard M. A., Lerman S. B. & Macivor J. S. (2017) Biodiversity in the City: Fundamental Questions for Understanding the Ecology of Urban Green Spaces for Biodiversity Conservation*.* *Bioscience* **67,** 799-807.

Lequeux H., Hermans C., Lutts S. & Verbruggen N. (2010) Response to copper excess in *Arabidopsis thaliana*: Impact on the root system architecture, hormone distribution, lignin accumulation and mineral profile*.* *Plant Physiology and Biochemistry* **48,** 673-82.

Levizou E., Zanni A. A. & Antoniadis V. (2019) Varying concentrations of soil chromium (VI) for the exploration of tolerance thresholds and phytoremediation potential of the oregano (*Origanum vulgare*)*.* *Environmental Science and Pollution Research* **26,** 14-23.

Li F., Shi W., Jin Z., Wu H. & Sheng G. D. (2017) Excessive uptake of heavy metals by greenhouse vegetables*.* *Journal of Geochemical Exploration* **173,** 76-84.

Ling W., Dang H. & Liu J. (2013) In situ gradient distribution of polycyclic aromatic hydrocarbons (PAHs) in contaminated rhizosphere soil: a field study*.* *Journal of Soils and Sediments* **13,** 677-85.

Lintern M., Anand R., Ryan C. & Paterson D. (2013) Natural gold particles in Eucalyptus leaves and their relevance to exploration for buried gold deposits*. Nature Communications* **4**.

Liu X., Song Q., Tang Y., Li W., Xu J., Wu J., Wang F. & Brookes P. C. (2013) Human health risk assessment of heavy metals in soil-vegetable system: a multi-medium analysis. *Science of the Total Environment* **463-464,** 530.

Lottermoser B. G., Munksgaard N. C. & Daniell M. (2009) Trace Element Uptake by Mitchell Grasses Grown on Mine Wastes, Cannington Ag–Pb–zinc Mine, Australia: Implications for Mined Land Reclamation*. Water, Air & Soil Pollution* **203,** 243-59.

Lu Q., He Z. L., Graetz D. A., Stoffella P. J. & Yang X. (2011) Uptake and distribution of metals by water lettuce (*Pistia stratiotes* L.). *Environmental Science and Pollution Research* **18,** 978-86.

Lu Q., Li J., Chen F., Lin L., Tang Y., Liang D., Xia H., Lai Y. & Wang X. (2017) Effects of mutual intercropping on the cadmium accumulation in accumulator plants *Stellaria media*, *Malachium aquaticum*, and *Galium aparine*. *Environmental Monitoring and Assessment* **189,** 622.

Ma T., Zhou L., Chen L. Li Z., Wu L., Christie P. & Luo Y. (2016) Oxytetracycline Toxicity and Its Effect on Phytoremediation by *Sedum plumbizincicola* and *Medicago sativa* in Metal-Contaminated Soil. *Journal of Agricultural and Food Chemistry* **64,** 8045-53.

Mackay A. K., Taylor M. P., Munksgaard N. C., Hudson-Edwards K. A. & Burn-Nunes L. (2013) Identification of environmental lead sources and pathways in a mining and smelting town: Mount Isa, Australia*. Environmental Pollution* **180,** 304-11.

Madejón P., Domínguez M. T., Gil-Martínez M., Navarro-Fernández C. M., Montiel-Rozas M. M., Madejón E., Murillo J. M., Cabrera F. & Marañón T. (2018) Evaluation of amendment addition and tree planting as measures to remediate contaminated soils: The Guadiamar case study (SW Spain). *CATENA* **166,** 34-43.

Mahmood-ul-Hassan M., Suthar V., Ahmad R. & Yousra M. (2017) Heavy metal phytoextraction-natural and EDTA-assisted remediation of contaminated calcareous soils by sorghum and oat. *Environmental Monitoring and Assessment* **189,** 591.

Mahmud J. A. L., Hasanuzzaman M., Nahar K., Rahman A., Hossain M. S. & Fujita M. (2017) γ-aminobutyric acid (GABA) confers chromium stress tolerance in *Brassica juncea* L. by modulating the antioxidant defense and glyoxalase systems. *Ecotoxicology* **26,** 675-90.

Maji M. D., Chattopadhyay S., Kumar P. & Saratchandra B. (2005) In vitro screening of some plant extracts against fungal pathogens of mulberry (*Morus* spp.). *Archives of Phytopathology and Plant Protection* **38,** 157-64.

Malandrino M., Abollino O., Buoso S., Giacomino A., La Gioia C. & Mentast E. (2011) Accumulation of heavy metals from contaminated soil to plants and evaluation of soil remediation by vermiculite. *Chemosphere* **82,** 169-178.

Mandzhieva S. S., Minkina T. M., Chaplygin V. A., Motuzova G. V., Sushkova S. N., Bauer T. V. & Nevidomskaya D. G. (2016) Plant contamination by heavy metals in the impact zone of Novocherkassk Power Station in the south of Russia. *Journal of Soils and Sediments* **16,** 1383-1391.

Manzoor S., Hameed A., Khan M. A. & Gul B. (2017) Seed germination ecology of a medicinal halophyte *Zygophyllum propinquum*: responses to abiotic factors. *Flora* **233,** 163-70.

Márquez-García B., Márquez C., Sanjosé I., Nieva F. J. J., Rodríguez-Rubio P. & Muñoz-Rodríguez A. F. (2013) The effects of heavy metals on germination and seedling characteristics in two halophyte species in Mediterranean marshes. *Marine Pollution Bulletin* **70,** 119-24.

Martinat S., Navratil J., Hollander J. B., Trojan J., Klapka P., Klusacek P. & Kalok D. (2018) Re-reuse of regenerated brownfields: Lessons from an Eastern European post-industrial city. *Journal of Cleaner Production* **188,** 536-45.

Martino L., Yan E. & LaFreniere L. (2019) A hybrid phytoremediation system for contaminants in groundwater*. Environmental Earth Sciences* **78,** 1-16.

McCutcheon S. C. & Schnoor J. L. (1997) Phytoremediation: Transformation and Control of Contaminants. Wiley.

McDonald A. G., Murray B. R., Krix D. W. & Murray M. L. (2021) Complex soil contamination severely impacts seed-sown crop viability in Australia. *Australian Journal of Crop Science* **15,** 531-537.

McLaughlin M. J., Hamon R. E., McLaren R. G., Speir T. W. & Rogers S. L. (2000) Review: A bioavailability-based rationale for controlling metal and metalloid contamination of agricultural land in Australia and New Zealand. *Australian Journal of Soil Research* **38,** 1037-1086.

Meeinkuirt W., Taeprayoon P., Pichtel J. & Thongchai A. (2019) Soil amendments for cadmium phytostabilization by five marigold cultivars. *Environmental Science and Pollution Research* **26,** 8737-47.

Meers E., Ruttens A., Hopgood M., Lesage E. & Tack F. M. G. (2005) Potential *of Brassic rapa*, *Cannabis sativa*, *Helianthus annuus* and *Zea mays* for phytoextraction of heavy metals from calcareous dredged sediment derived soils. *Chemosphere* **61,** 561-572.

Mehmood T., Bibi I., Shahid M., Niazi N. K., Murtaza B., Wang H., Ok Y. S., Sarkar B., Javed M. T. & Murtaza G. (2017) Effect of compost addition on arsenic uptake, morphological and physiological attributes of maize plants grown in contrasting soils. *Journal of Geochemical Exploration* **178,** 83-91.

Mielke H. W. & Zahran S. (2012) The urban rise and fall of air lead (Pb) and the latent surge and retreat of societal violence*.* *Environment International* **43,** 48-55.

Ministry for the Environment, New Zealand Government (MFE) (2013) Environmental Guideline Value (EGV) database. Wellington, New Zealand. Viewed 10th September 2018. <http://www.mfe.govt.nz/land/risks-contaminated-land/managing-contaminated-land/contaminated-land-management-guidelines-0>

Mitton F. M., Gonzalez M., Monserrat J. M. & Miglioranza K. S. B. (2018) DDTs-induced antioxidant responses in plants and their influence on phytoremediation process. *Ecotoxicology and Environmental Safety* **147,** 151-6.

Mohamed H. I. (2011) Molecular and Biochemical Studies on the Effect of Gamma Rays on Lead Toxicity in Cowpea (*Vigna sinensis*) Plants. *Biological Trace Element Research* **144,** 1205–1218.

Moon E. M. & Peacock C. L. (2011) Adsorption of Cu(II) to Bacillus subtilis: A pH-dependent EXAFS and thermodynamic modelling study. *Geochimica et Cosmochimica Acta* **75,** 6705-19.

Mosa K. A., Saadoun I., Kumar K., Helmy M. & Dhankher O. P. (2016) Potential biotechnological strategies for the cleanup of heavy metals and metalloids*. Frontiers in Plant Science* **7,** 303.

Mozafariyan M., Kamelmanesh M. M. & Hawrylak-Nowak B. (2016) Ameliorative effect of selenium on tomato plants grown under salinity stress*. Archives of Agronomy and Soil Science* **62,** 1368-80.

Munton D. (2002) Fumes, Forests and Further Studies: Environmental Science and Policy Inaction in Ontario*. Journal of Canadian Studies* **37,** 130.

Murray H., Thompson K. & Macfie S. M. (2009) Site- and species-specific patterns of metal bioavailability in edible plants. *Botany* **87,** 702-11.

Murray M. (2019) White’s Creek Wetland, UTS Subject 91159 Environmental Remediation, online lecture modules, UTS, Sydney, viewed 10 September 2019.

Mykolenko S., Liedienov V., Kharytonov M., Makieieva N., Kuliush T., Queralt I., Marguí E., Hidalgo M., Pardini G. & Gispert M. (2018) Presence, mobility and bioavailability of toxic metal(oids) in soil, vegetation and water around a Pb-Sb recycling factory (Barcelona, Spain). *Environmental Pollution* **237,** 569.

Nair P. M. G. & Chung I. M. (2015) Study on the correlation between copper oxide nanoparticles induced growth suppression and enhanced lignification in Indian mustard (*Brassica juncea* L.). *Ecotoxicology and Environmental Safety* **113,** 302-13.

Nafiu A., Agbenin John O. & Andreas B. (2011) Phytoavailability, human risk assessment and transfer characteristics of cadmium and zinc contamination from urban gardens in Kano, Nigeria. *Journal of the Science of Food and Agriculture* **91,** 2722-30.

Nakagawa K. & Tsunematsu T. (2010) Feasibility Study on Phytoremediation of Heavy Metal Contaminated Soil by Turnip *Brassica Rapa*. *Doboku Gakkai Ronbunshu* **2002,** 89-97.

National Environmental Protection Measure (NEPM) (2013) Schedule B1: Guideline on Investigation for Soil and Groundwater. Viewed 22 May 2018 <http://www.nepc.gov.au/system/files/resources/93ae0e77-e697-e494-656f-afaaf9fb4277/files/schedule-b1-guideline-investigation-levels-soil-and-groundwater-sep10.pdf>

Nature (2014) Seven Days: The news in brief, Business: Power Out. *Nature* **506,** (7488) 270-271, Viewed May 7 2020. <www.dx.doi.org/10.1038/506270a/>

Nie X., Dong F., Ding D., Liu N., Li G., Zhang D. & Liu M. (2015) Ability of *Pistia stratiotes* L. and *Eichhornia crassipes* for Remediation of Uranium-contaminated Waste Water. *Atomic Energy Science and Technology* **49,** 1946-1953.

Ning Z., He L., Xiao T. & Márton L. (2015) High Accumulation and Subcellular Distribution of Thallium in Green Cabbage (*Brassica oleracea* L. Var. *Capitata* L.). *International Journal of Phytoremediation* **17,** 1097-104.

Nkrumah P., Baker A., Chaney R., Erskine P., Echevarria G., Morel J. & Ent A. (2016) Current status and challenges in developing nickel phytomining: an agronomic perspective. *Plant and Soil* **406,** 55-69.

Olguin E. J., Garcia-Lopez D. A., Gonzalez-Portela R. E. & Sanchez-Galvan G. (2017) Year-round phytofiltration lagoon assessment using *Pistia stratiotes* within a pilot-plant scale biorefinery. *Science of the Total Environment* **592,** 326-33.

Olson P. E. & Fletcher J. S. (1999) Field Evaluation of Mulberry Root Structure with Regard to Phytoremediation. *Bioremediation Journal* **3,** 27-34.

Orians G. H. & Milewski A. V. (2007) Ecology of Australia: the effects of nutrient-poor soils and intense fires*. Biological Reviews* **82,** 393-423.

Pandey V. C. & Mishra T. (2018) Assessment of *Ziziphus mauritiana* grown on fly ash dumps: Prospects for phytoremediation but concerns with the use of edible fruit. *International Journal of Phytoremediation* **20,** 1250-6.

Panfili F., Manceau A., Sarret G., Spadini L., Kirpichtchikova T., Bert V., Laboudigue A., Marcus M. A., Ahamdach N. & Libert M. (2005) The effect of phytostabilization on Zn speciation in a dredged contaminated sediment using scanning electron microscopy, X-ray fluorescence, EXAFS spectroscopy, and principal components analysis. *Geochimica et Cosmochimica Acta* **69,** 2265-2284.

Parimala V., Krishnani K. K., Gupta B. P., Jayanthi M. & Abraham M. (2004) Phytoremediation of Chromium from Seawater Using Five Different Products from Coconut Husk. *Bulletin of Environmental Contamination and Toxicology* **73,** 31-37.

Parrish Z. D., Banks M. K. & Schwab A. P. (2004) Effectiveness of Phytoremediation as a Secondary Treatment for Polycyclic Aromatic Hydrocarbons (PAHs) in Composted Soil. *International Journal of Phytoremediation* **6,** 119-137.

Passioura J. B. (2006) The perils of pot experiments. *Functional Plant Biology* **33**, 1075-1079.

Paz-Alberto A., Celestino A. B. & Sigua G. C. (2014) Phytoremediation of Pb in the sediment of a mangrove ecosystem. *Journal of Soils and Sediments* **14,** 251-8.

Peer W. A., Mamoudian M., Lahner B., Reeves R. D., Murphy A. S. & Salt D. E. (2003) Identifying model metal hyperaccumulating plants: germplasm analysis of 20 Brassicaceae accessions from a wide geographical area. *New Phytologist* **159,** 421-30.

Pérez-Sanz A., Millán R., Sierra M. J., Alarcón R. García P., Gil-Díaz M., Vazquez S. & Lobo M. C. (2012) Mercury uptake by *Silene vulgaris* grown on contaminated spiked soils. *Journal of Environmental Management* **95,** S233-S23.

Phillips M. L. & Murray B. R. (2012) Invasiveness in exotic plant species is linked to high seed survival in the soil. *Evolutionary Ecology* **14,** 83-94.

Pigna M., Cozzolino V., Violante A. & Meharg A. A. (2009) Influence of Phosphate on the Arsenic Uptake by Wheat (*Triticum durum* L.) Irrigated with Arsenic Solutions at Three Different Concentrations. *Water, Air and Soil Pollution* **197,** 371-80.

Poschenrieder C., Bech J., Llugany M., Pace A., Fenés E. & Barceló J. (2001) Copper in plant species in a copper gradient in Catalonia (north east Spain) and their potential for phytoremediation. *Plant Soil* **230,** 247-256.

Prasad A., Singh A. K., Chand S., Chanotiya C. S. & Patra D. D. (2010) Effect of Chromium and Lead on Yield, Chemical Composition of Essential Oil, and Accumulation of Heavy Metals of Mint Species. *Communications in Soil Science and Plant Analysis* **41,** 2170-86.

Pulkownik A. (2000) Ecotoxicology of metals in estuarine plants. PhD Thesis, University of Technology, Sydney.

Qin F., Liu G., Huang G., Dong T., Liao Y. & Xu X. (2018) Zinc application alleviates the adverse effects of lead stress more in female *Morus alba* than in males. *Environmental and Experimental Botany* **146,** 68-76.

R Core Team (2019) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.

Rafati M., Khorasani N., Moattar F., Shirvany A., Moraghebi F. & Hosseinzadeh S. (2011) Phytoremediation Potential of *Populus Alba* and *Morus alba* for Cadmium, Chromuim and Nickel Absorption from Polluted Soil. *International Journal of Environmental Research* **5,** 961-70.

Rahman M. M., Azirun S. M., Boyce A. N. & Bennett M. (2013) Enhanced Accumulation of Copper and Lead in Amaranth (Amaranthus paniculatus), Indian Mustard (*Brassica juncea*) and Sunflower (*Helianthus annuus*). *PLoS ONE* **8,** e62941.

Rai P. K., Lee S. S., Zhang M., Tsang Y. F. & Kim K. (2019) Heavy metals in food crops: Health risks, fate, mechanisms, and management. *Environment International* **125,** 365-85.

Rede D., Santos L. H. M. L. M., Ramos S., Oliva-Teles F., Antão C., Sousa S. R. & Delerue-Matos C. (2016) Ecotoxicological impact of two soil remediation treatments in *Lactuca sativa* seeds. *Chemosphere* **159,** 193-198.

Reid M. S., Hoy K. S., Schofield J. R. M., Uppal J. S., Lin Y., Lu X., Peng H. & Le X. C. (2020) Arsenic speciation analysis: A review with an emphasis on chromatographic separations. *Trac-Trends in Analytical Chemistry* **123,** 115770.

Reynolds R. (2012) Guerrilla gardening -- why people garden without boundaries: Richard Reynolds at TEDxItaewon. TEDx Talks, 11 Aug 2012, Korea, Viewed 26 March 2020, *YouTube.* <https://youtu.be/mQPZsD8nKu4>.

Rezek J., der Wiesche C., Mackova M., Zadrazil F. & Macek T. (2009) Biodegradation of Pahs in Long-Term Contaminated Soil Cultivated with European White Birch (*Betula Pendula*) and Red Mulberry (*Morus Rubra*) Tree. *International Journal of Phytoremediation* **11,** 66-81.

Rickson R. J., Deeks L. K., Graves A., Harris J. A. H., Kibblewhite M. G. & Sakrabani R. (2015) Input constraints to food production: the impact of soil degradation. *Food Security* **7,** 351-64.

Rizwan M., Ali S., Rizvi H., Rinklebe J., Tsang D. C. W., Meers E., Ok Y. S. & Ishaque W. (2016) Phytomanagement of heavy metals in contaminated soils using sunflower: A review. *Critical Reviews in Environmental Science and Technology* **46,** 1498-528.

Roberts D. A., Paul N. A., Bird M. I. & de Nys R. (2015) Bioremediation for coal-fired power stations using macroalgae*. Journal of Environmental Management* **153,** 25-32.

Robertson A. W., Trass A., Ladley J. J. & Kelly D. (2006) Assessing the benefits of frugivory for seed germination: the importance of the deinhibition effect. *Functional Ecology* **20,** 58-66.

Robinson B., Green S., Mills T., Clothier B., van der Velde M., Laplane R., Fung L., Deurer M., Hurst S., Thayalakumaran T. & van den Dijssel C. (2003) Phytoremediation: using plants as biopumps to improve degraded environments. *Australian Journal of Soil Research* **41,** 599.

Robinson B. & Anderson C. (2007) Phytoremediation in New Zealand and Australia, *Methods in Biotechnology*, N. Willey, Humana Press Inc., Totowa, NJ. **23,** 455-468.

Rojas R. V., Achouri M., Maroulis J. & Caon L. (2016) Healthy soils: a prerequisite for sustainable food security. *Environmental Earth Sciences* **75,** 1-10.

Rojjanateeranaj P., Sangthong C. & Prapagdee B. (2017) Enhanced cadmium phytoremediation of *Glycine max* L. through bioaugmentation of cadmium-resistant bacteria assisted by biostimulation. *Chemosphere* **185,** 764-71.

Rouillon M., Harvey P. J., Kristensen L. J., George S. G. & Taylor M. P. (2017) VegeSafe: A community science program measuring soil-metal contamination, evaluating risk and providing advice for safe gardening. *Environmental Pollution* **222,** 557-66.

Roy M. & McDonald L. M. (2015) Metal Uptake in Plants and Health Risk Assessments in Metal-Contaminated Smelter Soils. *Land Degradation and Development* **26,** 785-92.

Rubio B., Nombela M. A. & Vilas F. (2000) Geochemistry of Major and Trace Elements in Sediments of the Ria de Vigo (NW Spain): an Assessment of Metal Pollution. *Marine Pollution Bulletin* **40,** 968-80.

Rufus L., Chaney J. Angle S., Li Y. & Baker A. J. M. (2007) Recovering Metals from Soils. US Patent 7268273, Justia 2020, Accessed 20 May 2020. <https://patents.justia.com/patent/7268273>

Ruscitti M., Arango M. & Beltrano J. (2017) Improvement of copper stress tolerance in pepper plants (*Capsicum annuum* L.) by inoculation with arbuscular mycorrhizal fungi. *Theoretical and Experimental Plant Physiology* **29,** 37-49.

Saeed W., Naqvi T. A., Shah M. M. & Ahmad R. (2015) Dietary Toxicity of Lead and Hyper-Accumulation in *Petroselinum crispum*. *Arabian Journal for Science and Engineering* **40,** 1819-24.

Saha R. & Pal P. (2018) Assessment of Arsenic Toxicity and Tolerance Characteristics of Bean Plants (*Phaseolus Vulgaris*) Exposed to Different Species of Arsenic. *Journal of Plant Nutrition* **41,** 340-7.

Salama A. K., Osman K. A. & Gouda N. A.-R. (2016) Remediation of lead and cadmium-contaminated soils. *International Journal of Phytoremediation* **18,** 364-367.

Sánchez-Rendón J., Reino-Molina J., Pernús-Alvarez M., Morales-Querol D. & Martín-Martín G. (2017) Effect of controlled conditions on the germination of five *Morus alba* L. varieties. *Pastos y Forrajes* **40,** 264+.

Sarwar N., Imran M., Shaheen M. R. Ishaque W., Kamran M. A., Matloob A., Rehim A. & Hussain S. (2017) Phytoremediation strategies for soils contaminated with heavy metals: Modifications and future perspectives*. Chemosphere* **171,** 710-21.

Säumel I., Kotsyuk I., Hölscher M., Lenkereit C., Weber F. & Kowarik I. (2012) How healthy is urban horticulture in high traffic areas? Trace metal concentrations in vegetable crops from plantings within inner city neighbourhoods in Berlin, Germany*. Environmental Pollution* **165,** 124-32.

Sethy S. & Ghosh S. (2013) Effect of heavy metals on germination of seeds. *Journal of Natural Science, Biology and Medicine* **4,** n/a.

Shahid M., Xiong T., Masood N*.,* Leveque T., Quenea K., Austruy A., Foucault Y. & Dumat C. (2014) Influence of plant species and phosphorus amendments on metal speciation and bioavailability in a smelter impacted soil: a case study of food-chain contamination*. Journal of Soils and Sediments* **14,** 655-65.

Shahid M., Dumat C., Khalid S., Schreck E., Xiong T. & Niazi N. K. (2017) Foliar heavy metal uptake, toxicity and detoxification in plants: A comparison of foliar and root metal uptake. *Journal of Hazardous Material* **325,** 36-58.

Song X., Hu X., Ji P., Li Y., Chi G. & Song Y. (2012) Phytoremediation of Cadmium-Contaminated Farmland Soil by the Hyperaccumulator *Beta vulgaris* L. var. *cicla*. *Bulletin of Environmental Contamination and Toxicology* **88,** 623-6.

Soudek P., Kotyza J., Lenikusova I., Petrova S., Benešová D. & Vaněk T. (2009) Accumulation of heavy metals in hydroponically cultivated garlic (*Allium sativum* L.), onion (*Allium cepa* L.), leek (*Allium porrum* L.) and chive (*Allium schoenoprasum* L.). *Journal of Food, Agriculture & Environment* **7,** 761-769.

Stamets P. E., Naeger N. L., Evans J. D*.,* Han J. O., Hopkins B. K., Lopez D., Moershel H. M., Nally R., Sumerlin D., Taylor A. W., Carris L. M. & Sheppard W. S. (2018) Extracts of Polypore Mushroom Mycelia Reduce Viruses in Honey Bees*. Scientific Reports* **8,** 13936.

State of New South Wales and Office of the Environment and Heritage (2013) *White Bay Power Station Complex*, State Heritage Register, Viewed 1 May 2020. <https://www.environment.nsw.gov.au/heritageapp/ViewHeritageItemDetails.aspx?ID=4500460>

Su-Lin T. (2016) Google in talks to develop landmark site as tech hub. *The Australian Financial Review* **29,** Fairfax Media Publications Pty Ltd., Melbourne.

Takahashi M., Kohama S., Shigeto J., Hase Y., Tanaka A. & Morikawa H. (2012) Mutants of *Ficus Pumila* Produced by Ion Beam Irradiation with an Improved Ability to Uptake and Assimilate Atmospheric Nitrogen Dioxide. *International Journal of Phytoremediation* **14,** 275-81.

Tang L., Hamid Y., Zehra A., Sahito Z. A., He Z., Hussain B., Gurajala H. K. & Yang X. (2019) Characterization of fava bean (*Vicia faba* L.) genotypes for phytoremediation of cadmium and lead co-contaminated soils coupled with agro-production. *Ecotoxicology and Environmental Safety* **171,** 190-8.

Tang X., Lim M. P. & McBride M. B. (2018) Arsenic uptake by arugula (*Eruca vesicaria*, L.) cultivars as affected by phosphate availability. *Chemosphere* **195,** 559-66.

Tanwar A., Aggarwal A., Charaya M. U. & Kumar P. (2015) Cadmium Remediation by Arbuscular Mycorrhizal Fungus–Colonized Celery Plants Supplemented with Ethylenediaminetetraacetic Acid. *Bioremediation Journal* **19,** 188-200.

Tchounwou P. B., Yedjou C. G., Patlolla A. K., Sutton D. J. & Luch A. (2012) Heavy Metal Toxicity and the Environment. *Molecular, Clinical and Environmental Toxicology. Experientia Supplementum* **101,** Springer Basel, Switzerland.

Temple J. B. (2008) Severe and Moderate Forms of Food Insecurity in Australia: Are They Distinguishable? *Australian Journal of Social Issues (Australian Council of Social Service)* **43,** 649-68.

Thijs S., Sillen W., Weyens N. & Vangronsveld J. (2017) Phytoremediation: State-of-the-art and a key role for the plant microbiome in future trends and research prospects. *International Journal of Phytoremediation* **19,** 23-38.

Tóth G., Hermann T., da Silva M. R. & Montanarella L. (2016) Heavy metals in agricultural soils of the European Union with implications for food safety. *Environment International* **88,** 299-309.

Turner B., Henryks J. & Pearson D. (2011) Community gardens: sustainability, health and inclusion in the city. *Local Environment* **16,** 489-92.

United Nations Environment Programme (2013) *Global Alliance to Eliminate Lead Paint: Business Plan*. Geneva. Viewed 14 June 2018. <http://drustage.unep.org/chemicalsandwaste/sites/unep.org.chemicalsandwaste/files/publications/GAELP_BusinessPlan.pdf>

UrbanGrowth NSW (2015) The Bays Precinct Sydney Transformation Plan. Accessed 14 May 2020. <https://thebayssydney.nsw.gov.au/assets/Document-Library/Reports-and-Plans-2014-/2015-Transformation-Plan.pdf>

Ursitti F., Vanderlinden L., Watson R. & Campbell M. (2004) Assessing and Managing Exposure from Arsenic in CCA-Treated Wood Play Structures. *Canadian Journal of Public Health* **95,** 429-33.

U.S EPA (1994) Method 200.8, Revision 5.4: Determination of Trace Elements in Waters and Wastes by Inductively Coupled Plasma – Mass Spectrometry. *United States Environmental Protection Agency*, Ohio, Accessed 18 June 2020. <https://www.epa.gov/sites/production/files/2015-08/documents/method_200-8_rev_5-4_1994.pdf>

U.S. EPA (1996) Method 3050B: Acid Digestion of Sediments, Sludges, and Soils. Revision 2, *United States Environmental Protection Agency*, Washington, DC.

Utsunomiya T. (1980) Japanese patent application publication. Japanese Patent 55-72959. Kokai, 57–190.

Vávra J., Daněk P. & Jehlička P. (2018) What is the contribution of food self-provisioning towards environmental sustainability? A case study of active gardeners. *Journal of Cleaner Production* **185,** 1015-23.

Vincent J. B. & Lukaski H. C. (2018) Chromium. *Advances in nutrition* **9,** 505-6.

Voutsa D., Grimanis A. & Samara C. (1996) Trace elements in vegetables grown in an industrial area in relation to soil and air particulate matter. *Environmental Pollution* **94,** 325-35.

Wang X., Sato T., Xing B., Tao S. (2005) Health risks of heavy metals to the general public in Tianjin, China via consumption of vegetables and fish. *Science of the Total Environment* **350,** 28-37.

Ware S., Johnstone C., Sparks K., Allan P., Bryant M., Murray A. (2018). *Power Plants*. Phytoremediation Gardens. Stage One Report. NSW Government, Landcom, Sydney, Australia.

World Health Organisation (2004) *Environmental Health Criteria 221:* zinc. Geneva. Viewed 28 May 2018. <http://www.who.int/ipcs/publications/ehc/ehc_221/en/>

World Health Organisation (2007) *Health risks of heavy metals from long-range transboundary air pollution*. Denmark. Viewed 2 April 2020. <http://www.euro.who.int/__data/assets/pdf_file/0007/78649/E91044.pdf>

World Health Organisation (2018) *Global Health Observatory (GHO) data*. Geneva. Viewed 9 May 2018. <http://www.who.int/gho/phe/chemical_safety/lead_paint_regulations/en/>.

Wright C. & Nyberg D. (2012) Working with passion: Emotionology, corporate environmentalism and climate change. *Human Relations* **65,** 1561-87.

Xiao R., Wang S., Li R., Wang J. J. & Zhang Z. (2017) Soil heavy metal contamination and health risks associated with artisanal gold mining in Tongguan, Shaanxi, China*. Ecotoxicology and Environmental Safety* **141,** 17-24.

Xiong Z. (1998) Lead Uptake and Effects on Seed Germination and Plant Growth in a Pb Hyperaccumulator *Brassica pekinensis* Rupr*. Bulletin of Environmental Contamination and Toxicology* **60,** 285-91.

Yale University (2018) *2018 EPI Report: Heavy Metals*. Environmental Performance Index. Viewed 18 June 2018. <https://epi.envirocenter.yale.edu/2018-epi-report/heavy-metals>

Yang L., Han Y., Wu D., Wang Y., Miaomiao L., Sutong W., Wenxin L., Meiyi L., Ying W. & Jinsheng S. (2017) Salt and cadmium stress tolerance caused by overexpression of the *Glycine Max* Na+/H+ Antiporter (GmNHX1) gene in duckweed (*Lemna turionifera* 5511). *Aquatic Toxicology* **192,** 127-35.

Yang Y., Ge Y., Tu P., Zeng H., Zhou X., Zou D., Wang K. & Zeng Q. (2019) Phytoextraction of Cd from a contaminated soil by tobacco and safe use of its metal-enriched biomass. *Journal of Hazardous Materials* **363,** 385-93.

Ying G., Rawson C. A., Kookana R. S., Peng P., Warne M. S. J., Tremblay L. A., Laginestra E., Chapman J. C. & Lim R. P. (2009) Contamination and screening level toxicity of sediments from remediated and unremediated wetlands near Sydney, Australia. *Environmental Toxicology and Chemistry* **28,** 2052.

Yousra M., Saman L., Ahmad R. & Mahmood-ul-Hassan M. (2020) Floriculture: alternate non-edible plants for phyto-remediation of heavy metal contaminated soils. *International Journal of Phytoremediation* **22,** 725-32.

Zhang H., Zheng Feei Ma, Luo X. & Li X. (2018) Effects of Mulberry Fruit (*Morus alba* L.) Consumption on Health Outcomes: A Mini-Review. *Antioxidants* **7,** 69.

Zhang L., Yin L. & Pan X. (2013) Germination, Survival, Growth Response of Eight Desert Plants to Oil Pollution and Removal Efficiency of Total Petroleum Hydrocarbon (TPHs). *Vegetos* **26,** 171-82.