Remediation of New Zealand sheep dip sites using biochar and phytoextraction technologies

A thesis presented in partial fulfilment of the requirements for the degree of

Doctor of Philosophy in Soil Science

Institute of Agriculture and Environment

College of Sciences, Massey University

Palmerston North, New Zealand

Samuel Gregory

2013

ABSTRACT

The practice of sheep dipping, which subjected livestock to inorganic and organic agricultural pesticides to eradicate pests such as lice and keds, is a historic practice; sheep dipping is no longer practiced in New Zealand today. Animals would be submerged in solid structures known as dips containing chemicals such as arsenicals and organochlorines with the leftover solution pumped onto surrounding soil. The use of pesticides such as these is now banned by law due to their persistence in the environment. Today an estimated 50,000 contaminated sheep dip sites exist in New Zealand representing perhaps the countries' most significant, but understated, environmental challenge.

To determine whether this historic agricultural practice had led to contamination of the environment, an investigation into the extent of contamination resulting from sheep dipping at a known historic dip site in Te Mahia, New Zealand was carried out. Characterisation of the site by arsenic soil concentration mapping revealed that 500 m^2 of agricultural land has been contaminated with this metalloid and that arsenic exists at varying high concentrations through the soil profile. Environmental risk from these historic pesticides was established by analysing plant and water samples below the dip site. Staple Maori food varieties such as watercress were significantly contaminated with arsenic while water samples taken from the stream below the dip returned spiked arsenic concentrations. Based on this, it was justified that arsenic/organochlorine contamination would need to be managed to reduce their effect on these food sources. The design of a coupled remediation strategy using phytoextraction and biochar was utilized to reduce remediation times and is the basis of this thesis.

Contaminated soil from the site was removed and amended with two types of biochar produced from willow feedstock. These biochars, known as 350°C and 550°C biochar were added into the soil at application rates of 30 t ha^{-1} and 60 t ha^{-1}. During a series of 180 d glasshouse trials, the phytoextraction of arsenic into *Lolium perenne* (ryegrass) shoot tissue was analysed along with growth parameters of shoot and root biomass and corresponding response to arsenic at the molecular level. In soil; microbial activity,

soil bacterial community, organochlorine concentration, and element dynamics were analysed as a function of biochar amendment.

Soil microbial activity, analysed using the dehydrogenase assay (DHA), was significantly increased ($P<0.01$) under all biochar treatments compared to the control after 180 d during two glasshouse trials. Metagenomic analysis of the soil bacterial community revealed that biochar amended soils were selecting for bacterial species such as Chryseobacterium, Flavobacterium and Dyadobacter and the family Pseudomonadaceae which are known bioremediators of hydrocarbons. This resulted in isomers of the organochlorine hexachlorocyclohexane (HCH), particularly alpha-HCH and gamma-HCH (lindane), undergoing 10-fold and 4-fold reductions in soil concentrations respectively (2.2 mg kg^{-1} and 0.4mg kg^{-1}) compared to the control (25 mg kg^{-1} and 1.6 mg kg^{-1} respectively). Amendment of soil with both biochars also caused a significant reduction ($P<0.01$) in soil DDT levels.

Biochar promoted a 2-fold increase in shoot dry weight (DW) and a 3-fold increase in root DW after 180 d during one glasshouse trial while during the second trial only ryegrass root biomass was significantly increased as a function of biochar amendment. This increase was attributed, at least in part, to the fertility value of biochar. No negative effect of biochar amendments on ryegrass germination was observed. All biochar amendments resulted in significant increases in arsenic concentrations within ryegrass shoot material. Through extrapolation, 350°C biochar amended soils was estimated to have the potential to increase ryegrass sward DW growth by 0.68 t ha^{-1} compared to ryegrass grown on unamended soils and would correspond to an increase in the extraction of total arsenic by 14,000 mg ha^{-1} compared to unamended soils and in doing so decrease soil remediation times by over 50 %.

Increased arsenic uptake as a function of biochar amendment resulted in increased enzymic activity of components of the antioxidant pathway including SOD and APX in most biochar treatments but across all treatments a reduction in GPX activity was observed. Analysis of specific metabolites utilizing metabolomics also suggest a definitive metabolite profile under biochar amendment compared to contaminated

control ryegrass samples. However, there was no significant difference ($P<0.05$) in chlorophyll content in response to the total arsenic concentration in ryegrass shoot tissue grown on contaminated soil. The observed increases in activity of SOD, APX and steady CAT activity is suggested to be efficiently catalysing the production of harmful ROS in this soil.

A 6-month field investigation into the effect of biochar amendment on the extraction of arsenic into a high biomass crop (*Salix sp*) resulted in significant increases of arsenic in stem biomass as a function of biochar amendment. When data was extrapolated to predict results of a long-term field trial and scale under willow treatment (stem) it was calculated that over 67.7 g of arsenic could be extracted in soils amended with 350°C biochar compared to 5.9 g extracted under control treatment. This could result - assuming a similar rate of extraction with time - in levels of arsenic concentration in soils reaching background concentrations in as little as 6 years, a reduction in remediation times of 92%.

ACKNOWLEDGEMENTS

The research detailed within this thesis could not have been completed without the support of a number of people. My sincerest gratitude goes to Dr Chris Anderson, Professor Michael McManus and Associate Professor Marta Camps for their knowledge and help over a multitude of different aspects relating to plant-soil interactions. I truly appreciate your time and expertise.

I would also like to acknowledge Jack Bowen (farm manager) for allowing continued access on the farm and always supporting the scientific nature of the research within this thesis.

I have been part of a number of lab groups over the past three years and would like to thank those that have helped along the way. To lab members of MTM, particularly Susanna Leung for technical guidance, I have thoroughly appreciated all the help you have given me. Soil Science Lab members; coming from a genetic and plant background, I struggled with the techniques carried out at first in your lab so my biggest thank you is to Bob Toes, Ian Furkert and Ross Wallace for their time. I would also like to mention Tao Wang and Roberto Calvelo-Pereira for their time in regards to biochar analysis. Thanks also to my friends at Massey, particularly Leigh, Matt, and Todd for your support, both at home and during glasshouse trials.

My studies were supported by a Ti Tipu Putaiao Fellowship, funded by the Ministry of Science and Innovation and I am also grateful for further travel support from both the Institute of Fundamental Sciences and Institute of Agriculture and Environment.

I am deeply grateful to my family for always supporting my ambitions in life, even though I have spent the good part of a decade in constant student-related poverty. Last but not least, to my wife Kate, for your constant support and help through the last three years has been immeasurable. I love you and you are my rock.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

AAS	Atomic Absorption Spectrometry
AOAC	Association of Analytical Communities
APX	Ascorbate Peroxidase
ARML	AgResearch Metabolomic Laboratory
As	Arsenic
ATP	Adenosine-5'-Triphosphate
BET	Brunauer–Emmett–Teller
bp	Base Pair
°C	Degrees Celsius
CaCO3	Calcium Carbonate
CAT	Catalase
CEC	Cation Exchange Capacity
cm	Centimetre
C_{org}	Organic Carbon
d	Day
DDD	Dichlorodiphenyldichloroethane
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
DHA	Dehydrogenase Activity
DM	Dry Matter
DNA	Deoxyribonucleic Acid
DW	Dry Weight
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic Acid
eV	Electronvolt
FIAS	Flow Injection Atomic Spectroscopy

FW	Fresh Weight
GC-MS	Gas Chromatography – Mass Spectrometry
GFAAS	Graphite Furnace Atomic Absorption Spectrometry
GPX	Guaiacol Peroxidase
H_2O_2	Hydrogen Peroxide
HCH	Hexachlorocyclohexane
H/C_{org}	Hydrogen / Organic Carbon Ratio
HILIC	Hydrophilic Interaction Liquid Chromatography
HNO_3	Nitric Acid
hr	Hour
IAA	Indole-3-acetic Acid
K_2PO_4	Potassium Phosphate
KI	Potassium Iodide
LC-MS	Liquid Chromatography – Mass Spectrometry
M	Molar
Meq	Milliequivalents
min	Minute
mL	Milli Litres
mmol	Millimoles
MQ	Milli Q
mV	Milli Volts
N_2	Nitrogen
NaCl	Sodium Chloride
NBT	Nitro-blue Tetrazolium
ng	Nanogram
O/N	Overnight
PC	Phytochelatin

PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PGPR	Plant Growth Promoting Rhizobacteria
PLS-DA	Partial Least Squares - Discriminant Analysis
ROS	Reactive Oxygen Species
rpm	Revolutions Per Minute
s.e	Standard Error
SOD	Superoxide Dismutase
sp.	Species
TGA	Thermogravimetric Analysis
t ha^{-1}	Tonnes per Hectare
WHC	Water Holding Capacity
WHO	World Health Organisation
w/v	Weight to Volume
w/w	Weight to Weight
XPS	X-ray Photoelectron Spectroscopy