USE OF HYDROGEL BASED SOIL AMENDMENTS TO PROMOTE SAFE USE OF WASTEWATER IN AGRICULTURE

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Table of Contents

List of Figures vii

List of Tables ix

List of Symbols and Abbreviations xi

Abstract xiii

Résumé xv

Acknowledgements xvii

Dedication xix

Thesis Format and Author Contributions xx

Chapter 1: Introduction 1

1.1 General Introduction 1

1.2 Study Objectives 3

1.3 Thesis Outline 3

Chapter 2: Literature Review 5

2.1 Global Freshwater Crisis Overview 5

2.2 Wastewater Irrigation 5

2.3 Heavy Metals in Wastewater 6

2.3.1 Harmful Effects 7

2.3.2 Environmental Occurrence 8

2.3.3 Environmental Fate 9

2.4 Super Absorbent Polymers (SAPs) 10

2.4.1 Classification 11

2.4.2 Agricultural Uses 12

2.4.3 Environmental Fate and Safety 13

2.4.4 Heavy Metal Removal 14

2.5 Biochar 16

2.5.1 Agricultural Uses 16

2.5.2 Removal of Heavy Metals........ 17

2.6 Heavy Metal Uptake by Plants........ 18

2.6.1 Adverse Effects on Plants 18

2.6.2 Uptake and Distribution Mechanism 18

2.6.3 Effect of pH and Plant Type on Plant Metal Uptake 19

2.6.4 Potatoes........ 20

2.6.5 Spinach........ 20

2.7 Adsorbent Sorption Capacity 20

2.8 Knowledge Gap 23

Connecting Text to Chapter 3 24

Chapter 3: Use of Polyacrylamide Superabsorbent Polymers and Plantain Peel Biochar to Reduce Heavy Metal Mobility and Uptake by Wastewater Irrigated Potato Plants........ 25

3.1 Abstract........ 25

3.2 Introduction........ 26

3.3 Methods and Materials........ 29

3.3.1 Characterisation of SAP and Biochar 29

3.3.2 Experimental Setup 31

3.3.3 Preparation of Synthetic Wastewater 32

3.3.4 Irrigation Scheduling........ 34

3.3.5 Soil and Leachate Sampling........ 34

3.3.6 pH Measurement 34

3.3.7 Heavy Metal Extraction and Quantification for Soil and Leachate Samples........ 34

3.3.8 Heavy Metal and Acrylamide Extraction and Quantification for Plant Tissue Samples 35

3.3.9 Data Analysis 36

3.4 Results........ 36

3.4.1 pH and Acrylamide Content 36

3.4.2 Heavy Metals in Soil 37

3.4.3 Heavy Metals in Plant Tissue 45

3.5 Discussion 48

3.6 Conclusions 51

3.7 Acknowledgement 52

Connecting Text to Chapter 4 54

Chapter 4: Effect of Hydrogel Based Soil Amendments on Heavy Metal Uptake by Spinach Grown with Wastewater Irrigation 55

4.1 Abstract 55

4.2 Introduction 56

4.3 Materials and Methods 58

4.3.1 Soil Amendments 58

4.3.2 Sorption experiment 59

4.3.3 Field study 61

4.3.4 Sample extraction and quantification 62

4.3.5 Data analysis and quality assurance 63

4.4 Results and Discussion 63

4.4.1 Sorption Experiment 63

4.4.2 Lysimeter Soil pH and CEC 69

4.4.3 Heavy metals in soil 70

4.4.4 Heavy metals in plant tissue 76

4.5 Conclusions 80

4.6 Acknowledgment 82

Connecting Text to Chapter 5 83

Chapter 5: Heavy Metal Uptake by Wastewater Irrigated Potato Plants Grown on Contaminated Soil Amended with Hydrogel and Biochar 84

5.1 Abstract 84

5.2 Introduction 85
5.3 Materials and Methods 87
5.3.1 Adsorbent material 87
5.3.2 Sorption experiment 88
5.3.3 Field study 88
5.3.4 Sample extraction and quantification 90
5.3.5 Data analysis and quality assurance 91
5.4 Results and Discussion 92
5.4.1 Sorption experiment 92
5.4.2 Soil pH and CEC 98
5.4.3 Heavy metals in soil 99
5.4.4 Heavy metals in plant tissue 104
5.5 Conclusions 108
5.6 Acknowledgment 110
Connecting Text to Chapter 6 111
Chapter 6: Effect of Hydrogel and Biochar Soil Amendments on Yield and Growth of Wastewater Irrigated Plants 112
6.1 Abstract 112
6.2 Introduction 113
6.3 Materials and Methods 114
6.3.1 Study Area 114
6.3.2 Soil Amendments 115
6.3.3 Design of Experiments 116
6.3.4 Synthetic Wastewater 118
6.3.5 Plant Yield and Physiological Parameters 119
6.3.6 Soil Moisture Content 120
6.3.7 Data Analysis 121

6.4 Results and Discussion 122
6.4.1 Soil Moisture Content 122
6.4.2 Plant Yield and Physiological Parameters 124
6.4.3 Root Structure Analysis 133
6.5 Conclusions 134
6.6 Acknowledgments 134
Chapter 7: Summary and Conclusions 135
7.1 Summary 135
7.2 Conclusions 135
7.2.1 Sorption Test 135
7.2.2 Soil pH and CEC 136
7.2.3 First Year Field Study with Potatoes 136
7.2.4 Second Year Field Study with Potato 137
7.2.5 Field Study with Spinach 138
7.2.6 Field Study – Plant Yield and Growth Parameters 139
7.3 Future Recommendations 140
Chapter 8: Contributions to Knowledge 142
References 143

List of Figures

Figure 2.1 Global freshwater withdrawal by sector (*FAO, 2016*)... 6

Figure 2.2 A schematic representation of SAP swelling (*adapted from Zohuriaan-Mehr and Kabiri, 2008*). 11

Figure 2.3 C_{is} (y axis) vs C_{iw} (x axis) curves showing different situations; Freundlich exponent, n=1 (a), n<1 (b and c), n>1 (d) (adapted from Schwarzenbach et al., 2005)……………………………………22

Figure 3.1 Schematic diagram of a lysimeter. 31

Figure 3.2 Mean concentrations of cadmium in surface soil samples after successive irrigations. 37

Figure 3.3 Mean concentrations of chromium in surface soil samples after successive irrigations. 39

Figure 3.4 Mean concentrations of copper in surface soil samples after successive irrigations. 40

Figure 3.5 Mean concentrations of iron in soil samples from the surface, 0.10 and 0.30 m depths after successive irrigations. 41

Figure 3.6 Mean concentrations of lead in surface and 0.10 m depth soil samples after successive irrigations. 43

Figure 3.7 Mean concentrations of zinc in surface and 0.10 m depth soil samples after successive irrigations (*Zn was not detected for first three irrigation events at 0.10 m depth below the surface*). 44

Figure 4.1 Sorption and desorption of heavy metal ions at different concentrations by treatments SAP+PBC, SAP and control 65-66

Figure 4.2 pH of multi-metal sorption solution at different concentrations for treatments SAP+PBC, SAP and control 67

Figure 4.3 Surface soil concentrations of heavy metals (a) Cd, (b) Cr, (c) Cu, (d) Fe, (e) Pb and (f) Zn, for all treatments and all irrigation events including background 73

Figure 4.4 Iron concentrations in soil samples collected from 0.10 m depth, after last irrigation event, for all treatments 75

Figure 5.1 Sorption and desorption of heavy metal ions at different concentrations by treatments SAP+GBC, SAP and control. 93-94
Figure 5.2 pH of multi-metal sorption solution at different concentrations for treatments SAP+PBC, SAP and control 95
Figure 5.3 Surface soil concentrations of heavy metals (a) Cd, (b) Cr, (c) Cu, (d) Fe, (e) Pb and (f) Zn, for all treatments and all irrigation events including background 100
Figure 5.4 Heavy metal concentrations in soil samples collected from 0.10 m depth after last irrigation event, for all treatments.. 104
Figure 6.1 Schematic diagram of lysimeter (taken from Dhiman et al., 2019)..................................... 114
Figure 6.2 Volumetric moisture content for different treatments and lysimeter soil sections at depths (a) 0-0.15 m, (b) 0.15-0.30 and (c) 0.30-0.45 m measured using time-domain reflectometry (TDR)............... 122
Figure 6.3 Water retention curves for treatments SAP, SAP+PBC, PBC (all mixed at 1% w/w with soil) and control 124
Figure 6.4 Relative chlorophyll content index (RCCI) for different treatments for potatoes grown in (a) 2016 and (b) 2015, and (c) spinach grown in 2016.. 128
Figure 6.5 Spinach (a) photosynthetic activity, (b) stomatal conductance and (c) transpiration rate values measured one day before irrigations for different treatments 130
Figure 6.6 Leaf temperature for (a) potato and (b) spinach plants grown in 2016 131
Figure 6.7 Normalized difference vegetative index (NDVI) for different treatments for (a) potato and (b) spinach plants grown in 2016 132
Figure 6.8 (a) Root surface area, (b) volume, (c) length density and (d) average diameter for spinach 2016 crop 133

List of Tables

Table 3.1 Physical and chemical properties of SUPERAB-A200 SAP. 29

Table 3.2 Physiochemical properties of gasified plantain peel biochar. 30

Table 3.3 Physical and chemical properties of lysimeter soil. 32

Table 3.4 Recipe for the preparation of synthetic wastewater for irrigation. 33

Table 3.5 pH values of the soil samples taken two days after sixth irrigation event for all treatments. 36

Table 3.6 Heavy metal uptake in different parts of potato plants in different treatments. 46

Table 4.1 Physiochemical properties of pyrolyzed plantain peel biochar (PBC). 59

Table 4.2 Summary of Freundlich isotherm constants for adsorption of heavy metal ions on soil amended with SAP+PBC and SAP. 67

Table 4.3 Soil pH for samples collected from surface and 0.10 m depth after last irrigation event. 69

Table 4.4 Cation exchange capacity, base saturation and pH_{CEC} of soil samples collected after last irrigation event from surface and 0.10 m depth. 70

Table 4.5 Summary of fixed effects for soil heavy metal concentration from repeated measures analysis. 74

Table 4.6 Concentration of heavy metals (mg kg^{-1}) in different parts of spinach plants. 77

Table 5.1 Summary of Freundlich isotherm constants for adsorption of heavy metal ions on soil amended with SAP+GBC and SAP. 95

Table 5.2 Soil pH for samples collected from surface and 0.10 m depth after the last irrigation event. 98

Table 5.3 Cation exchange capacity, base saturation and pHCEC of soil samples collected after the last irrigation event from surface and 0.10 m depth. 99

Table 5.4 Summary of fixed effects for surface soil heavy metal concentration from repeated measures analysis. 104

Table 5.5 Concentration of heavy metals (mg kg^{-1}) in different parts of potato plants grown in different treatments. 106

Table 6.1 Physio-chemical properties of lysimeter soil (taken from Dhiman et al., 2019). 115

Table 6.2 Physical and Chemical properties of SAP SUPERAB A200 (from Dhiman et al., 2019)........ 115

Table 6.3 Physiochemical properties of gasified and pyrolyzed plantain peel biochar. 116

Table 6.4 Design of experiments for years 2015 and 2016... 118

Table 6.5 Laboratory prepared synthetic wastewater recipe (taken from Dhiman et al., 2019). 119

Table 6.6 Summary of field data collected for different crops. 121

Table 6.7 Yield and plant growth parameters for potatoes grown in years 2015 and 2016 for different treatments. 125

Table 6.8 Yield and plant growth parameters for spinach grown in year 2016 for different treatments. . 125

Table 6.9 Summary of Fixed effects for plant physiological parameters from repeated measures analysis. 127

List of Symbols and Abbreviations

ANSI	American National Standards Institute
ASABE	American Society of Agricultural and Biological Engineers
ASTM	American Society for Testing and Materials
BET	*Brunauer-Emmet-Teller* surface area
BPA/F/S	Bisphenol A/F/S (*structural analogs*)
BS%	Percent base saturation
CCME	Canadian Council of Ministers of the Environment
CEC	Cation exchange capacity ($cmol(+)\ kg^{-1}$)
C_f	Final solution concentration ($mg\ L^{-1}$)
C_{is}	Adsorbate (*i*) concentration on solid phase ($mg\ g^{-1}$)
$C_{is\text{-}max}$	Sorption capacity ($mg\ g^{-1}$)
C_{iw}	Adsorbate (*i*) concentration in aqueous phase ($mg\ L^{-1}$)
C_o	Initial solution concentration ($mg\ L^{-1}$)
CRAAQ	Cultiver l'expertise diffuser le savoir
EBC	European Biochar Certificate
FAO	Food and Agriculture Organization
FW	Freshwater
GBC	Gasified plantain peel biochar
[*Heavy metal*]	Concentration of the specified heavy metal ($mg\ kg^{-1}$)
I.D.	Internal diameter (m)
IC-IMPACTS	India-Canada Centre for Innovative Multidisciplinary Partnerships to Accelerate Community Transformation and Sustainability
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma optical emission spectrometry
ISO	International Organization for Standardization
IWMI	International Water Management Institute
K_{iF}	Freundlich constant ($mg\ g^{-1}$)($L\ mg^{-1}$)$^{(1/n)}$

K_{iL}	Langmuir constant ($L\ mg^{-1}$)
LC-MS	Liquid chromatography mass spectrometry
LOD	Limit of detection
n	Freundlich exponent
NDVI	Normalized difference vegetative index
NHFPC	National Health and Family Planning of People's Republic of China
NIR	Near infrared radiation
NRCC	National Research Council of Canada
NSERC	Natural Sciences and Engineering Research Council of Canada
PAM	Polyacrylamide
PBC	Pyrolyzed plantain peel biochar
PVC	Polyvinyl chloride
R^2	Coefficient of determination
RCCI	Relative chlorophyll content index
SAP	Superabsorbent polymer
STATCAN	Statistics Canada
TDR	Time-domain reflectometry
TGA	Thermogravimetric analyzer
θ_{fc}	Field capacity
UN	United Nations
UNESCO	United Nations Educational, Scientific and Cultural Organization
USEPA	United States Environmental Protection Agency
WHO	World Health Organization
WW	Wastewater
WWTP	Wastewater treatment plant
WWF	World Wildlife Fund
ZPC	Zero point of charge

Abstract

Increasing food demand due to growing population has led to increase in irrigation water requirement. Since agriculture is the largest freshwater consumer, alternate sources of irrigation water, such as wastewater, could help alleviate the fresh water demand. However, wastewater contains contaminants such as heavy metals which could be taken up by food crops or could contaminate groundwater. Therefore, there is an urgent need to develop simple and cost-effective techniques to reduce contaminant mobility in soil and their translocation to crops. This study was conducted to determine the effect of polyacrylamide superabsorbent polymer (SAP) and SAP-plantain peel biochar mix soil amendments, on the mobility and uptake of heavy metals (Cd, Cr, Cu, Fe, Pb, Zn) by potato (*Solanum tuberosum* L.) and spinach (*Spinacia oleracea* L.) plants irrigated with synthetic wastewater, as well as on plant growth. Crops were grown in lysimeters (1.00 m long x 0.45 m I.D.), packed with sandy soil and arranged in a complete randomized design with these treatments. A non-amended control and a freshwater irrigation treatment were used for comparison. In the first year, SAP was mixed in the soil layer spanning from 0.15 to 0.25 m below the surface (1% w/w) to prevent its photodegradation, while biochar (SAP-biochar mix treatment) was mixed in top 0.10 m of soil (1% w/w). Based on the first year's results, it was decided to mix SAP in top 0.10 m of soil layer at the same rate, along with biochar, for the second year. Potatoes were grown in the first year. In second year, two separate experiments were conducted growing potato and spinach crops. Gasified plantain peel biochar (GBC) was used for potatoes, whereas pyrolyzed plantain peel biochar (PBC) was used for spinach. Soil samples from the surface and depths 0.10, 0.30 and 0.60 m were collected for heavy metal analysis. Yield and plant health parameters were also recorded. Upon harvest, heavy metal content in different plant parts was determined.

For the first year, SAP+GBC treatment retained higher amounts of Cd and Zn in topsoil ($p<0.05$). Both the treatments reduced Cd uptake in potato tubers ($p<0.05$), whereas SAP+GBC treatment also reduced Cu and Zn uptake ($p<0.05$). Acrylamide monomers were not detected in potato tuber flesh and peel samples for all the treatments. For second year, SAP-amended soil retained higher amounts of Cd, Cu, Fe and Zn ($p<0.05$), whereas SAP+GBC retained higher amounts of Cd, Cr and Fe ($p<0.05$) in topsoil, as compared to control. Both the treatments noticeably reduced uptake of Cd ($p<0.10$), Cr and Zn ($p<0.05$) by the tubers; SAP+GBC treatment also led to reduced Pb uptake ($p<0.05$). Higher metal concentrations were observed in tuber peels than in flesh. For spinach, SAP-treated soil retained higher amounts of Cr ($p<0.10$), Cu ($p<0.05$) and Fe ($p<0.10$) in topsoil, as compared to control. Both treatments exhibited potential to avoid increased uptake of Cr and Cu by the leaves, and SAP+PBC also avoided increased uptake of Cd in the leaves.

Potato tuber yield was found to be the lowest in SAP+GBC for both the years, but there were no significant differences in yield among other treatments. Higher spinach yield was observed in SAP+PBC as compared to other treatments ($p<0.05$). No significant differences were found amongst treatments for photosynthetic activity, stomatal conductance, transpiration rate, leaf temperature, NDVI, and root structure development for spinach plants as well as NDVI and leaf temperature for potatoes. Treatments SAP+GBC and SAP+PBC led to higher pH and CEC for surface soil as compared to control ($p<0.05$). All the treatments exhibited increased soil water retention as compared to control, which was also confirmed by a water retention characteristic experiment. In conclusion, SAP and SAP-plantain peel biochar amendments can reduce heavy metal mobility in soil and uptake by food crops, and thus promote safe use of wastewater in agriculture.

Résumé

L'augmentation de la demande alimentaire liée à la croissance démographique entraîne une hausse des besoins en eau d'irrigation. L'agriculture étant le plus grand consommateur d'eau douce, des sources alternatives d'eaux d'irrigation, tel les eaux usées, pourrait contribuer à alléger la demande en eau douce. Cependant, les eaux usées contiennent des contaminants, tels les métaux lourds, qui pourraient être assimilés par les cultures alimentaires ou contaminer les eaux souterraines. Il existe donc un besoin urgent de développer des techniques simples et rentables permettant de réduire la mobilité des contaminants dans le sol et leur assimilation par les cultures. La présente étude fut menée afin de déterminer l'effet d'amendements du sol avec un polymère superabsorbant à base de polyacrylamide (SAP), seul ou en combinaison avec du biocharbon de pelures de plantain, sur la mobilité dans le sol et l'assimilation de métaux lourds (Cd, Cr, Cu, Fe, Pb, Zn) par des cultures de pommes de terre (*Solanum tuberosum L.*) ou d'épinards (*Spinacia oleracea L.*), irrigués avec des eaux usées artificielles. La croissance et le rendement de ces cultures, cultivés dans des lysimètres (hauteur 1.00 m × diamètre interne 0.45 m) remplis d'un sol sablonneux fut étudié. Quatre traitements furent disposés en un protocole complètement aléatoire: Un témoin sans amendements, un témoin irrigué avec de l'eau douce, puis deux traitements irrigués avec des eaux usées, avec amendement du sol avec du SAP ou du SAP et biocharbon. Afin d'en prévenir la photodegradation le SAP fut initialement (An 1) mélangé avec la couche de sol entre 0.15 et 0.25 m sous la surface (1% w/w), tandis que le biocharbon (traitement SAP-biocharbon) fut mélangé au 0.10 m du sol arable (1% w/w). Vu les résultats de l'An 1, en l'An 2, le SAP et le biocharbon furent mélangés dans le 0.10 m de sol en surface (chacun à 1% w/w). En l'An 1 des pommes de terre furent cultivés, tandis qu'en l'An 2, deux expériences distinctes furent réalisées : une avec des pommes de terre, l'autre avec des épinards. Du biocharbon de pelures de plantain obtenu par gazéification servit pour les pommes de terre (GBC), tandis que du biocharbon de pelures de plantain obtenu par pyrolyse servit pour les épinards. Des échantillons de sol provenant de la surface et de profondeurs de 0.10, 0.30 and 0.60 m furent recueillis pour une analyse des métaux lourds. Le rendement et l'état de santé des plantes furent aussi notés. Suivant la récolte la teneur en métaux lourds de différentes parties des cultures furent déterminés.

En l'An 1, le traitement SAP+GBC retint des niveaux plus élevés de Cd et de Zn dans le sol arable que les autres traitements et témoins ($p<0.05$). Chacun des amendements réduisit

(p<0.05) l'assimilation de Cd par les tubercules de pomme de terre, mais le traitement SAP+GBC réduisit (p<0.05) aussi l'assimilation du Cu et du Zn. Pour tous les traitements, ni la chair ni les pelures de pomme de terre ne révélèrent la présence de monomères d'acrylamide. En l'An 2, comparé au témoin non-amendé, les sols ayant reçu un amendement de SAP retinrent des quantités plus élevées de Cd, Cu, Fe et Zn (p<0.05), tandis que le traitement SAP+GBC retint des quantités plus élevés Cd, Cr et Fe (p<0.05) dans la couche arable. Chacun des amendements réduit l'assimilation de Cd (p<0.10), Cr and Zn (p<0.05) par les tubercules, tandis que l'amendement SAP+GBC réduisit également l'assimilation du Pb (p<0.05). Des concentrations plus élevées de métaux lourds furent notés dans les pelures que dans la chair. Pour les épinards, le sol amendé de SAP retint plus de Cr (p<0.10), Cu (p<0.05) and Fe (p<0.10) dans la couche arable que les traitements témoins. Les deux traitements amendés démontrèrent un potentiel à réduire l'assimilation de Cr et Cu par les feuilles, et l'amendement SAP+PBC évita également l'assimilation de Cd par les feuilles.

Chaque année le plus faible rendement de pommes de terre (masse des tubercules) fut noté pour l'amendement SAP+GBC, tandis qu'aucune différence n'exista parmi les autres traitements. Par contraste, le rendement des épinards fut plus élevé (p<0.05) sous l'amendement SAP+PBC, par rapport aux autres traitements. L'activité photosynthétique, la conductance stomatique, le taux de transpiration, la température du feuillage, le NDVI, et le développement racinaire des plants d'épinards, ainsi que le NDVI et la température du feuillage des plants de pomme de terre ne montrèrent aucune différence significative entre traitements. Les amendements SAP+GBC et SAP+PBC menèrent à un pH et CEC de la couche arable plus élevé (p<0.05) par rapport aux témoins. Par rapport aux témoins, tous les amendements augmentèrent la rétention d'eau par le sol, ce qui fut confirmé en caractérisant le pouvoir de rétention d'eau des sols amendés. En conclusion, les amendements SAP and SAP-biocharbon de pelures de plantain peuvent réduire la mobilité des métaux lourds dans le sol et leur assimilation par les cultures alimentaires, permettant une utilisation agricole sécuritaire des eaux usées.

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Dedication

This thesis is dedicated to my parents - their love, sacrifices and selfless nature infused strength and motivation in me.

Thesis Format and Author Contributions

This thesis is written by the candidate in manuscript format, in accordance with guidelines outlined by McGill University's Graduate and Postdoctoral Studies. Research findings contained in the four manuscripts in this thesis have been presented at various reputable scientific conferences as oral presentations and posters, whereas the manuscripts have been submitted for publication to peer reviewed journals and/or are under preparation. The author of this thesis was responsible for all the stages of research and development, including the development of the concept, conducting laboratory and field studies, collection and analysis of data as well as discussion and documentation of results in the form of manuscripts presented here. The co-authors of the four manuscripts are; Prof. Shiv Prasher, Dr. Eman ElSayed, Dr. Ramanbhai Patel, Dr. Christopher Nzediegwu and Mr. Ali Mawof.

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- **Dhiman, J.**, Prasher, S., ElSayed, E., Nzediegwu, C., Mawof, A. and Patel, R. (2019, *under preparation*). Effect of Hydrogel Based Soil Amendments on Heavy Metal Uptake by Spinach Grown with Wastewater Irrigation.

- **Dhiman, J.**, Prasher, S., ElSayed, E., Nzediegwu, C., Mawof, A. and Patel, R. (2019, *under preparation*). Heavy Metal Uptake by Wastewater Irrigated Potato Plants Grown on Contaminated Soil Amended with Hydrogel and Biochar.

- **Dhiman, J.**, Prasher, S., ElSayed, E., Nzediegwu, C., Mawof, A. and Patel, R. (2019, *under preparation*). Effect of Hydrogel and Biochar Soil Amendments on Yield and Growth of Wastewater Irrigated Plants.

Following are the scientific presentations which highlighted the findings of research related to this thesis:

- **Dhiman, J.**, Prasher, S., ElSayed, E., Nzediegwu, C., Mawof, A. and Guan, P. (Jul 2019). Can we use Wastewater to Grow Food? 2019 Annual American Society of Agricultural and Biological Engineers (ASABE) Conference, Boston, Massachusetts, USA. Paper # 1901267.

- **Dhiman, J.**, Prasher, S., ElSayed, E., Nzediegwu, C. and Mawof, A. (Oct 2018). Use of Hydrogel and Biochar Technology to Reduce Heavy Metal Uptake in Wastewater Irrigated Potato Plants. Global Water Security Conference for Agriculture and Natural Resources – An ASABE and ISAE initiative, Hyderabad, India.

- **Dhiman, J.**, Prasher, S., ElSayed, E., Nzediegwu, C. and Mawof, A. (Aug 2018). Effect of Super Absorbent Polymers and Plantain Peel Biochar on Yield of Potato Grown with Wastewater Irrigation. 2018 Annual American Society of Agricultural and Biological Engineers (ASABE) Conference, Detroit, Michigan, USA. Paper # 1801735.

- **Dhiman, J.**, Prasher, S., ElSayed, E., Nzediegwu, C. and Mawof, A. (Jul 2018). Use of Hydrogels and Biochar to Reduce Heavy Metal Uptake by Wastewater Irrigated Potato and Spinach Plants. 2018 Annual Canadian Society for Bioengineering (CSBE) Conference, Guelph, Ontario, Canada. Paper #CSBE18108.

- **Dhiman, J.**, Prasher, S., ElSayed, E., Nzediegwu, C., Mawof, A. and Anderson, E. (Aug 2017). Uptake of Heavy Metals by Potato and Spinach Plants Irrigated with Wastewater in Hydrogel and Biochar Amended Soil. Canadian Society for Bioengineering (CSBE) Conference, Winnipeg, Manitoba, Canada.

- **Dhiman, J.**, Prasher, S., ElSayed, E., Nzediegwu, C. and Mawof, A. (Jul 2017). Uptake of Female Sex Hormones and Heavy Metals by Potato Irrigated with Wastewater in Hydrogel and Biochar Amended Soil. 2017 Annual American Society of Agricultural and Biological Engineers (ASABE) Conference, Spokane, Washington, USA. Paper #1701139.

- **Dhiman, J.**, Prasher, S., ElSayed, E., Nzediegwu, C. and Mawof, A. (Jun 2017). Safe Use of Wastewater in Irrigated Agriculture: Novel Methods of Reducing Heavy Metal Uptake by Potatoes. Annual Research Conference, India-Canada Centre for Innovative Multidisciplinary Partnerships to Accelerate Community Transformation and Sustainability (IC-IMPACTS), Vancouver, British Columbia, Canada.

- **Dhiman, J.**, Prasher, S., ElSayed, E. and Nzediegwu, C. (Jul 2016). Reduction of Cadmium Uptake by Potato Tubers Grown in Hydrogel Amended Soils. 2016 Annual Canadian Society for Bioengineering (CSBE) Conference, Halifax, Nova Scotia, Canada. Paper #CSBE16104.

- **Dhiman, J.**, Prasher, S., ElSayed, E. and Nzediegwu, C. (Jul 2016). Promoting Safe Use of Wastewater in Agriculture through Use of Hydrogels and Biochar. 2016 Annual American Society of Agricultural and Biological Engineers (ASABE) Conference, Orlando, Florida, USA. Paper #162462909.

- **Dhiman, J.**, Prasher, S., ElSayed, E. and Nzediegwu, C. (Jul 2016). Cadmium Uptake by Potato Plants in Soils Amended with Super Absorbent Polymer (SAP) and Plantain Peel Biochar (PPB). Canadian Society of Agronomy (CSA) Conference, Montreal, Quebec, Canada.

- **Dhiman, J.**, Prasher, S., ElSayed, E. and Nzediegwu, C. (Mar 2016). Use of Super Absorbent Polymers (SAP) (or hydrogels) and Plantain Peel Biochar to Reduce Bioavailability of Cadmium for Potato Plants (ePoster). AGM of the India-Canada Centre for Innovative Multidisciplinary Partnerships to Accelerate Community Transformation and Sustainability (IC-IMPACTS), Vancouver, British Columbia, Canada.

- **Dhiman, J.**, Prasher, S., ElSayed, E. and Nzediegwu, C. (Mar 2016). Use of Super Absorbent Polymers (SAP) (or hydrogels) and Biochar to Reduce Female Sex Hormone Pollution in Agriculture (ePoster). Research Conference, India-Canada Centre for Innovative Multidisciplinary Partnerships to Accelerate Community Transformation and Sustainability (IC-IMPACTS), Vancouver, British Columbia, Canada.

- **Dhiman, J.**, Prasher, S., Mannan, A., ElSayed, E. and Nzediegwu, C. (May 2015). Use of Superabsorbent Polymers (Hydrogels) to Promote Safe Use of Wastewater in Agriculture. Canadian Society for Civil Engineering (CSCE) 22nd Hydrotechnical Conference Event 2015, Montreal, Quebec, Canada. Paper #133.

Other presentations related to this thesis are as follows:

- **Dhiman J.** (Jul 2018). Promoting Safe Use of Wastewater for Irrigation: Innovative Strategies. Innovations in Sustainable Water Resource Management (ISWRM 2018), Ludhiana, India.

- **Dhiman J.**, S. Prasher, N. Sharma and R. Kanwar. (Jun 2018). Role of Biochar based Sustainable Green Technology in Water Treatment Systems. 2018 International Conference on Engaging Canada and India: Challenges of Sustainable Development Goals, hosted by Shastri Indo-Canadian Institute (SICI), New Delhi, India.

- **Dhiman J.** (Nov 2017). Use of Hydrogels and Biochar to Reduce Uptake of Heavy Metals in Food Crops.16th Annual BRACE Research Day Colloquium – Centre for Water Resources Management Event, Ste-Anne-de-Bellevue, Quebec, Canada.

- **Dhiman J.** (Jul 2017). Promoting Safe Use of Wastewater for Agriculture Using Hydrogels and Biochar. *Invited Talk*-Water: Challenges and Opportunities (WCO) workshop, University of Alberta, Edmonton, Alberta, Canada.

- **Dhiman J.** (Jun 2017). Use of Super Absorbent Polymers and Plantain Peel Biochar to Reduce Bioavailability of Cadmium in Potato Plants. 15th Annual BRACE Research Day – Centre for Water Resources Management Event, Montreal, Quebec, Canada.

- **Dhiman J.** (Jun 2017). Use of Biochar for Cleaner Water. Presented. Rapid fire presentation event, Summer Institute: Sustainable Communities in Low Resource Settings workshop hosted by India-Canada Centre for Innovative Multidisciplinary Partnerships to Accelerate Community Transformation and Sustainability (IC-IMPACTS), Vancouver, British Columbia, Canada.

- **Dhiman J.** (Mar 2016). Use of Super Absorbent Polymers and Plantain Peel Biochar to Reduce Bioavailability of Cadmium in Potato Plants. 14th Annual BRACE Research Day – Centre for Water Resources Management Event, Ste-Anne-de-Bellevue, Quebec, Canada.

Chapter 1: Introduction

1.1 General Introduction

The current world population is 7.3 billion and it is estimated to rise to 9.7 billion by the year 2050 (DeSA UN, 2015). This increase in population will cause increase in global food demand leading to an increased use of agricultural resources for food production. Increase in food demand caused by growing population will require increased freshwater supply for agriculture, stressing the existing freshwater resources. Freshwater is a very valuable resource, and it constitutes about 0.8% of the total water present on earth, neglecting the ice caps, glaciers and permanent snow (Gleick, 1993). It is estimated that by year 2025, two-thirds of the world's population may face water stress, and more than a billion people would face absolute water scarcity (WWF, 2016; Seckler et al., 1999). Even in the present times, about 80 countries in the world are experiencing water shortages, and about 2 billion people do not have access to clean water (Alois, 2007).

Since agriculture is the largest freshwater consumer, use of alternate sources of irrigation water, such as untreated wastewater, would help conserve freshwater resources in a cost-effective way. Use of wastewater for irrigation is proposed and highly encouraged by many researchers to tackle the problem of freshwater scarcity (Rusan et al., 2007; Al-Rashed and Sherif, 2000; Mohammad and Mazareh, 2003; Al-Salem, 1996). Apart from being an inexpensive alternative for irrigation in countries experiencing economic water stress (Rusan et al., 2007; Qadir et al., 2010), wastewater is also a source of many nutrients and organic matter required by soil to maintain its fertility (Weber et al., 1996). Due to increased wastewater production around the world, safe wastewater disposal in environment is also a major concern. As a common practice, wastewater is discharged openly into water bodies, leading to pollution especially in developing countries (Qadir et al., 2010). The use of wastewater for irrigation could thus tackle the problem.

However, contaminants present in untreated wastewater can pose problems related to human and animal health as well as the environmental issues (Qadir et al., 2007; Verlichhi et al., 2012; Rivera-Jaimes et al., 2017). Government agencies and farmers in many developing countries are not fully aware of the harmful impacts of wastewater contaminants on environment when it is used for irrigation (Qadir et al., 2010). Depending on the source, wastewater may contain a wide variety of contaminants, and heavy metals are one of the most common contaminants found in wastewater. Heavy metals are not easily degraded in the environment (Kirpichtchikova et al., 2006) and can be introduced in the environment from a variety of sources, such as industrial emissions, mining, disposal of wastes containing high quantities of heavy metals, sewage sludge and animal wastes, fertilizer application, pesticide application, wastewater irrigation and petrochemical spillages (Khan et al., 2008; Zhang et al., 2010). Most common heavy metal contaminants are lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), copper (Cu), mercury (Hg) and nickel (Ni) (Evanko and Dzombak, 1997). Wastewater irrigation may not only lead to accumulation of metals in soil, but also can result in excessive uptake of the contaminants by crops, affecting food quality, safety, and consequently human health (Muchuweti et al., 2001). Food chain contamination is one of the major pathways by which heavy metals enter human body (Khan et al., 2008). Persistent intake of heavy metal contaminated food by humans, may result in harmful impacts, symptoms of which may only be apparent after several years of exposure (Bahemuka and Mubofu, 1999; Ikeda et al., 2000). Intake of excessive amounts of metals have also been associated with reduced immune function, growth retardation, upper gastrointestinal cancer, disabilities related to malnutrition, and impaired psycho-social faculties (Iyengar and Nair, 2000; Türkdoğan et al., 2003).

Thus, there is a need to address the problem of heavy metal uptake in food crops, irrigated with wastewater, and their mobility in soil-water system. Hydrogels or super absorbent polymers (SAP) are network of loosely crosslinked polymer chains which are highly hydrophilic in nature and can absorb and retain water or aqueous solutions up to hundreds of times their own weight (Buchholz and Graham, 1998; Skouri et al., 1995; Zohuriaan-Mehr and Kabiri, 2008). Use of SAPs in soil as a water conservation technique has shown significant effects on crop yield. However, due to presence of high density of ionic and metal chelating groups, hydrogels can also be used to immobilize heavy metals in soil and thus reduce their bioavailability (Torres and Varennes, 1998; Varennes and Torres, 1999; Varennes and Queda, 2005; Yi et al., 2008). On the other hand, biochar can also be used as a soil amendment to stabilize heavy metals and thus reduce their uptake by crops (Lu et al., 2012; Al-Wabel et al., 2015; Park et al., 2011). Biochar is defined as a product of pyrolysis, carbonization and gasification of biomass (ANSI/ASABE, 2011). Biochar amendment to soil has been shown to increase crop yield and improve soil properties (Sohi et al., 2010; Lehmann et al., 2003; Glaser et al., 2001). Biochar can be produced from biomass feedstock which would otherwise be categorized as waste, such as from plantain peel, rice straw, rice husk, wheat straw, corn stover, corn cobs, barley straw, switchgrass, etc. In this study, polyacrylamide super absorbent polymers (SAP) and SAP-plantain peel biochar mix as soil amendments are proposed as a technique to reduce heavy metal mobility in soil and their uptake by plants grown with wastewater irrigation.

1.2 Study Objectives

The overall objectives of this study were to understand the fate and transport of heavy metals in sandy soils and to tackle the problem of heavy metal uptake by wastewater irrigated crops through incorporation of SAP and SAP-plantain peel biochar mix as soil amendments. The specific research objectives of the study were as follows:

- To assess the heavy metal sorption and desorption potential of SAP and SAP-plantain peel biochar mix as soil amendments for a sandy soil;
- To study the effect of SAP and SAP-plantain peel biochar mix soil amendments on heavy metal (Cd, Cr, Cu, Fe, Pb and Zn) mobility in a wastewater-irrigated sandy soil;
- To evaluate the role of SAP and SAP-plantain peel biochar mix soil amendments in heavy metal (Cd, Cr, Cu, Fe, Pb and Zn) uptake by wastewater-irrigated potatoes grown on a sandy soil; and
- To evaluate the role of SAP and SAP-plantain peel biochar mix soil amendments in heavy metal (Cd, Cr, Cu, Fe, Pb and Zn) uptake by wastewater-irrigated spinach grown on a sandy soil.

1.3 Thesis Outline

This thesis adheres to the guidelines set my McGill University's Department of Graduate and Postdoctoral Studies and follows a 'manuscript' format. The thesis contains eight chapters which follow the title page, table of contents, abstract (in English and French languages), acknowledgements, dedication and author contribution sections. The first chapter briefly introduces the proposed study and presents the research objectives, whereas the second chapter provides a review of relevant literature. Chapters 3 and 4 present the study on the effect of SAP and SAP-plantain peel biochar soil amendments in a sandy soil on heavy metal immobilization and uptake by wastewater irrigated potato and spinach plants respectively. Chapter 5 presents the study performed to understand the impact of the abovementioned soil amendments on heavy metal mobility and uptake by potato plants grown on already contaminated sandy soil for a second consecutive year. Chapter 6 explores the effect of the soil amendments on yield and growth parameters of wastewater irrigated potato and spinach plants grown on sandy soil across two years. Chapter 7 presents general conclusions and summary of the studies performed, whereas chapter 8 presents the contributions to knowledge and future recommendations for work related to the subject.

Chapter 2: Literature Review

2.1 Global Freshwater Crisis Overview

The availability of freshwater resources plays a pivotal role in the development of human civilization and would also continue to do so in the future. The current world human population is 7.3 billion and it is estimated to rise to 9.7 billion by the year 2050 (DeSA UN, 2015). This increase in population would cause an increase in global food demand leading to increased use of agricultural resources for food production. For instance, intensive adoption of rice – wheat cropping in Punjab region of north-western India led to over exploitation of groundwater resources resulting in lowering of water table (Dhiman et al., 2011; Dhiman et al., 2015). Seckler et al. (1999) emphasize the urgent need to tackle the problem of declining water resources in this region.

Freshwater is a very valuable resource and it constitutes about 0.8% of the total water present on earth, if the ice caps, glaciers and permanent snow are neglected (Gleick, 1993), of which agriculture is the prime user. Apart from agriculture, a large amount of freshwater is used in the industrial sector, and also required for drinking, basic sanitation and hygiene purposes which would lead to additional stress on present resources due to increasing population. It is estimated that by the year 2025, two-thirds of the world's population may face water stress (WWF, 2016) and more than a billion people would face absolute water scarcity by 2025 (Seckler et al., 1999). Even today, about 80 countries in the world are experiencing water shortages and about 2 billion people do not have access to clean water (Alois, 2007).

2.2 Wastewater Irrigation

It is also known that agriculture is the largest consumer of freshwater (figure 2.1). About 70% of the total available freshwater is withdrawn for agricultural purposes (FAO, 2016; UNESCO, 2016; Koehler, 2008). Therefore, effective and efficient freshwater use for agriculture is necessary for the conservation of the valuable resource.

The use of wastewater for irrigation is proposed and highly encouraged by many researchers to tackle the problem of freshwater scarcity (Rusan et al., 2007; Al-Rashed and Sherif, 2000; Mohammad and Mazareh, 2003; Al-Salem, 1996). Apart from being a cheaper alternative for irrigation in countries experiencing water stress (Rusan et al., 2007; Qadir et al., 2010), wastewater is also a source of many nutrients and organic matter required by soil to maintain its fertility (Weber et al., 1996). In many developing countries, urban and peri-urban farmers have no other choice than to use wastewater for irrigation due to physical and economical water shortage (Qadir et al., 2010). Wastewater is also intentionally used for irrigation as it is a source of nutrients to the plants and in some regions of the world it is cheaper than other water sources (Keraita et al., 2003; Scott et al., 2010).

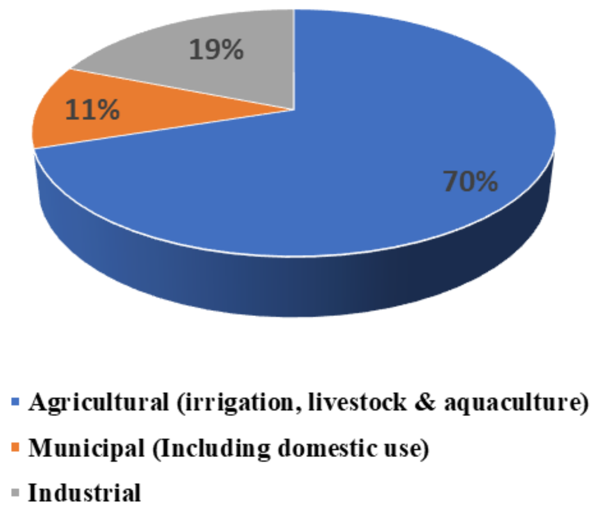


Figure 2.1 Global freshwater withdrawal by sector (*FAO, 2016*)

With growing urban population especially in developing nations, more and more freshwater is being diverted for industrial and commercial use owing to higher demand. This in turn leads to increase in wastewater generation (Lazarova and Bahri, 2004; Asano et al., 2007). Due to increased wastewater production around the world, safe wastewater disposal in environment is also of major concern. As a common practice, wastewater is discharged openly into water bodies leading to pollution especially in developing countries (Qadir et al., 2010). Due to lack of financial and technical resources, many developing countries face challenges in setting up wastewater collection and treatment plants (IWMI, 2006; WHO, 2006). Thus, it is important to devise interim low-cost short-term solutions to deal with the problem.

2.3 Heavy Metals in Wastewater

Despite the advantages of using wastewater for irrigation, the practice has its limitations. Contaminants present in untreated wastewater is a known threat to human and animal health, as well as the environment (Qadir et al., 2007; Wuana and Okieimen, 2011). Government agencies and farmers in many developing countries are not fully aware of the harmful impacts of wastewater contaminants, especially heavy metals, on the environment when it is used for irrigation (Qadir et al., 2010). Heavy metals can be introduced to environment from a variety of sources such as industrial emissions, mining, disposal of wastes containing high quantities of heavy metals, sewage sludge and animal wastes, fertilizer application, pesticide application, petrochemical spillages and wastewater irrigation (Khan et al., 2008; Zhang et al., 2010). Most of the municipal wastewater treatment plants around the world, especially in developing countries, are designed to primarily remove nutrients, and not heavy metals (Brown et al., 1973; Busetti et al., 2005). Removal of heavy metals from the effluents at biological wastewater treatment plants is considered as a side-benefit of the organic matter removal process; however, due to increased heavy metal loading in the environment, treated wastewater may also have high metal content (Karvelas et al., 2003; Teijon et al., 2010). Most common heavy metal contaminants in wastewater are lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), copper (Cu), mercury (Hg) and nickel (Ni) (Evanko and Dzombak, 1997). Unlike organic compounds, heavy metals are relatively less prone to microbial and chemical degradation (Kirpichtchikova et al., 2006) which allows the contaminants to maintain their concentrations in the environment for a long time.

2.3.1 Harmful Effects

Heavy metal contamination poses a serious threat to environment, human and animal health due to its toxicity, food chain accumulation and persistence in nature. Metals are generally essential for many living organisms but the presence of some heavy metals in excess amounts in environment could seriously affect animal as well as human health. Excess amounts of metals in humans, for example, can be deposited in internal organs and lead to serious ill-effects (Gavrilescu, 2004). Toxic metal ions could cause life threatening illnesses including irreversible damage to vital body systems (Malik, 2004). Wastewater irrigation may not only lead to accumulation of metals in soil, but also can result in excessive uptake of the contaminants by crops affecting food quality, safety and thus human health (Muchuweti et al., 2001). Heavy metal uptake by crops depends on the plant species as well (Rattan et al., 2005). Food chain contamination is one of the major pathways by which heavy metals can enter human body (Khan et al., 2008). Persistent intake of food contaminated with heavy metals in humans may result in harmful impacts, symptoms of which may only be apparent after several years of exposure (Bahemuka and Mubofu, 1999; Ikeda et al., 2000). Intake of excessive amounts of metals has also been associated with reduced immune function, growth retardation, upper gastrointestinal cancer, disabilities related to malnutrition and impaired psycho-social faculties (Iyengar and Nair, 2000; Türkdoğan et al., 2003).

Some of the important factors that determine health risk to humans when vegetables grown on contaminated soils are consumed are (G. F. Antonious and Kochhar, 2009; Sharma et al., 2008):

- The concentration of heavy metals in edible parts of vegetable crop and amount of vegetables consumed per person per day
- Washed vs. unwashed vegetable consumption (heavy metals can be deposited from the atmosphere as well)
- Toxicity of the heavy metal in vegetables (Cd and Pb are of great concern)
- Value of health risk index. The health risk index is calculated as
  (Vegetable consumption rate X mean heavy metal concentration in vegetable) / safe heavy metal intake limit). Value of risk index should preferably be less than one.

2.3.2 Environmental Occurrence

Release of heavy metals in the environment due to human activities has been increasing persistently because of intensive industrial operations and technological development around the globe (Gavrilescu et al., 2004). Effluents from mining and metal processing industries are major sources of heavy metal contamination in environment (Moore and Ramamoorthy, 1984). Most of the heavy metals found in wastewater are due to anthropogenic activities such as industrial processes (e.g. mining, electroplating, etc.) and urban inputs (Sun and Shi, 1998; Karvelas et al., 2003; Gavrilescu, 2004). Urban runoff to WWTPs, sewage water as well as surface and groundwater bodies may include heavy metals from household effluents, business effluents (e.g. car washes, electroplating industries, metal works, hosiery industries, etc.) and traffic related emissions (Karvelas et al., 2003; Sorme and Lagerkvist, 2002; Gagnon and Saulnier, 2003). Based on data collected by Xanthopoulos and Hahn (1993), concentration of heavy metals Pb, Cd, Zn, Cu and Ni in street runoff (Karlsruhe/Waldstadt region) was found to be 311, 6.4, 603, 108 and 57 µg/L respectively. Heavy metal release into the environment also occurs because of activities like weathering and flaking of paints, incineration of pharmaceutical products, batteries and electroplated goods as well use and decomposition of tires (Turner, 1990).

Rawat et al. (2003) estimated heavy metal concentrations in wastewaters and bed residues from open storm water drains around seven major industrial regions in New Delhi, India for years 1997-98. Samples were also collected downstream from a major river in India (Yamuna River). Average concentration range for Fe, Mn, Cu, Zn, Ni, Cr, Co, Cd and Pb were found to be 0.99-

212.00, 0.30-39.00, 0.15-20.00, 0.12-5.30, 0.60-11.00, 0.11-53.00, 0.36-0.55, 0.08-0.20 and 0.30-0.66 mg/L respectively; the same heavy metal mean concentrations in bed residues were found to be within the ranges of 29.00-84.06, 0.54-1.23, 0.06-8.13, 0.08-4.01, 0.03-1.58, 0.07-3.28, 0.02-0.05, 0.02-0.07 and 0.01-0.29 g/kg respectively. In the same study, mean concentrations of heavy metals (Fe, Mn, Cu, Zn, Ni, Cr, Co, Cd and Pb) in waters of River Yamuna downstream (Okhla region) were found to be 0.99, 0.32, 0.15, 0.12, 1.06, 0.11, 0.22, 0.20 and 0.36 mg/L respectively. Whereas, mean concentrations of the same heavy metals in bed sediment of the river at the same location where water was sampled, were found to be 29000, 540, 56, 76, 25, 68, 16, 23 and 12 mg/kg respectively.

2.3.3 Environmental Fate

Hydrogen ion concentration or pH can be considered as the most important factor in determining adsorption of metal ions on organic as well as inorganic surfaces (Nelson et al., 1981). This is because in acidic environment (low pH), metals exist as free ionic cations whereas at alkaline pH levels, these cations form insoluble oxides or hydroxides (Gadd and Griffiths, 1977). Availability and toxicity of heavy metal ions can also be affected by the presence of other ions in the environment. In the environment, heavy metals can be found as dissolved in water as well as in particulate matter form. Karvelas et al. (2003) investigated the distribution of heavy metals in the solid and aqueous phase in wastewater collected from a wastewater treatment plant (WWTP). The authors indicated that the phase distribution of metals in wastewater depends on the pH of the wastewater, as well as water solubilities of different chemical forms of the metal. Previous studies on phase distribution of heavy metals in sewage sludge have indicated that carbonate-bound, water-soluble and exchangeable fractions are major carriers of Ni and Zn, iron/manganese oxides and hydroxides are major carriers of Pb, Zn and Ni, sulphides and organic matter are significant carriers of Cr and Cu, and sludge particles are known to be bound to Fe and Pb heavy metals (Angelidis and Gibbs, 1989; Ščančar et al., 2000). In their study, Karvelas et al. (2003) found that most of the Ni and Mn were present in dissolved phase (80%-93% and 65%-85% of the total metal respectively) whereas Cu, Cr, Pb, Cd and Zn were mainly found in particulate phase (75%-95%). Lead heavy metal exhibited highest association with particulate matter (>95%) whereas iron exhibited moderate association with the same (58%-75%). The pH of the wastewater ranged from seven to nine in their study. The presence of microbes also affects the fate of heavy metals in environment. Microorganisms can uptake heavy metals from their environment thus reducing their toxicity. They can do so via adsorption of metals onto microbial cell surface or via cellular uptake for metabolic purpose (Gadd and Griffiths, 1977).

2.4 Super Absorbent Polymers (SAPs)

Super absorbent polymers (SAPs) are a network of loosely crosslinked polymer chains which are highly hydrophilic in nature and can absorb and retain water or aqueous solutions up to hundreds of times their own weight (Buchholz and Graham, 1998; Skouri et al., 1995; Zohuriaan-Mehr and Kabiri, 2008). They are also sometimes referred to as 'hydrogels'. Common SAPs are sugar like white or light yellow colored hygroscopic material. When the term "superabsorbent" is used without specifying the type of polymer, it refers to the most common anionic forms of polymer of acrylic acid (AA) or acrylamide (AM) (Zohuriaan-Mehr and Kabiri, 2008). These polymers are mostly used in hygienic and agricultural practices but have also been used in construction, food and electronics industries (Korpe et al., 2009). Polyacrylamide and polyacrylate are widely used super absorbent polymers in agricultural applications (Bai, et al., 2010).

The first water absorbent polymer was synthesized in 1938 via thermal polymerization of acrylic acid and divinylbenzene (Buchholz and Graham, 1998). The first generation of high water-holding capacity hydrogels were developed in the 1950s (Dayal et al., 1999), whereas first commercial SAP (hydrolyzed starch-graft-polyacrylonitrile or HSPAN) was synthesized at National Regional Laboratory of US Department of Agriculture (Buchholz and Peppas, 1994). Figure 2.2 shows mechanism of SAP (anionic) swelling (Zohuriaan-Mehr and Kabiri, 2008). The negatively charged groups on polymeric chain in SAP attract the water molecule via H-bonding. As the water attaches onto the polymeric chain, it swells and is enlarged as compared to collapsed chain configuration in dry state.

Modified hydrogels also have the potential to be used in designing water disinfection systems. Zeng et al. (2015), in their experiment with silver-reduced graphene oxide (Ag/rGO) hydrogel, synthesized via a facile hydrothermal reaction, showed that the polymer can be effectively used in disinfecting drinking water by inactivating *Escherichia coli (E. coli*) bacteria. Certain pharmaceuticals, such as antibiotics, can also be introduced to the environment through animal wastes, land application or leakage from storage structures (Kolpin et al., 2002). Presence of antibiotics in environment can lead to pathogenic microbes that are resistant to drugs and medicines (Chopra and Robert, 2001; Capone et al., 1996). More recently, it has also been shown that graphene-hydrogel adsorbent materials can be used in removing antibiotic contaminants such as Ciprofloxacin from aqueous solutions (Ma et al., 2015). Eco-friendly hydrogels, such as graphene oxide-chitosan hydrogels can also be used in developing column filtration/water purification systems to remove various cationic and anionic contaminants, including dyes and heavy metals, from water (Chen et al., 2013). Cellulose-based hydrogels have also been used in separating oil-water emulsions and thus can prove to be useful, especially in petroleum industries, in scenarios involving oil spills as well as separating trace amounts of water from crude oil (Rohrbach et al., 2014).

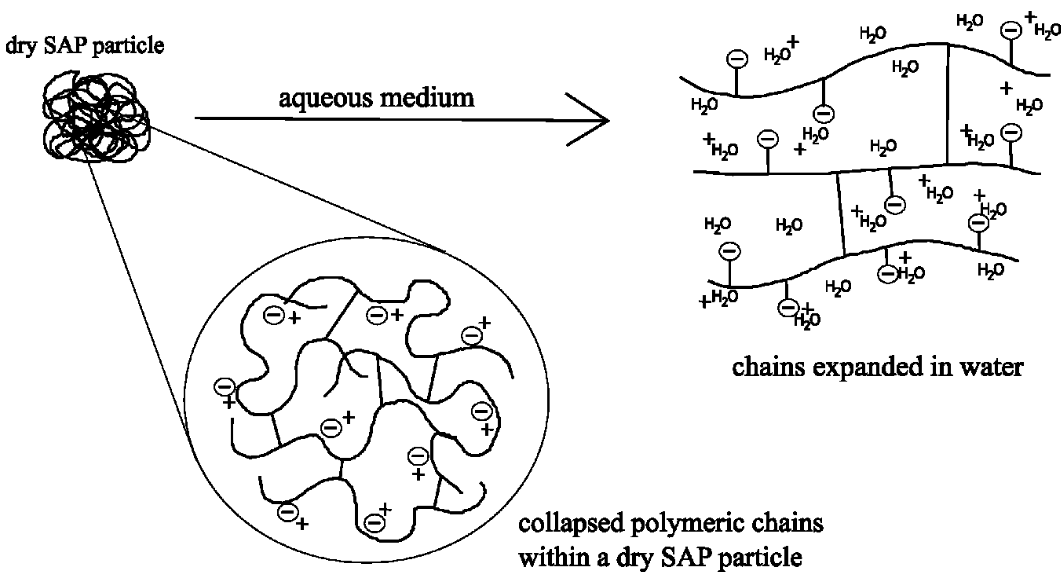


Figure 2.2 A schematic representation of SAP swelling (*adapted from Zohuriaan-Mehr and Kabiri, 2008*).

2.4.1 Classification

Based on the presence of an electrical charge in cross linked polymeric chains, SAPs can be classified in four different types (Zohuriaan-Mehr and Kabiri, 2008):

- Non-ionic
- Ionic (anionic or cationic)
- Amphoteric electrolyte, containing both acidic and basic group
- Zwitterionic, containing both anionic and cationic groups in each structural repeating unit.

Most of the commercial SAPs are anionic in nature (Zohuriaan-Mehr and Kabiri, 2008). Most of the common SAPs are also categorized based on their chemical structure or monomeric unit as follows (Zohuriaan-Mehr and Kabiri, 2008; Po, 1994):

- Polyacrylamides (PAM) or cross linked polyacrylates
- Hydrolyzed cellulose-polyacrylonitrile (PAN) or starch-PAN graft copolymers
- Maleic anhydride cross linked copolymers

SAPs can be either natural or synthetic, however, to improve properties of natural absorbents such as polysaccharides and polypeptides, some synthetic chemicals are often added (Zohuriaan-Mehr and Kabiri, 2008).

2.4.2 Agricultural Uses

The presence of liquid water in soil is very important for quality of plants and crops as it ensures uptake of nutrients. Use of SAPs as a soil amendment could help conserve water especially in sandy soils where they can act like tiny reservoirs of water and can hold water for plant use (Bakass et al., 2002). Apart from water conservation, SAPs also reduce irrigation frequency, soil compaction, soil erosion and water runoff as well as it helps improve soil aeration and microbial growth (El-Rehim et al., 2004). SAPs have also been used in agriculture as controlled release devices for nutrients. SAPs hold the nutrients tightly and can delay their dissolution while the plant gains more time to slowly uptake them improving their growth and quality (Liu et al., 2007; Bowman et al., 1990; Wu et al., 2008; Wu and Liu, 2008). However, Most of the SAPs used in agriculture are of anionic type and thus their swelling capacity reduces with presence of multivalent ions/salts present.

Abedi-Koupai and Asadkazemi (2006) investigated the effect of SAP incorporation on time taken to reach a permanent wilting point (PWP) of an ornamental plant, Cupressus arizonica under reduced irrigation situation. Treatments with 6 g/kg of SAP took 22 days to reach PWP as compared to control which took 12 days to reach same point. Anionic polyacrylamide (PAM) SAP is known to reduce soil erosion, enhance filtration and improve runoff water quality due to its stabilizing and flocculating properties. PAM is known to decrease sediment, total phosphorus, N-dissolved reactive phosphorus, chemical oxygen demand, pesticides, weed seeds and micro-organisms in runoff water (Sojka et al., 2010). In field studies, Sojka et al. (2010) showed that PAM was able to reduce sediments in runoff by 94% (average of 80%-99%) from furrow irrigation systems. Also, it is known to increase infiltration in fine textured soils by 15%-50% as compared to control.

2.4.3 Environmental Fate and Safety

Although SAPs are mostly prepared using toxic monomers, the process of polymerization for SAPs is irreversible, and therefore SAPs are considered to be non-toxic polymerization products (Buccholz and Graham, 1998; Doelker et al., 1990; Andrade, 1976; Po, 1994; Buccholz and Peppas, 1994). Also, SAPs such as cross-linked acrylamide and potassium acrylate copolymer are labelled as "Safe and Non-Toxic Materials" in MSDS sheets of the manufacturers (Zohuriaan-Mehr and Kabiri, 2008). In a study conducted by researchers at University of California (LA, USA) (Wallace et al., 1986), no toxic species were found to have been remained in soil after several years of SAP use. Moreover, the Agriculture Ministry of France has approved use of SAP in soil to be safe (APV 8410030). Published studies on toxicity of acrylate-based SAP consider these materials to be environmentally compatible (McGrath et al., 1993; Haselbach et al., 2000a; Haselbach et al., 2000b; Hamilton et al., 1995; Garay-Jimenez et al., 2008; Suresh et al., 2008).

Basanta et al. (2002) in their study showed that polyacrylate polymer had no adverse effect on microbial community in forest soils. In a separate experiment (Johnson, 1985), conducted to study degradation of polyacrylamide hydrogel in sandy soil for an extended period, no acrylamide monomers were detected in degradation products. It has been shown that in a natural environment, polyacrylamide-based hydrogels are not likely to degrade into toxic acrylamide, even in the presence of sunlight and chemicals such as pesticides (Ver Vers, 1999). Sojka et al. (2007) have reported that acrylamide monomer can be released when polyacrylamide is subjected to radiation below 300 nm. However, most of the UV radiation around this wavelength is absorbed by the atmospheric ozone layer and thus does not reach earth's surface (Diffey, 1991). Also, the half-life of acrylamide in agricultural soils in 18-45 hours (Lande et al., 1979), and since the toxic monomer is easily degraded and metabolized by micro-organisms, it is highly unlikely that it will bioaccumulate in the human food chain (Metcalf et al., 1973; Neely et al., 1974). Wallace et al. (1986) indicated that given the rates at which polyacrylamide hydrogels are used in agricultural applications, concentration of resulting toxic monomers from the hydrogels in soil will be negligible. The authors also mentioned that because of a very short half-life of acrylamide, the toxic monomer would quickly decompose to form propionamide and propionic acid; propionamide would eventually hydrolyze to form propionic acid, which is a harmless compound, often used in the food industry. The final degradation products from acrylamide would be ammonia, carbon-dioxide and water (Wallace et al., 1986).

However, in a study performed by Weston et al. (2009), it was established that use of anionic polyacrylamide in agriculture may contribute to aquatic toxicity, depending on the formulation of the conditioner used. The authors indicated that it was not polyacrylamide itself that caused toxicity, but other co-ingredients, such as surfactants and emulsifiers, used in certain formulations. To tackle the issue of synthetic SAPs biocompatibility, biodegradability and toxicity, researchers have also performed studies on SAPs based on biomaterials such as proteins, starch, cellulose and chitosan (Chang et al., 2010; Wu et al., 2012; Shi et al., 2014; Guilherme et al., 2015; Duquette and Dumont, 2018; Ni et al., 2018).

For acrylamide-based polymers, biological hydrolysis of amide into NH_3 may take place without damaging the carbon chain, depending on the soil microflora (Kay-Shoemake et al., 1998). The carbon chain of the polyacrylates can be degraded by microorganisms (Kawai, 1995) such as whit-rot fungi (Mai et al., 2004; Sutherland et al., 1997). White-rot fungi are known to be responsible for the degradation process of polyacrylate polymer and polyacrylate/polyacrylamide copolymer via solubilization and mineralization (Sutherland et al., 1997). The degradation rate of acrylate-based polymer hydrogel in municipal compost was investigated by 129 using radioactive labelling method. Under aerobic conditions, degradation rate was estimated to be 5.9% after 500 days.

2.4.4 Heavy Metal Removal

Hydrophilic crosslinked polymers which are able to absorb and retail large volumes of water, can also be used in water purification and separation processes (Finch, 1987). Modified polyacrylamide hydrogels are also commercially used in the purification of wastewater and metal extraction (Wu et al., 1991; Warshawsky, 1988). Porous structure and high water-content of superabsorbent polymers allows the solute to diffuse through the polymer's structure (Bell and Peppas, 1995). Hydrogels possess ionic functional groups (Yi et al., 2008) which may allow them to trap contaminants such as heavy metals. Also, because of high density of metal chelating groups present in superabsorbent polymers, these materials are well suited to bind heavy metals present in soil and reduce their bioavailability (Torres and Varennes, 1998; Varennes and Torres, 1999; Varennes and Queda, 2005).

Kasgoz et al. (2003) conducted an experiment with modified polyacrylamide-based hydrogels to determine the metal ion removal capacities. Types of polymers used were crosslinked polyacrylamide polymers, aminomethylated polyacrylamide superabsorbents (obtained from crosslinked polyacrylamide polymers via Mannich reaction) and superabsorbent polymers which are anionic derivatives of polyacrylamides (obtained via sulfomethylation reaction). Crosslinked polyacrylamide polymers were found to have Cu(II) ion removal capacities of 0.027 and 0.024 mmol/g respectively (pH=5.5). Owing to its higher amine value (AV) and equilibrium degree of swelling (EDS), aminomethylated polyacrylamide SAPs exhibited a higher copper ion removal capacity of 2.76 mmol/g at pH 5.5. However, the polymer product of sulfomethylation reactions had a high copper ion removal capacity of 4.07 mmol/g due to high basic group content (BGC) and high EDS. Polymer products of the same reaction also exhibited high metal removal capacities for Cd (0.62 mmol/g) and Pb (1.53 mmol/g) as well. Overall, superabsorbent polymers had higher metal adsorption capacities at higher pH of 4.5, than at pH of 3.0, as expected. This is because pH of the medium greatly affects the complex formation of heavy metal ion by a chelating ligand (Denizli et al., 1997). For instance, polymer products of Mannich reaction exhibited higher Cu ion removal capacity at pH of 4.5 than what it showed at pH of 3.0. This was attributed to interaction of the metal ions with unprotonated amine groups via chelation (Denizli et al., 2000). Adsorption rates were also determined in the same study. It was established that the process of adsorption was very fast in the first five minutes as equilibrium was approached. The adsorption process was slow after this period and saturation were reached within 20 minutes approximately for all metal ions. High initial rate of adsorption suggested that adsorption occurred mainly on the polymer surface.

Dhiman et al. (2015) in their experiment showed that polyacrylamide super absorbent polymer was able to reduce Cd concentration by 76.0% and 76.2% for the Cd-Pb cocktail and only Cd solution, respectively. Concentrations of Cd and Pb ions in the solutions were 5 ppm and 16 ppm respectively. In another study, adsorption capacities of prepared polyvinylpyrrolidone (PVP) and acrylic acid (AAc) copolymer (chelating hydrogel) superabsorbent were studied for Fe(III), Mn(II) and Cu(II) (Ali et al., 2003). Maximum adsorption of metal ions using the prepared polymer SAP for Fe, Cu and Mn ions was found to be 36.0, 23.0 and 14.0 mg/g respectively. Hydrogels (amidoximated hydrogel) also have the potential to separate trace amounts of uranium from water (Hazer and Kartal, 2010).

None of the previously conducted studies investigated the efficacy of SAPs in soil and their potential as a heavy metal sorbent in complex plant-soil-water environment, especially when the heavy metals are present along with other organic and inorganic contaminants including other heavy metals. Also, effect of SAP soil amendment on uptake of heavy metals by food crops irrigated with wastewater needs to be studied, which led us to use polyacrylamide SAPs as soil amendment in the research presented in this thesis.

2.5 Biochar

Biochar is defined as a product of pyrolysis, carbonization and gasification of biomass (ANSI/ASABE, 2011). Thermal decomposition of biomass (plant or animal derived) in partial or total absence of oxygen would lead to production of CO_2, combustible gases (H_2, CO, CH_4), volatile oils, tar as well as a carbon rich residue known as biochar (Sohi et al., 2010). Biochar consists of organic aromatic carbon which is generally more stable than carbon found in parent biomass, i.e. carbon in biochar cannot be readily converted into CO_2 even under favorable conditions such as those found in the soil environment (Sohi et al., 2010).

2.5.1 Agricultural Uses

Biochar is speculated to be deliberately used as far back in time as around 9000 ybp in the central Amazon region of *terra preta* to increase soil fertility (Sohi et al., 2010; Glaser et al., 2001). There is also evidence of historical use of charred products for increased soil fertility at other locations around the world, such as, Ecuador, Peru, Liberia of West Africa and savanna of South Africa (Lehmann et al., 2003). Biochar is known to improve crop yields. Several studies on impacts of charcoal addition in soils on different crop species, conducted in early 1980s and 1990s have been reviewed by Glaser et al. (2001).

Zhang et al. (2012) reported an increase in rice productivity, soil pH, soil organic carbon and total N in soils amended with wheat straw biochar. In a four-year study, Major et al. (2010) reported 28%, 30% and 140% increase in Maize crop yield for second, third and fourth year after wood biochar application (20 t/ha) as compared to control in Colombian savanna region. Micro nutrients such as Ca and Mg also exhibited increased availability (by 77%-320%) in biochar amended plots (under different application rates of 8 and 20 t/ha). In the same study, soil pH was also found to increase in plots amended with biochar. Improved fertilizer use efficiency is also reported to be the major reason for increased crop yields (Steiner et al., 2008). Biochar also contains ash, which may contain trace elements like phosphorus and potassium, which could have a short-term impact on crop growth (Lehmann, 2007; Steiner et al., 2007). Biochar also has the potential to increase water holding capacity of sandy soils, mainly due to its macro-porous nature which in turn is dependent on the cellular structure of the parent feedstock (Sohi et al., 2010). The effect may be neutral in medium textured soils and even can be negative in clay. Gaskin et al. (2007) showed that with addition of pine chip pellet biochar in loamy sand field soils at a rate of 88 t/ha, volumetric water content was almost doubled as compared to control at a potential of 0.10 MPa.

In a study, Park et al. (2011) applied chicken manure derived biochar (pyrolysis at 550°C, ground to <250μm) at 1% (w/w) rate in metal contaminated soil (Cd, Cu and Pb), and found that plant (Indian Mustard – *Brassica juncea*) dry biomass increased by 353% and 572% for shoot and root respectively). Al-Waleb et al. (2015) showed that addition of Concarpus biochar in metal contaminated soils (at different application rates of 0.0, 1.0, 3.0 and 5.0% w/w) increased shoot dry weight of maize plants (Zea mays) by 54.5-102% at 75% of field capacity and by 133-266% at 100% field capacity. The increase in dry biomass in plants could be attributed to the reduction of phytotoxicity of heavy metals due to biochar amendment.

2.5.2 Removal of Heavy Metals

Stabilization of heavy metals in soils amended with biochar could be possible because of several mechanisms explained by Lu et al., 2012. One of the reasons for metal stabilization can potentially be heavy metal exchange with Ca, Mg or other cations associated with biochar. Surface complexation of heavy metals in soil with various functional groups on biochar could be another possible mechanism. Physical adsorption and surface precipitation of heavy metals onto various available sites on biochar surface could be another probable mechanism of heavy metal stabilization.

In a study, application of Concarpus biochar in heavy metal contaminated soil (Fe, Mn, Zn, Cd. Cu and Pb) from a mining area led to an increase in metal immobilization, as well as reduced the metal uptake by maize plants (Zea mays) (Al-Wabel et al., 2015). The maximum reduction in heavy metal uptake by plant shoot, due to addition of the biochar in the soil was found out to be 60.5%, 28.0%, 60.0% and 53.2% for Mn, Zn, Cu and Cd, respectively (Soltanpour and Schwab, 1977). Park et al. (2011) in their experiment with chicken manure and green waste-derived biochar showed that applications of the biochar at a rate of 1% (by weight) significantly reduced NH_4NO_3 extractable Cd, Pb and Cu concentrations in the soils due to immobilization of metals.

2.6 Heavy Metal Uptake by Plants

Several studies indicate that food crops grown on contaminated soils accumulate heavy metals in their tissues (Liu, Zhao, Ouyang, Söderlund, and Liu, 2005; Muchuweti et al., 2006; Nabulo, Young, and Black, 2010; Sharma, Agrawal, and Marshall, 2007). For instance, it was shown that vegetables grown on sewage sludge amended land at selected locations in Zimbabwe, posed a health risk for humans (Muchuweti et al., 2006; Tandi, Nyamangara, and Bangira, 2004). Not all heavy metals are harmful to the plant. Some heavy metals such as Fe, Mn, Cu, Mo, Ni, Co and Zn are nutrients for the plants and are essential for proper plant growth (Casado-Vela et al., 2007; Fitter and Hay, 2012). However, some of the heavy metals, such as Zn, can be toxic at high levels and can be harmful if ingested through contaminated food, especially for people with Zn allergy. Excessive intake of Zn can also cause Zn poisoning in humans (Sharma, Agrawal, and Marshall, 2008).

2.6.1 Adverse Effects on Plants

Uptake of certain heavy metals can induce deficiency of some nutrients in plants. For instance, manganese uptake from contaminated soils (sewage applied to soils) can cause iron deficiency in plants (Somers and Shive, 1942). Some other heavy metals even at lower concentrations can induce chlorosis (Hewitt, 1953). Heavy metals affect plant growth adversely because of their toxicity. They can induce biochemical and/or physiological alterations in plants which can cause membrane damage, alteration of enzyme activities and inhibition of root growth. These effects can further cause deficiency of essential nutrients, inhibition of photosynthesis, etc. (Barceló and Poschenrieder, 1990). Metal toxicity also inhibits cell expansion and water conductivity. Thus, metal toxicity can prevent an adequate amount of water reaching different parts of the plants. This happens primarily because of cross-linking of pectin carboxyl groups in the wall by high concentrations of metals such as Cd and Al (Klimashevskii and Dedov, 1975; Matsumoto, Hirasawa, Torikai, and Takahashi, 1976).

2.6.2 Uptake and Distribution Mechanism

All plants can uptake and distribute metal ions of essential nutrients through their cell membranes (Fitter and Hay, 2012). Nutrients are taken up as ions in the aqueous phase. Movement of water occurs through the xylem due to the transpiration process. Nutrients can thus reach shoots and leaves. However, ions need specific proteins to be present in order to cross cell membranes. Thus, some ions are accumulated in roots, some are excluded, some move through cells because of specific proteins and some move in intercellular spaces. Root-soil interface also plays an important role in nutrient uptake. As the root tips elongate, root caps are pushed into the soil, where they form good soil-root interface due to secretions produced by the roots. Fibrous/hair like root structure leads to the maximization of area available for uptake of nutrients. Also, fungi such as mycorrhizal fungi can greatly increase soil root contact area. Nutrients become available as roots grow out of depletion zones and into new rich zones (interception). Nutrients in aqueous phase entering roots travel through cell cytoplasm and into vascular tissue. Movement of nutrients through plasmodesmata (cells of root epidermis linked to adjacent cells by plasmodesmata) is called symplastic transport, whereas movement through walls of root cells can also take place, and this is known as apoplastic transport. Metal ions can travel through root cell wall region until endodermis where they have to cross plasma membrane to enter cell symplasm. Water and metal ions may also enter cells via plasma membrane. The endodermis layer forms near root vascular tissue, and cells of the endodermis layer form a water impermeable barrier. Thus, metal ions can travel to vascular tissue through symplastic transport and not apoplastic transport (through walls of root cells). This allows for selectivity and filtration of ions. Metal ions can reach all parts of the plants as they are in aqueous solution. Metals can travel through the vascular portion of stem to shoots and leaves through xylem tubules. These can branch again to form leaf veins/fine tubules. As water evaporates from leaf surface, mineral ions can come out of these tubes into the leaf. The metals (in aqueous solution) permeate the leaf wall spaces from where cells can extract water as well as minerals. Areas of the plant undergoing rapid growth, such as fruits and tubers do not have high transpiration rate and thus xylem flow is low. Nutrients and metal ions reach these parts mainly through transport in phloem. Not all ions are phloem mobile. Ions can flow from xylem to phloem via transfer cell that lies between the two paths (Lack and Evans, 2005).

2.6.3 Effect of pH and Plant Type on Plant Metal Uptake

Transport and plant uptake of heavy metals depends on their concentration in soil and soil pH among various other factors. Low soil pH generally is associated with toxicity of metal ions; at low pH, high levels of metals like Al and Fe become soluble and can be toxic for the plant. Low pH is generally associated with the release of metal ions into solution (Fitter and Hay, 2012). At high pH, usually, metal ions form hydroxides and precipitate, and thus are less bioavailable to the plants. Usually, heavy metals are taken up by the food crop through the root system, however, in case of underground crops like potato, heavy metals can also be translocated from soil into potato tubers through diffusion as the tubers are in direct contact of contaminated soil (Angelova et al., 2010). On the other hand, some leafy vegetables like spinach are known to uptake heavy metals in excessive amounts (Ghosh et al., 2012). Thus, heavy metal uptake in underground crops, such as potato would vary from that in aboveground crops like spinach.

2.6.4 Potatoes

Like tomato, eggplant and pepper, potato (*Solanum tuberosum*) also belong to *Solanaceae* plant family and is the third most important food crop (Visser et al., 2009). Potatoes are also the most important non-grain food crop (Potato Genome Sequencing Consortium, 2011) in the world. More than half (~51%) of the world potato production in the year 2007 (325.30 million tons) was produced in developing countries such as India and China (FAO, 2008). Russet Burbank variety of potatoes was used for the study presented in this thesis (Chapters 3 and 5) as it is widely grown in North-America and is used fast food industries because of its excellent baking and processing qualities (Potato Association of America, 2016). To the best of authors' knowledge, information on heavy metal uptake by potatoes irrigated using wastewater in hydrogel-amended soil is not available in literature and thus needs to be investigated.

2.6.5 Spinach

Spinach (*Spinacia oleracea* L.), is an edible plant that belongs to *Amaranthaceae* family and is a leafy cool season vegetable that is native to central and southwestern Asia (Morelock et al., 2008; Suresh et al., 2015). Spinach is a good source of vitamin A, folate, vitamin C, calcium, iron phosphorous, sodium and potassium (Morelock et al., 2008, Suresh et al., 2015). It is also rich in antioxidants and has one of the highest ORAC (oxygen radical absorbance capacity) values in vegetables (Prior, 2003). China leads spinach production in the world, and in the year 2017, China accounted for approximately 92% of 27.90 million tonnes of world production. (FAOSTAT, 2019). Spinach is well known to uptake and accumulate heavy metals from contaminated soils, and it is classified as a hyper-accumulator of metals (Ghosh et al., 2012). Spinach was selected for the study presented in this thesis (Chapter 4) since information on the uptake of heavy metals by spinach plants irrigated with synthetic wastewater in the presence of hydrogel-based amendments was not found in the literature.

2.7 Adsorbent Sorption Capacity

The sorption capacities of sorbents can be evaluated by developing 'sorption isotherms'. It is important to know the equilibrium distribution of contaminants (chemical of interest *'i'*) between aqueous phase and the solid sorbent. We would like to know how the sorbate concentration on the solid (C_{is}) would depend on the chemical's concentration in the solution (C_{iw}). The relationship between these two concentrations describes the sorption isotherm. (Schwarzenbach, Gschwend, and Imboden, 2005).

The sorption capacity can be estimated by fitting experimental data to an empirical relationship; Freundlich isotherm (equation given below):

$$C_{is} = K_{iF} \, . \, C_{iw}^{n} \qquad (1)$$

Where, C_{is} is the concentration of the chemical i on solid phase (mg g^{-1}),
C_{iw} is the concentration of chemical i in the aqueous phase (mg L^{-1}),
K_{iF} is the Freundlich constant (capacity factor),
n is Freundlich exponent.

Cis can be determined using equation 2:

$$C_{is} = \frac{(C_o - C_f).V}{M} \qquad (2)$$

Where, C_o is the original/initial concentration of 'i' in solution (mg L^{-1}),
C_f is the final concentration of chemical i in the aqueous phase (mg L^{-1}),
V is the volume of aqueous solution (L),
M is the mass of the sorbent material (g).

The units of Freundlich constant depend on the value of Freundlich constant (n). When n=1, C_{is} varies linearly with C_{iw} (figure 2.4a), when n<1, the C_{is} vs C_{iw} graph is concave downwards (figure 2.4b and c) and when n>1, the graph is convex upwards (figure 2.4d). n=1 is an ideal condition, where the solute concentration in solution increases, sorption increases proportionally. In other words, if n=1, partition of the compound of interest is independent of the solution concentration (Desta, 2013; Komkiene and Baltrenaite, 2016). However, in case of sorbents such as biochar and SAP, as the concentration of solute in solution increases, C_{is} increases initially, showing sorption, and then starts to flatten out (figure 2.4b and c). This is because the binding sites in sorbents become saturated with increasing C_{iw} (Schwarzenbach et al., 2005). Figure 2.4c depicts an extreme case when compound concentration in solution is so high that all sorption sites are saturated and no further sorption occurs.

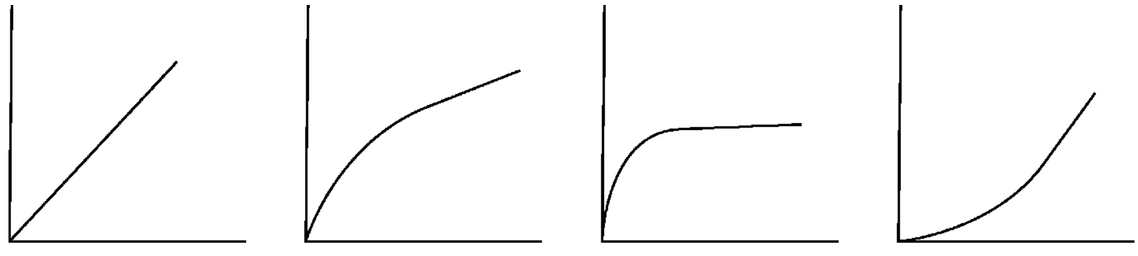


Figure 2.3 C_{is} (y axis) vs C_{iw} (x axis) curves showing different situations; Freundlich exponent, n=1 (a), n<1 (b and c), n>1 (d) (*adapted from Schwarzenbach et al., 2005*).

By using logarithmic values, Freundlich isotherm (equation 1) can be transformed to a linear form (equation 3), where the slope of the equation and antilog of the intercept term denote the Freundlich parameters n and K_{iF}. The coefficient of determinantion (R^2) value fairly close to 1 exhibits a good fit. Freundlich K and n determines the curvature and steepness of the isotherm respectively (Low, Lee, and Liew, 2000). Goldberg et al. (2005) suggested that sorption is a favorable process for values of n between 0.1 to 1 (or n between 1 to 10, if 1/n is used as exponent which is used in many studies). Freundlich K and n are also approximate indicators of sorption capaity and sorption intensity respectively (Komkiene and Baltrenaite, 2016; Hamdaoui and Naffrechoux, 2007).

$$\log C_{is} = n.logC_{iw} + logK_{iF} \qquad (3)$$

Langmuir isotherm can be developed from the sorption data to ascertain the maximum sorption capacity, $C_{is\text{-}max}$. Langmuir isotherms are ideally used in situations where C_{is} increases with C_{iw} upto a certain point and then C_{is} does not change even if C_{iw} is increased. This is because all the binding sites are considered to be saturated and no more compound is sorbed (figure 2.4c). The langmuir model is given by equation 4 (Schwarzenbach et al., 2005).

$$C_{is} = \frac{C_{is-max} \cdot K_{iL} \cdot C_{iw}}{1 + K_{iL} \cdot C_{iw}} \qquad (4)$$

Where, K_{iL} is Langmuir constant.

Langmuir model can be fitted by plotting $1/C_{is}$ vs $1/C_{iw}$. A linear regression equation '*y=mx+c*' can then be fitted. Upon solving for slope and intercept, $C_{is\text{-}max}$ and K_{iL} values are obtained (equation 5). $C_{is\text{-}max}$ (mg g^{-1}) gives the maximum sorption capacity of sorbent material. The coefficient of determinantion (R^2) value should be fairly close to 1 for a good fit.

$$\frac{1}{C_{is}} = \left(\frac{1}{C_{is-max} \cdot K_{iL}}\right)\frac{1}{C_{iw}} + \frac{1}{C_{is-max}} \qquad (5)$$

2.8 Knowledge Gap

Several investigations have been carried out to understand the fate and transport of heavy metals in soil. Studies have been performed to investigate the uptake of heavy metals by plants grown in contaminated soils. Studies have also been performed to understand the effect of SAP or hydrogel soil amendment on plant health and growth parameters. However, there is lack of knowledge regarding the fate of heavy metals in scenarios where untreated wastewater is used for irrigation in sandy soils, treated with polyacrylamide hydrogel-based amendments. To the best of the authors' knowledge, uptake of metals by plants grown on such soils, irrigated with wastewater containing heavy metals along with many other commonly found organic contaminants (steroidal sex hormones, antibiotics, pharmaceuticals, plastics and surfactant), nutrients and mineral sources, has not been studied, and thus needs thorough investigations. Therefore, a research investigation was proposed to understand the role of polyacrylamide SAP amendment (alone and with plantain peel biochar mix) to mitigate adverse effects of heavy metals, and to understand heavy metal transport and translocation to plants in order to develop techniques capable of promoting safe use of wastewater for irrigation.

Connecting Text to Chapter 3

Wastewater is used to irrigate crops in many parts of the world, and a large percentage (10% approx.) of irrigated surface area worldwide receives wastewater irrigation inadvertently. With a growing population, and consequent increase in urbanization and industrialization, more contaminants are being discharged into the environment which can potentially reach wastewater streams. These contaminants, including heavy metals can be taken up by food crops and end up in human food chain. Heavy metal ingestion can cause a variety of health issues in humans. It is known that polyacrylamide hydrogels or super absorbent polymers are used in agricultural applications and can also be potentially used as an adsorbent material for heavy metals. Biochar is also used for a variety of purposes in agriculture and it has been proven in several studies, that biochar can help immobilize heavy metals in soil. Plantain peel feedstock, which is considered as a waste-product in many parts of the world, can be converted into biochar through an inexpensive gasification/pyrolysis process and it can be used as a soil amendment to reduce heavy metal mobility. Chapter 3 provides insights into the role of polyacrylamide super absorbent polymer (SAP) and SAP-plantain peel biochar mix as soil amendments to reduce mobility and uptake of heavy metals by synthetic wastewater irrigated potato plants grown on sandy soil.

The following manuscript (Chapter 3) has been submitted to the Transactions of the American Society of Agricultural and Biological Engineers (ASABE) and is currently in press. The chapter follows the format of the accepted manuscript, which has been co-authored by Prof. Shiv Prasher (academic research supervisor), Dr. Eman ElSayed (Postdoctoral Fellow at McGill University's Bioresource Engineering Department at the time conducting research), Dr. Christopher Nzediegwu (Postdoctoral Fellow at University of Alberta's Department of Renewable Resources). Mr. Ali Mawof (PhD scholar at McGill University's Bioresource Engineering Department) and Dr. Ramanbhai Patel (Research Associate at McGill University's Bioresource Engineering Department). Studies and references cited are presented at the end under the '*References*' section.

Chapter 3: Use of Polyacrylamide Superabsorbent Polymers and Plantain Peel Biochar to Reduce Heavy Metal Mobility and Uptake by Wastewater Irrigated Potato Plants

3.1 Abstract

Increase in food demand, caused by a growing population, requires an increased supply of freshwater for agriculture, thereby amplifying stresses on freshwater resources. Use of alternate sources of irrigation water, such as wastewater, could help conserve the planet's precious resource. However, wastewater may contain contaminants like heavy metals (e.g., Cd, Cr, Cu, Fe, Pb and Zn) which could be taken up by plants and thereby enter animal and human food chains, leading to serious health and environmental issues. The ability of polyacrylamide super absorbent polymer (SAP) and a mixture of SAP and gasified plantain peel biochar (SAP+GBC) soil amendments to reduce heavy metal mobility in soil and deter their uptake by potato (*Solanum tuberosum* L.) plants irrigated with heavy-metal-bearing wastewater was tested. The experiment was carried out in lysimeters (1.0 m x 0.45 m I.D.) packed with sandy soil (1.35 Mg m^{-3}). The SAP was incorporated into the soil to a depth of 0.15-0.25 m from the surface, whereas GBC was mixed into the top 0.10 m of the soil at an application rate of 1% w/w. Potatoes were irrigated with laboratory-prepared synthetic wastewater as per crop water requirements. Composite soil samples were obtained for different depths (surface, 0.10, 0.30 and 0.60 m below the soil surface) for heavy metal analysis, two days after each irrigation event. Leachate from a drainage pipe was also collected for heavy metal analysis. Potato plants were harvested, and root, shoot, leaf, tuber flesh and tuber peel samples were subjected to heavy metal analysis. The samples were extracted using standard procedures and analyzed on ICP-OES and ICP-MS equipment. Tuber flesh and peels were also sampled and tested for acrylamide content using LC-MS/MS. The SAP+GBC treatment significantly ($p \leq 0.05$) reduced Cd, Cu and Zn uptake into potato tuber flesh tissue, as well as Cd uptake into tuber peels. The SAP treatment was also able to significantly ($p \leq 0.05$) reduce Cd uptake in the tuber. The SAP+GBC treatment retained significantly ($p \leq 0.05$) greater Cd and Zn in topsoil. Acrylamide monomers were not detected in potato tuber flesh and peel samples for all the treatments. Therefore, SAP and GBC incorporations in soils have the potential to reduce heavy metal leaching and uptake by plants.

3.2 Introduction

The current world population is 7.3 billion, and it is estimated to rise to 9.7 billion by the year 2050 (UN-DESA, 2015). Consequently, food production will have to be increased to feed the growing population. The world's freshwater resources are already under stress (WWF, 2016; Seckler et al., 1999). About 80 countries in the world are experiencing water shortages, and around 2 billion people do not have access to clean water (Alois, 2007). Therefore, an acute fresh water shortage can be expected in the near future. Since the agricultural sector is the largest freshwater consumer (FAO, 2016; UNESCO, 2016; Koehler, 2008), use of alternate sources for irrigation water, such as untreated wastewater, could supplement the freshwater demand in a cost-effective way. Use of wastewater for irrigation has been proposed and encouraged by many researchers to tackle the problem of freshwater scarcity (Rusan et al., 2007; Al-Rashed and Sherif, 2000; Mohammad and Mazareh, 2003). Apart from being an inexpensive alternative for irrigation in many developing countries experiencing water stress (Rusan et al., 2007; Qadir et al., 2010), wastewater is also a source of many nutrients as well as organic matter required to maintain soil fertility (Weber et al., 1996). On the other hand, with increased wastewater production around the world due to increased industrialisation and urbanisation, its safe disposal is also a major concern. Discharging untreated wastewater into water bodies and then using it for irrigation, a common practice in developing countries, is leading to the contamination of agricultural soils (Qadir et al., 2010). While using wastewater for irrigation serves the dual purpose of tackling the issue of its disposal and effectively supplying the rising water demand for food production, it can also contaminate agricultural soils.

Wastewater contains many contaminants, including heavy metals and organic compounds (Lester, 1987; Kurniawan et al., 2006; Khan et al., 2008; Ahluwalia and Goyal, 2007), which are harmful to human and animal health (Qadir et al., 2007). The most common heavy metal contaminants are lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), copper (Cu), mercury (Hg) and nickel (Ni) (Evanko and Dzombak, 1997). Unlike organic compounds, heavy metals are not prone to microbial or chemical degradation. Accordingly, increasing heavy metal contamination in soils is a serious environmental problem since soils are a major accumulator of heavy metals released through anthropogenic activities (Kirpichtchikova et al., 2006). Wastewater irrigation may not only lead to accumulation of heavy metals in soil but may also result in an excessive uptake of these contaminants by crops, thereby affecting food quality and human health

(Muchuweti et al., 2006). Food chain contamination is one of the major pathways by which heavy metals can enter the human body (Khan et al., 2008). Persistent intake of food contaminated with heavy metals may result in harmful impacts in humans, symptoms of which may only be witnessed after several years of exposure (Bahemuka and Mubofu, 1999; Ikeda et al., 2000). The intake of excessive amounts of heavy metals has been associated with weakened immune systems, growth retardation, upper gastrointestinal cancer, disabilities related to malnutrition and impaired psycho-social faculties (Iyengar and Nair, 2000; Türkdoğan et al., 2003). Heavy metals are also known to be phytotoxic to food crops [e.g., barley (*Hordeum vulgare* L.), soybean (*Glycine max* (L.) Merr.) and wheat (*Triticum æstivum* L.)] and may lead to reduced yields if present at higher concentrations (Page et al., 1972; Collins et al., 1976; Haghiri, 1973). Thus, it is imperative to both reduce heavy metal mobility in soil arising from wastewater irrigation and their uptake by food crops by adopting innovative and cost-effective ecofriendly techniques.

Superabsorbent polymers (SAPs), sometimes referred to as 'hydrogels', are highly hydrophilic networks of loosely crosslinked polymer chains, which can absorb and retain up to hundreds of times their own weight of water or aqueous solutions (Buchholz 1997; Skouri et al., 1995; Zohuriaan-Mehr and Kabiri, 2008). Mostly used in hygienic products, construction, food and electronic industries, these polymers have recently been used in agricultural practices for better water management (Korpe et al., 2009). Polyacrylamide and polyacrylate based polymers are the most widely used types of superabsorbent polymers in agricultural applications (Bai, et al., 2010). Studies on toxicity of acrylate based superabsorbent polymers have found these materials to be safe for the environment (McGrath et al., 1993; Haselbach et al., 2000a; Haselbach et al., 2000b; Hamilton et al., 1995; Garay-Jimenez et al., 2008). Hydrogels possess ionic functional groups (Yi et al., 2008) which may allow them to trap contaminants such as heavy metals (Dhiman et al., 2015); modified polyacrylamide-based hydrogels are also commercially used in the purification of wastewater and metal extraction (Wu et al., 1991; Warshawsky, 1988). Because of the high density of metal chelating groups present in some superabsorbent polymers, these materials are well suited to stabilising heavy metals in soil and reducing their bioavailability (Torres and Varennes, 1998; Varennes and Torres, 1999; Varennes and Queda, 2005). Superabsorbent polymers being hydrophilic and containing carboxylic functional groups, can bind heavy metals and therefore reduce the heavy metal uptake by plants (Huettermann et al., 2009).

Biochar is a product of pyrolysis, carbonisation and gasification of biomass (ANSI/ASABE, 2011). Thermal decomposition of biomass (plant or animal derived), in partial or total absence of oxygen, produces a carbon rich residue known as biochar (Sohi et al., 2010). Biochar production can also be treated as a value addition process as biomass feedstocks of little or no commercial value can be utilised. Biochar can be produced from residues of different crops such as barley (*Hordeum vulgare* L.) straw, rice (Oryza sativa L.) husk, cotton (*Gossypium hirsutum* L.) stalks, sugarcane (*Saccharum officinarum* L.) bagasse and plantain (*Musa* × *paradisiaca* L.) peels. Plantain is an important staple food in many tropical regions around the world and its peel accounts for about 40% of the fruit by weight (Tchango et al., 1999; Rubatzky and Yamaguchi, 1997). Plantain peel, which is otherwise considered a waste, could be used for sustainable biochar production. Biochar soil amendment is known to improve crop yields. Several studies conducted in the early 1980s and 1990s demonstrating the positive impacts of charcoal amendments on soils bearing different crop species have been reviewed by Glaser et al. (2001). Zhang et al. (2012) reported increase in rice productivity, soil pH, soil organic carbon and total N in soils amended with wheat straw biochar. Biochar also has the potential to increase the water holding capacity of sandy soils, mainly due to its macro-porous nature, which, in turn, is dependent on the cellular structure of the parent feedstock (Sohi et al., 2010). In addition to improving soil properties and increasing yield, several mechanisms could lead to the stabilisation of heavy metals in soils amended with biochar (Lu et al., 2012) through: (i) exchange of heavy metals with Ca, Mg or other cations associated with biochar, (ii) surface complexation of heavy metals in soil with various functional groups on biochar, and (iii) physical adsorption and surface precipitation of heavy metals onto various sites available on the surface of biochar. Amendment of contaminated soils with biochar is known to increase metal immobilisation as well as reduced plant uptake (Al-Wabel et al., 2015; Soltanpour and Schwab, 1977; Park et al., 2011). Generally, soils are good accumulators of heavy metals and application of biochar in the topsoil leads to further immobilisation of heavy metals. Due to their high specific surface area, pore structure and presence of functional groups, biochars are known to be effective in the adsorption of heavy metals (Liu and Zhang, 2009; Park et al., 2011). In soil, adsorption of metal cations on silicate minerals (Swift and McLaren, 1991), Fe, Al and Mn oxides (Schwertmann and Taylor, 1989) and humic matter (Stevenson and Fitch, 1986) can occur via nonspecific interactions (cation exchange) or more specific adsorption due to surface complexation (Msaky and Cavlet, 1990). Biochars could boost intrinsic sorption capacity of soils towards organic and inorganic contaminants such as heavy metals (Uchimiya et al., 2011), with the pH of the biochar playing an important role.

Considering the importance of wastewater irrigation due to scarcity of freshwater resources, the associated risk of heavy metal uptake by plants, and the unique characteristics of SAP and biochar, it appears that simple, innovative and cost-effective techniques could be developed for safe use of wastewater in agriculture by incorporating biochar and SAP in soil. Therefore, this study was conducted to quantify the effect of polyacrylamide SAP and plantain peel biochar incorporation in soil on the mobility of commonly found heavy metals (Cd, Cr, Cu, Fe, Pb and Zn) in untreated wastewater and their uptake by potato plants grown on sandy soil irrigated with laboratory prepared synthetic wastewater.

3.3 Methods and Materials

3.3.1 Characterisation of SAP and Biochar

A commercial cross-linked copolymer of potassium acrylate and acrylamide superabsorbent polymer, SUPER-AB-A200, was used in this study and was supplied by a Canadian environmental solutions company, Iramont Inc. The swelling ratio of the hydrogel was determined gravimetrically. Dry SAP (0.1 g) was kept in 200 mL of double deionised water for 24 hours. The swollen hydrogel was then taken out of water and blotting paper was used to wipe off excess water before weighing it again. Swelling ratio was determined by dividing the weight of swollen gel by that of dry SAP. Physical and chemical properties of the SAP are given in table 3.1. The MSDS sheet for the product indicates the material to be non-toxic and no hazardous decomposition products are formed upon use (Iramont, 2016).

Table 3.1 Physical and chemical properties of SUPERAB-A200 SAP.

CAS #	Appearance [a]	Specific gravity [a]	pH [a]	Solubility [a]	Particle size distribution (mm) [a]	Storage life (years) [a]	Life in soil (years) [a]	Toxicity [a]	Swelling Ratio
31212-13-2	White granular	1.2	6-7	Insoluble in aqueous solution	2-5: 61% 1-2: 20% <1: 19%	>7	>5	Non-toxic	200

[a] *Adapted from the manufacturer's website (Iramont, 2016).*

To add value to the waste, plantain peel biochar was produced from dried plantain peels. Green plantain fruit was purchased from Sami Fruits, Lasalle, QC, Canada. Fruits were peeled using standard kitchen knives and peels were dried in an isothermal oven for 48 hours (Lindberg Blue, Thermo Scientific, USA) at temperatures between 75°C and 80°C. Dried peel biomass was stored at room temperature. Biochar was produced in a gasifier (built at the Macdonald Campus Technical Service Building of McGill University, Sainte-Anne-de-Bellevue, Quebec, Canada) operating in a temperature range of 450°C to 500°C over a residence time of 20-25 min. The biochar was thoroughly mixed and stored in sealed plastic bags. Proximate and ultimate analysis of the biochar was performed at the CanmetENERGY Characterisation Laboratory (ISO 9001:2008 certified), Ottawa, ON, Canada. Hot nitric acid extraction method was used to determine heavy metal content in the biochar (Stephan et al., 2008). Biochar samples were also sent to the Materials Characterisation Laboratory at Department of Mining and Materials Engineering, McGill University, Montreal, Canada for Brunauer-Emmet-Teller (BET) surface area determination. Properties of the biochar are given in table 3.2.

Table 3.2 Physiochemical properties of gasified plantain peel biochar.

Parameter	Observed Value (wt %)	Method
Proximate Analysis		
Moisture Content [a]	9.88	ASTM D7582
Ash Content [a]	77.45	ASTM D7582
Volatile Content	18.09	ISO 562
Fixed Carbon	4.46	ASTM D7582
Ultimate Analysis		
Carbon	18.10	ASTM D5373
Hydrogen	0.48	ASTM D5373
Nitrogen	0.60	ASTM D5373
Total Sulfur	<0.05	ASTM D4239
Oxygen	3.37	By Difference
Specific Surface Area		
BET surface area (m^2 g^{-1})	1.9460	Lab Characterisation
Heavy Metal Analysis		
Heavy Metal	**Observed content ($mg\ kg^{-1}$)** (mean ± stdev)	**Allowable limit [b] ($mg\ kg^{-1}$)**
Cd	n.d.	1.50
Cu	7.11 ± 0.98	100.00
Cr	1.67 ± 0.17	90.00
Fe	669.12 ± 86.35	n.a.
Pb	0.04 ± 0.00	150.00
Zn	35.65 ± 1.39	400.00

[a] Estimated using a thermogravimetric analyzer (TGA).
[b] Based on European Biochar Certificate (EBC) guidelines (EBC, 2012).
Values are estimated on a dry weight basis.
n.d.- not detected, n.a.- not available.

3.3.2 Experimental Setup

The experiment was carried out in field lysimeters at Macdonald Campus of McGill University, Ste. Anne de Bellevue, QC, Canada. The lysimeters (0.45 m internal diameter × 1.0 m height) were filled with sandy soil (St. Amable complex) up to a height of 0.05 m from the top edge (figure 3.1). Physical and chemical properties of the soil used are given in table 3.3. A perforated pipe was installed at the bottom of each lysimeter for leachate collection (*i.e.*, 0.95 m depth of soil). At each of three different depths viz. 0.10, 0.30 and 0.60 m from the soil surface, four equidistant holes (10 mm dia.) were drilled radially through the lysimeter wall for soil sample collection.

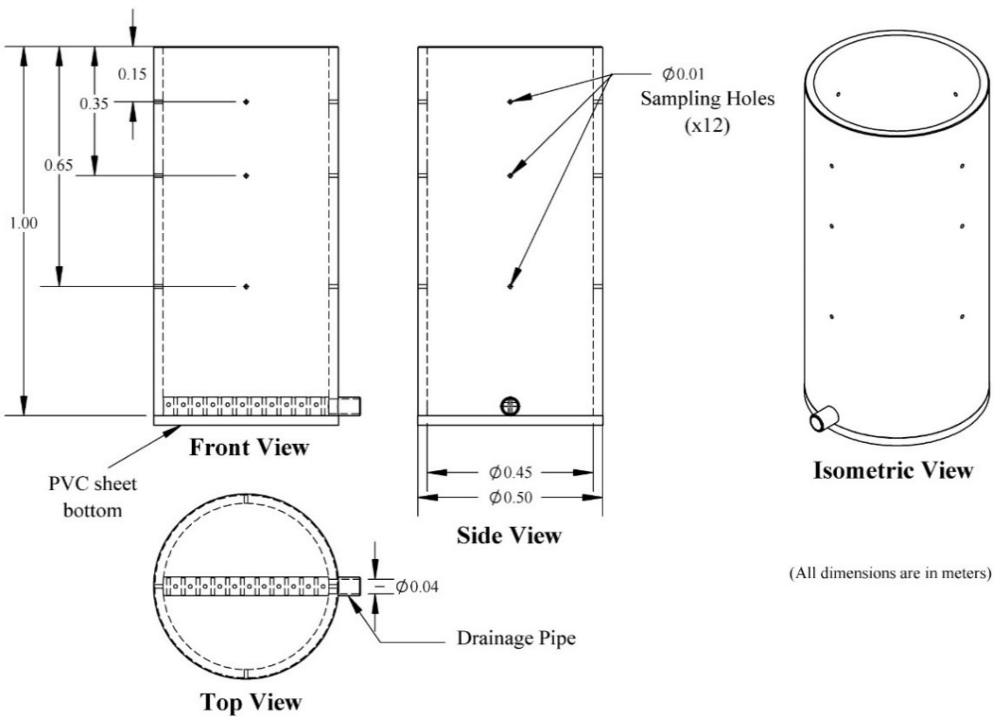


Figure 3.1 Schematic diagram of a lysimeter.

Three treatments (SAP, SAP+GBC and a non-amended control), replicated thrice, were randomly allocated to nine lysimeters. For the SAP treatment, SAP was mixed into the soil layer spanning 0.15 m to 0.25 m below the soil surface, at a rate of SAP:soil of 1% (w/w). In the SAP+GBC treatment, beyond the SAP application detailed above, biochar was mixed into the top 0.10 m of soil [biochar:soil 1% (w/w)]. The SAP was incorporated below the soil surface to prevent its photo-degradation. The lysimeter wall was tapped with a rubber mallet to prevent the formation of preferential flow paths along the lysimeter wall. A waterproof tent was set up above the lysimeters to prevent rainwater entry, allowing only a known volume of irrigation water to be used on a predetermined schedule. All lysimeters were brought to field capacity two days before planting. Russet Burbank potato tubers were obtained from Global Agri. Services Inc. (New Brunswick, Canada). A single tuber was planted in the center of each lysimeter at a depth of 0.10 m, with sprouts facing upward (Thompson-Morgan, 2015). Every alternate day 540 mL tap water was applied (1.70 mm day^{-1}) to establish plants before irrigating with wastewater started.

Table 3.3 Physical and chemical properties of lysimeter soil.

Type [a]	Sandy
Sand (%) [a]	92.20
Silt (%) [a]	4.30
pH [a]	5.50
Dry Bulk density (Mg m^{-3}) [a]	1.35
Organic matter (%) [a]	2.40±0.15
Saturated Hydraulic conductivity (m day^{-1}) [a]	1.67±0.45
Zero point of charge (ZPC) [a]	3.40
N (mg kg^{-1}) [b]	4.57±0.46
P (mg kg^{-1}) [b]	215.30±40.43
K (mg kg^{-1}) [b]	107.33±13.13
Ca (mg kg^{-1}) [b]	912.44±79.70
Mg (mg kg^{-1}) [b]	103.27±7.29
Al (mg kg^{-1}) [b]	1164.14±12.40

[a] *Adapted from a previous study conducted with soil from same source (ElSayed et al., 2013).*
[b] *NO_3-N was determined using KCl method (Carter and Gregorich, 2008), while P, K, Ca, Mg, and Al were determined using Mehlich III extraction procedure (Mehlich, 1984).*

A post-planting pre-emergence spray of the broadleaf and grass weed herbicide, SENCOR 480F (Bayer CropScience, ai: metribuzin, 480 g L^{-1}), was applied at the recommended rate (850 mL mixed in 100L ha^{-1}) for weed control in lysimeters (Hutchison, 2012). The soil was analyzed for available nutrients using Mehlich III method (table 3.3). Fertilisers were applied at the locally recommended rate of 314 kg N ha^{-1} and 280 kg K ha^{-1}; in each lysimeter, 7.4 g Muriate of potash (0-0-60) was applied at the surface in a single application on the day of planting, while 23.8 g of ammonium sulphate (21-0-0) was applied in three splits (2:1:1), on the day of planting, the 33rd day after planting and the 43rd day after planting, respectively.

3.3.3 Preparation of Synthetic Wastewater

To maintain the desired concentration of heavy metals in wastewater, synthetic wastewater was used to irrigate the potato plants. The recipe used to prepare the wastewater is given in table 3.4; to simulate a worst-case scenario, the highest concentrations in wastewater reported in the literature were used (Nopens et al., 2001; Aboulhassan et al., 2006; Li et al., 2008; Huang et al., 2009; Ahmed et al., 2011; Sim et al., 2011; Singh et al., 2014). All the analytical chemicals and standards for preparation of synthetic wastewater were procured from Sigma-Aldrich (St. Louis, MO, USA) and Fisher Scientific (Waltham, MA, USA). Tap water used for preparing the irrigation cocktail was stored in a container for one day prior to its use to remove possible chlorine content. The solutions of all ingredients (prepared in the laboratory) were thoroughly mixed in the required proportions, using a 200 L container to reach a final volume of 120 L of synthetic wastewater.

Table 3.4 Recipe for the preparation of synthetic wastewater for irrigation.

Ingredient	Category	Concentration (mg L^{-1})	Reference
Basic Wastewater Constituents			
Ammonium Chloride	Nitrogen Source	12.75	Nopens et al. (2001)
Peptone		17.41	
Urea		91.74	
Sodium Acetate	Carbon Source	79.37	Nopens et al. (2001)
Milk Powder		116.19	
Soy Oil		29.02	
Starch		122	
Yeast Extract		52.24	
Magnesium Phosphate	Phosphorus Source	29.02	Nopens et al. (2001)
Potassium Phosphate		23.4	
Calcium Chloride	Minerals	60	Nopens et al. (2001)
Magnesium Chloride		40	
Sodium Bicarbonate		100	
Wastewater Contaminants			
Cr	Heavy Metals	2	Ahmad et al. (2011)
Cd		5	
Pb		16	
Fe		120	
Zn		3	
Cu		8	
Estrone	Female Steroidal Sex Hormones	0.05 (8.15×10^{-3}) [a]	Sim et al. (2011)
17β-Estradiol		0.02 (0.634×10^{-3}) [a]	
Progesterone		0.02 (0.90×10^{-3}) [a]	Huang et al. (2009)
Oxytetracycline	Pharmaceuticals	19.5	Li et al. (2008)
Ibuprofen		0.0264	Singh et al. (2014)
Alkylphenyl polyethoxylate as Triton X-100	Surfactant	0.03	Aboulhassan et al. (2006)
BPA	Bisphenols	0.05	Based on LOD of instrument
BPF		0.05	
BPS		0.05	

[a] *Values in parentheses are the reported values whereas amount used in wastewater recipe. depends on limit of detection (LOD) of the instrument*

3.3.4 Irrigation Scheduling

The first wastewater irrigation was applied after the emergence of potatoes, on the 33rd day after planting. Subsequent irrigations were applied at ten-day intervals. Based on the potato crop water requirement of 635 mm (FAO, 2015; King and Stark, 2008), the amount of water provided to the plants before the start of wastewater irrigation (1.70 mm day^{-1} for 32 days) and lysimeter surface area (0.16 m^2), wastewater application rate for each of the eight irrigation events was calculated to be 11.5 L per lysimeter.

3.3.5 Soil and Leachate Sampling

Soil samples were collected through sampling ports two days after irrigation; two days were allowed for the soil to reach field capacity (θ_{fc}) and establish equilibrium between contaminants and amended soils. A composite soil sample was prepared for each depth of each lysimeter by taking equal amounts of soil from the four equidistant holes at the given depth. For surface soil sampling, soil samples were collected randomly from four locations at the soil surface. Samples were sealed in plastic bags, labelled and stored securely in a freezer (-20°C) until further analysis. During irrigation events, leachate was collected in a 1L amber colored glass bottles, attached to the outlet of drainage pipe of each lysimeter. Once the bottle was filled, it was replaced with another bottle; leachate was collected until flow stopped. The total volume of leachate from each lysimeter was measured, thoroughly mixed, and a 2 L subsample was collected. The samples were transported to the laboratory and extracted immediately for heavy metal analysis.

3.3.6 pH Measurement

Surface soil samples were collected from each lysimeter two days after the sixth irrigation for pH measurements. The pH of the soil samples was estimated as per guidelines outlined by Rayment and Higginson (1992), using an electrode pH meter (Accumet pH meter model AB15, Fisher Scientific, USA). For pH measurement of biochar, 1:30 w/v biochar:water solution was shaken for four hours on a vibratory shaker (Innova 2100 Platform Shaker, Eppendorf/New Brunswick Scientific Inc., NJ, USA), and pH of the supernatant was measured following the guidelines outlined by Zhang et al. (2015).

3.3.7 Heavy Metal Extraction and Quantification for Soil and Leachate Samples

Soil samples were extracted for heavy metals based on the method outlined by Stephan et al. (2008). Air-dried soil samples (0.16 g) were mixed with 2 mL trace metal grade nitric acid in 15 mL glass tubes and kept undisturbed for 24 h under a fume hood. Samples were then digested in a block digester (Isotemp Dry Bath Incubator, Fisher Scientific, USA) at 120°C for 5 hours. Extracts were then diluted using double deionised water to make a 50 mL volume and were then quantified using inductively coupled plasma optical emission spectrometry (ICP-OES) equipment (Vista-MPX CCD Simultaneous, Varian, CA, USA). Leachate samples were analyzed using the method outlined by Jung (2001). A volume of 200 mL of collected leachate sample was filtered through a 90 mm dia. (0.75 µm pore size) glass filter paper followed by a 47 mm dia. (0.45 µm pore size) glass filter paper via vacuum filtration. The apparatus was rinsed with deionised water after every filtration to prevent cross contamination. Concentrated nitric acid (trace metal grade) was added to the filtrate (1% v/v). About 40 mL of each of the filtered samples were stored in the refrigerator (4°C) in 50 mL plastic tubes for further analysis on ICP-OES equipment.

3.3.8 Heavy Metal and Acrylamide Extraction and Quantification for Plant Tissue Samples

The crop was harvested at maturity (117 days after planting). Aboveground biomass was cut with a standard steel knife. Roots and tubers were carefully harvested from the soil using a hand trowel. Potato tubers were washed with deionised water to remove soil and were peeled with a standard steel kitchen knife. Peel and flesh of the tubers were sampled separately for heavy metal analysis. Plant root, stem and leaves were sampled separately. Samples were cut in lengths of about 0.01 m using a knife and a chopping board. Sampling was performed on the day of harvest. Sampled plant parts were oven-dried at a temperature of 60°C for 48 hrs. The dried samples were then crushed with mortar and pestle and then ground with a coffee grinder. Care was taken to wash the equipment used between samples to prevent cross contamination. Samples were then digested using an acid block digestion method (Stephan et al., 2008). Quantification of the samples was carried out using inductively coupled plasma mass spectrometry (ICP-MS) equipment (ICP820-MS Varian, CA, USA) in the Bioresource Engineering laboratory, Macdonald Campus of McGill University, Canada.

Acrylamide content determination in plant tissue was only performed for the edible parts of the plant. Fresh potato tuber flesh and peel samples were cut, put in 50 mL Falcon tubes and immediately frozen using liquid nitrogen. Frozen samples were then stored in a -80°C freezer, then dried using a freeze dryer (BETA 2-8 LSC Plus, Christ, Germany) for 48 h. Freeze dried samples were then ground to powder using mortar and pestle. Samples were extracted for acrylamide determination using the method outlined by the U.S. Food and Drug Administration (2003) and were quantified at the Centre Régional de Spectrométrie de Masse laboratory, Université de

Montréal, Canada; a quantum triple quadrupole liquid chromatography–mass spectrometry (LC-MS; Thermo Fisher Scientific) system equipped with a YMC-Pack ODS-A column (100 × 4.6 mm, 3 µm, Waters) was used for the analysis.

3.3.9 Data Analysis

Data for heavy metal concentrations in soil were analyzed using a repeated measures (in time and space) statistical model to determine if the concentrations differed between treatments across time and varying depths. A one-way analysis of variance procedure was employed to test differences between mean heavy metal concentrations in different potato plant parts. Statistical tests were performed using JMP 13 (2017) statistical analysis and graphing software by SAS (JMP, SAS Institute Inc., Cary, NC).

3.4 Results

3.4.1 pH and Acrylamide Content

Measured values of pH of surface soil samples collected two days after the sixth irrigation showed no significant differences in pH between SAP and control treatments (table 3.5); however, pH was significantly higher under the SAP+GBC treatment than the control ($p \leq 0.05$). This can be attributed to the alkalinity of the biochar (biochar pH = 10.27 ± 0.06) used in the study. Biochars are generally alkaline in nature and their pH depends on the feedstock (Gaskin et al., 2008). Ash in biochar is mainly composed of inorganic minerals (Uchimiya et al., 2010; Mohan et al., 2014) and hydrolysis of salts of these minerals leads to an increase in pH of the soil (Tryon 1948; Gaskin et al., 2008). Similar results were reported by Uchimiya et al. (2010), where, an increase in soil pH was observed after loading the soil with biochar produced from broiler litter manure. Acrylamide monomers were not detected in potato tuber flesh or peel samples for SAP and SAP+GBC treatments. The finding was similar to that of Suresh et al. (2018), who did not find acrylamide monomers in cherry tomatoes grown in soil amended with polyacrylamide superabsorbent polymer (0.1% and 0.5% w/w).

Table 3.5 pH values of the soil samples taken two days after sixth irrigation event for all treatments.

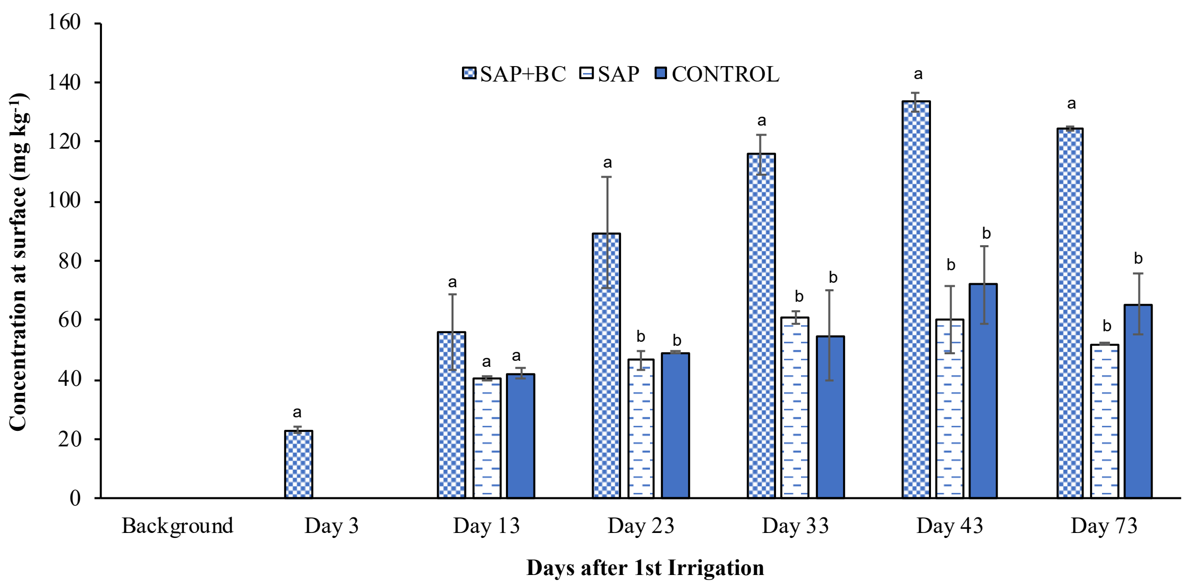
Treatment	pH
SAP	5.07 ± 0.29^{ab}
SAP+GBC	5.43 ± 0.21^{a}
Control	4.93 ± 0.38^{b}
Soil without amendment with fresh water application	5.40 ± 0.10^{ab}

Values with different letters down the column are significantly different from each other (α=0.05).

3.4.2 Heavy Metals in Soil

3.4.2.1 Cadmium

For the three treatments, the concentration of Cd ([Cd]) gradually increased with each additional irrigation event, while across all irrigation events the [Cd] was significantly ($p \leq 0.05$) greater in the SAP+GBC treatment than either the SAP or control treatments (figure 3.2). In all the treatments, [Cd] in the soil prior to the first wastewater irrigation was below the limit of detection (LOD) of 15.6 mg kg^{-1}. For the control, the mean [Cd] increased to 42.0, 49.1, 54.7, 71.9 and 65.1 mg kg^{-1} after the second, third, fourth, fifth and eighth irrigation, respectively; indicating that the sandy soil used in the experiment could hold roughly 65 mg kg^{-1} of Cd, when present with other heavy metals and contaminants at varying concentrations. This concurs with the findings of Elliott et al. (1986) who found maximum Cd sorption (competitive sorption: Cd, Cu, Pb and Zn; pH 6.0-6.5) in soils low in organic matter, ranging from about 56.20 to 67.44 mg kg^{-1}. Thus, further loading the soil with Cd through wastewater application would result in leaching of Cd to a deeper soil profile. For SAP, the mean [Cd] showed an increasing but non-significant ($p > 0.05$) trend, rising to 40.5, 46.4, 60.9, 60.0 and 52.0 mg kg^{-1}, respectively, after the same sequence of irrigations.



Different letters above the error bars (standard error) indicate significant differences in concentrations for a given event (α=0.05)

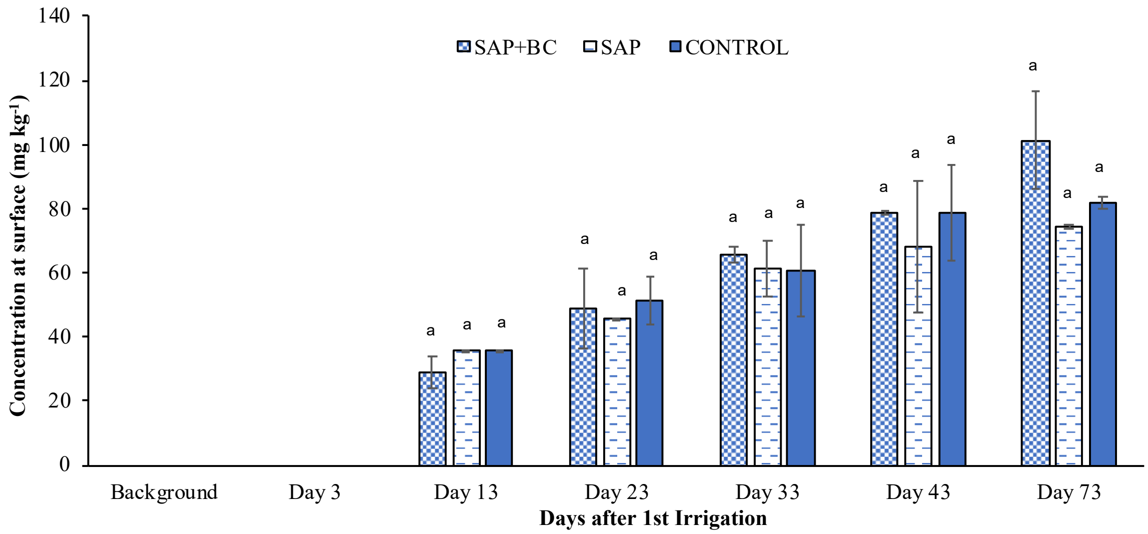
Figure 3.2 Mean concentrations of cadmium in surface soil samples after successive irrigations.

Overall there were no significant differences ($p > 0.05$) between the [Cd] in control and SAP treatments for all irrigation events. For both SAP and control treatments, cadmium was not detected immediately after the first irrigation. Based on Cd loading through irrigation and soil's Cd holding properties, theoretically, the depth of travel of the heavy metal after first irrigation would be around 4 mm for SAP and control treatments. In surface irrigation, a large quantity of water floods the surface in a short time. Therefore, water would move instantly to some depth beyond 4 mm, and heavy metals might not be adsorbed to the soil up to its holding capacity, resulting in a distribution of the heavy metal below its detection limit in topsoil after the first irrigation. The same effect was not seen for the SAP+GBC treatment, as biochar present in the topsoil would have held the heavy metal with a greater affinity, compared to the other two treatments.

The mean [Cd] in surface soil samples from the SAP+GBC treatment after the same sequence of irrigation events were 22.9, 55.8, 89.5, 115.9, 133.3, and 124.7 mg kg^{-1} respectively, indicating a gradual but significant ($p \leq 0.05$) increase in [Cd] with the application of wastewater irrigation. However, after the eighth irrigation there was a non-significant decrease in [Cd] compared to that after the fifth irrigation ($p > 0.05$). A similar but non-significant trend was also observed for the control and SAP treatments. Heavy metal concentrations after the second irrigation were significantly less than after the fifth and eighth irrigation ($p \leq 0.05$), showing a significant accumulation of the heavy metal with subsequent irrigations. From figure 3.2 it is evident that soil from the SAP+GBC treatment may hold up to about 125 mg Cd kg^{-1}. The heavy metal was not detected at deeper soil depths or in the leachate (LOD = 50 ppb).

3.4.2.2 Chromium

The concentration of Cr ([Cr]) increased with each irrigation event in all treatments, indicating its accumulation over time; however, it was not detected in background soil samples taken before the first wastewater irrigation (LOD = 15.6 mg kg^{-1}) (figure 3.3). Mean [Cr] in surface soil samples over the same sequence of irrigation events as for Cd, were 35.7, 51.1, 60.7, 78.5 and 81.9 mg kg^{-1}, respectively. The [Cr] after the fifth and eighth irrigations were significantly greater than those after the second irrigation ($p \leq 0.05$). However, [Cr] was not significantly different amongst treatments ($p > 0.05$). This suggests that the soil used in this study could hold Cr up to approximately 80 mg kg^{-1}. This is in accordance with the findings of Mapanda et al. (2005) who observed [Cr] in topsoil (87% sand, 1% organic matter and pH: 5.4 – 7.0) of a vegetable production site in Crowborough (Zimbabwe) receiving treated effluent from a sewage treatment plant as irrigation water since 1975, to range between 48 – 100 mg kg^{-1}.



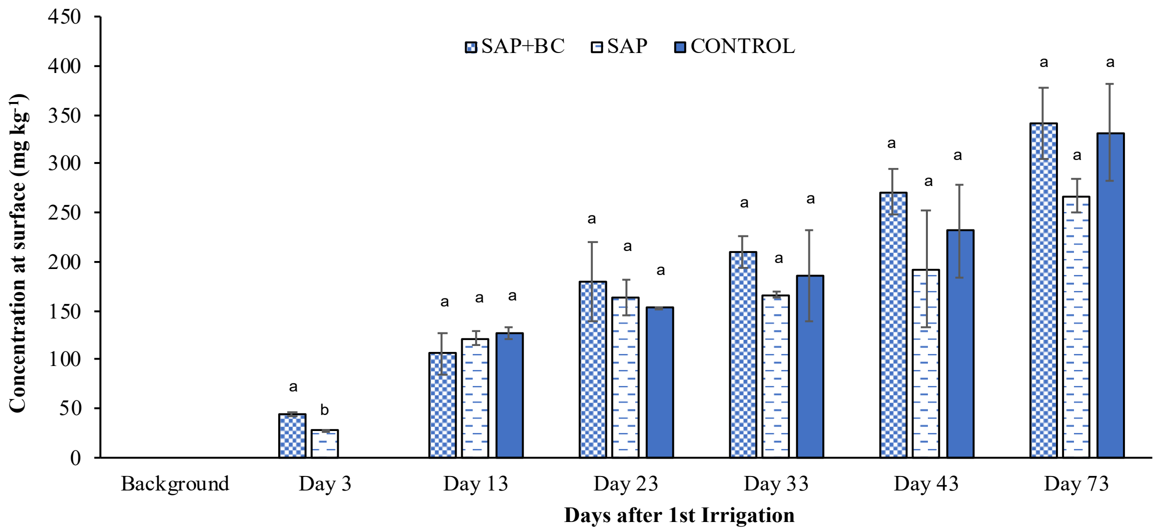
Different letters above the error bars (standard error) indicate significant differences in concentrations for a given event (α=0.05)

Figure 3.3 Mean concentrations of chromium in surface soil samples after successive irrigations.

Although chromium was added through wastewater irrigation, after the first irrigation event it was not detected in surface soil samples of any of the treatments. Theoretically, based on soil properties, Cr loading and lysimeter dimensions, Cr would move up to a depth of about 1.3 mm after the first irrigation, though in practice this depth would exceed 1.3 mm under surface irrigation. Moreover, the surface soil samples were collected from the top 10 mm in depth, which would mix soil from the depth where Cr had not yet reached, or which had very low [Cr], lowering the heavy metal concentration below its detection limit. This could explain why Cr was not detected at the soil surface after the first irrigation for any treatment. Moreover, there was no effect of either SAP or SAP+GBC amendment on the concentration of Cr in the topsoil layer. For all individual events, surface soil Cr concentrations for both amendment treatments were not significantly different ($p > 0.05$) from those of the control. However, numerically, compared to the other two treatments, [Cr] values were consistently higher in the SAP+GBC treatment after the fourth (65.7 mg kg^{-1}), fifth (78.7 mg kg^{-1}) and eighth (101.2 mg kg^{-1}) irrigation events. These higher [Cr] could be indicative of the presence of biochar in the topsoil. At no time was Cr detected at other soil depths or in the leachate (LOD = 50 ppb), indicating the soil's high affinity for Cr.

3.4.2.3 Copper

Copper was not detected in background soil samples (LOD = 15.6 mg kg^{-1}). Copper concentrations ([Cu]) in surface soils after the first irrigation event were significantly higher ($p \leq 0.05$) under the SAP+GBC treatment (44.9 mg kg^{-1}) than under the SAP treatment (27.3 mg kg^{-1}) (figure 3.4). This could be attributed to the presence of biochar in topsoil. In the control, the [Cu] after the second, third, fourth, fifth and eighth irrigation events were 127.2, 152.8, 186.0, 231.7 and 331.9 mg kg^{-1}, respectively. The [Cu] in SAP and SAP+GBC treatments were not significantly different ($p > 0.05$) from those of the control from the second irrigation onwards, though, numerically, [Cu] was greater under the SAP+GBC treatment than the SAP or control treatments after the third (179.4 mg kg^{-1}), fourth (209.9 mg kg^{-1}), fifth (271.2 mg kg^{-1}) and eighth (341.4 mg kg^{-1}) irrigations, possibly due to the presence of biochar in topsoil. Overall, treatments did not have a significant effect on [Cu] in topsoil of the lysimeters; however, the increase in [Cu] with irrigation in all the treatments was significant ($p<0.05$; figure 3.4). For all treatments, copper was found only in the surface soil, and not at subsequent soil depths or in the leachate (LOD = 50 ppb).

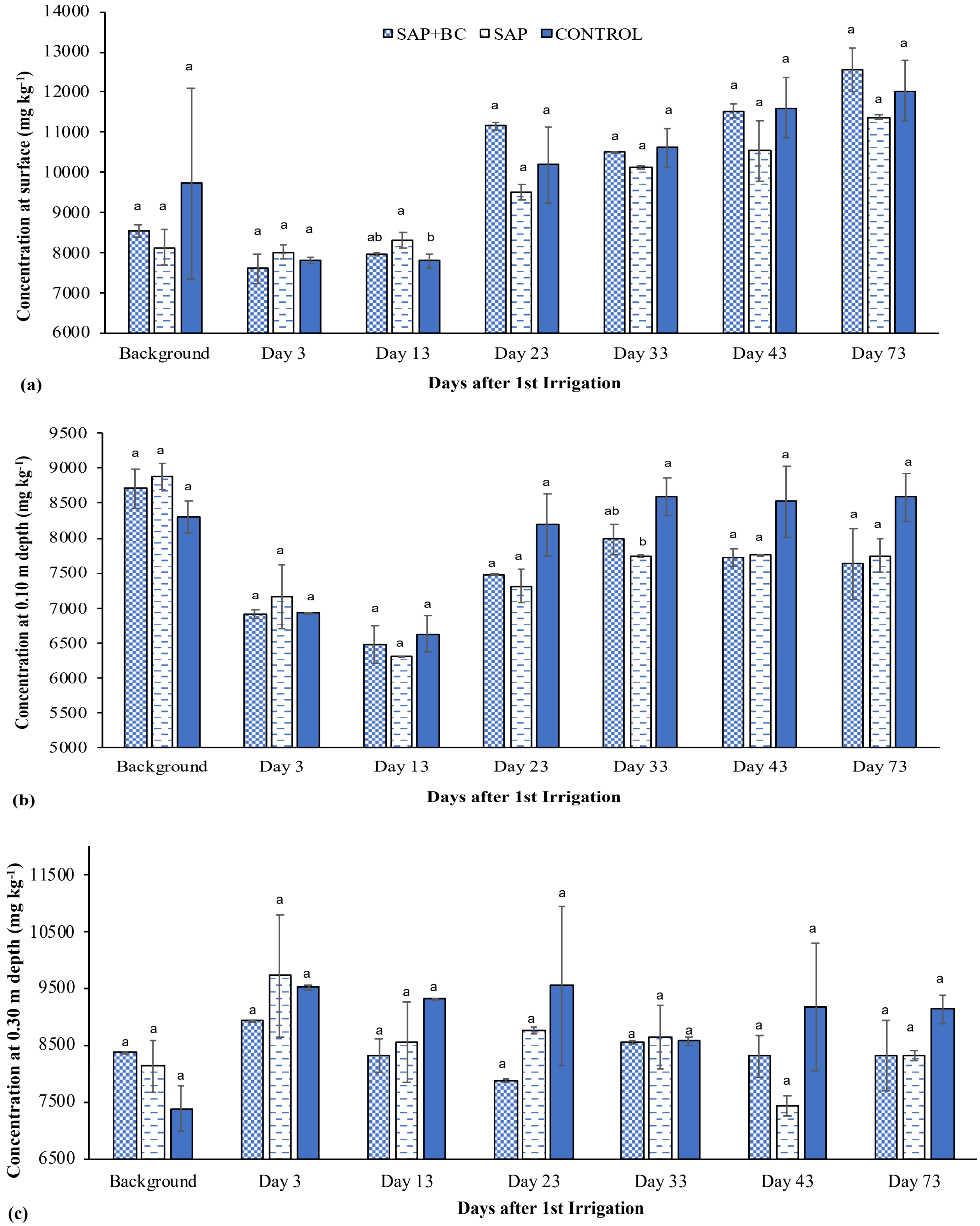


Different letters above the error bars (standard error) indicate significant differences in concentrations for a given event (α=0.05)

Figure 3.4 Mean concentrations of copper in surface soil samples after successive irrigations.

3.4.2.4 Iron

A plot of mean Fe concentrations ([Fe]) in surface, 0.10 m and 0.30 m depth soil samples (figure 3.5), shows that for all the treatments the background [Fe] in surface and 0.10 m depth samples was slightly higher than that after the first irrigation. This could possibly be the result of redistribution of Fe due to the disturbance caused while mixing of amendments and planting potato tubers. The [Fe] in surface soil between the first and eighth irrigation event ranged from 7785 to 12020 mg kg^{-1}, 7998 to 11369 mg kg^{-1} and 7589 to 12567 mg kg^{-1} in the control, SAP and SAP+GBC treatments, respectively. There was no significant effect of the SAP+GBC treatment on [Fe] when compared to the control, indicating that biochar addition in the surface soil did not increase the soil's sorption capacity for Fe. As expected, we observed no effect of the SAP treatment on [Fe] in surface soil as SAP was applied at 0.15 – 0.25 m below the surface.



Different letters above the error bars (standard error) indicate significant differences in concentrations for a given event (α=0.05)

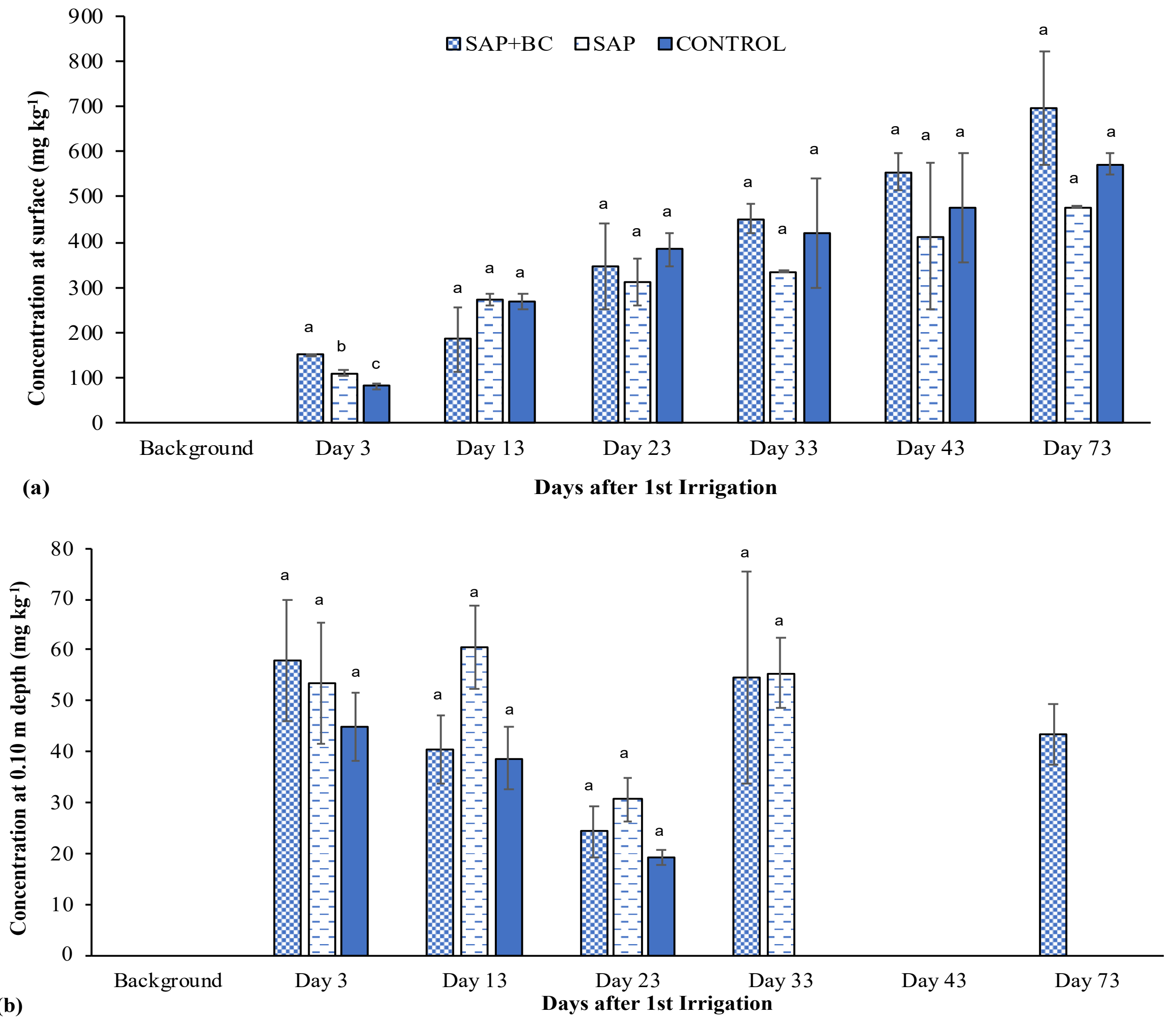
Figure 3.5 Mean concentrations of iron in soil samples from the surface, 0.10 and 0.30 m depths after successive irrigations.

There was a significant increase in [Fe] with application of successive irrigations in all the three treatments ($p \leq 0.05$) as Fe was added through wastewater application (figure 3.5). Across irrigation events, the concentration at a depth of 0.10 m was significantly lower than that at the surface ($p \leq 0.05$). This was expected as the surface soil would have adsorbed Fe, but some amount would have leached up to 0.10 m in depth as well. At a depth of 0.10 m, the [Fe] ranged from 6628.6 to 8583.5 mg kg^{-1}, 6310.8 to 7755.9 mg kg^{-1} and 6479.2 to 7985.9 mg kg^{-1} in the control, SAP and SAP+GBC treatments, respectively.

The [Fe] after the third, fourth, fifth and eighth irrigation events were significantly greater than they were after first and second irrigations ($p \leq 0.05$). The increase in [Fe] with the application of wastewater was also observed at a depth of 0.10 m, similar to the trend observed at the soil surface. At a depth of 0.30 m, there were no significant differences in [Fe] amongst treatments. Although, [Fe] at a depth of 0.30 m was significantly greater than at a depth of 0.10 m ($p \leq 0.05$), it did not increase over time (figure 3.5). This indicates that a minimal amount of Fe leaching from the surface and the first depth would have reached a depth of 0.30 m in the soil. Despite Fe being mobile in the liquid phase, it was not detected below 0.30 m depth and, in the leachate, (LOD= 50 ppb) collected from the drainage pipe at the bottom of the lysimeter. This observation corroborates the inference that Fe from wastewater would not have leached below 0.30 m in depth. As the SAP was applied at a depth of 0.15-0.25 m, its impact, if any, on sorption of Fe, would be reflected in the [Fe] under the SAP treatment being significantly different than that in the remaining treatments. However, no such differences were recorded; therefore, it is likely that application of SAP at a depth of 0.15-0.25 m might not have affected the mobility of Fe in soil.

3.4.2.5 Lead

Lead was neither detected at a depth of 0.30 m or in leachate throughout the experiment (LOD=50 ppb), nor in background soil samples from either the surface or at a depth of 0.10 m. Under the control treatment, the lead concentration [Pb] increased from 82.0 mg kg^{-1} after the first irrigation event to 572.5 mg kg^{-1} after the eighth irrigation event (figure 3.6). The corresponding changes in [Pb] under the SAP and SAP+GBC treatments were 111.0 to 477.1 mg kg^{-1} and 150.9 to 695.6 mg kg^{-1}, respectively. Clearly, in all cases there was a significant buildup of Pb over time ($p \leq 0.05$). At a depth of 0.10 m Pb was detected after the start of wastewater irrigations; however, the concentrations were within 60.6 mg kg^{-1} for all the treatments. Irrigation with contaminated water apparently led to trace amount of Pb reaching a depth of 0.10 m; however, the quantity was significantly lower than that accumulated at the surface ($p \leq 0.05$). There was no significant effect of either of the treatments on the concentrations at either depth.



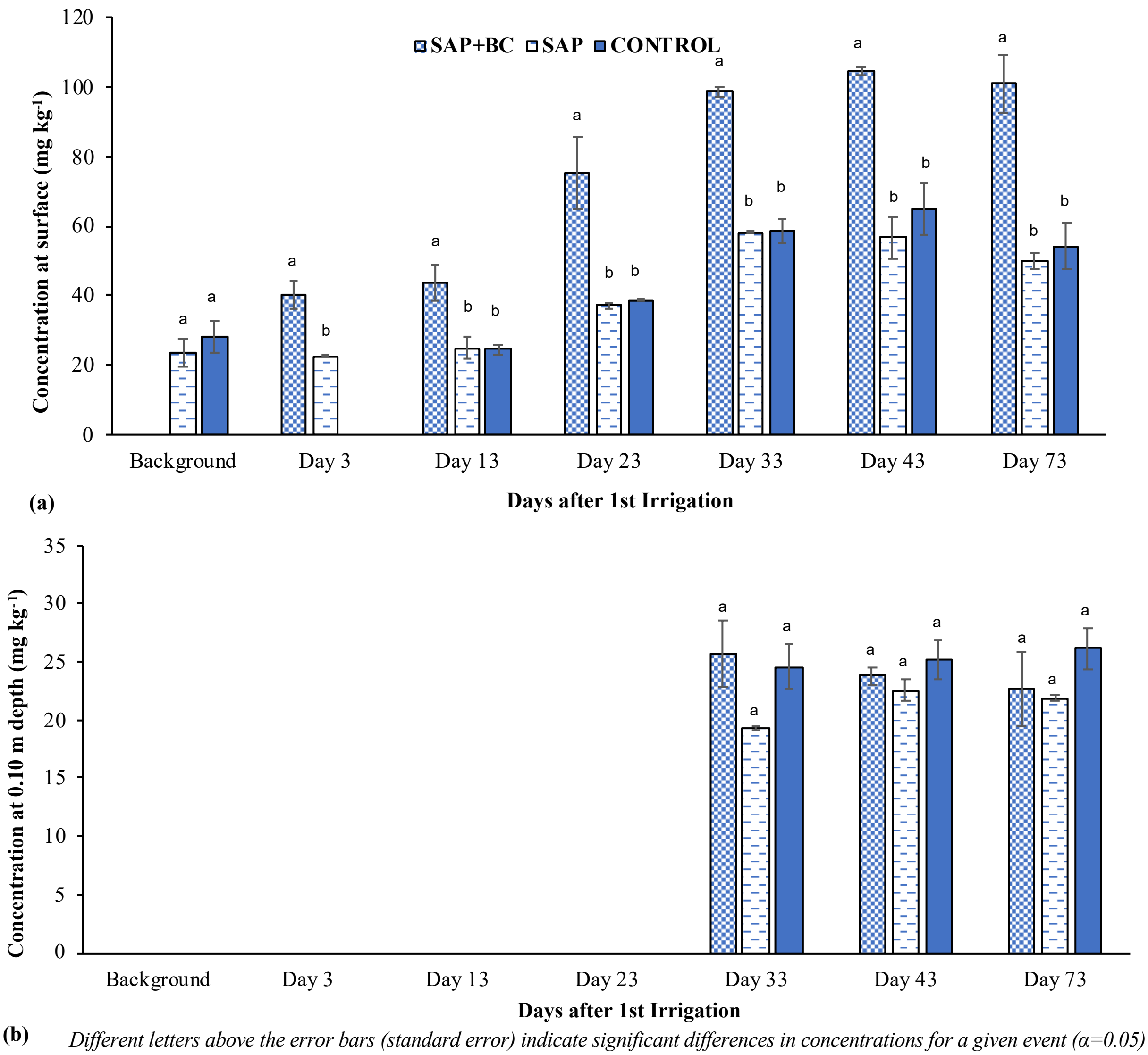
Different letters above the error bars (standard error) indicate significant differences in concentrations for a given event (α=0.05)

Figure 3. 6 Mean concentrations of lead in surface and 0.10 m depth soil samples after successive irrigations.

3.4.2.6 Zinc

The background level of Zn in the surface soil of non-amended control was relatively low (28.1 mg kg^{-1}). As in the control, Zn was not detected after the first irrigation (figure 3.7), it appears that the heavy metal was redistributed in the soil profile due to mixing in of the amendments or/and planting of the tubers. Under the SAP treatment also, the background Zn concentration ([Zn]) was relatively low (23.5 mg kg^{-1}), but gradually increased after subsequent irrigations, reaching 54.1

and 50.0 mg kg^{-1} in control and SAP treatments respectively, after the eighth irrigation event. No background Zn was detected in the soil for SAP+GBC treatment. A gradual increase in [Zn] with subsequent irrigations was also observed for the SAP+GBC treatment: the [Zn] increased from 40.2 mg kg^{-1} after the first irrigation event to 100.9 mg kg^{-1} after the eighth irrigation event (figure 3.7). There was a significant Zn buildup due to irrigation over time ($p \leq 0.05$) in this treatment as well. There were no differences between [Zn] in soils from control and SAP treatments. However, the [Zn] in surface soil from the SAP+GBC treatment was significantly higher than that of the control and SAP treatments, throughout the experiment ($p \leq 0.05$; figure 3.7).



Different letters above the error bars (standard error) indicate significant differences in concentrations for a given event (α=0.05)

Figure 3.7 Mean concentrations of zinc in surface and 0.10 m depth soil samples after successive irrigations (*Zn was not detected for first three irrigation events at 0.10 m depth below the surface*).

At a depth of 0.10 m, Zn was neither detected in background soil samples, nor in the samples collected after the first, second and third irrigations. However, after the fourth, fifth and eighth irrigation events, Zn was detected at low concentrations (19.3 to 26.1 mg kg^{-1}) in all treatments; there were no significant differences ($p > 0.05$) among the treatments. This indicates that Zn would move slowly downward in soil, reaching a depth of about 0.10 m. The lack of increase in [Zn] after either the fifth or eighth irrigation events, as compared to after the fourth irrigation event indicates that Zn would have moved to a limited depth beyond 0.10 m. Nevertheless, the [Zn] at a depth of 0.10 m were significantly lower than those at the surface for all treatments ($p \leq 0.05$). Zn was not detected in leachate for any irrigation event (LOD = 50 ppb).

3.4.3 Heavy Metals in Plant Tissue

Table 3.6 provides the concentrations of different heavy metals in potato plant root, tuber flesh, tuber peels, shoots and leaves. Concentrations of all the heavy metals were significantly higher in roots than in tuber peel, tuber flesh, shoot or leaves ($p \leq 0.05$). The concentrations of heavy metals in peel were significantly greater than in the tuber flesh ($p \leq 0.05$). Root, peel, flesh and leaf samples from plants grown on SAP+GBC amended soils had significantly lower [Cd] compared to matching samples collected from the non-amended control soil.

The SAP treatment significantly reduced Cd uptake in potato flesh, peel and leaf samples as compared to control ($p \leq 0.05$). The [Cd] concentration in potato flesh from the SAP+GBC treatment was 0.58 mg kg^{-1}, which was relatively closer to the permissible limit of 0.1 mg kg^{-1} defined by the CODEX standard for contaminants and toxins in food (CODEX STAN 193-1995), as compared to the other treatments. However, the concentration in potato flesh from the non-amended control was 2.89 mg kg^{-1}, which is significantly higher than that of either SAP+GBC or SAP treatments ($p \leq 0.05$). It may be noted that [Cd] was quite high (5 mg L^{-1}) in the synthetic wastewater. The [Cd] in potato peels from the SAP+GBC treatment was 3.42 mg kg^{-1}, some 17-fold lower than that measured in the control (59.53 mg kg^{-1}). The [Cd] in leaves and shoots of plants grown under the SAP+GBC treatment were the lowest among all treatments, indicating that the SAP+GBC treatment could reduce uptake of Cd by potatoes.

Table 3.6 Heavy metal uptake in different parts of potato plants in different treatments.

Heavy Metal	Treatment	Concentration in different plant parts (mg kg^{-1})				
		Root	Flesh	Peel	Shoot	Leaf
Cd	*SAP+GBC*	61.73 ± 11.54^{b}	0.58 ± 0.06^{b}	3.42 ± 0.84^{b}	10.89 ± 1.57^{a}	2.75 ± 0.10^{c}
	SAP	167.11 ± 23.42^{a}	1.35 ± 0.50^{b}	13.29 ± 8.59^{b}	16.84 ± 2.2^{a}	4.39 ± 0.40^{b}
	CONTROL	225.68 ± 31.25^{a}	2.89 ± 0.40^{a}	59.53 ± 7.24^{a}	16.18 ± 3.61^{a}	6.28 ± 0.57^{a}
Cr	*SAP+GBC*	11.20 ± 1.83^{a}	0.10 ± 0.02^{a}	1.76 ± 0.30^{a}	1.42 ± 0.33^{a}	1.32 ± 0.23^{a}
	SAP	4.43 ± 0.20^{b}	0.06 ± 0.02^{a}	1.61 ± 0.51^{a}	1.37 ± 0.29^{a}	1.42 ± 0.16^{a}
	CONTROL	8.31 ± 2.44^{ab}	0.06 ± 0.05^{a}	1.92 ± 0.31^{a}	1.73 ± 0.69^{a}	1.98 ± 0.46^{a}
Cu	*SAP+GBC*	65.59 ± 13.27^{a}	4.73 ± 0.68^{b}	16.06 ± 2.06^{b}	4.81 ± 0.89^{b}	7.30 ± 0.96^{b}
	SAP	24.74 ± 5.18^{a}	7.34 ± 1.61^{ab}	17.60 ± 3.98^{b}	4.78 ± 1.41^{b}	9.56 ± 1.74^{ab}
	CONTROL	56.65 ± 19.40^{a}	9.76 ± 1.18^{a}	32.09 ± 1.08^{a}	9.66 ± 0.06^{a}	13.86 ± 0.98^{a}
Fe	*SAP+GBC*	1014.16 ± 124.88^{a}	16.10 ± 0.99^{a}	253.73 ± 50.26^{a}	90.24 ± 19.16^{a}	332.35 ± 24.81^{a}
	SAP	1088.26 ± 110.45^{a}	19.82 ± 2.73^{a}	307.69 ± 71.19^{a}	84.62 ± 12.71^{a}	350.63 ± 43.03^{a}
	CONTROL	1431.67 ± 176.43^{a}	20.62 ± 1.71^{a}	381.68 ± 41.83^{a}	93.89 ± 32.24^{a}	389.91 ± 35.30^{a}
Pb	*SAP+GBC*	91.83 ± 17.65^{a}	0.03 ± 0.01^{a}	6.58 ± 2.00^{a}	8.30 ± 2.41^{a}	4.22 ± 1.25^{a}
	SAP	34.12 ± 4.96^{b}	0.03 ± 0.04^{a}	8.75 ± 4.19^{a}	7.07 ± 2.52^{a}	4.85 ± 1.39^{a}
	CONTROL	70.77 ± 20.98^{ab}	0.05 ± 0.02^{a}	17.66 ± 4.81^{a}	9.69 ± 4.14^{a}	3.34 ± 0.51^{a}
Zn	*SAP+GBC*	67.83 ± 10.57^{b}	18.12 ± 0.96^{b}	34.92 ± 3.07^{a}	52.72 ± 8.51^{a}	17.86 ± 1.49^{a}
	SAP	288.92 ± 27.00^{a}	22.46 ± 2.55^{ab}	116.24 ± 43.51^{a}	71.09 ± 8.89^{a}	17.35 ± 0.31^{a}
	CONTROL	379.79 ± 101.27^{a}	25.26 ± 2.19^{a}	117.19 ± 8.52^{a}	102.00 ± 22.77^{a}	19.34 ± 0.49^{a}

Different superscript letters for each heavy metal and plant part indicate significant differences amongst treatments (α=0.05).

It appears that, compared to the control, neither SAP nor SAP+GBC had any positive impact on reducing Cr, Fe or Pb uptake by any portion of the potato plant. While the quantity of Cr in potato flesh samples for all treatments was within the maximum permissible limit of 0.5 mg kg^{-1} (NHFPC, 2012), peel samples contained Cr in amounts exceeding this limit. This could be attributed to the high levels of the heavy metal present in the wastewater (2 mg L^{-1}). In the case of Fe, numerically, the concentrations were lowest in all the plant parts sampled from the SAP+GBC treatment, except shoots (non-edible part). Iron, a micronutrient, can be found in concentrations ranging from 9.8 to 29.1 mg kg^{-1} in conventionally grown Canadian potato tubers (Warman and Havard, 1998). Concentrations of Fe in tuber flesh samples were within this range for all the treatments. While the [Pb] in potato tuber flesh was also within the maximum permissible limit of 0.1 mg kg^{-1} (peeled potatoes) (CODEX STAN 193-1995), the [Pb] in potato peels was above the specified limit of 0.2 mg kg^{-1} (NHFPC, 2012). This may be attributed to its higher concentration (16 mg L^{-1}) in synthetic wastewater.

The SAP+GBC treatment significantly reduced Cu uptake by tuber flesh, peel, leaf and shoots as compared to the non-amended control ($p \leq 0.05$), whereas the SAP treatment resulted in a significant reduction of Cu uptake by peel and plant shoots as compared to the control ($p \leq 0.05$). Although not significantly different, the [Cu] in flesh and plant leaf samples from the SAP treatment were numerically lower than those of the control; more experiments are needed to draw inferences on the effect of SAP on Cu uptake by potato plants. The [Cu] in sampled plant parts from SAP and SAP+GBC treatments were not significantly different from one other ($p > 0.05$). In the case of surface soil samples, no significant difference of treatments was found in terms of Cu retention for either treatment. Biochar did not have any effect on retaining Cu at the soil surface soil (figure 3.4), while the effect of SAP could not be seen by analyzing surface soil samples as it was added 0.15 – 0.25 m below the surface. This indicates an effect of SAP alone in reducing Cu uptake by potato plants, as SAP was applied at a depth accessed by roots. Thus, both SAP and SAP+GBC were effective in reducing Cu uptake by potatoes. As a micronutrient, [Cu] in potato tubers can vary from 2.0 to 6.4 mg kg^{-1} in conventionally grown Canadian potato tubers (Warman and Havard, 1998). The mean [Cu] in tuber flesh grown in SAP+GBC amended soils (4.73 mg kg^{-1}) was within this range, whereas the [Cu] in tubers from the SAP (7.34 mg kg^{-1}) and control (9.76 mg kg^{-1}) treatments were higher than this range. Comparing the effect of soil type on the uptake of Cu by potatoes, Moore et al. (2013) found that Cu uptake by plants grown in sandy soil was greater than in those grown on a silt loam soil. Our results showed a relatively high uptake of Cu, which could be due to the sandy soil filling the lysimeters. Sandy soils are suspected of not binding Cu strongly and better promoting its translocation to plants when compared to silty or clay soils.

The SAP+GBC treatment significantly reduced Zn uptake by tuber flesh and plant roots as compared to the control ($p \leq 0.05$), although the effect was not evident in tuber peel, shoot or leaves. However, it may be noted that the [Zn] in tuber peel, shoot and leaves grown under the SAP+GBC treatment was numerically less than that of potatoes grown in non-amended soil. Under the SAP amended treatment, the [Zn] in all parts of the plant was less than that under the control treatment; however, the difference was not significant ($p > 0.05$). Zn is a micronutrient present in potato tubers in amounts ranging from 0.6 to 17.3 mg kg^{-1} (Warman and Havard, 1998). The [Zn] in the flesh of tubers grown on SAP+GBC amended soil (18.1 mg kg^{-1}) was comparable to this range. However, [Zn] in tubers grown in the control (25.3 mg kg^{-1}) and SAP amended soils tubers (22.5 mg kg^{-1}) were on the higher side.

3.5 Discussion

A general trend of accumulation in the topsoil with subsequent wastewater irrigations was observed for all heavy metals tested. Since heavy metals are both non-biodegradable and non-thermo-degradable, they are persistent in nature and tend to accumulate to toxic levels in soils (Bohn et al., 1985; Sharma et al., 2007). The levels of Cd, Cr and Cu were quantified in the topsoil layer across irrigation events. However, these metals were not detected in soil samples from a depth of 0.10 m, indicating their complete sorption in the topsoil layer for all treatments including the control. However, Fe, Pb and Zn were found in both surface soils and at a depth of 0.10 m. Presence of more than one heavy metal cation in a soil water system results in their competitive sorption (onto soil or any other sorbent material), favoring certain cations over others (Echeverria et al., 1998; Elliot et al., 1986; Gomes et al., 2001). Different mechanisms of competitive adsorption have been proposed to be at play: (i) preferential adsorption of hydrolyzed metal products and induced cation hydrolysis on the surface of hydroxides vs. unhydrolyzed products (James et al., 1975; Schwertmann and Taylor, 1989), (ii) adsorption based on the electronegativity of the cation (Hsu, 1989), or (iii) tendency of the cation to form a covalent bond based on its ionic radius (Sposito, 2008). McBride (1994) ordered metal cations according to their sorption preference based on electronegativity: Cu > Pb > Cd > Zn. Sposito (2008) ordered the cations according to their potential to form strong complexes by forming covalent bonds: Pb > Cd > Cu > Zn. The latter order concurred with the results of the present experiment, where Zn, along with Fe and Pb were found to leach to depth of 0.10 m, whereas Cd, Cu and Cr were not detected in soil at this depth. However, the Pb and Fe could have reached the 0.10 m depth because of their high concentration in wastewater (16 mg L 1and 120 mg L 1, respectively) as compared to other heavy metals.

Due to the range of depths at which the polymer was applied (0.15 – 0.25 m below the surface), heavy metal concentrations at the surface and at a depth of 0.10 m under the SAP treatment were not significantly different from those under control conditions. However, due to biochar's ability to bind metal cations (McBride, 1994; Sposito, 2008), the SAP+GBC treatment significantly retained Cd and Zn in the topsoil layer as compared to the control treatment. Biochar pH can also play an important role in the immobilisation of heavy metals in soil-water-plant systems. In a study on the effect of broiler litter derived biochar on immobilisation of heavy metal ions (Cu, Cd, Ni and Pb), Uchimiya et al. (2010) showed that an increase in pH due to the addition of biochar in soil increased the immobilisation of Ni and Cd. The immobilisation of metal ions was attributed to various mechanisms, including the formation of metal hydroxide, carbonate or phosphate precipitates resulting from an increase in pH. Soil solution pH affects metal ion speciation as well as surface charge density of carbonaceous material such as biochar (Sanchez-Polo and Rivera-Utrilla, 2002). Thus, pH has a direct effect on mobility of heavy metal ions in soil-water systems (Uchimiya et al., 2010). The plantain peel biochar used in this study was found to be strongly alkaline (pH = 10.27 ± 0.06) and its use as an amendment may have increased the immobilisation of heavy metals under the SAP+GBC treatment compared to the control.

The depth of travel for a heavy metal in soil would increase with the application of an additional quantity of the metal. Considering soil's Cd holding capacity to be 65 mg kg^{-1} (figure 3.2), the experimental soil's properties, Cd loading and lysimeter dimensions, it may be estimated that Cd could move to a depth of about 33 mm after the eighth irrigation. This suggests that Cd would not be detected at a depth of 0.10 m or at any further depths, which was reflected by the present observations. Similarly, considering Cd retention in the SAP+GBC amended soil to be 125 mg kg^{-1} (figure 3.2), the estimated depth of travel for Cd would be 17 mm by the end of the experiment. This depth is approximately half the depth to which Cd would have moved in soil without biochar. Different forms of Cr (chromate, bichromate and dichromate ions) are not strongly sorbed by soil under alkaline or slightly acidic conditions (Wittbrodt and Palmer, 1995). Potential adsorption sites in soil are also reduced in the presence of other ions (Banks et al., 2006). Based on the soil's Cr holding capacity being roughly 80 mg kg^{-1} (figure 3.3), the depth of travel after the eighth irrigation would be about 10.7 mm under the control treatment. A similar depth of travel could be estimated for the SAP treatment as well (10.7 mm). Given the maximum topsoil layer [Cr] under the SAP+GBC treatment (101.2 mg kg^{-1}), the depth to which Cr could theoretically have travelled was estimated to be about 8.5 mm, which is slightly less than its depth of travel under other treatments. This may explain why this heavy metal was not detected at a depth of 0.10 m. In the case of Cu, considering the final topsoil layer concentration to be 331.9 mg kg^{-1} (figure 3.4), the approximate depth of travel after eight irrigations was calculated to be around

10.3 mm for the control treatment. Similar depths of travel were calculated for the SAP (12.8 mm) and SAP+GBC treatments (10.0 mm), as the [Cu] under all treatments did not differ significantly.

Although Pb was detected at depth of 0.10 m, the concentration was below the maximum allowable limit of 70 mg kg^{-1} (for agricultural soils) specified by the Canadian soil quality guidelines for the protection of human and environmental health (CCME, 1999). Most of the Pb was held in the top 0.10 m of soil and could not be detected at a depth of 0.30 m below the surface or in the leachate from any of the irrigation events. Generally, Pb is considered to be relatively immobile in soil (USEPA, 2005) as it can form complexes with clay minerals and organic matter. In this study however, Pb was found to leach to a depth of 0.10 m in the soil profile. This can be attributed to the presence of a lower amount of material capable of binding lead (clay, organic matter, etc.) in sandy soil (NRCC, 1978). Also, complex rhizosphere chemistry as a result of interaction between soil, water, heavy metals and plant roots (USEPA, 2005) can potentially have contributed to the transport of Pb to a depth of 0.10 m. Conventionally, plants are known to decrease heavy metal leaching through processes such as the adsorption of the contaminant on root surfaces, its uptake by the plant itself and its microbial immobilisation in the root zone. However, in the rhizosphere, organic acids can be exuded by plant roots, leading to the decomposition of organic matter, and microbial activity can solubilise heavy metals, thereby increasing their leaching potential (USEPA, 2008). Due to change in behavior of lead adsorption induced by the presence of the amendments (SAP, SAP+GBC) used in the study, the mobility of Pb may have been increased in comparison to that under the non-amended control. This may explain why this heavy metal was detected at a depth of 0.10 m in SAP and SAP+GBC soil samples for days 33 and 73 but was not detected for the control samples (figure 3.6). Due to the presence of biochar in the topsoil layers, the SAP+GBC treatment retained a significantly greater amount of Zn in surface soil as compared to the SAP and control treatments (figure 3.7). The [Zn] did not increase with time at the 0.10 m soil depth, as Zn accumulated in the topsoil layer.

In concurrence with other studies (Dunbar et al., 2003; Moore et al., 2013), concentration of all the heavy metals was greater in roots than in other plant parts (table 3.5). Although potato plant roots are not consumed as food, heavy metals in the root could translocate to different parts of the plants, including the tubers. Moreover, it was found that heavy metal concentrations in peels of potato tuber were higher than in tuber flesh. Davies and Crews (1983) in their study on Pb and

Cd content in potato peel and flesh also found greater amounts of these heavy metals to be present in peels as compared to flesh. Reduced uptake of Cd into different parts of plants grown in SAP and SAP+GBC amended soils as compared to the non-amended control (table 3.5) can be attributed to the ability of SAP and biochar to retain this heavy metal. figure 3.2 shows that, due to the presence of biochar, the SAP+GBC treatment retained significantly greater levels of Cd in the soil's surface layer than under the control treatment. Park et al. (2011) showed that soil amendment with chicken manure or green waste biochars led to immobilisation of Cd, which led to a significant reduction in its uptake by Indian mustard [*Brassica juncea* (L.) Czern.]. The SAP treatment did not have a significant effect on [Cd] in the topsoil layer but did influence uptake into plant parts (tuber flesh, peel and leaf) due to the application depth of the polymer (0.15 – 0.25 m below surface), where the plant roots had access. Although [Cd] in plant roots did not differ significantly ($p > 0.05$) between the control and SAP treatments, numerically they were 26% lower in the SAP treatment (167.11 mg kg^{-1}) compared to the control (225.68 mg kg^{-1}). In general, mean Cd uptake into potato plant parts was least under the SAP+GBC treatment, followed by the SAP treatment and then the control. This can be due to the combined effect of SAP and biochar.

Reduced uptake of Zn in root and flesh samples grown on SAP+GBC amended soils as compared to the control (table 3.5) can be again be attributed to the biochar's ability to hold onto the heavy metal. This is evident in figure 3.7, where the SAP+GBC treatment's ability to retain significantly higher levels of Zn as compared to the control can be seen. Compared to the control, the SAP treatment was able to significantly reduce Cu uptake into potato peel samples. However, neither amendment treatment was able to significantly ($p > 0.05$) reduce Cr, Fe and Pb uptake by different plant parts as compared to the control. This is in accordance with the fact that these amendments also did not have any effect on the concentration of these heavy metals in soil (figures 3.3, 3.5 and 3.6). Acrylamide monomers were not detected in potato tuber flesh or peel samples for the SAP and SAP+GBC treatments.

3.6 Conclusions

A general trend of accumulation of all heavy metals in the topsoil was observed for all treatments. Heavy metals, Cd, Cr and Cu, were not detected in soil samples taken from depths of 0.10 m or below, but Fe, Pb and Zn were detected at a depth of 0.10 m due to preferential sorption by the amended soil. Of these, Fe was detected in samples from 0.30 m below the surface, likely due to its inherent presence in soil and its higher concentration in synthetic wastewater. For all treatments, the concentrations of Fe, Pb and Zn were significantly lower at a depth of 0.10 m than at the surface of the soil. Heavy metals were not detected in the leachate collected after any of the irrigations. There was no effect of SAP on the concentration of heavy metals in surface soil, or on the concentration of Fe, Pb or Zn at a depth of 0.10 m, as this amendment was incorporated in a soil layer, 0.15-0.25 m below the surface. However, due to biochar application in the top 0.10 m of soil, the SAP+GBC treatment retained significantly greater amounts of Cd and Zn in topsoil layer when compared to the non-amended control.

The SAP+GBC treatment significantly reduced Cd, Cu and Zn uptake in potato tuber flesh as well as Cd uptake in tuber peel as compared to the control. The SAP treatment significantly reduced Cd uptake into the edible parts of the plant (potato tuber flesh and peel) as well as Cu uptake into potato peel as compared to the non-amended control. Both these amendments were ineffective in reducing Cr, Fe and Pb uptake in any plant parts. Potato plant roots had significantly higher metal concentrations than any other parts of the plant for all the heavy metals, irrespective of treatments. Generally, the concentrations of heavy metals in tuber peel were significantly higher than those in the tuber flesh. It is therefore suggested that potatoes irrigated with wastewater should be peeled before consumption. Acrylamide monomer was not detected in any edible parts of the potato plant for any of the amendment treatments. Thus, potatoes grown in soil amended with SAP may be safe for consumption.

In this experiment SAP was incorporated in soil in a soil layer 0.15 – 0.25 m below the surface to prevent photo-degradation of the polymer. More experiments are needed to study the effect of SAP amendment depth on heavy metal immobilisation. Furthermore, in the present study 1% biochar was mixed into the topsoil; however, it is likely that addition of a higher proportion of biochar might further immobilise heavy metals and reduce their uptake by potatoes. Therefore, more experiments are needed to determine the effect of biochar quantity on heavy metal uptake by potatoes. Biochar produced from different feedstocks at different temperatures could also be tested to identify the most effective biochar and develop environment friendly, inexpensive and simple techniques to improve immobilisation of heavy metals from wastewater.

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Connecting Text to Chapter 4

In the previous chapter, role of polyacrylamide super absorbent polymer (SAP) and SAP-plantain peel biochar on heavy metal mobility and uptake by potato tubers was discussed. Potato tubers grow below the ground and are in direct contact with heavy metal contaminated soil, which is why potato plants were chosen for the study. However, in Chapter 4, it was decided to test the amendments efficacy on heavy metal uptake by an aboveground crop under similar circumstances (grown on sandy soil and wastewater irrigated). Therefore, spinach was chosen as the food crop for the study. Spinach is known to hyper-accumulate heavy metals, which makes it an ideal candidate for the study. Also, unlike the previous study, hydrogel amendments were mixed in top 0.10 m of the soil profile to ensure that it can contribute towards metal immobilization. Pyrolyzed plantain peel biochar (PBC) was utilized, instead of gasified plantain peel biochar (GBC) used in chapter 3, for SAP-biochar mix amendment. In line with the previous chapter, chapter 4 explains the role of SAP and SAP+PBC soil amendments on the mobility of some common heavy metals in soil profile as well as their uptake by wastewater irrigated spinach plants grown on sandy soil.

This chapter will soon be sent for publication in a refereed journal. The manuscript will be co-authored by Prof. Shiv Prasher (academic research supervisor), Dr. Eman ElSayed (Postdoctoral Fellow at McGill University's Bioresource Engineering Department at the time conducting research), Dr. Christopher Nzediegwu (Postdoctoral Fellow at University of Alberta's Department of Renewable Resources). Mr. Ali Mawof (PhD scholar at McGill University's Bioresource Engineering Department) and Dr. Ramanbhai Patel (Research Associate at McGill University's Bioresource Engineering Department). The original draft has been modified to maintain consistency with the format of this thesis, in accordance with McGill University's thesis guidelines. Studies and references cited are presented at the end under the '*References*' section.

Chapter 4: Effect of Hydrogel Based Soil Amendments on Heavy Metal Uptake by Spinach Grown with Wastewater Irrigation

4.1 Abstract

Untreated wastewater contains contaminants such as heavy metals, which can be taken up by food crops irrigated with wastewater and can potentially cause a variety of serious health ailments in humans. Use of polyacrylamide super absorbent polymers (SAP treatment) and SAP-pyrolyzed plantain peel biochar mix (SAP+PBC treatment) as soil amendments, is proposed to reduce heavy metal mobility in soil and uptake by wastewater irrigated spinach (*Spinacia oleracea* L.) plants. Sorption test was carried out to establish ability of the treatments to bind heavy metals (Cd, Cr, Cu, Fe, Pb and Zn). The experiment was conducted in field lysimeters packed with sandy soil. SAP was mixed in top 0.10 m of soil at the rate of 1% (w/w) for both the treatments, whereas the biochar was also mixed at the same rate in top 0.10 m of soil for treatment SAP+PBC; non-amended control (wastewater irrigated), along with freshwater irrigated non-amended lysimeters were also included in the study. Synthetic wastewater was used for irrigating spinach plants, for a total of four times at an interval of ten days. After each irrigation event, composite soil samples were obtained at different depths for heavy metal analysis. Spinach leaves were harvested twice, and during second and the final harvest, samples from plant root and stem were also collected for heavy metal analysis. Soil samples collected at the end of the experiment were subjected to pH and CEC analysis. Sorption test results showed that both the treatments acted as better sorbents for Cd, Cu and Zn heavy metals, compared to control. Treatment SAP+PBC exhibited significantly ($p<0.05$) higher pH and CEC for surface soil as compared to control. At the end of the experiment, compared to control, SAP treated soil was able to retain significantly higher amounts of Cr ($p<0.10$), Cu ($p<0.05$) and Fe ($p<0.10$) metals, whereas no significant differences were observed between SAP+PBC and control. Both the treatments were able to significantly ($p<0.05$) reduce Cu uptake in plant stem, as compared to control. SAP+PBC treatment was able to prevent a significant increase in uptake of Cd by the leaves from the second harvest due to wastewater irrigation. Concentrations of Cr and Cu in spinach leaves from second harvest were found to be significantly ($p<0.05$) higher in control treatment, as compared to FW treatment; the concentrations of these metals for SAP+PBC and SAP treatments were similar to FW treatment, highlighting the ability of the amendments to reduce the uptake of contaminants by spinach plants irrigated with wastewater.

4.2 Introduction

Agriculture is the largest consumer of freshwater with about 70% of the total available freshwater withdrawn for agricultural purposes (FAO, 2016; UNESCO, 2016; Koehler, 2008). Therefore, minimizing freshwater use for agriculture is necessary to alleviate stress from water reserves. Use of wastewater for irrigation is proposed and highly encouraged by many researchers to tackle the problem of freshwater scarcity (Rusan et al., 2007; Al-Rashed and Sherif, 2000; Mohammad and Mazareh, 2003; Al-Salem, 1996). In many developing countries, urban and peri-urban farmers have no other choice than to use wastewater for irrigation due to water scarcity and economical constrains (Qadir et al., 2010). Despite the advantages of using wastewater for irrigation, the practice has its limitations. Contaminants present in untreated wastewater, such as heavy metals, can harm human and animal health, as well as the environment (Qadir et al., 2007; Gupta et al., 2008). Heavy metals, being more persistent in nature as compared to other contaminants, can leach to shallow groundwater reserves and may lead to drinking water contamination for humans and animals (Alloway, 1990; Santona et al., 2006; Hashim et al., 2011; Al-Subu et al., 2003).

Wastewater irrigation can also result in excessive uptake of heavy metals by crops, affecting food quality and safety of human health (Muchuweti et al., 2006; Wang et al., 2005). Food chain contamination is one of the major pathways by which heavy metals can enter human body (Khan et al., 2008). Persistent intake of food and water, contaminated with heavy metals in humans, may result in harmful impacts, symptoms of which may only be apparent after several years of exposure (Bahemuka and Mubofu, 1999; Ikeda et al., 2000). Intake of excessive amounts of metals has also been associated with reduced immune function, growth retardation, upper gastrointestinal cancer, disabilities related to malnutrition and impaired psycho-social faculties (Iyengar and Nair, 2000; Türkdoğan et al., 2003). Thus, there is a need to develop a cost-effective and low-tech solution to reduce heavy metal mobility in soils subjected to wastewater irrigation, as well as reduce their uptake by plants grown on such soils.

SAPs are networks of loosely crosslinked polymer chains which are highly hydrophilic in nature and can absorb and retain water or aqueous solutions up to hundreds of times their own weight (Buchholz and Graham, 1998; Skouri et al., 1995; Zohuriaan-Mehr and Kabiri, 2008). Therefore, SAPs are used in agricultural applications, especially for irrigation water conservation (Bai, et al., 2010). Due to presence of high density of metal chelating groups in superabsorbent polymers, these materials are well-suited to stabilize heavy metals in soil and reduce their bioavailability (Torres and Varennes, 1998; Varennes and Torres, 1999; Varennes and Queda, 2005). Published studies on toxicity of acrylate-based superabsorbent polymers consider these materials to be environmentally compatible (McGrath et al., 1993; Haselbach et al., 2000a; Haselbach et al., 2000b; Hamilton et al., 1995; Garay-Jimenez et al., 2008). In two separate studies including growing potatoes and cherry tomatoes using polyacrylamide SAP amendment (1% and 0.1-0.5% w/w respectively), no acrylamide content was observed to be taken up by the plants (Dhiman et al., 2019; Suresh et al., 2018). SAP can be applied in granular (dry) or emulsified liquid (gel) form (Trenkel et al., 1996). Generally granular form such as polyacrylamide SAP is preferred for agricultural use as its handling is easy.

Biochar is a product of pyrolysis, carbonization and gasification of biomass (ANSI/ASABE, 2011). Biochar is widely used in agriculture and it has potential to increase crop yields (Zhang et al., 2012; Major et al., 2010; Steiner et al., 2008; Park et al., 2011) as well as improve soil properties (Sohi et al., 2010; Gaskin et al., 2007; Lehmann 2007). A large quantity of by-products of food processing industries is wasted. Plantain is a staple food for many countries in Asia, South America and Africa; plantain peels are considered as a waste from the fruit and can lead to disposal problems in the environment (Ogunjobi and Lajide, 2013), especially in developing countries like Nigeria which is one of the largest plantain producers in the world (FAO, 2004). Converting the peels to biochar for remediation purposes can be considered as adding value to waste. Plantain peel biochar amended soils have the potential to stabilize heavy metals and make them less bio-available (Lu et al., 2012; Nzediegwu et al., 2019).

Although SAPs and biochar have shown their usefulness in agriculture and pollution control, information on the effect of combination of these promising amendments on heavy metal uptake by wastewater irrigated spinach plants is not known. Use of hydrogels or super absorbent polymers (SAP) and SAP-biochar mixture as soil amendment may lead to reduced heavy metal mobility and uptake by plants grown with wastewater irrigation (Dhiman et al., 2019). Therefore, this study was carried out to determine the effect of polyacrylamide SAP and SAP mixed with plantain peel biochar soil amendments on the mobility of common wastewater borne heavy metals (Cd, Cr, Cu, Fe, Pb and Zn) in soil, as well as their uptake by spinach plants, irrigated with laboratory prepared synthetic wastewater. A similar study was conducted in the previous year by Dhiman et al. (2019) using potato plants which is a tuber crop. Irrigation of vegetables with wastewater is a very common practice in developing countries like India (Arora et al., 2008). Presently, several crops, such as potatoes, spinach, cauliflower, carrot and fenugreek are inadvertently irrigated using wastewater around the world (Dhiman et al., 2019; Arora et al., 2008). Even though spinach is known to bioaccumulate contaminants such as heavy metals from the soil (Romer and Keller, 2001; Intawongse and Dean, 2006; Mattina et al., 2003), it is irrigated with wastewater. Thus, it is necessary to determine the impact of wastewater irrigation on this food crop and to develop methods to minimize the ill-effects. To the best of the author's knowledge, study on evaluating the effects of SAP and SAP-biochar mix soil amendments on heavy metal mobility and uptake by synthetic-wastewater irrigated spinach plants has not been reported previously.

4.3 Materials and Methods

4.3.1 Soil Amendments

Super absorbent polymer (SAP) used in the present study is a cross-linked copolymer of potassium acrylate and acrylamide (commercial name: SUPERAB A200) in granular form and was procured from a Canadian environmental solutions company, *Iramont Inc*. General physical and chemical properties of the used SAP hydrogel are provided elsewhere (Dhiman et al., 2019). Pyrolyzed plantain peel biochar (PBC) was prepared from oven-dried plantain peels, using a pyrolyzer unit built at the *Macdonald Campus Technical Service Building of McGill University* in Sainte-Anne-de-Bellevue, Quebec, Canada. Plantain fruits were procured from *Sami Fruits*, Lasalle, Quebec, Canada and were peeled using standard kitchen knives. The peels were oven-dried at temperatures ranging from 75°C to 80°C for 48 hours. Dried peels were subjected to a temperature of about 460°C with a residence time of 10 min in the pyrolyzer unit to obtain PBC. Proximate and ultimate analysis of biochar was performed at the *CanmetENERGY Characterisation Laboratory* (ISO 9001:2008 certified), Ottawa, ON, Canada. Biochar samples were sent to the *Materials Characterisation Laboratory* at *Department of Mining and Materials Engineering, McGill University*, Montreal, Canada for *Brunauer-Emmet-Teller* (BET) surface area determination. For pH measurement of biochar, 1:30 w/v biochar:water solution was shaken for four hours on the vibratory shaker and pH of the supernatant was measured following the guidelines outlined by Zhang et al. (2015). Properties of the biochar used are provided in table 4.1.

Table 4.1 Physiochemical properties of pyrolyzed plantain peel biochar (PBC).

Parameter	Observed Value (wt %)	Method
Proximate Analysis		
Moisture Content*	5.68	ASTM D7582
Ash Content*	27.97	ASTM D7582
Volatile Content	31.32	ISO 562
Fixed Carbon	40.71	ASTM D7582
Ultimate Analysis		
Carbon	57.40	ASTM D5373
Hydrogen	3.18	ASTM D5373
Nitrogen	2.16	ASTM D5373
Total Sulfur	<0.05	ASTM D4239
Oxygen	9.32	By Difference
pH		
pH (mean ± stdev)	10.6 ± 0.10	Lab
Specific Surface Area		
BET Surface Area (m^2g^{-1})	0.7560	Lab
Heavy Metal Analysis		
Heavy Metal	**Observed content ($mg\ kg^{-1}$)** (mean ± stdev)	**Allowable limit$^{\Phi}$ ($mg\ kg^{-1}$)**
Cd	0.08 ± 0.01	1.50
Cu	11.68 ± 0.05	100.00
Cr	1.11 ± 0.15	90.00
Fe	649.01 ± 58.81	n.a.
Pb	0.04 ± 0.01	150.00
Zn	573.58 ± 33.69	400.00

** Estimated using thermogravimetric analyzer (TGA).*
$^{\Phi}$ Based on European Biochar Certificate (EBC) guidelines (EBC, 2012).
All measurements are made on a dry weight basis; n.a. - not available.

4.3.2 Sorption experiment

Sorption and desorption tests were conducted in laboratory to ascertain if SAP and SAP- pyrolyzed biochar amendments can act as effective adsorbents in soil. Treatments used were SAP+PBC, SAP, and soil (control). Treatment samples were prepared by mixing the amendments in the soil at the rate of 1% (w/w). Five concentrations (0.1, 0.2, 0.3, 0.4 and 0.5 mM) of multi-metal solutions (Cd, Cr, Cu, Fe, Pb and Zn) were prepared using deionized water and analytical chemicals procured from either Sigma-Aldrich (St. Louis, MO, USA) or Fisher Scientific (Waltham, MA, USA). Two grams of sample for each treatment was mixed with 30 mL of prepared solution for each concentration in 50 mL plastic centrifuge tubes (in triplicate) using a vortex shaker (MS2 Minishaker, IKA, China). The tubes were shaken for 24 hours on a vibratory shaker (Innova 2100 Platform Shaker, Eppendorf/New Brunswick Scientific Inc., NJ, USA) at room temperature to reach equilibrium. Next, the samples were subjected to centrifugation at 3500 rpm for 10 min (Sorvall Legent T, Thermo Scientific, MA, USA). Supernatant collected after filtration (0.45 µm pore size) was analyzed for heavy metal concentration using inductively coupled plasma employing optical emission spectrometry (ICP-OES) equipment (Vista-MPX CCD Simultaneous, Varian, CA, USA) to determine sorption. Electrode pH meter (Accumet pH meter model AB15, Fisher Scientific, USA) was used to estimate pH of the supernatant. For desorption, 30 mL of deionized water was mixed with the used adsorbent, collected from sorption experiment, in 50 mL plastic centrifuge tubes. Process of mixing, shaking, collecting supernatant and analyzing it on ICP equipment was repeated to determine the percentage of adsorbed metals released back into the solution.

Sorption behaviour of the treatments was evaluated by fitting the data using Langmuir (Langmuir, 1916) and Freundlich (Freundlich, 1906) adsorption isotherm models. The isotherm models provide a numerical relationship between the sorbate concentration on the solid (Cis) and the chemical's concentration in the solution (C_{iw}). Equation 1 represents the Freundlich isotherm model (Schwarzenbach et al., 2005).

$$C_{is} = K_{iF} \,.\, C_{iw}^{1/n} \qquad (1)$$

Where, C_{is} is the concentration of the chemical i on solid phase (mg g^{-1}),
C_{iw} is the concentration of chemical i in the aqueous phase (mg L^{-1}),
K_{iF} is the Freundlich constant (capacity factor; its units depend on exponent n)
1/n is Freundlich exponent.

C_{is} can be determined using equation 2:

$$C_{is} = \frac{(C_o - C_f).V}{M} \qquad (2)$$

Where, C_o is the original/initial concentration of 'i' in solution (mg L^{-1}),
C_f is the final concentration of chemical i in the aqueous phase (mg L^{-1}),
V is the volume of aqueous solution (L),
M is the mass of the sorbent material (g).

Logarithm form of equation 1 was used to fit the experimental data in linear form (equation 3) to estimate n and K_{if} from the slope and intercept values of the best fit line respectively.

$$\log C_{is} = 1/n. logC_{iw} + logK_{iF} \qquad (3)$$

Langmuir adsorption isotherm model (equation 4) was used to determine the maximum sorption capacity of a sorbent($C_{is\text{-}max}$; mg g^{-1}) (Schwarzenbach et al., 2005).

$$C_{is} = \frac{C_{is-max} \cdot K_{iL} \cdot C_{iw}}{1 + K_{iL} \cdot C_{iw}} \qquad (4)$$

Where, K_{iL} is Langmuir constant. Data was fitted linearly using the Langmuir model, by plotting $1/C_{is}$ vs. $1/C_{iw}$ (equation 5) and estimating K_{iL} and $C_{is\text{-}max}$ from slope and intercept of the best fit line, respectively.

$$\frac{1}{C_{is}} = \left(\frac{1}{C_{is-max} \cdot K_{iL}}\right)\frac{1}{C_{iw}} + \frac{1}{C_{is-max}} \qquad (5)$$

4.3.3 Field study

The study was carried out in field lysimeters situated at *Macdonald Campus of McGill University* in Ste. Anne de Bellevue, QC, Canada. Lysimeter used in this study was a hollow cylinder (1.00 m long x 0.45 m I.D.) made of from PVC material, and filled with sandy soil up to 0.05 m below the top edge. Four equidistant holes (10 mm dia.) were drilled at three different heights viz. 0.10, 0.30 and 0.60 m from the soil surface to facilitate soil sampling along the depth of the column. An outlet was provided at the bottom to collect leachate. Lysimeters were arranged in completely randomized design (in triplicate) with three wastewater irrigated treatments (SAP+PBC, SAP and a non-amended control), as well as a non-amended fresh water irrigation treatment. SAP was mixed in top 0.10 m of soil at the rate of 1% (w/w) in SAP and SAP+PBC treatment assigned lysimeters, whereas PBC was mixed in top 0.10 m of soil at the same rate in SAP+PBC treatment assigned lysimeters.

The lysimeters were brought to field capacity one day before planting. Spinach plants were obtained from a local farmer's market (Jean Talon Farmer's Market, Montreal, Canada) and were transplanted (three plants per lysimeter). Fertilizers (ammonium sulfate and potassium sulfate) were applied at locally recommended rates (CRAAQ, 2013). Ammonium sulfate (21-0-0) was applied in lysimeter soil at the rate of 120 kg N ha^{-1} (9.00 g per lysimeter) in two equal splits, on the day of planting and on the day of first wastewater irrigation (22^{nd} day after planting), whereas, 5.0 g of potassium sulfate (0-0-60) was applied at the rate of 163 kg K ha^{-1} on the day of planting. Based on the available nutrient content analysis of the lysimeter soil samples, it was established that phosphorus fertilization was not required. Every third day, 400 mL (2.5 mm day^{-1}) of freshwater was applied to each of the lysimeters for establishment until commencement of irrigation. Spinach plants were subjected to first irrigation on the 22nd day after transplantation. According to treatments, the lysimeters were irrigated with laboratory prepared synthetic wastewater or fresh water, four times at an interval of ten days at the rate of 4.00 L (~25 mm) per lysimeter per irrigation based on the crop water requirement for spinach (Sanders, 2001). Synthetic wastewater was used to maintain control over the level of contaminants being introduced to the lysimeters. Highest concentrations of some commonly found wastewater contaminants, as reported in literature, were used in preparation of synthetic wastewater, recipe for which is provided elsewhere (Dhiman et al., 2019).

Background soil samples from the surface were also taken for all treatments on the day of transplantation. Composite soil samples were collected two days after each irrigation, from the surface and the four sampling holes at each depth; two days were allowed for the soil to reach field capacity and establish equilibrium between contaminants and amended soils. Samples were sealed in plastic bags, labelled and stored securely in a freezer (-20°C) until further analysis. Leachate samples were not collected for this study as heavy metals were not detected in leachate samples collected for every irrigation event, in a similar study conducted during the previous year (Dhiman et al., 2019). Spinach plants were harvested twice; for the first harvest, leaves were picked on 42nd day after transplanting and second whole plant harvest was done on 64th day after transplanting. Root samples were also collected on the day of second harvest. All the plant tissues were separated, and samples were stored in airtight plastic bags, and stored securely in a freezer (-20°C) until further analysis.

4.3.4 Sample extraction and quantification

Laboratory electrode pH meter was used to estimate pH of soil samples collected from the surface, two days after the last irrigation event (4th), following the soil survey method guidelines outlined by Rayment and Higginson (1992). Soil samples, which were taken from the surface and 0.10 m depth, after the 4th irrigation event, were analyzed for estimation of cation exchange capacity (CEC) and percent base saturation (BS%) according to the $BaCl_2$ method outlined by Hendershot et al. (1993).

Hot nitric acid extraction method was used to determine heavy metal content in soil samples (Stephan et al., 2008) collected from the surface, two days after irrigation events. The glassware used for the study were washed using a laboratory grade detergent, soaked in 4% HNO_3 solution for 24 hours, rinsed using double deionized water and air-dried before use. Surface soil samples for irrigation events 1, 3 and 4 were extracted to determine heavy metal accumulation in topsoil due to wastewater irrigation. Soil samples collected from 0.10 m depth, two days after the last irrigation event, were also extracted for heavy metal analysis to check if the metals had leached down to this depth. The quality control samples (samples with known amount of heavy metals) were also extracted to establish reproducibility and reliability. For the analysis of heavy metals in the plant parts (leaf, stem and roots) the samples were oven-dried at a temperature of 60°C for 48 hrs, crushed with mortar and pestle and ground with a coffee grinder. The equipment was washed between samples to prevent cross contamination. Samples were then digested using an acid block digestion method (Stephan et al., 2008). The ICP-OES and ICP-MS (ICP820-MS Varian, CA, USA) equipment were employed for quantification of heavy metal content in extracted soil and plant tissue samples, respectively. Experimental blanks were also run in parallel with the test samples. Recovery percentage for all heavy metals was found to be more than 80% after extracting and testing the quality control samples. The method followed is described by Dhiman et al. (2019).

4.3.5 Data analysis and quality assurance

Sorption data were analyzed using Matlab R2018b (2018) computer software. Freundlich and Langmuir adsorption models were fitted on equilibrium sorption data using this software. Least square means difference statistical technique, using Student's t-test, was used for pairwise comparison of means for cation exchange capacity, percent base saturation, pH of solutions prepared for sorption analysis and pH of the soil samples for different treatments. Lysimeter soil heavy metal concentration data for different metals and irrigation events were analyzed using a repeated measures statistical model. The metal concentration was assigned as response variable, treatment and time were assigned as fixed effects, whereas lysimeter was assigned as subject and was nested within treatment. Statistical tests were performed using JMP 13 (2017) statistical analysis and graphing software by SAS (JMP, SAS Institute Inc., Cary, NC).

4.4 Results and Discussion

4.4.1 Sorption Experiment

Freundlich constant (K_{if}) and exponent (n) are given in table 4.2. Freundlich constant and exponent determine the curvature and steepness of the isotherm (Low et al., 2000), and are approximate indicators of sorption capacity and sorption intensity, respectively (Komkiene and Baltrenaite, 2016). Goldberg et al. (2005) suggested that sorption is a favorable process for values of n between 0.1 to 1 (or n between 1 to 10, since 1/n is used as an exponent). It is evident from table 4.2 that sorption exponent (n) for all metals are close to one for all treatments, signifying sorption varying linearly with solution concentration for the range of concentrations used. Percentage of Cd metal in solution, removed by treatments SAP+PBC, SAP and control, decreased as the concentrations increase from 0.1 to 0.5 mM (figure 4.1). This is also reflected by the Freundlich exponent values for the treatments SAP+PBC and SAP in table 4.2 (n>1). For Cd, SAP+PBC treatment performed slightly better than treatment SAP, sorbing more than 95.61-99.19% of the metal from the solution, whereas treatment SAP sorbed 70.49-92.57% of the metal from solutions across all concentrations (figure 4.1). However, both the treatments performed better compared to control, which was only able to sorb 7.21-45.05% of the metal from the solution (figure 4.1). Less than 0.5% of the mass sorbed was found to be desorbed during the desorption study for both the treatments; however, mass desorbed for control was found to vary from 0.99 to 14.54% of the desorbed amount, as the initial concentration of the solution varied from 0.1 to 0.5 mM respectively (figure 4.1). Similar adsorption and desorption trends were observed for Zn metal across treatments (table 4.2; figure 4.1).

Sorption percentage increased with increasing concentrations of Cr, Fe and Pb heavy metals for both the treatments, which is in line with the value of Freundlich exponent being less than one in table 4.2 (figure 4.1). In the case of Cr, control adsorbed 97.26-99.16% of the metal from solution and desorbed only 0.88-12.72% of the sorbed metal. The corresponding values for SAP+PBC were 82.30-98.93% and 3.39-31.12%, and for SAP were 73.33-96.24% and 2.59-34.93%, respectively; these values show that sorption decreased, and desorption increased due to SAP and biochar addition. This can be attributed to preferential sorption of different cations by the sorbent material, which depends on the properties and amounts of ions present in a multi-ion solution, pH, temperature and properties of the sorbent material (Echeverria et al., 1998; Elliot et al., 1986; Gomes et al., 2001).

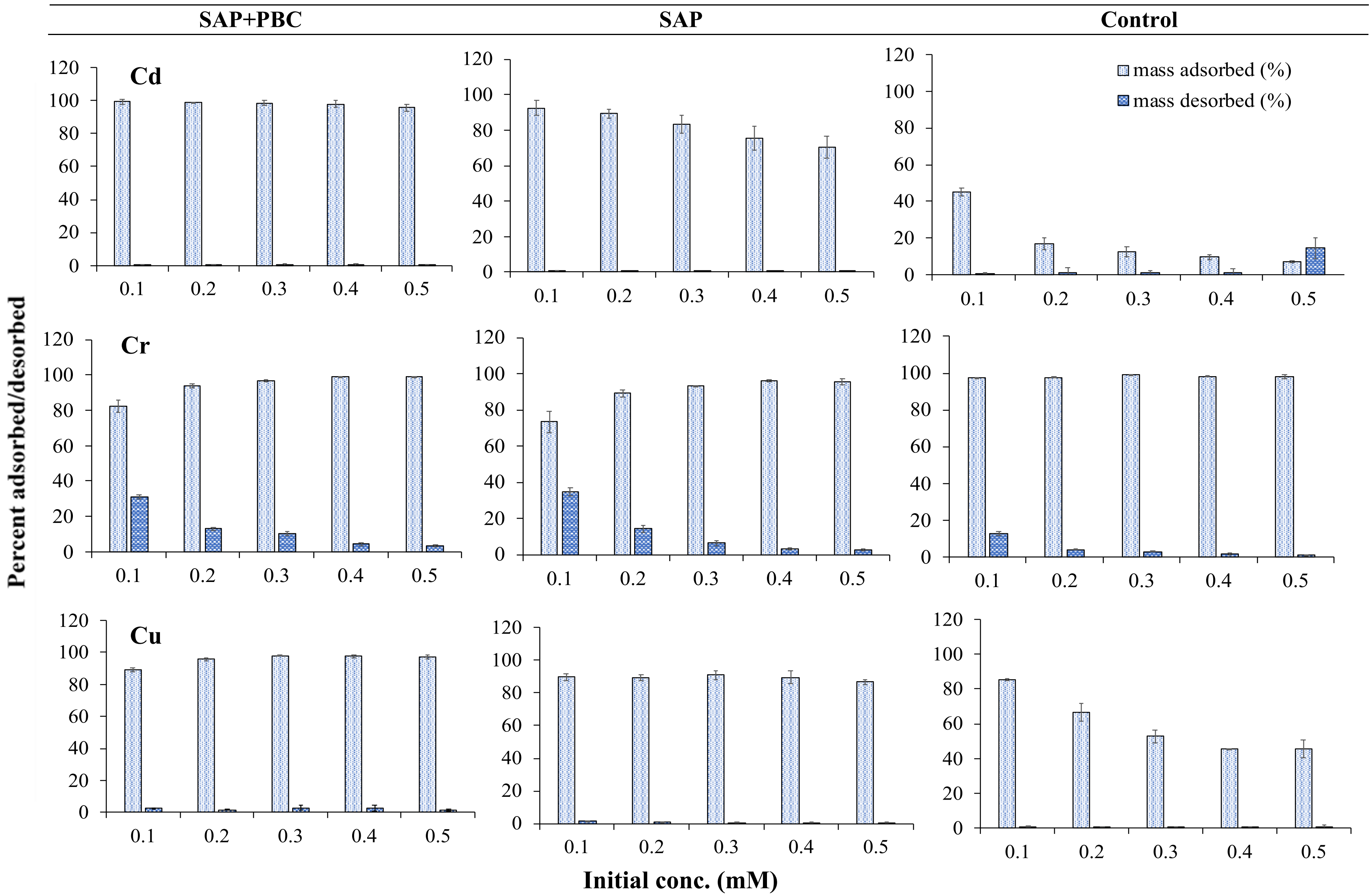


Figure 4.1 Sorption and desorption of heavy metal ions at different concentrations by treatments SAP+PBC, SAP and control *(cont'd)*.

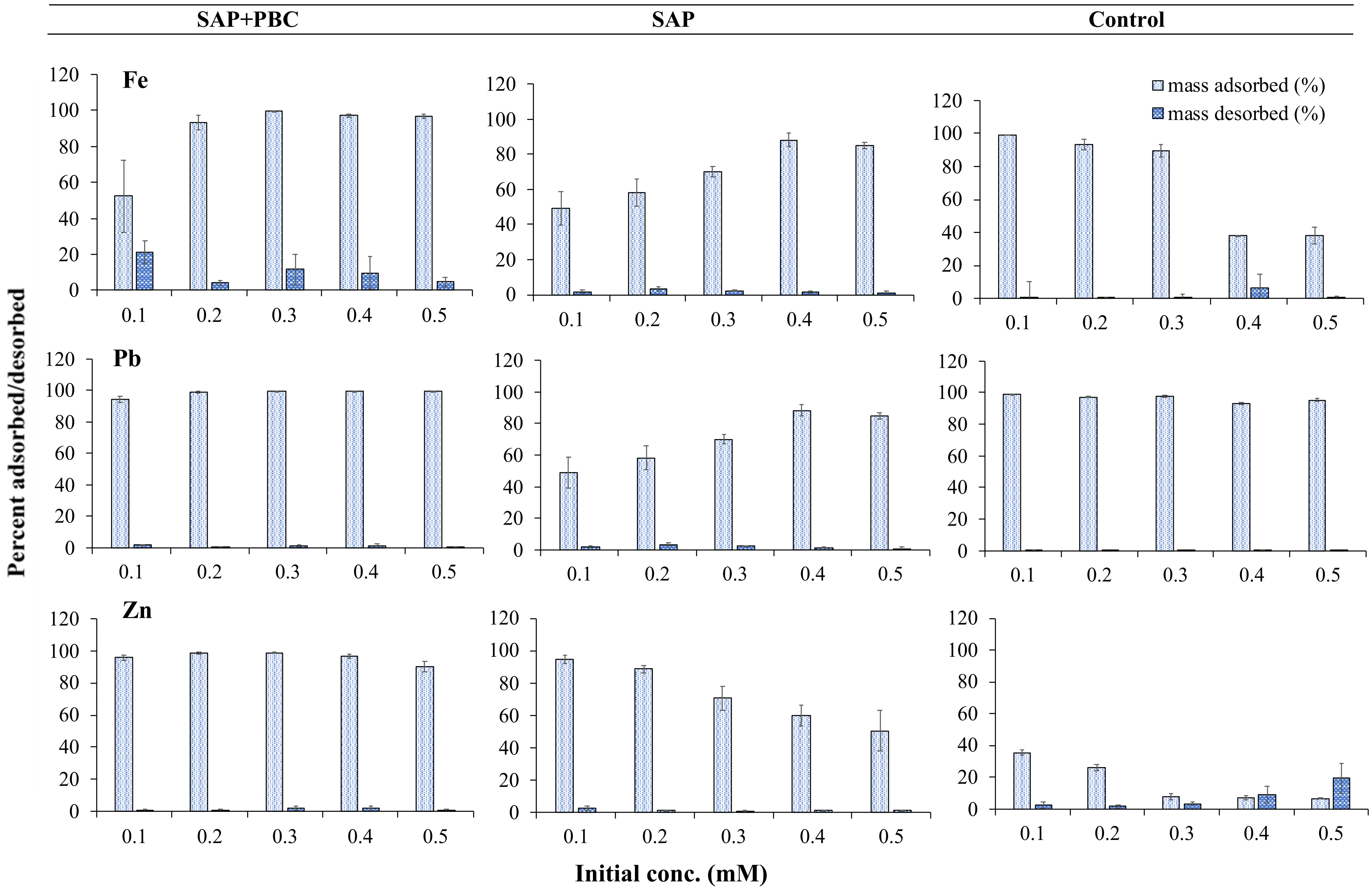


Figure 4.1 Sorption and desorption of heavy metal ions at different concentrations by treatments SAP+PBC, SAP and control.

Table 4.2 Summary of Freundlich isotherm constants for adsorption of heavy metal ions on soil amended with SAP+PBC and SAP.

Treatment	Cd		Cr		Cu		Fe		Pb		Zn	
	K_{if}	n	K_{if}	n	K_{if}	n	K_{if}	n	K_{if}	n	K_{if}	n
SAP+PBC	0.01550	1.01892	0.01031	0.89624	0.01210	0.94666	0.00469	0.72850	0.01271	0.96628	0.01521	1.02385
SAP	0.02140	1.20134	0.00854	0.85610	0.01372	1.01252	0.00367	0.72464	0.01295	0.97298	0.03200	1.64557

Units for Freundlich constant (K_{if}) is (mg g^{-1})(L mg^{-1})$^{1/n}$. Freundlich exponent n is dimensionless.

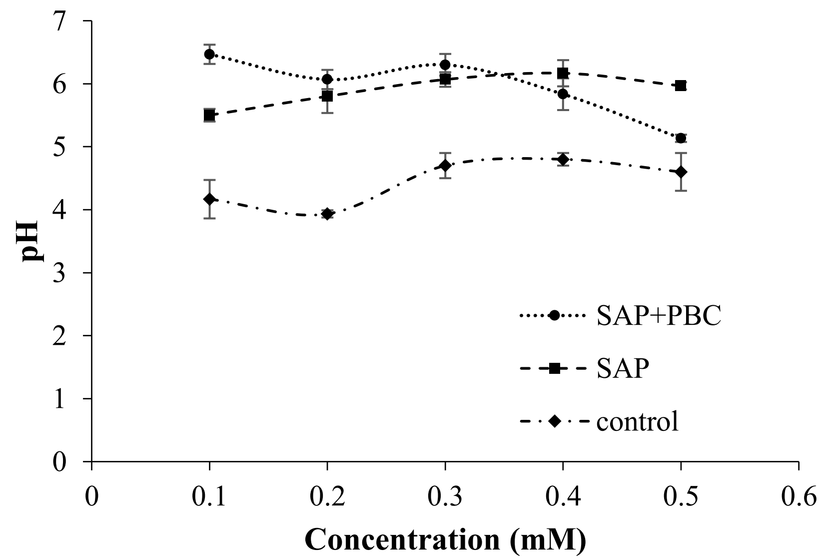


Figure 4.2 pH of multi-metal sorption solution at different concentrations for treatments SAP+PBC, SAP and control.

At pH range of 0-6, Cr(VI) usually exists as negatively charged hydrogen chromate ion ($HCrO_4^-$) and in this pH range, positive charges on an adsorbent surface can be induced via protonation which allows for electrostatic interaction between the anion and the sorbent (Shaikh and Kumar, 2017). Sorption increased, whereas desorption decreased with increasing initial concentration of metal solutions for all treatments in case of Cr(VI) metal. This can be due to various factors such as change in pH of the solution with increasing metal concentration (figure 4.2) as well as abundance of other metals in the solution. In a study conducted by Choppala et al. (2010), it was found that Cr(VI) adsorption was proportional to Fe and Al content in the soil system which can impart higher positive charges to the soil leading to increased sorption. With increasing concentration of the multi-metal solution, Fe concentrations also increased from 0.1 mM to 0.5 mM, which could explain better Cr sorption and lower desorption at higher concentrations. As the initial concentration of the multi-metal solution increases, more amount of metal cations is adsorbed on the sorbent neutralizing the negative charge present at the surface. This promotes sorption of anionic form of Cr, leading to increased sorption and reduced desorption (Anah and Astrini, 2017).

With increasing concentration of Fe in solution, proportions of Fe adsorbed by SAP+PBC and SAP treatments were found to increase in general; however, this trend was not observed for control. Treatment SAP+PBC was able to adsorb 52.46% and 96.89% of Fe from the 0.1 and 0.5 mM solutions, respectively, whereas for SAP, this range was observed to be 48.99% to 84.76%. In case of control, the percentage of Fe adsorbed by the non-amended soil decreased from 99.10% to 38.22% as concentration of the multi-metal solution increased from 0.1 to 0.5 mM. For SAP and SAP+PBC treatments, increase in sorption of Fe(II), with increasing solution concentration, can be attributed to the presence of acrylamide-based SAP in both treatments. Similar results were observed by Chauhan et al. (2008), who found that the percent adsorption of Fe(II) by acrylamide hydrogel increased with increase in ionic strength of the metal in solution. In case of control, the percentage of Fe adsorbed decreased with increasing initial solution concentration, which reflects decreasing availability of binding sites for the metal ion in the non-amended soil. For SAP+PBC, SAP and soil, desorption varied from 4.10%-21.39%, 0.93%-3.49% and 0.33%-6.52% of the amount of adsorbed metal, respectively, across solutions at different initial concentrations. Since desorption was found to be low in SAP and control, biochar may be the reason for relatively higher desorption of Fe in SAP+PBC treatment. One possible reason for the same could be high Fe content of the biochar (649.01 ± 58.81 mg kg^{-1}; table 4.1). All the treatments, including control, worked well with respect to Pb sorption, as more than 90% of the metal was sorbed and less than 2% of that was desorbed across all initial solution concentrations (figure 4.1). In case of Cu, both the treatments performed better than control. SAP+PBC and SAP treatments adsorbed 89.07%-98.01% and 86.61%-90.96% of the metal, respectively, whereas the non-amended soil was able to adsorb 45.46%-85.36% (figure 4.1). Again, this was due to the presence of the polymer and biochar in the treatments which provided extra binding sites for the metal, causing increased adsorption. As expected, a trend of decreasing adsorption percentage with increasing solution concentration was observed for control. Less than 3% of the adsorbed metal was desorbed in all the treatments, including control. Overall, treatments SAP+PBC and SAP performed better than the control with respect to sorption of Cd, Cu, Fe and Zn heavy metals. In case of Pb, all the treatments including control worked well, which can be attributed to its high potential to form strong complexes (Sposito, 2008). For Cr, control worked better than the treatments due to low pH of the sorption solution.

4.4.2 Lysimeter Soil pH and CEC

Soil pH has a direct effect on the mobility of heavy metal ions in soil-water systems. In a study it was shown that an increase in pH due to the addition of broiler litter derived biochar in soil increased the immobilisation of heavy metals Ni and Cd (Uchimiya et al., 2010). The immobilisation of metal ions was attributed to various mechanisms, including the formation of metal hydroxide, carbonate or phosphate precipitates resulting from an increase in pH. Soil solution pH affects metal ion speciation as well as surface charge density of carbonaceous material such as biochar (Sanchez-Polo and Rivera-Utrilla, 2002). Soil pH values for samples, collected from surface and 0.10 m depth two days after the fourth irrigation, are provided in table 4.3. At the surface, SAP+PBC treatment exhibited significantly higher pH value than that in control, whereas no significant difference was observed between SAP and control. Also, there was no significant difference between surface soil pH of SAP, control and FW treatments. This can be attributed to alkaline nature of the biochar (10.6 ± 0.10; table 4.1) used in SAP+PBC treatment. At 0.10 m depth, because of the application depth of amendments (top 0.10 m), no significant differences between soil pH was observed for all treatments.

Table 4.3 Soil pH for samples collected from surface and 0.10 m depth after last irrigation event.

Treatment	Depth	pH
SAP+PBC	**surface**	5.30 ± 0.78^{a}
SAP		4.73 ± 0.12^{ab}
Control		4.27 ± 0.55^{b}
FW		5.10 ± 0.10^{ab}
SAP+PBC	**0.10 m**	6.10 ± 0.53^{a}
SAP		5.33 ± 1.16^{a}
Control		5.57 ± 0.25^{a}
FW		5.67 ± 0.15^{a}

Values with different letters down the column are significantly different from each other (α=0.05) for each depth.

Cation exchange capacity (CEC) values are used to estimate heavy metal adsorption capability of soils (McBride et al., 1981). Plant heavy metal uptake is usually inversely related to CEC of the soil, as it reflects the capacity of soil to adsorb metal ions (John et al., 1972; Hinesly et al., 1982; Haghiri 1974; Liphadzi et al., 2005). This is because soils with low CEC have fewer binding sites to adsorb heavy metals, thus making them more available to plants compared to soils with higher CEC (Fergusson, 1990). CEC was estimated for soil samples collected two days after last irrigation from the surface and 0.10 m depth. Results for estimated values of CEC for the soil samples and pH of the CEC solutions (pH_{CEC}) are presented in table 4.4. CEC of surface soil for SAP+PBC treatment was significantly higher than that of control; however no significant difference was found between surface soil CEC values for SAP and control. Increase in CEC on surface of SAP+PBC treatment can be attributed to incorporation of biochar on top 0.10 m of soil (Liang et al., 2006). No significant differences were found between CEC of soil at 0.10 m depth of lysimeter for all treatments.

Table 4.4 Cation exchange capacity, base saturation and pH_{CEC} of soil samples collected after last irrigation event from surface and 0.10 m depth.

Treatment	Depth	CEC (cmol(+) kg^{-1})	pH_{CEC}
SAP+BC		10.71±0.18[a]	4.73±0.47[a]
SAP	**surface**	5.85±0.03[ab]	4.53±0.31[a]
Control		5.68±2.05[b]	4.27±0.12[a]
SAP+BC		9.90±2.47[a]	5.03±0.24[a]
SAP	**0.10 m**	10.34±5.43[a]	5.00±0.64[a]
Control		5.97±0.06[a]	4.80±0.28[a]

Values with different letters down the column are significantly different from each other (α=0.05) for each depth.

4.4.3 Heavy metals in soil

Concentration of heavy metals in surface soil samples ([*heavy metal*]) collected for all treatments and irrigation events are given in figure 4.3. All the heavy metals (Cd, Cr, Cu, Fe, Pb and Zn) were found in the samples collected from the surface during the experiment due to wastewater irrigation. Figure 4.4 represents [Fe] found in 0.10 m soil samples for all treatments. Except Fe, no heavy metal was detected in soil samples collected from 0.10 m depth for all treatments.

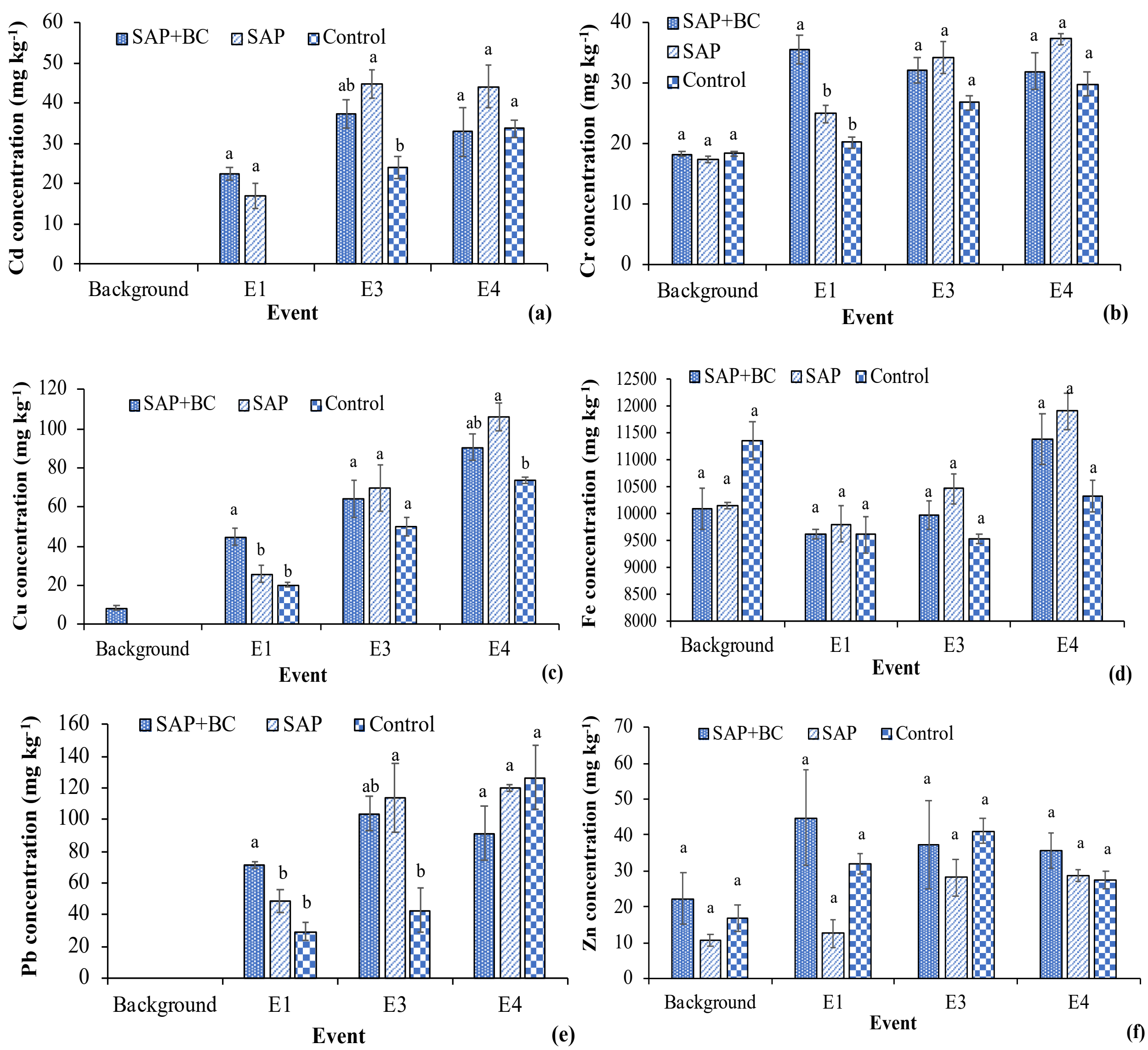
In case of Cd, a trend of accumulation can be observed, as [Cd] increases with subsequent irrigations (figure 4.3a). The heavy metal concentration for irrigation 3 and 4 were found to be significantly higher than that of background and event 1. Also, [Cd] was not detected in background samples, but was found in the two amended treatments after first irrigation, which led to the term ‘event’ have an overall significant effect in the repeated measures analysis (table 4.5). For all treatments, [Cd] was below the detection limit of 15.6 mg kg^{-1} for background surface soil samples. The concentration gradually increased with the application of wastewater irrigation. For the first irrigation, although [Cd] was below the detection limit in case of control, for SAP+PBC and SAP treatments it was found to be 22.45 and 16.91 mg kg^{-1}, respectively. It is likely that Cd would have distributed to a greater soil depth in the absence of amendments (in control). Theoretically, based on heavy metal input, the soil bulk density, LOD (15.6 mg kg^{-1}), and dimensions of the lysimeter, Cd can potentially travel through top 0.60 m of the soil profile without being detected, which is a very small distance. It is quite probable that after first irrigation, no heavy metal was detected in topsoil, since the heavy metal may have redistributed itself in topsoil below detection limit. Similar observation was also made by Dhiman et al. (2019), who conducted a similar lysimeter study with potato plants. For control, [Cd] increased to 24.13 and 33.61 mg kg^{-1} after 3rd and 4th irrigations, respectively. The corresponding concentrations for SAP+PBC were 37.44 and 32.84 mg kg^{-1}, and for SAP were 44.71 and 44.15 mg kg^{-1} respectively. Thus, the concentration significantly increased with subsequent wastewater irrigations, as confirmed from repeated measures analysis (table 4.5).

After 1st irrigation, the concentration of Cd was significantly higher for both SAP+PBC and SAP, as compared to control ($p<0.05$; figure 4.3a). The higher accumulation could be attributed to the presence of soil amendments; sorption study corroborates this assertion. There was no significant difference between the [Cd] for SAP+PBC and SAP. A similar trend was observed after 3rd irrigation (figure 4.3a). However, the concentration was relatively higher for SAP and SAP+PBC, as compared to control ($p<0.05$ and $p<0.10$ respectively). After 4th irrigation, the concentration continued to increase in control, but not in the amended treatments. This could be due to the heavy metal's competition with other metal ions for sorption sites, as well as saturation of the amended soil with Cd after third irrigation. The results hint at the SAP+PBC and SAP treatment's ability to quickly capture Cd from the start (event 1) as compared to control, where the heavy metal concentration increased relatively gradually. Consequently, no significant differences between [Cd] for the treatments were observed for event 4.

Average background concentration for Cr in surface soil for all the treatments was similar and ranged from 17.28 (SAP) to 18.27 mg kg^{-1}(control) (figure 4.3b). Trend of accumulation of the heavy metal was observed in case of control, where [Cr] increased from 18.27 to 20.12 mg kg^{-1} after first irrigation, and to 29.71 mg kg^{-1} after four irrigations. Similar trend was also observed for SAP treatment where [Cr] increased from background concentration of 17.28 mg kg^{-1} to 24.83

and 37.14 mg kg^{-1} after one and four irrigations, respectively. However, this trend was not observed for SAP+PBC treatment, for which [Cr] increased from 18.20 to 35.43 mg kg^{-1} after first irrigation but did not significantly change after subsequent irrigations. It can be said that SAP+PBC treated soil got saturated with the heavy metal after first irrigation because of the biochar's ability to quickly bind the heavy metal as compared to other treatments. Consequently, after the first irrigation, [Cr] was significantly ($p<0.05$) higher in SAP+PBC, as compared to other two treatments. After first irrigation, mean [Cr] in SAP treated soil was found to be higher than that of control, but the difference was not statistically significant. However, with subsequent irrigations, SAP treated soil exhibited noticeably higher [Cr] than control, after third and fourth irrigations ($p<0.10$). After third and fourth irrigations, mean [Cr] in SAP+PBC was numerically higher than that of control, but the difference was not statistically significant, possibly because of accumulation of the metal in control with wastewater irrigations and saturation of SAP+PBC amended soil after first irrigation, as mentioned earlier. No significant difference was observed between [Cr] for SAP and SAP+PBC treatments after third and fourth irrigations, which is in accordance with the findings of the sorption test.

In case of Cu metal, background concentration was found to be 8.38 mg kg^{-1} for SAP+PBC treatment and the metal was not detected for other two treatments (figure 4.3c). With subsequent irrigations, Cu was found to accumulate in topsoil, which is also in accordance with the results of repeated measures analysis (table 4.5). After first irrigation, [Cu] for SAP+PBC (44.60 mg kg^{-1}) was found to be significantly ($p<0.05$) higher than that of SAP (25.44 mg kg^{-1}) and control (20.22 mg kg^{-1}); however, this difference was not observed after third and fourth irrigations. This again reflects biochar's ability to rapidly bind the metal. No significant difference was observed in [Cu] between SAP and control treatments after first irrigation. Also, no significant differences were observed between all treatments after third irrigation. With further loading however, [Cu] in SAP significantly increased ($p<0.05$), compared to control, after four irrigations, whereas no significant difference was observed between SAP+PBC and SAP treatments. It is interesting to note that no significant difference was observed between SAP+PBC and control, in terms of [Cr] and [Cu], after fourth irrigation, whereas, SAP exhibited significantly higher concentration of the metals, compared to control. This is contrary to what is expected; addition of an extra sorbent material, biochar, along with SAP would increase metal retention in soil compared to SAP alone and control. This may be explained by binding of sorption sites in polymer by water soluble salts (Bowman et al., 1990; Horkay et al., 2000) originating from the ash content (27.97%; table 4.1) of biochar (Vassilev et al., 2013), thus reducing their availability towards heavy metals within the complex soil-water-plant environment. This can offset the added benefit of addition of biochar as a sorbent material.



E1, E2, E3 and E4 corresponds to irrigation events; soil samples were collected two days after each irrigation event. Different letters above the error bars indicate significant difference in concentrations for a given event (α=0.05).

Figure 4.3 Surface soil concentrations of heavy metals (a) Cd, (b) Cr, (c) Cu, (d) Fe, (e) Pb and (f) Zn, for all treatments and all irrigation events including the background.

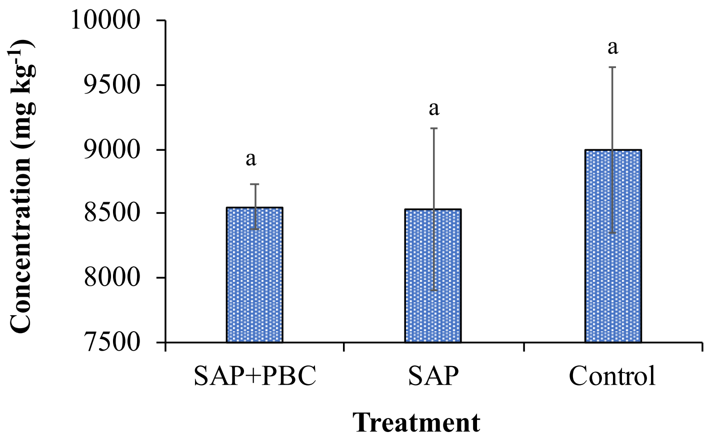
Table 4.5 Summary of fixed effects for soil heavy metal concentration from repeated measures analysis.

Fixed effects	Heavy Metal					
	Cd	Cr	Cu	Fe	Pb	Zn
Treatment	*	*	-	-	*	-
Event	*	*	*	*	*	*
Treatment x Event	-	*	-	*	-	*

** Denotes statistically significant effects (p<0.05)*

- Denotes no significant effect (p≥0.05).

Mean background [Fe] in surface soil were found to be greater than that after first wastewater irrigation for all treatments (figure 4.3d). This observation corroborates with a similar study performed by Dhiman et al. (2019), where reduction of [Fe] after first wastewater irrigation was attributed to redistribution of the metal due to disturbance in the soil caused by planting. No significant differences were observed between [Fe] values for all treatments and after first irrigation. However, mean [Fe] values were found to be numerically higher in SAP+PBC (9601 mg kg^{-1}) and SAP (9797 mg kg^{-1}) treatments, as compared to control (9595 mg kg^{-1}); this difference increased with subsequent irrigations (3^{rd} and 4^{th}). SAP treatment exhibited considerably higher [Fe] than control, after third and fourth irrigations ($p<0.10$). Even though no significant differences were observed between [Fe] in SAP+PBC and SAP treatments after third and fourth irrigations, [Fe] in the former treatment was also not significantly different than that in control. This can again be attributed to the binding of sorption sites in hydrogel by water soluble minerals in biochar ash, offsetting the overall sorption capacity in a complex soil-water-plant system, as observed in case of Cr and Cu metals. No significant difference was observed between [Fe] after fourth irrigation, in all the treatments at 0.10 m depth (figure 4.4). However, mean [Fe] in control (8992 mg kg^{-1}) was found to be numerically higher than that in the SAP+PBC (8551 mg kg^{-1}) and SAP (8532 mg kg^{-1}) treatments (figure 4.4), which is in accordance with the observed concentration of the metal in surface soil (figure 4.3d); since mean [Fe] at surface for control was found to be numerically lower than that of the other two treatments for all irrigation events, the metal must have leached down and caused an increase in [Fe] at 0.10 m depth.



Different letters above the error bars indicate significant difference in concentrations for a given event (α=0.05).

Figure 4.4 Iron concentrations in soil samples collected from 0.10 m depth, after last irrigation event, for all treatments.

Lead heavy metal was not detected in background surface soil samples for all treatments (figure 4.3e). However, with subsequent irrigations, the concentration of the metal in surface soils increased for all treatments, which is in accordance with the results of repeated measures analysis (table 4.5); the term 'event' had a significant effect ($p<0.05$) on response (metal concentration). Mean [Pb] in SAP+PBC after first, third and fourth irrigation events was observed as 71.51, 103.68 and 91.32 mg kg^{-1} respectively. Corresponding [Pb] for SAP treatment were found to be 48.43, 113.72 and 120.31 mg kg^{-1} respectively, and for control, these values were observed to be 29.18, 42.82 and 126.69 mg kg^{-1} respectively. In case of SAP+PBC and SAP treatments, considerable increases in [Pb] was observed after first and third irrigations (relative to previous events), whereas for control, a significant increase in [Pb] was also observed after fourth irrigation in addition to first and third events. No significant change in [Pb] was observed for the two treatments after fourth irrigation, as compared to the concentration observed after third irrigation event. This hints at ability of the amendments to bind and retain Pb metal relatively quickly, compared to control. After first irrigation, [Pb] in SAP+PBC treatment was significantly higher ($p<0.05$) than that of SAP treatment and control. Also, after first irrigation, [Pb] in SAP treatment was noticeably higher than that in control ($p<0.10$). After third irrigation, difference between [Pb] in SAP+PBC and SAP treatments was reduced and was not statistically different from each other, but [Pb] in SAP and SAP+PBC treatments were still considerably higher than that in control ($p<0.05$ and $p<0.10$

respectively). With further loading via wastewater irrigation, [Pb] increased for control and no significant differences were observed between all treatments.

In case of Zn, mean background concentrations in surface soil were found to be 22.28, 10.83 and 16.93 mg kg^{-1} for SAP+PBC, SAP and control, respectively; these were not significantly different from each other. No significant differences in [Zn] were observed between treatments for all events, which exhibits the ineffectiveness of the amendments in retaining the metal at topsoil compared to control. A gradual increase in [Zn] was observed with each irrigation for SAP and control; however, a small decrease was noticed for control after last irrigation, which could hint at leaching of the metal to further depth in the soil profile. For SAP+PBC amended soil, [Zn] increased from 22.28 mg kg^{-1} at background to 44.75 mg kg^{-1} after first irrigation and did not significantly change with subsequent irrigations. This also hints at biochar's ability to rapidly adsorb the metal after first irrigation. Despite this, the effect of biochar could not be established conclusively, because the differences in [Zn] between SAP+PBC and both SAP or control were not significant after first irrigation. In general, concentrations of the heavy metals in topsoil for this study was less than what was observed in a similar study conducted with potato plants the previous year (Dhiman et al., 2019). This can be due to several reasons. In the present study, the plants were only subjected to four wastewater irrigations at the rate of 4.00 L per irrigation, leading to input of lesser amounts of heavy metals to soil, as compared to eight irrigations at the rate of 11.50 L per irrigation in the previous study.

4.4.4 Heavy metals in plant tissue

Heavy metal concentration values in different parts of spinach plants for all treatments as well as for plants grown using freshwater are presented in table 4.6. Roots are crucial plant parts that come into direct contact with contaminants and regulate various physiological processes including transport of heavy metals to other parts of the plant (Nedelkoska and Doran, 2000). Thus, it is important to study metal concentrations in plant roots. Concentrations of heavy metals Cd, Cr, Cu, Fe, Pb and Zn in the roots of plants grown with freshwater (FW) were observed to be 0.81, 0.56, 8.63, 198.39, 0.98 and 49.27 mg kg^{-1} respectively. These values are comparable to the results of a different study, where the concentrations of Cu, Pb and Zn in roots of spinach plants grown in uncontaminated soil, were found to be 21.2, 0.7 and 67.2 mg kg^{-1} respectively (Intawongse and Dean, 2006). In separate studies, Cd and Cr contents in roots of spinach plants grown on uncontaminated soil was found to vary from 0.95 to 1.33 mg kg^{-1} (Chunilall et al., 2003) and 0.86

to 2.64 mg kg^{-1} (Srikanth and Reddy, 1991) respectively, which is also comparable to the results of this study.

Table 4.6 Concentration of heavy metals (mg kg^{-1}) in different parts of spinach plants.

Plant Part	Heavy Metal	Treatment			
		SAP+BC	SAP	WW	FW
Leaf - Harvest 1	Cd	17.75±13.78^{a}	5.05±1.46^{ab}	6.21±0.12^{ab}	1.34±0.11^{b}
	Cr	7.68±6.68^{a}	2.36±2.02^{a}	1.35±1.12^{a}	2.50±2.35^{a}
	Cu	21.99±15.08^{a}	9.13±2.95^{a}	12.05±3.62^{a}	9.05±1.48^{a}
	Fe	1674.51±17.81^{a}	417.73±440.88^{ab}	285.36±121.87^{b}	1043.26±1110.92^{ab}
	Pb	46.41±46.54^{a}	12.24±10.08^{a}	2.61±1.19^{a}	1.85±1.31^{a}
	Zn	110.01±10.08^{a}	112.39±69.87^{a}	133.36±42.13^{a}	79.91±13.91^{a}
Leaf - Harvest 2	Cd	12.50±5.02^{ab}	21.84±15.45^{a}	19.96±8.80^{a}	1.80±0.30^{b}
	Cr	3.85±4.24^{ab}	3.01±0.13^{ab}	7.00±3.03^{a}	1.46±0.69^{b}
	Cu	21.96±11.65^{ab}	18.82±0.32^{ab}	33.89±11.97^{a}	14.17±1.08^{b}
	Fe	381.03±170.23^{a}	715.96±362.37^{a}	798.58±266.05^{a}	322.80±61.20^{a}
	Pb	29.31±35.42^{a}	20.20±0.18^{a}	51.80±33.18^{a}	2.28±0.70^{a}
	Zn	83.73±6.92^{a}	89.45±55.11^{a}	116.55±25.48^{a}	103.39±61.55^{a}
Root	Cd	12.57±12.54^{a}	10.21±3.62^{a}	7.85±0.86^{a}	0.81±0.57^{a}
	Cr	2.68±2.26^{a}	3.07±2.89^{a}	2.45±0.89^{a}	0.56±0.02^{a}
	Cu	14.97±0.29^{ab}	18.64±5.87^{a}	20.76±3.82^{a}	8.63±0.74^{b}
	Fe	285.60±153.92^{a}	337.46±2.99^{a}	343.02±197.63^{a}	198.39±52.95^{a}
	Pb	12.24±11.47^{ab}	5.42±0.53^{ab}	14.68±3.45^{a}	0.98±0.30^{b}
	Zn	85.91±38.49^{a}	34.53±1.48^{ab}	29.79±3.76^{b}	49.27±37.17^{ab}
Stem	Cd	8.23±0.58^{a}	8.84±2.75^{a}	9.42±0.08^{a}	1.81±0.07^{b}
	Cr	2.20±0.06^{a}	1.09±0.89^{ab}	1.84±0.56^{a}	0.58±0.05^{b}
	Cu	7.10±2.16^{b}	6.82±1.61^{bc}	10.85±0.42^{a}	4.26±0.13^{c}
	Fe	148.01±4.10^{b}	138.80±14.92^{b}	211.13±42.63^{a}	77.14±8.49^{c}
	Pb	5.83±6.00^{ab}	5.23±5.46^{ab}	12.06±0.12^{a}	0.38±0.09^{b}
	Zn	108.97±55.79^{a}	80.19±42.47^{a}	83.72±18.50^{a}	82.96±23.50^{a}

Different superscript letters for each heavy metal and plant part indicate significant differences amongst treatments (α=0.05).

No significant difference was observed in concentrations of Cd, Cr and Fe in spinach roots of plants grown using wastewater as compared to plants grown using freshwater (FW). Also, concentrations of these metals in roots of plants from all the wastewater irrigated treatments were similar. This could be due to minimal addition of heavy metals in soil due to four wastewater irrigations. However, based on the sorption test results, it may be stated that after further loading the soil with metals via wastewater irrigation, especially in case of Cd, SAP+PBC and SAP treatments may exhibit reduction in plant uptake as compared to control (figure 4.1). In case of Cu and Pb, concentrations of both the metals were numerically lower in the plant roots from SAP+PBC and SAP treatments as compared to control, but the differences were not significant statistically. Root concentrations of Cu and Pb in SAP+PBC treatment were not found to be significantly different from concentrations of the metals in FW treatment. Also, Pb concentration in SAP treatment was not significantly different from that of FW treatment. On the other hand, control treatment exhibited significantly ($p<0.05$) higher concentrations of Cu and Pb in plant roots compared to FW treatment. Thus, it could be argued that SAP+PBC soil amendment prevented increase in uptake of Pb and Cu, whereas SAP amendment prevented increased uptake of Pb by the plant roots. The finding also corroborates with the result of the sorption test where it was observed that SAP+PBC and SAP treatment adsorbed greater amount of Cu and Pb (figure 4.1). No significant differences in root content of Zn were observed between the wastewater irrigated treatments and FW.

Concentrations of Cd and Fe were significantly ($p<0.05$) higher in stems of the plants grown with wastewater irrigation as compared to FW treatment (table 4.6). Amounts of these metals in roots of the plant were not significantly different, which signifies higher translocation of the metals from roots to other parts of the plant grown under wastewater irrigation. Amount of Zn heavy metal in the stem was similar in all the treatments (including FW), which is also in accordance with Zn content in soil (figure 4.3) and roots of the plant. No significant differences were observed between SAP and FW treatments for Cr and Cu contents of plant stem, whereas concentrations of these metals were found to be higher ($p<0.05$) in SAP+PBC and control treatments as compared to FW treatment. This shows that addition of SAP in soil prevented increased uptake of Cr and Cu in stem of spinach plant from wastewater irrigated soil; sorption test (figure 4.1) substantiates the effect of SAP. Concentration of Pb in plant stem for control was significantly higher ($p<0.05$) than that of FW treatment; however, the metal concentrations in SAP+PBC and SAP were similar to that of FW. This shows the potential of both the amendments in reducing Pb uptake by spinach plants grown on wastewater irrigated soil.

Amounts of heavy metals in edible parts of spinach (leaves) are of utmost importance as these parts can introduce contaminants to the human body and can cause wide range of health problems. Permissible levels of Cd, Cr, Cu, Pb and Zn for leafy vegetables such as spinach are 0.20, 0.50, 40.00, 0.30 and 60.00 mg kg^{-1} respectively (CODEX, 1984 and 1995; NHFPC, 2012).

Concentrations of Cd, Cr, Pb and Zn in leaves of spinach plants grown with freshwater irrigation, from both the harvests exceeded the permissible limits (table 4.6), which is of concern. It is possible that FW treatment plants picked up the metals form the soil in which they were grown. The concentrations of Cr and Zn in soil before start of the experiment were in the range of 17.28-18.27 mg kg^{-1} 10.83-22.28 mg kg^{-1} respectively (figures 4.3b and 4.3f), whereas Cd and Pb metals were not detected in the soil (figures 4.3a and 4.3e). Even though Cd and Pb were not detected, they could be present in the soil below their detection limit (15.60 mg kg^{-1}) and can be taken up by the plants. Similar results have been observed in other studies. For instance, concentration of Pb and Zn in leaves of spinach plant grown with freshwater irrigation on uncontaminated soils was found to be 3.12 mg kg^{-1} (Chunilall et al., 2003) and 70.20 mg kg^{-1} (Intawongse and Dean, 2006) respectively, which is also above the recommended levels. Levels of Cu in leaves were found to be below the permissible limit for all the treatments including FW treatment. Iron is also considered a micronutrient, and spinach is a good source of Fe for human consumption. Fe in second spinach harvest from FW was observed 322.80 mg kg^{-1}; this was comparable to the concentration in the range of 200-250 mg kg^{-1} reported by Arora et al. (2008) for spinach grown with freshwater.

In leaf samples from the first harvest, no significant differences were observed in concentrations of heavy metals Cr, Cu, Pb and Zn among all the treatments including FW treatment. Similar observation was made in case of Fe, Pb and Zn metal content in leaves from the second harvest. No significant differences were observed between Cd concentration in leaves from first harvest for SAP+PBC, SAP and control treatments; however, treatment SAP+PBC exhibited significantly ($p<0.05$) higher Cd concentration as compared to FW treatment. For second harvest, Cd concentration in spinach leaves from plants grown on SAP+PBC amended soil was not significantly different than that of FW treatment. Whereas, SAP treatment and control exhibited higher ($p<0.05$) Cd levels in leaves, as compared to FW treatment. This could be due to activation of biochar in soil with time; activation transforms biochar into an efficient adsorbent, which can reduce plant uptake of contaminants such as heavy metals. Fresh biochar added to the soil is thermodynamically unstable initially (Macias and Arbestain, 2010), and complex processes like redox reactions, hydration, hydrolysis, carbonation and decarbonation weather the biochar and alter its properties over time (Joseph et al., 2010) as well as lead the deprotonated organic acids to form complexes with metals present in the soil (Violante and Gianfreda, 2000). The concentrations of all heavy metals in leaves from SAP+PBC treatment was numerically lower in second harvest as compared to the first, which corroborates activation of biochar with time. All the treatments under wastewater irrigation had Fe content which, for the first harvest, was not significantly different from that of the FW treatment; however, SAP+PBC treatment exhibited significantly higher concentration of the metal than control, which may be because of Fe content of the biochar itself (649.01 mg kg^{-1}; table 4.1); this is in accordance with the findings of the sorption test. Concentrations of Cr and Cu in spinach leaves from second harvest were found to be significantly ($p<0.05$) higher in control as compared to FW; however, concentrations of these metals for SAP+PBC and SAP were not found to differ significantly than FW. This again shows ability of the amendments to limit the uptake of heavy metals by wastewater irrigated spinach plants.

4.5 Conclusions

Sorption test indicated that SAP+PBC and SAP treated soils were better sorbents for Cd, Cu and Zn, as compared to non-amended control. On the other hand, control showed higher sorption of Cr than SAP+PBC and SAP. With increasing ionic strength of the multi-metal solution, both treatments were found to adsorb increasing proportions of Fe, whereas the opposite trend was observed for control. At higher Fe concentrations, although both SAP+PBC and SAP treatments showed higher sorption than control, desorption was the least in case of SAP.

Due to biochar's alkalinity and ability to facilitate cation exchange in soil, SAP+PBC treatment exhibited significantly higher ($p<0.05$) pH and CEC for surface soil, as compared to control, at the end of the experiment. Owing to application depth of the amendments, no substantial differences were observed between treatments at 0.10 m depth. Treatments, SAP+PBC and SAP, were able to quickly capture Cd and Pb heavy metals from the start (event 1), as compared to control, where the heavy metal concentration increased relatively gradually with subsequent irrigations. Due to presence of biochar, SAP+PBC treatments exhibited higher ($p<0.05$) concentrations of Cr and Cu metals after first irrigation, as compared to the other two treatments. After the first irrigation, SAP treatment also exhibited higher Pb concentration, as compared to control ($p<0.10$). However, the differences decreased with subsequent irrigations and, at the end of the experiment, only SAP was able to retain higher amounts of Cr ($p<0.10$) and Cu ($p<0.05$) in topsoil, as compared to control. At the end of the experiment, surface Pb concentration was found to be similar for all treatments. No significant differences were observed for topsoil Zn concentration between all treatments and for all events. Except Fe, none of the heavy metals were detected at 0.10 m depth below the surface. No significant differences were observed between treatments for Fe concentration in soil at 0.10 m depth. After third and fourth irrigations, surface soil Fe concentrations were considerably higher in SAP treatment, as compared to control ($p<0.10$). At the end of the experiment, no significant differences were observed between surface heavy metal concentrations for SAP+PBC treatment and control for all metals, possibly because of binding of sorption sites in polymer by water soluble salts originating from the ash content of biochar, thus reducing their availability for heavy metals and offsetting overall sorption capacity of SAP+PBC treated soil in a complex soil-water-plant system.

No significant increase was observed in concentrations of Cd, Cr and Fe in spinach roots from plants grown using wastewater, as compared to plants grown using freshwater (FW). Despite higher Cu and Pb concentration in soil, SAP+PBC did not allow its increased uptake by plant roots, as compared to control; treatment SAP also prevented increased uptake of Pb in roots, as compared to control. Addition of SAP in soil also prevented increased uptake of Cr in the stem of spinach plant due to irrigation with contaminated wastewater, as compared to control. For SAP+PBC and SAP treatments, the Cu concentration in plant stem was found to be significantly ($p<0.05$) less than that of control. Concentration of Pb in plant stem was found to be similar for SAP+PBC, SAP and FW treatments; however, it was found to be significantly higher in control ($p<0.05$), as compared to FW treatment. This highlights the potential of the amendments to avoid Pb uptake by wastewater irrigated spinach plants.

Levels of Cu in spinach leaves were found to be below the permissible limit for all the treatments. In leaf samples from first harvest, no significant differences in concentrations of heavy metals Cr, Cu, Pb and Zn were observed among all the treatments including FW treatment. The same was observed for Fe, Pb and Zn metals for second harvest. The concentrations of all heavy metals in leaves from SAP+PBC treatment were numerically lower in second harvest, as compared to the first, possibly due to activation of biochar in soil with time. Also, SAP+PBC treatment was able to avoid increased uptake of Cd in leaves from the second harvest due to wastewater irrigation, as compared to control. Concentrations of Cr and Cu in spinach leaves from second harvest were found to be significantly ($p<0.05$) higher in control treatment, as compared to FW treatment; however, the concentrations in SAP+PBC and SAP treatments were similar to those in FW treatment. Thus, the study highlighted the ability of the amendments to reduce Cr and Cu uptake, in addition to Pb, by spinach plants grown using wastewater irrigation.

4.6 Acknowledgment

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Connecting Text to Chapter 5

In chapter 3, role of polyacrylamide super absorbent polymer (SAP) and SAP-plantain peel biochar soil amendment on heavy metal mobility in soil and uptake by wastewater irrigated potato tubers grown on sandy soil, was investigated. However, hydrogel amendments were mixed at a depth of 0.15-0.25 m below the soil surface to protect the polymer against photodegradation. As a result, SAP's effect on heavy metal immobilization was not witnessed because none of the metals, except iron, were detected below 0.10 m depth for all treatments, including control. Therefore, in the study presented in chapter 5, used SAP was removed from the lysimeters and fresh SAP was mixed in top 0.10 m of the soil profile for both amendments (SAP and SAP+GBC); whereas, no new biochar was added for the planned study. The soil was already contaminated from the previous year's study which was performed on the same lysimeters. Chapter 5 provides an insight into the role of SAP and SAP+GBC soil amendments on heavy metal immobilization and uptake by wastewater irrigated potato plants grown on contaminated sandy soil.

This chapter will soon be sent for publication in a refereed journal. The manuscript will be co-authored by Prof. Shiv Prasher (academic research supervisor), Dr. Eman ElSayed (Postdoctoral Fellow at McGill University's Bioresource Engineering Department at the time conducting research), Dr. Christopher Nzediegwu (Postdoctoral Fellow at University of Alberta's Department of Renewable Resources). Mr. Ali Mawof (PhD scholar at McGill University's Bioresource Engineering Department) and Dr. Ramanbhai Patel (Research Associate at McGill University's Bioresource Engineering Department). The original draft has been modified to maintain consistency with format of this thesis, in accordance with McGill University's thesis guidelines. Studies and references cited are presented at the end under the '*References*' section.

Chapter 5: Heavy Metal Uptake by Wastewater Irrigated Potato Plants Grown on Contaminated Soil Amended with Hydrogel and Biochar

5.1 Abstract

Heavy metal uptake by food crops and its potential for groundwater contamination are of major concern in areas where untreated wastewater is used for irrigation. To minimize heavy metal uptake by wastewater irrigated food crops and to minimize its transport to deeper soil layers, use of polyacrylamide super absorbent polymer (SAP) and SAP-gasified plantain peel biochar mix (SAP+GBC) as soil amendments is proposed in this study. Sorption test was conducted to determine ability of the treatments to adsorb heavy metals (Cd, Cr, Cu, Fe, Pb and Zn). The field experiment was conducted by growing synthetic wastewater irrigated potato plants (*Solanum tuberosum* L.) in lysimeters packed with sandy soil. Lysimeters employed in this study were used in performing a similar study during the previous year, and thus were contaminated with heavy metals. For SAP+GBC treatment, GBC was mixed in the top 0.10 m of soil [biochar:soil 1% (w/w)] during the previous year's experiment, whereas SAP was mixed in soil layer spanning from 0.15-0.25 m below the surface for both the amended treatments. For the present study, SAP used in the previous year's experiment was carefully replaced with fresh SAP, that was mixed in the top 0.10 m of soil at the rate of 1% (w/w). Irrigation was carried eight times, at an interval of 10 days, based on the crop water requirement. Non-amended freshwater irrigated lysimeters were also included in the study for comparison. After every irrigation, soil samples were collected from different depths for heavy metal analysis. Upon maturity, potato tubers, plant root, stem, leaf, tuber flesh and tuber peel tissues were sampled separately for heavy metal analysis. Soil samples, collected at the end of the experiment, were also subjected to pH and CEC analysis. Sorption test results suggest that the treatments performed well in sorbing heavy metals. Addition of SAP+GBC amendment in soil led to noticeable ($p<0.10$) increase in surface soil pH, as well as in CEC of soil at surface and at 0.10 m depth ($p<0.05$). Compared to control, treatment SAP+GBC was able to retain significantly higher amount of Cd, Cr and Fe in topsoil ($p<0.05$), whereas, SAP treatment retained significantly higher amount of Cd, Cu, Fe and Zn in topsoil ($p<0.05$). SAP treatment performed better than SAP+GBC in case of Cu, Fe and Zn heavy metals. Higher levels of metals were found in potato peels, as compared to tuber flesh tissue, for all the treatments. Both the treatments were able to significantly reduce Cd uptake in tuber flesh and peel tissue, as compared to control ($p<0.10$). SAP+GBC treatment was also able to significantly ($p<0.05$) reduce Cr uptake

in tuber flesh tissue, whereas both the treatments significantly ($p<0.05$) reduced Cr concentration in tuber peels, as compared to control.

5.2 Introduction

Agricultural practices utilize 70% of the total water withdrawn from freshwater resources (FAO, 2016; UNESCO, 2016; Koehler, 2008), which makes it the largest consumer of freshwater. Use of wastewater for irrigation can thus help in minimizing freshwater use for agriculture. Use of wastewater for irrigation is proposed and highly encouraged by many researchers to tackle the problem of freshwater scarcity (Rusan et al., 2007; Al-Rashed and Sherif, 2000; Mohammad and Mazareh, 2003; Al-Salem, 1996). Apart from being a cheaper alternative for irrigation in countries experiencing water stress (Rusan et al., 2007; Qadir et al., 2010), wastewater is also a source of many nutrients and organic matter required by soil to maintain its fertility (Weber et al., 1996). Wastewater is also intentionally used for irrigation as it is a source of nutrients to the plants and, in some regions of the world, it is cheaper than other water sources (Keraita et al., 2003; Scott et al., 2010). Wastewater irrigation is practiced on about 10% of the total irrigated surface area worldwide (Jiminez, 2006). With growing urban population, especially in developing nations, more and more freshwater is being diverted for industrial and commercial use, owing to higher demand. This, in turn, leads to increase in wastewater generation (Lazarova and Bahri, 2004; Asano et al., 2007). Due to increased wastewater production around the world, safe wastewater disposal in environment is also of major concern. As a common practice, wastewater is discharged openly into water bodies, leading to pollution especially in developing countries (Qadir et al., 2010). Due to lack of financial and technical resources, many developing countries might face challenges in setting up wastewater collection and treatment systems in the near future (IWMI, 2006; WHO, 2006). Use of untreated wastewater for agriculture can thus contribute towards preserving our freshwater reserves as well as tackle the problem of wastewater disposal.

However, contaminants present in untreated wastewater can harm human as well as the environment (Qadir et al., 2007). Depending on the source, wastewater may contain a wide variety of contaminants, ranging from organic contaminants such as antibiotics, sex hormones (Kolpin et al., 2002) and pesticides (Fernandez et al., 2001), to inorganic contaminants such as heavy metals (Khan et al., 2008). Heavy metals are not prone to microbial or chemical degradation, which makes them accumulate in soil and contaminate groundwaters (Kirpichtchikova et al., 2006). Wastewater irrigation may not only lead to accumulation of metals in soil, but also can result in excessive uptake of the contaminants by crops, affecting food quality, safety and thus human health (Muchuweti et al., 2006; Dhiman et al., 2019). Food chain contamination is one of the major pathways by which heavy metals can enter human body (Khan et al., 2008). Heavy metals can also leach to shallow groundwater reserves and may lead to drinking water contamination for humans and animals (Alloway, 1990; Santona et al., 2006; Hashim et al., 2011; Al-Subu et al., 2003). Human consumption of food and water, contaminated with heavy metals, may result in harmful impacts, symptoms of which may only be apparent after several years of exposure (Bahemuka and Mubofu, 1999; Ikeda et al., 2000). Intake of heavy metals have also been associated with reduced immune function, growth retardation, upper gastrointestinal cancer, disabilities related to malnutrition, and impaired psycho-social faculties (Iyengar and Nair, 2000; Türkdoğan et al., 2003). Therefore, it is required to develop a cost-effective solution which can reduce heavy metal mobility in soil as well as their uptake by crops utilizing wastewater for irrigation.

In previous studies it was shown that amending soils with hydrogels or super absorbent polymers (SAP) and SAP-biochar mixture can reduce heavy metal mobility and uptake by potatoes grown with wastewater irrigation (Dhiman et al., 2019). Hydrogels or SAPs are network of loosely crosslinked hydrophilic polymer chains which can absorb and retain aqueous solutions, up to hundreds of times their own weight (Buchholz and Graham, 1998; Skouri et al., 1995; Zohuriaan-Mehr and Kabiri, 2008), and thus they are used in agriculture for water conservation. Most commonly-used hydrogels in agriculture are polyacrylamide and polyacrylate SAPs (Bai et al., 2010), and due to high density of metal chelating groups, these SAPs are well-suited to stabilize heavy metals in soil and reduce their bioavailability (Torres and Varennes, 1998; Varennes and Torres, 1999; Varennes and Queda, 2005). Published studies on toxicity of acrylate-based superabsorbent polymers consider these materials to be environmentally compatible (McGrath et al., 1993; Haselbach et al., 2000a; Haselbach et al., 2000b; Hamilton et al., 1995; Garay-Jimenez et al., 2008). In two separate studies, including growing potatoes (Dhiman et al., 2019) and cherry tomatoes (Suresh et al., 2018) on polyacrylamide SAP-amended soils (1% and 0.1-0.5% w/w respectively), no acrylamide content was observed in edible parts of the plants. Biochar is also widely used in agriculture, as it has potential to increase crop yields (Zhang et al., 2012; Major et al., 2010; Steiner et al., 2008; Park et al., 2011) as well as improve soil properties (Sohi et al., 2010; Gaskin et al., 2007; Lehmann 2007). Biochar is defined as a product of pyrolysis, carbonization and gasification of biomass (ANSI/ASABE, 2011). It has been shown that biochar amended soils also have the potential to stabilize heavy metals and reduce their bioavailability for plants (Lu et al., 2012; Nzediegwu et al., 2019).

This study was carried out to study the effect of amending soil with SAP and SAP-gasified plantain peel biochar mix (SAP+GBC) on mobility of heavy metals (Cd, Cr, Cu, Fe, Pb and Zn) and their uptake by wastewater irrigated potato plants, grown in a contaminated soil (from previous year's experiment). In previous experiment (Dhiman et al., 2019), SAP was applied at a depth of 0.15-0.25 m below the surface for both the treatments (SAP and SAP+GBC). In this study, potato plants were grown in soils, contaminated from previous year's wastewater irrigation, and with hydrogel applied in top 0.10 m of soil. Hydrogel used in this study was polyacrylamide SAP, since it is one of the most commonly used SAPs in agricultural applications (Zohuriaan-Mehr and Kabiri, 2008; Bai et al., 2010). Biochar used in this study was produced from plantain peels, which are considered a waste and can lead to disposal problems in the environment (Ogunjobi and Lajide, 2013), especially in developing countries like Nigeria which is one of the largest plantain producers in the world (FAO, 2004). Plantain is a staple food in many Asian, African and South American countries, and converting plantain peels to biochar can be seen as a value-addition process. Potatoes were chosen for this study as they are the third most important food crop (Visser et al., 2009) and the most important, as well as a widely consumed non-grain food crop (PGSC, 2011). Investigation on the effectiveness of SAP and SAP-biochar mix as soil amendments for controlling the heavy metal mobility and uptake by potatoes from wastewater irrigated contaminated soil is important.

5.3 Materials and Methods

5.3.1 Adsorbent material

A cross-linked copolymer of potassium acrylate and acrylamide (commercial name: SUPERAB A200) hydrogel/SAP, used in this study was procured from a Canadian environmental solutions company, *Iramont Inc*. Biochar used in this study was produced via gasification of oven-dried plantain peels using a gasifier unit (built at *Macdonald Campus Technical Service Building of McGill University*, Sainte-Anne-de-Bellevue, Quebec, Canada). The production process of gasified plantain peel biochar (GBC) as well as physiochemical properties of SAP and biochar used in this study are provided elsewhere (Dhiman et al., 2019).

5.3.2 Sorption experiment

Laboratory sorption and desorption tests were carried out to find out if SAP and SAP-gasified biochar amendments used in this study can improve soil's heavy metal retention properties. Samples for treatments, SAP+GBC, SAP and soil (control), were prepared in by mixing the amendments in soil at the rate of 1% (w/w). Details of the laboratory experiment are provided elsewhere (Dhiman et al., 2019). In summary, five different concentration solutions (0.1, 0.2, 0.3, 0.4 and 0.5 mM) of multi-metal solutions (Cd, Cr, Cu, Fe, Pb and Zn) were used along with two grams of each sample (SAP+GBC, SAP and control) in triplicate. Samples were introduced to 30 mL of each solution in centrifuge tubes and were shaken for 24 hours at room temperature, before collecting the supernatant for heavy metal analysis on inductive coupled plasma employing optical emission spectrometry (ICP-OES) equipment (Vista-MPX CCD Simultaneous, Varian, CA, USA). Electrode pH meter (Accumet pH meter model AB15, Fisher Scientific, USA) was used to estimate pH of the supernatant. For desorption, 30 mL of deionized water was mixed with the used adsorbent collected from sorption experiment in centrifuge tubes. Process of mixing, shaking, collecting supernatant and analyzing it on ICP equipment was repeated to determine the percentage of adsorbed metals desorbed in solution. Sorption behaviour of the treatments was evaluated by fitting the data using Langmuir (Langmuir, 1916) and Freundlich (Freundlich, 1906) adsorption isotherm models.

5.3.3 Field study

The study was carried out in field-lysimeters situated at Macdonald Campus of McGill University in *Ste. Anne de Bellevue*, QC, Canada. Lysimeter used in this study was a hollow cylinder (1.00 m long x 0.45 m I.D.) made of from PVC material, and filled with sandy soil. It had four equidistant holes (10 mm dia.), each at three different heights viz. 0.10, 0.30 and 0.60 m from the soil surface, to facilitate soil sampling along the depth of the column. An outlet was provided at the bottom to collect leachate. This field study was extension of the experiment conducted during the previous year using the same lysimeters (Dhiman et al., 2019). In the first year, SAP was mixed into the lysimeter soil layer spanning 0.15 m to 0.25 m below the soil surface at a rate of 1% (w/w) for SAP and SAP+GBC treatments. For the SAP+GBC treatment, GBC was mixed into the top 0.10 m of soil [biochar:soil 1% (w/w)]. SAP was incorporated below the soil surface to prevent its photo-degradation. Treatments were randomly assigned to lysimeters, replicated thrice. Potato plants were grown in the designated lysimeters for the first year and irrigated with laboratory-prepared synthetic wastewater. For comparison purpose, three lysimeters were randomly chosen to grow potato plants using freshwater (FW) on non-amended soil. A post-planting pre-emergence spray of the broadleaf and grass weed herbicide, SENCOR 480F (Bayer CropScience, ai: metribuzin, 480 g L^{-1}), was applied at the recommended rate (850 mL mixed in 100L ha^{-1}) for weed control in lysimeters (Hutchison, 2012). Fertilizers (muriate of potash and ammonium sulphate) were applied at recommended rates (Stark et al., 2004). The field study was conducted under a water proof tent to prevent entry of any rainwater, allowing only a known volume of irrigation water to be used at a predetermined schedule.

The objective of the first-year study was to determine the effect of treatments on heavy metal mobility in soil and it's uptake by wastewater irrigated potato plants. No heavy metal except Fe was detected below 0.10 m depth of the soil profile after eight wastewater irrigations (11.5 L per lysimeter, per irrigation event). Thus, effect of hydrogel/SAP amendment on mobility of heavy metals in soil could not be ascertained. Therefore, in the present study, fresh SAP was mixed in the top 0.10 m of soil at the rate of 1% (w/w), for SAP and SAP+GBC treatments, one day before planting potato tubers. To prevent influence of the left-over SAP, soil from 0.15-0.25 m depth was carefully replaced with fresh soil (from same source) in all lysimeters. The 12 randomly assigned lysimeters, which were used for the previous year's study (for treatments SAP+GBC, SAP, control and FW; in triplicate), were used with same assignment of treatments for this study as well. All lysimeters were brought to field capacity, one day before planting. Russet Burbank potato tubers were obtained from Global Agri. Services Inc. (New Brunswick, Canada). A single tuber was planted in the center of each lysimeter at a depth of 0.10 m, with sprouts facing upward (Thompson-Morgan, 2015). Fertilizers were applied at the same rate as in the first-year study; in each lysimeter, 7.4 g Muriate of potash (0-0-60) was applied at the surface in a single application on the day of planting, while 23.8 g of ammonium sulphate (21-0-0) was applied in three splits (2:1:1), on the day of planting, the 52nd day after planting and the 62^{nd} day after planting, respectively. Before commencing wastewater irrigation, 225 mL fresh water was applied to each plant, every second day, starting from 14^{th} day after planting.

Laboratory prepared synthetic wastewater was used for irrigation of potato plants to maintain the desired concentration of heavy metals in water. Highest concentrations of some commonly found contaminants in wastewater, as reported in literature, were used to prepare the irrigation water. Wastewater recipe and details of its preparation are provided elsewhere (Dhiman et al., 2019). First wastewater irrigation was applied on 42^{nd} day after planting, and a total of eight wastewater irrigations were applied at the rate of 11.5 L per lysimeter per irrigation event (~72 mm) at ten-day intervals, based on the crop water requirement (FAO, 2015; King and Stark, 2008). Composite soil samples were collected from the four sampling holes at each depth along the lysimeter column as well as the surface, two days after 1^{st}, 3^{rd}, 6^{th} and 8^{th} irrigation; two days were allowed for the soil to reach field capacity and establish equilibrium between contaminants and amended soils. Background soil samples from the surface were also taken for all treatments, one day before planting potato tubers. Samples were sealed in plastic bags, labelled and stored securely in a freezer (-20°C) until further analysis. Leachate samples were not collected for this study as heavy metals were not detected in leachate samples collected for every irrigation event, in the study conducted during the previous year (Dhiman et al., 2019). The crop was harvested at maturity (119^{th} day after planting). Aboveground biomass was cut with a standard steel knife. Roots and tubers were carefully harvested from the soil using a hand trowel. Potato tubers were washed with deionised water to remove soil and were peeled with a standard steel kitchen knife. Peel and flesh of the tubers were sampled separately for heavy metal analysis. Plant root, stem and leaves were sampled separately. Samples were cut in lengths of about 0.01 m, using a knife and a chopping board. Sampling was performed on the day of harvest. Samples were securely sealed in plastic bags and stored in freezer (-20°C).

5.3.4 Sample extraction and quantification

Lysimeter soil samples collected from surface and 0.10 m depth, two days after last irrigation (eighth); they were analyzed for cation exchange capacity (CEC), base saturation (BS%) and pH, as per standard guidelines (Hendershot et al., 1993; Rayment and Higginson, 1992). Cation Exchange Capacity is a measure of soil particles' ability to adsorb and retain positively charged ions (cations), such as plant nutrients and heavy metals, due to the presence of negative charges on its surface (Brady and Weil, 2008). It is calculated as the sum of all major cations, expressed in cmol(+) kg^{-1} (amount of exchangeable positive charge per unit mass of soil). Percent base saturation (B.S. %) is calculated as ratio of the sum of basic cations (Ca, Mg, Na, K; cmol(+) kg^{-1}) and CEC, expressed as a percentage (Hendershot et al., 1993). Soil samples, collected from the surface for background and two days after first, third, sixth and eighth irrigation events, were analyzed for heavy metal content. Soil samples, collected from 0.10 m depth, on last day of soil sampling, were also extracted for heavy metal analysis to check if metals had leached down after two seasons of growing potatoes, irrigated with heavy metal contaminated wastewater. Hot nitric acid extraction method was used to extract soil samples (Stephan et al., 2008) and ICP-OES equipment was employed for quantification of heavy metal content in the extracted samples. The method has been described in detail by Dhiman et al. (2019). Plant tissue samples (tuber flesh, tuber peel, root, stem, leaf) for treatments, SAP+GBC, SAP and control, were oven-dried at a temperature of 60°C for 48 hrs and were crushed with mortar and pestle before grinding them with a standard kitchen coffee grinder. Care was taken to wash the equipment used, between samples, to prevent cross-contamination. Prepared samples were then digested using the acid block digestion method, outlined by Stephan et al. (2008). Quantification of the samples was carried out using inductively coupled plasma mass spectrometry (ICP-MS) equipment (ICP820-MS Varian, CA, USA). Samples of edible parts (tuber flesh and tuber peel) of the plants grown with freshwater (FW treatment) were also digested and extracted for comparison of heavy metal content.

5.3.5 Data analysis and quality assurance

Matlab R2018b (2018) computer software was employed in the analysis of sorption data. Freundlich and Langmuir adsorption models were fitted on equilibrium sorption data using the software. Least square means difference statistical technique, using Student's t-test, was used for pairwise comparison of means of different treatments for cation exchange capacity, percent base saturation, pH of solutions prepared for sorption analysis and pH of the soil samples. For heavy metal analysis in soil, a repeated measures statistical model was used; metal concentration was assigned as the response variable, treatment and time (irrigation events) were assigned as fixed effects, whereas lysimeter was assigned as subject and was nested within treatment. All the statistical tests were performed using JMP 13 (2017) statistical analysis and graphing software by SAS (JMP, SAS Institute Inc., Cary, NC). All laboratory and field experiments were replicated thrice and quality control samples (samples with a known amount of heavy metals) as well as experimental blanks were run in parallel with the test samples using ICP equipment to establish reproducibility and reliability. Mean recovery percentage values, established by analyzing quality control samples, were found to be above 80% for all metals. All glassware in this study underwent standard cleaning procedure before use; they were washed using a laboratory grade detergent, soaked in 4% HNO_3 solution for 24 hours, rinsed using double deionized water and air-dried.

5.4 Results and Discussion

Langmuir (Langmuir, 1916) and Freundlich (Freundlich, 1906) adsorption isotherm models were used to fit sorption equilibrium data collected for all treatments and metals; however, the data was only able to successfully fit on Freundlich model for SAP and SAP+GBC treatments. Because of presence of the polymer and biochar, the treatments contributed a variety of surfaces towards adsorption. It is also known that the Langmuir model assumes a monolayer of adsorbate molecules surrounding a homogeneous solid surface (Hanaor et al., 2014), which may explain why the data did fit the model. Equilibrium sorption data for only SAP+GBC and SAP treatments fitted the Freundlich isotherm model, and not for the non-amended control. Freundlich model is more suitable in explaining adsorption involving heterogeneous surfaces and a multilayer adsorption process (McKay, 1995) as it considers non-uniform distribution of adsorption energies spread across heterogeneous adsorbent surface; as the stronger adsorption sites are filled with adsorbates first, adsorption energies reduce leading towards equilibrium (Foo and Hameed, 2010).

5.4.1 Sorption experiment

Estimated values of Freundlich constant and Freundlich exponent for treatments SAP+GBC and SAP, for all heavy metals, are provided in table 5.1. Coefficient of determination (R^2) values, calculated during model fitting, exceeded 0.99 for all treatments and metals. Figure 5.2 depicts the pH of the sorption solution for all the treatments at different solution concentrations. It was observed that in SAP+GBC and SAP treatments, adsorption of Cd, Cu, Fe and Zn heavy metals was higher than those in the control (figure 5.1).

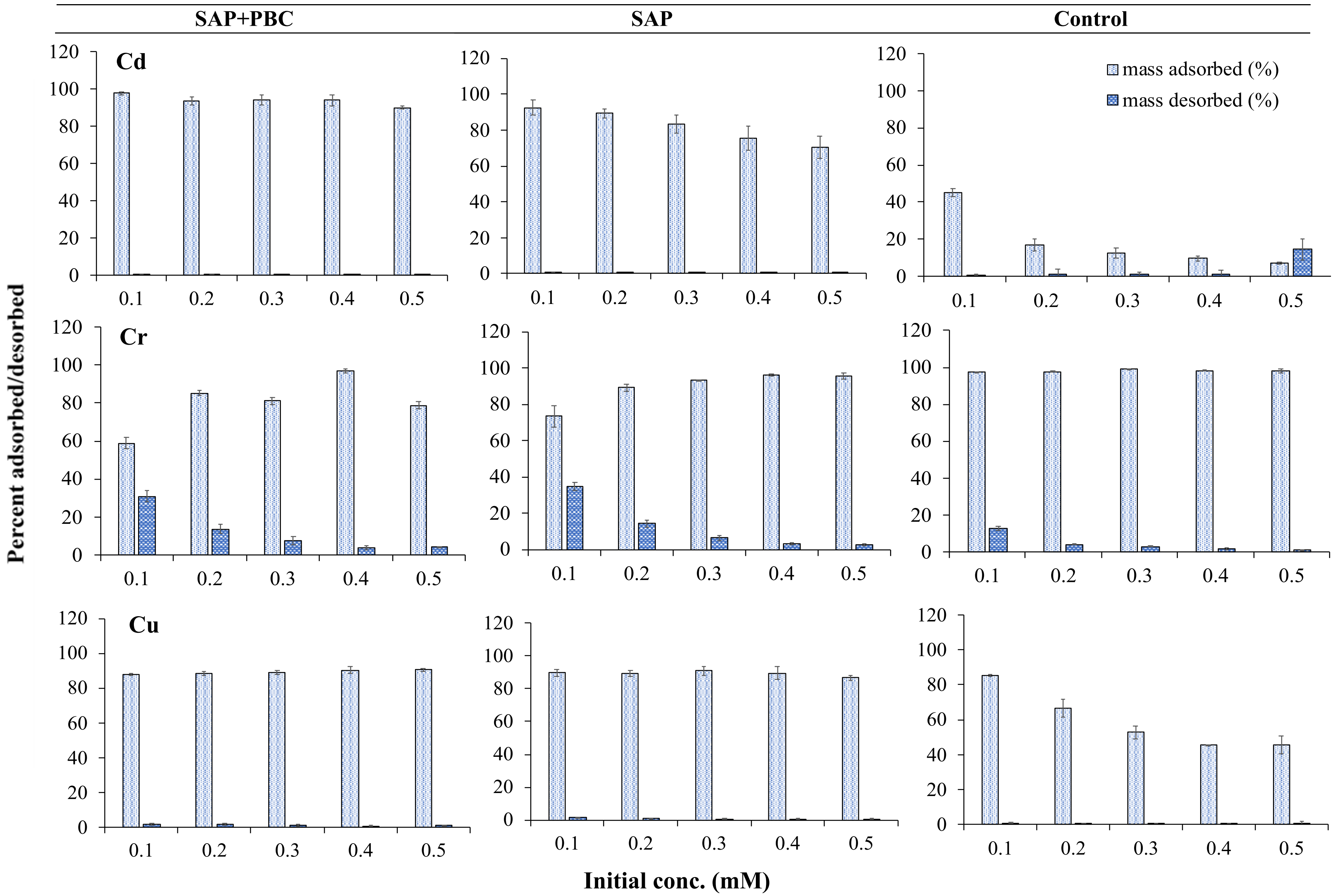


Figure 5.1 Sorption and desorption of heavy metal ions at different concentrations by treatments SAP+GBC, SAP and control *(cont'd)*.

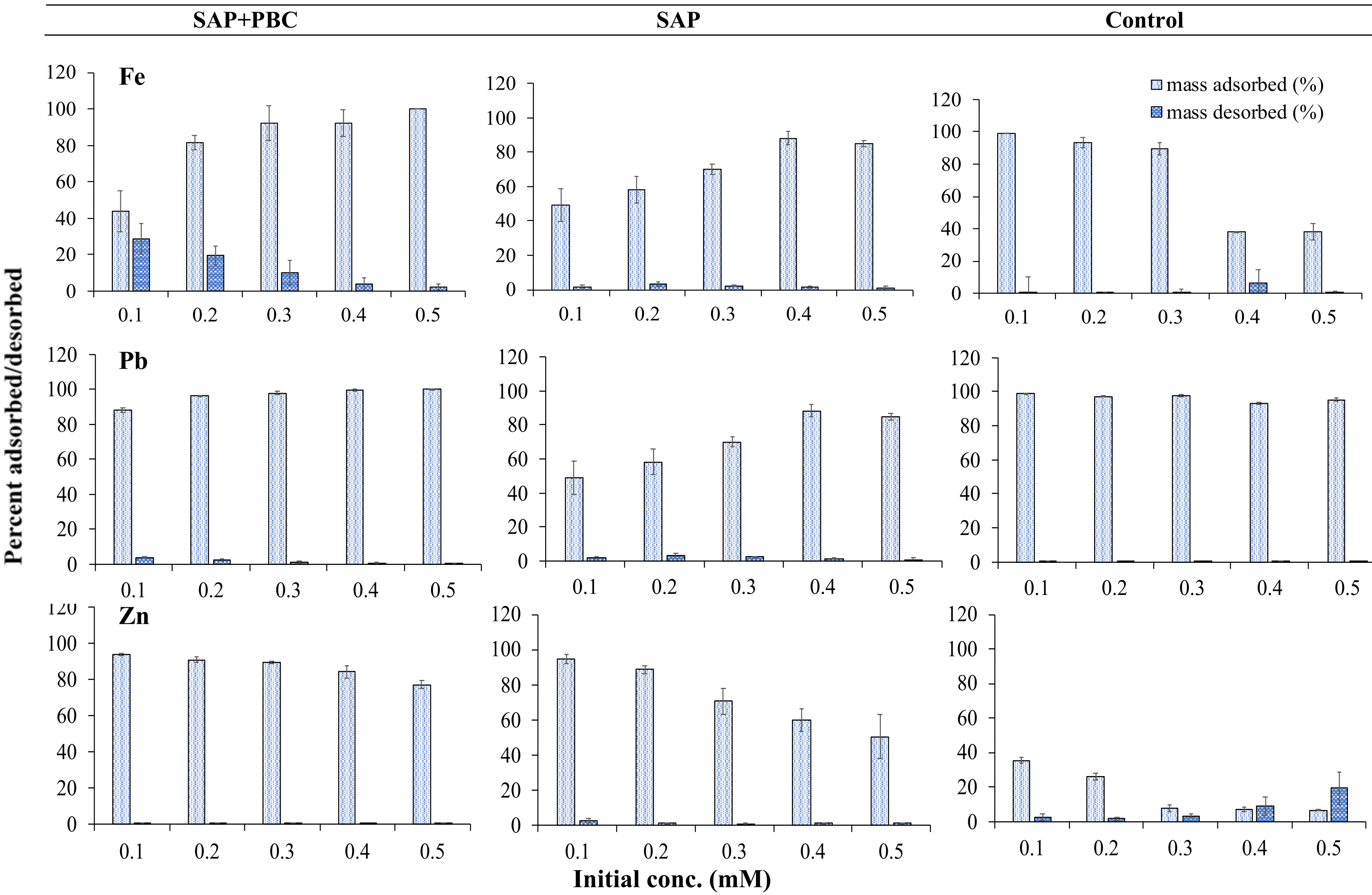


Figure 5.1 Sorption and desorption of heavy metal ions at different concentrations by treatments SAP+GBC, SAP and control.

Table 5.1 Summary of Freundlich isotherm constants for adsorption of heavy metal ions on soil amended with SAP+GBC and SAP.

Treatment	Cd		Cr		Cu		Fe		Pb		Zn	
	K_{if}	1/n	K_{if}	1/n	K_{if}	1/n	K_{if}	1/n	K_{if}	1/n	K_{if}	1/n
SAP+GBC	0.01587	1.04069	0.00665	0.82062	0.01257	0.98152	0.00310	0.66879	0.01053	0.92890	0.01745	1.11856
SAP	0.02140	1.20134	0.00854	0.85610	0.01372	1.01252	0.00367	0.72464	0.01295	0.97298	0.03200	1.64557

Unit for Freundlich constant (K_{if}) is (mg g^{-1})(L mg^{-1})1/n. Freundlich exponent 1/n is dimensionless.

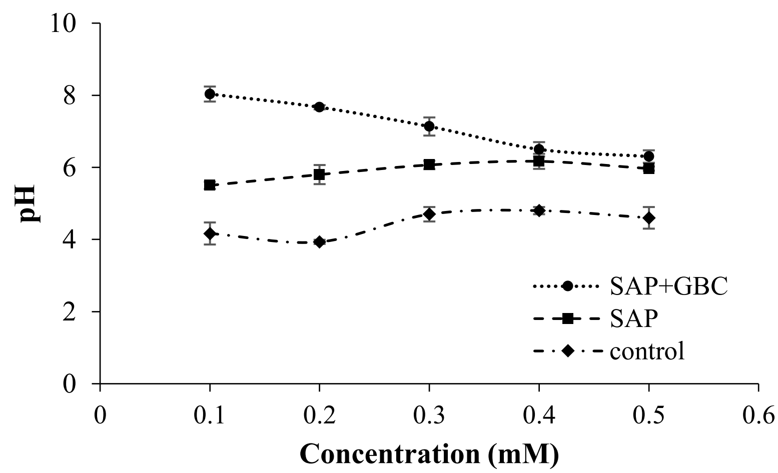


Figure 5.2 pH of multi-metal sorption solution at different concentrations for treatments SAP+PBC, SAP and control.

Freundlich constant (K_{if}) is an approximate indicator of sorption capacity, whereas Freundlich exponent (n) reflects sorption intensity (Komkiene and Baltrenaite, 2016). Thus, both model parameters determine the curvature and steepness of the isotherm (Low et al., 2000). Sorption is a favorable process for values of the exponent between 0.1 to 1 or between 1 to 10 in case 1/n is used as an exponent (Goldberg et al., 2005). Proportion of Cd and Zn metals in solution, removed by SAP+GBC, SAP and control, decreased as the concentrations increased from 0.1 to 0.5 mM (figure 5.1), which is in accordance with the Freundlich exponent values for these metals (1/n>1, for treatments SAP+GBC and SAP; table 5.1). Overall, both the treatments performed better than control, which removed 45.05% Cd and 35.23% Zn at 0.1 mM solution concentration, and 7.21% Cd and 6.64% Zn at 0.5 mM concentration (figure 5.1). Treatment SAP+GBC removed 89.78-97.42% and 77.33-93.90% of Cd and Zn, respectively, whereas SAP treatment removed

70.49-92.57% and 50.32-94.81% of the respective metals from the solution across the given range of concentrations. Also, SAP+GBC treatment desorbed 0.10-0.46% and 0.23-0.81% of adsorbed Cd and Zn, respectively, whereas SAP treatment desorbed 0.13-0.48% and 0.70-2.43% of the adsorbed metals, respectively, across the range of different solution concentrations. Non-amended control treatment exhibited higher desorption rates, as compared to the two treatments, for Cd (0.99-14.54%) and Zn (1.48-19.15%) heavy metals, especially at higher solution concentrations. It can be stated that presence of high density of sorption sites in SAP+GBC and SAP treatments led to better sorption properties of the treatments, as compared to control, in case of Cd and Zn heavy metals.

Value of Freundlich exponent (1/n) for Cr, Fe and Pb is less than one for SAP+GBC and SAP treatments (table 5.1), which shows increase in the proportion of the metal being sorbed with increasing solution concentrations. In case of Cr, the non-amended soil (control) performed the best, as it sorbed 97.26-99.16% and desorbed only 0.88-12.72% of the metal, across all concentrations. SAP+GBC treatment sorbed 58.72-96.86% and desorbed 4.16-30.86% of the metal, whereas the SAP treatment was able to remove 73.33-96.24% of the metal from the solution and desorbed 2.59-34.93%, across the range of concentrations.

Several factors affect sorption of metal ions from a multi-metal solution, viz. preferential sorption of different cations by the sorbent material depending on the properties and amounts of ions present in a multi-ion solution, temperature, pH and properties of the sorbent material (Echeverria et al., 1998; Elliot et al., 1986; Gomes et al., 2001). Non-amended control exhibited the lowest range of pH values (3.93-4.80) for different solution concentrations (figure 5.2), which may explain better performance of control in sorbing Cr metal, as compared to other treatments. Cr (VI) exists as negatively charged hydrogen chromate ion ($HCrO_4^-$) at pH range of 0-6, and, within this pH range, protonation at the adsorbent surface can impart a positive charge, leading to electrostatic interaction between the anion and the sorbent material (Shaikh and Kumar, 2017). Above pH 6.0, theoretically, abundance of negatively charged hydrogen chromate ion in solution is reduced (Butler, 1964) which can lead to reduced sorption. Griffin et al. (1977) showed that sorption of Cr(VI) on clay minerals reduced, as pH of the solution increased from 1.0 to 9.0, which is reflected by the fact that lowest sorption was shown by SAP+GBC treatment, as it exhibited highest pH range (6.3-8.03) out of the three treatments (figure 5.2). The proportion of Cr metal being sorbed by the sorbents increased, whereas desorption decreased, with increasing solution concentrations (figure 5.1). This can be attributed to the abundance of other metals in the solution. It is known that Cr(VI) adsorption by soil is proportional to Fe and Al content in the soil system which can impart greater amounts of positive charges to the soil surface, leading to increased sorption of the metal (Choppala et al., 2010). As the multi-metal solution concentration increased, Fe concentration also increased from 0.1 to 0.5 mM, which may explain better Cr sorption and lower desorption at higher concentrations. As the initial concentration of the multi-metal solution increases, more amount of metal cations is adsorbed on the sorbent, neutralizing the negative charge present at the surface. This reduces hindrance to sorption of anionic form of Cr, leading to increased sorption and reduced desorption (Anah and Astrini, 2017).

Treatment SAP+GBC sorbed 43.65% of Fe(II) from 0.1 mM solution, and with increase in concentration, Fe sorption increased to 99.82% for 0.5 mM concentration solution (figure 5.1). A similar trend was observed for the SAP treatment, which adsorbed 48.99%-88.18% of the metal from multi-metal solutions of varying concentrations. In case of non-amended control, however, an opposite trend was observed; 99.10% Fe was sorbed from 0.1 mM concentration solution and as the concentration increased, the proportion of metal sorbed by soil decreased to 38.22% for 0.5 mM solution. In a different study, it was found that proportion of Fe(II), adsorbed by acrylamide hydrogel, increased with increase in ionic strength of the metal in solution (Chauhan et al., 2008). However, in the case of control, percentage of Fe adsorbed also decreased because of decreasing availability of binding sites with increasing concentrations. Desorption of Fe varied from 2.00%-28.63%, 0.93%-3.49% and 0.33%-6.52% for SAP+GBC, SAP and control treatments, respectively. Higher desorption was observed in the SAP+GBC treatment, which may be attributed to high Fe content of the biochar (669.12 ± 86.35 mg kg^{-1}; table 3.2 in Dhiman et al., 2019). In case of Cu, SAP+GBC and SAP treatments performed better than control, possibly because of extra binding sites at the adsorbent material surface, imparted by presence of hydrogel and biochar. Sorption percentage for Cu varied from 87.93% to 90.38%, 86.61% to 90.96% and 45.46% to 85.36% for SAP+GBC, SAP and control treatments. Similar to the results observed for Fe metal, with increasing solution concentration, adsorption percentage for Cu was found to decrease in case of the non-amended soil. Desorption percentage (Cu) was below 2% for all treatments and across all concentrations. In case of Pb, performance of all the treatments, including control, was comparable. Treatments SAP+GBC, SAP and control sorbed 88.30% to 99.96%, 94.44% to

98.99% and 93.00% to 99.03% of Pb, respectively, from the tested solution concentrations, whereas less than 4% desorption was observed. All the treatments exhibited similar performance in the case of Pb, while the control worked better than both the treatments in case of Cr, due to variation in the pH of the adsorbent-solution mixture. Similar sorption test results were also obtained when SAP+PBC (pyrolyzed plantain peel biochar) was used instead of SAP+GBC (*Chapter 4*).

5.4.2 Soil pH and CEC

Soil pH is an important factor that affects heavy metal mobility in soil-water systems (Uchimiya et al., 2010). Various pH dependant mechanisms that lead to immobilization of metal ions are suggested by Sanchez-Polo and Rivera-Utrilla (2002), namely, formation of metal hydroxide, carbonate and phosphate precipitates as well as interactions between changing surface charge densities of adsorbent material and metal ion species. Soil pH values for samples, collected after eighth irrigation event, from the surface and 0.10 m depth, are presented in table 5.2. Compared to SAP and control, treatments, SAP+GBC exhibited the highest surface soil pH ($p<0.10$). This could be explained by alkalinity of the biochar used in this study (pH = 10.27 ± 0.06; Dhiman et al., 2019). No significant differences were observed at 0.10 m depth.

Table 5.2 Soil pH for samples collected from surface and 0.10 m depth after the last irrigation event.

Treatment	Depth	pH
SAP+GBC	surface	5.10 ± 0.20^{ab}
SAP		4.17 ± 0.15^{c}
Control		4.57 ± 0.59^{bc}
FW		5.23 ± 0.06^{a}
SAP+GBC	0.10 m	5.13 ± 0.25^{a}
SAP		4.87 ± 0.32^{a}
Control		4.80 ± 0.44^{a}
FW		5.33 ± 0.21^{a}

Values with different letters down the column are significantly different from each other (α=0.05) for each depth.

Cation exchange capacity (CEC) of the soil has been considered as an important factor governing heavy metal immobilization in soil (McBride et al., 1981), as it reflects the capacity of soil to adsorb metal ions, thus reducing plant metal uptake (John et al., 1972; Hinesly et al., 1982; Haghiri 1974; Liphadzi et al., 2005). It is known that soils with lower CEC have fewer binding sites to immobilize heavy metals, making them available for the plant uptake (Fergusson, 1990).

Observed values for CEC and pH of the CEC solutions (pH_{CEC}) for soil samples, collected from the surface and 0.10 m depth, two days after the eighth irrigation event, are presented in table 5.3. Treatment SAP+GBC exhibited significantly higher ($p<0.05$) soil CEC at surface and 0.10 m depth, as compared to control, whereas no significant differences were observed between SAP and control treatments for both sampling depths. It can be stated that incorporation of biochar led to an increase in CEC of the soil (Liang et al., 2006) in the SAP+GBC treatment, as compared to the other treatments.

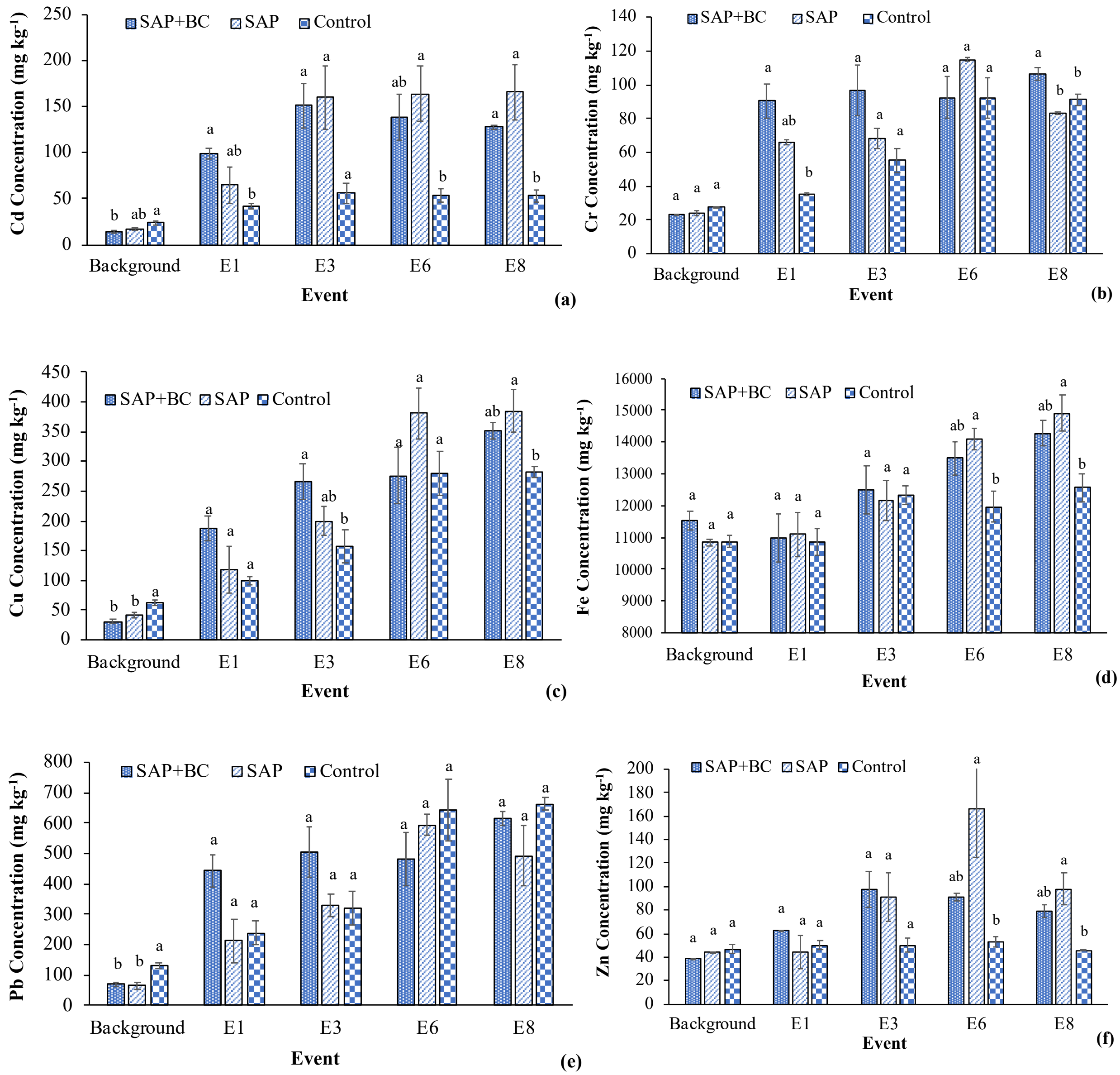
Table 5.3 Cation exchange capacity, base saturation and pHCEC of soil samples collected after the last irrigation event from surface and 0.10 m depth.

Treatment	Depth	CEC (cmol(+) kg^{-1})	pH_{CEC}
SAP+GBC		$3.63±0.43^{a}$	$4.13±0.12^{a}$
SAP	**surface**	$2.92±0.69^{ab}$	$4.33±0.12^{a}$
Control		$2.40±0.16^{b}$	$4.40±0.08^{a}$
SAP+GBC		$5.66±0.68^{a}$	$4.27±0.05^{a}$
SAP	**0.10 m**	$3.69±0.10^{b}$	$4.13±0.05^{b}$
Control		$2.82±0.09^{b}$	$3.90±0.00^{c}$

Values with different letters down the column are significantly different from each other (α=0.05) for each depth.

5.4.3 Heavy metals in soil

Concentrations of all the heavy metals ([*heavy metal*]) in soil samples, collected from the surface and from 0.10 m depth, for SAP+GBC, SAP and control treatments are given in figures 5.3 and 5.4, respectively. Except for Fe, no other heavy metal was detected at 0.30 m depth below the surface, which is in accordance with the study conducted in the previous year (Dhiman et al., 2019). Summary of the fixed effects for soil heavy metal concentrations from repeated measures analysis is provided in table 5.4. Heavy metal accumulation trend was observed in the case of Cd, as [Cd] increased with subsequent irrigation events, up to event 3 (figure 5.3a). Consequently, the fixed effect, ‘event’, was found to be significant ($p<0.05$; table 5.4). Treatment was also found to have a significant effect in the overall statistical model, as SAP+GBC and SAP treatments exhibited significantly higher ($p<0.05$) [Cd] than control in the surface soil layer across all events. Significantly higher [Cd] was observed ($p<0.05$) in control (23.90 mg kg^{-1}) for the background surface soil samples, as compared to SAP+GBC (14.26 mg kg^{-1}), but no significant difference was observed between control and SAP (17.33 mg kg^{-1}) treatments. After the first irrigation however, SAP+GBC treatment retained significantly higher ($p<0.05$) amount of the heavy metal (98.89 mg kg^{-1}) in topsoil, compared to control (41.44 mg kg^{-1}). SAP treatment also retained higher amount of Cd in topsoil (64.83 mg kg^{-1}), as compared to control, but statistically the difference was not significant.



E1, E3, E6 and E8 corresponds to irrigation events; soil samples were collected two days after each irrigation event. Different letters above the error bars indicate significant difference in concentrations for a given event (α=0.05).

Figure 5.3 Surface soil concentrations of heavy metals (a) Cd, (b) Cr, (c) Cu, (d) Fe, (e) Pb and (f) Zn, for all treatments and all irrigation events, including the background.

Cadmium metal concentration seemed to stabilize in all treatments after the third irrigation, as it varied from 127.78-151.49 mg kg^{-1}, 159.96-165.83 mg kg^{-1} and 52.97-55.85 mg kg^{-1} for SAP+GBC, SAP and control treatments, respectively. After the third irrigation, both SAP treatments retained higher amounts of the metal, as compared to control ($p<0.10$). SAP treatment retained a significantly higher amount of Cd, as compared to control, after sixth irrigation event ($p<0.05$), whereas SAP+GBC also indicated higher [Cd], as compared to control ($p<0.10$). At the end of the experiment, both SAP treatments retained significantly higher ($p<0.05$) amounts of Cd as compared to control. This can be attributed to the presence of sorbent materials (hydrogel and biochar) in the soil, which led to an increase in soil CEC, as compared to control (table 5.3). The heavy metal was not detected (LOD=15.6 mg kg^{-1}) at 0.10 m depth below the surface in both SAP treatments (figure 5.4), as most of the metal would have adsorbed/retained by the topsoil. At the end of the experiment, SAP+GBC and SAP treatments retained 141.23% and 213.06% more Cd in the topsoil, respectively, as compared to control. The results for Cd are in accordance with a similar study, conducted with spinach plants (*Chapter 4*).

Background [Cr] in topsoil for all the treatments ranged from 23.29 to 27.57 mg kg^{-1} and did not differ significantly. A trend of accumulation of the metal in surface soil was observed in case of control and SAP treatments, where [Cr] gradually increased with subsequent irrigation events, from 27.57 and 23.88 mg kg^{-1}, before the start of the experiment, to 91.22 and 83.11 mg kg^{-1}, respectively, after the last irrigation event (figure 5.3b). The findings are in accordance with the repeated measures analysis results (table 5.4). However, this trend was not observed in case of SAP+GBC treatment, possibly because of biochar's ability to rapidly bind the metal after the first irrigation itself and getting saturated. As a result, no significant increase in surface soil [Cr] was observed after third, sixth and eighth irrigations. Consequently, the interaction term in the repeated measures statistical model was found to be significant ($p<0.05$; table 5.4), i.e., effect of treatment (SAP+GBC) varied significantly with irrigation events. After first and third irrigations, [Cr] in the topsoil of SAP+GBC treatments was found to be noticeably greater ($p<0.05$ and $p<0.10$ respectively) than that in control. As [Cr] accumulated gradually in SAP and control treatments with subsequent irrigations, no significant difference was observed between any of the treatments after the sixth irrigation event. Owing to the presence of biochar, at the end of the experiment (after eighth irrigation), [Cr] in the surface soil of SAP+GBC treatment was found to be significantly greater ($p<0.05$) than that of SAP and control treatments. However, Cr was detected in soil at 0.10 m depth for both SAP+GBC (19.25 mg kg^{-1}) and control (18.70 mg kg^{-1}) treatments (figure 5.4), but it was not detected for treatment SAP. A possible reason could be that while preparing the lysimeters before commencing wastewater irrigation, the metal could be redistributed below the detection limit (15.6 mg kg^{-1}) while removing SAP amended soil from the previous year and mixing new hydrogel in the topsoil.

In case of Cu, a trend of metal accumulating in topsoil with wastewater irrigations was observed for all the treatments, which is also evident from the fact that the term 'event' was found to be statistically significant in the repeated measures analysis model ($p<0.05$; table 5.4). Background [Cu] for topsoil ranged from 30.34 to 62.15 mg kg^{-1} for all the treatments; control was found to have highest mean [Cu] in topsoil, as compared to the other two treatments ($p<0.05$) (figure 5.3c). No significant differences were observed between surface soil [Cu] for the treatments after first irrigation event; however, after third irrigation, SAP+GBC exhibited significantly higher ($p<0.05$) amount of Cu in the surface soil (265.58 mg kg^{-1}), as compared to control (156.34 mg kg^{-1}). As discussed before, this can be attributed to presence of additional sorption sites in the amended soil, imparted by the hydrogel and biochar. After third irrigation, although [Cu] for SAP (199.96 mg kg^{-1}) was higher than that in control, the difference was not significant. With subsequent irrigations, surface soil [Cu] in control (280.31 mg kg^{-1}) and SAP (380.68 mg kg^{-1}) treatments, after the sixth irrigation, increased substantially, as compared to a small increase in [Cu] observed for the SAP+GBC treatment (275.85 mg kg^{-1}); no significant differences were observed among all the treatments after the sixth irrigation. At the end of the experiment, SAP treated soil exhibited significantly higher ($p<0.05$) [Cu] at the surface (385.13 mg kg^{-1}) compared to control (281.47 mg kg^{-1}), which can be attributed to polymer's ability to bind heavy metals (based on sorption test results). No significant difference was observed between SAP+GBC (352.01 mg kg^{-1}) and control, as well as between SAP+GBC and SAP, which is in accordance with the sorption test results. Copper metal was not detected at 0.10 m depth in all the treatments (figure 5.4).

Iron metal was found to accumulate in topsoil with subsequent irrigations for all the treatments (figure 5.3d), which led to the term 'event' having a significant effect ($p<0.05$) on topsoil [Fe] in the repeated measures statistical model (table 5.4). By the end of the eighth irrigation, [Fe] in surface soil increased by 23.70%, 37.33% and 15.56% for SAP+GBC, SAP and control treatments, respectively (figure 5.3d). No significant differences were observed among surface soil [Fe] for all treatments until the third irrigation event. Treatment SAP exhibited significantly higher [Fe], as compared to control ($p<0.05$), after sixth and eighth irrigations.

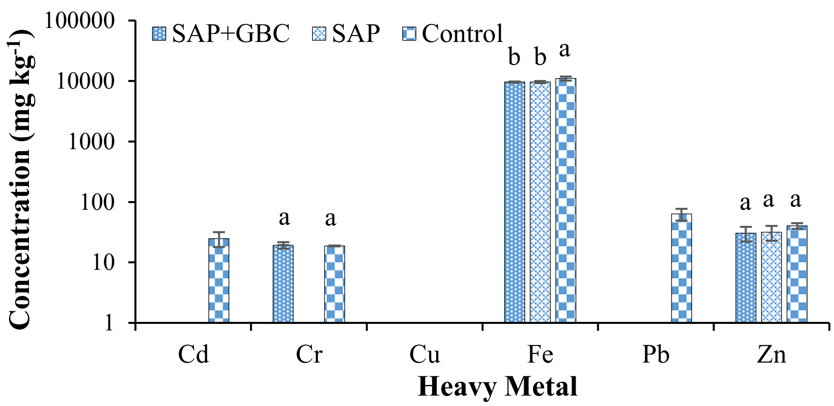
Treatment SAP+GBC also indicated higher [Fe] in surface soil, as compared to control, after sixth and eighth irrigations ($p<0.10$). It can be said that after eight irrigations, both the treatments performed better, as compared to control, with SAP performing slightly better than the SAP+GBC treatment. Consequently, at 0.10 m depth, control exhibited significantly higher ($p<0.05$) [Fe] in soil (10990 mg kg^{-1}), as compared to SAP+GBC (9596 mg kg^{-1}) and SAP (9604 mg kg^{-1}) treatments (figure 5.4). A similar trend was also observed in the case of Pb, as it accumulated in topsoil with subsequent irrigations (table 5.4; figure 5.3). Background [Pb] was found to be significantly higher in control (131.33 mg kg^{-1}), as compared to SAP+GBC (70.98 mg kg^{-1}) and SAP (64.87 mg kg^{-1}) treatments (figure 5.3e). After first irrigation, no significant difference in topsoil [Pb] was observed between SAP and control treatments. The SAP+GBC treatment also exhibited considerably higher (85.69%) [Pb], as compared to control ($p<0.10$). This can be attributed to the presence of biochar, which rapidly sorbed the heavy metal after the first irrigation. No significant [Pb] increase was observed in topsoil for SAP+GBC treatment with further irrigations. As the topsoil for other treatments received more Pb through wastewater irrigations, this difference was diminished, and as a result, no significant differences were observed between any of the treatments after third, sixth and eighth irrigations. At the 0.10 m depth, Pb was not detected (LOD=15.6 mg kg^{-1}) for SAP+GBC and SAP treatments, whereas [Pb] in the control treatment was found to be 62.98 mg kg^{-1}.

No significant increase in [Zn] was observed with subsequent irrigations in the case of control, as it only varied within 14% of background concentration (46.79 mg kg^{-1}). Metal accumulation was observed in the other two treatments; [Zn] increased from 38.72 (background) to 79.29 mg kg^{-1} and from 43.78 to 97.84 mg kg^{-1}, after eight irrigations, for SAP+GBC and SAP treatments, respectively (figure 5.3f). No significant differences were observed amongst treatments for surface soil [Zn] at background, as well as after first and third irrigations. However, with further irrigations, SAP treatment exhibited significantly greater [Zn], as compared to control (211.86% higher; $p<0.05$). After the sixth irrigation, mean [Zn] for SAP+GBC treatment was also found to be greater than that in control (53.19 mg kg^{-1}) by 71.03%, but statistically the difference was not significant. A similar trend was also observed at the end of the experiment (after the eighth irrigation). It may be noted that the SAP treatment showed slightly higher metal content than SAP+GBC treatment for Cu, Fe, and Zn, which could be attributed to the high ash content of biochar (77.45% d.b.; table 3.2 of Dhiman et al., 2019). A possible explanation could be that polymer's sorption sites were occupied by soluble salts (Bowman et al., 1990; Horkay et al., 2000), originating from high ash content of the biochar (Vassilev et al., 2013), thus reducing their availability for interacting with heavy metals, which can offset the added benefit of addition of biochar as an extra sorbent material.

Table 5.4 Summary of fixed effects for surface soil heavy metal concentration from repeated measures analysis.

Fixed effects	Heavy Metal					
	Cd	Cr	Cu	Fe	Pb	Zn
Treatment	*	-	-	-	-	*
Event	*	*	*	*	*	*
Treatment X Event	*	*	-	-	-	-

** Denotes statistically significant effects (p<0.05)*
- Denotes no significant effect (p≥0.05).



Different letters above the error bars indicate significant difference in concentrations for a given event (α=0.05).

Figure 5.4 Heavy metal concentrations in soil samples collected from 0.10 m depth after the last irrigation event, for all treatments.

5.4.4 Heavy metals in plant tissue

Observed heavy metal concentrations in edible and non-edible parts of the plants are provided in table 5.5 for all treatments. In case of edible plant parts (potato tuber flesh and peel), concentration of all metals for plants grown on FW treatment is also provided. Plant roots come in direct contact with contaminants in soil, and they also regulate physiological processes that are involved in transport of heavy metals to other plant parts (Nedelkoska and Doran, 2000). Therefore, it is important to study concentrations of various metals in plant roots. In accordance with other studies (Dunbar et al., 2003; Moore et al., 2013; Dhiman et al., 2019), it was found that concentrations of all the heavy metals were significantly higher in roots than in other parts of the plants ($p<0.05$) for all metals and in all treatments. None of the amendments was able to reduce the uptake of heavy metals by plant roots, as compared to wastewater irrigated control.

In case of freshwater irrigated (FW) potato flesh tissue, the concentrations of metals Cd, Cr, Cu, Fe, Pb and Zn were found to be 0.07, 0.05, 2.15, 26.92, 0.04 and 7.16 mg kg^{-1}, respectively, whereas, for tuber peels, they were found to be 0.25, 0.30, 5.51, 100.26, 0.26 and 15.31 mg kg^{-1}, respectively. Concentrations of Cd and Cu were found to be comparable to the range of values observed for potato tubers from 16 different cultivars, grown with freshwater in a different study (Ozturk et al., 2011). However, concentration of Fe, Pb and Zn in tuber flesh tissue (for FW treatment) in the present study was found to be below the range of values observed by Ozturk et al. (2011). This can be due to the fact that, in the cited study, heavy metal analysis was performed on whole tubers including peel, and not peel and tuber flesh tissue separately. In the present study, peels of potato tubers from FW treatment were found to have significantly higher metal concentrations for all the metals, as compared to flesh tissue samples.

Similar to the observation in the case of FW treatment, peels of potato tubers grown using wastewater (SAP+GBC, SAP and WW treatments) exhibited significantly higher metal concentrations as compared to tuber flesh samples, for all metals ($p<0.05$). This is in accordance with previous studies (Davies and Crews, 1983; Dhiman et al., 2019) where it was determined that heavy metal content in potato peels was generally higher than that of tuber flesh tissue. As expected, concentrations of Cd in tuber flesh and peel tissue for wastewater irrigated treatments were found to be significantly higher ($p<0.05$) than that of plants grown with freshwater irrigation (FW). However, both SAP+GBC and SAP treatments exhibited lower [Cd] in tuber flesh and peel samples, as compared to control($p<0.10$), which is in accordance with sorption test (figure 5.1) and soil Cd concentration results (figure 5.3a). Concentrations of Cd in tubers from wastewater irrigated treatments were higher than the permissible limit of 0.1 mg kg^{-1} (for food products), suggested by CODEX standard for contaminants and toxins in food (CODEX STAN 193-1995). Both the treatments (SAP and SAP+GBC) reduced Cd uptake in plant leaf as compared to control ($p<0.05$), but no significant differences were observed in case of stem tissue of plants grown on different treatments.

Table 5.5 Concentration of heavy metals (mg kg^{-1}) in different parts of potato plants grown in different treatments.

Plant Part	Treatment	Heavy Metal					
		Cd	Cr	Cu	Fe	Pb	Zn
Flesh	FW	0.07±0.01^{b}	0.05±0.00^{a}	2.15±0.01^{a}	26.92±0.66^{a}	0.04±0.00^{c}	7.16±0.13^{b}
	SAP+GBC	1.54±0.63^{a}	0.02±0.02^{a}	1.61±0.37^{a}	15.86±0.16^{c}	0.05±0.01^{b}	8.65±1.54^{b}
	SAP	1.98±0.36^{a}	0.07±0.05^{a}	2.92±0.91^{a}	25.11±0.78^{a}	0.08±0.00^{a}	8.30±0.01^{b}
	WW	2.63±0.46^{a}	0.06±0.01^{a}	2.72±1.41^{a}	21.21±0.88^{b}	0.09±0.00^{a}	15.28±2.72^{a}
Peel	FW	0.25±0.04^{b}	0.30±0.05^{b}	5.51±0.51^{b}	100.26±4.66^{a}	0.26±0.01^{b}	15.31±1.98^{b}
	SAP+GBC	23.89±16.43^{a}	0.33±0.02^{b}	9.41±5.21^{ab}	80.03±17.26^{a}	1.64±0.18^{b}	31.75±13.93^{ab}
	SAP	20.75±0.67^{a}	0.40±0.11^{b}	6.89±0.40^{ab}	117.58±15.14^{a}	2.61±0.53^{ab}	33.85±7.91^{ab}
	WW	43.87±4.54^{a}	0.62±0.01^{a}	18.69±7.02^{a}	124.41±2.82^{a}	4.99±1.69^{a}	43.07±10.59^{a}
Leaf	SAP+GBC	14.85±2.10^{b}	0.47±0.01^{a}	6.67±3.44^{a}	176.24±40.68^{a}	2.20±0.19^{a}	10.95±1.99^{a}
	SAP	8.21±3.50^{b}	0.89±0.01^{a}	6.36±1.18^{a}	285.32±8.09^{a}	4.87±0.27^{a}	13.68±1.63^{a}
	WW	28.74±0.70^{a}	1.09±0.53^{a}	10.93±0.73^{a}	228.46±78.50^{a}	5.70±3.42^{a}	13.08±1.07^{a}
Stem	SAP+GBC	37.61±1.57^{a}	0.20±0.06^{a}	1.00±1.03^{a}	19.20±2.87^{a}	2.04±1.79^{a}	82.35±17.92^{a}
	SAP	68.29±27.22^{a}	1.30±0.89^{a}	5.85±4.36^{a}	89.36±49.70^{a}	10.73±8.39^{a}	151.45±43.40^{a}
	WW	69.88±15.75^{a}	0.39±0.13^{a}	7.26±6.13^{a}	45.95±7.51^{a}	2.84±0.64^{a}	121.60±14.53^{a}
Root	SAP+GBC	266.50±131.29^{a}	16.63±7.80^{a}	138.71±86.93^{a}	1808.11±858.99^{a}	197.30±106.91^{a}	320.31±102.49^{a}
	SAP	285.34±63.30^{a}	12.41±0.34^{a}	114.22±52.46^{a}	1453.75±888.31^{a}	152.72±53.11^{a}	351.73±60.64^{a}
	WW	366.50±109.62^{a}	5.04±0.16^{a}	114.91±3.29^{a}	988.75±3.20^{a}	162.48±55.16^{a}	332.06±26.49^{a}

Values with different letters down the column (for different treatments) are significantly different from each other at α=0.05 for the specified plant part and heavy metal.

No significant differences were observed between Cr uptake by potato tuber flesh tissue samples from all the treatments, including FW. However, both treatments (SAP+GBC and SAP) significantly reduced Cr uptake in tuber peel tissue, as compared to control ($p<0.05$). It is to be noted that [Cr] in tuber flesh tissue was found to be within the permissible limit of 0.5 mg kg^{-1} for all treatments (NHFPC, 2012), whereas for tuber peel tissue, [Cr] was found to lie within this limit for FW, SAP+GBC and SAP treatments only, not for control; this highlights the ability of the amendments to reduce Cr uptake in potato peels. This observation was not in line with the findings of the sorption test which showed that non-amended soil worked best in case of Cr adsorption, as compared to the two other treatments (figure 5.1). Generally, Cr(VI) adsorption on soil decreases as the pH increases within the range of 1.0 to 9.0 (Griffin et al., 1977). In the field, when plants are also grown on soils subjected to heavy metal contamination, organic acids exuded by plant roots can also affect pH around the roots where uptake of metal takes place (USEPA, 2008). The complex relationship between plant, soil and amendments, along with the fact that many other ingredients were present in the wastewater, apart from heavy metals (unlike sorption test solutions), may have led to reduced Cr uptake by potato tuber flesh and peels, as compared to the control. Concentrations of the metal in plant leaf and stem tissues were found to be similar for all treatments.

No significant difference was observed between Cu uptake in tuber flesh tissue for all treatments, including FW. In the case of potato tuber peel tissue, Cu uptake was not significantly higher in both the treatments, as compared to the FW treatment, but it was significantly higher in the control than that in the FW treatment ($p<0.05$). Also, both SAP+GBC and SAP treatments exhibited lower [Cu] in plant leaf, as compared to control ($p<0.10$). This is in accordance with findings of the sorption test, where it was observed that SAP+GBC and SAP treatments performed better in sorbing the metal, as compared to non-amended control (figure 5.1). It is suspected that sandy soils are not able to bind Cu strongly and thus can lead to translocation of the metal to plants grown on such soils (Moore et al., 2013). These observations hint at the amendments' potential to reduce Cu uptake by wastewater irrigated potato plants grown on sandy soils.

Mean iron content in potato tuber flesh samples was found to vary between 15.86 to 26.92 mg kg^{-1} for all the treatments, which is within the range of Fe found in conventionally grown Canadian potato tubers (9.80 to 29.10; Warman and Harvard, 1998). In all the other parts of the plant, no significant difference was observed for [Fe] in all the treatments.

In case of Pb, the concentration of the metal in potato tuber flesh for plants, grown with wastewater irrigation, was found to be significantly higher than that of FW treatment ($p<0.05$); however, [Pb] values were found to be within the permissible limit of 0.1 mg kg^{-1} for peeled potato tubers (CODEX STAN 193-1995). Concentration of Pb in peel tissue for FW treatment was found to be very close to the permissible limit of 0.2 mg kg^{-1} (NHFPC, 2012), whereas for wastewater irrigated treatments, [Pb] was significantly higher than this limit. This may be attributed to high Pb concentration in the synthetic wastewater (16 mg L^{-1}). SAP+GBC significantly reduced uptake of Pb in potato tuber flesh and peel tissue, as compared to control ($p<0.05$). The SAP amendment in soil was able to prevent significant increase in the uptake of the metal by potato peel tissue, and maintained it to a level comparable to that of the FW treatment. No significant effect of the treatments was observed for [Pb] in stem and leaf tissue samples of the plants.

Zinc is a micronutrient present in potato tubers and generally its concentration ranges from 0.6 to 17.3 mg kg^{-1} (Warman and Havard, 1998). In the current study, concentration of the metal was found to be within this range for all the treatments in case of tuber flesh tissue but not for peel tissue samples; mean [Zn] in tuber flesh samples from control treatment, however, was found to be on the higher side (15.28 mg kg^{-1}). Both SAP+GBC and SAP treatments significantly reduced Zn uptake in potato tuber flesh tissue, as compared to control ($p<0.05$). Also, both these treatments prevented increased Zn uptake in tuber peel tissue due to wastewater irrigation; unlike the amount observed for control, the concentrations were similar to that of the FW treatment. No significant differences in Zn uptake by leaf and stem tissues of plants were observed amongst all wastewater irrigated treatments.

5.5 Conclusions

Owing to the presence of high density of sorption sites in SAP+GBC and SAP treatments, the sorption test suggested that the two treatments adsorbed higher quantity of Cd, Cu, Fe and Zn, as compared to control, across different tested concentrations. Less than 0.5% and 2.50% of the adsorbed metals, Cd and Zn, respectively, were desorbed by these treatments; these rates were lower than the desorption rates observed for the non-amended control. The two treatments also adsorbed higher amount of Fe and Cu, as compared to control, however, SAP+GBC treatment exhibited higher desorption (2.00-28.63%) of adsorbed Fe, as compared to SAP and control treatments, possibly due to high Fe content of the biochar (669.12 ± 86.35 mg kg^{-1}) and the higher adsorbed Fe amount. In the case of Cu, less than 2% of the adsorbed metal was found to desorb for all treatments and across all concentrations. SAP+GBC and SAP treatments desorbed less than 35% of the adsorbed Cr, whereas control desorbed less than 13% of the metal. Non-amended control performed better than the other two treatments, in terms of Cr adsorption, because of low adsorbent-solution mixture pH. For Pb, the performance of all the treatments, including control, was comparable, and less than 4% desorption was observed for all the treatments; this indicated that the amendments were not effective in improving binding of Pb heavy metal.

Due to alkalinity of biochar (pH = 10.27 ± 0.06), addition of SAP+GBC amendment in soil led to increase ($p<0.10$) in surface soil pH, as compared to SAP and control treatments, whereas no significant differences were observed at 0.10 m depth. Also, SAP+GBC treatment significantly increased soil CEC at the surface and at 0.10 m depth as compared to SAP and control ($p<0.05$). All metals were found to accumulate in topsoil with subsequent irrigations. Overall, the SAP+GBC treatment retained significantly higher amount of Cd, Cr and Fe in topsoil, as compared to control ($p<0.05$). The SAP amendment in soil also retained significantly higher amount of Cd, Cu, Fe and Zn in topsoil, as compared to control ($p<0.05$) at the end of the experiment. Because of the presence of biochar, SAP+GBC amended soil quickly bound and retained Cr and Pb in topsoil, as compared to other two treatments after first irrigation; with further irrigations, changes in concentration of these heavy metals were not significant. Compared to control, SAP was ineffective in holding on to Cr heavy metal in topsoil, whereas neither of the two types of amendments were effective in retaining Pb in topsoil. After eight irrigations, the SAP+GBC treatment did not exhibit an increased topsoil [Zn], as compared to control. Concentration of Cu, Fe and Zn heavy metals in soil were higher in SAP than in SAP+GBC treatment. Thus, using SAP as a soil amendment would be better than SAP+GBC when these heavy metals are present in the wastewater to be used for irrigation.

It was found that peel tissue of potato tubers generally showed significantly higher levels of heavy metals, as compared to tuber flesh. Both SAP+GBC and SAP treatments significantly reduced Cd uptake in tuber flesh and peel tissue, as compared to control ($p<0.10$). Also, both the treatments significantly reduced Cd uptake in leaf tissue of plants, as compared to control ($p<0.05$). Treatment SAP+GBC also significantly reduced Cr uptake in tuber peel tissue, as compared to control ($p<0.05$). No significant differences were observed in Cr uptake by tuber flesh among all treatments (including FW) which indicated that amendments may not be effective in reducing Cr uptake by tubers. However, tuber flesh tissue [Cr] for all treatments was within the permissible limits (0.5 mg kg^{-1}) for potatoes. No significant differences were observed between tuber flesh [Cu] for all treatments, including the FW treatment, which showed that Cu uptake may not be dependent on its concentration in sandy soil within a certain limit. However, both the treatments prevented significant increase in Cu uptake by tuber peel tissue, as seen in the case of control, when compared to FW treatment. [Cu] in plant leaf tissue was found to be considerably low in SAP+GBC and SAP treatments, as compared to control ($p<0.10$). Mean Fe content in tuber flesh samples was found to be comparable to the range of Fe content found in conventionally grown Canadian potato tubers (15.86 to 26.92 mg kg^{-1}). In case of Pb, concentration of the metal in tuber flesh for all treatments was found to be within the permissible limit of 0.1 mg kg^{-1}. SAP+GBC treatment significantly reduced Pb uptake in tuber flesh and peel tissue, as compared to control ($p<0.05$). The SAP amendment in soil prevented significant increase in Pb uptake by potato peel tissue, as [Pb] for SAP was similar to that of the FW treatment. Both SAP+GBC and SAP treatments significantly reduced Zn uptake in tuber flesh tissue, compared to control ($p<0.05$). Also, both these treatments were able to prevent significant increase in Zn uptake by tuber peel tissue, since concentrations of the metal in peel tissue for these two treatments were similar to that of the FW treatment; In control, however, [Zn] was found to be significantly higher compared to FW. Concentrations of all heavy metals in plant stem and root tissues were not found to differ significantly amongst treatments. The concentrations of all the heavy metals were significantly higher in roots than in other parts of the plants ($p<0.05$) for all the metals and treatments. The present study indicates that the amendments have the potential to reduce Cd, Cu, Pb and Zn uptake by wastewater irrigated potato tubers grown on sandy soil.

5.6 Acknowledgment

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Connecting Text to Chapter 6

Studies presented in chapters 3, 4 and 5 investigates the role of SAP-based soil amendments on heavy metal immobilization and plant uptake. Chapter 6 provides an insight into the effect of these amendments on plant health, growth and yield when wastewater is utilized for irrigation. Plant growth parameters and yield observations across two years for wastewater irrigated potato, and for one year in case of wastewater irrigated spinach grown on sandy soil, are presented in chapter 6.

This chapter will soon be sent for publication in a refereed journal. The manuscript will be co-authored by Prof. Shiv Prasher (academic research supervisor), Dr. Eman ElSayed (Postdoctoral Fellow at McGill University's Bioresource Engineering Department at the time conducting research), Dr. Christopher Nzediegwu (Postdoctoral Fellow at University of Alberta's Department of Renewable Resources). Mr. Ali Mawof (PhD scholar at McGill University's Bioresource Engineering Department) and Dr. Ramanbhai Patel (Research Associate at McGill University's Bioresource Engineering Department). The original draft has been modified to maintain consistency with the format of this thesis, in accordance with McGill University's thesis guidelines. Studies and references cited are presented at the end under the '*References*' section.

Chapter 6: Effect of Hydrogel and Biochar Soil Amendments on Yield and Growth of Wastewater Irrigated Plants

6.1 Abstract

Global human population is expected to rise to 9.7 billion by the year 2050, leading to a significant increase in food demand. Increased irrigation water requirement for enhancing food production will place additional pressure on our already stressed freshwater (FW) resources. Since agriculture is the largest freshwater consumer in the world, alternate sources of irrigation water, such as untreated wastewater, would help in the conservation of planet's precious resource in a cost-effective manner. However, wastewater contains contaminants which can adversely affect plant growth and health. The aim of this study was to determine the effect of super absorbent polymer (SAP), alone and mixed with two types of biochars, SAP-gasified plantain peel biochar (GBC) and pyrolyzed plantain peel biochar (PBC), on yield and growth of wastewater (WW) irrigated potatoes (*Solanum tuberosum* L.) and spinach (*Spinacia oleracea* L.) plants. In 2015 and 2016, potato plants were grown in lysimeters (1.00 m long x 0.45 m dia.) packed with sandy soil in a complete randomized design (three replicates) with treatments: SAP+WW, SAP+GBC+WW, WW (no amendment, wastewater irrigated) and FW (no amendment, freshwater irrigated). The amendments were incorporated in soil at the rate of 1% (w/w). In 2016, spinach plants were grown in the lysimeters, following a similar design of experiment, however, PBC was used instead of GBC. Plants were irrigated with laboratory prepared synthetic wastewater, and were harvested at maturity. Observations on yield and plant health parameters, viz. photosynthetic activity, stomatal conductance, normalized difference vegetative index (NDVI), relative chlorophyll content index (RCCI), leaf temperature, root surface area, root volume, average root thickness and root length density were recorded. Potato tuber yield was the lowest in SAP+GBC+WW treatments during both the years, whereas no significant differences were found in yields amongst other treatments. On the other hand, spinach yield in SAP+PBC+WW treatment was found to be significantly higher than that in other treatments ($p<0.05$). No significant differences in photosynthetic activity, stomatal conductance, transpiration rate, leaf temperature, NDVI and root structure development for spinach plants as well as leaf temperature and NDVI values for potato plants were found due to treatments. Results showed that wastewater could potentially be used for irrigating potatoes and spinach plants in sandy soils, without any reduction in yield; however, tuber yield may be decreased due to GBC amendment.

6.2 Introduction

Currently, about 80 countries in the world are experiencing water shortages and about 2 billion people do not have access to clean water (Alois, 2007). Since agriculture is the largest freshwater consumer (FAO, 2016; UNESCO, 2016; Koehler, 2008), exploitation of alternate sources of irrigation water such as untreated wastewater could partly compensate for the increased freshwater demand. Use of wastewater for irrigation has been proposed and highly encouraged by researchers to tackle the problem of freshwater scarcity (Rusan et al., 2007; Al-Rashed and Sherif, 2000; Mohammad and Mazareh, 2003; Al Salem, 1996). Apart from being a cost-effective alternative for irrigation in countries experiencing economic water stress (Rusan et al., 2007; Qadir et al., 2010), wastewater is also a source of many nutrients required by soil to maintain its fertility (Weber et al., 1996). However, contaminants present in untreated wastewater can be harmful to humans and the environment (Qadir et al., 2007). Depending on the source, wastewater may contain a wide variety of contaminants such as steroidal sex hormones, pharmaceuticals, plastics and heavy metals. Wastewater contaminants, such as heavy metals, are known to adversely affect health and growth of food crops [e.g., barley (*Hordeum vulgare* L.), soybean (*Glycine max* (L.) Merr.) and wheat (*Triticum æstivum* L.)], and may lead to reduced yields if present at higher concentrations in the soil-water system (Zhang and Yang, 1994; Page et al., 1972; Collins et al., 1976; Haghiri, 1973). Thus, it is necessary to develop an effective technique to promote safe use of wastewater for agriculture, without compromising on plant health and crop yield.

Addition of amendments like super absorbent polymers (SAPs) and biochar have been associated with better plant growth and yield. SAPs or hydrogels are hydrophilic networks of loosely crosslinked polymeric chains which can absorb and retain water or aqueous solutions up to hundreds of times their own weight (Buchholz and Graham, 1998; Skouri et al., 1995; Zohuriaan-Mehr and Kabiri, 2008). These polymers have been used in hygienic products, construction, food and electronics industries, and more recently in agriculture for efficient use of water resources (Korpe et al., 2009). Polyacrylamide and polyacrylate polymers are some commonly used SAPs in agricultural applications (Bai, et al., 2010). Hydrogel incorporation in soil can potentially lead to improved plant health and yield (Baasiri et al., 1986; Gu et al., 1996; El-Sayed et al., 1991; Suresh et al., 2018) through improvement of certain soil properties such as increased soil-water holding capacity and improved soil structure leading to reduction in soil penetration resistance (John et al., 2005; Busscher et al., 2009). Biochar is defined as a product of pyrolysis, carbonization and gasification of biomass (ANSI/ASABE, 2011). Biochar soil amendment is known to have a positive impact on soil physio-chemical properties, fertilizer and water use efficiencies as well as crop yield (Glaser et al., 2001; Zhang et al., 2012; Major et al., 2010; Steiner et al., 2008). SAPs and biochar soil amendments are also known to adsorb some of the commonly found contaminants in wastewater such as hormones, pharmaceuticals and heavy metals, thus making them unavailable for the plants to uptake and leading to improved crop yields even in contaminated soils (Park et al., 2011; Al-Wabel et al., 2015; Samrah et al., 2010; Torres and Varennes, 1998; Varennes and Torres, 1999; Varennes and Queda, 2005; Dhiman et al., 2019).

Wastewater could potentially have a negative impact on plant health, growth and yield. To the best of the authors' knowledge, information on the effects amending the soil with SAP and a mixture of SAP and biochar, on growth, health and yield of synthetic-wastewater irrigated plants is not available. Therefore, this study was conducted to determine the effect of incorporating polyacrylamide SAP and SAP-plantain peel biochar mixture in the soil, on plant health, growth and yield parameters for potatoes and spinach grown across two years (2015 and 2016) using synthetic wastewater.

6.3 Materials and Methods

6.3.1 Study Area

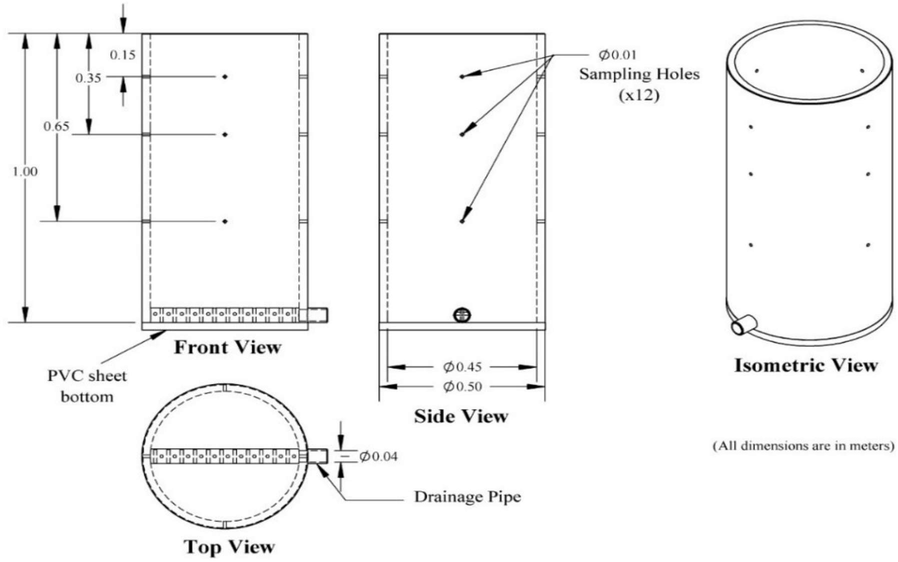


Figure 6.1 Schematic diagram of lysimeter (*taken from Dhiman et al., 2019*).

The study was carried out in a field-lysimeters situated at *Macdonald Campus of McGill University* in Ste. Anne de Bellevue, QC, Canada. Lysimeter used in this study was a hollow cylinder (1.00

m long x 0.45 m I.D.) made of from PVC material, and filled with sandy soil (soil from *Ste-Amable complex*, QC, Canada) levelled at 0.05 m from the top (figure 6.1). The lysimeter had four equidistant holes (10 mm dia.), each at three different heights viz. 0.10, 0.30 and 0.60 m from the soil surface. The holes are drilled to provide for soil sampling sites along the depth of the column. An outlet was provided at the bottom to collect the leachate. Values for physical and chemical characteristics of the soil are provided in table 6.1.

Table 6.1 Physio-chemical properties of lysimeter soil (*taken from Dhiman et al., 2019*).

Type [a]	Sandy
Sand (%) [a]	92.20
Silt (%) [a]	4.30
pH [a]	5.50
Dry Bulk density (Mg m^{-3}) [a]	1.35
Organic matter (%) [a]	2.40±0.15
Saturated Hydraulic conductivity (m day^{-1}) [a]	1.67±0.45
Zero point of charge (ZPC) [a]	3.40
N (mg kg^{-1})	n.a.
P (mg kg^{-1})	215.30±40.43
K (mg kg^{-1})	107.33±13.13
Ca (mg kg^{-1})	912.44±79.70
Mg (mg kg^{-1})	103.27±7.29
Al (mg kg^{-1})	1164.14±12.40

Nutrients P, K, Ca, Mg and Al were determined using Mehlich III extraction procedure (Mehlich, 1984). n.a.- not available.
[a] *Adapted from a previous study conducted with soil from same source (ElSayed et al., 2013).*

6.3.2 Soil Amendments

Super absorbent polymer (SAP) used in our study was a cross-linked copolymer of potassium acrylate and acrylamide (commercial name: SUPERAB A200). It was procured from a Canadian environmental solutions company, *Iramont Inc*. General physical and chemical properties of the used SAP hydrogel are given in table 6.2.

Table 6.2 Physical and Chemical properties of SAP SUPERAB A200 (*from Dhiman et al., 2019*).

CAS #	Appearance [a]	Specific gravity [a]	pH [a]	Solubility [a]	Particle size distribution (mm) [a]	Storage life (years) [a]	Life in soil (years) [a]	Toxicity [a]	Swelling Ratio
31212-13-2	White granular	1.2	6-7	Insoluble in aqueous solution	2-5: 61% 1-2: 20% <1: 19%	>7	>5	Non-toxic	200

[a] *Adapted from manufacturer's website (Iramont, 2016).*

For the first-year (2015), plantain peel biochar (GBC) was prepared from oven-dried plantain peels using a gasifier unit (built at *Macdonald Campus Technical Service Building of*

McGill University, *Sainte-Anne-de-Bellevue*, Quebec, Canada). Plantain was procured from *Sami Fruits*, Lasalle, Quebec, Canada (for both years), and were peeled using standard kitchen knives. The peels were dried in a conventional oven at temperatures ranging from 75°C to 80°C. Biochar production was carried out at temperatures, varying from 450°C to 500°C, with a residence time of 20-25 min. Due to low yield of biochar obtained using gasification process, a pyrolyzer unit fabricated at Macdonald Campus's Mechanical Workshop was used for the second year (2016) to produce pyrolyzed plantain peel biochar (PBC). Proximate and ultimate analysis of biochar was performed at the *CanmetENERGY Characterisation Laboratory* (ISO 9001:2008 certified), Ottawa, ON, Canada. Biochar samples were sent to the *Materials Characterisation Laboratory at Department of Mining and Materials Engineering, McGill University*, Montreal, Canada for Brunauer-Emmet-Teller (BET) surface area determination. Properties of the two types of biochar used are provided in table 6.3.

Table 6.3 Physiochemical properties of gasified and pyrolyzed plantain peel biochar.

Parameter	**Observed Value (wt %)**		**Method**
	GBC	**PBC**	
Proximate Analysis			
Moisture Content*	9.88	5.68	ASTM D7582
Ash Content*	77.45	27.97	ASTM D7582
Volatile Content	18.09	31.32	ISO 562
Fixed Carbon	4.46	40.71	ASTM D7582
Ultimate Analysis			
Carbon	18.10	57.40	ASTM D5373
Hydrogen	0.48	3.18	ASTM D5373
Nitrogen	0.60	2.16	ASTM D5373
Total Sulfur	<0.05	<0.05	ASTM D4239
Oxygen	3.37	9.32	By Difference
Specific Surface Area			
BET Surface Area (m^2g^{-1})	1.9460	0.7560	Lab Characterization

** Estimated using thermogravimetric analyzer (TGA). All measurements are made on dry weight basis.*

6.3.3 Design of Experiments

The design of experiments for both the years is presented in table 6.4. For year 2015, SAP was mixed into the lysimeter soil layer spanning 0.15 m to 0.25 m below the soil surface, at a rate of 1% (w/w) for SAP and SAP+GBC treatments. For SAP+GBC treatment, GBC was mixed into the top 0.10 m of soil [biochar:soil 1% (w/w)]. The SAP was incorporated below the soil surface to prevent its photo-degradation. The field study was conducted under a water proof tent to prevent the entry of any rainwater, allowing only a known volume of irrigation water to be used at a predetermined schedule. All lysimeters were brought to field capacity, two days before planting. Potato plants were grown in the designated lysimeters for the first year. Russet Burbank potato tubers were obtained from *Global Agri. Services Inc*. (New Brunswick, Canada). Potato tuber was planted in the center of each lysimeter at a depth of 0.10 m, with sprouts facing upward (Thompson-Morgan, 2015). Every alternate day, about 500 mL of tap water was applied to establish plants before irrigating with wastewater started. A pre-emergence spray of the broadleaf and grass weed herbicide, *SENCOR 480F* (*Bayer CropScience*, *ai*: metribuzin, 480 g L^{-1}), was applied at the recommended rate (850 mL mixed in 100L ha^{-1}) for weed control in lysimeters (Hutchison, 2012). Fertilizers (ammonium sulfate and muriate of potash) were applied at locally recommended rates in each lysimeter. Plants in the three lysimeters under SAP+GBC+WW treatment did not emerge and therefore the three potato seedlings were replaced (transplanted) by reserve potato plants which were grown in other lysimeters (with soil from the same source) in the field on 27^{th} day after initial planting. Seeds for the reserve potato plants were also planted at the same time as other plants in the experiment and were given the same treatment until the start of wastewater irrigation. It was suspected that non-emergence was caused due to crusting and hardening of the topsoil layer because of biochar addition. It was expected that breaking of crust while replanting would not adversely affect the plant. The first wastewater irrigation (11.5 L per lysimeter) was applied 33 days after planting (post-emergence). Subsequent eight irrigations of the same volume were applied at ten-day intervals. Crop was harvested on maturity.

For the year 2016, 12 new lysimeters were randomly assigned to different treatments for spinach crop (table 6.4). SAP was mixed in top 0.10 m of the lysimeter soil at the rate of 1% w/w for treatments SAP and SAP+PBC, whereas PBC was also mixed in top 0.10 m of the soil profile at the same rate, for SAP+PBC treatment. Spinach plants were obtained from a local farmers market (*Jean Talon Farmer's Market, Montreal, Canada*) and were transplanted (three plants per lysimeter) in the lysimeters which were brought to field capacity a day before planting. Fertilizers (ammonium sulfate and muriate of potash) were applied at locally recommended rates. Every third day, about 400 mL of freshwater was applied to each of the lysimeters for sustenance, before commencing wastewater irrigation. Spinach plants were subjected to first wastewater irrigation (4.0 L) on the 22^{nd} day after transplantation. In total, lysimeters were irrigated four times at an interval of ten days between subsequent irrigations. For potato plants, the 12 lysimeters that were used in year 2015, were used again for the year 2016 (with same assignment of treatments). However, fresh SAP was mixed in the top 0.10 m of soil at the rate of 1% (w/w), for SAP and SAP+GBC treatments. Soil from 0.15-0.25 m depth was replaced with fresh soil (from the same source) in all lysimeters to replace used SAP from year 2015. This was done to study the effect of SAP incorporation in topsoil on the movement of contaminants found in wastewater, which was an objective of a different study (*Chapter 5*). Potato planting, irrigation and harvesting activities were performed in same way as in the previous year. However, first wastewater irrigation was performed on 42nd day after planting; a total of eight irrigations were performed with ten-day intervals. No herbicide was used for either crop in year 2016.

Table 6.4 Design of experiments for years 2015 and 2016.

Year	Crop	Treatments [a]	Assignment of Treatments	Replicates	Number of lysimeters
2015	Potato	SAP+WW SAP+GBC+WW WW FW	Completely randomized	3	12
2016	Potato	SAP+WW SAP+GBC+WW WW FW	Completely randomized	3	12
	Spinach	SAP+WW SAP+PBC+WW WW FW	Completely randomized	3	12

[a] *SAP+WW – hydrogel amendment, wastewater irrigation*
SAP+GBC+WW – hydrogel and gasified plantain peel biochar amendment, wastewater irrigation
SAP+PBC+WW - hydrogel and pyrolyzed plantain peel biochar amendment, wastewater irrigation
WW – non-amended control, wastewater irrigation;
FW – non-amended control, freshwater irrigation.

6.3.4 Synthetic Wastewater

Wastewater used for irrigation during both the years was prepared in laboratory to maintain control over the level of contaminants being introduced. Highest concentrations of some commonly found wastewater contaminants, as reported in literature, were used in preparation of the synthetic wastewater (table 6.5). All the chemicals used for preparation were procured from either *Sigma-Aldrich* (St. Louis, MO, USA) or *Fisher Scientific* (Waltham, MA, USA). Regular tap water was stored in a container for one day to remove possible chlorine content and then used for the preparation of wastewater.

Table 6.5 Laboratory prepared synthetic wastewater recipe (taken from Dhiman et al., 2019).

Ingredient	Category	Concentration (mg L^{-1})	Reference
Basic Wastewater Constituents			
Ammonium Chloride	Nitrogen Source	12.75	Nopens et al. (2001)
Peptone		17.41	
Urea		91.74	
Sodium Acetate	Carbon Source	79.37	Nopens et al. (2001)
Milk Powder		116.19	
Soy Oil		29.02	
Starch		122	
Yeast Extract		52.24	
Magnesium Phosphate	Phosphorus Source	29.02	Nopens et al. (2001)
Potassium Phosphate		23.4	
Calcium Chloride	Minerals	60	Nopens et al. (2001)
Magnesium Chloride		40	
Sodium Bicarbonate		100	
Wastewater Contaminants			
Cr	Heavy Metals	2	Ahmad et al. (2011)
Cd		5	
Pb		16	
Fe		120	
Zn		3	
Cu		8	
Estrone	Female Steroidal Sex Hormones	0.05 (8.15×10^{-3}) [a]	Sim et al. (2011)
17β-Estradiol		0.02 (0.634×10^{-3}) [a]	
Progesterone		0.02 (0.90×10^{-3}) [a]	Huang et al. (2009)
Oxytetracycline as OXYVet	Pharmaceuticals	19.5	Li et al. (2008)
Ibuprofen		0.0264	Singh et al. (2014)
Alkylphenyl polyethoxylate as Triton X-100		0.03	Aboulhassan et al. (2006)
BPA	Bisphenols	0.05	Based on LOD of instrument
BPF		0.05	
BPS		0.05	

[a] Values in parentheses are the reported values whereas amount used in wastewater recipe depends on limit of detection (LOD) of the instrument.

6.3.5 Plant Yield and Physiological Parameters

Normalized difference vegetation index (NDVI) was measured using an active crop canopy sensor; *Crop Circle ACS-430* (Holland Scientific Inc., Nebraska, USA) for potato and spinach plants grown in the year 2016. NDVI measurements were made after first wastewater irrigation (weeks

3-8 and weeks 7-12 after planting for spinach and potato plants, respectively; 2-3 times per week) for plants grown in 2016. Relative chlorophyll content index (RCCI) for spinach and potato plants was estimated (non-destructive method) using a chlorophyll meter; *SPAD-502 Plus* (Konica Minolta, Europe) (Ling et al., 2011). For year 2015 potato plants, RCCI was estimated only for weeks 12, 13 and 14 after planting (four times per week), whereas for year 2016 crops, RCCI was measured before, on and after wastewater irrigation events. Similar trend was also followed while measuring leaf temperature using an infrared thermometer for potato and spinach plants grown in year 2016. Photosynthetic activity, stomatal conductance and transpiration rates for spinach plants were also estimated, one day before each wastewater irrigation, using *Li-cor 6400* (LI-COR, Nebraska, USA) instrument. On maturity, potato tubers were harvested, weighed and stored securely (117th and 120th day after planting for years 2015 and 2016, respectively). For spinach plants, two harvests were performed. Leaves were picked on 42nd day after transplanting. Second and final harvest was made on 64th day after transplanting; leaves were weighed and stored securely. Aboveground biomass weight and root weight was measured for all the plants. For spinach, root from one of the three plants per lysimeter was randomly chosen for estimating root surface area, length density, volume and average diameter using *WinRHIZO 2007 root analyzer* (Regent Instruments, QC, Canada) software and scanner.

6.3.6 Soil Moisture Content

To understand the effect of the soil amendments on soil moisture content, three additional lysimeter (mimicking the treatments SAP, SAP+GBC and non-amended control) were set up in the year 2015. Potato plants were grown using freshwater on these lysimeters and *Moisturepoint* time domain reflectometer (TDR) equipment/probes (Environmental Sensors Inc., Canada) were installed to estimate moisture content at depths 0-0.15, 0.15-0.30 and 0.30-0.45 m. Moisture content readings were recorded for weeks 4-10, with three to four observations made per week. In 2016, water retention curves for soil-amendment mixtures were developed for 0, 0.10, 0.33, 0.50 and 1.00 bar pressures using a pressure plate apparatus (*Soilmoisture Equipment Corp.*, CA, USA), based on the methodology outlined by Tuller et al. (2004). Soil samples from the field were procured and mixed with SAP and SAP+PBC amendments at the rates used for spinach plants (1% w/w). In addition, PBC alone was mixed in soil at the same rate, and the resultant sample was also analyzed for comparison with other two treatments and the non-amended control. Based on the bulk density of the soil, samples were packed in PVC retainer rings and saturated before use. Water loss was determined gravimetrically, and the analysis was conducted in three replicates.

6.3.7 Data Analysis

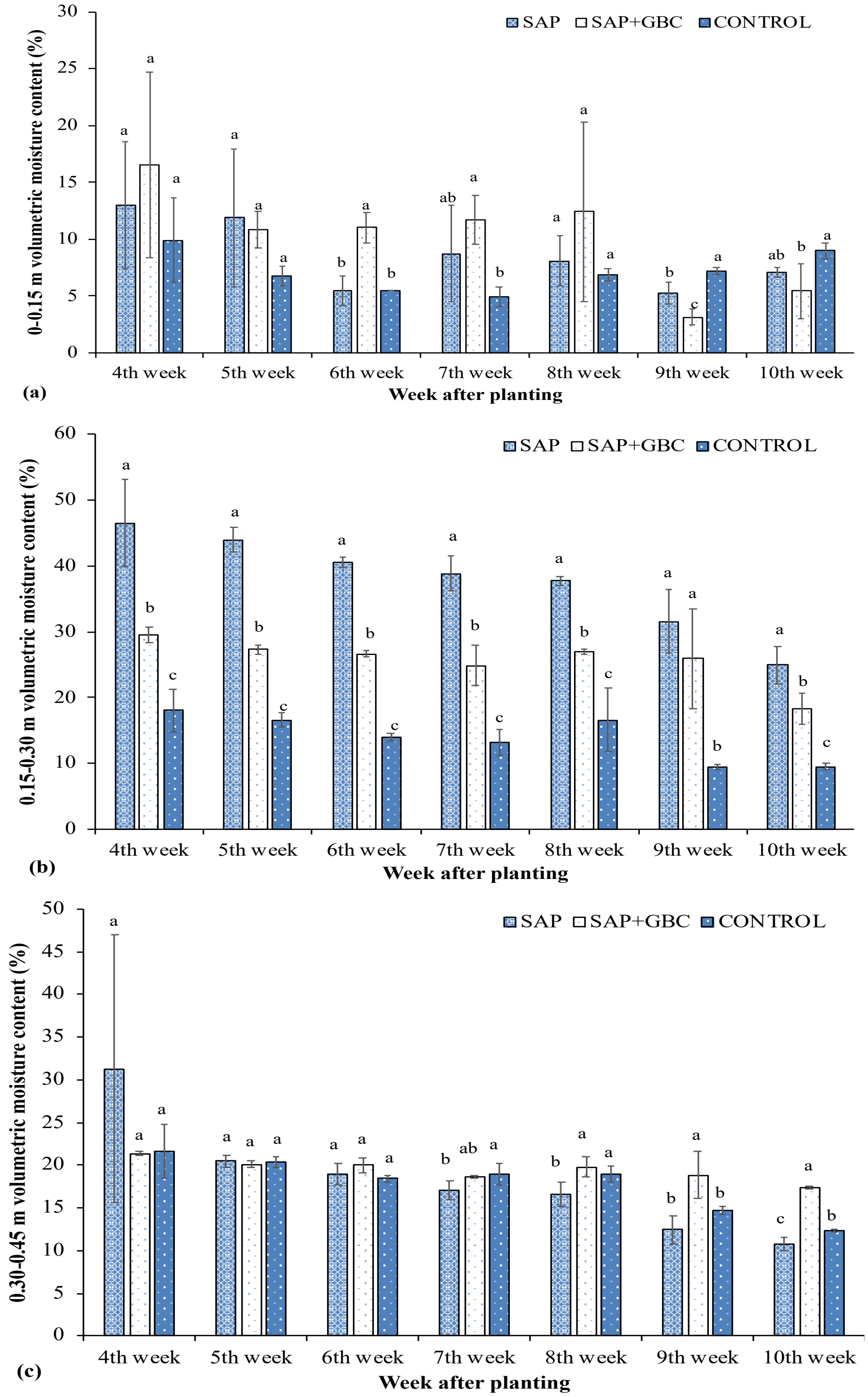
Photosynthetic activity, stomatal conductance and transpiration rate data from *Li-cor 6400* equipment and NDVI data from *Crop Circle ACS-430* equipment were extracted using shell script programming (PowerShell, 2018) and Matlab R2018b (2018) computer software. Data for plant physiological parameters (photosynthetic activity, stomatal conductance, transpiration rate, NDVI, RCCI and leaf temperature) were analyzed using a repeated measures statistical model. The plant physiological parameter was assigned as response variable, treatment and time were assigned as fixed effects, whereas lysimeter was assigned as subject and was nested within treatment. Data pertaining to plant yield and harvest parameters (yield, root weight, aboveground biomass weight and total biomass weight), volumetric moisture content, soil water retention curve and root analysis were subjected to least square means difference pairwise comparisons, using Student's t-test. Statistical tests were performed using JMP 13 (2017), a statistical analysis and graphing software by SAS (JMP, SAS Institute Inc., Cary, NC). Summary of the collected data for different crops is provided in table 6.6.

Table 6.6 Summary of field data collected for different crops.

Type	**Field parameter**	**Crop**		
		Potato 2015	**Potato 2016**	**Spinach 2016**
Soil moisture content	TDR volumetric moisture content	✓	×	×
Plant physiological parameters	RCCI	✓	✓	✓
	Photosynthetic activity	×	×	✓
	Stomatal conductance	×	×	✓
	Transpiration rate	×	×	✓
	Leaf temperature	×	✓	✓
	NDVI	×	✓	✓
	Root structure analysis	×	×	✓
Harvest parameters	Yield	✓	✓	✓
	Root weight	✓	✓	✓
	Aboveground biomass weight	✓	✓	✓
	Total biomass weight	✓	✓	✓
	Root Structure Analysis	×	×	✓

6.4 Results and Discussion

6.4.1 Soil Moisture Content



Different letters above the error bars indicate significant differences in mean moisture content amongst treatments for a given week (α=0.05).

Figure 6.5 Volumetric moisture content for different treatments and lysimeter soil sections at depths (a) 0-0.15 m, (b) 0.15-0.30 and (c) 0.30-0.45 m measured using time-domain reflectometry (TDR).

The TDR moisture content, recorded during year 2015 experiment, is shown in Fig. 6.2. It is apparent from Fig. 6.2a that surface moisture content in SAP+GBC amended lysimeter were numerically higher than the non-amended lysimeter until 8 weeks, although statistically not different. It is evident that SAP application at 0.15-0.25 m depth of the soil led to significantly higher retention of water in treatments SAP and SAP+GBC, as compared to the non-amended control, for the 0.15-0.30 m depth (figure 6.2b). This observation was expected because of SAP's ability to absorb large amounts of water, leading to increased soil-water retention (Lu et al., 2003). At 0.15-0.30 m depth, moisture content across all the weeks for SAP+GBC treatment was found to be lower than that of SAP treatment but still significantly higher than control. This can be attributed to the high ash content of GBC (77.45 %; table 6.3). Biomass ash is mostly composed of mineral content, containing water soluble salts (Vassilev et al., 2013), which may lead to decreased absorption of water by SAP (Bowman and Evans, 1991; Horkay et al., 2000; Bowman et al., 1990). Also, for weeks 8-10, significantly higher moisture content was found in 0.30-0.45 m soil depth for treatment SAP+GBC, as compared to SAP treatment (figure 6.2c), which may account for the water that was not absorbed by the SAP at its application depth. From figure 6.2a, it is evident that moisture content values in 0-0.15 m soil layer for SAP+GBC treatment was found to be numerically higher than that of SAP treatment for most of the weeks (weeks 4, 6, 7, and 8, respectively), but the difference was not always statistically significant. Biochar addition can potentially lead to increase in soil water content (Karhu et al., 2011; Basso et al., 2013). Yu et al. (2013) in their study showed that average water holding capacity in loamy sand soil increased linearly from 16±0.7% to 32.3±1.4% when yellow pine scrap biochar soil amendment rate was increased from 0% to 10% (w/w), respectively. However, in the present study, the change in average soil water holding capacity was small, which increased from 16.0% to 16.8%, with 1% biochar soil amendment. This could explain why surface moisture content of SAP+GBC treatment was, at times, not significantly different than that of the other two treatments since biochar application rate used in present study was also 1% (w/w).

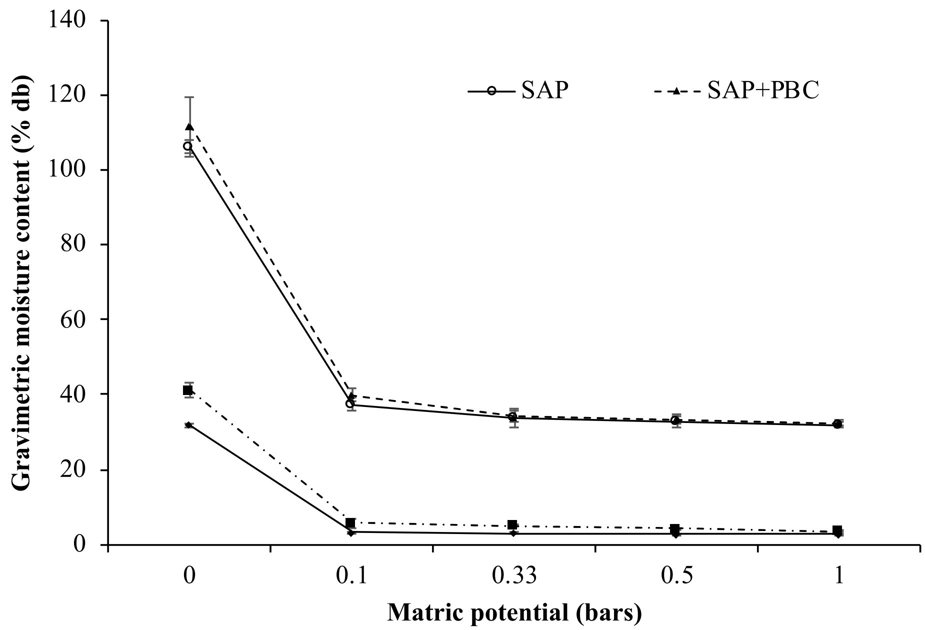


Figure 6.3 Water retention curves for treatments SAP, SAP+PBC, PBC (all mixed at 1% w/w with soil) and control.

In the water retention experiment, at saturation as well as at potentials 0.1, 0.33, 0.5 and 1 bar, water content in SAP+PBC and SAP treatments were significantly higher than that of PBC treatment and non-amended control (figure 6.3). Although, numerically, water content in SAP+PBC treatment was higher than that of SAP treatment at all matric potentials, they were not statistically different. This can again be explained by the small amount of biochar application rate, as discussed above. Similarly, PBC water content was numerically higher than that of non-amended control at all pressure values, the difference was significant only at saturation and 0.1 bar potential.

6.4.2 Plant Yield and Physiological Parameters

For potato crop grown in year 2015, tuber yields for treatments SAP+WW (0.78±0.27 kg plant^{-1}), WW (0.69±0.16 kg plant^{-1}) and FW (0.83±0.38 kg plant^{-1}) were statistically not different from each other. Similar observation was made for 2016 potato crop, where tuber yields for SAP+WW (0.51±0.07 kg plant^{-1}), SAP+GBC+WW (0.17±0.07 kg plant^{-1}) and WW (0.24±0.05 kg plant^{-1}) treatments were not significantly different than that of FW (0.26±0.17) (table 6.7). In case of spinach, yields for treatments SAP+WW (57.25±16.07 g plant^{-1}) and WW (41.48±22.20 g plant^{-1}) were not significantly different than that of FW (28.11±16.58 g plant^{-1}). These results indicate that wastewater can potentially be used for irrigating potato and spinach plants without compromising on yield.

Table 6.7 Yield and plant growth parameters for potatoes grown in years 2015 and 2016 for different treatments.

Year	Treatment	Parameter				
		Tuber yield (kg plant^{-1})	Tuber yield (metric ton ha^{-1})	Root weight (kg plant^{-1})	Above ground biomass (kg plant^{-1})	Total biomass weight (kg plant^{-1})
2015	SAP+WW	0.78±0.27^{ab}	39.89±6.39^{ab}	0.06±0.01^{a}	1.27±0.23^{a}	2.11±0.48^{a}
	SAP+GBC+WW	0.32±0.13^{b}	16.38±3.12^{b}	0.05±0.01^{a}	0.98±0.35^{a}	1.36±0.37^{b}
	WW	0.69±0.16^{ab}	35.14±3.85^{ab}	0.05±0.01^{a}	0.98±0.19^{a}	1.72±0.06^{ab}
	FW	0.83±0.38^{a}	42.10±9.05^{a}	0.05±0.01^{a}	0.85±0.11^{a}	1.73±0.48^{ab}
2016	SAP+WW	0.51±0.07^{a}	25.72±3.58^{a}	0.06±0.00^{a}	0.66±0.27^{a}	1.37±0.07^{a}
	SAP+GBC+WW	0.17±0.07^{b}	8.40±3.60^{b}	0.04±0.02^{ab}	0.72±0.55^{a}	0.60±0.23^{b}
	WW	0.24±0.05^{ab}	11.97±2.35^{ab}	0.02±0.01^{bc}	0.25±0.09^{a}	0.48±0.14^{b}
	FW	0.26±0.17^{ab}	12.99±8.67^{ab}	0.02±0.01^{c}	0.28±0.14^{a}	0.55±0.32^{b}

Values with different letters down the column are significantly different from each other (α=0.05).

Table 6.8 Yield and plant growth parameters for spinach grown in year 2016 for different treatments.

Treatment	Parameter		
	Total biomass weight (g plant^{-1})	Root weight (g plant^{-1})	Aboveground biomass weight (g plant^{-1})
SAP+WW	58.70±16.45^{b}	1.45±0.49^{ab}	57.25±16.07^{b}
SAP+PBC+WW	158.03±79.35^{a}	2.78±1.49^{a}	155.25±77.88^{a}
WW	42.58±22.78^{b}	1.11±0.59^{b}	41.48±22.20^{b}
FW	29.10±17.10^{b}	0.99±0.54^{b}	28.11±16.58^{b}

Values with different letters down the column are significantly different from each other (α=0.05).

Average per hectare potato production in Canada for years 2002-2011 was within the range of 27.5 to 31.7 tonnes (STATCAN, 2011), which is lower compared to the potato (2015) yields for SAP+WW, WW and FW treatments. However, yields for 2016 potato crop were low, as compared to 2015 crop, for all treatments. This could be due to differences in weather conditions. Treatment SAP+GBC+WW (2015 potato) exhibited lowest tuber yield which can be attributed to either transplant shock to the plants and/or high ash content of the biochar. Due to non-emergence, all the plants for this treatment were replaced. Plant roots are susceptible to injury during transplanting, causing a disturbance in water uptake and transpiration balance which may lead to water stress (Mountain and McKeen, 1965; Li et al., 2016). Transplant injury in rice, for example, can also lead to disturbance in various plant metabolic processes (Sasaki and Gotoh, 1999). Lower tuber yield in 2015 and 2016 crops for SAP+GBC+WW treatment can also be attributed to the biochar's high pH (10.27) and high ash content (77.45%), which is higher than most of the biochars (Singh et al., 2010). In a study by Smider and Singh (2014), it was concluded that, while soil application of high ash biochar is expected to have a positive effect on plant growth and yield due to its high nutrient content and pH, it can have a phytotoxic effect on plants grown in poorly buffered soils such as sandy soils. It was shown that green waste biochar application to sandy soil, at rates of 0.5% and 1.5% (w/w), caused a significant reduction in shoot dry matter and yield for corn plants. This was a direct result of high ash content (56.2%) and high pH (12.1) of the biochar, leading to OH^- toxicity and high osmotic potential due to presence of excess soluble salts. Soil used in the present study was classified as sandy soil (table 6.1).

No significant difference was observed in terms of root weight for potato plants grown in year 2015 (table 6.7). However, in year 2016, fresh root weights for SAP+WW and SAP+GBC+WW treatments were found to be significantly different than that of WW and FW respectively. This can be attributed to the fact that SAP was applied at top 0.10 m soil layer in year 2016, compared to its application at soil layer 0.15-0.25 m below the surface in year 2015. Highest root density for Russet Burbank potato pants is reported to be in top 0.10 m and 0.10-0.20 m of soil profile (Lesczynski and Tanner, 1976). Therefore, in year 2016, SAP was applied in high root density zone, compared to year 2015, where only half of the applied SAP (application depth of 0.15-0.25 m) was present in this zone. It is known that hydrogel polymer application in soil can lead to better plant root development (Hütterman et al., 1999; Orikiriza et al., 2009; Dehgan et al., 1994, Suresh et al., 2018). No significant difference was observed between aboveground biomass for all treatments for both 2015 and 2016 potato plants (table 6.7).

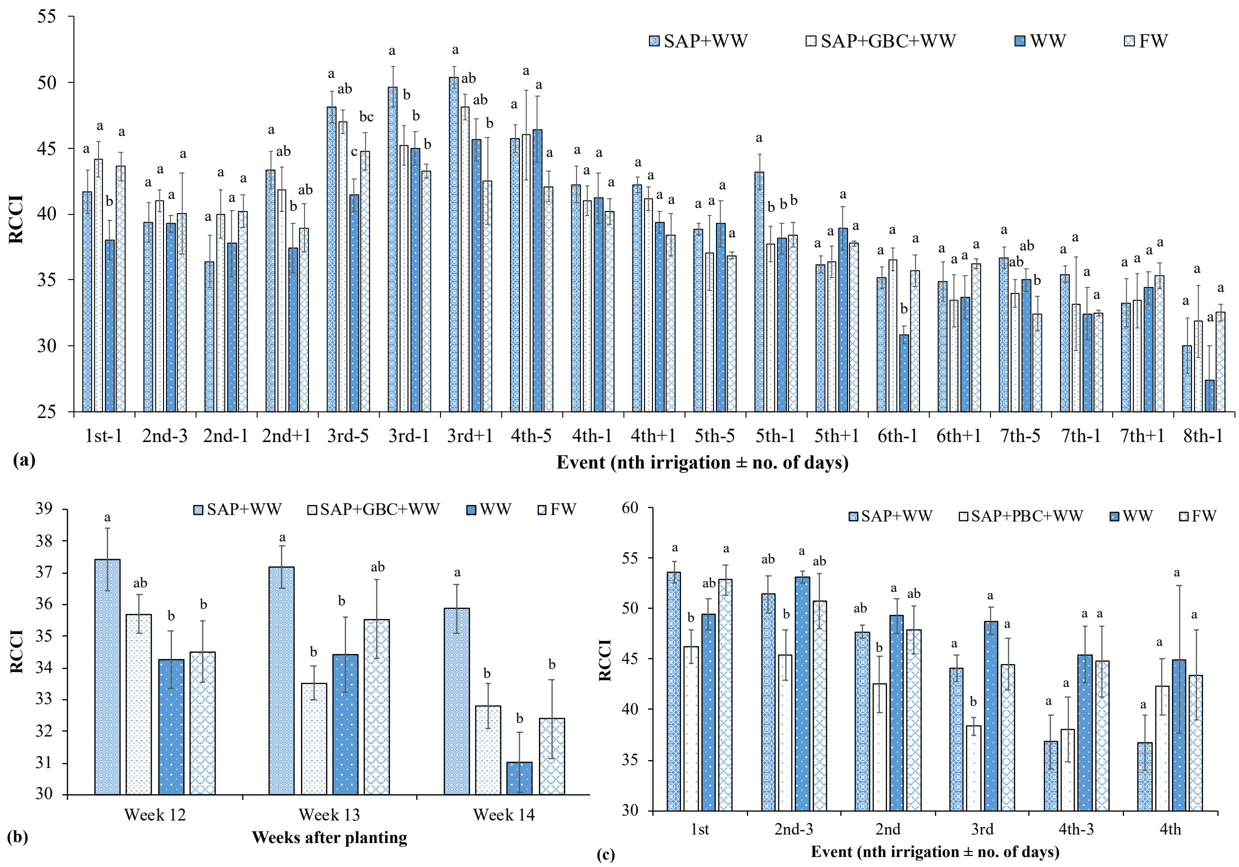
In case of spinach, yield for SAP+PBC+WW treatment (155.25±77.88 g $plant^{-1}$) was significantly higher than all other treatments (table 6.8). Similar trend was also observed for fresh root weight; the weight was significantly higher in SAP+PBC+WW treatment (2.78 g $plant^{-1}$), as compared to WW (1.11 g $plant^{-1}$) and FW (0.99 g $plant^{-1}$) treatments. However, no significant difference was observed between SAP+WW and SAP+PBC+WW treatments. Improved yields in spinach plants can be attributed to PBC incorporation in soil. Depending on the soil type, biochar incorporation can lead to addition and/or increased availability of certain nutrients, improved aeration and improved soil water holding capacity (Lehmann and Rondon, 2006; FFTC, 2001). In previous field and pot studies, biochar incorporation in soil has shown an increase in spinach yield (Milla et al., 2013; Zhang et al., 2011).

Table 6.9 Summary of Fixed effects for plant physiological parameters from repeated measures analysis.

Fixed effects	Potato 2015	Potato 2016			Spinach 2016					
	RCCI	RCCI	Leaf temperature	NDVI	RCCI	Photosynthetic activity	Stomatal conductance	Transpiration rate	Leaf temperature	NDVI
Treatment	*	*	-	-	*	-	-	-	-	-
Time	*	*	*	-	*	*	*	*	*	*
Treatment x Time	-	*	-	-	-	-	-	*	-	-

** Denotes statistically significant effects (p<0.05)*
- Denotes no significant effect (p≥0.05).

SPAD readings have been shown to be positively correlated to the nitrogen content of leaves, as well as photosynthetic and transpiration rates (Piekkielek and Fox, 1992). Effects of both, treatment and time, were significant on RCCI readings for all the crops (table 6.9). Significant effect of time on RCCI readings as well as on other physiological parameters, such as photosynthetic activity, stomatal conductance and transpiration rate, can be expected as values for these parameters change with time (Sestak, 1963; Piekkielek and Fox, 1992). In case of 2015 potato crop, mean RCCI value for plants grown in SAP+WW treatment was significantly higher than that of WW and FW treatments for all three weeks, and for weeks 12 and 14 respectively (figure 6.4b). SAP+GBC+WW treatment had significantly lower RCCI values than that of SAP+WW treatment. This can be explained by the transplantation shock that the plants grown in SAP+GBC+WW treatment had to withstand. However, higher chlorophyll content in SAP+WW treatment plants did not translate into higher tuber yields. This can be because of high aboveground biomass weight in SAP+WW treatment plants (table 6.7) as it indicates low dry matter distribution/allocation for the tubers (Balamani and Poovaiah, 1985).



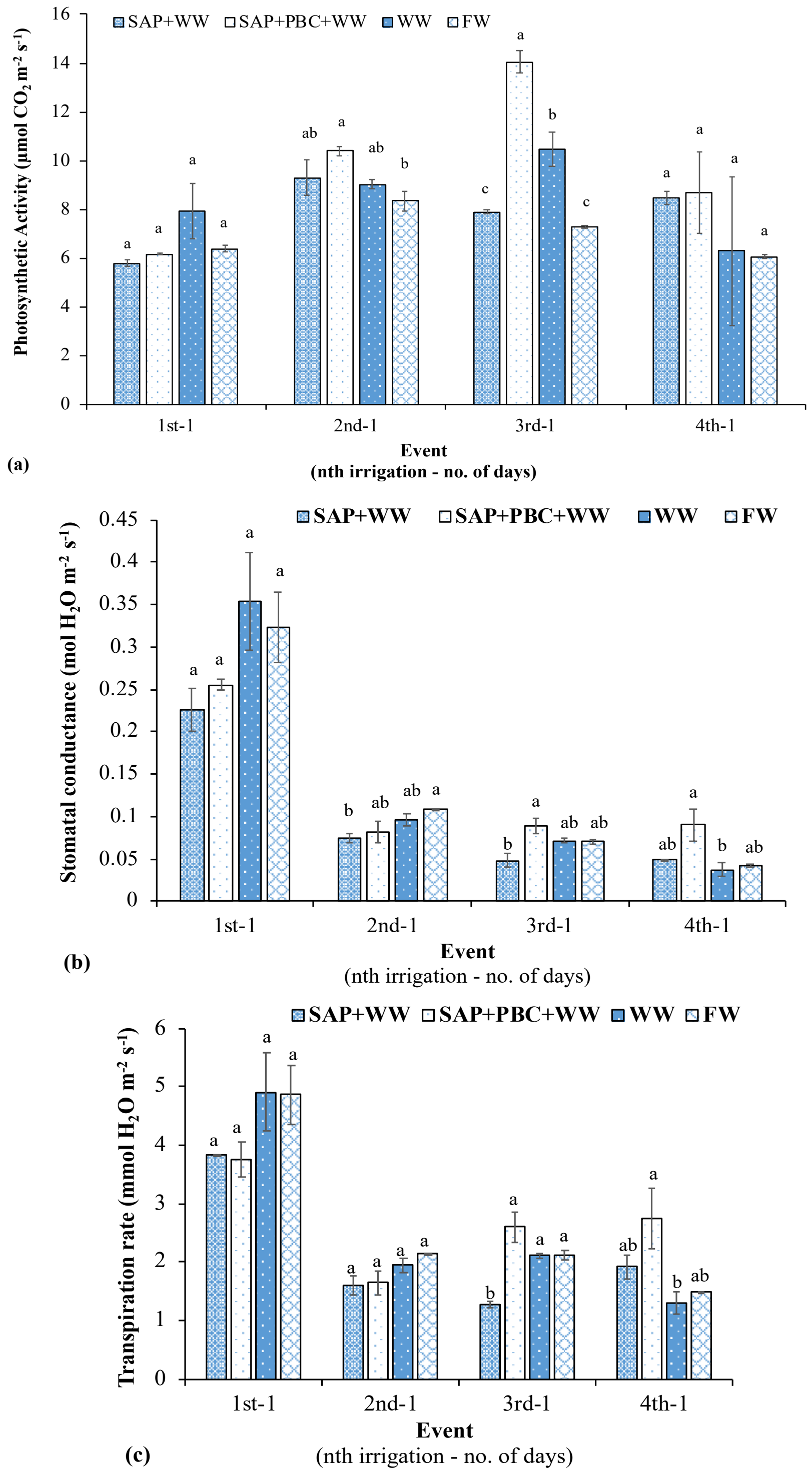
Different letters above the error bars indicate significant differences in mean RCCI amongst treatments for a given event/week (α=0.05).

Figure 6.4 Relative chlorophyll content index (RCCI) for different treatments for potatoes grown in (a) 2016 and (b) 2015, and (c) spinach grown in 2016.

For potatoes grown in 2016, RCCI values for WW treatment were found to be significantly lower than that of the SAP+WW treatment for several events (*1st-1, 2nd+1, 3rd-5, 3rd-1, 5th-1 and 6th-1*; events are coded as *nth irrigation ± no. of days*), but for most of the events, no significant difference amongst treatments was observed (figure 6.4a). Consequently, effect of *treatment x time* interaction on RCCI was found to be significant (table 6.9), implying that the effect of treatment on chlorophyll content is not constant across time. In the overall statistical model, SAP+GBC+WW treatment's mean RCCI value (39.42) was significantly higher than that of FW treatment (38.31), which, in turn, was significantly higher than WW treatment's RCCI reading (37.04). Mean RCCI value for SAP+WW treatment plants was found to be the highest numerically (40.10) but was not significantly different than any of the other treatments. Thus, it can be stated that SAP+GBC amendment has the potential to maintain increased nitrogen content in wastewater irrigated potato plants.

Treatment was also found to have a significant effect on SPAD readings for spinach crop (table 6.9), with WW treatment plants having highest overall mean RCCI value (48.75) which was significantly higher than that of SAP+WW (45.26) and SAP+PBC+WW (42.16) treatments. There was no significant difference between WW and FW treatments (47.88), as well as between FW and SAP+WW treatments. Despite statistically significant differences amongst treatments, numerical differences between them were small, and thus the differences have little practical importance. In a previous study (Liu et al., 2006), large differences between SPAD readings for spinach plants were observed based on nitrogen fertilizer application rates, with plants receiving greater amounts of N, exhibiting eight times higher SPAD values compared to the treatment receiving no N application. However, in this study, equal amounts of N fertilizer were applied to all treatments, which could explain smaller differences among SPAD readings between treatments.

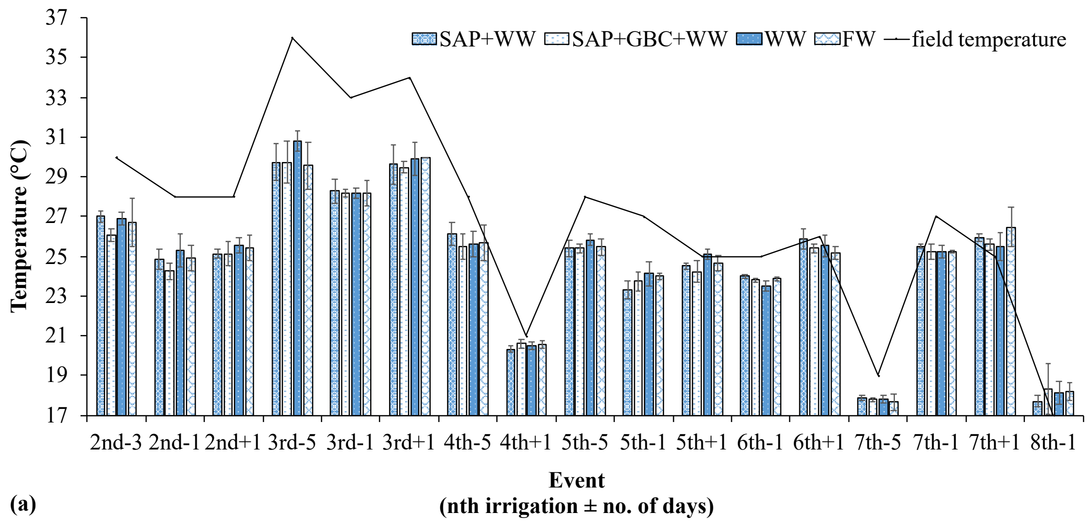
Treatments were not found to have a statistically significant effect on photosynthetic activity, stomatal conductance and transpiration rate for spinach plants (table 6.9). Stomatal conductance and transpiration rate decreased significantly after the first irrigation (figure 6.5b and 6.5c). Mean stomatal conductance and transpiration rate values for all treatments were within the range of 0.226 to 0.354 mol H_2O $m^{-2}s^{-1}$ and 3.74 to 4.91 mmol H_2O $m^{-2}s^{-1}$, respectively, on the day before first wastewater irrigation. On the day before second wastewater irrigation, the values dropped within the ranges of 0.074-0.109 mol H_2O $m^{-2}s^{-1}$ and 1.587-2.135 mmol H_2O $m^{-2}s^{-1}$ for stomatal conductance and transpiration rates, respectively. Also, maximum photosynthetic activity value was found to be 14.05 µmol CO_2 $m^{-2}s^{-1}$ (SAP+PBC+WW treatment, on the day before 3rd irrigation; figure 6.5a). Contrary to the observations made in the present study, Giri et al. (2016) in their study showed that photosynthetic activity (estimated using *Li-cor* 6400 equipment) in spinach plants grown under ambient conditions, 25 days after transplanting can be around 60 µmol CO_2 $m^{-2}s^{-1}$. This difference could be because of a fungal infection/wilt, since leaves turned yellowish prematurely for most plants.



Different letters above the error bars indicate significant differences in means amongst treatments for a given event (α=0.05).

Figure 6.5 Spinach (a) photosynthetic activity, (b) stomatal conductance and (c) transpiration rate values measured one day before irrigations for different treatments.

It is known that a higher leaf temperature is concurrent with lower photosynthetic rate, transpiration rate and ultimately productivity in a plant (Eller, 1977; Brown and Escombe, 1905). Non-availability of sufficient amount of water or water-stress in plants can lead to decreased transpiration rate which can cause elevated leaf temperature (Wiegand and Namken, 1966). In the present study, treatments did not have any significant effect on leaf temperatures for both potato and spinach plants grown in 2016 (table 6.9). As expected, leaf temperature varied proportionally with the ambient temperature (figure 6.6).



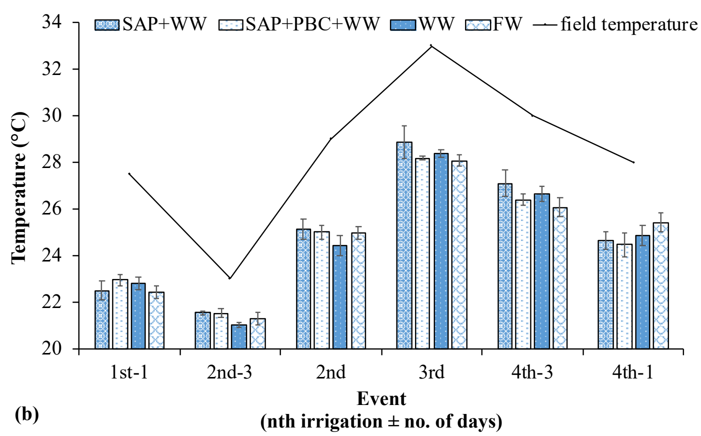
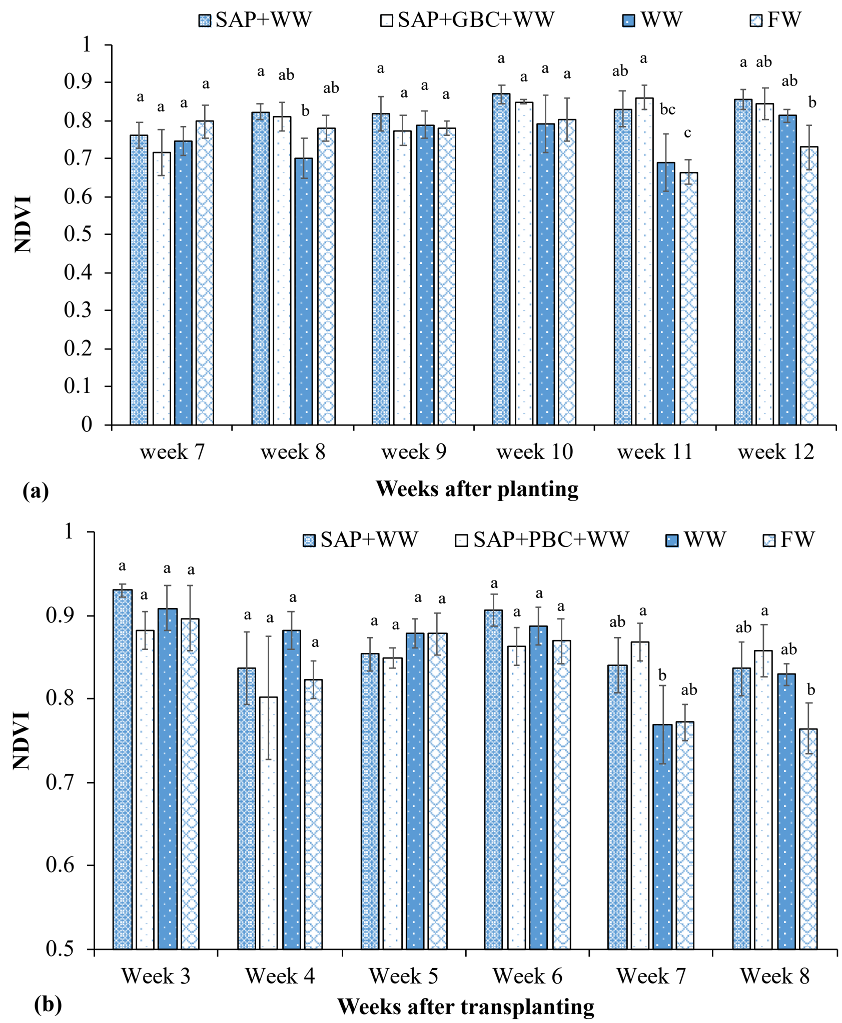


Figure 6.6 Leaf temperature for (a) potato and (b) spinach plants grown in 2016.

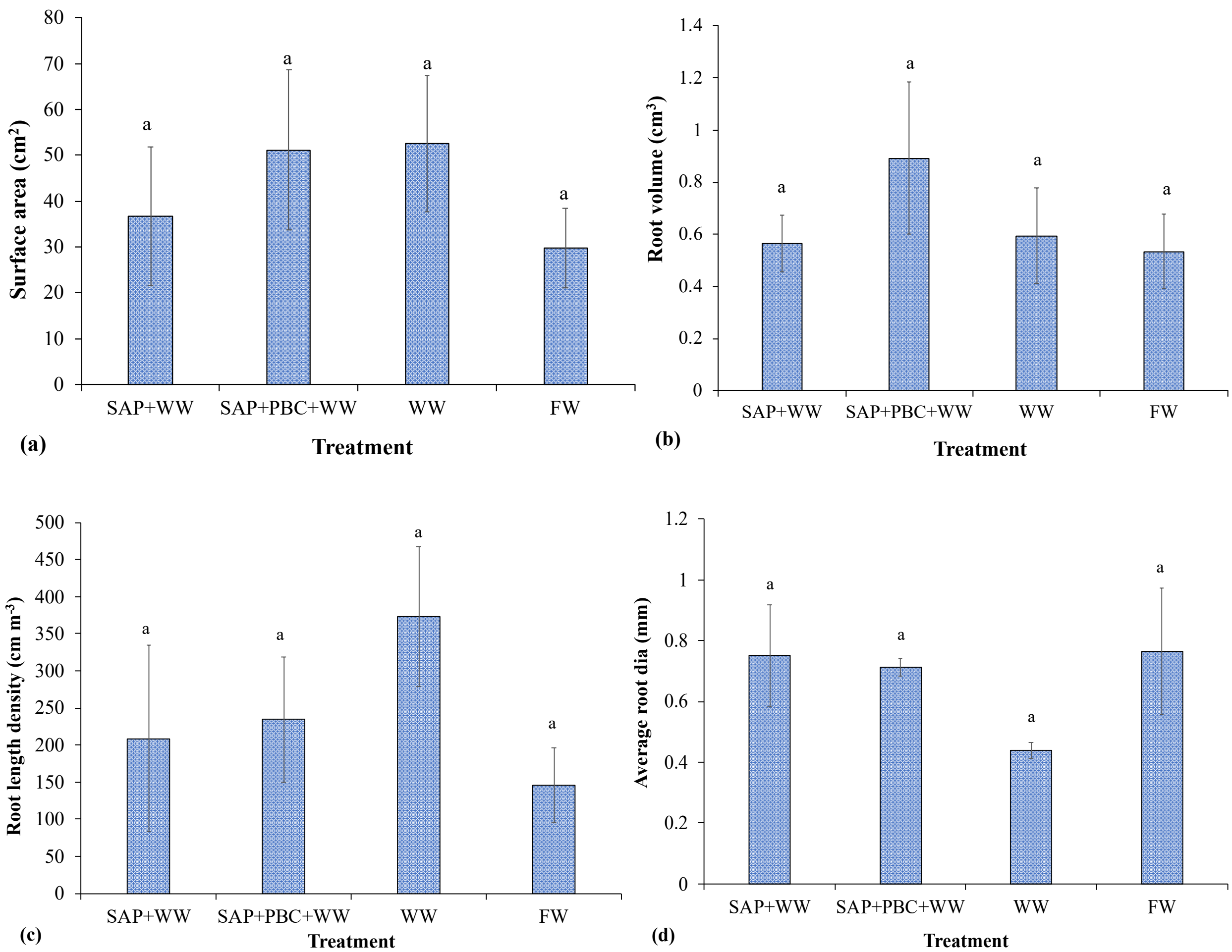


Different letters above the error bars indicate significant differences in mean NDVI amongst treatments for a given week (α=0.05).

Figure 6.7 Normalized difference vegetative index (NDVI) for different treatments for (a) potato and (b) spinach plants grown in 2016.

NDVI value for plants correspond to different wavelengths of light reflected by the leaves, and this spectral index is widely used for assessing plant health, growth, yield, stresses, and physiological parameters (Aparicio et al., 2000; Nilsson, 1995; Asrar et al., 1984). In healthy plants, pigments in leaves such as chlorophyll and xanthophylls mostly absorb visible light (Vis) and reflect majority of the near-infrared light (NIR), resulting in higher NDVI values (NDVI=(NIR-Vis)/(NIR+Vis)). In response to various stresses, photosynthetic rate in a plant can decrease, accompanied by higher visible light and lower NIR light reflectance leading to lower NDVI value (Kim et al., 2010). In general, positive correlations between plant growth, yield and NDVI values have been observed (Aparicio et al., 2000). No overall significant effect of the treatments on NDVI was observed for both potato and spinach plants grown in 2016 (table 6.9). However, week 10 onwards, numerically the NDVI values for treatments SAP+WW and SAP+GBC+WW for potatoes were found to be greater than that of non-amended WW and FW treatments (figure 6.7a). Similar observation was made for spinach plants for treatments SAP+WW and |SAP+PBC+WW treatments, week 7 onwards.

6.4.3 Root Structure Analysis



Different letters above the error bars indicate significant differences amongst treatments (α=0.05).

Figure 6.8 (a) Root surface area, (b) volume, (c) length density and (d) average diameter for spinach 2016 crop.

Root surface area, root volume, thickness and length density are important root characteristics that represent root growth and structure and can also affect plant health (Kerk and Sussex, 2012; Gamalero et al., 2002; Gunawardena et al., 2001; Aljuaifari et al., 2018). No significant difference was found for these parameters among different treatments for spinach plants (figure 6.8). Since nutrients (fertilizer) and water were applied at recommended rates, it might be possible that the differences due to treatment effects on root growth were not conclusive.

6.5 Conclusions

Through the findings of this study, it was concluded that wastewater could potentially be used for irrigating potato and spinach crop without any adverse effect on yield. However, use of biochar with high ash content and high pH can lower tuber yield for wastewater-irrigated potato plants grown in sandy soils. It was found that, pyrolyzed biochar (PBC) incorporation in soil lead to significantly higher spinach yield in SAP+PBC+WW treatment compared to other treatments. The study also highlighted the effect of SAP and SAP-biochar mix on soil water retention. SAP application led to higher water retention in soil. However, if SAP and biochar mixture is applied to the soil, higher percentage of ash in biochar leads to reduced water absorption by SAP because of water-soluble salts present in biochar ash.

Apart from yield, effect of treatments on plant growth parameters was also studied. RCCI values for SAP+WW treatment in potato plants (2015) was found to be significantly higher than WW and FW treatments. However, because of transplantation shock, RCCI values for SAP+GBC+WW treatment was lower than that of SAP+WW treatment in year 2015. For year 2016 potato plants, mean RCCI value was found to be significantly higher in SAP+GBC+WW treatment compared to WW and FW treatments. No significant differences were found amongst treatments for photosynthetic activity, stomatal conductance, transpiration rate, leaf temperature, NDVI and root structure development for spinach plants as well as leaf temperature and NDVI values for potato plants (2016). For future studies it is recommended that, the beneficial effects of SAP and SAP biochar mix amendments could potentially be further explored by inducing water stress to the plants through limiting watering frequency and rate of fertilizer application.

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Chapter 7: Summary and Conclusions

7.1 Summary

Water has played a pivotal role in the development of human civilization and it will continue to do so in the future. There has been an increase in demand of freshwater due to increasing human population. The global human population is projected to reach 9.6 billion by the year 2050 and this would also lead to further increase in demand for freshwater for urbanization, industrialization and agriculture. Thus, there is a need to increase our water use efficiency to meet the increasing water demand in the future. Agriculture is the biggest consumer of freshwater, as it utilizes approximately 70% of the drawn freshwater. Therefore, using wastewater from urban and industrial settings for irrigation is encouraged and even practiced in some parts of the world. However, wastewater is known to be a source of contaminants such as heavy metals, which can be taken up by food crops or leach down to water table and harm human health. In order to address the issue of heavy metal uptake in edible parts of wastewater irrigated plants, use of polyacrylamide super absorbent polymer (SAP) and SAP-plantain peel biochar mix as soil amendments was investigated. Various field and laboratory studies were also carried out to test the effectiveness of these amendments.

7.2 Conclusions

7.2.1 Sorption Test

Findings of laboratory sorption test are summarised below:

- Owing to the presence of high density of sorption sites in SAP, SAP+GBC and SAP+PBC amended soils, results of the sorption test suggest that the three treatments performed better in sorbing Cd, Cu, Fe and Zn, as compared to control across different concentrations used. Less than 0.5% and 2.50% of the adsorbed metals, Cd and Zn respectively, were desorbed by the treatments, which was lower than the desorption rates observed for non-amended control.
- The treatments also performed better in sorbing Fe and Cu, as compared to control. However, SAP+GBC and SAP+PBC treatments exhibited higher desorption (2.00-28.63% and 4.10-21.39%, respectively) of the adsorbed Fe metal, as compared to SAP and control treatments, possibly due to high Fe content of the biochar (669.12 ± 86.35 mg

kg^{-1} and 649.01 ± 58.81 mg kg^{-1} for GBC and PBC, respectively). For Cu, less than 2% of the adsorbed metal was found to desorb for all treatment and across all concentrations.

- Non-amended control performed better than other treatments for Cr adsorption because of low adsorbent-solution mixture pH, exhibited by control. Treatments desorbed less than 35% of the adsorbed metal, whereas the control desorbed less than 13% of Cr.
- In the case of Pb, performance of all the treatments, including control, was comparable and less than 4% desorption was observed for all treatments.

7.2.2 Soil pH and CEC

Due to alkalinity of biochar and its ability to facilitate cation exchange, treatment of soil with SAP+GBC and SAP+PBC amendments led to noticeable increase in surface soil pH ($p<0.10$ and $p<0.05$, respectively) and cation exchange capacity (CEC), as compared to SAP and control treatments ($p<0.05$). SAP treatment had no significant effect on soil pH and CEC, as compared to control.

7.2.3 First Year Field Study with Potatoes

- All heavy metals were found to accumulate in soil with subsequent irrigations.
- None of the heavy metals were detected in soil below 0.30 m of the surface as well as in the leachate for all treatments. Cd, Cr, and Cu were also not detected at 0.10 m below the surface for all treatments, including control. Also, except Fe, no other metal was detected at soil samples taken from 0.30 m depth below the surface.
- For metals which were detected at 0.10 m depth below soil surface (Fe, Pb and Zn), concentrations at surface were significantly higher than at 0.10 m depth for all treatments.
- Because of SAP application at depth of 0.15-0.25 m below the surface, no significant differences were observed in metal concentrations between SAP and control treatments at surface and at depth of 0.10 m. However, due to biochar application in the top 0.10 m of soil, the SAP+GBC treatment retained significantly greater amounts of Cd and Zn in topsoil layer, compared to the non-amended control ($p<0.05$).
- Plant roots exhibited significantly higher concentrations of metals in general, as compared to other parts ($p<0.05$), irrespective of the treatment. Also, higher amounts of metals were accumulated in tuber peels as compared to tuber flesh tissue in all treatments.

- SAP treatment significantly reduced Cd uptake into the edible parts of the plant (potato tuber flesh and peel) as well as Cu uptake into potato peel as compared to the non-amended control ($p<0.05$).
- SAP+GBC treatment significantly reduced Cd, Cu and Zn uptake in potato tuber flesh as well as Cd uptake in tuber peel as compared to the control ($p<0.05$).
- Acrylamide monomer was not detected in any edible parts of potatoes for any of the SAP amended treatments.

7.2.4 Second Year Field Study with Potato

- All heavy metals were found to accumulate in soil with subsequent irrigations.
- SAP treatment of soil led to significantly higher concentration of Cd, Cu, Fe and Zn metals in topsoil at the end of the experiment, as compared to control ($p<0.05$).
- Compared to control, SAP+GBC treatment was also able to retain significantly higher amounts of Cd, Cr and Fe in topsoil ($p<0.05$) at the end of the experiment (after 8 wastewater irrigations).
- Compared to control, treatments SAP and SAP+GBC were ineffective in sorbing significantly higher amounts of Cr and Zn, respectively. Also, both treatments were not able to retain higher amounts of Pb, as compared to control, after eight irrigation events. Overall, SAP treatment performed better than SAP+GBC treatment in retaining Cu, Fe and Zn in topsoil.
- Similar to the findings of the previous year, plant roots exhibited significantly higher concentrations of metals in general, as compared to other parts ($p<0.05$). Higher amounts of metals were accumulated in tuber peels, as compared to tuber flesh tissue, for all treatments.
- Both SAP+GBC and SAP treatments were able to considerably reduce Cd uptake in tuber flesh and peel tissue, as compared to control ($p<0.10$).
- SAP+GBC treatment was also able to significantly reduce Cr and Pb uptake in tuber flesh and peel tissue, as compared to control ($p<0.05$). No significant differences were observed for Cr uptake in tuber flesh tissue for all treatments, including the FW treatment. However, Cr concentration in tuber flesh was found to be within permissible limits (0.5 mg kg^{-1}) for all treatments. SAP and SAP+GBC treatments were able to significantly reduce Cr concentration in tuber peels to lie within the permissible limit as compared to control

($p<0.05$). The SAP amendment in soil was able to prevent significant increase in the uptake of Pb by potato peel tissue and maintained it to a level comparable to that of the FW treatment.

- SAP+GBC and SAP treatments were able to significantly reduce Zn uptake in tuber flesh tissue, compared to control ($p<0.05$). Also, both these treatments were able to prevent increased Zn uptake in tuber peel tissue and maintained the concentration of the metal similar to that found for FW treatment.
- Copper concentrations in tuber peel tissue for SAP+GBC and SAP treatments were comparable to that in FW treatment; whereas, wastewater control exhibited significantly higher metal concentration as compared to FW treatment ($p<0.05$), highlighting the amendments' potential to prevent increased Cu uptake by wastewater irrigated potato plants.
- Mean Fe and Pb content in tuber flesh tissue samples was found to lie within the permissible limits for all treatments.
- Both SAP and biochar applications are done in the topsoil, just below the soil surface, therefore, they could persist and continue to remain effective for a good number of years.

7.2.5 Field Study with Spinach

- SAP treatment was able to retain considerably higher amounts of Cr ($p<0.10$) and Cu ($p<0.05$) in topsoil, as compared to control, at the end of the experiment.
- Except Fe, no other metal was detected at 0.10 m depth below the soil surface. No significant differences were observed between treatments for Fe concentration in soil at 0.10 m depth.
- After the last irrigation event, no significant differences were observed between topsoil metal concentration in SAP+PBC and SAP treatments, as well as between SAP+PBC and control, for all metals. This may be attributed to binding of sorption sites in the polymer by water soluble salts, originating from the ash content of biochar, thus reducing their availability for binding heavy metals and offsetting overall sorption capacity of SAP+PBC treated soil in a complex soil-water-plant system.
- SAP amended soil was able to retain significantly higher amount of Fe in topsoil, as compared to control, at the end of the experiment.

- SAP treatment was able to avoid the increased uptake of Cr in stems of spinach plant due to irrigation with contaminated wastewater, when compared to control. As compared to control, SAP and SAP+PBC treatments were able to significantly reduce Cu uptake by plant stem ($p<0.05$). Since Pb concentrations in the plant stem tissue for both the treatments were found to be similar to that in FW treatment; however, control exhibited significantly higher plant stem Pb concentration as compared to FW ($p<0.05$). This highlights the ability of the amendments in preventing increased Pb uptake by the plants
- Copper metal was found to be within permissible limits in spinach leaves for all treatments, including control.
- In leaf samples from first harvest, no significant differences were observed between concentrations of heavy metals Cr, Cu, Pb and Zn for all the treatments, including the FW treatment. Same observation was also made for second harvest leaves in case of Fe, Pb and Zn metals.
- Mean concentrations of all heavy metals in leaves from SAP+PBC treatment was numerically lower in the second harvest, compared to the first, possibly because of stabilization and activation of biochar in soil with time. Also, SAP+PBC treatment was able to avoid increased uptake of Cd in leaves from the second harvest due to wastewater irrigation, as compared to control.
- SAP+PBC treatment exhibited significantly higher concentration of Fe than control ($p<0.05$) for leaves collected during the first harvest, which may be because of the Fe content of the biochar itself (649.01 mg kg^{-1}).
- Concentrations of Cr and Cu in spinach leaves from the second harvest were found to be significantly higher in the control treatment, as compared to the FW treatment ($p<0.05$); however, concentrations of these metals for SAP+PBC and SAP treatments were not found to differ significantly than the FW treatment, highlighting the ability of the amendments to avoid increased uptake of contaminants by spinach plants irrigated with wastewater.

7.2.6 Field Study – Plant Yield and Growth Parameters

- Amending the soil with SAP led to higher soil water retention as compared to control; however, because of water-soluble salts present in biochar, SAP+GBC treatment exhibited reduced soil water retention as compared to SAP treatment.

- Amending the soil with high ash content and high pH biochar can potentially reduce potato tuber yield in plants grown on sandy soils.
- Pyrolyzed biochar (PBC) incorporation in soil led to significantly higher spinach yield in SAP+PBC treatment, as compared to other treatments.
- Relative chlorophyll content index (RCCI) in the first-year potato plants, grown on SAP amended soil, was found to be significantly higher than that of control and FW treatment. Potentially due to transplantation shock, the observed RCCI values for SAP+GBC treatment was found to be lower than that of the SAP treatment. However, for second-year potato plants, RCCI values of plants grown on SAP+GBC amended soil were found to be significantly higher, as compared to control and FW treatment ($p<0.05$).
- No significant differences were found amongst treatments for photosynthetic activity, stomatal conductance, transpiration rate, leaf temperature, NDVI and root structure development for spinach plants as well as leaf temperature and NDVI values for potato plants.
- Wastewater could potentially be used for irrigating potato and spinach crop without any adverse effect on yield.

7.3 Future Recommendations

This study provides an insight into the role of biochar and polyacrylamide super absorbent polymer-based soil amendments on heavy metal immobilization in soil and reduced uptake in wastewater irrigated food crops grown on sandy soils. However, more studies are required to broaden the horizon of understanding about the role and effects of hydrogel-based soil amendments in agricultural fields receiving wastewater irrigation, as well as to address the limitations of the present study. The following suggestions are recommended to design future studies:

1. Unlike organic compounds, heavy metals are relatively less prone to microbial and chemical degradation. Therefore, irrigating agricultural fields with wastewater for prolonged periods may lead to significant build up of metals in hydrogel and biochar amended soils, and consequently, render the land unfit for food production. A study to determine the ‘effective’ time period for the soil amendments, in combination with the use of phytoremediation (with non-edible crops) to remove accumulated metals from the soil, should be carried out. The

frequency of employing phytoremediation techniques should also be ascertained, as metals may leach down and contaminate groundwater reserves, once the soil becomes saturated with heavy metals due to prolonged wastewater irrigation.

2. Use of sewage sludge derived biochar in contaminant mobility and plant uptake studies (similar to those presented in this thesis) should be undertaken, since this feedstock is readily available in developing countries, where wastewater irrigation can lead to serious environmental issues.
3. Effects of different kinds of hydrogel and biochar soil amendments on soil microbiome in the rhizosphere should be studied.
4. Effect of hydrogel-based soil amendments on reducing uptake of commonly found wastewater contaminants other than heavy metals (such as pharmaceuticals, sex hormones, pesticides, plastics and antibiotics) should be studied.
5. This study can be modified to investigate role of SAP-based soil amendments on heavy metal mobility in soil and uptake by wastewater irrigated food crops under varying water stress levels.
6. Similar studies can also be performed on other soil types for different crops, to assess the efficacy of SAP-based amendments and construct recommendations based on soil and crop type. These studies should be performed for a minimum of two seasons to account for variability in weather conditions.
7. Hydrogels synthesized using natural products (such as cellulose) can also be used in similar future studies, which will alleviate concerns regarding safe use of synthesized superabsorbent polymers.
8. Varying application rates of hydrogel-based amendments should be investigated to identify the most optimum rate to use.
9. Cost-benefit analysis of using hydrogel-based soil amendments on a large scale should also be performed. Findings from such a study would be useful in drafting government policies, such as providing subsidies to low-income farmers to adopt this technology.

Chapter 8: Contributions to Knowledge

To the best of the author’s knowledge, no study has yet been performed to investigate the effect of polyacrylamide super absorbent polymer (SAP)/hydrogel and SAP-plantain peel biochar mix as soil amendments for remediation of heavy metals which are introduced through untreated wastewater irrigation, and which co-exist in wastewater along with other contaminants such as antibiotics, steroidal sex hormones, bisphenol plastics, pharmaceuticals as well as other heavy metals. It was shown that polyacrylamide SAP and biochar amendments could be used in parts of the world, inadvertently utilizing untreated wastewater for irrigation, in order to mitigate the effects of heavy metal uptake by food crops. The study led to the following contributions to knowledge:

1. Use of polyacrylamide SAP and SAP-plantain peel biochar soil amendments can not only lead to reduced uptake of heavy metals by wastewater irrigated crops (both underground and aboveground) grown on sandy soils, but also reduce heavy metal mobility in the soil. Amending topsoil with SAP alone exhibited greater heavy metal retention. Sorption-desorption tests performed in laboratory also confirmed that SAP and SAP-plantain peel biochar amendments could immobilize co-existing heavy metals present in wastewater. Once adsorbed, it is quite likely that the heavy metals will not desorb in appreciable amounts.
2. Wastewater irrigated potatoes may have higher concentrations of heavy metals in tuber peels, as compared to tuber flesh, even when they are grown on hydrogel and biochar amended soils. Therefore, when the source of irrigation water is unknown, it is advisable to peel off the potatoes before consumption.
3. Acrylamide monomers were not detected in potato tubers grown using polyacrylamide-based hydrogel soil amendments. Therefore, it appears to be safe to use acrylamide-based hydrogels in agriculture.
4. SAP-biochar soil amendment can increase soil water retention in sandy soils.

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