THE ROLE OF BIOCHAR IN ENHANCING SAFE USE OF UNTREATED WASTEWATER IN AGRICULTURE

Christopher Nzediegwu

Department of Bioresource Engineering

McGill University

Montreal Canada

December, 2018

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Doctor of Philosophy

Abstract

THE ROLE OF BIOCHAR IN ENHANCING SAFE USE OF UNTREATED WASTEWATER IN AGRICULTURE

In many developing countries, water scarcity and the growing population are becoming problematic. Therefore, the reuse of wastewater for irrigation provides an alternative management option. Irrigation with poorly treated or untreated wastewater could, however, pose risk to human health due to the presence of a wide range of contaminants, including heavy metals, which can move into the edible parts of various crops such as potatoes (Solanum *tuberosum L.*) and spinach (Spinacia *oleracea L.*).

The study aimed to investigate biosorbent role in the remediation of heavy metals in soil and crops irrigated with untreated wastewater. Both aboveground and belowground crops were selected to better assess the effect of rooting system on the plant uptake of heavy metals.

To achieve this goal, a field lysimeter experiment was undertaken to elucidate the fate and transport of six water-borne heavy metals (Cd, Cr, Cu, Fe, Pb and Zn) in irrigation water applied to potatoes (cv. Russet Burbank) and spinach grown on a sandy soil. Plantain peel biochar (1% w/w) was incorporated in the top 0.1 m of soil. All the control and biochar treatments were replicated three times in a completely randomized design carried out on nine outdoor PVC lysimeters (1.0 m height × 0.45 m diameter).

In a two-year study, potatoes were planted, irrigated at 10-day intervals, leachate samples were collected, followed by soil samples collected two days after each irrigation. Results showed that all heavy metals accumulated in the top soil; Fe, Pb and Zn were detected at 0.1 m depth; while

only Fe was detected at 0.3 m depth. No heavy metals were detected in the leachate. Matured potatoes were harvested and separated into flesh, peel, leaf, stem and root. Results indicated that heavy metals translocated to all parts of the potato plant. The heavy metals were relatively low in the potato parts under freshwater (vs. wastewater). Biochar-amended-soil significantly ($p<0.05$) reduced only Cd and Zn in tuber flesh (69% and 33%, respectively) and peels compared to the non-amended wastewater control. Interestingly, biochar amendment, after the second season significantly ($p<0.05$) reduced Cd, Cu, Cr, Pb and Zn in the edible flesh.

As an example of aboveground crops, spinach was planted in lysimeters, irrigated every 10 days and harvested twice for heavy metal analysis. Results showed that biochar amendment improved CEC and increased the pH of the soil, which resulted in a 42% reduction of Zn in spinach leaves. The impact of biochar on translocation of other heavy metals (Cd, Cu, Cr, Fe, and Pb) to spinach leaves was at minimum, possibly due to competition with other compounds in the soil solution.

The effect of biochar on potato yield was also studied for the two seasons. It was found that: (1) In the first season, the yield was significantly less in the biochar treatment, possibly due to germination delay in the biochar amended lysimeters. (2) In the second season with no germination delay observed, the yield was similar in the presence and absence of biochar. Of note, yields were not affected even though significantly higher ($p<0.05$) heavy metals were taken up by different parts of the potato plants under wastewater irrigation (vs. freshwater). This can be alarming to some degree as the farmer may be getting the expected yield but with unhealthy potatoes, and not realizing this at all. It was concluded that effect of plantain peel biochar on the plant health parameters and yield of potatoes was not significant.

Overall, biochar amendment, with improved pH and CEC, showed high potential in the immobilization of heavy metals in soil, thereby reducing their uptake by plants. Therefore, the application of biochar as soil amendment could result in safer use of wastewater irrigation for crops. The accumulation of heavy metals in soil and uptake by plant parts, however, were crop-dependent.

Résumé

Face aux pénuries en eau et une rapide croissance démographique, chacun problématique dans plusieurs pays en voie de développement, la réutilisation des eaux usées pour l'irrigation offre une alternative de gestion permettant de conserver les ressources en eau. Cependant, l'irrigation avec des eaux usées insuffisamment ou non traitées présente un risque à la santé humaine puisque plusieurs contaminants, dont les métaux lourds, peuvent se retrouver dans ou sur les parties comestibles de diverses cultures tels les pommes de terre (*Solanum tuberosum* L.) et épinards (*Spinacia oleracea* L.).

La présente étude visa à éclaircir le rôle des sorbants biologiques dans la restauration des sols et cultures contaminés avec des métaux lourds par voie de leur irrigation avec des eaux usagées non-traitées. Des cultures comestibles de surface ou souterraines furent choisis afin de mieux évaluer l'effet du système racinaire sur l'assimilation de métaux lourds par une culture. Une étude lysimétrique en champ fut entreprise afin d'élucider le destin et le transport de six métaux lourds (Cd, Cr, Cu, Fe, Pb and Zn) présents dans des eaux usées servant à irriguer des pommes de terre (cv. Russet Burbank) ou des épinards cultivés sur un sol sablonneux. Du biochar de pelures de plantain fut incorporé dans la couche de 0.10 m en surface du sol à un taux de 1% (pondéré selon le poids). Les traitements témoins et d'ajout de biochar furent répétés trois fois dans un protocole complétement aléatoire distribué à travers 9 lysimètres en PVC (hauteur, 1.0 m × diamètre, 0.45 m), situés à l'extérieur.

Les pommes de terre furent plantées, irrigués à un intervalle de 10 jours, des échantillions de lixiviat prélevés, puis des échantillons de sol prélevés 2 jours après chaque irrigation. Tous les métaux lourds s'accumulèrent dans la couche supérieure du sol: le Fe, Pb, et Zn étant détectés à

une profondeur de 0.1 m, puis le Fe détecté jusqu'à une profondeur de 0.3 m. Aucun métal lourd ne fut détecté dans le lixiviat. Les pommes de terre furent récoltées à leur complète maturité et séparés en chair, pelure, feuille, tige et racine. On retrouva des métaux lourds à travers toutes les plants de pomme de terre, mais à des niveaux plus bas pour les plants irrigués avec de l'eau douce plutôt que des eaux usées. Par rapport au témoin n'ayant reçu aucun biochar, l'ajout de biochar au sol diminua de façon significative ($p<0.05$) les teneurs en Cd et Zn de la chaire des tubercules (69% et 33%, respectivement) ainsi que celle des pelures. Singulièrement, après la seconde saison de culture, l'ajout de biochar diminua de façon significative ($p<0.05$) les teneurs en Cd, Cu, Cr, Pb and Zn de la chair comestible.

Comme exemple de culture en surface, des épinards furent plantés dans les lysimètres. Cette culture fut irriguée aux 10 jours et les feuilles échantillonnées à deux occasions pour une analyse de métaux lourds. L'ajout de biochar au sol améliora son CEC et augmenta son pH, donnant lieu à une réduction de 42% du Zn dans les feuilles. L'effet d'un ajout de biochar au sol n'eut qu'un effet minime sur le mouvement des autres métaux lourds (Cd, Cu, Cr, Fe, and Pb) vers les feuilles d'épinards, le résultat probable de leur compétition avec d'autres cations dans la solution du sol.

L'effet d'un ajour de biochar sur le rendement des pommes de terre fut évalué sur une période de deux saisons. Lors de la première saison, le rendement avec ajout de biochar (*vs.* le témoins sans ajout) fut significativement moins élevé, possiblement à cause d'une germination retardée dans les lysimètres ayant reçu du biochar. Par contraste, lors de la seconde saison, aucun retardement de la germination ne fut noté, et les rendements en présence/absence de biochar furent semblables. Il est à noter que ce manque de différence en rendements (avec/sans biochar) eu lieu même si les différentes parties des plants ayant reçu des eaux usées montrèrent tous une accumulation de métaux lourds significativement ($p<0.05$) plus élevé que dans les plants irrigués avec de l'eau douce. Ceci pourrait être préoccupant, puisqu'un producteur pourrait atteindre le rendement attendu, tout en ayant produit des pommes de terre insalubres sans le savoir. Il est à conclure que l'ajout de biochar de pelures de plantain au sol à un taux de 1% n'eut aucun effet significatif, ni sur l'aspect santé des pommes de terre, ni sur leur rendement. L'ajout du biochar, augmenta le pH et la CEC du sol, mais ces impacts positifs furent masqués par une suffisance d'éléments nutritifs dans le sol.

En général, l'ajout de biochar démontra un potentiel élevé pour l'immobilisation des métaux lourds dans le sol, réduisant ainsi leur assimilation par les plantes. L'accumulation de métaux lourds dans le sol et leur assimilation et distribution dans différentes parties de la plante différa selon l'espèce cultivée. Potentiellement, l'ajout de biochar au sol permettrait donc une utilisation plus sécuritaire des eaux usées pour l'irrigation de certaines cultures.

Acknowledgements

I express my sincere gratitude to my supervisor, Dr. Shiv Prasher, for responding to my email back in 2014 when I was in search of a supervisor in a reputable institution of learning to pursue a PhD. Since then, he has presented himself as not just my supervisor, but also my academic role model. His consistent guidance through personal and group meetings, emails and even phone conversation has in no doubt sharpened and enabled me in the whole process of my thesis.

Special thanks goes to Dr. Eman ElSayed, a post-doctoral fellow, and Dr. Ramanbhai Patel, a research associate, who served as great guides. I am grateful with regards to several discussions with Dr. Danielle Donnelly, Dr. Asim Biswas, Dr. Valerie Gravel, Dr. Kebba Sabally, Dr. Yogendra Narasimhamurthy Kalenahalli, Dr. Samson Sotocinal, Dr. George Dodds and Dr. Maryam Kargar.

I will not fail to thank Ms. Helene Lalande, Dr. Michael Ngadi and Dr. Benoit Cote for making their labs available even during holidays, when I needed to catch-up with deadlines. I am also thankful to Mr. Guy Rimmer, and Mr. Michael Bleho for access to their greenhouse and cold room, respectively. Also, I am thankful to Mr. Paul Meldrum, and Ms. Chantal Charette who provided fertilizers, pesticides and oxyvet used in the field study.

My thanks goes to Ms. Sedigheh Zarayan, the manager of soil and water quality lab and to our administrative staff, Ms. Susan Gregus and Ms. Christine Trudeau, for their great support.

Graduate students in soil and water quality lab, namely Jaskaran Dhiman, Ali Mawof, Sadananda Sharma, Joba Purkaystha, Negar Sharifi Mood, Harmanjot Kaur, Rahul Suresh, Sukhjot Mann, Michael Saminsky, Peter Miele, Tian’ai Zhou, Peining Guan, and Sara Zeidan deserve my accolades for their support.

Also, I am thankful to Anishaben Patel, Abu Mahdi Mia, Shubhanker Joshi, Lakshitaa Lugun and Emma Anderson, who were undergraduate students (summer interns) with soil and water quality lab.

I appreciate the financial support from the Tertiary Education Trust Fund (TETFund) Nigeria and the India-Canada Centre for Innovative Multidisciplinary Partnerships to Accelerate Community Transformation and Sustainability (IC-IMPACTS).

I will also like thank the entire community of Nigerian students and other friends in McGill University, especially those at Macdonald campus, for their social and moral support. My thanks also goes to the entire Restoration House family for their spiritual support.

The last but not the least goes to Ms. Blessing Nzediegwu, my lovely wife, for her emotional support; to our lovely son, Oluomachi Zenith Nzediegwu; to my brother, Emmanuel Nzediegwu and to other family members, especially my late father, Deacon Joshua Nzediegwu, for their love, support and understanding all these while.

Dedication

This thesis is dedicated to God Almighty for his infinite mercies and love throughout my studies.

Thesis format and contribution of authors

In accordance to McGill University thesis presentation guidelines, this thesis is written by the candidate in manuscript format. The thesis consists of four manuscripts with chapters presented at several reputable scientific conferences in the form of abstracts and posters. Chapter 3 has been published in the Journal of Environmental Management. Chapters 4 and 5 are respectively under review in the Journal of Environmental Management and Water Research, while Chapter 6 is ready for submission to Agriculture, Ecosystems and Environment. The following served as co-authors in the four manuscripts: Dr. Shiv Prasher, as the supervisor; Dr. Eman ElSayed, as academic and technical advisor; Dr. Ramanbhai Patel, as academic and technical advisor; Mr. Jaskaran Dhiman, as laboratory and field assistant; and Mr. Ali Mawof, as laboratory and field assistant.

The candidate was fully involved at every stage of the thesis including concept development, laboratory and field experimentation, data analysis, result discussion and manuscript preparation. Dr. Shiv Prasher as the thesis supervisor was deeply involved in providing scientific advice and technical supervision. He also edited and reviewed the prepared manuscripts.

Dr. Ramanbhai Patel as a research associate ensured that the manuscripts met technical standards by posing several technical questions and suggestions. He was also involved in data verification and consistency.

Dr. Eman ElSayed as a post-doctoral fellow through her wealth of experience provided technical and academic supervision including experimental design. She was involved in reviewing the prepared manuscripts.

Mr. Jaskaran Dhiman as a PhD scholar assisted in all field and laboratory work. His participation made clear several technical steps from our supervisor.

Mr. Ali Mawof as a PhD scholar assisted in all field and laboratory work including site preparation, sample collection and batch equilibrium experiments, which were tedious and time consuming.

Publications related to the thesis are listed as follows:

- **Nzediegwu, C**., Prasher, S., Elsayed, E., Dhiman, J., Mawof, A. & Patel, R. (2019). Effect of Biochar on Heavy Metal Accumulation in Potatoes from Wastewater Irrigation. Journal of Environmental Management. 232, 153-164. doi.org/10.1016/j.jenvman.2018.11.013
- **Nzediegwu, C**., Prasher, S., Elsayed, E., Dhiman, J., Mawof, A. & Patel, R. (2018, under review). Impact of Soil Biochar Incorporation on the Uptake of Heavy Metals Present in Wastewater by Spinach Plants. Journal of Environmental Management.
- **Nzediegwu, C**., Prasher, S., Elsayed, E., Dhiman, J., Mawof, A. & Patel, R. (2018, under review). Role of Aging of Biochar in Reducing Metal Uptake by Potato Plants Irrigated with Wastewater. Water Research.
- **Nzediegwu, C.,** Prasher, S., Elsayed, E., Dhiman, J., Mawof, A. & Patel, R. (2018, to be submitted). Effect of Biochar on the Yield of Potatoes Cultivated under Wastewater Irrigation. Agriculture, Ecosystems and Environment.

Scientific presentations related to the thesis are listed as follows:

- **Nzediegwu, C**., Prasher, S., Elsayed, E., Dhiman, J., & Mawof, A. (2018, July). Effects of Biochar on the Yield of Potatoes Grown with Wastewater. Paper presented at the American Society of Agricultural and Biological Engineers Annual International Meeting, Detroit, Michigan. Paper #1800567
- **Nzediegwu, C**., Prasher, S., Elsayed, E., Dhiman, J., & Mawof, A. (2018, July). Screening Environmental Data for Outliers using JMP Software: A Time-Saving and Unbiased Approach. Paper presented at the American Society of Agricultural and Biological Engineers Annual International Meeting, Detroit, Michigan. Paper #1800277
- **Nzediegwu, C**., Prasher, S., Elsayed, E., Dhiman, J., & Mawof, A. (2018, July). Effect of Crop-Type on Biochar Functionality as a Biosorbent in Wastewater Agriculture. Paper Presented at the Canadian Society for Bioengineering (CSBE), Guelph, Ontario, Canada

- **Nzediegwu, C**., Prasher, S., Elsayed, E., Dhiman, J., Mawof, A., & Anderson, E. (2017, August). Effect of Plantain Peel Biochar on the Uptake of Cd and Zn in Spinach Irrigated with Wastewater. A Poster presented at the Canadian Society for Bioengineering (CSBE) Conference, Winnipeg, Manitoba, Canada

- **Nzediegwu, C**., Prasher, S., Elsayed, E., Dhiman, J., & Mawof, A. (2017, July). Uptake of Heavy Metals in the Peel and Flesh of Potatoes Irrigated with Untreated Wastewater in the Presence of Plantain Peel Biochar. Paper presented at the 19th International Botanical Congress, Shenzhen, China. Paper #T1-31-02

- **Nzediegwu, C**., Prasher, S., Elsayed, E., Dhiman, J., & Mawof, A. (2017, July). Heavy Metal Presence in the Edible Parts of Potatoes Irrigated with Untreated Wastewater in the Presence of Plantain Peel Biochar. Paper presented at the American Society of Agricultural and Biological Engineers Annual International Meeting, Spokane, Washington. Paper #1700421

- **Nzediegwu, C**., Prasher, S., Elsayed, E., Dhiman, J., & Mawof, A. (2017, July). Impact of Plantain Peel Biochar Addition in Soil on Soil CEC and pH under Untreated Wastewater Irrigation. Paper presented at the American Society of Agricultural and Biological Engineers Annual International Meeting, Spokane, Washington. Paper #1700823

- **Nzediegwu, C**., Prasher, S., Elsayed, E., Dhiman, J., & Mawof, A. (2017, June). A Novel Plant-Waste Adsorbent for removing Pb in Wastewater. Poster Presented at the AGM of the India-Canada Centre for Innovative Multidisciplinary Partnerships to Accelerate Community Transformation and Sustainability (IC-IMPACTS), Montreal, Canada

- **Nzediegwu, C**., Prasher, S., Elsayed, E., & Dhiman, J. (2016, July). Effect of Plantain Peel Biochar in Reducing the Contamination of Heavy Metals in Wastewater Irrigated Potato. Paper Presented at the American Society of Agricultural and Biological Engineering (ASABE) Annual International Meeting, Orlando, Florida. Paper #162456855

- **Nzediegwu, C**., Prasher, S., Elsayed, E., & Dhiman, J. (2016, July). Uptake of Heavy Metals in Potatoes Irrigated with Untreated Wastewater. Poster Presented at the Canadian Society of Agronomy (CSA), Montreal, Canada

- **Nzediegwu, C**., Prasher, S., Elsayed, E., & Dhiman, J. (2016, March). Cadmium Loading in Soil and Potato Tubers Irrigated with Untreated Wastewater. Poster Presented at the AGM of the India-Canada Centre for Innovative Multidisciplinary Partnerships to Accelerate Community Transformation and Sustainability (IC-IMPACTS), Montreal, Canada

- **Nzediegwu, C**., Prasher, S., Mannan, A., Elsayed, E., & Dhiman, J. (2015, April and May). Role of Plantain Peel Biochar in Enhancing Safe Use of Untreated Wastewater. Paper presented at the 22nd Hydrotechnical Conference, Montreal

Other presentations related to the thesis are listed as follows:

- **Nzediegwu, C.** (2018, February). SciComm Workshop Bonus 3: Designing pipes, benefits of essential oils and reducing metals in crops. A podcast assessable at https://soundcloud.com/broad-science/sets/scicomm-workshop-stem-students

- **Nzediegwu, C.** (2017, November). Deciding a Suitable Sorbent for Soil and Water Contaminant Immobilization: An Experimental Approach. A Slide presented at the 16th Brace Research Day, Macdonald Campus, McGill University

- **Nzediegwu, C.** (2017, June). Attenuation of Heavy Metals in Wastewater using Plantain Peel Biochar. A slide presented at the 15th Brace Research Day, New Residence Hall, McGill University

- **Nzediegwu, C.** (2017, March). Combatting Heavy Metals in Soil. A Slide presented at McGill-Wide 3MT Competition, Thompson Ballroom, McGill University, March 2017. Available at https://www.youtube.com/watch?v=alxfJg8BO3k

- **Nzediegwu, C.** (2017, January). War against Heavy Metals: Biochar an "Active Soldier". A Slide presented at the Lister Family Engaged Science Initiative 3MT Competition, Macdonald Campus, McGill University

Table of Contents

Contents

List of Figures

List of Tables

Chapter 1: Introduction

1.1 General introduction

Freshwater is a fundamental resource for humans and other life forms in the environment (Milà I Canals et al., 2009). Worldwide, there has been an increase in freshwater depletion since the past 100 years due to rise in human population, economic growth, improved standard of living and industrialization. Among all the sectors, such as industrial, domestic and agriculture, involved in the depletion of freshwater, agriculture is the major driver contributing to almost 70% of the world's freshwater withdrawal (Connor et al., 2017). To reduce the total reliance of agriculture on freshwater, the use of wastewater is seen as the only lasting solution (Farahat and Linderholm, 2015). Wastewater agriculture is more economical than freshwater agriculture as it performs a dual purpose of water and nutrient replenishment, simultaneously (Connor et al., 2017; Farahat and Linderholm, 2015). Although in developed countries, wastewater receives some level of treatment before being used for irrigation, however, it is not the case in most developing countries. In developing countries, farmers irrigate crops with the untreated wastewater (Mohan et al., 2011), thereby polluting the soil directly and the water bodies indirectly with contaminants, including heavy metals. Most of these contaminants are known to be hazardous to human, plants and animals, and thus, require appropriate measures to control their movement in the environment.

Heavy metals belong to the transition element group of the periodic table. Although they occur naturally in the environment (Alloway, 2013b), their occurrence could also be traced to anthropogenic sources such as industries and agriculture (Mohan et al., 2011). Wastewater discharge from industries is a major source of heavy metal in soil and water bodies (Ahmad et al., 2011; Gokhale et al., 2008). Other anthropogenic sources are fossil fuel combustion and transportation (Alloway, 2013b). Concentration of Cr as high as 16.3 mg L^{-1} (326 times above the permissible limit of 0.05 mg L^{-1} in drinking water) was measured in wastewater discharged to the river Ganges at Kanpur, India (Mohan et al., 2011). The ability of heavy metals to cause various health disorder in humans (Gokhale et al., 2008), and their recalcitrant nature, make them an environmental concern that demands attention.

Biochar, the carbon-rich product obtained from the thermal breakdown (Inyang et al., 2015) or pyrolysis of plant-based materials (Lehmann and Joseph, 2009), possess many agronomical (Alburquerque et al., 2014; Alburquerque et al., 2013) and environmental (Abit et al., 2012; Kookana et al., 2011) benefits. For instance, biochar application led to enhanced wheat grain yield when applied in conjunction with mineral fertilizer to a nutrient-poor soil (Alburquerque et al., 2013). Environmentally, laboratory- and field-scale, biochar application has shown effectiveness in reducing the mobility of organic (Cabrera et al., 2014; Chen and Chen, 2009) and inorganic contaminants in soil and water media. In a pot study (Lu et al., 2014), heavy metals (Cd, Cu, Pb and Zn) significantly adsorbed onto biochar-amended soils. The benefits derived from the use of biochar depend largely on the type of feedstock (Abit et al., 2012; Alburquerque et al., 2014), the production method, the production temperature (Angin and Şensöz, 2014), and application rate (Sarmah et al., 2010). Furthermore, the effectiveness of biochar depends on soil type and plant species (Alburquerque et al., 2014) used for the study. For example, the chemical and surface properties of a single feedstock biochar pyrolized at two different temperatures, 400 and 700^{o}C, showed significant difference in their fixed carbon content (Angin and Şensöz, 2014); this could potentially affect their contaminant sorption behavior as well.

Since feedstock plays an important role in the functioning of biochar (Abit et al., 2012; Alburquerque et al., 2014), it is worth evaluating sustainable feedstock, such as plantain peel.

Plantain, a staple food rich in starch, is predominantly cultivated in Africa, Asia and Latin America (Tchango Tchango et al., 1999). In 2014, about 30 Tg of plantain was produced globally (FAO, 2017). In all forms of plantain processing, peel always remains a waste product (Tchango Tchango et al., 1999). With plantain peel accounting for about 40% of plantain fruit (e.g., 12 Tg globally in 2014) (Rubatzky and Yamaguchi, 1997), it could constitute a sustainable feedstock for biochar production. Modified plantain peel (Agarry et al., 2013), plantain peel residue (Idowu et al., 2011) and plantain peel activated carbon (Inam et al., 2016) have been studied as sorbent materials. To the best of my knowledge, the potency of plantain peel biochar on the simultaneous immobilization of Cd, Cr, Cu, Fe, Pb and Zn in untreated wastewater when applied to soil as irrigation water for cop cultivation has not been studied thus far.

1.2 Research objectives

The overall objective of this study was:

- To study the fate and transport of heavy metals in sandy soils and their translocation to crops when irrigated with untreated wastewater in the presence of a novel plant-based bio-sorbent

The specific objectives were:

- To evaluate the sorption and desorption potential of plantain peel biochar through batch sorption study in a sandy soil substrate;
- To study the role of plantain peel biochar for heavy metal (Cd, Cr, Cu, Fe, Pb and Zn) immobilization in sandy soils irrigated with untreated wastewater;
- To evaluate the effect of plantain peel biochar amendment on heavy metal loading to potato tubers irrigated with untreated wastewater;

- To evaluate the effect of plantain peel biochar amendment on heavy metal loading of spinach irrigated with untreated wastewater; and
- To verify the stability of aging biochar in a sandy soil under untreated wastewater irrigation.

The above objectives were met by conducting both laboratory- and field-scale experiments on sandy soils.

1.3 Thesis outline

The thesis is separated into nine chapters. Chapter 1 introduces the research topic, followed by the objective statements and scope of this investigation. Chapter 2 provides a general review of relevant literature on the subject matter. Chapter 3 addresses the effect of biochar on heavy metal accumulation in potatoes from wastewater irrigation. Chapter 4 presents the impact of soil biochar incorporation on the uptake of heavy metals, present in wastewater, by spinach plants. Chapter 5 discusses the stability of aging biochar in the second year of its application to soil cultivated with potatoes under wastewater irrigation. Chapter 6 deals with the agronomic benefits of biochar after two years of field study with potatoes under wastewater irrigation. Chapter 7 provides a general summary and conclusion. Chapter 8 presents the contributions to knowledge and recommendations for future research work. Chapter 9 contains the references.

Chapter 2: Literature Review

2.1 Wastewater

Wastewater is any water originating from households, industries, and agricultural fields that has been polluted as a result of anthropogenic or natural interference (Lin and Lee, 2007). Given the burden placed on freshwater by agricultural sector, which accounts for 70% global freshwater withdrawal, wastewater appears to be an alternative (Hettiarachchi et al., 2018). Although in developed countries, wastewater receives some level of treatments before disposal, however, in developing countries, only little proportion of wastewater (≤8%) is treated before discharge, possibly due to lack of infrastructures, technical know-how and money (Connor et al., 2017). To worsen the situation, both the treated and untreated wastewater find their way to water bodies such as rivers, lakes and canals that serves as major source of irrigation water to farmers downstream. A lasting solution would have targeted at treating wastewater at the point of generation, however, this is not practiced, particularly in the developing countries, where there are institutional challenges, failure of legal systems and cultural issues (Connor et al., 2017). As such, wastewater ends up on agricultural land as irrigation water.

Of note, wastewater, especially the untreated, has become "blessing in disguise" to several farmers who have reported some benefits from its use. Such benefits include (Hettiarachchi et al., 2018; Jaramillo and Restrepo, 2017): (1) increased crop yield possibly due to the high nutrient loading of the wastewater, (2) increased income by reducing additional cost on fertilizers, and (3) reduced cost on energy required to pump water from groundwater wells. In fact, more than 20 million hectares of arable land are already receiving wastewater globally. This number is expected to increase given the demand for water by agriculture to feed the estimated 9 billion people in 2050 (United Nations, 2017).

However, wastewater serves as a major pathway for contaminant (e.g. heavy metals) influx into the environment including soil and crops. Depending on the soil physicochemical properties, such as texture, pH and cation exchange capacity (CEC), heavy metal species in wastewater could accumulate on the soil surface and be available for plant uptake or leach to the groundwater. For instance, Pb, Cd, Zn and Fe accumulation was noticed in a loamy soil irrigated with wastewater (Kiziloglu et al., 2008), while in another study (Hartley et al., 2004), Pb and Cd were measured in the leachate of soils contaminated with coal fly ash deposits. Abdu et al. (2011) raised a concern of Cd and Zn leaching to groundwater following wastewater irrigation. There is a dire research need towards immobilizing the contaminants from wastewater for its effective and efficient use in agriculture, and at the same time ensuring that crops and groundwater are, as much as possible, contaminant-free.

2.1.1 Characteristics of wastewater

A larger proportion of wastewater is water, while much smaller portion (0.06%) is comprised of suspended and dissolved solids. Suspended and dissolved solids and their different fractions such as total solid, total suspended solid, total dissolved solid, fixed suspended solid and volatile suspended solid are useful in classifying wastewater into treated and untreated (raw).

Untreated wastewater is classified as wastewater with total dissolved solid in the range of 250 to 850 mg kg^{-1} (Lin and Lee, 2007). As the name implies, untreated wastewater receives no intentional treatment resulting in high levels of nutrients such as nitrogen (25 to 85 mg L^{-1}) and phosphorus (2 to 20 mg L^{-1}), which makes wastewater valuable in agriculture (Lin and Lee, 2007).

2.1.2 Synthetic wastewater

Conducting research with raw wastewater is challenging due to its dynamic nature as the amounts of suspended and dissolved solids as well as contaminants change with every discharge even from the same source. To overcome this, researchers have developed several recipes to simulate any kind of wastewater called synthetic wastewater (LaPara et al., 2006; Nopens et al., 2001). Synthetic wastewater is of importance as it ensures uniformity and repeatability of experimental parameters such as contaminant concentrations and pH. Moreover, several studies have been conducted using simulated wastewater designed to meet specific needs (Peligro et al., 2016; Senthil Kumar, 2014). Once the concentrations of the contaminants of interest are known (from the literature), a careful laboratory approach is ultimately followed to prepare the wastewater, taking safety into considerations.

2.1.3 Contaminants in wastewater

Depending on the source (domestic, industrial or agricultural), wastewater contains contaminants broadly classified as organic and inorganic with the later comprising of heavy metals such as Pb, Cd, Cu, Cr, Zn and Fe, which are of environmental concerns due to their persistent nature. Representing elements with atomic mass greater than 5 g cm^{-3} (Alloway, 2013b), heavy metals serve as components of industrial processes and products (Table 2.1) finding their way into the environment mainly through indiscriminate discharge of wastewater from industries.

Upon irrigation with contaminated wastewater, heavy metals get accumulated in soil and further translocate to edible crops. Depending on the concentration, heavy metals can pose health challenges when present in the food chain (Table 2.1). Therefore, appropriate steps are needed to reduce their bioavailability in soil.

Table 2.1. Heavy metals in the environment

Metal	Usage and potential pathway to environment	Health effects	References
Cr	Electroplating, metal finishing, magnetic tapes, pigments, leather tanning, wood protection, chemical manufacturing, electrical and electronic equipment, catalysis,	Cr(VI) Nausea, diarrhea, liver and kidney damage, dermatitis, internal hemorrhaging and respiratory problem	(Devi et al., 2014; Fraga and Oteiza, 2002; Macomber and Imlay, 2009; Mohan et al., 2011; Siegel, 2002; Sigel et al., 2013)
Cd	Electroplating, mining, nickel-cadmium battery industry, smelter operation, Combustion of Fossil fuels (coal and oil), cadmium pigment in paint, neutron absorber, anti-corrosive coating of metals	Endocrine disruptor, kidney diseases	
Pb	Combustion of leaded Gasoline, Fertilizers, antiknock agents, lead-acid battery, pipes, pigments, glassware and ceramics, mining	Affects brain and nerve tissue, endocrine disruptor	
Cu	Roofing, pigments, alloys, miming, kitchenware, fertilizers, traffic, water pipes	Alzheimer, Indian childhood cirrhosis	
Zn	Anti-corrosion coating, batteries, cans, rubber industry, paints, soldering and welding fluxes	Cu deficiency, epigastric pain, impaired immune function	
Fe	Cast iron, machine manufacturing, wrought iron, steel, transportation, alloys	Cancer, liver and heart disease, diabetics	

2.2 Biochar

2.2.1 General characteristics of biochar

Biochar is a carbon rich solid by-product of thermal carbonization of organic feedstocks, including agricultural wastes, in the absence of air. With feedstocks containing different proportions of cellulose, lignin and hemicellulose, which undergo chemical bond alterations at high heating temperature (200 to 700^{o}C), biochar properties change likewise (Table 2.2). Nevertheless, biochar is characterized by high pH, and relatively high specific surface area making it useful for both agronomic and environmental purposes.

Table 2.2. Selected properties of biochar as affected by feedstocks

Feedstock	**pH**	**SSA (m^2 g^{-1})**	**Ash content (%)**	**Carbon content (%)**	**References**
Wood (mixed)	9.1	-	13.1	75.9	(Bruun et al., 2014)
Mulberry Wood	10.2-11.1	16.6-58.0	7.52-9.82	67.9-80.1	(Zama et al., 2017)
Wheat straw	10.7	-	40.1	52.2	(Bruun et al., 2014)
Sugar Cane Straw	10.2	5.0	13.4	68.8	(Puga et al., 2015)
Corn cob	10.0-10.5	12.44-47.63	6.11-13.2	69.6-82.1	(Zama et al., 2017)
Sesame Straw	10.1	289.2	-	72.6	(Park et al., 2016)
Poultry manure	9.6-10.4	3.33-8.97	71.4-79.6	21.3-24.1	(Zama et al., 2017)

2.2.2 Agronomical benefits

Biochar affects soil physicochemical properties, including water holding capacity, nutrient use efficiency, pH, and CEC to the extent that crop yield is affected (Bruun et al., 2014; Pandit et al., 2018). Different types of biochar have been tested to ascertain their agronomic impacts, some of which are positive, while others are neutral or even negative. For instance, straw- and wood-biochar improved water retention capacity of coarse sandy soil under spring barley cultivation (Bruun et al., 2014). In another study, addition of wood biochar increased plant water availability and soil water retention by 23% and 18%, respectively (Pandit et al., 2018). Similarly, the pH and the CEC of biochar-amended soil were increased resulting in increased maize yield (Pandit et al., 2018). Biochar has also shown potentials in increasing soil available nutrients including K, which is very much present in biochar`s ash (Gautam et al., 2017; Pandit et al., 2018). On the contrary, addition of Castanea sativa wood biochar to soil showed no effect on the growth and harvest yield of strawberry, barley and potato, although increased availability of K was noticed (Jay et al., 2015). In a 4-year plot study, oak wood biochar showed no effect on the yield of corn and cotton, although there was an increase in the yield of peanut studied in the same period (Lamb et al., 2018). As such, there are variabilities in the function of biochar necessitating more research.

2.2.3 Environmental benefits

Biochar is receiving increasing attention as an organic sorbent to reduce the transport of soil-laden contaminants including heavy metals and hormones, which are predominantly present in wastewater. The ability of biochar to act as a sorbent has been associated with its specific surface area, pH, mineral content and functional groups, which in turn control the availability of contaminants especially heavy metals in soil solutions. By increasing soil`s pH, rice straw derived biochar immobilized Pb, Cd and Zn in a 90-day incubation pot study (Dang et al., 2018). Biochar derived from pigeon pea reduced the bioavailability of Cd and copper (Cu) to vegetables possibly due to soil pH change due to biochar (Coumar et al., 2016a; Coumar et al., 2016b). Accordingly, in a 2-year field study, wheat straw biochar applied to a Cd-contaminated soil reduced the uptake of Cd in rice grain by 45% and 62% in 2009 and 2010, respectively (Cui et al., 2011). Biochar derived from sugar cane straw reduced the uptake of Cd, Pb (65%) and Zn (58%) by jack bean (Canavalia ensiformis) grown on a Zn mining contaminated soil (Puga et al., 2015). This reduction was a consequence of biochar reducing the availability of Cd (56%), Pb (50%) and Zn (54%) in the soil solution as compared to the no-biochar control. In these and other known cases, biochar is used for the remediation of an already contaminated soil where the biochar is not loaded continuously with contaminants and may thus have different behavior when it is loaded continuously with contaminants—as applicable in wastewater irrigation.

2.2.4 Mechanism of operation

The underlying sorption mechanism of biochar for heavy metals are electrostatic interaction, precipitation, surface complexation and ion exchange, which are enhanced by biochar's physicochemical properties such as pH, pore size, ash content, elemental composition and surface area. Biochar's physicochemical properties vary with feedstock and pyrolysis temperature such that as pyrolysis temperature increases, water loss and volatile compound disappearance increases resulting typically in increased pore size, which characterizes any biochar (Li et al., 2017). As temperature changed from 500 to 900^{o}C, pore size of biosolid-derived biochar increased from 0.056 to 0.099 cm^3 g^{-1} resulting in surface area increase from 25.4 to 67.6 m^2 g^{-1} (Chen et al., 2014), suggesting that increased pore size is associated with increased surface area.

2.2.4.1 Complexation

Heavy metals undergo complexation with functional groups such as carboxylic (-COOH), carbonyl (-CO), hydroxylic (-OH) and amino ($-NH_2$) groups, which are abundantly present on the surfaces of biochar and play an important role in their sorption (Uchimiya et al., 2012). Such reactions render toxic metal species immobile, thereby making them less available in solution. Following FTIR spectra analysis, a good correlation was noticed between biochar sorption capacity for Cr and the number of oxygen-containing functional groups on the biochar suggesting complexation as a likely mechanism for Cr sorption onto biochar (Pan et al., 2013). After Pb's sorption to mulberry wood biochar, X-ray Photoelectron Scanning showed fewer peaks, which suggest complexation between Pb and the biochar's functional groups (Zama et al., 2017).

2.2.4.2 Ion exchange

During pyrolysis, mineral components such as Ca, K, Mg, and P of biochar increase as the ash content increases. When biochar is mixed with soil, heavy metals in the soil solution can be precipitated or exchanged with these minerals. A property that quantifies the exchangeable cation in organic materials including biochar is the cation exchange capacity, which varies with feedstock and production temperature (Zama et al., 2017). A sorption study with broiler litter biochar reveals that metal cations (Cd^{2+}, Cu^{2+}, Ni^{2+} and Pb^{2+}) displaced base cations (Ca^{2+}, Mg^{2+}, Na^{+} and K^{+}) in the exchange complex (Uchimiya et al., 2010b), which suggest cation exchange as one of the mechanisms for their immobilization. In another study, cation exchange was responsible for the sorption of Pb to poultry manure, irrespective of the production temperature (Zama et al., 2017).

2.2.4.3 Precipitation

Minerals such as crystalline calcite ($CaCO_3$), Quartz (SiO_2) and Gonnardite [$(Na, Ca)_2$ $(Si, Al)_5O_{10}.3H_2O$] present in biochar potentially release anions such as carbonates (CO_3^{2-}) and phosphates (PO_4^{3-}) into solutions (Zama et al., 2017). These and other anions form precipitates with metallic cations, such as Cd^{2+}, Cu^{2+}, Ni^{2+} and Pb^{2+}, already present in contaminated soil or water. An XRD study of biochars derived from peanut shell, mulberry wood and buckwheat husk, reveals that Pb was precipitated as $PbCO_3$, $PbCO_3(OH)_2$, $Pb_3(PO_4)_2$, $Pb_9(PO_4)_6$, and $Pb_3(CO_3)_2(OH)_2$ in sorption solution (Zama et al., 2017). In the same study, XPS spectra reveals the presence of $PbSO_4$ and $PbCl_2$. Precipitation is not peculiar to Pb, as other heavy metals such as Cd have reportedly precipitated out of sorption solution as $CdCO_3$ and $Cd_9(PO_4)_6$ in the presence of biochar. Being responsible for metal speciation, the pH of the solution, controlled by the pH of the biochar, determines the type of precipitate formed.

Although discussed separately, these mechanisms do not occur in isolation, rather they occur simultaneously once biochar is in contact with soil, including contaminated soil. Also, the feedstock type determines the dominant mechanism.

2.2.5 Feedstock

Feedstock represents the material, mostly organic, from which biochar is produced. As research done on the use of biochar as soil conditioner increases, several feedstocks spanning from wood, plant waste, sludge and animal manure are being tested. Irrespective of the type, feedstock undergoes some form of pre-treatment, such as drying and shredding. Drying helps to reduce the moisture content (<10%), while shredding helps to achieve size reduction to (<4 mm) depending on the pyrolyzing unit. Among other factors such as production temperature, the feedstock type has been identified as a major factor that controls the properties of biochar, which in-turn affects its performance (Table 2.2).

As much as possible, the choice of feedstock should be guided by factors such as availability, sustainability, alternative usability in addition to cell wall composition (i.e., lignin, cellulose and hemicellulose content), which of course is inherent in any organic material. For instance, farmers may not be willing to produce biochar from a feedstock that already serves as food for their animals, irrespective of the quality and function of biochar that could be produced. Also, how much of the feedstock material is produced annually determines its sustainability and should be taken into consideration.

2.2.5.1 Plantain peel as a feedstock

In Africa, Asia and Latin America, plantain serves as a major staple food. In 2007, the global production of plantain and banana was estimated as 100 Tg (International Institute of Tropical

Agriculture, 2009). More recently, in 2014, more than 30 Tg of plantain was produced globally (FAO, 2017). Moreover, these estimates only considered 15% of plantain and banana that get to international market, whereas the other 85% consumed locally were not considered. This high production of plantain directly implies high production of wet peel, accounting for up to 40% by weight of the fruit (e.g., 12 Tg globally in 2014) (Rubatzky and Yamaguchi, 1997). As such, plantain peel, a cellulose-rich material (Agarry et al., 2013), is a sustainable agricultural feedstock abundantly present as waste in developing countries like Uganda, Rwanda, Ghana and Nigeria, which are among the top four producers of plantain in West Africa. Modified plantain peel has been utilized as sorbent material for the sorption of 2,6-dichlorophenol in aqueous solution (Agarry et al., 2013), whereas plantain peel residue (Idowu et al., 2011) and plantain peel activated carbon (Inam et al., 2016) have been studied as sorbent materials. Moreover, plantain peel is a clean product (i.e., free from heavy metals; Table 2.3), however, it has received less attention. To further add value to plantain peel, and reduce the menace caused by its presence in the environment (Tchango Tchango et al., 1999), transforming it to biochar and utilizing it as a soil amender for agronomical and environmental benefit is proposed. To the best of our knowledge, nothing has been done with plantain peel biochar.

2.3 Soil as a substrate

In biochar studies, the soil, which houses the biochar is of importance. Soil is a collection of materials such as sand, silt, clay, organic matter, carbonates, oxides, water, air and bacteria in different proportions giving rise to different soil composition (Yong et al., 2012). A change in the soil composition would result in a significant change in the behavior of the biochar to function as a sorbent.

Table 2.3. Concentrations of selected heavy metals in plantain parts

	concentration in mg kg^{-1}			
	Cd	Cr	Pb	As
plantain fruit	0.0042±0.0002	0.3175±0.0274	0.0250±0.0009	0.0044±0.0003
plantain peel	0.0051±0.0002	0.1180±0.1324	0.2236±0.1452	0.0084±0.0012
MPL*	0.1	1.0	6.0	1.4

MPL is maximum permissible limit; *CODEX STAN 193-1995(amended: 2010)

2.3.1 Soil classification

Although different soil classifications exist (Yong et al., 2012), our focus is on the particle size and textural soil classification system. In this system, the particles size distribution of the soil (i.e., sand (2 to 0.05 mm), silt (0.05 to 0.002 mm) and clay (<0.002 mm)) determines the class of the soil as shown in Figure 2.1. Accordingly, 12 textural classes are identified including sand, which supposedly has the least percent clay size (<10%). Clay size content correlates with clay mineral content, which possesses net negative charges having specific surface area as high as 800 m^2 g^{-1} for montmorillonite (Yong and Warkentin, 1975). The impact of clay minerals on sorption of heavy metals have been studied extensively with clay showing high affinity for the heavy metals (Abollino et al., 2003; Malandrino et al., 2006). Having this in mind, making a right choice of soil, which will not buffer the effect of the intended soil conditioner—biochar in this case—is imperative. As a rule of thumb, sandy soil has served as a suitable substrate for contaminant transport study.

2.4 Crop type

Crops are broadly categorized according to their use as food, feed, fuel, and fiber. They continue to form the basis of human existence and sustenance. They are mainly grown on soil where majority of their nutrients such as N, P and K are stored. Although with different rooting systems, crops undergo metabolic process involving nutrient and water uptake from the soil.

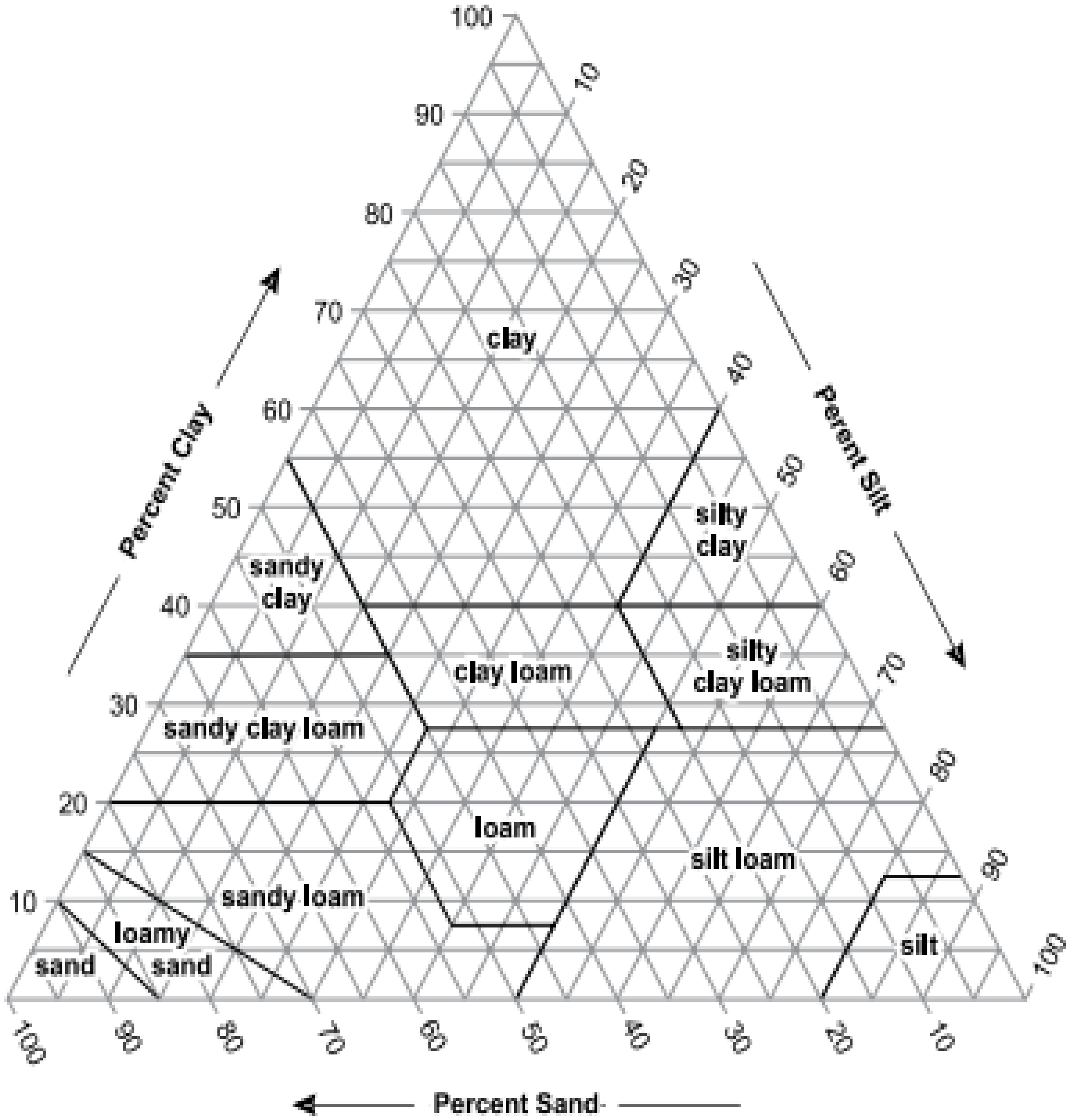

Figure 2-1. USDA soil texture triangle (adapted from Buol et al. (2011))

In this process, if the soil is contaminated, the crops could get contaminated depending on how resistant it is to contaminant uptake. Such contaminated crops, when consumed, are a major pathway for heavy metals in human bodies. However, the level of contamination may depend partly on the proximity of the crop to the contaminated soil—i.e., whether below ground (e.g., tuber such as potatoes) or above ground (e.g., vegetables such as spinach) and partly on the type of root exudate in the rhizosphere.

2.4.1 Potato

A vital source of antioxidants, protein and starch (Brown, 2005), potato (Solanum *tuberosum* L.) is the most widely grown tuber crops in the world (Chandrasekara and Josheph Kumar, 2016). Their high nutritional value has led to a rise in their cultivation, especially in developing countries like China and India (FAO, 2017). As a shallow rooted crop, mostly cultivated on light soils, potato is very sensitive to water stress, and therefore require regular irrigation to achieve higher yields (King and Stark, 1997).

2.4.2 Spinach

Spinach (Spinacia *oleracea* L.), a fast-growing vegetable crop, is rich in essential nutrients such as vitamin C, protein, and β-carotene (Kusuma et al., 2016; Tressler et al., 1936), making it increasingly important in diets (Kamruzzaman et al., 2016) across the world. Since the past 15 years, the global annual production of spinach has doubled to about 24 million tons (FAO, 2017), and this trend is expected to continue. However, since 60% of world arid lands exist in continents that houses the world top producers of spinach (FAO, 2017; Thomas, 2011), one of the challenges its production (as in other crops) will face is water stress (Leskovar and Piccinni, 2005). Water stress can affect leaf quality, carbohydrate metabolism and marketable yield of spinach (Leskovar and Piccinni, 2005; Zrenner and Stitt, 1991). For instance, spinach grown with limited water (50% evapotranspiration rate) resulted in more yellow leaves (indication of bad quality), and less marketable yield than those grown with sufficient water (100% evapotranspiration rate) (Leskovar and Piccinni, 2005). Therefore, to mitigate the effects of water stress in spinach, applying irrigation is necessary, especially in these arid regions.

2.4.3 Root exudation

The behavior of biochar, when mixed with soil on the rhizosphere, may vary from plant to plant given the different biochemical activities that take place in the rhizosphere. One such activity is the excretion of root exudates by plants. Root exudates are composed of organic compounds especially organic acids, control cations and oxyanions; they differ from plant to plant (Balendres et al., 2016; Li et al., 2013). For instance, root exudates from potatoes differ from cabbage (Dechassa and Schenk, 2004) and peanuts (Li et al., 2013) in their types and compositions. Given their compositions, root exudates control certain physicochemical properties of the soil-root interface such as pH, redox potential and the formation of stable complexes (Balendres et al., 2016; Kabata-Pendias and Pendias, 1984), which affect the availability and uptake of nutrients (Dechassa and Schenk, 2004). Nutrients have similar uptake mechanisms as heavy metals (Moreno-Jiménez et al., 2012). For instance, root exudates from potatoes and cabbage affected the availability of phosphorus to their root tissues (Dechassa and Schenk, 2004), while root exudates from spinach enhanced the uptake of Cu and Zn (Degryse et al., 2008). Additionally, root exudate from lettuce affected the availability of arsenic in a lettuce grown soil (Bergqvist et al., 2014). Therefore, given the role of this plant-dependent process (root exudation) in nutrient availability and uptake mechanism, it could affect the performance of biochar as a sorbent. Therefore, it is important to study whether crop type will affect the performance of biochar.

2.5 Sorption

Sorption provides an answer to the question of whether an ion or a molecule will remain in the liquid or solid phase at equilibrium, which is very important in predicting the fate and transport of such ion or compound in soil. During sorption, one of the following phenomena could take place: adsorption, absorption, precipitation or polymerization. However, when the actual mechanism of ion attachment on a solid phase is unknown, it is generally referred to as sorption (Sparks, 1995).

The following terms are associated with sorption: sorbate, sorbent, and sorptive. Sorbate is the material (ion or compound) that are attached on the solid surface, sorbent is the solid surface (e.g., soil, biochar, clay, etc) where the sorbate is attached, while the sorptive is the material (ion or molecule) that are in the solution or liquid phase. When the attachment phenomenon is adsorption, the sorbate, sorbent and sorptive are otherwise called adsorbate, adsorbent and adsorptive, respectively (Sparks, 1995).

2.5.1 Batch sorption experiment

Sorption experiment is conducted to determine the distribution of ions in solution at constant temperature and pressure (Sparks, 1995). Also, the sorption capacity, which depicts the number of ions that can saturate the surface of a sorbent, can be determined. A known mass of the sorbent (M_s in kg) is bathed with a known volume of solution (V_s in L), which contains a known initial concentration of the sorptive (C_i in mg L^{-1}) of interest—heavy metal ions in this case. The mixture is shaken until equilibrium (a steady state) is reached after which the solid phase is separated from the liquid phase either by centrifugation or by filtration. The final concentration of the sorptive at equilibrium (C_e in mg L^{-1}) is determined. The concentration of sorbate (C_s in mg kg^{-1}) attached on the sorbent is then calculated according to mass balance equation (Equation 2.1).

$$C_s = \frac{(C_i - C_e) * V_s}{M_s} \quad (2.1)$$

2.5.2 Sorption isotherm

Isotherm is a plot of the concentration of sorbate (Cs) on the ordinate versus the equilibrium concentration (Ce) on the abscissa. It is used to describe the sorption process. It is pertinent to note that the sorption isotherm does not in any way convey the mechanism of the process (i.e., it does not tell whether it is adsorption or precipitation (Sparks, 1995)). However, to understand the mechanism, spectroscopic techniques such as FTIR are applied. Depending on the sorbent and sorbate interactions, four different shapes of isotherms have been identified as: C-curve, S-curve, L-curve and H-curve (Figure 2.2), where the C-curve is generally known as the linear sorption isotherm with slope equal to the distribution coefficient (Kd) given as C_s/C_e (Sparks, 1995).

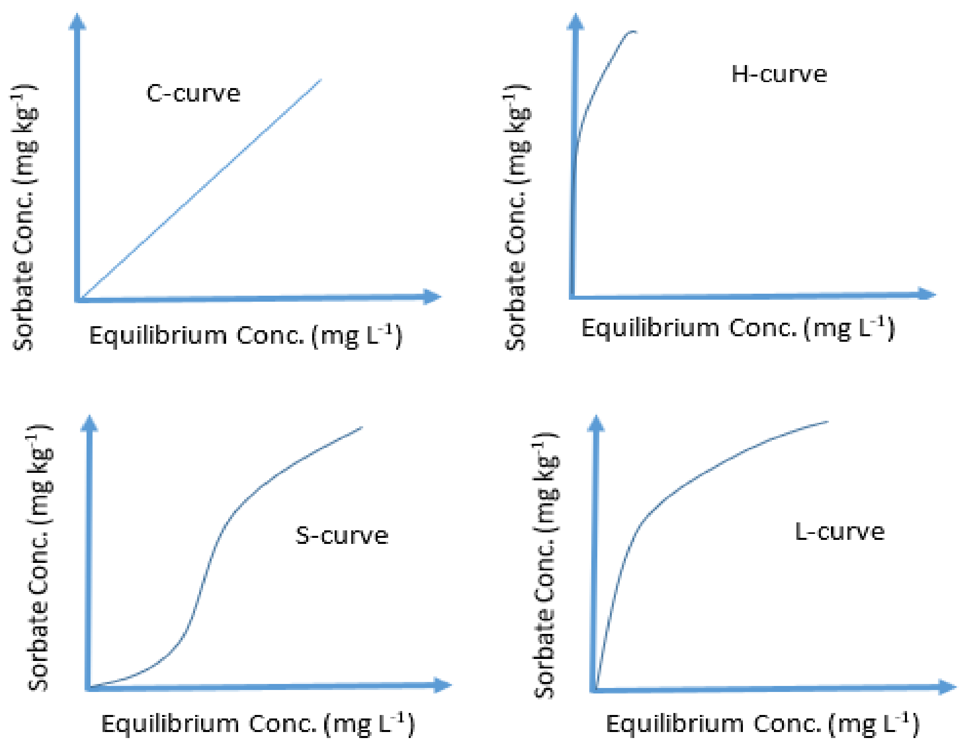

Figure 2-2. Generalized shapes of isotherm

The Kd gives an indication of the leachability of the ion (Gomes et al., 2001; Park et al., 2016) and it is often used to choose selectivity sequence when multiple metals are tested. The larger the Kd value, the more likely the ion will be attached to the sorbent. On the other hand, small Kd values indicate availability of the ion in solution. When the plot of Cs against Ce does not yield a straight line, it becomes difficult to get a single Kd value (i.e., the slope). As such, concentration dependent Kd (i.e., Kd at each concentration) was used. This was the case in the sorption of heavy metals (Cu, Cd, Cr, Pb and Zn) onto sesame straw biochar (Park et al., 2016), where it was attributed to lack of strong competition at low concentration below 10 mg L^{-1}. Likewise, Gomes et al. (2001) had similar observations in the competitive sorption of Cu, Cd, Cr, Ni, Pb and Zn onto soils.

2.5.3 Equilibrium-based sorption models

Empirical and non-empirical models or equations have been used to describe sorption of contaminants on sorbent materials. The most popularly used models are Freundlich, and Langmuir although several modifications of Freundlich and Langmuir such as Sips, Redlich-Peterson and Toth do exist (Ayawei et al., 2017).

2.5.3.1 Freundlich model

Originally developed to explain adsorption onto gas and solute phase, the Freundlich model has been used extensively to describe sorption onto solid phases, including soils and biochars (Park et al., 2016; Sangiumsak and Punrattanasin, 2014). It is mathematically expressed as (Eq. 2.2):

$$C_s = K_d {C_e}^{1/n} \qquad (2.2)$$

Apart from 1/n, which is a dimensionless adsorption intensity (Sangiumsak and Punrattanasin, 2014), the other terms have been defined in previous sections.

Put in its linear form, Eq. 2.2 becomes (Eq. 2.3):

$$\mathrm{Log}\ C_s = \mathrm{Log}\ K_d + \frac{1}{n}\mathrm{Log}\ C_e \qquad (2.3)$$

A plot of Log C_s on the ordinate against Log C_e on the abscissa, gives a straight-line curve with slope as 1/n and intercept as Log K_d. From Eq. 2.2, if 1/n is 1, then K_d, the distribution coefficient will be equal to the partition coefficient (Eq. 2.1). One major pitfall of the Freundlich model is that it does not predict the maximum sorption capacity. More so, having just one Kd term in the Freundlich model shows that the adsorption energy is not related to the surface coverage.

2.5.3.2 Langmuir model

Developed in 1918 by Irving Langmuir to account for gas molecule adsorption on planar surfaces, the Langmuir model has been used widely to describe sorption of molecules or ions on colloidal surfaces. It has as well been applied to describe sorption on heterogeneous surfaces such as soils and biochars (Park et al., 2016; Sangiumsak and Punrattanasin, 2014), although it has sometimes failed due to its inherent underlying assumptions stated as follows (Sparks, 1995): (1) sorption occurs only on planar surfaces with fixed number of sorption sites. (2) Adsorption is not irreversible. (3) Flow of molecules on the surfaces is not lateral. (4) The energy of sorption for all sites is similar and not related to surface coverage. Given the heterogeneous nature of soil, most of these assumptions are not applicable. As such, Langmuir model should be used solely to quantify and describe sorption when dealing with soils and related materials such as biochar. Langmuir model is expressed mathematically as (Eq. 2.4):

$$C_s = \frac{kC_e b}{(1+kC_e)} \qquad (2.4)$$

Where k (in L mg^{-1}) is a constant associated with the binding energy between the sorbate and sorbent, and b (in mg kg^{-1}) is the maximum sorption capacity for monolayer coverage. Put in its linear form, Eq. 4 becomes (Eq. 2.5):

$$C_e/C_s = 1/kb + C_e/b \qquad (2.5)$$

A plot of C_e/C_s on the ordinate against Ce on the abscissa, yields a straight line with slope as 1/b and intercept as 1/kb.

Despite the shortcoming noted by Sparks (1995), Langmuir model as well as Freundlich are the most widely used models for describing adsorption of contaminants onto soils and biochars in the literature (Bogusz et al., 2017; Melo et al., 2016).

2.6 Desorption

Desorption study is performed to intentionally detached the already attached sorbate under equilibrium conditions. As reported by Sparks (1995), some researchers do not perform desorption as part of sorption study, however others, especially recently, do (Bogusz et al., 2017; Melo et al., 2016). Desorption is very important as it reveals long-term affinity potential of a sorbent material, which is very important in understanding the true fate of a contaminant (Sparks, 1995). In fact, Melo et al. (2016) observed through desorption study that sorption of Cd on soil amended with sugarcane straw biochar was reversible, possibly because they used acidified water (pH 4.5) which could have increased the solubility of Cd. On the contrary, Bogusz et al. (2017), who used distilled water, noticed that heavy metals (Ni and Zn) adsorbed to soil amended with willow biochar desorbed less as compared to the control without biochar amendment. In a contaminant fate study, desorption is planned based on the purpose for which the sorbent will be used and as such consideration is given to the shaking solution, which could either be water or acidic water. For the case of an agricultural soil that will be receiving wastewater irrigation and sometimes rainfall, it will be worthwhile to perform desorption study with ionic-strength adjusted water. For a sorbent washing purpose, desorption study can be done with a more aggressive solution such as acidic water. In any of the cases above, the desorption process is performed maintaining similar conditions as the sorption process—same temperature, same equilibration time, same mixing speed and same adsorbent dosing (Bogusz et al., 2017).

2.7 Knowledge gap

Given the institutional breakdown, lack of technical skills and lack of money that is ravaging several developing countries (Connor et al., 2017), the use of untreated wastewater for irrigation remains the most sustainable alternative to reduce the burden placed by agriculture on freshwater withdrawal. Untreated wastewater contains contaminants, including heavy metals, at high concentrations capable of causing detrimental health challenges to humans who consume crops cultivated using such water. To alleviate the ill-effects posed by untreated wastewater usage for irrigation, innovative and affordable techniques, which can be deployed by farmers to reduce contaminant transport into crops, are required. Phytoremediation, composting and liming are several techniques being considered. Adiloğlu et al. (2016) studied phytoremediation of nickel with canola plant (Brassica *napus* L.), while Wieshammer et al. (2007) studied the phytoremediation of Zn and Cd using Salix spp. and Populus spp. Although identifying a plant for phytoremediation is an inexpensive approach, however, not only that it takes longer time for plants to establish, unintended introduction of invasive plants could be detrimental to the ecosystem (Mahar et al., 2016). Biochar (described in section 2.2) with additional benefit of sequestering carbon (reducing CO_2 emission (Lehmann and Joseph, 2009)) could offer a better option. In a batch sorption study, Uchimiya et al. (2010b) observed simultaneous removal of Ni, Cd, Cu and Pb from soil and water using broiler litter biochar. In a pot study, Lu et al. (2014) found heavy metals (Cd, Cu, Pb and Zn) significantly adsorbed onto sandy-loam soil amended with bamboo and rice straw biochars as compared to the control (without biochar). In another pot study, Namgay et al. (2010) found that modified wood biochar reduced concentration of As, Cd and Cu was observed in maize shoot after 3 weeks of biochar amendment, while levels of Pb and Zn was unpredictable. Also, in the soil, the concentration of Pb reduced, although the concentration of As and Zn increased, Cu did not change and Cd was inconsistent. In these and other recent studies (Son et al., 2018; Vamvuka et al., 2018; Wagner and Kaupenjohann, 2015), biochar was either used in an aqueous solution or used in the remediation of an already contaminated soil, where the biochar was not loaded continuously with contaminants; biochar may thus have different behavior when it is loaded continuously with contaminants—as applicable in wastewater irrigation. As such, there is a research need to understand the behavior of biochar when continuously loaded with contaminants, especially co-existing, which is obtainable in real life. When heavy metals co-exist in the presence of a sorbent, they compete for sorption sites.

Although the findings of the above research indicate heavy metal sorption in cultivated soils to a different extent when amended with biochar produced from different feedstock, these studies did not report translocation of contaminants to crops cultivated in biochar-amended soils, especially under wastewater irrigation. Such studies are very much needed given the current interest in wastewater irrigation, especially in developing countries.

Since feedstock plays an important role in characteristics of biochar, it is worth evaluating sustainable feedstock, such as plantain peel, which is wasted otherwise. To the best of my knowledge, the potency of plantain peel biochar on the simultaneous immobilization of Cd, Cr, Cu, Fe, Pb and Zn in this type of complex mixture when applied to soil as irrigation water is not known.

Connecting Text to Chapter 3

Biochar has shown potential of immobilization of wastewater-borne heavy metals when amended with soil. Biochar needs to be produced from sustainable feedstock for agricultural and environmental application. Plantain peel, considered as a waste, could be converted to biochar. It may prove to be a sustainable feedstock for biochar production in certain parts of the world, if found effective. It is also imperative that sandy soils are prone to a greater degree of transport of contaminants when irrigated with wastewater. Heavy metal translocation from wastewater to a few aboveground agricultural crops is documented. Underground crops, such as potatoes, come in direct contact with contaminants, and therefore such crops are liable to greater risk of heavy metal translocation. Chapter 3 provides information about the fate and transport of heavy metals in a sandy soil amended with biochar. Biochar was produced from plantain peel feedstock, and it was characterized. In the field experiment, the biochar was mixed with soil, and potatoes were grown using synthetic wastewater irrigation. The accumulation of heavy metals in soil and their uptake by different part of the potato plant was studied.

This chapter, Effect of Biochar on Heavy Metal Accumulation in Potatoes from Wastewater Irrigation, has been published in the Journal of Environmental Management. The manuscript is co-authored by Dr. Shiv Prasher, my supervisor, Dr. Eman ElSayed, a post-doctoral fellow in the department, Mr. Jaskaran Dhiman, a PhD scholar in the department, Mr. Ali Mawof, a PhD scholar in the department and Dr. Ramanbhai Patel, a research associate in the department. To ensure consistency with the thesis format, the original draft has been modified, and the cited references are listed in the reference section (Chapter 9).

Chapter 3: Effect of Biochar on Heavy Metal Accumulation in Potatoes from Wastewater Irrigation

3.1 Abstract

In many developing countries water scarcity has led to the use of wastewater, often untreated, to irrigate a range of crops, including tuber crops such as potatoes (Solanum *tuberosum* L.). Untreated wastewater contains a wide range of contaminants, including heavy metals, which can find their way into the portion of the crop which is consumed, thereby posing a risk to human health. An experiment was undertaken to elucidate the fate and transport of six water-borne heavy metals (Cd, Cr, Cu, Fe, Pb and Zn), applied through irrigation water to a potato (cv. Russet Burbank) crop grown on sandy soil, having either received no biochar amendment or having top 0.10 m of soil amended with 1% (w/w) plantain peel biochar. A non-amended control, irrigated with tap water, along with the two contaminated water treatments were replicated three times in a completely randomized design carried out on nine outdoor cylindrical PVC lysimeters (1.0 m × 0.45 m). The potatoes were planted, irrigated at 10-day intervals, then leachate was collected. Soil samples collected two days after each irrigation showed that all heavy metals accumulated at 0.0 m depth; Fe, Pb and Zn were detected at 0.1 m depth, while only Fe was detected at 0.3 m depth. The heavy metals were not detected in the leachate. Matured potatoes were harvested and separated into flesh, peel, leaf, stem and root. Heavy metals translocated to all portions of the potato plant (tuber flesh, peel; root, stem and leaf). Biochar-amended-soil significantly reduced only Cd and Zn concentrations in tuber flesh (69% and 33%, respectively) and peel compared to the non-amended wastewater control ($p<0.05$). Heavy metal concentrations in the tuber flesh were significantly

lower than those in the peel, suggesting that when consuming potatoes grown under wastewater irrigation, the peel poses a higher health risk than the flesh.

Keywords: plantain peel biochar, synthetic wastewater, contaminants, potatoes, irrigation, lysimeters

3.2 Introduction

Facing a scarcity of freshwater (Mekonnen and Hoekstra, 2016), a number of developing countries have resorted to wastewater, particularly untreated wastewater, for irrigation (Hussain et al., 2013; Melloul et al., 2001; Yang et al., 2006). Irrigation with untreated wastewater risks contaminating soils and receiving water bodies with dangerous levels of heavy metals (Ahmad et al., 2011; Gokhale et al., 2008). Depending on the soil physicochemical characteristics (e.g., texture, pH, cation exchange capacity), heavy metal species in untreated wastewater could accumulate at the soil surface and be available for plant uptake or leach to the groundwater. For instance, lead (Pb), cadmium (Cd), zinc (Zn) and iron (Fe) accumulation was detected in a loamy soil irrigated with untreated wastewater (Kiziloglu et al., 2008), whereas Pb and Cd were measured in the leachate of soils contaminated with coal fly ash deposits (Hartley et al., 2004). Abdu et al. (2011) raised concerns of Cd and Zn leaching to the groundwater following wastewater irrigation.

Crops grown with heavy metal contaminated irrigation water can take up heavy metals through their roots, and translocate them to different edible portions, where they may accumulate to toxic levels. For instance, Hussain et al. (2013) reported the uptake of copper (Cu), Zn, Pb, chromium (Cr) and Fe in vegetable crops grown in wastewater irrigated soils, and their eventual translocation to leaves and stems. In another study, Roy and McDonald (2015) reported high concentrations of Cd and Zn in carrot (Daucus *carota* L.) and radish (Raphanus *sativus* L.) grown in a heavy metal contaminated soil. Likewise, potatoes grown in soil receiving sewage water irrigation had high concentrations of Cd, and Pb, far above permissible limits for potatoes (0.1 mg kg^{-1}) (Sadiq Butt et al., 2005).

Consumption of heavy metal contaminated food may result in serious health problems. For example, Pb poisoning is linked to kidney disease (Wang et al., 2009), while diseases such as Alzheimer's and "itai-itai", are linked to Cu and Cd toxicity, respectively (Brewer, 2009). By displacing calcium, Cd accumulates in bones and softens them (Wang and Bhattacharyya, 1993). Moreover, both Cd and Pb have long been classified as endocrine disruptors (Colborn et al., 1993), while Zn, though regarded as an essential micro nutrient (Lichtfouse et al., 2009), can, at toxic levels, lead to a shortage of other nutrients (e.g., Cu and Fe), leading to anæmia, neutropænia and poor immune function (Fosmire, 1990). Therefore, it is essential to reduce the bioavailability of these heavy metals to crops, especially root and tuber crops which comes in direct contact with contaminated soil and water. There is an urgent need to develop innovative low-cost, simple and easy-to-use methods to reduce the risk of heavy metal translocation from irrigated wastewater to food crops.

Biochar, a carbon-rich product obtained from the thermal breakdown (Inyang et al., 2015) or pyrolysis of plant based materials (Lehmann and Joseph, 2009), has shown some environmental benefits when applied to soil (Abit et al., 2012; Kookana et al., 2011). Not only is it useful in carbon sequestration and reducing greenhouse gas emissions, but it may also prove to be a versatile product for reducing contaminant transfer from soil-water media to food crops. For instance, laboratory- and field-scale studies have shown biochar's effectiveness in reducing the mobility and bioavailability of heavy metals from soil and water media (Park et al., 2016; Zhang et al., 2016). In a batch sorption study, Uchimiya et al. (2010a) observed simultaneous removal of nickel, Cd, Cu and Pb in soil and water using broiler litter biochar. Namgay et al. (2010) investigated the role of modified wood biochar in the uptake of Cd, Cu, Pb and Zn by maize (Zea *mays* L.) shoots. They observed reduced concentrations of Cd and Cu in maize shoots 3 weeks after biochar amendment of the soil. The effectiveness of biochar in reducing heavy metal movement largely depends on the type of feedstock used for its production (Abit et al., 2012; Alburquerque et al., 2014). For instance, Fellet et al. (2014) conducted a bioavailability study using three different feedstocks (orchard pruning residues, fir tree pellets and manure plus fir tree pellet). They found the biochar originating from manure and fir tree pellets to have outperformed the others in terms of Cd and Pb immobilization.

Biochar can be produced from agricultural waste and food by-products, such as plantain peel. Plantain, a staple food rich in starch, is predominantly cultivated in Africa, Asia and Latin America (Tchango Tchango et al., 1999). In 2014, about 30 Tg of plantain was produced globally (FAO, 2017). In all forms of plantain processing, peel always remains a waste product (Tchango Tchango et al., 1999). With plantain peel accounting for about 40% of plantain fruit (e.g., 12 Tg globally in 2014) (Rubatzky and Yamaguchi, 1997), it could constitute a sustainable feedstock for biochar production. Modified plantain peel (Agarry et al., 2013), plantain peel residue (Idowu et al., 2011) and plantain peel activated carbon (Inam et al., 2016) have been studied as sorbent materials. Accordingly, the transformation of plantain peel to biochar and its use as a biosorbent could be a very viable option.

Some studies have investigated the benefits of soil amendment with biochar on potato production (Collins et al., 2013; Jay et al., 2015; Liu et al., 2014; Upadhyay et al., 2014; Walter and Rao, 2015; Yang et al., 2015), while others (Akhtar et al., 2015a; Akhtar et al., 2015b) have studied the role of biochar in improving water uptake by potatoes grown with saline water irrigation. Although studies have shown that heavy metals are taken up by potatoes from wastewater irrigation, the use of biochar to mitigate their uptake from wastewater has not been investigated. Such investigation is necessary for potatoes being a vital source of antioxidant as well as being sensitive to water stress (Brown, 2005; King and Stark, 1997). Moreover, potato peel has been reported to contains more antioxidants than the flesh (Brown, 2005), and many consumers eat their potatoes unpeeled. However, peels would be subject to greater exposure to heavy metal contaminated water and soil, and would likely take up greater quantities of heavy metals (Havre and Underdal, 1976). Thus, there is a need to develop techniques to minimize transport of wastewater borne several co-existing heavy metals in light soils, and to control their translocation to tuber developing while in direct contact of contaminants. The present study was conducted to understand the effect of plantain peel biochar amendment to sandy soil on the transport of wastewater borne six co-existing heavy metals (Cd, Cr, Cu, Fe, Pb and Zn) in soil, and to evaluate the effect on uptake of these heavy metals by potato roots, and subsequent translocation to edible parts—flesh and peel, and non-edible parts—stem and leaves.

3.3 Materials and methods

3.3.1 Biochar production and characterization

Green plantain fruits were purchased from Sami Fruits, Ville LaSalle (Montreal), Canada. The fruits were peeled with stainless steel knives and the peels dried in an oven at 80^{o}C for two days. After drying, the peels were stored at room temperature. The peels were then gasified in a student-type gasifier (Kwofie and Ngadi, 2016) located in McGill University, Macdonald Campus Technical Service Building (Sainte-Anne-de-Bellevue, Quebec, Canada) at a temperature between 450° and 500^{o}C. The biomass residence time in the gasifier ranged from 18 to 25 mins. The ash content of the biochar was determined following the method described by Enders et al. (2012); trace metal analysis was performed following the hot nitric acid extraction method described in the soil extraction section. A 1:30 (w/w) biochar: deionized water suspension was prepared, and placed on a reciprocating shaker for 4 h (Zhang et al., 2015). The pH was then measured using an electrode type pH meter (Accumet AB 15, Fisher Scientific). Afterwards, the electrical conductivity (EC) of the suspension was measured using an EC meter (DiST 6 EC/TDS/Temperature Tester, Hanna Instruments, Woonsocket, Rhode Island, USA). Other proximate and ultimate analysis of the biochar, such as volatile matter, fixed carbon, organic carbon, hydrogen, nitrogen and oxygen content (Table 3.1), were quantified at the Canmet ENERGY/Characterization Laboratory (ISO 9001:2008, FS 64051), Ottawa, Ontario, Canada.

Table 3.1. Properties of Gasified Plantain Peel Biochar on dry weight basis

Proximate Analysis	%dry weight	Method
Moisture TGA	9.88	ASTM 7582
Ash	77.5	ASTM 7582
Volatile matter	18.1	ISO 562
Fixed Carbon	4.0	ASTM 7582
Ultimate Analysis		Method
Carbon	18.1	ASTM D5373
Hydrogen	0.48	ASTM D5373
Nitrogen	0.6	ASTM D5373
Total Sulphur	<0.05	ASTM D4239
Oxygen	3.37	By Difference

TGA-thermogravimetric analysis

The removal percentage $[\text{i.e.}, (\text{initial conc.} - \text{equilibrum conc.}) * 100/\text{initial conc.}]$ of the biochar in a multi-metal solution was tested. For this, a 100 mg L^{-1} stock solution each of Cd, Pb and Cr was prepared using reagent grade salt: $Cd(NO_3)_2.4H_20$, $Pb(NO_3)$ and $K_2Cr_2O_7$, respectively. Multi-metal solutions representing low (2.5, 8 and 1 mg L^{-1}), medium (5, 16 and 2 mg L^{-1}) and high (10, 32 and 4 mg L^{-1}) concentrations of Cd, Pb and Cr, respectively, were prepared in 5% nitric acid (heavy metal grade); the medium concentrations were based on typical wastewater concentrations of these heavy metals in developing countries (Ahmad et al., 2011). Replicated three times, biochar-multi-metal solution mixtures (1:20 w/w) were prepared in a 50-mL Falcon tube and capped; it was vortexed, and kept on a rotary shaker for 24 h. Afterwards, the resulting solutions were vacuum filtered (Whatman No. 1) and the filtrates were analysed using ICP-OES (Varian, Vista-MPX CCD Simultaneous).

3.3.2 Experimental procedure

Nine cylindrical lysimeters (1.0 m x 0.45 m) built of PVC (Fig. 3.1) were packed with sandy soil to a bulk density of 1.35 Mg m^{-3}. Soil properties are shown in Table 3.2. The three treatments were:

- wastewater applied to soil having received no biochar amendment (control, WW-B),
- wastewater applied to soil where the 0.10 m of topsoil has been amended with 1% (w/w) plantain peel biochar (WW+B) (Sarmah et al., 2010), and
- freshwater application to soil without biochar (FW-B).

The WW+B to WW-B comparison served to evaluate the effect of biochar on the spatial and temporal mobility of the contaminants, while the FW-B to WW-B comparison served to determine the overall bioavailability of heavy metals to potatoes.

Treatments were replicated three times in a completely randomized design implemented on nine outdoor PVC lysimeters (1.0 m × 0.45 m). Four soil sampling ports per depth were drilled radially in the lysimeter wall at depths of 0.15, 0.35 and 0.65 m from the top of the lysimeter and sealed with plastic stoppers. Russet Burbank potato tubers were procured from Global Agri Services, Grand Falls, New Brunswick, Canada. The lysimeter soils were brought to field capacity, and then sprouted potato tubers were planted 60 to 100 mm below the soil surface. The quantity of fertilizer applied per lysimeter was based on recommendations from Idaho Extension (http://www.extension.uidaho.edu/nutrient). Potassium sulphate (0-0-60) was broadcasted at the rate of 280 kg K ha^{-1} (7.42 g/lysimeter) on the day of planting. Ammonium sulphate (21-0-0) was applied on the soil surface at an overall rate of 314 kg N ha^{-1} (23.8 g/lysimeter), half on the day of planting (Day 1), and a quarter each on Days 33 and 43, corresponding to the potatoes' bulking period, when nitrogen requirements are high (Ojala et al., 1990). Herbicide (Sencor® 480 F) was applied, pre-emergence, at the rate of 2.25 L ha^{-1}. Irrigation water was not applied for 20 days after planting until the potatoes began emerging. Prior to irrigation with wastewater, from Day 20 to 33, tap water was applied every two days at a rate of 1.7 mm d^{-1} (270 mL/lysimeter) to encourage potato growth.

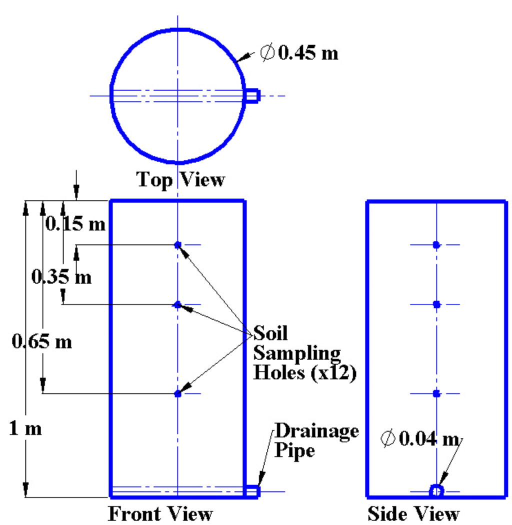

Figure 3-1. Orthographic view of a one-meter tall aboveground lysimeter

Table 3.2. Physical and chemical properties of soil

Soil Properties	
Sand (%)	92.2
Silt (%)	4.3
Clay (%)	3.5
pH	5.5
CEC (cmol(+) kg^{-1})	3.3±0.4
Organic matter (%)	2.4±0.15
Hydraulic conductivity (m day^{-1})	1.67±0.45
ZPC	3.4
P (mg P kg^{1})	215.30±40.43
K (mg K kg^{-1})	107.33±13.13
N (mg NO_3-N kg^{-1})	4.57±0.46
Ca (mg Ca kg^{-1})	912.44±79.70
Mg (mg Mg kg^{-1})	103.27±7.29
Al (mg Al kg^{-1})	1164.14±12.40
Cd (mg Cd kg^{-1})	<LOD
Cr (mg Cr kg^{-1})	17.86±0.38
Cu (mg Cu kg^{-1})	<LOD
Fe (mg Fe kg^{-1})	11109.64±238.68
Pb (mg Pb kg^{-1})	<LOD
Zn (mg Zn kg^{-1})	16.70±2.28

LOD: limit of detection; ZPC: zero point of charge; CEC: cation exchange capacity; the heavy metals Cd, Cr, Cu, Fe, Pb and Zn were determined following hot acid extraction (Stephan et al., 2008) and quantified by ICP-OES. The LOD was 50 µg L^{-1} (15.6 mg kg^{-1}) for all the metals. P, K, Ca, Mg, and Al were determined following Mehlich III extraction (Mehlich, 1984), while N was determined following 2.0 M KCl method (Carter and Gregorich, 2008). Other soil properties were adapted from a previous study (ElSayed et al., 2013).

Made with tap water, stored overnight in an open container to release chlorine, the synthetic wastewater employed in the present study combined basic synthetic wastewater ingredients (LaPara et al., 2006; Nopens et al., 2001), along with a number of additional contaminants (e.g., heavy metals, hormones, pharmaceuticals, surfactants and plasticizers) at concentrations based on worst case reports from a number of countries in the developing world, or at concentrations sufficient for residual or translocated concentrations to be above detection limits (Table 3.3). Soluble salts of the contaminant metals of interest (Sigma Aldrich, Oakville, Ontario) were mixed with the dechlorinated water, basic synthetic wastewater ingredients and other contaminants, to prepare the synthetic wastewater applied to the WW-B and WW+B treatments at every irrigation event (Table 3.3).

A total of eight irrigations occurred at 10-day intervals; 11.5 L of water was applied as irrigation to each lysimeter at the just ponding rate. Porous cheese cloth was placed on the top of each lysimeter to maintain uniform distribution of irrigation water on the soil surface and avoid spot erosion.

3.3.3 Leachate analysis

After each irrigation, leachate exiting each lysimeter's drainage outlet was collected in one-liter bottles until flow stopped (Figure 3.1). After total leachate volume was measured, a single 1.0 L composite leachate sample was prepared, brought to the lab, and immediately extracted for heavy metals. Leachate samples were filtered through a 90 mm preconditioned glass filter (Advantec® GF-75) followed by a 47 mm diameter filter (0.45 µm pore size; Maine 1215548; Maine Manufacturing, LLC) (American Public Health Association, 2005). The filtrate was acidified to 1% (v/v) with concentrated nitric acid (trace metal grade, 70% pure) and heavy metals analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES) (Varian, Vista-MPX CCD Simultaneous).

3.3.4 Soil analysis

Composite soil samples (≈5 g) were collected from each depth of the soil profile (0.0, 0.10, 0.30 and 0.60 m): prior to planting (Day-BP), and two days after each irrigation event. These two days were allowed for soil to attain field capacity.

Table 3.3. Recipes for synthetic wastewater.

Purpose	Substance/ Compounds	Country	Concentration **(mg L^{-1})** (µg L-1)	**Wastewater Recipe Source** Contaminant reporter
		Basic synthetic wastewater ingredients		
C source	Sodium Acetate		**79.37**	Nopens et al. (2001)
	Milk powder		**116.19**	Nopens et al. (2001)
	Soy Oil		**29.02**	Nopens et al. (2001)
	Starch		**122**	Nopens et al. (2001)
	Yeast Extract		**52.24**	Nopens et al. (2001)
N Source	Ammonium Chloride		**12.75**	Nopens et al. (2001)
	Peptone		**17.41**	Nopens et al. (2001)
	Urea		**91.74**	Nopens et al. (2001)
P Source	Magnesium phosphate		**29.02**	Nopens et al. (2001)
Minerals	$CaCl_2$		**60**	LaPara et al. (2006)
	$MgCl_2$		**40**	LaPara et al. (2006)
	$NaHCO_3$		**100**	LaPara et al. (2006)
	K_3PO_4		**30**	LaPara et al. (2006)
	Additional contaminants concentrations based on worst case reports or need to exceed LOD			
Heavy Metals	Potassium dichromate (Cr)	India	**2**	Ahmad et al. (2011)
	Cadmium Nitrate (Cd)	India	**5**	Ahmad et al. (2011)
	Lead Nitrate (Pb)	India	**16**	Ahmad et al. (2011)
	Iron Sulphate (Fe)	India	**120**	Ahmad et al. (2011)
	Zinc Nitrate (Zn)	India	**3**	Ahmad et al. (2011)
	Copper Nitrate (Cu)	India	**8**	Ahmad et al. (2011)
Hormones	Estrone (E1)	Korea	8.15 (50)	Sim et al. (2011) — LOD
	Estradiol (E2)	Korea	0.634 (20)	Sim et al. (2011) — LOD
	Progesterone	China	0.90 (20)	Huang et al. (2009) — LOD
Pharmaceuticals	Oxytetracycline	China	19.5	Li et al. (2008)
	Ibuprofen	India	26.45	Singh et al. (2014)
Surfactant	Triton X-100 or alkylphenyl polyethoxylate	Morocco	30	Aboulhassan et al. (2006)
Plasticizers	Bisphenol A		(50)	Based on LOD
	Bisphenol S		(50)	Based on LOD
	Bisphenol F		(50)	Based on LOD

Values in parentheses are actual concentrations used

The soil samples were brought to the lab and stored in a freezer (-24^{o}C) until analysis. The topsoil's pH was measured at the end of experiment following the standard soil survey test method (pHWC2/2) (Rayment and Higginson, 1992). The soil was air-dried for two days and passed through a 2-mm sieve. One gram of the air-dried soil sample was weighed into a 15 mL bottle and 5 mL of deionised water was added (soil: water ratio, 1:5). The solution was shaken for 1 h, and then the pH of the suspension measured using an electrode type pH meter (Accumet AB 15). The cation exchange capacity (CEC) and the organic matter content of the soil samples (surface and 0.1 m below surface) was measured following $BaCl_2$ (Carter and Gregorich, 2008) and loss-on-ignition methods (Rowell and Coetzee, 2003), respectively.

Heavy metals were recovered from the soil by a hot nitric acid extraction method (Kargar et al., 2013; Stephan et al., 2008). About 3 g of soil was weighed into an aluminum weighing dish and air dried for 2 days. The air-dried soil was crushed using a ceramic mortar and pestle, and homogenized by passing it through a 2 mm sieve (Fisher Scientific Co., U.S. Standard Series). The crushed soil subsamples (0.16 g each) were weighed into 15 mL digestion tubes, and 2 mL of concentrated nitric acid (trace metal grade, 70% pure) was added (Kargar et al., 2013). The solution was kept overnight under a fume hood. The next day, the samples were place on a block digester (Fisher Scientific®, dry batch incubator) instrumented with a thermometer. The temperature was gradually increased to 80°C and kept at this temperature until fuming stopped. The temperature was further increased, gradually, to 120±5°C. At this temperature, the solution was further digested for 5 hours. Afterwards, the samples were removed from the digester and allowed to cool for 15 min. The digested solution was transferred into a 50 mL falcon tube, the digestion tube rinsed five times with double deionized water, and the volume of the solution in the falcon tube adjusted to 50 mL. Aliquots (15 mL) of the digested solutions were analyzed by ICP-OES. To ensure quality control, reference materials (SED 98-04 and SED 92-03, Environment Canada) and method blanks were added in all batches. Recovery percentage of the SED 98-04 and SED 92-03 were as follows: 83.4, 83.9% for Cr; 82.4, 106.3% for Cu; 61.0, 83.6% for Fe; 95.1, 90.8% for Pb; and 87.7, 82.4% for Zn; it was not available for Cd as concentrations in the reference material were below detection limit. The detection limits, converted to mg kg^{-1} from mg L^{-1} using same proportion as digested samples, were 15.6 for Cd, Cu, Cr, Zn and Pb; and 156.5 for Fe.

3.3.5 Potato analysis

The potatoes were harvested at Day 120. The potato tissues (peel, flesh, root, stem, and leaf) were separated and washed (3 times) with deionized water to ensure no soil particles were retained. The peeled tubers were dissected longitudinally on a plastic chopping board, and one half was further diced into 10 mm × 10 mm cubes. All tissues were sampled and oven dried (60^{o}C) for 2 days.

The oven-dried samples were crushed with a coffee grinder followed by a ceramic mortar and pestle. Crushed subsamples (0.16 g) were weighed and placed into 15 mL digestion tubes, and the procedure detailed for soil analysis performed. The concentration of heavy metals in the potato tissues was expected to be low, therefore, quantification was done by inductively coupled plasma mass spectrometry (ICP-MS) (Varian ICP820-MS or Analytik-Jena). Peach leaf Standard Reference Material, NIST-1547, as well as method blanks were added in all batches. Recovery percentage of the NIST-1547 were as follows: 198.3% for Cd, 85.6% for Cr, 98.9% for Cu, 82.2% for Fe, 91.1% for Pb, and 105.8% for Zn. The detection limits converted to mg kg^{-1} using same proportion as digested samples were as follows: 0.010, 0.011, 0.016, 0.046, 0.008 and 0.032 for Cd, Cr, Cu, Fe, Pb and Zn, respectively.

3.3.6 Data analysis

3.3.6.1 Accumulation factor

The accumulation factor, also known as the bio-accumulation factor, is defined as the ratio of concentration of heavy metal in plant tissue to its concentration in soil (Zhuang et al., 2009). The accumulation factor of the different heavy metals was calculated for peel, flesh and root. The depth of heavy metal distribution from the soil surface was determined. The concentration of heavy metals in soil (weighted average) for an effective root zone depth of 0.30 m was used (Opena and Porter, 1999).

3.3.6.2 Statistical analysis

All data were subjected to repeated measure analysis with PROC Mixed procedure in the Statistical Analysis System (SAS, v. 9.4, SAS Institute, Cary, North Carolina). Treatment, days and depth were assigned as fixed effects, lysimeter served as subject, and was nested within treatment, while the concentration served as the response variable. For heavy metal concentrations in plant tissues, data were subjected to least square means difference comparisons for each pair (WW+B and WW-B; WW-B and FW-B) using student t-test in SAS-JMP® 13.0.0 (Copyright © 2016 SAS Institute Inc.).

3.4 Results and discussion

3.4.1 Biochar characterization

The ash content of the biochar was 77.5% (Table 3.1). Ash content is indicative of inorganic materials such as carbonates, sulphates and phosphates (Downie et al., 2009); inorganic compounds form precipitates with heavy metals and are important in their sorption (Qian et al., 2016). The EC exceeded 17.02 dS m^{-1} (exceeding the instrument's maximum reading), denoting the presence of a high level of dissolved ions. This was to be expected since the ash content was high (Gai et al., 2014). At 10.27±0.05 pH, the biochar was alkaline. Adsorption of heavy metals to soil and their bioavailability to plants is strongly tied to soil pH, which affects the type of charge on soil particle surfaces (Andersson and Nilsson, 1974). Mixing of the biochar with soil could increase the soil's pH to such a level as would reduce the bioavailability of heavy metals in the soil solution. According to the International Biochar Initiative, the concentrations of heavy metals (e.g., Cd, Cu, Cr, Fe, Pb, and Zn) in the biochar were within acceptable limits for its use as a soil amendment to be deemed safe with respect to potential heavy metal contamination (Table 3.4; http://www.biochar-international.org/characterizationstandard).

Table 3.4. Heavy metal concentrations (mean±standard error) in gasified plantain peel biochar and International Biochar Initiative (IBI) allowable threshold concentrations for biochar.

Heavy Metal	**Biochar heavy metal concentrations (mg kg^{-1})**	
	Plantain Peel Biochar	**IBI Allowable Thresholds**
Cd	not detected	1.4-39
Cu	7.11±0.98	63-1500
Cr	1.67±0.17	64-1200
Fe	669.12±86.35	not available
Pb	0.043±0.004	70-500
Zn	35.65±1.39	200-7000

3.4.2 Mass balance of the heavy metals

Each irrigation event added heavy metals to soil. The total mass of Cd, Cr, Cu, Fe, Pb and Zn applied to each lysimeter, after eight wastewater irrigations, was 460, 184, 736, 11040, 1472 and 276 mg, respectively. However, the uptake of heavy metals varied with the treatments, with plants grown in biochar-amended (*vs.* non-amended) soil bearing lesser heavy metal concentrations. The heavy metals, Cd, Cr, Cu, Fe, Pb and Zn, taken up by the potato plants represented 1.2%, 0.2%, 0.4%, 0.5%, 0.1% and 6.5%, respectively, for the WW-B treatment and 0.6%, 0.2%, 0.3%, 0.3%, 0.2% and 1.6% for the WW+B treatment. This could be attributable to the immobilization of the metals in soil as a result of pH increase caused by biochar amendment.

3.4.3 Heavy metals in soil

Concentrations of heavy metals (Cd, Cu, Cr, Fe, Pb and Zn) measured in soil samples drawn at the surface, at 0.1 m below the soil surface and at 0.3 m below the soil surface (e.g. $[\mathrm{Cd}]_{\mathrm{soil}}^{\mathrm{surf}}$, $[\mathrm{Cd}]_{\mathrm{soil}}^{0.1}$, $[\mathrm{Cd}]_{\mathrm{soil}}^{0.3}$, respectively) during the experimental period are shown in Fig. 3.2, while the results of the statistical analysis are summarized in Table 3.5. Irrespective of the treatment, all the heavy metals were detected in the upper soil profile, their adsorption probably due to the presence of soil organic matter, which plays a vital role in the immobilization of heavy metals in soil (He and Singh, 1993). The soil organic matter ranged from 3.8±0.3% at the surface to 2.3±0.1% at a 0.1 m depth; a range above the 2% threshold necessary for heavy metal fixation in soil (Kabata-Pendias, 2010). Biochar amendment did not contribute to soil organic matter content due to slower biodegradability of carbon in biochar (Cross and Sohi, 2011). Another soil property that could account for the binding of the heavy metals in the soil profile is the presence of fine particles in the form of clay (3.5%; Table 2), which are known to possess high specific surface area (Macht et al., 2011). Time had a significant positive effect ($p \leq 0.05$) on soil Cd concentration at the surface ($[\mathrm{Cd}]_{\mathrm{soil}}^{\mathrm{surf}}$). In the WW-B treatment, the $[\mathrm{Cd}]_{\mathrm{soil}}^{\mathrm{surf}}$ gradually increased from non-detectable on Day 3 to 65.1 mg kg^{-1} on Day 73, compared to a rise in $[\mathrm{Cd}]_{\mathrm{soil}}^{\mathrm{surf}}$ from non-detectable to 102.4 mg kg^{-1} over the same period for the WW+B treatment. This increase in $[\mathrm{Cd}]_{\mathrm{soil}}$ upon the application of wastewater concurred with the observations of Yang et al. (2006).

The $[\mathrm{Cd}]_{\mathrm{soil}}^{\mathrm{surf}}$ in the WW+B treatment was significantly higher ($p \leq 0.05$) than that under the WW-B throughout the experiment.

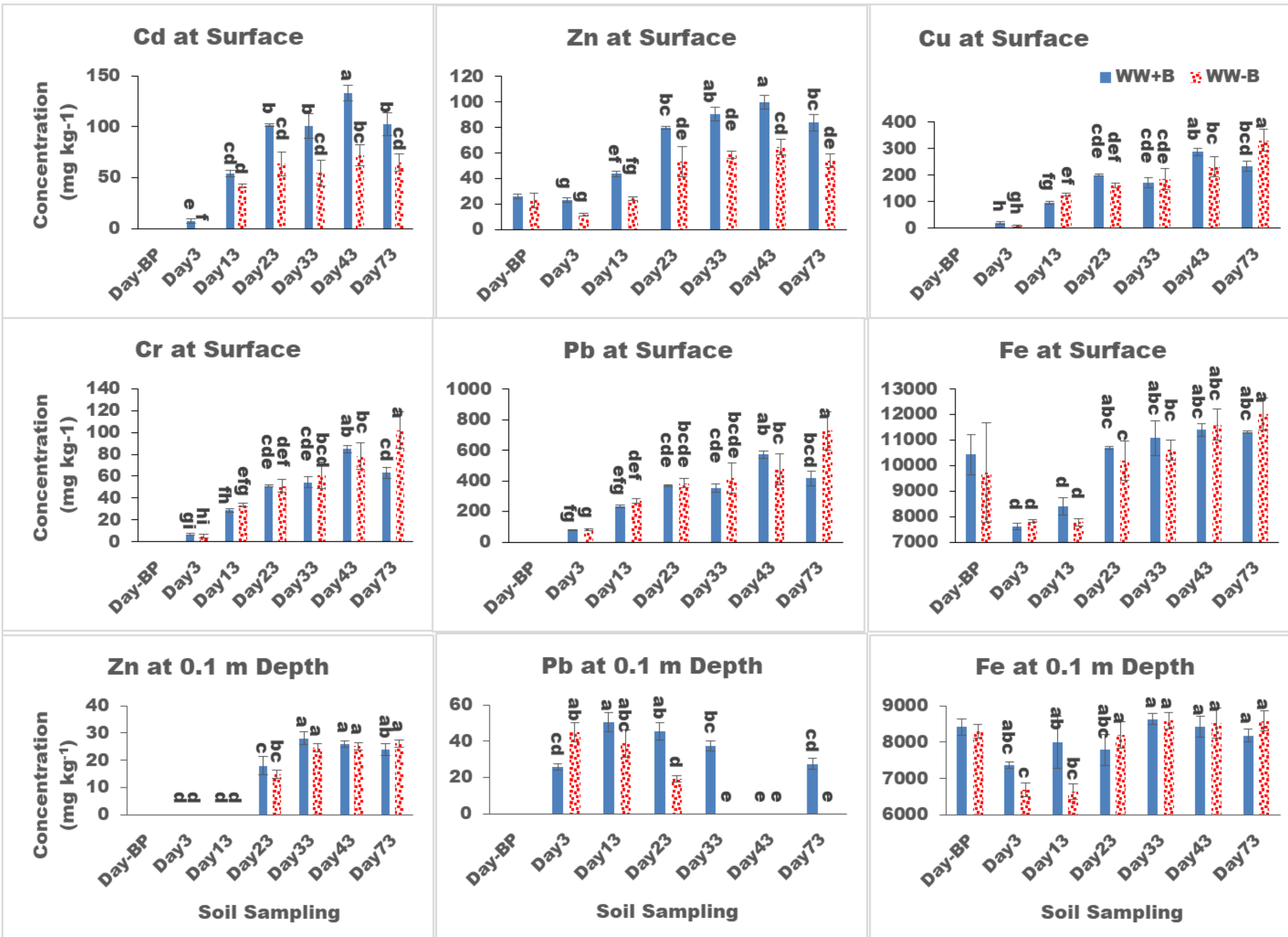

Figure 3-2. Concentrations of Cd, Zn, Cu, Cr, Pb and Fe at the soil surface and 0.1 m depth; plots for Cd, Cu and Cr at 0.1 m depth are not shown because concentrations were below the detection limit. Day-BP is the day before planting. Error bars represent standard errors of 3 replicates; different letters above the error bars indicate significant difference across time and across treatment ($p<0.05$) as per repeated measures analysis of variance.

Although in aqueous solution, a laboratory study conducted to evaluate sorption of Cd by the biochar used in this experiment showed 98% removal percentage for Cd (Nzediegwu et al., 2015); the biochar's addition to the soil significantly altered the soil's pH (from 4.9±0.18 to 5.2±0.05) and the soil's CEC by 64%. Bian et al. (2013) similarly reported an increase in soil's pH with biochar amendment. Soil becomes increasingly negatively charged with increase in pH above its zero point of charge (ZPC, pH = 3.4; (Sposito, 2008)).

Table 3.5. Repeated measures analysis of variance for the heavy metals in soil.

Effects	Cd	Cr	Cu	Zn	Pb	Fe
Treatment	*	ns	ns	*	ns	ns
Days	*	*	*	*	*	*
Depth	-	-	-	*	*	*
Treatment x Days	ns	ns	*	ns	ns	ns
Treatment x Depth	-	-	-	*	ns	ns
Treatment x Depth x Days	-	-	-	ns	ns	ns

* represents significant difference ($p<0.05$); ns, no significant difference; - represents not applicable.

An increase in negative charges results in greater electrostatic adsorption (Jiang et al., 2012b) and alters the soil's cation exchange mechanism—as in this present study—in a manner which favors Cd adsorption (Sposito, 2008). Accordingly, a slight increase in pH could have a significant effect on bioavailability of metals (Jay et al., 2015; Kelly et al., 2014). The fact that 57% more Cd was accumulated in the biochar-amended (than non-amended) soil receiving wastewater irrigation, suggests that the biochar may reduce the risk of Cd transport into plants by fixing it in the soil.

Although no Zn was detected at the soil surface in the WW-B treatment after the first irrigation (Day 3), a $[\mathrm{Zn}]_{\mathrm{soil}}^{\mathrm{surf}}$ of 24.4 mg kg^{-1} was detected after the second irrigation (Day 13), which then gradually increased after each subsequent irrigation, reaching 54.1 mg kg^{-1} after the 8th irrigation event (Day 73), when the highest $[\mathrm{Zn}]_{\mathrm{soil}}^{\mathrm{surf}}$ was measured. It appears that Zn accumulated at the soil surface over the growing season. Mapanda et al. (2005) showed a gradual increase in topsoil Zn in the range of 14 to 225 mg kg^{-1} in soils that received wastewater over a period of 10 years. The slight decrease in $[\mathrm{Zn}]_{\mathrm{soil}}^{\mathrm{surf}}$ between the day before planting (day-BP) and the first irrigation (Day

3), could be attributed to the redistribution of Zn when the soil was disturbed during the planting of potato tubers and the mixing of biochar into the topsoil.

For the WW+B treatment, a $[Zn]_{soil}^{surf}$ of 23.3 mg kg^{-1} was observed after the first irrigation. As in the case of the WW-B treatment, $[Zn]_{soil}^{surf}$increased over the growing season, such that after eight irrigations, a $[Zn]_{soil}^{surf}$ of 83.8 mg kg^{-1} was measured. Statistical analysis showed a significant positive effect of repeated wastewater irrigations on $[Zn]_{soil}^{surf}$ ($p \leq 0.05$; Table 3.5). The highest concentration of $[Zn]_{soil}^{surf}$ in both WW-B (64.9 mg kg^{-1}) and WW+B (99.8 mg kg^{-1}) treatments occurred after the 5th irrigation (Day 43; Fig. 3.2). Although the $[Zn]_{soil}^{surf}$ in both treatments were similar after the first two irrigations, the$[Zn]_{soil}^{surf}$was significantly greater ($p \leq 0.05$) under the WW+B (vs.WW-B) treatment after all successive irrigations. This showed that biochar adsorbed Zn strongly as compared to soil alone. Qian et al. (2016) reported that high ash content biochar released carbonates and hydroxides which precipitated Zn from soil solution. While the $[Zn]_{soil}^{surf}$ under the WW+B treatment could be high, and despite the accumulation of Zn at the soil surface under both WW-B and WW+B treatments, the $[Zn]_{soil}^{surf}$ remained below the Canadian Council of Ministers of the Environment's (CCME; http://st-ts.ccme.ca/en/index.html) permissible limit for Zn in an agricultural soil of 200 mg kg^{-1}.

After the first irrigation, under the WW-B treatment, $[Cu]_{soil}^{surf}$ and $[Cr]_{soil}^{surf}$ were below the detection limit, while $[Pb]_{soil}^{surf}$ reached 82.0 mg kg^{-1}; however, after the eighth irrigation, they had reached 332, 102, and 727 mg kg^{-1}, respectively (Figure 3.2), indicating that these metals accumulated in the topsoil following application of wastewater. Sadiq Butt et al. (2005) also reported accumulation of Cu and Pb in soil irrigated with wastewater. Irrigation with untreated wastewater irrigation accordingly raises great concerns with regard to human health, especially in the case of Pb, one of the most toxic heavy metals, and a known endocrine disruptor (Antonious and Snyder, 2007a; Colborn et al., 1993). For the WW+B treatment, while measurable $[\mathrm{Cu}]_{\mathrm{soil}}^{\mathrm{surf}}$ and $[\mathrm{Pb}]_{\mathrm{soil}}^{\mathrm{surf}}$ were noted after the first irrigation, biochar amendment did not show any significant effect on either of these heavy metals' concentrations when compared to the WW-B treatment (Table 3.5). This could be due to competition for available sorption sites in the soil-biochar mix by different constituents of the synthetic wastewater (Table 3.3). Xu et al. (2013) reported that Zn outcompeted Cu for sorption sites in a multi-metal experiment with rice (Oryza sativa L.) husk biochar. It could also be that some of the heavy metals, especially Pb and Cu, were not favored for cationic adsorption due to the soil's increased negative charge when its pH increased above the ZPC (Sposito, 2008). Cheng et al. (2006) noted that the surfaces of fresh biochar — as was used in the present experiment — have fewer negative charges and are hydrophobic. However, through oxidation and hydrolysis in the soil environment over time, biochar surfaces became more negatively charged due to the formation of carboxylate and phenolic groups (Cheng et al., 2006). Similarly, Lehmann (2007) noted that as biochar ages, its cation retention capacity increases. Accordingly, the effect of biochar on adsorption of Cu, Cr and Pb was not apparent.

Laboratory studies have shown that, in both single and multi-metal systems, Pb consistently outcompetes Cd or Zn for sites on biochar (Jiang et al., 2012b; Trakal et al., 2011; Xu et al., 2013). Furthermore, a preliminary laboratory sorption test showed that plantain peel biochar alone had a 94% removal percentage for Pb (Nzediegwu et al., 2015). However, the present study did not show any effect of soil-incorporated biochar on the sorption of Pb. This could be due to the complexities of the soil-biochar-plant-wastewater system in which the crop was grown. Potato roots exude organic compounds into the rhizosphere which could affect the adsorption behavior of heavy metals in soil (Balendres et al., 2016).

Under the WW-B treatment, $[Fe]_{soil}^{surf}$ varied from 7816 to 12020 mg kg^{-1} between Day 03 and Day 73 (Figure 3.2), showing that Fe accumulated at the soil surface with irrigation. Although $[Fe]_{soil}^{surf}$ in the lysimeter soil prior to planting was as high as 9728 mg kg^{-1} (day-BP; Figure 3.2), the Fe could have been redistributed in the soil profile following the addition of freshwater (Al-Nakshabandi et al., 1997), as freshwater was briefly used to irrigate all potatoes after they emerged. It could also be that mixing the soil at the time of biochar application and potato planting redistributed Fe in the soil. After planting, but before the first wastewater irrigation, the $[Fe]_{soil}^{surf}$ had declined to 7926 mg kg^{-1}. Similar to WW-B, the $[Fe]_{soil}^{surf}$ rose from 7598 on Day 03 and stabilized at 11320 mg kg^{-1} on Day 73 under the WW+B treatment. Biochar amendment had no significant effect on $[Fe]_{soil}^{surf}$ ($p > 0.05$; Table 3.5). The $[Fe]_{soil}^{0.1}$ ranged from 6628 mg kg^{-1} at the start of the season to 8633 mg kg^{-1} towards the end of the season (Fig. 3.2).

Neither Cd, Cu nor Cr were found at the 0.1 m as well as 0.3 m soil sampling depths, implying that untreated wastewater irrigation might contribute to their build-up in surface soil (64% higher organic matter than at 0.1 m depth) which could facilitate their transport to plants. On the other hand, Zn, Pb and Fe were detected at 0.1 m depth (Fig. 3.2). Transport of Zn through the soil was reflected by $[Zn]_{soil}^{0.1}$ values of 18.04 mg kg^{-1} to 28.07 mg kg^{-1} after the first two irrigations in the WW-B and WW+B treatments, respectively. This difference was not significant ($p > 0.05$). However, the $[Zn]_{soil}^{0.1}$ remained virtually unchanged after subsequent irrigations. The Zn may have been translocated into portions of the potato, although the Zn was expected to be low in potatoes, and therefore the amount translocated would be minimal as compared to the overall amount in soil. For Pb, a concentration of 50.57 mg kg^{-1} was measured, suggesting that Pb was slightly more mobile under the WW+B treatment than the WW-B treatment. This could possibly explain the concentrations measured on day 33 and day 73 at a depth of 0.1 m. Statistical analysis showed that $[\mathrm{Pb}]_{\mathrm{soil}}^{0.1}$ under the WW-B treatment was not significantly different from that under the WW+B treatment (Table 3.5). Moreover, $[\mathrm{Pb}]_{\mathrm{soil}}^{0.1}$ were low and well within the agricultural soil permissible limit of 70 mg kg^{-1} (CCME; http://st-ts.ccme.ca/en/index.html). Accordingly, these concentrations ($[\mathrm{Pb}]_{\mathrm{soil}}^{0.1}$) had no practical importance in a system receiving only a single season's irrigation. The lower $[\mathrm{Fe}]_{\mathrm{soil}}^{0.1}$ after the first two irrigations (Day 3 and Day 13) might reflect the mobility of Fe toward lower soil profiles (i.e., 0.3 m below surface; Figure 3.2). There was no significant effect of biochar at the 0.1 m depth either; however, overall $[\mathrm{Fe}]_{\mathrm{soil}}$ concentration did significantly decrease with depth ($p \leq 0.05$; Table 3.5).

The concentrations of $[\mathrm{Pb}]_{\mathrm{soil}}$ and $[\mathrm{Zn}]_{\mathrm{soil}}$ declined significantly with depth and were below the instrument detection limit of 50 µg L^{-1} (i.e., 15.6 mg kg^{-1} considering the mass of soil used for extraction), at depths exceeding 0.10 m. Although it might have been expected — given the differences in $[\mathrm{Pb}]_{\mathrm{soil}}^{0.1}$ and $[\mathrm{Zn}]_{\mathrm{soil}}^{0.1}$ between the WW+B and WW-B (Fig. 3.2) — that Pb and Zn would move to depths exceeding 0.10 m; however, over the entire season they were not detected at a soil depth of 0.3 m. The accumulation of Pb and Zn at lower depths were likely minimal over a single season; however, continuous application of wastewater, from year after year, would likely raise their concentrations in lower strata of the soil profile, particularly if some Pb and Zn transport occurred through preferential flow of drainage water towards the groundwater table. Iron was detected up to 9772 mg kg^{-1} at this depth (Figure A). The $[\mathrm{Fe}]_{\mathrm{soil}}^{0.3}$ slightly exceeded the $[\mathrm{Fe}]_{\mathrm{soil}}^{0.1}$ values on occasion. This may be attributable to the greater root density at the shallower depth, typical of shallow-rooted crops like potatoes, allowing a greater proportion of the Fe to be taken up and translocated into portions of the potato plant than would occur at greater and less root-dense depths.

None of the heavy metals tested (Cd, Cu, Cr, Fe, Pb and Zn) were found in the leachate at any time throughout the season, suggesting that untreated wastewater irrigation might not be a major pathway for groundwater contamination with these heavy metals. This observation regarding the mobility of heavy metals in soil is consistent with the work of Knechtenhofer et al. (2003), who, in a study on the fate of heavy metals in acidic shooting range soils, reported that Cu only accumulated in the top soil layer. Nevertheless, it appears that there is transport of some heavy metals in soil, and as heavy metals do not degrade, they are liable to appear in drainage and/or ground water if the land is irrigated with wastewater over an extended period.

3.4.4 Heavy metals in potato flesh

The concentrations of heavy metals (Cd, Zn, Cu, Pb, Fe and Cr) in flesh, peel, root, stem and leaf of potatoes (e.g. $[\mathrm{Cd}]_{\mathrm{plant}}^{\mathrm{flesh}}$, $[\mathrm{Cd}]_{\mathrm{plant}}^{\mathrm{peel}}$, $[\mathrm{Cd}]_{\mathrm{plant}}^{\mathrm{root}}$, $[\mathrm{Cd}]_{\mathrm{plant}}^{\mathrm{stem}}$, $[\mathrm{Cd}]_{\mathrm{plant}}^{\mathrm{leaf}}$, respectively) are presented in Table 3.6. The $[\mathrm{Cd}]_{\mathrm{plant}}^{\mathrm{flesh}}$ under the FW-B treatment was 0.2 mg kg^{-1}, a level comparable to 0.5 mg kg^{-1}, the upper level of $[\mathrm{Cd}]_{\mathrm{plant}}$ measured in potato tubers grown in a non-contaminated soil (Antonious and Snyder, 2007a). As Cd is ubiquitously in the environment (Ronchetti et al., 2016) it can be taken up by crops grown on non-contaminated soils. At 2.9 mg kg^{-1} and 0.9 mg kg^{-1}, respectively, the $[\mathrm{Cd}]_{\mathrm{plant}}^{\mathrm{flesh}}$ under WW-B and WW+B treatments were significantly different, and both above the CODEX permissible limit of 0.1 mg kg^{-1} (Codex Alimentarius Commission, 1995). The high $[\mathrm{Cd}]_{\mathrm{plant}}^{\mathrm{flesh}}$ under the WW-B treatment might have resulted from the greater availability of Cd in soil that received wastewater (Fig. 3.2). Nevertheless, soils amended with biochar appears to have held Cd strongly, making it less available for plant uptake (Fig. 3.2). Nonetheless, irrigation with wastewater can lead to high uptake of heavy metals such as Cd by crops like potatoes (Sadiq Butt et al., 2005).

Table 3.6. Concentrations (mg kg^{-1}) of heavy metals in potato flesh, peel, root, stem and leaf irrigated with untreated wastewater or freshwater.

Potato Tissue	Treatment	Cadmium	Zinc	Copper	Lead	Iron	Chromium
Flesh	**WW+B**	0.9±0.09b	16.9±1.19b	5.2±0.48c	0.042±0.02a	18.8±1.9b	0.07±0.005b
	WW-B	2.9±0.33a	25.3±1.79a	9.8±0.96b	0.049±0.01a	20.6±1.4ab	0.06±0.004b
	FW-B	0.2±0.01b	18.0±0.96b	13.1±0.59a	0.055±0.01a	26.4±0.7a	0.14±0.020a
Peel	**WW+B**	8.6±2.73b	42.9±5.54b	23.6±4.87a	10.3±3.76a	279.8±34.9a	3.08±0.969a
	WW-B	59.5±5.91a	117.2±6.96a	32.1±0.88a	17.7±3.93a	381.7±34.2a	1.92±0.254a
	FW-B	0.5±0.03b	36.2±2.48b	20.1±2.57a	0.6±0.11b	279.3±41.6a	1.15±0.175a
Root	**WW+B**	43.3±4.90b	81.9±20.10b	26.0±3.87a	35.4±2.45a	601.6±21.9b	4.94±0.282a
	WW-B	225.7±25.52a	379.8±82.69a	56.7±15.84a	70.8±17.13a	1431.7±144.1a	8.31±1.996a
Stem	**WW+B**	15.4±1.08a	41.1±3.12a	7.0±0.10b	13.3±0.72a	118.8±3.6a	2.31±0.244a
	WW-B	16.2±2.95a	102.0±18.60a	9.7±0.05a	9.7±3.38a	93.9±26.3a	1.73±0.565a
Leaf	**WW+B**	2.9±0.14b	19.4±0.95a	9.2±0.14b	5.8±0.24a	360.2±36.6a	1.52±0.019a
	WW-B	6.3±0.47a	19.3±0.40a	13.9±0.80a	3.3±0.44a	389.9±28.8a	1.98±0.376a

The values are the mean ± standard error of 3 replicates; for each potato tissue category, different letters signify a significant difference ($p \leq 0.05$). The permissible limits of the heavy metals in plant tissue (mg kg^{-1}) are Cd (0.1*, 0.3), Zn (50), Cu (10), Pb (0.1*), Fe (not available) and Cr (1.5). *signifies CODEX standard (Stan 193-1995), while the others are from WHO (Nazir et al., 2015).

Irrigation of potatoes using wastewater containing Cd as high as in the present synthetic wastewater could pose a threat to human health. Despite a significantly greater $[\mathrm{Cd}]_{\mathrm{soil}}$ in WW+B (vs.WW-B) treated soil (Fig. 3.2), Cd uptake and translocation into potato flesh was significantly lower under the WW+B treatment. The high $[\mathrm{Cd}]_{\mathrm{soil}}$ in biochar-amended soil could be due to tight bonding of Cd with biochar, thereby reducing Cd availability to potatoes. In terms of effectiveness, under waste water irrigation, the biochar amendment showed a 69% reduction in the $[\mathrm{Cd}]_{\mathrm{plant}}^{\mathrm{flesh}}$ compared to the non-amended treatment. Bian et al. (2013) reported a reduction in Cd in rice grains ranging from 20% to 90% due to the application of biochar on a Cd-contaminated soil.

Given that potatoes, as tubers are directly exposed to soil contaminants, reduction to such an extent is very important. Biochar amendment to soil appears to provide an inexpensive and feasible technique to alleviate Cd contamination risk in potatoes grown with heavy metal contaminated water.

The $[\mathrm{Zn}]_{\mathrm{plant}}^{\mathrm{flesh}}$ under the FW-B was 18.0 mg kg^{-1}, a concentration similar to the $[\mathrm{Zn}]_{\mathrm{plant}}$ measured in potatoes irrigated with uncontaminated well water (Zavadil, 2009). Zinc exists naturally in the parent material of soils (Morrison et al., 2004); accordingly, the $[\mathrm{Zn}]_{\mathrm{soil}}$ of 16.70 mg kg^{-1} in background lysimeter soil (Table 3.2) might have arisen naturally from the soil. The highest $[\mathrm{Zn}]_{\mathrm{plant}}^{\mathrm{flesh}}$ among all treatments, 25.3 mg kg^{-1}, was observed in the WW-B treatment. Dvorak et al. (2003) reported similar concentrations of Zn, in potato tubers grown in soils treated with sludge and Zn-rich fertilizers. Similar observations were made by Antonious and Snyder (2007a), who found Zn in potato tubers grown on soils receiving sewage water to significantly ($p<0.05$) exceed that of potatoes tubers grown on soils not having received sewage water. In the present study, over the experimental period, $[\mathrm{Zn}]_{\mathrm{soil}}$ was much higher in treatments receiving wastewater than in the freshwater irrigated treatment (Fig. 3.2, Table 3.2). Accordingly, it was expected that the $[\mathrm{Zn}]_{\mathrm{plant}}$ under the WW-B treatment would be higher than in potatoes under the FW-B treatment. Although Zn is an essential micronutrient necessary for the development of the plumule and radicle in plants (Lichtfouse et al., 2009), high Zn are hazardous to human health (Fosmire, 1990). Numerically, $[\mathrm{Zn}]_{\mathrm{plant}}^{\mathrm{flesh}}$ was the lowest under the WW+B treatment, although not significantly different from that under the FW-B treatment (Table 3.6). Mixing biochar with soil (WW+B) reduced Zn uptake by potatoes significantly (33%, $p \leq 0.05$) as compared to the non-amended soil (WW-B). Although

$[\mathrm{Zn}]_{\mathrm{soil}}$ under WW+B was significantly higher than for the WW-B treatment (Fig. 3.2), it appears that biochar significantly reduced Zn uptake into the edible flesh. The high ash content of biochar would have contributed to reducing the availability of Zn in the soil solution. Nevertheless, in all the treatments the $[\mathrm{Zn}]_{\mathrm{plant}}$ remained within the permissible limit of 50 mg kg^{-1}, and as such, wastewater irrigation for a season might not pose a threat in terms of Zn uptake into potato flesh. However, persistent wastewater irrigation could build up Zn in soil, and could be taken up by potatoes at concentrations adversely affecting human health. So, where wastewater irrigation is practiced, biochar amendment to soil would be worthwhile.

The $[\mathrm{Cu}]_{\mathrm{plant}}^{\mathrm{flesh}}$ under the FW-B treatment was 13.1 mg kg^{-1} (Table 3.6). LeRiche et al. (2009) working in eastern Canada reported $[\mathrm{Cu}]_{\mathrm{plant}}^{\mathrm{flesh}}$ of up to 10.4 mg kg^{-1} in potato flesh of several cultivars presumably receiving freshwater irrigation. This suggests that some Cu is present in the flesh of potatoes irrigated with freshwater. Copper concentrations in crops are affected by the soil's parent material and agronomic practices (Haase et al., 2007). Although $[\mathrm{Cu}]_{\mathrm{plant}}^{\mathrm{flesh}}$ was slightly above the permissible limit of 10 mg kg^{-1}, Cu is one of the essential elements naturally present in potato tubers (LeRiche et al., 2009). At toxic levels, Cu can present serious toxicity issues to humans, including cognitive decline and Alzheimer's in elderly people (Brewer, 2009). The $[\mathrm{Cu}]_{\mathrm{plant}}^{\mathrm{flesh}}$ under the WW-B treatment was 9.8 mg kg^{-1}, significantly lower than under the FW-B treatment ($p \leq 0.05$). The uptake of Cu could have been inhibited by the antagonistic presence of higher $[\mathrm{Zn}]_{\mathrm{soil}}$ in the WW-B soil than the FW-B soil (Fosmire, 1990). Under the WW+B treatment, $[\mathrm{Cu}]_{\mathrm{plant}}^{\mathrm{flesh}}$ was 5.2 mg kg^{-1}; significantly lower than in either FW-B or WW-B treatments ($p \leq 0.05$). Although, there was no overall difference in $[\mathrm{Cu}]_{\mathrm{soil}}$ between the two wastewater treatments, biochar amendment did significantly reduced Cu uptake by potatoes as compared to its absence ($p \leq 0.05$; Table 3.6). The antagonistic higher presence of $[\mathrm{Zn}]_{\mathrm{soil}}$ under the WW+B treatment, compared to the WW-B treatment (Figure 3.2) could have reduced the availability of Cu for uptake by potatoes (Fosmire, 1990).

The $[\mathrm{Pb}]_{\mathrm{plant}}^{\mathrm{flesh}}$ under the FW-B treatment was 0.055 mg kg^{-1} (Table 3.6), similar to the concentration reported for well-water irrigated potatoes (Sadiq Butt et al., 2005). Although Pb is not an essential element, it is reportedly present in crops including potatoes at very low concentrations due to its uptake from soils (Kabata-Pendias and Pendias, 1984). Although Pb was undetectable in soil under the FW-B treatment, the $[\mathrm{Pb}]_{\mathrm{plant}}^{\mathrm{flesh}}$ detected under this treatment could have resulted from uptake from the soil, which had a higher detection limit than potatoes. The similarity in $[\mathrm{Pb}]_{\mathrm{plant}}^{\mathrm{flesh}}$ under the WW-B and WW+B treatments (0.049 and 0.042 mg kg^{-1}, respectively) likely reflects the lack of difference in $[\mathrm{Pb}]_{\mathrm{soil}}$ between these treatments (Fig. 3.2). The $[\mathrm{Pb}]_{\mathrm{plant}}^{\mathrm{flesh}}$ in potatoes receiving wastewater was similar to that of potatoes receiving freshwater. Although $[\mathrm{Pb}]_{\mathrm{soil}}$ under wastewater (WW-B and WW+B) treatments were relatively high as compared to freshwater-irrigated soil, similar translocation was observed. This may be due to a low rate of translocation of Pb to potato flesh (Khan et al., 2008; Lee et al., 1981).

The $[\mathrm{Fe}]_{\mathrm{plant}}^{\mathrm{flesh}}$ did not differ significantly between treatments, being 26.4, 20.6, and 18.8 mg kg^{-1} under the FW-B, WW-B, and WW+B treatments, respectively (Table 3.6). Iron is commonly present in nature, and apart from aluminum, no other heavy metal is as common (Fontecave and

Pierre, 1993). The pre-planting $[\mathrm{Fe}]_{\mathrm{soil}}$ in our lysimeter topsoil was 11109 mg kg^{-1} (Table 3.2); therefore, the presence of Fe in crops grown with uncontaminated water is not uncommon. Hussain et al. (2013) reported the Fe in the edible parts of onion (Allium *cepa* L.) and garlic (Allium *sativum* L.) grown in wastewater irrigated soils to be similar to those observed in freshwater irrigated controls. Biochar amendment did not show a significant effect on Fe uptake.

The $[\mathrm{Cr}]_{\mathrm{plant}}^{\mathrm{flesh}}$ were 0.14, 0.06, and 0.07 mg kg^{-1} under the FW-B, WW-B, and WW+B treatments, respectively. Although statistically different, these concentrations were at least 10-fold below the permissible limit of 1.5 mg kg^{-1}. Therefore, the differences have no practical importance; the presence of Cr in the potatoes is not a major concern. Although irrigation with wastewater would have gradually increased $[\mathrm{Cr}]_{\mathrm{soil}}$, its uptake did not increase. This was likely due to interference with other heavy metals, especially Fe, present in the soil (Fig. 3.2). For example, when Fe is present at high concentrations, it may reduce the availability of Cr for plant uptake (Offenbacher and Pi-Sunyer, 1988). Moreover, Stasinos and Zabetakis (2013) reported lower $[\mathrm{Cr}]_{\mathrm{plant}}$ in potatoes irrigated with Cr-laden water as compared to freshwater. Surdyk et al. (2010) found that Cr in potatoes was not related to the Cr in irrigation water, but rather to the presence or absence of other — potentially antagonistic — heavy metals in the soil.

3.4.5 Heavy metals in peels

Potato peel represents between 3 and 6% of the tuber, depending on the peeling method used, and serves as a protection for the flesh (Woolfe and Poats, 1987). It comes in direct contact with wastewater and contaminated soil, therefore, one would expect its contaminant concentrations to be higher than those in the flesh. Contaminant uptake and translocation into the peel followed the same trends as in flesh for most of the heavy metals. The $[\mathrm{Cd}]_{\mathrm{plant}}^{\mathrm{peel}}$ was 8.6, 59.5 and 0.5 mg kg^{-1} under the FW-B, WW-B and WW+B treatments, respectively (Table 3.6). The $[\mathrm{Cd}]_{\mathrm{plant}}^{\mathrm{peel}}$ was significantly higher ($p \leq 0.05$) in the WW-B than the WW+B treatment, and likewise, greater under the WW-B than the FW-B treatment ($p \leq 0.05$). The wastewater irrigation-driven accumulation of Cd in soil (Figure 3.2) was expected to raise $[\mathrm{Cd}]_{\mathrm{plant}}^{\mathrm{peel}}$ in both WW-B and WW+B treatments; however, the addition of biochar resulted in an 86% decrease of $[\mathrm{Cd}]_{\mathrm{plant}}^{\mathrm{peel}}$ compared to its absence. Despite significant differences in $[\mathrm{Cd}]_{\mathrm{plant}}^{\mathrm{peel}}$, it was frequently above the permissible limit of 0.1 mg kg^{-1}. This suggests that untreated wastewater irrigation could be a major pathway for Cd in potatoes, but that soil amendment with biochar could decrease Cd uptake.

The $[\mathrm{Zn}]_{\mathrm{plant}}^{\mathrm{peel}}$ under the FW-B treatment was 36.2 mg kg^{-1}, a concentration within the permissible limit of 50 mg kg^{-1} according to WHO (Nazir et al., 2015). In WW-B, it was 117.2 mg kg^{-1}, while in WW+B it was 42.9 mg kg^{-1}. The $[\mathrm{Zn}]_{\mathrm{plant}}^{\mathrm{peel}}$ under the WW-B treatment (117.2 mg kg^{-1}) was above the permissible limit and significantly higher than under the FW-B treatment. The significant increase of $[\mathrm{Zn}]_{\mathrm{plant}}^{\mathrm{peel}}$ under the WW-B treatment suggests that wastewater irrigation is a major pathway for Zn to contaminate potato peel. The $[\mathrm{Zn}]_{\mathrm{plant}}^{\mathrm{peel}}$ under the WW+B treatment was within the permissible limit, and, despite the Zn being the same in all wastewater, $[\mathrm{Zn}]_{\mathrm{plant}}^{\mathrm{peel}}$ was significantly lower under the WW+B than under the WW-B treatment. Although it was expected that $[\mathrm{Zn}]_{\mathrm{plant}}^{\mathrm{peel}}$ under the WW+B treatment would be higher than under the FW-B treatment, no significantly difference ($p > 0.05$) was found. It appears that biochar is quite effective in controlling the transfer of Zn from wastewater to potato peels. This agrees with the high retention rate for Zn noted in the soil under biochar amendment, which eventually became less available for uptake. In potato flesh, such a trend was observed.

Although addition of biochar to soil restricted translocation of Zn and Cd, the concentration of $[\mathrm{Cu}]_{\mathrm{plant}}^{\mathrm{peel}}$ was not significantly different ($p > 0.05$) among the FW-B, WW-B and WW+B treatments: 20.1 mg kg^{-1}, 32.1 mg kg^{-1} and 23.6 mg kg^{-1}, respectively (Table 3.6). Accordingly, there was no marked impact of wastewater on the uptake of Cu, although a slight increase occurred with wastewater vs. freshwater. The $[\mathrm{Pb}]_{\mathrm{plant}}^{\mathrm{peel}}$ under FW-B, was 0.6 mg kg^{-1}, a concentration similar to that reported by Sadiq Butt et al. (2005) for potatoes irrigated with uncontaminated canal water. While the $[\mathrm{Pb}]_{\mathrm{plant}}^{\mathrm{peel}}$ under the WW-B treatment (17.7 mg kg^{-1}) was not significantly different ($p > 0.05$) than that under the WW+B treatment (10.3 mg kg^{-1}; Table 3.6), reflecting the lack of difference in $[\mathrm{Pb}]_{\mathrm{soil}}$ between these treatments, both types of wastewater irrigated potatoes had significantly higher $[\mathrm{Pb}]_{\mathrm{plant}}^{\mathrm{peel}}$ than freshwater-irrigated potatoes. Irrigation with wastewater increased $[\mathrm{Pb}]_{\mathrm{plant}}^{\mathrm{peel}}$ above the permissible limit. Sadiq Butt et al. (2005) also reported that potatoes irrigated with wastewater showed greater Pb uptake than freshwater-irrigated potatoes.

The $[\mathrm{Fe}]_{\mathrm{plant}}^{\mathrm{peel}}$ ranged from 279.3 mg kg^{-1} to 381.7 mg kg^{-1} (Table 3.6), and showed no significant difference ($p > 0.05$) among treatments. Fe is naturally present in soil and it translocated to potato peel and flesh under irrigation with either wastewater or fresh water. Biochar may not be effective in reducing Fe uptake.

The $[\mathrm{Cr}]_{\mathrm{plant}}^{\mathrm{peel}}$ ranged from 1.15 mg kg^{-1} under the FW-B treatment to 3.08 mg kg^{-1} under the WW+B treatment. But even with a more than 2-fold difference between these treatments, overall there was no significant difference in $[\mathrm{Cr}]_{\mathrm{plant}}^{\mathrm{peel}}$ between any treatments ($p > 0.05$, Table 3.6). The $[\mathrm{Cr}]_{\mathrm{plant}}^{\mathrm{peel}}$ in the present study was comparable to those which Antonious and Snyder (2007a) found for potatoes grown in both contaminated and non-contaminated soils. Despite there being no significant difference amongst treatments, $[\mathrm{Cr}]_{\mathrm{plant}}^{\mathrm{peel}}$ under the FW-B treatment was within the permissible limit of 1.5 mg kg^{-1}, while those under wastewater irrigation exceeded this limit. This suggests that wastewater irrigation could lead to the build-up of Cr in potato peel as compared to freshwater irrigation. Mixing biochar into the soil did not reduce Cr build-up in the peel. Although Cr accumulated in the soil following wastewater irrigation (Figure 3.2, Table 3.2), its uptake by potato tubers was minimal, indicating that the translocation of Cr from soil to plant was quite low (Khan et al., 2008; Lee et al., 1981).

Overall, for both wastewater and freshwater irrigated potatoes, all the heavy metals in the peels were more than a two-fold greater than the heavy metals in the flesh (Table 3.6), suggesting that consuming potato peels would increase the amount of the heavy metals that get to the food chain. Potato peels have direct contact with soils and are therefore expected to bear higher heavy metal concentrations compared to the flesh. Therefore, removing the peels before consumption can reduce the amount of Cr that enters the food chain under wastewater irrigation.

3.4.6 Heavy metals in roots

Given the concentration of heavy metals in potatoes was relatively low under the FW-B treatment, heavy metals in roots, stem and leaves were not analyzed for this treatment. However, the concentrations of heavy metals — especially Cd, Zn and Pb — in potatoes, especially in peels were high under wastewater than freshwater irrigation. Accordingly, roots, stem and leaves were analyzed to determine if heavy metals could accumulate in roots and be transported to above-ground potato plant tissues, and whether biochar affects these processes.

Higher concentrations of heavy metals were found in roots than other portions of the potato plant, including tubers (Table 3.6). This corroborates the findings of Gichner et al. (2006), who reported higher concentration of heavy metals, especially Pb, Cd, Cu and Zn, in potato roots compared to aboveground parts. The $[\mathrm{Cd}]_{\mathrm{plant}}^{\mathrm{root}}$ and $[\mathrm{Zn}]_{\mathrm{plant}}^{\mathrm{root}}$ under the WW+B treatment was significantly (6- and 5-fold, respectively) lower ($p \leq 0.05$) than under the WW-B treatment. As stated earlier, the upward shift in soil pH following biochar amendment could have reduced the bioavailability of Cd to the roots of the WW+B treatment.

The $[\mathrm{Cu}]_{\mathrm{plant}}^{\mathrm{root}}$, $[\mathrm{Pb}]_{\mathrm{plant}}^{\mathrm{root}}$, and $[\mathrm{Cr}]_{\mathrm{plant}}^{\mathrm{root}}$ showed no significant differences between the WW+B and WW-B treatments; $[\mathrm{Fe}]_{\mathrm{plant}}^{\mathrm{root}}$ was significantly higher in the WW-B than WW+B treatment, although there was no significant difference among treatments for either peel or flesh. It is likely that greater amount of soil Fe would have translocated into and accumulated in roots under the WW-B treatment than under the WW+B treatment. Moreover, greater $[\mathrm{Cu}]_{\mathrm{plant}}^{\mathrm{root}}$, $[\mathrm{Pb}]_{\mathrm{plant}}^{\mathrm{root}}$, $[\mathrm{Cr}]_{\mathrm{plant}}^{\mathrm{root}}$, $[\mathrm{Fe}]_{\mathrm{plant}}^{\mathrm{root}}$ were observed in the root tissue than in either the peel or flesh. This could be attributable to the roots' direct contact with the contaminated soil; particularly since the roots are responsible for the plant's water and nutrient supply (Eshel and Beeckman, 2013).

3.4.7 Heavy metals in stems

Under both wastewater treatments, all heavy metals were transported to the stem (Table 3.6). Biochar seemingly had no effect on transport of heavy metals to stem, except Cu, where the

$[\mathrm{Cu}]_{\mathrm{plant}}^{\mathrm{stem}}$ was significantly lower for the WW+B than the WW-B treatment ($p \leq 0.05$). Uptake of heavy metals from contaminated soils and their translocation to the potato stem has been reported (Gichner et al., 2006). The $[\mathrm{Cu}]_{\mathrm{plant}}^{\mathrm{stem}}$ and $[\mathrm{Zn}]_{\mathrm{plant}}^{\mathrm{stem}}$ found in the present study, were comparable to those reported for potato stems grown in contaminated soils (Antonious et al., 2011). The present study showed that growing potatoes with untreated wastewater (vs. freshwater) could result in higher heavy metals concentration in both roots and stems.

3.4.8 Heavy metals in leaves

As in the other parts of the potato plants, $[\mathrm{Cd}]_{\mathrm{plant}}^{\mathrm{leaf}}$ was significantly lower under the WW+B treatment than the WW-B treatment (Table 3.6). This was expected, since Cd in the leaf would have arisen through Cd translocation from the root where $[\mathrm{Cd}]_{\mathrm{plant}}^{\mathrm{root}}$ was lower under the WW+B than the WW-B treatment. With large $[\mathrm{Zn}]_{\mathrm{plant}}^{\mathrm{root}}$ values and treatment differences in the root, one would have expected similar significant differences in $[\mathrm{Zn}]_{\mathrm{plant}}^{\mathrm{leaf}}$; however, no such differences were detected between the WW+B and WW-B treatments. It appears that translocation of Zn in potatoes beyond the roots was rather slow. The $[\mathrm{Cu}]_{\mathrm{plant}}^{\mathrm{leaf}}$ was significantly lower under the WW+B than the WW-B treatment, perhaps because Cu taken up by the root is easily translocated to leaf and stem. As observed in other portions of the potato plant, particularly the roots, $[\mathrm{Pb}]_{\mathrm{plant}}^{\mathrm{leaf}}$ did not differ significantly ($p > 0.05$) among treatments (Table 3.6). Similarly, the $[\mathrm{Fe}]_{\mathrm{plant}}^{\mathrm{leaf}}$ and $[\mathrm{Cr}]_{\mathrm{plant}}^{\mathrm{leaf}}$ did not differ significantly between the WW+B and WW-B treatments. The concentrations of $[\mathrm{Zn}]_{\mathrm{plant}}^{\mathrm{leaf}}$ and $[\mathrm{Cu}]_{\mathrm{plant}}^{\mathrm{leaf}}$ were comparable to those reported in previous studies (Antonious et al., 2011), whereas the leaf concentrations of the other heavy metals were higher in our study than in the literature. As stated earlier, this greater uptake might be due to high concentrations of these heavy metals in the soil (Figure 3.2). The uptake of heavy metals by plants depends on the amount available in soil solution (Alloway, 2013a; Antonious et al., 2011). Overall, concentrations of heavy metals (Cd, Pb, Cr and Fe) in leaves were relatively high. Since the leaves of potatoes are responsible for producing energy via photosynthesis, the presence of heavy metals in wastewater could have negative effects on photosynthesis (Pietrini et al., 2010).

3.4.9 Accumulation factors

Accumulation factors (AF) are useful in predicting the relative ease with which a compound accumulates in one of two environmental compartments (receiving and source). Accumulation factors for all heavy metals in the following combinations: flesh/soil ($\mathrm{AF}_{\mathrm{soil}}^{\mathrm{flesh}}$), peel/soil ($\mathrm{AF}_{\mathrm{soil}}^{\mathrm{peel}}$), and root/soil ($\mathrm{AF}_{\mathrm{soil}}^{\mathrm{root}}$), are presented in Figures 3.3A, 3.3B and 3.3C, respectively. An AF < 1.0 indicates that the given compound's accumulation (e.g., heavy metals) in the receiving compartment (flesh, peel or root) is less than that in the source compartment (soil). When this is true, it implies that the compound has more affinity for the source compartment than the receiving compartment, and as such, its uptake would be low. On the other hand, an AF > 1 indicates a concern; the level of concern depending on the particular heavy metal, e.g. for same accumulation factor, concern would be greater for Pb than Fe. Figure 3.3A shows that $1.37\times10\text{-}4 \leq \mathrm{AF}_{\mathrm{soil}}^{\mathrm{flesh}} \leq 0.624$ across all treatments, while both $\mathrm{AF}_{\mathrm{soil}}^{\mathrm{peel}}$ and $\mathrm{AF}_{\mathrm{soil}}^{\mathrm{root}}$ were less than 1.0 (Figures 3.3B, 3.3C, respectively) for all heavy metals, with the exception of Zn and Cd under the WW-B treatment. This concurs with the findings of Khan et al. (2008), who reported that AF values for Cr, Cu, and Pb in vegetables irrigated with wastewater were all below 1. Given that the accumulation of Cu, Cr, Fe and Pb in root, peel and flesh of potatoes might be low under untreated wastewater irrigation, these heavy metals may pose a lesser threat.

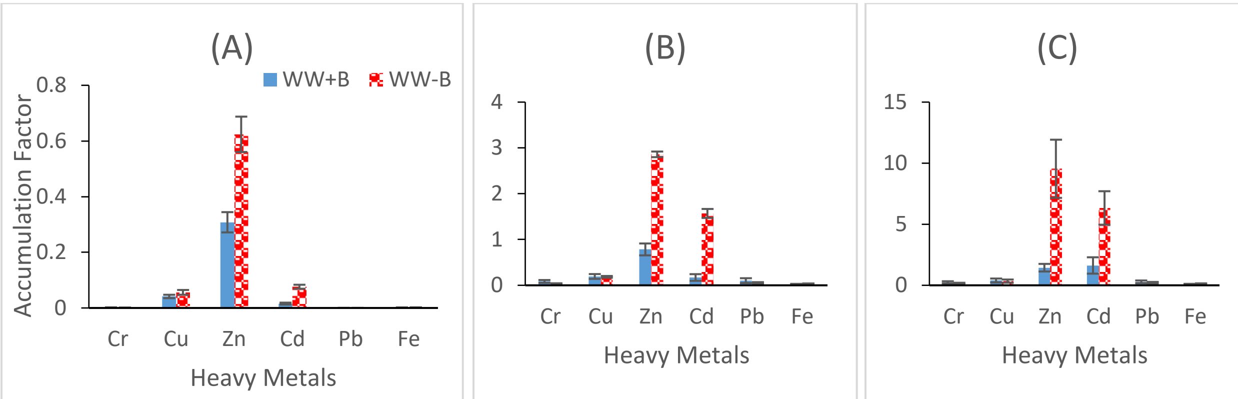

Figure 3-3. Accumulation factors: (A) Flesh/Soil, (B) Peel/Soil, and (C) Root/Soil, of heavy metals (Cr, Cu, Zn, Cd, Pb and Fe). Please note the scales are variable. Error bars represent standard error for 3 samples

However, Zn and Cd accumulation per unit weight was higher in both root and peel as compared to soil, which poses a risk because there would be greater translocation of these heavy metals to potato tissues. This risk is higher when the soil is not amended with biochar (Figure 3.3). The order of accumulation (in terms of potato plant portions) for all heavy metals was root>peel>flesh; this is in the order of flow of the soil solution from the soil to the plant shoot. Biochar, employed as a soil amendment, adsorbed these heavy metals, and their uptake was reduced. Therefore, the accumulation of heavy metals in root, peel and flesh were less under the WW+B than under the WW-B treatment.

3.5 Conclusions

Irrigating potatoes with untreated wastewater resulted in accumulation of heavy metals (Cd, Cr, Cu, Fe, Pb and Zn) at the surface of the receiving soil. Apart from Zn, Pb and Fe, heavy metals were not detectable at a depth of 0.1 m from the surface, and only Fe was detected at a depth of

0.3 m below the soil surface. Across the full growing season, none of the heavy metals was detected in the leachate. Heavy metals were found to have translocated to all the portions of the potato plant (flesh, peel, root, stem and leaf). Mixing soil with high ash content (77.5%) gasified plantain peel biochar significantly adsorbed Cd and Zn in the soil, thereby reducing their uptake into the flesh of potatoes by 69% and 33%, respectively, compared to the non-amended soil. Apart from Cd, heavy metal concentrations in tuber flesh under both WW-B and WW+B treatments were below permissible limits. Results suggest that the amendment of soil with plantain peel biochar could present a viable technique for reducing Cd and Zn uptake by potatoes under wastewater irrigation. Moreover, biochar amendment of the soil significantly reduced Cd and Zn in potato peel compared to the lack of biochar amendment. The concentrations of all the heavy metals detected in flesh were much lower than in the peel, suggesting that when consuming potatoes grown under wastewater irrigation, the peel poses the greatest health risks.

3.6 Acknowledgements

The authors express gratitude to the Natural Science and Engineering Research Council of Canada (NSERC), Tertiary Education Trust Fund, Nigeria and the India-Canada Centre for Innovative Multidisciplinary Partnerships to Accelerate Community Transformation and Sustainability for providing fund for the research. The authors would also like to thank Sedigheh Zarayan, our Lab Supervisor.

Connecting Text to Chapter 4

After observing that plantain peel biochar immobilized Cd and Zn, the concern of whether it can reduce translocation of these heavy metals to an aboveground crop—spinach in this case—was investigated. A batch equilibrium study on the sorption and desorption potential of the biochar is presented as well.

The chapter, Impact of Soil Biochar Incorporation on the Uptake of Heavy Metals Present in Wastewater by Spinach Plants, has been prepared as a manuscript and it is currently under review by the Journal of Environmental Management. The manuscript is co-authored by Dr. Shiv Prasher, my supervisor, Dr. Eman ElSayed, a post-doctoral fellow in the department, Mr. Jaskaran Dhiman, a PhD scholar in the department, Mr. Ali Mawof, a PhD scholar in the department and Dr. Ramanbhai Patel, a research associate in the department. To ensure consistency with the thesis format, the original draft has been modified with the cited work listed in the reference section (Chapter 9).

Chapter 4: Impact of Soil Biochar Incorporation on the Uptake of Heavy Metals Present in Wastewater by Spinach Plants

4.1 Abstract

The effect of plantain-peel biochar on the biosorption of six heavy metals (Cd, Cr, Cu, Fe, Pb and Zn) to spinach (Spinacia *oleracea* L.) irrigated with untreated wastewater was investigated. Arranged in a complete randomized design with three replicates, the treatments were: Biochar + Wastewater (WW + B), Wastewater (WW − B) and Freshwater (FW − B). Nine outdoor lysimeters (0.45 m diameter x 1.0 m height) were packed with sandy soil (bulk density 1.35 Mg m^{-3}) and brought to field capacity one day before starting the experiment. Biochar (1% w/w) was mixed in the top 0.10 m of soil under WW+B treatment. Spinach were planted in each lysimeter, irrigated (every 10 days for 4 times in total), harvested (harvest-1 and harvest-2) and analyzed for the heavy metals. The biochar amendment improved CEC and increased the pH of soils which could have resulted in a 42% reduction of translocation of Zn in spinach leaves. Assuming daily spinach consumption of 200 g per person, Zn in spinach grown in soil amended with biochar would be below the provisional maximum tolerable daily intake limit for adults (20 mg) as prescribed by WHO. Consumption of spinach grown with wastewater in soil without biochar amendment may not be safe because of Zn toxicity. There was no noticeable impact of biochar on translocation of other heavy metals (Cd, Cu, Cr, Fe, and Pb) to spinach leaves, possibly due to competition with other compounds in the soil solution or due to alterations imposed by the possible presence of root exudates in the rhizosphere.

Keywords: Spinach; wastewater irrigation; plantain peel biochar; lysimeters; heavy metals; rhizosphere

4.2 Introduction

Biochar is a carbon-rich solid by-product produced by charring organic wastes in oxygen limited conditions (Lehmann and Joseph, 2009), and it is becoming a viable tool for immobilization of contaminants, such as heavy metals, in soils. During charring, the porosity as well as the specific surface area of the biochar increases, which improves its biosorbent characteristics (Thies and Rillig, 2009). Likewise, the presence of inorganic minerals in the charred organic wastes results in the high alkalinic pH of biochar (Petter and Madari, 2012; Vines, 1898). Notably, the effects of biochar's pH on soil (by controlling heavy metal speciation) may be the underlying mechanism for the reduction in heavy metal uptake by plants. For example, biochar derived from pigeon pea reduced the bioavailability of cadmium (Cd) and copper (Cu) to vegetables possibly due to the increase in soil pH by the biochar (Coumar et al., 2016a; Coumar et al., 2016b). In our previous study, plantain peel biochar with high alkalinic pH (~10.2), after mixing with soil, reduced the Cd and zinc (Zn) uptake by potatoes (Nzediegwu et al., 2019).

The behavior of biochar, when mixed with soil in the rhizosphere, may vary from plant to plant given that different biochemical processes, which could affect heavy metal availability and their uptake, take place in the rhizosphere (Dechassa and Schenk, 2004; Degryse et al., 2008). Therefore, it is important to study the effectiveness of biochar (e.g., plantain peel) for a given crop, especially those aboveground leafy vegetables like spinach, which is increasingly used in modern cuisine (Heine, 2004).

Spinach (Spinacia *oleracea* L.), a fast-growing vegetable crop, is rich in essential nutrients, such as vitamin C, protein, and β-carotene (Kusuma et al., 2016; Tressler et al., 1936), making it increasingly important in human diets (Kamruzzaman et al., 2016) around the world. In the past

15 years, the global annual production of spinach has doubled to about 24 million tons (FAO, 2017), and it is expected to continue to increase. However, one of the challenges with spinach production is water stress (Leskovar and Piccinni, 2005). Water stress may affect leaf quality, carbohydrate metabolism and the marketable yield of spinach (Leskovar and Piccinni, 2005; Zrenner and Stitt, 1991). For instance, spinach grown with limited water (50% evapotranspiration rate) resulted in more yellow leaves (indication of bad quality), and less marketable yield than spinach grown with sufficient water (100% evapotranspiration rate) (Leskovar and Piccinni, 2005). Therefore, to mitigate the effects of water stress in spinach, irrigation is necessary, especially in arid and semi-arid regions.

Due to the rapid depletion of freshwater in arid regions, including those in developing countries, its availability for the irrigation of crops is limited (Liesch and Ohmer, 2016; Richter et al., 2013). Increasing demand for freshwater in other sectors, such as power generation, as well as industrial and domestic needs (Blackhurst et al., 2010; Keraita and Drechsel, 2004; Rodríguez-Liébana et al., 2014) is further diminishing the water supply for irrigated agriculture. Thus, the use of wastewater, especially when it is untreated (Rodríguez-Liébana et al., 2014), offers a feasible alternative for irrigation (Chen et al., 2004; Yang et al., 2006). Many farmers choose to use untreated wastewater for diverse reasons but this is largely the result of experience, cost of treatment, and availability (Arora et al., 2008; Keraita and Drechsel, 2004). Despite this widespread use, untreated wastewater contains high concentrations of contaminants, especially heavy metals which come mostly from industrial discharge (Ahmad et al., 2011; Gokhale et al., 2008; Scott et al., 2004).

Untreated wastewater irrigation could result in heavy metal build up in soil and the subsequent uptake by plants, even to a toxic level. For instance, spinach leaves were affected by the toxicity of lead (Pb), Cd and Zn in a simulated wastewater irrigation study (Alia et al., 2015). The consumption of vegetables, contaminated by heavy metals, can cause health issues for human, thereby, posing a global environmental and public health concern. For instance, kidney, skeletal and renal damage in humans have been linked to Cd exposure through ingested food. Cd exposure can be a major cause of prostate and kidney cancer (Järup, 2003). Lead, on the other hand, accumulates in blood and the skeleton, and has been linked with brain damage in children, who are more susceptible to Pb toxicity due to the high permeability of their blood-brain barrier (Järup, 2003). Thus, it is important to reduce the translocation of heavy metals into crops irrigated with untreated wastewater.

To date, only a few studies have looked at reducing the uptake of heavy metals in spinach, especially after applying biochar amendment (Coumar et al., 2016a; Coumar et al., 2016b; Younis et al., 2015). Younis et al. (2015) investigated the ability of cotton stock biochar to reduce the uptake of Cd and Ni, while other researchers (Coumar et al., 2016a; Coumar et al., 2016b) studied the ability of pigeon pea biochar to reduce the mobility of Cu, as well as Cd. These studies showed that both biochars were able to reduce the mobility of heavy metals to the edible part of spinach when compared to a control. However, these studies focused on the uptake of heavy metals from soil which was already contaminated and where the biochar was not loaded continuously with the wastewater-laden contaminants. Moreover, these studies did not include the effect of biochar when several heavy metals co-existed, especially with organic compounds. When heavy metals co-exist, they respond differently to the available sorption sites as compared to their lone existence (Echeverría et al., 1998; Elliott et al., 1986). Moreover, the type of biochar used is a factor to consider, since it has been established that biochar's properties depend mainly on the type of feedstock used for its production (Abit et al., 2012; Alburquerque et al., 2014). With 12 TG produced as waste in 2014 (FAO, 2017), plantain peel could be a suitable feedstock in many parts of the world. Therefore, this study was conducted to investigate the effect of plantain peel biochar on the fate of six wastewater borne co-existing heavy metals (Cd, Cu, Cr, Zn, Fe, and Pb) in soil and their bioavailability to spinach. The study aimed specifically to assess: (1) whether or not irrigating with untreated wastewater leads to the accumulation of heavy metals in a sandy soil where spinach was grown, (2) whether or not biochar amendment significantly reduces the uptake of the heavy metals by spinach tissues when irrigated with untreated wastewater, and (3) which spinach parts accumulate the most heavy metals.

4.3 Materials and methods

4.3.1 Biochar characterization

Oven-dried plantain peel from green plantain fruit, purchased from Sami Fruits (LaSalle, Montreal in Canada), was pyrolized at 460^{o}C for 10 min to produce biochar. The ash content of the biochar (Table 4.1) was determined in a muffle furnace, following the method described by Enders et al. (2012). Air-dried biochar was homogenized by passing through a 2-mm sieve; the muffle furnace was brought to a temperature of 105^{o}C at the rate of 5^{o}C min^{-1}. Six empty crucibles were placed inside the furnace for curing; the temperature was raised from 105^{o}C to 750^{o}C at the rate of 5^{o}C min^{-1} and left for 10 min. After curing, the furnace was cooled from 750^{o}C to 105^{o}C. Then, the furnace was opened, and the crucibles were quickly transferred into the desiccator to prevent absorption of moisture from the environment during cooling. Cooling time was recorded, and the weight of the crucibles was measured. One gram of sample was weighed using an electronic weighing balance (±0.0001 precision) into the crucibles; the crucibles were transferred into the muffle furnace again; the temperature of the furnace was raised from 105^{o}C to 750^{o}C at a rate of

5°C min^{-1} and left to ash for 6 h. Afterwards, the furnace was brought to a temperature of 105°C, and the samples were transferred into a desiccator. The weight of the ash was recorded; the ash content was calculated as percentage dry weight of the biochar.

Table 4.1. Properties of pyrolized plantain peel biochar on dry weight basis

Proximate Analysis	**%dry weight**	**Method**			
Moisture TGA	5.68	ASTM 7582	**SSA**	0.756±0.014 m^2 g^{-1}	
Ash content	27.97	ASTM 7582	**pH****	10.6±0.1	
			EC**	7.06 dS m^{-1}	
Volatile matter	31.32	ISO 562	**Bulk density**	0.21 Mg m^{-3}	
Fixed Carbon	40.71	ASTM 7582	**Heavy Metals**	**Concentrations (mg kg^{-1})**	**IBI Allowable Thresholds (mg kg^{-1})**
Ultimate Analysis	**%dry weight**	**Method**	**Cd**	0.08±0.01	1.4-39
Carbon	57.4	ASTM D5373	**Cr**	1.11±0.15	63-1500
Hydrogen	3.18	ASTM D5373	**Cu**	11.68±0.05	64-1200
Nitrogen	2.16	ASTM D5373	**Fe**	649.01±58.81	not available
Total Sulphur	<0.05	ASTM D4239	**Pb**	0.04±0.01	70-500
Oxygen	9.32	By Difference	**Zn**	573.58±33.69	200-7000

TGA-thermogravimetric analysis; SSA: specific surface area; EC: electrical conductivity; *: Ash content determined by Enders method is 28.7±0.6. **EC and pH were measured for 1:30 (w/w) biochar: deionized water solution. The ± values are standard error.

The pH and electrical conductivity (EC) of the biochar (Table 4.1) were measured using electrode type meters (Accumet AB 15 and DiST 6 EC/TDS/Temperature Tester, Hanna Instruments, Rhodes Island, USA, respectively). A 1:30 (w/w) biochar:deionized water solution was prepared, placed on a shaker (200 rpm) for 4 hours (Zhang et al., 2015), and then the pH and EC of the suspension was measured. Other proximate analysis (volatile matter and fixed carbon content) and elemental analysis (Table 4.1) were performed at the Canmet ENERGY/Characterization

Laboratory (ISO 9001:2008, FS 64051), Ottawa, Ontario, Canada, whereas the BET (Brunauer Emmet Teller) specific surface area (Table 4.1) was measured with Tristar 3000 (Micrometric) at McGill University Materials Characterization Laboratory, Montreal, Canada. For surface area measurement, the biochar samples (~0.2750 g), degassed overnight (120^{o}C), were exposed to nitrogen (77.350K; used as a probe molecule) at a relative pressure (P/P^{o}) range of 0.06 to 0.20. The specific surface area was calculated from BET isotherm and assumed the molecular area of nitrogen as 16.2 Å^{2} (Amziane and Collet, 2017; Jiang et al., 2012a). The morphology of the biochar was determined by scanning electron microscopy (SEM) (Hitachi TM 3000) at two magnifications (X500 and X1000).

Heavy metal concentrations in the biochar (Table 4.1) were determined following the hot nitric acid method described in the soil analysis (Section 4.3.3). The International Biochar Initiative (IBI) allowable threshold of the heavy metals in biochar (Table 4.1; http://www.biochar-international.org/characterizationstandard) are also reported. All the heavy metals in the biochar were within the permissible limit, and thus, the biochar quality was acceptable.

To ascertain the heavy metal holding capacity of the biochar, sorption and desorption tests were performed in line with Sarmah et al. (2010). Soil samples were collected from the lysimeters, filled with soil from a field at McGill University, Macdonald Campus, Canada (45^{o}24'48.48" N, 73^{o}56'28.06" W). The soil samples were air-dried for two days and passed through a 2-mm sieve. Stock solutions of all the heavy metals were prepared using reagent grade salts (Table 4.3) in 4% nitric acid (heavy metal grade). Cocktails (0.1 mM, 0.2 mM, 0.3 mM, 0.4 mM, and 0.5 mM) of all the heavy metals (Cd, Cr, Cu, Fe, Pb and Zn) were prepared in 0.001 M $NaNO_3$ solution; the 0.001 M $NaNO_3$ solution was used to account for ionic strength of the sorption solution (Jiang et al., 2012a). Performed in triplicate, the treatments were: non-amended soil (WW-B) and biochar-amended soil (WW+B). Two grams of the air-dried soil was weighed into a 50-mL falcon tube, followed by addition of 1% (w/w) air-dried biochar (passing 2-mm sieve). Then 30 mL of the cocktail solution was added to each of the falcon tubes. The falcon tubes were capped, vortexed (1800 rpm; 30 secs), placed on a shaker (205 rpm; 24 h), and then centrifuged (3500 rpm; 10 min). The supernatant was carefully decanted. About 15 mL aliquot of the supernatant was collected for heavy metal analysis on inductively coupled plasma optical emission spectrometry (ICP-OES, Varian, Vista-MPX CCD Simultaneous). The pH of the remaining aliquot was measured. For desorption, 30 mL of double deionized water was added to the residual soil in the 50-mL falcon tube. The procedure from vortexing, as described in the sorption test, was repeated. The sorption data were presented as coefficient of distribution, Kd being $\text{mass adsorbed}/\text{equilibrum concentration}$, while the overall %adsorbed (%A) and overall %desorbed (%D) heavy metals were calculated using molar mass weighted average method as follows:

$$\%\mathrm{A} = \frac{1}{\mathrm{MW_T}} \sum_{\mathrm{i}=1}^{\mathrm{n}} (\%\mathrm{A_i} * \mathrm{MW_i}) \qquad (4.1)$$

$$\%\mathrm{D} = \frac{1}{\mathrm{MW_T}} \sum_{\mathrm{i}=1}^{\mathrm{n}} (\%\mathrm{D_i} * \mathrm{MW_i}) \qquad (4.2)$$

Where, i from 1 to n, are the heavy metals in the sorption solution, MW is the molar mass of a heavy metal, and MW_T is the sum of molar masses of all the heavy metals in the cocktail.

4.3.2 Field lysimeter study

Nine PVC cylindrical lysimeters (1.0 m height x 0.45 m diameter; Fig. 4.1) were packed with a sandy soil to a bulk density of 1.35 Mg m^{-3}. The physicochemical properties of the soil are presented in Table 4.2. A 5-cm deep clearance was left from the top of the lysimeter to the soil surface to prevent water overflowing the lysimeter during irrigation. Four holes (0.1 m diameter) were drilled radially through wall at depths 0.15 m, 0.35 m and 0.65 m for collecting composite soil samples. The soil sampling holes on the lysimeters were sealed with rubber stoppers, and then the lysimeters were brought to field capacity. The lysimeters were arranged in a completely randomized design. Performed in triplicate, the 3 treatments were: (i) wastewater without biochar (WW-B), (ii) wastewater with biochar (WW+B), and (iii) freshwater without biochar (FW-B). Three lysimeters under WW+B were amended with the biochar in upper 0.1 m layer at the rate of 1.0% of soil (w/w) (Sarmah et al., 2010). The remaining six lysimeters were not amended with biochar. The WW-B served as a control for WW+B to check whether biochar amendment has any effect on the spatial and temporal mobility of the heavy metals in soil and translocation to different parts of spinach. The concentration of heavy metals in the in soil and in spinach tissues (roots, stems and leaves) was determined for samples from FW-B, which served as a control for WW-B.

Spinach (purchased from the Jean Talon Farmers Market, Montreal, Canada) was transplanted in all lysimeters. Three spinach plants were planted at the apex of a 6 cm equilateral triangle traced on the surface of each lysimeter soil. Fertilizer was applied according to the recommended dose in Quebec (http://collections.banq.qc.ca/ark:/52327/bs2012495): 5.0 g potassium sulphate (0-0-60) was spread per lysimeter to apply 163 kg K ha^{-1} on the day of planting; 9.0 g ammonium sulphate (21-0-0) was applied on the soil surface equivalent to 120 kg N ha^{-1} in two splits, half each on the day of planting (Day 1) and on the day of the first wastewater irrigation (Day 22). Immediately after transplanting, freshwater was applied at a rate of 2.5 mm day^{-1} (400 mL/lysimeter); this was continued every alternate day until 20 days when development of new leaves was noticed on the spinach. New leaf development after transplanting signifies that the plant has stabilized (Ziv, 1989).

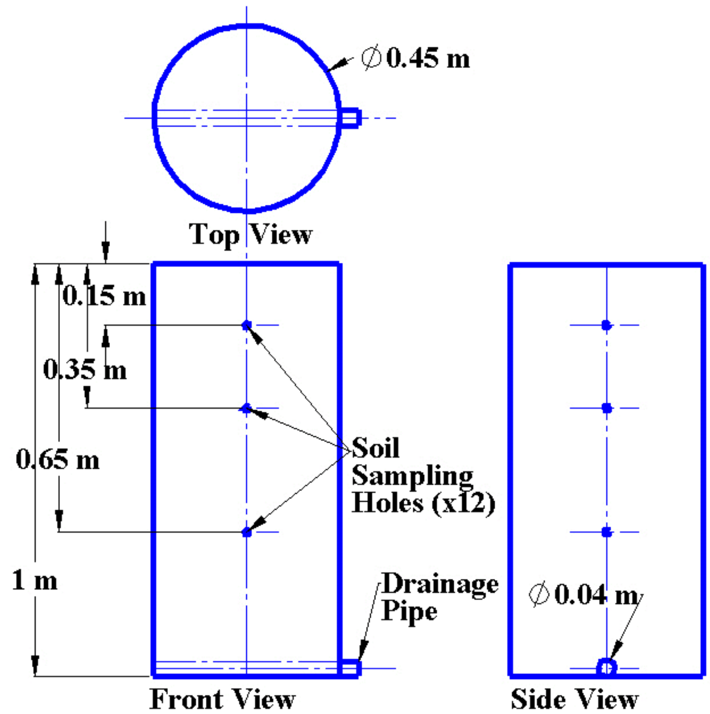

Figure 4-1. Orthographic view of one-metre tall aboveground lysimeter

Highest concentrations of Cd, Cr, Cu, Fe, Pb and Zn, representing the worst-case scenario of untreated wastewater in different parts of developing countries, were adopted for synthesizing wastewater (LaPara et al., 2006; Nopens et al., 2001). For preparation of wastewater, tap water was stored overnight in an open container for dechlorination. Soluble salts of compounds (purchased from Sigma Aldrich) were mixed with other contaminants to prepare synthetic wastewater on every irrigation event, following the recipe given in Table 4.3. A total of four wastewater irrigations were scheduled at a 10-day interval; each irrigation of 25 mm (4 L/lysimeter) was applied (Sander, 2012). Porous cheese cloth was placed on the top of lysimeter to maintain the uniform distribution of irrigation water on the soil surface. Leachate from the bottom of the lysimeter was not collected for analysis as heavy metals are adsorbed in the upper soil layer and do not leach into the drainage water (Nzediegwu et al., 2019).

Table 4.2. Physicochemical properties of soil before biochar amendment

Soil Properties	
Sand (%)	92.2
Silt (%)	4.3
Clay (%)	3.5
pH	5.5
Organic matter (%)	2.4±0.15
Hydraulic conductivity (m day^{-1})	1.67±0.45
ZPC	3.4
P (mg kg^{-1})	215.30±40.43
K (mg kg^{-1})	107.33±13.13
N (mg kg^{-1})	4.57±0.46
Ca (mg kg^{-1})	912.44±79.70
Mg (mg kg^{-1})	103.27±7.29
Al (mg kg^{-1})	1164.14±12.40

ZPC: zero point of charge; P, K, Ca, Mg, and Al were determined according to Mehlich (1984), N was determined following 2 M KCl method (Carter and Gregorich, 2008). Other soil properties were adapted from ElSayed et al. (2013).

Composite soil samples (~5 g) were collected before the first irrigation (Day-BP), and two days after each irrigation event from the surface and sampling holes. Two days were allowed for the soil to attain field capacity. The soil samples were brought to the lab and were stored in a freezer (-24^{o}C) until analysis. Spinach leaves were harvested after the first 2 irrigations with wastewater (42 days after transplanting), and then at the end of the growing season. The spinach tissues (leaves, stems and roots) were separated and properly washed (3 times) with deionized water to ensure no soil particles were retained on them (Zheng et al., 2016). The tissues were sampled and oven dried (60^{o}C) for 2 days and stored for heavy metal analysis.

Table 4.3. Synthetic wastewater recipe. Values in brackets are actual concentrations used in the synthetic wastewater adjusted so the level of the residual or translocated substance of interest would be above the detection limit.

Purpose	Substance/ Compounds	Country	Concentration **(mg L^{-1})**	**Wastewater Recipe Source** Contaminant reporter
			Basic synthetic wastewater ingredients	
C source	Sodium Acetate		**79.37**	Nopens et al. (2001)
	Milk powder		**116.19**	Nopens et al. (2001)
	Soy Oil		**29.02**	Nopens et al. (2001)
	Starch		**122.00**	Nopens et al. (2001)
	Yeast Extract		**52.24**	Nopens et al. (2001)
N Source	Ammonium Chloride		**12.75**	Nopens et al. (2001)
	Peptone		**17.41**	Nopens et al. (2001)
	Urea		**91.74**	Nopens et al. (2001)
P Source	Magnesium phosphate		**29.02**	Nopens et al. (2001)
Minerals	$CaCl_2$		**60**	LaPara et al. (2006)
	$MgCl_2$		**40**	LaPara et al. (2006)
	$NaHCO_3$		**100**	LaPara et al. (2006)
	K_3PO_4		**30**	LaPara et al. (2006)
	Additional contaminants levels based on worst case reports or need to exceed LOD			
Heavy Metals	Potassium dichromate (Cr)	India	**2**	Ahmad et al. (2011)
	Cadmium Nitrate (Cd)	India	**5**	Ahmad et al. (2011)
	Lead Nitrate (Pb)	India	**16**	Ahmad et al. (2011)
	Iron Sulphate (Fe)	India	**120**	Ahmad et al. (2011)
	Zinc Nitrate (Zn)	India	**3**	Ahmad et al. (2011)
	Copper Nitrate (Cu)	India	**8**	Ahmad et al. (2011)
Hormones	Estrone (E1)	Korea	8.15 (50) µg L^{-1}	Sim et al. (2011) — LOD
	Estradiol (E2)	Korea	0.634 (20) µg L^{-1}	Sim et al. (2011) — LOD
	Progesterone	China	0.90 (20) µg L^{-1}	Huang et al. (2009) — LOD
Pharmaceuticals	Oxytetracycline	China	19.5	Li et al. (2008)
	Ibuprofen	India	26.45 µg L^{-1}	Singh et al. (2014)
Surfactant	Triton X^{-}100 or alkylphenyl polyethoxylate	Morocco	30 µg L^{-1}	Aboulhassan et al. (2006)
Plasticizers	Bisphenol A		(50 µg L^{-1})	Based on LOD
	Bisphenol S		(50 µg L^{-1})	Based on LOD
	Bisphenol F		(50 µg L^{-1})	Based on LOD

4.3.3 Soil analysis

Heavy metals were recovered following the hot nitric acid extraction method (Kargar et al., 2013). Soils (~3.0 g) were weighed into low-form aluminum weighing dishes and air-dried for 2 days. The air-dried soils were crushed using a ceramic mortar and pestle; it was homogenized by passing through a 2-mm sieve (Fisher Scientific Co., U.S. Standard Series). The homogenized soil samples (0.16 g) were weighed into 15-mL digestion tubes; and 2 mL of concentrated nitric acid (trace metal grade, 70% pure) was added. The solution was kept overnight under the fume hood. The next day, the samples were placed on a block digester (Fisher Scientific®, dry batch incubator) with a thermometer attached to one of the slots to measure the temperature. The temperature was gradually increased to 80°C and kept at this temperature until fuming stopped. Care was taken at this stage to prevent splash. The temperature was further increased, gradually, to 120±5°C. At 120°C, the solution was digested for 5 h. Afterwards, the samples were removed from the digester and cooled for 15 min. The digested solution was transferred into 50-mL falcon tubes. The digestion tube was rinsed five times with double deionized water and water was transferred to the falcon tube. Finally, double deionized water was added until the solution in the falcon tube reached the 50-mL mark. About 15 mL aliquot of the digested solution was analyzed for the heavy metals using ICP-OES (Varian, Vista-MPX CCD Simultaneous). In all batches, reference materials (SED98-04 and SED92-03, Environment Canada) and method blanks were added. Percent recovery of the SED98-04 and SED 92-03 were as follows: 83.4, 83.9% for Cr; 82.4, 106.3% for Cu; 61.0, 83.6% for Fe; 95.1, 90.8% for Pb; and 87.7, 82.4% for Zn; it was not available for Cd as concentrations in the reference material were below detection limit. The detection limits, converted to mg kg^{-1} from mg L^{-1} using same proportion as digested samples, were 15.6 for Cd, Cu, Cr, Zn and Pb; and 156.5 for Fe.

4.3.4 Measurement of soil pH

The pH of the soil (surface and 0.1 m depth), sampled 2 days after each irrigation event, was measured following the soil survey standard test method (pHWC2/2) (Rayment and Higginson, 1992). The soil was air-dried for two days and passed through a 2-mm sieve. One gram of the air-dried soil sample was weighed into a 15-mL bottle and 5 mL of deionized water was added (soil: water ratio, 1:5). The solution was shaken for 1 h, and then the pH of the suspension was measured using an electrode type pH meter (Accumet AB 15).

4.3.5 Measurement of soil CEC

The CEC of the soil samples (surface and 0.1 m below the surface) collected at the end of the season was measured following the $BaCl_2$ method (Carter and Gregorich, 2008). One gram of the air-dried soil (<2 mm) was weighed in a 50-mL falcon tube. Then, 25 mL of 0.1M $BaCl_2$ was added and shaken slowly on an end-to-end shaker (15 rpm, 2 h). Afterwards, it was centrifuged (700 g, 15 mins) and filtered (Fisher Q8; particle retention 20 to 25 µmm). The supernatant was collected and diluted (x20 with milli pure water). Then, 100 mg La L^{-1} and 100 mg Cs L^{-1} was added to a 10-mL aliquot of the diluted supernatant for Ca, K and Mg analyses. The remaining portion of the diluted supernatant was divided in twos. The first portion was used for Na, Al, Fe, and Mn analyses, while the second portion was used for pH determination. All cations (Ca, K, Mg, Na, Al, Fe and Mn) were quantified in Atomic Absorption Spectrometer (Varian, SpectrAA 220FS). The CEC was calculated as $[\mathrm{Ca^{2+} + Mg^{2+} + K^{+} + Na^{+} + Al^{3+} + Fe^{3+} + Mn^{2+}}]$ in cmol (+) kg^{-1}.

4.3.6 Analysis of heavy metals in spinach

The oven-dried spinach samples were crushed with a coffee grinder and a ceramic mortar and pestle. Then, 0.16 g of the homogenized samples were weighed in 15-mL digestion tubes, and the procedure, as detailed for soil digestion described above, was performed. Aliquot of the digested solution was analyzed for the heavy metals using inductively coupled plasma mass spectrometry (ICP-MS, Varian ICP820-MS or Analytik-Jena). Standard Reference Material, NIST-1547, as well as method blanks were added in all batches. Recovery percentage of the NIST-1547 were as follows: 92.7% for Cd, 79.2% for Cr, 104.0% for Cu, 83.4% for Fe, 100.7% for Pb, and 106.4% for Zn. The detection limits converted to mg kg^{-1} using same proportion as digested samples were as follows: 0.033, 0.032, 0.071, 0.239, 0.051 and 0.382 for Cd, Cr, Cu, Fe, Pb and Zn, respectively.

4.3.7 Statistical data analysis

Repeated measure analysis with the PROC Mixed procedure in SAS was used to analyze the soil pH and the soil heavy metal concentrations data. Other data were subjected to one-way analysis of variance using SAS-JMP® 13.0.0 (Copyright © 2016 SAS Institute Inc.). Where applicable, outliers were removed [assuming Huber distribution (Maier et al., 2017)] using SAS-JMP® 13.0.0 (Copyright © 2016 SAS Institute Inc.).

4.4 Results and discussion

4.4.1 Morphology of biochar

Figure 4.2 presents the SEM of the biochar in two magnifications (X500 and X1000), meant to reveal the surface pore distribution. The SEM suggests that the biochar has pores of different sizes and shapes formed due to thermochemical restructuring of the hemicellulose, lignin and cellulose in the plantain peel (Agarry et al., 2013; Kabenge et al., 2018) as the water and the volatile materials evaporate during pyrolysis (Li et al., 2017). Pores could enhance the potential of biochar to hold heavy metals by increased surface functional groups.

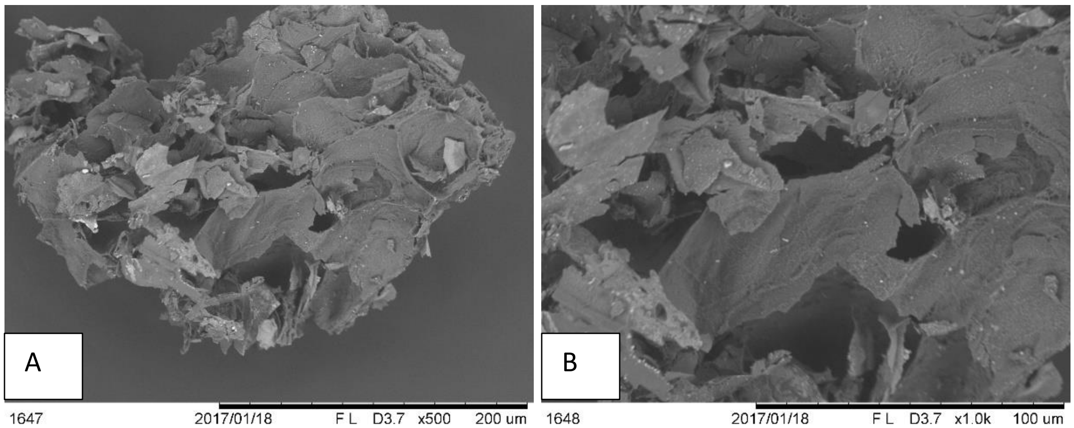

Figure 4-2. Scanning electron microscopy of biochar at two magnifications (A) X500 and (B) X1000

4.4.2 Sorption and desorption

Distribution coefficients of heavy metals (Cd, Cr, Cu, Fe, Pb and Zn) in WW+B and WW-B and the corresponding overall adsorbed and desorbed percentages are presented in Table 4.4. There was a considerable variation among Kd values of different heavy metals for the two treatments at different initial concentrations. A plot of mass adsorbed versus equilibrium concentration did not fit any isotherm, including linear, Langmuir and Freundlich models for most of the heavy metals. In a competitive sorption study of six heavy metals (Cd, Cr, Cu, Ni, Pb and Zn) to soil, Gomes et al. (2001) observed a similar trend. They suggested an evaluation of sorption characteristics based on Kd at a given initial concentration when plot of mass adsorbed versus equilibrium concentration do not follow a straight line due to competitive sorption (Gomes et al., 2001). The $[\mathrm{Kd}]_{\mathrm{WW-B}}^{\mathrm{Cd}}$ ranged from 0.0012 to 0.0120 L g^{-1}, while that of the $[\mathrm{Kd}]_{\mathrm{WW+B}}^{\mathrm{Cd}}$ ranged from 0.0086 to 0.2445 L g^{-1}.

Table 4.4. Distribution coefficient, Kd (L g^{-1}), of heavy metals under different treatments

Treatment	Conc. added (mM)	Cd	Cr	Cu	Fe	Pb	Zn	%A	%D
WW-B	0.1	0.0122	0.5267	0.0866	1.6951	1.5154	0.0081	78.9	2.8
	0.2	0.0030	0.6154	0.0295	0.2109	0.5342	0.0052	68.8	1.5
	0.3	**0.0021**	**1.7443**	**0.0166**	**0.1272**	**0.5968**	**0.0013**	64.1	1.5
	0.4	0.0016	0.8422	0.0124	0.0559	0.1973	0.0011	59.7	2.2
	0.5	0.0012	0.8644	0.0125	0.1132	0.3038	0.0011	61.0	5.4
WW+B	0.1	0.2445	0.0266	0.0836	0.0121	0.1468	0.0879	83.1	4.0
	0.2	0.0391	0.1542	0.0676	0.2048	0.5700	0.0229	85.3	2.3
	0.3	**0.0294**	**0.0907**	**0.0905**	**0.3501**	**0.9115**	**0.0207**	84.4	2.8
	0.4	0.0112	1.0916	0.0464	6.6202	14.5587	0.0072	77.6	1.0
	0.5	0.0086	0.0618	0.0444	8.2790	30.7574	0.0059	74.1	1.4

WW-B: non-amended soil; WW+B: biochar amended soil. Values in bold font were used for comparison; %A: percent adsorbed; %D: percent desorbed.

The Kd for WW+B at all added concentrations was higher as compared to WW-B, suggesting larger amounts of Cd could bind to the WW+B as compared to the WW-B. Therefore, availability of Cd in the soil solution under WW+B for plant uptake would be low. As expected, both $[\mathrm{Kd}]_{\mathrm{WW+B}}^{\mathrm{Cd}}$ and $[\mathrm{Kd}]_{\mathrm{WW-B}}^{\mathrm{Cd}}$ decreased with an increase in the solution concentration (Table 4.4), possibly due to saturation of the available adsorption sites with the increased concentration of heavy metals (Saeed et al., 2005). The $[\mathrm{Kd}]_{\mathrm{WW-B}}^{\mathrm{Cr}}$ ranged from 0.5267 to 1.7443 L g^{-1}, while the $[\mathrm{Kd}]_{\mathrm{WW+B}}^{\mathrm{Cr}}$ ranged from 0.0266 to 1.0916 L g^{-1}. The higher $[\mathrm{Kd}]_{\mathrm{WW-B}}^{\mathrm{Cr}}$, as compared to $[\mathrm{Kd}]_{\mathrm{WW+B}}^{\mathrm{Cr}}$ (apart from 0.4 mM), could be due to the competing presence of the other heavy metals, which could have reduced the adsorption of Cr to WW+B. According to Liu et al. (2013), in multi-metal adsorption experiment, the adsorption capacity of heavy metals, including Cr, decreased in the presence of other competing metals. Moreover, no established trend between Kd and the added concentration was noticed. The high $[\mathrm{Kd}]_{\mathrm{WW+B}}^{\mathrm{Cr}}$ at 0.4 mM could be associated with the higher presence of Fe (in WW+B), which possibly caused Cr precipitation via reduction reaction (Wittbrodt and Palmer, 1996). The $[\mathrm{Kd}]_{\mathrm{WW-B}}^{\mathrm{Cu}}$ ranged from 0.0124 to 0.0866 L g^{-1}, while $[\mathrm{Kd}]^{\mathrm{Cu}}_{\mathrm{WW+B}}$ ranged from 0.0444 to 0.0905 L g^{-1}. At the lowest concentration (0.1 mM), the Kd was almost the same for both treatments. For all other concentrations, the $[\mathrm{Kd}]^{\mathrm{Cu}}_{\mathrm{WW+B}}$ was higher than that of $[\mathrm{Kd}]^{\mathrm{Cu}}_{\mathrm{WW-B}}$, suggesting that Cu could be less bioavailable in the WW+B treatment as compared to the WW-B treatment. The $[\mathrm{Kd}]^{\mathrm{Cu}}_{\mathrm{WW-B}}$ decreased with increased solution concentration. This was expected as the sorption sites become saturated with an increase in concentration. However, the $[\mathrm{Kd}]^{\mathrm{Cu}}_{\mathrm{WW+B}}$ showed no established trend; possibly, it could indicate that Cu is held, in the presence of biochar, by other sorption mechanisms (other than adsorption), such as surface complexation. The $[\mathrm{Kd}]^{\mathrm{Fe}}_{\mathrm{WW-B}}$ ranged from 0.0559 to 1.6951 L g^{-1}, while $[\mathrm{Kd}]^{\mathrm{Fe}}_{\mathrm{WW+B}}$ ranged from 0.0121 to 8.2790 L g^{-1}. Apart from the initial concentrations (i.e., 0.1 and 0.2 mM), the $[\mathrm{Kd}]^{\mathrm{Fe}}_{\mathrm{WW+B}}$ was greater than $[\mathrm{Kd}]^{\mathrm{Fe}}_{\mathrm{WW-B}}$. There was a decrease in $[\mathrm{Kd}]^{\mathrm{Fe}}_{\mathrm{WW-B}}$ with the increase in added concentrations till 0.4 mM, implying saturation of WW-B treatment at lower Fe concentrations; however, at the concentration beyond 0.4 mM (Table 4.4) there could be precipitation of Fe in the solution phase. The $[\mathrm{Kd}]^{\mathrm{Fe}}_{\mathrm{WW+B}}$ increased with the increase in concentration, implying that for the range of concentration studied, Fe was not saturated on the soil-biochar surface. This is important as the concentration of Fe, in the environment, is relatively high as compared to other heavy metals. Thus, amending the soil with biochar could be an effective technique to alleviate Fe transport in the soil. The $[\mathrm{Kd}]^{\mathrm{Pb}}_{\mathrm{WW-B}}$ ranged from 0.1973 to 1.5154 L g^{-1}, while $[\mathrm{Kd}]^{\mathrm{Pb}}_{\mathrm{WW+B}}$ ranged from 0.1468 to 30.7574 L g^{-1}. Although there was no established trend for $[\mathrm{Kd}]^{\mathrm{Pb}}_{\mathrm{WW-B}}$, it was observed that $[\mathrm{Kd}]^{\mathrm{Pb}}_{\mathrm{WW+B}}$ increased with the added concentration (Table 4.4). It appears that within the range of concentration studied, the available adsorption site on the soil-biochar surface remained unsaturated. Higher $[\mathrm{Kd}]^{\mathrm{Pb}}_{\mathrm{WW+B}}$ (vs. $[\mathrm{Kd}]^{\mathrm{Pb}}_{\mathrm{WW-B}}$) denotes that Pb could be less mobile in WW+B as compared to WW-B. This corroborates the findings of Trakal et al.

(2011), who in a multi-metal sorption study reported that the adsorption of Pb onto soil was enhanced by biochar amendment. The $[\mathrm{Kd}]_{\mathrm{WW-B}}^{\mathrm{Zn}}$ decreased from 0.0081 to 0.0011 L g^{-1}, while $[\mathrm{Kd}]_{\mathrm{WW+B}}^{\mathrm{Zn}}$ decreased from 0.0879 to 0.0059 L g^{-1}. As expected, both $[\mathrm{Kd}]_{\mathrm{WW-B}}^{\mathrm{Zn}}$ and $[\mathrm{Kd}]_{\mathrm{WW+B}}^{\mathrm{Zn}}$ decreased with an increase in the added concentration possibly due to saturation of the adsorption sites. $[\mathrm{Kd}]_{\mathrm{WW+B}}^{\mathrm{Zn}} \geq 5\ \mathrm{X}\ [\mathrm{Kd}]_{\mathrm{WW-B}}^{\mathrm{Zn}}$ might suggest that Zn could bind more to WW+B as compared to WW-B.

Based on $[\mathrm{Kd}]_{0.3\mathrm{mM}}$ (average initial concentrations ≈ 25mg L^{-1} used by Gomes et al. (2001)), the selectivity of the heavy metals for WW-B was $\mathrm{Cr} > \mathrm{Pb} > \mathrm{Fe} > \mathrm{Cu} > \mathrm{Cd} > \mathrm{Zn}$, whereas for WW+B, it was $\mathrm{Pb} > \mathrm{Fe} > \mathrm{Cr} > \mathrm{Cu} > \mathrm{Cd} > \mathrm{Zn}$. The selectivity order looks similar for both treatments, except that Cr and Pb interchanged positions, which is in accordance with the observations of Fontes and Gomes (2003), who reported a selectivity order of $\mathrm{Cr} > \mathrm{Pb} > \mathrm{Cu} > \mathrm{Ni} > \mathrm{Cd} > \mathrm{Zn}$ and $\mathrm{Pb} > \mathrm{Cr} > \mathrm{Cu} > \mathrm{Ni} > \mathrm{Cd} > \mathrm{Zn}$ for various Brazilian soils in a competitive sorption experiment (without Fe though). Although the selectivity order for both treatments did not follow the electronegativity order (i.e., $\mathrm{Pb}\ (2.33) > \mathrm{Cu}(1.9) > \mathrm{Fe}(1.83) > \mathrm{Cd}(1.69) > \mathrm{Cr}(1.66) > \mathrm{Zn}(1.65)$) reported in the literature (McBride, 1994), it appears that with biochar amendments, the position of Pb was maintained. This is important given the toxic nature of Pb to humans.

The overall %adsorbed and overall %desorbed heavy metals are presented in Table 4.4. Although with little deviation, percent adsorbed decreased with an increase in concentration for both WW-B and WW+B. For all concentrations, the WW+B held more heavy metals as compared to the WW-B further suggesting a better sorption potential with soil-biochar mix as compared to no biochar amended soil. Although with no established trend, the amount of heavy metal desorbed after attaching to either the amended (WW+B) or non-amended soil (WW-B) was very minimal as compared to the amount adsorbed, suggesting high affinity of the heavy metals for the sorption sites on biochar as well as soil. Nevertheless, it was noteworthy that at high initial concentrations (0.4 and 0.5 mM), the amount desorbed from WW+B was quite low as compared to WW-B, suggesting better sorption potential of biochar, which would minimize transport of heavy metals in soil beyond the amendment zone, especially in a soil with high heavy metal contamination.

4.4.3 Effect of biochar amendment on soil CEC and soil pH

The exchangeable cations and the CEC of the soil samples (WW-B and WW+B) collected after the last irrigation are presented in Table 4.5 along with the pH of CEC solution (pH_{cec}). The CEC of the surface soil was significantly (84%) higher for WW+B as compared to WW-B, indicating that biochar's incorporation into the soil increased the number of sites for exchangeable cations. This is obvious, particularly for K, where the exchangeable cation was significantly higher ($p < 0.05$) in WW+B (vs. WW-B) possibly due to the concentration of K (13.5%) in the biochar. Although statistically not significant ($p > 0.05$), it is evident that the exchangeable cations were numerically higher in most cases for WW+B as compared to WW-B. Increase in soil CEC—as in this present study—could potentially mean an increase in the adsorption of heavy metals. Jiang et al. (2012a), in an incubation study, reported that biochar's addition to soil increased soil CEC (similar range as in this present study), which, in turn, increased sorption of Pb. Moreover, at lower depths (i.e., 0.1 m below), the soil CEC was 58% higher in WW+B (vs. WW-B), indicating a greater likelihood of more sorption in WW+B as compared to WW-B. Although not significant, the numerically higher presence of the other exchangeable cations (Ca, Mg, Na, Al, Fe and Mn) in WW+B at 0.1 m depth (Table 4.5) as compared to WW-B could imply more cation exchange reactions between these cations and heavy metal cations (present in soil solution) in the ion exchange complex (Joseph et al., 2010; Lu et al., 2012).

Table 4.5. Exchangeable cations and cation exchange capacity (and its associated pH) of biochar amended, and non-amended soil irrigated with wastewater, and their statistical analysis

Exchangeable cations (cmol (+) kg^{-1})	**Surface**		**0.1 m depth**		**Effects**		
	WW-B	WW+B	WW-B	WW+B	Treatment	Depth	Interaction
Ca	3.54±0.95	4.31±0.34	4.52±0.59	5.06±0.71	ns	ns	ns
Mg	0.78±0.23	1.21±0.20	0.45±0.07	0.70±0.11	ns	ns	ns
K	0.33±0.05^{b}	4.08±1.40^{a}	0.12±0.02^{b}	2.23±0.16^{a}	*	ns	ns
Na	0.52±0.13^{a}	0.65±0.15^{a}	0.08±0.01^{b}	0.15±0.04^{b}	ns	*	ns
Al	0.37±0.16	0.06±0.02	0.03±0.01	0.04±0.02	ns	ns	ns
Fe	0.05±0.01^{a}	0.02±0.01^{a}	0.01±0.00^{b}	0.01±0.01^{b}	ns	*	ns
Mn	0.09±0.01^{a}	0.08±0.01^{a}	0.01±0.003^{b}	0.02±0.007^{b}	ns	*	ns
CEC	5.68±1.18^{b}	10.42±1.55^{a}	5.22±0.62^{b}	8.22±0.97^{a}	*	ns	ns
pH_{cec}	4.3±0.1	4.8±0.3	4.8±0.3	5.0±0.3			

* and ns denote significance and non-significance at $p<0.05$, respectively. Different superscript letters indicate significant difference in the mean values of two treatments at a given depth; pH_{cec} is the pH of the CEC solution.

Figure 4.3 presents the pH of the soil (surface and 0.1 m below i.e., $[pH]_{Soil}^{Surf}$ and $[pH]_{Soil}^{0.1}$, respectively), with, and without, biochar amendment, irrigated with wastewater. Overall, the $[pH]_{Soil}^{Surf}$ did not change with time in both treatments, with the exception that pH decreased at the end of the experiment (Day 54), possibly due to continuous wastewater irrigation; the wastewater had a pH of 4.6±0.07. At 0.1 m depth, the $[pH]_{Soil}^{0.1}$ in WW-B gradually increased from 5.0 (day24) to 5.6 (day 54). The pH in WW+B was 5.5 before wastewater irrigation (i.e., before adding biochar); it increased to 6.2 after the first irrigation and stabilized at about 6.0 until the end of experiment. So, at the surface and at 0.1 m depths, the pH in biochar treatment was significantly higher than that without biochar ($p<0.05$). In agreement with previous studies (Coumar et al., 2016b; Puga et al., 2016), mixing biochar (pH 10.6) in the soil caused a significant increase in the soil's pH possibly through dissolution of soluble salts (Joseph et al., 2010). This is important as pH controls the availability of contaminants (in soils) including heavy metals to crops.

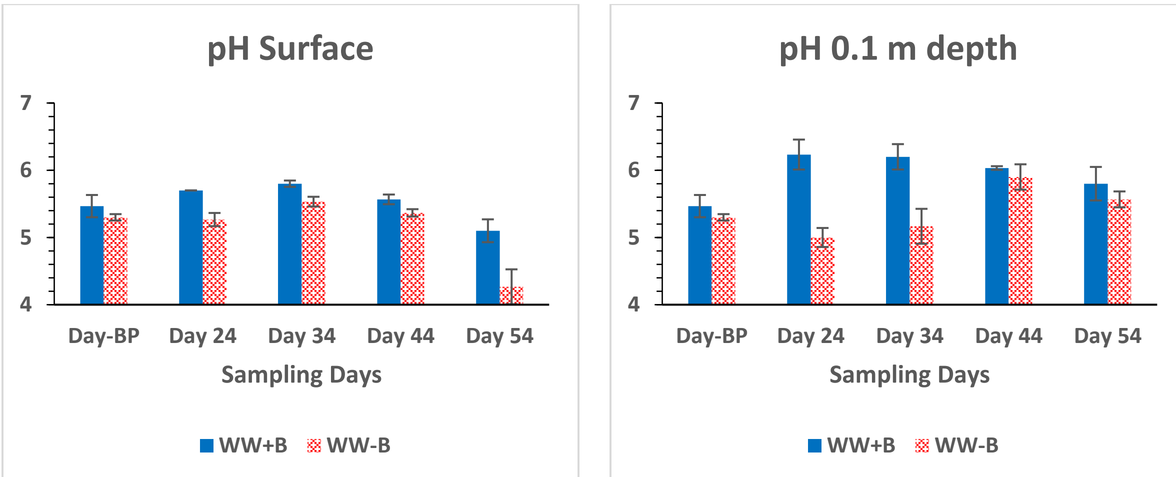

Figure 4-3. pH of the soil irrigated with wastewater. Day-BP is the day before planting. The error bar is standard error for three replicates.

4.4.4 Heavy metals in soil

Figure 4.4 presents the concentrations of heavy metals (Cd, Cu, Cr, Fe, Pb and Zn) measured in the soil samples collected at the soil surface ($[\mathrm{Cd}]_{\mathrm{Soil}}^{\mathrm{Surf}}$) during the experiment. In the WW-B treatment, the concentration of $[\mathrm{Cd}]_{\mathrm{Soil}}^{\mathrm{Surf}}$ increased gradually from below the detection limit (15.6 mg kg^{-1}) on Day24 to 33.6 mg kg^{-1} at the end of the season (Day54). Likewise, the concentration of $[\mathrm{Cd}]_{\mathrm{Soil}}^{\mathrm{Surf}}$ for the WW+B treatment increased from below the detection limit on Day24 to 41.4 mg kg^{-1} at the end of the season. Thus, there was an increase in concentration of Cd over time; repeated measure analysis showed a significant increase ($p<0.05$; Fig. 4.4) due to contaminated water irrigation. Irrigation with contaminated water tends to increase the concentration of Cd in soil (Aydin et al., 2015).

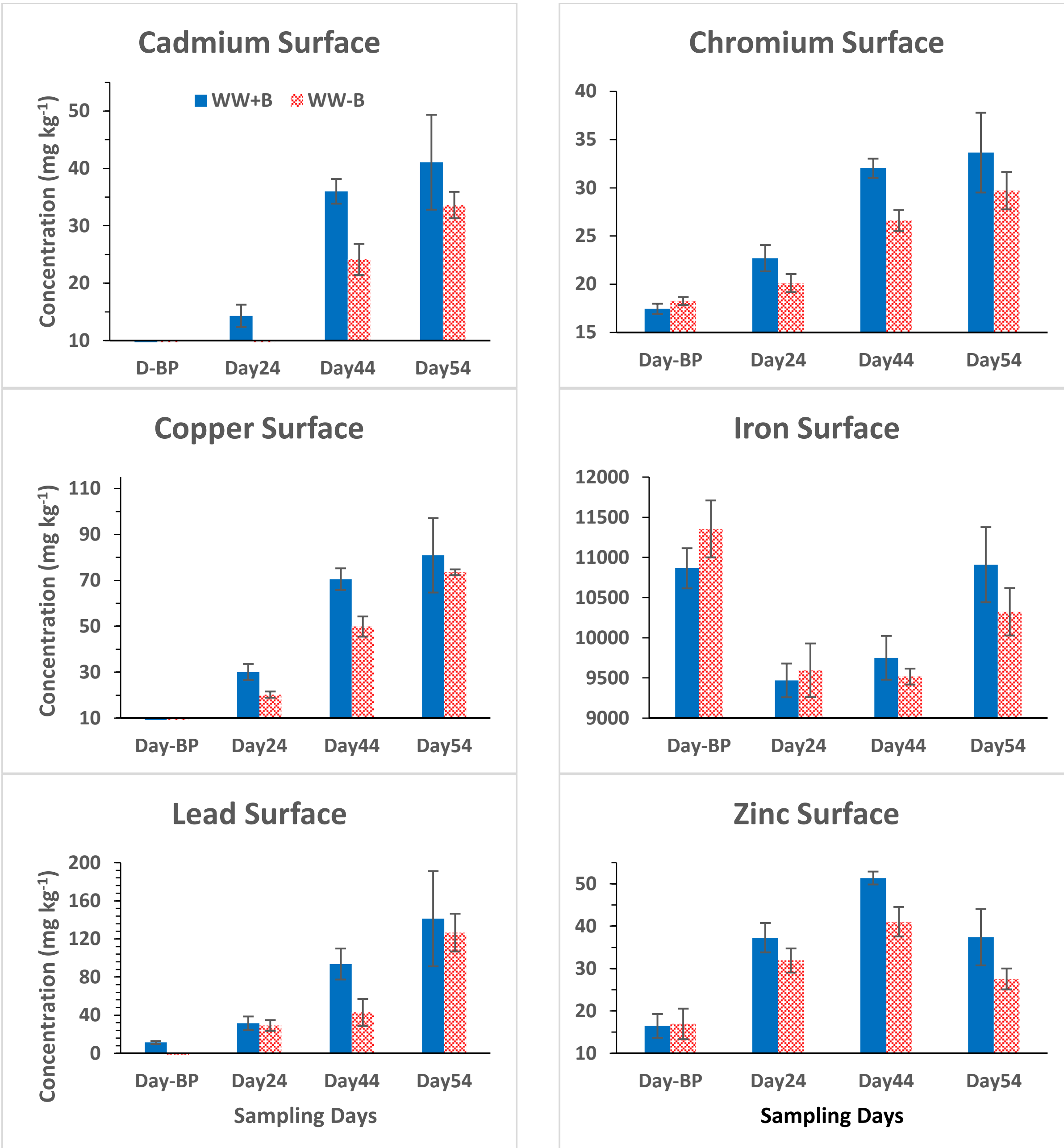

Figure 4-4. Concentrations of Cd, Cr, Pb, Cu, Fe and Zn in the soil surface irrigated with wastewater. Day-BP that signifies the day before planting. The error bar represents standard error of three replicates.

Although statistically not significant ($p>0.05$), $[Cd]_{soil}$ was numerically higher in WW+B (vs. WW-B) throughout the season (Figure 4.4). Given that $[Kd]^{Cd}_{WW+B} \geq 8 \text{ X } [Kd]^{Cd}_{WW\text{-}B}$, more adsorption of Cd in WW+B treatment as compared to WW-B was expected. Moreover, it was noticed that the addition of biochar to the soil increased the soil's pH (Figure 4.3), which resulted in 84% (i.e., from 5.68 to 10.42 cmol (+) kg^{-1}) increase in the soil's CEC (Table 4.5). Although not conclusive, it appears that biochar tends to increase Cd sorption, therefore, further investigation is warranted.

Chromium was detected in both treatments (WW-B and WW+B) for all the events, including the background (day-BP; Figure 4.4). Since Cr is ubiquitous, its presence on day-BP is not unusual (Kabata-Pendias and Pendias, 1984). In the WW-B treatment, the concentration of $[\mathrm{Cr}]_{\mathrm{Soil}}^{\mathrm{Surf}}$ increased gradually from 18.3 mg kg^{-1} on day-BP to 29.7 mg kg^{-1} on day 54, while the concentration of $[\mathrm{Cr}]_{\mathrm{Soil}}^{\mathrm{Surf}}$ for the WW+B treatment rose from 17.4 mg kg^{-1} on day-BP and peaked at 33.6 mg kg^{-1} on day 54. This increase in $[\mathrm{Cr}]_{\mathrm{Soil}}^{\mathrm{Surf}}$, for the entire season, resulting from wastewater irrigation corroborates the findings of Liang et al. (2014), who reported the accumulation of Cr in soil following contaminated river water irrigation. Although the Kd suggests that Cr would be less mobile in WW-B compared to WW+B, possibly due to competition with other heavy metals; however, there was no significant difference ($p > 0.05$) in both treatments. It appears that the sorption behavior of Cr was affected by the additional presence of a cultivated crop, such as spinach, in the rhizosphere (Semhi et al., 2012). Overall, $[\mathrm{Cr}]_{\mathrm{Soil}}^{\mathrm{Surf}} \gg 1.4$ mg kg^{-1} (CCME permissible limit in agricultural soil), which implies that wastewater irrigation is a concern.

Apart from day-BP, $[\mathrm{Cu}]_{\mathrm{soil}}$ was detected in both treatments (WW-B and WW+B) for the entire season (Figure 4.4). The concentration of $[\mathrm{Cu}]_{\mathrm{Soil}}^{\mathrm{Surf}}$ in WW-B rose from 20.2 mg kg^{-1} (day 24) to a maximum of 73.5 mg kg^{-1} (day 54), while the concentration of $[\mathrm{Cu}]_{\mathrm{Soil}}^{\mathrm{Surf}}$ for the WW+B treatment increased from 30 mg kg^{-1} (day 24) to a maximum of 80.9 mg kg^{-1} (day 54). With more than twice the significant increase ($p < 0.05$) in $[\mathrm{Cu}]_{\mathrm{Soil}}^{\mathrm{Surf}}$ for the entire season in both treatments, it was clear that Cu accumulated in the soil surface, which is not unusual (Sadiq Butt et al., 2005). Guided by the Kd value (Table 4.4), it was expected that Cu should bind more in the WW+B (vs. WW-B); however, the differences in Cu concentrations between treatments was not significant ($p > 0.05$). It appears that the plant root in the rhizosphere produced compounds that affected the sorption behavior of Cu for both treatments. Degryse et al. (2008) observed that Cu's mobility was affected by organic ligands (i.e., exudates) released by spinach root. Overall, $[\mathrm{Cu}]_{\mathrm{Soil}}^{\mathrm{Surf}}$ > CCME permissible limits for agricultural soils (i.e., 63 mg kg^{-1}) is a concern.

The concentration of $[\mathrm{Fe}]_{\mathrm{Soil}}^{\mathrm{Surf}}$ in WW-B treatment after the first irrigation with wastewater (i.e., Day24) was 9594 mg kg^{-1}, while at the end of the season (Day54), it was 10323 mg kg^{-1}. Likewise, in the WW+B treatment, the concentration of $[\mathrm{Fe}]_{\mathrm{Soil}}^{\mathrm{Surf}}$ on Day24 was 9469 mg kg^{-1}, while on Day54 it was 10909 mg kg^{-1}. Although $[\mathrm{Fe}]_{\mathrm{Soil}}^{\mathrm{Surf}}$ in the soil before wastewater irrigation (Day-BP) was high as compared to $[\mathrm{Fe}]_{\mathrm{Soil}}^{\mathrm{Surf}}$ after irrigation (i.e., Days 24, 44 and 54; Fig. 4.4), it could have been redistributed during spinach planting and biochar mixing when the soil was disturbed; biochar was mixed in the top 0.1 m. Similar observations were made in our previous experiment (Nzediegwu et al., 2019) where the soil was disturbed during potato planting and biochar mixing. The high concentration of Fe measured in the soil is not uncommon because Fe is ubiquitous in the environment. Apart from aluminum, Fe is the most common heavy metal in the earth's crust (Fontecave and Pierre, 1993), including soil. Soil amendment with biochar had no significant effect ($p > 0.05$) in immobilizing Fe as compared to WW-B; the Kd value also suggests the same.

The concentration of $[\mathrm{Pb}]_{\mathrm{Soil}}^{\mathrm{Surf}}$, in WW-B treatment, increased gradually from below the detection limit on Day-BP to 126.69 mg kg^{-1} at the end of the season (Day54). It indicates that Pb accumulated in the soil following wastewater irrigation, which corroborates the observations of

Sadiq Butt et al. (2005), who reported that Pb from wastewater irrigation accumulated in the soil surface. Similarly, the concentration of $[\mathrm{Pb}]_{\mathrm{Soil}}^{\mathrm{Surf}}$, in WW+B treatment, increased from below the detection limit on Day-BP to 141.19 mg kg^{-1} at the end of the season. This suggests that mixing the soil with biochar (WW+B) showed no significant effect on Pb immobilization as compared to the control (WW-B). Overall, the concentrations of $[\mathrm{Pb}]_{\mathrm{Soil}}^{\mathrm{Surf}}$ was greater than the permissible limits for agricultural soils (70 mg kg^{-1}; CCME). Thus, being one of the most toxic heavy metals (Antonious and Snyder, 2007b), Pb poses a health risk as it could be taken up by plants, thereby, entering the human food chain; plantain peel biochar may not be effective in reducing Pb risk.

Zinc was detected in the soil before irrigation (Day-BP), which is not unusual as it could have come from parent materials (Kabata-Pendias and Pendias, 1984). Having received wastewater for the entire season (Day24 to Day54), the concentration of $[\mathrm{Zn}]_{\mathrm{Soil}}^{\mathrm{Surf}}$, in WW-B and WW+B, stabilized at 27.56 mg kg^{-1} and 41.4 mg kg^{-1}, respectively. Kumar Sharma et al. (2007) measured similar Zn concentrations in soils that received wastewater. Towards the end of the growing season (Day44 to Day54) $[\mathrm{Zn}]_{\mathrm{Soil}}^{\mathrm{Surf}}$ dropped. Given the chemical similarities (Group IIB) that exist between Cd and Zn (Narwal et al., 1993), coupled with the fact that this happened when spinach matured leaves were just harvested (suggesting the need for more nutrients), it could be that $[\mathrm{Cd}]_{\mathrm{Soil}}^{\mathrm{Surf}}$ presence (Figure 4.4; Day44 to Day54) enhanced the availability of Zn for plant uptake (McKenna et al., 1993). Therefore, Zn [a micronutrient (Rout and Das, 2009)] might have been aggressively taken up by spinach leaves to meet a metabolic need (Frossard et al., 2000). Although not significant ($p > 0.05$), the numerical value of $[\mathrm{Zn}]_{\mathrm{Soil}}^{\mathrm{Surf}}$ was higher in WW+B as compared to WW-B, signifying that soil amended with biochar could potentially bind more Zn compared to non-amended soil. Moreover, $[\mathrm{Kd}]_{\mathrm{WW+B}}^{\mathrm{Zn}} \geq 5 \mathrm{X} [\mathrm{Kd}]_{\mathrm{WW\text{-}B}}^{\mathrm{Zn}}$ suggests the same.

Below 0.1 m, Cd, Cr, Cu, Pb and Zn were not detected. This is not unusual as it has been reported that heavy metals, such as Cu, were not mobile in the soil profile (Knechtenhofer et al., 2003); nevertheless, they could accumulate in the root zone of shallow rooted crops like spinach. It is important to mention that in our previous study with potatoes (Nzediegwu et al., 2019), Pb and Zn were detected at 0.1 m below. Since potatoes are a tuber crop, causing soil to loosen, it could be that Pb as well as Zn moved through preferential flow (Kim et al., 2008). Moreover, Pb and Zn may have moved because more irrigation water (i.e., more concentration of Pb and Zn) was applied for potato (vs. spinach) cultivation. Therefore, the type of crop under wastewater irrigation could influence the transport of heavy metals in soil, either by affecting the soil physical structure or by the water requirement. Transport of Cd, Cr, Cu, Pb and Zn, in the soil profile for one season of spinach cultivation under wastewater irrigation might not be a concern; however, the concern might increase with wastewater irrigation given the growing demands for spinach (Kamruzzaman et al., 2016)—requiring year round production. Although not significant ($p > 0.05$), transport of Fe in the soil profile was revealed by $[\mathrm{Fe}]_{\mathrm{Soil}}^{0.1}$ of 8992.29±368.90 and 9775.95±520.41 mg kg^{-1} measured in WW-B and WW+B, respectively, at the end of the season (Day54). In our previous experiment with potatoes, Fe mobility in soil was observed as well; thus, it is not uncommon that Fe moved to lower depths. High concentration of Fe in wastewater as compared to the other heavy metals (Table 4.3) could have resulted in the transport. Such transport is a concern given that most vegetables, including spinach, have their roots distributed in this zone.

Although given the results, it was not conclusive that biochar amendment increased adsorption of different wastewater borne co-existing heavy metals in field conditions, but it indicated such a tendency, especially for Cd, Cu and Zn. It may be possible that biochar amendment may reduce the transport of heavy metals if the amount of biochar is increased or if the biochar is activated before amendment.

4.4.5 Heavy metals in spinach parts

Heavy metal (Cd, Cr, Cu, Fe, Pb and Zn) concentrations in root, stem, and leaf after first and second harvests are summarized in Table 4.6.

Table 4.6. Concentrations (mg kg^{-1}) of heavy metals in the roots, stems, and leaves (harvest 1 and 2) of spinach irrigated with both wastewater and freshwater

Spinach Parts	Treatments	Cd	Cr	Cu	Fe	Pb	Zn
Root	FW-B	0.8±0.27b	0.7±0.14b	10.7±1.74b	198.4±24.96a	1.0±0.14b	49.3±17.52a
	WW+B	13.3±1.24a	5.5±2.25a	21.0±0.89a	311.9±12.83a	13.9±5.03a	36.8±1.79a
	WW-B	11.4±2.90a	2.4±0.42ab	20.8±1.80a	343.0±93.16a	14.7±1.62a	29.8±1.77a
Stem	FW-B	1.8±0.03b	0.6±0.02b	4.3±0.06b	77.1±4.00b	0.4±0.04b	83.0±11.08a
	WW+B	8.0±1.28a	2.3±0.45a	8.9±1.36a	134.1±26.10b	12.3±3.19a	51.5±5.59b
	WW-B	9.8±0.32a	1.8±0.26ab	10.9±0.20a	211.1±20.10a	10.7±1.10a	83.7±8.72a
Harvest-1 Leaf	FW-B	1.3±0.05b	2.5±1.11a	9.1±0.70a	1043.3±523.69a	1.8±0.62b	79.9±4.64b
	WW+B	3.7±1.02ab	1.8±0.19a	9.6±0.61a	437.7±7.12a	7.0±1.64a	61.1±2.09b
	WW-B	6.2±0.04a	0.7±0.13a	12.1±1.71a	285.4±57.45a	2.6±0.40ab	133.4±19.86a
Harvest-2 Leaf	FW-B	1.8±0.14b	1.5±0.32b	14.2±0.51b	508.2±152.72a	2.3±0.33b	103.4±29.01a
	WW+B	20.1±4.52a	8.0±2.16a	36.6±6.38a	503.9±71.43a	71.6±16.95a	68.0±9.61a
	WW-B	20.0±4.15a	7.0±1.43a	33.9±5.64a	798.6±125.42a	51.8±15.64a	116.5±12.01a

The values are the mean ± standard error of 3 replicates; for each spinach tissue category, different letters signify significant difference among mean concentrations ($p \leq 0.05$). The permissible limits of the heavy metals in plant tissue (mg kg^{-1}) are: Cd (*0.3), Zn (50), Cu (10), Pb (*0.2), Fe (not available) and Cr (1.5). *signifies CODEX standard [Stan 193-1995;(Codex Alimentarius Commission, 1995)], while the others are from WHO (Nazir et al., 2015).

The concentrations of Cd, Cr, Cu, Fe, Pb and Zn in spinach roots in FW-B were 0.8, 0.7, 10.7, 198.4, 1.0 and 49.3 mg kg^{-1}, respectively. These concentrations are not unusual, given the high concentration of heavy metals, especially Zn and Fe, in the soil (Figure 4.4). Comparable concentrations of heavy metals (Cd, Cr, Fe, Pb and Zn) have been reported in the roots of spinach cultivated with freshwater in soil (Bahmanyar, 2008; Pathak et al., 2013). For WW-B, the corresponding concentrations were 11.4, 2.4, 20.8, 343.0, 14.7 and 29.8 mg kg^{-1}, respectively. The concentration of Cd, Cu, and Pb were significantly higher in WW-B as compared to FW-B, which signifies that irrigation with wastewater significantly increased the uptake of these heavy metals in the root. Although statistically not significant ($p>0.05$), the concentrations of Cr and Fe in WW-B were also numerically higher; this trend indicates that wastewater irrigation may increase the uptake of these heavy metals also. This is of great concern, as it is likely that greater amounts of these heavy metals may translocate to the leaves, the edible part, when the concentrations are high in the root. Soil amendment with biochar (WW+B) showed no significant effect on the root's heavy metal uptake, which was expected, given that no effect of biochar was evident on the heavy metal concentrations in soil (Figure 4.4).

In FW-B, the concentrations of Cd, Cr, Cu, Fe, Pb and Zn in the stems were 1.8, 0.6, 4.3, 77.1, 0.4 and 83.0 mg kg^{-1}, respectively. It indicates that all of these heavy metals could translocate to the stem. The corresponding concentrations in WW-B were 9.8, 1.8, 10.9, 211.1, 10.7 and 83.7 mg kg^{-1}, respectively, indicating $\geq 50\%$ increase for all the heavy metals. The increase in concentration of Cd, Cu, Fe and Pb was significantly higher as compared to the freshwater irrigated stem (FW-B). Although the stem is not the edible part, increased heavy metal levels in this part of the plant could be a great health concern since it serves as a water and nutrient transport channel, carrying nutrients, including heavy metals from the root to the leaves (Sperry, 1995). There was no difference in concentration of Zn between FW-B and WW-B (Table 4.6). Thus, it appears that naturally occurring Zn could translocate to spinach, and Zn in wastewater may not affect the uptake when a certain level of Zn is present in the soil. The concentrations of Cd, Cr, Cu, Fe, Pb and Zn in stems in WW+B were 8.0, 2.3, 8.9, 134.1, 12.3 and 51.5 mg kg^{-1}, respectively. It is evident that amendment with biochar significantly reduced ($p < 0.05$) the transport of Fe and Zn to stems as compared to that without biochar amendment, which could reduce the amount of Fe and Zn that would get to the leaves.

Spinach is grown for its edible leaves, which are harvested several times in a growing season. Overall, higher concentrations of all heavy metals were detected in the leaves (harvest-2) as compared to the other parts (stem and root; Table 4.6). Pathak et al. (2013) made similar observations in spinach irrigated with paper mill waste. Since this was true for freshwater and wastewater irrigated treatments, it could be that spinach, which is a leafy vegetable, stores more nutrients, including heavy metals, in the leaves as compared to the roots and stems (Frossard et al., 2000). For the FW-B treatment, comparable concentrations of the heavy metals were measured between harvests, except for Fe, which had a greater variation in harvest-1 (i.e., 50% RSD). The heavy metals in the leaves under FW-B possibly came from the soil even though the soil`s Cd, Cu and Pb were below detection limits of 0.05 mg L^{-1} (Figure 4.4). The concentrations of all of the heavy metals in the spinach leaves of FW-B treatment were higher than the CODEX and WHO permissible limits (Codex Alimentarius Commission, 1995; Nazir et al., 2015). This is a serious health concern given that spinach is cultivated under freshwater as well as wastewater irrigation in different parts of the world. After the first harvest (Table 4.6), all of the heavy metals were detected in the leaves (apparently comparable for both freshwater and wastewater); with further irrigation, the heavy metal concentrations increased considerably in leaves under wastewater (WW-B), and not under freshwater (FW-B) irrigation till the end of the season (i.e., harvest-2), except for Zn that showed a slight decrease. In WW-B, the concentrations of Cd, Cr, Cu, and Pb in the leaves at the second harvest were 20.0, 7.0, 33.9, and 51.8 mg kg^{-1}, respectively. These concentrations were significantly higher ($p < 0.05$) than in FW-B (Table 4.6). The concentrations of Fe and Zn in WW-B were 798.6 and 116.5 mg kg^{-1}, respectively; these were numerically higher than FW-B, although statistically not different. Overall, wastewater irrigation resulted in much higher heavy metal loading in the leaves than freshwater irrigation. As expected, treatments of plants with high heavy metals in their roots resulted in high heavy metals in their leaves (particularly harvest-2; Table 4.6). Several studies reported an increase in the leaves' heavy metals due to wastewater irrigation (Gupta et al., 2012; Rattan et al., 2005), and our results corroborate this for Cd, Cr, Cu, Pb and Zn. Apparently, given similar soil heavy metal concentrations in both treatments (WW-B and WW+B; Figure 4.4), biochar amended treatment (WW+B) showed no significant effect ($p > 0.05$) as compared to the wastewater control (WW-B). It could be that the biochar surface was not fully oxidized within the short growing season of spinach. The biochar used in this experiment was fresh and it may require more time to be activated in the soil (Lehmann, 2007). Although this field study (one season) indicates that biochar was not as effective as a sorbent, the Kd values of the heavy metals, particularly Cd and Zn, the pH, the morphology (Figure 4.2) and the fixed carbon content (Table 4.1) suggest that the biochar could be useful in reducing heavy metal bioavailability, especially as depicted by the >41% numerically lower Zn concentration in leaves of WW+B as compared to WW-B (Table 4.6). It appears that biochar amendment would reduce Zn in spinach leaves where irrigation water contains high concentrations of Zn. Moreover, assuming daily vegetable consumption (including spinach) of 200 g per person (Li et al., 2006), Zn in spinach grown in soil amended with biochar would be below the provisional maximum tolerable daily intake limit for adults (20 mg) prescribed by WHO (World Health Organization (WHO) et al., 1996).

4.5 Conclusions

This study investigated the effect of pyrolized plantain peel biochar on the bioavailability of six heavy metals (Cd, Cr, Cu, Fe, Pb and Zn) to spinach irrigated with wastewater and freshwater.

Apart from Fe that moved down the soil profile, the other heavy metals were only detected in the top soil. Although there was no treatment effect in the soil, all the heavy metals subsequently translocated to different parts of the spinach (leaves, stem and root) with the leaves accumulating more heavy metals compared to the stem and root. The alkaline biochar used was dominated by micropores with high fixed carbon content. The Kd values suggest that biochar is a good adsorbent for heavy metals, particularly Cd and Zn. Moreover, improved soil properties, such as CEC and pH, were noticed in soils with biochar amendment as compared to no biochar amendment. Overall, lower concentrations of the heavy metals were detected in the freshwater irrigated spinach as compared to their wastewater counterparts, which had much higher concentrations in harvest-2 (vs. harvest-1). Biochar amendment resulted in 42% reduction of Zn in spinach leaves, thus, keeping it below WHO provisional maximum tolerable daily intake limit for adults as compared to the no biochar amendment. For the other heavy metals (Cd, Cu, Cr, Fe, and Pb), there was no noticeable impact of biochar, possibly due to competition with other compounds in the soil solution or due to alterations imposed by the possible presence of root exudates in the rhizosphere, and possibly, plantain peel biochar may not be effective. Nevertheless, further investigations are warranted to confirm these findings.

4.6 Acknowledgements

The authors express gratitude to NSERC, Tertiary Education Trust Fund, Nigeria and the India-Canada Centre for Innovative Multidisciplinary Partnerships to Accelerate Community Transformation and Sustainability for providing fund for the research. The authors would also like to thank Sedigheh Zarayan, our Lab Supervisor.

Connecting Text to Chapter 5

Long-term effectiveness is an important characteristic to consider in recommending a particular sorbent-soil amendment. Having documented that biochar immobilized heavy metals in soil and reduced their subsequent translocation to potato parts in 2015 (as documented in chapter 3), it was not known whether the biochar would remain active and effective after one-year of being in the soil.

Chapter 5, Role of Aging of Biochar in Reducing Metal Uptake by Potato Plants Irrigated with Wastewater, documents the effect of biochar on soil`s chemical properties in the second year, thereby immobilizing effect of additional heavy metals in the soil. The effect on the uptake of the heavy metals in potato parts is also reported. Furthermore, sorption and desorption characteristics of the soil and soil-biochar mix, through batch equilibrium study, is also reported.

The manuscript, submitted to Water Research, is co-authored by Dr. Shiv Prasher, my supervisor, Dr. Eman ElSayed, a post-doctoral fellow in the department, Mr. Jaskaran Dhiman, a PhD scholar in the department, Mr. Ali Mawof, a PhD scholar in the department and Dr. Ramanbhai Patel, a research associate in the department. To ensure consistency with the thesis format, the original draft has been modified by listing the cited work in the reference section (Chapter 9).

Chapter 5: Role of Aging Biochar in Reducing Metal Uptake by Potato Plants Irrigated with Wastewater

5.1 Abstract

The potential of biochar to enhance the safe use of wastewater, especially untreated, in the second year of its application was tested. Biochar amendment was applied in 2015 and potatoes were grown in lysimeters with synthetic wastewater irrigation. In 2016, potatoes were again grown in the same lysimeters by applying wastewater irrigation, and the effectiveness of biochar after ageing was evaluated. The other treatments included soil without biochar amendment that was irrigated with wastewater or freshwater. The analysis of the soil showed that all the heavy metals accumulated in the top soil, while only Zn, Pb and Fe moved to a depth of 0.1 m. Mixing of soil with biochar significantly increased its pH and CEC, thereby, immobilizing all the heavy metals, except Fe, as compared to the control with no biochar. The heavy metals translocated to all potato parts (flesh, peel, root, stem and leaves). While the concentration of heavy metals were relatively low in the potato parts under freshwater (flesh and peel), concentrations in the wastewater irrigated potatoes (flesh, peel, root, stem and leaves) were relatively high. However, biochar amendment, after the second year of being mixed in the soil, significantly reduced ($p<0.05$) the concentration of Cd, Cu, Cr, Pb and Zn in the edible flesh. The results showed that biochar was effective in the second year of its amendment to soil in immobilizing wastewater-laden heavy metals.

Keywords: lysimeters; biochar; wastewater; heavy metals; potatoes; plantain peel

5.2 Introduction

Biochar, a solid byproduct of organic waste(s) pyrolysis, may reduce the uptake of wastewater-borne heavy metals—known to be toxic when found in the human food chain (Chen, 2012)—in crops grown on contaminated soils. The availability of soil heavy metals to the plant may be explained by several mechanisms including surface adsorption due to the presence of net negative charges on the biochar surface (Dang et al., 2018). Other supporting mechanisms are precipitation, cation exchange and complexation (Zama et al., 2017). With adsorption being a surface phenomenon and biochar (like other sorbents) having a constant specific surface area (Rees et al., 2014), it is likely that biochar`s active surface might become saturated, thereby, making biochar sorption effectiveness temporal. With increasing interest in the use of biochar for heavy metal immobilization, field studies are imperative to evaluate whether biochar can act as a sorbent for heavy metal immobilization in the second year after its amendment to soil.

Several studies on heavy metal immobilization by biochar have been conducted on a short-term basis of less than one year (Beesley and Marmiroli, 2011; Dang et al., 2018), while only a few others have been conducted for a relatively long term of more than one year (Cui et al., 2016; Cui et al., 2011). In a one-week study on the bioavailability of heavy metals (Pb, Cu, Zn and Ni) in a biochar-amended soil, Rees et al. (2014) found that the increased soil pH caused a significant reduction in availability of all heavy metals as compared to the soil without biochar. In an eight-week incubation study, Cd and Zn were immobilized in the presence of hardwood biochar-amended soil, which further led to their reduction in the leachate (Beesley and Marmiroli, 2011), whereas, in a 90-day pot study, soil amended with rice straw derived biochar immobilized Pb, Cd and Zn due to the increased pH (Dang et al., 2018). On the other hand, in a 2-year field study, wheat straw biochar applied to Cd-contaminated soil reduced the uptake of Cd in rice grain by 45% and 62% in the first and second year, respectively (Cui et al., 2011). In a 5-year field study, Cui et al. (2016) noticed that the ability of biochar to immobilize Cd and Cu in a wastewater contaminated soil decreased with time; their bioavailability were suppressed in the first three years of amendment, whereas there was no difference in the last two years relative to a non-amended control. Given such variability in biochar's performance with time after amendment, crop type and feedstock, it is imperative to undertake further studies to evaluate a given biochar for its field application.

Moreover, this is even more important when soil receives wastewater, which serves as a sustainable alternative to depleting freshwater (Yang et al., 2006) and contains "microbial-loving" carbon compounds, such as starch and peptone (Nopens et al., 2001) that could further cause metabolic reactions in the rhizosphere as these carbon compounds degrade. Studies meant to focus on the aforementioned scenario only looked at remediation (with biochar) of sites that have been contaminated due to wastewater discharge on land or due to long-term wastewater irrigation (Schweiker et al., 2014; Wagner and Kaupenjohann, 2014; Wagner and Kaupenjohann, 2015). Thus, there is a lack of information on the interaction of biochar amendment on soil and wastewater irrigation for multiple seasons.

Therefore, this study was undertaken to ascertain the effect of biochar on the transport of heavy metals in the second year of its application in order to understand the stability and effect of plantain peel biochar. Furthermore, transport of heavy metals (Cd, Cr, Cu, Fe, Pb and Zn) in soil and their translocation to potato tissues (peel, flesh, root, stem and leaves) was also studied.

5.3 Materials and methods

5.3.1 Biochar characterization

The plantain peel biochar has a pH (in water) and BET (Brunauer Emmett Teller) surface area of 10.3 and 1.946 m^2 g^{-1}, respectively. The morphology was determined using scanning electron microscopy (Hitachi TM3000). Other relevant information of the biochar is given in Table 5.1.

Table 5.1. Properties of gasified plantain peel biochar on dry weight basis

Proximate Analysis	%dry weight	Method	Ultimate Analysis	%dry weight	Method
Moisture TGA	9.88	ASTM 7582	**Carbon**	18.1	ASTM D5373
Ash content	77.45	ASTM 7582	**Hydrogen**	0.48	ASTM D5373
Volatile matter	18.09	ISO 562	**Nitrogen**	0.6	ASTM D5373
Fixed Carbon	4.02	ASTM 7582	**Total Sulphur**	<0.05	ASTM D4239
			Oxygen	3.37	By Difference

TGA-thermogravimetric analysis

5.3.2 Batch equilibrium study

A batch equilibrium study was conducted following the procedures described by Sarmah et al. (2010). Soil samples (see Table 5.2 for physicochemical properties), collected from lysimeters, filled with soil collected from the Macdonald Campus Farm of McGill University (45º24’48.48” N, 73º56’28.06” W), were air-dried for two days and homogenized by passing through a 2-mm sieve. Reagent grade salts of the heavy metals (Table 5.3) were used to prepare the stock solution in 4% heavy metal grade nitric acid. Five cocktails of concentrations, 0.1, 0.2, 0.3, 0.4, and 0.5 mM, of all the heavy metals (Cd, Cr, Cu, Fe, Pb and Zn) were prepared in 0.001 M $NaNO_3$ solution; the $NaNO_3$ solution helped to correct the ionic strength (Jiang et al., 2012a). The two treatments, replicated three times, were: non-amended soil (WW-B) and biochar-amended soil (WW+B).

5.3.2.1 Sorption

Two grams of the air-dried soil as well as 0.02 g of the biochar (1% w/w), weighed in a 50-mL falcon tube, were mixed with 30 mL of the cocktail solution. The falcon tubes were capped, vortexed (1800 rpm) for 30 sec, shaken (205 rpm) for 24 h, and then centrifuged (3500 rpm) for 10 min. The liquid portion was carefully decanted and then 15 mL aliquot was used for heavy metal analysis on inductively coupled plasma optical emission spectrometry (ICP-OES, Varian, Vista-MPX CCD Simultaneous). The pH of the remaining liquid portion was measured.

5.3.2.2 Desorption

Desorption was performed using the solid portion of the sorbent from the sorption study. For this, 30 mL double deionized water was added and the steps, from vortexing described in the sorption test, were repeated.

The sorption data were presented as coefficient of distribution, Kd determined as $\text{mass adsorbed}/\text{equilibrum concentration}$ (Gomes et al., 2001; Park et al., 2016), while the overall mass adsorbed (MA) and mass desorbed (MD) were also calculated using molar mass weighted average method, given as:

$$\mathrm{MA} = \frac{1}{\mathrm{MW_T}} \sum_{i=1}^{n} (\mathrm{MA_i} * \mathrm{MW_i}) \qquad (5.1)$$

$$\mathrm{MD} = \frac{1}{\mathrm{MW_T}} \sum_{i=1}^{n} (\mathrm{MD_i} * \mathrm{MW_i}) \qquad (5.2)$$

Where, i (from 1 to n) is the number of heavy metal in the sorption solution and MW_T is the sum of molar masses of the heavy metals.

Table 5.2. Soil physicochemical properties

Soil Properties	**mg kg^{-1}**	**Soil Properties**	
P	215.30±40.43	**Sand (%)**	92.2
K	107.33±13.13	**Silt (%)**	4.3
NO_3-N	4.57±0.46	**Clay (%)**	3.5
Ca	912.44±79.70	**pH**	5.5
Mg	103.27±7.29	**Organic matter (%)**	2.4±0.15
Al	1164.14±12.40	**Hydraulic conductivity (m day^{-1})**	1.67±0.45
Cd	<LOD	**ZPC**	3.4
Cr	17.86±0.38		
Cu	<LOD		
Fe	11109.64±238.68		
Pb	<LOD		
Zn	16.70±2.28		

LOD: limit of detection; ZPC: zero point of charge; the heavy metals Cd, Cr, Cu, Fe, Pb and Zn were determined following hot acid extraction (Kargar et al., 2013) and quantified by ICP-OES. The LOD was 50 µg L^{-1} (15.6 mg kg^{-1}) for all the metals. P, K, Ca, Mg, and Al were determined following Mehlich III extraction (Mehlich, 1984), while N was determined following 2.0 M KCl method (Carter and Gregorich, 2008). Other soil properties were adapted from a previous study (ElSayed et al., 2013). Where applicable, the values are the mean ± standard error of 3 replicates.

5.3.3 Field study

In 2015, a sandy soil (Table 5.2) was packed in nine field lysimeters (0.45 m diameter by 1.0 m height) up to 0.95 m allowing 0.05 m clearance from the top. The PVC lysimeter had four equally spaced holes, drilled at depth 0.15, 0.35 and 0.65 m, for soil sampling. Arranged in a completely randomised design, the three treatments were: wastewater with biochar (WW+B), wastewater without biochar (WW-B) and freshwater without biochar (FW-B). For the three lysimeters under WW+B treatment, biochar (1% w/w) was mixed in the top 0.1 m of the soil. In the first year, 2015, following the planting and emergence of potatoes, each lysimeter under WW+B and WW-B received a total of 92 L of synthesized wastewater (11.5 L every 10 days for 8 times in each lysimeter), prepared following the recipe in Table 5.3, and lysimeters under FW-B received the same amount of fresh water. At maturity, the potatoes were harvested, and the lysimeters were protected from rain water and snow over the winter period to prevent dilution of the soil column until the next planting season in 2016. In year 2016, the soil was brought to field capacity and background soil samples were collected for heavy metal analysis. No new biochar was mixed with the soil in 2016 and the experiment was repeated along the same lines as in 2015.

Russet Burbank potato tubers, procured from Global Agri Services, Grand Falls, New Brunswick, Canada, after sprouting, were planted. As in 2015, fertilizer was applied following the recommendations from Idaho Extension (http://www.extension.uidaho.edu/nutrient). Potassium sulphate (0-0-60) was broadcasted at the rate of 280 kg K ha^{-1} (7.42 g/lysimeter) on the day of planting. Ammonium sulphate (21-0-0) was applied on the soil surface at an overall rate of 314 kg N ha^{-1} (23.8 g/lysimeter), half on the day of planting (Day 1), and a quarter each on Days 52 and 62, corresponding to the potatoes' bulking period, when nitrogen requirements are high (Ojala et al., 1990). Before irrigation with wastewater, from Day 14 to 41, tap water was applied every two days at a rate of 0.7 mm d^{-1} (118 mL/lysimeter) to ensure potato growth.

Made with tap water, stored overnight in an open container for dechlorinating, the synthetic wastewater employed in the present study combined basic synthetic wastewater ingredients (LaPara et al., 2006; Nopens et al., 2001) along with a number of additional contaminants (e.g., heavy metals, hormones, pharmaceuticals, surfactants and plasticizers) at levels based on the worst case reports from a number of countries in the developing world (Table 5.3). Soluble salts of the contaminant metals of interest (Sigma Aldrich, Oakville, Ontario), basic synthetic wastewater ingredients and other contaminants were mixed with the dechlorinated water, to prepare the synthetic wastewater.

Table 5.3. Recipes for synthetic wastewater.

Purpose	Substance/ Compounds	Country	Concentration (mg L^{-1})	Wastewater Recipe Source Contaminant reporter
	Basic synthetic wastewater ingredients			
C source	Sodium Acetate		**79.37**	Nopens et al. (2001)
	Milk powder		**116.19**	Nopens et al. (2001)
	Soy Oil		**29.02**	Nopens et al. (2001)
	Starch		**122**	Nopens et al. (2001)
	Yeast Extract		**52.24**	Nopens et al. (2001)
N Source	Ammonium Chloride		**12.75**	Nopens et al. (2001)
	Peptone		**17.41**	Nopens et al. (2001)
	Urea		**91.74**	Nopens et al. (2001)
P Source	Magnesium phosphate		**29.02**	Nopens et al. (2001)
Minerals	$CaCl_2$		**60**	LaPara et al. (2006)
	$MgCl_2$		**40**	LaPara et al. (2006)
	$NaHCO_3$		**100**	LaPara et al. (2006)
	K_3PO_4		**30**	LaPara et al. (2006)
	Additional contaminants levels based on worst case reports or need to exceed LOD			
Heavy Metals	Potassium dichromate (Cr)	India	**2**	Ahmad et al. (2011)
	Cadmium Nitrate (Cd)	India	**5**	Ahmad et al. (2011)
	Lead Nitrate (Pb)	India	**16**	Ahmad et al. (2011)
	Iron Sulphate (Fe)	India	**120**	Ahmad et al. (2011)
	Zinc Nitrate (Zn)	India	**3**	Ahmad et al. (2011)
	Copper Nitrate (Cu)	India	**8**	Ahmad et al. (2011)
Hormones	Estrone (E1)	Korea	8.15 (50) µg L^{-1}	Sim et al. (2011) — LOD
	Estradiol (E2)	Korea	0.634 (20) µg L^{-1}	Sim et al. (2011) — LOD
	Progesterone	China	0.90 (20) µg L^{-1}	Huang et al. (2009) — LOD
Pharmaceuticals	Oxytetracycline	China	19.5	Li et al. (2008)
	Ibuprofen	India	26.45 µg L^{-1}	Singh et al. (2014)
Surfactant	Triton X^{-}100 or alkylphenyl polyethoxylate	Morocco	30 µg L^{-1}	Aboulhassan et al. (2006)
Plasticizers	Bisphenol A		(50 µg L^{-1})	Based on LOD
	Bisphenol S		(50 µg L^{-1})	Based on LOD
	Bisphenol F		(50 µg L^{-1})	Based on LOD

Values in parentheses are actual concentrations used in the synthetic wastewater adjusted to simulate worst case scenario.

A total of eight irrigations were applied at 10-day intervals; for each irrigation event, 11.5 L of water was applied as irrigation to each lysimeter at the just ponding rate. Porous cheese cloth was placed on top of the lysimeter to maintain uniform distribution of irrigation water on the soil surface and prevent soil erosion.

Soil samples were collected, from depths 0.0 and 0.1 m, two days after irrigation and stored in -24ºC freezer for further analysis. At maturity (i.e., 120 days after planting), the potatoes were harvested, washed and separated into flesh and tuber. The roots, stems and leaves were collected as well.

5.3.4 Heavy metal analysis

A 0.16 g each of oven-dried potato tissues (flesh, peel, leaf, stem and root) as well as 0.16 g of oven-dried soil samples were digested separately in 2 mL of hot nitric acid in a block digester following the procedure by Kargar et al. (2013). The heavy metals in solution were quantified using ICP-MS and ICP-OES for plant tissue extract and soil sample extract, respectively. To ensure quality control, reference materials—NIST-1547 (for plant tissues) and SED 92-03 (for soil; Environment Canada)—and method blanks were added to all batches. Recovery percentage of the NIST-1547 were as follows: 92.7% for Cd, 79.2% for Cr, 104.0% for Cu, 83.4% for Fe, 100.7% for Pb, and 106.4% for Zn, while the recovery percentage of the SED 92-03 were as follows: 83.9% for Cr; 106.3% for Cu; 83.6% for Fe; 90.8% for Pb; and 82.4% for Zn; it was not available for Cd as concentrations in the reference material were below detection limit. Accordingly, the detection limits, converted to mg kg^{-1} from mg L^{-1} using same proportion as digested samples, were: Cd (0.033, 15.6), Cr (0.032, 15.6), Cu (0.071, 15.6), Fe (0.239, 156.5), Pb (0.051, 15.6) and Zn (0.382, 15.6).

5.3.5 Cation Exchange Capacity (CEC), Soil Organic Matter (SOM), pH and FTIR Spectra

The effective cation exchange capacity and the soil organic matter content of soil samples collected at the last sampling day was determined following $BaCl_2$ (Carter and Gregorich, 2008) and loss-on-ignition methods (Rowell and Coetzee, 2003), respectively. The pH of the soil samples was measured following dissolution in water (1:5; biochar:water) using an electrode type meter (Accumet AB 15) (Rayment and Higginson, 1992). Attenuated total reflectance Fourier transform infrared (FTIR) spectra was performed according to Uchimiya et al. (2010b). For this, air-dried soil samples, collected in 2015 (called before) and in 2016 (called after), were homogenized by passing through a 150-µm sieve (ASTM E11 NO. 100). With a resolution of 4 cm^{-1}, spectra were collected on triplicate samples (64 scans each) from 650 to 4500 cm^{-1}.

5.3.6 Data analysis

Repeated measures analysis of variance with time assigned as the repeated factor was performed for both the soil's heavy metal concentration. One-way ANOVA was performed on the potato tissue heavy metal, the soil's pH and soil's CEC data to test for mean differences. All analysis were performed in SAS-JMP® 13.0.0 (Copyright © 2016 SAS Institute Inc.).

5.4 Results and discussion

5.4.1 Surface morphology

The SEM of the biochar for two magnifications, presented in Figure 5.1, depicts the presence of pores which seem to be covered by tar-like materials and ash residues. Chowdhury et al. (2016) made similar observations in durian wood sawdust biochar. This may have resulted from a collapse of the carbon lattice during the biochar production and could further explain, visually, the high ash content (Table 5.1) of the biochar, which depicts the presence of inorganic minerals.

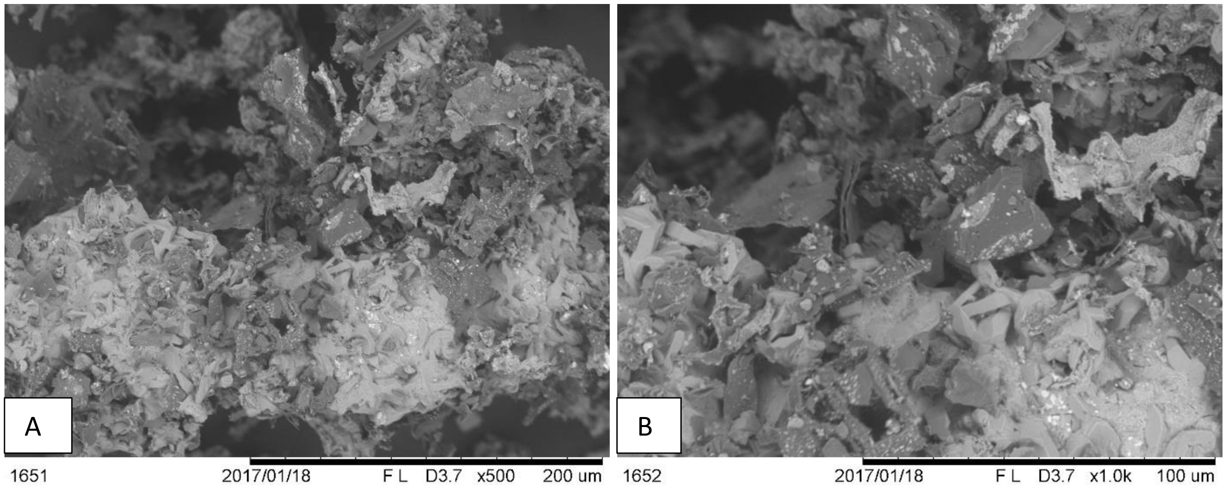

Figure 5-1. Scanning electron microscopy of gasified plantain peel biochar for two magnifications: x500 (A) and x1000 (B)

Such inorganic minerals (phosphate, sulphates and carbonates) are important in the adsorption process as they can form precipitate or exchange with heavy metal cations in soil solution (Uchimiya et al., 2010b; Zama et al., 2017), making them unavailable.

5.4.2 Effect on soil properties

In the first year (2015), biochar amendment increased the soil pH by 0.3 units. Although additional biochar was not amended in the second year (2016), the pH of the non-amended (WW-B) and biochar-amended (WW+B) soils were 4.70±0.37 and 5.43±0.11, respectively, showing a net increase in pH of 0.73. The pH is a very important chemical property of soil that controls the fate and transport of contaminants including heavy metals; generally heavy metals are displaced in soil solution as pH increases from 5.0 to 7.5 (Chen, 2012). With such an increase in pH, the biochar-amended soil could still be active for the immobilization of heavy metals even after one year of mixing in the soil. The top soil's CEC of WW+B was slightly higher (2.60±0.04 cmol(+) kg^{-1}) than WW-B (2.40±0.09 cmol(+) kg^{-1}); however, the difference was not significant ($p>0.05$), possibly due to the transport of cations with the irrigation water travelling through the soil column or due to mass flow of cations to plant roots. At 0.1 m depth, the CEC for WW-B and WW+B were 3.25±0.36 and 5.37±0.49 cmol(+) kg^{-1}, signifying that biochar's presence up to this depth significantly increased the number of cations at the soil's exchange complex as compared to soil without biochar (WW-B); higher CEC is important for heavy metal immobilization. Being an indication of stability in soil, the biochar maintained similar percent increase in CEC for both years; in 2015 it was 75%, whereas in 2016 it was 65%.

Figure 5.2 presents the FTIR spectra of the soil and soil-biochar mix collected in 2015 (WW-B Before and WW+B After) and 2016 (WW-B After and WW+B After).

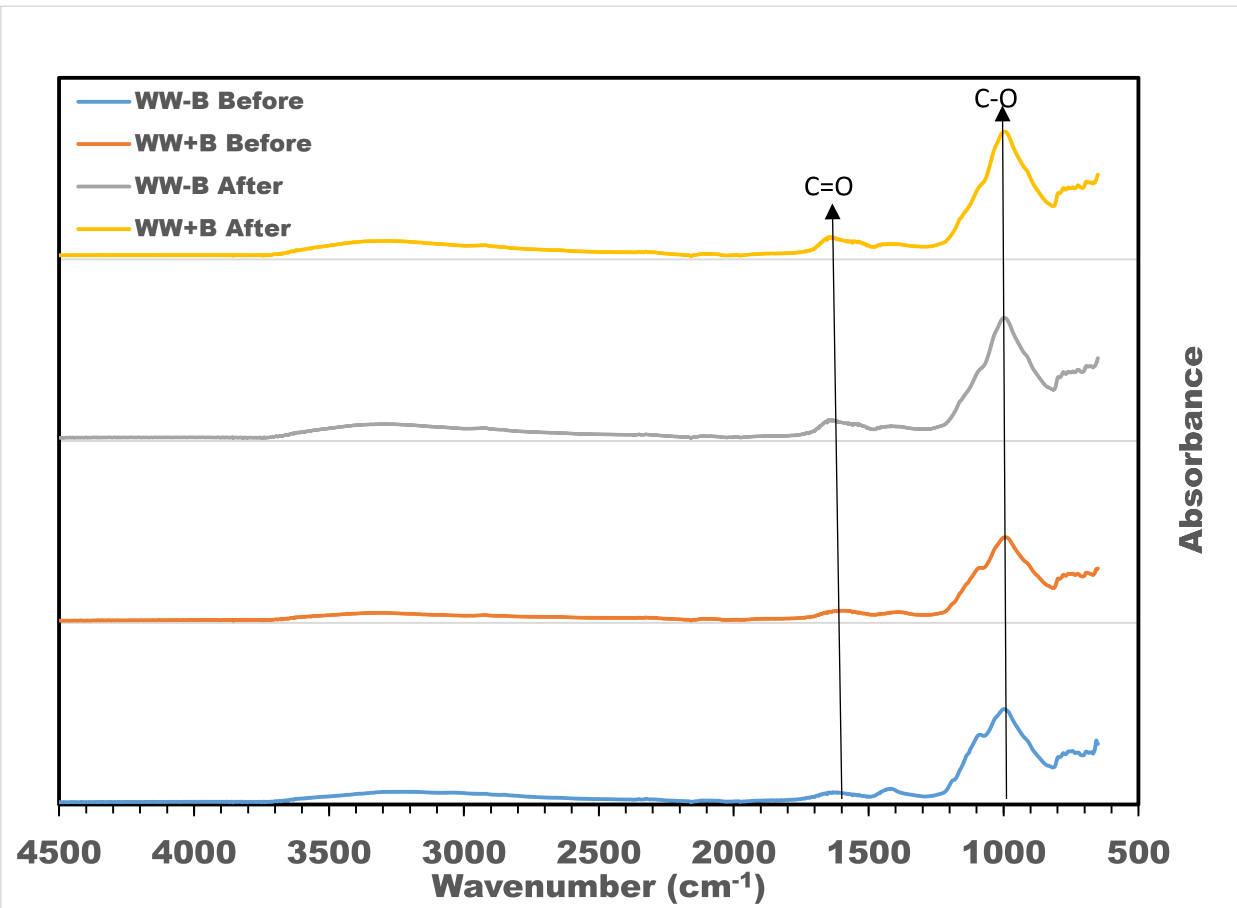

Figure 5.2. FTIR spectra of soil and soil-biochar mix collected in 2015 (before) and 2016 (after)

With identical shapes, two peaks respectively at wavenumbers 1000 and 1650 cm^{-1} corresponding to C-O and C=O stretching of carbonyl and carboxylic functional groups were identified (Uchimiya et al., 2012). Such oxygen-containing functional groups are responsible for binding and complexing heavy metals in soil (Uchimiya et al., 2011). After two years, no new functional groups were noticed; however, there was a relative increase in the existing functional groups. Such increase, especially in the biochar amended soil (WW+B), may have resulted from the formation of organo-metallic complexes, and could indicate biochar's stability in the soil (Haberhauer et al., 1998; Uchimiya et al., 2011). There was also a disappearance of the peaks initially at wavenumber 1070 cm^{-1} for WW-B and WW+B possibly due to saturation of the soil (Haberhauer et al., 1998) by heavy metals and other compounds present in the soil receiving wastewater.

5.4.3 Sorption and desorption

The batch equilibrium results at different initial concentrations, presented as a distribution coefficient, mass adsorbed, and mass desorbed are given in Table 5.4.

Table 5.4. Distribution coefficient, Kd (L kg^{-1}), overall mass adsorbed, and overall mass desorbed for each added heavy metal concentration for the different treatments

Treatment	Conc. Added (mM)	Cd	Cr	Cu	Fe	Pb	Zn	MA (mg kg^{-1})	MD (mg kg^{-1})
WW-B	0.1	12.2	526.7	86.6	1695.1	1515.4	8.1	157.4	2.7
	0.2	3.0	615.4	29.5	210.9	534.2	5.2	284.3	1.9
	0.3	**2.1**	**1744.3**	**16.6**	**127.2**	**596.8**	**1.3**	411.7	2.4
	0.4	1.6	842.2	12.4	55.9	197.3	1.1	516.9	3.7
	0.5	1.2	864.4	12.5	113.2	303.8	1.1	659.1	3.9
WW+B	0.1	2889.1	150.5	180.1	38.4	729.0	410.6	179.7	8.1
	0.2	530.8	522.8	1397.5	3352.0	10795.7	496.0	368.8	7.4
	0.3	**49.2**	**1218.4**	**2103.7**	**5045.8**	**47246.1**	**65.9**	528.2	8.3
	0.4	21.8	4383.8	1642.8	6620.2	14477.1	35.9	675.2	12.7
	0.5	18.8	7553.1	274.2	8279.0	18920.4	19.6	827.8	7.5

WW-B: non-amended soil; WW+B: biochar amended soil. Values in bold font were used for comparison; MA: mass adsorbed; MD: mass desorbed

Unable to fit any of the conventional isotherms—linear, Freundlich and Langmuir, the Kd values for both treatments (WW-B and WW+B) calculated across the concentrations were inconsistent apart from Cd, which showed a gradual decrease with increased concentration. Therefore, concentration dependent Kd approach was adopted (Gomes et al., 2001) to describe the heavy metal selectivity. Considering a concentration of 0.3 mM, corresponding to the environmental relevant concentration for most of the heavy metals, the selectivity order for WW-B and WW+B were: Cr > Pb > Fe > Cu > Cd > Zn and Pb > Fe > Cu > Cr > Zn > Cd, respectively. The presence of biochar in the soil evidently altered the affinity of the heavy metals and placed Pb in the foremost position and sent Cr back from the first to the third position. The effect of biochar on the pH of the sorption solution, which affects the selectivity of co-existing heavy metals (Yong and Phadungchewit, 1993), could explain such displacement. Overall, the mass of heavy metals adsorbed ranged from 157.4 to 659.1 mg kg^{-1} and 179.7 to 827.8 mg kg^{-1} for WW-B and WW+B, respectively. Irrespective of the treatment, the amount of heavy metals adsorbed increased with an increase in the added concentration signifying unsaturation of the sorption sites for the tested concentrations. At each initial concentration, mass adsorbed was higher in WW+B treatment as compared to the WW-B treatment. It implies that biochar-amended soil held more heavy metals as compared to the non-amended soil and depicts an improvement in the soil sorption potential because of the biochar`s presence. On the other hand, the amount of heavy metal desorbed ranged from 1.9 to 3.9 mg kg^{-1} and 7.4 to 12.7 mg kg^{-1} for WW-B and WW+B, respectively. Although, WW+B (vs. WW-B) appear to desorb more heavy metals, the overall mass retained (i.e., mass adsorbed minus mass desorbed) was higher in the biochar amended soil for each added concentration (Table 5.4). It is likely that adsorbents desorb more when they adsorb more; however, what matters should be the overall mass retained on the adsorbent, which accounts for the portion of the adsorbate (e.g. heavy metals) that will not be bioavailable. This is important in describing the fate and transport of heavy metals as it shows the strength of heavy metal bonding on the adsorbent.

5.4.4 Heavy metals in soil

Heavy metal concentrations in the top soil for the entire season including the background are presented in Figure 5.3, while the statistical analysis are presented in Table 5.5. There was an accumulation of all heavy metals in the top soil for the entire season with progressive wastewater irrigation. It signifies the effect of wastewater, which contains these heavy metals at relatively high concentrations (Table 5.3). The SOM at this depth, being 3.7±0.17% for WW-B treatment, could be responsible for the binding of the heavy metals (Kabata-Pendias, 2010). Biochar amendment showed no significant effect on the SOM, probably due to biochar's recalcitrant nature (Hernandez-Soriano et al., 2016). Cadmium and Zn were significantly higher ($p<0.05$; Table 5.5) in WW+B as compared to WW-B, indicating lesser mobility due to biochar`s presence. Although not significant, the concentrations of the other heavy metals (Cr, Cu, Fe and Pb) were numerically higher as well. This is not unusual, given the evidence from the batch equilibrium study where the biochar-amended soil demonstrated more sorption affinity for the heavy metals. Moreover, an increase in the soil's pH and the soil's CEC, noticed in the biochar-amended soil (vs. soil without biochar), suggested the same. By increasing the soil's pH, biochar amendment to soil has, on several occasions, immobilized heavy metals, such as Cd, Cu, Pb and Zn in soil (Dang et al., 2018; Rees et al., 2014). In another study (Bolan et al., 2003), Cr immobilization in a Cr-contaminated soil was associated with an increase in the soil's CEC due to the addition of organic amendments.

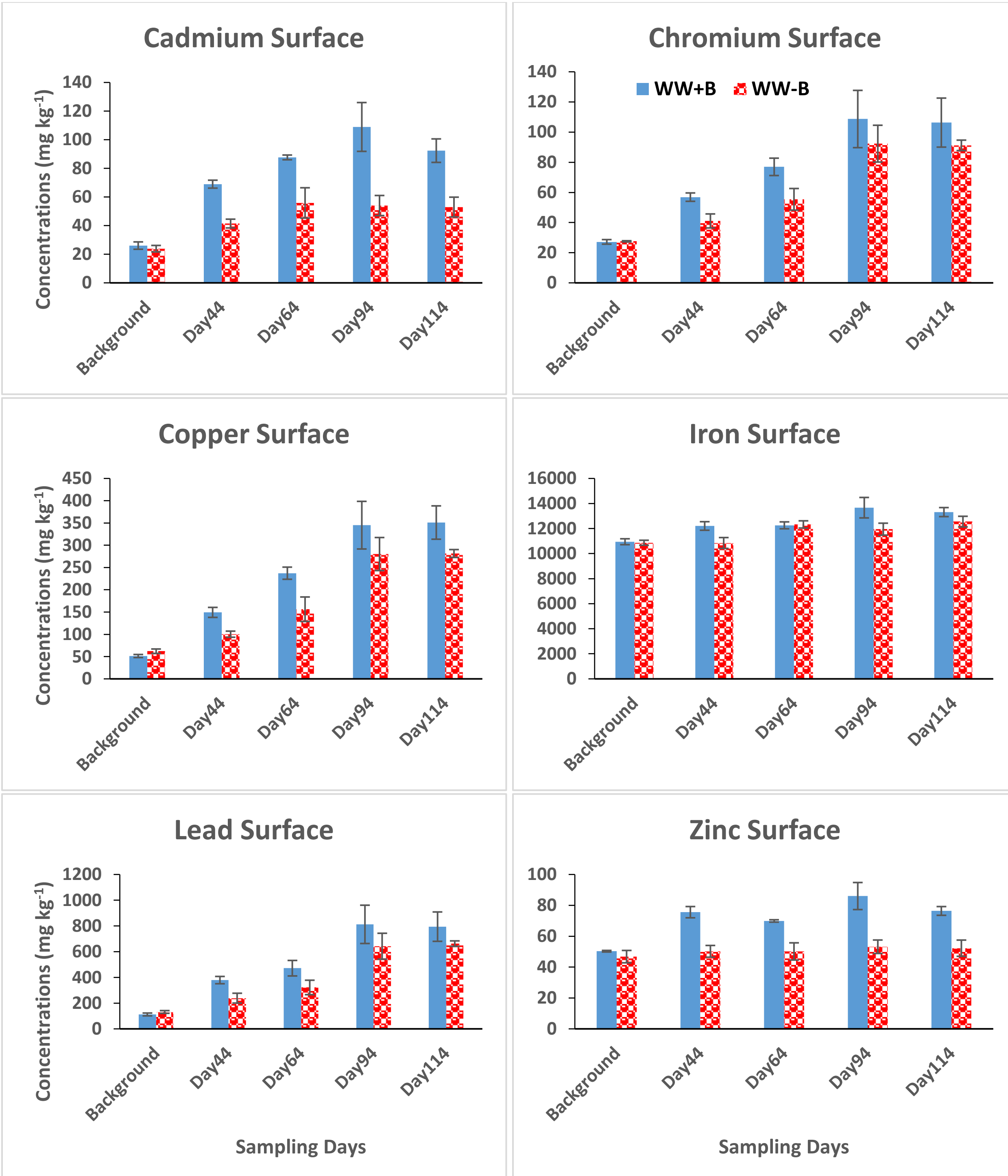

Figure 5.3. Concentrations of Cd, Zn, Cu, Cr, Pb and Fe at the soil surface of a sandy soil irrigated with untreated wastewater. Background is the day before planting. The error bar represents standard error of three replicates.

Table 5.5. Repeated measures analysis of the heavy metals in soil.

Effects	Cd	Cr	Cu	Fe	Pb	Zn
Treatment	*	ns	ns	ns	ns	*
Days	*	*	*	*	*	*
Treatment*Days	ns	ns	ns	ns	ns	ns

ns means not significant; * means significant; level of significance is 0.05

Of note, the biochar used in this study, having remained in the soil for more than one year, was improved, as depicted by the CEC and FTIR spectra of the biochar amended soil, possibly due to reactions such as dissolution and oxidation (Cheng et al., 2006). Also, the pores of the biochar, which appeared to be clogged by ash or tar-like material (Figure 5.1), would have opened and became accessible to the heavy metals. Organo-mineral micro-agglomerates formed on the pores of aging biochar reportedly immobilized heavy metals such as Zn in a Zn-contaminated soil (Kumar et al., 2018).

At 0.1 m depth, Cd, Cu and Cr were not detected in both treatments. However, under WW-B, Fe, Pb, and Zn were detected at reasonably high concentrations of 10990±484, 63.0±8.1, and 40.2±2.6 mg kg^{-1}, respectively. In WW+B treatment, the corresponding concentrations were 9748±366, 55.7±6.0, and 24.2±3.4 mg kg^{-1}, respectively. These were all lower than the corresponding concentration in WW-B. Although the concentrations of Pb and Zn at this depth were within the CCME acceptable limits in agricultural soils of 70 and 200 mg kg^{-1}, respectively, their presence, especially Zn, which was significantly higher ($p<0.05$) in WW-B (vs. WW+B), were perhaps an indication of transport under WW-B. This might have resulted in more uptake of these heavy metals by potato tissues. However, without the presence of Cd, Cu and Cr at 0.1 m depth, even after the second year of receiving wastewater, this provides possible insight that wastewater irrigation might not be a pathway for their transport to the subsoil and subsequently, to groundwater.

5.4.5 Heavy metals in plant

The heavy metal concentrations in potato tissues with or without biochar amendment and irrigated with freshwater or wastewater are presented in Table 5.6. For freshwater treatment (FW-B), all of the heavy metals (Cr, Cu, Zn, Cd, Pb and Fe) were detected below CODEX and WHO acceptable limits in the flesh (Codex Alimentarius Commission, 1995; Nazir et al., 2015).

Table 5.6. Heavy metal concentrations (mg kg^{-1}) in potato tissues under wastewater and freshwater irrigation after two years of biochar amendment

Potato Tissue	Treatment	Cr	Cu	Zn	Cd	Pb	Fe
Flesh	FW-B	0.04±0.01ab	2.9±0.63a	8.3±0.91b	0.1±0.02c	0.05±0.016ab	23.6±2.77a
	WW+B	0.026±0.001b	0.3±0.27b	7.3±0.79b	1.2±0.17b	0.05±0.004b	18.0±1.39a
	WW-B	0.06±0.01a	2.7±0.82a	15.3±1.57a	2.6±0.26a	0.09±0.001a	24.4±2.65a
Peel	FW-B	0.30±0.03b	5.5±0.29b	15.3±1.15b	0.3±0.02b	0.23±0.027b	116.7±13.58a
	WW+B	0.41±0.08ab	5.7±1.08b	19.9±3.61b	11.8±5.72b	2.59±1.047ab	90.7±16.57a
	WW-B	0.55±0.05a	18.7±4.1a	43.1±6.12a	43.9±2.62a	4.99±0.973a	153.6±23.83a
Root	WW+B	7.68±1.25a	69.7±13.9a	178.9±7.77a	163.4±3.63a	84.57±17.247a	931.9±92.23a
	WW-B	9.96±4.02a	149.1±28.0a	557.0±184.05a	366.5±63.29a	162.48±31.846a	1383.1±322.03a
Stem	WW+B	1.61±1.01a	5.5±4.80a	57.7±4.46a	35.1±10.14a	12.33±8.207a	86.7±46.35a
	WW-B	1.22±0.68a	7.3±3.54a	222.5±82.68a	69.9±9.10a	9.45±5.407a	87.3±33.96a
Leaf	WW+B	0.75±0.09a	4.2±0.69b	9.8±0.94a	7.3±1.08b	2.73±0.204a	191.8±36.00a
	WW-B	1.09±0.30a	10.9±0.42a	21.7±7.07a	38.3±7.80a	5.70±1.976a	228.5±45.32a

The values are the mean ± standard error of 3 replicates; for each potato tissue category, different letters signify a significant difference ($p \leq 0.05$). The permissible limits of the heavy metals in plant tissue (mg kg^{-1}) are Cd (0.1*, 0.3), Zn (50), Cu (10), Pb (0.1*), Fe (not available) and Cr (1.5). *signifies CODEX standard (Stan 193-1995), while the others are from WHO (Nazir et al., 2015).

The presence of heavy metals in potatoes grown with uncontaminated water is not uncommon given the ubiquitous nature of these metals, which sometimes are present as micronutrients in crops, including potatoes. Moreover, these heavy metals are present in uncontaminated soils (Alloway, 2013b)—as in this present study, especially for Cr, Fe and Zn (Table 5.2)—and could be available for plant uptake. Irrigation with wastewater resulted in an increase in the heavy metal concentrations, especially for Cd and Zn with up to 2500 and 84% increase, respectively (Table 5.6).

Known for its toxic effects, Cd concentration in potato flesh, far above the CODEX permissible limit of 0.1 mg kg^{-1} (Codex Alimentarius Commission, 1995), is of major concern particularly with no biochar amendment (WW-B). Although still above the CODEX permissible limit, amendment with biochar (WW+B) resulted in two times reduction of potatoes' Cd content, as compared to the no biochar amendment (WW-B). The concentration in FW-B was at par with the limit, which indicates that Cd would be translocated to potatoes if soil has trace amounts (Table 5.2 and Figure 5.3).

Although Cr, Cu, Zn, and Fe were numerically higher in FW-B than WW+B, the difference were not statistically significant ($p>0.05$) with the exception of Cu, which is a micro-nutrient. Moreover these concentrations were within CODEX and WHO permissible limit in potato flesh and as such the differences have no practical significance. In the first year of biochar amendment to soil (2015), only Cd and Zn were significantly reduced in the flesh of potatoes (Nzediegwu et al., 2019). However, after one year (2016) with no additional biochar amendment to the soil, there was a significant reduction ($p<0.05$) of Cd, Cr, Cu, Pb and Zn in the flesh (Table 5.6). When applied to soil, biochar's surfaces become more negatively charged through oxidation and hydrolysis, thereby, increasing the soil's cation retention capacity (Cheng et al., 2006; Lehmann, 2007) as noted in this present study. This is of importance since the one-time application of biochar to soil appears to be environmentally viable for more than one season, therefore, offsetting any cost spent on its application while enhancing safer use of wastewater.

With possible uptake from soil (Table 5.2) (Alloway, 2013b), all the heavy metals were detected in potato peels under freshwater irrigation (FW-B; Table 5.6). Being taken up from soil, heavy metals in potato peels under freshwater irrigation have been reported previously (Sadiq Butt et al., 2005); therefore, it is not unusual. Apart from Pb which was just slightly higher than the CODEX acceptable limit of 0.1 mg kg^{-1} in potato tubers, the concentrations of the other heavy metals were within their permissible limits. As expected, irrigation with wastewater significantly increased the concentration of all heavy metals [but Fe, which is naturally high in soil (Figure 5.3)] in the peel as compared to freshwater irrigation. Moreover, the peels had direct contact with the wastewater irrigated soil and would have absorbed these heavy metals from the contaminated soil. Similar observations (> heavy metals in wastewater versus freshwater) were noticed in potato tubers that received contaminated wastewater (Sadiq Butt et al., 2005). The concentration of all the heavy metal in the peel was higher than in the flesh. Although the peel makes up only 6% of the tuber (Woolfe and Poats, 1987), the ratio of the concentration in the peel to concentration in the flesh for these heavy metals varied from 1.8 in Zn to as high as 7.5 in Cr and 2.8 in Zn to 55.4 in Pb in freshwater and wastewater irrigated potatoes, respectively. Accordingly, this can raise the tuber (peel and flesh) concentration by 5% to 39% and 11% to 327% of the only flesh concentration in freshwater and wastewater irrigated potatoes, respectively. This poses a concern, especially for wastewater irrigated potatoes, given that the potato peel is consumed alongside the flesh. Such a practice of eating the peel would potentially increase the amount of these heavy metals making their way into the food chain. However, the peels under biochar amendment (WW+B) showed less heavy metal presence with Cd, Cu and Zn significantly reduced ($p<0.05$), whereas Cr, Pb and Fe were numerically less as compared to the control (WW-B). Therefore, having been in the soil for up to two years, the biochar remains active and effective for heavy metal immobilization.

Heavy metals in the roots, stems and leaves of FW-B treatment were not determined given that the concentrations were relatively low in the tubers, especially the peel. Therefore, subsequent discussion will focus on WW-B and WW+B treatments.

All the heavy metals were detected in the potato roots that received wastewater (WW-B). This was expected since the roots had direct contact with the contaminated soil and play an active role in the uptake of nutrients, which have similar uptake mechanisms as for heavy metals. Moreover, there was an accumulation of these heavy metals in the soil depth where the roots of the potato were distributed. Although with no significant difference, mixing biochar in the soil numerically reduced the uptake of the heavy metals as compared to the no biochar treatment. The ratio of reduction (WW-B:WW+B) across the heavy metals was in this order: Zn (3.1) > Cd (2.2) > Cu (2.1) > Pb (1.9) > Fe (1.5) > Cr (1.3). Of interest was the 2 times reduction noticed in Cd, which is very toxic when it gets into the human food chain (Järup, 2003). This also supports the significantly low concentration of Cd noticed in the flesh as less uptake led to less translocation. Similarly, this was true for the other heavy metals. Given that biochar maintained its effectiveness as seen from the reduced uptake, it could be concluded that biochar as a soil conditioner was stable for immobilizing heavy metals, such as Cd and Zn.

Heavy metal uptake in the stem was as high as 222.5 mg kg^{-1} for Zn and as low as 1.22 mg kg^{-1} for Cr. The presence of heavy metals in potato stems is not unusual since the stem serves as a channel for nutrient and water movement. Moreover, heavy metals are relatively soluble (Chuan et al., 1996) and as such, seems to be taken along with the nutrients in soil solution. As suggested by the inverse translocation factor (TFI: concentration in roots/concentration in stem): Cu (20.4) > Pb (17.2) > Fe (15.8) > Cr (8.2) > Cd (5.2) > Zn (2.5), lower concentrations were measured in the stem as compared to the root implying that after being taken up by the root, translocation to the stem slowed down. Cu showed the least readiness to translocate from root to stem, while Zn showed the highest readiness. Mixing biochar with the soil seems not to affect the presence of the heavy metals in the stem as there was no significant difference between the treatments for all the heavy metals. This is understandable as the stem could just be a channel for water and nutrient transport and as such, may not store the heavy metals in favor of any treatment—WW-B or WW+B. Moreover, the potato stem is not edible, thereby, offering minimal concern to human health.

The concentration of heavy metals in the leaf under WW-B ranged from 1.09 mg kg^{-1} for Cr to 228.5 mg kg^{-1} for Fe. For the most toxic metals, such as Cd and Pb, it was 38.3 mg kg^{-1} and 5.70 mg kg^{-1}, respectively. The leaf, being the chlorophyll containing part of plants, including potatoes, requires some nutrients for nourishment (Palta, 1990). Since these nutrients are available in the soil solution, which simultaneously contains heavy metals (Figure 5.3), it is not unusual to have heavy metals in the leaf. Although potato leaves are not edible, they could be composted for nutrient recycling in agricultural fields making the presence of heavy metals at a high concentration a challenge. However, mixing the soil with biochar reduced this concern as much lower concentrations were measured in the leaves under biochar amended soil. Cu and Cd had significantly lower concentrations ($p<0.05$) under biochar amendment. As such, biochar remained active in the soil after two years of application.

Overall, with heavy metal concentrations following the order of root > stem > leaf, it appears that the proximity of the potato parts to the point of contaminant loading (the soil) played a role in their uptake. The TFI for the leaf with the order: Pb (28.5) > Zn (25.7) > Cu (13.7) > Cd (9.6) > Cr (9.1) > Fe (6.1) and the TFI for the stem showed that translocation patterns from root to stem and from root to leaf were consistently greater than 1 implying a higher tendency of the heavy metals to remain in the root.

5.5 Conclusions

The biochar would enhance the safety against translocation of heavy metals under wastewater irrigation, especially untreated, for at least a period of 2 years. Biochar was amended in soil once and potatoes were grown applying wastewater for two years. The soil sampled in the second year showed that all the heavy metals accumulated in the top soil, while only Zn, Pb and Fe moved to the 0.1 m depth. Mixing the soil with biochar significantly increased the soil's pH and the soil's CEC, thereby, immobilizing the heavy metals (except Fe), as compared to the control with no biochar. The FTIR spectra suggested that the oxygen-containing functional groups in the soil and soil-biochar mix increased with time and were responsible for binding the heavy metals. The heavy metals translocated to all the potato parts (flesh, peel, root, stem and leaves). While the concentration of the heavy metals were relatively low in potato parts under freshwater (flesh and peel), concentrations in the wastewater irrigated potatoes (flesh, peel, root, stem and leaves) were relatively high. However, biochar amendment, after the second year of being mixed in the soil, significantly reduced ($p<0.05$) the concentration of Cd, Cu, Cr, Pb and Zn in the edible flesh. Therefore, it appears that biochar is effective as a viable sorbent for immobilizing wastewater laden heavy metals for at least two years.

5.6 Acknowledgements

The authors express gratitude to NSERC, Tertiary Education Trust Fund, Nigeria and the India-Canada Centre for Innovative Multidisciplinary Partnerships to Accelerate Community Transformation and Sustainability for providing fund for the research. The authors would also like to thank our Lab Supervisor, Ms. Sedigheh Zarayan.

Connecting Text to Chapter 6

While implementing biochar amendment as a technique for immobilization of heavy metals and improving the quality of crops, it is necessary to investigate how crop yield is affected by wastewater irrigation and biochar amendment.

Chapter 6, Effect of Biochar on the Yield of Potatoes Cultivated under Wastewater Irrigation for Two Years, documents the yield of potatoes cultivated in both 2015 and 2016 in biochar amended soil.

This chapter has been prepared as a manuscript and it is ready for submission to Agriculture, Ecosystems and Environment. The manuscript is co-authored by Dr. Shiv Prasher, my supervisor, Dr. Eman ElSayed, a post-doctoral fellow in the department, Mr. Jaskaran Dhiman, a PhD scholar in the department, Mr. Ali Mawof, a PhD scholar in the department and Dr. Ramanbhai Patel, a research associate in the department. To ensure consistency with the thesis format, the original draft has been modified by listing the cited work in the reference section (Chapter 9).

Chapter 6: Effect of Biochar on the Yield of Potatoes Cultivated under Wastewater Irrigation for Two Years

6.1 Abstract

This study makes investigations into the effects of biochar, produced from plantain peel (~12 Tg wasted annually), on the yield of potatoes (Solanum *tuberosum* L.) irrigated with wastewater in two consecutive years. Potatoes were grown in 2015 and 2016 in nine lysimeters (1.0 m x 0.45 m), packed with sandy soil to a bulk density of 1.35 Mg m-3. The lysimeters were arranged in a completely randomised design with three replicates. The three treatments were: (i) wastewater with biochar, (ii) wastewater without biochar (control), and (iii) freshwater without biochar. The soil with biochar treatments was amended in 2015 with an application rate of 1% (w/w) on the top 0.1 m of soil. After 33 days of planting, the potatoes were irrigated 8 times, on a 10-day irrigation interval, with freshwater or wastewater that was synthesized to represent a typical wastewater in developing countries. Plant health parameters such as (i) greenness, (ii) photosynthetic rate, transpiration rates, stomatal conductance, and (iii) plant reflectance (normalized difference vegetative index- NDVI) were measured. After 120 days of planting, the potato tubers were harvested. The fresh weight was measured, and the tubers were counted. It was found that plant health parameters, greenness, photosynthesis rate, stomatal conductance and transpiration rate varied with time but there were no significant differences. There were no differences in NDVI among treatments; these measurements started after 43 days when plant canopy was already developed. In 2015, there was marginally higher moisture content at 0.1 m depth in biochar-amended soil than in control, although it was significantly higher in control at the surface; however there was no significant difference in moisture content due to treatments. There was no

effect of biochar on leaf temperature. In 2015, the fresh weight and number of tubers were significantly less in the biochar treatment, possibly due to germination delay in the biochar amended lysimeters. However, in 2016, no germination delay was noticed, and the yield was similar in all the treatments. Yields were not affected even though significantly higher ($p<0.05$) heavy metals were taken up by different parts of the potato plants under wastewater irrigation (vs. freshwater). This can be alarming to some degree as the farmer may be getting the expected yield but with unhealthy potatoes, and not realizing this at all. Thus it was concluded that there was no effect of plantain peel biochar on the plant health parameters as well as yield of potatoes. It was observed that the biochar raised the pH and the CEC of the soil, however it is likely that these positive impacts would have been masked by enough plant nutrients in soil. Thus, further investigations under controlled nutrient environment are required.

Keywords: Potato yield; wastewater irrigation; lysimeters; plantain peel biochar; sandy soils

6.2 Introduction

With a current value of 7.3 billion, the world population is expected to reach 11.2 billion by 2100 (Teytelboym, 2016; United Nations, 2017). Accordingly, more water will be required to produce more food for growing population. This will potentially exert more pressure on freshwater, especially in agriculture sector, since 70% of its withdrawal is currently used in agriculture worldwide to sustain food production (Aquasat, 2014). Therefore, there is an urgent need to seek for alternative sources of water (for agriculture) that will reduce the burden on current freshwater resources and still increase crop yield.

Wastewater could be a viable option to supply supplemental irrigation water given that it is quite available and, in addition, is reportedly rich in macro nutrients such as N, P and K (Qian and

Mecham, 2005; Raschid-Sally and Jayakody, 2009; Singh et al., 2012), which could reduce cost of inorganic fertilizers. For instance, Singh et al. (2012) reported an increase in soil available N, P and K, as well as, organic carbon following wastewater irrigation. Despite its benefits, wastewater irrigation could have detrimental effects on soil properties such as pH, which could affect the availability of plant's essential nutrients in soil solution and consequently reduce crop yield. Furthermore, wastewater irrigation could release antagonistic heavy metals in soil (Angin et al., 2005), depending on the wastewater source. Therefore, incorporating soil conditioners, such as biochar, which can increase soil pH and CEC (which translates to higher crop yield), in addition to serving as contaminant sorbent (Puga et al., 2016) as contaminants present in wastewater could be restrained from being transmitted deep in soil and getting translocated into food crops.

Biochar, a solid product of organic waste pyrolysis, is reportedly rich in soluble salts, which translates to high soil pH (Lehmann and Joseph, 2009; Singh et al., 2010). When incorporated into soil (acidic), biochar raised the soil's pH (Alling et al., 2014). For instance, hardwood hickory-derived biochar increased soil pH (15%), as compared to no-biochar amended soil (Laird et al., 2010); the study also reported that biochar significantly increased the surface area of the soil. However, establishing clear relationships between soil physiochemical properties (e.g. pH) and biochar application as a soil amendment is difficult due to the variability in properties of different biochars and site-specific interactions with soil and vegetation (Alburquerque et al., 2013; Xie et al., 2014).

Variability in biochar characteristics often results from different feedstocks used for its production (Alburquerque et al., 2013). A few studies have shown conflicting results—either positive (Artiola et al., 2012; Butnan et al., 2015; Rodríguez-Vila et al., 2015) or neutral (Borchard et al., 2014; Jay et al., 2015) with respect to crop yield when different types of biochars were used. For instance, Artiola et al. (2012) reported that biochar derived from pine forest waste increased soil's pH, and subsequently increased Bermuda grass production. Likewise, Alburquerque et al. (2013) showed that addition of wheat straw and olive tree pruning biochar to soil increased available P, and thus increased wheat grain yield. On the contrary, biochars derived from wood (Fagus species and Picea species) had no effect on maize yield (Borchard et al., 2014), and biochar from another wood (Castanea sativa) also had no effect on the yield of potatoes (Jay et al., 2015).

Plantain peel (40% of plantain fruit (Rubatzky and Yamaguchi, 1997) could be a sustainable feedstock for transforming into biochar since it ends up as a waste in most countries where plantain is produced (Tchango Tchango et al., 1999), thereby adding value to the waste, while protecting the environment from nuisance. In a previous study, Nzediegwu et al. (2019) found plantain peel biochar (mixed with sandy soil under potato cultivation) to adsorb wastewater-laden Cd and Zn as compared to no-biochar soil. It is also known that the effect of biochar is feedstock- and crop-specific. Potato ranks first among other tuber crops (Consortium, 2011); its demand (globally) is increasing, possibly due to its nutritional value (Brown, 2005; FAO, 2017), and the investigation on the effect of biochar are valuable. A few types of biochars have been tested for growing potatoes (Akhtar et al., 2015a; Collins et al., 2013; Hien et al., 2017; Koga et al., 2017; Liu et al., 2017; Liu et al., 2014; Walter and Rao, 2015); biochars from bamboo, wood, rice husk and kunai grass have shown either positive (Hien et al., 2017; Liu et al., 2014; Walter and Rao, 2015), neutral (Jay et al., 2015; Koga et al., 2017) or negative (Liu et al., 2017) impact on potato yield. It needs to be investigated whether or not plantain peel biochar affect crop yield. Moreover, it is not clear what would be the interaction of wastewater irrigation and biochar in soil, and consequently the effect on potato yield. Therefore, our goal was to conduct a 2-year field-study to understand the effect of plantain peel biochar on the yield of potatoes, cultivated in a lysimeter field soil and irrigated with synthetic wastewater. Specifically, different yield parameters such as tuber weight and number of tubers were measured for two years (2015 and 2016).

6.3 Materials and methods

6.3.1 Biochar characterization

Detailed description of the biochar used in this study has been documented elsewhere (Nzediegwu et al., 2019). Briefly, the biochar has a pH of 10.3, determined following dissolution of the biochar (1:30 w/w) in deionised water, shaken for 4 h, and measured with an electrode type pH meter (Accumet AB 15); it has mineral ash content of 77.45%, determined following ASTM 7582. The total C and total N, determined as per ASTM D5373, were 18.1 and 0.6%, respectively resulting in a C:N ratio of 30.2. The total metal (essential and non-essential; Table 6.1) contents were determined by nitric acid digestion method (Kargar et al., 2013), where 0.16 g of oven-dried biochar was digested with 2 mL of 70% nitric acid on a block digester, gradually increased to 120^{o}C and holding at this temperature for 5 h. The soluble metals in the digested solution (diluted 50 times with deionized water) were then analyzed using inductively coupled plasma mass spectrometry (Varian ICP820-MS or Analytik-Jena).

6.3.2 Experimental set-up

The experiment was conducted in 2015 and 2016, between the months of June and October, when outside temperature was above 10^{o}C (Food and Agriculture Organization FAO, 2008). Class A evaporation pan was installed in the field to record pan evaporation and compute daily reference evapotranspiration, following standard procedures (Doorenbos and Pruitt, 1975). A BIOS thermometer/hydrometer (Model: tr415) was installed outside to record temperature for the experimental period. Sandy soils were packed to a bulk density of 1.35 Mg m^{-3} in nine outdoor lysimeters, having four 10 mm holes drilled radially at depths of 0.15 and 0.3 m for soil sampling.

Table 6.1. Physical and chemical properties of biochar and soil

Properties	Soil	Biochar
Sand (%)	92.2	n.a.
Silt (%)	4.3	n.a.
Clay (%)	3.5	n.a.
pH	5.5	10.3±0.1
Organic matter (%)	2.4±0.15	n.a.
Hydraulic conductivity (m day^{-1})	1.67±0.45	n.a.
ZPC	3.4	n.a.
P (mg P kg^{-1}	215.30±40.43	n.a.
K (mg K kg^{-1})	107.33±13.13	n.a.
N (mg NO_3-N kg^{-1})	4.57±0.46	n.a.
Ca (mg Ca kg^{-1})	912.44±79.70	1947.0±46.7
Mg (mg Mg kg^{-1})	103.27±7.29	2063.1±69.8
Al (mg Al kg^{-1})	1164.14±12.40	83.6±1.1
Cd (mg Cd kg^{-1})	<LOD	<LOD
Cr (mg Cr kg^{-1})	17.86±0.38	1.67±0.17
Cu (mg Cu kg^{-1})	<LOD	7.11±0.98
Fe (mg Fe kg^{-1})	11109.64±238.68	669.12±86.35
Pb (mg Pb kg^{-1})	<LOD	0.043±0.04
Zn (mg Zn kg^{-1})	16.70±2.28	35.65±1.39

LOD: limit of detection; ZPC: zero point of charge; n.a.: not available; the heavy metals Cd, Cr, Cu, Fe, Pb and Zn were determined following hot acid extraction (Kargar et al., 2013) and quantified by ICP-OES. The LOD was 50 µg L^{-1} (15.6 mg kg^{-1}) for all the metals. P, K, Ca, Mg, and Al were determined following Mehlich III extraction (Mehlich, 1984) while N was determined following 2.0 M KCl method (Carter and Gregorich, 2008). Other soil properties were adapted from a previous study (ElSayed et al., 2013).

Properties of the soil are presented in Table 6.1. The lysimeters were arranged in a completely randomized design with 3 replicates. The three treatments studied were: wastewater and biochar (WW+B), wastewater with no biochar (WW-B) and freshwater without biochar (FW-B). The WW-B served as control for the biochar treatment (WW+B), while FW-B served as control for the wastewater treatment (WW-B). The soil was brought to field capacity one day before biochar mixing. The biochar was mixed in the three lysimeters under WW+B at the top soil profile (0 to

0.1 m depth) at a rate of 13.5 t ha^{-1}. Afterwards, potatoes were procured and planted. The procedures for potato procurement, planting, fertilization, and irrigation have been documented elsewhere (Nzediegwu et al., 2019). Five weeks after planting, when the potatoes had emerged and stabilized, as visualized by new leaf development, synthesized wastewater (see Table 6.2 for recipe) as well as freshwater was applied (11.5 L $lysimeter^{-1}$) at a 10-day interval for eight times. Soil samples were collected from the surface, 0.1 m and 0.3 m depth two days after irrigation and stored in -24^{o}C freezer for further analysis.

Plant health parameters were monitored after the plant canopy had developed (~5 weeks after planting) till the end of growing season. All foliage measurements were taken from the fourth petiole (Stark et al., 2004) on days one, three, and nine after each irrigation, which correspond to when the potatoes were either water stressed or not. Setting a photon flux density of 800 μmol m^{-2} s^{-1} (i.e., twice the value used in greenhouse studies (Robredo et al., 2007)), photosynthesis rate, transpiration rate and stomatal conductance were measured using LI-COR portable photosynthesis system (LI-6400, LI-COR Inc., Lincoln, Nebraska). Greenness, a measure of leaf chlorophyll level, was measured with Minolta chlorophyll meter (SPAD-502, MINOLTA Co. Ltd., Japan). The leaf temperature, a measure of water stress, was measured using Hylogy Infrared Digital thermometer (Model: MD-H4). Typically, a clearance of 0.03 m was allowed between the leaf surface and the infrared thermometer. Plant reflectance [normalize difference vegetative index (NDVI)], which serves as a growth monitoring parameter (Prasad et al., 2006), was measured with an active canopy crop sensor (Crop Circle, ACS-430, Holland Scientific, Lincoln Nebraska USA). Clearance between plant canopy and the sensor was maintained typically at 0.25 m for each lysimeter.

Table 6.2. Recipes for synthetic wastewater.

Purpose	Substance/ Compounds	Country	Concentration **(mg L^{-1})**	**Wastewater Recipe Source** Contaminant reporter
	Basic synthetic wastewater ingredients			
C source	Sodium Acetate		**79.37**	Nopens et al. (2001)
	Milk powder		**116.19**	Nopens et al. (2001)
	Soy Oil		**29.02**	Nopens et al. (2001)
	Starch		**122**	Nopens et al. (2001)
	Yeast Extract		**52.24**	Nopens et al. (2001)
N Source	Ammonium Chloride		**12.75**	Nopens et al. (2001)
	Peptone		**17.41**	Nopens et al. (2001)
	Urea		**91.74**	Nopens et al. (2001)
P Source	Magnesium phosphate		**29.02**	Nopens et al. (2001)
Minerals	$CaCl_2$		**60**	LaPara et al. (2006)
	$MgCl_2$		**40**	LaPara et al. (2006)
	$NaHCO_3$		**100**	LaPara et al. (2006)
	K_3PO_4		**30**	LaPara et al. (2006)
	Additional contaminant levels based on worst case reports or set to exceed LOD			
Heavy Metals	Potassium dichromate (Cr)	India	**2**	Ahmad et al. (2011)
	Cadmium Nitrate (Cd)	India	**5**	Ahmad et al. (2011)
	Lead Nitrate (Pb)	India	**16**	Ahmad et al. (2011)
	Iron Sulphate (Fe)	India	**120**	Ahmad et al. (2011)
	Zinc Nitrate (Zn)	India	**3**	Ahmad et al. (2011)
	Copper Nitrate (Cu)	India	**8**	Ahmad et al. (2011)
Hormones	Estrone (E1)	Korea	8.15 (50) μg L^{-1}	Sim et al. (2011) — LOD
	Estradiol (E2)	Korea	0.634 (20) μg L^{-1}	Sim et al. (2011) — LOD
	Progesterone	China	0.90 (20) μg L^{-1}	Huang et al. (2009) — LOD
Pharmaceuticals	Oxytetracycline	China	19.5	Li et al. (2008)
	Ibuprofen	India	26.45 μg L^{-1}	Singh et al. (2014)
Surfactant	Triton X-100 or alkylphenyl polyethoxylate	Morocco	30 μg L^{-1}	Aboulhassan et al. (2006)
Plasticizers	Bisphenol A		(50 μg L^{-1})	Based on LOD
	Bisphenol S		(50 μg L^{-1})	Based on LOD
	Bisphenol F		(50 μg L^{-1})	Based on LOD

Values in parentheses are concentrations used in the synthetic wastewater adjusted to represent worst-case scenario.

After 120 days of planting, the potatoes were harvested. The root weight, shoot length, number of tubers, above ground biomass weight, and total tuber weight were recorded. The yield per lysimeter (alias yield per plant) for each treatment was calculated as the average tuber weight. With a row spacing of 36 in and a within row spacing of 12 in (Bohl et al., 1995), a plant density (i.e., number of plant per hectare) of 35,880 plant ha^{-1} (Canadian Food Inspection Agency CFIA, 2017) was used to calculate the total yield (t ha^{-1}) (i.e., $\text{plant density} * \text{yield (t) per plant}$). The potato tubers were graded by passing them, one after the other, through a 50-mm diameter hole (Shiri-e-Janagard et al., 2009; USDA, 1983).

The gravimetric moisture content of the soil samples, collected depth-wise (0.0 m and 0.1 m), two days after each irrigation, were determined following the standard oven-dry method (Hollinger and Isard, 1994). Wet soil samples were weighed and heated to105ºC in an oven for 24 h. The moisture content was calculated as $((\text{weight of wet soil} - \text{weight of dry soil}) / \text{weight of wet soil}) * 100\%$. The exchangeable cations and cation exchange capacity (CEC) of the soil samples were determined following $BaCl_2$ method (Carter and Gregorich, 2008), while the pH of the soil was determined following the standard soil survey method (Rayment and Higginson, 1992), where air-dried soil was bathed with deionized water (1:5 w/w soil: water), shaken for 1 h, and the suspension measured with an electrode type meter (Accumet AB 15).

6.3.3 Data analysis

Greenness, leaf temperature, LI-COR and NDVI data were analyzed with compound multivariate analysis of variance using two layers of repeated measures, namely irrigation days and measurement day after irrigation. The soil moisture data was subjected to one layer repeated measures analysis, while the yield related data, exchangeable cations and CEC were subjected to one-way analysis of variance. All analysis were performed using SAS-JMP® 13.0.0 (Copyright © 2016 SAS Institute Inc.).

6.4 Results and discussion

6.4.1 Leaf greenness

The greenness values, measured at the bulking stage in 2015, are presented in Figure 6.1 along with the 2016 greenness values, segmented by three levels, 1, 3, and 9 days after irrigation, for the vegetative season of the potatoes. Although with no significant treatment effect ($p>0.05$), the 2015 SPAD revealed a slight decrease in greenness from about 35 in the beginning to about 31 towards the end of season. Similar observations were noticed by Minotti et al. (1994) and Vos and Bom (1993) who used SPAD data to access the foliar N of potatoes. Likewise, in 2016, after application of second split N fertilization on day 51, the greenness increased for about two weeks, and then gradually decreased with the growing season. This was expected since N concentration decreases with increase in biomass (Bélanger et al., 2001). Irrigation with wastewater showed no significant difference ($p>0.05$) in greenness as compared to the corresponding greenness in freshwater treatment (Figure 6.1). Although it was expected that irrigation with wastewater (vs. freshwater) should increase greenness, given that it contains sources of nutrients (Table 6.2), however, it appeared the nutrients were not readily available, possibly due to high presence of contaminants, such as Fe in the wastewater (Table 6.2) or due to less time for mineralization (da Fonseca et al., 2005). With both treatments receiving inorganic fertilizer—as in present study—for two years, the chlorophyll content in maize leaf was not significantly affected by wastewater (sewage effluent) irrigation as compared to freshwater irrigation (da Fonseca et al., 2005). Overall, biochar amendment did not affect leaf greenness for both the years. It could be that biochar did not improve soil's N availability. Moreover, Nelson et al. (2011) reported that biochar addition to soil either suppressed or did not affect N availability when applied at 2% and 0.2% (w/w) rates, respectively.

Figure 6-1. Greenness measured using SPAD for 2015 and 2016. For 2015, Day93 IR7 and Day103 IR8 signify irrigations 7 and 8, respectively. The error bars represent the standard error.

6.4.2 Leaf and ambient temperature

The leaf and ambient temperature values, measured in 2016 and segmented according to days after irrigation, are presented in Figure 6.2. Leaf temperature is an indication of plant's water stress, with higher values signifying more water stress (Loveys et al., 2008). For the entire monitoring period, the leaf temperature ranged from 17.5 to 30.6^{o}C.

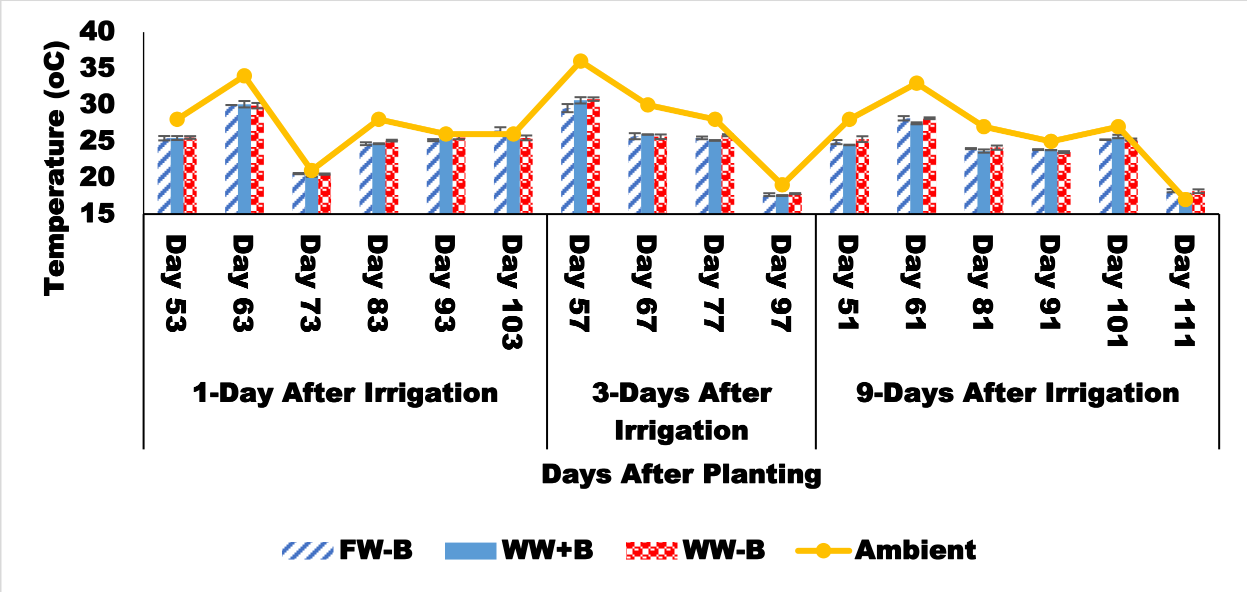

Figure 6-2. Leaf temperature measured in 2016 and segmented by levels of days after irrigation. Ambient is the ambient air temperature in tent. The error bars represent standard error.

There was effect of days, driven mainly by the ambient air temperature (Figure 6.2). In corroboration with Leuzinger et al. (2010), who also noticed similarity between ambient and leaf temperatures of Pinus sylvestris, this is not unusual since the experiment was conducted in the field. Overall, neither irrigation water quality nor biochar amendment showed significant effect on the leaf temperature.

6.4.3 Stomatal conductance, transpiration and photosynthesis rate

Being a measure of the rate of gaseous exchange (CO_2 and water vapor) through plant's stomata, stomatal conductance controls photosynthesis and transpiration rate (Meidner and Mansfield, 1968) and it is important in describing plant's health. The photosynthesis rate, stomatal conductance and transpiration rate of potatoes, measured in 2016, are presented in Figure 6.3. Photosynthesis indicated a general trend of decrease with time for all three measurement days, although statistically not significant ($p>0.05$). It ranged from 14.16 µmol CO_2 m^{-2} s^{-1} few days after the first irrigation to 2.21 μmol CO_2 m^{-2} s^{-1} toward the end of the season; the range was similar to that observed by Chandra et al. (2008) in cannabis. However, there was no effect of treatments. It could be that photosynthesis rate decreased with the development of potatoes; evidently, slight color change with time was noticed on the potato leaves, especially towards maturity. No treatment effect signifies that neither the irrigation water nor the biochar amendment had an effect on photosynthesis rate. Since all the treatments received same level of fertilization as recommended for potatoes, it could be that the soil condition necessary for biochar to show its agronomic effectiveness was buffered. Moreover, there have been evidence that biochar shows agronomic benefits mainly in nutrient-depleted soils (Hussain et al., 2017; Kimetu et al., 2008), whereas not much agronomic benefits are noticed in nutrient-rich soils —as in our case (Hussain et al., 2017).

Stomatal conductance and transpiration rate followed similar pattern as that of photosynthesis rate for the whole measurement period. This is not unusual given that transpiration is associated with stomatal opening and occurs throughout a plant's growing season (Meidner and Mansfield, 1968). It appears that the stomatal conductance decreased with days after irrigation, especially between days 61 to 97, which corresponds to the tuber bulking period (He et al., 2012); however, the analysis showed no significant differences as compared to the rest of the season. The results were statistically similar in all treatments suggesting that biochar as well as irrigation water quality did not alter the growth parameters of potatoes. This is in accordance with the observation of other plant health parameters such as greenness.

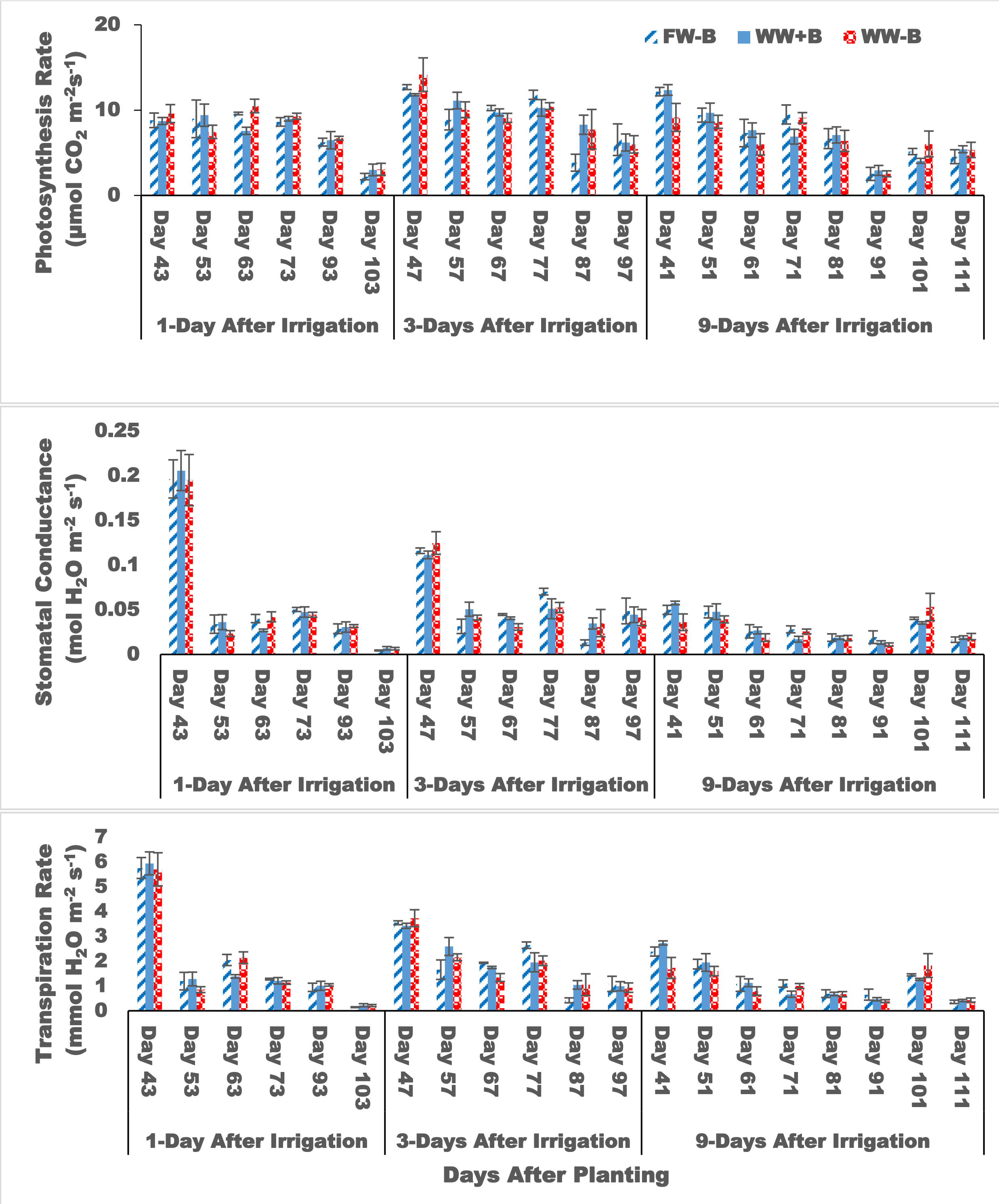

Figure 6-3. Photosynthesis rate, stomatal conductance and transpiration rate measured in 2016 and segmented according to measurement days after irrigation. The error bars represent standard error.

6.4.4 Normalized difference vegetative index (NDVI)

Normalized difference vegetative index (NDVI), measured during the vegetative period in 2016 and segmented according to days after irrigation, is presented in Figure 6.4. Overall, the NDVI ranged from 0.61 (day 53) to 0.86 (day 73), showing that the potato plants grew vigorously (Patil et al., 2014). Similar NDVI values have been reported for potatoes grown under rain-fed and irrigated water management (Shamal and Weatherhead, 2014). However, there was no treatment effect, implying that wastewater irrigation as well as freshwater irrigation had similar impact on potatoes' vigor. This is important as wastewater can serve as an alternative for potato irrigation in arid and semi-arid regions where freshwater is scarce. Amendment with biochar (WW+B) did not alter the NDVI, as in the case of the other plant health parameters.

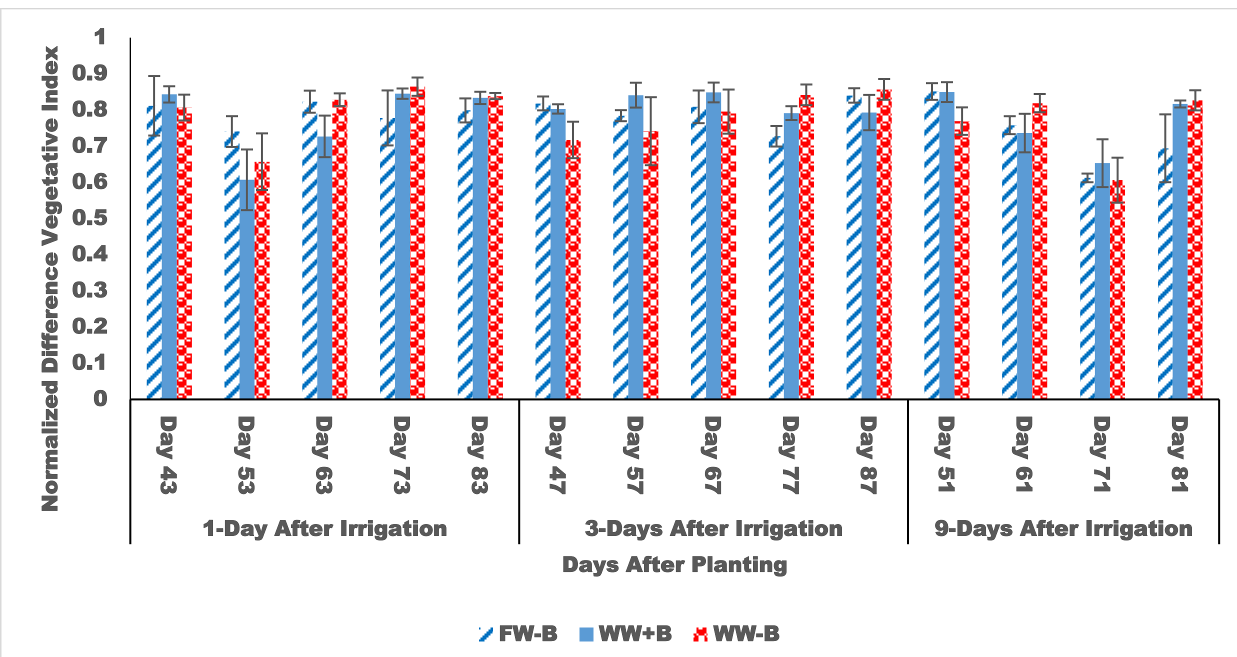

Figure 6-4. Normalized difference vegetative index (NDVI) measured in 2016 and segmented according to days after irrigation. The error bars represent standard error.

6.4.5 Soil moisture

Figure 6.5 presents the moisture content (gravimetric) of the soil sampled at the surface and 0.1 m below (i.e., $[\mathrm{MC}]_{\mathrm{soil}}^{\mathrm{surf}}$ and $[\mathrm{MC}]_{\mathrm{soil}}^{0.1}$, respectively) for both years (2015 and 2016).

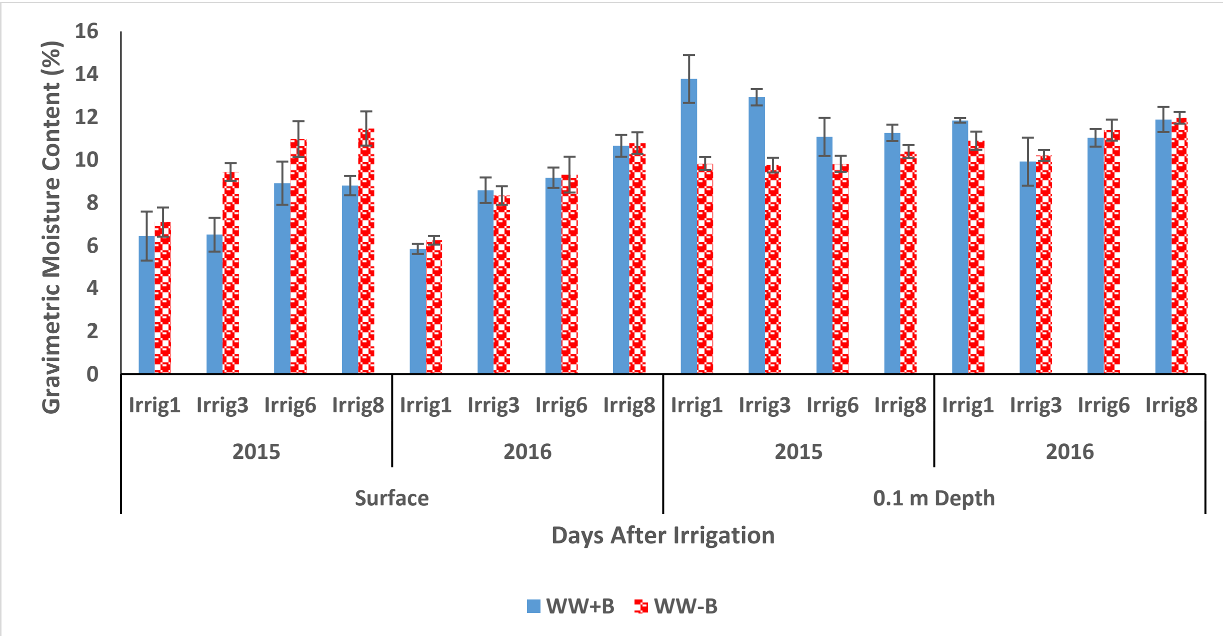

Figure 6-5. Gravimetric soil moisture measured in 2015 and 2016, two days after irrigation, from soil sampled at the surface and 0.1 m depth. Error bars are standard error.

Table 6.3. Repeated measures analysis of variance for moisture content

Effects	**Surface**		**0.1 m Depth**	
	2015	2016	2015	2016
Treatment	ns	ns	*	ns
Time	*	*	ns	*
Treatment*Time	ns	ns	*	ns

ns: not significant; *: significant; significant level is 0.05; -: not applicable

In the first year (2015), lowest $[\mathrm{MC}]_{\mathrm{soil}}^{\mathrm{surf}}$of 7.1% was measured in WW-B after first irrigation, whereas after eight irrigations, the highest moisture (11.5%) was measured, depicting an increase in moisture with irrigation. In 2016, a similar trend was noticed, where $[\mathrm{MC}]_{\mathrm{soil}}^{\mathrm{surf}}$ increased with irrigation. This was true also for biochar-amended treatment (WW+B), mainly in 2016. Interestingly, it seems that in 2015 while WW-B held more water at the surface, WW+B held more water at the 0.1 m depth, where water is mostly required by plant roots, including potatoes, for metabolism (Clothier and Green, 1994). At 0.1 m depth, irrigation appeared not to affect soil moisture as $[\mathrm{MC}]_{\mathrm{soil}}^{0.1}$ in WW-B stayed almost same for the entire season and for the both years. This was expected given the nature of the soil (sandy), which had low water retention potential (J. Rawls et al., 1982), thereby drains water quickly. However, $[\mathrm{MC}]_{\mathrm{soil}}^{0.1}$ was slightly altered by biochar amendment (WW+B), especially in 2015, suggesting an improvement in the soil water retention properties, which could potentially result in higher potato yield.

6.4.6 Exchangeable cations and cation exchange capacity

Table 6.4 presents the exchangeable cations, cation exchange capacity and base saturation of the soil, with or without biochar. The abundance of exchangeable cations, otherwise known as CEC, in soil is very vital as it plays an active role in the bioavailability of nutrients which exist mostly in their ionic forms for plant uptake. The CEC of the surface soil for both treatments were apparently similar. However, at 0.1 m depth, the CEC was significantly higher ($p<0.05$) in WW+B (vs. WW-B) with 65% increase. This signifies a great potential for biochar to increase the cation exchange activities in the exchange complex of the soil, which could result in the availability of soil nutrients. Accordingly, the base saturation, showing the percentage of base cations occupying the exchange complex, was significantly higher ($p>0.05$) in WW+B as compared to WW-B. This is in line with the higher base cations (Ca, Mg, K and Na) measured in WW+B as compared to WW-B (except Mg). Increase in base cations results in pH increase (Ste-Marie and Paré, 1999), which is vital for nutrient availability and could ultimately affect crop yield.

Table 6.4. The exchangeable cations, cation exchange capacity and base saturation of the soil with or without biochar

Exchangeable cations (cmol(+) kg^{-1})	Surface		0.1 m depth	
	WW-B	**WW+B**	**WW-B**	**WW+B**
Ca	0.96±0.04a	1.41±0.13a	1.23±0.36a	3.85±0.47b
Mg	0.54±0.00a	0.46±0.09a	0.15±0.02a	0.77±0.09b
K	0.20±0.01a	0.25±0.03a	0.20±0.03a	0.48±0.09a
Na	0.24±0.02a	0.22±0.01a	0.11±0.00a	0.16±0.01b
Al	0.40±0.04b	0.19±0.03a	1.50±0.05b	0.09±0.05a
Fe	0.06±0.01a	0.06±0.00a	0.02±0.00b	0.00±0.00a
Mn	0.01±0.00a	0.02±0.00b	0.05±0.008b	0.02±0.004a
CEC	2.40±0.09a	2.60±0.04a	3.25±0.36a	5.37±0.49b
BS (%)	80.49±1.06a	89.82±1.29b	50.16±5.28a	97.53±1.43b
pH_{CEC}	4.4±0.1	4.1±0.0	3.9±0.0	4.4±0.1

BS is base saturation; depth with different letters implies significant difference at α=0.05; pHcec is the pH associated with the CEC solution

6.5 Potato yield

With 28 mm < potato size ≤ 50 mm, irrespective of treatment (WW-B, WW+B and FW-B), the potatoes were considered as marketable in line with Shiri-e-Janagard et al. (2009). As such, the potatoes were pooled together regardless of the size for tuber weight measurement. Figure 6.6 presents the yield parameters of potatoes under wastewater [with biochar (WW+B) or without biochar (WW-B) amendment] and freshwater (FW-B) irrigation for two years. The tuber weight per plant being a measure of the yield varied with years. In 2015, the tuber weight in FW-B was 0.83 kg/plant, whereas in 2016, the tuber weight was 0.26 kg/plant, showing a 69% decrease. Potato yield decrease had been associated with climatic factors (high temperature), agronomic factors (low quality seed) and disease infestation (Haverkort and MacKerron, 1995).

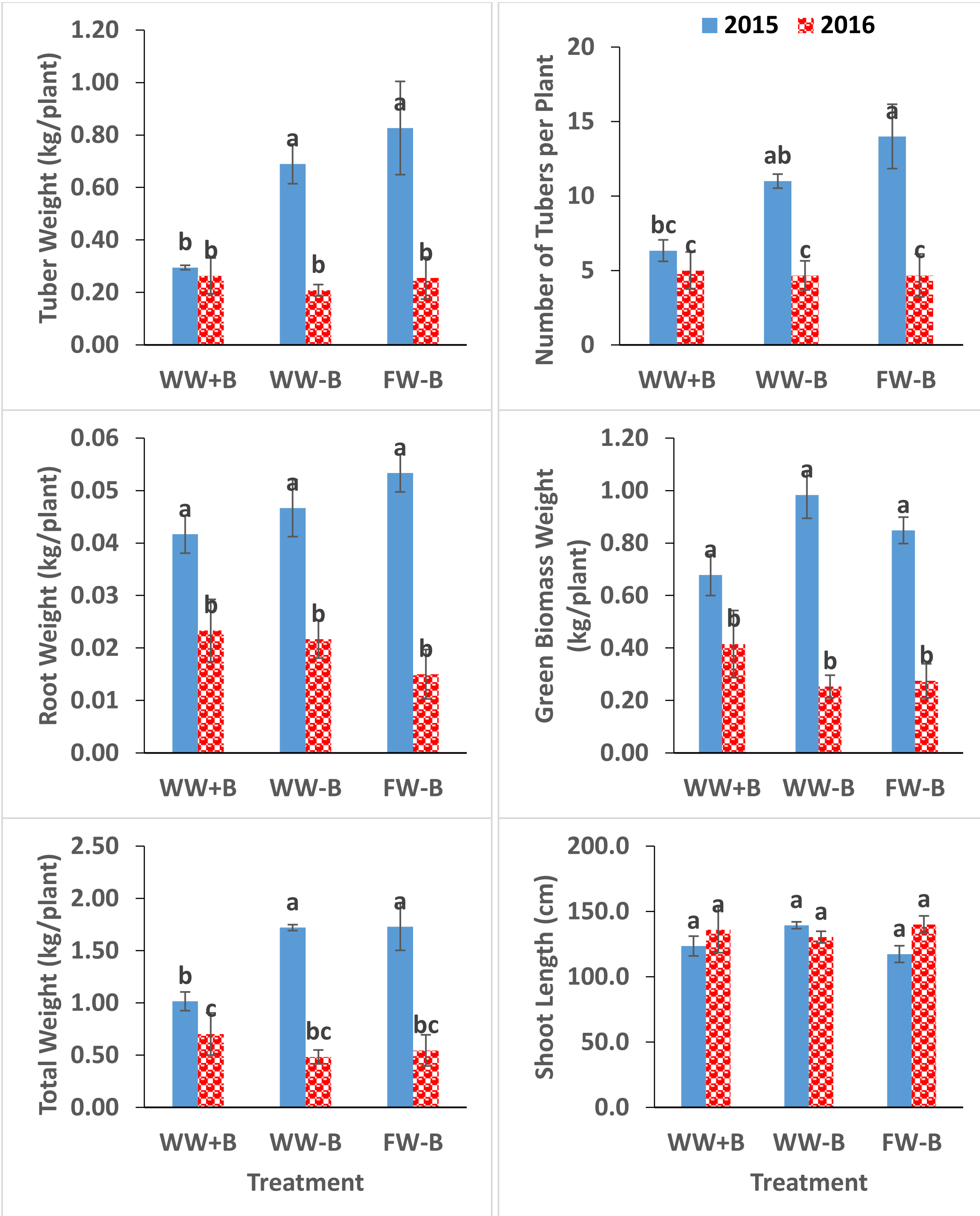

Figure 6-6. Yield parameters of potatoes under wastewater [with (WW+B) and without (WW-B) biochar amendment] and freshwater (FW-B) irrigation for two years. The error bars represent standard error. Bars with the same letters are not significantly different (α=0.05)

Since the potato seeds used in both years were sourced from the same place, and since disease infestation was not noticed for both years, the last two possible causes were not responsible for the yield drop. Therefore, climatic factors could be responsible. Although conducted during the same period of both years (under a tent), yearly variation in climate cannot be ignored. Specifically, monthly temperature data (daily average; Table 6.5) from neighboring weather station, Sainte Anne de Bellevue (45°25'38.00" N, 73°55'45.00" W), obtained from Environment Canada (http://climate.weather.gc.ca/index_e.html) for both years (2015 and 2016) suggests differences in the mean monthly temperature, especially for August corresponding to the tuber initiation and bulking period.

Table 6.5. Climatic data for Sainte Anne de Bellevue for the months of June to October in 2015 and 2016 adapted partly from Environment Canada (http://climate.weather.gc.ca/index_e.html)

Climatic data	June		July		August		September		October	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
Max temperature (ºC)	22.2	24.3	25.9	26.8	25.2	27.3	24.0	22.6	12.7	13.7
Min temperature (ºC)	11.6	12.6	15.8	15.8	15.6	16.5	12.2	10.7	2.5	4.8
Average temperature (ºC)	17.0	18.5	20.9	21.3	20.4	21.9	18.1	16.7	7.6	9.3
E_{pan} (mm day^{-1})	2.9	3.6	2.5	2.8	2.8	3.0	2.6	2.1	n.a.	n.a.
K_{pan}	0.80	0.75	0.80	0.80	0.80	0.80	0.80	0.80	n.a.	n.a.
ET_o (mm day^{-1})	2.4	2.7	2.0	2.2	2.3	2.4	2.1	1.7	n.a.	n.a.

E_{pan} is pan evaporation; K_{pan} is pan coefficient; and ET_o is reference evapotranspiration. The K_{pan} was estimated according to Doorenbos and Pruitt (1975) using hourly relative humidity and wind speed data from Environment Canada.

Although in a simulated study, Ingram and McCloud (1984) reported 80% drop in tuber yield as temperature increased from 22 to 25ºC. Temperature increase—as in this study—affected partitioning of assimilates between foliage and tuber (Haverkort and MacKerron, 1995; Ingram and McCloud, 1984; Timlin et al., 2006). Moreover, it is on record that temperature increase delayed tuber initiation (Haverkort and MacKerron, 1995; Timlin et al., 2006), which perhaps could have affected the potato yield. Evidently, the temperature increase was supported by reference evapotranspiration measured for both years, which increased with temperature increase with the exception of September (Table 6.5). This observation was not peculiar to FW-B treatment as WW-B showed almost similar (70%) decrease as well. There was no significant difference in tuber weight between the wastewater irrigated potatoes and their freshwater counterpart for both the years. This is quite important given the scarcity of freshwater, especially in arid and semiarid regions. Irrigating with wastewater would obviously serve as an alternative while maintaining same yield. For the biochar-amended treatment (WW+B), the tuber weight in 2015 was quite less as compared to the WW-B treatment. Notably, in 2015, when biochar (1% w/w) was added to the soil, there was delayed germination in all the biochar-amended treatment, which resulted in transplanting of the potatoes (planted on same day in extra lysimeters) to the biochar amended treatments. The biochar's ash content was relatively high (77.45%); however, given the biochar application rate (1% w/w of soil), this translates to <8 g (ash) kg^{-1} (soil)—a rate that did not affect the germination of rapeseed (Brassica *napus* L.) (Nabeela et al., 2015). Moreover, in 2016, the delayed germination was not noticed as all the potatoes germinated and emerged typically at the same time; there was no treatment effect in the tuber weight across treatments. Thus, the delayed germination in 2015 could be a mere happenstance, and it probably explains the less yield noticed in WW+B as compared to the other treatments (WW-B and FW-B). When crops, including potatoes, are transplanted, they are subjected to shock, which affects their agronomic cycle (Rowell et al., 1986; Shonnard and Peloquin, 1991). Having increased the soil's CEC, it was expected that the biochar would improve potato's yield, however it was not so probably because the cation exchange favoured the contaminant adsorption and not nutrient availability. Koga et al. (2017) had similar observations where wood derived biochar, having improved the porosity of soil, showed no significant effect on potato tuber yield.

Like tuber weight, the average number of tubers varied with years. In 2015, it was 14 in FW-B, while in 2016 it was 5, signifying a 64% decrease. Similarity in the decrease between the number of tubers and the tuber weights is a clear indication of the effect of growing season on the yield. A similar trend was observed in WW-B. There was no significant difference ($p>0.05$) between the number of tubers produced that received either freshwater or wastewater irrigation. Although the number of tubers in biochar treatment was significantly less in 2015; however, in 2016 (with no germination delay noticed), the number of tubers were similar in WW+B as compared to the other treatments (WW-B and FW-B). This was expected given the no treatment effect noticed in the plant health parameters as well as the yield.

Overall, the green biomass and root weight, which represents the non-edible part of the potatoes, were statistically the same across treatments, including year 2015 when WW+B showed significantly less yield than WW-B (Figure 6.6). Again, it could be that the delayed germination, which perhaps shortened the cropping days (120) of the potatoes (Russet Burbank) used in this experiment, affected the potato growth stages, especially tuber initiation, bulking and maturation, which occur towards the later part of potato growth (He et al., 2012). Potatoes, after planting, have five developmental stages, which are sprout development, vegetative growth, tuber initiation, tuber bulking and maturation (Johnson and Powelson, 2008). Shortened cultivation days—as noticed in this study—had no effect on potato biomass production, which occurs at the early growth stage (He et al., 2012). Moreover, as stated previously, in 2016 with no germination delay, the green biomass and root weight as well as tuber weight were typically similar across treatments (i.e., FW-B, WW-B and WW+B). Although some studies have revealed that biochar improves crop yield (Uzoma et al., 2011a), however, our yield results in addition to the plant health parameters such as greenness and stomatal conductance, measured in 2016, did not indicate any effect. Since biochar's agronomic effects are feedstock specific (Alburquerque et al., 2013), plantain peel biochar did not improve potato yield. Plantain peel biochar is more likely to improve the yield of potatoes with increase in application rate as observed by Uzoma et al. (2011b), therefore requires further investigations. Mixing the biochar with soil increased the soil's pH by 0.7 and then maintained it for two years. It is expected that this would have positive effect on nutrient availability and as a result yield would increase yield parameters and/or would indicate positive impact, however it appeared that the effect was masked by sufficient availability of nutrients present in the soil. Nevertheless, the C:N ratio and the high mineral ash of the biochar coupled with the elevated CEC of the biochar amended soil suggest its potential for agricultural uses.

The shoot length, representing the length of the longest stem, remained unchanged for both years (2015 and 2016) implying that neither cultivation year nor treatment had effect on it (Figure 6.6). It appears that shoot length does not have direct relationship with tuber production.

In another study by the authors (see Chapters 3 and 5), it was, however, found that biochar amendment reduces heavy metal uptake by potato tubers, both in potato flesh and potato peels. On one hand, this is a good news that we can produce relatively healthier potatoes with biochar amendment and these yields are comparable to those obtained with freshwater irrigation. However, since there are no yield differences between with and without biochar amendment, when irrigated with wastewater, farmers may not realize anything problematic and falsely believe that the potatoes grown with wastewater irrigation contain significantly higher levels of heavy metals and they could be unhealthy to eat.

6.6 Conclusions

It was found, after two years of field investigation, that potatoes grown in a sandy soil, amended with plantain peel biochar in the top 0.1 m and irrigated with wastewater, did not show significant differences in yield than in un-amended soil either irrigated with wastewater or freshwater. Total fresh tuber weights as well as the total number of tubers were similar in all treatments. This was further confirmed by the no significant difference noticed in the plant health parameters such as leaf greenness, plant canopy reflectance, and photosynthesis and transpiration rates measured in 2016. However, the biochar showed a positive effect on the pH and the CEC of the soil. This is of interest given that the biochar, being environmentally relevant (from previous studies; Chapters 3 and 5), presents no detrimental effect on potato production and reduces uptake of wastewater borne contaminants in potatoes. It is likely that increased application rate may improve potato and other crop yields, however further investigations are needed.

6.7 Acknowledgements

The authors express gratitude to NSERC, Tertiary Education Trust Fund, Nigeria and the India-Canada Centre for Innovative Multidisciplinary Partnerships to Accelerate Community Transformation and Sustainability for providing fund for the research. The authors would also like to thank our Lab Supervisor, Ms. Sedigheh Zarayan.

Chapter 7: Summary and General Conclusions

The use of wastewater for irrigating crops appears to be a sustainable alternative to alleviating the depletion of limiting freshwater resources in the world, especially in the arid and semi-arid regions. Wastewater contains nutrients which can replenish soil fertility, thereby increasing yield as well as reducing fertilizer expenses. However, one of the biggest challenges associated with wastewater reuse, especially in developing countries where wastewater receives little or no treatment before discharge and where household and industrial effluents are not separately handled, is the presence of contaminants, including heavy metals. These contaminants could accumulate in the soil and could subsequently be taken up by plants under cultivation. To reduce contaminant translocation to the edible parts of crops, after wastewater irrigation, laboratory and field studies were conducted over two seasons.

7.1 Laboratory-Scale studies

Sorption and desorption experiments were performed to investigate the sorption behavior of heavy metals in the presence and absence of plantain peel biochar. The coefficient of distribution (Kd) and sorption-desorption efficiency of the soil and biochar-soil mix were determined following batch equilibrium studies. Additionally, the properties of biochar, such as specific surface area, pH and SEM images were determined. The following conclusions were drawn from this study:

1. The plantain peel biochar was highly alkaline with relatively low specific surface area.
2. The biochar was dominated by micropores.
3. The Kd values of the heavy metals were relatively higher in the biochar-amended soil suggesting the biochar to be a good adsorbent for co-existing heavy metals.

7.2 Field-Scale studies

The field studies were categorized into four as reported below:

7.2.1 Wastewater irrigation in potatoes – year 1

The effect of biochar, a soil conditioner and a biosorbent, on the transport of wastewater-laden heavy metals (Cd, Cr, Cu, Fe, Pb and Zn) in soil and their subsequent uptake by potato tubers and plant tissues, was investigated. The biochar, produced via gasification (450-500^{o}C for 18-25 min) was applied one-time to soil and received periodic irrigation with freshwater and synthetic wastewater. Soil samples (0.0, 0.1 and 0.3 m below), leachate, as well as plant tissues (flesh, peel, root, stem and leaf) were sampled and analyzed for the heavy metals. The following conclusions were drawn from this study:

1. Irrigating with untreated wastewater resulted in accumulation of heavy metals (Cd, Cr, Cu, Fe, Pb and Zn) in the surface of the receiving soil.
2. Apart from Zn, Pb and Fe, heavy metals were not detected at 0.1 m depth; while only Fe was detected at 0.3 m depth.
3. Over the growing season, none of the heavy metals was detected in the leachate.
4. The heavy metals translocated to all the parts of the potato plant (flesh, peel, root, stem and leaf).
5. Mixing soil with plantain peel biochar significantly adsorbed Cd and Zn in the soil, consequently reducing their uptake into the flesh of potatoes by 69% and 33%, respectively, compared to the no-biochar amended soil. Heavy metal concentrations in tuber flesh in the presence and absence of biochar were below permissible limits except for Cd.

6. Biochar amendment of the soil significantly reduced Cd and Zn in potato peel compared to that without biochar amendment.
7. The concentrations of all the heavy metals detected in flesh were much lower than in the peel, suggesting that when consuming potatoes grown under wastewater irrigation, the peel poses the greatest health risks.
8. Results suggest that the amendment of soil with plantain peel biochar could present a viable technique for reducing Cd and Zn uptake by potatoes under wastewater irrigation.

7.2.2 Wastewater irrigation in spinach

Following the results of study 1, the fate and transport of the heavy metals in wastewater irrigation of spinach, an aboveground vegetable, was studied. Spinach was planted in soil, with biochar (pyrolized at 460^{o}C for 10 min) mixed in top 0.1 m. Irrigation frequency was based on spinach's water requirement. Soil samples (0.0 and 0.1 m) and spinach tissues were collected and analyzed for the heavy metals. The following conclusions were drawn from this study:

1. While the other heavy metals were retained in the top soil, only Fe moved down in the soil profile.
2. Heavy metals translocated to different parts of spinach (leaves, stem and root), with the leaves accumulating more heavy metals as compared to the stem and root.
3. Overall, higher concentrations of the heavy metals were detected in the wastewater irrigated spinach as compared to their freshwater counterparts, especially in harvest-2 (vs. harvest-1).
4. Biochar amendment resulted in 42% reduction of Zn in spinach leaves, thus maintaining the quality under the WHO provisional maximum tolerable daily intake limit for adults as compared to the no biochar amendment. For the other heavy metals (Cd, Cu, Cr, Fe, and Pb),

there was no noticeable impact of biochar, possibly due to competition with other contaminants in the soil solution.

5. Moreover, improved soil properties such as CEC and pH were evident in soils with biochar amendment as compared to no biochar amendment.

7.2.3 Wastewater irrigation in potatoes – year 2

Maintaining the same set-up as in study 1, the field experiment was repeated for another year, without adding additional biochar to the soil. The potential of biochar as a sorbent material after one year of being mixed with the soil under wastewater irrigation was tested. The CEC and pH of the soil and soil-biochar mix were measured. The fate and transport of the heavy metals in the soil were determined from the soil sampled after all irrigations. The uptake of the heavy metals by the potato tubers and other tissues was also studied. The following conclusions were drawn from this study:

1. Aging biochar significantly increased the soil`s pH and the soil`s CEC, thereby immobilizing the soil's heavy metals (except Fe), as compared to the control with no biochar.
2. Accordingly, the heavy metals translocated to all the potato parts (flesh, peel, root, stem and leaves).
3. While the concentration of the heavy metals were relatively low in potato parts under freshwater (flesh and peel), concentrations in the wastewater irrigated potatoes (flesh, peel, root, stem and leaves) were relatively high.
4. Biochar amendment, after the second year of being there in the soil, significantly reduced ($p<0.05$) the concentration of Cd, Cu, Cr, Pb and Zn in the edible flesh. Therefore, for two

years of potato cultivation with untreated wastewater, biochar has been demonstrated as a viable sorbent for immobilizing wastewater-laden heavy metals.

7.2.4 Effect of wastewater irrigation on crop yield

While implementing biochar amendment as a technique for immobilization of heavy metals and improving the quality of crops, it was necessary to investigate how crop yield (e.g. potato) is affected by wastewater irrigation and biochar amendment for the two consecutive seasons. For this, tuber weight, average number of tubers, biomass weight as well as plant health parameters were collected. The following conclusions were drawn from this study:

1. Potato yield under freshwater and wastewater irrigation were similar. This can be alarming to some degree as the farmer may be getting the expected yield but with unhealthy potatoes, and not realizing this at all.
2. Overall, biochar amendment showed no noticeable effect on the yield.
3. There was no treatment effect in the plant health parameters, such as leaf greenness, plant canopy reflectance, and photosynthesis and transpiration rates.
4. Biochar showed a positive effect on the pH and the CEC of the soil. This is of interest given that the biochar, being environmentally relevant (see Studies 1 and 3), presents no detrimental effect on potato production and reduces uptake of wastewater borne contaminants in potatoes. It is likely that increased application rate may improve potato and other crop yields, however further investigations are needed.

Chapter 8: Contributions to Knowledge and Recommendations for Future Studies

This study has led to the following contributions to knowledge:

1. To the best of author's knowledge, this study is the first of its kind to investigate the effect of plantain peel biochar as soil amendment on the remediation of several co-existing heavy metals from untreated wastewater irrigation. Reusing agricultural wastes (e.g. plantain peels) by converting them to biochar via gasification or pyrolysis presents a promised solution to countries where plantain is majorly cultivated. The study showed the increased environmental values of reusing the waste in agricultural practices as powerful tool for the remediation of heavy metals.
2. Reusing untreated wastewater for irrigation is a common practice in many developing countries. This study questioned the safety aspects of this practice by growing potatoes and spinach with wastewater, coupled with adding plantain peel biochar as amendment and sorbent. Results showed higher retention of heavy metals in soil and consequently lesser heavy metal residues in the tubers. Interestingly, the concentrations of heavy metals were higher in the peels as compared to the flesh with significant reduction in the concentrations of Cd, Cr, Cu, Pb and Zn in the flesh; suggesting peeling potatoes as safe measure when consuming potatoes irrigated with untreated wastewater.
3. Plantain peel biochar`s function as a sorbent is crop-dependent. It reduces Cd and Zn in potato peel/flesh, whereas it reduces only Zn in spinach leaves.
4. The study has revealed that the plantain peel biochar is stable in soil and would potentially be more effective with aging.

5. Laboratory sorption-desorption studies indicated the high affinity of plantain peel biochar amended soil to heavy metals. These findings were confirmed by field experiments where biochar provided high retention of heavy metals when coexisting with other contaminants in the wastewater. This can help in providing decision-making tool, in the form of a model, for the prediction of the fate of heavy metals in untreated wastewater. Therefore, it could serve as agricultural decision making tool to ensure the safety measures of crops irrigated with poorly treated or untreated wastewater.

Suggestions for further research

Based on the work done in this study, the following points are suggested for future research:

i) The application rate of the biochar to identify the optimum rate needed for both agronomic and environmental performance should be studied.

ii) The effect of plant root exudates on the behavior of biochar as a sorbent should be investigated.

iii) The study should be done on other soils with different soil texture and organic matter content.

iv) Investigations should be made to develop “designer” biochar that can do the specific job at hand more effectively and efficiently.

v) The effectiveness of biochar could be evaluated over an extended period of time in order to understand the potential of biochar as soil amendment, especially in situations where wastewater is used for irrigation.

Chapter 9: References

Abdu N., Abdulkadir A., Agbenin J.O., Buerkert A. Vertical distribution of heavy metals in wastewater-irrigated vegetable garden soils of three West African cities. Nutrient Cycling in Agroecosystems 2011; 89: 387-397.

Abit S.M., Bolster C.H., Cai P., Walker S.L. Influence of feedstock and pyrolysis temperature of biochar amendments on transport of Escherichia coli in saturated and unsaturated soil. Environmental Science and Technology 2012; 46: 8097-8105.

Abollino O., Aceto M., Malandrino M., Sarzanini C., Mentasti E. Adsorption of heavy metals on Na-montmorillonite. Effect of pH and organic substances. Water Research 2003; 37: 1619-1627.

Aboulhassan M.A., Souabi S., Yaacoubi A., Baudu M. Removal of surfactant from industrial wastewaters by coagulation flocculation process. Int. J. Environ. Sci. Technol. International Journal of Environmental Science & Technology 2006; 3: 327-332.

Adiloğlu S., Turgut Sağlam M., Adiloğlu A., Süme A. Phytoremediation of nickel (Ni) from agricultural soils using canola (Brassica napus L.). Desalination and Water Treatment 2016; 57: 2383-2388.

Agarry S., Owabor C., Ajani A. Modified plantain peel as cellulose-based low-cost adsorbent for the removal of 2, 6-dichlorophenol from aqueous solution: adsorption isotherms, kinetic modeling, and thermodynamic studies. Chemical Engineering Communications 2013; 200: 1121-1147.

Ahmad A., Ghufran R., Zularisam A.W. Phytosequestration of metals in selected plants growing on a contaminated Okhla industrial areas, Okhla, New Delhi, India. Water, Air, & Soil Pollution 2011; 217: 255-266.

Akhtar S.S., Andersen M.N., Liu F. Biochar mitigates salinity stress in potato. Journal of Agronomy and Crop Science 2015a; 201: 368-378.

Akhtar S.S., Andersen M.N., Liu F. Residual effects of biochar on improving growth, physiology and yield of wheat under salt stress. Agricultural Water Management 2015b; 158: 61-68.

Al-Nakshabandi G.A., Saqqar M.M., Shatanawi M.R., Fayyad M., Al-Horani H. Some environmental problems associated with the use of treated wastewater for irrigation in Jordan. Agricultural Water Management 1997; 34: 81-94.

Alburquerque J.A., Calero J.M., Barrón V., Torrent J., del Campillo M.C., Gallardo A., et al. Effects of biochars produced from different feedstocks on soil properties and sunflower growth. Journal of Plant Nutrition and Soil Science 2014; 177: 16-25.

Alburquerque J.A., Salazar P., Barrón V., Torrent J., Del Campillo M.D.C., Gallardo A., et al. Enhanced wheat yield by biochar addition under different mineral fertilization levels. Agronomy for Sustainable Development 2013; 33: 475-484.

Alia N., Sardar K., Said M., Salma K., Sadia A., Sadaf S., et al. Toxicity and bioaccumulation of heavy metals in spinach (Spinacia oleracea) grown in a controlled environment. International Journal of Environmental Research and Public Health 2015; 12: 7400-7416.

Alling V., Hale S.E., Martinsen V., Mulder J., Smebye A., Breedveld G.D., et al. The role of biochar in retaining nutrients in amended tropical soils. Journal of Plant Nutrition and Soil Science 2014.

Alloway B.J. Heavy metals in soil: trace metals and metalloids in soil and their bioavailability. Reading, UK: Springer 2013a.

Alloway B.J. Sources of heavy metals and metalloids in soils. In: Alloway BJ, editor. Heavy Metals in Soils: Trace Metals and Metalloids in Soils and their Bioavailability. Springer Netherlands, Dordrecht, 2013b, pp. 11-50.

American Public Health Association. Standard methods for the examination of water and wastewater. 3030 preliminary treatment of samples*#(67). The American Public Health Association (APHA), the American Water Works Association (AWWA), and the Water Environment Federation (WEF), Washington, DC, USA, 2005.

Amziane S., Collet F. Bio-aggregates based building materials: state-of-the-art report of the RILEM Technical Committee 236-BBM. Vol 23: Springer, 2017.

Andersson A., Nilsson K. Influence of lime and soil pH on Cd availability to plants. Ambio 1974: 198-200.

Angin D., Şensöz S. Effect of pyrolysis temperature on chemical and surface properties of biochar of rapeseed (Brassica napus L.). International Journal of Phytoremediation 2014; 16: 684-693.

Angin I., Yaganoglu A.V., Turan M. Effects of long-term wastewater irrigation on soil properties. Journal of Sustainable Agriculture 2005; 26: 31-42.

Antonious G.F., Dennis S.O., Unrine J.M., Snyder J.C. Ascorbic acid, β-carotene, sugars, phenols, and heavy metals in sweet potatoes grown in soil fertilized with municipal sewage sludge. Journal of Environmental Science and Health - Part B Pesticides, Food Contaminants, and Agricultural Wastes 2011; 46: 112-121.

Antonious G.F., Snyder J.C. Accumulation of heavy metals in plants and potential phytoremediation of lead by potato, Solanum tuberosum L. Journal of Environmental Science and Health, Part A 2007a; 42: 811-816.

Antonious G.F., Snyder J.C. Accumulation of heavy metals in plants and potential phytoremediation of lead by potato, Solanum tuberosum L. J Environ Sci Health A Tox Hazard Subst Environ Eng 2007b; 42: 811-6.

Aquasat. Water withdrawal by sector, around 2007. 2017, 2014.

Arora M., Kiran B., Rani S., Rani A., Kaur B., Mittal N. Heavy metal accumulation in vegetables irrigated with water from different sources. Food Chemistry 2008; 111: 811-815.

Artiola J.F., Rasmussen C., Freitas R. Effects of a biochar-amended alkaline soil on the growth of romaine lettuce and bermudagrass. Soil Science 2012; 177: 561-570.

Ayawei N., Ebelegi A.N., Wankasi D. Modelling and interpretation of adsorption isotherms. Journal of Chemistry 2017; 2017.

Aydin M.E., Aydin S., Beduk F., Tor A., Tekinay A., Kolb M., et al. Effects of long-term irrigation with untreated municipal wastewater on soil properties and crop quality. Environmental Science and Pollution Research 2015; 22: 19203-19212.

Bahmanyar M.A. Cadmium, nickel, chromium, and lead levels in soils and vegetables under long-term irrigation with industrial wastewater. Communications in Soil Science and Plant Analysis 2008; 39: 2068-2079.

Balendres M.A., Nichols D.S., Tegg R.S., Wilson C.R. Metabolomes of potato root exudates: compounds that stimulate resting spore germination of the soil-borne pathogen Spongospora subterranea. Journal of Agricultural and Food Chemistry 2016; 64: 7466-7474.

Beesley L., Marmiroli M. The immobilisation and retention of soluble arsenic, cadmium and zinc by biochar. Environmental Pollution 2011; 159: 474-480.

Bélanger G., Walsh J.R., Richards J.E., Milburn P.H., Ziadi N. Critical nitrogen curve and nitrogen nutrition index for potato in Eastern Canada. American Journal of Potato Research 2001; 78: 355-364.
Bergqvist C., Herbert R., Persson I., Greger M. Plants influence on arsenic availability and speciation in the rhizosphere, roots and shoots of three different vegetables. Environmental Pollution 2014; 184: 540-546.
Bian R., Chen D., Liu X., Cui L., Li L., Pan G., et al. Biochar soil amendment as a solution to prevent Cd-tainted rice from China: Results from a cross-site field experiment. Ecological Engineering 2013; 58: 378-383.
Blackhurst B.M., Hendrickson C., Vidal J.S.i. Direct and indirect water withdrawals for U.S. industrial sectors. Environmental Science & Technology 2010; 44: 2126-2130.
Bogusz A., Oleszczuk P., Dobrowolski R. Adsorption and desorption of heavy metals by the sewage sludge and biochar-amended soil. Environmental Geochemistry and Health 2017.
Bohl W.H., Nolte P., Kleinkopf G.E., Thornton M.K. Potato seed management: seed size and age: University of Idaho, College of Agriculture, Cooperative Extension System, Agricultural Experiment Station, 1995.
Bolan N.S., Adriano D.C., Natesan R., Koo B.J. Effects of organic amendments on the reduction and phytoavailability of chromate in mineral soil. Journal of Environmental Quality 2003; 32: 120-128.
Borchard N., Siemens J., Ladd B., Möller A., Amelung W. Application of biochars to sandy and silty soil failed to increase maize yield under common agricultural practice. Soil and Tillage Research 2014; 144: 184-194.
Brewer G.J. The risks of copper toxicity contributing to cognitive decline in the aging population and to Alzheimer's disease. Journal of the American College of Nutrition 2009; 28: 238-242.
Brown C.R. Antioxidants in potato. American Journal of Potato Research 2005; 82: 163-172.
Bruun E.W., Petersen C.T., Hansen E., Holm J.K., Hauggaard-Nielsen H. Biochar amendment to coarse sandy subsoil improves root growth and increases water retention. Soil Use and Management 2014; 30: 109-118.
Butnan S., Deenik J.L., Toomsan B., Antal M.J., Vityakon P. Biochar characteristics and application rates affecting corn growth and properties of soils contrasting in texture and mineralogy. Geoderma 2015; 237: 105-116.
Cabrera A., Cox L., Spokas K., Hermosín M.C., Cornejo J., Koskinen W.C. Influence of biochar amendments on the sorption–desorption of aminocyclopyrachlor, bentazone and pyraclostrobin pesticides to an agricultural soil. Science of The Total Environment 2014; 470–471: 438-443.
Canadian Food Inspection Agency CFIA. Seed potato crop inspection. 2017, 2017.
Carter M.R., Gregorich E.G. Soil sampling and methods of analysis. [Pinawa, Manitoba]; Boca Raton, FL: Canadian Society of Soil Science ; CRC Press, 2008.
Chandra S., Lata H., Khan I.A., Elsohly M.A. Photosynthetic response of Cannabis sativa L. to variations in photosynthetic photon flux densities, temperature and CO2 conditions. Physiol Mol Biol Plants 2008; 14: 299-306.
Chandrasekara A., Josheph Kumar T. Roots and tuber crops as functional foods: a review on phytochemical constituents and their potential health benefits. International Journal of Food Science 2016; 2016: 15.

Chen B., Chen Z. Sorption of naphthalene and 1-naphthol by biochars of orange peels with different pyrolytic temperatures. Chemosphere 2009; 76: 127-133.
Chen J.P. Decontamination of heavy metals : processes, mechanisms, and applications. Baton Rouge, UNITED STATES: Chapman and Hall/CRC, 2012.
Chen T., Zhang Y., Wang H., Lu W., Zhou Z., Zhang Y., et al. Influence of pyrolysis temperature on characteristics and heavy metal adsorptive performance of biochar derived from municipal sewage sludge. Bioresource Technology 2014; 164: 47-54.
Chen Y., Wang C., Wang Z., Huang S. Assessment of the contamination and genotoxicity of soil irrigated with wastewater. Plant and Soil 2004; 261: 189-196.
Cheng C., Lehmann J., Thies J.E., Burton S.D., Engelhard M.H. Oxidation of black carbon by biotic and abiotic processes. Organic Geochemistry 2006; 37: 1477-1488.
Chowdhury Z., Karim M., Aqeel Ashraf M., Khalisanni K. Influence of carbonization temperature on physicochemical properties of biochar derived from slow pyrolysis of durian wood (Durio zibethinus) sawdust. Vol 11, 2016.
Chuan M.C., Shu G.Y., Liu J.C. Solubility of heavy metals in a contaminated soil: Effects of redox potential and pH. Water, Air, and Soil Pollution 1996; 90: 543-556.
Clothier B.E., Green S.R. Rootzone processes and the efficient use of irrigation water. Agricultural Water Management 1994; 25: 1-12.
Codex Alimentarius Commission. Codex general standard for contaminants and toxins in food and feed. Codex stan. 193, 1995.
Colborn T., vom Saal F.S., Soto A.M. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environmental Health Perspectives 1993; 101: 378-384.
Collins H.P., Streubel J., Alva A., Porter L., Chaves B. Phosphorus uptake by potato from biochar amended with anaerobic digested dairy manure effluent. Agronomy Journal 2013; 105: 989-998.
Connor R., Renata A., Ortigara C., Koncagül E., Uhlenbrook S., Lamizana-Diallo B.M., et al. The United Nations World Water Development Report 2017. Wastewater: The Untapped Resource. The United Nations World Water Development Report 2017.
Consortium P.G.S. Genome sequence and analysis of the tuber crop potato. Nature 2011; 475: 189-195.
Coumar M.V., Parihar R.S., Dwivedi A.K., Saha J.K., Lakaria B.L., Biswas A.K., et al. Pigeon pea biochar as a soil amendment to repress copper mobility in soil and its uptake by spinach. BioResources 2016a; 11: 1585-1595.
Coumar M.V., Parihar R.S., Dwivedi A.K., Saha J.K., Rajendiran S., Dotaniya M.L., et al. Impact of pigeon pea biochar on cadmium mobility in soil and transfer rate to leafy vegetable spinach. Environmental monitoring and assessment 2016b; 188: 31.
Cross A., Sohi S.P. The priming potential of biochar products in relation to labile carbon contents and soil organic matter status. Soil Biology and Biochemistry 2011; 43: 2127-2134.
Cui H., Fan Y., Fang G., Zhang H., Su B., Zhou J. Leachability, availability and bioaccessibility of Cu and Cd in a contaminated soil treated with apatite, lime and charcoal: A five-year field experiment. Ecotoxicology and Environmental Safety 2016; 134: 148-155.
Cui L., Li L., Zhang A., Pan G., Bao D., Chang A. Biochar amendment greatly reduces rice Cd uptake in a contaminated paddy soil: A two-year field experiment. BioResources 2011; 6: 2605-2618.

da Fonseca A.F., Melfi A.J., Montes C.R. Maize growth and changes in soil fertility after irrigation with treated sewage effluent. I. plant dry matter yield and soil nitrogen and phosphorus availability. Communications in Soil Science and Plant Analysis 2005; 36: 1965-1981.

Dang V.M., Joseph S., Van H.T., Mai T.L.A., Duong T.M.H., Weldon S., et al. Immobilization of heavy metals in contaminated soil after mining activity by using biochar and other industrial by-products: the significant role of minerals on the biochar surfaces. Environmental Technology 2018: 1-16.

Dechassa N., Schenk M.K. Exudation of organic anions by roots of cabbage, carrot, and potato as influenced by environmental factors and plant age. Journal of Plant Nutrition and Soil Science 2004; 167: 623-629.

Degryse F., Verma V.K., Smolders E. Mobilization of Cu and Zn by root exudates of dicotyledonous plants in resin-buffered solutions and in soil. Plant and Soil 2008; 306: 69-84.

Devi C.B., Nandakishore T., Sangeeta N., Basar G., Devi N., Jamir S., et al. Zinc in human health. Journal of Dental and Medical Sciences 2014; 13: 18-23.

Doorenbos J., Pruitt W.O. Guidelines for predicting crop water requirements. Food and Agriculture Organization of the United Nations. Rome, Irrigation and Drainage Paper. 1975; 24.

Downie A., Crosky A., Munroe P. Physical properties of biochar. Biochar for environmental management: Science and technology 2009: 13-32.

Dvorak P., Tlustos P., Száková J., Cerny J., Balik J. Distribution of soil fractions of zinc and its uptake by potatoes, maize, wheat and barley after soil amendment by sludge and inorganic Zn salt. Plant Soil and Environment 2003; 49: 203-212.

Echeverría J.C., Morera M.T., Mazkiarán C., Garrido J.J. Competitive sorption of heavy metal by soils. Isotherms and fractional factorial experiments. Environmental Pollution 1998; 101: 275-284.

Elliott H.A., Liberati M.R., Huang C.P. Competitive Adsorption of Heavy Metals by Soils1. Journal of Environmental Quality 1986; 15: 214-219.

ElSayed E.M., Prasher S.O., Patel R.M. Effect of nonionic surfactant Brij 35 on the fate and transport of oxytetracycline antibiotic in soil. Journal of Environmental Management 2013; 116: 125-134.

Enders A., Hanley K., Whitman T., Joseph S., Lehmann J. Characterization of biochars to evaluate recalcitrance and agronomic performance. Bioresource Technology 2012; 114: 644-653.

Eshel A., Beeckman T. Plant roots: the hidden half: CRC press, 2013.

FAO. Food and Agriculture Organization of the United Nations. FAOSTAT database, Rome, Italy, 2017.

Farahat E., Linderholm H.W. The effect of long-term wastewater irrigation on accumulation and transfer of heavy metals in Cupressus sempervirens leaves and adjacent soils. Science of The Total Environment 2015; 512–513: 1-7.

Fellet G., Marmiroli M., Marchiol L. Elements uptake by metal accumulator species grown on mine tailings amended with three types of biochar. Science of The Total Environment 2014; 468–469: 598-608.

Fontecave M., Pierre J.L. Iron: metabolism, toxicity and therapy. Biochimie 1993; 75: 767-773.

Fontes M.P.F., Gomes P.C. Simultaneous competitive adsorption of heavy metals by the mineral matrix of tropical soils. Applied Geochemistry 2003; 18: 795-804.

Food and Agriculture Organization FAO. The potato: cultivation, 2008.

Fosmire G.J. Zinc toxicity. The American journal of clinical nutrition 1990; 51: 225-227.

Fraga C.G., Oteiza P.I. Iron toxicity and antioxidant nutrients. Toxicology 2002; 180: 23-32.

Frossard E., Bucher M., Mächler F., Mozafar A., Hurrell R. Potential for increasing the content and bioavailability of Fe, Zn and Ca in plants for human nutrition. Journal of the Science of Food and Agriculture 2000; 80: 861-879.

Gai X., Wang H., Liu J., Zhai L., Liu S., Ren T., et al. Effects of feedstock and pyrolysis temperature on biochar adsorption of ammonium and nitrate. PLoS ONE 2014; 9: e113888.

Gautam D.K., Bajracharya R.M., Sitaula B.K. Effects of biochar and farm yard manure on soil properties and crop growth in an agroforestry system in the Himalaya. 2017 2017; 6.

Gichner T., Patková Z., Száková J., Demnerová K. Toxicity and DNA damage in tobacco and potato plants growing on soil polluted with heavy metals. Ecotoxicology and Environmental Safety 2006; 65: 420-426.

Gokhale S.V., Jyoti K.K., Lele S.S. Kinetic and equilibrium modeling of chromium (VI) biosorption on fresh and spent Spirulina platensis/Chlorella vulgaris biomass. Bioresource Technology 2008; 99: 3600-3608.

Gomes P.C., Fontes M.P.F., da Silva A.G., de S. Mendonça E., Netto A.R. Selectivity sequence and competitive adsorption of heavy metals by Brazilian soils. Soil Science Society of America Journal 2001; 65: 1115-1121.

Gupta N., Khan D.K., Santra S.C. Heavy metal accumulation in vegetables grown in a long-term wastewater-irrigated agricultural land of tropical India. Environmental Monitoring and Assessment 2012; 184: 6673-6682.

Haase T., Schüler C., Heß J. The effect of different N and K sources on tuber nutrient uptake, total and graded yield of potatoes (Solanum tuberosum L.) for processing. EURAGR European Journal of Agronomy 2007; 26: 187-197.

Haberhauer G., Rafferty B., Strebl F., Gerzabek M.H. Comparison of the composition of forest soil litter derived from three different sites at various decompositional stages using FTIR spectroscopy. Geoderma 1998; 83: 331-342.

Hartley W., Edwards R., Lepp N.W. Arsenic and heavy metal mobility in iron oxide-amended contaminated soils as evaluated by short- and long-term leaching tests. Environmental Pollution 2004; 131: 495-504.

Haverkort A.J., MacKerron D.K.L. Potato ecology and modelling of crops under conditions limiting growth : Proceedings of the Second International Potato Modeling Conference, held in Wageningen 17-19 May, 1994. 1995.

Havre G.N., Underdal B. Lead contamination of vegetation grown close to roads. Acta Agriculturae Scandinavica 1976; 26: 18-24.

He Q.B., Singh B.R. Effect of organic matter on the distribution, extractability and uptake of cadmium in soils. Journal of Soil Science 1993; 44: 641-650.

He Z., Larkin R.P., Honeycutt W. Sustainable potato production : global case studies. 2012.

Heine P. Food culture in the Near East, Middle East, and North Africa. Westport, Conn.: Greenwood Press, 2004.

Hernandez-Soriano M.C., Kerré B., Kopittke P.M., Horemans B., Smolders E. Biochar affects carbon composition and stability in soil: a combined spectroscopy-microscopy study. Scientific reports 2016; 6: 25127-25127.
Hettiarachchi H., Caucci S., Ardakanian R. Safe use of wastewater in agriculture: the golden example of nexus approach. In: Hettiarachchi H, Ardakanian R, editors. Safe Use of Wastewater in Agriculture: From Concept to Implementation. Springer International Publishing, Cham, 2018, pp. 1-11.
Hien T.T.T., Shinogi Y., Mishra A., Viet D.D. The effect of bamboo Biochar on Crop's productivity and quality in the field condition. Journal of the Faculty of Agriculture, Kyushu University 2017; 62: 477-482.
Hollinger S.E., Isard S.A. A soil moisture climatology of Illinois. Journal of Climate 1994; 7: 822-833.
Huang X., Lin J., Yuan D., Hu R. Determination of steroid sex hormones in wastewater by stir bar sorptive extraction based on poly(vinylpyridine-ethylene dimethacrylate) monolithic material and liquid chromatographic analysis. Journal of Chromatography A 2009; 1216: 3508-3511.
Hussain A., Alamzeb S., Begum S. Accumulation of heavy metals in edible parts of vegetables irrigated with waste water and their daily intake to adults and children, District Mardan, Pakistan. Food chemistry 2013; 136: 1515-1523.
Hussain M., Farooq M., Nawaz A., Al-Sadi A.M., Solaiman Z.M., Alghamdi S.S., et al. Biochar for crop production: potential benefits and risks. Journal of Soils and Sediments 2017; 17: 685-716.
Idowu S.O., Oni S.O., Adejumo A.A. Biosorption of chromium (VI) from aqueous solution by biomass of plantain (Musa Paradisiaca) peel residue. Afr J Med Phy, Biomed Eng & Sc. 2011; 3: 22-27.
Inam E.I., Etim U.J., Akpabio E.G., Umoren S.A. Simultaneous adsorption of lead (II) and 3,7-Bis(dimethylamino)-phenothiazin-5-ium chloride from aqueous solution by activated carbon prepared from plantain peels. Desalination and Water Treatment 2016; 57: 6540-6553.
Ingram K.T., McCloud D.E. Simulation of potato crop growth and development1. Crop Science 1984; 24: 21-27.
International Institute of Tropical Agriculture. Banana & plantain. 2016, 2009.
Inyang M.I., Gao B., Yao Y., Xue Y., Zimmerman A., Mosa A., et al. A review of biochar as a low-cost adsorbent for aqueous heavy metal removal. Critical Reviews in Environmental Science and Technology 2015: 1-28.
J. Rawls W., L. Brakensiek D., E. Saxtonn K. Estimation of soil water properties. Transactions of the ASAE 1982; 25: 1316.
Jaramillo M., Restrepo I. Wastewater Reuse in Agriculture: A Review about Its Limitations and Benefits. Sustainability 2017; 9: 1734.
Järup L. Hazards of heavy metal contamination. British Medical Bulletin 2003; 68: 167-182.
Jay C.N., Fitzgerald J.D., Hipps N.A., Atkinson C.J. Why short-term biochar application has no yield benefits: Evidence from three field-grown crops. Soil Use and Management 2015; 31: 241-250.
Jiang T.-Y., Jiang J., Xu R.-K., Li Z. Adsorption of Pb(II) on variable charge soils amended with rice-straw derived biochar. Chemosphere 2012a; 89: 249-256.

Jiang T., Jiang J., Xu R., Li Z. Adsorption of Pb (II) on variable charge soils amended with rice-straw derived biochar. Chemosphere 2012b; 89: 249-256.
Johnson D.A., Powelson M.L. Potato health management. St. Paul, MN: APS Press, 2008.
Joseph S.D., Camps-Arbestain M., Lin Y., Munroe P., Chia C.H., Hook J., et al. An investigation into the reactions of biochar in soil. Soil Research 2010; 48: 501-515.
Kabata-Pendias A. Trace elements in soils and plants: CRC press, 2010.
Kabata-Pendias A., Pendias H. Trace elements in soils and plants. Vol 315: CRC press Boca Raton, 1984.
Kabenge I., Omulo G., Banadda N., Seay J., Zziwa A., Kiggundu N. Characterization of banana peels wastes as potential slow pyrolysis feedstock. Journal of Sustainable Development 2018; 11: 14.
Kamruzzaman M., Khatun S., Rakib A., Hoque M., Rani M. Temporal variation in seed quality of Indian spinach preserved in different containers. International Journal of Agricultural Research, Innovation and Technology 2016; 5: 51-57.
Kargar M., Jutras P., Clark O.G., Hendershot W.H., Prasher S.O. Trace metal contamination influenced by land use, soil age, and organic matter in Montreal tree pit soil. Journal of Environmental Quality 2013; 42: 1527-1533.
Kelly C.N., Peltz C.D., Stanton M., Rutherford D.W., Rostad C.E. Biochar application to hardrock mine tailings: Soil quality, microbial activity, and toxic element sorption. Applied Geochemistry 2014; 43: 35-48.
Keraita B., Drechsel P. Agricultural use of untreated urban wastewater in Ghana. Wastewater use in irrigated agriculture: Confronting the livelihood and environmental realities 2004: 101-112.
Khan S., Cao Q., Zheng Y.M., Huang Y.Z., Zhu Y.G. Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. Environmental Pollution 2008; 152: 686-692.
Kim Y., Steenhuis T.S., Nam K. Movement of heavy metals in soil through preferential flow paths under different rainfall intensities. CLEAN – Soil, Air, Water 2008; 36: 984-989.
Kimetu J.M., Lehmann J., Ngoze S.O., Mugendi D.N., Kinyangi J.M., Riha S., et al. Reversibility of soil productivity decline with organic matter of differing quality along a degradation gradient. Ecosystems 2008; 11: 726.
King B.A., Stark J.C. Potato irrigation management: University of Idaho, Cooperative Extension System, College of Agriculture, 1997.
Kiziloglu F.M., Turan M., Sahin U., Kuslu Y., Dursun A. Effects of untreated and treated wastewater irrigation on some chemical properties of cauliflower (Brassica olerecea L. var. botrytis) and red cabbage (Brassica olerecea L. var. rubra) grown on calcareous soil in Turkey. Agricultural Water Management 2008; 95: 716-724.
Knechtenhofer L.A., Xifra I.O., Scheinost A.C., Flühler H., Kretzschmar R. Fate of heavy metals in a strongly acidic shooting-range soil: small-scale metal distribution and its relation to preferential water flow. Journal of Plant Nutrition and Soil Science 2003; 166: 84-92.
Koga N., Shimoda S., Iwata Y. Biochar impacts on crop productivity and greenhouse gas emissions from an andosol. Journal of Environmental Quality 2017; 46: 27-35.
Kookana R.S., Sarmah A.K., Van Zwieten L., Krull E., Singh B. Biochar application to soil. agronomic and environmental benefits and unintended consequences. Advances in Agronomy. 112, 2011, pp. 103-143.

Kumar A., Joseph S., Tsechansky L., Privat K., Schreiter I.J., Schüth C., et al. Biochar aging in contaminated soil promotes Zn immobilization due to changes in biochar surface structural and chemical properties. Science of the Total Environment 2018; 626: 953-961.
Kumar Sharma R., Agrawal M., Marshall F. Heavy metal contamination of soil and vegetables in suburban areas of Varanasi, India. Ecotoxicology and Environmental Safety 2007; 66: 258-266.
Kusuma D.S., Vanhanen L.P., Savage G.P. Evaluation of extraction parameters for total oxalate determination in spinach using Design of Experiment analysis. Journal of Food Composition and Analysis 2016; 51: 9-14.
Kwofie E.M., Ngadi M. Sustainable energy supply for local rice parboiling in West Africa: The potential of rice husk. Renewable and Sustainable Energy Reviews 2016; 56: 1409-1418.
Laird D.A., Fleming P., Davis D.D., Horton R., Wang B., Karlen D.L. Impact of biochar amendments on the quality of a typical Midwestern agricultural soil. Geoderma 2010; 158: 443-449.
Lamb M.C., Sorensen R.B., Butts C.L. Crop response to biochar under differing irrigation levels in the southeastern USA. Journal of Crop Improvement 2018; 32: 305-317.
LaPara T.M., Klatt C.G., Chen R. Adaptations in bacterial catabolic enzyme activity and community structure in membrane-coupled bioreactors fed simple synthetic wastewater. Journal of Biotechnology 2006; 121: 368-380.
Lee C., Sturgis T., Landin M. Heavy metal uptake by marsh plants in hydroponic solution cultures. Journal of Plant Nutrition 1981; 3: 139-151.
Lehmann J. Bio-energy in the black. Frontiers in Ecology and the Environment 2007; 5: 381-387.
Lehmann J., Joseph S. Biochar for environmental management : science and technology. London; Sterling, VA: Earthscan, 2009.
LeRiche E.L., Wang-Pruski G., Zheljazkov V.D. Distribution of elements in potato (Solanum tuberosum L.) tubers and their relationship to after-cooking darkening. HortScience 2009; 44: 1866-1873.
Leskovar D.I., Piccinni G. Yield and leaf quality of processing spinach under deficit irrigation. HortScience 2005; 40: 1868-1870.
Leuzinger S., Vogt R., Körner C. Tree surface temperature in an urban environment. Agricultural and Forest Meteorology 2010; 150: 56-62.
Li D., Yang M., Hu J., Ren L., Zhang Y., Li K. Determination and fate of oxytetracycline and related compounds in oxytetracycline production wastewater and the receiving river. Environmental Toxicology and Chemistry 2008; 27: 80-86.
Li H., Dong X., da Silva E.B., de Oliveira L.M., Chen Y., Ma L.Q. Mechanisms of metal sorption by biochars: Biochar characteristics and modifications. Chemosphere 2017; 178: 466-478.
Li X., Zhang T., Wang X., Hua K., Zhao L., Han Z. The composition of root exudates from two different resistant peanut cultivars and their effects on the growth of soil-borne pathogen. Int J Biol Sci 2013; 9: 164-173.
Li Y., Wang Y.-b., Gou X., Su Y.-b., Wang G. Risk assessment of heavy metals in soils and vegetables around non-ferrous metals mining and smelting sites, Baiyin, China. Journal of Environmental Sciences 2006; 18: 1124-1134.

Liang Q., Gao R., Xi B., Zhang Y., Zhang H. Long-term effects of irrigation using water from the river receiving treated industrial wastewater on soil organic carbon fractions and enzyme activities. Agricultural Water Management 2014; 135: 100-108.
Lichtfouse E., Hamelin M., Navarrete M., Debaeke P. Sustainable agriculture: Springer, 2009.
Liesch T., Ohmer M. Comparison of GRACE data and groundwater levels for the assessment of groundwater depletion in Jordan. Hydrogeology Journal 2016; 24: 1547-1563.
Lin S.D., Lee C.C. Water and wastewater calculations manual (2nd Edition) (2). New York, USA, US: McGraw-Hill, 2007.
Liu C., Liu F., Ravnskov S., Rubæk G.H., Sun Z., Andersen M.N. Impact of wood biochar and its interactions with mycorrhizal fungi, phosphorus fertilization and irrigation strategies on potato growth. Journal of Agronomy and Crop Science 2017; 203: 131-145.
Liu W., Wang T., Borthwick A.G.L., Wang Y., Yin X., Li X., et al. Adsorption of Pb2+, Cd2+, Cu2+ and Cr3+ onto titanate nanotubes: competition and effect of inorganic ions. Science of The Total Environment 2013; 456: 171-180.
Liu Z., Chen X., Jing Y., Li Q., Zhang J., Huang Q. Effects of biochar amendment on rapeseed and sweet potato yields and water stable aggregate in upland red soil. Catena 2014; 123: 45-51.
Loveys B.R., Theobald J.C., Jones H.G., McCarthy M.G. An assessment of plants-based measures of grapevine performance as irrigation scheduling tools. International Society for Horticultural Science (ISHS), Leuven, Belgium, 2008, pp. 421-427.
Lu H., Zhang W., Yang Y., Huang X., Wang S., Qiu R. Relative distribution of Pb2+ sorption mechanisms by sludge-derived biochar. Water Research 2012; 46: 854-862.
Lu K., Yang X., Shen J., Robinson B., Huang H., Liu D., et al. Effect of bamboo and rice straw biochars on the bioavailability of Cd, Cu, Pb and Zn to Sedum plumbizincicola. Agriculture, Ecosystems and Environment 2014.
Macht F., Eusterhues K., Pronk G.J., Totsche K.U. Specific surface area of clay minerals: Comparison between atomic force microscopy measurements and bulk-gas (N2) and -liquid (EGME) adsorption methods. Applied Clay Science 2011; 53: 20-26.
Macomber L., Imlay J.A. The iron-sulfur clusters of dehydratases are primary intracellular targets of copper toxicity. Proceedings of the National Academy of Sciences 2009; 106: 8344-8349.
Mahar A., Wang P., Ali A., Awasthi M.K., Lahori A.H., Wang Q., et al. Challenges and opportunities in the phytoremediation of heavy metals contaminated soils: A review. Ecotoxicology and Environmental Safety 2016; 126: 111-121.
Maier C., Loos C., Hasenauer J. Robust parameter estimation for dynamical systems from outlier-corrupted data. Bioinformatics 2017; 33: 718-725.
Malandrino M., Abollino O., Giacomino A., Aceto M., Mentasti E. Adsorption of heavy metals on vermiculite: Influence of pH and organic ligands. Journal of Colloid and Interface Science 2006; 299: 537-546.
Mapanda F., Mangwayana E.N., Nyamangara J., Giller K.E. The effect of long-term irrigation using wastewater on heavy metal contents of soils under vegetables in Harare, Zimbabwe. Agriculture, Ecosystems & Environment 2005; 107: 151-165.
McBride M.B. Environmental chemistry of soils. New York: Oxford University Press, 1994.
McKenna I.M., Chaney R.L., Williams F.M. The effects of cadmium and zinc interactions on the accumulation and tissue distribution of zinc and cadmium in lettuce and spinach. Environmental Pollution 1993; 79: 113-120.

Mehlich A. Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. Communications in Soil Science and Plant Analysis 1984; 15: 1409-1416.
Meidner H., Mansfield T.A. Physiology of stomata: Tata Mcgraw-Hill Publishing Company Limited; Bombay, 1968.
Mekonnen M.M., Hoekstra A.Y. Four billion people facing severe water scarcity. Science Advances 2016; 2.
Melloul A.A., Hassani L., Rafouk L. Salmonella contamination of vegetables irrigated with untreated wastewater. World Journal of Microbiology and Biotechnology 2001; 17: 207-209.
Melo L.C.A., Puga A.P., Coscione A.R., Beesley L., Abreu C.A., Camargo O.A. Sorption and desorption of cadmium and zinc in two tropical soils amended with sugarcane-straw-derived biochar. Journal of Soils and Sediments 2016; 16: 226-234.
Milà I Canals L., Chenoweth J., Chapagain A., Orr S., Antón A., Clift R. Assessing freshwater use impacts in LCA: Part I - Inventory modelling and characterisation factors for the main impact pathways. International Journal of Life Cycle Assessment 2009; 14: 28-42.
Minotti P.L., Halseth D.E., Sieczka J.B. Field chlorophyll measurements to assess the nitrogen status of potato varieties. HortScience 1994; 29: 1497-1500.
Mohan D., Rajput S., Singh V.K., Steele P.H., Pittman C.U. Modeling and evaluation of chromium remediation from water using low cost bio-char, a green adsorbent. Journal of hazardous materials 2011; 188: 319-333.
Moreno-Jiménez E., Esteban E., Peñalosa J.M. The fate of arsenic in soil-plant systems. In: Whitacre DM, editor. Reviews of Environmental Contamination and Toxicology. Springer New York, New York, NY, 2012, pp. 1-37.
Morrison G., Fatoki O., Linder S., Lundéhn C. Determination of heavy metal concentrations and metal fingerprints of sewage sludge from eastern cape province, South Africa by inductively coupled plasma–mass spectrometry (ICP-MS) and laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS). Water, Air, and Soil Pollution 2004; 152: 111-127.
Nabeela F., Murad W., Khan I., Mian I.A., Rehman H., Adnan M., et al. Effect of wood ash application on the morphological, physiological and biochemical parameters of Brassica napus L. Plant Physiol Biochem 2015; 95: 15-25.
Namgay T., Singh B., Singh B.P. Influence of biochar application to soil on the availability of As, Cd, Cu, Pb, and Zn to maize (Zea mays L.). Australian Journal of Soil Research 2010; 48: 638-647.
Narwal R.P., Singh M., Singh J.P., Dahiya D.J. Cadmium-zinc interaction in maize grown on sewer water irrigated soil. Arid Soil Research and Rehabilitation 1993; 7: 125-131.
Nazir R., Khan M., Masab M., Rehman H.U., Rauf N.U., Shahab S., et al. Accumulation of heavy metals (Ni, Cu, Cd, Cr, Pb, Zn, Fe) in the soil, water and plants and analysis of physico-chemical parameters of soil and water collected from Tanda Dam kohat. Journal of Pharmaceutical Sciences and Research 2015; 7: 89-97.
Nelson N.O., Agudelo S.C., Yuan W., Gan J. Nitrogen and phosphorus availability in biochar-amended soils. Soil Science 2011; 176: 218-226.
Nopens I., Capalozza C., Vanrolleghem P.A. Stability analysis of a synthetic municipal wastewater. Universiteit Gent Belgium, 2001, pp. 22.

Nzediegwu C., Prasher S., Elsayed E., Dhiman J., Mawof A., Patel R. Effect of biochar on heavy metal accumulation in potatoes from wastewater irrigation. Journal of Environmental Management 2019; 232: 153-164.

Nzediegwu C., Prasher S., Mannan A., Elsayed E., Dhiman J. Role of plantain peel biochar in enhancing safe use of untreated wastewater. 22nd Hydrotechnical Conference, Montreal, 2015.

Offenbacher E.G., Pi-Sunyer F. Chromium in human nutrition. Annual review of nutrition 1988; 8: 543-563.

Ojala J., Stark J., Kleinkopf G. Influence of irrigation and nitrogen management on potato yield and quality. American Potato Journal 1990; 67: 29-43.

Opena G.B., Porter G.A. Soil management and supplemental irrigation effects on potato: II. root growth. Agronomy Journal 1999; 91: 426-431.

Palta J.P. Leaf chlorophyll content. Remote sensing reviews 1990; 5: 207-213.

Pan J., Jiang J., Xu R. Adsorption of Cr(III) from acidic solutions by crop straw derived biochars. Journal of Environmental Sciences 2013; 25: 1957-1965.

Pandit N.R., Mulder J., Hale S.E., Martinsen V., Schmidt H.P., Cornelissen G. Biochar improves maize growth by alleviation of nutrient stress in a moderately acidic low-input Nepalese soil. Science of The Total Environment 2018; 625: 1380-1389.

Park J., Ok Y.S., Kim S., Cho J., Heo J., Delaune R.D., et al. Competitive adsorption of heavy metals onto sesame straw biochar in aqueous solutions. Chemosphere 2016; 142: 77-83.

Pathak C., Chopra A.K., Srivastava S. Accumulation of heavy metals in Spinacia oleracea irrigated with paper mill effluent and sewage. Environmental Monitoring and Assessment 2013; 185: 7343-7352.

Patil V., Al-Gaadi K., Wahb-Allah M., Saleh A., Marey S., Samdani M., et al. Use of saline water for greenhouse bell pepper (Capsicum annuum) production. American Journal of Agricultural and Biological Sciences 2014; 9: 208.

Peligro F.R., Pavlovic I., Rojas R., Barriga C. Removal of heavy metals from simulated wastewater by in situ formation of layered double hydroxides. Chemical Engineering Journal 2016; 306: 1035-1040.

Petter F.A., Madari B.E. Biochar: agronomic and environmental potential in Brazilian savannah soils. Revista Brasileira de Engenharia Agrícola e Ambiental 2012; 16: 761-768.

Pietrini F., Zacchini M., Iori V., Pietrosanti L., Ferretti M., Massacci A. Spatial distribution of cadmium in leaves and its impact on photosynthesis: examples of different strategies in willow and poplar clones. Plant Biology 2010; 12: 355-363.

Prasad A.K., Chai L., Singh R.P., Kafatos M. Crop yield estimation model for Iowa using remote sensing and surface parameters. International Journal of Applied Earth Observation and Geoinformation 2006; 8: 26-33.

Puga A.P., Abreu C.A., Melo L.C.A., Beesley L. Biochar application to a contaminated soil reduces the availability and plant uptake of zinc, lead and cadmium. Journal of Environmental Management 2015; 159: 86-93.

Puga A.P., Melo L.C.A., de Abreu C.A., Coscione A.R., Paz-Ferreiro J. Leaching and fractionation of heavy metals in mining soils amended with biochar. Soil and Tillage Research 2016; 164: 25-33.

Qian T., Wang Y., Fan T., Fang G., Zhou D. A new insight into the immobilization mechanism of Zn on biochar: the role of anions dissolved from ash. Scientific Reports 2016; 6: 33630.

Qian Y.L., Mecham B. Long-term effects of recycled wastewater irrigation on soil chemical properties on Golf Course Fairways This report was financed in part by the U.S. Department of the Interior, Geological Survey, through the Colorado Water Resources Research Institute and Grant no. 01HQGR0077. The views and conclusions contained in this document are those of the authors and should not be interpreted as necessarily representing the official policies, either expressed or implied, of the U.S. government. Agronomy Journal 2005; 97: 717-721.

Raschid-Sally L., Jayakody P. Drivers and characteristics of wastewater agriculture in developing countries: Results from a global assessment. Vol 127: IWMI, 2009.

Rattan R.K., Datta S.P., Chhonkar P.K., Suribabu K., Singh A.K. Long-term impact of irrigation with sewage effluents on heavy metal content in soils, crops and groundwater—a case study. Agriculture, Ecosystems & Environment 2005; 109: 310-322.

Rayment G., Higginson F.R. Australian laboratory handbook of soil and water chemical methods: Inkata Press Pty Ltd, 1992.

Rees F., Simonnot M.O., Morel J.L. Short-term effects of biochar on soil heavy metal mobility are controlled by intra-particle diffusion and soil pH increase. European Journal of Soil Science 2014; 65: 149-161.

Richter B.D., Abell D., Bacha E., Brauman K., Calos S., Cohn A., et al. Tapped out: how can cities secure their water future? Water Policy 2013; 15: 335-363.

Robredo A., Pérez-López U., de la Maza H.S., González-Moro B., Lacuesta M., Mena-Petite A., et al. Elevated CO2 alleviates the impact of drought on barley improving water status by lowering stomatal conductance and delaying its effects on photosynthesis. Environmental and Experimental Botany 2007; 59: 252-263.

Rodríguez-Liébana J.A., Mingorance M.D., Peña A. Role of irrigation with raw and artificial wastewaters on pesticide desorption from two Mediterranean Calcareous Soils. Water Air Soil Pollut Water, Air, & Soil Pollution 2014; 225.

Rodríguez-Vila A., Covelo E.F., Forján R., Asensio V. Recovering a copper mine soil using organic amendments and phytomanagement with Brassica juncea L. Journal of Environmental Management 2015; 147: 73-80.

Ronchetti S.A., Novack G.V., Bianchi M.S., Crocco M.C., Duvilanski B.H., Cabilla J.P. In vivo xenoestrogenic actions of cadmium and arsenic in anterior pituitary and uterus. Reproduction 2016; 152: 1-10.

Rout G.R., Das P. Effect of metal toxicity on plant growth and metabolism: I. zinc. In: Lichtfouse E, Navarrete M, Debaeke P, Véronique S, Alberola C, editors. Sustainable Agriculture. Springer Netherlands, Dordrecht, 2009, pp. 873-884.

Rowell A.B., Ewing E.E., Plaisted R.L. Comparative field performance of potatoes from seedlings and tubers. American Potato Journal 1986; 63: 219-227.

Rowell M.J., Coetzee M.E. The measurement of low organic matter contents in soils. South African Journal of Plant and Soil 2003; 20: 49-53.

Roy M., McDonald L.M. Metal uptake in plants and health risk assessments in metal-contaminated smelter soils. Land Degradation & Development 2015; 26: 785-792.

Rubatzky V.E., Yamaguchi M. Plantain, starchy banana, breadfruit, and jackfruit. World Vegetables: Principles, Production, and Nutritive Values. Springer US, Boston, MA, 1997, pp. 253-276.

Sadiq Butt M., Sharif K., Ehsan Bajwa B., Aziz A. Hazardous effects of sewage water on the environment. Management of Environmental Quality: An International Journal 2005; 16: 338-346.

Saeed A., Iqbal M., Akhtar M.W. Removal and recovery of lead(II) from single and multimetal (Cd, Cu, Ni, Zn) solutions by crop milling waste (black gram husk). Journal of Hazardous Materials 2005; 117: 65-73.

Sander D. Spinach. North Carolina State University. North Carolina Cooperative Extension Service. Horticulture Information Leaflets. 2001, 2012.

Sangiumsak N., Punrattanasin P. Adsorption behavior of heavy metals on various soils. Polish Journal of Environmental Studies 2014; 23.

Sarmah A.K., Srinivasan P., Smernik R.J., Manley-Harris M., Antal Jr M.J., Downie A., et al. Retention capacity of biochar-amended New Zealand dairy farm soil for an estrogenic steroid hormone and its primary metabolite. Australian Journal of Soil Research 2010; 48: 648-658.

Schweiker C., Wagner A., Peters A., Bischoff W.A., Kaupenjohann M. Biochar reduces zinc and cadmium but not copper and lead leaching on a former sewage field. Journal of Environmental Quality 2014; 43: 1886-1893.

Scott C., Faruqui N., Raschid-Sally L. Wastewater use in irrigated agriculture : confronting the livelihood and environmental realities. Cambridge, GB: CAB International, 2004.

Semhi K., Clauer N., Chaudhuri S. Variable element transfers from an illite-rich substrate to growing plants during a three-month experiment. Applied Clay Science 2012; 57: 17-24.

Senthil Kumar P. Adsorption of lead (II) ions from simulated wastewater using natural waste: a kinetic, thermodynamic and equilibrium study. Environmental Progress & Sustainable Energy 2014; 33: 55-64.

Shamal S.A.M., Weatherhead K. Assessing spectral similarities between rainfed and irrigated croplands in a humid environment for irrigated land mapping. Outlook on Agriculture 2014; 43: 109-114.

Shiri-e-Janagard M., Tobeh A., Abbasi A., Jamaati-e-Somarin S., Hassanzadeh M., Zabihi-e-Mahmoodabad R. Effects of water stress on water demand, growth and tuber grade of potato (Solanum tuberosum L.) crop. Research Journal of Environmental Sciences 2009; 3: 476-485.

Shonnard G.C., Peloquin S.J. Performance of true potato seed families. 2. Comparison of transplants vs. seedling tubers. Potato Research 1991; 34: 409-418.

Siegel F.R. Environmental geochemistry of potentially toxic metals. Vol 32: Springer, 2002.

Sigel A., Sigel H., Sigel R.K. Cadmium: from toxicity to essentiality. Vol 11: Springer, 2013.

Sim W., Lee J., Shin S., Song K., Oh J. Assessment of fates of estrogens in wastewater and sludge from various types of wastewater treatment plants. Chemosphere 2011; 82: 1448-1453.

Singh B., Singh B.P., Cowie A.L. Characterisation and evaluation of biochars for their application as a soil amendment. Australian Journal of Soil Research 2010; 48: 516-525.

Singh K.P., Rai P., Singh A.K., Verma P., Gupta S. Occurrence of pharmaceuticals in urban wastewater of north Indian cities and risk assessment. Environ Monit Assess Environmental Monitoring and Assessment : An International Journal Devoted to Progress in the Use of Monitoring Data in Assessing Environmental Risks to Man and the Environment 2014; 186: 6663-6682.

Singh P.K., Deshbhratar P.B., Ramteke D.S. Effects of sewage wastewater irrigation on soil properties, crop yield and environment. Agricultural Water Management 2012; 103: 100-104.
Son E.B., Poo K.M., Chang J.S., Chae K.J. Heavy metal removal from aqueous solutions using engineered magnetic biochars derived from waste marine macro-algal biomass. Science of the Total Environment 2018; 615: 161-168.
Sparks D.L. Environmental soil chemistry. San Diego: Academic Press, 1995.
Sperry J.S. 5 - Limitations on stem water transport and their consequences A2 - Gartner, Barbara L. Plant Stems. Academic Press, San Diego, 1995, pp. 105-124.
Sposito G. Chemistry of soils (2). Cary, GB: Oxford University Press, USA, 2008.
Stark J.C., Westermann D.T., Hopkins B. Nutrient management guidelines for Russet Burbank potatoes: University of Idaho, College of Agricultural and Life Sciences, 2004.
Stasinos S., Zabetakis I. The uptake of nickel and chromium from irrigation water by potatoes, carrots and onions. Ecotoxicology and Environmental Safety 2013; 91: 122-128.
Ste-Marie C., Paré D. Soil, pH and N availability effects on net nitrification in the forest floors of a range of boreal forest stands. Soil Biology and Biochemistry 1999; 31: 1579-1589.
Stephan C.H., Courchesne F., Hendershot W.H., McGrath S.P., Chaudri A.M., Sappin-Didier V., et al. Speciation of zinc in contaminated soils. Environmental Pollution 2008; 155: 208-216.
Surdyk N., Cary L., Blagojevic S., Jovanovic Z., Stikic R., Vucelic-Radovic B., et al. Impact of irrigation with treated low quality water on the heavy metal contents of a soil-crop system in Serbia. Agricultural water management 2010; 98: 451-457.
Tchango Tchango J., Bikoï A., Achard R., Escalant J., Ngalani J. Plantain: post-harvest operations. Centre de Recherches Regionales sur Bananiers et Plantains, Cameroon (CRBP). Post Harvest Compendium 1999: 4-9.
Teytelboym A. Population growth. NATURE 2016; 530.
Thies J.E., Rillig M.C. Characteristics of biochar: biological properties. Biochar for environmental management: Science and technology 2009: 85-105.
Thomas D.S. Arid environments: their nature and extent. Arid Zone Geomorphology: Process, Form and Change in Drylands, Third Edition 2011: 1-16.
Timlin D., Lutfor Rahman S.M., Baker J., Reddy V.R., Fleisher D., Quebedeaux B. Whole plant photosynthesis, development, and carbon partitioning in potato as a function of temperature. Agronomy Journal 2006; 98: 1195-1203.
Trakal L., Komárek M., Száková J., Zemanová V., Tlustoš P. Biochar application to metal-contaminated soil: evaluating of Cd, Cu, Pb and Zn sorption behavior using single-and multi-element sorption experiment. Plant Soil Environ 2011; 57: 372-380.
Tressler D., Mack G., King C. Vitamin C content of vegetables. I. Spinach. Journal of Food Science 1936; 1: 3-7.
Uchimiya M., Bannon D.I., Wartelle L.H. Retention of Heavy Metals by Carboxyl Functional Groups of Biochars in Small Arms Range Soil. Journal of Agricultural and Food Chemistry 2012; 60: 1798-1809.
Uchimiya M., Chang S., Klasson K.T. Screening biochars for heavy metal retention in soil: Role of oxygen functional groups. Journal of Hazardous Materials 2011; 190: 432-441.
Uchimiya M., Lima I.M., Klasson K.T., Wartelle L.H. Contaminant immobilization and nutrient release by biochar soil amendment: Roles of natural organic matter. Chemosphere 2010a; 80: 935-940.

Uchimiya M., Lima I.M., Thomas Klasson K., Chang S., Wartelle L.H., Rodgers J.E. Immobilization of heavy metal ions (CuII, CdII, NiII, and PbII) by broiler litter-derived biochars in water and soil. Journal of Agricultural and Food Chemistry 2010b; 58: 5538-5544.

United Nations. World population projected to reach 9.8 billion in 2050, and 11.2 billion in 2100 – says UN. 2017, 2017.

Upadhyay K.P., George D., Swift R.S., Galea V. The influence of biochar on growth of lettuce and potato. Journal of Integrative Agriculture 2014; 13: 541-546.

USDA. United States standards for grades of potatoes for processing. In: United States Department of Agriculture, editor, 1983.

Uzoma K.C., Inoue M., Andry H., Fujimaki H., Zahoor A., Nishihara E. Effect of cow manure biochar on maize productivity under sandy soil condition. Soil Use and Management 2011a; 27: 205-212.

Uzoma K.C., Inoue M., Andry H., Zahoor A., Nishihara E. Influence of biochar application on sandy soil hydraulic properties and nutrient retention. Journal of Food, Agriculture and Environment 2011b; 9: 1137-1143.

Vamvuka D., Dermitzakis S., Pentari D., Sfakiotakis S. Valorization of meat and bone meal through pyrolysis for soil amendment or lead adsorption from wastewaters. Food and Bioproducts Processing 2018; 109: 148-157.

Vines S.H. An elementary text-book of botany. 1898.

Vos J., Bom M. Hand-held chlorophyll meter: a promising tool to assess the nitrogen status of potato foliage. Potato Research 1993; 36: 301-308.

Wagner A., Kaupenjohann M. Suitability of biochars (pyro- and hydrochars) for metal immobilization on former sewage-field soils. European Journal of Soil Science 2014; 65: 139-148.

Wagner A., Kaupenjohann M. Biochar addition enhanced growth of Dactylis glomerata L. and immobilized Zn and Cd but mobilized Cu and Pb on a former sewage field soil. European Journal of Soil Science 2015; 66: 505-515.

Walter R., Rao B.K.R. Biochars influence sweet-potato yield and nutrient uptake in tropical Papua New Guinea. Journal of Plant Nutrition and Soil Science 2015; 178: 393-400.

Wang C.H., Bhattacharyya M.H. Effect of cadmium on bone calcium and 45Ca in nonpregnant mice on a calcium-deficient diet: evidence of direct effect of cadmium on bone. Toxicology and Applied Pharmacology 1993; 120: 228-239.

Wang L.K., Chen J.P., Hung Y., Shammas N.K. Heavy metals in the environment: CRC Press, 2009.

Wieshammer G., Unterbrunner R., García T.B., Zivkovic M.F., Puschenreiter M., Wenzel W.W. Phytoextraction of Cd and Zn from agricultural soils by Salix ssp. and intercropping of Salix caprea and Arabidopsis halleri. Plant and Soil 2007; 298: 255-264.

Wittbrodt P.R., Palmer C.D. Effect of temperature, ionic strength, background electrolytes, and Fe(III) on the reduction of hexavalent chromium by soil humic substances. Environmental Science & Technology 1996; 30: 2470-2477.

Woolfe J.A., Poats S.V. The potato in the human diet: Cambridge University Press, 1987.

World Health Organization (WHO), Food Agriculture Organization of the United Nations (FAO), International Atomic Energy Agency (IAEA). Trace elements in human nutrition and health. Geneva: World Health Organization, 1996.

Xie T., Reddy K.R., Wang C., Yargicoglu E., Spokas K. Characteristics and applications of biochar for environmental remediation: A review. Crit. Rev. Environ. Sci. Technol 2014.

Xu X., Cao X., Zhao L., Wang H., Yu H., Gao B. Removal of Cu, Zn, and Cd from aqueous solutions by the dairy manure-derived biochar. Environmental Science and Pollution Research 2013; 20: 358-368.

Yang Q.W., Lan C.Y., Wang H.B., Zhuang P., Shu W.S. Cadmium in soil–rice system and health risk associated with the use of untreated mining wastewater for irrigation in Lechang, China. Agricultural Water Management 2006; 84: 147-152.

Yang Y., Ma S., Zhao Y., Jing M., Xu Y., Chen J. A field experiment on enhancement of crop yield by rice straw and corn stalk-derived biochar in Northern China. Sustainability (Switzerland) 2015; 7: 13713-13725.

Yong R.N., Nakano M., Pusch R. Environmental soil properties and behaviour: CRC Press, 2012.

Yong R.N., Phadungchewit Y. pH influence on selectivity and retention of heavy metals in some clay soils. Canadian Geotechnical Journal 1993; 30: 821-833.

Yong R.N., Warkentin B.P. Chapter 2 - clay minerals in soils. In: Yong RN, Warkentin BP, editors. Developments in Geotechnical Engineering. 5. Elsevier, 1975, pp. 21-70.

Younis U., Qayyum M.F., Shah M.H.R., Danish S., Shahzad A.N., Malik S.A., et al. Growth, survival, and heavy metal (Cd and Ni) uptake of spinach (Spinacia oleracea) and fenugreek (Trigonella corniculata) in a biochar-amended sewage-irrigated contaminated soil. Journal of Plant Nutrition and Soil Science 2015; 178: 209-217.

Zama E.F., Zhu Y.-G., Reid B.J., Sun G.-X. The role of biochar properties in influencing the sorption and desorption of Pb(II), Cd(II) and As(III) in aqueous solution. Journal of Cleaner Production 2017; 148: 127-136.

Zavadil J. The effect of municipal wastewater irrigation on the yield and quality of vegetables and crops. Soil Water Res 2009; 4: 91-103.

Zhang G., Guo X., Zhao Z., He Q., Wang S., Zhu Y., et al. Effects of biochars on the availability of heavy metals to ryegrass in an alkaline contaminated soil. Environmental Pollution 2016.

Zhang J., Lü F., Zhang H., Shao L., Chen D., He P. Multiscale visualization of the structural and characteristic changes of sewage sludge biochar oriented towards potential agronomic and environmental implication. 2015; 5: 9406.

Zheng W., Wiles K.N., Dodge L. Uptake and accumulation of pharmaceuticals and hormones in vegetables after irrigation with reuse water. Champaign, IL: Illinois Sustainable Technology Center, 2016.

Zhuang P., McBride M.B., Xia H., Li N., Li Z. Health risk from heavy metals via consumption of food crops in the vicinity of Dabaoshan mine, South China. Science of The Total Environment 2009; 407: 1551-1561.

Ziv M. Enhanced shoot and cormlet proliferation in liquid cultured gladiolus buds by growth retardants. Plant Cell, Tissue and Organ Culture 1989; 17: 101-110.

Zrenner R., Stitt M. Comparison of the effect of rapidly and gradually developing water-stress on carbohydrate metabolism in spinach leaves. Plant, Cell & Environment 1991; 14: 939-946.

Appendix

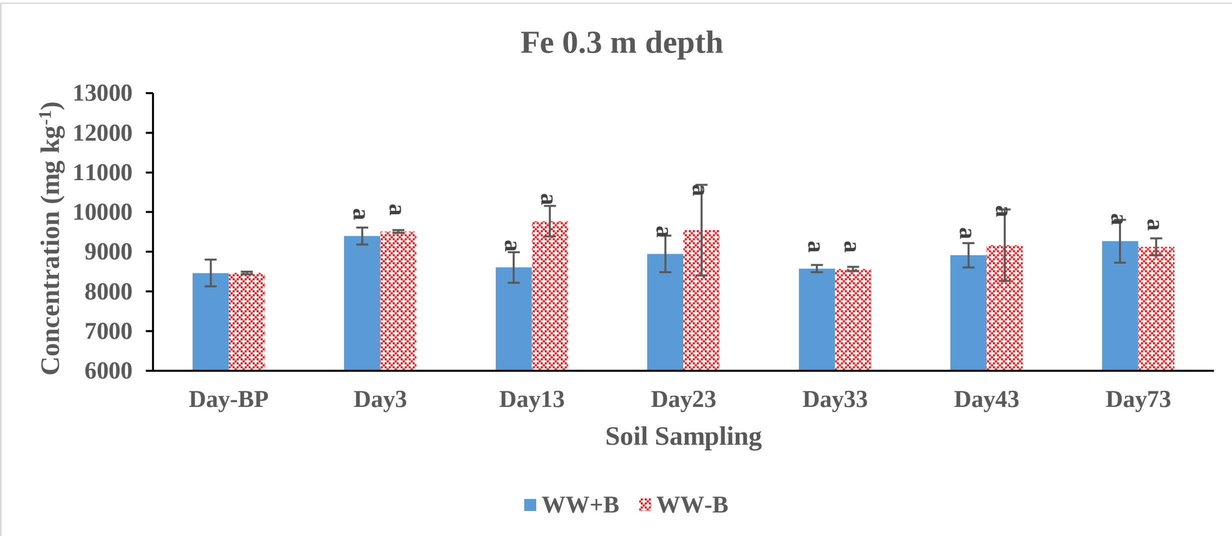

Figure A. Concentrations of Fe at 0.3 m depth of a sandy soil irrigated with untreated wastewater. Day-BP is the day before planting; the error bar represents standard error; bars with different letters signifies significant difference across both time and treatment (p<0.05).