HEAVY METAL ACCUMULATION MANAGEMENT IN BLACK SOLDIER FLY LARVAE RAISED ON CONTAMINATED WASTE USING SELECTED BIOCHAR.

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DECLARATION AND APPROVAL

Declaration

I hereby declare that this thesis is the result of my own original research work except for the duly acknowledged citations and quotations. No part of this work has been previously or concurrently presented for an award of another degree in this university or elsewhere.

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DEDICATION

This thesis is dedicated to the memory of my late father, Mwana mulena Kaiba C. Mutanekelwa, who unfortunately did not stay long enough in this world to see me graduate but his values of hard work and commitment saw this process through to its completion.

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Contents

ACRONYMS/ ABBREVIATIONS/ SYMBOLS

AAS	Atomic absorption spectrophotometer
ANOVA	Analysis of Variance
BAF	Bioaccumulation factor
BSF	Black soldier fly
BSFL	Black soldier fly larvae
Cd	Cadmium
Cd^{2+}	Cadmium
CS	Coconut shells
CSB	Coconut shells biochar
EFSA	European food safety agency
H_2SO4.	Sulphuric acid
IBI	International Biochar Initiative
FAO	Food and Agriculture Organization
LSD	Least significant difference
NaOH	Sodium hydroxide
PAHs	Polyromantic hydrocarbons
PCBs	Polychlorinated biphenyls
PFO	Pseudo – first order
PPM	Parts per million
PSO	Pseudo - second order
RH	Rice husks
RHB	Rice husks biochar
SDGs	Sustainable Development Goals
SEM	Scanning Electron Microscope
TLUD	Top Lid Updraft Drum
MR	Meticulous Research
WHO	World Health Organization
± SD	Plus or minus standard deviation

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ABSTRACT

The capacity of Black soldier fly (BSF) larvae to feed on organic matter and yield a protein rich biomass which can naturally be consumed by animals, offers an innovative opportunity of using waste rapidly generated in cities to produce tons of BSF for livestock feed. Efforts to recycle such waste using this insect has gained popularity but there is a public health concerns on the quality of larvae raised from such substrate to be used in the food system as feed ingredients. Contrary to microbial contaminants which are inactivated by BSF larvae, chemical contaminants like heavy metals are a threat to this industry as they are reported to accumulate in the BSFL posing a threat to consumers along the food chain. Heavy metals naturally occur in the earth crust and are released via natural disasters but a greater percentage of their release to the environment is largely attributed to anthropogenic factors. A study was conducted to investigate the effect of biochar introduction to BSF feed as a feed additive on heavy metal accumulation in BSF larvae. Biochar is a carbonaceous product of pyrolysis which is nontoxic and previous studies have reported its efficiency in binding toxins and general animal health when ingested as supplements. The objective of this study was to establish the adsorptive capacity of locally produced biochar in managing heavy metal accumulation associated with BSF reared on contaminated municipal organic wastes. This being so, optimization studies on rice husks biochar (RHB) and coconut shells biochar (CSB) capacity to adsorbed Cadmium metal were conducted. Adsorption behavior of Cadmium (Cd) onto the RHB and CSB with respect to the various parameters of influence was studied. The equilibrium data from the batch adsorption experiments was used to design kinetic and isotherms models. The isotherms showed a best fit of Freundlich model for RHB and CSB which informs heterogeneity kind of adsorption and exponential distribution of adsorption sites and their energies. Kinetic data indicated a chemisorption process of adsorption for both biochar types and this was best described by the PSO model. Furthermore, the remediation studies for Cd accumulation in BSFL using biochar feed additive was done in a CRD experiment. BSF larvae were fed on Irish potato peels spiked at varied Cd concentration levels and an equal amount of biochar with respect to type was mixed to the feed as treatment. Thereafter, the harvested BSF larvae were acid digested and analyzed for Cd using an AAS which detected only a small fraction of Cd^{2+} in the BSF larvae as well as in the food remains. This showed that the substantial amount of Cd^{2+} which was unaccounted for were retained in the biochar. These results indicate that RHB and CSB are suitable adsorbent which can effectively manage heavy metals. Findings from this study can therefore provide a basis for using biochar as a low-cost adsorbent to effectively remediate the heavy metal accumulation threat in BSF reared on contaminated organic waste.

Keywords: Black soldier fly, Food security, Bioaccumulation, Cadmium, Biochar.

CHAPTER ONE: INTRODUCTION

1.1 BACKGROUND

The current food system is not making progress toward cutting down the number of undernourished people especially in low-income countries. This situation is worsening as the demand for animal protein continue to escalate making them expensive and less accessible to many families in most developing countries. On the other hand, as food production increases to meet the demand, a third of this food goes to waste through food loss and usually dumped haphazardly making an environmental nuisance (WFP 2020). In the quest to ensure this valuable nutrient (protein) is made available, researchers have explored other food resources like the nutritional potential of edible insects. Edible insects have high crude protein levels of 40-75%, all essential amino acids, rich in fatty acids and have a high proportion of dietary fiber. Besides, insects have chitin as part of their exoskeleton which has many health benefits like antibiotic properties along with a number of minerals (Dickie *et al*., 2019).

Entomophagy however is challenged by inconsistency supply as most cultures access edible insects from the wild, a method that is greatly challenged by seasonality. Moreover, overexploitation, urbanization and land conversion have led to decline of wild catch among other obstacles like regulations promoting conservation of natural ecosystems. As a result, several investigations have been made towards controlled farming of edible insects and it is evident that insects can be reared. Thus, different technologies of insect farming have since been developed and promoted as a sustainable way to improve quality and supply of edible insects. Among the successfully reared insects, the Black soldier fly *Hermetia illucens* is more pronounced as one that is easy and cheap to rear. The black soldier fly (BSF), is a true fly that is native to America though widely distributed, in the temperate and tropics. The capacity of larval stage of this insect to utilize low-value organic waste as feed and yield high-value protein bodies, makes it the most used insect species to produce feed for animals (Cadinu *et al*., 2020). Apparently adults of this insect live on water only for about 8 days and tend to shy away from humans, they do not sting neither are they pests nor vectors of any specific disease (Wang & Shelomi, 2017). The larvae of BSF feed on a wide range of rotting organic matter and have been used in waste management using different substrates like municipal waste, animal offal, manure, brewers' waste, vegetable and fruit residues, fecal sludge, kitchen waste amongst others (Banks *et al*., 2014; Gold *et al*., 2018; Nyakeri., 2018; Wang & Shelomi, 2017).

The diversity of the substrates that BSF larvae can process and their superior feed conversion ratios presents a cheap and easy production system of the much-needed protein. In fact, a number of studies have not just suggested BSF larvae as practical to rear for feed production but also as a suitable and cheap tool to valorize municipal wastes. Whereas this innovation (using waste to raise BSF larvae for feed) seems to be a solution to poor waste disposal and livestock feed industry, safety concerns on its utilization as food and feed have been raised. Generally, waste is reported to have diverse contaminants which may be passed on to the larvae and en route to the food chain if the larvae is to be used as feed or feed ingredient thus posing a safety concern on the consumer.

Recently Mausi *et al*. (2014) reported a threat of heavy metal contamination in fruit samples collected from randomly selected market sites in Eldoret town of Kenya. Similarly, Karanja *et al.* (2012) detected an elevated of heavy metal content in kale leaves which was attributed to the use of untreated industrial wastewater for irrigation in peri-urban Nairobi. It was further reported that the highest loading of heavy metals in vegetables were demonstrated to be from chicken manure, mainly due to use of commercial feeds in chicken rearing. All these substances stand as potential contributors to diverse contaminants in waste and uncontrolled management of such fruit and vegetable waste raises concern on using municipal waste for BSF rearing. As a result, all potential hazards (microbial hazards, chemical hazards, allergenic potential, environmental hazards, and impact of processing and storage) as identified in the EFSA must be considered in farmed insects for food and feed especially the BSF larvae which can easily thrive on all forms of organic waste (Schrögel & Wätjen, 2019).

Interestingly, microbial hazards are reported not to be a challenge in BSF larvae (Bessa et al., 2020). Wang & Shelomi, (2017) reported that the larvae of BSF can reduce microbial load in the feed substrates by lowering the concentrations of bacteriophages and bacteria such as *Salmonella enteritidis* and E*nterococcus coli*. However, hygiene practices and treating the meal against microbes, parasites and bacterial spores may reduce the chance of microbial contamination to a greater extent. Similarly, Bosch *et al*., (2017); Lalander *et al*., (2016) and Purschke *et al*., (2017) reported a reduction in mycotoxins, pesticides and pharmaceuticals whose half-lives shortened after BSF treatment in the residual compost.

On the other hand, information on the safety with respect to heavy metals in insects reared for food and feed is limited (van der Fels-Klerx *et al*., 2018). However, there are a number of recent studies (Bessa *et al.*, 2020; Proc *et al*., 2020; Wu *et al*., 2020) that have shown accumulation of heavy metals in BSF at levels higher than the initial concentration level in the feed, thus making heavy metals a potential contaminant in BSF farming. These studies suggest that heavy metals may be present in the waste used as feed for the insect thus the accumulation in the body of the BSF. Therefore, feeds originally derived from insects reared in such conditions pose a threat to public health as contaminants move from waste into BSF and eventually in the food chain (van der Fels-Klerx *et al*., 2018).

Heavy metals are naturally occurring elements that have a high atomic weight and a density compared to that of water (van der Fels-Klerx *et al*., 2018). Their various application in industrial, domestic, agricultural, medical and technological sectors has led to their wide distribution in the environment; raising concerns over their potential effects on human health, livestock and the environment (Ismail &

Moustafa, 2016; Tchounwou *et al*., 2012). Heavy metals are considered systemic toxicants that are known to induce multiple organ damage, even at lower levels of exposure. Thus owing to their high degree of toxicity, metals like Arsenic, Cadmium, Chromium, Lead, and Mercury rank among the priority metals that are of public health concern as they are classified as human carcinogens by the U.S. Environmental Protection Agency and the International Agency for Research on Cancer (Tchounwou *et al*., 2012).

Presence of heavy metals in the organic waste suitable for BSF rearing limits the viability of the BSF industry as it requires careful examination of waste to be used as feed. Apparently most small holder farmers cannot afford the cost and procedure of heavy metal analysis. So far, a number of studies have focused on accumulation potential of these metals in the edible insects and Cadmium is reported as a persistent metal that accumulate in BSF larvae at levels higher than in the substrates (Diener *et al*., 2015). Recently, Diener *et al*. (2015) and Van Der Fels-Klerx *et al*. (2016) confirmed Cadmium bioaccumulation in BSF larvae through analysis of both the larvae and substrate. Wang & Shelomi, (2017) reported different heavy metals like Chromium, Arsenic, Nickel and Mercury to accumulate in the larvae of the BSF. While accumulation potential of toxic metals in edible insects particularly the BSF larvae is well documented, research on their remediation is scarce.

This informed the overall objective of this study which is to remediate heavy metal in BSF, thereby reducing the risks associated by minimizing the detrimental effects of heavy metal exposure and their accumulation in BSF larvae. Generally, insects get rid of most harmful substances which they may have consumed through excretion of the gut lining during the process of fasting prior to harvesting. The other way is through molting as they transform from one phase to another thus the reason why heavy metals are reported to accumulate the highest in larvae and prepupa of the BSF than the adults. However, the larval stage of BSF is the most targeted stage in feed industry due to their highest crude protein levels.

The larvae of BSF accumulate Cadmium at bioaccumulation factor (BAF) greater than 1 and the same is reported in other fly species like fruit flies and marsh mosquitoes (van der Fels-Klerx *et al*., 2018). This entails that the accumulation in the organism becomes greater than that of the medium from which organism was taken from. Insects of this family have the capacity to store Cadmium through the metallothionein genes which bind this metal and prevent its excretion. Additionally, Cadmium can be transported by Calcium ions channels in heated conditions thus allowing its passage directly through the body tissues (Diener *et al*., 2015; Van Der Fels-Klerx *et al*., 2016). This explains the persistent accumulation of this metal in BSF larvae considering their high Calcium content compared to other insects' species. Overall previous reports observed the highest BAF value for cadmium in BSFL, followed by arsenic and lead (Alagappan *et al*., 2022).

This study employed adsorption technique where bioavailability and accumulation of the contaminant heavy metal Cadmium was studied in the larvae of BSF using biochar as remediate adsorbent. Biochar which is a cost effective and an ecofriendly carbonaceous product of pyrolysis was incorporated in BSF larvae feed spiked with different levels of Cadmium (Lyu *et al*., 2016). Biochar has unique physicochemical properties, with high affinity to binding inorganic and organic contaminants in different ecosystems capable of adsorbing various pollutants such as heavy metals, mycotoxins, pesticides (Mahdi, 2019). A number of studies have confirmed the potential effectiveness of biochar in remediating heavy metals in aqueous solutions and soils (Komkiene & Baltrenaite, 2016; Li *et al*., 2017; Lyu *et al*., 2016; Tan *et al*., 2015; Wu *et al*., 2018).

According to Sakhiya *et al*. (2020) Biochar can effectively immobilize, adsorb and sequestrate a number of heavy metals including Chromium, Lead, Cadmium, Nickel, Mercury, Copper, and Zinc from soils and water. Recently, impressive discoveries of blending biochar with organic matter like manure, cattle urine and compost have been made by various researchers (Kalus *et al*., 2019; Schmidt *et al*., 2019). This prompted studies on not only mixing biochar with manure, but also its inclusion in animal feeding systems. These results indicated an improvement in nutrient intake efficacy and a general animal health improvement. This suggests that there are plenty of potential relationships between various parameters that biochar can achieve given its remarkable properties (Kalus *et al*., 2019).

This study investigated the adsorption potential of biochar from rice husks and coconut shells produced using the Top Lid Updraft Drum (TLUD) technology. Rice husks and Coconut shells are lignified type of agricultural waste that take long to decompose thus they are abundantly available. Using them however, as feedstock for biochar production for different purposes is a solution that can prevent heaping of underutilized large masses of such waste. Moreover, the thermal decomposing technology employed for biochar production in this study is simple and less expensive, making it affordable for farmers of different classes. The TLUD is not just recommended for its simple and inexpensive design but its capacity to burn pyrolytic gases such as carbon monoxide, methane and aerosols up to 75% thus it is ecofriendly (Mathu *et al*., 2017).

Results from the optimization studies conducted here were used as guiding conditions in spiking BSF larvae substrate/feed correspondence with the average Cadmium concentration levels present in organic wastes (supposedly feed for the BSF larvae) around some selected parts of Kenya. Thereafter the bioaccumulation of Cd metal in the harvested BSF larvae and the frass (residue) after feeding was studied through monitoring and comparing the Cd concentration levels before and after feed with respect to the biochar type.

1.2 STATEMENT OF THE PROBLEM

Utilization of organic waste poorly disposed especially in cities to raise BSF larvae for animal feed, presents an innovative strategy that answers to the food feed competition surrounding soya beans and fish besides addressing the environmental concerns of waste accumulation.

However, using organic waste (municipal waste) for BSF rearing as feed/ingredient is challenged by the food safety concern which presents the diversity of contaminants in the waste. Of major concern are the heavy metals reported to accumulate in BSFL at levels higher than the initial concentration spiked in the feed (Diener *et al*., 2015). Particularly, Cadmium has been reported to accumulate more (91.3%) in BSFL than other metals like Cu (Nan 2020). These metals can easily enroute into the targeted waste (organic) through poor disposal, as other types of waste like electronic waste (E waste), Phosphate fertilizers, Polyvinylchloride plastics, paints and batteries in which contaminants like Cadmium are used as stabilizers get mixed with municipal organic waste (Genchi et al., 2020).

While there is growing body of research assessing the heavy metal accumulation in BSF larvae, little is known about remediation strategies to rid this industry of the heavy metal risk and constant monitoring of heavy metal content in the feed is technically unattainable by small holder farmers. Thus, this study fills the knowledge gap by quantifying the potential of biochar as a low-cost adsorbent that can adsorb Cadmium from BSF feed waste.

1.3 OBJECTIVES

1.3.1 Main objective

To contribute towards management of heavy metals in BSF larvae using selected agricultural waste biochar for feed safety.

1.3.2 Specific objectives

To determine the adsorption behavior of RHB and CSB for Cadmium remediation in BSF Larvae fed on contaminate organic Waste.

To evaluate the effect of selected biochar on Cadmium accumulation in BSF larvae.

1.4 HYPOTHESES

H_0: Rice husks and Coconut shells biochar are not potential adsorbents of Cadmium

H_0: Rice husks and Coconut shells biochar have no effect on Cadmium accumulation in BSF larvae

1.5 JUSTIFICATION

The potential bioaccumulation of heavy metals in BSFL fed on contaminated organic waste limits the substrates to be used in BSFL rearing. Considering the threat that heavy metal contamination in BSFL poses to food safety and food security, addressing the issue of heavy metal bioaccumulation justifies the need for this study. This study sought to remediate Cadmium reported to accumulate in the larvae using biochar. Hence two types of agricultural wastes that is rice husks and coconut shells often considered to be of less economical value and nuisance to the environment were used for biochar production. This provides a basis for promoting utilization of highly lignified agricultural wastes which take long to decompose for biochar production which can then be used as remediator for heavy metals in BSF larvae feed or other media like soil.

Moreover, the use of biochar as a feed additive in animal farming is well documented. Despite most results and experience being concentrated in cattle and chicken, biochar is also administered to other livestock like sheep, goat, horses, pigs cats, rabbits ,dogs and fish farming (Kammann *et al*., 2017) and a great potential to improve animal health, feed efficiency and livestock housing climate like reducing greenhouse gas emissions is reported. Results enhances food security by suggesting a remediation measure that is not just affordable and eco-friendly but reduces the risk of using untreated waste.

1.7 SIGNIFICANCE OF THE STUDY

This study contributes to achieving three of the United Nations Sustainable Development Goals (UN SDGs). It focuses on combating the chemical food safety threat in BSF rearing industry using organic waste polluting the environment in cities, there by contributing to ending hunger, good health and well-being as well as ensuring city sanitation. The results from this study can help in sensitization, monitoring and policy formulation supporting the use municipal waste in black soldier fly rearing for food and feed. It provides a basis for promoting the use of various organic waste types accumulating in cities as feed substrates for BSF larvae rearing. This study contributes to the understanding of heavy metals in edible insects particularly the BSF and suggest remediation measure thus enhancing food safety by breaking the en routing of heavy metals along the food stream using an afford remediate. The study brings to the body of knowledge and literature for other scholars interested in heavy metal contamination in, not just edible insects but other ecosystems.

CHAPTER TWO: LITERATURE REVIEW

2.1 INTRODUCTION

The intensification of agriculture to meet the increasing demand for food by half the world's population found in urban areas has presented a tremendous increase of solid waste in cities. Despite the substantial efforts to reduce waste accumulation in cities by the relevant authorities, waste management still remains one of the pressing issues of global concern. This impacts many aspects of social and economic development thus addressing this issue is a step towards achieving 50% of the Sustainable Development Goals (SDGs). Waste management has revolutionized from traditional methods like open air burning and dumping to programs that emphasize reuse and recycling of resources as well as reduction of consumption (Ashikuzzaman & Howlader, 2019; Mohammad, 2020; WHO, 2021)

However, despite the greater percentage of solid waste in many developing countries being organic, recycling programs have been directed towards the inorganic wastes like scrap metal due to the perceived high value of the products obtained there of when compared to organic waste products (Joly & Nikiema, 2019). Researchers have since explored various technologies and processes of managing waste materials which Lohri *et al*., (2017) groups into four major categories namely direct use, biological treatment, physico-chemical treatment and thermo-chemical treatment. Of these technologies, biological treatment particularly, Blac soldier fly bioprocessing stands out to be the most promising technology capable of solving environmental pollution and provide an alternative animal protein within a short time at low cost (Charlton *et al*., 2015; Diener *et al*., 2015; Lohri *et al*., 2017).

2.2 BLACK SOLDIER FLY

The black soldier fly, *Hermetia illucens* (*Linnaeus*) is a non-pest Diptera of the *Stratiomyidae* family native to the neotropics also known as South America region, one of the six major biogeographic areas of the world. Currently, BSF is distributed in tropical and subtropical regions around the World between 46^{o} N and 42^{o} S (Diener, 2010). These insects occur naturally in some African countries like Zambia, South Africa, Guinea and Ghana whereas in other countries like Kenya, it was introduced as brood stocks, imported for rearing purposes following the rising attention that it has continually received (Nyakeri,2018). The capacity of the BSFL to bio convert organic waste into a protein rich biomass leaving a very rich frass/manure valuable for soil fertilization has made it popular in the recent past (Tomberlin *et al*., 2002). In fact, Mutafela, (2015) presented the use of BSF in waste management as being more viable economically than direct using of raw manure. A comparison study on this, presented BSFL by products (protein, fat and compost) as being 100- 200 times more economically valuable at US$200 per ton with unprocessed or raw manure which stood at US$10-20 per ton.

2.2.1 The life cycle of BSF larvae

The Black soldier fly undergo a complete metamorphosis i.e., it passes through 4 distinct stages (egg, larvae, pupa and adult) to complete its life cycle. Under optimal rearing conditions, the cycle is estimated to be complete within 40 -43 days period otherwise it can be extended up to 6 months(Mutafela, 2015; Nyakeri., 2018). Just like any other insect, the cycle starts with mating and this is achieved by males congregating in certain areas away but near the substrate and call to the females. This is so because females need to oviposit their eggs near a food source for the off springs to thrive on. Each female lays a cluster of between 500-900 eggs into dry cracks and cervices which then hatches into larvae 3-4 days after (Diener, 2010).

The larvae turn into prepupa in 14 days and within the same period of time, the prepupa changes into pupa which burrows into dry medium where it undergoes further exoskeletal casing for at least two weeks. The cycle is closed by emergence of an adult fly from the casing which breaks at the tip allowing its release. The newly emerged flies are slightly larger, greenish bodies with undeveloped folded wings which gradually unfold within 2-3 hours and 2 days later, these become adults which are able to mate. Interestingly, the adult BSF do not have functional feeding parts hence they do not feed instead live on stored fat during their life span of 5-12 days(Mutafela, 2015).

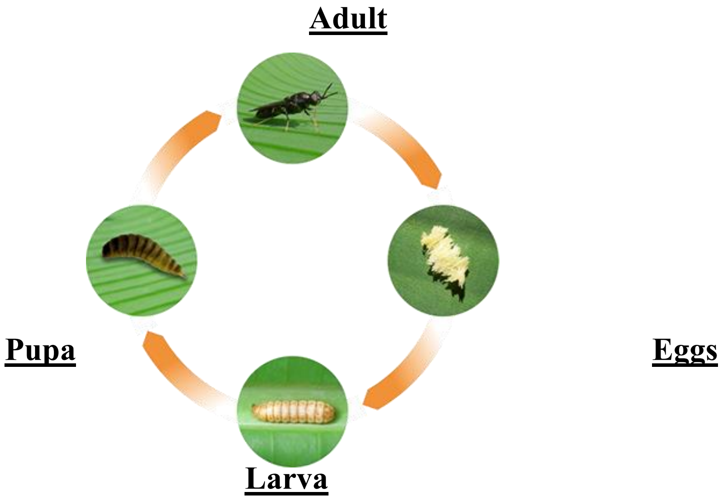


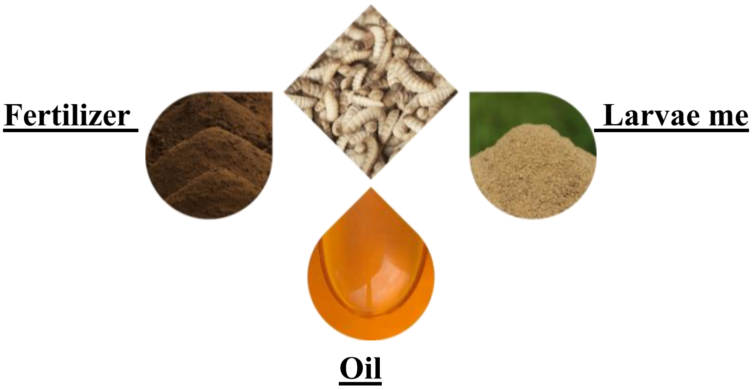
Figure 1: Life cycle of the Black soldier fly. Source: Entofood Sdn Bhd, (2016)

2.2.2 Importance of Black soldier fly

As the world population continues to grow, resources become scarcer, posing humanity with a challenge of providing the growing population with healthy diets from sustainable food systems. Apparently, the global food production of energy dense foods has kept pace with the rising population but a good number of people still face protein crisis and suffer a double burden of overweight with hidden hunger and malnourishment and the bigger percentage is mostly women and children. This informed programs like the Scaling Up Nutrition (SUN) Movement, a Global initiative uniting multisectoral efforts to end Under- Nutrition in women of productive age and child stunting. In this regard, governments have sought to promote sustainable production of nutrient dense foods and insect farming presents a sustainable solution to the protein crisis with limited carbon prints among other benefits like insect frass to support another soil nutrition (FAO, 2021)

The BSF is one insect that have so far presented versatile economic benefits. Black soldier fly larvae are rich in lipids, proteins, polysaccharides and Calcium hence their great potential to be used in animal feed. BSF meal which is a whole BSF processed into a meal product is among the common marketable BSF ingredients, Besides, the BSF can also be extracted and separated, a defatted protein meal with a protein content up to 60% and an extracted oil part of on average 30%, remain. When extracting the oil and protein part of the BSF, some byproducts are released. These by-products mainly consist of enzymes, chitins and antimicrobial peptides (Bakker, 2020).

From the defatted BSF exoskeleton is one of the fast growing biopolymer called chitin which is said to have a positive effect on the functioning of the immune system (Huis, 2012). The chitin can further be processed into chitosan another high value product that has potential application in agriculture as pest-control and other industries like textiles, water treatment, food preservation and many more (Meticulous Research, 2020). Additionally, after rearing, residue by-product called frass remain. The residue mainly includes fecal matter, residues of organic waste and BSF skins which has great potential to be used as biofertilizer (Zahn, 2017).



Economic products of the Black soldier fly

Black soldier fly larvae can solve environmental problems associated with organic waste thus reducing the cost of waste disposal and treatment. The composition of BSFL's gut microbiota is made in such a way that it can consume and break down different organic types thus reducing the waste biomass and prevent environmental contamination (Mutafela, 2015). So many studies have shown the potential of this insect in waste reduction and how it can be used in waste processing to overcome the challenge of waste accumulation in cities (Diener, 2010; Joly & Nikiema, 2019). Furthermore, colonization of BSF on organic matter is said to successfully reduce offensive odors and control population of vectors like common house flies by 94-100% (Mutafela, 2015).

2.3 FOOD SAFETY CONCERNS RELATED TO THE USE OF BSF LARVAE AS FEED

Entomophagy of some insects like the BSF larvae has been challenged on safety grounds despite its potential to contribute greatly to human nutritional requirements where malnutrition is a challenge (Belluco *et al*., 2013; Imathiu, 2020; van der Fels-Klerx *et al*., 2018). Top on the list of concerns is the issue of microbiological and chemical healthy risks that insect derived foods pose along the food chain. Such hazards present a challenge to the food industry when legislators and food safety authorities use them as critical points to protect the general public through restrictions of certain activities (Belluco *et al*., 2015).

Research on BSF has significantly gained attention over the past few years due to versatile benefits that the BSF offers. Most important of them all is the ability of the larvae of this species to bio convert organic waste into feed source rich in essential amino acids and fatty acids among other micronutrients vital for animal nutrition (Biancarosa, Liland, Biemans, *et al*., 2017). Owing to the fact that BSF thrive inclusively on a diet that is high in number and diversity of microbes, safety concerns in relation to its use as food or feed have been raised. So many studies present the larvae of this insect as one that can scavenge on different organic matter including human excreta (Banks *et al*., 2014; Diener, 2010; Nguyen *et al*., 2015).

Transfer and accumulation of microbial and chemical substances that the BSF substrate is said to be rich in poses a challenge to this industry(Lalander *et al*., 2013; Wang & Shelomi, 2017). Waste material that have so far been reported to be suitable for BSF rearing are either animal based (manure, blood) and risk having pharmaceuticals or fruits and vegetables wastes which may also have some pesticides residues (Gold *et al*., 2018; Lalander *et al*., 2016). The same applies to poorly stored grains as they may harbor mycotoxins whereas municipal waste may have heavy metals and other toxins like polyromantic hydrocarbons (PAHs), dioxins, polychlorinated biphenyls (PCBs) etc.

2.3.1 Microbial contaminants of BSFL

Interestingly BSFL is noted to reduce microbiological contaminants in their substrates(Wang & Shelomi, 2017) .According to Lalander *et al*., (2013) and Wang & Shelomi, (2017) the larval activity of this insect sanitizes the waste by inactivating some zoonotic *Enterobacteriaceae* such a *Salmonella* spp. and *Escherichia coli*. Additionally, previous researchers reported that BSFL do not accumulate mycotoxins, pharmaceuticals, dioxins, PCBs, PAHs and some selected pesticides (Bosch *et al*., 2017; Lalander *et al*., 2016; Purschke *et al*., 2017). This shows that BSFL are free of microbial contaminants, actually Lalander *et al*., (2013) said that inoculating BSF substrate with 9omicrobes increases the larval performance process. However, Wang & Shelomi, (2017) cautions that hygiene practices must be observed for the larvae can be contaminated after harvesting.

2.3.2 Chemical contaminants of BSFL

On the contrary chemical hazards especially heavy metals accumulation in the larvae has recently been raised as an issue of concern in BSF farming. According to Wang & Shelomi, (2017) different heavy metals like Chromium, Arsenic, Nickel and Mercury were reported to accumulate in the larvae of the BSF but the levels of Cadmium were reported to be higher than what was contained in food substrate. Similarly, Van Der Fels-Klerx *et al*., (2016) conducted a study where Lead, Arsenic and Cadmium were spiked in the BSF substrate. Cadmium and Arsenic were found in the larvae at concentration higher that the European Commission limit for feed material.

However, the bioaccumulation factor (BAF) of Arsenic in BSF was less than one and the majority of Arsenic consumed was excreted. On the contrary, Van Der Fels-Klerx *et al*., (2016) reported that Cadmium accumulate in the larvae at BAF greater than one and it is also said to accumulate in other fly species like fruit flies and marsh mosquitoes. Members of this family have the capacity to store Cadmium through the metallothionein genes which bind this metal and prevent its excretion. Additionally, Cadmium can be transported by heat which shocks proteins thus allowing Cadmium to pass through Calcium ions channels (Diener *et al*., 2015; Van Der Fels-Klerx *et al*., 2016). This explains the persistent accumulation of this metal in BSF larvae considering their high Calcium content compared to other insect species.

These results concur with the findings from other studies where BSF was provided with feeding substrate spiked with Cadmium (Charlton *et al*., 2015; Diener *et al*., 2015; Gao *et al*., 2017). According to Biancarosa *et al*., (2017) Cadmium concentrations in the substrate have a direct effect on the levels of its accumulation in the BSF larvae. In their study, they reported a simultaneous increase of Cadmium levels in the larvae to increasing concentrations in the seaweed enriched feeding media. Diener *et al*.,(2015) and Van Der Fels-Klerx *et al*., (2016) reported a higher Cadmium accumulation than in the case of Biancarosa *et al*., (2017). However, the variation was due to exposure of higher Cadmium levels or spiked media having much more available Cadmium than sea weed enriched media (Biancarosa, Liland, & Araujo, 2017).

2.4 REMEDIATION OF HEAVY METALS IN INSECTS

Contaminants (microbes and chemical) present in the waste used to rear BSFL may remain in the gut or even tissues of this valuable larvae even after harvest and they may be taken up by livestock if used as feed (Diener *et al*., 2015). This poses a health risk to the general public as these toxic substances may accumulate in the food chain and eventually reach humans as consumers. Therefore, effective elimination measures of toxic persistent contaminants like heavy metals are imperative to this new industry of BSF larvae for feed if it is to stand the test of time and be approved by the government.

According to Diener *et al*., (2015) defecation has so far been reported as an effective way of eliminating heavy metals in insects particularly the metals that accumulate in the lining of the gut. Meal worms are a best example of insects where this has worked effectively for, they discard the mid gut epithelium after four days. However, Schrögel & Wätjen, (2019) urges that accumulation of heavy metal ions occur to a varying extent depending on the insect species, metal type and stage of development. Hence the need for specific species information regarding different types of heavy metal accumulation and elimination. Moreover, some metals ions go beyond the tissues lining in the gut and therefore, defecation alone may not be an effective method of eliminating heavy metals in edible insects. In fact, Schrögel & Wätjen, (2019) recorded separation technique of metal ions along the gut from those in the body tissues by starvation from the contaminated diet to uncontaminated one for two days or starving the larvae prior to harvesting. With respect to the above heavy metals elimination techniques Gao *et al*., (2017) reported that heavy metal accumulation in BSFL may have no removal strategies. Hence harvesting of this insect at pupa stage was recommended rather than the prepupa or larval stages due to exceedingly higher heavy metal accumulation in the latter stages compared with China (GB 2762-2012) and European Regulations (EC, 2003). Considering the consistency in reports about Cadmium accumulation in BSFL (Imathiu, 2020) and presented benefits of BSF larvae to the feed industry and waste management, innovative remediation measures are needed.

2.4.1 Biochar as an adsorbent for remediation of Cadmium

Biochar is a charcoal like substance that is made by burning organic materials from agricultural and forestry waste(biomass) in a controlled process called pyrolysis. In agriculture, biochar has for some time been recommended for soil amendment and mixing it with bulky organic manure has shown even better crop yield across a broader spectrum of soil types and climate (Kammann *et al.*, 2017; Schmidt *et al*., 2019; Woolf, 2008). Owing to the vast potential application and non-toxicity of biochar, researchers have sort to use it as feed additive and a general improvement in animal health (Inyang *et al*., 2015; Kalus *et al*., 2019; Man *et al*., 2020; Schmidt *et al*., 2019; European Biochar Foundation (EBC), 2012). The non-digestibility of biochar makes it effective in preventing assimilation of orally ingested harmful toxins by having them attached to the adsorption sites there by passing through the gut.

Recent studies have shown that biochar has high adsorption capacity for different toxins like mycotoxins, plant toxins, pesticides, toxic metabolites and pathogen in animal feed (Schmidt *et al*., 2019). Several studies have since used biochar in animal husbandry as feed, veterinary and bedding. However, there is limited information on the use of biochar in insect farming. This study therefore, used biochar as an insect feed additive to remediate Cadmium in BSF feed, a persistent heavy metal that accumulates in the BSF larvae. Cadmium has strong toxicity, solubility, mobility and biological accumulation which can led to kidney and bone damage after long exposure (Tan *et al*., 2015; Wu *et al*., 2018). In insects, particularly BSF, this metal increases the duration and pupation rate (Gao *et al*., 2017).

Considering the potential toxicity of Cadmium, different methods have been used to get rid of it. Of the common methods used to remediate heavy metals, adsorption is considered the simplest, most economical and effective way of removing heavy metals even at low concentrations (Seng, 2018; Tan *et al.*, 2015). However, the efficiency of biochar depends largely on the type of the parent stock used for making this biochar. The parent stock influence physicochemical properties like, active functional groups, aromatic components, cation exchange capacity, microporous structure and a relatively high pH which are vital to adsorption (Wang *et al*., 2019; Woldetsadik *et al*., 2016). To a greater extent, these properties are affected by feedstock used for biochar production (Liu *et al*., 2015).

Thus, the reason for using two feed stocks namely rice husks and coconut shells. These feed stocks complied to the International Biochar Initiative (IBI) standards which recommend making biochar from toxic free stock with no competition for other vital options like food. The IBI recommends crop residue, forestry wastes and animal manures for biochar production rather than burning or leaving them to rot thus releasing greenhouse gases (carbon dioxide and methane) in the atmosphere. Coconut shells and rice husks are among the many agro wastes that attract very little management strategies in Africa. Despite a number of products that can be obtained from coconut shells, they are considered a nuisance once the edible inner pulp is removed. For instance, Amoa, (2016) reported a total of 200 to 300 thousand metric tons of coconut waste yearly that are littered around the streets, backyards or burnt in open spaces in Ghana.

Similarly, rice husks which comprise of about 20% of the whole grain weight are often heaped around the milling areas. Masoud *et al*. (2016) recorded an estimation of 100 million tons of husks from 500 million tons of rice produced every year in developing countries. Traditionally, rice husks have a low–value application (chicken litter, animal roughage) owing to their poor nutritive value, indigestibility and abrasive character. Hence, their availability exceeds the local need for use and are still in excess. Therefore, without proper utilization options in place, rice husks pose a growing challenge of environmental pollution and space (Masoud *et al*., 2016). Biochar from coconut shells and rice husks have been used for heavy metals remediation in a number of studies.

A study by Prapagdee *et al*.(2016) reported that rice husks biochar can effectively remove Cadmium up to 97 % within the contact time of 180 minutes from aqueous solution. According to Suman & Shalini, (2017) converting coconut shells to biochar is a potential option which can lead to utilization of these wastes for sustainable purposes. Recently Chen *et al*. (2019) reported that coconut derived biochar performed better than rice straw biochar on Cadmium remediation. However, biochar amount increment for both types yielded the same results in the long run. Liu *et al*. (2015) also reported similar results where coconut and bamboo biochar showed a much higher capacity to adsorb Cadmium than rice husks biochar.

CHAPTER THREE: MATERIALS AND METHODS

3.1 STUDY AREA

Morphological characterization of RHB and CSB was conducted at the National Centre for Nanostructured materials, Council for Scientific and Industrial Research (CSIR) in South Africa. CSIR is a scientific and technology research organization covers a wide spectrum of Scientific and Technological clusters that impacts towards accelerating socioeconomic prosperity. Adsorption capacity of the aforementioned adsorbents on Cadmium was thereafter investigated through adsorption optimization studies which were conducted at the Chemistry laboratory of Technical University of Kenya (TUK). TUK has equipped facilities that offer different, industrial and analytical chemistry laboratory services. Results from the optimization studies conducted here were used as guiding conditions in the applicative remediation

part (BSF feeding experiment) of this study which was conducted at Jaramogi Oginga Odinga University of Science and Technology (JOOUST) insect farm and the research laboratory. JOOUST is located in the township of Bondo in Siaya County of Western Kenya at geographical coordinates of 0.09°S, 34. 26°E.These coordinates provide warmer conditions which are most favorable for BSF rearing.

3.2 MATERIALS

3.2.1 Biochars

The agricultural waste used as biomass for Biochar production in this study were rice husks (RH) and coconut shells (CS). These materials were cleaned by washing with tap water and dried to removed impurities from the dumping site. Thereafter, they were sun dried for 4 days before pyrolysis. Rice husks (RH) and coconut shells (CS) were collected from Ahero in Kisumu County and the coast of Mombasa respectively. The method used for pyrolysis in this study was Top Lid Updraft Drum (TLUD) technology under limited oxygen conditions. This technology is mostly preferred for its efficiency to pyrolyze feedstock without much smoke released to the atmosphere. Moreover, the TLUD is simple and its design is inexpensive thus highly recommendable to small holder farmers who cannot afford sophisticated machines (Van *et al*., 2020).

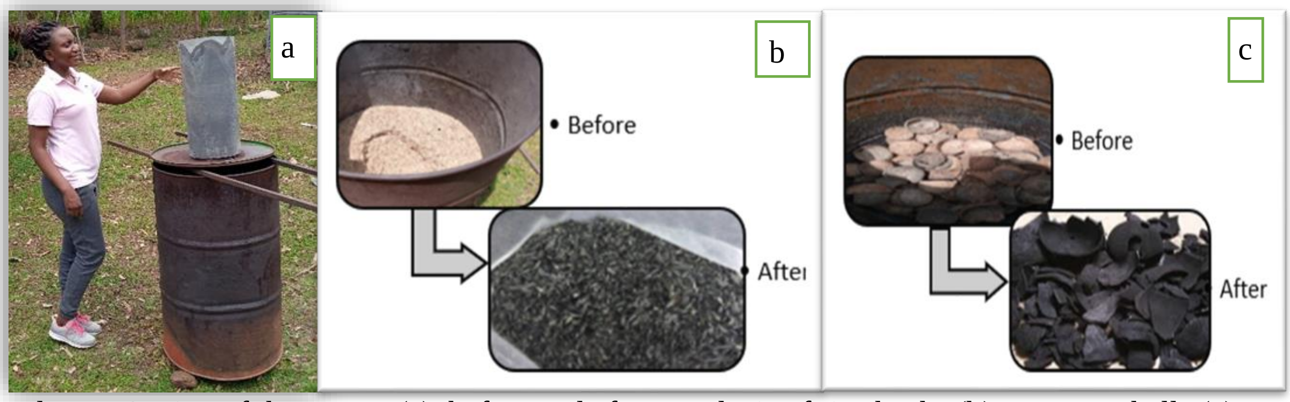


Plate 1: Setting of the TLUD (a), before and after pyrolysis of rice husks (b), coconut shells (c)

The TLUD technology burns pyrolytic gases such as carbon monoxide, methane and aerosols up to 75 % (Mathu *et al*., 2017). In the present study, this equipment was constructed using a 200 L metallic drum, perforated at the base as a primary air intake section, a piece of iron sheet for chimney which was fitted on the drum lid and two iron bars as shown in Figure 2 below. The TLUD was filled with selected agricultural wastes, one type at a time and fire was started at the top material. Once flames were well established, the drum was covered to reduce oxygen supply and biochar started forming under limited oxygen. Combustion started with white smoke for about 30 seconds and there after it was entirely “clean combustion” (i.e., no smoke).

The process was closely observed by the intensity of the flames which eventually became low while tossing some water at the sides of the drum. The tossing of water was to indicate that the biochar was ready at instant puffs of steam as well as to prevent ash formation by maintain temperature. Pyrolysis process took 2 hours and 1-hour 30 mins for rice husks and coconut shells respectively, thereafter Biochar was cooled by sprinkling water as described by Van *et al*. (2020) and sun dried before crushing them and labelling them according to the feedstock parent material used.

3.2.2 Reagents and instrumentation

High purity Cadmium standard 1000ppm was purchased from ChromAfrica operations in Kenya sulphuric acid (98%), Nitric acid (69%), Hydrochloric acid (37%) and Sodium hydroxide (analytical grade). All solutions preparations and rinsing of used apparatus was done using distilled water. An analytical weighing balance was used to measure sample mass, magnetic mechanical shaker was used for stirring and laboratory heat digestor was used for sample digestion. Samples were filtered before running them on with an Atomic Absorption Spectrophotometer (AAS: Shimadzu AA-7000) to measuring Cadmium concentration. Whatman filter paper number 1 was used, pH adjustments were done using AP85. Plastic tins (18×9) were purchased from shoppers to serve as BSF feeding trays, 20 liters plastic buckets were used for feed collection and packaging. Box of gloves, a sieve and a wooden stand was purchased within Bondo.

3.2.3 Black soldier fly

Black soldier fly larvae (BSFL) used in this study were obtained from the black soldier fly unit maintained at JOOUST. Female black soldier flies were attracted to lay eggs on the cardboard using fruit waste. Eggs laid on the cardboards within 4 days were collected and transferred to a plastic vessel container containing chick mash for hatching. The hatched neonates were fed on chick mash for five days in order to speed up their growth rate. A wire mesh of diameter 1.2mm was used to separate (sieve) the larvae and the residues on the 6^{th} day of age. The counted larvae were used for the experiment according to the respective treatments.

3.3 EXPERIMENTAL METHODS

3.3.1 Activation of Biochar

Prior to adsorption studies, both coconut shell and rice husks biochar were activated in an acid. This was simply to improve the physical properties of biochar in order to increase its adsorption capacity.

Despite the potential that biochar has to adsorb heavy metal ions from contaminated solutions, its efficacy is often enhanced by activation. In fact, recent studies have focused on biochar activation to enhance its capacity to achieve desired results. According to Sakhiya *et al*. (2020) activation is the process of increasing specific surface area and pore density which consequently improves adsorption. Several methods can be used to activate biochar but the present study employed physical and chemical activation methods.

Biochar was ground using an electric grinder into smaller particle size. With respect to the nature of the study where biochar was used as a feed additive, grinding the biochar to fine particles was necessary so that it can easily be scooped at the required dosages and incorporated into the substrate. Furthermore, small size particle adsorbents tend to have increased surface area and can easily interact with the substrate. Then biochar was later chemically activated using sulfuric acid where both types i.e., coconut shells and rice husks biochar were soaked in 1 molar sulphuric acid for 24 hours. Thereafter, the samples were washed with distilled water to remove the excess acid from the surface before oven drying them at 150 °C. The oven dried biochar was packed in air tight containers for adsorption experiments.

3.3.2 Morphological characterization of rice husks and coconut shells biochar

The surface properties of the selected biochar were investigated using scanning electron microscope (SEM: JEOL JSM-7500F). The biochar samples were selected and carefully mounted on aluminum studs using conductive glue. Preconditioning was done by coating with a thin layer of carbon thereafter analyzed in a vacuum chamber, then the whole area of the biochar was scanned and photographed for analysis. SEM provides high resolution electronic images of the sample surface whose contrast provides information about pores, fissures, and other surface materials depending on the characteristic electron emitted (Novak & Johnson, 2019). Since adsorption of porous materials like biochar happens in the pores of the adsorbent, understanding the pore distribution and volume give characteristic information on the ability of adsorbent material to adsorb molecules thus an important parameter in adsorption studies.

3.3.3 Adsorption behavior of RHB and CSB for Cadmium remediation in BSF Larvae fed on contaminate organic Waste.

3.3.3.4 Calibration studies

Cadmium working standard of 1 ppm was prepared from 1000 ppm by measuring 0.5ppm into a 500 mL volumetric flask and this was diluted to the final volume of the flask with distilled water. The formulated Cd working standard was used to determine the capacity of Coconut shells biochar (CSB) and Rice husks Biochar (RHB) to adsorb Cadmium at different parameters including biochar dosage, solution pH, contact time and temperature.

Plate 2:Preparation of Cd working standard

3.3.3.5 Batch adsorption studies

Adsorption studies were caried out by first optimizing the parameters that influence adsorption such as contact time, pH, temperature and adsorbent dosage. The effect of contact time on Cd removal was studied by shaking 5 g of each the adsorbents in 50 mL of Cd working standard solution at intervals of 5 mins, 10 mins, 20 mins, 30 mins, 60 mins and 120 mins keeping other parameters constant. Furthermore, adsorption kinetics which describes the influence of reaction time governing rate of adsorbent uptake was investigated on both CSB and RHB using Pseudo-first order and Pseudo-second order models. The values of linear regression coefficients (R^2) obtained from Equation 1 (PFO) and 2 (PSO) were used to predict the most suited kinetic model and isotherm for the process

$$\ln(q_e - q_t) = \ln q_e - k_1 t \quad \ldots\ldots (1)$$

$$\frac{t}{q_t} = \frac{1}{k_2\, q_e^2} + \frac{t}{q_e} \quad \ldots\ldots (2)$$

Where q_e is the amount of Cd adsorbed at equilibrium (mg/g), and q_t is the amount of Cd adsorbed at time t (mg/g). k_1 (min^{-1}) and k_2 ($g\ mg^{-1} min^{-1}$) are the equilibrium rate constants.

The effect of biochar dosage on Cadmium adsorption, was studied by agitating different amounts of each biochar (0.25 g, 1 g, 2 g, 5 g and 10 g) in 50 mL of Cd standard solution. An analytical weighing balance was used to measure the required amounts of the adsorbent.

The effect of solution temperature was investigated at intervals of 10 °C, 20 °C, 25 °C (room temperature), 30 °C and 40°C. The effect of pH on Cd removal was investigated on varying solution pH at 1.5, 3, 6, 7 and 12. The adsorbents (CSB and RHB) were agitated for 60 minutes and 20 minutes respectively with 50 ml of 1 ppm Cd standard solution. The pH adjustments were made using either 1 M of NaOH or 5 M of H_2SO_4. The effect of initial concentration on adsorption capacity of the two selected biochar types was monitored by varying Cadmium concentrations from 0.25 to 3 ppm keeping other parameters constant. This experiment was conducted at the recorded optimum contact time for each biochar type that was 30 minutes and 60 minutes for Rice husks biochar and coconut shells biochar respectively. A magnetic mechanical shaker was used to stir the mixtures for the required time before filtration.

The adsorption isotherms for the adsorptive removal of Cadmium by the two types of biochar was obtained using the Langmuir and Freundlich isotherm models. Generally, the Langmuir model describes a monolayer adsorption of the adsorbate onto the localized adsorption sites. It assumes energies of adsorption as uniform with no transmigration of the sorbate in the plane of the surface. The linearized form of Langmuir isotherm is expressed as;

$$\frac{1}{q_e} = \frac{1}{K_L\, q_{max}} \cdot \frac{1}{C_e} + \frac{1}{q_{max}} \qquad (3)$$

Where q_{max} represents that maximum adsorption capacity (mg/g) and K_L (L/mg) is the Langmuir 's isotherm constant which shows the binding bond between Cadmium and biochar.

The dimensionless Langmuir constant also known as the separation factor (R_L) was calculated using equation 4 below;

$$R_L = \frac{1}{1 + C_i \times K_L} \qquad (4)$$

Where R_L is the Langmuir constant which indicate the adsorption possibility as favorable if its ($0 < R_L > 1$), unfavorable ($R_L > 1$), linear ($R_L = 1$) or irreversible ($R_L = 0$).

Equation 5 represents the linearized form of Freundlich isotherm model which describes adsorption processes that is non- ideal and reversible. The model applies to multilayer adsorption with non-uniform distribution of energies and affinities over heterogeneous surfaces (Foo & Hameed, 2012).

$$\text{Log } Q_e = \text{Log } K_f + \frac{1}{n} \text{Log } C_e \qquad (5)$$

Where K_f is the Freundlich's constant and used to measure the adsorption capacity and 1/n is the adsorption intensity. The value of 1/n demonstrates the adsorption process either favorable or unfavorable if $0.1 < 1/n < 0.5$ and $1/n > 2$ respectively.

All adsorption experiments were performed in 3 replicates under same conditions and the means plus or minus (±) standard deviation were presented as data. The mixtures of biochar and the Cd solution were shaken at 30 rpm and removed at respective times and thereafter, biochar residues for both biochar type at all parameters were separated from the filtrates using Whatman filter paper No.1. Concentration of the filtrates was determined using the flame Atomic Absorption Spectrophotometer (AAS) shown below (Plate 3).

Plate 3:Atomic Absorption Spectrophotometer
(AAS: Shimadzu AA- 7000)

3.3.3.6 Data analysis

Adsorption at equilibrium q_e (mg/g), was calculated from the mass balance equation as in eq. 6;

$$q_e = \left(\frac{c_o - c_e}{m}\right) \times v \qquad (6)$$

Where C_o is the initial concentration in (ppm), C_e is the Cd concentration at equilibrium (ppm), V is the solution volume (L) and m is the mass of biochar dosage (g).

The percentage of Cadmium ion removal was calculated as in equation (7) below;

$$\text{Removal } (\%) = \left(\frac{c_o - c_t}{c_o}\right) \times 100 \qquad (7)$$

where, C_o and C_t are the initial and the final Cd concentration (mg/l) at time t, respectively.

Origin lab software version 9.1 was used for statistical analysis, model fitting and graphical representation of the effect of sorbent dosage, contact time, pH, solution temperature and initial metal concentration onto the two types of biochar. Regression analysis was used to evaluate the best fit isotherm and kinetics models.

3.3.5 Effect of RHB and CSB on Cadmium accumulation in BSF larvae.

3.3.5.1 Experimental set up

To assay the effect of selected biochar on Cadmium accumulation in BSF larvae, a completely randomized design experiment with a 2× 2 factorial arrangement in 3 replications was conducted. The experimental treatments included (I) biochar factor at 2 levels of type that is coconut and rice biochar, (ii) metal factor at 3 concentration levels, 0,1 and 3 ppm. The concentration of Cadmium used in this study was determined based on the typical wastewater concentration of Cadmium metal in developing countries. On a dry weight basis, 5g biochar with respect to type was blended in the 115g Irish potato peels meal making a 3 days feed ration of 120g for 200 larvae as per experimental unit. This mixture was then spiked with Cadmium metal solution according to the levels of treatment. Weighing, blending of biochar and feed substrate as well as spiking of the feed was done at Jaramogi Oginga Odinga University of Science and Technology post graduate research laboratory. The feeding trial experiment was conducted at the university insect farm in the BSF larvarium and a wooden cage was made to hold the feeding trays according to the treatments. Feeding went on for 16 days and just before harvesting the insects were starved for 24 hours to prevent gut content interference with data collection.

3.3.5.2 Sampling and analysis

Simple random sampling was used to pick samples according to the experimental units which were later blanched and sun dried. The dried samples, food as well as frass residue as per treatment were packaged in plastic zip lock and kept in the refrigerator, for Cd analysis. To prepare samples for analysis 5g of the dried BSF larvae was digested using acid digestion method where in a mixture of Hydrochloric and Nitric acid was added to the samples before placing them in a laboratory microwave digester for two hours.

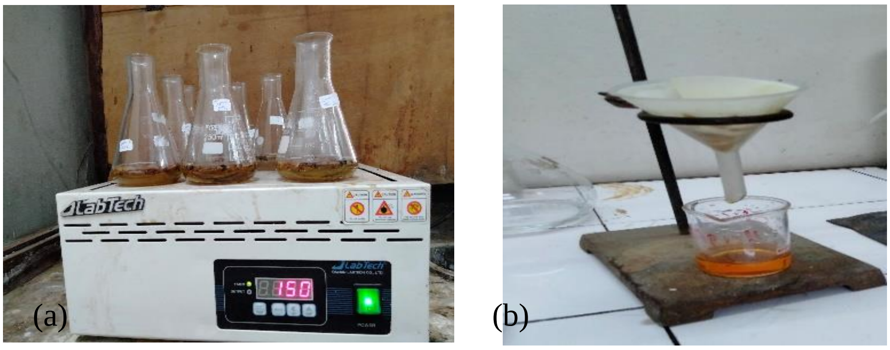


Plate 4:Sample digestion (a) and filtration of digests (b)

After digestion, the sample was filtered to remove the remaining residue and an orangish solution was diluted with distilled water then analyzed using the atomic absorption spectrophotometer (AAS). The bioaccumulation factor was calculated according to Walker (1990);

$$\mathbf{BAF} = \frac{\text{CONCETRATION IN ORANISM (C1)}}{\text{CONCETRATION IN WASTE SUBSTRATE (C2)}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(8)$$

3.3.5.3 Statistical analysis

Statistical analysis of experimental data was conducted using Origin Lab software version 9.1 a. One way ANOVA test was done to determine if there was any variation in the means of RHB and CSB adsorption capacities across Cadmium concentration levels. Fishers' LSD post hoc test analysis was then carried out as a mean separation procedure to indicate where the difference lies. All tests were performed with confidence level of 95% ($\alpha = 0.05$)

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1. Scanning Electron Microscope (SEM) analysis

The morphological features of RHB and CSB were examined using SEM and the micrographs of the respective biochars at X5000 magnifications are shown in Figure 2. From these results, it can be seen that the surface of RHB (Fig. 2a) had plenty of clear irregular crystals which covered the surface while a hollow nature of uneven pattern was observed from CSB (Fig. 2b) image. The roughness and coarse irregular crevices of distinct dimensions observed in both biochar types, indicate the presence of unevenly distributed different sized pores which are imminent adsorption sites. These cavities also serve as channels of adsorption giving access to the active adsorption sites of meso and micropores to interact with the adsorbate thereby allowing for more adsorption (Asuquo *et al*., 2017).

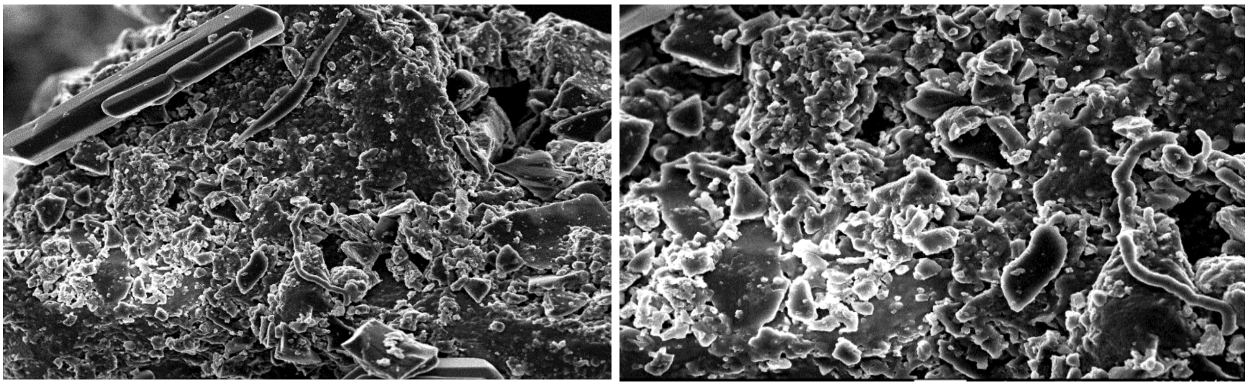


Figure 2 (a): SEM images for rice husks biochar (RHB)

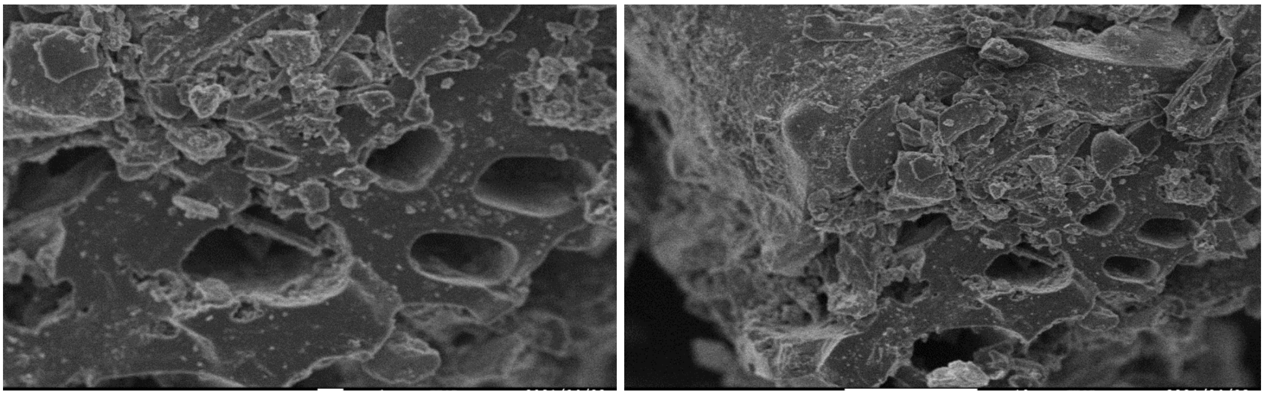


Figure 2 (b): SEM images for coconuts shells biochar (CSB)

Despite being subjected to same pyrolytic conditions, CSB developed a well-defined pore structure whereas RHB showed more irregular pore structure with different sizes and shapes. This could be due to lignin richness content of the biomass material. According to Mahdi *et al*. (2018), highly lignified feedstocks tends to produce macroporous biochars than feedstocks rich in cellulose which produce microporous and in this case, coconut shells are more lignified than rice husks thus one reason for the variation. Moreover, previous studies attributed increased porosity of biochar to formation of internal pores as biomass volatilize during pyrolysis (Mahdi, 2019). However, both biochars portrayed a heterogeneous distribution of pores and rough texture which is in agreement with Freundlich isotherm observed under kinetics studies in this work.

4.2 ADSORPTION BEHAVIOR OF Cd^{2+} ON RHB AND CSB FOR HEAVY METAL REMEDIATION IN BSF LARVAE FED ON CONTAMINATED ORGANIC WASTE

4.1.1 Effect of contact time

The adsorption of Cadmium onto RHB and CSB was monitored at varying time intervals of 5 mins, 10 mins, 20 mins, 30 mins, 60 mins and 120 mins keeping other parameters constant. Cadmium was rapidly adsorbed onto RHB and CSB in the first 30 minutes as observed in figure 3. Thereafter, CSB showed a relatively low increase in the subsequent absorption of Cd before reaching saturation or equilibrium at 60 minutes while RHB showed a slight reduction before levelling up.

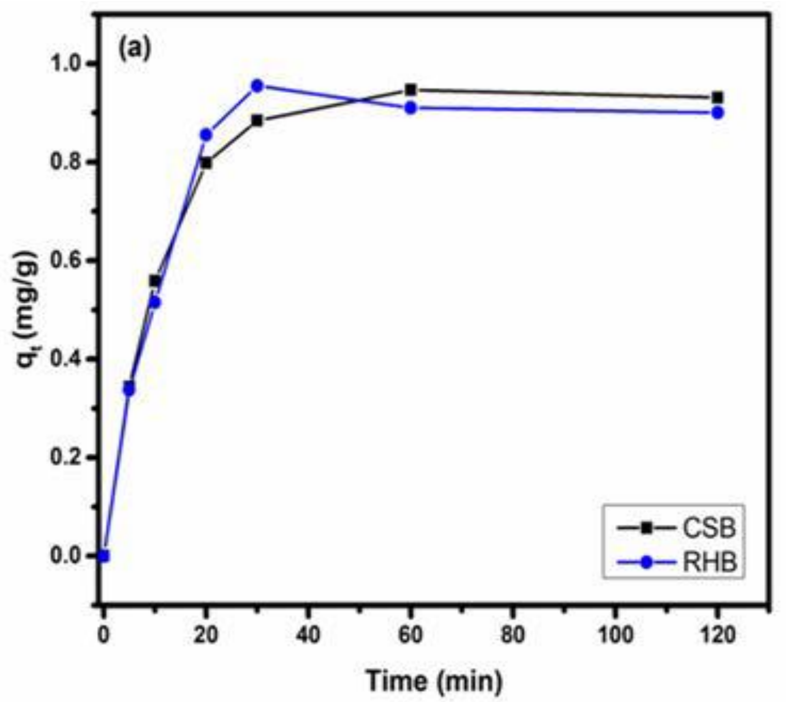


Figure 3: Effect of contact time on adsorption capacity of Cd, plotted based on the mean ± SD of the 3 replicates used.

This trend in adsorption is ascribed to two-phase adsorption phenomena that is the prominently rapid phase and the relatively low phase. The explanation owing to the two-phase adsorption phenomenon is the abundant availability of active adsorption sites on adsorbent surface which gradually become saturated with time (Chaukura *et al.*, 2017; Lou *et al*., 2016; Zhao *et al*., 2020). However, a slight decrease in adsorption shown by RHB from 30 minutes before saturation was attributed to desorption of weakly bounded Cd ions as adsorption sites on the adsorbent decreases. Similar results were reported for Pine sawdust biochar on Cu (II) ions (Lou *et al*., 2016). In this study, RHB was more effective in adsorption of Cadmium than CSB because it took lesser time. Despite uniform experimental conditions that both adsorbents were subjected to, RHB and CSB reached adsorption equilibrium at different times of 30 and 60 minutes respectively. This means that CSB needed longer time (60 minutes) to reach equilibrium adsorption while RHB adsorption of Cadmium only took 30 minutes at slightly higher Qt than that of CSB. One reason for variation in reaching equilibrium of the two biochars produced under same pyrolytic conditions is difference in pore structure as RHB show a more porous structure than CSB.

4.1.2 Effect of dosage

The effect of RHB and CSB dosages on adsorption of Cadmium was as shown in Figure 4. Cadmium adsorption increased with increase in dosage of the respective adsorbents. This can be attributed to an increase in the total number of adsorption sites present on the surface of the adsorbents which, in turn, increases the overall binding of Cadmium ions. While this remained true for rice husks biochar, coconut shells biochar only increased adsorption up to 2 g before a slight reduction of approximately 0.001 and thereafter remained constant. This could mean that there were no more Cadmium ions in the working standard that extra sorbent could bind, rather the metal ions in the solution were less than the exchangeable sites on the biochar thus less Cadmium uptake (Ramalingham *et al.*, 2020). Considering the SEM micrographs of the two adsorbents, RHB showed numerous irregular pores than CSB. Therefore, increasing the dosage directly increases adsorption sites thus the reason for continued increase in adsorption of RHB.

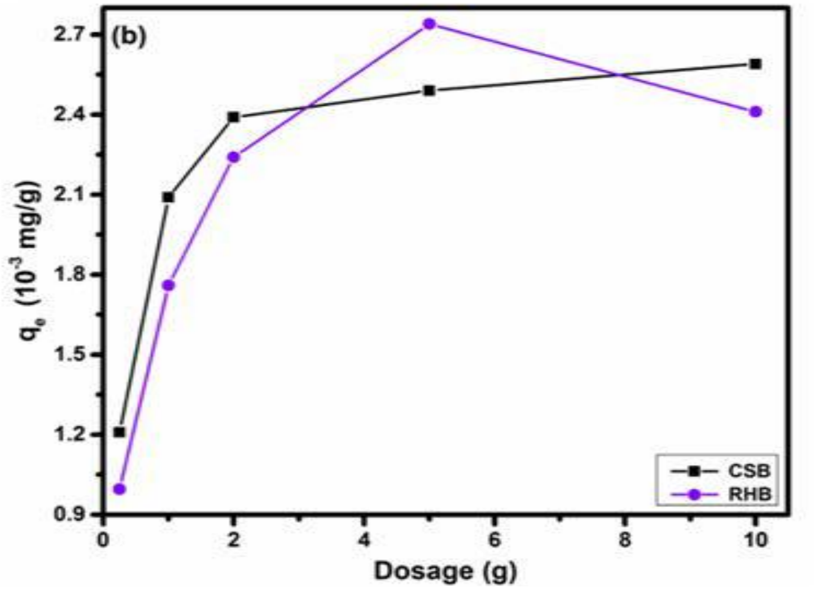


Figure 4:Effect of adsorbent dosage on Cd adsorption, plotted data presents the mean ± SD of three replicates

4.1.3 Effect of pH

The adsorption of Cd^{2+} on RHB and CSB was studied over a pH range of 1.5 to 12 and the results are illustrated in Figure 5. Adsorption of Cd was relatively low at lower pH value of 1.5 to 2 but a rapid increase in Cd^{2+} removal was observed when pH increased from pH 3. However, increasing pH to 12 yielded no large changes for both biochar types due to possible reduction of the adsorption sites at basic pH. The Cd removal of CSB and RHB at acidic pH that is less than 2 was low but increasing pH from 3 to 5 and 6 respectively increased the rate of adsorption before reaching equilibrium. This was as a result of high competition between metal ions and hydrogen ions for the sorbent sites that happens at low pH thus decreased overall removal of the contaminant (Jia *et al*., 2018).

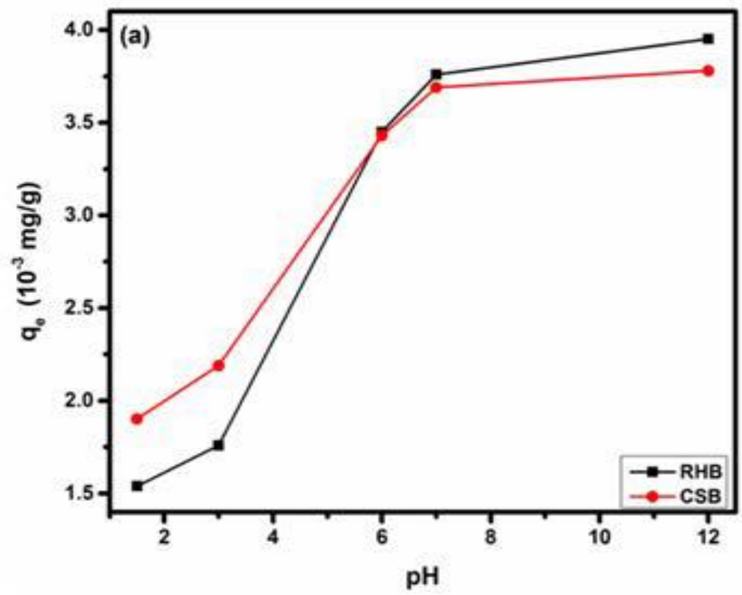


Figure 5:Effect of pH on adsorption of Cd, plotted on the mean ± SD of three replicates

The rapid adsorption observed with increasing pH value (from 3) was due to reduced competition between metal ions and hydrogen ions for the sorbent sites as deprotonation of functional groups of the adsorbents discharged additional binding sites resulting in higher adsorption of the adsorbate (Mahdi, 2019). The graph though showed a reduction in the rate of adsorption as pH values approached 12, this entails that at higher pH, adsorption is less due to precipitation which leads to formation of hydroxide complexes whose solubility is much lower (Kołodyn'ska *et al*., 2012).

4.1.4 Effect of adsorption temperature

Temperature is another parameter that affects adsorption capacity and kinetics rate of any adsorbent (Ramalingham *et al*., 2020). Thus, adsorption capacity of Cadmium on CSB and RHB was determined as a function of temperature. The sorption capacities of both biochars increased with increasing temperature which is an indication of endothermic type of adsorption process as presented in Figure 6. While both biochar types portrayed a endothermic adsorption process, the best performance on CSB and RHB was observed at 40 ˚C. Taghlidabad *et al*. (2020) reported similar results where the optimum temperature for best performance on the used biochars was 40 °C.

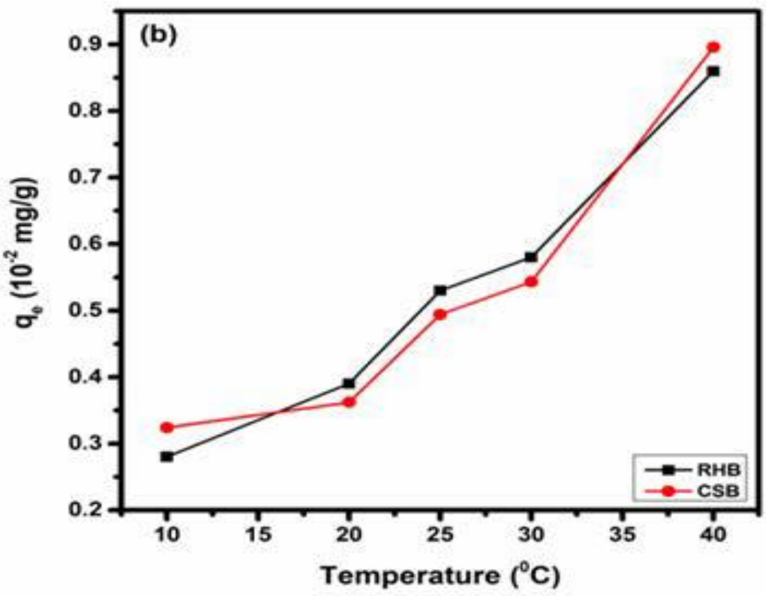


Figure 6:Effect of temperature on adsorption of Cd, plotted data is based on mean ± SD of three replicates

4.1.5 Effect of initial concentration

Cadmium adsorption onto RH and CS biochar showed an increase with increasing metal ion concentration, thus the removal capacity of the total metal ion increased from the lowest concentration as shown in Figure 7. This behavior can be attributed to an increase in the mass transfer driving force which is as a result of increasing number of collisions between the sorbent and the metal ions as the concentration increases (Zhao *et al*., 2020). Therefore, at optimum conditions, it could be concluded that the highest Cadmium adsorption occurred at the highest initial metal concentration which was 3 ppm. These results concur with previous work of Gebrekidan & Mihretu, (2020).

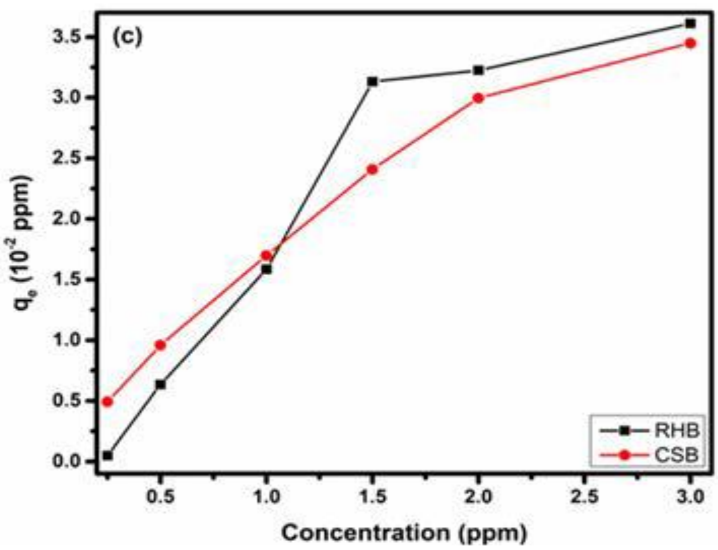


Figure 7:Effect of initial concentration on adsorption of CD, data is plotted based on the mean ± SD of three replicates

4.1.6 Application of Isotherms

4.1.6.1 Adsorption isotherms

This study employed Langmuir and Freundlich models to fit the equilibrium data. The models were constructed using effect of biochar on Cadmium initial concentration data at equilibrium points of other parameters of influence. From these results and based on the experimental data for Cadmium adsorption onto RHB and CSB, Freundlich model best fitted the adsorption process for both biochar types. The isotherm parameters for both models determined in this study are presented in Table 2.

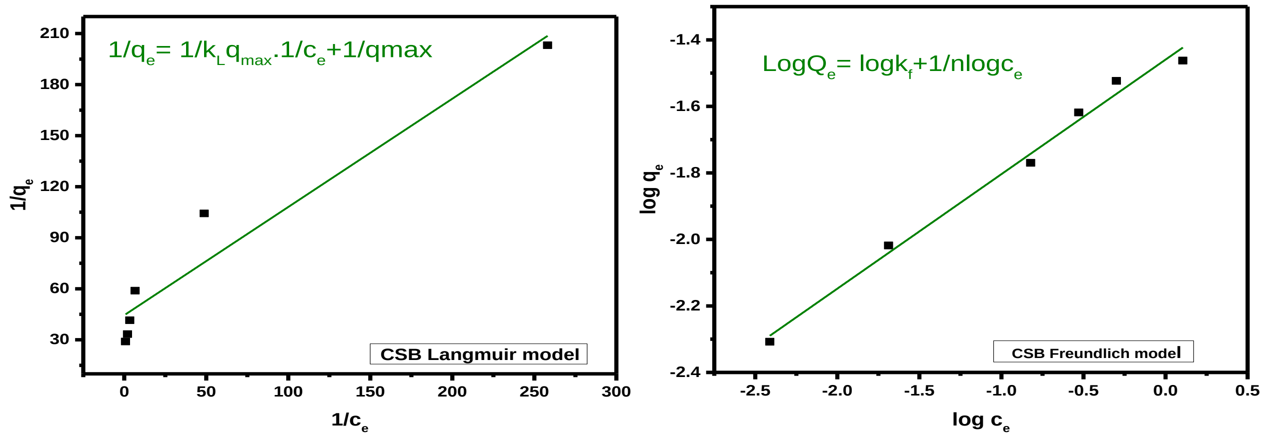


Figure 8: Coconut shells biochar adsorption isotherm models

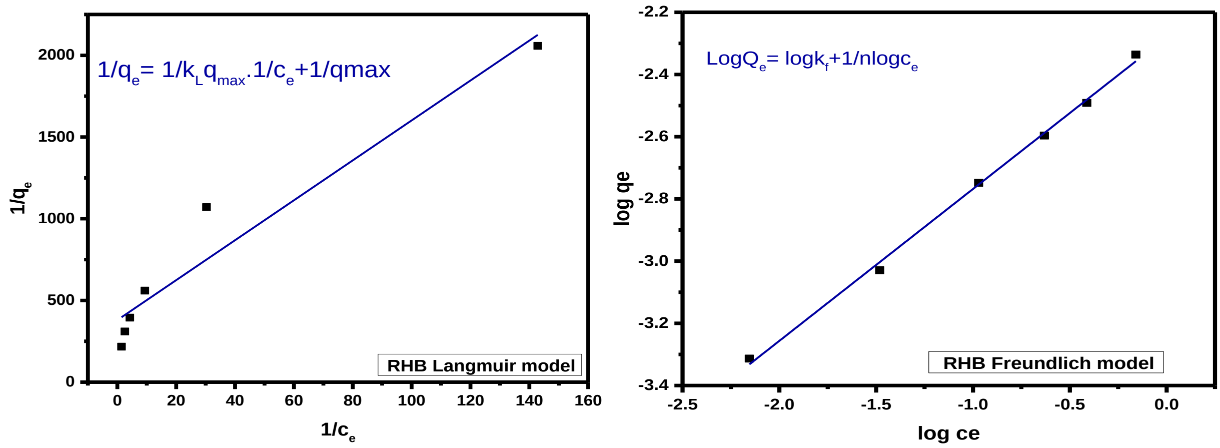


Figure 9:Rice husks biochar adsorption isotherm models.

These parameters together with the linear regression values indicated that Freundlich model fits the data better than Langmuir model. Furthermore, the 1/n value which is the heterogeneity factor of CSB and RHB was in the range 0 and 1 which is also an indication of favorable adsorption (Van, Hien *et al*., 2020). This trend was also reported by Taghlidabad *et al*. (2020) for biochar produced from apple and grape pruning residues, Puglla *et al*. (2020) also reported similar findings for Cadmium and lead onto peanut shells, Chonta pulp and corn cob biochar.

Table 1:Langmuir and Freundlich isotherm parameters and correlation coefficients for Cd adsorption onto CSB and RHB

Langmuir					**Freundlich**		
Biochar	q_{max}	k_l	R_l	R^2	1/n	K_f	R^2
RHB	0.0023	4640.43	95.9547	0.9205	0.4882	0.0052	0.9967
CSB	0.0225	28.2924	12.1191	0.9222	0.3444	0.0347	0.9877

Previously Doumer *et al*. (2016) described a similar trend for biochar produced from five different feedstocks namely sugarcane bagasse, eucalyptus forest residues, castor meal, green pericarp of coconut, and water hyacinth. Despite that, these results were opposite of other previous biochar adsorption studies which supported Langmuir isotherm model like Wu *et al*.(2019) who conducted adsorption studies using biochar from mushroom substrate. Khan *et al*.(2017) used rice husks biochar on Cadmium from soils with different water conditions and their results fitted on Langmuir model In this case the variation to our rice husks findings could be attributed to pyrolysis condition activation and experimental conditions.

This therefore informs that biochar from different feedstocks produced at different temperatures have different adsorption capacities and mechanisms with regard to sorbate of interest (Hien *et al.*, 2020; Khan *et al*., 2017). In this study, Cadmium adsorption on rice husks and coconut shells biochar was best described or fitted using the Freundlich model, which implied that adsorption process occurred on a heterogeneous multilayered surface. This can be seen from the 1/n and R^2 values. The value of 1/n for both biochar types demonstrates the adsorption process favorable to the Freundlich model as 1/n is less than 2 in the both rice and coconut shells biochar while as the R^2 in Freundlich are greater than those in Langmuir.

4.1.6.2 Adsorption kinetics

The adsorption kinetic parameters of Cadmium on CSB and RSB was further evaluated using Pseudo 1^{st} order and Pseudo 2^{nd} Order equations. The results of the fitting of both kinetic models to the Cadmium adsorbed are shown in Figure 10 and 11. The corresponding results of the kinetic parameters are presented in table 1.

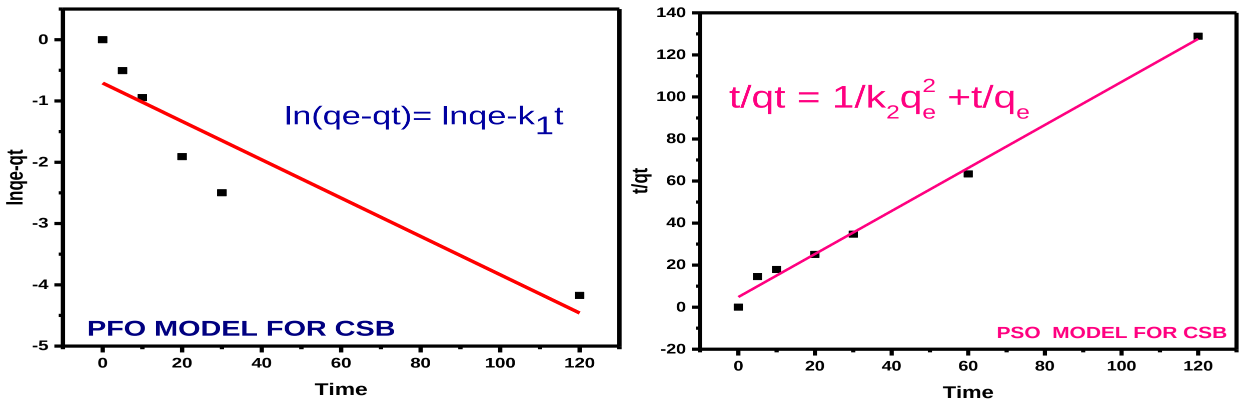


Figure 10: Adsorption kinetic models for Coconut shells biochar

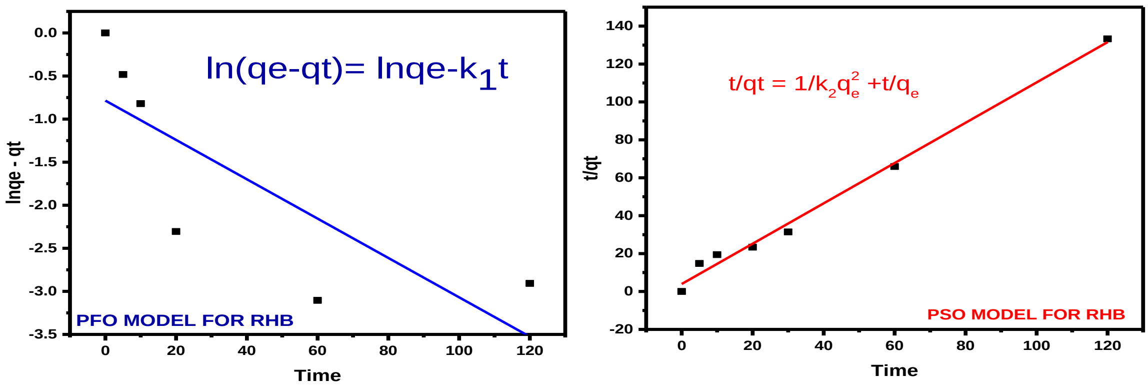


Figure 11: Adsorption kinetic models for rice husk biochar

Pseudo second order kinetics was observed to be the best fitted model in explaining the kinetics of Cadmium adsorption on both biochars. This model showed higher value of linear regression coefficient (R^2) of 0.9904 and 0.9934 for RHB and CSB respectively when compared to Pseudo first order model with respective R^2 values of 0.5441 and 0.8102. Furthermore, pseudo second order model was also shown by the closeness of the calculated adsorption capacity (q_e) and the experimental (q_e). Based on these results, adsorption of Cd on coconut shells biochar and rice husks biochar occurred through chemisorption mechanism which is controlled by chemical processes like valence forces sharing or exchanging electrons between adsorbent and adsorbate (Zhao *et al.*, 2019) and electrostatics.

Table 2: Data for linear PFO and PSO models of adsorption kinetics

Order of reaction	Parameters	RHB	CSB
	$q_{, exp.}$(mg/g)	0.8345±0.008	0.8461±0.057
Pseudo 1st order	$q_{e, cal.}$(mg/g)	0.3926±0.008	0.4391±0.057
	k_1 (min-1)	-0.0010	-0.0004
	R^2	0.5441	0.8102
Pseudo 2nd order	$q_{e, cal.}$(mg/g)	0.8399±0.008	0.8484±0.057
	k2 (g mg-1min-1)	0.1857	0.1232
	R2	0.9904	0.9934

4.2 EFFECT OF RHB AND CSB ON CADMIUM ACCUMULATION IN BSF LARVAE.

Two types of biochar were used in this study to investigate the absorbance of Cd from waste spiked with Cadmium. The initial concentration level used to spike the feed substrate were 3 ppm, 1 and 0 ppm. From the initial concentration levels, RHB removed 98.4 % from 1 ppm and 92.1% from 3ppm whereas CSB adsorbed 99.3 % from 1 ppm and 94.7% from 3 ppm as shown in Figure 12.

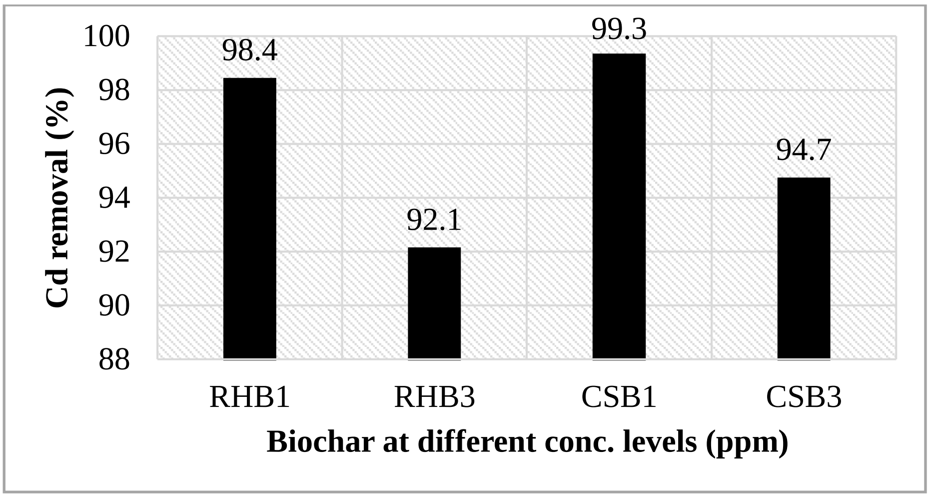


Figure 12:Cadmium removal percentages across the concentration levels

Analysis of variance (ANOVA) results presented in table 3, indicated that at α = 0.05, the means of adsorption capacity of the two biochar types at different concentration levels were significantly different. At 95% confidence level, Fisher's LSD post hoc test confirmed a significant difference in biochar adsorption across the concentration levels but no significant difference was found between the biochar types (Table 4). This is also confirmed by the error bars indicated in figure 13 below which overlapped at 3ppm concentration of both biochar types and the same is observed at 1 ppm concentration treatment. However, the error bars did not overlap across the concentration levels at each biochar type application indicating a significant difference in the amount of Cd removed at the respective concentration levels.

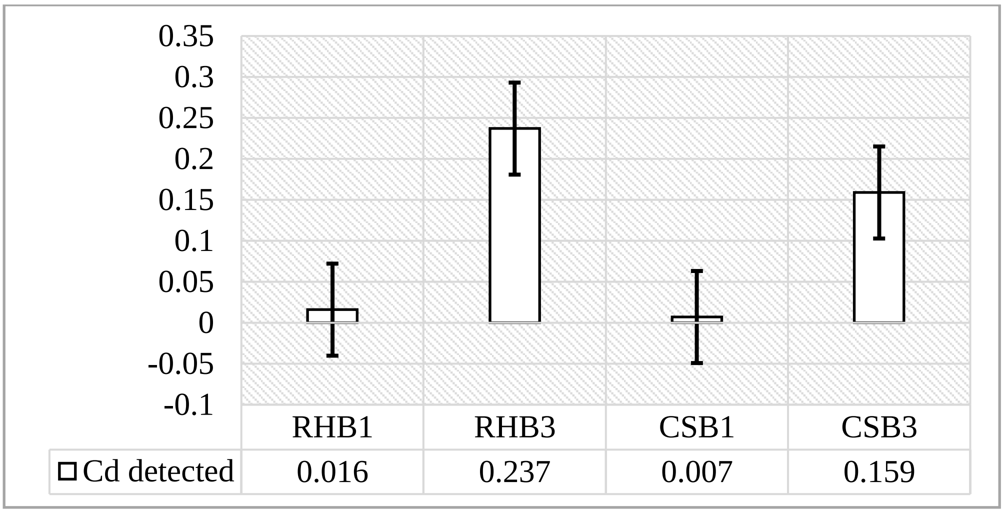


Figure 13: Error bars showing difference in adsorption capacities of RHB and CSB, plotted data is based on mean ± SD of three replicates.

The results from this study proves the effectiveness of both biochar types in adsorbing Cadmium from the waste used a BSF feed thus making it unavailable for accumulation in the harvested larvae. The effectiveness of biochar in reducing the levels of heavy metals in water and soils where the majority of animal feed is obtained is well documented. Abedinzadeh *et al*., (2020) observed a reduction in Cd and Pb uptake of maize plants with application of biochar at various irrigation water quality which entails that the Cd and Pb spiked in the soil was adsorbed by the biochar and thus not accessible to plants.

Despite both biochars proving effective in adsorbing Cd at 5g dosage, a decrease in adsorption capacity of both biochar types with increasing Cadmium ions from 1 to 3 ppm was observed. Similar findings were reported by previous biochar adsorption studies like Van *et al*., (2020) who reported a gradual decrease in the adsorption of bamboo, wood and rice husk biochars at higher metal concentrations of zn^{2+} when the dosage for all the biochar was constant. This shows a direct proportional relationship between the adsorbate and the adsorbent which is in agreement with the findings of Abhishek *et al*., (2020) who reported a higher removal efficiency with the increasing biochar loading.

According to Lao & Mbega, (2020) the mechanism behind this biochar potential to adsorb heavy metals is ascribed to electrostatic interaction between biochar, heavy metal and conditions of the media of application. When added to the soil, Peng *et al*., (2011) reported that biochar induces the cation exchange capacity which increases the negatively charged ions and because the metals have positively charged ions, they then get bound to the biochar surface areas. The variation in removal percentages between the lower (1ppm) and higher(3ppm) concentration levels can be attributed adsorption sites of the 5g of the adsorbents being occupied thus a reduction in adsorption with increasing concentration of Cd.

This explains why a dosage of 5 g biochar used in this study showed a higher Cadmium removal at 1 ppm and a slight reduction in the adsorption percentage when Cadmium ions increased to 3ppm. The two biochar types have the potential to remediate Cadmium in BSF reared on contaminated waste. The batch experiments confirmed the capacity of these adsorbents in binding the Cd metal and the same trend was described by the application part of the study. Moreover, bio accumulative factor (BAF) was lower than 1 for the BSF samples as well as the frass suggesting no accumulation of Cadmium in the BSF tissue (Vanda *et al*., 2020).

CHAPTER 5: CONCLUSION AND RECOMMENDATIONS

5.1 GENERAL CONCLUSIONS

The Agrowaste (rice husks and coconut shells) used in this study showed great potential towards management of Cadmium accumulation in BSF larvae reared on contaminated waste. The adsorption studies were done to determine the behavior of selected biochar on removal of Cadmium ions from waste effluents at different adsorption parameters and it was found to be influenced by pH, biochar dosage, contact time, temperature and metal initial concentration. However, both biochar types showed a two-phase adsorption behavior at increasing temperature of up to 40 °C and the results well fitted Freundlich isotherm model which suggested a non-uniform type of adsorption. This was supported by SEM images which showed potential of heterogeneous adsorption by the representation of the adsorption sites. The adsorbents had scattered and irregular adsorption sites which required 30 to 60 minutes contact time with respect to concentration levels and dosage of the adsorbent.

The RHB and CSB used in this study showed a high adsorption capacity of the targeted Cd metal ion. Despite an insignificant statistical difference ($p > 0.05$) in the adsorption capacity of both biochars, RHB presented a removal rate of about 98.4 to 92.1 % from feed spiked with 1 and 3 ppm respectively while CSB 99.3 and 94.7% removal at the respective concentrations. The difference in adsorption percentages was found to be statistically significant ($p < 0.05$) across the concentration effect. Generally, the tested and applied high adsorption capacity of the selected biochars confirmed their suitability for Cadmium management in BSF if contaminated municipal organic waste is to be used for BSF farming. The remediation results for the heavy metal risk in BSF provides a pathway that works towards reducing the food-feed competition which has skyrocketed livestock production.

5.2 RECOMMENDATIONS

Based on the findings of this study and the nutritional improvement properties that biochar presented in other livestock from previous studies we recommend utilization of highly lignified agricultural waste like coconut shells and Rice husks in formation and inclusion of biochar for remediating of heavy metals like cadmium in untreated substrate that are used for BSF rearing.

Further research in;

- The effect of biochar as a feed additive on growth and nutritional parameters of BSF at different life stages.
- Different other management strategies to remediate Cadmium as well as other heavy metals accumulation in BSF larvae.

REFERENCES

Abedinzadeh, M., Etesami, H., Ali, H., & Sha, S. (2020). Combined use of municipal solid waste biochar and bacterial biosorbent synergistically decreases Cd (II) and Pb (II) concentration in edible tissue of forage maize irrigated with heavy metal – spiked water. *Heliyon*, *6*(January). https://doi.org/10.1016/j.heliyon.2020.e04688

Abhishek, P., Bishnu, A., & Aitazaz, F. (2020). We are IntechOpen , the world ’ s leading publisher of Open Access books Built by scientists , for scientists TOP 1 % Biochar-Assisted Wastewater. *IntechOpen*. https://doi.org/DOI: http://dx.doi.org/10.5772/intechopen.92288

Alagappan, S., Rowland, D., Barwell, R., Cozzolino, D., Mikkelsen, D., Olarte Mantilla, S. M., James, P., Yarger, O., & Hoffman, L. (2022). Organic side streams (bioproducts) as substrate for black soldier fly (Hermetia illucens) intended as animal feed: Chemical safety issues. *Animal Production Science*. https://doi.org/10.1071/AN22155

Amoah, Y. D. (2016). *Analysis of emission and energy content of coconut husks.* [Kwame Nkrumah University of Science and Technology (KUMASI)]. https://www.idin.org/sites/default/files/resources/KNUST Coconut Husk Emissions Spring 2016.pdf

Ashikuzzaman, M., & Howlader, M. H. (2019). *Sustainable Solid Waste Management in Bangladesh*. *December*, 35–55. https://doi.org/10.4018/978-1-7998-0198-6.ch002

Asuquo, E., Martin, A., Nzerem, P., Siperstein, F., Fan, X., & Martin, A. (2017). Adsorption of Cd (II) and Pb (II) ions mesoporous activated carbon from aqueous solutions using Equilibrium , kinetics and adsorbent : characterisation studies . *Journal of Environmental Chemical Engineering 2017*, *5*(No.1), 679–698. https://doi.org/10.1016/j.jece.2016.12.043

Bakker, L. (2020). Product market applications of the Black Soldier Fly. *Wageningen University & Research*, 1–45. https://edepot.wur.nl/520307

Banks, I. J., Gibson, W. T., & Cameron, M. M. (2014). Growth rates of black soldier fly larvae fed on fresh human faeces and their implication for improving sanitation. *Tropical Medicine & International Health*, *19*(1), 14–22. https://doi.org/10.1111/tmi.12228

Belluco, S., Losasso, C., Maggioletti, M., Alonzi, C. C., Paoletti, M. G., & Ricci, A. (2013). Edible insects in a food safety and nutritional perspective: A critical review. *Comprehensive Reviews in Food Science and Food Safety*, *12*(3), 296–313. https://doi.org/10.1111/1541-4337.12014

Belluco, S., Losasso, C., Maggioletti, M., Alonzi, C., Ricci, A., & Paoletti, M. G. (2015). Edible insects : a food security solution or a food safety concern ? *Comprehensive Reviews in Food Science and Food Safety*, *5*(2), 25–30. https://doi.org/10.2527/af.2015-0016

Bessa, L. W., Pieterse, E., Marais, J., & Hoffman, L. C. (2020). Why for feed and not for human consumption? The black soldier fly larvae. *Comprehensive Reviews in Food Science and Food*

Safety, *19*(5), 2747–2763. https://doi.org/10.1111/1541-4337.12609

Biancarosa, I., Liland, N. S., & Araujo, P. (2017). Uptake of heavy metals and arsenic in black soldier fly (Hermetia illucens) larvae grown on seaweed-enriched media. *Journal of the Science of Food and Agriculture, September*. https://doi.org/10.1002/jsfa.8702

Biancarosa, I., Liland, N. S., Biemans, D., Araujo, P., Bruckner, C. G., Waagbø, R., Torstensen, B. E., Lock, E. J., & Amlund, H. (2017). Uptake of heavy metals and arsenic in black soldier fly (Hermetia illucens) larvae grown on seaweed-enriched media. *Journal of the Science of Food and Agriculture*, *98*(6), 2176–2183. https://doi.org/10.1002/jsfa.8702

Bor, S. L. (1988). Rice Hulls. In *Rice* (pp. 269–270). © Springer Science+Business Media New York 1991 269.

Bosch, G., Van Der Fels-Klerx, H. J., De Rijk, T. C., & Oonincx, D. G. A. B. (2017). Aflatoxin B1 tolerance and accumulation in black soldier fly larvae (hermetia illucens) and yellow mealworms (tenebrio molitor). *Toxins*, *9*(6), 1–10. https://doi.org/10.3390/toxins9060185

Cadinu, L. A., Barra, P., Torre, F., Delogu, F., & Madau, F. A. (2020). Insect rearing: Potential, challenges, and circularity. *Sustainability (Switzerland)*, *12*(11). https://doi.org/10.3390/su12114567

Charlton, A. J., Dickinson, M., Wakefield, M. E., Fitches, E., Kenis, M., Han, R., Zhu, F., Kone, N., Grant, M., Devic, E., Bruggeman, G., Prior, R., & Smith, R. (2015). Exploring the chemical safety of fly larvae as a source of protein for animal feed. *Journal of Insects as Food and Feed*, *1*(1), 7–16. https://doi.org/10.3920/JIFF2014.0020

Chaukura, N., Murimba, E. C., & Gwenzi, W. (2017). Synthesis, characterisation and methyl orange adsorption capacity of ferric oxide–biochar nano-composites derived from pulp and paper sludge. *Applied Water Science*, *7*(5), 2175–2186. https://doi.org/10.1007/s13201-016-0392-5

Chen, P., Wang, H., Zheng, R., Zhang, B., & Sun, G. (2018). Long-term effects of biochar on rice production and stabilisation of cadmium and arsenic levels in contaminated paddy soils. *Earth and Environmental Science Transactions of The Royal Society of Edinburgh*, *109*(3–4), 415–420. https://doi.org/https://doi.org/10.1017/S175569101800049X

Dickie, F., Miyamoto, M., & (Tilly) Collins, C. M. (2019). The Potential of Insect Farming to Increase Food Security. In *Edible Insects* (pp. 1–10). https://doi.org/10.5772/intechopen.88106

Diener, S. (2010). Valorisation of Organic Solid Waste using the Black Soldier Fly , Hermetia illucens , in Low and Middle - Income Countries.[Doctoral dissertation]. In *ETH Zürich.* (Issue 19330). https://doi.org/10.3929/ethz-a-6559779

Diener, S., Zurbrügg, C., & Tockner, K. (2015). Bioaccumulation of heavy metals in the black soldier fly, Hermetia illucens and effects on its life cycle. *Journal of Insects as Food and Feed*, *1*(4), 261–270. https://doi.org/10.3920/JIFF2015.0030

Doumer, M. E., Rigol, A., Vidal, M., & Mangrich, A. S. (2016). Removal of Cd , Cu , Pb , and Zn from aqueous solutions by biochars. *Environmental Science and Pollution Research - Springer*, 2684–2692. https://doi.org/10.1007/s11356-015-5486-3

EC, E. commission. (2003). *Opinion of the Scientific Committee on Animal nutrition on undesirable substances in feed* (Issue May 2002).

FAO. (2021). Looking at edible insects from a food safety perspective,Challenges and opportunities for the sector. Rome. In *Looking at edible insects from a food safety perspective*. https://doi.org/10.4060/cb4094en

Gao, Q., Wang, X., Wang, W., Lei, C., & Zhu, F. (2017). Influences of chromium and cadmium on the development of black soldier fly larvae. *Environmental Science and Pollution Research*, *24*(9), 8637–8644. https://doi.org/10.1007/s11356-017-8550-3

Gebrekidan, A., & Mihretu, L. D. (2020). Removal of heavy metals from aqueous solutions using Eucalyptus Camaldulensis : An alternate low cost adsorbent. *Cogent Chemistry*, *May*. https://doi.org/10.1080/23312009.2020.1720892

Gold, M., Tomberlin, J. K., Diener, S., Zurbrügg, C., & Mathys, A. (2018). Decomposition of biowaste macronutrients, microbes, and chemicals in black soldier fly larval treatment: A review. *Waste Management*, *82*, 302–318. https://doi.org/10.1016/j.wasman.2018.10.022

Hien, N. Van, Valsami-jones, E., Cong, N., & Thi, T. (2020). *Bioresource Technology Reports E ff ectiveness of di ff erent biochar in aqueous zinc removal : Correlation with physicochemical characteristics*. *11*(February). https://doi.org/10.1016/j.biteb.2020.100466

Huis, A. Van. (2012). 7 . Insects as animal feed. In *Edible Insects - Future prospects for food and feed security* (pp. 89–97). FAO.

Imathiu, S. (2020). Benefits and food safety concerns associated with consumption of edible insects. *NFS Journal*, *18*(November 2019), 1–11. https://doi.org/10.1016/j.nfs.2019.11.002

Inyang, M. I., Gao, B., Yao, Y., Xue, Y., Zimmerman, A., Mosa, A., Pullammanappallil, P., Ok, Y. S., & Cao, X. (2016). A Review of Biochar as a Low-Cost Adsorbent for Aqueous Heavy Metal Removal. *Critical Reviews in Environmental Science and Technology*, *3389*(September). https://doi.org/10.1080/10643389.2015.1096880

Ismail, I., & Moustafa, T. (2016). Biosorption of heavy metals. *Heavy Metals: Sources, Toxicity and Remediation Techniques*, *3*, 131–174. https://doi.org/10.1201/9781315364339-6

Jia, Y., Shi, S., Liu, J., Su, S., Liang, Q., Zeng, X., & Li, T. (2018). Study of the effect of pyrolysis temperature on the Cd2+ adsorption characteristics of biochar. *Applied Sciences (Switzerland)*, *8*(7). https://doi.org/10.3390/app8071019

Joly, G., & Nikiema, J. (2019). *Global Experiences on Waste Processing with Black Soldier Fly (Hermetia illucens):From Technology to Business*. CGIAR Research Program on Water, Land

and Ecosystems (WLE). 62p.(Resource Recovery and Reuse Series 16). doi:%0A10.5337/2019.214

Kalus, K., Koziel, J. A., & Opaliński, S. (2019). A review of biochar properties and their utilization in crop agriculture and livestock production. *Applied Sciences (Switzerland)*, *9*(17). https://doi.org/10.3390/app9173494

Kammann, C., Ippolito, J., Hagemann, N., Borchard, N., Cayuela, M. L., Estavillo, J. M., Fuertes-Mendizabal, T., Jeffery, S., Kern, J., Novak, J., Rasse, D., Saarnio, S., Schmidt, H. P., Spokas, K., & Wrage-Mönnig, N. (2017). Biochar as a tool to reduce the agricultural greenhouse-gas burden–knowns, unknowns and future research needs. *Journal of Environmental Engineering and Landscape Management*, *25*(2), 114–139. https://doi.org/10.3846/16486897.2017.1319375

Karanja, N., Njenga, M., Mutua, G., Lagerkvist, C., Kutto, E., & Okello, J. (2012). Concentrations of Heavy Metals and Pesticide Residues in Leafy Vegetables and Implications for Peri-urban Farming in Nairobi, Kenya. *Journal of Agriculture, Food Systems, and Community Development*, *3*(1), 255–267. https://doi.org/10.5304/jafscd.2012.031.003

Khan, M. A., Khan, S., Ding, X., Khan, A., & Alam, M. (2017). The effects of biochar and rice husk on adsorption and desorption of cadmium on to soils with different water conditions (upland and saturated). *Chemosphere*. https://doi.org/10.1016/j.chemosphere.2017.11.110

Kołodyn'ska, D., Wnetrzak, R., Leahy, J. J., Hayes, M. H. B., Kwapin'ski, W., & Hubick, Z. (2012). Kinetic and adsorptive characterization of biochar in metal ions removal. *Chemical Engineering Journal*, *197*, 295–305. https://doi.org/10.1016/j.cej.2012.05.025

Komkiene, J., & Baltrenaite, E. (2016). Biochar as adsorbent for removal of heavy metal ions [Cadmium(II), Copper(II), Lead(II), Zinc(II)] from aqueous phase. *International Journal of Environmental Science and Technology*, *13*(2), 471–482. https://doi.org/10.1007/s13762-015-0873-3

Lalander, C., Senecal, J., Gros Calvo, M., Ahrens, L., Josefsson, S., Wiberg, K., & Vinnerås, B. (2016). Fate of pharmaceuticals and pesticides in fly larvae composting. *Science of the Total Environment*, *565*(September), 279–286. https://doi.org/10.1016/j.scitotenv.2016.04.147

Lalander, Cecilia, Diener, S., Elisa, M., Zurbrügg, C., Lindström, A., & Vinnerås, B. (2013). Science of the Total Environment Faecal sludge management with the larvae of the black soldier fl y (Hermetia illucens) — From a hygiene aspect. *Science of the Total Environment, The*, *458–460*, 312–318. https://doi.org/10.1016/j.scitotenv.2013.04.033

Lao, E. J., & Mbega, E. R. (2020). Biochar as a feed additive for improving the performance of farm animals. *Malaysian Journal of Sustainable Agriculture (MJSA)*, *4*(2), 86–93. https://doi.org/http://doi.org/10.26480/mjsa.02.2020.86.93

Li, H., Dong, X., Evandro, B., Oliveira, L. M. De, Chen, Y., & Ma, L. Q. (2017). Chemosphere

Mechanisms of metal sorption by biochars : Biochar characteristics and modi fi cations. *Chemosphere*, *178*, 466–478. https://doi.org/10.1016/j.chemosphere.2017.03.072

Liu, J. ., Yang, X., Lu, K. ., Zhang, X. ., Huang, H. ., & Wang, H. . (2015). Effect of bamboo and rice straw biochars on the transformation and bioavailability of heavy metals in soil. *Acta Sci. Circumstantiae*, *35,* 3679–3687.

Lohri, C. R., Stefan, D., Imanol, Z., Adeline, M., & Christian, Z. (2017). Treatment technologies for urban solid biowaste to create value products : a review with focus on low- and middle- income settings. *Review in Environmental Science and Bio/Technology*, 81–130. https://doi.org/10.1007/s11157-017-9422-5

Lou, K., Rajapaksha, A. U., Ok, Y. S., & Chang, X. S. (2016). Sorption of copper(II) from synthetic oil sands process-affected water (OSPW) by pine sawdust biochars: effects of pyrolysis temperature and steam activation. *Journal of Soils and Sediments*, *16*(8), 2081–2089. https://doi.org/10.1007/s11368-016-1382-9

Lyu, H., Gong, Y., Gurav, R., & Tang, J. (2016). Potential Application of Biochar for Bioremediation of Contaminated Systems. In *Biochar Application*. Elsevier Inc. https://doi.org/10.1016/B978-0-12-803433-0.00009-6

Mahdi, Z. A. (2019). Single and Multicomponent Heavy Metal Ion Adsorption from Aqueous System Using Biochar Derived from Date Seed S Biomass. *Griffith Research Online*. https://doi.org/https://doi.org/10.25904/1912/670

Mahdi, Z., Yu, Q. J., & El, A. (2018). Removal of lead (II) from aqueous solution using date seed - derived biochar : batch and column studies. *Applied Water Science*. https://doi.org/10.1007/s13201-018-0829-0

Man, K. Y., Chow, K. L., Man, Y. B., Mo, W. Y., & Wong, M. H. (2020). Technology Use of biochar as feed supplements for animal farming. *Critical Reviews in Environmental Science and Technology*, *0*(0), 1–31. https://doi.org/10.1080/10643389.2020.1721980

Masoud, M. S., El-saraf, W. M., Abdel, A. M., Ali, A. E., Mohamed, E. A., & Hasan, H. M. I. (2016). Rice husk and activated carbon for waste water treatment of El-Mex Bay , Alexandria Coast , Egypt. *Arabian Journal of Chemistry*, *9*, S1590–S1596. https://doi.org/10.1016/j.arabjc.2012.04.028

Mathu, I., Nishanth Cheruvu, Cristian Birzer, & Paul Medwell. (2017). *Biochar production and characterisation- a field study*.

Mausi, G., Simiyu, G., & Lutta, S. (2014). Assessment of selected heavy metal concentrations in selected fresh fruits in Eldoret Town, Kenya. *Journal of Environment and Earth Science*, *4*(3), 1–8.

Meticulous Research®, (MR). (2020). Black Soldier Fly Market by Product Type (Protein Meal, Whole Dried Larvae, Oil, Biofertilizer, Live Insect, Chitin/Chitosan), Application (Animal Feed, Agriculture, Pet Food, Pharmaceutical, and Cosmetics), and Geography- *Global Forecast to 2030.*

Mutafela, R. N. (2015). High Value Organic Waste Treatment via Black Soldier Fly Bioconversion : Onsite Pilot Study. *Trita-Im-Ex 2015:16*, 1–64.

Nguyen, T. T. X., Tomberlin, J. K., & Vanlaerhoven, S. (2015). Ability of Black Soldier Fly (Diptera: Stratiomyidae) Larvae to Recycle Food Waste. *Environmental Entomology*, *44*(2), 406–410. https://doi.org/10.1093/ee/nvv002

Novak, J, M., & Mark, G, J. (2019). Elemental and Spectroscopic Characterization of Low-Temperature Based Designer Biochars and Their Use as Soil Amendments. In *Biochar from Biomass and Waste* (Issue 350 C, pp. 37–58). Elsevier Inc. https://doi.org/10.1016/B978-0-12-811729-3.00003-0

Mohammad, S. (2020). Hazardous Waste Management -Reuse, Recycling and Reclamation . *Hazardous Waste Management -Reuse, Recycling and Reclamation , November*, 1–10. https://doi.org/10.13140/RG.2.2.16054.93765

Nyakeri. (2018). *Optimization of production of Black soldier fly (Hermetia illucens, L) for fish feed formulation*. Jaramogi Oginga Odinga University of Science and Technology.

Peng, X., Ye, L. L., Wang, C. H., Zhou, H., & Sun, B. (2011). Temperature- and duration-dependent rice straw-derived biochar : Characteristics and its effects on soil properties of an Ultisol in southern China. *Soil & Tillage Research*, *112*, 159–166. https://doi.org/10.1016/j.still.2011.01.002

Prapagdee, S., Piyatiratitivorakul, S., & Petsom, A. (2016). Physico-chemical Activation on Rice Husk Biochar for Enhancing of Cadmium Removal from Aqueous Solution. *Asian Journal of Water, Environment and Pollution*, *13*(1), 27–34. https://doi.org/10.3233/AJW-160004

Proc, K., Bulak, P., Wiącek, D., & Bieganowski, A. (2020). Hermetia illucens exhibits bioaccumulative potential for 15 different elements – Implications for feed and food production. *Science of the Total Environment*, *723*. https://doi.org/10.1016/j.scitotenv.2020.138125

Puglla, E. P., Diana, G., Cristhian, T., Francisco, O., & García-Ruiz, M. J. (2020). Biochar from Agricultural by-Products for the Removal of Lead and Cadmium from Drinking Water. *Water*, 1–16.

Purschke, B., Scheibelberger, R., Axmann, S., Adler, A., & Jäger, H. (2017). Impact of substrate contamination with mycotoxins, heavy metals and pesticides on the growth performance and composition of black soldier fly larvae (Hermetia illucens) for use in the feed and food value chain. *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment*, *34*(8), 1410–1420. https://doi.org/10.1080/19440049.2017.1299946

Ramalingham, S., Mogili, D., & Prasad, R. (2020). Sorption of Heavy Metals onto Biochar. In A. Adbelhafez & A. Mohamed (Eds.), *Applications of Biochar for Environmental Safety*. IntechOpen. https://doi.org/http://dx.doi.org/10.5772/intechopen.92346

Sakhiya, A. K., Anand, A., & Kaushal, P. (2020). Production, activation, and applications of biochar in recent times. *Biochar*, *2*(3), 253–285. https://doi.org/10.1007/s42773-020-00047-1

Schmidt, H. P., Hagemann, N., Draper, K., & Kammann, C. (2019). The use of biochar in animal feeding. *PeerJ*, *2019*(7). https://doi.org/10.7717/peerj.7373

Schrögel, P., & Wätjen, W. (2019). Insects for food and feed-safety aspects related to mycotoxins and metals. *Foods*, *8*(8), 1–28. https://doi.org/10.3390/foods8080288

Seng, L. K. O. K. (2018). Adsorption of heavy metals using banana peels in wastewater treatment. *International Conference on Research in Education and Science, 2,* 312–317.

Suman, S., & Shalini, G. (2017). Pyrolysis of coconut husk biomass: Analysis of its biochar properties. *Energy Sources, Part A: Recovery, Utilization, and Environmental Effects*.

Taghlidabad, R. H., Sepehr, E., Khodaverdiloo, H., & Samadi, A. (2020). Characterization of cadmium adsorption on two cost-effective biochars for water treatment. *Arabian Journal of Geosciences*. https://doi.org/https://doi.org/10.1007/s12517-020-05477-6

Tan, C., Zeyu, Z., Rong, H., Ruihong, M., Hongtao, W., & Wenjing, L. (2015). Adsorption of cadmium by biochar derived from municipal sewage sludge : Impact factors and adsorption mechanism. *Chemosphere*, *134*, 286–293. https://doi.org/10.1016/j.chemosphere.2015.04.052

Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. (2012). Molecular, clinical and environmental toxicicology Volume 3: Environmental Toxicology. *Molecular, Clinical and Environmental Toxicology*, *101*, 133–164. https://doi.org/10.1007/978-3-7643-8340-4

Tomberlin, J. K., Sheppard, D. C., Joyce, J. A., Tomberlin, J. K., Sheppard, D. C., & Joyce, J. A. (2002). Selected Life-History Traits of Black Soldier Flies (Diptera : Stratiomyidae) Reared on Three Artificial Diets. *Entomological Society of America*, *95*(3), 379–386.

Van, Hien, N., Valsami-Jones, E., Vinh, N. C., Phu, T. T., Tam, N. T. T., & Lynch, I. (2020). Effectiveness of different biochar in aqueous zinc removal: Correlation with physicochemical characteristics. *Bioresource Technology Reports*, *11*. https://doi.org/10.1016/j.biteb.2020.100466

van der Fels-Klerx, H. J., Camenzuli, L., Belluco, S., Meijer, N., & Ricci, A. (2018). Food Safety Issues Related to Uses of Insects for Feeds and Foods. *Comprehensive Reviews in Food Science and Food Safety*, *17*(5), 1172–1183. https://doi.org/10.1111/1541-4337.12385

Van Der Fels-Klerx, H. J., Camenzuli, L., Van Der Lee, M. K., & Oonincx, D. G. A. B. (2016). Uptake of cadmium, lead and arsenic by Tenebrio molitor and Hermetia illucens from contaminated substrates. *PLoS ONE*, *11*(11). https://doi.org/10.1371/journal.pone.0166186

Vanda, molnar É., Simon, E., Ninsawat, S., Béla, T., & Szilárd, S. (2020). Pollution Assessment Based on Element Concentration of Tree Leaves and Topsoil in Ayutthaya Province, Thailand. *International Journal of Environmental Research and Public Health, 17(14):5165*. https://doi.org/10.3390/ijerph17145165

Wang, Y.-S., & Shelomi, M. (2017). *Review of Black Soldier Fly (Hermetia illucens) as Animal Feed and Human Food*. https://doi.org/10.3390/foods6100091

Wang, Y., Gu, K., Wang, H., & Shi, B. (2019). Remediation of heavy-metal-contaminated soils by biochar : a review. *Environmental Geotechnics*, 1–14. https://doi.org/https://doi.org/10.1680/jenge.18.00091

WHO. (2021). Solid waste management, air pollution and health. *Urban Health Initiative a Model Process for Catalysing Change*, *2015*(April). https://www.who.int/publications/i/item/WHO-HEP-ECH-AQH-2021.8

Woldetsadik, D., Drechsel, P., Keraita, B., Marschner, B., Itanna, F., & Gebrekidan, H. (2016). Effects of biochar and alkaline amendments on cadmium immobilization , selected nutrient and cadmium concentrations of lettuce (Lactuca sativa) in two contrasting soils. *SpringerPlus*, *2016*. https://doi.org/10.1186/s40064-016-2019-6

Woolf, D. (2008). Biochar as a soil amendment : A review of the environmental implications . *Organic Eprints*, *January*, 1–31. https://www.researchgate.net/publication/321831639

Wu, C., Li, Y., Chen, M., Luo, X., Chen, Y., Belzile, N., & Huang, S. (2018). Adsorption of Cadmium on Degraded Soils Amended with Maize-Stalk-Derived Biochar. *International Journal of Environmental Research and Public Health Article*, *15*(2331), 1–17. https://doi.org/10.3390/ijerph15112331

Wu, N., Wang, X., Xu, X., Cai, R., & Xie, S. (2020). Effects of heavy metals on the bioaccumulation, excretion and gut microbiome of black soldier fly larvae (Hermetia illucens). *Ecotoxicology and Environmental Safety*, *192*(November 2019), 110323. https://doi.org/10.1016/j.ecoenv.2020.110323

Wu, Q., Xian, Y., He, Z., Zhang, Q., Wu, J., Yang, G., & Zhang, X. (2019). Adsorption characteristics of Pb (II) using biochar derived from spent mushroom substrate. *Scientific Reports*, *Ii*, 1–11. https://doi.org/10.1038/s41598-019-52554-2

Zahn, N. H. (2017). The effects of insect frass created by Hermetia illucens on Spring onion growth and soil fertility. *ResearchGate*, *May*, 1–65. https://www.researchgate.net/publication/321831639

Zhao, S., Ta, N., & Wang, X. (2020). Absorption of Cu(II) and Zn(II) from aqueous solutions onto biochars derived from apple tree branches. *Energies*, *13*(13). https://doi.org/10.3390/en13133498

APPENDICES

Table 3: The analysis of variance (ANOVA) table for the absorbance of Cd ions at different concentration levels and biochar type

Source of variation	DF	Sum of Squares	Mean Sum of Squares	F	P-Value
Concentration in ppm	1	0.007790	0.007790	76.202	2.32e-05 *
Biochar	1	0.000154	0.000154	1.507	0.2545
Residual	8	0.000818	0.000102		

* implies significance at 5% level

Table 4:summary statistics for adsorption levels at different concentration levels factors of comparison

Concentration in ppm	Mean ± SE
0	0.0000±0a
1	0.0276±0.008b
3	0.0627±0.019c

Note: different LSD letters (least significant difference) indicates significant difference of absorbance at various concentration levels