

# The potential use of black soldier fly larvae residue as a fertilizer.

*An-Marthe Ingelaere*



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## Abstract

In sub-Saharan Africa (SSA), the primary constraint of agriculture productivity has consistently been ascribed to soil fertility. A common approach to improve soil fertility is the use of fertilizers, both organic and inorganic. Though recently interest is increasing in the use of newer alternatives like biostimulants. Biostimulants are found to contribute to plant growth as well as to plant health. There are different forms of biostimulants, such as phytohormones, humic substances amongst others, and it can be applied from different sources, for example vermicompost. For similar reasons to the earthworms in the vermicomposting process, the Black Soldier Fly (BSF) received growing attention. The larvae of the BSF can convert organic waste into valuable products. The larvae themselves can be used as feed for pigs, poultry and fish, or can be further processed to obtain biodiesel, chitin and/or essential fatty acids. The residue of the bioconversion process has the potential to be used as a fertilizer. However, previous literature showed mixed results regarding the effect on the plant and on the characteristics of the residue. This study confirmed the assumption that the quality of the residues depend on the substrate fed to the Black Soldier Fly larvae (BSFL). Furthermore, the study compared the physico-chemical characteristics of the BSFL residues with other fertilizers, a commercial inorganic fertilizer, a commercial organic fertilizer, chicken manure and vermicompost. Based on the NPK content, the BSFL residues could not replace the commercial inorganic fertilizer. However, based on other physico-chemical characteristics, like the pH, EC or OM content, the BSFL residues can have an advantage over the inorganic fertilizer. Compared to the other organic fertilizers, the BSFL residues of the study have remarkably higher OM content. Though regarding the other characteristics, the data found for the BSFL residues are not remarkably higher nor remarkably lower than of the other organic fertilizers. Besides the characteristics of a fertilizer, several other factors determine the use of it. Socio-economic characteristics, like the cost of a fertilizer, the availability, and the lack of knowledge regarding a fertilizer, but also specific household characteristics, such as household size and composition, education level, gender, and ownership of livestock and/or poultry, can either constrain or promote the use of a certain fertilizer. Moreover, all these factors are interlinked. As for each fertilizer, BSFL residue has its' own advantages and disadvantages. A SWOT-analysis combining the bio-physical and socio-economic factors regarding BSFL residues as a fertilizer is included in the study.

Even though there is a major potential to use BSFL residues as a fertilizer, further research should be done. Further research should be done to the characteristics, in particular to the presence of biostimulants in the residues. Furthermore, the effect of the residues on soil fertility should be determined in the mid and long-term.

*Keywords: organic waste, fertilizer, black soldier fly, residue, physico-chemical characteristics, sub-Saharan Africa, socio-economic constraints*

## Foreword

After completing the master Bioscience engineering technology: agriculture and horticulture at the University of Ghent in Belgium, I enrolled in the master Agroecology at the Swedish University of Agricultural Sciences. Although there is no simple definition, agroecology is seen as a science, a practice, and a social movement, focusing on shifting towards a more sustainable food system. By enrolling in the master Agroecology, I got to broaden my view on agriculture. Coming from an agricultural science background, I was used to the reductionism approach of the present-day agriculture system, also referred to as 'conventional' or 'industrial' agriculture. However, I learned that the holistic approach of agroecology is a more accurate representation of reality and in that way, principles, concepts, and strategies can be provided that are more oriented towards sustainable agroecosystems.

Within the Agroecology master I learned to develop a more holistic view on agriculture, and it gave me a better understanding of sustainability issues related to agriculture.

The holistic approach of agroecology is also reflected in my master thesis. The first two research questions fall within natural sciences, whereby the focus is on the physico-chemical characteristics of Black Soldier Fly Larvae (BSFL) residues and other fertilizers. While the last research question focuses on the socio-economic context.

## Abbreviations

ANOVA	Analysis of Variance
BS	Biostimulants
BSF	Black Soldier Fly
BSFL	Black Soldier Fly larvae
BW	Brewery waste
BW+MW	Brewery waste and market waste (50/50 mixture)
EC	Electrical conductivity
HS	Humic substances
K	Potassium
MW	Market waste
N	Nitrogen
OM	Organic matter
P	Phosphorus
SOM	Soil organic matter
SSA	Sub-Saharan Africa
PGPR	Plant growth-promoting rhizobacteria
PH	Protein hydrolysates

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# 1. Background and literature review

## 1.1. Introduction

Poverty and food insecurity are major problems in sub-Saharan Africa (SSA). Currently, more than half of the extreme poor worldwide live in SSA (The World Bank Group, 2019) and food insecurity is much higher in Africa than other parts of the world (FAO et al., 2019). In countries across SSA the agricultural sector is a large part of the total GDP, on average 15%, and employs more than half of the labour force (OECD/FAO, 2016). Moreover, the agricultural sector (including crops, livestock, fisheries and forest products) is the only source of food (Jones, 2008). Therefore, improving agricultural productivity is essential to reduce poverty and food insecurity in SSA. In many parts of the world the industrial agriculture model, that relies heavily on external inputs such as mineral fertilizers, pesticides, genetically modified varieties and mechanisation, was able to increase agricultural productivity during the latter half of the twentieth century (Gliessman, 2015). In Africa, however, the development to this model has been largely absent (Gachene & Kimaru, 2003). Even though the industrial agriculture model boosted productivity, the model has major consequences on the basic foundations of agriculture. The model has degraded natural resources, essential for agriculture, and it made agriculture depended on non-renewable resources, like fossil fuels. Furthermore, the model has social consequences, it benefits a few and leaves out many small-scale farmers. In other words, the industrial agriculture model is not sustainable (Gliessman, 2015). Sustainable in terms of endurance over time and regarding the three pillars of sustainability, the ecological, the economical and the social pillar (Altieri & Nicholis, 2005; Gliessman, 2015). Thus, it is not only needed to increase productivity in SSA, but it needs to be done in a way that does not lead to drawbacks.

In agriculture, the soil plays a crucial role. Besides providing plants an anchorage place, it also provides plants with water and nutrients. It is a complex, dynamic and living component of the agroecosystem. Management practices can either degrade or improve the soil, which in turn can either decrease or increase crop production (Gliessman, 2015).

Soil degradation, “a change in the soil health status resulting in a diminished capacity of the ecosystem to provide goods and services for its beneficiaries” (FAO, 2019) is a major challenge globally. It is most severe in sub-Saharan Africa, where around 65% of the land area is classified as degraded (Vlek et al., 2008). Soil degradation processes, either physically, chemically or biologically, interact and influence one another. Physical degradation processes are soil erosion, surface sealing, soil compaction and reduced capacity to store water. Chemically, nutrient depletion, acidification, dispersion/alkalization, salinization and toxic contamination can degrade the soil.

Biological degradation can occur through depletion of soil organic matter, loss of soil biological diversity and loss of plant, animal and microbial biomass (Tully et al., 2015). All these forms of degradation lead to a decrease in soil fertility, which is the ability of the soil to support plant growth, and thus productivity (Gachene & Kimaru, 2003). In SSA, nutrient depletion, the net loss of nutrients from the soil due to higher nutrient outputs than nutrient inputs (Drechsel et al., 2001), is the main form of soil degradation (Tully et al., 2015). Output of nutrients from the system is not only through harvesting of the crops. It can also happen through leaching, the downward movement of soluble nutrients (Lehmann & Schroth, 2003), and/or soil erosion, the detachment and transport of the top layer of the soil (Ashman & Puri, 2001). Likewise, there are different forms of nutrient input. There is the addition of nutrients through rainfall, fallow and/or fertilizers (Drechsel et al., 2001).

## 1.2. Fertilizers

In the past, land was abundant, and the maintenance of soil nutrients was managed by periodic fallowing of land. Since land became more scarce, fallow periods became shorter and shorter and new management practices emerged (Bigot et al., 1987; Henao & Baanante, 2006). Currently, soil nutrients are mainly managed by application of fertilizers, organic and/or inorganic. In SSA however, fertilizer use is low, only about 16.2 kg/ha/year. While worldwide, the average fertilizer use is going up to 140.6 kg/ha/year (The World Bank Group, 2016). Figure 1 visualises the difference in fertilizer use worldwide. The three maps show the average use of N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O per area of cropland, which is the sum of arable land and permanent crops, in a time series from 2002 to 2017 (FAO, 2017). The figure only shows the use of chemical and mineral NPK, which is clearly much lower in SSA than many other parts in the world.

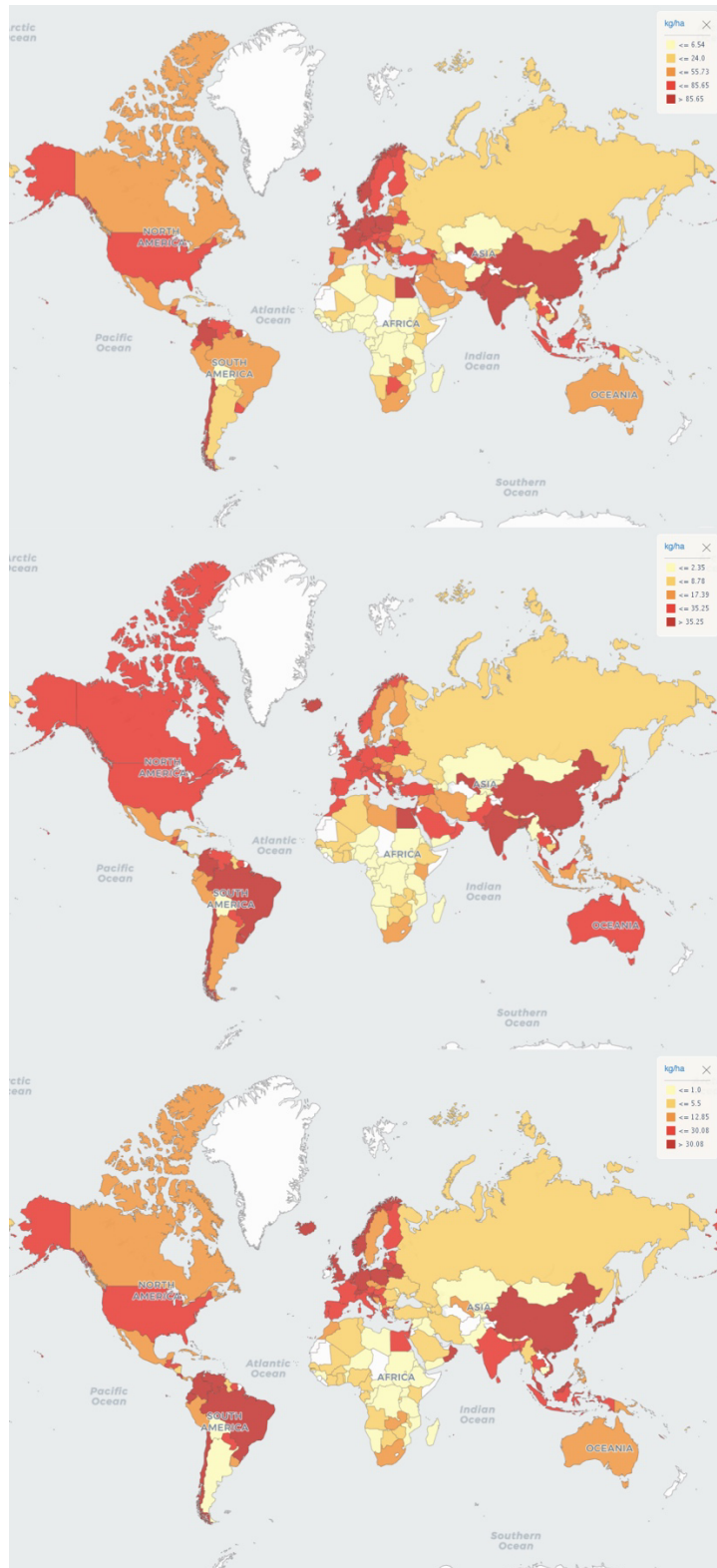


Figure 1. Maps showing the average use of chemical and mineral N (top), P<sub>2</sub>O<sub>5</sub> (middle) and K<sub>2</sub>O (bottom) per area of cropland, which is the sum of arable land and permanent crops, in a time series from 2002 to 2017 (retrieved from FAO, 2017).

### 1.2.1. Inorganic fertilizers

The use of inorganic fertilizers, also called mineral or synthetic fertilizers, has played a significant role in increasing global agricultural production (Bindraban et al., 2015; Gliessman, 2015). The nutrient content of mineral fertilizers is known and usually in concentrated form. Therefore, relatively low amounts of mineral fertilizers are enough to address specific nutrient needs. Since mineral fertilizers release nutrients rapidly, it is a good practice for urgent, short term nutrient needs. However, there are some disadvantages with regards to mineral fertilizers. Due to the quick release, nutrients easily get lost by leaching and volatilization, which has consequences for the environment (Gliessman, 2015). Additionally, many inorganic fertilizers are deficient in micro-nutrients (Bindraban et al., 2015). Other concerns are, the high energy consumption of mineral fertilizer production (Gellings & Parmenter, 2004), the possible effect on the nutrient balance and acidity of the soil, and the deterioration of soil structure (Gachene & Kimaru, 2003). Furthermore, in SSA, mineral fertilizers are expensive and low in consistency and quality (Bold et al., 2015; Luswata & Mbowa, 2015; Toro, 2015).

### 1.2.2. Organic fertilizers

Organic fertilizers, such as animal manure, household wastes, compost, leguminous cover crops, green manure, are environmentally better than inorganic fertilizers. Organic fertilizers raise the level of soil organic matter (SOM), which plays a key role in the physical, chemical and biological composition of the soil (Omotayo & Chukwuka, 2009). Soil structure, aeration, water holding capacity and other soil characteristics can be improved by using organic fertilizers (Gachene & Kimaru, 2003). Organic fertilizers also enhance soil biological activity; encouraging the growth of beneficial microorganisms and earthworms (Chen, 2006). While the nutrient content of inorganic fertilizers is concentrated and known, organic fertilizers usually have lower nutrient content which are usually unknown and which can vary between batches. Furthermore, nutrients from an organic source are released unpredictably. Nutrients are less prone to leaching, since the release is regulated and protected by biological soil processes (Sanginga & Woomer, 2009). However, without control over the release of nutrients, nutrients may become available at improper moments. On the other hand, organic fertilizers contain sufficient micronutrient besides the macronutrient content (Bindraban et al., 2015). Regarding the acquisition and cost, organic fertilizers have their own advantages and disadvantages. Organic fertilizers are less expensive for farmers, though they often require more labour for the production/collection, transportation, storage and application. For example, animal manure needs to be collected, which is particularly labour intensive when animals are free ranged. In addition, organic resources are not only functional as soil improver, thus there are competing uses for the nutrient source. For example, animal manure can also be used to produce domestic fuel (Bayu et al., 2005; Gachene & Kimaru, 2003).

As both types of fertilizers have their advantages and disadvantages, it is generally recommended to farmers to use a combination of organic and inorganic fertilizers (Bayu et al., 2005; Chen, 2006; Gachene & Kimaru, 2003). While inorganic fertilizers provide readily available nutrients for rapid plant up-take, organic fertilizers are an important input of organic matter to the soil, which plays an essential role in several soil processes (Gachene & Kimaru, 2003). In sub-Saharan Africa (SSA), this strategy is in particular promising for resource-poor small-scale farmers (Bayu et al., 2005). Additionally, more and more interest goes to newer alternatives like biostimulants.

### 1.3. Biostimulants

Until recently the main aim of fertilizer application was the addition of nutrients for plant growth. Nutrient input is necessary given the above mentioned nutrient depletion issue in SSA (Tully et al., 2015). General mineral nutrients, like N, P and K, are building blocks for cellular structures and for components such as nucleic acids, ATP, enzymes, etc. and are thus crucial in plant growth. However, plant growth is not only dependent on nutrients. The regulation of plant growth is determined by various factors, both abiotic and biotic. Basically, plant growth is the result of cell proliferation, which is the combination of cell growth and cell division. For cell division to occur, the cell needs to go through the entire cell cycle. The entire cell cycle consists of four distinct phases; G1 (postmitotic interphase), S (DNA synthetic phase), G2 (post synthetic interphase) and M (mitosis), in addition to the G0 phase in which cells are in a quiescent state. The cell cycle is governed by checkpoints, between G1 and S and between G2 and M (figure 2).

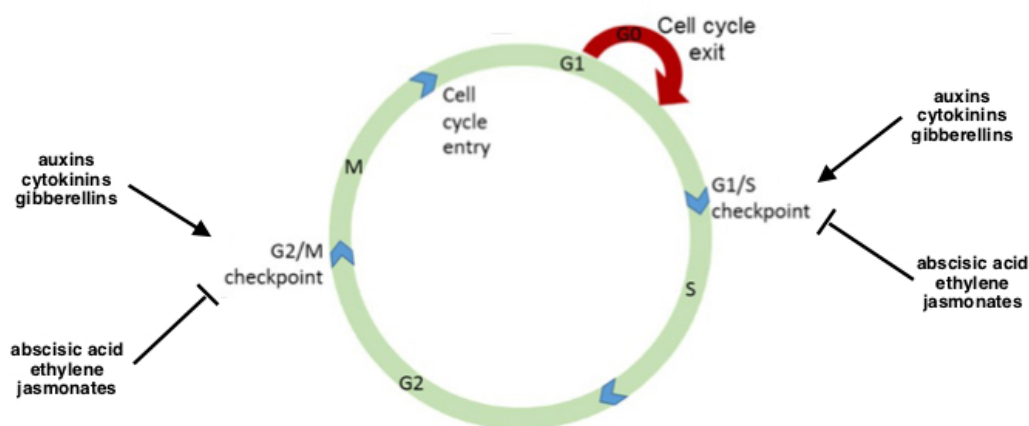


Figure 2. Schematic diagram of the plant cell cycle. (adapted from Wong et al., 2015)

Although different factors, such as availability of resources and environmental conditions, exert control over the cell cycle in varying degrees, phytohormones are the dominant regulators, more specifically at the checkpoints (Wong et al., 2015). Phytohormones or plant hormones, are naturally occurring small molecules which influence physiological processes in plants. According to structural and chemical diversity, several classes of phytohormones are identified, including auxins, cytokinins (CK), gibberellins (GA), abscisic acid (ABA), ethylene, brassinosteroids (BR), salicylates (SA), jasmonates (JA) and strigolactones (SL) (Chu et al., 2017; Dilworth et al., 2017). Each of these hormones differ in their conditions of biosynthesis, transport and function. Phytohormones do not act in isolation, they can have synergistic, additive and antagonistic effects on each other (Wolters & Jürgens, 2009). In the cell cycle, cytokinins, auxins, and gibberellins generally play positive regulatory roles, while ethylene, abscisic acid and jasmonates are the phytohormones with inhibitory roles (figure 2). Besides their role in the cell cycle, phytohormones play a role in various other processes. They, for example, regulate the opening of stomata and chloroplast production (Wong et al., 2015), which in turn have an effect on photosynthesis and thus plant growth (Toh et al., 2018). Other processes then plant growth, like development and plant defence, are also influenced by phytohormones. As mentioned before, phytohormones do not act in isolation and per process there is a complex network of interactions between different phytohormones. On top, the influence of phytohormones can vary. Different phytohormone interactions and responses were found for different plant species and for different environmental conditions, such as the type of attacker (Checker et al., 2018). For example, the jasmonic acid (JA) pathway is



primarily activated by chewing and mining herbivores, necrotrophic pathogens, bacteria and nematodes, whereas the salicylic acid (SA) pathway is mainly activated by sucking herbivores, biotrophic pathogens and viruses. Variation in interaction between the two important defence pathways are also found, though it is often reciprocal antagonistic. (Moreira et al., 2018; Schweiger et al., 2014). Even though all phytohormones are of interest, cytokinins are thought to have a pivotal role in both plant growth and plant defence (Checker et al., 2018; Giron et al., 2013; Wong et al., 2015).

The importance of phytohormones is clear, therefore increasing interest has gone to it in plant production. Though phytohormones are not the only aspect that gained interest. There is also an increasing interest in other materials promoting plant production without being nutrients, soil improvers, or pesticides. These are grouped in the term biostimulant (BS). "A plant biostimulant is any substance or microorganism applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrients content. By extension, plant biostimulants also designate commercial products containing mixtures of such substances and/or microorganisms." (du Jardin, 2015, p. 7). Besides phytohormones, there are several other biostimulants. There are the humic substances (HS), humins, humic acids and fulvic acids, which are the natural constituents of the soil organic matter. Humic substances are formed by decomposition of plant, animal and microbial residues, but also by microbial metabolism. The influence of HS on plant productivity can be indirectly and directly. HS are essential contributors to soil fertility through modification of soil characteristics. Direct effect of HS is attributed to physical and metabolic plant processes (Rose et al., 2014). Often the beneficial effects of HS are ascribed to positive changes in the root architecture. Beneficial effects are also ascribed to induced H<sup>+</sup>-ATPase activity which results in enhancement of nutrient uptake, to the influence on primary and secondary metabolism and to the stress alleviation (Canellas et al., 2015). Though inconsistent, the effects of HS are generally positive. Factors contributing to the variability in effects are the source of HS, the environmental conditions, the receiving plant and the dose and manner of HS application (Rose et al., 2014). There are also the protein hydrolysates (PH) which consist of peptides and amino acids obtained from chemical and/or enzymatic hydrolysis of proteins from plant and animal origin. Protein hydrolysates are known to increase nutrient uptake through several mechanisms, like increase in soil microbial and enzymatic activity, enhanced mobility and solubility of micronutrients, modification of the root architecture, and increase in reductase, glutamine synthetase. Furthermore, PH application has been found to contribute to the mitigation of environmental stresses. The effects of PH on plant productivity is not only attributed to the peptides and amino-acids. Other compounds, such as fats, carbohydrates, phenols, mineral elements, phytohormones and other organic compounds also play a role (Colla et al., 2015). Besides the previously mentioned biostimulants, seaweed extracts and botanicals, like chitosan and other biopolymers, and inorganic compounds, are other biostimulant substances. However, as the definition

states, biostimulants can also be of microbial nature. These are the beneficial fungi and bacteria. A commonly mentioned fungal biostimulant is the mycorrhizal fungi, a symbiotic fungi mainly known for its role in plant nutrition (especially P). Other fungi distinct from the mycorrhizal species, for example *Trichoderma* spp., can also be regarded as biostimulant. The mutualistic, endosymbionts of the type *Rhizobium* and the mutualistic, rhizospheric plant growth-promoting rhizobacteria (PGPRs) are the two main types of beneficial bacteria. *Rhizobium* spp. are the well-studied bacteria forming nitrogen-fixing associations with legumes. While *Rhizobia* mainly enhance nutrient acquisition, plant growth-promoting rhizobacteria (PGPR) influence several aspects, including growth, morphogenesis and development and response to biotic and abiotic stress. When it comes to biostimulants of microbial nature, they are often referred to as biofertilizers (du Jardin, 2015).

Even though the precise mechanisms are not always clear, biostimulants contribute to both plant growth and plant health. Given these beneficial functions of biostimulants and the growing demand for quality food, in addition to the growing concern to the environment, there is an increased interest in the production and application of biostimulants. Biostimulants can be applied in its single active form, for example application of protein hydrolysates extracted from fish by-products, or through products containing biostimulants, like vermicompost.

#### 1.4. Vermicompost

Vermicomposting is a non-thermophilic process whereby organic waste is converted into compost by the interaction of earthworms and microorganisms residing within the worms (Arancon & Edwards, 2005). The result of the process, called vermicompost, consists of decayed organic matter and worm casts (Ramnarain et al., 2019). In the process the earthworms are responsible for physically conditioning the substrate and altering biological activity. By grinding, mixing, aggregating, aerating and gut passing the earthworms create the conditions for microorganisms which are responsible of the biochemical degradation of the organic waste (Aira et al., 2002; Venkatesh & Eevera, 2008). The vermicomposting process can be done with different earthworm species. In general, epigeic species are used because of their greater potential as waste decomposers than anecics and endogeics (Gajalakshmi & Abbasi, 2004). *Eisenia fetida* and *E. andrei* are the epigeic earthworms most commonly used in vermicomposting (Domínguez & Edwards, 2010). Likewise, different types of organic wastes, such as animal manure, food waste and sewage sludge, can be the substrate for the vermicomposting process. By converting the waste into a valuable product, vermicomposting contributes to efficient and sustainable waste management. On top, it helps to reduce the occurrence of human pathogens in organic waste, including faecal coliforms, *Salmonella* spp., enteric viruses and helminthes (Eastman et al., 2001; Edwards & Subler, 2010). Due to its characteristics vermicompost is an interesting

fertilizer. Application of vermicompost showed numerous benefits, including increased plant growth (Edwards et al., 2006; Joshi et al., 2015; Theunissen et al., 2010; Xu & Mou, 2016), improved soil properties (Joshi et al., 2015; Theunissen et al., 2010; Xu & Mou, 2016) and alleviation of biotic (Edwards et al., 2006; Joshi et al., 2015; Simsek-Ersahin, 2011) and abiotic stress conditions (Benazzouk et al., 2020; Chinsamy et al., 2014; Kiran, 2018). These beneficial effects are not only attributed to the plant available nutrient content, but also the presence of different biostimulants. Vermicompost contains a large and diverse amount of microorganisms, humic substances, chitinase and phytohormones (Allardice et al., 2015; Aremu et al., 2015; Joshi et al., 2015; Wong et al., 2020; Yasir et al., 2009).

Recently an insect, the Black Soldier Fly (BSF), received growing attention for similar reasons as the earthworms in the vermicomposting process. BSF also has the ability to convert waste into valuable products and can thus play a role in efficient and sustainable waste management.

## 1.5. Black Soldier Fly

### 1.5.1. Black Soldier Fly in general

The Black Soldier Fly (BSF), *Hermetia illucens*, is a widespread fly of the dipteran family Stratiomyidea. It is native of tropical, subtropical and temperate zones of America, but through human-mediated dispersal, the fly is nowadays present worldwide, between the latitude 40° south and 45° north (Devic & Fahmi, 2014; Dortmans et al., 2017). The species is holometabolous, undergoing a complete metamorphosis from egg, larva, pupa to adult. Eggs are about 1 mm long and have an ovoid shape (Devic & Fahmi, 2014). A cluster of eggs (around 400-800) are laid in dry cracks and cavities close to moist, decomposing organic matter and hatch after four days. The emerged larvae are barely a few millimetres in size, but can grow up to 25 mm by feeding voraciously on the decomposing organic matter (Dortmans et al., 2017). More importantly, the larval stage is the only stage of feeding. The larvae are found to be polyphagous and can efficiently digest organic materials derived from plants, animals and humans (Kim et al., 2011). The BSF goes through different larval stages by moulting. The first five larval stages have a cream-like colour and are difficult to differentiate. The final larval stage, the prepupa, is characterized by a dark brown to greyish colour. Additionally, the final larval stage is characterized by a modification of the mouthpart into a hook-shaped structure. This modification facilitates the migration from the moist food source towards dry, warm and sheltered areas. The larva stage requires approximately 14-16 days under optimal conditions. Thereafter the pupation stage lasts around two to three weeks. The dark coloured pupa stays immobile and stiff during that period. At the end of the pupation stage, the fly emerges from its pupa shell (Dortmans et al., 2017). The adult fly is black, wasp-like and 15-20 mm long. Unlike a larva, an adult does not feed, except on water or nectar

to stay hydrated, which has an influence on its lifespan. Approximately, an adult fly lives for 5 to 14 days (Devic & Fahmi, 2014). Like the adult stage, the duration of the whole life cycle is highly variable and depends on various factors, such as temperature, humidity and diet (Sheppard et al., 2002).

### 1.5.2. Black Soldier Fly larvae

The uniqueness of the insect is that it is not considered a pest, nor a vector for pathogens. The Black Soldier Fly larvae (BSFL) are even found to reduce pathogenic bacteria. Erickson and colleagues (2004) observed the reduction of *Escherichia coli* and *Salmonella enterica* in chicken manure and Liu and colleagues (2008) the reduction of *E. coli* in dairy manure. *Salmonella* spp. was also found to be reduced in human faeces, though the BSFL were found to have no impact on *Enterococcus* spp. and *Ascaris suum* ova. (Lalander et al., 2013). On the other hand, Awasthi and colleagues (2020) did find a reduction of *Enterococcus* spp. besides the reduction of other pathogenic bacteria including *Bacillus*, *Salmonella* and *Vibrio* spp. On top, they reduce housefly (*Musca domestica*) population through larval competition and inhibition of housefly oviposition (Newton et al., 2005). Therefore, the presence of the insect is not harmful, in fact rearing BSFL can be useful in several ways. It is an efficient way of disposing organic wastes such as food wastes, animal manure and even human excreta (Diener, Zurbrügg, et al., 2011). By feeding on the waste, the larvae reduce the waste substantially. For instance, swine manure could be reduced by 56% (Newton et al., 2005). While reduction rates of 65-75% were found in trials with household waste (Diener, Zurbrügg, et al., 2011). The reduction of the waste is thus variable, which was also found by Lopes and colleagues (2020). They found reduction rates between 54 and 67 % depending on the different proportions of bread waste and aquaculture waste. It was concluded that BSFL cannot consume fat-rich fish carcasses alone. It was furthermore confirmed that the type of waste influences the growth of the larvae and their composition. In the process, the larvae convert the waste into protein-rich and fat-rich biomass, valuable for several purposes. Due to their favourable characteristics, prepupae of the BSF can be used to feed pigs, poultry and fish. Further processed, other products such as, biodiesel, chitin and/or essential fatty acids can be produced (Newton et al., 2005). It is thus an excellent waste management method which is in line with the concept of circular economy by creating products with economic value. Furthermore, the residue of the bioconversion process have the potential to be used as organic fertilizer (Alattar et al., 2016; Beesigamukama et al., 2020; Choi et al., 2009; Xiao et al., 2018).

### 1.5.3. Black Soldier Fly Larvae residue

The BSFL residue or frass is a mixture of undigested feeding material, larvae faeces, larvae exuviae and other parts from other BSF stages. Metabolic products, for example,

hormones, enzymes or antibiotics, and other organisms, such as bacteria, fungi, protozoa and yeasts, are also associated with the mixture (Vickerson et al., 2015). The term frass, though, is generally used to refer to larvae excrements only (Kagata & Ohgushi, 2012; Makkar et al., 2014). The rearing of BSFL has been a growing technique, and so has the research on the topic. Yet, most research has been on the production process itself and the nutrient content of the larvae, while only recently more interest has gone to the residue. Experiments done with maize (Beesigamukama et al., 2020), Chinese cabbage (Choi et al., 2009), spring onion (Zahn, 2017), lettuce (Suantika et al., 2017), basil, sudan grass (Newton et al., 2005) and other vegetables (Temple et al., 2013) showed positive effects of residue application on the growth of the plants. Under field conditions, BSFL residues derived from brewery spent grains, performed better than commercial organic and inorganic fertilizers. While all fertilizers influenced maize plant height, chlorophyll concentration and macronutrient uptake, the increase was found to be higher in plots treated with BSFL residue. Furthermore, maize yield and nitrogen use efficiency were improved with BSFL residue application (Beesigamukama et al., 2020). In the pot experiment with Chinese cabbage, the growth rate as well as the chemical composition of the cabbages were compared between cabbages grown on BSFL residue and on commercial fertilizer. To obtain the BSFL residue, the larvae were fed with food waste. Both, chemical composition and growth rate, were almost identical between the two treatments. On top, the chemical composition of the BSFL residue and the commercial fertilizer were similar. Only the electrical conductivity (EC) of the residue was slightly higher, most likely because of the higher amount of sodium (Na) found in the residue (Choi et al., 2009). Although not statistically significant, the height and weight of spring onions in a pot experiment were positively affected by the addition of BSFL residue, except for the application rate of 20 t/ha whereby the effect was negative. The BSFL residue was obtained by the bioconversion of a 1:1:1 mixture of avocados, bananas and mangos (Zahn, 2017). An experiment with lettuce also showed positive results. Even though the biomass of the lettuce, fresh and dry, was lower than with cow dung, it was significantly higher compared to the treatment with coffee husk. In the same experiment, nutrient utilization was also analysed. Except for nitrogen utilization, phosphorus and potassium utilization were highest with BSFL residue, compared to the other two treatments. In this case, the residue was derived from the bioconversion of coffee husk by BSFL (Suantika et al., 2017). Growing rates of basil and sudan grass in similar experiments showed the possible value of BSFL residue, bioconverted swine manure, as soil amendment. Though, the growing rates were not as good as for plants growing in commercial potting soil. As in the pot experiment with spring onion, in higher amounts, in this case mixtures of 40 and 50% BSFL residue with sand, the application affected the growth adversely (Newton et al., 2005). This finding, regarding high application rates, was also observed in field trials with BSFL residue derived from food waste. Field application rates of 10 t/ha and 15 t/ha gave optimal yields for lettuce and squash, respectively bok choy, onions, beans and tomatoes. While above these application rates, above ground biomass reduced. Besides

these findings, the field trials showed occasional protection against wireworm by BSFL residue application. The connection between wireworm protection and BSFL residue application was not certain and neither was the mechanism. Yet, some assumptions were made. Increased vigour of the plants by BSFL residue application may have allowed the plants to withstand wireworm attacks. The association of certain fungus with BSFL residue application may be having some effect. Another assumption made, was ascribed to chitin, chitosan and/or chitinases, due to presence of shed insect integument in the residue (Temple et al., 2013). The functionality of chitosan, a deacetylated of the biopolymer chitin, and the chitinase enzymes in plant protection has been described (du Jardin, 2015; Hadwiger, 2013; Kramer & Muthukrishnan, 1997). Furthermore, the pest control activity of the BSFL residue has been observed again. It was found that three species of wireworms (*Agriotes lineatus*, *A. obscurus* and *Limonius canus*) were killed by applying BSFL residue. A protective effect was also found against European chafer and cabbage root maggots (Vickerson et al., 2015). Additionally, trials demonstrated that BSFL residue, obtained by the bioconversion of a fruit and vegetable mixture, prevent the accumulation of *Fusarium oxysporum*, *Rhizoctonia solani* and *Phytophthora myriotylum*. At the same time, the growth of Valentino Green Bush Bean Plants was greater in treatments with the residue compared to both a control, just soil, and the treatments with a chemical fertilizer (Choi & Hassanzadeh, 2019). Another interesting finding is that through the bioconversion process by BSFL, the half-life of pharmaceuticals and pesticides is shortened without accumulation of the products in the larvae. Thus, converting organic wastes using BSFL could reduce the risk of spread of pharmaceuticals and pesticides in the environment (Lalander et al., 2016). Moreover, heavy metal concentration decreased after BSFL bioconversion (Sarpong et al., 2019). These are beneficial characteristics besides the above mentioned reduction of pathogenic bacteria and housefly population. Still caution is urged given that in some studies still amounts of heavy metals, like Cu and Cd (Wu et al., 2020) or pathogenic bacteria, for example *Salmonella* (Wynants et al., 2019) were detected in the residue.

By contrast to the positive effects, Alattar, Alattar and Popa (2016) found that the residue, applied untreated in a ratio of one part residue to two parts soil, stunted corn plant growth. This may have been due to toxic ammonium levels, though this was not proven. As mentioned before, at higher levels, BSFL residue application can have adverse effects (Newton et al., 2005; Temple et al., 2013; Zahn, 2017). Given these finding, optimum application rate, method and timing should be considered for specific crops and soil conditions. In consideration of the possible phytotoxicity it might not be enough to look at optimum application, though also to processing methods. The residue can be processed in three ways. The residue can be composted for a period of two months and marketed as an organic fertilizer. The residue can also be fed to worms, resulting in a stable and mature vermicompost. For the residue with high moisture content, it can also be

processed in an anaerobic digester to produce biogas (Dortmans et al., 2017). Alternatively, co-conversion with the BSFL gut bacteria *Bacillus subtilis* followed by aerobic fermentation results into residue suitable as organic fertilizer (Xiao et al., 2018).

In addition to research on growth effects on plants, some literature sources are found dealing with basic physico-chemical characteristics of the BSFL residue. In these literature sources, the production of the BSFL residues is done under different circumstances and with different starting substrates. Food waste is a generally used starting substrate (AgriProtein, 2017; Choi et al., 2009; Temple et al., 2013), though in those literature sources the composition was not specified. Given that BSFL can process both plant based as animal based, the composition can contain both. In Kawasaki and colleagues (2020) the composition was mentioned, namely 17% cabbage, 17% carrot, 16% potato, 10% horse mackerel, 8% ground pork, 5% apple pomace, 5% banana peel, 4% grapefruit pomace, 4% orange pomace, 3% rice, 3% bread, 3% wheat noodle, 3% Chinese noodle and 2% eggshell. The food waste used by Liu and colleagues (2020) consisted of noodles, rice, cabbage and pork in a ratio of 13:10:10:5. Three other sources used plant-based substrates, like maize straw (Gao et al., 2019), coffee husk (Suantika et al., 2017) and a combination of wheat (50%), alfalfa meal (30%) and corn meal (20%) (Setti et al., 2019). Besides food waste, Liu and colleagues (2020) used sewage sludge as substrate for the BSFL. In another case, even manure was used for the production of the residue, more specifically swine manure was used (Newton et al., 2005). Table 1 shows the variance in data of the different literature sources. Assumedly, this variance is due to the difference in substrate, given that the frass of *Mamestra brassicae* (L.) had different quality depending on the substrate fed (Kagata & Ohgushi, 2012). Additionally variation can be ascribed to the variation in production system. Furthermore, it should be noted that the different methods were used for the chemical analyses.

Table 1. Physico-chemical characteristics of BSFL residues derived from different substrates.

Feeding material	Food waste (not specified)	Food waste (not specified)	Food waste (not specified)	Food waste (specified above)	Food waste (specified above)	Maize straw	Coffee husk	Wheat, alfalfa and corn meal	Brewery spent grains	Sewage sludge	Swine manure
Moisture content (%)	n.d.	n.d.	n.d.	55.60	62.99	38.23	n.d.	n.d.	30.1	75.32	n.d.
pH	6.9	5.5	7.0	7.40	7.36	8.03	n.d.	8.8	7.7	7.93	7
EC (dS/m)	21	44	0.5	9.67	3.51	n.d.	n.d.	8.5	2.7	0.36	n.d.
OM (%)	68.8 <sup>b*</sup>	73.79 <sup>b*</sup>	3.3 <sup>a,O</sup>	61.64 <sup>b*</sup>	88.69	84.87	53.49 <sup>b</sup>	60.54 <sup>b</sup>	60.54 <sup>b*</sup>	46.98	0.73 <sup>b*</sup>
C:N	16	9	n.d.	16.61	16.50	78.14	24.49	8.0	16.8	13.67	10.22
C (%)	40	42.9	1.93 <sup>b,O</sup>	35.84	51.48 <sup>c</sup>	49.23 <sup>b</sup>	31.10	35.2	35.2	27.20 <sup>c</sup>	0.4232 <sup>e</sup>
N (%)	3.6	4.54	n.d.	2.16	3.12 <sup>a</sup>	0.63	1.27	4.4	2.1	1.99 <sup>a</sup>	0.0414 <sup>e</sup>
NO <sub>3</sub> -N (mg/kg)	n.d.	538	8.7	1000 <sup>a</sup>	n.d.	n.d.	n.d.	n.d.	1.39	n.d.	n.d.
NH <sub>4</sub> -N (mg/kg)	n.d.	9675	186.7	8800 <sup>a</sup>	n.d.	n.d.	n.d.	n.d.	74.4	n.d.	n.d.
P (%)	1.6	1.23	n.d.	0.05	1.14 <sup>a</sup>	1.11 <sup>d</sup>	0.2 <sup>d</sup>	2.27 <sup>d</sup>	1.16	1.11 <sup>a</sup>	0.0378 <sup>e</sup>
K (%)	1.4	2.44	n.d.	0.07	0.84 <sup>a</sup>	1.73 <sup>d</sup>	2.32 <sup>d</sup>	3.42 <sup>d</sup>	0.17	0.51 <sup>a</sup>	0.0169 <sup>e</sup>
Ca (%)	0.00017 <sup>a</sup>	0.64	n.d.	1.00	n.d.	n.d.	n.d.	4.5	0.19	n.d.	0.0425 <sup>e</sup>
Mg (%)	0.00003 <sup>a</sup>	0.13	n.d.	0.09	n.d.	n.d.	n.d.	0.8	0.16	n.d.	0.0176 <sup>e</sup>
Na (%)	n.d.	1.67	n.d.	0.08	n.d.	n.d.	n.d.	0.3	n.d.	n.d.	0.0048 <sup>e</sup>
S (mg/kg)	355	4900 <sup>a</sup>	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	44.44 <sup>e</sup>
Fe (mg/kg)	6286	471	n.d.	2400 <sup>a</sup>	n.d.	n.d.	n.d.	600.0	n.d.	n.d.	6.8 <sup>e</sup>
Mn (mg/kg)	241.6	13	n.d.	100 <sup>a</sup>	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	6.02 <sup>e</sup>
Zn (mg/kg)	78.2	49	n.d.	100 <sup>a</sup>	n.d.	n.d.	n.d.	140.0	n.d.	n.d.	12.91 <sup>e</sup>
Cu (mg/kg)	11.3	11	n.d.	100 <sup>a</sup>	n.d.	n.d.	n.d.	46.1	n.d.	n.d.	8.05 <sup>e</sup>
B (mg/kg)	12.2	7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.16 <sup>e</sup>
Cd (mg/kg)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.5	n.d.	n.d.	n.d.
Ni (mg/kg)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	6.1	n.d.	n.d.	n.d.
Cr (mg/kg)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.6	n.d.	n.d.	n.d.
Hg (mg/kg)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<0.2	n.d.	n.d.	n.d.
Pb (mg/kg)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<0.1	n.d.	n.d.	n.d.
References	(AgriProtein, 2017)	(Temple et al., 2013)	(Y.-C. Choi et al., 2009)	(Kawasaki et al., 2020)	(Liu et al., 2020)	(Gao et al., 2019)	(Suantika et al., 2017)	(Setti et al., 2019)	(Beesigamukama et al., 2020)	(Liu et al., 2020)	(Newton et al., 2005)



<sup>a</sup> from mg/kg to percentage: divide by 10000 (thus from g/kg to percentage: divide by 10); from percentage to mg/kg: multiply by 10000

<sup>b</sup> OM (%) = total organic carbon (%) x 1.72 ; organic C (%) = OM (%) x 0,58 → assuming 58% of organic matter exists as carbon (Burt, 2011)

<sup>c</sup> note of caution: here the OM was derived from the value of C

<sup>d</sup> derived from C:N

<sup>e</sup> from P<sub>2</sub>O<sub>5</sub> to P: divide by 2.29; from K<sub>2</sub>O to K: divide by 1.2

<sup>e</sup> from ppm to percentage: divide by 10000; 1 ppm = 1 mg/kg

<sup>o</sup> outlier

Besides the variance in nutrient content, the table also shows a higher concentration of NH<sub>4</sub><sup>+</sup>-N than NO<sub>3</sub>-N, which is the opposite of animal manure. The bioconversion by BSF larvae does not include the microbial fermentation process associated with animal digestion and deposition. While this results in less concern of potential NO<sub>3</sub>-N accumulation, and thus less health risks, NH<sub>4</sub><sup>+</sup>-N can volatilize as ammonia or nitrite gas when it comes into contact with alkaline or acidic soil respectively (Kawasaki et al., 2020). Moreover, high levels of ammonium can be toxic for plants (Alattar et al., 2016). On the other hand, ammonia has an antimicrobial effect on certain bacteria such as *Salmonella* sp. (Erickson et al., 2004). Furthermore, not only the nutrient content varies, but also the proportions. The C:N ratio seems to vary on a range from 8 up to 80. While a C:N ratio between 1 and 15 results in mineralization, and thus a release of N for plant uptake, a C:N ratio that is higher than 35 results in microbial immobilization, which means that microbes utilize and tie up nitrogen. For an equilibrium state between mineralization and immobilization, a C:N ratio between 20 and 30 is required (Brust, 2019). This was only found in the research by Suantika and colleagues (2017), whereby the C:N ratio of approximately 24 is even the optimal ratio for microbial growth (Brust, 2019).

Apart from physico-chemical characteristics, BSFL residue is found to have some biostimulant properties. As the residue is a mixture of undigested feeding material and insect derivatives, such as faeces and exuviae, it contains chitin. Chitin, found amongst others in insect exoskeletons, and metabolites are known to act as a protective barrier against diseases and pathogens (Choi & Hassanzadeh, 2019; Schmitt & de Vries, 2020). In addition to chitin, antimicrobial peptides produced by BSF exhibit various inhibitory effects on pathogens (Jiang et al., 2019). Another category of plant biostimulants found in BSFL residue is the beneficial bacteria category. Nitrifying and nitrogen-fixing bacteria, which participate in the nitrogen cycle and plants' uptake of nitrogen, were found in the residue (Choi & Hassanzadeh, 2019). Nitrogen-fixing bacteria, transform atmospheric nitrogen (N<sub>2</sub>) into biologically available nitrogen, ammonia (NH<sub>3</sub>). The best known nitrogen-fixing bacteria are the endosymbionts of the genus *Rhizobium*. Nevertheless, there are also free-living bacteria (and archaea) which can fix nitrogen, for example species of the genera *Azotobacter*, *Clostridium*, *Pseudomonas*, etc. The nitrifying bacteria, in their turn, convert ammonia (NH<sub>3</sub>) into nitrite (NO<sub>2</sub><sup>-</sup>) and then into nitrate (NO<sub>3</sub><sup>-</sup>)

), which can be taken up by plants. This nitrification process comprises two distinct steps that are carried out by distinct types of nitrifying bacteria. The first step, which is the oxidation of ammonia to nitrite, can be carried out by a few types of bacteria in the genera *Nitrosomonas*, *Nitrosospira*, and *Nitrosococcus* and by ammonia-oxidizing Archeae. The second step, which is the oxidation of nitrite to nitrate, can be carried out by a different group of bacteria, whereby some are of the genera *Nitrospira*, *Nitrobacter*, *Nitrococcus*, or *Nitrospina* (Bernhard, 2010). Besides nitrogen-fixing and nitrifying bacteria, other bacteria were found in BSFL residue. Kawasaki and colleagues (2020) found *Bacillaceae*, *Sporosarcina* and *Xanthomodaceae* as the most abundant bacteria in the residue. Although the family *Xanthomodaceae* includes two genera, *Xanthomonas* and *Xylella* which can cause diseases in certain plants, the cultivation test with *Brassica rapa* var. *perviridis* showed no pathogens. Furthermore, it was found that *Escherichia*, *Lactobacillales* and *Carnobacterium* were present in much lower abundance in the BSFL residue than in the starting substrate. On the other hand, certain bacteria were only detected in the BSFL residue. These findings suggest that the changes in bacterial abundance were caused by larval processing. Similarly, Wynants and colleagues (2019) found a significant correlation between the larvae and residues for the average number of fungi, lactic acid bacteria and endospores. While, conversely, no correlations of the average microbial counts were found between the substrates, on the one hand and of the larvae or residue at the other hand. Other research that included analyses of the gut microbiome, suggests that the microbial community of the residue can be reshaped by passing through the gut of the BSFL. The microbial community was found to be the closest in similarity to those of the substrate, food waste, during the first days of the bioconversion, while at the end it was more similar to the gut microbiome. At the same time, the microbial community of the gut changed over time. Moreover, the microbial community of the BSFL residue and the BSFL gut became more and more similar. Hence, the findings suggest that the substrate contributes to the composition of the microbial community of the gut and residue (Jiang et al., 2019), confirming previous findings (Boccazzi et al., 2017; Jeon et al., 2011). Similarly, it was found that feeding substrates that were spiked with the heavy metals, Cu and Cd, remarkably altered the gut microbiome of BSFL (Wu et al., 2020). However, it is assumed that other factors besides substrate type, also contribute to the microbial composition (Wynants et al., 2019). The research by Jiang and colleagues (2019), furthermore, showed a correlation between metabolic functions and microbes. For example, *Corynebacterium*, *Enterococcus*, *Vagococcus*, *Anaerococcus* and *Providencia*, whose relative abundances were higher in the BSFL residue than in the substrate and natural composting of the substrate, correlated positively with the associated genes and metabolic function groups that had higher abundance in the residue, such as carbohydrate-active enzymes, hydrogen metabolism, nitrogen cycle, and sulphur compound metabolism. This example additionally illustrates the possible variation in microbial composition of BSFL residue, given that Kawasaki and colleagues (2020)

observed *Bacillaceae*, *Sporosarcina* and *Xanthomodaceae* as the most abundant bacteria.

## 2. Thesis framework

The study was in association with Makerere University, Kampala and ProTeen. ProTeen is a business rearing Black Soldier Fly larvae (BSFL) on urban-organic waste in Kampala, Uganda. The idea of ProTeen was initiated in 2017 after the founders uncovered sustainability challenges farmers throughout Uganda were facing. One of the main challenges of farmers, in particular poultry farmers, was the lack of qualitative and affordable feed. Fishmeal, the primary source of poultry feed, has become scarce, expensive and low in quality due to overfishing in Lake Victoria. By rearing BSFL, ProTeen wants to offer high-quality protein feed to farmers. Using this technique, the social and environmental impacts of production of current feed sources, like grain, corn and soya, are reduced. At the same time, it contributes to a solution for challenges in waste management (ProTeen, 2019). In Kampala, approximately 65% of the waste is collected. The rest of the waste is openly dumped or burned, which has several consequences, such as the risk for diseases, flooding and environmental pollution. Given that a big part of the waste is organic (Godfrey et al., 2019; Kaza et al., 2018), BSFL rearing can have a big impact on waste management. ProTeen is currently working on the upscaling and optimization of the production system with the available resources. Thereby the focus is on the production of the larvae, though the by-product of the production system, the residue, has great potential as well. Therefore, the focus of this study is on the potential use of the BSFL residue.

## 3. Objectives

The main objective of the thesis is to assess the potential of BSFL residue as a fertilizer. To facilitate this assessment, three research questions were defined.

Research questions & hypotheses:

- Does the substrate that is used to feed the BSFL have an effect on the physico-chemical characteristics of the BSFL residue?  
The hypothesis is that the type of substrate will affect the physico-chemical characteristics of the BSFL residue.
- Does BSFL residues differ from other fertilizers (mineral NPK (YaraMila UNIK 17), commercial organic fertilizer (Fertiplus Cow), chicken manure and vermicompost)?  
The hypothesis is that the characteristics of BSFL residues will be different from the characteristics of other fertilizers.

- Does the socio-economic context of sub-Saharan Africa (SSA) affect the use of fertilizer?

The hypothesis is that the socio-economic context of SSA will have an influence on the use of certain fertilizers, either positive or negative.

## 4. Material and methods

### 4.1. Assessment of the effect of the substrate on the physico-chemical characteristics of BSFL residue

In order to understand if the substrate affects the residues, first Black Soldier Fly larvae (BSFL) residues had to be produced from different starting substrates. The term substrates refer to the waste materials that are used to feed the BSFL. The residue is a mixture of undigested substrate, larvae faeces, larvae exuviae and other parts from other BSF stages after the conversion of the substrate. The substrates were selected based on feasibility to rear BSFL and accessibility. The by-product of breweries, or brewery waste, is composed of spent grains, brewer's yeast and molasses. It's shown to be a good substrate to grow BSF, it is readily available in large quantities and available throughout the year (Chia et al., 2018). Brewery waste was used as one of the substrates. Food waste is a commonly used substrate to rear BSFL. In addition, the use of food waste as substrate is a way to alleviate issues related to dumping and burning of waste. Given the challenges regarding waste collection in Kampala (Kaza et al., 2018), it was opted to use market waste as second substrate. The waste is gathered in one place and it is daily available. A 50/50 mix of brewery waste and the collected market waste, was used as third substrate.

Once the residues were produced, physico-chemical analyses were done of the starting substrate and the different residues. The analyses were done in the soil lab of Makerere University. Due to several technical challenges encountered in Uganda, lacking of certain chemical reagents to conduct chemical analysis locally, machinery issues, etc., the analyses was restricted to following parameters, moisture content, pH, electrical conductivity (EC), organic matter (OM) and nitrogen (N), phosphorus (P) and potassium (K) values.

By setting up an experiment whereby the starting substrate was the only variable, and other factors, such as production parameters and lab analyses, were kept constant, the effect of the substrate on the characteristics of the residue could be assessed. The different substrates used for production of BSFL residues, were brewery waste (BW), market waste (MW), and a 50/50 mix of brewery waste with market waste (BW+MW). Even though poor facilities hindered to produce the different BSFL residues in controlled conditions, the conditions were the same for all the replicates (n=6) of the three treatments. Similarly, in the lab, all samples were analysed using the same method per parameter.

#### 4.1.1. Production of the residues

##### *Material:*

- Brewery waste
- Market waste (tomato, watermelon, cabbage, citrus and jackfruit)
- Black soldier fly larvae (BSFL)
- Scale → manual scale (accurate to 100 g) and digital scale (accurate to 0.01 g)
- 18 plastic basins
- Ziploc bags
- Freezer
- Electric heater
- Thermometer
- Hygrometer
- Sieve

##### *Treatments:*

- Brewery waste (BW)
- Market waste (MW) (a mixture of 18% tomato, 12% watermelon, 25% cabbage, 22% citrus and 23% jackfruit)
- 50/50 mix of brewery waste and market waste (BW + MW)

##### *Set-up:*

Per treatment there were six replicates that were randomised as seen in table 2. The brewery waste and market waste were the control substrates for the rearing of the larvae. For each replicate, 5 kg of substrate was used to rear 10 000 BSFL larvae.

*Table 2. Randomised set-up of the six replicates of the three objects; brewery waste (BW), market waste (MW) and 50/50 mix of brewery waste & market waste (BW+MW)*

MW+BW5	BW6	MW1	MW+BW3	BW5	MW5	MW+BW6	BW4	MW4
BW2	MW2	MW+BW2	BW1	MW3	MW+BW4	BW3	MW6	MW+BW1

##### *Method:*

For the production of the residues, the three types of substrate were prepared and divided over plastic basins. The brewery waste (BW) was ready to use and six clean basins were filled with the substrate, 5.0 kg in each basin. For the market waste (MW), a big mixture of tomato, watermelon, cabbage, citrus and jackfruit was made before dividing it over the six basins. The fruits and vegetables were chosen based on availability throughout the year and the moisture content. Before mixing them together, they were shredded into

small pieces and excessive moisture was pressed out of the tomatoes, the watermelon and the citrus. The total mixture, after shredding and removing excessive moisture, consisted of 10.8 kg tomato, 7.3 kg watermelon, 15.4 kg cabbage, 13.4 kg citrus and 14.1 kg jackfruit. Again, six clean basins were filled with 5.0 kg of the market waste mixture. For the third treatment (BW+MW), the basins were filled and mixed with 2.5 kg of brewery waste and 2.5 kg of the market waste mixture to have a total of 5.0 kg of substrate in each basin. From each substrate, three samples were taken and stored in a freezer. The substrate was elevated in the middle of the basins to prevent the escape of the larvae.

In each basin about 10 000 of 10-day-old BSFL, which were pre-fed with a mixture of BW and maize bran, were placed in the middle of the basin onto the substrate. As counting of all the larvae would have been too much work, the average weight of 10 000 larvae was derived from the average weight of 100 larvae. The average weight of 100 larvae, 0.11 g, was determined by counting and weighing 100 larvae for 15 times. This was done following the method of Miranda, Cammack and Tomberlin (2020).

The basins were then placed in a room. Because of limited facilities, the temperature and relative humidity could not be controlled in the room. To restrict variations in temperature, an electric heater with a heat sensor was placed in the room. The average, maximum and minimum temperature and humidity were recorded daily. Additionally, observations were made throughout the duration of the process.

After 14 to 16 days the larvae were, as much as possible, separated from the residues, mainly using a sieve. Both, larvae and residues were weighed and stored in the freezer.

#### 4.1.2. Lab analyses

##### *Material:*

The lab analyses were done according to Okalebo, Gathua and Woomer (2002). Therefore, the materials used for the different analyses can be found in "Laboratory Methods of Soil and Plant Analysis: A Working Edition." by Okalebo *et al.* (2002).

##### *Treatments:*

- Brewery waste (BW) (substrate)
- Market waste (MW) (substrate)
- 50/50 mix of brewery waste and market waste (BW + MW) (substrate)
- BW residue
- MW residue
- BW + MW residue

### *Set up:*

Per substrate three samples (n=3) were taken to conduct the lab analyses. For the residues there were six replicates (n=6) for the analyses.

### *Method:*

To determine the moisture content, small cans were made out of aluminium and weighed ( $W_e$ ). Fresh samples were added in the cans and weighed ( $W_f$ ). These samples were weighed again after being oven dried for 24 hours at 105 °C ( $W_d$ ).

$$\text{moisture (\%)} = \frac{W_f - W_d}{W_d - W_e} \times 100$$

For the other measurements, samples were oven dried (60 °C), crushed and sieved with a 2 mm sieve. For the pH and electrical conductivity (EC) values, 20.0 ml of distilled water was added to 2.00 g of sample and shook for 15 minutes. pH was measured with a Mettler Toledo pH-meter and EC with a MRC conductivity meter. Organic matter (OM) was estimated through loss on ignition. Dry crucibles ( $W_c$ ) were filled with small amounts of oven dried samples and weighed ( $W_{c+s}$ ). The crucibles were put in a muffle furnace at 550 °C for at least 8 hours and were weighed after they cooled in a desiccator ( $W_a$ ).

$$\text{ash (\%)} = \frac{W_a - W_c}{W_{c+s} - W_c} \times 100$$

$$\text{organic matter (\%)} = 100 - \text{ash (\%)}$$

To determine total nitrogen (N), total phosphorus (P) and total potassium (K), first digest solutions were made using 2.00 g of oven dried (60 °C), crushed and sieved samples. The production of the digest solutions, as well as the determination of total N, P and K was done according to Okalebo *et al.* (2002). For the determination of total P, a spectrophotometer at 880 nm wavelength was used instead of a colorimeter.

#### 4.1.3. Statistical analyses

The results from the lab analyses were analysed using Statistical Product and Service Solutions (SPSS) software. One-way analysis of variance (ANOVA), after confirmation of the homogeneity of variance and normality assumptions, were conducted. Significant means were separated by Tukey HSD test at  $p < 0.05$ . When the assumptions were not confirmed, a Kruskal-Wallis test followed by a Dunn post-hoc test was performed.

Furthermore paired t-tests or Wilcoxon sign tests, as non-parametric alternative, were performed between the substrate and residue of each treatment and one-sample t-tests or Wilcoxon signed rank tests to compare with mean values for BSFL residues from previous literature.

## 4.2. Comparison of BSFL residues with other fertilizers

To further assess the potential of the residues as a fertilizer, the residues were compared with other fertilizers. A mineral NPK fertilizer (YaraMila UNIK 17), a commercial organic fertilizer (Fertiplus Cow), chicken manure and vermicompost.

### *Material:*

- YaraMila UNIK 17
- Fertiplus Cow
- Chicken manure
- The three different residues (produced within the scope of the previous research question)
- The lab analyses were done according to Okalebo, Gathua and Woomer (2002). Therefore, the materials used for the different analyses can be found in "Laboratory Methods of Soil and Plant Analysis: A Working Edition." by Okalebo *et al.* (2002).

### *Treatments:*

- BW residue
- MW residue
- BW + MW residue
- Mineral NPK fertilizer YaraMila UNIK 17
- Commercial organic fertilizer Fertiplus Cow
- Chicken manure
- Vermicompost

### *Set-up:*

A statistical comparison was done on the data collected from the different treatments.

### *Method:*

The data on the different residues was already derived within the scope of the first research question. In order to conduct analyses on the other fertilizers, the mineral fertilizer (YaraMila UNIK 17) and Fertiplus Cow were bought in a local store while the chicken manure was obtained from a neighbouring chicken farmer. One sample was taken from the mineral fertilizer, the commercial organic fertilizer and the chicken manure, these were analysed in the lab as described in the previous part. For the comparison with vermicompost, it was opted to use data from literature.



For the statistical comparison, multiple one-sample t-tests were conducted. When the normality assumption was not confirmed, the non-parametric alternative, a Wilcoxon signed rank test was conducted.

#### 4.3. Assessment of the socio-economic constraints/challenges related to fertilizer use in sub-Saharan Africa (SSA)

To assess the socio-economic aspect of fertilizer use in SSA, it was opted to do a literature review. This decision was made based on several challenges with other methods such as surveys, semi-structured interviews, groups discussion, etc. As a European student there would have been a language and cultural barrier with the possible correspondents, mainly farmers. In addition the stay in Uganda was only limited to a short duration.

### 5. Results and discussion

#### 5.1. Assessment of the effect of the substrate on the physico-chemical characteristics of BSFL residue

In general, the physico-chemical parameters were significantly different between BW residue, MW residue and BW+MW residue ( $p < 0.05$ ). For part of the parameters, moisture content, organic matter (OM), and nitrogen (N) content, all three treatments were significantly different. Compared to the BW residue, the moisture content of the MW residue and the BW+MW residue is significantly higher and the moisture content of BW+MW residue is significantly lower than moisture content of the MW residue. The moisture content of the MW residue is thus the highest, thereafter is the moisture content of the BW+MW residue and the moisture content of the BW residue is the lowest. The same trend is seen for moisture content of the different feeding substrates. In contrast to the sequence of the moisture content of the residues the OM content and N content of the MW residue is lowest, that of the BW residue is the highest and the OM and N content of the BW+MW residue is intermediate.

For the other parameters, namely pH, electrical conductivity (EC), phosphorus (P) and potassium (K) content, the difference was significant between only two treatments. These statistical results are indicated with different alphabet characters in table 3. For the P content, BW residue and BW+MW residue were not significantly different, though they were significantly higher than the P content of the MW residue. For the pH, EC and for the K content it was found that BW residue and MW residue were mutually different, but they both showed no significant difference with BW+MW residue. Regarding the EC, this outcome is also visible when looking at the means and standard error. The EC value of the BW+MW residue is in the middle of the EC values of BW residue with the lowest EC value and MW residue with the highest EC value, and it has a high standard error. The

high standard error means that there is a large difference between the replicates which can be attributed to the variability in market waste. The trend found for the EC values of the different residues was also found for the pH and K values of the residues, however, this outcome is less visible in table 3. This is because the findings for these parameters are based on the non-parametric Kruskal-Wallis test, followed by a post-hoc Dunn test, which is less powerful than the one-way ANOVA and post-hoc Tukey test.

In table 3, the mean values for the different substrates were also added and compared with each other. The results of the one-way ANOVA and Tukey tests, indicate that there is a difference between the three waste-streams for the moisture content, OM. As mentioned above the differential trend of the moisture content of the different substrates is the same as for the residues. This is also correct for the OM parameter, even though the trend is different. For the other parameters Kruskal-Wallis followed by Dunn tests were conducted. The results indicate that the N and K content of the BW substrate is significantly different from that of the MW substrate, though neither of these two substrates showed significant difference with the BW+MW substrate. While the N content of the BW substrate is higher than the other treatments, the K content is lower, which was also found for the residue for the respective treatment. There is thus a clear link between the characteristics of the feeding substrate and the characteristics of the residues. For the remaining parameters, namely pH, EC and P content, no significant difference between the three substrates was found at all. It should, however, be noted that for the analyses of the substrates only three samples were taken. For the K content of the BW+MW substrate, it was even less because one sample got missing during the process. Additionally the samples were stored in a normal freezer for approximately one month.

Table 3. Physico-chemical characteristics of the substrates and BSFL residues for brewery waste (BW), market waste (MW) and a 50/50 mix of brewery waste and market waste (BW+MW), (mean  $\pm$  standard error). Different alphabet characters indicate significant difference (for substrate and residues separately) (one-way ANOVA + Tukey HSD test or Kruskal-Wallis + Dunn post hoc test,  $p < 0.05$ ).

	BW		MW		BW+MW	
	substrate	residue	substrate	residue	substrate	residue
moisture content (%)	326.64 <sup>a</sup> $\pm$ 5.65	65.03 <sup>x</sup> $\pm$ 9.23	587.33 <sup>b</sup> $\pm$ 22.24	437.48 <sup>y</sup> $\pm$ 10.60	438.85 <sup>c</sup> $\pm$ 8.00	215.28 <sup>z</sup> $\pm$ 18.74
pH (1:10 H <sub>2</sub> O)	3.83 <sup>a</sup> $\pm$ 0.06	6.31 <sup>x</sup> $\pm$ 0.02	4.03 <sup>a</sup> $\pm$ 0.04	9.63 <sup>y</sup> $\pm$ 0.16	4.00 <sup>a</sup> $\pm$ 0.03	6.42 <sup>xy</sup> $\pm$ 0.02
EC (dS/m)	1.90 <sup>a</sup> $\pm$ 0.95	5.09 <sup>x</sup> $\pm$ 0.05	6.12 <sup>a</sup> $\pm$ 0.17	12.82 <sup>y</sup> $\pm$ 0.37	2.80 <sup>a</sup> $\pm$ 1.39	8.46 <sup>xy</sup> $\pm$ 2.35
OM (%)	96.94 <sup>a</sup> $\pm$ 0.15	93.62 <sup>x</sup> $\pm$ 0.14	92.42 <sup>b</sup> $\pm$ 0.37	78.96 <sup>y</sup> $\pm$ 0.45	94.91 <sup>c</sup> $\pm$ 0.11	89.33 <sup>z</sup> $\pm$ 0.89

N (%)	5.55 <sup>a</sup> ± 0.12	5.86 <sup>x</sup> ± 0.12	1.49 <sup>b</sup> ± 0.05	2.08 <sup>y</sup> ± 0.07	3.92 <sup>ab</sup> ± 0.16	3.34 <sup>z</sup> ± 0.08
P (%)	0.78 <sup>a</sup> ± 0.02	1.88 <sup>x</sup> ± 0.08	0.38 <sup>a</sup> ± 0.07	0.43 <sup>y</sup> ± 0.07	0.96 <sup>a</sup> ± 0.34	1.67 <sup>x</sup> ± 0.06
K (%)	0.86 <sup>a</sup> ± 0.12	0.55 <sup>x</sup> ± 0.02	18.52 <sup>b</sup> ± 1.77	45.06 <sup>y</sup> ± 1.67	6.88 <sup>ab</sup> ± 0.55	19.67 <sup>xy</sup> ± 0.75

### 5.1.1. Moisture content

The moisture content was significantly different between the three substrates, however all of them were greater than 100%. While moisture contents below 40% hinder larvae development (Cammack & Tomberlin, 2017) and limits microbial activity (Liang et al., 2003), too high moisture content creates anaerobic conditions and a foul odour. Anaerobic conditions are hostile for the larvae which results in food to remain untouched and thus reducing larvae yield and inhibiting waste reduction. In addition, anaerobic environments lead to larval mortality (Diener, Studt Solano, et al., 2011; Diener, Zurbrügg, et al., 2011; Lalander et al., 2020). For larval development the most suitable moisture content is between 70 and 80% (Dortmans et al., 2017). Anaerobic decomposition, furthermore reduces organic nitrogen (N) to organic acids and ammonia (NH<sub>3</sub>). On the one hand, these have a phytotoxic effect (Bernal et al., 2009; Möller & Müller, 2012), on the other hand ammonia has an antimicrobial effect on certain bacteria such as *Salmonella* sp. (Erickson et al., 2004). Another reason for avoiding anaerobic conditions is the emission of greenhouse gases (GHGs) such as methane gas (CH<sub>4</sub>), carbon dioxide (CO<sub>2</sub>) and nitrous oxide (N<sub>2</sub>O), during anaerobic decomposition (Luo et al., 2014).

Even though the moisture content significantly reduced over time (paired sample t-test, p<0.05), which was in line with observations by others (Kawasaki et al., 2020; Liu et al., 2020), only the residue derived from BW had a moisture content under 100%. The BW residue had a mean moisture content of 65.03% (figure 3). The high moisture content of the MW (figure 4) and BW+MW residues hampered an easy separation of the residues by sieving. For residue separation by sieving the moisture content of the residue should be 50% (Cheng et al., 2017). Even though the moisture content of the BW residue exceeded the 50%, it was still possible to separate the residue from the larvae by sieving. Unlike the other two treatments, whereby the separation was done partly with a self-harvester and partly manually. As mentioned above, the larvae of the BSF will migrate away from the moist food source in the final larval stage. A self-harvester is used for this process to happen.

It is clear that the moisture content of the starting substrate will affect the final moisture content of the BSFL residue. This was also suggested when looking at data found for moisture content in different papers, whereby BSFL residues were derived from different waste sources (see table 4). However, given that the moisture content of the BW residue (65.03%) is significantly higher than the moisture content BSFL residue (30.1%) which

was also derived from brewery waste (Beesigamukama et al., 2020), suggests that the production system also plays a role.

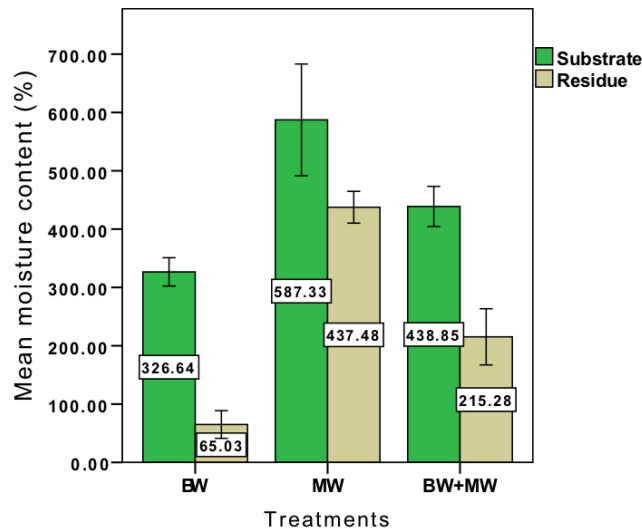


Figure 3. Mean moisture content (%) with standard error bars for the substrate ( $n=3$ ) and residue ( $n=6$ ) of each treatment; brewery waste (BW), market waste (MW) and a 50/50 mix of brewery waste and market waste (BW+MW). ( $t$ -test,  $p<0.05$ )

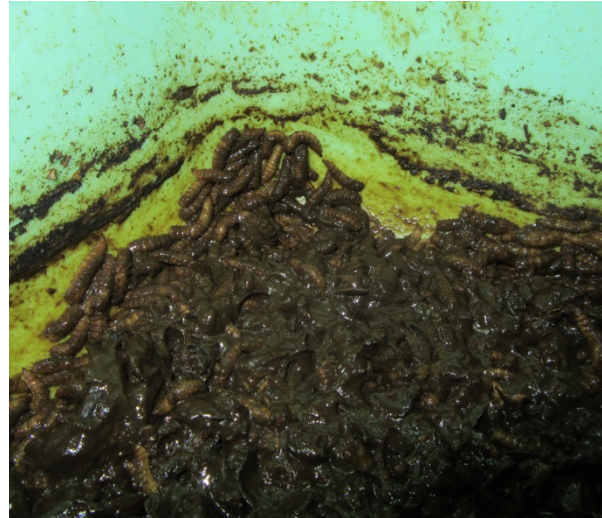


Figure 4. Mixture of larvae and residues from the market waste (MW) treatment, just before harvesting.

Since the production of the residues was done in closed basins, the excess water could not be drained. Therefore, it could be assumed that the reduction of moisture content was mainly by evaporation. In order to further decrease moisture content, evaporation could be increased by increasing ventilation (Lalander et al., 2020) and temperature (Bernal et al., 2009). Lalander and colleagues (2020) found that it would be possible to regulate ventilation based on substrate properties to achieve a residue moisture level suitable for easy separation. While Cheng and colleagues (2017) concluded that the maximum moisture content of the substrate in order to get a sievable residue is 75%, Lalander and colleagues (2020) found that with adequate ventilation even substrates with a moisture content up to 90% could be used for BSFL treatment. However, even with regulated ventilation the results suggested that substrates with moisture content over 90% were not suitable for BSFL treatment. Alternatively, the substrate could be pre-treated to reduce the moisture content. Since this has the disadvantage of generating wastewater, another option could be to mix waste streams with different moisture contents. As seen in this study the BW+MW residues had significantly lower moisture content than the residues from MW alone. Still it is important to take into account other properties of the substrates. For example, Lopes and colleagues (2020) found that waste rich in fat, like aquaculture waste, can decrease evaporation by forming a lipid layer on top, and thus maintaining a high moisture content.

### 5.1.2. pH

In contrast to the moisture content, the pH of the different waste-streams was not significantly different. All of the substrates had an acidic pH, with a mean around 4.0. Such a low initial pH was previously found to have a negative influence on the growth and development of BSFL (Ma et al., 2017). The low pH value in combination with the high moisture content, make the substrates used in the study unfavourable for BSFL production. Therefore, it can already be recommended to mix the used substrates with other organic waste streams to become a moisture content of 70-80% and a pH 6.0 to 8.0, which is the recommended initial pH according to Ma and colleagues (2017). On top, a higher pH would be beneficial given that most bacteria grow best around neutral pH values (6.5 – 7.0) (S. Choi & Hassanzadeh, 2019).

As in earlier research, the pH increased during the bioconversion process (figure 5) (Kawasaki et al., 2020; Liu et al., 2020; Newton et al., 2005; Sarpong et al., 2019). By comparing the data for the substrates with the data for the residues with a paired t-test, the increase in pH was found to be statistically significant for each treatment. The significant increase in pH can be ascribed to the production of ammonia during the digestion of proteins by the larvae, but it is also hypothesised to be produced by the indigenous microflora of the substrate (Erickson et al., 2004; Lalander et al., 2015). While the pH of the BW and BW+MW residues were still slightly acidic, with a mean pH of 6.31 and 6.41 respectively, the residues derived from MW was basic (mean pH of 9.63). Hence, the MW residue could be useful for repairing acidic soils, which is a current issue in sub-Saharan Africa (Tully et al., 2015). Such a high pH is remarkable. Even though different values were found for the pH of BSFL residues (table 4), most values range around 7 and 8. On the other hand, a remarkable low pH of 5.5 was found by Choi and colleagues (2009).

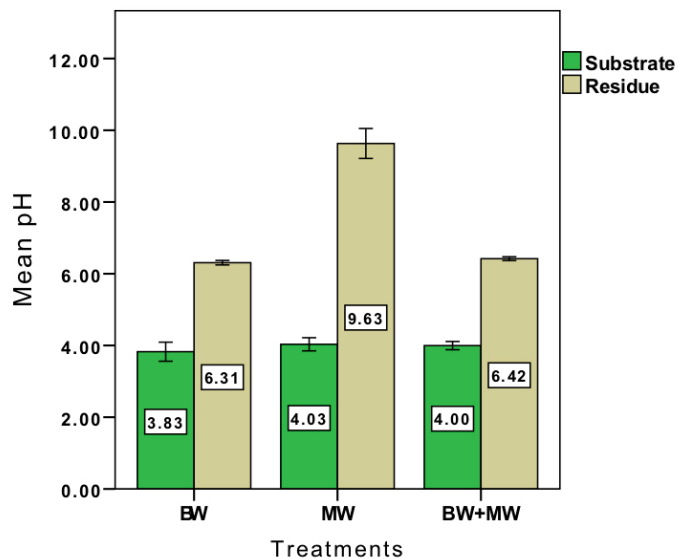


Figure 5. Mean pH with standard error bars for the substrate (n=3) and residue (n=6) of each treatment; brewery waste (BW), market waste (MW) and a 50/50 mix of brewery waste and market waste (BW+MW). (t-test,  $p < 0.05$ )

a mean pH of 6.31 and 6.41 respectively, the residues derived from MW was basic (mean pH of 9.63). Hence, the MW residue could be useful for repairing acidic soils, which is a current issue in sub-Saharan Africa (Tully et al., 2015). Such a high pH is remarkable. Even though different values were found for the pH of BSFL residues (table 4), most values range around 7 and 8. On the other hand, a remarkable low pH of 5.5 was found by Choi and colleagues (2009).

It is important to look at the pH given that the pH controls many chemical processes that take place in the soil, specifically plant nutrient availability. Most plant nutrients are optimally available to plants within a 6.5 to 7.5 range (Division of Agriculture and Natural Resources, University of California, 2020).

### 5.1.3. EC

The electrical conductivity (EC) is commonly used to measure soluble salt concentrations, which is influenced by the presence of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{CO}_3^{2-}$ ,  $\text{HCO}_3^-$ ,  $\text{NO}_3^-$  ions and some micronutrients (Gondek et al., 2020). However, it is also an important indicator for soil fertility. Like the pH, the EC of the soil affects plant nutrient availability and microbial populations. While too low EC levels limit plant growth due to nutrient deficiency, too high EC levels induces salinity stress which also inhibits plant growth (Ding et al., 2018).

While composting, in general, reduces EC levels (Gondek et al., 2020), the bioconversion by BSFL resulted in an increase of the EC (figure 6). Even though statistical analysis showed that the EC difference between substrate and residue was only significant for the MW treatment, it should be considered that the paired t-test was conducted with only 3 values. Furthermore, a significant increase in EC was also found by other researchers (Jiang et al., 2019; Kawasaki et al., 2020; Liu et al., 2020). The increase in EC may be ascribed to the release of ions by the decomposition of organic matter (Jain et al., 2019).

The difference in EC level between the treatments is only significant between the BW residue and MW residue. The EC value found for the BW+MW treatment does not significantly differ from the other two treatments. This is the same trend as for the EC levels of the substrates. The difference in EC level between the residues is thus originated from the difference in EC level of the feeding substrates, whereby market waste which is a mixture of 18% tomato, 12% watermelon, 25% cabbage, 22% citrus and 23% jackfruit has a higher EC than brewery waste. While in this study the EC variation is not so pronounced, the range of EC levels found in other papers is very broad. Liu and

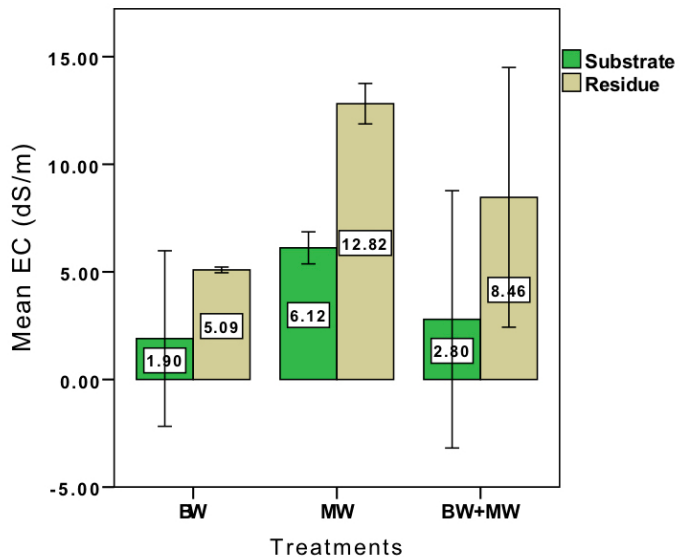


Figure 6. Mean electrical conductivity (EC) (dS/m) with standard error bars for the substrate ( $n=3$ ) and residue ( $n=6$ ) of each treatment; brewery waste (BW), market waste (MW) and a 50/50 mix of brewery waste and market waste (BW+MW). ( $t$ -test,  $p<0.05$ )

colleagues (2020), for example, found that BSFL residue derived from sewage sludge had an EC level of 0.36 dS/m. In contrast, Temple and colleagues (2013) studied BSFL residues with an EC level of 44 dS/m. Excluding the two highest EC values found in previous research (21 dS/m by Agriprotein *et al.* (2017) and 44 by Temple *et al.* (2013)), the values found in this study are rather high.

Given these rather high EC values of the residues, applying the residues as a soil amendment could have a negative impact on the soil and yield, especially in arid and semiarid regions. However, it has been shown that application of compost with EC values of > 5 dS/m enhanced plant growth. Assumptions were made that compost with high EC values and limited Na<sup>+</sup> and Cl<sup>-</sup> concentrations does not limit plant growth as long as the mixture of compost and soil or media does not exceed an overall EC of 5 dS/m (Gondek *et al.*, 2020). Furthermore in a study with BSFL residues, application generally resulted in drops of the soil EC (Zahn, 2017). Thus, even though caution should be taken, further research should indicate the effect of residue application on the soil and plant growth. In addition, the type of crop and environmental conditions, like soil texture should be taken into account as the optimal EC value depends on these factors (Ding *et al.*, 2018).

#### 5.1.4. OM

BSFL can convert organic waste, from different origin, into biomass rich in protein and fat. At the same time, it reduces the organic waste substantially. In this study the weight of the substrate was 5000 g for each replicate, the mean weight left after conversion was 756.96 g for the BW treatment, 541.35 g for the MW treatment and 713.07 g for the BW+MW treatment. Hence, the mean reduction ranged from 84.86% for BW, 85.74% for BW+MW to 89.17% for MW. Besides the significant reduction in biomass, the organic matter content reduced during the process. The reduction of OM ranged from 3.42% for the BW treatment, 5.88% for the BW+MW treatment, to 14.56% for the MW treatment. Compared to the results from Liu and colleagues (2020), these degrees of reduction are rather low. The OM reduction of the food waste, consisting out of noodle, rice, cabbage and pork, by the BSFL was 21.98%. For the sewage sludge treatment, an OM reduction of 14.51% was found. This reduction may be ascribed to decomposition, whereby dead organic matter is broken down into constituent parts, like CO<sub>2</sub> and inorganic ions (Liu *et al.*, 2020; Robertson & Paul, 2000). Subsequently, this reflects the increase in EC discussed above.

Besides the low reduction level in this study compared to previous studies, the OM content of the BSFL residues in this study is significantly higher than most values found in other papers. Excluding the two extreme low values (Choi *et al.*, 2009; Newton *et al.*, 2005), the mean OM content based on values from previous papers is 66.59% with a standard error of 4.61. All three BSFL residues in this study have significantly higher OM content than

66.59%. Yet, two other studies found OM contents above 80% (Gao et al., 2019; Liu et al., 2020).

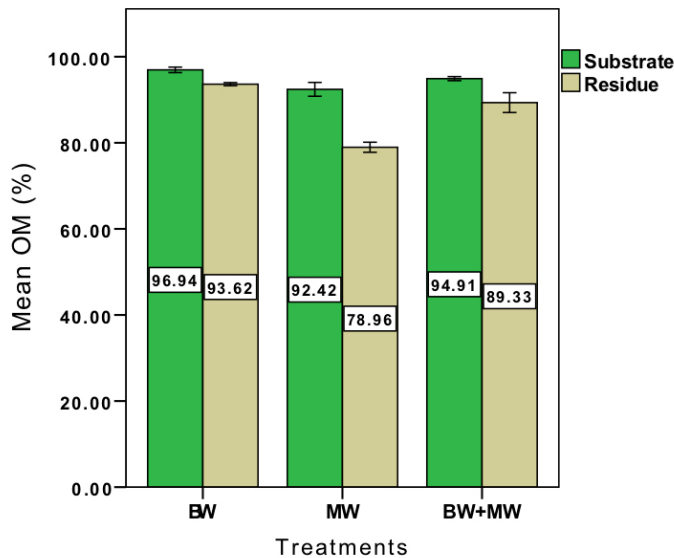


Figure 7. Mean organic matter (OM) (%) with standard error bars for the substrate ( $n=3$ ) and residue ( $n=6$ ) of each treatment; brewery waste (BW), market waste (MW) and a 50/50 mix of brewery waste and market waste (BW+MW). ( $t$ -test,  $p<0.05$ )

Thus, even though the organic matter (OM) content reduced, the OM content of the residues is still high (figure 7). This is an advantage given that OM is an important aspect for soil fertility. It plays a key role in the physical, chemical and biological processes of the soil. OM does not only promotes the growth of soil organisms, OM also improves soil structure, which in turn improves, soil drainage, water infiltration, aeration and water holding capacity (Gachene & Kimaru, 2003). Hence, the application of the residues from the bioconversion process by the BSFL could have a beneficial impact on soil fertility and yield, especially in SSA where often soils are low in organic matter (Chivenge et al., 2011).

On the other hand, an OM content above 65% may indicate that the process has not been thoroughly completed and that part of the OM is unstable. If this is applied to the soil, the unstable OM will be lost as  $\text{CO}_2$  by rapid decomposition (Sullivan et al., 2018). That the process might be incomplete, was also suggested by Kawasaki and colleagues (2020) based on the chemical composition. Hence, post-treatment is required to obtain mature compost. Three different processes were mentioned before. The residues could simply be composted for approximately two months or it could be composted by feeding it to worms, which result in vermicompost. As mentioned above, vermicompost has beneficial characteristics because of the biostimulant effect. A third option is to feed the residues into an anaerobic digester (Dortmans et al., 2017). This option could be used for the MW and BW+MW residues given their high moisture content. Since the BW residue has a significantly lower moisture content, the anaerobic digestion is not a suitable post-treatment.



### 5.1.5. NPK

The content of the macronutrients nitrogen (N), phosphorus (P) and potassium (K) vary in the different residues. The N content differs significantly between all three treatments. For the P content, it was found that the BW residue and BW+MW residue has similar P content, which is significantly different from the P content of the MW residue. The statistical analyses for the K content showed a significant difference between BW residue and MW residue, though neither are significantly different with the BW+MW residues. Even though the lab analyses were done equally for all the samples, the K content of the MW and BW+MW samples, both substrate and residues, were remarkably high. As well as the K content data of the analysed commercial fertilizers compared to the content stated on the label. Thus, it is advised to be cautious with the data.

When the nutrient content of the residues was compared with those of the substrates, mixed results were found. According to the results of the Wilcoxon sign test, the N content of the substrate and the residue were equal per treatment. It should however be noted that the statistical test conducted is not very powerful and on top, the test only compared three data. The graph (figure 4) suggests that the N content increased during the bioconversion process for BW and MW treatment, while for the BW+MW treatment the N content reduced. Likewise, mixed results were found in the literature. Liu and colleagues (2020) found in their study that the total N content of the BSFL

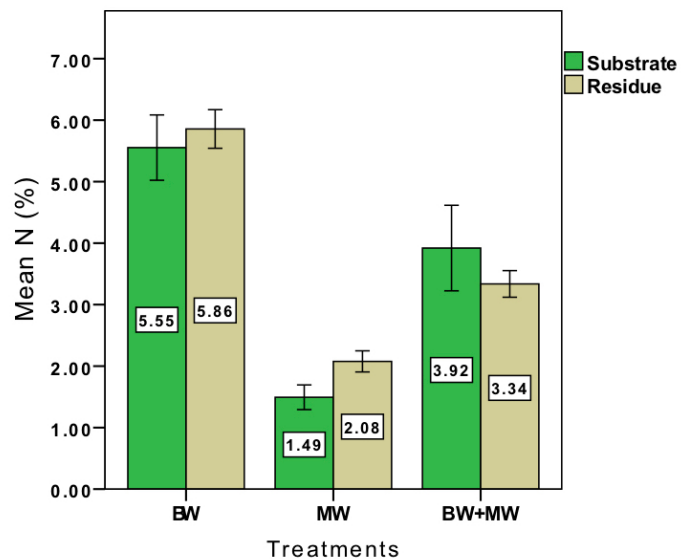


Figure 8. Mean nitrogen (N) content (%) with standard error bars for the substrate ( $n=3$ ) and residue ( $n=6$ ) of each treatment; brewery waste (BW), market waste (MW) and a 50/50 mix of brewery waste and market waste (BW+MW). (Wilcoxon sign test,  $p<0.05$ )

residue derived from sewage sludge was lower than the sewage sludge itself. On the other hand, they found that the bioconversion of food waste by BSFL resulted in an increase in N content. Furthermore, some researchers found that N increased with the bioconversion process (Kawasaki et al., 2020; Sarpong et al., 2019), which could be due to the biochemical activities of the larvae and the activity of microbes, like nitrifying bacteria (Sarpong et al., 2019). Other researchers found, by contrast, that the N content decreases

(Jiang et al., 2019; Newton et al., 2005; Suantika et al., 2017), due to N used by the BSFL for metabolic processes (Suantika et al., 2017).

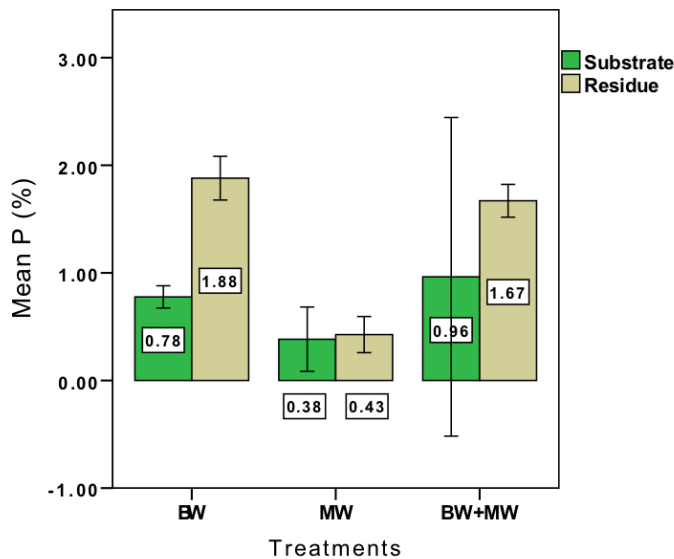


Figure 9. Mean phosphorus (P) content (%) with standard error bars for the substrate (n=3) and residue (n=6) of each treatment; brewery waste (BW), market waste (MW) and a 50/50 mix of brewery waste and market waste (BW+MW). (t-test,  $p < 0.05$ )

For the P content, the paired sample t-test indicated an increase for the BW treatment. For the MW and BW+MW treatments, the paired t-tests did not indicate a significant difference between substrate and residue, whilst the mean P contents for the BW+MW treatment suggest otherwise. Due to the high variance between the data of the BW+MW substrate, the difference was not detected as significantly (figure 5). While some studies also found increases in the P content (Liu et al., 2020; Sarpong et al., 2019; Suantika et al., 2017), other studies indicated that the bioconversion process reduced the P content (Kawasaki et al., 2020; Newton et al., 2005).

In the bioconversion process with BW, the K content approximately remained unchanged. Similar to the results of the P content for the BW+MW treatment, the means suggest a difference, while the paired t-test did not indicate a significant difference. For the MW treatment, however, the increase in K content was found to be significant (figure 6). As with the N and P content, some researchers found that the K content increased (Liu et al., 2020; Sarpong et al., 2019), while others found that the K content decreased (Kawasaki et al., 2020; Newton et al., 2005; Suantika et al., 2017).

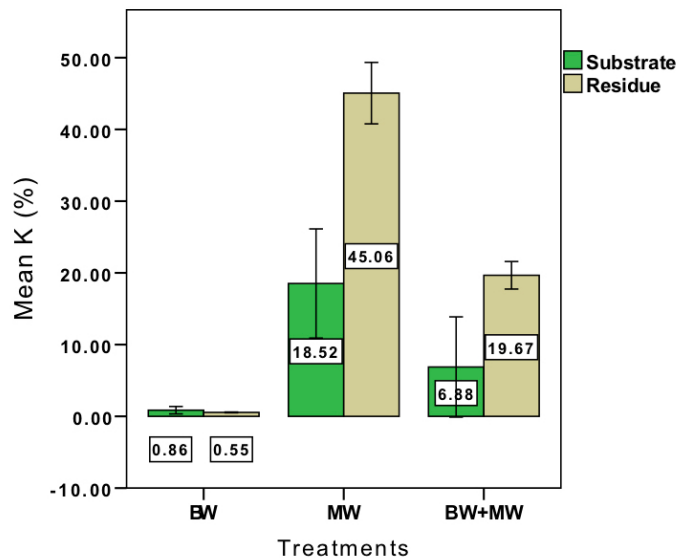


Figure 10. Mean potassium (K) content (%) with standard error bars for the substrate (n=3) and residue (n=6) of each treatment; brewery waste (BW), market waste (MW) and a 50/50 mix of brewery waste and market waste (BW+MW). (t-test,  $p < 0.05$ )

As mentioned before, the K content of the MW and BW+MW residue are extremely high and are most likely not accurate. In contrast to the K content of these residues, the K content found for the BW residue is significantly lower. Furthermore, the K value of the BW residue falls within the range of K values (0.02 - 3.42) found in previous papers. Thus, this value might be more accurate. That the K content of the MW and BW+MW residues is higher than the residue of the BW treatment could be explained by the fact that fruits and vegetables, the composition of the market waste, is high in potassium. Compared to the mean K value from previous literature ( $1.29 \pm 0.37$ ), the K value from the BW residue is significantly lower. On the other hand, the K content is significantly higher than the K content of BSFL residue also derived from brewery waste found by Beesigamukama and colleagues (2020). Likewise, the N and P content found for BSFL residue derived from brewery waste in this study are significantly higher than the values found by Beesigamukama and colleagues (2020). As for the K content, mean values based on previous literature were calculated for N and P. An average N content of 2.39% and an average P content of 0.99% was found. Multiple one-sample t-tests showed that the BW and BW+MW residue has significantly higher N and P content than the average values, while the N and P content of the MW residue are significantly lower than the average values.

Table 4. Physico-chemical characteristics of BSFL residues from this study and from different literature. The average is calculated from the values from the literature (excluding some outliers).

Feeding material	Brewery waste	Market waste (specified above)	Brewery waste + market waste	Food waste (not specified)	Food waste (not specified)	Food waste (not specified)	Food waste (specified above)	Food waste (specified above)	Maize straw	Coffee husk	Wheat, alfalfa and corn meal	Brewery spent grains	Sewage sludge	Swine manure	AVERAGE of the values from literature
Moisture content (%)	65.03	437.48	215.28	n.d.	n.d.	n.d.	55.60	62.99	38.23	n.d.	n.d.	30.1	75.32	n.d.	52.45 ± 8.20
pH	6.31	9.63	6.42	6.9	5.5	7.0	7.40	7.36	8.03	n.d.	8.8	7.7	7.93	7	7.36 ± 0.28
EC (dS/m)	5.09	12.82	8.46	21	44	0.5	9.67	3.51	n.d.	n.d.	8.5	2.7	0.36	n.d.	4.21 ± 1.63 (without EC values 21 & 44)
OM (%)	93.62	78.96	89.33	68.8 <sup>b*</sup>	73.79 <sup>b*</sup>	3.3 <sup>a,○</sup>	61.64 <sup>b*</sup>	88.69	84.87	53.49 <sup>b</sup>	60.54 <sup>b</sup>	60.54 <sup>b*</sup>	46.98	0.73 <sup>b*</sup>	66.59 ± 4.61 (without EC values 3.3 & 0.73)
N (%)	5.86	2.08	3.34	3.6	4.54	n.d.	2.16	3.12 <sup>a</sup>	0.63	1.27	4.4	2.1	1.99 <sup>a</sup>	0.04 <sup>e</sup>	2.39 ± 0.48
P (%)	1.88	0.43	1.67	1.6	1.23	n.d.	0.05	1.14 <sup>a</sup>	1.11 <sup>d</sup>	0.2 <sup>d</sup>	2.27 <sup>d</sup>	1.16	1.11 <sup>a</sup>	0.04 <sup>e</sup>	0.99 ± 0.23
K (%)	0.55	45.06	19.67	1.4	2.44	n.d.	0.07	0.84 <sup>a</sup>	1.73 <sup>d</sup>	2.32 <sup>d</sup>	3.42 <sup>d</sup>	0.17	0.51 <sup>a</sup>	0.02 <sup>e</sup>	1.29 ± 0.37
References	own data	own data	own data	(AgriProtein, 2017)	(Temple et al., 2013)	(Y.-C. Choi et al., 2009)	(Kawasaki et al., 2020)	(Liu et al., 2020)	(Gao et al., 2019)	(Suantika et al., 2017)	(Setti et al., 2019)	(Beesigamukama et al., 2020)	(Liu et al., 2020)	(Newton et al., 2005)	

n.d.: not determined

<sup>a</sup> from mg/kg to percentage: divide by 10000 (thus from g/kg to percentage: divide by 10); from percentage to mg/kg: multiple by 10000

<sup>b</sup> OM (%) = total organic carbon (%) x 1.72 ; organic C (%) = OM (%) x 0,58 → assuming 58% of organic matter exists as carbon (Burt, 2011)

\*note of caution: here the OM was derived from the value of C

<sup>d</sup> from P<sub>2</sub>O<sub>5</sub> to P: divide by 2.29; from K<sub>2</sub>O to K: divide by 1.2

<sup>e</sup> from ppm to percentage: divide by 10000; 1 ppm = 1 mg/kg

<sup>○</sup> outlier

For all the parameters, the values of the BW+MW residue lays in between the values of the BW residue and the MW residue. Given that BW+MW is a mix of both BW and MW, this trend indicates that the starting substrate to some level affects the characteristics of the BSFL residues. However, the significant difference between the values of this study and a previous study (Beesigamukama et al., 2020), suggest that other factors, like production system, plays a role. Furthermore, this confirms the variability in nutrient content with organic fertilizers, which is a huge disadvantage. This variability is further demonstrated by the standard errors (table 3), which represent the variance between replicates of the same treatment.

## 5.2. Comparison of BSFL residues with other fertilizers

If the BSFL residues are to be used as a fertilizer, it is interesting to compare them with fertilizers that are currently used. The three different residues were compared with one inorganic and three organic fertilizers, namely Fertiplus Cow, chicken manure and vermicompost. Table 7 contains the main values of the residues and the physico-chemical values of the other fertilizers. Given that the values of the inorganic fertilizer (YaraMila UNIK 17), Fertiplus Cow and the chicken manure were obtained from just one analysis, the data should be interpreted with caution. As seen in the table, for the two commercial the data from the analysis and the label fertilizers do not always comply, especially the K content highly differs. The high difference in K content was also find for chicken manure between the data from the analysis and an average value derived from different papers (table 5).

Table 5. Physico-chemical characteristics of chicken manure retrieved from different papers. The values were used to get average values (mean  $\pm$  standard error) for chicken manure.

								AVERAGE
Moisture content (%)	14.21	25.23	74.53	13.4	20.9	n.d.	n.d.	29.65 $\pm$ 11.43
pH	8.30	8.10	n.d.	8.43	7.4	n.d.	n.d.	8.06 $\pm$ 0.23
EC (dS/m)	7.27	6.76	n.d.	3.63	n.d.	n.d.	n.d.	5.89 $\pm$ 1.14
OM (%)	n.d.	76.92	67.37	68.10	n.d.	n.d.	n.d.	70.80 $\pm$ 3.07
N (%)	3.23	5.13	5.9	3.45	3.04 <sup>a</sup>	3.52	0.60	3.55 $\pm$ 0.64
P (%)	0.11	2.32	0.65	0.72	2.02 <sup>a</sup>	0.86	0.34	1.00 $\pm$ 0.32
K (%)	0.19	2.14	2.38	1.44	2.65 <sup>a</sup>	1.53	0.44	1.54 $\pm$ 0.36
References	(Kawasaki et al., 2020)	(Dede & Özer, 2018)	(Quiroga et al., 2010)	(El-Haggar, 2007)	(Sistani et al., 2008)	(Zayed et al., 2013)	(Asiriwuwa et al., 2013)	

n.d.: not determined

<sup>a</sup> from g/kg to percentage: divide by 10

The data for the vermicompost were also retrieved from literature. The vermicomposting process is, like the BSFL process, used to compost different types of organic waste. Table 6 contains the values that were found for vermicompost derived from different wastes, such as food waste, manure, paper waste and others. For each parameter an average value was calculated based on all the different values. These average values were used to make the comparison with the BSFL residues. The values of the vermicompost produced from brewers' spent grain, which is the main residue of the brewing process (Saba et al., 2019), were used for the comparison with the BW residue. Since the market waste in this study was a mix of fruits and vegetables, more specific from tomato, watermelon, cabbage, citrus and jackfruit, the MW residue characteristics were compared with the characteristics of vermicompost derived from fruit and vegetable waste. The values found in table 7 for the vermicompost derived from fruit and vegetables are the average values from the data found by Jadia and Fulekar (2008) and Huang and colleagues (2012). Like with the BSFL residues and the chicken manure, the values for the physico-chemical characteristics that were found vary.

Table 6. Physico-chemical characteristics for vermicompost retrieved from different papers. The values were used to get average values (mean  $\pm$  standard error) for vermicompost (in general) and for vermicompost derived from fruit and vegetable waste.

Feeding material	Not specified	Not specified	Food waste (not specified)	Food waste (not specified)	Vegetable waste (cabbage, French bean, cauliflower, ladyfinger, spinach, and carrot)	Fruit & vegetable waste (banana peels, cabbage, lettuce, potato, watermelon peels)	Brewer spent grain	Municipal solid waste	Household solid wastes	Cow manure	Cow manure + rice straw and/or grass clipping	Animal manure + waste material (grasses, brewed black tea leaf and dry tree leaf)	Paper waste	Paper waste	Biological sludge from wood & paper industries	Chemical sludge from wood & paper industries	AVERAGE
moisture content (%)	n.d.	n.d.	n.d.	n.d.	70.15	66.16	n.d.	n.d.	28.3	n.d.	n.d.	47.70	n.d.	n.d.	32.2	21.2	44.29 $\pm$ 8.35
pH	8.1	7.22	6.53	n.d.	6.8	8.46	5.6	6.31	8.2	n.d.	6.50	7.40	6.41	n.d.	8	7.7	7.17 $\pm$ 0.25
EC (dS/m)	1.7	6.88	2.68	n.d.	10.55	3.73	n.d.	1.29	0.55	n.d.	3.71	3.90	2.96	n.d.	0.36	0.64	3.25 $\pm$ 0.85
OM (%)	33	n.d.	n.d.	n.d.	n.d.	n.d.	59.3	n.d.	65.8	n.d.	n.d.	46.50	n.d.	n.d.	67.8	63	55.9 $\pm$ 5.52
N (%)	1.5	3.5	1.86	1.3	1.33	0.69 <sup>a</sup>	5.10	1.59	2.2	1.9	1.36	2.30	1.60	1.0	2.6	2.2	2.00 $\pm$ 0.27
P (%)	1.8	0.71	0.52	2.7	0.47	0.44 <sup>a</sup>	n.d.	0.40	0.72	4.7	0.58	0.96 <sup>a</sup>	0.26	1.4	0.5	0.6	1.12 $\pm$ 0.31
K (%)	1.2	0.10 <sup>b</sup>	2.98	9.2	n.d.	n.d.	n.d.	2.65	n.d.	1.4	0.56	0.99 <sup>a</sup>	2.45	6.2	n.d.	n.d.	2.77 $\pm$ 0.90
References	(Adamipour et al., 2019)	(Kalanitari et al., 2010)	(Soobhany et al., 2017)	(Arancon et al., 2005)	(Jadia & Fulekar, 2008)	(Huang et al., 2012)	(Saba et al., 2019)	(Soobhany et al., 2017)	(Amouei et al., 2017)	(Arancón et al., 2005)	(Ramnarain et al., 2019)	(Ugur et al., 2019)	(Soobhany et al., 2017)	(Arancon et al., 2005)	(Amouei et al., 2017)	(Amouei et al., 2017)	

n.d.: not determined

<sup>a</sup> from mg/kg to percentage: divide by 10000; from percentage to mg/kg: multiple by 10000

<sup>b</sup> from P<sub>2</sub>O<sub>5</sub> to P: divide by 2.29; from K<sub>2</sub>O to K: divide by 1.2

Table 7. Physico-chemical characteristics for the different BSFL residues and four other different fertilizers, namely the commercial inorganic fertilizer YaraMila UNIK17, the commercial organic fertilizer Fertiplus Cow, chicken manure and vermicompost.

	BW residue	MW residue	BW+MW residue	inorganic fertilizer		Fertiplus Cow		chicken manure		vermi-compost		
				analysis	label	analysis	label	analysis	average	average	brewers' spent grain	fruit & vegetable waste
moisture content (%)	65.03	437.48	215.28	1.64		12.16	10-12	16.52	29.65	44.29		68.16
pH	6.31	9.63	6.42	5.02		6.68	6.1	7.75	8.06	7.17	5.6	7.36
EC (dS/m)	5.09	12.82	8.46	91.60		7.20		5.12	5.89	3.25		7.14
OM (%)	93.62	78.96	89.33	ND		75.83	65	49.30	70.80	55.9	59.3	
N (%)	5.86	2.08	3.34	11.06	17	3.92	4.2	1.54	3.55	2.00	5.10	1.01
P (%)	1.88	0.43	1.67	6.75	17	1.40	1.31	1.23	1.00	1.12		0.46
K (%)	0.55	45.06	19.67	51.70	17	14.58	2.33	10.45	1.54	2.77		

n.d.: not determined

### 5.2.1. Moisture content

The moisture content of the different residues was, as seen above, significantly different. Though, at the same time, all three residues had significantly higher moisture contents than the inorganic fertilizer, Fertiplus Cow and chicken manure. The moisture content of the vermicompost, both the average and the fruit and vegetable derived vermicompost, had statistically the same moisture content as the BW residue. The BW residue was the treatment with the lowest moisture content and was the only residue that could be separated through sieving. As discussed before, the high moisture content of the residue hampers the easy separation of the residue from the larvae. Furthermore, the advantage of a lower moisture content is that it makes it easier to transport and handle.

### 5.2.2. pH

The multiple one-sample t-tests showed that there is a significant difference between the residues and the other fertilizers. The MW residue is the only basic residue and has a significantly higher pH than all the other fertilizer. Only the average pH value for chicken manure is also slightly basic, though the pH of the MW residue is still significantly higher.



Even though the pH of the BW residue and BW+MW residue is significantly different from Fertiplus Cow and the analysed value of chicken manure, they all range around a neutral pH. The average pH value found for vermicompost and the pH value for vermicompost derived from fruit and vegetable waste also range around a neutral pH. The inorganic commercial fertilizer and the vermicompost from brewers' spent grain, have the lowest pH, 5.02 and 5.6 respectively. The low pH of the fertilizer could reinforce soil acidification, which is already an issue in sub-Saharan Africa (Tully et al., 2015).

### 5.2.3. EC

The electrical conductivity (EC) value that was found for the inorganic fertilizer was remarkable high, much higher than the different residues and the other fertilizers. This is in contrast with the findings of Choi and colleagues (2009), whereby the EC level of the BSFL residue was found to be slightly higher than the EC of a chemical commercial fertilizer. As discussed before, high EC levels could induce salinity stress and inhibit plant growth. Furthermore, it could have a negative impact on the soil, especially in arid and semiarid regions (Gondek et al., 2020). The high EC level in combination with the acid pH, might make it undesirable to apply the inorganic fertilizer. In contrast, the average EC value found for vermicompost is 3.25 dS/m, which is significantly lower than the EC of the three different residues.

Compared to the other fertilizers, there were mixed results. The EC value of the BW residue is significantly lower than the EC value of Fertiplus Cow, the average EC value found for chicken manure and the EC found for vermicompost derived from fruit and vegetable waste. Compared to the EC value of chicken manure from the analysis, the EC value of the BW residue is not significantly different. For the BW+MW residue, Wilcoxon signed rank tests showed no difference in EC level with Fertiplus Cow, neither with the chicken manure (both values) and the fruit and vegetable waste vermicompost. Whilst for the MW residue, one-sample t-tests showed that the mean EC of the MW residue was significantly higher than all the organic fertilizers analyzed. Similarly, Kawasaki and colleagues (2020) found that BSFL residues had higher EC compared to cow, horse and poultry manure. The variance indicates a difference in nutrient content.

### 5.2.4. OM

One of the advantages of organic fertilizers over inorganic fertilizers, is the organic matter (OM) content. In the previous part it was seen that the OM content is significantly different between the three residues. Similarly, the OM content of the residues is significantly different from the OM content of the organic fertilizers. All the three residues had higher OM content than Fertiplus Cow and both values found for chicken manure and vermicompost. This might be an advantage, however, an OM content above 65% may

indicate that the process has not been thoroughly completed and that part of the OM is unstable. Therefore, different post-treatments were suggested, which were discussed above. One of the options is to feed the residues to earthworms to become vermicompost. Based on the values that were found for the OM content of different vermicomposts (table 6), it can be assumed that feeding the residues to earthworms could be an effective way to further reduce the OM content.

#### 5.2.5. NPK

Unlike previous findings that the nutrient content of BSFL residue is similar to a commercial inorganic fertilizer (Choi et al., 2009), the multiple one-sample t-tests and Wilcoxon signed rank tests (for the K content) in this study suggest that the NPK content of all the three BSFL residues is significantly lower than the NPK content of the commercial inorganic fertilizer YaraMila UNIK 17. For the N and P content this was found for both the values found during the analyses and the data from the label. For the K content, the values for the residues were significantly lower than the K value found during the analysis of the inorganic fertilizer. Compared to the value on the label, namely 17, the MW residue and BW+MW residue had significantly higher K content. The comparison of the NPK content of the BSFL residues with the commercial organic fertilizer Fertiplus Cow, the chicken manure and vermicompost, showed mixed results.

Regarding the N content, the BW residue has higher N values than Fertiplus Cow (both values), while for the MW residue and BW+MW residue the N content is significantly lower than that of Fertiplus Cow (both values). Compared to the value from the analysis of chicken manure, the N content of all three residues was significantly higher. This is in accordance with the results found by Temple and colleagues (2013), whereby more N was found in BSFL residue than in composted poultry litter. The difference was approximately 60%, whereas the difference with chicken manure found in this research ranged between 0,54% for the MW residue and 4,32% for the BW residue. However, compared to the average N value found for chicken manure, the BW residue has higher N content, MW residue has lower N content and the N content of BW+MW residue is statistically not different. Similar to this last finding, Kawasaki and colleagues (2020) found that the N content of BSFL residue is similar to poultry manure. In that same study, the BSFL residue was found to have higher N content than cow and horse manure (Kawasaki et al., 2020). Therefore, it could be assumed that the BSFL residues would have higher N content than the Fertiplus Cow, given that Fertiplus Cow is made out of cow manure. However, only the BW residue had higher N content, while the MW residue and BW+MW residue had significantly lower N content. On the other hand, a study with swine manure indicated that the swine manure had significantly higher nutrient contents than the BSFL residue derived from it (Newton et al., 2005).

For vermicompost, the average N value that was found based on multiple papers is 2.00%. This value is statistically not different from the N content of MW residue, but is significantly lower than the N content of the other two BSFL residues (BW and BW+MW). The N value found for vermicompost derived from brewers' spent grain, is higher than the average value found and significantly higher than the N values from the MW residue and BW+MW residue. The N content of the BW residue, on the other hand, is significantly higher than the N value for vermicompost derived from brewers' spent grain, though the difference is small. In both cases, the BSFL residue and vermicompost, the highest N content was found when brewery waste was used as the feeding material. Similarly, both the BSFL residue and the vermicompost derived from fruit and vegetable waste has lower N content than the BSFL residues and vermicompost derived from other waste. The N value found for vermicompost derived from fruit and vegetable waste was significantly lower than the three residues.

For the P content, both the BW residue and BW+MW residue had significantly higher P content than all the other organic fertilizers (each time for both values). The MW residue, on the other hand, had a lower mean P content than Fertiplus Cow (both values), chicken manure (both values) and the average P value for vermicompost. Besides the average value for vermicompost, an average P value was found for vermicompost derived from fruit and vegetable waste. Compared to the P content of the BSFL residue derived from market waste, which was a mixture of fruit and vegetable waste, this value was statistically the same. For the vermicompost derived from brewers' spent grains, no value for the P content was found. In previous studies, the P content of BSFL residue was found to be lower than that of (composted) poultry litter (Kawasaki et al., 2020; Temple et al., 2013) and of cow manure (Kawasaki et al., 2020).

As for the K content, it was mentioned before that the K values for YaraMila UNIK 17 and Fertiplus Cow found during the analyses are much higher than the K values on the label. Likewise, the K value found for chicken manure during the analysis is much higher than the average K value that was derived from several papers. Hence, the comparisons of K content between the residues and other fertilizers should be interpreted with caution.

If only the values from the analyses are taken into account, the commercial inorganic fertilizer (YaraMila UNIK 17) has significantly higher K content than all the residues. However, if compared to the K value from the label, the K content of the inorganic fertilizer is only higher than the BW residue, yet lower than the K content of the MW and BW+MW residue. Compared to the organic fertilizers, Wilcoxon signed ranked tests showed that the K content of the BW residue is significantly lower than the K content of Fertiplus Cow, both analysed and labelled value, lower than the K content of chicken manure, both values, and lower than the average K value found for vermicompost. For the K content, there were no values found for vermicompost specifically derived from brewers' spent grain or fruit and vegetable waste. Multiple one-sample t-tests indicated a higher K content

for the MW residue and BW+MW residue compared to both values of Fertiplus Cow, chicken manure and vermicompost. While in this study BSFL residue had significantly different K content from other fertilizers, Kawasaki and colleagues (2020) found no difference between the K content of BSFL residue and cow, horse and poultry manure. Even though Temple and colleagues (2013) found a higher K value for the BSFL residue than for the composted poultry litter, the difference was much smaller and not even statistically proven.

#### 5.2.6. Way-forward

Based on the NPK content, the BSFL residues could not replace the commercial inorganic fertilizer. Besides the higher nutrient content, the commercial inorganic fertilizer has the advantage that the nutrients are immediately available. On top, the nutrient content does not vary as it does with the BSFL residues derived from different substrates. However, based on other physico-chemical characteristics, like the pH, EC or OM content, the BSFL residues can have an advantage over the inorganic fertilizer. Furthermore, previous studies showed a disease and pest suppressing effect of BSFL residue application (Choi & Hassanzadeh, 2019; Temple et al., 2013; Vickerson et al., 2015).

Compared to the other organic fertilizers, BSFL residues' advantage is its high OM content. However, if there is no post-treatment, the high moisture content of the BSFL residues hampers the use of it. Regarding the NPK content, the BSFL residues are not remarkable higher neither remarkable lower in nutrient content.

Compared to the vermicomposting process, the bioconversion of organic waste by BSFL is much shorter. In optimal rearing conditions, the larva stage of the BSF takes 14-16 days (Dortmans et al., 2017). In contrast, the vermicomposting process takes minimum around 1 month. Furthermore, BSFL can also digest waste from animal origin like carcasses, which is not the case for the vermicomposting process. On the other hand, vermicompost is proven to contain biostimulants, like humic substances and phytohormones (Aremu et al., 2015; Wong et al., 2020). Though given the several positive results of BSFL residue application, it can be assumed that BSFL residues also contain biostimulants.

Thus BSFL residue, just like other fertilizers, has its own advantages and disadvantages. Hence, it could be considered to combine the use of BSFL residues with other fertilizers. In any case, it is generally recommended to use a combination of organic and inorganic fertilizers to sustain crop yields and soil fertility. While small amounts of inorganic fertilizers can provide readily available nutrients for rapid plant-uptake, organic fertilizers release nutrients slower and are an important input of organic matter to the soil (Gachene & Kimaru, 2003). Given that soil organic matter plays an essential role in several soil processes, it is important that it is maintained or even improved. Kaur, Kapoor and Gupta (2005) found that the integrated use of organic manures and inorganic fertilizers resulted in soil organic matter. In addition, a more active microflora was associated with the

combination of both fertilizers. Similarly, Dutta and colleagues (2003) observed that the application of organic fertilizers together with inorganic fertilizers had a positive effect on microbial biomass. Furthermore, studies showed increased crop productivity as a result of combined application (Bokhtiar & Sakurai, 2005; Chand et al., 2006).

Besides the beneficial effect of the integrated use of organic and inorganic fertilizers on crop productivity and soil fertility, it is economically more feasible. Especially for small-scale farmers in sub-Saharan Africa which in general cannot afford to purchase significant amounts of inorganic fertilizers (Bayu et al., 2005).

### 5.3. Assessment of the socio-economic constraints/challenges related to fertilizer use in sub-Saharan Africa (SSA)

The socio-economic assessment is of interest given that this thesis is part of the master Agroecology. Even though there is no simple definition, agroecology is seen as a science, a practice and a social movement, focusing on shifting towards a more sustainable food system (Gliessman, 2015; Wezel et al., 2009). The holistic approach of agroecology is reflected in the master thesis.

Agriculture productivity has an important role in the reduction of poverty and food insecurity, two of the major problems in sub-Saharan Africa (SSA). In SSA, the primary constraint of agriculture productivity has consistently been ascribed to soil fertility (Onduru et al., 2007; Stewart et al., 2019), which is the soil's capacity to "receive, store and transmit energy to support plant growth" (FAO, 2019). Therefore it is no surprise that there is a lot of focus on the management of soil fertility, still soil fertility management remains a challenge (Onduru et al., 2007). Even though there is plenty literature on soil fertility approaches, most of it exclusively covers the biophysical aspects. As a result, there is limited implementation of soil fertility approaches (Stewart et al., 2019). There is thus a lack of a holistic approach that integrates the biophysical factors with social and economic factors.

An important approach in improving soil fertility is the use of fertilizer. As mentioned before, fertilizer use in SSA is very low. Smallholder farmers in SSA are usually restricted to the use of organic fertilizers, like manure because of their low cost and on-farm availability. However, the quantities of available organic fertilizers alone is not sufficient to meet the nutrient needs of crops (Morris et al., 2007; Raimi et al., 2017). There is thus a need to additionally fertilize the soil. Commercial fertilizers can be both organic and inorganic, though organic fertilizers are mostly obtained from the farm itself while the market is the main source of inorganic fertilizers (Okoboi & Barungi, 2012). Since commercial fertilizers are rarely used, it is valuable to determine and understand social and economic barriers for fertilizer use.

There are several socio-economic characteristics that influence the use of fertilizers. The quality of a fertilizer is the most important attribute for farmers (Komakech et al., 2015). Organic fertilizer have varying and often uncertain quality (Sanginga & Woomer, 2009). While inorganic fertilizer are deemed to have a certain and consistent quality, several literature show that this is not always the case in SSA (Bold et al., 2015; Luswata & Mbowa, 2015; Toro, 2015). Although the quality of a fertilizer is rather a biophysical aspect, it is related with socio-economic factors. The low quality of the commercial fertilizers can be due to several reasons, such as bad storage, inappropriate handling procedures, but it can also be ascribed to adulteration and tampering (Bold et al., 2015). In SSA, most of the fertilizers on the market are imported. In fact, in Uganda all inorganic fertilizer that are used are imported (Okoboi & Barungi, 2012). Since fertilizers are commonly imported in big batches and most farmers in SSA are smallholder farmers, it is inevitable for agro-dealers to open and re-pack the fertilizers in smaller quantities. Within this process, it is likely that the fertilizers get tampered, resulting in underweight bags and/or loss of nutrients. Besides untruthful weights and nutrient contents, moisture contents that exceed the acceptable limits is another indicator for bad quality. The increased moisture content is a result of poor storage and the re-packing into smaller quantities (Luswata & Mbowa, 2015). As with commercial fertilizers, poor storage can affect the quality of manure (Nkonya et al., 2002). Quality deterioration of commercial fertilizers occurs at different points along the entire supply chain. In some cases even at importer level, which means that it is possible that overseas manufacturers deliberately produce poor quality fertilizers (Luswata & Mbowa, 2015).

That most of the commercial fertilizers are imported also has an impact on the cost of fertilizers. Compared to Europe, North America or Asia, fertilizers are two to six times more expensive in Africa (Sanchez, 2002). Because fertilizers are imported they are subject to high transportation costs (Gregory & Bumb, 2006), inconsistent foreign exchange rates (Raimi et al., 2017) and high governmental input taxes (Luswata & Mbowa, 2015). The fertilizer market in SSA is small and fragmented, its underdeveloped structure does not enable efficient pricing and competition. Furthermore, investors are discouraged by the unfavourable business environment. There are redundant regulations that needs to be followed, taxes and fees are excessively high and rental costs are also high (Raimi et al., 2017). With this structure, fertilizer traders pass on the high costs to the farmers (Luswata & Mbowa, 2015). In consequence, demand stays low which also discourages to invest in fertilizer trade. Hence, the fertilizer market stays small and prices stay high. On top of the high prices, smallholder farmers in SSA often don't have the cash and/or cannot obtain credit to purchase fertilizers (Morris et al., 2007; Muzari et al., 2012). Up to 60% of the smallholder farmers are unable to afford the high priced fertilizers (Raimi et al., 2017). The ability to purchase is further related to other factors. It is related to education level, gender, household size and composition, and ownership of livestock and/or poultry. More educated farmers are more likely to be able to afford commercial

fertilizers because they are more likely to have additional income from outside the farm. The ability to purchase fertilizers is also affected by the gender of the farmers' household head. Compared to male-headed households, female-headed households are relatively poor. This factor is also linked with education level, as women in sub-Saharan countries tend to have a lower education level. Household size and composition, more specifically the share of adult members in the household, affects labour availability and in turn affects the possibility to purchase fertilizers. The labour availability also affects the use of on-farm available fertilizers since collecting and applying manure is labour intensive. The ownership of livestock and/or poultry is on the one hand a source of manure and on the other hand it is a way to generate income. Thus, farmers who own livestock and/or poultry are more likely to purchase commercial fertilizer since those farmers are wealthier compared to those without livestock (Okoboi & Barungi, 2012). Yet, even if farmers do have the cash or credits to afford commercial fertilizers, the wedge between the high price of fertilizer and the low price of crops makes it unprofitable to purchase it anyway (Morris et al., 2007).

As previously mentioned, on-farm organic fertilizers are insufficiently available to meet nutrient needs. Similarly, the availability of inorganic fertilizers is inadequate or they are not available at the right time of the year. On top of that the inorganic fertilizers that are available are often not the type required for the cultivated crops (Chianu et al., 2012; Morris et al., 2007). These are consequences of input markets that are malfunctioning because of a lack of effective market information systems. Besides the lack of information for importers and wholesalers, farmers have even less market information (Morris et al., 2007). This is linked with weak agricultural extension services (Chianu et al., 2012; Nkonya et al., 2002; Okoboi & Barungi, 2012) As with the ability to purchase fertilizers, the lack of extension services is in turn linked with other factors. A positive correlation was found between level of education and access to information and advice from extension workers. Due the fact that extension services are mainly conducted by men who lack gender-awareness, women receive less extension services (Okoboi & Barungi, 2012). In addition to the lack of market information, many farmers in SSA have poor access to the fertilizer markets. To get to fertilizer markets is often a struggle due to distance and poor road infrastructure (Nkonya et al., 2002; Okoboi & Barungi, 2012). In Uganda, for example, fertilizer trade is concentrated in and around Kampala (Luswata & Mbowwa, 2015). Thus to access the fertilizer markets is harder for those farmers who live further away from the capital. Furthermore, the transportation is an additional cost for the farmers (Morris et al., 2007).

Another major constraint regarding fertilizer use is the lack of knowledge. Irrespective of fertilizer type, farmers are often not aware, or are at least distrustful of the benefits of

fertilizer use (Luswata & Mbowe, 2015; Okoboi & Barungi, 2012; Omamo, 2003). In contrast, Bold and colleagues (2015) do believe that farmers are aware of the benefits, however they also believe that farmers lack the knowledge to distinguish low and high quality fertilizers. Furthermore, lack of knowledge can be in terms of application. Often farmers are constraint in their knowledge about the correct application (Luswata & Mbowe, 2015). The lack of knowledge is not only on commercial fertilizers. According to Raimi et al. (2017) the available manure is partly wasted due to minimal technical expertise and poor management. As with the lack of market information, the lack of knowledge about the benefits, quality and application of fertilizers is linked to the poorly developed extension services. It can also be linked to the education level of the farmer. In addition, the distrust of fertilizers could be linked to the inconsistent results due to the bad quality of commercial fertilizers in SSA.

The above discussed challenges regarding fertilizer use are all interlinked and they are also linked with household socio-economic characteristics. Relations were made with, amongst others, household size and composition, education level, gender, and ownership of livestock and/or poultry. Another example of a household specific characteristic affecting fertilizer use is age. Though, mixed results were found. On one hand, young farmers may have restricted economic abilities, limited access to extension services and limited labour availability, all of which may restrict fertilizer use. On the other hand, young farmers may be more open to try new inputs (Okoboi & Barungi, 2012). Correlations are thus varying and can even be context specific. For example, in the northern region of Uganda the two decade lord resistance army conflict disrupted the agricultural system with low fertilizer use as a result (Luswata & Mbowe, 2015).

Figure 11 is a mind-map that gives an overview of the multiple factors influencing the use of a certain fertilizer based on the literature found. It does not only include the web of socio-economic factors (blue), but also the bio-physical factors (green) and other factors (grey).



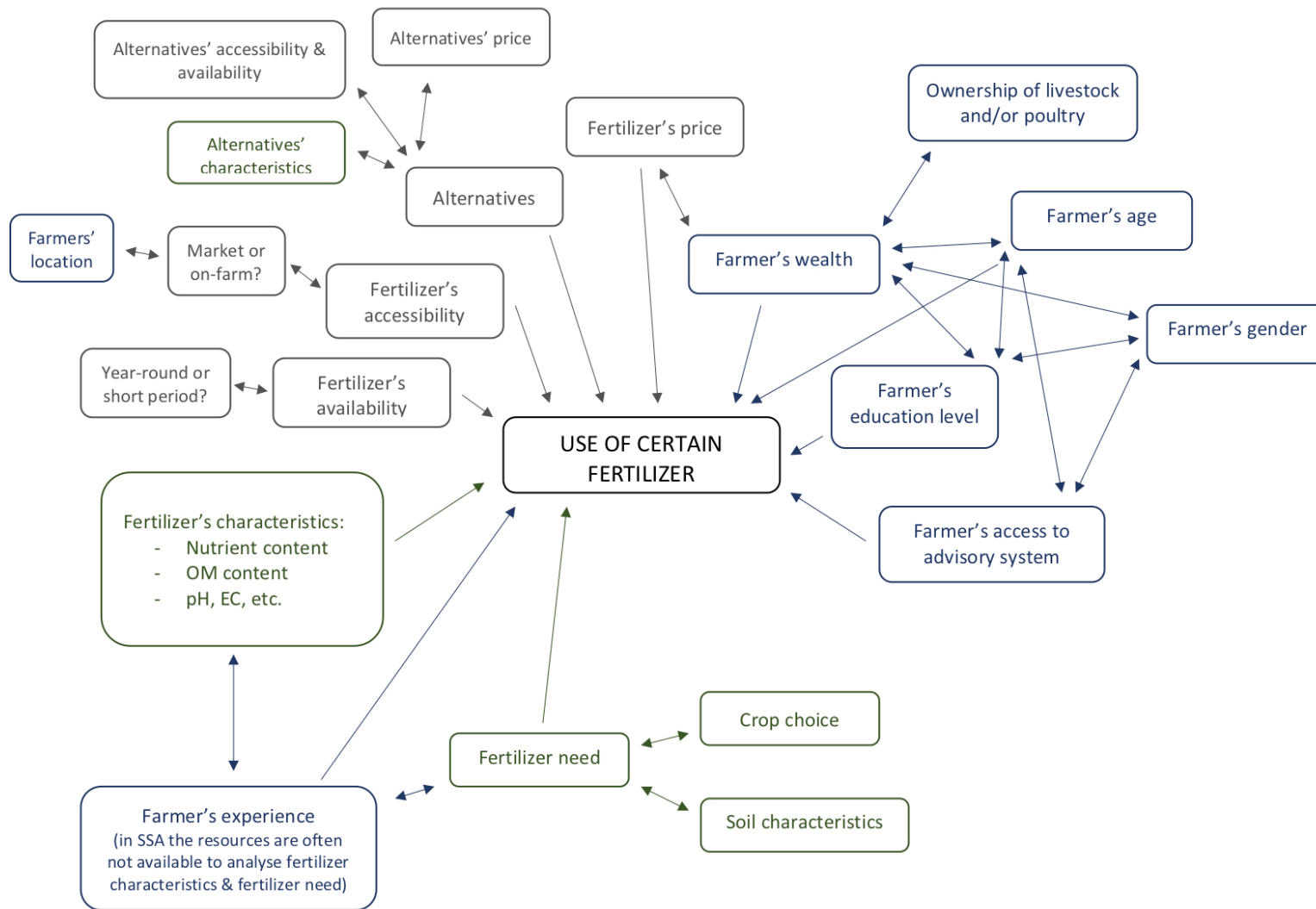


Figure 11. Mind-map of the multiple factors influencing the use of a certain fertilizer based on the literature found. Socio-economic factors are in blue), bio-physical factors are in green and other factors in grey

With the current soil fertility status and the challenges regarding the use of fertilizers in SSA, it has become increasingly interesting to look at alternative practices and products in soil management. The use of black soldier fly larvae (BSFL) residue as fertilizer is a promising alternative. It does not only have promising nutrient values, it could also overcome some of the previously mentioned challenges. While the production of inorganic fertilizers in SSA is rather uneconomically and unsustainable due to power challenges (Raimi et al., 2017), production of BSFL residue is rather easy in SSA given that the ideal temperature for the larvae is between 24 and 30°C. In addition, the main input of the production process is organic waste (Dortmans et al., 2017), which is abundantly available. Consequently, many of the challenges associated with fertilizers being imported may be less pronounced. The cost for the production process is not as high as producing inorganic fertilizers and therefore the residue is more affordable for farmers. The availability of the product is also less of a challenge if it doesn't have to be imported. Organic waste is year-round available and thus production is continuously possible. It is even possible for farmers to produce the larvae and residue themselves with on-farm waste. On the other hand, consistent quality and knowledge might still be challenges. As seen in the results above, the composition of the residue varies along different starting substrates. Hence, it may be a challenge to access consistent waste streams or it may be a challenge to apply residues with varying compositions to the field. Lack of knowledge remains a challenge as farmers are not familiar with the production and use of BSFL residues. Furthermore is the adoption of new technologies, similar to fertilizer application, influenced by certain factors. With new technology, defined as the means and methods of producing goods and services, including organizational methods and physical technique that are new to a particular place or group of people, or represents a new way of using a technology (Loevinsohn et al., 2013).

Whether and how farmers' adopt a new technology is determined by the dynamic interaction between characteristics of the technology itself and the variety of conditions and circumstances (Loevinsohn et al., 2013). The precondition of adoption is the characteristic of the technology. Trialability or the degree to which the technology can be tried out on a small scale first is an important factor determining adoption. Another major determinant is the farmers' perception about the performance of the technology. If farmers perceive the technology being compatible to their needs and environment, they are more likely to use the technology (Mwangi & Kariuki, 2015). For example, the adoption of biogas failed in Ethiopia because the design was not appropriate for the socio-economic and cultural context. In contradiction to SSA countries, the adoption rate of biogas in Asia is higher (Mwirigi et al., 2014). This already shows the interaction of the technology's characteristics and the context specific factors. As discussed with the challenges regarding fertilizer use, there are several factors, that are intertwined, that determine the use of a technology. It is important though to be aware that these factors can have

ambiguous influences. Mwangi and Kariuki (2015) discussed that the relationship between farm size and adoption can be positive, negative or even neutral. Trialability is higher with farmers with large farm size given that they can afford to devote part of their land to try a new technology. Higher trialability positively influences adoption. On the other hand, small farm size may encourage the adoption of a technology, especially in case of land-saving technologies. Still other studies have shown insignificant or neutral interaction. A major constraint to technology adoption is the cost. Therefore, the net gain from adoption, inclusive all costs of using the new technology, will determine the adoption of the technology. Related with the cost of the technology and its' use, is the income of the farmer, especially income from outside the farm. Off-farm income often has a positive influence on technology adoption because it is important to overcome cash constraints. However when it comes to labour intensive technologies, a negative relationship was found because less labour is available on the farm itself. As well as off-farm income, access to credit is an important strategy to overcome liquidity constraints and hence to promote technology adoption. Since the cost of production and thus also purchase of BSFL residues are not as high as inorganic fertilizers. These factors have less impact on the adoption of it. Other factors influencing the adoption of new technologies are access to knowledge through social groups or extension services and household specific characteristics such as, education level, age, gender, household size and composition. Extension services play a major role in technology adoption. Access to extension services positively influences adoption as they are an important source of knowledge (Mwangi & Kariuki, 2015). The poorly developed extension services in SSA (Muzari et al., 2012) may thus constraint the adoption of BSFL residue as fertilizer. Another source of knowledge is other farmers. Farmers learn from each other the existence, usage and benefits of technologies and are consequently more likely to adopt these technologies. Though free-riding behaviour within a social group may negatively impact technology adoption (Mwangi & Kariuki, 2015). Furthermore, farmers often lack the knowledge to appropriately assess the potential and practical use of new technologies, leading to limited adoption (Lybbert et al., 2017). In general, educational level has a positive influence on technology adoption, although some insignificant or negative influences were also found. The positive effect of education is by increasing farmers' ability to obtain, process and use information (Mwangi & Kariuki, 2015). Indirectly, educational level affects adoption as educated farmers are more likely to have off-farm income (Okoboi & Barungi, 2012). As with fertilizer use, age has a mixed impact on the adoption of new technologies. Older farmers have more experience and gained knowledge over the years which helps them to evaluate technology information. On the other hand, older farmers become more risk-averse and are less interested in long-term investments. The relationship of technology adoption with gender and with household size and composition, is rather indirect (Mwangi & Kariuki, 2015). In general, women have less access to credit, extension services and are usually less educated (Okoboi & Barungi, 2012). Household size and composition reflects labour availability, which, as discussed before, has an influence on adoption (Mwangi & Kariuki,

2015). Like with factors determining fertilizer use, all these factors are interacting and influence one another. Furthermore, the availability and accessibility of alternative options has an impact on the adoption and on the relationship between the different factors and adoption rate. For example, the adoption of biogas digesters is, in Uganda, negatively influenced by educational level because with their higher income educated people can afford other sources of energy like electricity (Mwirigi et al., 2014). In case of BSFL residue, the adoption will thus also be influenced by the accessibility of alternative fertilizers such as inorganic fertilizers, animal manure, vermicompost, etc. However, as discussed before, these alternatives have their own constraints.

To promote the use of black soldier fly larvae (BSFL) residue as fertilizer, it is important to consider all these factors. It needs a holistic approach integrating both bio-physical and socio-economic factors. Furthermore it is important to keep in mind that in certain situations the use of BSFL residue is not the best or only option.

The following SWOT-analysis (table 8) brings the bio-physical and socio-economic factors regarding BSFL residues as fertilizers together. In the SWOT-analysis the strengths (S), weaknesses (W), opportunities (O), and threats (T) of BSFL residue are included. Strengths and weaknesses are defined as internal characteristics, while opportunities and threats are external factors (FAO, 2006).

*Table 8. SWOT-analysis on BSFL residue as fertilizer. Including both bio-physical and socio-economic factors.*

<p><b>STRENGTHS</b></p> <ul style="list-style-type: none"> <li>- High OM content</li> <li>- Improves yield</li> <li>- Disease and pest suppressing effect</li> <li>- Low cost</li> <li>- Can be produced by farmers themselves</li> <li>- Besides the residues, the protein-rich and fat-rich larvae can be used as for several purposes, like feed</li> </ul>	<p><b>WEAKNESSES</b></p> <ul style="list-style-type: none"> <li>- Variability in characteristics (NPK content)</li> <li>- NPK content not as high as inorganic fertilizers</li> </ul>
<p><b>OPPORTUNITIES</b></p> <ul style="list-style-type: none"> <li>- Part of waste management</li> <li>- In line with the concept of circular economy</li> </ul>	<p><b>THREATS</b></p> <ul style="list-style-type: none"> <li>- Other fertilizers (both inorganic and organic)</li> <li>- Lack of knowledge</li> <li>- Alternative uses for organic waste e.g. vermicomposting, biogas</li> </ul>

## 6. Conclusion

The variance between the three treatments in this study confirms that the starting substrate affects the physico-chemical characteristics of the Black Soldier Fly larvae (BSFL) residue. The BSFL residues were derived from brewery waste (BW), market waste (MW) consisting of tomato, watermelon, cabbage, citrus and jackfruit, and a 50/50 mixture of the brewery waste and the market waste mixture (BW+MW). For each parameter, the BW residue and MW residue were significantly different. The BW+MW residue was significantly different from the other two treatments for the parameters moisture content, organic matter (OM) content and nitrogen (N) content, while for the parameters pH, electrical conductivity (EC) and potassium (K) content the BW+MW residue did not differ significantly from the BW and MW residues. Regarding the phosphorus (P) content, the BW+MW residue did not differ from the BW residue, but did differ significantly from the MW content. For all the parameters, the values for the BW+MW residue, which was derived from a 50/50 mixture of BW and MW, laid in between the values of the BW residue and the MW residue. Despite these findings, the starting substrate is not the only factor that has an influence on the physico-chemical characteristics of the residues. Comparing the moisture content in this study with previous research showed that the production system, more specifically ventilation, affects this parameter. Yet, even if all parameters in the production system are kept constant, the characteristics of the BSFL residue will still vary, especially OM content and nutrient content (NPK). The high variability of fertilizers derived from organic material is a major disadvantage.

Besides the variability, fertilizers from organic material in general contain less inorganic mineral nutrients (NPK) than inorganic fertilizers. In this study the BSFL residue had significantly lower NPK content than the commercial inorganic fertilizer. Compared to other organic fertilizers, mixed results were found. Even though the low mineral nutrient content, organic fertilizers sometimes show similar growth promotion effects. This effect can be attributed to trace natural biostimulants. In order to better understand the functionality, a new criterion of plant nutrition was developed: A-NPK, where the A refers to “the active ingredients (total pool of biostimulants) present in organic materials, soils and irrigation waters. It includes phytohormones and several other growthpromoting compounds (microProteins, amino acids, humic acids, fulvic acids, unknown compounds).” The presence of biostimulants was already proven in vermicompost. Further research should investigate the “A” of A-NPK in BSFL residues and the biostimulant effect of BSFL residue on plant growth and plant health. Furthermore, studies need to be done to determine the mid and long-term effects of BSFL residue application on soil fertility.

Even though further research should be done, BSFL residue has the potential to be used as a fertilizer. Not only based on its characteristics and the shown positive effects on plant growth and plant health, but also because of certain socio-economic aspects. Literature

shows that in sub-Saharan Africa, the majority of the farmers do not have access to inorganic fertilizers. On top, it was seen that often the quality of commercial inorganic fertilizers is not consistent. In contrast, BSFL residues are low in cost, given that it is derived from waste, and farmers could even produce it themselves. Furthermore the process does not only result in the residues but also the protein-rich and fat-rich larvae are valuable for several purposes, like feed. Thus reducing the costs of feed for farmers with livestock and/or poultry.

In this study the focus was on the residues and it became clear that certain types of waste or mixtures of waste can be given preference in order to get qualitative residues. On the other hand, many researches has been done with the focus on the larvae, regarding the growth and survival of the larvae but also regarding the composition of the larvae. It could be interesting to compare the ideal starting substrate when focused on the residues and the ideal starting substrate when focused on the larvae. Are these consistent or are these completely opposite?

Hence, the bioconversion of organic waste by BSFL is interesting in many respects. However, further research is required.

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