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Using Black Soldier Fly for waste recycling and effective *Salmonella* spp. reduction

Project Thesis of

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Table of contents:

1. Introduction

Due to changing economic conditions, rapid urbanisation and demographic changes, decision makers are confronted with new issues such as providing a proper waste management system as well as providing conditions for economic growth. Even though many partly sustainable solutions have already been found and integrated, much of the municipal solid waste - which is supposed to increase globally from current amount 1.3 billion tons per year up to 2.2 billion tons in 2025 (Hoornweg and Bhada-Tata, 2012) - is left untreated in low-income countries, meaning that untreated leachate can get into water bodies causing eutrophication, contribute to increasin greenhouse gas esmissions and being a base for spread of diseases (Hoornweg and Bhada-Tata, 2012). While valuable fractions of municipal waste such as paper, metal, plastic, glass etc. lead to an income for households in low- and middle-income countries by selling it to recycling firms or any kind of waste pickers, the organic waste fraction is mostly regarded as worthless (Diener, 2010).

The organic content in municipal solid waste can be as high as >90 % in low-income countries (Hoornweg and Bhada-Tata, 2012). In countries of East Asian and Pacific region for instance, the household waste comprises 62 % of organic compounds in average (Hoornweg and Bhada-Tata, 2012). Since infrastructure for waste and wastewater management is lacking or insufficient in many low- and middle-income countries, generated waste is treated by the informal sector and due to its low value end up at illegal and/or open dumping sites or is combusted at roadsides, private properties and landfills (UN-HABITAT, 2010).

In addition to insufficient waste management UN-HABITAT (2010) reported, that the lack of infrastructure and sanitation (both based on weak economic situation in general) in most countries of Asia, Africa and Latin America causes the appearance of widespread diseases such as malaria and increases human mortality. According to UNICEF/WHO (2012) 2.6 billion people have no access to improved sanitation; several hundred million are practicing open defecation. In combination with missing waste collection and management system refuse, human excreta and animal manure (chicken, cow, goat, pig), well known as a source of pathogens (Pell, 1997) - remain closed by inhabited areas. This inevitably leads to contact between waste and disease vectors such as rats and dogs.

However, many existing waste management systems all over the world lack one point: recycling of nutrients. Organic residues are high in organic matter and vital macronutrients such as phosphorous, nitrogen and potassium, as well as micronutrients (copper, zinc, manganese etc.). Phosphorous for example is essential and cannot be replaced, but this nutrient is not available or usable at the end of

certain waste treatments since it enters water bodies or landfills. Nutrients need to be reused to a greater extent saving resources and allowing proper nutrition of increasing global population in the next decades. It is desirable to reutilise organic residues as a fertiliser and, thus, to close the nutrient loop and to amend stressed soils (Newton et al., 2005).

To avoid cross-contamination of crops when human excreta, organic waste or manure are used as a fertiliser, it has to be ensured that pathogens are eliminated in the material before application. Otherwise it is possible to use such organic fertiliser for tree crops preventing direct contact between crop and contaminated material. The pathogen - disease causing organisms - *Salmonella* spp. achieve gastrointestinal illness or typhoidal fever. Crump et al. (2004) reported that the global case-fatality rate is 1 % due to typhoid fever. Hence, contaminated organic residues could be a health risks for composting plant workers or consumer of products treated with organic fertilisers (Déportes et al., 1995). In the composting process, pathogens can be removed if maintained correctly, although all material may not be inactivated (Droffner and Brinton, 1995). It is difficult to create right conditions that guarantee a temperature of >50 °C for one week or more, the requirement for ensuring good inactivation. The same problem occurs in anaerobic digestion of treating organic material: the temperature is not sufficient to remove all pathogens in most cases (Strauch and Ballarini, 1994). Additional options of hygienisation are drying and/or gassing with ammonia (Himathongkham and Riemann, 1999), application of sodium carbonate and of alkali conditions (Arthurs et al., 2001). Those treatments may work in high-income countries, but low- and middle-income countries mostly do not have sufficient waste collection systems or financial means to implement such comparatively expensive systems.

Besides from already well-established processes such as digestion and composting, the use of insects to treat organic waste and faeces/manure and create usable products is one field of current research. Several investigations of using one insect species, the Black Soldier Fly (BSF), has been suggested (Sheppard et al., 1994, Newton et al., 1977, Bradley and Sheppard, 1983, Tomberlin, 2002). BSF larvae feed on organic material such as organic waste, human faeces and animal manure (Diener et al., 2011b, Lalander et al., 2013, Tomberlin et al., 2002). Lalander et al. (2013) observed that BSF treatment had a negative influence on the concentration of *Salmonella* Typhimurium in faecal sludge, while Erickson et al. (2004) and Liu et al. (2008) reported this for *Escherichia coli* in dairy and poultry manure, respectively. According to Winfield and Groisman (2003), *Salmonella* spp. is able to infect a large number of animal species and survives in the soil, in water and on many surfaces, thus, *Salmonella* spp. is a great risk for animal husbandry since an affected farm can be shut down for weeks.

1.1 Objects of the study

The objective of this study was to evaluate the influence BSF composting has on the concentration of four different *Salmonella* strains with different origins and impacts in society and in agriculture, in order to establish whether BSF composting is a reliable *Salmonella* inactivation method.

2. Background

2.1 Black Soldier Fly larvae composting

Black Soldier Fly (BSF), *Hermetia illucens* (Diptera: Stratiomyidae), originates from the American continent and has spread throughout the tropics and subtropics between 45 °N and 40 °S, with preference to warmer areas (Diener et al., 2011a). Under optimal conditions it takes two weeks for BSF larvae to reach the sixth and final larval stage (instar), in which the so called prepupae crawl out of the material in the search for a dry and dark place to pupate; the larvae are thus self-harvesting, which means that the prepupae and feed does not have to be separated manually. In case of cold temperatures or food scarcity the larval stage can be extended for several months. During their short life, the main focus is breeding. BSF do not eat and rely on their body fat reserve and are thus not a vector of diseases (Diener et al., 2011b).

Fly composting is a comparatively easy management system, in which organic material is converted into high value protein and organic fertiliser; as a result it closes and shortens the nutrient loop (two steps instead of three) (Fig. 1). One advantage of BSF treatment is that it is a decentralised system that could be implemented more or less easily in low- and middle-income countries.

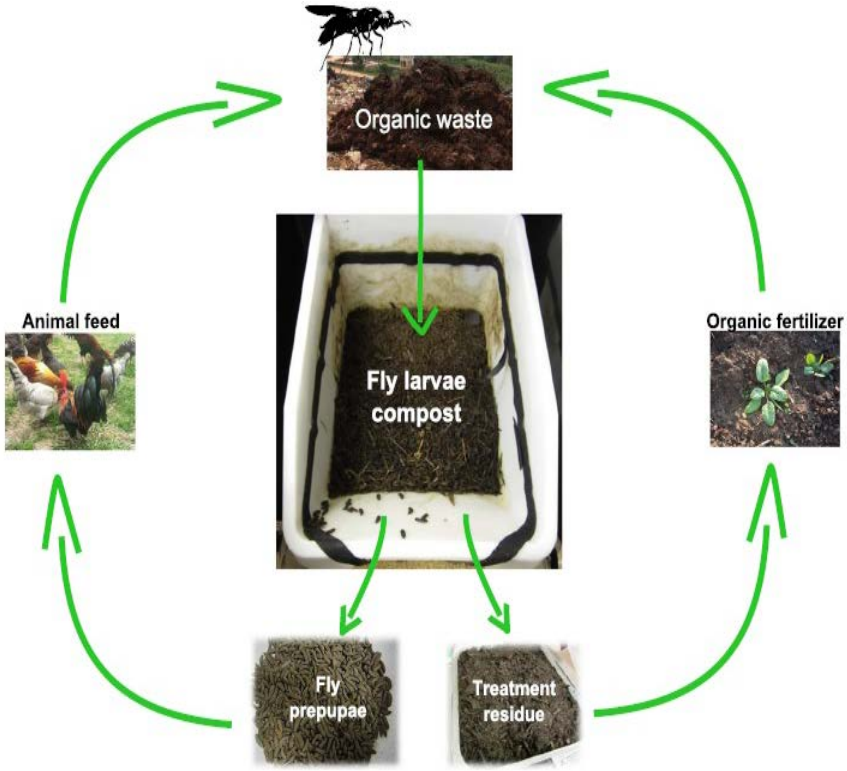

Fig. 1 graphical representation of the concept of fly larvae composting (Lalander et al., 2014): fly larvae consume organic waste and the prepupae, larvae in the final larval stage, migrate out of the compost, which can be used as organic fertiliser. The prepupae can be used as animal feed. Animal manure, human faeces and food waste serve as substrate for fly composting and the loop is closed.

Due to larval movement, the material is aerated, which prevents anaerobic conditions. As a result, less fatty acid compounds arise, with a reduction of odour as a consequence. An additional benefit is the BSF larvae ability to prevent females of the common domestic house fly (*Musca domestica*)- a vector of diseases and cause of many problems in low- and middle-income countries – to place their eggs in the material (Bradley and Sheppard, 1983).

BSF prepupae are composed of around 40 % protein and 30 % fat - depending on the substrate they consume - and include essential amino and fatty acids (Sheppard et al., 2002), making them a desirable alternative to conventionally produced feed. According to Kroeckel et al. (2012), fish meal can be replace up to 50 % by BSF meal in aquacultures without reducing the productivity of the system, which could decrease the pressure on the overfished wild fish populations. BSF prepupae can also be used as a protein source for poultry and pig (Newton et al., 1977). Furthermore, studies by Li et al. (2011) have shown the potential of producing biodiesel of BSF prepupae using the protein-rich residue as foodstuff after extracting grease.

By implementing BSF treatment as organic waste management strategy, occurrence of open dumping and illegal combustion could be reduced and, thus, greenhouse gas emissions (especially methane) could be saved. Additionally, the mixture of organic waste, solid waste and human and animal manure would not be discarded at roadsides anymore, or at places where it generally could attract vectors of disease. Eutrophication and spreading of diseases could thus decrease and potentially be fully prevented, which greatly enhances living conditions of many people.

The BSF technology has a great potential of treating any kind of organic material. But certain limitations exist, such as the appearance of pathogens, increasing within the nutrient loop and enhancing the risk of horizontal transmission (Erickson et al., 2004). Before the BSF treatment residue can be used as a fertiliser on agricultural land it has to fulfil certain standards from a hygienic point of view. Hence, it is vital to know whether an additional sanitisation step is necessary; such as composting, digestion and chemical hygienisation (Droffner and Brinton, 1995, Strauch and Ballarini, 1994, Arthurs et al., 2001).

2.2 Legal situation

Another point of BSF treatment and its implementation is the legal situation. In the European Union insects are not precisely considered in the feedstuff legislation, they are considered as production animals and abide under the by-product regulation EC 1069/2009. Therefore, BSF larvae cannot be

fed on animal by-products, such as organic waste and animal manures. Furthermore, BSF larvae or prepupae need to be killed in certified slaughterhouses. Even though it is forbidden to use dead production animals to produce further animals, it is allowed to feed living fly larvae to animals.

Smith and Pryor (2013) reported that under the EU regulation EC 56/2013 proteins from insects are allowed to be fed to aquaculture species, but that it is currently forbidden to feed farmed animals on waste and on manure, respectively. The situation in low- and middle-income countries as well as China, is more favourable, because there are no or only few laws and regulations regarding the production and use of insects in the feed and food industry. Due to less administrative barriers, using BSF as waste management strategy is more likely in those countries in the near future. According to Binkert and Gürtler (2014), insect s are not only used as animal feedstuff but also for human consumption (e.g. Thailand and Nigeria).

2.3 *Salmonella* spp. as a risk factor

Salmonella spp. is a motile, gram-negative bacterium in the family *Enterobacteriaceae,* as is also *Escherichia coli* and *Shigella* spp. The genus of *Salmonella* spp. contains more than 2400 genotypes (Du, 2011) and occurs worldwide in both cold- and warm-blooded animals, in humans and in the environment. *Salmonella* spp. are zoonotic bacteria, i.e. humans can be infected by animal through direct transmission and vice versa. *Salmonella* spp. survive in the soil, in water and on several surfaces (Winfield and Groisman, 2003), as a consequence cross-contamination of *Salmonella* spp. between animals, humans and groceries often occurs. Contagion takes place through the faecal-oral route and through contact with contaminated water (mostly due to contact with sewage).

According to FDA (2012), *Salmonella* spp. can cause two kinds of illness: gastrointestinal and typhoidal. Gastrointestinal diseases cause diarrhoea, cramps, fever and vomiting, but symptoms usually subside by themselves after a couple of days. Many kinds of food can get contaminated with *Salmonella* spp., e.g. meats, vegetables, fruits and even dry foods such as spices or nuts. Typhoidal illness caused by sewage-contaminated water, causes headache, aches, diarrhoea and lethargy. People with weak immune systems are specially exposed because *Salmonella* spp. can spread to other organs and can cause serious illness. *Salmonella* Typhimurium for example is strictly a human pathogen, which causes invasive enteric fever, whereas other types mainly lead to gastrointestinal illness without suffering bloodstream (Braden, 2006).

Apart from human illnesses, *Salmonella* spp. infection of animals is no less important. On farms where groceries such as meat, milk or eggs are produced, the risk of spreading salmonellosis and contagion inside the farm is high. Stables for animal husbandry can get contaminated with *Salmonella* spp. due to many vectors such as purchase of animals from outside, pets, wild birds, insects, personnel or the use of contaminated water and/or feed (Sagherian, 2011).

According to Braden (2006), the risk of cross-contamination due to consumption of eggs is especially high, since eggs are frequently consumed raw, for instance they are used for ice-cream, salad dressings, mayonnaise or health drinks. Funk and Gebreyes (2004) investigated risk factors for *Salmonella* spp. infection on swine farms and concluded that stricter hygienic requirements such as separate rooms for changing clothes and footwear lead to less occurrence of *Salmonella* spp. Fenestrated flooring instead of flush-gutter flooring is recommended to minimise contact time between porcine faeces and animal, thus, reducing risk of contamination. Cleaning and disinfection lower the risk of *Salmonella* spp. appearance in high concentrations, but it is hard to remove the contamination totally, thus, salmonella organisms remain as contaminants on the floor (Funk et al., 2001). Studies of Davies and Wray (1996) has shown that using formaldehyde as disinfectant reduces but does not eliminate *Salmonella* spp. in poultry husbandry. However, both the benefits and the health risks of formaldehyde as disinfectant should be considered (Funk and Gebreyes, 2004).

In addition, management strategies on farms have an important role as well. The so called *all in-all out* production management that keeps animals together in groups as they move through the phases of production and the provided areas for personnel to change clothing and boots lead to a one third emerge of *Salmonella* spp. on Danish farms (Lo Fo Wong et al., 2004). Contrary to that, Vaessen et al. (1997) found out that contact between different cow herds was, unexpectedly, preventive for *Salmonella* Dublin infection. To avoid a contamination, all stakeholders have to satisfy certain hygienic conditions (cleaning, providing shower and changing room facilities for workers and visitors etc.) to ensure a safe environment and should control the flocks for husbandry regularly since *Salmonella* spp. is transmitted vertically (e.g. via egg from parent to chick) (Sagherian, 2011).

Altogether, *Salmonella* spp. contamination can cause serious diseases for animals as well as for humans, and if a farm is once contaminated it is very costly and time-consuming to eliminate salmonella organisms completely; the whole flock needs to be slaughtered and as a result economic disadvantages due to factory shut down and missing gains will appear.

However, professional management systems and high hygienic standards occur in high-income countries, while it is unlikely to implement a similar system in low- and middle-income countries.

Therefore, an option needs to be provided that is better adapted to the conditions and reduces appearance of contamination in combination with giving a better and simpler maintenance.

3. Materials and methods

All experiments were carried out at the Swedish University of Agriculture Uppsala (SLU), Sweden. All substrates used for the microbial analyses were purchased from the National Institute of Veterinary Medicine (SVA), Uppsala, Sweden.

For the material feeding trials, two substrates were used, poultry feed and human faeces. The dry poultry feed was mixed with water to a 40 % dry matter. Human faeces (22 % dry matter) were collected fresh in plastic bags during October 2013 and March 2014 at SLU Uppsala, Sweden. Until its use the material was stored at -18 °C. In preparation for the trials, the faecal samples were thawed at the laboratory, manually mixed, divided into feeding portions of 75 g respectively 110 g and again kept at -18 °C till final use.

All experiments were carried out by using the SmartStore™ Classic 2 treatment reactor with a lid including net for aeration, that were 21x17x11 cm (Fig. 2).

Fig. 2 The SmartStore™ Classic 2 treatment reactor with lid including net for aeration

3.1 Experimental design

Two set of experiments took place:

1) Feeding trials, in which two substrates were tested as larval feed for BSF composting;

2) *Salmonella* inactivation trials, in which the inactivation of four different Salmonella spp. strains by BSF treatment was followed.

Both experiments were done in triplicates.

In order to evaluate how the feeding activity of BSF larvae influence waste conversion rate and inactivation of pathogens: larval growth; prepupal weight; material reduction; waste conversion rate and pH development in material, caused by larval feeding, were monitored in the feeding trials.

In the *Salmonella* inactivation trials, equal conditions as in the feeding trails were provided to draw inferences from larval activity about the concentration [CFU/g] of four *Salmonella* spp. strains, described in more depth in section 3.2.. The four *Salmonella* strains used were:

- *Salmonella* Senftenberg (ATCC®8400™)
- *Salmonella* Dublin (ATCC®39184™)
- *Salmonella* Typhimurium DT104 (ATCC®700408™)
- *Salmonella enterica* subspecies 1 serovar Typhimurium phage type 178 isolated from sewage sludge referring to Sahlström et al. (2004)

At the end of the feeding trials, one of the tested materials was selected for use in the *Salmonella* spp. Inactivation trial; the aim was to exclude an influence of pH on inactivation of *Salmonella* spp.

3.2 *Salmonella* strains

All *Salmonella* strains were cultured in nutrient broth (Oxoid AB, Sweden) for at least 12h at 37 °C before inoculated into the feed portions.

***Salmonella* Senftenberg (ATCC®8400™)**

Salmonella Senftenberg is mostly found in poultry, but it also appears in other animals and is one of the US top five salmonella serovar isolated from food due to cross-contamination. It has been associated with several foodborne outbreaks of salmonellosis in humans. It has been suggested that this serovar is more resistant to stresses such as low pH, heating, desiccation and irradiation than

other *Salmonella* serovars (Pedersen et al., 2008). *Salmonella* Senftenberg is widely distributed and has been found in North America, Europe and Asia.

***Salmonella* Dublin (ATCC®39184™)**

Salmonella Dublin is adapted to cattle, which is the primary host, but it also can infect and cause diseases in different hosts, including humans. This strain has been reported in North America, South America and Europe and is responsible for over 40 % over all cattle outbreaks in the USA (AHDC, 2013), thus, *Salmonella* Dublin is of special interest in bovine industry.

***Salmonella enterica* subspecies 1 serovar Typhimurium phage type 178**

This strain is isolated from Swedish sewage sludge (Sahlström et al., 2004).It infects both humans and animals and is widely distributed. *Salmonella* Typhimurium is the second most common serovar isolated from humans in the USA and in Europe; although it is distributed worldwide and has been found in many animal species also (cattle, poultry, swine, wild animals and insects). *S.* Typhimurium has been used as model to understand the pathogenicity of *Salmonella* spp.

***Salmonella* Typhimurium DT 104 (ATCC®700408™)**

This serovar has similar characteristics compared with related *S.*Typhimurium strains, but it is furthermore multiresistant to ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracyclines (ACSSuT resistance type) (Threlfall, 2000). This multiple resistance makes DT 104 a serious threat to bovine, swine and poultry husbandry and thereby also to the consumer.

3.3 Physicochemical analyses

For determination of total solids, the material was dried at 105 °C for 24 h and 4 h at 550 °C for volatile solids (the organic content), respectively. A radiometer electrode was used for pH measurement at room temperature. The samples were prepared by diluting 5 g of material in 25 mL deionized water and were left to settle for at least 30min before measurement.

3.4 Microbiological analyses

For preparing samples, 5 g of material were diluted with 45 mL of buffered NaCl peptone water with Tween 80 at pH 7, which was also used for further dilution. A 100 µL volume of selected dilution was

spread on xylose lysine desoxycholate agar (XLD) (Oxoid AB, Sweden) (Fig.3a) and incubated at 37 °C for at least 12 h for *Salmonella* spp. enumeration. The plates were enumerated with a detection limit of 100 CFU mL^{-1}.

Figure 3a *Salmonella* spp. colonies grown on XLD plate: the sample is spread on the ager, which comprises sodium thiosulfate. *Salmonella* spp. metabolise this compound to hydrogen sulphide; that turns the agar black.

To lower the detection limit of Salmonella spp. concentration, the sample was enriched the following way: 5 g per unit was immersed in 45 mL buffered peptone water, which was also used for further dilution, and incubated at 37 °C for 24 h. A drop (10 µL) was immersed into Modified Semisolid Rappaport–Vassiliadis medium (MSRV) (Fig.3b) and incubated at 41.5 °C for 16 h. Positive results were evaluated by spreading 100 µL on a XLD plate and incubated at 37 °C for at least 12 h.

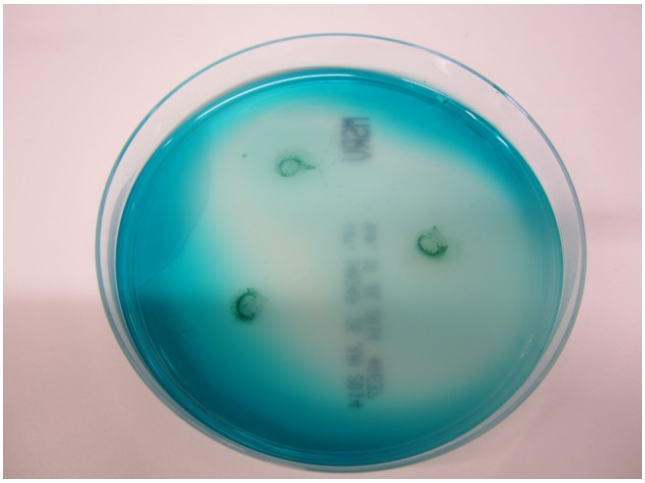

Figure 3b Evidence of *Salmonella* spp. by using MSRV plate: three drops of enriched *Salmonella* dilution were put into the semisolid agar. Salmonellae migrate through the selective material and produce opaque halos of growth. Gram-positive bacteria are suppressed due to Novobiocin, low pH, high magnesium chloride concentration and malachite green.

3.5 Calculation

The waste-to-biomass conversion rate (WBC) on a dry matter basis was calculated as follows:

$$\mathrm{WBC} = \frac{\mathrm{Mig.PP_{DM}}}{\mathrm{Waste_{DM}}} \text{ x } 100 \qquad (1)$$

where $\mathrm{Mig.\,PP_{DM}}$ and $\mathrm{Waste_{DM}}$ were the total solids in the migrating prepupae and waste, respectively.

The material reduction on a DM basis was calculated as:

$$\mathrm{Mat_{DM.Red}} = \left(1 - \frac{\mathrm{Residue_{DM}}}{\mathrm{Waste_{DM}}}\right) \text{ x } 100 \qquad (2)$$

where $\mathrm{Residue_{DM}}$ and $\mathrm{Waste_{DM}}$ were the total solids in the treatment residue and the inflow waste, respectively.

3.6 Statistical Analyses

One-way analysis of variance (ANOVA) with a 95 % confidence interval was used to show whether a significant difference occurred concerning WBC and material reduction in the feeding trials. Linear regression with *t* test was used in the *Salmonella* trials to establish if the reduction of *Salmonella* spp. was statistically significant in the treatments compared to the controls. All analyses were conducted in Minitab 17.

3.7 Feeding trials

For each trial, 200 larvae - with a total starting weight of 0.20 g (trail 1), 0.21 g (trial 2) and 0.28 g (trial 3) fed on faecal sludge and 0.6 g (trial 1), 0.7 g (trial 2) and 0.75 g (trial 3) fed on poultry feed - were placed in opaque boxes (Fig.2) with a starting ratio of feed. The treatment reactors were stored in an incubator at 28 °C. The feeding rate was 8 g total solids of poultry feed per sample (40 mg DM per larva and day); all trials were carried out for four weeks.

Three times a week, 10 larvae were removed, washed, dried and weighed to measure their growth. The larvae were returned to the treatment and fresh food was added to the buckets. Once a week, additional samples for pH, dry matter and volatile solids were taken from the treatments. As soon as

the first prepupa emerged from the material, a pupation site was offered by putting the reactor into a larger bucket with shredded paper at the bottom. From that date, only the escaped prepupae were removed from the trials, counted, weighed and frozen. From the point when 50 % of larvae had reached prepupal state, feeding was stopped and the trials were continued till two or three sampling days without emerged prepupae.

3.8 *Salmonella* trials

To provide constant conditions compared to the feeding trials, 200 larvae were placed in opaque boxes (Fig. 2) together with a starting ratio of faeces. Faeces were used as feed, to ensure that the pH stayed >5 when stored. *Salmonella* is inactivated at pH<4. In consequence of problems regarding hatching eggs and production of young larvae, the composition regarding age and weight had to be changed within the triplicates as shown in Table 1 below.

Table 1 Larval composition and the weight at the beginning of each *Salmonella* trial

Trial No.	1			2		3
Larval Composition [number x age]	40 x 4 d	80 x 11 d	80 x 13 d	24x 6 d	176 x 10 d	200 x 7 d
S. Dublin	0.04 g	3.68 g	4.83 g	0.26 g	9.78 g	2.53 g
S. Typhimurium DT104	0.04 g	4.24 g	4.93 g	0.29 g	9.23 g	2.61 g
S. Senftenberg	0.03 g	4.29 g	4.92 g	0.23 g	10.51 g	2.85 g
S. Typhimurium DT 178	0.03 g	3.95 g	4.83 g	0.29 g	10.45 g	2.99 g

Salmonella inoculation took place one day after the start of the trial; 1 mL of the *Salmonella* inoculate was mixed into the material of each unit.

To evaluate the influence of larval activity, control replicates with equal conditions without larvae were installed; the controls were mixed manually. The experiments lasted until the day when the concentration of *Salmonella* spp. was under the detection limit of 1 CFU per 5 gram (CFU=colony-forming unit); which happened within 7-13 days and 6-10 sample occasions.

4. Results and discussions

4.1 Feeding trials

The pupation rate of larvae fed on human faeces was 91.8 %, while 93 % of the larvae fed on poultry feed were found as prepupae. The material reduction of human faeces was 51.3 %; the waste-to-biomass conversion rate (WBC) was 9 %. It took 14 days until the first prepupa emerged from the reactors, 5 days later more than 50 % of the initial larvae had emerged. The reduction of poultry feed was found to be 84.9 % on dry matter base, while the WBC was 17.7 %. The first prepupa emerged after 12 days; four days later more than 50 % of the initial larvae emerged (Table 2).

Table 2 The pupation rate, material reduction and waste-to-biomass conversion rate (WBC) of BSF fed on poultry feed and human faeces, respectively, presented are the average values and standard deviation (SD); material reduction and WBC are given on a dry matter basis

	Human faeces		Poultry feed	
	Average	SD	Average	SD
Pupation rate [%]	91.8	4.5	93.0	2.9
Material reduction [%]	51.3	1.5	84.9	3.6
WBC [%]	9.0	0.6	17.7	3.3
Time until 1st PP emerged [d]	14	0	12	0
Time until 50% of larvae emerged [d]	19	0	16	0

4.1.1 Feeding trials with faeces

The pH was found to increase with time, from 7.2 in the inflow material up to 8.7 in the outflow material. There was a tendency of a reduction of the organic content (from 83.8 % to 81.6 %). Total solids in the inflow faeces were 21.6 %, while total solids in the outflow were 27.6 %. The prepupae fed on faeces were 61.8 % moisture and 17.39 % ash.

The same inflow material was used throughout the feeding trials; hence, no standard deviation is given (Tables 3 and 4).

Table 3 The pH and percentage total solids and volatile solids (organic content) in the inflow and outflow material and prepupae; presented are average values and standard deviation (SD) of the triplicates fed on faeces.

	pH		Total solids [%]		Organic content [% of DM]	
	Average	SD	Average	SD	Average	SD
Inflow material	7.2	-	21.6[a]	-	83.8	-
Outflow material	8.7	0.2	27.3[a]	1.3	81.8	0.4
Prepupae			39.2	0.3	82.6	2.3

[a] Significant difference ($p<0.05$) between in- and outflow material

4.1.2 Feeding trials with poultry feed

The organic content decreased in the process, from 90 % in the inflow material to 77 % in the outflow material. Furthermore, the pH was found to increase within the four weeks long trial; the pH of the inflow material was 6.3, while the average pH in the outflow material was 7.3. Total solids in the inflow poultry feed was 41.2 % and decreased in the process to 31.1 %. The prepupae were 63.1 % moisture.

Table 4 The pH and percentage total solids and volatile solids (organic content) in the inflow and outflow material and prepupae; presented are average values and standard deviation (SD) of the triplicates fed on poultry feed.

	pH		Total solids [%]		Organic content [% of DM]	
	Average	SD	Average	SD	Average	SD
Inflow material	6.3	-	41.2[a]	-	90.0[a]	-
Outflow material	7.3	1.3	31.1[a]	5.4	77.0[a]	3.4
Prepupae			36.9	1.7	n.a.	-

n.a. not analysed

[a] Significant difference ($p<0.05$) between in- and outflow material

4.2 Material reduction and waste-to-biomass conversion

The material reduction and WBC in the faecal feeding trial were over 50 % and over 9 %, respectively and in accordance to previous findings (Lalander et al., 2014, Myers et al., 2008, Sheppard et al., 1994). Banks et al. (2013) reported a WBC of 22.9 % in a small-scale system of 10 BSF larvae fed one lump sum of human faeces (lump amount feeding), while the WBC decreased enormously to 1.6 %, when 100 larvae were fed on the same amount of material per larvae and day. Furthermore, it was evaluated whether the feeding regime could have an influence on larval growth; the lump amount feeding lead to larger prepupae, and most efficient feed conversion rate (2 %), while incremental feeding (every second day fresh material was added) caused higher material reduction in two out of three cases. As a result Banks et al. (2013) pointed out larval density and the feeding regime as parameters for optimising BSF treatment. This conforms with findings of Diener et al. (2011a), suggesting a larval density of 5 larvae cm^{-2}. It was also recommended to install a drainage system in case of liquid accumulation in the reactors since the reduction of municipal organic waste increased from 65.5 % to 72.7 % on dry weight (Diener et al., 2011a).

Diener et al. (2011a) reported a material reduction of over 70 % for mixed organic waste, while Gobbi et al. (2013) found similar results for BSF larvae fed on poultry feed. This conforms to findings of this study; material reduction of poultry feed was nearly 85 % and the corresponding WBC was 17 %. These higher rates can be explained by a greater amount of available nutrients in the poultry feed compared to that in human faeces.

In summary, it can be said, that there are several parameters for optimising BSF larvae treatment such as feeding regime, humidity and larval density within the reactor. Although the results of this study base on trials that were conducted in batch mode, the high waste-to-biomass conversion rates show that BSF larvae are effective at converting human faeces and other organic material into animal protein. In combination with the high prepupae yield of over 90 %, the high material reduction and WBC rates support the use of BSF larvae for faecal waste management.

4.3 Salmonella trials

The chosen material for evaluation of *Salmonella* spp. reduction was human faeces due to its pH neutrality. During the feeding trials poultry feed occasionally reached an acidic value of 4.5, while pH of faeces was between 7.1 and 8.8. Hence, an influence of pH on the inactivation of *Salmonella* spp. was excluded, as declared in Section 3.1.

The concentration of all four *Salmonella* spp. strains decreased drastically within the process. The average inflow concentration of all trials was around 10^8 CFU g^{-1} and dropped below the detection limit of 1 CFU per 5 g within 7-13 day in all treatment reactors, while the concentration of the controls of *Salmonella* Senftenberg and both *Salmonella* Typhimurium DT 104 and DT 178 were found to be positive on the last day of each trial (Figs. 4a-c).

The concentration of *S*. Senftenberg, *S*. Typhimurium DT 178 and *S*. Typhimurium DT 104 was significantly ($p<<0.05$) reduced both with time and due to treatment (Figs. 4a-c), while the concentration of *Salmonella* Dublin was not affected by BSF larvae treatment as the reduction was as great in the control (Fig. 4d). However, the concentration of *S.* Dublin was below the detection limit after 7 days.

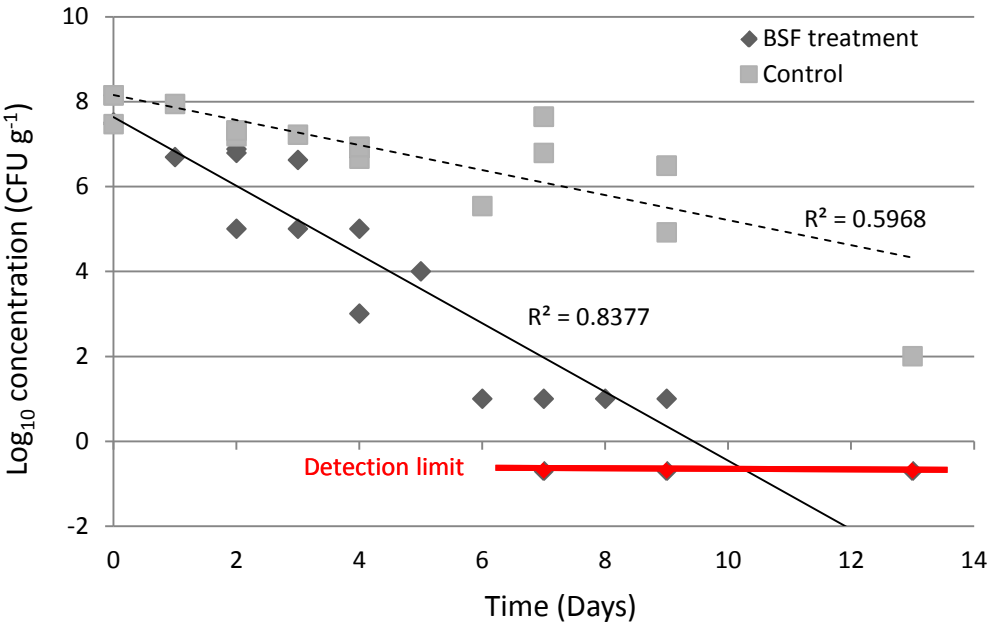

Figure 4a The $\log_{10}$ concentration of *Salmonella* Typhimurium DT 104 (CFU g^{-1}) in the treatment (*diamond*) and control (*square*) within a timeframe of 13 days.

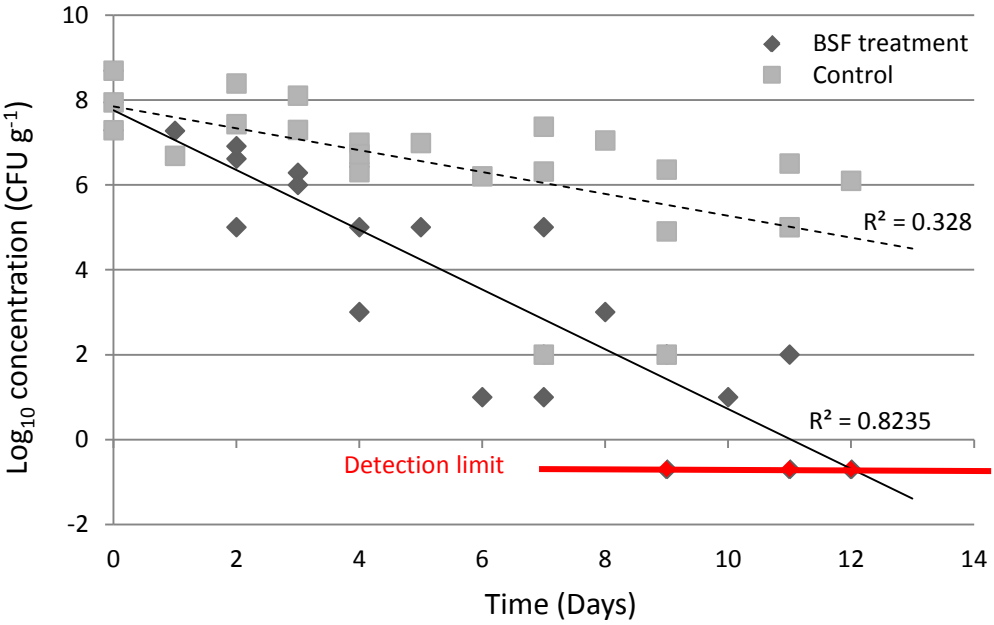

Figure 4b The $\log_{10}$ concentration of *Salmonella* Senftenberg (CFU g^{-1}) in the treatment (*diamond*) and control (*square*) within a timeframe of 12 days.

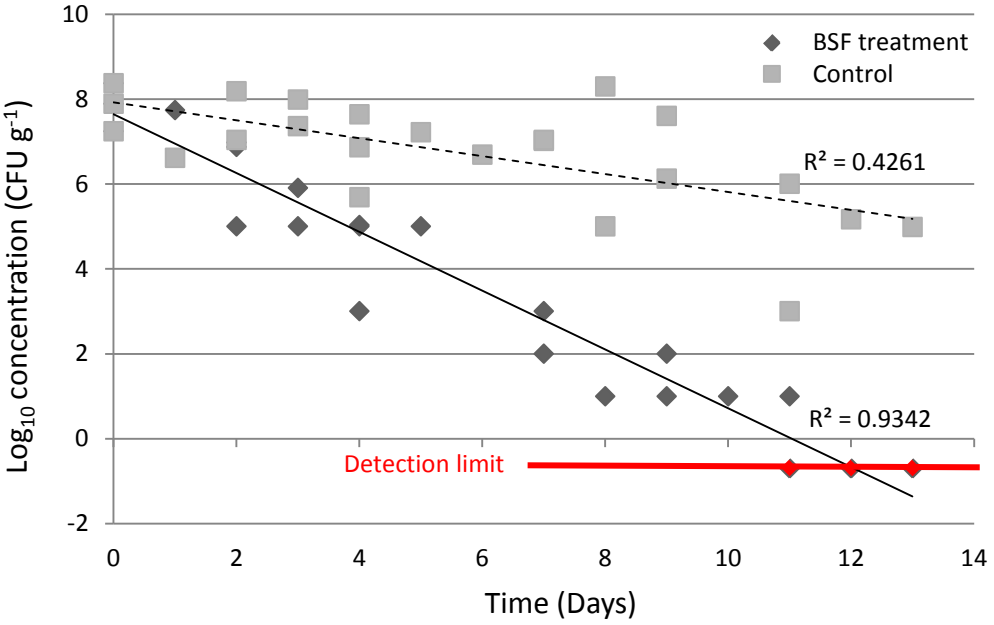

Figure 4c The $\log_{10}$ concentration of *Salmonella* Typhimurium DT 178 (CFU g^{-1}) in the treatment (*diamond*) and control (*square*) within a timeframe of 13 days.

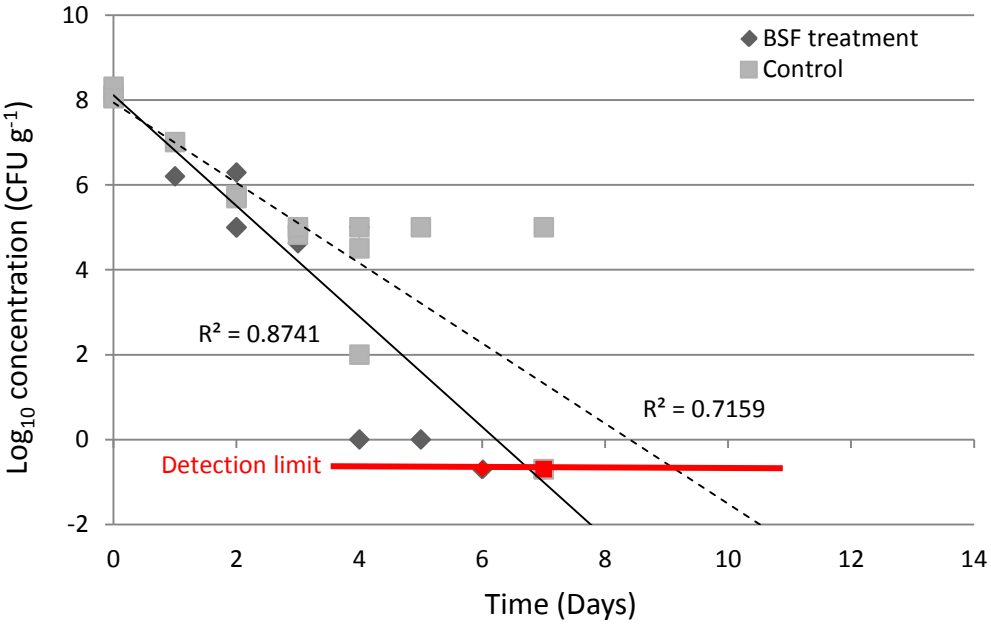

Figure 4d The $\log_{10}$ concentration of *Salmonella* Dublin (CFU g^{-1}) in the treatment (*diamond*) and control (*square*) within a timeframe of 7 days.

4.4 *Salmonella* spp. reduction

The reduction of the four *Salmonella* spp. strains was found to be 8 $\log_{10}$ within this experiment; that corresponds with findings of Lalander et al. (2014), where a 7 $\log_{10}$ reduction was monitored in a continuous flow reactor using a feed mixture of pig manure, human faeces and dog food. Erickson et al. (2004) reported a 3 $\log_{10}$ reduction of *Salmonella Enteritidis* in chicken manure treated by BSF larvae within 3 days. The fact that BSF larvae have a significant influence on the reduction of different *Salmonella* spp. strains, which only infect certain animal species and that are antibiotic multiresistant, reduces the risk of infection between humans and animals and between different animals.

It could be assumed that BSF larvae fed on highly contaminated food and used as animal feed would be a vector of disease; however, according to Sheppard et al. (1994) and Lalander et al. (2013) BSF larvae empty their gut prior to migration. The *Salmonella* spp. concentration in the prepupae gut was <0.5 CFU g^{-1}, while the concentration in the bath water, the prepupae have been washed with, was found to be <1.0 CFU g^{-1}, the same value found in the gut of BSF larvae. Thus, the risk of re- and

cross-contamination, respectively, due to BSF larvae fed on contaminated material and used as animal feed, is low; it could be even lower by further treatment of the prepupae such as drying, which could be done in the sun in low-income countries.

Although, a reduction in the risk of infection between animals and humans with the BSF treatment due to *Salmonella* inactivation, BSF prepupae and the residue - if to be used as organic fertiliser - cannot be regarded as a completely sanitised. If no additional hygienisation is included, hygienic safety can be increased by introducing additional barriers such as the extension of the retention time, the use of a combination between mineral and organic fertiliser and the selection of crops. For example the residue could be without further treatment to fertilise tree crops, as there would be no direct contact between the crop and the contaminated material.

5. Conclusion

The conducted feeding trials have shown the enormous potential of using *Hermetia illucens* in organic waste management. The material reduction of human faeces was 51.3 %, while the corresponding WBC was 9 %.

The inactivation of *Salmonella* Senftenberg and the two types of *Salmonella* Typhimurium (the widespread DT 178 and the antibiotic multiresistant DT 104) was found to be accelerated in the BSF larvae treatment, while the reduction in the concentration of *S*. Dublin was as rapid as in the control.

BSF larvae composting is an innovative strategy for treatment of organic waste since it produces animal feed that is abundant in fat and protein, and an organic fertiliser, that can be used in agriculture. It closes the local nutrient loop and is a comparatively easy management system, which has the potential of being self-financing. Waste management and food and feed production are linked by Black Soldier Fly treatment. This strategy can improve human and animal nutrition as it is declared as a major issue for future life. In summary, BSF treatment could contribute in reducing health risks in low- and middle-income countries by progressing sanitation. Generally, decision makers and local inhabitants need to be convinced by the idea to implement Black Soldier Fly treatment as waste management strategy, thus, further research is needed.

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