Science of the Total Environment 458–460 (2013) 312–318

Contents lists available at SciVerse ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Faecal sludge management with the larvae of the black soldier fly (*Hermetia illucens*) — From a hygiene aspect

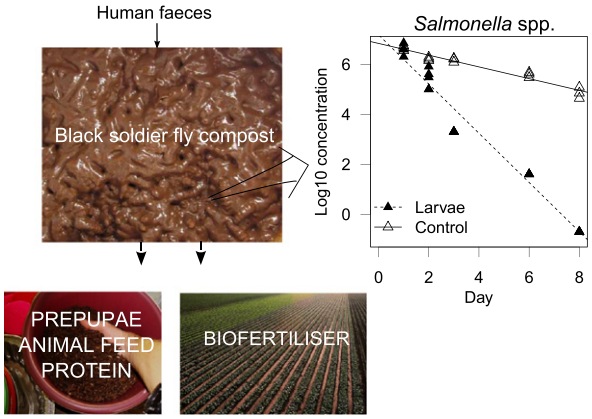
Cecilia Lalander [a,*], Stefan Diener [b], Maria Elisa Magri [a,c], Christian Zurbrügg [b], Anders Lindström [d], Björn Vinnerås [a,d]

[a] Department of Energy and Technology, Swedish University of Agricultural Sciences, Uppsala, Sweden
[b] Eawag: Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland
[c] Universidade Federal de Santa Catarina, Florianópolis, Brazil
[d] National Veterinary Institute, Uppsala, Sweden

HIGHLIGHTS

- A system for simultaneous treatment for human waste and animal feed protein production was investigated in the presented study.
- Black soldier fly larvae feed on organic waste and are high in proteins and fat, which make them ideal as feed for animals.
- Focus of the study was the fate of pathogen and indicator microorganisms in the insect driven treatment system.
- The concentration of Salmonella spp. greatly reduced, but no effect on Ascaris suum ova was established.
- A post-treatment step is recommended, but this is greatly simplified by the significant mass reduction.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 22 December 2012
Received in revised form 25 March 2013
Accepted 10 April 2013
Available online xxxx

Keywords:
Animal feed
Black soldier fly
Faecal management
Reuse agriculture
Protein source

ABSTRACT

Inadequate and lacking sanitation and wastewater treatment systems can lead to the spreading of diarrhoeal diseases. One contributing factor in the lack of such treatment systems is the lack of economic incentives for stakeholders throughout the service chain. However, the organic fraction of the waste is high in valuable plant nutrients and could be reused in agriculture and as animal feed. For example, grown larvae of the black soldier fly, *Hermetia illucens* L. (Diptera: Stratiomyidae), make an excellent protein source in animal feed, while the feeding activity of the larvae substantially reduces the dry mass of the treated material. This study examined the effect of black soldier fly larvae on the concentration of pathogenic microorganisms in human faeces and found a 6 $\log_{10}$ reduction in *Salmonella* spp. in human faeces in eight days, compared with a <2 $\log_{10}$ reduction in the control. No increased reduction was observed for *Enterococcus* spp., bacteriophage ΦX174 or *Ascaris suum* ova.

1. Introduction

Diarrhoea caused by infection with enteric disease agents is widespread throughout low- and mid-income countries. Diarrhoeal diseases and their medical consequences, cause an estimated 2.2 million deaths per year and are the leading cause of death in children under the age of

* Corresponding author. Tel.: +46 762215361.
E-mail address: cecilia.lalander@slu.se (C. Lalander).

0048-9697/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.scitotenv.2013.04.033

five in sub-Saharan Africa (WHO and UNICEF, 2000). A major factor in the spread of such diseases is lack of sanitation. In Africa, more than 60% of the population does not have access to improved sanitation, with 40% of the rural population practising open defecation (Morella, 2008). Moreover, the safe collection and treatment of faecal sludge from on-site sanitation systems such as latrines and septic tanks is often not guaranteed and faecal sludge is discarded directly into water bodies or nearby fields. The effects of these practices are many; they contribute to the pollution of groundwater, contamination of agricultural produce and spreading of diseases such as diarrhoea, cholera and helminthiasis (Nguyen-Viet et al., 2009).

One reason for the current situation is the lack of economic incentives for stakeholders throughout the faecal sludge service chain. The cost of emptying and safe disposal of faecal sludge to a treatment plant often has to be paid by the household. The management of solid waste is also an increasing problem in urban environments in low and middle-income countries. As a consequence, large volumes of waste are thrown into the streets causing blockages, flooding and spread of diseases (Zurbrügg, 2002). In low-income countries in Africa, 50–90% of the solid waste fraction consists of organic matter such as food left-overs, rotten fruit and vegetables, crop residues and animal excreta (Asomani-Boateng and Haight, 1999). The collection and treatment of waste is often neglected, especially in the urban context. The common practise in rural areas of feeding organic household waste to chickens and pigs is not practised to the same extent in the cities. This leads to open dumping, where the waste attracts disease vectors (rats, dogs) and is cross-contaminated with human faeces. Animal manure (cow, pig, chicken, goat), a known source of many zoonotic pathogens (Pell, 1997), comprises a large fraction of the urban waste.

All these fractions of organic wastes are high in valuable plant nutrients and can be reused in agriculture as fertiliser and as a soil amendment. At the same time they are high in pathogenic microorganisms and therefore require adequate sanitisation prior to use (Albihn and Vinnerås, 2007; Winker et al., 2009). Technologies that sanitise organic wastes and convert them into valuable products are well known: thermophilic composting, anaerobic digestion and desiccation on drying beds are common practices. However, the collection and treatment costs often cannot be covered by selling the products generated (Murray et al., 2011; Zurbrügg et al., in press). New and financially attractive waste management strategies should thus be explored and fostered. Similar to the well-established recycling and resource recovery sector for inorganic material (glass, plastics or metal), collection and recovery of municipal organic waste and faecal matter from on-site sanitation systems can contribute to generate value added if appropriate valorisation technologies are provided (Fluitman, 2000; UN-HABITAT, 2010; Wang et al., 2008).

Organic waste has a high nutritional and energetic potential and can be used as a feed substrate for insect larvae in general, and the larvae of the black soldier fly (BSF), *Hermetia illucens* L. (Diptera: Stratiomyidae), in particular. Grown larvae make an excellent protein source in animal feed and their feeding activity reduces the dry mass of the waste significantly. The insect protein could be used in animal feed to replace fishmeal, in February 2013 was priced at USD 1883 per metric tonne (IMF, 2013). In this way, a high value product derived from organic waste could be produced, which could contribute to the economic viability of the treatment system.

BSF is a tropical fly found in warmer regions worldwide. Larvae feed on decomposing organic material such as fruit and vegetable waste, as well as human and animal manure (Diener et al., 2011b; Bradley, 1930; Tomberlin et al., 2002). The larvae can consume large amounts of waste and have been demonstrated to reduce the dry matter content of manure by 58% (Myers et al., 2008) and that of municipal organic waste by 70% (Diener et al., 2011a). Furthermore, BSF colonisation can eliminate breeding by the common house fly, a key vector in spreading disease (Bradley and Sheppard, 1984). Given ideal conditions (temperatures around 25–30 °C and with an ample supply of food) the BSF larvae can develop into prepupae within two weeks, but development within three to four weeks is not uncommon. The prepupae migrate out of the waste to find a dark, dry site for pupation (Tomberlin et al., 2009). This habit facilitates the collection of the prepupae, which consist of 36–48% protein and 31–33% fat (Diener et al., 2009; St-Hilaire et al., 2007b). BSF prepupae have long been known as possible protein sources in aquaculture (Bondari and Sheppard, 1981) and in swine production (Newton et al., 1977). A more recent study has endorsed previous findings (St-Hilaire et al., 2007a).

Besides the reduction in biomass, proper treatment of organic waste includes the sanitisation of the material. Antibacterial activity in excretion/secretion of insect larvae is known and its effect even in maggot therapy for wound debridement (Parnes and Lagan, 2007). There is also evidence that BSF larval activity sanitises waste. Inactivation of Enterobacteriaceae such as *Salmonella* spp. and *Escherichia coli* has been demonstrated (Erickson et al., 2004; Liu et al., 2008). On the other hand, little is known about their influence on other pathogenic bacteria, viruses and parasitic organisms.

The objective of this study was to assess the effect of feeding activity by BSF larvae on the concentration of bacteria, viruses and parasites in human excreta and the concentrations in which the same microorganisms would attach to the surface of the migrated prepupae or into the prepupae. Two bacteria (*Salmonella* spp. and *Enterococcus* spp.), a bacteriophage (ΦX174) and a parasite (*Ascaris suum*) were investigated.

2. Materials and methods

2.1. Materials

Human faeces were collected fresh in plastic bags during a three-week period and stored at −20 °C immediately upon collection. At the laboratory, these bulk faeces samples were thawed, pooled (TS 36–38%), divided into feeding portions and kept at −20 °C until use. Human faeces used for the *Ascaris* experiment were collected from a dry toilet in Uppsala, Sweden. Excess toilet paper was removed to give a total solid content of 36%.

A. suum, which infects pigs, is often used as model for *Ascaris lumbricoides* that infects humans (Johnson et al., 1998). Adult *A. suum* worms were collected from the intestines of slaughterhouse pigs and their eggs harvested by dissection of the posterior 2 cm of the worm uterus. Washing of the eggs was conducted as described in Eriksen et al. (1996), aside from the use of sodium hypochlorite. Collected eggs (10,000 eggs mL^{-1}) were stored in physiological saline solution (0.9% wt/vol) at 4 °C until the start of the experiment. The initial viability was verified by immersing 1 mL of the *A. suum* solution in 4 mL 0.1 M H_2SO_4 followed by incubation at 28 °C for 30 days, and was found to be 86–88%.

The bacterial inoculate solutions consisted of 10^8 CFU mL^{-1} *Enterococcus faecalis* (ATCC 29212) and 10^9 CFU mL^{-1} *Salmonella enterica* subspecies 1 serovar Typhimurium phage type 178 (isolated from sewage sludge; Sahlström et al. (2004)) grown in unselective bacterial nutrient medium (NB, Oxoid AB, Sweden).

Propagation of bacteriophage ΦX174 to a concentration of 10^9 PFU mL^{-1} was performed in unselective bacterial nutrient medium (NB, Oxoid AB, Sweden) using the host strain *E. coli* (ATCC 13706). The phage was collected by centrifuging the solution at 2000 ×*g* for 10 min followed by sterile filtration and kept at 4–6 °C until use.

BSF larvae were obtained from a colony in operation for more than 20 generations at Eawag, the Swiss Federal Institute of Aquatic Science and Technology in Switzerland.

2.2. Experimental set-up

Three sets of experiments were conducted in order to investigate: 1) the reduction in bacteria and bacteriophages in larval-treated material; 2) the reduction/inactivation of *A. suum* ova in larval-treated

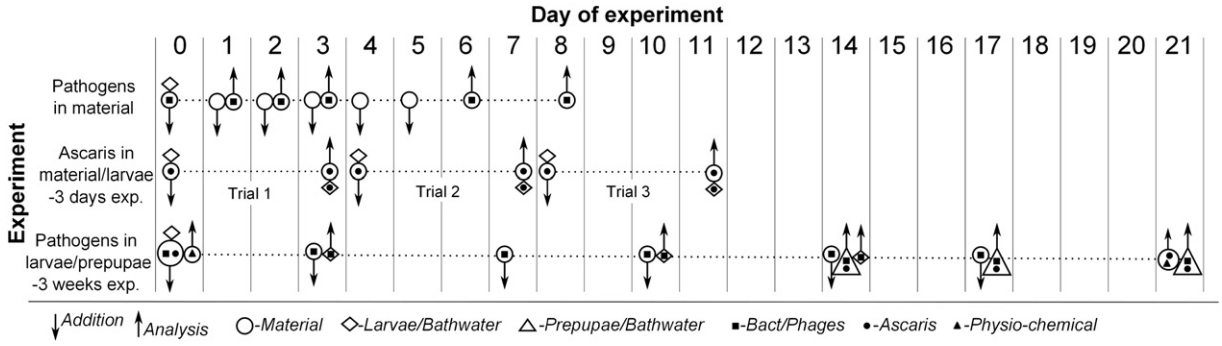


Fig. 1. Schematic representation of the fractions added and analysed in the three sets of experiments conducted.

material and the presence of *A. suum* ova in larvae; and 3) the presence of bacteria, bacteriophages and *A. suum* ova in larvae and prepupae (Fig. 1). Negative controls without larvae were included in the two first sets of experiments. The pathogen concentration in larval-treated material was compared with that in the control with no larvae, in order to verify whether a significant difference between the two could be established. All experiments were conducted in triplicate unless otherwise stated.

The first set of experiments – investigating whether the feeding activity of the larvae could inactivate pathogens in the material – was conducted during nine days in total, starting with 10- to 11-day-old larvae. The first material into which the larvae were placed was contaminated with bacteria (10^7–10^6 CFU g^{-1}) and bacteriophages (~10^6 PFU g^{-1}), while no pathogens were inoculated to the subsequent material added as extra feed throughout the experiment. It was not possible to add all materials at the start of the experiment, as the surface of the uppermost material would dry out, preventing further larval feeding.

The second set of experiments – investigating the potential of using BSF larvae for inactivation of *A. suum* ova in the material – was conducted in three trials (three days each) when the feeding activity of the larvae was expected to be at its highest. Thus trial 1 used 10-day-old larvae; trial 2 used 14-day-old larvae and trial 3 used 18-day-old larvae.

In order to investigate whether the pathogens inoculated into the material could be found in the larvae and the prepupae, a third set of experiments was conducted over the course of three weeks. The larvae were added into material heavily contaminated with bacteria (10^7–10^6 CFU g^{-1}), bacteriophages (~10^6 PFU g^{-1}) and *A. suum* ova (300 ova g^{-1} faeces at the first feeding). Throughout the experiment the larvae were fed with material heavily contaminated with bacteria and bacteriophages, while the *A. suum* ova were only added at the first feeding. Around five larvae, and later prepupae, were collected and immersed in buffer containing a surfactant (Tween 80). The Tween buffer in which the larvae/prepupae were immersed is hereafter referred to as bathwater. After immersion in Tween buffer the larvae/prepupae were immersed in ethanol upon which they were opened and their gut content analysed for bacteria, bacteriophages and *A. suum* ova. The same parameters were analysed in the bathwater, in order to establish whether a measurable change in pathogen concentration/activity in material attached to the larval/prepupal bodies could be found.

Experimental sets 1 and 3 were conducted in 13 cm × 12 cm × 15 cm opaque plastic containers, while 200 mL transparent plastic beakers covered with opaque black plastic were used in set 2. In order to prevent larval escape the containers were covered with perforated aluminium foil. The containers were kept in a controlled environment at 60–70% RH at 20–25 °C.

2.2.1. Pathogen reduction in material

On day zero, 65 g of unspiked fekal material was placed in containers. Eight containers were prepared: five treatments with larvae and three controls without larvae. Into the treatment containers, 500 10–11-day-old larvae were added. On day one, 65 g faeces spiked with bacteria (*S.* Typhimurium (~10^6 CFU mL^{-1}); *E. faecalis* (~10^6 CFU mL^{-1})) and bacteriophage ΦX174 (~10^6 PFU g^{-1}) were added to each container. On days two and three, 65 g unspiked fekal material was added to each container. The containers with larvae were not mixed as larval movement rendered complete material mixing. The materials in the controls were thoroughly mixed. Samples were taken from all containers. On days four and five, 65 g uncontaminated fekal material was added to each container but no samples were taken. Day five was the last feeding day. The larvae were left in the treatment units without adding any additional feed and samples were taken on days six and eight. The experiment was concluded on day eight.

2.2.2. A. suum in material/larvae

All six containers used in this part of the experiment received 30 of human faeces and 10,000 *A. suum* ova; 100 BSF larvae were added to the three treatment units following the methods previously described, while nothing further was added to the three control units. The containers were covered with perforated aluminium foil and incubated in darkness at 25 °C at 60% RH for three days. The procedure was performed three times, with 11-, 14- and 18-day-old larvae. The initial *A. suum* ova concentration was around 300 ova g^{-1} faeces. This concentration was chosen arbitrarily as a concentration that would be extractable. There is no mean concentration of *Ascaris* ova excreted by an infected individual, as it depends on the intensity of infection and can vary greatly from around 10 ova g^{-1} faeces to >100,000 ova g^{-1} faeces (Anderson et al., 1993; Carneiro et al., 2002).

2.2.3. Pathogens in larvae/prepupae

Three containers were filled with 50 g fekal matter spiked with *S. typhimurium* (10^6 CFU mL^{-1}), *E. faecalis* (10^6 CFU mL^{-1}), bacteriophage ΦX174 (10^6 PFU mL^{-1}), 10,000 *A. suum* ova and 200 larvae. The plastic containers were placed onto a wood chip bed and inclined at a 45° angle, to allow the prepupae to independently crawl out of the material. Every third or fourth day, 80 g of fekal matter inoculated with all organisms listed above except *A. suum* ova, were added to each container. Three larvae were taken out of each container and washed in Tween containing buffer followed by immersion into ethanol. The larvae were opened and their gut immersed into buffer and homogenised for

Table 1
Degradation of the larval-treated material and the control compared with the start material. The faeces were treated with BSF larvae (larvae) or without (control) for a total of three weeks, fresh material was added on days 0 (start), 3, 4, 7, 10, 14 and 17. The faeces were kept at 25 °C throughout the course of the experiment.

	Total weight (g)	TS (%)	VS (%)	Total TS (g)	Total VS (g)	Reduction total TS (%)	Reduction total VS (%)
Start	370	36 ± 1.3	91 ± 0.3	133	337		
Larvae	118 ± 4	30 ± 1.2	86 ± 0.6	30	26	73	75
Control	228 ± 2	41 ± 1.3	86 ± 0.4	93	196	30	34

Table 2
Physico-chemical parameters (±SE) of the start material (faeces), larval-treated faeces and the control.

	pH	NO_3^- (mg g^{-1} ash)	TAN (mg g^{-1} ash)
Start	6.0 ± 0.0	0.8 ± 0.1	4.6 ± 0.7
Larvae	7.5 ± 0.0	0.8 ± 0.1[a]	20.3 ± 0.5[b]
Control	7.5 ± 0.1	1.0 ± 0.2[a]	15.1 ± 0.5

[a] No significant difference from start material.
[b] Significant difference from control.

analysis of bacteria and bacteriophages. The same procedure was repeated on selected days. The prepupae found in the wood chips were collected and one subsample analysed for bacteria/bacteriophages. Another subsample of the prepupae was analysed for presence of *A. suum* ova. The analysis was conducted in the same way as described above for the larvae, with both the prepupal gut and bathwater being analysed. Within three weeks, most of the prepupae had crawled out of the containers and the experiment was terminated. In total around 20 larvae and 20 prepupae (five per sampling occasion) per replicate were analysed for bacteria and bacteriophages. Around 20 prepupae per replicate were analysed for presence of *A. suum* ova. The remaining prepupae were collected and stored at −20 °C.

2.3. Microbiological sampling

The buffer used for all experiments was buffered NaCl peptone water with Tween 80 at pH 7, hereafter referred to as Tween buffer. One gram was extracted and further diluted in Tween buffer. *Enterococcus* spp. were grown on Slanetz–Bartley agar (Oxoid AB, Sweden) and incubated at 44 °C for 48 h. *Salmonella* spp. were grown on xylose lysine desoxycholate agar (XLD) (Oxoid AB, Sweden) containing 0.15% sodium-novobiocin and incubated at 37 °C for 12 h. For sampling of ΦX174, the host was cultured in unselective microbial medium (NB, Oxoid AB, Sweden) at 37 °C for 4–12 h; 1 mL of sample of suitable dilution was mixed with 2 mL soft agar and 1 mL host solution and poured onto blood agar base (BAB) plates (Oxoid AB, Sweden). The plates were incubated at 37 for 7 ± 2 h.

2.4. A. suum ova extraction

The viability of *A. suum* ova was monitored: i) in faeces that were consumed by larvae; ii) in water used to wash the larvae upon removal from material (bathwater) and iii) in controls. The viability of *A. suum* ova in bathwater was analysed in order to establish whether it would be lower for ova found on the larval bodies compared with ova found in the material. *A. suum* ova extraction was conducted according to the procedure of the United States Environmental Protection Agency (EPA/625/R-92/013), but using 1/10 of the volumes stated in that method. Prior to incubation, 15–20 of the extracted *A. suum* ova were verified. The tubes were incubated at 28 °C for 30 days in 0.1 M H_2SO_4.

After approximately 30 days the incubated material was analysed under microscope (≥100 ova per sample). A 1 mL Sedwick Rafter Counting Cell was used for ova counting and pre-larvae and larvae were counted as viable. For the examination of *A. suum* ova in larvae and prepupae, only flotation and the subsequent steps were performed. In this case the viability of the ova was not investigated.

2.5. Physico-chemical analyses

The content of total solids (TS), total volatile solids (VS), pH, total ammonium-nitrogen (TAN) and nitrate (NO_3^-) was analysed.

2.5.1. TS and VS

The material was dried at 105 °C for 14 h for TS determination and at 550 °C for 6 h for VS determination.

2.5.2. pH

A radiometer electrode was used to measure pH. All analyses were conducted at room temperature (RT) on 10 g of sample diluted with 50 mL deionised water and left to settle for 1 h at RT prior to analysis.

2.5.3. TAN and NO_3^-

In a closed container, 5 g sample was mixed with 100 mL deionised water. The material was allowed to settle for 2 h, after which 25 mL of the supernatant was extracted and a spatula tip of activated charcoal (powder) was added. The solution was stirred with a magnetic stirrer for 10 min, diluted 1:5 or 1:10 in deionised water and filtered through a fluted filter. Spectroquant© test kit number 114544 was used for TAN analyses and kit number 114764 for NO_3^- analyses.

2.6. Statistical analysis

One-way analysis of co-variance (ANCOVA) with 95% confidence interval was used to establish whether a statistical significant difference occurred between the regression of pathogens in the larval-treated material and the control. One way analysis of variance (ANOVA) with 95% confidence interval was used to establish whether a statistically significant difference occurred in *A. suum* ova concentration and chemical parameters before and after the treatment. In cases where a statistically significant difference was found, Tukey multiple comparisons of means (95% family-wise confidence level) was performed. All analyses were conducted in R (R Development Core Team, 2011).

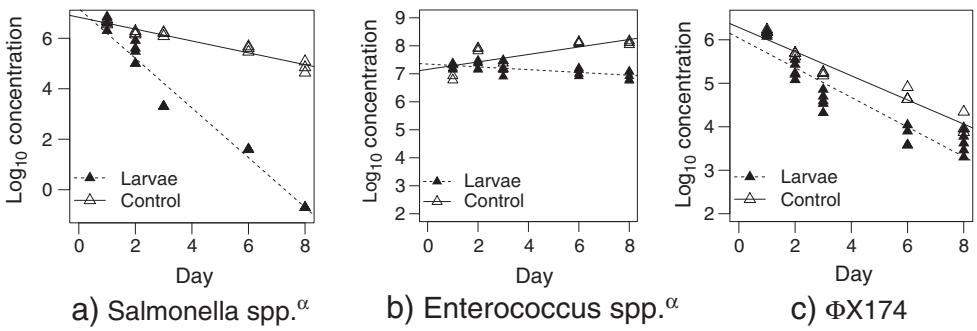


Fig. 2. Log_{10} concentration of a) *Salmonella* spp. (CFU g^{-1}), b) *Enterococcus* spp. (CFU g^{-1}) and c) ΦX174 (PFU g^{-1}) in the treatment (▲) and the control (△) over the course of the experiment. *a* indicates a significant difference in the rate of reduction between control and larvae treatment.

3. Results and discussion

3.1. Physico-chemical analysis of material

A comparison of the degradation of material in the larval-treated faeces and the control is presented in Table 1. The reductions in total TS and VS were calculated from the average total weight, TS and VS of the respective treatment. The weight of the material was reduced by 50% ww in the larval-treated material, with a 73% reduction in total dry mass and 75% reduction in total volatile solids. The only significant difference in physico-chemical parameters between the larval-treated material and the control was the TAN concentration (Table 2). The concentration of nitrate was not significantly different between the start material and the larval-treated material and control, while the pH, TS, VS and TAN had altered significantly.

The larvae greatly reduced the fekal mass, by 73% on a dry matter basis compared with 30% in the control. The concentration of nutrients (per g ash) was not significantly different between the larval-treated material and the control, but a large fraction of the total nutrients in the start material was removed in the larval-treated material and assimilated into the larval biomass. The higher TAN concentration observed for the larval-treated material is most likely due to the higher degradation of organically bound nitrogen. The nitrate concentration was still low after the treatment, indicating that the material had not been stabilised. An additional maturation step is likely to be required if the treatment residue is to be used as fertiliser.

3.2. Pathogen reduction in material

The concentration of *Salmonella* spp., *Enterococcus* spp. and ΦX174 was recorded over time in faeces consumed by larvae and in the control without larvae. In the treated faeces, a 6 $\log_{10}$ reduction in the concentration of *Salmonella* spp. was achieved in 8 days, while less than a 2 $\log_{10}$ reduction was achieved in the control (Fig. 2a). There was a small reduction in the *Enterococcus* spp. concentration (<1 $\log_{10}$) in the treatment, but the concentration of *Enterococcus* spp. increased by 1 log10 in the control (Fig. 2b). There was no significant difference in the rate of reduction of ΦX174 between the treatment and the control (total reduction >2 $\log_{10}$) (Fig. 2c).

The fitted regression lines displayed in Fig. 2 were statistically significant for all organisms (Table 3).

Previous studies have demonstrated that the feeding action of BSF larvae can accelerate the reduction of Enterobacteriaceae (Erickson et al., 2004; Liu et al., 2008), which is confirmed by the findings of this study. However, the larvae appeared to have little reducing effect on *Enterococcus* spp. and bacteriophage ΦX174.

3.3. A. suum ova reduction in faeces

The viability of *A. suum* ova was initially slightly lower in the control than in the treated faeces and the bathwater (Table 4). After three weeks the viability of the *A. suum* ova had decreased to 37–44% in both the treated material and the control. However, the number of samples analysed was not sufficient to establish whether the small difference in viability between the treatment and the control was statistically significant.

Table 3
R^2- and p-values of the fitted regression lines.

	Salmonella spp.		*Enterococcus* spp.		ΦX174	
	R^2	p	R^2	p	R^2	p
Larvae treatment	0.96	2.2×10^{-16}	0.46	0.0002	0.86	3.8×10^{-11}
Control	0.94	2.3×10^{-9}	0.58	0.001	0.94	2.3×10^{-9}

Table 4
Viability and concentration of *A. suum* ova in faeces treated with BSF larvae, in the larval bathwater and in the control.

	Larval-treated faeces	Larval bathwater	Control
Percentage viability (±SE) 3 day exp.	94 ± 0.6	92 ± 1.1	88 ± 1.4
Estimated concentration (ova g^{-1} ww)	320		150
Percentage viability 3 week exp.	37		44
Estimated concentration (ova g^{-1} ww)	4		2

The concentration of *A. suum* ova was not satisfactorily established in the control, as the ova adhered to faecal particles to a greater extent in the control compared with the treated material, making the counting a far greater challenge and with underestimation of *A. suum* ova concentration as a consequence. The estimated mean ova concentrations of ova in the treated material and the controls are shown in Table 4. The mass reduction of the treated material has not been taken into account in the values presented.

When taking the mass reduction into account and estimating the total number of ova found in the material, the differences were not as significant as shown in Table 4. In total 4800, ova were found in the treated material compared with 4200 in the control. However, for the treated samples great numbers of ova were found inside the larvae and in the bathwater. The percentage of eggs thus unaccounted for was 20% in the treated material and 58% in the control (Fig. 3).

The BSF larvae did not increase the inactivation rate of *A. suum* ova. A higher viability was observed amongst the ova in the treated faeces, but the difference compared with the control was small and could be due to larval consumption of non-viable ova. The inactivation thus appeared to be unaffected by the presence of the BSF larvae. Similarly, Nordin et al. (2009) observed a small percentage reduction in unamended faeces from source-separated toilets (~50% after 35 days at 24 °C). Furthermore, the ova found on the larvae (bathwater) did not demonstrate lower viability, which suggests that the larvae had no proximity related impact on the ovum viability.

The reduction and inactivation of *A. suum* ova did not reach 1 $\log_{10}$ in the BSF treatment. A 3 $\log_{10}$ reduction in viability of *A. suum* ova is required for acceptable treatment of raw wastewater in ascariasis-hyperendemic areas (1000 ova mL^{-1}) according to WHO guidelines (WHO, 2006; Mara et al., 2010). Thus, BSF treatment cannot be considered an adequate sanitisation method for faecal matter intended for reuse in agriculture in areas with prevalence of ascariasis. An additional treatment step would be required if the treatment residue was to be used as fertiliser for food crops. Ammonia sanitisation is a reliable and inexpensive treatment which ensures not only a hygienically safe product but also a product with increased fertiliser value (Vinnerås, 2007).

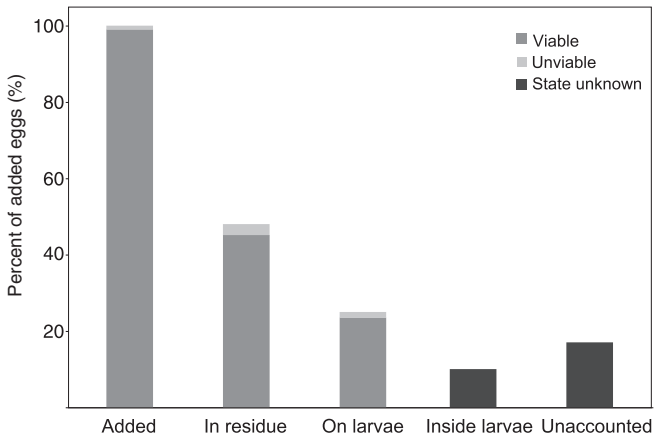


Fig. 3. Fate of *A. suum* ova in the larval-treated samples.

Table 5
Concentrations of pathogenic organisms in larvae and prepupae and the bathwater used to clean each.

		Larval		Prepupal	
	Material (CFU/PFU g^{-1})	Bathwater (CFU/PFU mL^{-1})	Larval gut (CFU/PFU g^{-1})	bathwater (CFU/PFU mL^{-1})	Prepupal gut (CFU/PFU g^{-1})
Enterococcus spp.	10^8	10^7	10^6	10^6	10^5
Salmonella spp.	10^5–<1[a]	<1	<1	<1	<0.5
ΦX174	70,000	8500–4000	~1000	100–10	<2.5

[a] Was 10^5 CFU g^{-1} at the first sampling but quickly decreased and was below the detection limit for the remainder of the experiment.

Table 6
Concentration of *A. suum* ova larvae and prepupae and the bathwater to clean each.

3 days experiment			3 weeks experiment		
Material (ova g^{-1} ww)	Larval bathwater (ova $larva^{-1}$)	Larvae (ova $larva^{-1}$)	Material (ova g^{-1} ww)	Prepupal bathwater (ova $prepupa^{-1}$)	Prepupae (ova $prepupa^{-1}$)
320	25	10	3	0.05	2

The subsequent treatment step would be greatly facilitated by the great mass reduction brought about by larval feeding.

3.4. Pathogens in larvae and prepupae

The concentrations of all microorganisms analysed were lower in the gut of prepupae and the prepupal bathwater than in the larval gut and larval bathwater (Table 5). The bathwater was analysed in order to establish whether the concentration of bacteria/bacteriophages would be significantly lower in material that had been in proximity to the larvae/prepupae. The *Salmonella* spp. concentration was 10^5 CFU mL^{-1} at the start of the experiment, quickly decreased and was below the detection limit in the material throughout the run of the experiment, even though freshly inoculated material was continuously added.

A. suum ova were found inside the larvae and the prepupae (Table 6).

The lower concentrations of organisms found inside the gut of the prepupae compared with the gut of the larvae suggest that the prepupae empty their gut prior to migration. This observation confirms previous findings by Sheppard et al. (1994)). However, May (1961) found traces of food in the gut when prepupae were left in coloured fly medium for three days, indicating that prepupae take up food when there is no opportunity for migration.

As microorganisms were found in the prepupal gut, a treatment or processing step is recommended before feeding the prepupae to animals. This would be of particular importance if the treated material contained pathogens that could infect more than one animal, e.g. *Salmonella* spp. can infect humans, cattle, pigs and birds. In this study, *Salmonella* spp. was found inside some but not all of the prepupae analysed. In samples where it was found, the concentration was very low. Nonetheless, an additional treatment step, such as drying, is recommended.

4. Conclusion

BSF larvae accelerated the reduction of *Salmonella* spp. in faeces, suggesting that BSF treatment removes zoonotic Enterobacteriaceae and thus decreases the risk of disease transmission to animals and humans when treatment residues are used as fertiliser in agriculture.

No significant impact on the change in concentrations of *Enterococcus* spp. and ΦX174 over time was established. Furthermore, BSF larvae were demonstrated to have no impact on the destruction/inactivation of *A. suum* ova. Therefore if the treatment residues are intended for use as a fertiliser for food crop production, an additional treatment step, e.g. ammonia sanitisation, is recommended.

Before using the prepupae in animal feed, further processing, such as drying, would be required.

Acknowledgement

The study was supported by the Swedish Ministry of Foreign Affairs as part of its special allocation on global food security. Particular thanks to Alex Johansson for all his help in the laboratory.

References

Albihn A, Vinnerås B. Biosecurity and arable use of manure and biowaste — treatment alternatives. Livest Sci 2007;112(3):232–9. [12th International Conference of the Ramiran-Network SEP 11-13, 2006 Aarhus, Denmark].
Anderson TJC, Zizza CA, Leche GM, Scott ME, Solomons NW. The distribution of intestinal helminth infections in a rural village in Guatemala. Mem Inst Oswaldo Cruz 1993;88(1):53–65.
Asomani-Boateng R, Haight M. Reusing organic solid waste in urban farming in African cities — a challenge for urban planners. Third World Plan Rev 1999;21(4):411–28.
Bondari K, Sheppard DC. Soldier fly larvae as feed in commercial fish production. Aquaculture 1981;24:103–9.
Bradley GH. *Hermetia illucens* L. A pest of sanitary privies in Louisiana. J Econ Entomol 1930;23(6):1012–3.
Bradley SW, Sheppard DC. Housefly oviposition inhibition by larvae of *Hermetia illucens*, the black soldier fly. J Chem Ecol 1984;10(6):853–9.
Carneiro FF, Cifuentes E, Tellez-Rojo MM, Romieu I. The risk of *Ascaris lumbricoides* infection in children as an environmental health indicator to guide preventive activities in Caparao and Alto Caparao, Brazil. Bull World Health Organ 2002;80(1):40–6.
Diener S, Zurbrugg C, Tockner K. Conversion of organic material by black soldier fly larvae: establishing optimal feeding rates. Waste Manag Res 2009;27(6):603–10.
Diener S, Studt Solano N, Roa Gutiérrez F, Zurbrügg C, Tockner K. Biological treatment of municipal organic waste using black soldier fly larvae. Waste Biomass Valorization 2011a;2(4):357–63.
Diener S, Zurbrügg C, Gutiérrez DH, Nguyen-Viet H, Morel A, Koottatep T. Black soldier fly larvae for organic waste treatment — prospects and constraints. In: Alamgir M, Bari QH, Rafizul IM, Islam S, Sarkar G, Howlader MK, editors. Proceedings of the WasteSafe — 2nd International Conference on Solid Waste Management in the Developing Countries. Khulna, Bangladesh; 2011b.
Erickson MC, Islam M, Sheppard C, Liao J, Doyle MP. Reduction of *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Enteritidis in chicken manure by larvae of the black soldier fly. J Food Prot 2004;67(4):685–90.
Eriksen L, Andreasen P, Ilsoe B. Inactivation of *Ascaris suum* eggs during storage in lime treated sewage sludge. Water Res 1996;30(4):1026–9.
Fluitman F. Training and Work in the Informal Sector of Developing Countries: Issues and Good Practice. Tech. rep., International Labour Organization; 2000.
IMF. Fishmeal prices; April 2013. http://www.quandl.com/IMF-International-Monetary-Fund/PFISH_USD-Fishmeal-Price, Last visited 26/4/2013.
Johnson PW, Dixon R, Ross AD. An in-vitro test for assessing the viability of *Ascaris suum* eggs exposed to various sewage treatment processes. Int J Parasitol 1998;28(4):627–33.
Liu Q, Tomberlin JK, Brady JA, Sanford MR, Yu Z. Black soldier fly (Diptera: Stratiomyidae) larvae reduce *Escherichia coli* in dairy manure. Environ Entomol 2008;37(6):1525–30.
Mara D, Hamilton A, Sleigh A, Karavarsamis N. Discussion paper: options for updating the 2006 WHO guidelines. Tech. rep., World Health Organization; 2010.
May B. The occurrence in New Zealand and the life history of the soldier fly *Hermetia illucens* (L.) (Diptera: Stratiomyidae). N Z J Sci 1961;4:55–65.
Morella E, Foster V, Banerjee SG. Climbing the ladder: the state of sanitation in sub-Saharan Africa. Tech. rep., Africa Infrastructure Country Diagnostic (AICD); 2008.
Murray A, Cofie O, Drechsel P. Efficiency indicators for waste-based business models: fostering private-sector participation in wastewater and faecal-sludge management. Water Int 2011;36:505–21.

Myers HM, Tomberlin JK, Lambert BD, Kattes D. Development of black soldier fly (Diptera: Stratiomyidae) larvae fed dairy manure. Environ Entomol 2008;37(1):11–5.
Newton GL, Booram CV, Barker RW, Hale OM. Dried *Hermetia illucens* larvae meal as a supplement for swine. J Anim Sci 1977;44(3):395–400.
Nguyen-Viet H, Zinsstag J, Schertenleib R, Zurbrügg C, Obrist B, Montangero A, et al. Improving environmental sanitation, health, and well-being: a conceptual framework for integral interventions. Ecohealth 2009;6(2):180–91.
Nordin A, Nyberg K, Vinnerås B. Inactivation of *Ascaris* eggs in source-separated urine and feces by ammonia at ambient temperatures. Appl Environ Microbiol 2009;75(3):662–7.
Parnes A, Lagan KM. Larval therapy in wound management: a review. Int J Clin Pract 2007;61(3):488–93.
Pell AN. Manure and microbes: public and animal health problem? J Dairy Sci 1997;80(10):2673–81.
R Development Core Team. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.; 2011 [URL http://www.R-project.org].
Sahlström L, Aspan A, Bagge E, Danielsson-Tham ML, Albihn A. Bacterial pathogen incidences in sludge from Swedish sewage treatment plants. Water Res 2004;38(8):1989–94.
Sheppard DC, Newton GL, Thompson SA, Savage S. A value added manure management system using the black soldier fly. Bioresour Technol 1994;50(3):275–9.
St-Hilaire S, Cranfill K, McGuire MA, Mosley EE, Tomberlin JK, Newton L, et al. Fish offal recycling by the black soldier fly produces a foodstuff high in omega-3 fatty acids. J World Aquacult Soc 2007a;38(2):309–13.
St-Hilaire S, Sheppard C, Tomberlin J, Irving S, Newton L, McGuire M, et al. Fly prepupae as a feedstuff for rainbow trout, *Oncorhynchus mykiss*. J World Aquacult Soc 2007b;38(1):59–67.
Tomberlin JK, Sheppard DC, Joyce JA. Selected life-history traits of black soldier flies (Diptera: Stratiomyidae) reared on three artificial diets. Ann Entomol Soc Am 2002;95(3):379–86.
Tomberlin JK, Adler PH, Myers HM. Development of the black soldier fly (Diptera: Stratiomyidae) in relation to temperature. Environ Entomol 2009;38(3):930–4.
UN-HABITAT. Solid waste management in the world's cities: water and sanitation in the world's cities 2010. Tech. rep., United Nations Human Settlements Programme; 2010.
Vinnerås B. Comparison of composting, storage and urea treatment for sanitising of faecal matter and manure. Bioresour Technol 2007;98(17):3317–21.
Wang J, Han L, Li S. The collection system for residential recyclables in communities in Haidian District, Beijing: a possible approach for China recycling. Waste Manag 2008;28(9):1672–80.
WHO. WHO guidelines for the safe use of wastewater, excreta and greywater — volume 4: excreta and greywater use in agriculture. WHO press; 2006.
WHO, UNICEF. Global water supply and sanitation assessment 2000 report. Tech. Rep. 92 4 156202 1, World Health Organisation and United Nations Children's Fund; 2000.
Winker M, Vinnerås B, Muskolus A, Arnold U, Clemens J. Fertiliser products from new sanitation systems: their potential values and risks. Bioresour Technol 2009;100(18):4090–6.
Zurbrügg C. Urban solid waste management in low-income countries of Asia — how to cope with the garbage crisis. Urban solid waste management review session; 2002.
Zurbrügg C, Gfrerer M, Ashadi H, Brenner W, Küper D. Determinants of sustainability in solid waste management — the Gianyar Waste Recovery Project in Indonesia. Waste Manag 2013:2126–33.