DISS. ETH NO. 19330

Valorisation of Organic Solid Waste using the Black Soldier Fly, *Hermetia illucens*, in Low and Middle-Income Countries

A dissertation submitted to

ETH ZURICH

for the degree of

Doctor of Sciences

presented by

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2010

This Ph.D. thesis was conducted at the Swiss Federal Institute of Aquatic Science and Technology (Eawag) in Dübendorf and was financed by the Velux Stiftung

Illustrations title page: Schremmer 1986

Dank – Acknowledgments – Agradecimientos

An erster Stelle möchte ich mich bei meinem direkten Betreuer Chris Zurbrügg bedanken. Er machte es möglich, dass ich mich während vier Jahren intensiv mit organischem Abfall und Fliegenmaden beschäftigen konnte. Eine Thematik, wie sie meinen Interessen und Kenntnissen nicht besser angepasst hätte sein könnte. Seine präzisen Fragen im saloppen Stil und seine immense Erfahrung im Entwicklungskontext trugen massgeblich zur Qualität meiner Arbeit und auch zu meiner beruflichen Entwicklung bei. Ausserdem stellte er sicher, dass ich neben der Kopfarbeit auch körperlich herausgefordert wurde, sowohl auf dem Surfbrett in den pazifischen Wellen als auch auf dem Badmintonfeld in Stettbach. Einen besonderen Dank richte ich an meine beiden Doktorväter Klement Tockner und Jukka Jokela. Klement hat diese Arbeit sehr gut begleitet und mir immer wieder wichtige inhaltliche Impulse gegeben. Jukka kam durch seine späte Involvierung ins Projekt leider die undankbare Aufgabe zu, erst das Endprodukt beurteilen zu dürfen/müssen. Für das entgegengebrachte Vertrauen und den thematischen Freiraum bin ich ihnen sehr dankbar.

I would also like to thank Jeff Tomberlin, THE authority when it comes to Black Soldier Flies. I am very lucky that he shared his knowledge so openly with me and I am honoured that he accepted to be my external co-examiner.

En Costa Rica podía contar con el apoyo incondicional de Floria Roa Gutiérrez. Como supervisora estaba siempre dispuesta a escucharme. También estableció la base para contactos importantes y encuentros interesantes con instituciones locales y centros de investigación. Además me gustaría expresar mi agradecimiento a Marian Pérez Gutiérrez y Elías Rosales por su hospitalidad, gran interés en mi trabajo y discusiones estimulantes. El departamento de Ingeniería Agrícola del Instituto Tecnológico de Costa Rica, encabezado por Milton Solórzano, y la Escuela de Agricultura de la Región Tropical Húmeda (Universidad EARTH) han contribuido de manera significativa al éxito de mi trabajo. Edgar Alvarado, quien siempre trató mis preocupaciones con suma paciencia y abrió de par en par las puertas del campus para mí, merece mención especial. También doy las gracias a Johan y Annie Perret, que siempre crearon con su personalidad afectuosa y hospitalidad las condiciones ideales para distracciones ocasionales. El trabajo en el larvario no hubiera sido posible sin la ayuda de Nandayure Studt Solano. Su dedicación e ideas han contribuido en gran medida al éxito de los experimentos.

I am grateful to Eawag's Department of Water and Sanitation in Developing Countries (Sandec) for letting me carry out my research in an always inspiring and challenging atmosphere. My repeated thanks go to Caterina Dalla Torre, who looked after my fly colony during my absence abroad despite the unpleasant odours; and to Sylvie Peter for transforming my written output into a literary masterpiece.

In this context, I gratefully acknowledge the financial support of VELUX FOUNDATION, which allowed me to focus exclusively on my work.

Schliesslich möchte ich mich bei meiner Familie für ihre Geduld und ihre moralische Unterstützung im Allgemeinen und bei Petra im Speziellen bedanken. Du begleitest mich in die verregnetste Stadt Lateinamerikas, zeigst Verständnis wenn ich in Hundekadavern am Strassenrand stochere, baust mich auf wenn meine Maden einer Zinkvergiftung erliegen und die Gespräche mit Dir sind immer wieder höchst inspirierend.

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Summary

In recent years, solid waste management awareness in low and middle-income countries has gradually increased, resulting in improved collection coverage and reduced dumpsites and landfills. Especially valorisation of recyclables enjoys a growing popularity, be it through a motley crew of scavengers or via official recycling services run by local government authorities. However, since the organic fraction of municipal organic waste is still considered less valuable than other waste products, such as paper, glass or metal, it is often excluded from this value-added chain. Despite its energy content, it ends up on streets or accumulates on dumpsites, where it attracts vector diseases and produces greenhouse gases. The organic waste treatment technology, using larvae of the black soldier fly, *Hermetia illucens*, paves the way to a financially sustainable treatment option, assigning organic refuse its inherent value to compete against other recyclables. The feeding activity reduces up to 80% of the biomass of organic waste products, such as market/kitchen waste, animal manure or even human faeces. The last larval stage, the so-called prepupa, consists of ~40% protein and ~30% fat, making it a valuable alternative to fishmeal in animal feed.

Past research, though sound and consistent, has neglected the potential use of *H. illucens* as municipal organic waste treatment option with focus on developing countries. The objectives of this thesis were therefore to broaden the knowledge of the biological and physico-chemical processes during the life cycle of *H. illucens,* with special focus on heavy metal contamination of municipal organic waste. To this end, laboratory experiments under controlled conditions were conducted, *inter alia,* using standardised feed spiked with different concentrations of cadmium, lead, and zinc to assess potential restrictive uses of the prepupae in animal feed in order to preclude bioaccumulation. Furthermore, the study also acquired experience in field application of this technology by in situ experiments in Costa Rica.

The laboratory experiments confirmed the larvae's expected potential to reduce waste and produce protein. Moreover, the results obtained in Costa Rica even surpassed our expectations. Compared to about 40% household waste reduction obtained in the laboratory, the in situ experiments reached a 65% to 75% waste reduction. Even prepupal weight measured under field conditions was 32% higher than in the laboratory. The experiments on the fate of heavy metals revealed different accumulation patterns dependent on the existing heavy metals, i.e. cadmium was accumulated, lead suppressed and zinc was kept at a constant level.

The study confirmed the potential of the black soldier fly to act as an ecological engineer in low and middle-income countries. Municipal organic waste provides a suitable food source for prepupae production, thereby allowing management of a healthy soldier fly colony under appropriate environmental conditions. However, since this technology also revealed deficiencies, especially its susceptibility to disruptive factors, further research will be necessary. Sound information on the biological treatment processes and environmental conditions required by these insects constitute the greatest challenge to achieving enhanced performance and financial gain from the treatment facility. This will strengthen resilience of the treatment plant and waste management plan in which the BSF unit is embedded.

Zusammenfassung

Die Situation im Bereich des Abfallmanagements in Schwellen- und Entwicklungsländern hat sich in den letzten Jahren massgeblich verbessert. Vor allem wiederverkäufliche Materialien landen heute anstatt auf improvisierten Müllkippen oder im Strassengraben öfters in Recyclingzentren, sei es durch lose organisierte Truppen von Müllsammlern, oder durch offizielle Sammeldienstleistungen der Gemeinden. Allerdings wird dem organischen Anteil im Haushaltsabfall oft jeglicher Wert abgesprochen und es werden ausschliesslich „wertvolle“ Materialien wie Papier, Glas oder Metall eingesammelt. Trotz seines Energiegehalts bleibt organischer Müll in Strassen, Wasserläufen oder Abfallhalden liegen, wo er Krankheitsvektoren anzieht und durch unkontrollierte anaerobe Prozesse Klimagase produziert. Die Methode, organische Wertstoffe mittels Larven der Waffenfliege, *Hermetia illucens*, in ein protein- und fettreiches Produkt umzuwandeln, gibt diesem bisher vernachlässigten Anteil des Haushaltabfalls einen Wert und ermöglicht auch für organische Abfälle eine ökonomisch nachhaltige Abfallwirtschaft. Die Fressaktivität der Larven reduziert Abfallstoffe wie Markt- und Küchenabfälle, Tiermist oder sogar menschliche Fäkalien um bis zu 80%. Das letzte Larvenstadium, die sogenannte Prä-Puppe, besteht aus ca. 40% Protein und ca. 30% Fett und stellt dadurch eine wertvolle Alternative zum heute in Tierfutter verwendeten Fischmehl dar.

Die bisherige Forschung, wenn auch fundiert und solide, hat das Potential von *H. illucens* als Abfallveredler in Schwellen- und Entwicklungsländern vernachlässigt. Das Ziel der vorliegenden Arbeit war daher, die wissenschaftlichen Grundlagen bezüglich der biologischen und physikalisch-chemischen Prozesse im Lebenszyklus von *H. illucens* zu erweitern. Ein spezieller Fokus lag dabei auf einer potentiellen Akkumulierung von in Haushalt- und Marktabfällen vorhandenen Schwermetallen in den Prä-Puppen sowie auf der Aufskalierung der Technologie vom Labormassstab zur Pilotanlage unter Feldbedingungen. Zu diesem Zweck wurden Laborversuche unter kontrollierten Bedingungen durchgeführt, u.a. mit standardisiertem Futter, welches mit Cadmium, Blei und Zink in verschiedenen Konzentrationen versetzt wurde. Ausserdem wurden in Costa Rica durch Feldversuche mit organischen Haushaltsabfällen neue Erkenntnisse gesammelt.

Die Laborversuche bestätigten das Potential der Larven, die Masse organischer Abfälle signifikant zu reduzieren und daraus eine beträchtliche Menge proteinreicher Prä-Puppen zu produzieren. Diese ermutigenden Laborergebnisse wurden durch die Feldversuche in Costa

Rica sogar noch übertroffen. Im Vergleich zu der im Labor erzielten Trockengewichtsreduktion von 40%, erreichten die Feldexperimente eine Abfallverminderung von 65% bis 75%. Selbst das Gewicht der Prä-Puppen fiel rund 32% höher aus als im Labor. Die Schwermetallversuche ergaben, abhängig vom verwendeten Element, unterschiedliche Akkumulationsmuster. So wurde Cadmium in den Prä-Puppen aufkonzentriert, Blei hingegen unterdrückt und Zink wurde auf einem mehr oder weniger konstanten Niveau gehalten.

Die Studie bestätigte das Potenzial von *Hermetia illucens,* in Schwellen- und Entwicklungsländern organische Siedlungsabfälle in Wertstoffe umzuwandeln. Selbst dieses heterogene Substrat stellt ein geeignetes Futter für eine Prä-Puppen-Produktion dar, vorausgesetzt die äusseren Umstände, wie z.B. Temperatur, Luftfeuchtigkeit oder Fütterungsplan, entsprechen den Bedürfnissen der Tiere. Allerdings hat die Studie auch Schwachstellen dieser Technologie aufgezeigt. Vor allem die Anfälligkeit gegenüber Störfaktoren verlangt nach fundierterem Wissen und wirft neue Forschungsfragen auf, denn: eine stabile, resiliente Population ist die Voraussetzung für eine konstante Abfallabbauleistung und eine ergiebige Prä-Puppenernte und liefert die Basis für eine ökonomisch attraktive Abfallbewirtschaftung in Entwicklungsländern.

1

General Introduction

Waste management in developing countries

Due to rapid urbanisation, changes in demographics and consumer behaviour, municipalities and decision makers are confronted with new challenges in solid waste management. Over the past decade, numerous cities have increased their efforts at finding sustainable solid waste management solutions, especially in developing integrated solid waste management strategies, including construction and operation of sanitary landfills. To cover part of the increasing waste management costs, it comes as no surprise that scavenging, or in other words, valorising recycling activities, has turned into an income-generating activity conducted by the formal resource management authorities or performed jointly with the informal sector. In Ankara, Turkey, for example, scavengers collect and sell to middle men 50% of the recyclables produced by households, commerce and trade, yielding a total of about USD 50,000/day (Ali, 2002), and at least 150,000 waste pickers throughout Delhi's waste management system divert more than 25% of all waste generated into recyclables, thus saving the municipal authorities substantial costs (UN-HABITAT, 2010).

Scavenging comprises more than just waste picking and is generally regarded as an "income-generating activity of separating recyclables from waste products", is either performed by waste pickers working and living on landfills (Figure 1-1), by scavengers rummaging through garbage bags on streets or by household owners separating their waste before selling it to itinerant waste buyers. In waste management systems based on a pay-as-you-throw principle, the definition of "waste scavenging" can even comprise attempts by the households to refrain from buying superfluous packaging material or may include products, which basically lead to minimising the disposal fee and, thus, to income generation. Waste scavenging can therefore be considered the backbone of sustainable, integrated solid waste management. Since it is based on a purely money-driven process, it is not surprising that waste products already utilised in an existing market have a higher recycling rate than products to date regarded as worthless, such as the municipal organic waste fraction. In Quezon City, Philippines, for example, 33% (240,000 tonnes) of the total annual waste generated is reused by the formal and informal sectors. However, the recycled organic fraction accounts for only 14,000 tonnes (UN-HABITAT, 2010) – an example typical of countless municipalities in developing countries. The main reason for this lack of interest is the effort required to collect, separate and transform organic waste, eventually leading to a relatively small profit. With compost, for example, the product generated is handicapped by high transport costs in relation to product value, a competitive chemical fertiliser market or unfair regulations and policies (e.g. subsidies for chemical fertilisers) (Rouse et al., 2008). Generation of biogas from municipal organic waste is also a widespread technology at household level, particularly in India and China. However, economically viable middle and large-scale projects are often hindered by a lack of framework conditions for use and sale of the gas itself or its secondary products (electricity, heat). Efforts are therefore necessary to impart not only an idealistic value to the organic fraction, focusing on its nutrient recovery and environmental protection potential, but also to emphasise its inherent economic value.


Figure 1-1: Waste scavenger on a municipal landfill in Siquirres, Costa Rica

Waste treatment by black soldier fly larvae

Conversion of organic waste by larvae of the black soldier fly, *Hermetia illucens* L. (Diptera: Stratiomyidae) into versatile prepupae is an interesting recycling technology, with a potential to give waste the aforementioned value.

The black soldier fly, *Hermetia illucens*, is widespread in tropical and warmer temperate regions between about 45°N and 40°S (Üstüner et al., 2003). Its larvae feed on different decaying organic material, such as rotting fruits and vegetables, animal manure and human excreta. The last larval stage, the so-called prepupa, migrates from the feed source in search of a dry and protected pupation site. Pupation occurs within the larval skin and the adult emerges after about 14 days (Figure 1-2). The adults are rather lethargic and poor flyers. Females mate two days after emerging and oviposit into dry cracks and crevices adjacent to a feed source. Due to the relatively long period between oviposition and eclosion (3–4 days), eggs are never laid directly onto the moist rotting material. During its adult stage, *H. illucens* does not feed and relies solely on its body fat reserve. Consequently, the fly does not come into contact with any degrading or fresh organic material including foodstuffs, and can therefore not be regarded as unsanitary or a vector of diseases.

Once hatched, larvae start to feed on the waste, thus achieving a dry mass volume waste reduction of ~55% (Myers et al., 2008; Newton et al., 1995; Sheppard, 1983). Due to high larval densities and the voracious appetite of the larvae, fresh material is processed extremely fast and bacteria growth suppressed or restrained, thereby reducing production of bad odour to a minimum.

An additional advantage of *H. illucens* is its capacity to repel oviposition of female house flies (Bradley & Sheppard, 1984), a serious disease vector especially in developing countries, where open defecation and inappropriate sanitation account for dangerous

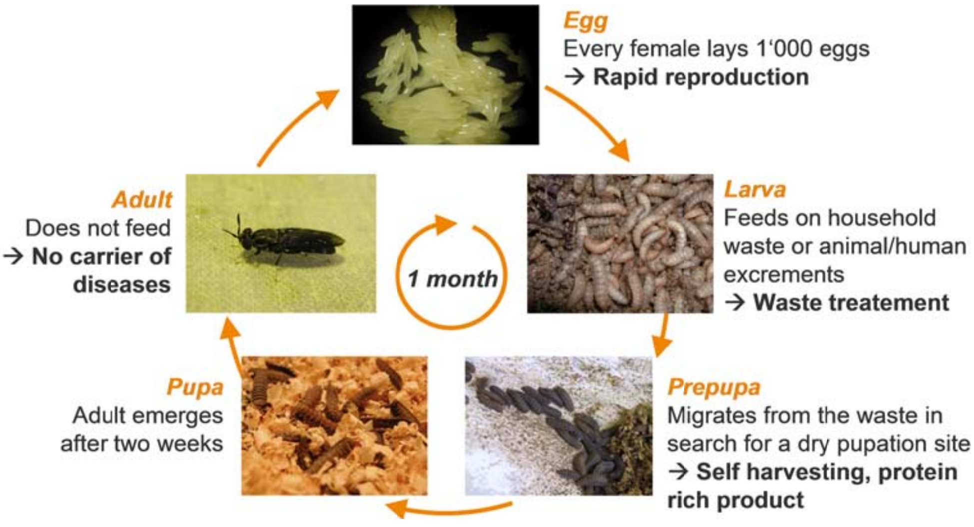

Figure 1-1: Life cycle of the black soldier fly, *Hermetia illucens*

sources of pathogens (Graczyk et al., 2001). Under ideal conditions with abundant food sources (i.e. waste deposits), larvae can mature in two weeks. However, food shortage and low temperatures can extend the larval period up to four months (Furman et al., 1959). This great flexibility in their life cycle can be very helpful in managing populations during periods of waste shortage and in storing larvae to incubate a new population.

The prepupae, the last immature stage, show a pronounced migratory habit. They need to leave the food source to successfully pupate into adult flies. At this stage, the prepupae are at their maximum size, exhibiting large protein (36–48%) and fat (31–33%) contents to sustain them through metamorphosis (Hale, 1973; St-Hilaire et al., 2007b; Stamer, 2005). This final instar shows slight morphological changes compared to the feeding larva. Its labrum, for example, is bent down like the beak of an eagle. It is used as a hook to pull them to a suitable pupation site (Schremmer, 1986). In facilities designed for waste management and prepupae collection, larvae climb up a ramp (30–45°) out of a rimmed container to eventually end in a collecting vessel attached to the end of the ramp. The yield of these energy-rich prepupae renders this technology so appealing to waste managers.

Historical divagation

The black soldier fly's propensity to revolutionise waste management as an ecological engineer was already foreseen as early as 1916. L.H. Dunn describes the corpse of a young man found in the Canal Zone (today known as Panama). He writes: *"When found, the remains were covered with the long dark larvae of* H. illucens*. They were in such great numbers that some parts of the body, and even places on the sodden clothing, were covered with crawling masses of larvae almost half an inch deep"* (Dunn, 1916). A few years later, G.H. Bradley not only described another favorite food source, but also portrayed the prepupae's migratory habit as follows: *"The flies … were breeding in the privy pits, and whenever a seat cover was raised they would come out in swarms. In some cases a few sluggish individuals would crawl unnoticed into the underwear of the person using the privy"* (Bradley, 1930). However, first experiments did not focus mainly on waste reduction but more on inhibiting house fly oviposition in poultry manure (Furman et al., 1959). Others did not recognise the potential of this fly and tried to eradicate it for rather odd reasons: *"The actions of the larvae in the manure produce an unsightly condition, increase the problem of unpleasant odors, and sometimes cause the manure to spread onto the walkways. … Therefore, data were obtained on the effectiveness of common larviciding chemicals against the larvae of* H. illucens *in poultry manure"* (Axtell & Edwards, 1970). Fortunately, O.M. Hale took up the cudgels for the black soldier fly and eventually described the promising effects of *Hermetia illucens* for waste management and protein production: *"It is certainly plausible to assume that this common soldier fly can be used to convert waste materials into usable, high quality nutrient supplements. Such biological degradation and recycling of organic waste materials could be one way of alleviating some of the problems of waste disposal"* (Hale, 1973).

Products

Today, animal feed production strongly relies on protein and fat derived from forage fishery. In 2002, fishmeal and fish oil were primarily used worldwide for intensive food production, i.e. 24% for pigs, 22% for poultry and 46% for aquaculture (Alder et al., 2008). However, civil society and retailers are increasing their pressure on aquaculturists and poultry farmers to improve overall sustainability of fishery resources. Consequently, diminishing global supplies of wild forage fish and rising market prices for fishmeal have prompted the animal feed industry in recent years to look for alternative protein sources. Research aimed at finding new raw materials to replace protein began in the 1970s and has developed considerably in recent years. Various protein sources have been tested as replacements with ambiguous results. For example, single cell organisms (algae, bacteria, yeast) and most slaughterhouse waste lack certain essential amino acids. Moreover, slaughterhouse waste exhibits low palatability. The main disadvantages of plant sources are their low protein percentage and high fibre and carbohydrate content (Sanz et al., 2000).

However, early studies with *Hermetia illucens* larvae and prepupae fed to poultry, pig and fish revealed promising results with regard to replacing fishmeal (Bondari & Sheppard, 1987; Hale, 1973; Newton et al., 1977). A recent study by St-Hilaire et al. (2007b) working with rainbow trout, *Oncorhynchus mykiss*, endorsed these early findings but also found that the fish contained reduced levels of omega-3 fatty acids. Newton et al. (2005b) even replaced 50% of commercial fish food with prepupae without negative effects on the growth of channel catfish fingerlings. However, all authors identified the need for improvement concerning the formulation of the feed, especially as regards the protein, fat and fibre ratio. However, the different compounds (protein, fat, chitin) could be fractionated and sold separately instead of using prepupae as one product. This would not only make formulation of animal diets easier, the different fractions could also be sold on specific markets, fetching higher prices. Newton et al. (2005a) propose, for example, to extract the fat and convert it into biodiesel. They state that if the oil from soldier fly prepupae raised on pig manure were converted into biodiesel, it would yield as much energy as methane production from the same amount of manure.

Chitin is a further valuable fraction. The cuticle of insects is composed of chitin in a matrix with cuticular protein, lipids and other compounds. Chitin is of commercial interest due to its high percentage of nitrogen (6.9%) compared to synthetically substituted cellulose (1.25%). This makes chitin a useful chelating agent for products in medicine, cosmetics and even biotechnology (Kumar, 2000). Yet, the economic feasibility of extracting chitin from soldier fly prepupae has still to be assessed.

Besides the yield of prepupae, the black soldier fly treatment process generates a second product, i.e. the residue or digestate. Larval and bacterial activity reduces not only the dry mass but also several nutrient contents such as nitrogen or phosphorus. In pig manure, 80.5% of total nitrogen and 75.7% of phosphorus are removed (NC State University, 2006). Experiments with cow manure showed a nitrogen reduction of 43%, and 67% of the phosphorous was transformed into larval biomass (Myers et al., 2008). A possible designated use of this residue is application in agriculture, similar to compost or subsequent processing in a biogas facility. However, first growing experiments with larva residue from pig manure did not reveal promising results pertaining to performance of basil (*Ocimum basilicum*) and Sudan grass (*Sorghum sudanese*) grown on mixtures of BSF residue with either clay or sand (Newton et al., 2005a). The question is to what extent can the product “residue” contribute to the revenue of a soldier fly treatment facility, or should it be regarded as a necessary evil whose impact on the environment has to be kept to a minimum.

Research gaps

Combination of waste treatment capacity together with generation of a valuable product makes the black soldier fly technology a highly promising tool for waste management in low and middle-income countries. It offers small entrepreneurs the possibility of income generation without high investment costs, and concurrently reduces the environmental impact. Yet, despite ample research on various topics geared to commercial waste treatment using *H. illucens*, initiatives for upscaling this technology are scarce and only few studies centre on developing countries (Hem et al., 2008; Lardé, 1990b) or examine food scraps or household waste as a feed source (Newby, 1997; Warburton & Hallman, 2002).

The objectives of this thesis are therefore to:

i) understand the key biological and physico-chemical processes during the life cycle of the black soldier fly, *Hermetia illucens*, especially its applicability in a waste processing unit.

ii) assess possible obstacles and limitations in the context of low and middle-income countries.

iii) facilitate replication and dissemination of this technology by acquiring experience with medium-scale studies in Costa Rica in close collaboration with local researchers and implementation partners.

Consequently, chapter 2 looks into the question of perfecting the waste treatment process. To optimise this technology, a balance has to be found between providing the larvae with enough food to achieve a high prepupal weight and preventing an overload of the system to ensure proper waste stabilisation and high waste reduction.

Chapter 3 examines the faith of heavy metals present in organic waste in developing countries. To assess the risk of bioaccumulation and transfer of heavy metals along the food chain, soldier fly larvae were fed contaminated food.

Finally, chapter 4 makes the leap from lab-scale experiments to medium-scale trials under natural environmental conditions in Costa Rica. Some 50 kg of municipal organic waste was transformed daily into prepupal biomass, thereby meeting our expectations concerning the significant potential of this technology, but also shows its limitations and reveals other questions requiring further research.

2

Conversion of Organic Material by Black Soldier Fly Larvae – Establishing Optimal Feeding Rates

:: abstract

Larvae of the black soldier fly, Hermetia illucens (Diptera: Stratiomyidae), are voracious feeders of organic material and may thus be used in simple engineered systems to reduce organic waste in low and middle-income countries. Controlled feeding experiments with standard fodder were conducted to assess the optimum amount of organic waste to be added to a CORS system (Conversion of Organic Refuse by Saprophages). A daily feeding rate of 100 mg chicken feed (60% moisture content) and larva resulted in an optimum trade-off between material reduction efficiency (41.8%, SE 0.61) and biomass production (prepupal dry weight: 48.0 mg, SE 2.0). Applied to market waste and human faeces, this corresponds to a potential daily feeding capacity of 3–5 kg/m2 and 6.5 kg/m2, respectively. In addition, H. illucens prepupae quality was assessed to determine their suitability to substitute fishmeal in animal feed production. The chitin-corrected crude protein content ranged from 28.2 to 42.5%, depending on the amount of food provided to the larvae. Based on our study, a waste processing unit could yield a daily prepupal biomass of 145 g (dry mass) per m2. We conclude that larvae of the black soldier fly are potentially capable of converting large amounts of organic waste into protein-rich biomass to substitute fishmeal, thereby contributing to sustainable aquaculture.

This chapter has been published as: **Diener, S.**, Zurbrügg, C., Tockner, K., 2009, Conversion of organic material by black soldier fly larvae – Establishing optimal feeding rates. *Waste Management and Research*, 27: 603–610

:: introduction

Low waste collection coverage and inadequate treatment of waste lead to unbearable conditions in villages and cities of low and middle-income countries. A global survey by the United Nations Development Program (UNDP) in 151 cities revealed that poor solid waste disposal faced by city dwellers was considered the second most serious problem after unemployment (UNDP, 1997). It impacts human health, the environment, and is also a major obstacle to economic development. In contrast, well functioning informal waste management systems are inherently linked to job opportunities and income generation. Specifically, recycling of inorganic material (e.g. glass, metal, plastics) from municipal solid waste is already an important source of income and employment of the informal sector - especially in low and middle-income countries (e.g. Ali, 1999; WASTE & Skat, 2006). However, one to two thirds of the solid waste is not collected in developing countries. In particular, organic waste reuse is still in its embryonic stage despite its high recovery potential. Organic material can make up more than 50% of the total waste production (e.g., Henry et al., 2006; Ojeda-Benitez et al., 2003; Sharholy et al., 2006). For lack of alternatives, solid waste as well as human and animal excreta are often dumped arbitrarily in streets and drains. Heavy rainfall may clog the drains and lead to urban and suburban flooding. Furthermore, organic waste deposits will not only form breeding places for disease transmitting insects and rodents, but also cause odour nuisance. Given the large fraction of organic waste, simple and efficient collection and organic waste treatment systems may significantly mitigate these problems and enhance recycling of organic matter and nutrients.

Conversion of Organic Refuse by Saprophages (CORS) has been an issue over the past decades (Barnard et al., 1998; Beard & Sands, 1973; El Boushy, 1991; Elissen et al., 2006; Ramos-Elorduy et al., 2002). The most familiar example of CORS is vermicomposting, i.e. the conversion of organic waste by worms and microorganisms into black, earthy-smelling, nutrient-rich humus. Similarly, larvae of *Hermetia illucens* L. (Diptera: Stratiomyidae), better known as the black soldier fly, have been propagated as converters of organic waste, but also as a nutritious feed for chicken, pig breeding and aquaculture (Bondari & Sheppard, 1981; Booram et al., 1977; Lardé, 1990b; Newby, 1997; Newton et al., 1977; Sheppard et al., 1994). This non-pest fly originates from the southern US and has spread throughout the tropics and subtropics. Its larvae feed on organic waste, however, since the adult fly does not take up any food, surviving only on its reserves, it does not pose any disease transmission risks. Mating takes place two days after emergence, and oviposition occurs two days after fertilisation. Larval development requires about two weeks, depending on temperature and food availability. The prepupae crawl out of the organic material in search of a dry pupation site. During this migratory phase, prepupae can easily be harvested by simply constricting their migration paths (Sheppard, 2002; Tomberlin et al., 2002). The prepupae are protein-rich, similar to commercial fishmeal, and may therefore be used as animal feed.

Previous studies focused on the degradation of cow, chicken or pig manure by *Hermetia illucens* larvae in cost and maintenance-intensive systems of high-income countries. However, little information is available on the capability of the larvae to digest municipal organic solid waste (MOSW) or to process agricultural waste (e.g. coffee, banana), as well as excreta and faecal sludge from on-site sanitation facilities (e.g. pit latrines) – all waste products whose disposal and management cause serious challenges in low and middle-income countries. Newby (1997) investigated the activity of *H. illucens* in a compost-type system at household level in Australia. Lardé (1990b) tested the conversion of coffee pulp in a small-scale experiment. Bradley already noted in 1930 that *H. illucens* infested privies (i.e. pit latrines), thus indicating their potential to treat faeces (Bradley, 1930). Though all the aforementioned studies showed promising results, the further development of this technology from an experimental scale into a practicable full-scale plant has not yet been tested.

In fact, organic waste continues to constitute tremendous problems in low and middle-income countries, as no valid solution has yet been identified. Development from experimental to full-scale waste treatment facilities, using the larvae of the black soldier fly, offers several advantages. Since such facilities can be developed and operated at low cost (low building and maintenance costs; independent from power supply), they are more adapted to the economic potential of developing countries. Furthermore, creating additional value chains and generating a surplus income through the sale of harvested prepupae or their use in animal husbandry can strengthen the economic resilience of farmers or small entrepreneurs to natural hazards or market fluctuations. Agricultural studies in Africa revealed that the high cost of feed is an important factor for smallholder poultry production (Sonaiya, 1993). Strategies to counteract high feed prices could consist in switching to other poultry species such as waterfowls, which may be raised with other feed (snails, water hyacinths). Another option would be to supplement the feed with alternative materials produced locally or even by the farmers themselves to ease the financial burden of the smallholder. Prein and Ahmed (2000) pointed out the advantages of integrated agriculture-aquaculture (IAA) systems by providing successful examples from Africa (Malawi, Ghana) and Asia (Bangladesh, Philippines). Ahmed and Lorica (2002) described the positive effects of small-scale aquaculture on household income, employment and consumption. Therefore, the use of a black soldier fly CORS system of appropriate design and feasible operation can meet the requirements of such extensive cultures, as the yield in prepupae can be used directly. In low and middle-income countries, the fly can act as an ecological engineer.

Table 2-1: Relative protein and fat content (% of dry weight) of different feed sources

	Protein	**Crude fat**	**Source**
	%	%	
Insect meal			
Black Soldier Fly, *Hermetia illucens*			
• prepupa	44	33	(St-Hilaire et al., 2007b)
• larva	42–45	31–35	(Booram et al., 1977; Hale, 1973)
House fly, *Musca domestica*			
• pupa	63	15	(Calvert et al., 1969; Ravindran & Blair, 1993)
• larva	38	20	(Ogunji et al., 2007)
Mealworm, *Tenebrio molitor*	48–58	29–38	(Ng et al., 2001; Ramos-Elorduy et al., 2002)
Fishmeal	62–70	8.9–9.3	(Sauvant et al., 2004)
Soybean meal	43–47	1.5–1.9	(Sauvant et al., 2004)

The high protein and fat content of dried soldier fly prepupae reinforces its high potential as flymeal in animal feed production (Table 2-1). A very attractive market for dried soldier fly prepupae is the rapidly growing aquaculture industry. Between 2002 and 2004, this economic activity grew worldwide by 6.1% on average. Some low and middle-income countries such as Chile, Iran or Viet Nam even showed growth rates as high as 11.2%, 16.5% and 30.6%, respectively. Myanmar exhibited the highest growth rate with 40.1% (FAO, 2007). Fishmeal and fish oil are the main food sources for farmed aquatic species. The rapid spread of aquaculture leads not only to an increase in demand for fishmeal derived from wild fish stocks, but to a rise in fishmeal price and pressure on the natural fish populations. Therefore, alternative protein sources, ideally of animal origin, may turn out to be a highly attractive alternative for farmers currently depending on fishmeal. The prepupae of *Hermetia illucens* may serve as such an alternative protein source.

A CORS system combining waste treatment with concurrent protein production has to meet two objectives: i) to achieve a high waste reduction efficiency, and ii) to yield a maximum prepupal biomass. Therefore, it is necessary to determine the maximum amount of waste that can be processed by larvae per day. Waste is considered fully processed when it has been subject to one larval digestion cycle. The resulting residues can be used as nutritious soil amendment in agriculture. Digestion stabilizes the waste, reduces odour emissions as well as fungal and bacterial growth. In addition, for a CORS system to be economically viable, it must generate prepupae of attractive quality and quantity.

This paper experimentally tests the hypothesis that there is a threshold in food supply, where larval dry weight does not increase further and larval development time is no longer reduced by an increase in food supply. The experiments conducted identify the maximum amount of waste larvae can cope with within their development period:

The specific research questions are:

i) What is the daily maximum food quantity (i.e. organic waste) digestible by *Hermetia illucens?* We determine the highest overall material reduction rate in relation to larval development time.

ii) What is the daily optimum food quantity for black soldier fly prepupae to reach maximum dry mass within the shortest development time?

iii) Does the protein content of prepupae differ with varying feeding rates?

:: material and methods

Larvae of the black soldier fly *Hermetia illucens* L. (Diptera: Stratiomyidae) were obtained from a population reared at constant temperature (27 °C, 67% RH) in a container (3 m x 2 m) in Dübendorf, Switzerland. The container was fitted with two windows, as direct sunlight is required for successful mating (Tomberlin & Sheppard, 2002). The larvae were reared on chicken feed (UFA 625) thoroughly mixed with water (60% moisture). All feeding experiments were conducted in a temperature-controlled, insulated container (T = 26 °C (SE: 0.02), RH = 67% (SE 0.14), 12 h photoperiod).

Each treatment (three replicates per treatment) contained 200 larvae (hand counted) fed with five different daily food rates: 12.5, 25, 50, 100, and 200 mg/day/larva (wet weight, 60% moisture content, chicken feed UFA 625, apparent metabolisable energy for poultry = 11.7 MJ/kg), and are henceforth referred to as class 12.5, 25, 50, 100, and 200, respectively. The 6-day old larvae were initially placed onto the prepared food within plastic boxes (14 x 7.5 x 7 cm) and covered by black cardboard to protect them from light disturbance. The lid of the boxes contained holes to allow air circulation. To avoid oviposition of other flies into the experimental box, a mosquito mesh was clamped between box and lid. Food rations were prepared, weighed, and kept frozen 24 h before use.

Sampling and feeding was performed every two to three days. While sampling and feeding, larvae were transferred into another box containing the next food ration. Residual material of the previous box was dried for at least 24 h at 105 °C to determine its dry mass. At each transfer, five randomly selected larvae were removed for further analysis. The selected larvae were weighed (wet mass), lyophilised to measure dry weight and ground for nitrogen analysis (CHNS-O, EA 1108-Elemental Analyser, Carlo Erba). Assuming proteins from animal tissue to contain about 16 percent nitrogen (Jones, 1931), crude protein content was calculated by multiplying the total nitrogen content with the conversion factor 6.25. However, as the nitrogen content in chitin amounts to 6.89%, such a conversion may lead to an overestimation of the calculated protein content. To take this factor into account, the chitin content of the prepupae was determined using the formic acid method (Lovell et al., 1968). The chitin-derived nitrogen content of the prepupae was subtracted from the total nitrogen content before protein calculation.

Prepupae were removed and placed into a box containing wood shavings to allow pupation in a dry environment. Feeding of larvae was continued until more than 50% of the larvae in a box had developed into prepupae. Five prepupae were sampled from each box and treated the same way as the larvae (see above).

The emerging adults were killed using ethyl acetate. Sex was determined and the individuals were lyophilised to assess their dry weight.

To take into account not only the overall material reduction but also the time the larvae require to reduce this amount of food, a waste reduction index (WRI, Eq. 1) was calculated using the overall degradation *D* (Eq. 2) divided by the number of days the larvae fed on the material.

$$WRI = \frac{D}{t} \times 100 \qquad (1) \qquad\qquad D = \frac{W - R}{W} \qquad (2)$$

W represents the total amount of organic material applied during the time *t* and *R* the residue after the time *t*. The factor 100 is used to give the index a practical value. High WRI values indicate good reduction efficiency.

Quantitative nutritional aspects are based on the terminology of Scriber and Slansky (1981): B = (I–F)–M and ECD = B/(I–F), where B = assimilated food used for growth (measured as prepupal biomass), I = total food offered during the experiment, F = residue in experimental boxes (undigested food + excretory products), M = assimilated food metabolised (calculated by mass balance). All figures are given in mg dry weight. ECD = efficiency of conversion of digested food. High ECD values indicate high food conversion efficiency.

One-way ANOVAs, with subsequent Tukey HSD tests were conducted to determine the differences between the various treatments ($P < 0.05$).

Table 2-2: Larval development time (egg to prepupa, in days), total feeding days during the experiment, relative material reduction (%) during the entire experiment, relative waste reduction index (WRI, overall reduction/number of days), and efficiency of conversion of digested food (ECD, %). Daily feeding rates: 12.5, 25, 50, 100, and 200 mg layer hen feed (60% moisture) per larva and day.

		12.5		**25**		**50**		**100**		**200**	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Development time	days	42.2a	0.32	32.7b	0.66	20.1c	0.14	16.6d	0.31	15.9d	0.02
# feeding days	days	36.2	0.19	26.7	0.18	14.1	0.07	10.6	0.07	9.9	0.05
Overall reduction	%	39.7ab	1.10	37.3b	0.55	43.2a	1.41	41.8ab	0.61	26.2c	1.40
WRI		1.1a	0.03	1.3a	0.02	3.1b	0.10	3.8c	0.06	2.4d	0.13
ECD	%	38.0a	0.60	33.3a	1.40	26.2b	2.10	24.4b	0.43	25.8b	1.00

Mean values followed by the same letter in the same row do not vary significantly ($P<0.05$)

:: results and discussion

Waste Reduction Efficiency: A food supply of 12.5, 25, 50, and 100 mg/day/larvae led to a 37.3%–43.2% material reduction (dry weight) (Table 2-2). A supply of 200 mg resulted in a 26.2% reduction. Class 100 showed the highest WRI value, indicating that 100 mg of food per larva and day is the most suitable feeding rate to efficiently reduce waste. Material reduction identified in the present study coincides well with the reported 39–56% cow manure reduction (NC State University, 2006) and the 50% chicken manure reduction (Sheppard et al., 1994)Between 17.1% (class 200) and 32.6% (class 50) of the material fed to the larvae was metabolised (Figure 2-1). Only a small fraction (6.0–16.1%) was transformed into prepupal biomass. The remaining part (55.9–76.9%) was left as residual matter, which had either passed through the gut of the larvae or had not been eaten at all. The efficiency in converting digested food (ECD) averaged 29.6% (SE 1.48, range 24.4–38.0%) (Table 2-2).

The ECD value indicates how efficient *H. illucens* converts food into biomass. Previous data on quantitative food processing by insects unfortunately do not provide data on H. illucens (Slansky & Scriber, 1982; Wiegert & Petersen, 1983). To quantify more accurately the food conversion efficiency of the black soldier fly, its assimilation efficiency (AD) is required (Scriber & Slansky, 1981). This factor indicates the digestibility of a specific food by a certain organism. However, AD has not been determined in this study. Nevertheless, estimates can be used for H. illucens feeding on different waste sources, which can then be used further to adjust operation of a CORS facility.

We should mention that the food added to the feeding boxes may have lost water between individual feeding events. We can, however, not clearly determine to what extent this phenomenon has influenced our results. It is likely that especially the small food rations experienced a stronger dehydration due to the unfavourable surface area to volume ratio, and that the drier food could subsequently not be converted to the same extent than under constant moisture conditions.

Weight Gain and Development Time: Larvae fed with 200 mg chicken feed (wet weight, 60% moisture) per day revealed the highest prepupal dry weight (mean 63.3 mg, SE 6.36).

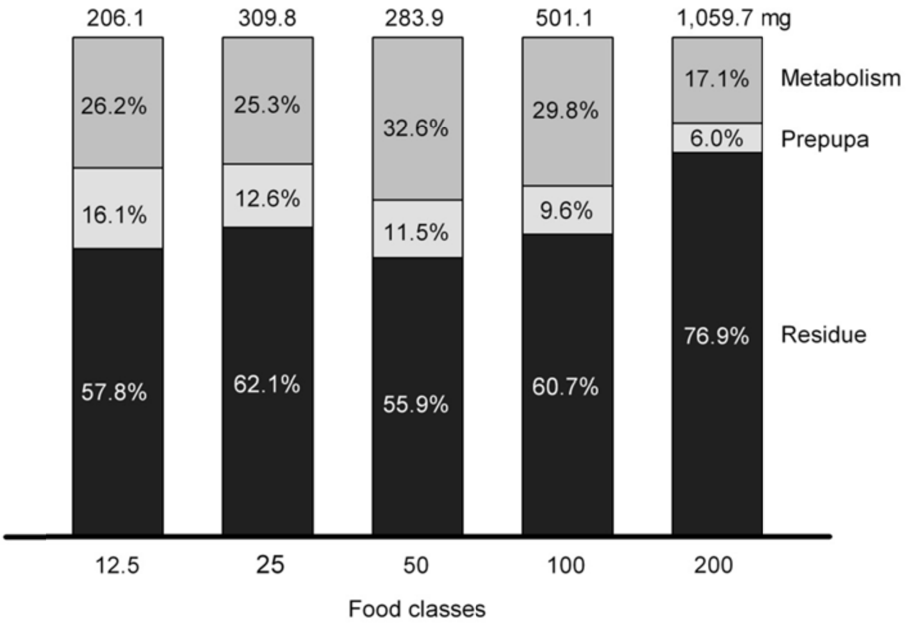

Figure 2-1: The relative proportion and the absolute amount of food (five food classes; mg dry weight) that is converted into biomass (prepupal weight, mg in top row), used for metabolisms, and remains as residuals. Metabolism is calculated as the difference between residue and prepupal weight and the total amount of food provided during the entire development time.

These larvae required 15.9 days (SE 0.02) development time. Larvae fed with 100 mg/day exhibited a prepupal weight of 48.0 mg (SE 2.00) and a development time of 16.6 days (SE 0.31). Food supply of less than 100 mg/day resulted in a similar prepupal weight but in a longer larval development time (Table 2-2 and Figure 2-2). From the life-cycle viewpoint, the feeding rate of 100 mg food/larva/day marks the threshold where additional daily food supply does no longer accelerate larval development time (Figure 2-3).

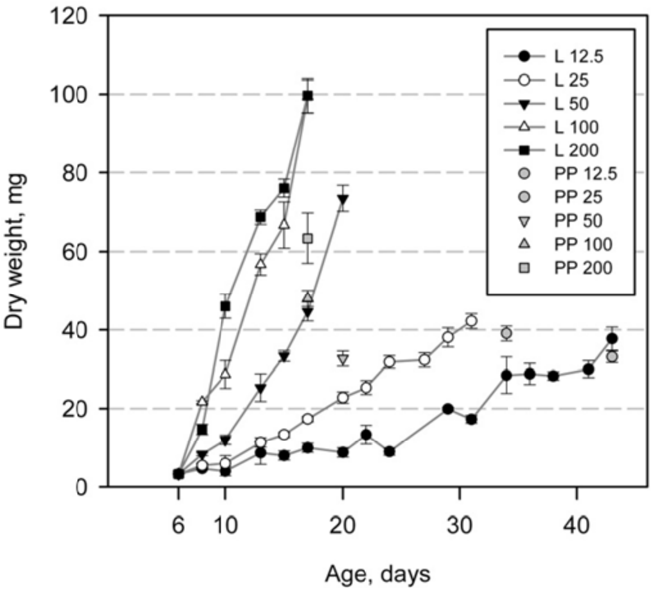

Figure 2-2: Increase in dry weight (mean±SE) of *H. illucens* larvae with development time, and final weight of the prepupae. Five different feeding treatments: 12.5 to 200 mg chicken feed/larva/day (60% moisture). L 12.5–L 200: dry weight of larvae; PP 12.5–PP 200: dry weight of prepupae

In the event of food shortage, the larvae are capable of prolonging their development time. This biological trait may simplify operation of a waste management system, as larvae can cope with varying food supply. The lower threshold, where lack of food eventually leads to the death of the larva, was not determined in this study.

Prepupal dry weight was similar among classes 12.5, 25 and 50 but differed from class 200 (Table 2-3). Our results show that larvae provided with limited food migrate out of the waste as soon as their prepupal weight reaches ~35 mg DW. We can conclude that in the event of food shortage, larvae will feed until they have achieved the minimum energy reserve required to perform pupal development. This critical weight is defined as the minimal weight at which further feeding and growth are not required for a normal metamorphosis and pupation (Nijhout & Williams, 1974). There is a time lag from achieving critical weight to secretion of the prothoracicotropic hormone (PTTH), which causes the larva to stop feeding. The weight gain within this period may explain the differences in prepupal weight between the various feeding types.

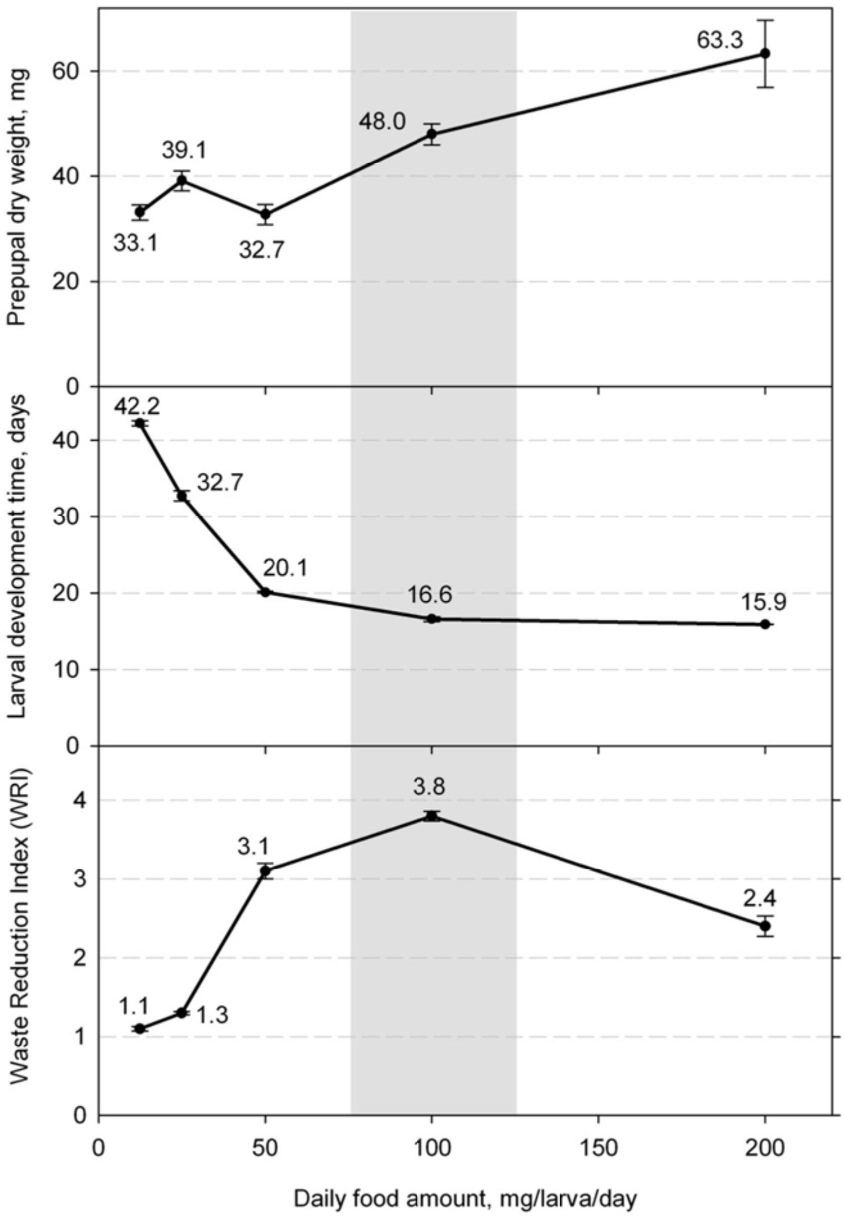

Figure 2-3: Prepupal dry weight, mg, larval development time, days, and Waste Reduction Index (WRI), dependent on daily food quantity. Grey area indicates optimal feeding rate.

As expected, adults that underwent food stress during their larval development (classes 12.5, 25 and 50) showed reduced dry weights compared to the other two classes (Table 2-3). Adult females weighed 1.3–1.5 times more than males, typically for arthropods.

Protein Content: The relative protein content of prepupae ranged from 31.9% (SE 1.74, class 200) to 46.3% (SE 1.88, class 12.5) (Table 2-4). The chitinous fraction of prepupae was 8.72% DW (SE 0.71). Hence, the chitin-corrected protein contents ranged from 28.2% (SE 1.74, class 200) to 42.5% (SE 1.88, class 12.5).

The protein contents of the well nourished prepupae obtained in this study turned out to be slightly lower than the 42–44% observed by Booram et al. (1977), Hale (1973) and St-Hilaire et al. (2007a). Nevertheless, the protein content obtained approves the applicability of dried prepupal meal in the feed industry.

Table 2-3: Prepupal and adult weight (mg) of *Hermetia illucens* fed with five different amounts of chicken food (12.5, 25, 50, 100, and 200 mg/larva/day, 60% moisture)

		12.5		**25**		**50**		**100**		**200**	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Prepupae											
Wet weight	mg	88.5a	4.47	103.0ab	5.64	98.7a	3.84	121.3b	2.40	157.3c	8.19
Dry weight	mg	33.1ab	1.44	39.1ab	1.90	32.7a	1.92	48.0b	2.00	63.3c	6.36
Adults											
♂♂ dry weight	mg	12.7a	0.48	16.8b	1.69	16.6b	0.24	24.8c	0.66	29.7d	0.70
♀♀ dry weight	mg	17.9a	0.43	24.4b	1.17	25.1b	0.30	33.5c	1.89	37.7c	0.72

Mean values followed by the same letter in the same row do not vary significantly ($P<0.05$)

Table 2-4: Larval, prepupal and adult protein content (% of dry weight) of *Hermetia illucens* fed with different amounts of chicken food daily (12.5, 25, 50, 100, and 200 mg/larva/day, 60% moisture). Protein calculation is based on total nitrogen x 6.25, except for prepupae (chitin-corrected values, see text)

		12.5		**25**		**50**		**100**		**200**	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Larvae	%	42.4a	1.29	38.9ab	0.84	38.6ab	1.53	37.3ab	1.27	34.9b	0.97
Prepupae											
Total	%	46.3a	1.88	36.4b	1.35	36.6b	0.33	38.1b	0.61	31.9b	1.74
Chitin corrected	%	42.5a	1.88	32.6b	1.35	32.8b	0.33	34.4b	0.61	28.2b	1.74
Adults											
♂♂	%	52.9a	0.71	48.7a	2.31	51.4a	0.23	49.0a	0.88	47.9a	0.59
♀♀	%	52.4a	0.93	47.7b	1.10	51.8ac	0.71	49.0abc	0.83	48.4bc	0.58

Mean values followed by the same letter in the same row do not vary significantly ($P<0.05$)

:: conclusions

We conclude that a daily rate of 100 mg chicken feed per larva meets both the demand for a nutrient-rich prepupal output and high organic matter degradation within a short time (Figure 2-3).

Using the digestible energy as a reference value, our results can be projected to different kinds of organic waste (Table 2-5). Depending on larval density and waste source, a daily load of 3–8 kg per m^2 can be applied to a black soldier fly CORS system. Needless to say that the calculated feeding rates presented in Table 2-5 are based on rough estimations and have to be interpreted carefully as they depend on other factors such as material moisture or fibre content.

Assuming a larval density of 5 larvae/cm^2, a prepupal dry mass of 2.5 kg could be harvested per life cycle and m^2 (145 g/m^2/day) when fed with a waste equivalent of 100 mg chicken feed/larva/day.

Table 2-5: Projected daily waste loading rates for a *Hermetia illucens* waste management system based on the energy values of different organic waste sources; calculation basis: 100 mg chicken feed/larva/day (60% moisture)

	Gross energy	Digestible energy	Daily feeding rate	Waste loading rate/m^2	Energy data source
	MJ/kg	MJ/kg	mg/larva/day	kg[§]	
Chicken feed[#]	*–*	*11.7*	*100*	*5.00*	
Kitchen waste	22.0	19.3[†]	61	3.05	(ALP, 2007)
Vegetable waste	17.6	11.9[†]	98	4.90	(ALP, 2007)
Green banana	17.8	11.4[‡]	103	5.15	(Ulloa et al., 2004)
Pig manure	17.8	7.4[‡]	158	7.90	(Ulloa et al., 2004)
Poultry manure	14.6	6.7[‡]	175	8.75	(Ulloa et al., 2004)
Human faeces	21.6	–	130[◊]	6.50	(Diem & Lentner, 1968)

[§] Assumption: 50,000 larvae/m^2

[#] UFA 625 Alleinfutter für Legehennen UNIVERSAL

[†] Digestible energy for pigs

[‡] Potential digestible energy for fish, calculated according to digestible energy coefficients for *Ictalurus punctatus* (channel catfish)

[◊] Calculation assumed the same gross energy/digestible energy ratio as for pig manure

For farmers and small entrepreneurs in low and middle-income countries, biomass produced by black soldier flies can be an additional income derived from this low-tech based waste treatment. It has been shown that even an irregular waste input (e.g. due to seasonal or personal fluctuations) larvae population remain alive albeit with reduced metabolic activity until the next food/waste input.

This study has taken a major step towards the quantification of a black soldier fly CORS system. However, our results were obtained from small-scale laboratory experiments. Implementation at a larger scale in situ will lead to new challenges, especially under changing environmental conditions (e.g. humidity, incident solar radiation, predators/parasitoids), the design of the treatment system (operability and usability), and the fate of problematic waste contents (e.g. heavy metals, pathogens) will be new elements to tackle.

:: acknowledgements

We would like to thank Simone Blaser, Maline Elumelu, Silvana Kaeser, and Livia Baumgartner for assisting during the experiments. We are grateful to Jeff Tomberlin for having introduced us to his flies and to Craig Sheppard for supplying us with *Hermetia illucens* eggs. This study was funded by the Velux Foundation and by the Swiss Federal Institute of Aquatic Science and Technology.

3

Bioaccumulation of Heavy Metals and Life Cycle Effects

:: abstract

In developing countries, effective waste management strategies are constrained by high collection costs and lack of adequate treatment and disposal options. The organic fraction in particular, constitutes a great, yet mostly neglected, reuse potential. Concomitantly, the demand for alternative protein sources by the livestock feed industry is sharply increasing. A technology that effectively transforms organic waste into valuable feed is therefore a timely option. Larvae of the non-pest black soldier fly, *Hermetia illucens* L. (Diptera: Stratiomyidae), may be used to reduce significantly the mass of organic waste. Concurrently, larval feeding converts organic waste into high-protein prepupae. The sale of this animal feed may cover the waste collection costs and thus promote innovative, small-scale entrepreneurs to establish a profitable business niche. Organic waste, however, often contains persistent pollutants, such as heavy metals that may accumulate in the larvae and prepupae of black soldier flies and consequently in the food chain. In this study, we fed black soldier fly larvae with feed spiked with heavy metals (cadmium, lead and zinc) to examine the extent of metal accumulation in the larvae and the effect on the life cycle determinants and bilateral symmetry of adult flies. The cadmium accumulation factor in prepupae ranged between 2.32 and 2.94; however, the lead concentration remained well below its initial concentration in the feed. The bioaccumulation factor of zinc in prepupae decreased with increasing zinc concentration in the feed (from 0.97 to 0.39). None of the three heavy metal elements had significant effects on the life cycle determinants (prepupal weight, development time, sex ratio) nor on the bilateral symmetry of the adult flies.

This chapter is in preparation for publication as: **Diener, S.**, Zurbrügg, C., Tockner, K., The potential use of the black soldier fly (Diptera: Stratiomyidae) as animal feed: Bioaccumulation of heavy metals and effects on the life cycle, *Environmental Entomology*

:: introduction

Urban poverty is a fundamental challenge in low and middle-income countries associated with rapid urban sprawl (Moore et al., 2003). The urban poor suffer most from inadequate sanitary services and deficient municipal solid waste management (MSWM) leading to increased health risks and impaired household resilience. While informal collection and recycling systems of inorganic material with a market value are currently available, the organic waste fraction often remains uncollected and untreated. Indiscriminately dumped organic waste accumulates along streets, clogs storm water drains, pollutes water bodies, rots, and attracts disease-transmitting vectors (e.g. flies, rodents), thus posing serious direct or indirect health risks to local residents. Local authorities, community-based organizations, non-governmental organizations (NGOs) and research institutions have recognized this deficiency and identified the need for simple, environmentally and economically sustainable organic waste treatment solutions in urban areas (Fluitman, 2000; Zurbrügg et al., 2007).

In many low and middle-income countries, the mass of organic waste may be substantially reduced using larvae of the non-pest black soldier fly, *Hermetia illucens* L. (Diptera, Stratiomyidae) (Diener et al., 2009). *H. illucens* larvae feed voraciously on decaying organic leftovers from markets and restaurants, animal droppings and on human feces. Myers et al. (2008) and Sheppard et al. (1994) reported a 33–58% reduction in organic matter from cow manure and 50% from chicken manure respectively. According to Diener et al. (2009), black soldier flies can transform on average 7.9 kg of pig manure, 8.8 kg of poultry manure or 6.5 kg of human feces per m^2 and day.

In its final larval instar, *H. illucens* leaves the food source in search of a dry pupation site. This dispersing habit facilitates harvesting the prepupae which consist of 35–44% raw protein and 33–35% crude fat (Booram et al., 1977; Diener et al., 2009; St-Hilaire et al., 2007b). Hence, larval feeding converts organic waste into a highly valuable protein that may be used as a substitute for fishmeal thus contributing to cover the waste collection costs as well as allowing innovative, small-scale entrepreneurs to establish a profitable business niche.

In developing countries, organic waste is abundant but underused. Concurrently, the demand for alternative protein sources by the animal feed industry is growing rapidly. A technology is required which transforms efficiently organic waste into valuable feedstuff and also provides an attractive economic niche for small entrepreneurs. The price for fishmeal, a key component of industrial animal feed, has doubled from 2004 to 2008 (Naylor et al., 2009) and may be expected to remain high due to declining wild fish stocks and the ongoing boom in aquaculture. Insect protein can be a viable food resource in aquaculture, chicken and frog farms. The housefly, *Musca domestica* L. (Diptera, Muscidae) larvae and pupae have already shown their potential in chicken production (Calvert et al., 1969; Ocio & Vinaras, 1979). Dried black soldier larvae have successfully been used as feed for rainbow trout, *Oncorhynchus mykiss*, chickens and bullfrogs (Newton et al., 2005a; St-Hilaire et al., 2007b). In the Republic of Guinea, a land-locked region with limited access to fish and fishmeal, Hem et al. (2008) have set up a production facility for black soldier fly larvae converting palm kernel meal into food for tilapia, *Oreochromis niloticus*.

Organic waste often contains persistent pollutants such as heavy metals that may accumulate in larvae and prepupae and therefore in the food chain. While terrestrial organisms ingest contaminants orally (biomagnification), aquatic organisms also enrich pollutants in their biomass through diffusion (bioconcentration). Bioaccumulation refers to both bioconcentration and biomagnification (Walker, 1990). The bioaccumulation factor (BAF) is the concentration of a pollutant in organisms divided by its concentration in the diet.

Table 3-1: Heavy metal concentration in municipal solid waste and vegetables (based on dry weight) compared to the legal maximum threshold level allowed in animal feed, human food and compost (LMIC = Low and Middle-Income Countries; n/a = not applicable)

	Cd	Pb	Zn	Reference
	mg/kg	mg/kg	mg/kg	
Heavy metal content in municipal solid waste				
Sweden	0.16–0.6	2.4–26	49–165	(Eklind et al., 1997)
Dhaka, Bangladesh	5.0	n/a	226	(Rytz, 2001)
Heavy metal content in vegetables				
Garden vegetables, rural village, Bangladesh	0.05–0.4	0.2–1.7	11–54	(Alam et al., 2003)
Market vegetables, Delhi, India	1.0–5.5	0.3–2.2	41–150	(Marshall, 2003)
Field vegetables, Varanasi, India	0.5–4.3	3–16	3–41	(Sharma et al., 2007)
Heavy metal limits in animal feed				
European Union	2	10	n/a	(European Union, 2002)
Heavy metal limits in human food				
European Union	0.05–1.0	0.02–1.0	n/a	(European Union, 2001)
India	0.1–1.5	0.2–10	5–100	(Government of India, 1954)
Heavy metal limits in compost from household waste				
European Union	0.7	45	200	(European Union, 1991)
Proposed standard in LMIC	3	150	300	(Hoornweg et al., 1999)

Heavy metals enter the waste stream in various ways, be it through atmospheric emissions or inappropriate disposal of heavy metal-containing refuse. Heavy metal concentrations and their legal thresholds in organic waste can therefore vary widely in different countries (Table 3-

1) depending on the effectiveness of air pollution control and local waste management practices. The lack of separate battery collection, for example, remains a main source of organic waste contamination by heavy metals (European Commission, 2002). Hence, segregation and separation of contaminating material from the organic waste fraction will generally lower the contamination level.

A stable black soldier fly population generating viable eggs and producing healthy offspring are prerequisites for running a sustainable organic waste treatment facility using black soldier flies. However, heavy metals in organic waste may influence the life history traits. For example Cu- and Pb-contaminated host plants negatively affected the fecundity and intrinsic rate of natural increase (r_m) in the cabbage aphid, *Brevicoryne brassicae* L. (Görür, 2006). Reduced bodyweight in the offspring of the carabid beetle, *Pterostichus oblongopunctatus*, inhabiting a metal-polluted environment has been observed (Lagisz & Laskowski, 2008).

In this study, the larvae of the black soldier fly, *Hermetia illucens*, were fed with chicken feed contaminated by different levels of cadmium, lead and zinc to investigate the following research questions:

i) to what extent do cadmium, lead and zinc – fed at different concentrations – accumulate in the prepupae of the black soldier fly?

ii) does heavy metal in the food influence the life cycle determinants of the flies? (i.e. development time, body weight sex ratio)?

iii) do heavy metals affect the bilateral symmetry of the adult flies?

:: material and methods

Animals. Black soldier flies, *Hermetia illucens* L. (Diptera: Stratiomyidae), were obtained from a laboratory colony grown in an indoor cage (1.5 m x 1.5 m x 2 m) at constant temperature (26.5 °C ± 0.05, 60.8% RH ± 0.8). The room was fitted with two windows as direct sunlight is crucial for successful mating (Tomberlin & Sheppard, 2002).

The newly hatched larvae used for the experiments were reared on chicken feed (UFA 625, digestible energy: 11.7 MJ/kg, 60% moisture). A detailed description of the breeding and hatching facility is given in Diener et al. (2009).

The experiments conducted in Switzerland did not violate Swiss law (e.g. Animal Protection Law, Animal Husbandry Act) or any of the provisions or regulations stipulated in these laws.

The experiments also met the International Guiding Principles for Biomedical Research Involving Animals as issued by the Council for the International Organizations of Medical Sciences (CIOMS, 1985).

Experimental setup. Larvae were fed with chicken feed pellets moistened (final moisture level: 60%) with either pure deionized water (control) or a solution of deionized water containing heavy metal ions (three concentration levels for each metal). The 2% HNO_3 solutions used for feedstock preparation contained cadmium (1,000 ppm), lead (1,000 ppm) or zinc (10,000 ppm). The nominal concentrations in the food were: Cd: 0.0 µg/g (control), 2.0 µg/g, 10.0 µg/g, 50.0 µg/g; Pb: 0.0 µg/g (control), 5.0 µg/g, 25.0 µg/g, 125.0 µg/g; Zn: 0.0 µg/g (control), 100 µg/g, 500 µg/g, 2,000 µg/g. Low concentrations corresponded to the metal concentrations typical for market vegetables in India or Bangladesh (Alam et al., 2003; Marshall et al., 2003; Sharma et al., 2007). Middle concentrations for cadmium and lead corresponded to concentrations typical for organic waste in Bangladesh or Sweden (Eklind et al., 1997; Rytz, 2001). Metal concentrations in the control groups were derived from the chicken feed itself. Unfortunately, the low concentration series of zinc was contaminated during the experiment with food containing a high concentration of zinc and the results for this series had to be discarded. However, since the background concentration of zinc in the chicken feed used for the control series (145.3 mg/kg, SE 10.1) was comparable to the zinc concentration in the original food for the low concentration series (177.4, SE 3.8), the remaining two treatment groups (medium and high concentration) could still be compared with the control groups, which were then used to represent the low-zinc series.

Each replicate (three replicates per treatment) contained 200 7-day old larvae placed in plastic containers (14 x 7.5 x 7 cm) and covered with nylon tulle held in place by the lid of the box. The lids with nine holes (ø 15 mm) allowed air circulation. The food and larvae were covered with a re-sealable polyethylene bag containing a black cardboard to shield larvae from light. The pre-prepared meal portions were packed into separate polyethylene bags and kept frozen until use. The quantity of the diet was calculated based on 100 mg food (wet weight) per larva and day. The larvae were fed three times a week. Feeding stopped when 50% of the larvae in the box metamorphosed into prepupae to avoid overfeeding of the remaining larvae.

Sampling and analysis. The samples (larvae, larval exuviae, prepupae, pupal exuviae, and adults) were washed with deionized water, weighed, lyophilized to measure dry weight, and ground in an agate mortar for heavy metal analysis. The food samples and the remains at the end of the experiments, the so-called residue, were treated the same way except for the washing. Larvae and prepupae were killed by freezing (–10 °C), while adults were killed with ethyl acetate. To prepare the samples for the analyses, ~50 mg of the ground material was digested in polytetrafluorethylene (PTFE) beakers (HPR-300/10). The material was moistened with deionized water. Approximately 4 ml HNO_3 and 1 ml H_2O_2 were added before the sample was heated in a laboratory microwave digester (MLS 1200 MEGA). The clear solution was diluted with deionized water (10x for Cd and 100x for Pb and Zn) and analyzed with the HR-ICP-MS (Element II Thermo Fisher Scientific). The standard solutions were made using Merck ICP multi-element standard solution IV: 10, 100, 1,000, 5,000 and 10,000 ng/l. The natural river water standard SLRS-4 and the TM-28.3 trace elements fortified calibration standard were used as a control. The detection limit for these elements was 10 ng/l.

The bioaccumulation factor (BAF) was calculated according to Walker (1990) as:

$$BAF = \frac{concentration\ in\ organism\ (C_i)}{concentration\ in\ \ food\ and\,/\,or\ water\ ingested\ (C_o)}$$

In the present case, C_o consisted solely of the heavy metal concentration in the food.

The effect of heavy metal exposure on development was estimated quantitatively based on fluctuating asymmetry (FA), the non-directional deviation from bilateral symmetry, proposed as an environmental quality assessment method (Clarke, 1993; Nunes et al., 2001), as well as a predictor for growth, fecundity and survival of animal and plant populations (Leary & Allendorf, 1989; Møller, 1999). Departures from bilateral symmetry relative to an appropriate control is assumed to show effects of environmental stress such as elevated temperatures, crowding, polluted habitats or food sources. A more detailed description can be found in Palmer (1986). The length of the basal radial cell (br) of the adult flies treated with cadmium and lead were measured. Left and right wings of ten randomly selected adult flies per treatment, replicate and sex were fixated with scotch tape, and the basal radial cell's length was measured with 25x magnification (minimum resolution 0.04 mm). To assess measurement errors, two different examiners simultaneously measured the cells of the control group and the animals treated with cadmium. A paired sampled t-test revealed a significant difference between the measurements made by the two different examiners. However, since the two data sets strongly correlated (Pearson correlation = 0.967, N = 452), a constant measurement error was assumed and further FA calculations were performed with the data assessed by one examiner. Two different FA indices were calculated to detect differences in the magnitude of FA among heavy metal concentrations: i) FA1 as the absolute value of the difference between right and left, $|(L_i–R_i)|$ and ii) FA2 as the ratio of the measurements from each side of an individual, (L_i/R_i).

Statistical analyses. Statistical analyses were performed using SPSS Statistics 17.0 software. Relations between the heavy metal concentrations of the different fractions were tested using Kendall's tau rank correlation. ANOVA was used to reveal significant differences between the various treatments. For some data, a violation of the Levene Homogeneity of Variances was calculated. However, as the groups are equal in size, ANOVA is very robust to this violation.

Table 3-2: Cadmium concentration in soldier flies, *Hermetia illucens*, at different life stages based on dry weight, in digested material (residue) and in food source. Larvae fed with chicken feed (100 mg/larva/day, 60% moisture) spiked with cadmium (three different concentrations; SE = standard error; n.d. = not detected)

	Control		**Low cadmium**		**Medium cadmium**		**High cadmium**	
	Mean (mg/kg)	SE	Mean (mg/kg)	SE	Mean (mg/kg)	SE	Mean (mg/kg)	SE
Food	0.2 a	0.02	2.7 b	0.2	13.3 b	0.7	61.5 b	2.3
Residue	0.2 a	0.01	2.9 b	0.1	16.0 bc	0.4	89.8 c	2.1
Larvae	0.2 a	0.02	7.0 d	0.3	32.5 d	0.6	170.5 d	8.5
Prepupae	n.d.	-	7.9 d	0.6	36.2 d	1.9	142.9 e	8.3
Adults	n.d.	-	0.6 a	0.04	1.9 a	0.2	7.8 a	0.4
Larval exuviae	0.1 a	0.03	2.2 ab	0.3	18.8 bc	4.1	54.2 b	3.7
Pupal exuviae	0.5 b	0.1	5.2 c	0.7	22.9 c	2.6	94.1 c	12.1

Mean values followed by the same small letter in the same column do not vary significantly ($P<0.05$)

:: results

Heavy metal accumulation. In all development stages (larvae, prepupae, and adults), the metal concentration generally increased significantly with increasing metal concentration in the food (Table 3-2, Table 3-3, Table 3-4 and Table 3-5). However, the bioaccumulation factor (BAF), i.e. the ratio of the amount of metal in the body compared to that in the food varied among the different metal elements and development stages (Table 3-6). In prepupae, the BAF ranged from 2.32 to 2.94 for cadmium, independent of the concentration in the food, while the BAF remained <1 (0.25–0.74) for lead. In adults, the BAF was very low for both cadmium and lead concentrations (BAF: 0.12–0.21). For zinc, the BAF decreased with increasing concentration in the food (prepupae: from 0.97 to 0.39; adults: from 0.98 to 0.19). The EU threshold value for cadmium (2 mg/kg) in animal feed was exceeded in prepupae even at low cadmium concentration (7.9 mg/kg SE 0.6). Only prepupae from the low lead concentration group (1.5

mg/kg SE 0.7) met the EU concentration limit for lead (10 mg/kg) in animal feed (European Union, 2002).

Table 3-3: Lead concentration in soldier flies, *Hermetia illucens*, at different life stages based on dry weight, in the digested material (residue) and in food source. Larvae fed with chicken feed (100 mg/larva/day, 60% moisture) spiked with lead at three different concentrations (SE = standard error; n.d. = not detected)

	Control		**Low lead**		**Medium lead**		**High lead**	
	Mean (mg/kg)	SE	Mean (mg/kg)	SE	Mean (mg/kg)	SE	Mean (mg/kg)	SE
Food	1.1 ab	0.4	5.9 bc	0.3	34.3 c	1.8	142.9 b	2.9
Residue	0.1 a	0.01	7.8 cd	0.6	53.2 d	3.3	267.9 c	12.8
Larvae	n.d.	-	3.8 ab	0.4	22.8 b	1.6	141.7 b	17.2
Prepupae	n.d.	-	1.5 a	0.7	25.3 bc	1.9	40.1 ab	3.7
Adults	n.d.	-	n.d.	-	5.9 a	0.57	17.3 a	1.36
Larval exuviae	5.9 c	1.3	11.3 e	0.01	87.7 e	4.8	312.9 c	74.1
Pupal exuviae	3.7 bc	0.1	9.3 de	1.0	24.2 bc	2.1	66.7 ab	3.6

Mean values followed by the same small letter in the same column do not vary significantly ($P<0.05$)

Table 3-4: Zinc concentration in soldier flies, *Hermetia illucens*, at different life stages based on dry weight, in the digested material (residue) and in food source. Larvae fed with chicken feed (100 mg/larva/day, 60% moisture) spiked with zinc at three different concentrations. Samples from the series "Low zinc" were contaminated during the experiment and could not be used for interpretation (SE = standard error; n/a = not applicable)

	Control		**Low zinc**		**Medium zinc**		**High zinc**	
	Mean (mg/kg)	SE	Mean (mg/kg)	SE	Mean (mg/kg)	SE	Mean (mg/kg)	SE
Food	145.3 b	10.1	177.4	3.8	616 b	37.8	2,044 c	16.2
Residue	192.3 b	13.1	n/a	–	1,196 c	66.7	3,313 d	240.9
Larvae	165.8 b	19.9	n/a	–	596 b	88.0	866 b	141.9
Prepupae	138.9 b	24.3	n/a	–	513 ab	44.5	801 ab	32.1
Adults	141.4 b	9.2	n/a	–	272 ab	22.8	389 ab	37.6
Larval exuviae	275.5 c	13.5	n/a	–	1,514 c	240.2	1,883 c	104.8
Pupal exuviae	35.1 a	4.1	n/a	–	145 a	39.2	334 a	56.5

Mean values followed by the same small letter in the same column do not vary significantly ($P<0.05$)

Table 3-5: Kendall's tau rank correlation between the heavy metal concentration in food and the concentration values in larvae, prepupae, larval exuviae, and adults of the black soldier fly, *Hermetia illucens*

	Metal concentration larvae			Metal concentration prepupae			Metal concentration larval exuviae			Metal concentration adults		
	r	p	N	r	p	N	r	p	N	r	p	N
Cadmium	0.778*	0.004	12	0.833*	0.002	9	0.722*	0.007	11	0.741*	0.000	18
Lead	0.778*	0.004	9	0.833*	0.002	9	0.778*	0.004	12	0.671*	0.001	17
Zinc	0.667*	0.012	9	0.611*	0.022	9	0.833*	0.002	9	0.647*	0.000	18

*** p<0.01**

Table 3-6: Bioaccumulation factor (BAF) for larvae, prepupae and adults of the black soldier fly, *Hermetia illucens* fed heavy metal contaminated food at three different concentrations (Table 3-2, Table 3-3 and Table 3-4). BAF for "Low concentration, Zinc" was calculated using data from control group (see explanation in text). Because the concentrations of cadmium and lead in the control group were so low, the analytical error had the effect of providing inaccurate BAFs, and are therefore not shown here (SE = standard error; n/a = not applicable)

	Low concentr.		Medium concentr.		High concentr.	
	BAF	SE	BAF	SE	BAF	SE
Cadmium						
• Larvae	2.65	0.10	2.46	0.11	2.79	0.24
• Larval exuviae	0.86	0.19	1.41	0.31	0.88	0.06
• Prepupae	2.94	0.09	2.75	0.25	2.32	0.09
• Adults	0.21	0.01	0.15	0.01	0.13	0.01
Lead						
• Larvae	0.66	0.09	0.67	0.07	0.99	0.10
• Larval exuviae	1.9	0.11	2.56	0.01	2.21	0.56
• Prepupae	0.25	0.12	0.74	0.03	0.28	0.03
• Adults	n/a	–	0.17	0.01	0.12	0.01
Zinc						
• Larvae	1.14	0.09	0.97	0.14	0.42	0.07
• Larval exuviae	1.92	0.19	2.45	0.32	0.92	0.04
• Prepupae	0.97	0.20	0.84	0.09	0.39	0.01
• Adults	0.98	0.08	0.45	0.04	0.19	0.02

Effects of heavy metals on life cycle determinants. Prepupae treated with cadmium were significantly heavier than the control group. No significant effects were found in prepupae treated with lead and zinc (Table 3-7). Development time from hatching of the larva to the prepupal stage generally increased with heavy metal concentration although the increase was statistically insignificant (Table 3-8). Average development time until pupation amounted to 15.2 days (SE 0.1) and did not differ significantly between treatments or sexes. Heavy metals had no influence on the sex ratio of adults (average males/females ratio: 0.98, SE 0.02).

Bilateral symmetry. Heavy metal had no significant effect on the symmetry of the basal radial cell (br) of adult flies (FA1) aside from females treated with lead when clustered by gender groups. However, this difference could only be seen in FA1 (|L–R|) with a mean of 88.0µm (SE = 13.9; N = 30) in the control group and 44.0µm (SE = 8.2; N = 30) at high lead concentration. FA1 was surprisingly the highest in the control group.

Table 3-7: Prepupal dry weight of *Hermetia illucens* fed with chicken feed (100 mg/larva/day, 60% moisture) spiked with three different heavy metals at different concentrations (SE = standard error; n/a = not applicable)

	Control		Low		Medium		High	
	mg	SE	mg	SE	mg	SE	mg	SE
Cadmium	55.9 a	2.3	96.2 c	10.0	75.3 b	2.0	83.6 bc	3.3
Lead	55.9 ab	2.3	61.5 b	6.8	51.3 a	2.1	59.1 ab	0.6
Zinc	55.9 a	2.3	n/a	–	64.8 a	5.7	59.2 a	4.8

Mean values followed by the same small letter in the same row do not vary significantly ($P<0.05$)

Table 3-8: Effects of heavy metal concentration in food on development time (eclosion from egg to prepupa) of *Hermetia illucens* larvae (SE = standard error; n/a = not applicable)

	Control		Low		Medium		High	
	days	SE	days	SE	days	SE	days	SE
Cadmium	18.4 ab	0.5	18.0 a	0.5	18.8 ab	0.4	19.3 b	0.6
Lead	18.4 a	0.5	18.8 ab	0.3	19.4 b	0.4	20.7 c	0.2
Zinc	18.4 a	0.5	n/a	–	18.9 a	0.5	20.1 b	0.6

Mean values followed by the same small letter in the same row do not vary significantly ($P<0.05$)

:: discussion

The black soldier fly, *Hermetia illucens*, fed with cadmium, lead and zinc, exhibits different accumulation patterns. The level of accumulated cadmium in larvae and prepupae was higher than the original cadmium concentration in the food, yet, the incorporation of lead and zinc was below the corresponding concentrations in the food. These findings are consistent with literature data (Figure 3-1). In the literature reports, the BAF of cadmium uptake by detritivorous insects averages 2.86 (SE 0.30, range 0.46–6.09) (Gintenreiter et al., 1993; Kazimirova & Ortel, 2000; Kramarz, 1999; Lindqvist, 1992; Maryanski et al., 2002; Ortel, 1995). Cellular cadmium uptake probably occurs through Ca^{2+} channels. Due to their very similar ionic radii, Cd^{2+} ions can easily enter the cell via Ca^{2+} channels, independent of endocytosis or an ATP requiring ion pump (Braeckman et al., 1999). Moreover, Braeckman et al. (1999) found a protein of the HSP70-family induced by elevated cadmium concentrations in the environment of *Aedes albopictus* (Diptera, Culicidae) cells. Production of this protein, which protects other proteins from denaturation, may also explain the low effect of contaminated food on life-cycle parameters such as development time or fluctuating asymmetry despite the observed bioaccumulation of cadmium (cf. present study).

In contrast to cadmium, the BAF for zinc decreased with increasing zinc concentration in the food, which suggests active regulation of zinc within the body (Table 3-6). Similarly, larvae of the house fly, fed with zinc-contaminated food (from 61 to >7,000 mg/kg) accumulated zinc only up to a maximum level of 216 mg/kg (Kramarz, 1999; Maryanski et al., 2002). Even though the mean zinc concentration in the literature data for *M. domestica* is lower than that found in prepupae of *H. illucens* of the present study (484 mg/kg, SE 97.5), it is possible that the two organisms possess a similar regulation mechanism.

Active regulation of zinc in insects has been described previously (Lindqvist, 1995; Mason et al., 1983). Zinc is an essential, yet potentially toxic element. Therefore, it is not surprising that its intracellular uptake is actively regulated. Especially the metal-responsive-element-binding transcription factor-1 (MTF-1) is a key regulator in higher eukaryotic cells. It is responsible for the activation of several genes involved in intracellular zinc sequestration and transport (Laity & Andrews, 2007).

Though larvae and prepupae contained low lead concentrations in the present study, the larval exuviae accumulated lead. Lead tends to be stored in granular, metal-containing structures of the cells before it is transported to and immobilized in the exoskeleton (Hare, 1992). Similar to terrestrial insects, lead is most likely disposed of during molting (Roberts & Johnson, 1978).

Heavy metal concentration in adults was significantly lower than in prepupae. We assume that this phenomenon occurs mainly because animals defecate before pupation or shortly after adult emergence. Yet Sheppard et al. (1994) reported without supporting data that prepupae had an empty gut when migrating. Conversely, Aoki & Suzuki (1984) describe an over 50% loss of the larva’s cadmium content due to defecation in newly emerged flesh flies within the first two days following emergence. In the present study, prepupae were collected 1–3 days after transformation. We assume that defecation had not occurred during this period, and cadmium was removed during the later prepupal phase, i.e. during pupation, or after emergence. Therefore, toxic substances and pathogens present in the waste may remain in the gut of the harvested prepupae and in this way may be taken up by fishes or poultry fed with the prepupae. Future studies have to determine the period between initiation of the last larval instar (prepupa) and defecation, including the potential loss of feedstuff energy due to such a protraction.

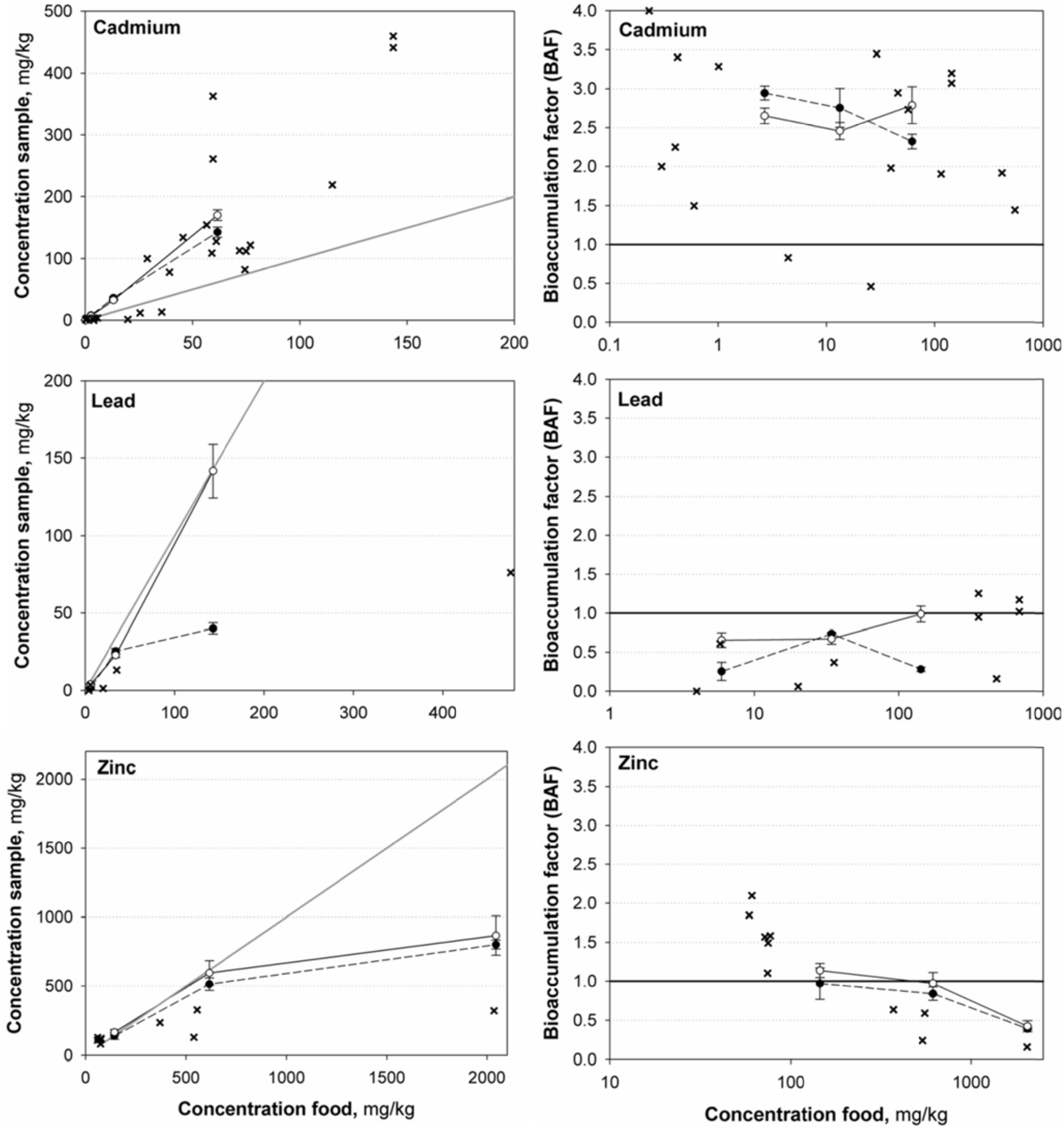

Figure 3-1: Concentration and bioaccumulation factor (BAF) of different insect larvae (○) and prepupae (●) fed with heavy metal contaminated food compared with literature data (x). 1:1 line shown for reference (–) Missing BAF values are attributed to undetectable concentrations in control groups. References: Cd: (Kazimirova & Ortel, 2000; Kramarz, 1999; Lindqvist, 1992; Maryanski et al., 2002; Ortel, 1995), Pb: (Gintenreiter et al., 1993; Kazimirova & Ortel, 2000; Ortel, 1995), Zn: (Kramarz, 1999; Maryanski et al., 2002)

The effective elimination of heavy metals by defecation has been described for larvae of the social paper wasp *Polistes dominulus* (Hymenoptera: Vespidae) (Urbini et al., 2006). However, even if heavy metals accumulate in the cell lining of the alimentary canal, they may be rejected after a short time. For example, *Tenebrio molitor* (Coleoptera: Tenebrionidae) discards cells of the midgut epithelium after four days (Lindqvist & Block, 1995; Thomas & Gouranto, 1973). Heavy metal accumulated in these cells will therefore be rejected with defecation.

The aforementioned detoxification strategies may explain the minor effects of heavy metal exposure on development time and morphology. The basal radial cells did not reveal an asymmetry with increasing heavy metal concentration. These results are promising with regard to establishing a healthy and reproductive adult colony used for egg production in a BSF waste treatment plant. FA has been applied and tested to measure environmental stress factors (heavy metals, pesticides, genetically modified organisms) in previous research studies, although with very variable results. Besides significant effects of stress factors on FA (e.g., Clarke, 1993; Liu et al., 2005; Nunes et al., 2001) and no effects on FA (e.g., Maryanski et al., 2002; Rabitsch, 1997), an inconsistent pattern between stress factor and FA can often be found (e.g., Dobrin & Corkum, 1999; Görür, 2006; Hardersen, 2000; Hardersen & Wratten, 1998).

Certainly, the method of fluctuating asymmetry allows us to make predictions of the fecundity and health status of future generations. However, a correlation between environmental stress factors, fluctuating asymmetry and fecundity does not necessarily preclude that environmental stresses that do not affect the fluctuating asymmetry do not have an effect on future generations just the same. Therefore, to what degree the future fitness of a population is, in fact, affected by heavy metal contaminated food needs to be addressed in future studies.

:: conclusions

Our studies reveal that lead and zinc do not accumulate in larvae or prepupae. Furthermore, the three heavy metal elements examined had only minor effects on the development of the black soldier fly even at very high concentrations. Yet, since cadmium accumulated in the prepupae, it could potentially limit the use of prepupae in the production of animal feed. In the case of lead and zinc, concerns about the use of prepupae in animal feed are less critical. The waste treatment technology using black soldier flies may contribute to reducing the burden of an animal protein shortage in the animal feed market and provide new income opportunities for small entrepreneurs in low and middle-income countries.

:: acknowledgements

We wish to express our thanks to Simone Blaser and Mauro Esposito for assisting during the experiments, David Kistler for supporting us in lab analysis and Alejandra Teresita Arroyo for her statistical advice. We appreciate the comments by Janet Hering on an early version of the manuscript, and are grateful to Sylvie Peter for linguistic editing support. The authors would like to acknowledge the financial support by the Velux Foundation, Eawag: the Swiss Federal Institute of Aquatic Science and Technology, the Swiss National Centre of Competence in Research (NCCR North South) and the Swiss Agency for Development and Cooperation (SDC).

4

Feasibility in Practice

:: abstract

Valorisation of municipal organic waste through larval feeding activity of the black soldier fly, *Hermetia illucens*, constitutes a potential benefit, especially for low and middle-income countries. Besides waste reduction and waste stabilisation, the product in form of the last larval stage, the so-called prepupae, offers a valuable additive in animal feed, opening new economic niches for small entrepreneurs in developing countries. We have therefore evaluated the feasibility of the black soldier fly larvae to digest and degrade mixed municipal organic waste in a medium-scale field experiment in Costa Rica, and explored the benefits and limitations of this technology. We achieved an average prepupae production of 252 g/m^2/day (wet weight) under favourable conditions. Waste reduction ranged from 65.5% to 78.9% depending on the daily amount of waste added to the experimental unit and presence/absence of a drainage system. Three factors strongly influenced larval yield and waste reduction capacity: i) high larval mortality due to elevated zinc concentrations in the waste material and anaerobic conditions in the experimental trays; ii) lack of fertile eggs due to zinc poisoning and iii) limited access to food from stagnating liquid in the experimental trays. This study confirmed the great potential of this fly as a waste manager in low and middle-income countries, but also identified knowledge gaps pertaining to biological larvae requirements and process design to be tackled in future research.

This chapter has been accepted for publication as: **Diener, S.**, Studt Solano, N.M., Roa Gutiérrez, F., Zurbrügg, C., Tockner, K., Biological treatment of municipal organic waste using black soldier fly larvae, *Waste and Biomass Valorization*

:: introduction

Management of municipal solid waste (MSW) in low and middle-income countries remains a challenging and neglected key issue. Especially in urban and peri-uban areas, the household waste often remains uncollected on streets and drains, thereby attracting disease vectors and causing water blockages (Diaz et al., 1996; Zurbrügg et al., 2007). Compared to other waste components, such as glass, metal and paper, the organic fraction, often amounting to 80% of the total municipal waste, is frequently looked upon as a waste fraction without market value and therefore ignored by the informal waste recycling sector. Even if collected, MSW typically ends up in a landfill or on a more or less uncontrolled dumpsite where the material decomposes in large heaps under anaerobic conditions. Around 6.8% of Africa's greenhouse gas emissions are generated by waste activities, primarily methane released from open dumps (Couth & Trois, 2009). To reduce the environmental burden and improve public health, new and financially attractive waste management strategies should be explored and fostered. Similar to the well-established recycling and resource recovery sector for inorganic material, such as glass, PET or metal, collection and recovery of municipal organic waste (MOW) can contribute to generating additional value if appropriate valorisation technologies are provided (UN-HABITAT, 2010).

Examples of CORS technologies (Conversion of Organic Refuse by Saprophages), such as vermicomposting or faecal sludge treatment by the aquatic worm *Lumbriculus variegatus,* could offer promising alternatives of nutrient recovery from organic waste (Elissen, 2007). Organic waste digestion by the larvae of the black soldier fly, *Hermetia illucens* L. (Diptera: Stratiomyidae) is another CORS solution combining nutrient recovery and income generation. The larvae of this non-pest fly feed on and thereby degrade organic material of different origin. Domestic waste, chicken, pig and cow manure and even human excreta were found to be easily processed by the larvae. The last larval stage, the prepupa, migrates from the feed source to pupate and can therefore easily be harvested. Since prepupae contain on average 44% crude protein and 33% fat (St-Hilaire et al., 2007b), it is an appropriate alternative to fishmeal in animal feed with a potential market value of USD 330/tonne dry weight (Newton et al., 2005b). Conversion of organic waste into high nutritional biomass opens new economic opportunities for municipalities and small entrepreneurs in the MSW sector.

Over the past 20 years, the biology of *H. illucens* has been studied extensively, especially regarding its use in waste management and protein production (Diener et al., 2009; Sheppard et al., 1994; Tomberlin & Sheppard, 2002; Tomberlin et al., 2002). Additional research focused on the prepupae's potential as animal feed (St-Hilaire et al., 2007b; Stamer, 2005) and its limitations in waste management (Diener et al., 2011; Erickson et al., 2004).

So far, the BSF technology has been successfully tested in the laboratory. However, it remains unclear to which extent the result from laboratory studies can be transferred to the field – a prerequisite for future industrial application of this technology. Sheppard et al. (1994) modified a caged layer house fitted with a concrete basin below the cage batteries. The basin allowed easy harvesting and quantification of migrating prepupae. However, it did not allow controlling the feeding rate, and the waste reduction had to be estimated based on the depth of the accumulated chicken droppings. Newton et al. (2005b) employed the faeces of 12 pigs in an automated system using a dewatering belt to transport the material to the larva treatment bed, thus achieving a 56% fresh material reduction.

Bioconversion of palm kernel meal into fish feed in the Republic of Guinea and in Indonesia is so far the only known application of the soldier fly technology in tropical countries (Fléchet, 2008; Hem et al., 2008). However, both these cases did not take advantage of the self-harvesting habit of the prepupae. They rather separated the prepupae together with immature larvae from the processed material by filtering and cleaning with water. The aforementioned studies all used homogenous agricultural waste products as feedstock. Application of the results obtained from this past research to the treatment of an inhomogeneous feeding source like municipal organic waste remains to be understood and proven.

The main objective of the present study was consequently to evaluate the feasibility of black soldier fly larvae to digest and degrade mixed municipal organic waste in a medium-scale field experiment in Costa Rica, and to explore the benefits and limitations of this technology. Furthermore, this study will provide recommendations for future research and application of this promising waste treatment process in low and middle-income countries.

:: materials and methods

Study site. The research study site was located on the campus of the EARTH University (Escuela de Agricultura de la Región Tropical Húmeda) in Guácimo, Costa Rica. The experiments were carried out in a former chicken pen (30 x 8 m) roofed by a corrugated metal sheet and enclosed by a wire net.

Fly colony. A population of the black soldier fly, *Hermetia illucens* L., was maintained in a small green house (2 x 3 x 2.5 m), referred to as "moscario", roofed with transparent plastic foil fitted with a sun shading net and nylon netted sidewalls. The moscario was placed on a meadow, exposed daily to direct sunlight for about eight hours. In the moscario, a black plastic foil-covered tray (1 x 2 m) containing organic waste was used to attract ovipositing females. The method for collecting eggs was adapted from Tomberlin et al. (**2002**). Strips of corrugated cardboard (20 x 4 cm, flute opening 2 x 5 mm) were wrapped around skewers and tucked into rings of bamboo (∅ 10–15 cm x 5 cm) placed into the tray. The cardboard strips were collected at least every second day and inserted into nylon covered plastic cups containing wetted rabbit feed. These hatching containers were stored in a dark and warm environment (24 °C, 93% RH). Larvae hatched approximately three days after oviposition and dropped from the cardboard strips into the wetted rabbit feed. The young larvae were then used at an age of 4–6 days to inoculate the experimental setup. To supply the colony with new adults, ~500 prepupae from every experiment's harvest were placed into plastic bowls (∅ 35 cm x 10 cm), where they pupated in a mixture of hay, wood shavings and pieces of an empty nest of arboreal termites (*Nasutitermes* spp). The bowls were covered with nylon netting and the emerging adults released into the moscario.

Experimental setup. The experiments were conducted in trays (80 cm x 200 cm x 30 cm), the so-called "larveros", built with zinc-coated steel sheets (Figure 4-1). For exiting prepupae, two ramps at a 28° angle led from the base plate (100 x 80 cm) to the upper end of each shorter side panel. A plastic pipe (∅ 11 cm x 94 cm) was fixed along the top of this edge. A slit (5 x 80 cm) cut into the pipe allowed migrating soldier fly prepupae to enter and crawl along the pipe leading to downspouts at each end of the pipe from where they fell into harvesting containers.

To avoid ant invasion, the larveros had to be placed on pieces of bamboo (∅ 10–15 cm x 25 cm) standing in water-filled plastic pots.

Organic waste generated by the residents of the EARTH university campus was used for the experiments. The content of several bags of the source-separated organic waste (approximately 20 kg/day) was mixed thoroughly to achieve certain homogeneity and then added to the larveros according to the defined feeding regime.

We designed a full-factorial experiment to assess: (i) the effect of the larvero cover (black sheet or shading net), (ii) the effect of food application (surface-fed or mixed with residue) and (iii) the effect of food amount (low and high amounts).

Since the factor “cover” was found to have no influence whatsoever on the results, the data analysed originated from a practically full-factorial experiment (two-level, two-factor) with two repetitions.

The factor “application” was chosen to assess whether waste reduction and larval growth benefited from fresh food mixed with already digested material or if surface application led to an enhanced performance from improved access of the larvae to fresh food and increased oxygen supply. Fresh waste was folded in manually using a shovel. Regarding the factor “amount”, larveros that were allocated the attribute “low” received on average 1.5 kg of fresh organic waste per day. Larveros with the attribute “high” were fed daily with 4.6 kg of fresh organic waste.

Besides the eight experimental larveros, a ninth larvero, set up and inoculated once with fresh larvae produced from 20 egg clutches, received a surface-fed intermediate amount of food (2.1 kg/day). The content of this “control” larvero was mixed thoroughly several times throughout the project period.

To ensure a coequal establishment of the larval population, all eight experimental larveros were fed daily with 1.1 kg fresh, untreated waste for the first 21 days. During this starting phase, fresh food was placed on top of the larvero’s residual material, independently from the subsequent feeding regime. Six days after the first prepupal crawl-off, the feeding regime was altered according to the assigned factors and run for another 34 days. Feeding and harvesting were conducted at least every second day.

To test the influence of a drainage system on the larvae’s performance, three larveros were equipped with a perforated plastic tube (∅ 5 cm x 80 cm, holes ∅ 6 mm) placed at the bottom of the larvero leading to a tap mounted onto the lower end of the larvero’s side panel. These drained larveros together with three non-drained larveros were fed daily an average of 4.0 kg fresh waste for 23 days.

Samples of waste, prepupae and residue were dried at 80 °C for 72 hours for analysis and determination of the dry mass. Linear regression and one-way ANOVAs with subsequent Tukey

HSD tests were conducted to determine relations and differences between the various treatments ($P \leq 0.05$).

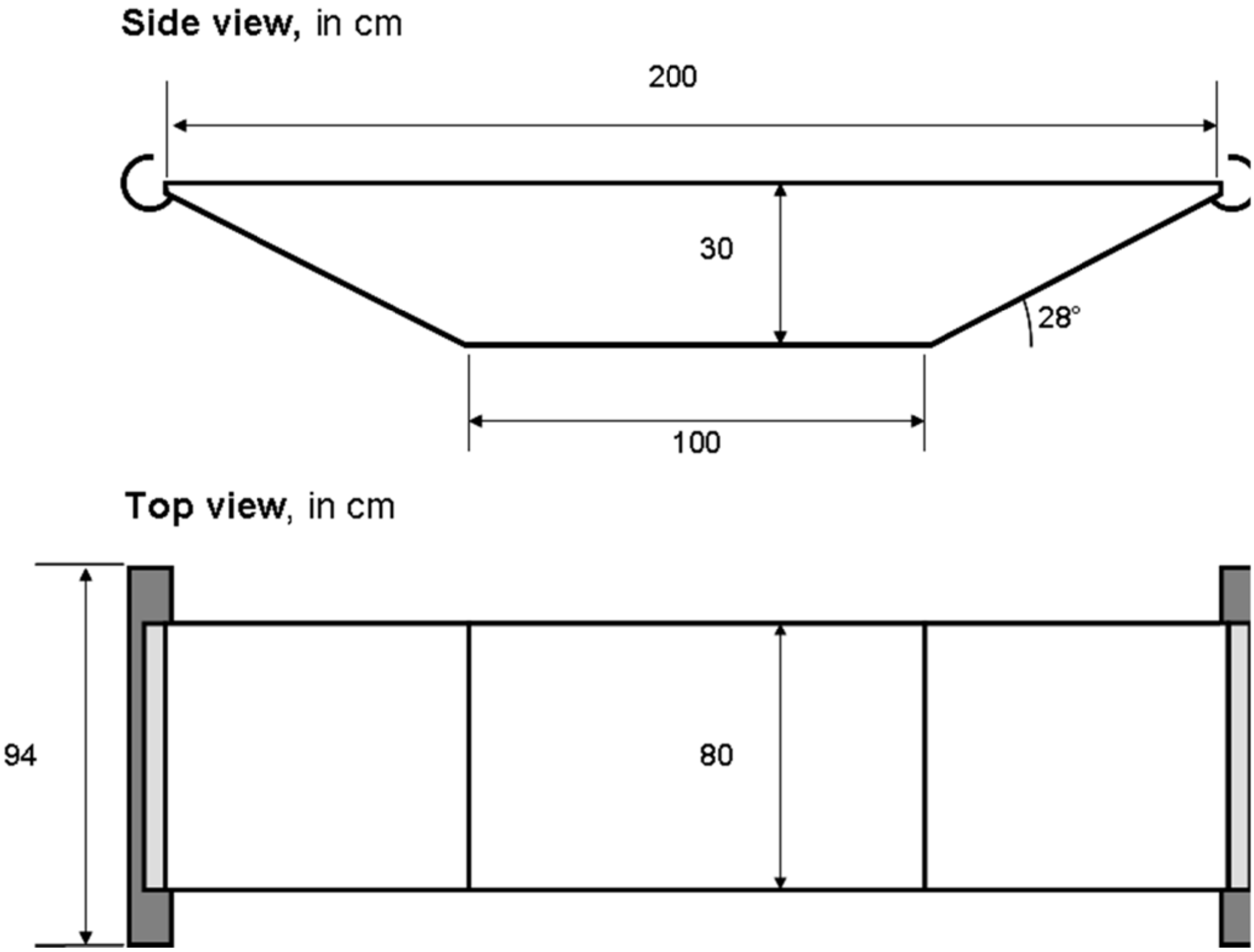

Figure 4-1: Design of a larvero for medium-scale experiments

:: results and discussion

Life history traits. We successfully established a stable black soldier fly colony. A mean daytime temperature of 31.8 °C provided optimal conditions for reproduction, and *H. illucens* flies were capable of tolerating a wide range of temperatures in the moscario (recorded range: 15–47 °C). Similarly, Booth and Sheppard (1984) reported that 99.6% of the oviposition occurred within a temperature range of 27.5–37.5°C. In our experiments, the egg yield (~40 egg clutches daily) from the about 500 adults released to the moscario every day was, however, far lower than expected under the prevailing environmental conditions. We assume that zinc poisoning, caused by the zinc-coated larveros could be responsible for the reduced fecundity. Residual material in the larveros revealed average zinc concentrations of 4,120 mg/kg dry mass (range:

1,550–8,810 mg/kg). There is no literature describing zinc-related fecundity deficiency in terrestrial dipterans, however, Beyer & Anderson (1985) found that zinc concentrations in soil litter exceeding 1,600 mg/kg led to a reduced life span, offspring number and survival rate of the offspring of woodlice, *Porcellio scaber* (Isopoda: Porcellionidae). Other literature describes the reduced fecundity of ground beetles either fed with zinc-contaminated house fly larvae (Kramarz & Laskowski, 1997) or raised on zinc-contaminated ground (Lagisz & Laskowski, 2008). If applicable to dipteran organisms in general and to *H. illucens* in particular, these zinc findings explain the low egg yield of the colony. The actual prepupal harvest, which was far lower than expected in relation to the number of young larvae added to the larveros, is also likely to be attributed to the elevated zinc concentration. Each larvero had been inoculated with freshly hatched larvae originating from a daily average of 4.5 egg clutches. Tomberlin et al. (2002) found an average of 1,160 eggs per egg clutch deposited into corrugated cardboard similar to the one used in the present study. Taking this number as a measure, a prepupal harvest of 178,000 prepupae would have resulted throughout the 34 monitored days in the present study. However, in the high-fed larveros receiving 4.6 kg of food per day, we harvested only 26,000 prepupae or 6.8 times less than expected. In the low-fed larveros receiving 1.5 kg/day, the factor was even 11.7. This phenomenon is not only caused by the reduced hatchability of eggs laid by zinc-contaminated females and the, thereby, overestimated amount of young larvae inoculated as described above, but possibly also due to an increased mortality of young larvae feeding on zinc-contaminated material. Zinc in food offered to house fly larvae is known to lead to a 60% mortality rate in larvae and 70% in pupae. Borowska et al. (2004) attribute this increased larval mortality rate to the larvae's reduced density of haemocytes, an indicator for the fitness of the immune defence in insects. A linear regression revealed that the amount of food and zinc concentration in the residue was strongly related to the prepupal harvest (Table 4-1, Table 4-2). Yet, when interpreting this regression analysis, one has to bear in mind that sample size N = 9 was very small, and the correlation between amount of food and zinc concentration amounted to −0.664. Nevertheless, future experimental setups and full-scale treatment facilities should avoid zinc-coated sheeting but rather use plastic or concrete instead.

Table 4-1: Regression model for mean prepupal yield during the last week of the experiment as a function of "application" (where fresh waste is placed in the larvero), "amount" (daily dry weight of waste) and "zinc concentration" (Zn concentration in the residue at the end of the experiment)

Factor		Unstandardised coefficients		Standardised coefficients	
		B	Std Error	Beta	Sig
Constant		241.4	49.5		0.005
Application*	Surface = 1; Mixed = 2	−46.8	16.5	−0.355	0.036
Amount	kg dry weight/day	29.4	6.8	0.671	0.008
Zinc concentration	mg/kg dry weight	−0.016	0.009	−0.282	0.152

Dependent variable: Mean prepupal yield during the last week of the experiment, g of fresh weight/day; N = 9; Adj R^2 = 0.910; F = 27.96; df = 3; Model Sig = 0.001

Table 4-2: Pearson's correlation of mean prepupal yield during the last week of the experiment as a function of "application" (where is the fresh waste placed in the larvero?), "amount" (daily dry weight of waste) and "zinc concentration" (Zn concentration in the residue at the end of the experiment); N = 9

Factor		Prepupal yield		Application		Amount		Zinc conc.	
		r	Sig	r	Sig	r	Sig	r	Sig
Prepupal yield	g fresh weight/day	1.000	–	−0.408	0.138	0.834	0.003	−0.850	0.002
Application*	Surface = 1; Mixed = 2			1.000	–	0.066	0.433	0.347	0.180
Amount	kg dry weight/day					1.000	–	−0.664	0.025
Zinc conc.	mg/kg dry weight							1.000	–-

Prepupal yield. Average prepupal yield during the last experimental week amounted to 252 g/day (wet weight, SE 23.9) in the high-fed larveros receiving 4.6 kg/day and 134 g/day (wet weight, SE 22.6) in the low-fed larveros (Table 4-3). Compared to the two high/surface larveros, the residue of the high/mixed larveros revealed a higher zinc concentration, thereby possibly explaining the lower prepupal yield. The undisturbed surface loading probably allowed larvae to feed partially on uncontaminated food, thus leading to a higher prepupal harvest.

During the 55-day experimental study, the larveros never reached a steady state, with the amounts of prepupae harvested still augmenting and showing a strong fluctuation in daily prepupal harvests. To what extent this can be attributed to zinc contamination or to heterogeneity of the municipal organic waste still remains uncertain. As a matter of fact, other studies also showed strong variations in prepupal crawl-off and rarely established a constant and predictable harvest (NC State University, 2006; Sheppard, 1983; Sheppard et al., 1994). Days with high prepupal yield were followed by low prepupae production. Interestingly, all the larveros followed a synchronised pattern of high and low harvest, implying an overall trigger for larval migration being either of exogenic (humidity, temperature) or endogenic (pheromones) nature.

Waste reduction efficiency. The relative dry weight reduction in the different treatments varied from 66.4% to 78.9% (Table 4-4). It is not surprising that waste reduction was highest in the larveros receiving less food. However, the effective waste material reduction (effective dry weight eliminated per day) was significantly higher in the larveros receiving a high feeding rate, i.e.740 g/m^2/day if the fresh waste was mixed completely with the residuum, and 780 g/m^2/day if applied on the surface of the residuum. Despite a relatively low larval density, a fairly high relative dry weight reduction was achieved demonstrating that the larvae were able to cope with the daily amount of waste fed. With a 0.86 larvae/cm^2 density, we achieved a daily feeding rate of 507 mg/larva/day, which is far higher than the 61 mg of kitchen waste proposed by Diener et al. (**2009**). Under ideal environmental conditions and with a 5-larvae/cm^2 density, the loading capacity of the system could be a severalfold of the 4.6 kg/day, as fed in the present study.

During the experiments, a considerable amount of stagnating liquid accumulated in the larveros, thus creating an anaerobic, foul-smelling environment. Larvae avoided these areas, causing submerged food to remain untouched. Installation of a drainage system in the larveros improved the waste reduction efficiency from 65.5% to 72.2% for dry weight and from 38.9% to 50.8% for wet weight reduction, though both at a statistically insignificant degree (Sig. = 0.094 and 0.058, respectively). However, the drainage system used in this study revealed some shortcomings: the tap installed to control the effluent often clogged and larvae crawled through the openings. Use of an appropriate filter material (coarse sand, synthetic filter mat) and installation of an s-shaped water seal are possible solutions to these problems.

During the experiments, larvae of the green hover fly, *Ornidia obesa* F. (Diptera: Syrphidae) were often found among the harvested *H. illucens* prepupae. They showed the same migratory habit and were of similar shape and weight (208 mg, SE 4.5). However, in comparison to the 252 g/day harvest of *H. illucens*, the daily *O. obesa* harvest of 8 g/day is negligible. The fact that *O. obesa* had been found in decaying material, such as coffee pulp or pig carcasses (Lardé, 1990a; Martins et al., 2010) and apparently with a similar larval development as *H. illucens,* makes the green hover fly a possible protagonist in organic waste treatment. Further research has to assess its nutritional value and to appraise possible constraints and risk potential for humans and animals.

Table 4-3: Prepupal weight and prepupal harvest resulting from black soldier fly treatment units fed different amounts of municipal organic waste. Low = low amount of food; High = high amount of food; Surface = fresh food applied directly on top of residue; Mixed = fresh food mixed thoroughly with residue; Control = no continuing inoculation with fresh larvae; Numbers in brackets = standard error of the mean

ID	Feed amount		Zinc concentration*	Individual prepupal weight		Prepupal harvest**	
	kg/day		mg/kg dw	mg		g/m²/day	
	ww	dw		ww	dw	ww	dw
Low-Surface	1.50	0.37	4,660	160 (5.3)	64 (2.1)	161 (41.9)	64 (16.8)
Low-Mixed	1.50	0.37	5,310	138 (4.4)	55 (1.8)	107 (16.1)	43 (6.4)
High-Surface	4.60	1.11	2,590	220 (8.0)	88 (3.2)	286 (37.4)	114 (15.0)
High-Mixed	4.60	1.11	4,350	195 (4.6)	78 (1.8)	218 (28.0)	87 (11.2)
Control	2.05	0.50	5,540	171 (4.5)	68 (1.8)	186 (19.8)	74 (7.9)

* Zinc concentration in the residue at the end of the experiment

** Average of the last seven days of the experiment

Table 4-4: Waste reduction in black soldier fly treatment units fed different amounts of municipal organic waste. Low = low amount of food; High = high amount of food; Surface = fresh food applied directly on top of residue; Mixed = fresh food mixed thoroughly with residue; Control = no continuing inoculation with fresh larvae; Numbers in brackets = standard error of the mean

ID	Feed amount		Relative waste reduction		Absolute waste reduction	
	kg/day		%		kg/m²/day	
	ww	dw	ww	dw	ww	dw
Low-Surface	1.50	0.37	75.5 (3.3)	78.4 (1.7)	1.2 (0.05)	0.29 (0.01)
Low-Mixed	1.50	0.37	75.8 (4.4)	78.9 (4.4)	1.2 (0.07)	0.29 (0.02)
High-Surface	4.60	1.11	51.8 (1.1)	69.6 (3.1)	2.4 (0.05)	0.78 (0.04)
High-Mixed	4.60	1.11	46.2 (3.1)	66.4 (1.8)	2.1 (0.15)	0.74 (0.02)
Control	2.05	0.50	55.1 (–)	69.5 (–)	1.3 (–)	0.39 (–)

:: conclusions and recommendations

Despite the zinc contaminated and hostile environment for the larvae in the larveros and subsequent reduced fecundity of the adults, this technology revealed its great potential for organic waste reduction and protein production. Even though the system did not reach a steady state during the observed period, the larveros produced a remarkable average of 252 g (SE 20.2) of fresh prepupae per day and m^2 during the last week of the experiment and reduced the dry matter by 68%. Prepupal harvest per digested food, the so-called feed conversion rate (FCR), correlates with the few other medium-scale studies using fly larvae for waste treatment and protein production. However, waste reduction in the present study is far higher than in the other aforementioned studies (Table 4-5).

Table 4-5: Biomass yield and waste reduction of different pilot-scale systems. MOW = Municipal organic waste; DW = dry weight; WW = wet weight; FCR = Feed conversion ratio

Species	Feed source	Total amount of feed	Residue	Waste reduction	Yield	FCR	Source
H. illucens	MOW	151 kg DW	48 kg DW	68% DW	17.8 kg DW	14.5	This study
H. illucens	Pig manure	68 kg DW	42 kg DW	~39% DW	~2.7 kg DW	9.6	(Newton et al., 2005a)
H. illucens	Chicken manure	5,240 kg WW	~2,620 WW	~50% WW	196 kg WW	13.4	(Sheppard et al., 1994)
M. domestica	Chicken and cow manure	125 kg WW	95 kg WW	25% WW	3 kg WW	10.0	(Morgan & Eby, 1975)

Three factors strongly influenced larval yield and waste reduction capacity: i) lack of fertile eggs due to zinc poisoning; ii) high larval mortality due to the hostile environment in the larveros (zinc concentration, anaerobic conditions) and iii) limited access to food due to stagnating liquid in the larveros. Though elimination of these stumbling blocks is simple, future research will have to concentrate on two main aspects: the biological key factors as well as design and operation of the treatment facility. Enhanced knowledge of the environmental and nutritional requirements of *H. illucens* will significantly improve resilience of the treatment system. Thanks to the larvae's natural habit to colonise feed sources undergoing changes in time, *H. illucens* has developed several peculiarities to warrant survival of the population. During food shortage or unfavourable conditions (oxygen deficiency or low temperatures), the larvae reduce or cease to feed. Under other conditions, when survival of the individual is endangered (e.g. high temperature, toxic conditions), the larvae try to abandon the feed source. For a successful soldier fly treatment system, it is therefore of utmost importance to determine what triggers cessation of food intake or mass migration of immature larvae.

Design and operation of the treatment facility is subject to local context as well as existing habits and requirements. The nature of the waste products and availability of labour and machinery strongly influence construction of the facility. However, the following recommendations are generally applicable: i) a regular, well-balanced food supply prevents bad odours and guarantees a consistent and efficient feeding activity; ii) a drainage system is required when working with wet material (household waste, pig manure) or in a humid climate and iii) use of a ramp for self-harvesting proved of great value and its further development should be pursued. Based on the aforementioned prerequisites, at least 15 kg of fresh municipal organic waste can be added daily to an area of one m^2 yielding a prepupal harvest of 0.8–1.0 kg.

:: acknowledgements

This study was funded by the Velux Foundation and the Swiss Federal Institute of Aquatic Science and Technology. We are grateful to the Department of Ingeniería Agrícola of the Instituto Tecnológico de Costa Rica headed by Milton Solórzano, and to the Universidad EARTH (especially Edgar Alvarado). These two institutions hosted the pilot project in Costa Rica. We would also like to thank Petra Kohler and Roger Lütolf for assisting in the olfactory challenging experiments and Sylvie Peter for linguistic editing support.

5

Conclusions and Outlook

:: general conclusions

The aim of this study was to pave the way to a financially sustainable organic waste management alternative in low and middle-income countries using larvae of the black soldier fly, *Hermetia illucens*. A combination of laboratory experiments under controlled conditions and applied research under field conditions was chosen to reach this objective.

Laboratory experiments confirmed, on the one hand, the predicted larvae's potential to reduce waste and produce protein, thus allowing a balanced trade-off between high prepupal weight and high material reduction. On the other hand, the lab results relating to absolute prepupal weight and waste reduction were lower than the values given in the literature, thereby turning operation of an economically sustainable treatment facility into a rather questionable issue.

Fortunately, these concerns were unfounded as revealed by the successful field experiments conducted in Costa Rica. Waste reduction exceeded all our expectations despite local stumbling blocks, such as a fly colony weakened by zinc poisoning and a far lower larval density. Instead of waste reduction values around 40% as achieved in the laboratory, the household waste could be reduced from 65% to 75%. Even the prepupal weight attained under field conditions was far higher than the value assessed in the laboratory. Prepupae grown on municipal organic waste weighed 195–220 mg (wet weight) – even prepupae fed in the laboratory experiments with the highest waste ration weighed 25% less (157 mg).

Laboratory experiments on the fate of heavy metals present in the feed revealed highly interesting accumulation patterns varying according to metal type and concentration. To put it in a nutshell: cadmium was accumulated, lead suppressed and zinc was kept at a more or less constant level. Accumulation of heavy metals and the way the insects deal with them are of special concern, particularly as regards sustainability of this technology, which aims at being productive while generating a non-toxic product. Many insects possess a natural detoxification mechanism requiring, however, additional energy spent at the cost of growth and/or health. This is being reflected in an alteration of at least one of the important life history traits, such as decreased body mass, life span, reproduction, resistance to other stress factors or decreased resilience in general (Maryanski et al., 2002). The consequences of such a fight against disruptive factors were observed during the field experiments in Costa Rica, where the fly population struggled with a high zinc concentration. It somehow managed to survive but seemed extremely week and susceptible to additional stress factors. Avoiding contaminated food sources, though highly recommended, may not always be possible. Therefore, to provide a somewhat optimum environment, any further perturbing factors should be avoided.

Use of prepupae and residue derived from heavy metal contaminated sources may understandably be restricted. Concentrations will possibly accumulate along the food chain, either via the prepupae fed to chickens, fish or pigs or through the residue ending up in commercially sold vegetables. A process allowing separation of the heavy metals from the products (prepupae and residue) should be developed. In the case of the prepupae, separating the different fractions, such as protein, fat and chitin and using only the uncontaminated parts could provide a possible solution.

The residue from the field trials fell short of our expectations. The material was very wet (82–86% H_2O) and gave off a foul-smelling odour. Whole pieces of organic waste, such as mangoes or bananas, remained untouched due to the stagnating liquid at the bottom of the larveros, thus creating anaerobic conditions. Such conditions hinder larval access to the food source, thus, reducing yield and inhibiting waste reduction. Furthermore, handling of the residue is hampered due to its sticky consistency and foul odour. The system's vulnerability is also enhanced due to ongoing and unstable biological processes. Only the combination of a well designed drainage system and appropriate feeding regime (chopped food, small quantities with high loading frequency) will lead to a steady treatment process and an appropriate residual product. Yet, the residue will most likely have to be post-treated through composting/vermicomposting to ensure biological stability prior to its application as a soil conditioner, or its remaining energy potential possibly exploited by feeding it to a biogas plant.

:: challenges of future research

The study at hand confirms the application potential of the black soldier fly in solid waste management. However, we are dealing here with a technology whose delicate equilibrium is based on living organisms. However, *Hermetia illucens* is an extremely resistant species capable of dealing with demanding environmental conditions, such as drought, food shortage or oxygen deficiency. Owing to their robustness, survival of the species as a whole will not be endangered within a region, yet, a waste source turning anaerobic, temperatures reaching lethal values or elevated heavy metal concentrations exceeding a certain threshold level may prove fatal to the affected larva population. Even a partial collapse of a treatment plant's population can have serious economic and environmental consequences. Contracts with waste suppliers and product purchasers may be unfulfilled and, thus, the collected waste disposed of untreated. The challenge of future research will therefore be to provide sound data on the biological treatment processes and the environmental conditions required by the insects to enhance performance of the treatment facility and, thus, increase financial output. This will strengthen resilience of the treatment plant and the waste management plan in which the BSF unit is embedded. Such research must occur iteratively, involving plant operators, researchers and regional planners, with closely knit information exchange networks to ensure rapid response to emerging new developments and challenges.

The treatment process itself and its effectiveness are crucial elements of the entire treatment system requiring special attention in future research work. However, on either side of the treatment process, several components in the treatment chain should not be neglected and also form part of upcoming research activities. On the input side, the waste products have to conform to certain nutritional and sanitary standards, and their regular supply has to be guaranteed, possibly in combination with an appropriate collection regime. On the other hand, the treatment system generates two valuable products, i.e. the prepupae and residue. Besides technological research questions, such as post-processing of the residue, preservation of the prepupae and/or extraction of the different components (protein, fat, chitin) from the prepupae, it is important to also assess market demand and susceptibility of a product whose origin is a combination of organic waste and dipteran larvae. It is hard to believe, but there may still be a certain bias against this technology.

It is not quite clear yet if organic waste management using larvae of the black soldier fly will ever turn into an economically self-sustaining business. Based on experimental data, it is indeed easy to calculate waste conversion rates, prepupal yield, fish meal equivalents or replaced fertiliser, and extrapolate this data to plan and design systems treating tonnes of organic waste per day. These promising calculations should rather be regarded as a motivation for further researchers not to abandon their mission and not as a promise for investors or municipal officers planning a full-scale treatment plant. The black soldier fly technology still has to undergo several developmental stages. Yet, the advance of this emerging treatment tool is most likely to follow the development of commercial biogas production units. Though known for centuries, waste treatment using biodigestors became widespread on a small scale only in the middle of the 20th century, when developing countries, such as India and China, embraced this technology by implementing especially small-scale units. Europe, North America and the Soviet Union became involved in research and implementation a few years later. Unfortunately, a lack of knowledge of the exact processes and system operation led to numerous failures. Asian countries and US farm digesters reported 50% and 80% failure rates, respectively (Verma, 2002). Today, biogas reactors are run on a professional, economic sustainable level at different scales also as a result of tenacious efforts, pioneer research work and experience acquired through failure. To sum up: we shall not expect instant miracles, but shall persist to continue research and enjoy forming part of a technological evolution.

6

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Publications

Peer reviewed publications

4. **Diener, S.**, Zurbrügg, C., Tockner, K., 2009, Conversion of organic material by black soldier fly larvae – Establishing optimal feeding rates. *Waste Management and Research*, 27: 603–610

3. Müller, A., **Diener, S.**, Schnyder, S., Stutz, K., Sedivy, C., Dorn, S., 2006, Quantitative pollen requirements of solitary bees: Implications for bee conservation and the evolution of bee-flower relationships, *Biological Conservation*, 130(4): 604–615

Book chapters and reports

2. Zurbrügg, C., Rothenberger, S., Vögeli, Y., **Diener, S.**, 2007, Organic solid waste management in a framework of Millennium Development Goals and Clean Development Mechanism, In: Diaz, L.F., Eggerth, L.L., Savage, G.M. (Ed.), Management of Solid Wastes in Developing Countries, CISA, Padova, Italy, pp 430

1. Morel, A., **Diener, S.**, 2006, Greywater Management in Low and Middle-Income Countries, Review of different treatment systems for household or neighbourhoods. Swiss Federal Institute of Aquatic Science and Technology (Eawag), Dübendorf, Switzerland, pp 96

Publications in preparation

Diener, S., Studt Solano N., Roa Gutiérrez F., Zurbrügg C., Tockner, K., Biological treatment of municipal organic waste using black soldier fly larvae, *Waste and Biomass Valorization (in press)*

Diener, S., Zurbrügg, C., Tockner, K., The potential use of the black soldier fly (Diptera: Stratiomyidae) as animal feed: Bioaccumulation of heavy metals and effects on the life cycle (Submitted to *Environmental Entomology*)

Referee experience

Water Science & Technology, Journal of Insect Science, Resources, Conservation & Recycling, WaterLines

Competitive research funding

2006–2010	Velux Foundation funding 4 year PhD study "From Waste to Value – Using Black Soldier Flies as ecological engineers in low and middle-income countries", CHF 270,000 (USD 240,000)

Presentations

Invited speaker

2010 Oct., Research Institute of Organic Agriculture, speaker in the course "Ecological Aquaculture"

2010 June, London School of Hygiene and Tropical Medicine. Seminar as part of the "Bugging You" series. "The potential of the Black Soldier Fly for organic waste management in low and middle-income countries"

2008 Feb., Leibniz-Institute of Freshwater Ecology and Inland Fisheries. "From Waste to Value – Using Black Soldier Flies as ecological engineers in low and middle-income countries"

2007 Aug., Swiss Federal Institute for Forest, Snow and Landscape Research. "From Waste to Value"

Presentations at conferences

2011 Feb. 13-15, WasteSafe 2011, 2nd International Conference on solid Waste Management in Developing Countries, Khulna, Bangladesh. **Oral presentation**: "Black Soldier Fly Larvae for Organic Waste Treatment – Prospects and Constraints"

2009 Oct. 5–9, Twelfth International Waste Management and Landfill Symposium, Cagliari, Sardinia, Italy. **Oral presentation**: "Are larvae of the black soldier fly – *Hermetia illucens* – a financially viable option for organic waste management in Costa Rica?"