ASSESSMENT OF THE ROLE OF FOUR SPECIES OF EARTHWORM IN THE BIOREMEDIATION OF ALLELOPATHIC WEEDS SALVINIA AND IPOMOEA

Thesis submitted
For the award of the degree of

DOCTOR OF PHILOSOPHY
in
Environmental Technology

by
Channgam Khamrang K, MSc, M. Phil

Under the supervision of

Prof. S. A. Abbasi, PhD, DSc, FNASc, FIIChE, FIE, PE
UGC Emeritus Professor

Centre for Pollution Control and Environmental Engineering
Pondicherry (Central) University
Puducherry 605014, India
September 2017

PONDICHERRY
UNIVERSITY
R. V. Nagar, Kalapet
Pondicherry 605 014

CENTRE FOR
POLLUTION CONTROL
AND ENVIRONMENTAL
ENGINEERING

S. A. Abbasi PhD, DSc, FNASc, FIIChE, FIE
UGC Emeritus Professor

prof.s.a.abbasi@gmail.com
Mobile: 94432-65262
www.prof-abbasi.com

CERTIFICATE

This is to certify that **Channgam Khamrang K** has herself carried out the work embodied in her thesis ***Assessment of the role of four species of earthworm in the bioremediation of allelopathic weeds salvinia and ipomoea*** being submitted to Pondicherry University for the award of the degree of **Doctor of Philosophy** in **Environmental Technology**. She has complied with all the relevant academic and administrative regulations, and the thesis embodies a bonafide record of the work done by her under my supervision. The work is original and has not been submitted for the award of any certificate, diploma or degree of this or any other university.

Prof S. A. Abbasi

DECLARATION

I hereby affirm that this thesis entitled ***Assessment of the role of four species of earthworm in the bioremediation of allelopathic weeds salvinia and ipomoea*** submitted to Pondicherry University for the award of the degree of **Doctor of Philosophy** in **Environmental Technology** is a record of original work done by me under the guidance of **Prof S. A. Abbasi** ,UGC Emeritus Professor, Centre for Pollution Control and Environmental Engineering, Pondicherry University, and that it has not formed the basis for the award of any other degree, diploma, certificate, or any other title by any university or institution before.

Date:
Place: Puducherry **Channgam Khamrang**

ACKNOWLEDGEMENT

I Thank the Almighty God for His blessings and mercy for enlightening me all throughout the course of my work.

I am immensely grateful to my supervisor **Prof S. A. Abbasi**, for the astute guidance, insightful suggestions, constructive criticisms and unwavering support and dedication during the different stages of my study. His unfaltering patience, constant motivation and confidence in me kept me going. Without his contributions, this work would not have been possible.

My deepest gratitude to **Dr. Tasneem Abbasi**, Assistant Professor, Centre for Pollution Control & Environmental Engineering, Pondicherry University for her suggestions and encouragement throughout the course.

My sincere thanks to Doctoral Committee members **Prof G. Poyamoli** and **Dr S. Gajalakshmi** and for their valuable comments.

I sincerely thank **Dr. Naseer Hussain** for his guidance in all aspects of my work. I particularly thank him for all his support and encouragement in my research. I am also thankful to **Dr M. Premalatha and Ms Anandajothi** for their support throughout my work.

A special thanks to **Banu** and **Rafiq** for their consistent help throughout my PhD work. I am also thankful to **Mrs Patnaik, Mr Prem Aravind, Prabu anna, Mathu anna** and all my seniors and juniors for providing me unflinching encouragement and support in various ways.

I wish to thank my family members, especially my parents, my sister and all my freinds, for their prayers, love, affection and encouragement. Words will be insufficient to acknowledge and appreciate their moral, financial and emotional support.

Lastly, my heartfelt and sincere gratitude to all the colleagues, friends and the staff, whose names I could not mention here. Thank you all.

Thank you all.

Channgam Khamrang

Dedicated to my parents

CONTENT

List of tables

Chapter 2

Chapter 3

Chapter 4

List of figures

Chapter 3

Chapter 4

Chapter 5

Chapter 6

:

Chapter 1

Introduction to the thesis: assessment of the role of four species of earthworm in the bioremediation of allelopathic weeds salvinia and ipomoea

1.1 Backdrop of the present work

Every year invasive plants like salvinia *(Salvinia molesta)* and ipomoea *(Ipomoea carnea)* generate billions of tons of phytomass across the world (Abbasi and Abbasi 2010). As the leaves and then the plants die, they undergo degradation.When the degradation occurs aerobically, it generates CO_2 which is a global warming gas (GWG). But a large proportion of the degradation occurs anaerobically, especially in case of salvinia which is an aquatic weed. As ipomoea is amphibious, a large part of its biomass also degrades anaerobically in the anoxic zones of wetlands. This anaerobic degradation leads to the generation of a broadly 3:1 (v/v) mixture of methane (CH_4) and CO_2. As methane is 25 time more potent GHG ─ molecule to molecule ─ than CO_2 this contributes massively to global warming (Abbasi *et al.,* 2011, 2012).

Invasive plants also, in general, seriously harm biodiversity by elbowing out other vegetation and monopolizing the use of soil nutrients, land, and water. Additionally, weeds like ipomoea and parthenium are not only allelopathic but also possess mammalian toxicity (Hussain *et al.*, 2016).

Since several decades extensive efforts have been made across the world to destroy the invasive plants or at least control their proliferation. A vast variety of chemical, biological, and mechanical methods have been tried, singly or in combination. But all such efforts have at best only achieved partial or temporary success. In most situations a “controlled” invasive has either come back or has paved the way for some other invasive to move in. Moreover all the three approaches of combating invasive plants have their own serious downside and at times the attempted remedy ends up being worse than the disease (Abbasi and Nipaney 1993).

Attempts have also been made to find ways of utilizing invasive plants as possible source of paper pulp chemicals (including medicinals and cosmetics), animal feed, mulch, artifects, etc. But none has been able to replace pre-existing products which are better in quality as also, often, less expensive to mass-produce (Abbasi and Nipaney 1993).

1.2 Potential of vermicomposting in utilizing ‘waste’ phytomass

In nature earthworms scavenge upon plant debris and other organic matter. Indeed alongside ants and termites they play a major role in mineralizing organic debris and rejuvenating the soil ─ comminuting it by their burrowing, ingestion, casting, and improving its water retention capacity while bringing its pH to near neutral (Abbasi *et al.*, 2015). Epigeic (phytophagous) ─ and, to a lesser extent anecic (geophytophagous) ─ earthworms are specially tuned to scavenging organic matter. If this natural ability can be harnessed in controlled vermireactors, it can be possible to convert the huge quantities of biomass that is generated by invasive species like salvinia and ipomoea into organic fertilizer.

1.2.1 The challenges involved

But, till recently no technology existed with which phytomass ─ especially crop waste and weeds ─ could be directly converted to vermicompost. Attempts were, of course, made by several authors in the past (reviewed in Abbasi *et al.,* 2015) to vermicompost phytomass but the conventional vermireactors used for the purpose, which have been successful in vermicomposting zoomass (animal manure), were unable to process phytomass. To get round this problem most authors resorted to either pre-compost the phytomass after blending it with animal manure (mainly cow dung) and/or vermicompost phytomass after adding to it animal manure to the extent of 50% or more of the feed. This approach has several limitations, including the following:-

i) Supplementing phytomass with large proportions of animal manure, especially cowdung, entails two major disadvantages. First is that to process the very large quantities of phytomass that are available, equally large quantities of animal manure shall be needed. But it is not possible to find so much manure because of numerous competitive uses of manure already in existence (Abbasi *et al.,* 2012; Tauseef *et al.,* 2013). The second major disadvantage is that unlike waste phytomass, animal manure is not available free of cost. Hence dependence on animal manure makes the process economics highly unfavorable.

ii) Collection and transport of animal manure are among the operations which lead to massive emissions of global warming gases methane and nitrous oxide (Abbasi *et al.,* 2013; Tauseef *et al.,* 2013), besides other pollutants, like ammonia. Large-scale use of manure in vermicomposting of phytomass will add to global warming and pollution (Tabassum-Abbasi *et al.,* 2016).

iii) The reported processes, as summarized by Abbasi *et al.,* (2015), have all been very slow, taking 2 months or more to achieve substantial conversion of phytomass to vermicast (unless pre-composting had been done). As the rate of any process is directly related to its efficiency, hence economics, this aspect further diminishes the utilizability of reported processes.

iv) When pre-composting had to be done, it further adds time and cost to the overall process, eroding its economic viability still further.

v) There is absence of a logical criterion to define what the product of a vermireactor is, and how to ascertain that the reactants have been fully converted to that product. Due to the absence of this criterion, there are no pointers available with which the vermireactor operation can be engineered to enhance the efficiency and the economics of the process. Nearly all past attempts have been on batch reactors with very long, and unfounded, solid retention times (SRTs).

1.2.2 The high-rate vermicomposting process

To meet this challenge S. A. Abbasi had conceived the idea of high-rate vermicomposting during late 1990s (Abbasi *et al.,* 2015). Subsequently he and co-workers developed and refined the concept based on extensive experimentation and modeling (Abbasi *et al.,* 2009; 2015). Appropriate machinery was also developed and its patent claims published (Abbasi *et al.,* 2011b; Tauseef *et al.,* 2013). These initiatives have made it possible to directly vermicompost phytomass ─ without any pretreatment or manure supplementation ─ and at rates 2-3 times faster than conventional vermicomposting systems (Ganeshkumar *et al.,* 2014; Nayeem-Shah *et al.,* 2015).

The concept of high-rate vermicomposting is based on the following premises:

a) Vermicomposting is a process very different form composting. Whereas the latter is a quintessential batch process, the former is amenable to continuously-fed operation (Abbasi *et al.,* 2009). The microbiology and biochemistry of the two processes also has several major differences (Gajalakshmi and Abbasi, 2008).

b) The most rational and the most easily quantifiable criteria with which vermicomposting systems can be designed, optimized, and monitored is the vermicast production. This is because vermicast is the finished product of vermicomposting and fresh vermicast is believed to be more soil friendly and plant-friendly than aged vermicast (Edward *et al.,* 2011; Karthikeyan *et al.*, 2014a, b).

c) Based on the earthworm species and the nature of substrate, it takes only 6-18 h for vermicomposting to occur because this is the time that is taken between the commencement of ingestion of a substrate by an earthworm and its exit as the vermicast (Abbasi and Ramasamy, 2001). Hence the upper limit of the speed of a vermireactor, defined as the time taken to convert a feed into vermicast, is only 12 ± 6 h. But conventional vermireactors take 8-12 weeks for converting most of the feed to vermicast and this indicates something basically flawed in the way conventional vermicomposting systems have been designed and operated.

d) Unless very complex instrumentation and control is done, it is not possible to have a vermicomposting system which will be able to separate from the vermireactor each grain of vermicast as it is generated. Hence the upper limit of vermireactor speed is not achievable in practice. But it appears possible to significantly enhance vermireactor efficiency by taking it closer to its theoretical limit without compromising on simplicity (hence better economics) of vermireactor operation.

The high-rate vermicomposting paradigm has the following attributes:

i) It relies on a reactor geometry that has been chosen to maximize earthworm-substrate contact as well as ease of cast deposition. To this end, a high surface area-to-volume ratio is set for the reactors. This also ensures mixing and aeration of the reactor content by the earthworm movements thereby preventing anaerobic pockets from developing.

ii) The vermicast harvesting is also made easier by the low aspect-ratio because many species deposit their cast on top of the substrate while some others do it at the bottom.

iii) Low substrate column height makes it possible to maintain, with relative ease, uniformity in the moisture content across the reactor depth. There is little, if any, accumulation of water in the reactor bottom, saving on the need for recycling.

iv) The sand-gravel ‘vermibed’, which occupies over 25% space in conventional vermireactors, is replaced by moist jute cloth. This maximizes the use of reactor volume, proportionately reducing the system cost.

v) Earthworm density is maximized to achieve highest sustainable population for a given feed. The high earthworm: feed ratio further helps mixing and aeration of the substrate due to earthworm movement.

1.3 But is phytomass-derived vermicompost utilizable as a fertilizer?

The existing knowledge of the virtues of vermicompost as a fertilizer is almost entirely based on experience with manure-based vermicompost. A few studies also exist on phytomass but in all these reports phytomass had been vermicomposted with cow dung supplementation and it is not possible to say with certainly whether the phytomass part had any positive role or whether the source of the benefit is entirely the animal manure part (Suthar and Sharma, 2013; Karthikeyan *et al.,* 2014).

The question whether phytomass−derived vermicompost is utilizable as a fertilizer becomes even more poignant when we consider vermicompost derived from highly invasive (weedy) species like ipomoea (*Ipomoea carnea*), parthenium (*Parthenium hysterophorus*), lantana (*Lantana camara*), or salvinia (*Salvinia molesta*). All these species possess strong allelopathy and all, except salvinia, are also toxic to animals and other plants in various other ways. Will their vermicompost retain these hostile characteristics? If not, whether the vermicompost will have attributes beneficial to soil and plants? If yes, to what extent? And if earthworms are able to bioremediate allelopathic weeds, is the attribute species−dependent? If yes, to what extent?

1.4 The present work

The present world is an outcome of the efforts to seek answers to the questions posed above. The work has focused on two of the world's most intransigent and widespread of invasive plants — salvinia and ipomoea. Of these salvinia is aquatic and ipomoea is amphibious. Both are strongly allelopathic (Karthekiyan *et al*., 2014; Rajiv *et al*., 2013; Hussain and Abbasi, 2015; Devi *et al*., 2014). Ipomoea is also known to generate toxic exhudates (Rios *et al*., 2008; Bevilacqua *et al*., 2011; Knox *et al*., 2011; Patel, 2011) which kill seeds of other species and contain chemicals which toxify animals who graze upon them (Maishi *et al*., 1998; Ahmed *et al*., 2007; Oudhia, 2000).

We have first presented studies on the direct vermicomposting of salvinia and ipomoea by four species of earthworm: *Eisenia andrei, Perionyx sansibaricus*, *Lumbricus rubellus* and *Drawida willsi*. No pre-composting or manure supplementation was done. The vermireactors were operated without interruption for several months to establish the viability of the high-rate vermicomposting paradigm.

We then studied the rate of vermicomposting achieved by the second and the third generation of earthworms ─ born and grown in weed-fed reactors ─ in comparison to the first generation animals which had been raised to adulthood on cowdung before being introduced into weed-fed vermireactors. These were to see whether there is adaptive response and if yes, to what extent.

Detailed studies on the transformations that occur when salvinia or ipomoea get vermicomposted by the four earthworm species were then carried out with the aid of UV-Visible spectrophotometry, Fourier transform infrared spectrometry (FTIR), thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), and scanning electron microscopy (SEM).

1.5 Summary

This Chapter sets the context of the present thesis, briefly telling why the work described in the thesis was attempted.

References

1. “Abbasi, S. A., 1993. World’s Worst Weed: Impact and Control. International Book Distributors Dehradun, xi + 226 Pages.

2. Abbasi, S. A., Nayeem-Shah, M., Tasneem Abbasi., 2015. Vermicomposting of phytomass: limitations of the past approaches and the emerging directions. J. Clean. Prod. 93, 103-114.

3. Abbasi, T., Tauseef, S. M., Abbasi, S. A., 2012. Anaerobic digestion for global warming control and energy generation: An overview. Renew. Sustain. En*er*. Rev. 16, 3228-3242.

4. Abbasi, T., Tauseef, S. M., Abbasi, S. A., 2012. Biogas Energy, Springer, New York & London, xiv+169 Pages; ISBN 978-1-4614-1039-3.

5. Abbasi, T., Tauseef, S.M., Abbasi, S. A., 2011, Global Warming and the Role of Wetlands Lap Lambart Academic, Germany; ISBN 978-3846556009. xiv+264 pages.

6. Abbasi, T., Tauseef, S. M., Abbasi, S. A., 2013. Ozone hole spreads father: its history and its lessons, Int. J. Environ. Sci. Eng. Res, *4*, 69-75.

7. Abbasi, S. A., Ramasamy, E. V., Khan, F. I., 2001. Control of suspended particulate matter (SPM) by greenbelts, J. Inst. Public Health Eng, (3), 25-29.

8. Ahmed, R., Jilani, G., Arshad, M., Zahir, Z. A., Khalid, A., 2007. Bio-conversion of organic wastes for their recycling in agriculture: and overview of perspectives and prospects, Annals of Micorbiol, 57(4) 471-479.

9. Abbasi, T., Abbasi, S. A., 2010. Production of clean energy by anaerobic digestion of phytomass - new prospects for a global warming amelioration technology. Renew. Sust. Energ Rev. 14, 1653–1659.

10. Abbasi, T., Gajalakshmi, S., Abbasi, S. A., 2009. Towards modeling and design of vermicomposting systems: Mechanisms of composting/vermicomposting and their implications, Ind. J. Biotechnol, *8,* 177-182.

11. Bevilacqua, G., Czakon, M., van Hameren, A., Papadopoulos, C. G., Worek, M., 2011. Complete off-shell effects in top quark pair hadroproduction with leptonic decay at next-to-leading order. J. High Energ. Phys. 2, 1-30.

12. Chari, K. B., Abbasi, S. A., 2005. Map viewer: a new add-on tool for geographic information system, Inf. Technol. J.4, 469-475.

13. Devi, O. I., Dutta, B. K., Choudhary, P., 2014. Allelopathic effects of some weed species on the growth of tomato plants (*L. Solanum lycopersicum*). J. Int. Acad. Res. Multidisciplinary, 2:110–117.

14. Edwards, C. A., Subler, S., Arancon, N. Q., 2011. Vermiculture technology—earthworms, organic wastes and environmental management. CRC Press, Boca Raton.

15. Ganeshkumar, T., Premalatha, M., Gajalakshmi, S., Abbasi, S. A., 2014. A new process for the rapid and direct vermicomposting of the aquatic weed salvinia *(Salvinia molesta)*. Bioresour. Bioproc, 1(26), 1-5.

16. Hussain, N., Abbasi, T., Abbasi, S. A., 2016. Vermicomposting-mediated conversion of the toxic and allelopathic weed ipomoea into a potent fertilizer, Process Saf. Environ. Protect, 103, 97-106.

17. Hussain, N., Abbasi, T., Abbasi, S. A., 2015. Vermicomposting eliminates the toxicity of Lantana (*Lantana camara*) and turns it into a plant friendly organic fertilizer, J. Hazard. Mat, 298, 46-57.

18. Karthikeyan, M., Gajalakshmi, S., Abbasi, S. A., 2014. Effect of storage on the properties of vermicompost generated from paper waste: with focus on pre-drying and extent of sealing. Int. J. Energ. Environ. Eng, 5:135; 1-11.

19. Karthikeyan, M., Gajalakshmi, S., Abbasi, S. A., 2014. Ingestion of sand and soil by phytophagous earthworm *Eudrilus eugeniae*: a finding of relevance to earthworm ecology as well as vermitechnology. Arch. Agron. Soil Sci, 60(12); 1795-1804.

20. Knox, C., Law, V., Jewison, T., Liu, P., Ly, S., Frolkis, A., Djoumbou, Y., 2011. Drug Bank 3.0: a comprehensive resource for ‘omics’ research on drugs. Nucleic acids Res, 39 (suppl 1), D1035-D1041.

21. Maishi AI, Ali PKS, Chaghtai SA, Khan G. A., 1998. Proving of *Parthenium hysterophorus*, L. Brit Homoeopath J. 87:17– 21.

22. Nayeem-Shah, M., Gajalakshmi, S., Abbasi, S. A., 2015. Direct, rapid and sustainable vermicomposting of the leaf litter of neem *(Azadirachta indica*).Appl. Biochem. Biotechnol, 2(175), 792-801.

23. Oudhia, P., 2000. Germination and seedling vigour of Kodomillet as affected by allelopathy of *Ipomoea carnea* Jacq. Ind J. Plant Physiol. 5 (4), 383-384.

24. Patel, S., 2011. A weed with multiple utility: *Lantana camara.* Rev. Environ, Sci. Biotechnol, 10, 341-351.

25. Rajiv, P., Rajeshwari, S., Yadav, H., Rajendran, V. R., 2013. Vermiremediation: detoxification of parthenin toxin from parthenium weed. doi:10.1016/j.jhazmat.2013.08.075

26. Rios, E., Cholich, L., Silva, J., Acosta de Pérez, O., 2008. Histopathological lesions in central nervous system of goats poisoned by *Ipomoea Carnea***.** Intox. Cabras. Rev. vet. 19:130–134.

27. Gajalakshmi, S., Abbasi, S.A., 2008. Solid waste management by composting: state of the art, Crit. Rev. Environ. Sci. Technol, (CRC Press) *38,* 311–400.

28. Gajalakshmi, S., Ramasamy, E.V., Abbasi, S.A., 2001. Screening of four species of detritivorous earthworms for sustainable vermicomposting. Environ. Technol. 22, 679-685.

29. Suthar, S., Sharma, P., 2013. Vermicomposting of toxic weed—*Lantana camara* biomass: chemical and microbial properties changes and assessment of toxicity of end product using seed bioassay. Ecotoxicol. Environ. Saf, *95*,179-187.

30. Tabassum-Abbasi, T., Abbasi, T., Abbasi, S, A., 2016. Reducing the global environmental impact of livestock production: the mini livestock option, J. Clean. Prod, 112, 1754-766.

31. Tauseef, S. M., Premalatha, M., Abbasi, T., Abbasi, S. A., 2013. Methane capture from livestock manure, J. Environ. Manag. 117, 187-207.”

Chapter 2

Phytowaste and vermicomposting as a potential route for its environmentally compatible utilization

2.1 Phytomass of invasive plants or weeds

2.1.1 How phytomass becomes a ‘waste’

Generation of ‘phytomass’ ─ which is the biomass of botanical species or ‘plants’ ─ is the sole means of converting solar energy into a source of animal food; in other words a means of primary production and primary consumption. If a species of phytomass in a region can not perish at the rate at which it is generated, it can lead to a progressive excess of that species in that region. When such an excess begins to harm other species of plants in a location, out-competing them, and begins to monopolize the use of soil, water, and other natural resources of that location, it becomes a nuisance and is called a weed. A patch of land covered with a weed is similar in nature to a patch of land on which municipal solid waste has been dumped: harmful and undesirable.

But no species of plant is intrinsically harmful or worthless. Indeed several species have been highly useful and essential in their regions of origin and have become serious pests only after they were introduced in other regions. Prosopis (*Prosopis juliflora)* is a major example. It has been considered a ‘wonder tree’, and perceived as a great blessing in its native desert environments (Patnaik 2017; Patnaik *et al.,* 2017). But upon being introduced in other regions it has become a very serious pest. Even olive tree, which is lovingly grown in most parts of the world, has become a weed in Australia as it has begun to invade and colonize lands which were not meant for it. *Hydrocotyle asiatica* is eaten as a vegetable in several regions of Asia while *Alternanthrea sessilis* and *Eclipta postrata*are keystone medicinal plants in the Ayurvedic and Homoeopathic systems of medicine (Swapna *et al.,* 2011; Abhang *et al.,* 2015; Jain *et al.*, 2015; Himaja and Neelufar, 2015; Chung *et al.,* 2017). Yet all the three are regarded as weeds because the extent of their growth is lot more than that which being is utilized. Indeed nearly every species presently recognized as a weed had been used in traditional medicine, or as animal feed, or as food, or as an ornamental plant (Hussain, 2016; Banupriya, 2017). But the quantities thus utilized have been miniscule in comparison to the quantities generated.

2.1.2 Some common attributes of the invasives

Not all exotic plants become weeds. Those who become, have certain defining traits which include one or more of the following:

1. A very strong ability to reproduce and regenerate. Most invasive species have large seed banks with high viability. Most have the ability to regenerate sexually as well as vegetatively.
2. A high tolerance towards agro-climatic variations. Such plants can thrive in widely verying soil types, ambient humidity, ambient temperature, water availability, etc. They also trend to adapt to new environments quicker than other species do.
3. Great resilience and hardiness: they can withstand attempts at their eradication and keep coming back strongly.
4. Allelopathy and propensity to carry/release chemicals toxic to soil, animals (including humans), and other plants. This helps them elbow out other vegetation and hasten their colonization.
5. A pronounced ability to invade new regions and rapidly proliferate in them.
6. Presence of very few, if any, grazers or natural enemies. Plants which become invasive in exotic locales have enemies and grazers in their natural habitat which

keep their spread in check. When such plants are taken to regions where those natural enemies do not exist, the plants proliferate unchecked.

2.1.3 Aggravation due to anthropogenic factors

The above mentioned traits are aided and abetted by anthropogenic interventions such as:

1. Increasing conversion of natural forests into mono-cultures for commercial purposes
2. Environmental pollution
3. Interventions such as damming of rivers
4. Habitat fragmentation caused by unplanned and runaway 'development' in the form of roads, buildings, transmission lines, etc

These and similar other forms of anthropogenic tampering of the environment reduces bio-diversity and makes it increasingly difficult for the remaining of the sensitive species to survive. This paves the way for a few hardy and domineering species to invade more and more areas and colonize them.

2.1.4 The impact

The impact of colonization of land or water by invasive plants is to worsen and hasten the eco-degradation already set in motion by the anthropogenic tampering with the environment. The impact manifests itself in the following ways:

i) Colonization of large areas by one or the other of these species which eliminates most other vegetation. With it are eliminated a large number of animals associated with those species of vegetation. It is also common to see 2-3 weeds like ipomoea, salvinia, and water hyacinth pressing upon each other to gain accidency in an area.

ii) There is increasing pressure on land and water resources, besides soil nutrients, which are monopolized by one or more of these weeds.

iii) The habitat or food loss suffered by smaller animals ─ who had depended on the vegetation since repelled by these weeds ─ adversely effects the animals higher in the food chain. It also favours a small number of hardly animals capable of surviving in the weed monocultures, allowing them to proliferate and dominate at the cost of more sensitive and niche-specific animals, thereby harming the biodiversity even further.

iv) Serious jeopardies to the aesthetics of the water−spreads, water holding capacity of the lakes and reservoirs, water quality, fisheries, navigation, water sports, etc, caused when the invasives happen to be an aquatic weed like salvinia *(Salvinia molesta)*, or an amphibious weed like ipomoea *(Ipomoea carnea)*.

The cumulative losses caused by these weeds in terms of loss of forest and arable land, water quality and quantity, soil nutrients, biodiversity, ecosystem services, and habitats are estimated to run into billions of rupees per year.

The phytomass represented by weeds can be regarded as ‘phytowaste’ for the following reasons:

i) It does not perform ecosystem services as numerous other species (which are not directly used by humans but indirectly contribute to ecosystem health) do. On the other hand it interferes with and harms those species which perform ecosystem services.

ii) It either has no direct utility at all, or has much lesser utility in comparison to its availability.

iii) By eliminating or discouraging most sensitive species of plants and animals, it facilitates proliferation of a few hardy species which can be disease vectors. For example salvinia provides habitat for mosquitoes which can cause malaria, dengue and elephantiasis. It also facilitates the species of snail which causes chistomiasis.

iv) When it falls on soil/water in the form of dead leaves/twigs or dead whole plants it degrades aerobically or anaerobically ─ mostly latter. The former generates CO_2 while the latter emits a 1:3 mixture of CO_2 and CH_4 (methane). Given that each molecule of CH_4 causes 25 times more global warming than each molecule of CO_2 (34 times by the estimate of Shindell *et al.*, 2009), this type of degradation in

> nature amounts to a plant contributing more global warming gas (GWG) than it had earlier fixed (in the form of atmospheric or aquatic CO_2) in the course of photosynthesis.

Viewed from this perspective, stands of weeds like salvinia or ipomoea are veritable ‘waste factories’ because they keep spreading and consolidating thereby contributing more and more ‘waste’ phytomass. Salvinia, alongside water hyacinth *(Echhoria crassipes)*, is the most productive of all plants, attaining net primary production of the order of 60 ash-free dry tonnes per hectare, per year (Abbasi and Nipancy, 1995).

2.1.4 Huge streams of other phytowaste

Massive streams of phytowaste are also generated in the course of agriculture, especially horticulture. An example is the biodegradable waste generated to the tune of 105 million tonnes per year (Al-Juhaimi *et al.,* 2013; Nayeem-Shah 2014) in the course of cultivation of date palm trees (*Phoenix dactylifera).* As is the case with the weed phytomass, these streams are also largely unutilized at present and degrade in the open, releasing a more potent greenhouse gas ─ CH_4 ─ than the one they had fixed earlier, viz. CO_2.

2.2 Past efforts to eradicate or control the weeds

In a recent state-of-the-art review from this author’s group, Banupriya (2017) has noted as follows:

Quote:

“Classically, infestation by any plant is controlled by any one or the combinations of the following means:

i) Physical removal
ii) Killing by chemicals
iii) Biological control

Since over a hundred years, especially during the second half of the 20th century, very strong efforts have been made all over the world to find ways to destroy unwanted plants (Abbasi and Nipaney 1993). Indeed minor weeds can be, and are, controlled by periodic manual removal and large number of chemicals have been tried with which to kill seeds and seedlings of the weeds. In like manner biological methods, mainly revolving round weed-specific grazers, have been tried in profusion. But no major invasive has even been controlled, let alone destroyed till now. Once in a while a temporary subduing of an infestation is achieved but either the targeted invasive itself stages a comeback, or some other equally pernicious invasive takes over.

Worse, chemical and biological methods carry the grave risk of harming non-target species as also toxifying the environment. Moreover a biological control agent can itself go out of control and become a pest itself.

For all these reasons invasive plants, as a group, have not only withstood all attempts at controlling them but they are colonizing more and more areas with time. The same is very much true of salvinia and ipomoea".

2.3 Impact of infestation by salvinia and ipomoea

Salvinia (*Salvinia molesta,* D. S. Mitchell) is an exceedingly invasive and dominant aquatic weed, capable of multiplying and growing faster than most other known botanical species. It reproduces vegetatively; a tiny bit of salvinia leaf can lead to daughter plants which then multiply so rapidly that a bank-to-bank coverage of a water body by salvinia can occur in a matter of a few weeks. After spreading horizontally, salvinia mats thicken vertically as the weed's leaves are pushed upward and can get packed into mats up to 1 meter thick (Bhat, 2016). This enables salvinia to attain biomass productivity of the order of 60 dry (ash free) tonnes per hectare (Abbasi and Nipaney, 1993). This level of primary production puts salvinia at par with water hyacinth ─ known to be the most productive of all plants (Abbasi and Nipaney, 1993; Crites *et al.,* 2006).

With huge tracts of wetlands colonized by salvinia in South America, Africa, South Asia, and Australia (Abbasi and Nipaney, 1993; Bhat, 2016), billions of tonnes of salvinia biomass is generated every year across the world. As no method exists to utilize any sizeable fraction of the enormous salvinia biomass (Bhat, 2016), it remains unharvested, causing serious harm to the wetlands (Abbasi and Nipaney 1993).

Indeed due to its highly invasive and colonizing attributes salvinia has been included in the list of “100 of the world’s worst invasive alien species” (Luque *et al.*, 2013; GISD, 2017). This has happened after the International Union for Conservation of Nature (IUCN), assessed more than 10,000 invasive species from the world’s largest databases for their capacity to spread and for their potential ecological or economic impact. According to Luque *et al.*, (2013), “more than 650 experts from 63 countries then voted on the ten candidate species that were shortlisted, and selected the giant salvinia (*Salvinia molesta*) native to Brazil, this fern has spread throughout the tropics and subtropics. It doubles in abundance within days, forming thick, floating mats that block light from expanses of water, reduce its oxygen content and degrade water quality. They also impede water-based transport, clog irrigation and power generation systems, and harm local fisheries. Now in the global spotlight, this new entrant to the IUCN list is set to increase public awareness of the harm caused by invasive species and to stimulate more discussion in science and policy circles”.

There have been instances wherein rapidly growing spreads of salvinia have been halted and repelled by the use of biological agents (Room *et al.*, 1981) but such successes have been few and far between. The same biocontrol agent which might have been effective in a particular situation has been found wanting in other situations (Abbasi and Nipaney 1993). Chemical and mechanical methods have seen even lesser success in controlling salvinia.

Ipomoea *(Ipomoea carnea* Jacq., also called *Ipomoea fistulosa)* is an evergreen, perennial, fast growing, amphibious shrub. It attains heights ranging from 1.1 to 3 m and stem diameter between 1.5 and 6 cm. It was initially used to make fences — its violet flowers being an attraction — but has since, metaphorically, crossed all fences to invade and colonize landmasses and wetlands everywhere. Ipomoea is able to adapt to very diverse terrestrial as well as aquatic habitats (Mohanty and Mishra, 1963). Its hardiness, rapid growth rate and high regenerative capacity has made it into one of the most dominant and harmful of the weeds that have infested the world’s tropical and sub-tropical regions (Shaltout *et al.,* 2010; Rafiq Kumar *et al.,* 2015; Kumar *et al.,* 2014). It colonizes vast tracts of land masses and water bodies thereby posing serious threat to ecosystem functioning (Rafiq Kumar *et al.,* 2015; Kumar *et al.,* 2014). The losses it causes in terms of harm to water quality, 'theft' of soil nutrients and other means, run into several billion rupees per annum (Chari *et al.,* 2005; Abbasi, and Chari, 2008*).*

Ipomoea possesses several alkaloids, particularly swansonine, which are known to cause a chronic neurologic disease characterized by weight loss, depression, altered behavior, infertility, birth defects and death of animals which graze on its leaves (Panter *et al.,* 1999; Hueza *et al., 2003;* Armien *et al.,* 2007; Rios *et al.,* 2008; Cook *et al.,* 2009, 2015). It is also known to contain allelopathic compounds, which repel or toxify other vegetation, thereby preventing their growth (Abbasi and Abbasi, 2010; Ganesh *et al.,* 2008).

2.4 Attempts at utilization of salvinia and ipomoea

There have been concerted attempts to find ways and means of utilizing weeds and other phytowaste, including salvinia and ipomoea. Table 2.1 summarizes the initiatives taken for salvinia. Similar initiatives have been taken for ipomoea (Banupriya, 2017). It reveals that the attempts encompass a wide range, and include utilization as:

- Antimicrobial agents
- Insecticides, helminthicides, fungicides
- Source of drugs
- Source of other useful chemicals
- Source of activated carbon
- Feedstock in pyrolysis and gasification plants
- As biosorbents (other than as activated carbon)
- Source of biofuels
- Agents for biomimetic nanoparticle synthesis
- Source of heat transfer fluids
- Agents for phytoremediation

- Agents for phytoextraction
- Corrosion inhibitors
- Source of antoxidants
- Compost
- Source of energy precursors in the form of volatile fatty acids
- Source of fermentable sugars
- Paper pulp
- Additive to cow-dung in generating vermicompost.

But, none of these methods have proved economically viable so far. Secondly even if one or other of the above mentioned options become viable, the quantities of the weeds which would be utilizable will be insignificant.

2.5 The potential of vermicomposting

As brought out by Abbasi *et al.*, (2015), earthworms process enormous quantities of leaf litter and other forms of plant debris in nature by ingesting them and converting them into vermicast. The latter is widely recognized as a soil-friendly and plant-friendly organic fertilizer. Earthworms also consume animal droppings but the quantities of plant biomass processed by earthworms are several times greater than the quantities of zoomass they handle. Yet, when controlled vermicomposting is done to process biodegradable solid waste, terrestrial/aquatic weeds, or crop waste is rarely vermicomposted on a large scale.

As noted earlier, weeds like salvinia and ipomoea generate billions of tonnes of biomass per year. This ever-increasing biomass remains unutilized. Besides harming the environment in many ways this also contributes to global warming as the debris and dead plants of the weeds degrade in the open, generating CO_2 or CO_2–CH_4 mixtures depending on whether the degradation occurs aerobically or anaerobically. Given this context, developing an inexpensive and clean process with which huge quantities of the weed biomass can be profitably utilized is a major challenge (Abbasi and Abbasi, 2010; Abbasi *et al.*, 2015).

2.6 The reasons why the potential of generating fertilizers from the vermicomposting of phytomass was unutilized so far

Abbasi *et al.,* (2015) have enumerated the reasons why vermicomposting is arguably the best option for utilization of biodegradable solid waste, especially phytomass:

Quote:

a) "There is a rapidly growing interest in the vermicomposting of waste phytomass, especially since the turn of the present century. It is perhaps due to the increasing appreciation that vermicomposting is a phytomass utilization option which can generate good quality organic fertilizer. There is also an increasing realization that other options of phytomass utilization, such as composting and anaerobic digestion are not only more cumbersome and expensive but incapable of handling phytomass (Abbasi and Abbasi,2010; Abbasi *et al.,* 2012). Sanitary landfills, which at present handle larger volumes of biodegradable solid waste than any other process does, are already overburdened, besides being inherently unsuitable for phytomass (Annepu, 2012; UNSTAT, 2011). Moreover at best only 60% of the methane that is generated by a landfill ─ often much lesser or none can be captured while the rest gets released into the atmosphere (Ritzkowshi and Stegmaun, 2007; Zamorano *et al.,* 2007). These aspects put sanitary landfills among of the world's major sources of global warming gases (Abbasi *et al.,* 2012). This is more so because each molecule of methane contributes 34 times to the global warming as compared to a molecule of carbon dioxide (Shindell *et al.,* 2009)".

b) "In contrast, vermicomposting is an aerobic process and only about 40% of the carbon contained in the phytomass is released as CO_2 (Nayeem-Shah, 2014). The rest is returned to the soil as vermicompost. As the CO_2 released from the phytomass comes from carbon that had already been sequestered, vermicomposting results in additional carbon sequestration".

In this back-drop Abbasi *et al*. (2015) have identified the reasons why past attempts at vermicomposting of phytomass have been unviable.

Quote:

a) “Despite the advantages potentially associated with vermicomposting, its application in phytomass utilization has not gone beyond laboratory-scale attempts at feasibility studies. There are several factors which have given rise to this situation, all of which emanate from the inherent unsuitability of the conventional batch-fed vermireactors which are characterized by low surface area-height ratios in handling phytomass. Attempts of various authors to circumvent this problem has led to the dependence on animal manure supplementation and/or pre-composting for achieving vermicomposting of phytomass. This makes the entire process more cumbersome and time-consuming, hence potentially costlier, than direct vermicomposting. It also severely limits the quantities of phytomass that can be utilized as vermireactor feed because much lesser quantities of animal manure are available for proportionate supplementation”. Unquote.

In brief, conventional vermicomposting technology is besieged with the following problems:-

a) *Slowness of the existing process designs:* It takes 4-6 months for the input feed to be converted to vermicast.
b) *Heavy reliance on animal manure:* Animal manure, especially cowdung and buffalo dung, have been the substrates traditionally used to generate vermicompost. Several authors have used other substrates like vegetable waste, garden trimmings and waste paper as vermireactor feed but always as a supplement to animal manure. But animal manure has several other remunerative uses, especially in developing countries, and is also a preferred feed for anaerobic digesters. If the coverage of vermicomposting has to be expanded its reliance on animal manure must be drastically reduced so that

other types of feedstock can be employed in vermireactors with little or no necessity of blending it with animal manure.

c) *Time - consuming nature of pre-composting:* Plant biomass is generally pre-composted, this adds up time and cost overall, affecting the economy of the process.

d) *Hazards in the collection and transport of animal manure:* This leads to emission of global warming gases and nitrous oxide (Tauseef *et al.,* 2013) and other pollutants like ammonia.

2.7 Special attributes of high-rate vermicomposting which enables utilization of invasive plants

As noted in Abbasi *et al.,* (2015);

Quote:

a) "In recent years the first author S.A. Abbasi and coworkers have developed the concept of high-rate vermicomposting and associated know-how (Gajalakshmi *et al.,* 2002, 2005; Abbasi *et al.,* 2009; Ganesh *et al.,* 2009; Abbasi *et al.,* 2011; Tauseef *et al.,* 2013a,b). As detailed in this paper, high-rate vermireactors are distinguished by high surface area-to-volume ratios, high earthworm densities, and pulse-fed operation. The utilization of the reactor space is maximized while there is much better substrate agitation, more uniform distribution of moisture, and almost no generation of leachate. The conditions also totally preclude formation of anaerobic pockets that besiege conventional vermireactors".

b) "The applicability of the high-rate vermicomposting technology has since been tested extensively in achieving direct and rapid vermicomposting of phytomass. Substrates including aquatic weeds salvinia (*Salvinia molesta*) and water hyacinth (*Eichhornia crassipes*); terrestrial weeds ipomoea (*Ipomoea carnea*), lantana, (*Lantana camara*), and parthenium (*Parthenium hystophorus*); other forms of lignocellulosic waste such as the one that emanates from the cultivation of date palm (*Phoenix dactylifera*), etc, have been vermicomposted without any pre-composting or manure supplementation (Table 6 and references sited therein). High rate vermireactors are also seen to vermicast paper waste with much lesser (7-10%) manure addition and at much faster rate, than achieved in past reports (Tauseef *et al.,* 2013a, b; Kathikeyan *et al.,* 2014). Even more significantly, the rate of vermicomposting achieved in these reactors is 2-3 times faster than the rate achievable in conventional vermireactors". Unquote.

2.8 The paradigm of high-rate vermicomposting and its essential features

In this backdrop Prof S. A. Abbasi and coworkers have developed the paradigm of high-rate vermicomposting and associated technology (Gajalakshmi *et al.,* 2002, 2005; Abbasi *et al.,* 2009; Ganesh *et al.,* 2009; Abbasi *et al.,* 2011; Tauseef *et al.,* 2013a,b) which enables direct and efficient vermicomposting of phytomass Abbasi *et al.,* (2015) have stated these five attributes of 'high-rate vermicomposting' paradigm which enables direct vermicomposting of phytomass. Quote:

i) "It relies on a reactor geometry that has been chosen to maximize earthworm-substrate contact as well as ease of cast deposition. To this end, a high surface area-to-volume ratio is set for the reactors. This also ensures mixing and aeration of the reactor content by the earthworm movements thereby preventing anaerobic pockets from developing."

ii) "The vermicast harvesting is also made easier by the low aspect-ratio because many species deposit their cast on top of the substrate while some others do it at the bottom."

iii) "Low substrate column height makes it possible to maintain, with relative ease, uniformity in the moisture content across the reactor depth. There is little, if any, accumulation of water in the reactor bottom, saving on the need for recycling."

iv) "The sand-gravel 'vermibed', which occupies over 25% space in conventional vermireactors, is replaced by moist jute cloth. This maximizes the use of reactor volume, proportionately reducing the system cost."

v) "Earthworm density is maximized to achieve highest sustainable population for a given feed. The high earthworm: feed ratio further helps mixing and aeration of the substrate due to earthworm movement. "Unquote.

Abbasi and coworkers have also demonstrated the success of their technology by direct and efficient vermicomposting of a large number of phytomass species (Abbasi *et al.,* 2014; Tauseef *et al.,* 2014; Nayeem-Shah *et al.,* 2015).

2.9 Past attempts at vermicomposting salvinia and ipomoea

All past reports on the vermicomposting of salvinia, ipomoea, and others weeds have been summarized in Abbasi *et al.,* (2015). As has been shown all past authors have relied on pre-composting and/or substantial cow-dung supplementation. Further, as detailed in Abbasi *et al.,* (2015) most past authors have not used any standard criteria to decide what exactly a vermicompost is and when does the process of vermicomposting get completed. To quote from Abbasi *et al*., 2015:

Quote:

a) "In only six of the studies, of which four are by these authors and coworkers, extent of vermicomposting has been quantified on the basis of fraction of substrate converted to vermicast within a given period of time. In all other studies no criterion has been used to ascertain whether the vermicomposting has been complete or how close to completion it is. In most (51) of the studies the authors have stopped their experiments after the C: N ratio of the reactor contents had declined to go below 20. In another 11 studies, vermicomposting was deemed to have been completed when the C: N ratio had fallen in to the 20-30 range. Thus in 62 of the 85 (or in 73%) studies vermicomposting has been assumed to have occurred on the basis of C: N ratio of the mixed reactor content. The concerned authors have justified it on the grounds that they continued vermicomposting till the contents of their vermireactors attained a C: N ratio one seeks in a compost. But composting is a process very different from vermicomposting (Abbasi *et al.,* 2009) and the compost of any substrate has widely different characteristics than its vermicompost. For example, vermicompost is distinguished not just by the C:N ratio or the high bioavailability of the nutrients it contains, as compost is, but also contains several of the enzymes, plant growth hormones, and pest repellants which a compost does not (Abbasi and Ramasamy; 2001 Edwards *et al.,* 2011). Hence the logic that is applicable to judge completion of composting, or suitability of a compost, cannot be directly extended to vermicomposting or vermicompost. Moreover C:N ratio basically indicates the concentration of total nitrogen in relation to total carbon and even though it is indicative of the progressive stabilization of a biowaste, it does not necessarily provide a quantitative measure of change in the bioavailability of the nutrients present in the substrate. Surprisingly none of the authors have continued their studies till C: N ratio had reached a steady state and have only *assumed* that vermicomposting had been completed once the C:N ratio had dropped below a premeditated level."

b) "A few of the authors have used even more subjective criterion to judge the occurrence of vermicomposting. These include change in the color of the substrate, or appearance of casting without quantifying the casting."

c) "Several authors (for example Singh and Suthar, 2012, and Suthar and Sharma, 2013) have periodically 'homogenised' the vermireactor contents in their study. This act would mix the vermicast, which is an easily distinguishable and quantifiable product of vermicomposting, with unreacted substrate. Due to this, in the subsequent vermireactor operation, the earthworms will have to perforce ingest portions of the vermicast that they had earlier produced, and will process that much of the reactants lesser. This would work against the efficiency of the process." Unquote.

This context makes it very difficult to work out as to what exactly was the product called 'vermicompost' in the reports of the past studies and how to design/ control reactors for process optimization.

Hence, and as detailed in Abbasi *et al.*, (2015), we have treated vermicast as the quantifiable and controllable product of vermicomposting. Accordingly we have used vermicast synonymously with vermicompost.

Table 2.1: Attempts at utilization of *Salvinia molesta*

S.no	*Type of use*	*Plant component used*	*Type of experiment*	*Key findings*	*Reference*
1	Phytoremediation	Whole plant	Assessment of *S. molesta* in detoxifying coal mine effluent	i) *S. molesta* removed Pb -96.96% > Ni - 97.01% > Cu- 96.77% > Zn- 96.38% >Mn- 96.22% > Fe- 94.12% > Cr- 92.85% > Cd- 80.99% in 10 days. ii) Impact of coal mine exposure on chlorophyll content showed a significant decrease of 42.49% from the control.	Lakra *et al.*, 2017
		Whole plant	Assessment of *S. molesta* in treating fish farm wastewater	i) *S. molesta* significantly removed 95% phosphate, and other parameters such as ammonia, turbidity and total suspended solids were within the standards in just 2 days.	Ng *et al.*, 2017
		Whole plant	Assessment of salvinia for the removal of color and chemical oxygen demand (COD) from pulp and paper mill effluent	Salvinia plan efficiently removed 49.72% color and 100% COD from the effluent.	Ahmad *et al* .,2017
		Whole plant	Assessment of S. *molesta* in treating palm oil mill effluent	*S. molesta* achieved 95% phosphate removal efficiency from the wastewater it also increased the biomass, which is superior in biochemical content that has its economic value.	Ng *et al.*,2017
		Not stated	Assessment of S. *molesta* in removal of	i) Heavy metals contents (less than 10 ppm) as within the permissible levels, except for	Ranjitha *et al* .,2016

S.no	*Type of use*	*Plant component used*	*Type of experiment*	*Key findings*	*Reference*
			heavy metals from industrial effluent.	chromium and lead. ii) *S. molesta* can grow healthy with the accumulation of these metals.	
		Root	Assessment of salvinia in heavy metals removal	Salvinia removed 102% of Fe and all the parameters such as BOD, COD, DO, pH, turbidity, oil and greese, nitrate and nitrite were within permissible limits.	Razak *et al.*, 2013
		Roots	Assessment of *S. molesta* for heavy metal remediation.	Salvinia could successfully be used for phytoremediation of mining tin tailings	Ashraf *et al.*, 2012
		Whole plant	Assessment of *S. molesta* for heavy metal remediation.	Successfully be used for phytoremediation of mining tin tailings	Ashraf *et al* ., 2011
		Not stated	Assessment of *S. molesta* for the removal of polar micro contaminants.	Salvinia contributes to the elimination capacity of micro contaminants in wetlands through biodegradation and uptake processes.	Matamoros *et al.*, 2012
		Root	Assessment of *S. molesta* and their potential as the heavy metals removal in root zone via phytogreen system.	Salvinia removed 102% of Fe and the contaminant is successfully absorbed by the root in order to stabilize the industrial wastewater	Abdul and Sulaiman, 2014
2	Wastewater treatment	Whole plant	Assessment of *S. molesta* to treat textile effluent	Salvinia plant significantly reduce the values of COD, BOD_5 and ADMI by 76%, 82% and 81%	Chandanshive *et al.*,2016

S.no	*Type of use*	*Plant component used*	*Type of experiment*	*Key findings*	*Reference*
				considering initial values 1185, 1440 mg/L and 950 units, respectively.	
		Not stated	Assessment of *S. molesta* to treat effluents from Nile tilapia culture ponds	*S. molesta* 72.1% of total phosphorus and 42.7% of total nitrogen indicating that the treated effluents may be reused in the aquaculture activity.	Henry and Camargo, 2006
		Not stated	Assessment of the nutritive value of *S. molesta* used in a Nile tilapia waste treatment and the species biomass potential uses.	i) Aerial part of salvinia observed 64.2% crude protein, 9.1% soluble carbohydrates, 18.7 mg.g-1 dry mass and lipids 4.5 %. ii)*S. molesta* aerial biomass have nutritive values with potential use for ruminant feeding or as ration ingredients.	Henry and Monteiro, 2002
		Root	Assessment of *S. molesta* to treat the effluent of a giant river prawn	*S. molesta* wetland suspended total inorganic nitrogen 19.8%, total Kjeldahl nitrogen (TKN) 30.9%, P-orthophosphate (PO4-P) 23.8% and efficient in treating pond effluent due to the root surface which forms an extensive area favorable to retention and adsorption of debris and absorption of nutrients.	Henares *et al.*,2014
		Leaves	Assessment of *S. molesta* to treat wastewaters containing zinc(II), and the subsequent conversion of the harvested	The uptake of zinc by the weed was very efficient - 50% zinc being removed within 15 days and 90% within 30 days of growth. The average gas yield from uncatalyzed salvinia is 30.4 L/kg (fresh weight). The 35-day average yield in presence of zinc (I1) works out to be	Abbasi and Nipaney, 1985

S.no	*Type of use*	*Plant component used*	*Type of experiment*	*Key findings*	*Reference*
			weed into energy (biogas),	40.3 L/kg (fresh weight) thus 33% enhancement in yield in the presence of zinc (I1*)*.	
			Assessment of *S. molesta* as a potential for the removal of heavy metals in highly polluted water	Salvinia efficiently removed nitrogen, phosphorus and zinc	Finlayson *et al.,*1984
		Not stated	Assessment of *S. molesta* to treat the wastewater	When nutrient concentrations are high, it can be predicted that 5.11 g N m -2 day -1 and 0.85 g P m -2 day -1 can be removed at a water temperature of 25°C, but only 1.1 g N m -2 day -1 and 0.18 g P m -2 day -1 at 12°C. This has a direct bearing on the design and costing of waste-water treatment ponds using salvinia for excess nutrient removal.	Toerien *et al.,*1983
		Not stated	Assessment of *S. molesta* for the removal of chromium from tannery effluents by phytoremediation	Salvinia have great potential to remove chromium, which ranges from 36-99% in 10 days.	Mishra *et al.*, 2010
		Not stated	Assessment of *S. molesta* for the removal of chromium from wastewater by phytoremediation	Chromium removal from spiked solutions ranged from 40-99% in 7 days.	Shiny *et al.*, 2004
		Not stated	Assessment of *S. molesta*	Salvinia showed ultrastructural changes at 0.1	Gupta and

S.no	*Type of use*	*Plant component used*	*Type of experiment*	*Key findings*	*Reference*
			for the absorption of cadmium from water	ppm and can be considered as an indicator of Cd in water	Devi, 1995
			Assessment of *S. molesta* for the removal of chromium and nickel from wastewater	The rate of percentage removal of metal ions was observed to be 56-96 and 18-72% after the first 2 and 14 days and the nickel and cadmium-enriched solution the biomass growth of Salvinia was high	Srivastav *et al.*,1994
			Assessment of *S. molesta* for treating aquaculture effluent	i)N and P concentrations were significantly higher ($P<0.05$) in the inflow (mean of 0.66 mg L–1 and 233.6 mg L–1, respectively) than in the outflow of the tanks (mean of 0.38 mg L–1 and 174.7 mg L–1, respectively) ii)*S. molesta*, biomass gain was 135.2 and 143.1 g DM.m2, in the higher and lower concentrations, respectively	Henares and Camargo, 2014.
3	Oil absorption	Leaves and hairy roots	Assessment of *S. molesta* for oil absorption capacity	i) Salvinia are super hydrophobic and super oleophilic, and selectively absorb oil while repelling water. ii)*S. molesta* improved artificial bioinspired oil absorbents.	Zeiger *et al.*,2016
		Not stated	Assessment of *S. molesta* for the sorption of oils onto the dry biomass and the results were compared with	i)The *S. molesta* biomass was a better sorbent for oil than Peat Sorb (for a crude oil, 4.8 against 2.7 g of oil sorbed per g of biomass). ii)Main factors that control the sorption process were the hydrophobicity of the biomass, particle	Ribeiro *et al.*,2000

S.no	*Type of use*	*Plant component used*	*Type of experiment*	*Key findings*	*Reference*
			commercial oil sorbent, peat Sorb, a processed peat.	size, the chemico-physical composition of the plant and the sorbate, and the capillary suction displayed by the plant biomass	
		Not stated	Assessment of *S. molesta* for the oil removal and retention capabilities of the biomass sorbents which included kapok fiber, cattail fiber, Salvinia sp.,	i)The mass of oil sorbed for salvinia was greater than 70% i) Oil selectivity (hydrophobic properties) and physical characteristics of the sorbents are the two main factors that influence the oil sorption capability.	Khan *et al.*,2004
4	Synthesize of nanoparticle	Leaves	Assessment of *S. molesta* in synthesis of silver nanoparticles (AgNPs), which is tested for its antimicrobial efficacy.	The synthesized AgNPs were found to be an effective antibacterial agent against both gram positive and gram negative bacteria.	Verma *et al.*,2016
		Whole plant	Assessment of *S. molesta* in synthesis of gold nanoparticles (AuNPs).	The synthesized AgNPs were found to be stable and used as a bioagent.	Abbasi *et al* 2016
5	Antioxidant activity	Leaves	Assessment of *S. molesta* for its antioxidant activity using extracts of aqueous, ethanol, methanol, chloroform, and petroleum ether by the diphenyl-2-	i) Among the five different solvents, the maximum antioxidant activity of *S. molesta* was found in the ethanolic extract 90.3% followed by other solvents ii) *S. molesta* possess significant antioxidant activity and used as a potent therapeutic agent	Nithya *et al.*,2016

S.no	*Type of use*	*Plant component used*	*Type of experiment*	*Key findings*	*Reference*
			picrylhydrazyl assay		
6	Source of forage	Leaves	Assessment of *S. molesta* for its potential as a source of feed stuff influencing meat characteristics in ducks	*S. molesta* can be used as a dietary source of fatty acids for the production of healthy duck meat.	Dwiloka *et al.*, 2015
		Not stated	Assessment of *S. molesta* and its potential as a source of local duck feed	15% *S. molesta* to the local duck ration resulted in an increase in the body weight and feed conversion ratio , as well as increasing the income over feed cost by approximately IDR 2,468.65.	Santoso and Setiadi, 2016
		Not stated	Assessment of *S. molesta* as a feed for the herbivorous fish, tilapia (*Oreochromis niloticus Linneus*)	After 23 days the fish growth was (7.3 g per fish). Salvinia could be used as a feed supplement or ingredient in tilapia diets.	King *et al.*,2004
		Leaves	Assessment of *S. molesta* as a source of forage for ruminants.	Salvinia contain crude ash (17.3% in DM) and of lignin (13.7%) and tannins (0.93%) as a potential feed source for ruminants	Moozhiyil and Pallauf, 1986
7	Antibacterial activity	Leaves	Assessment of *S. molesta* for its antibacterial activity *using* leaf extract of 20 ml ethanol (75%), acetone, chloroform, aqueous and petroleum	*S. molesta* can be used as complete therapeutic agents since it possess significant activities ranging from antibacterial to immune-modulator.	Nithya *et al.*, 2015

S.no	*Type of use*	*Plant component used*	*Type of experiment*	*Key findings*	*Reference*
			ether		
8	Extraction of cytotoxic compounds	Not stated	Assessment of *S. molesta* for its cytotoxic potential using ethanol extract.	Bioactive compounds from salvinia, particularly salviniol have promising potential in the drug development for cancer.	Li *et al*., 2013
9	Source of bioactive compound	Leaves	Assessment of *S. molesta* for its phytochemical potential using extracts of petroleum ether, ethyl acetate, methanol, chloroform, acetone, benzene and water.	*S. molesta* extracts show the presence of many bioactive compounds after extensive investigation.	Mithraja *et al*., 2011
11	Lipid extraction	Not stated	Assessment of *S. molesta* for lipid extraction using methanol:chloroform in 2:1 ratio.	A lipid yield of 92.4% was obtained at the optimized conditions of temperature (85°C), solvent to biomass ratio (20:1), and time (137 min), whereas a predicted lipid yield of 93.5 % with regression model.	Mubarak *et al*.,2016
12	Determination of heavy metals	Leaves	Assessment of *S.molesta* for heavy metals accumulation.	i) Heavy metal content (less than 10ppm) was within the permissible levels, except cadmium and lead. ii) *S. molesta* can grow healthy with the accumulation of these metals and used for the production of biodiesel.	Sandhyasree *et al*., 2015
		Not stated	Assessment of *S. molesta* for heavy metals	The plant species identified could be useful for revegetation and erosion control in metals-	Ashraf *et al* ., 2010

S.no	*Type of use*	*Plant component used*	*Type of experiment*	*Key findings*	*Reference*
			accumulation and tolerance in plants growing on ex-mining area.	contaminated ex-mining sites.	
13	Source of plant harmones	Not stated	Assessment of *S. molesta* for its mineral content.	Leachate collected on days 7 and 14 had biological activity indicating that auxin-like compounds were released from *S. molesta* upon decomposition.	Arthur *et al*., 2007
		Whole plant	Assessment of *S. molesta* for detecting plant harmones using the soybean callus bioassay	Cytokinin-like activity was detected in the culture medium in which the ferns had been growing and activity co-eluted with the same cytokinins found in the plant material.	Stirk and Van, 2003
14	Removal of heavy metals	Not stated	Assessment of *S. molesta* for removal of trace metals in river water under laboratory conditions.	Salvinia plant showed to possess different affinity for the incorporation of the metals in its biomass and metal abatement in dilute wastewaters.	Espinoza *et al.*,2005
		Whole plant	Assessment of *S. molesta*, as green leaf manure in rice (Oryza sativa L.) nursery.	*S. molesta* obtained grain yield 51.9 g/ha to nursery.	Raju and Gangwar, 2004
15	Isolation of phenolic compound	Whole plant	Assessment of *S. molesta* to isolated the phenolic compound	i)Two glycosides, 60-O-(3,4-dihydroxy benzoyl)-b-D-glucopyranosyl ester (1), and 4-O-b-D-glucopyranoside-3-hydroxy methyl benzoate	Choudhary *et al.*,2008

S.no	*Type of use*	*Plant component used*	*Type of experiment*	*Key findings*	*Reference*
				(2), along with five known compounds methyl benzoate (3), hypogallic acid (4), caffeic acid (5), paeoniflorin (6) and pikuroside (7) were isolated for the first time from a fresh water fern *S. molesta*. ii)These compounds showed a potent antioxidant radical scavenging activity in a non-physiological assay	
		Leaves	Assessment of *S. molesta* to extract antioxidant activities and total phenolic contents using acetone/methanol	i) *S. molesta* exhibited the high antioxidant activity with IC50 value of 27.75±0.15 µg mL-1 ii) Nariginin was the major phenolic compounds (65.56-68.71 mg g-1 of crude extract) found in the extracts followed by myricetin (1.34-17.05 mg g-1 of crude extract) from *S. molesta* and	Chantiratikul *et al.*,2009
16	Biofuel	Whole plant	Assessment of *S. molesta* for biogas production	*S. molesta* can be successful used as biofuel production	Abbasi and Nipaney, 1984
		Whole plant	Assessment of *S. molesta* for the production of methane	*S. molesta* yield energy (methane) of the order of 108 Kcal ha- ^{1} year-^{1}.	Abbasi *et al.*,1990
		Whole plant	Assessment of *S. molesta* as bioagent for treating wastewaters	i)Salvinia can weed can grow upto 4-5 days in 100 ppm of nickel and cadmium ii)Anaerobic digestion of the weed spiked with low concentrations (1.18 mg L-1) of each of the metals revealed that all metals enhance biogas	Abbasi and Nipaney, 1994.

S.no	*Type of use*	*Plant component used*	*Type of experiment*	*Key findings*	*Reference*
				yield except chromium, The stimulatory effect followed the trend Cu (51%) >Mo (45%) >Zn (30%) >Hg (24.4%)^{t} = Cd (23.8%) > Ni (14%)	
17	Nanoscale biomimetics	Leaves	Assessment of *S. molesta* for enhancing air retention	The results indicate that the air-retaining property was greatly enhanced using the salvinia structure	Yang *et al.*,2013
		Not stated	Assessment of *S. molesta* for long-term air-retention	The complex elastic eggbeater-shaped hairs with a coating of SU-8 photoresist can support a droplet water of 1 ml. This work offered a new simple method to mimic the properties of *S. molesta* surface.	Tengfei *et al.*,2016
		Fern hair	Assessment of *S. molesta* to mimic the air trapping ability	A novel methodology for the fabrication of microstructures mimics the water-pinning and air-trapping ability of *S. molesta*. Water contact angle, water roll angle and adhesive force of the new microstructure and water fern are study.	Hunt and Bhushan, 2011.

2.10 Summary

This Chapter presents a brief overview of the way huge quantities of phytomass are generated by invasive plants all over the world and the harm it causes to biodiversity and other aspects of environmental health. The manner in which it contributes to global warming is also brought out.

References

1. “Abbasi, S. A., Nipaney, P.C., 1984. Generation of biogas from *Salvinia molesta* (Mitchell) on a commercial biogas digester. Environ Technol, 5(1-11), 75-80.

2. Abbasi, S.A., Nayeem-Shah, M., Abbasi, T., 2014. Vermicomposting of phytomass: Limitations of the past approaches and the emerging directions, J. Clean. Prod. 93, 103-114

3. Abbasi, T., Tauseef, S. M., Abbasi, S. A., 2012. Anaerobic digestion for global warming control and energy generation: An overview. Renew. Sust. Ener. Rev. 16, 3228-3242.

4. Abbasi, T., Tauseef, S. M., Abbasi, S. A., 2012. Bio. Ener, Springer, New Yark Londan, xiv+169 Pages; ISBN 978-1-4614-1039-3.

5. Abbasi, T., Tauseef, S.M., Abbasi, S. A., 2011, Global Warming and The Role of Wetlands Lap Lambart Academic, Germany; ISBN 978-3846556009. xiv+264 pages.

6. Abbasi, S.A., Nayeem-Shah, M., Abbasi, T., 2015. Vermicomposting of phytomass: limitations of the past approaches and the emerging directions. J. Clean. Prod. 93, 103-114.

7. Abbasi S.A., 1993. World's Worst Weed: Impact and Control. International Book Distributors Dehradun, xi + 226 Pages.

8. Abbasi, S.A., Chari, K.B., 2008. Environmental Management of Urban Lakes: With Special Reference to Oussudu. Discovery Publishing House, New Delhi, 269 pp.

9. Abbasi S.A., Nipaney P.C., 1993. Modelling and Simulation of Biogas Systems Economics. Ashish Publishing House, New Delhi, xviii + 356 Pages.

10. Abbasi, S. A., Nipaney, P.C., 1994. Potential of aquatic weed *Salvinia molesta* (Mitchell) for water treatment and energy recovery.

11. Abbasi, S. A., Nipaney, P.C., 1985. Wastewater treatment using aquatic plants. Survivability and growth of *Salvinia molesta* (Mitchell) over waters treated with zinc (II) and the subsequent utilization of the harvested weeds for energy (biogas) production. Resour. conserv, 12(1), 47-55.

12. Abbasi, S.A., Nipaney, P.C., 1995. Productivity of aquatic weed Salvinia (Salvinia molesta, Mitchell) in natural waters, Ecology Environ. Conerv. 1 (1-4), 11-12.

13. Abbasi, T., Chari, K.B., Abbasi, S.A., 2008. Oussudulake, Pondicherry, India: A survey on socio-economic interferences, Ind. Geog. J. 83, (2) 149-162.

14. Abbasi, T., Tauseef, S.M Abbasi, S.A., 2012. Anaerobic digestion for global warming control and energy generation: An overview. Renewable and Sustainable Energy Rev. 16, 3228-3242.

15. Abbasi, S.A., Nipaney, P.C. Schaumberg, G.D., 1990. Bioenergy potential of eight common aquatic weeds. Biol. wastes, 34(4), 359-366.

16. Abbasi, S.A., Ramasamy, E.V., 2001. Solid Waste Management with Vermitechnology, xiv. Discovery Publishing House, New Delhi, p. 178.

17. Abbasi, T., Abbasi, S. A., 2010. Biomass energy and the environmental impacts associated with its production and utilization, Renew. Sust. Ener. Review.14**,** 919–937.

18. Abbasi, T., Anuradha, J., Abbasi, S.A., 2016. Utilization of the pernicious aquatic weed salvinia (*Salvina molesta* DS Mitchell) in generating gold nanoparticles. Indian J.Biotechnol.15, 382-391

19. Abbasi, T., Gajalakshmi, S., Abbasi, S.A., 2009. Towards modeling and design of vermicomposting systems: mechanisms of composting/vermicomposting and their implications. Indian J. Biotechnol.8, 177-182.

20. Abdul Syukor bin, A.R., Sulaiman, S.B., 2013 Treatment of i{dotless}ndustrial wastewater using Eichornia crassipes, Pistia Stratiotes and *Salvinia Molesta* in phytogreen system. Energy Education Science and Technology Part A: Ener. Sci. Research.32, (1) 339-346.

21. Abhang, A. R., Pathare, S. A., Rohokale, P. G., 2015. Traditional uses of medicinal plants by tribal and rural folk from Mula river valley of Rahuri and its adjoining area of Parner and Sangamner tahsils of Ahmednagar District (M.S.). Inter. J.Pharma. Bio Sci. 6(1), P140-P148.

22. Ahmad, J., Abdullah, S.R.S., Hassan, H.A., Rahman, R.A.A., Idris, M., 2017.Screening of tropical native aquatic plants for polishing pulp and paper mill final effluent. Malaysian J. Analytical Sci. 21(1), 105-112.

23. Al−Juhaimi, F. Y., Hamad, S. H., Al−Ahaideb, S., Al−Otaibi, M. M., Ghafoor, K., Abbasi T., Abbasi, S. A., 2014. Biogas production through the anaerobic digestion of date palm tree waste−process optimization. Bioresour. *9,* 3323−3333.

24. Almeida Rios, S. D., Paes, D., Borém, A., 2009. Adaptability and stability of carotenoids in maize cultivars. Crop Breeding Appl. Biotechnol. *9*(4), 313-319.

25. Annepu, R. K., 2012. Sustainable solid waste management in India. Columbia University, New York, 2(01).

26. Anuradha, J., Abbasi, T., Ganaie, S. U., Abbasi, S. A., 2015. Gainful utilization of the highly intransigent weed ipomoea in the synthesis of gold nanoparticles. J. King Saud Univ. Sci. 27, 15-22.

27. Armien, A.G., Tokarnia, C.H., Peixoto, P.V., Frese, K., 2007. Spontaneous and experimental glycoprotein storage disease of goats induced by *Ipomoea carnea* (Convolvulaceae). Vet. Pathol. 44, 170-184.

28. Arthur, G.D., Stirk, W.A., Novák, O., Hekera, P., Van Staden, J., 2007. Occurrence of nutrients and plant hormones (cytokinins and IAA) in the water fern *Salvina molesta* during growth and composting. Environ. Exper. Bot, 61(2), 137-144

29. Ashraf, M.A., Maah, M. J., Yusoff, I., 2011. Heavy metals accumulation in plants growing in ex tin mining catchment. Inter. J. Environ. Sci. Technol, 8(2), 401-416.

30. Ashraf, M.A., Maah, M.J., Yusoff, I., 2012. Assessment of phytoextraction efficiency of naturally grown plant species at the former tin mining catchment. Fresen. Environ.Bull, 21(3), 523-533.

31. Ashraf, M.A., Maah, M.J., Yusoff, I., Gharibreza, M.M., 2010. Heavy metals accumulation and tolerance in plants growing on ex-mining area, Bestari Jaya, Kuala

Selangor, Peninsular Malaysia. Environ. Eng. Appli. *(ICEEA)*, International Conference on, 267-271.

32. Banupriya, D., 2017. Towards development of mechanizable high-rate vermicomposting systems for coverting biodegradable solid waste into organic fertilizers, Pondicherry University thesis.

33. Bhat, M. A., 2016. Wastewater treatment with a novel clean-green bioreactor SHEFROL®. Pondicherry University thesis.

34. Chandanshive, V.V., Rane, N.R., Gholave, A.R., Patil, S.M., Jeon, B.H., Govindwar, S.P., 2016. Efficient decolorization and detoxification of textile industry effluent by *Salvina molesta* in lagoon treatment. Environ. Research, 150, 88-96.

35. Chantiratikul, P., Meechai, P., Nakbanpotec, W., 2009. Antioxidant activities and phenolic contents of extracts from *Salvinia molesta* and *Eichornia crassipes*. Res. J. Biol. Sci, 4(10), 1113-1117.

36. Chari, S. T., Leibson, C. L., Rabe, K. G., Ransom, J., De Andrade, M., Petersen, G. M., 2005. Probability of pancreatic cancer following diabetes: a population-based study. Gastroenterol, 129(2), 504-511.

37. Choudhary, M.I., Naheed, N., Abbaskhan, A., Musharraf, S.G., Siddiqui, H., 2008. Phenolic and other constituents of fresh water fern *Salvinia molesta*. Phytochem, 69(4), 1018-1023.

38. Chung, I., Rahuman, A. A., Marimuthu, S., Kirthi, A. V., Anbarasan, K., Padmini, P., Rajakumar, G., 2017. Green synthesis of copper nanoparticles using eclipta prostrata leaves extract and their antioxidant and cytotoxic activities. Exp.Ther. Med, *14*(1), 18-24. doi: 10.3892/etm.2017.4466.

39. Cook, D., Gardner, D.R., Ralphs, M.H., Pfister, J.A., Welch, K.D.,Green, B.T., 2009. Swainsonine concentrations and endophyte amounts of *Undifilum oxytropis* in different plant parts of *Oxytropis sericea*. J. Chem. Ecol. 35, 1272–1278.

40. Cook. B. I., Ault, T. R., Smerdon, J. E., 2015. Unprecedented 21st century drought risk in the American Southwest and Central Plains, 10.1126/sciadv.1400082.

41. Crites, R. W., 2006. Natural Wastewater Treatment Systems. Boca Raton, FL: CRC Press.

42. Dwiloka, B., Setiadi, A., Santoso, S.I., Suprijatna, E., Susanti, S., 2015. Effects of duck feed supplemented with invasive giant salvinia (*Salvina molesta*) on duck meat characteristics. Turk. J. Vet. Anim. Sci. 39(6), 668-675.

43. Edwards, C.A., Norman, Q. A., Sherman, R., 2011. Vermiculture Technology, Earthworms, Organic Waste Environ. Manag. CRC Press, 17-19.

44. Espinoza-Quinones, F.R., Zacarkim, C.E., Palacio, S.M., Obregon, C.L., Zenatti, D.C., Galante, R.M., Rossi, N., Rossi, F.L., Pereira, I.R.A., Welter, R.A. Rizzutto, M.A., 2005. Removal of heavy metal from polluted river water using aquatic macrophytes Salvinia sp. Braz. J. Phys. 35(3B), 744-746.

45. Finlayson, C.M., Farrell, T.P., Griffiths, D.J., 1984. Studies of the hydrobiology of a tropical lake in north-western Queensland. III. Growth, chemical composition and potential for harvesting of the aquatic vegetation. Mar. Freshwat. Res, 35(5), pp.525-536.

46. Gajalakshmi, S., Ramasamy, E.V Abbasi, S.A., 2002. High-rate composting – vermicomposting of water hyacinth, Bioresour. Technol. 83, 235-239.

47. Gajalakshmi, S., Ramasamy, E.V., Abbasi, S.A., 2005. Composting-vermicomposting of leaf litter ensuing from the trees of Magnifera indica, Bioresour. Technol. 96, 1057-1061.

48. Ganesh, P.S., Sanjeevi, R., Gajalakshmi, S., Ramasamy, E.V.,Abbasi, S.A., 2008. Recovery of methane-rich gas fromsolid-feed anaerobic digestion of ipomoea (*Ipomoea carnea*). Bioresour. Technol. 99, 812–818.

49. Ganesh, P.S., Gajalakshmi, S., S.A. Abbasi, S.A., 2009. Vermicomposting of the leaf litter of acacia (Acacia auriculiformis): possible roles of reactor geometry, polyphenols, and lignin. Bioresour. Technol. 100, 1819–1827.

50. GISD., 2017. http://www.iucngisd.org/gisd/search.phpAccessed13January2017]

51. Gupta, M., Devi, S., 1995. Uptake and toxicity of cadmium in aquatic ferns. J. Environ. Biol. 16(2), 131-136.

52. Hussain, N., Abbasi, T., Abbasi, S. A., 2016. Vermicomposting-mediated conversion of the toxic and allelopathic weed ipomoea into a potent fertilizer, Process Saf. Environ Protect, 103, 97-106.

53. Henares, M.N.P., Camargo, A.F.M., 2014. Estimating nitrogen and phosphorus saturation point for Eichhornia crassipes (Mart.) Solms and *Salvinia molesta* Mitchell in mesocosms used to treating aquaculture effluent. Acta Limnol. Bras. 26(4), 420-428.

54. Henares, M.N.P., Camargo, A.F.M., 2014. Treatment efficiency of effluent prawn culture by wetland with floating aquatic macrophytes arranged in series. Braz. J. Biol, 74(4), 906-912.

55. Henry-Silva, G.G., Monteiro, C.A.F., 2002. Nutritive value of free-floating aquatic macrophytes (*Eichhornia crassipes, Pistia stratiotes* and *Salvinia molesta*), used in aquaculture waste treatment. Acta Sci. Biolog. Health Sci, 24(2), 519-526.

56. Henry-Silva, G.G., Camargo, A.F.M., 2006. Efficiency of aquatic macrophytes to treat Nile tilapia pond effluents. Scientia Agricola, *63*(5), 433-438.

57. Himaja, N., Neelufar Shama, S., 2015. Herbal wealth for hepatotoxicity: A review. Asian J.Pharm.Clin.Res, 8(1), 3-9.

58. Hueza I.M., Dagli M.L., Gorniak S.L. Paulino C.A. 2003. Toxic effect of pre-natal *Ipomoea carnea* administration to rats. Vet. Hum. Toxicol. 45:298-302.

59. Hunt, J., Bhushan, B., 2011. Nanoscale biomimetics studies of *Salvinia molesta* for micropattern fabrication. J. colloid. Interface sci, 363(1), 187-192.

60. Jain, S., Choudhary, G. P., Jain, D. K., 2015. Medicinal plants with potential anti-fertility activity: A review. Int. J. Green Pharm. *9*(4), 223-228.

61. Karthikeyan, M., Hussain, N., Gajalakshmi, S., Abbasi, S. A., 2014. Effect of vermicast generated form an allelopathic weed lantana *(Lantana camara)* on seed germination, plant growth, and yield of cluster bean *(Cyamopsis tetragonoloba)*. Environ. Sci. Pollut. Res. 21*:*12539–12548.

62. Khan, E., Virojnagud, W., Ratpukdi, T., 2004. Use of biomass sorbents for oil removal from gas station runoff. Chemos, 57(7), pp.681-689.

63. King, C., McIntosh, D., Fitzsimmons, K., 2004, September. Giant Salvinia (*Salvinia molesta*) as a partial feed for Nile Tilapia (*Oreochromis niloticus*). In Proc. 6th Int. Symp. Tilapia in Agric (12-16).

64. Kumar, M. R., Tauseef, S. M., Abbasi, T., Abbasi, S. A., 2015. Control of amphibious weed ipomoea (*Ipomoea carnea*) by utilizing it for the extraction of volatile fatty acids as energy precursors. J. Adv. Res. *6*(1), 73-78.

65. Kumar, S., Pandey, S., Pandey, A. K., 2014. In vitro antibacterial, antioxidant, and cytotoxic activities of Parthenium hysterophorus and characterization of extracts by LC-MS analysis. Bio. Med. Res. Inter.

66. Lakra, K.C., Lal, B., Banerjee, T.K., 2017. Decontamination of coal mine effluent generated at the Rajrappa coal mine using phytoremediation technology. Int. J. phytoremediat, 19(6), 530-536.

67. Li, S., Wang, P., Deng, G., Yuan, W., Su, Z., 2013. Cytotoxic compounds from invasive giant salvinia (*Salvina molesta*) against human tumor cells. Biorg. Med. Chem. Lett. *23*(24), 6682-6687.

68. Luque, A.E., Corales, R., Fowler, R.J., Dimarco, J., van Keken A., Winters, P., Keefer, M.C., Fiscella, K., 2013 .Bridging the digital divide in HIV care: a pilot study of an iPod personal health record. J. Inter. Assoc. Physicians AIDS Care. 12:117–121. doi: 10.1177/1545109712457712.

69. Matamoros, V., Nguyen, L.X., Arias, C.A., Salvadó, V., Brix, H., 2012. Evaluation of aquatic plants for removing polar micro contaminants: a microcosm experiment. Chemosphere, 88(10), 1257-1264.

70. Mishra, R., Dwivedi, S., Misra, S., 2010. Chromium removal from tannery effluent by phytoremediation. Pollut. Res, 29, 69-71.

71. Mithraja, M.J., Marimuthu, J., Mahesh, M., Paul, Z.M., Jeeva, S., 2011. Phytochemical studies on Azollapinnata R. Br., Marsileaminuta L. and *Salvina molesta* Mitch. Asian Pac. J. Trop. Biomed. 1(1), S26-S29.

72. Mohanty, P. K., Mishra, D., 1963. Stomatal distribution in relation to xeromorphy in aquatic plants. *Nature*, *200*(4909), 909-910.

73. Moozhiyil, M., Pallauf, J., 1986. Chemical composition of the water fern, *Salvinia molesta,* and its potential as feed source for ruminants. Econ. Bot, 40(3), 375-383.

74. Mubarak, M., Shaija, A., Suchithra, T.V., 2016. Optimization of lipid extraction from *Salvina molesta*. Environ. Sci. PolluT. Res, 23(14), 14047-14055.

75. Nayeem-Shah, M., Gajalakshmi, S., Abbasi, S.A., 2014. Direct vermicomposting of vegetable waste using the concept of high-rate vermireactor operation. Int. J. Environ. Sci. Eng. Res.4, 59–65.

76. Nayeem-Shah, M., Gajalakshmi, S., Abbasi, S. A., 2015. Direct, rapid and sustainable vermicomposting of the leaf litter of neem *(Azadirachta indica).*Appl. Biochem. Biotechnol. 2(175), 792-801.

77. Ng, Y.S. Chan, D.J.C., 2017. Wastewater phytoremediation by *Salvina molesta*. J. Water Process Eng. 15, 107-115.

78. Ng, Y.S., Samsudin, N.I.S., Chan, D.J.C., 2017. Phytoremediation Capabilities of *Spirodela polyrhiza* and *Salvina molesta* in Fish Farm Wastewater: A Preliminary Study.

79. Nitha, T.G., Jayanthi, J., Ragunathan, M.G., 2016. Antioxidant activity, total phenol, flavonoid, alkaloid, tannin, and saponin contents of leaf extracts of *Salvina molesta*. Asian J. Pharm.Clin. Res. 9(1), 200-3.

80. Nithya, T.G., Jayanthi, J., Raghunathan, M.G., 2015. Phytochemical, Antibacterial and GC MS analysis of a floating fern *Salvina molesta* DS Mitchell 1972. Int. J. Pharm.Tech Res. *8*(9), 85-90.

81. Panter, K.E., L.F. James, D.R. Gardner. 1999**.** Lupines, poison–hemlock and *Nicotiana* spp: toxicity and teratogenicity in

82. Patnaik, P., 2017. Towards a clean technology for the total utilization of the invasive xerophyte prosopis (*prosopis juliflora*, swartz, dc.), Pondicherry University thesis.

83. Patnaik, P., Abbasi, T., Abbasi, S. A., 2017. Prosopis *(Prosopis juliflora):* blessing and bane, Trop. Ecol. in press.

84. Raju, R.A., Gangwar, B., 2004. Utilization of potassium-rich green-leaf manures for rice (*Oryza sativa*) nursery and their effect on crop productivity. Ind. J. Agronomy, 49(4), 244-247.

85. Ranjitha, J., Raj, A., Kashyap, R., Vijayalakshmi, S., Donatus, M., 2016. Removal of heavy metals from Industrial Effluent using *Salvinia molesta*. Int. J. Chem. Tech. Res, *9*(05), 608-613.

86. Razak, A.S.B.A., bin Ab Wahid, Z., bin Zakaria, I., Said, M.I.B.M., 2013. Treatment of industrial wastewater at Gebeng area using *Eichornia Crassipes* Sp.(Water Hyacinth), *Pistia Stratiotes* Sp.(Water Lettuce) and *Salvinia molesta* Sp.(Giant Salvinia). Adv. Environ. Biol, *7*(12), 3802-3808.

87. Ribeiro, T.H., Smith, R.W., Rubio, J., 2000. Sorption of Oils by the Nonliving Biomass of a Salvinia sp. Environ. Sci. Technol, 34(24), 5201-5205.

88. Ritzkowski, M., Stegmann, R., 2007. Controlling greenhouse gas emissions through landfill in situ aeration. Int. J. Greenhouse Gas Control, 1(3), 281-288.

89. Room, P. M., Harley, K. L. S., Forno, I. W., Sands, D. P. A., 1981. Successful biological control of the floating weed salvinia. Nature, 294(5836), 78-80.

90. Rios, E., Cholich, L., Silva, J., Acosta de Pérez, O. Histopathological lesions in central nervous system of goats poisoned by Ipomoea Carnea. Intox. Cabras. Rev. vet. 2008; 19:130–134.

91. Sandhyasree, M., Kruthika, D.L., Priyanka, B., Vijayalakshmi, S., 2015. Ranjitha, J.b Microwave-assisted digestion for determination of Pb, Mg, Mn, Cd and Zn in *Salvina molesta* by flame atomic absorption spectrometry. Int. J. Pharma. Sci. Rev Res. 32, 95-97

92. Santoso, S.I., Setiadi, A., 2016. Profitable Utilization of Giant Salvinia, *Salvinia molesta*, as Local Duck Feed. Int. J. Poult. Sci. 15(4), 121.

93. Shaltout, K. H., Al-Sodany, Y. M., Eid, E. M., 2010. Growth behaviour of the invasive species *Ipomoea carnea* in the Nile Delta, Egypt. Hydrobiologia, 656(1), 187-197.

94. Shindell, D., Faluvegi, G., 2009. Climate response to regional radiative forcing during the twentieth century. Nat. Geosci. 2(4), 294.

95. Shiny, K.J., Remani, K.N., Jalaja, T.K., Sasidharan, V.K., 2004. Removal of chromium by two aquatic pteridophytes. J. Environ. Sci. Eng. 46(3), 249-251.

96. Singh, D., Suthar, S., 2012. Vermicomposting of herbal pharmaceutical industry waste: earthworm growth, plant-available nutrient and microbial quality of end materials. Bioresour. Technol. 112, 179-185.

97. Srivastav, R.K., Gupta, S.K., Nigam, K.D.P. and Vasudevan, P., 1994. Treatment of chromium and nickel in wastewater by using aquatic plants. Water Res, 28(7), 1631-1638.

98. Stirk, W.A., van Staden, J., 2003. Occurrence of cytokinin-like compounds in two aquatic ferns and their exudates. Environ. Exper. Bot, 49(1), 77-85.

99. Suthar, S., Sharma, P., 2013. Vermicomposting of toxic weed—*Lantana camara* biomass: chemical and microbial properties changes and assessment of toxicity of end product using seed bioassay. Ecotoxicol. Environ Saf, *95*, 179-187.

100. Swapna, M.M., Prakashkumar, R., Anoop, K.P., Manju, C.N., Rajith, N.P.,2011. A review on the medicinal and edible aspects of aquatic and wetland plants of India. J. Med. Plants Res.5(33):7163-76.

101. Abbasi. T., Tauseef, S. M., Abbasi, S. A., 2011. The Inclined Parallel Stack Continuously Operable Vermireactor, Offi. J. Patent. 22 9571.

102. Tauseef, S. M., Abbasi, T., Banupriya, G., Banupriya, D., Abbasi S. A., 2014. A new machine for clean and rapid separation of vermicast, earthworms and undigested substrate in vermicomposting systems, J. Environ. Sci. Eng. 56(4), 495-498.

103. Tauseef, S. M., Abbasi, T., Banupriya, G., Banupriya, D., Abbasi S. A., 2013b. A new machine for clean and rapid separation of vermicast, earthworms and undigested substrate in vermicomposting systems, Compost Sci.communicated.

104. Tauseef, S. M., Abbasi, T., Banupriya, D., Vaishnavi, G., Abbasi, S. A., 2013a. HEVSPAR: A novel vermireactor system for treating paper waste, Off. J. Patent Office, *24* 12726.

105. Tengfei, Z., Chaohui, W., Baogang, M. Zhuangde, J., 2016. Biomimetics studies of *Salvinia molesta* for fabrication. Micro & Nano Letters, 11(6), 291-294.

106. Toerien, D.F., Cary, P.R., Finlayson, C.M., Mitchell, D.S., Weerts, P.G.J., 1983. Growth models for *Salvinia molesta*. Aquat. Bot. 16(2), 173-179.

107. UNSTATS, 2011. Environmental Indicators. Available online at URL: https://unstats.un.org/unsd/environment/municipalased.htm (accessed October 2012).

108. Verma, D.K., Hasan, S.H., Banik, R.M., 2016. Photo-catalyzed and phyto-mediated rapid green synthesis of silver nanoparticles using herbal extract of *Salvina molesta* and its antimicrobial efficacy. J. Photochem. Photobiol. *B: Biology*, *155*, 51-59.

109. Yang, C.Y., Sung, C.K., 2013. Enhancing air retention by biomimicking *Salvinia molesta* structures. Jap. J.Appl. Phys, 52(6S), 06GF08.

110. Zamorano, M., Pérez, J. I. P., Pavés, I. A., Ridao, Á. R., 2007. Study of the energy potential of the biogas produced by an urban waste landfill in Southern Spain. Renew. Sustain Energy Rev, 11(5), 909-922.

111. Zeiger, C., da Silva, I.C.R., Mail, M., Kavalenka, M.N., Barthlott, W., Holscher, H., 2016. Microstructures of superhydrophobic plant leaves-inspiration for efficient oil spill cleanup materials. Bioinspir. Biomim, 11(5), 056003.”

Chapter 3

Direct vermicomposting of salvinia and the influence on it of three generations of earthworms

The chapter presents studies wherein salvinia was directly vermicomposted, without any pre-composting, manure supplementation, or any other pre-treatment. Four species of earthworms were, separately, studied for the purpose.

The first series of experiments had adult earthworms which had been born in cow-dung fed cultures and had grown to adulthood in them. The second series utilized earthworms born and raised in salvinia-fed cultures. Their next generation was then used for the third series of experiments. The objective was to see whether the second and the third generations display increasing adaptation to, and comfort with, the salvinia feed.

3.1 Introduction

As elaborated in Chapter 2, the reliance of the existing phytomass vermicomposting processes on cow-dung supplementation, besides their slowness, have been two of the prime reasons why phytomass vermicomposting has not come to be in vogue as animal manure vermicomposting has been. It was also brought out that the ‘high-rate vermicomposting’ paradigm introduced by S. A. Abbasi and coworkers (Abbasi *et al.*, 2009; 2011; 2014; 2015; Tauseef *et al.*, 2013 a, b) has the potential to remedy the lacunae.

In this chapter are presented studies on vermicomposting of salvinia as per the ‘high-rate vermicomposting’ paradigm. The reactors were operated without interruption for 160 days in each experiment to demonstrate the robustness and long-term sustainability of the process. Another major feature of the study was to assess the performance of three successive generations of earthworms and to explore whether there is adaptive response and increasing liking of the unitary salvinia feed by the earthworm.

3.1.1 Choice of earthworm species

The four species of earthworms chosen for the study were *Eisenia andrei, Perionyx sansibaricus, Lumbricus rubillus,* and *Drawida willsi*. The average relative sizes, and the morphological features of the four species are depicted in Figure 3.1. Some aspects of the biology and ecology of the four species, relevant to their use in vermicomposting, are summarized in Table 3.1. The first three species are of epigeic or phytophagous earthworms while *D. willsi* is anecic (geophytophagous). The epigeics are all exotic while *D.willsi* is endemic to the study area.

As of now *E. fetida* and *Eudrilus eugeniae* are the most extensively studied of epigeics *vis a vis* vermicomposting (Edward *et al.,* 2011; Abbasi *et al.,* 2015; Hussain, 2016) while the potential of *E. andrei, P. sansibaricus,* and *L. rubillus* has been relatively much less expired. Likewise very few anecic species have been tried in vermicomposting. All these considerations led us to short-list the four species of earthworms we have utilized in this study.

3.2 Materials and method

3.2.1 Substrate and vermicomposting

Whole plants of salvinia were collected from water-bodies situated near the place of author’s work (Pondicherry University campus). They were rinsed with tap water to remove adhering muck and invertebrates – if any – and gently wiped before loading them into the vermireactors. No chopping, pruning, soaking, or any other form of pre-treatment was done.

Two sizes of vermireactors were used for two different types of experiments involving different quantities of feed input. The larger of the reactors were rectangular plastic containers of 45 x 30 cm surface area and 15 cm height. The smaller of the reactors had 10 x 10 cm surface area and 7 cm height. Both types were provided with jute cloth sheets of 3-mm thickness, saturated with water, at the bottom to serve as bedding for the earthworms. The feed was laid over the jute cloth.

In order to quantify the vermicast generation per adult worm, the modules were operated in the *pseudo-discretized continuous reactor operation* (*PDCOP*) mode, conceived by Prof S. A. Abbasi and coworkers, and described elsewhere (Gajalakshmi and Abbasi, 2003,2004; Ganesh *et al.,* 2009). Its defining features are as summarized below:

- PDCOP enables an operation which is not really continuous but creates an ambience of a continuous reactor operation.

- In it, the reactors are started with a certain fixed quantity of the substrate and a fixed number of adult earthworms. After a set duration, say 20 days, the contents are removed and the extent of conversion of the substrate to vermicast and fecundity (in terms of number of juveniles and cocoons generated) are quantified. Within minutes, the reactors are restarted with fresh substrate and the same adult earthworms that were employed initially.

- In this way, it is possible to record the rate of vermicast production per adult earthworm as a function of time.

- By removing unconsumed substrate - which would otherwise biodegrade even without the action of the earthworms - the impact of happenings other than ingestion by the earthworms is minimized.

- The earthworms are always grazing upon totally fresh, or nearly fresh, substrate as they would be in a truly continuous vermireactor.

- Since the juveniles that are produced are removed before they grow significantly big to consume significant quantities of substrate, it is possible to dampen their influence on the reactor performance as well.

As stated above, two types of reactors were operated simultaneously:

i) In the first, larger type, of reactors 2 Kg (fresh weight) of salvinia was maintained as the substrate and 50 adult earthworms were engaged to feed upon it. The focus of these reactors was to assess vermicast production in each 20-day pulse and use it to calculate the production per worm, per day.

ii) In the second, smaller type, of reactors 500 g of (fresh weight) of salvinia was maintained as the substrate and 10 adult earthworms were employed to feed upon it. The focus was to assess the fecundity in terms of juveniles and cocoons generated per worm, per 20 days.

Theoretically it was possible to do both types of studies in either of the reactors. Yet, we designed the experiments as above because it is difficult to accurately count the juveniles (which look like pieces of black/brown thread) and cocoons (which can be mistaken for lumps of vermicast). In the first type of reactors the odds of wrong census are higher. On the other hand the second type of reactors have the drawback of generating lesser vermicast in each 20-day pulse than the larger reactor. Due to this, much greater percentage errors can occur in quantifying vermicast generated in it, in comparison to the other, four-times larger, reactor. For these reasons larger reactors were used to assess vermicast production while the smaller reactors were used to estimate fecundity. Moreover, several studies with triplicate and quadruplicate reactors in the laboratory where the author has worked, have shown that even as vermicast output in individual runs of 20 days may vary within replicates to the extent of ± 20%, the overall average output in the replicates is remarkably similar, agreeing within ± 3% (Kumar, 2016; Banupriay, 2017; Patnaik, 2017). The same trait was not seen with juveniles and cocoons. Due to this reason larger reactors were not duplicated while the smaller reactors were.

Both types of reactors were started for each of the species of the earthworms by releasing healthy, adult, animals, picked for this purpose randomly from cow-dung fed cultures maintained by the author. In the first run, all reactors were allowed to function for 20 days after which their contents were removed and placed in separate containers for the quantification of vermicast (in case of larger reactors) and production of juveniles and cocoons (in case of smaller reactors). Within a few minutes, fresh reactors were started with everything else the same as at the start except that from the earthworms removed from the previous run, only the adults were reintroduced into the corresponding reactors. Subsequent runs were also of 20–day duration and were continued till 160 days had elapsed from the start.

During the course of the experiments, all the modules were kept under the same ambient conditions of 30°C ± 4°C temperature and 60% ± 10% relative humidity. Their water content was maintained at 65 ± 5%. Mass balance of feed input and vermicast output was done on the basis of respective dry weights taken after oven-drying their randomly–picked and pooled samples at 105°C to constant weight. The castings were sieved through a 3-mm mesh to separate other particles. In this manner, it was possible to assess the vermicast output of the ‘parent’ worms as a function of time, without competition from offspring. It also ensured that the unutilized feed did not accumulate, and possibly biodegrade, in the modules.

3.3 Results and discussion

3.3.1 Vermicast production by the pioneers

The findings on the conversion of salvinia into vermicast by four species of earthworms are summarized in Table 3.1. The reactors running on 25 earthworms per kg of salvinia, led to 13.5 to 15.6% of salvinia vermicompost per 20-day pulse; in other words at the solids retention time (SRT) of 20 days.

Considering that 50 ± 10% of organic carbon contained in any feed is either converted to worm zoomass or is lost as CO_2 (due to respiration by earthworms and microorganisms present in the feed) in the course of vermicomposting, these figures reflect conversion of about twice as much feed as the vermicast produced. Hence the effective conversion of feed to vermicast per 20 days in reactors with 25 earthworms per kg of salvinia is in the range 27 -31%. But in all the reactors, there is a rising trend in vermicast production with time (Figure 3.1). It means that vermicast output per pulse is set to rise. Secondly had we not been removing the juveniles and cocoons from the reactors, they would be utilizing substantial parts of the feed. The combination of both these factors are likely to have caused much more than 27 - 31% utilization of salvinia per 20 days and the actual vermicast yield would have approached its theoretical maximum at 30 day SRT. This rate is several times faster than the 90-120 days that are taken by conventional vermireactors. Equally importantly, this rate has been achieved without any pre-composting, cow-dung supplementation, or even any pre-treatment of the salvinia feed.

The vermiconversion efficiencies of *E. andrei*, *L. rubillus,* and *D. willsi* were close to each other (Table 3.1). All the three species generated vermicast at higher rate than *P. sansibaricus*, the difference being statistically significant at ≥ 99% confidence level.

Table 3.1: Screening of four different earthworm species in pulse-fed reactors maintained with 2 kg salvinia per pulse and 50 adult earthworms

Number of days from start of the reactors	*E. andrei*		*P. sansibaricus*		*L. rubillus*		*D. willsi*	
	Vermicast generated as a fraction of dry weight equivalent of feed mass %	*Vermicast per worm, per day (mg)*	*Vermicast generated as a fraction of dry weight equivalent of feed mass %*	*Vermicast per worm, per day (mg)*	*Vermicast generated as a fraction of dry weight equivalent of feed mass %*	*Vermicast per worm , per day (mg)*	*Vermicast generated as a fraction of dry weight equivalent of feed mass %*	*Vermicast per worm, per day (mg)*
0-20	9.5	19.4	9.2	18.7	12.7	25.7	9.6	19.5
21-40	12.5	25.2	12.2	24.6	12.5	25.3	12.1	24.5
41-60	13.6	27.6	10.7	21.6	15	30.3	13.4	27.2
61-80	15.2	30.8	13.7	27.7	16	33.5	15.8	32
81-100	16.1	32.6	14.8	29.9	15.9	32.2	15.4	31.2
101-120	17.2	34.7	14.3	28.9	16	32.4	17.2	34.8
121-140	17.5	35.5	13.7	27.8	18.2	36.8	15.5	31.3
141-160	17.3	34.9	15.1	30.6	13.7	27.8	15.1	30.6
Average ± SD	15.6±2	31.6±4	13.5±2.1	27.3±3.2	15.4±1.9	31.2±3.8	14.9±2.4	30.2±3.4

**The reading of first 20 days have been excluded as it was a phase when the earthworm were acclimatizing with the feed and the reactor*

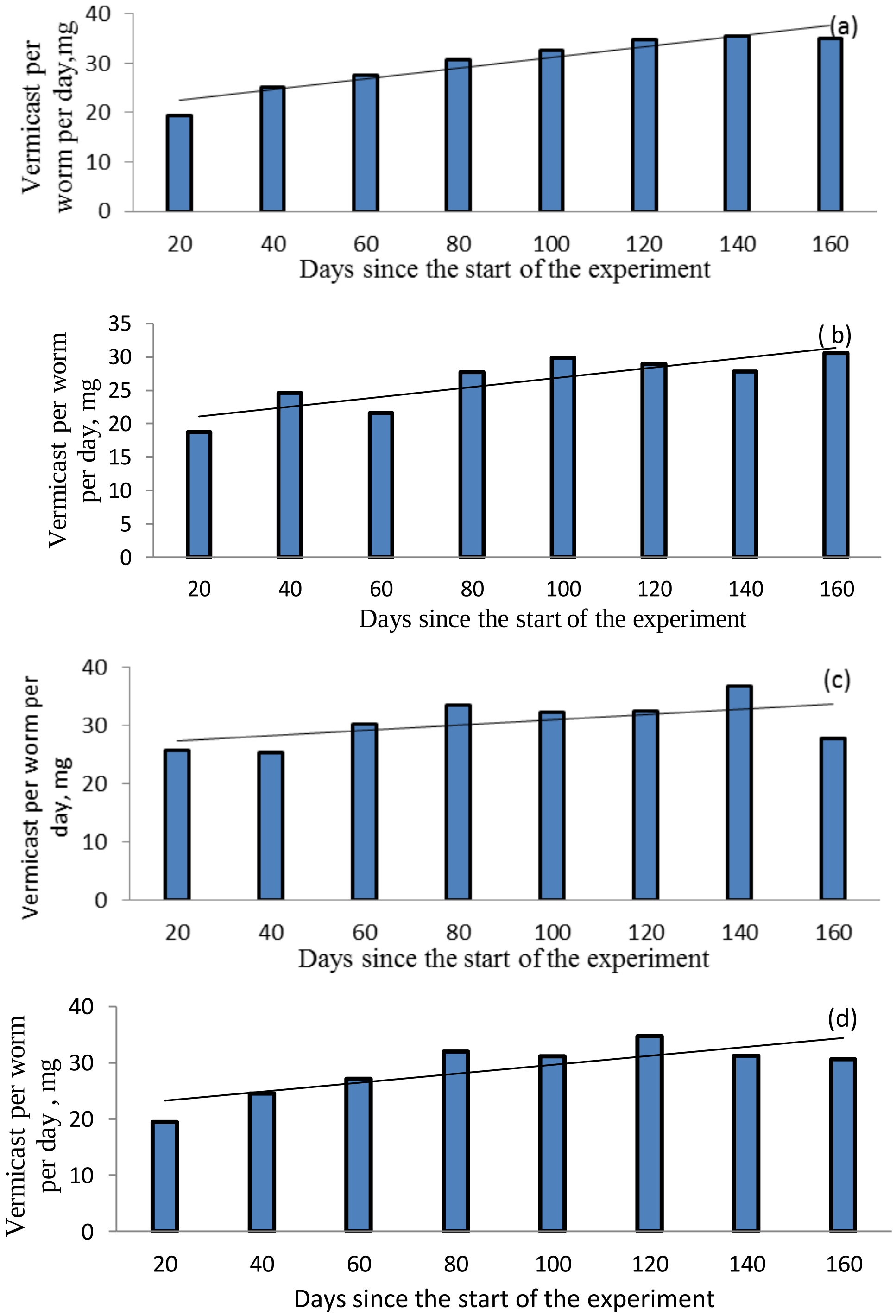

Figure 3.1: Vermicast generated in pulse-fed, semi-continuous reactors operated with a) *E. andrei* b) *P. sansibaricus* c) *L. rubillus* and d) *D. willsi* earthworms and fed with fresh salvinia. Trend lines are also shown.

In terms of juvenile production *E.andrei* matched the fecundity of *L. rubillus*; both produced nearly thrice as many juveniles as *P. sansibaricus* or *D. willsi* did (Table 3.2). The trend was similar in cocoon production (Table 3.3). The gap between the fecundities of *E. andrei/L. rubillus* and *P. sansibaricus/ D. willsi* was less winder than in case of juveniles, yet highly significant at >99% confidence level.

Table 3.2: Juveniles produced by four different earthworm species in pulse-fed reactors maintained on 500 g salvinia per pulse and 10 adult earthworms

No of days from start of the reactors	*E. andrei*			*P. sansibaricus*			*L. rubillus*			*D. willsi*		
	Reactor 1	*Reactor 2*	*Average ±SD*	*Reactor 1*	*Reactor 2*	*Average ±SD*	*Reactor 1*	*Reactor 2*	*Average ±SD*	*Reactor 1*	*Reactor 2*	*Average ±SD*
0-20	8	12	10±2.8	2	4	3±1.4	8	7	7.5±0.7	3	2	2.5±0.7
21-40	16	13	14.5±2.1	5	4	4.5±0.7	11	9	10±1.4	3	5	4±1.4
41-60	13	12	12.5±0.7	4	5	4.5±0.7	16	12	14±2.8	3	6	4.5±2.1
61-80	16	14	15±1.4	5	7	6±1.4	18	13	15.5±3.5	4	6	5±1.4
81-100	13	12	12.5±0.7	5	5	5±0	13	14	13.5±0.7	6	3	4.5±2.1
101-120	15	14	14.5±0.7	6	7	6.5±0.7	11	12	11.5±0.7	6	4	5±1.4
121-140	16	15	15.5±0.7	4	5	4.5±0.7	13	15	14±1.4	5	5	5±0
141-160	14	15	14.5±0.7	6	6	6±0	12	14	13±1.4	6	5	5.5±0.7
Average ± SD	14.7±1	13.6±1	14.1±1.3	5±1.3	5.4±1	5±1.1	13.4±2	12.7±2	13.1±2	4.7±1	4.8±1	4.8±0.5

**The reading of first 20 days have been excluded as it was a phase when the earthworm were acclimatizing with the feed and the reactor*

These findings indicate that over longer term operation of vermireactors in which juveniles and cocoons are not removed, the reactors operated with *E. andrei* and *L. rubillus* will overtake the reactors operated with *P. sansibaricus* or *D. willsi* because the former will create many more mouths to feed upon salvinia than the latter.

Table 3.3: Cocoons produced by four different earthworm species in pulse-fed reactors maintained on 500 g salvinia per pulse and 10 adult earthworms

Number of days from start of the reactors	*E. andrei*			*P. sansibaricus*			*L. rubillus*			*D. willsi*		
	*Reactor 1**	*Reactor 2**	*Average ±SD**	*Reactor 1*	*Reactor 2*	*Average ±SD*	*Reactor 1*	*Reactor 2*	*Average ±SD*	*Reactor 1*	*Reactor 2*	*Average ±SD*
0-20	4	8	6±2.8	2	3	2.5±0.7	6	9	7.5±2.1	2	2	2±0
21-40	7	9	8±1.4	4	5	4.5±0.7	9	5	7±2.8	5	5	5±0
41-60	10	7	8.5±2.1	4	4	4±0	9	6	7.5±2.1	4	6	5±1.4
61-80	8	10	9±1.4	6	4	5±1.4	10	6	8±2.8	4	5	4.5±0.7
81-100	12	9	10.5±2	5	4	4.5±0.7	9	8	8.5±0.7	5	6	5.5±0.7
101-120	11	10	10.5±0.7	5	6	5.5±0.7	8	7	7.5±0.7	6	4	5±1.4
121-140	12	7	9.5±3.5	5	7	6±0.7	9	7	8±1.4	7	5	6±1.4
141-160	10	9	9.5±0.7	4	6	5±1.4	7	10	8.5±2.1	4	8	6±0.8
Average ± SD	10±1.9	8.7±1.2	9.3±0.9	4.4±1.2	4.9±1.4	4.6±0.9	8.4±1.3	7.3±1.7	7.8±0.5	5±1.1	5.6±1.3	5.3±0.5

**The reading of first 20 days have been excluded as it was a phase when the earthworm were acclimatizing with the feed and the reactor*

Table 3.4: Test of significance in the difference of juvenile production by four difference species of earthworms fed on salvinia

Species	*Nature of the change in juvenile production*	*Confidence level (%) at which the difference was significant*
E. andrei in comparison to *L. rubillus*	increase	90%
L. rubillus in comparison to *P. sansibaricus*	increase	99%
P. sansibaricus in comparison to *D. willsi*	increase	75%

Table 3.5: Test of significance in the difference of cocoon production by four difference species of earthworms fed on salvinia

Species	*Nature of the change in cocoon production*	*Confidence level (%) at which the difference was significant*
E. andrei in comparison to *L. rubillus*	increase	99%
L. rubillus in comparison to *P. sansibaricus*	increase	99%
P. sansibaricus in comparison to *D. willsi*	decrease	97%

Table 3.6: Performance of the second and third generation of *E. andrei* born and grown in salvinia-fed vermireactors in comparison to the performance of manure-reared *E. andrei* with which the vermireactor were started

Number of days of reactor operation	*First generation**		*Second generation*		*Third generation*	
	Vermicast generated as a fraction of dry weight equivalent of feed mass %*	*Vermicast per worm , per day* (mg)*	*Vermicast generated as a fraction of dry weight equivalent of feed mass %*	*Vermicast per worm , per day (mg)*	*Vermicast generated as a fraction of dry weight equivalent of feed mass %*	*Vermicast per worm , per day (mg)*
20	9.5	19.4	19	38.4	20.8	42
40	12.5	25.2	17.6	35.7	23.1	46.7
60	13.6	27.6	23.8	48.2	23.9	48.4
80	15.2	30.8	22.5	45.6	21.8	44
100	16.1	32.6	19.1	38.7	21.7	43.9
120	17.2	34.7	23.4	47.4	23.1	46.7
140	17.5	35.5	19	38.4	19.9	40.2
160	17.3	34.9	20	40.6	22.5	45.6
Average ± SD	15.6±2	31.6±4	20.6±2.3	41.6±4.7	22.1±1.3	44.7±2.7

**The data of first 20 days have been excluded as it was a phase when the earthworm were acclimatizing with the feed and the reactor*

The performance of the second and the third generation of *E. andrei,* born and grown in salvinia-fed vermireactors in comparison to the performance of manure-reared first (pioneer) generation in vermicomposting salvinia is summarized in Table 3.6. There is a quantum jump in the average rate of vermicast production by the second generation of *E. andrei* compared to the first: of the order of 32%. The third generation has still higher (by 7.5%) vermicomposting efficiency, which is not as dramatically different from the second generation as the second generation's is from the first, yet statistically significant at 97% confidence level. It is also seen that whereas the pioneers took time to acclimatize with salvinia feed, as reflected in negligible feeding in the first 20 days, the second and the third generation earthworms did not require any priming and began generating near-average vermicast from the outset. The third generation of *E. andrei* produced larger number of juveniles (Table 3.7) and cocoons (Table 3.8) than the second generation and the second generation did so better than the first generation. The differences were significant at ≥ 97% confidence level most of the time (Tables 3.4 and 3.5).

3.3.2 Performance of successive generations of E. andrei in vermicomposting salvinia

These findings reveal that:

i) Successive generations of *E. andrei* can be raised with salvinia as the sole feed.
ii) The animals grown on salvinia are as healthy and reproductive as the ones grown on animal manure are known to be.
iii) Successive generations get increasingly acclimatized to salvinia and display increasing efficiency in vermicomposting salvinia.
iv) The reproductive ability of *E. andrei* in salvinia-fed reactors increases as it produces its second and third generation in it.

Table 3.7: Juveniles produced by the second and third generation of *E. andrei* born and grown in salvinia-fed vermireactors in comparison to the performance of manure-reared *E. andrei* with which the vermireactor were started

Number of days from start of the reactors	*Number of juvenile produced by first generation worms**			*Number of juveniles produced by second generation worms*			*Number of juveniles produced by third generation worms*		
	*Reactor 1**	*Reactor 2**	*Average ±SD **	*Reactor 1*	*Reactor 2*	*Average ±SD*	*Reactor 1*	*Reactor 2*	*Average ±SD*
20	8	12	10±2.8	18	16	17±1.4	18	20	19±1.4
40	16	13	14.5±2.1	15	18	16.5±2.1	22	18	20±2.8
60	13	12	12.5±0.7	17	16	16.5±0.7	17	21	19±2.8
80	16	14	15±1.4	19	18	18.5±0.7	16	17	16.5±0.7
100	13	12	12.5±0.7	17	15	16±1.4	20	15	17.5±3.5
120	15	14	14.5±0.7	15	19	17±2.8	18	16	17±1.4
140	16	15	15.5±0.7	19	18	18.5±0.7	17	21	19±2.8
160	14	15	14.5±0.7	18	15	16.5±2.1	19	20	19.5±0.7
Average ± SD	14.7±1	13.6±1.3	14.1±1.3	17.2±2	16.9±2	17.1±1	18.4±2	18.5±2.3	18.4±1.3

**The data of first 20 days have been excluded as it was a phase when the earthworm were acclimatizing with the feed and the reactor*

Table 3.8: Cocoons produced by the second and third generation of *E. andrei* born and grown in salvinia-fed vermireactors in comparison to the performance of manure-reared *E. andrei* with which the vermireactor were started

Number of days from start of the reactors	*Number of Cocoons produced by first generation worms**			*Number of Cocoons produced by second generation worms*			*Number of Cocoons produced by third generation worms*		
	*Reactor 1**	*Reactor 2**	*Average ±SD**	*Reactor 1*	*Reactor 2*	*Average ±SD*	*Reactor 1*	*Reactor 2*	*Average ±SD*
20	4	8	6±2.8	7	9	8±1.4	13	10	11.5±2.1
40	7	9	8±1.4	12	8	10±2.8	10	12	11±1.4
60	10	7	8.5±2.1	9	11	10±1.4	13	11	12±1.4
80	8	10	9±1.4	8	12	10±2.8	10	12	11±1.4
100	12	9	10.5±2.1	13	9	11±2.8	9	13	11±2.8
120	11	10	10.5±0.7	9	13	11±2.8	11	8	9.5±2.1
140	12	7	9.5±3.5	10	9	9.5±0.7	13	12	12.5±0.7
160	10	9	9.5±0.7	10	11	10.5±0.7	10	13	11.5±2.1
Average ± SD	10±1.9	8.7±1.2	9.3±0.9	9.8±2	10.3±1.6	10±1	11.1±1.6	11.4±2	11.3±0.9

**The data of first 20 days have been excluded as it was a phase when the earthworm were acclimatizing with the feed and the reactor*

Whereas the trend-line of vermicast production by the pioneer (first generation) of *E. andrei* has a clearly rising slope, reflective of increasing adaptation of salvinia feed by the earthworms who had been reared to adulated on cow-dung, the slope pertaining to the second generation has only a mild rise (Figure 3.2). The trend line pertaining to the third generation is almost flat. These patterns indicate that vermicomposting efficiency had almost peaked by the third generation and higher generations would perform similar to the third generation.

In case of *P. sansibaricus*, the second generation produced, on an average, about 25% more vermicast per unit time than the first generation (Table 3.9). The third generation recorded an advantage of 14% in this respect over the second generation. Whereas there was a long acclimatization period before the pioneers (first generation) began feeding upon salvinia to their capacity, no such priming was seen to be required by the second and the third generation.

The average number of juveniles and cocoons (Table 3.10 and 3.11) that were produced by *P. sansibaricus* followed the order third generation > second generation > first generation. The differences were significant at ≥ 96% confidence level in all but one case (Tables 3.12 and 3.13).

Table 3.9: Performance of the second and third generation of *P. sansibaricus* born and grown in salvinia-fed vermireactors in comparison to the performance of manure-reared *P. sansibaricus* with which the vermireactor were started

Number of days of reactor operation	*First generation**		*Second generation*		*Third generation*	
	Vermicast generated as a fraction of dry weight equivalent of feed mass %*	*Vermicast per worm, per day* (mg)*	*Vermicast generated as a fraction of dry weight equivalent of feed mass %*	*Vermicast per worm, per day (mg)*	*Vermicast generated as a fraction of dry weight equivalent of feed mass %*	*Vermicast per worm, per day (mg)*
20	9.2	18.7	16	32.4	19	38.2
40	12.2	24.6	18.7	37.8	18.1	36.6
60	10.7	21.6	17	34.3	19.9	40.2
80	13.7	27.7	15	30.4	21.6	43.7
100	14.8	29.9	16	32.3	17.92	36.3
120	14.3	28.9	17.6	35.6	18.7	37.8
140	13.7	27.8	17.1	34.5	18.9	38.2
160	15.1	30.6	17.6	35.7	19.8	40
Average ± SD	13.5±1.6	27.3±3.2	16.9±1.2	34.3±2.4	19.3±1.2	39±2.5

**The data of first 20 days have been excluded as it was a phase when the earthworm were acclimatizing with the feed and the reactor*

Table 3.10: Juveniles produced by the second and third generation of *P. sansibaricus* born and grown in salvinia-fed vermireactors in comparison to the performance of manure-reared *P. sansibaricus* with which the vermireactor were started

Number of days from start of the reactors	*Number of juvenile produced by first generation worms**			*Number of juveniles produced by second generation worms*			*Number of juveniles produced by third generation worms*		
	*Reactor 1**	*Reactor2 **	*Average ±SD**	*Reactor 1*	*Reactor 2*	*Average ±SD*	*Reactor 1*	*Reactor 2*	*Average ±SD*
20	2	4	3±1.4	6	7	6.5±0.7	7	9	8±1.4
40	5	4	4.5±0.7	7	6	6.5±0.7	8	8	8±0
60	4	5	4.5±0.7	8	6	7±1.4	9	11	10±1.4
80	5	7	6±1.4	6	9	7.5±2.1	8	9	8.5±0.7
100	5	5	5±0	9	7	8±1.4	10	8	9±1.4
120	6	7	6.5±0.7	7	9	8±1.4	8	9	8.5±0.7
140	4	5	4.5±0.7	8	5	6.5±2.1	10	10	10±0
160	6	6	6±0	7	7	7±0	11	8	9.5±2.1
Average ± SD	5±0.8	5.4±1.2	5±1	7.2±1	7±1.4	7.1±0.6	8.9±1.4	9±1.1	8.9±0.8

**The data of first 20 days have been excluded as it was a phase when the earthworm were acclimatizing with the feed and the reactor*

Table 3.11: Cocoons produced by the second and third generation of *P. sansibaricus* born and grown in salvinia-fed vermireactors in comparison to the performance of manure-reared *P. sansibaricus* with which the vermireactor were started

Number of days from start of the reactors	*Number of Cocoons produced by first generation worms**			*Number of Cocoons produced by second generation worms*			*Number of Cocoons produced by third generation worms*		
	*Reactor 1**	*Reactor 2**	*Average ±SD**	*Reactor 1*	*Reactor 2*	*Average ±SD*	*Reactor 1*	*Reactor 2*	*Average ±SD*
20	2	3	2.5±0.7	5	6	5.5±0.7	5	7	6±2.1
40	4	5	4.5±0.7	7	6	6.5±0.7	9	8	8.5±2.1
60	4	4	4±0	6	8	7±1.4	9	10	9.5±1.4
80	6	4	5±1.4	9	7	8±1.4	8	9	8.5±1.4
100	5	4	4.5±0.7	8	4	6±2.8	9	7	8±1.4
120	5	6	5.5±0.7	6	7	6.5±0.7	8	10	9±0.7
140	5	7	6±0.7	8	9	8.5±0.7	9	11	10±0.7
160	4	6	5±1.4	7	8	7.5±0.7	7	8	7.5±0.7
Average ± SD	4.4±1.2	4.9±1.4	4.6±0.9	7±1.3	6.9±1.6	6.9±1	8±1.4	8.7±1.5	8.4±1.2

**The data of first 20 days have been excluded as it was a phase when the earthworm were acclimatizing with the feed and the reactor*

Table 3.12: Test of significance in the difference of juvenile production by three generations of earthworms fed on salvinia

Earthworm species	*Nature of the change in juveniles production in the second generation compared to the first*	*Confidence level(%) at which the difference was significant*	*Nature of the change in juveniles production in the third generation compared to the second*	*Confidence level(%) at which the difference was significant*
E. andrei	increase	90%	increase	99%
L. rubillus	increase	99%	increase	99%
D. willsi	increase	90%	increase	90%
P. sansibaricus	increase	99%	increase	99%

Table 3.13: Test of significance in the difference of cocoon production by three generations of earthworms fed on ipomoea

Earthworm species	*Nature of the change in cocoons production in the second generation compared to the first*	*Confidence level(%) at which the difference was significant*	*Nature of the change in cocoons production in the third generation compared to the second*	*Confidence level(%) at which the difference was significant*
E. andrei	Increase	99%	increase	96%
L. rubillus	Increase	97%	increase	99%
D. willsi	Increase	99%	Increase	99%
P. sansibaricus	increase	96%	increase	99%

Table 3.14: Performance of the second and third generation of *L. rubillus* born and grown in salvinia-fed vermireactors in comparison to the performance of manure-reared *L. rubillus* with which the vermireactor were started

Number of days of reactor operation	*First generation**		*Second generation*		*Third generation*	
	Vermicast generated as a fraction of dry weight equivalent of feed mass %*	*Vermicast per worm , per day* (mg)*	*Vermicast generated as a fraction of dry weight equivalent of feed mass %*	*Vermicast per worm , per day (mg)*	*Vermicast generated as a fraction of dry weight equivalent of feed mass %*	*Vermicast per worm , per day (mg)*
20	12.7	25.7	17.2	34.7	21.2	42.9
40	12.5	25.3	18.9	38.2	23.1	46.7
60	15	30.3	20	40.4	19.8	40
80	16.	33.5	20.5	41.5	21.9	44.4
100	15.9	32.2	20	42.4	21.7	43.8
120	16	32.4	19.9	40.3	23.8	48.2
140	18.2	36.8	22.9	46.4	22.8	46.2
160	13.7	27.8	19.8	40	22.5	45.6
Average ± SD	15.4±1.9	31.2±3.8	20±1.6	40.5±3.4	22.1±1.2	44.7±2.6

**The data of first 20 days have been excluded as it was a phase when the earthworm were acclimatizing with the feed and the reactor*

Table 3.15: Juveniles produced by the second and third generation of *L.rubillus* born and grown in salvinia-fed vermireactors in comparison to the performance of manure-reared *L. rubillus* with which the vermireactor were started

Number of days from start of the reactors	*Number of juvenile produced by first generation worms**			*Number of juveniles produced by second generation worms*			*Number of juveniles produced by third generation worms*		
	*Reactor 1**	*Reactor 2**	*Average ±SD**	*Reactor 1*	*Reactor 2*	*Average ± SD*	*Reactor 1*	*Reactor 2*	*Average ±SD*
20	8	7	7.5±0.7	12	15	13.5±2.1	15	20	17.5±3.7
40	11	9	10±1.4	13	15	14±1.4	15	16	15.5±0.7
60	16	12	14±2.8	14	13	13.5±0.7	17	13	15±2.8
80	18	13	15.5±3.5	11	16	13.5±3.5	15	12	13.5±2.1
100	13	14	13.5±0.7	17	15	16±1.4	16	18	17±1.4
120	11	12	11.5±0.7	16	15	15.5±0.7	14	18	16±2.8
140	13	15	14±1.4	16	16	16±0	20	17	18.5±2.1
160	12	14	13±1.4	12	14	13±1.4	16	18	17±1.4
Average ± SD	13.4±2.6	12.7±2	13.1±1.8	13.9±2.2	14.9±1	14.4±1.2	16±1.9	16.5±2.7	16.3±1.6

**The data of first 20 days have been excluded as it was a phase when the earthworm were acclimatizing with the feed and the reactor*

The second generation of *L. rubillus* produced about 30% greater vermicast from salvinia per worm per day than its pioneer (first) generation. The third generation recorded a still 10% higher vermiconversion efficiency (Table 3.14). The trend of third generation being superior to the second and the second being superior to the first was manifest in the production of juveniles (Table 3.15) and cocoons (Table 3.16) as well.

Table 3.16: Cocoons produced by the second and third generation of *L. rubillus* born and grown in salvinia-fed vermireactors in comparison to the performance of manure-reared *L. rubillus* with which the vermireactor were started

Number of days from start of the reactors	*Number of Cocoons produced by first generation worms**			*Number of Cocoons produced by second generation worms*			*Number of Cocoons produced by third generation worms*		
	*Reactor 1**	*Reactor 2**	*Average ±SD**	*Reactor 1*	*Reactor 2*	*Average ±SD*	*Reactor 1*	*Reactor 2*	*Average ±SD*
20	6	9	7.5±2.1	7	9	8±2.1	7	10	8.5±2.1
40	9	5	7±2.8	10	8	9±2.1	9	12	10.5±2.1
60	9	6	7.5±2.1	8	10	9±1.4	9	11	10±1.4
80	10	6	8±2.8	9	8	8.5±1.4	8	10	9±1.4
100	9	8	8.5±0.7	10	7	8.5±1.4	9	7	8±1.4
120	8	7	7.5±0.7	9	8	8.5±0.7	12	11	11.5±0.7
140	9	7	8±1.4	10	7	8.5±0.7	9	10	9.5±0.7
160	7	10	8.5±2.1	8	10	9±0.7	13	12	12.5±0.7
Average ± SD	8.4±1.3	7.3±1.7	7.8±0.5	8.9±1.1	8.4±1.2	8.6±0.3	9.5±2	10.4±1.6	9.9±1.5

**The data of first 20 days have been excluded as it was a phase when the earthworm were acclimatizing with the feed and the reactor*

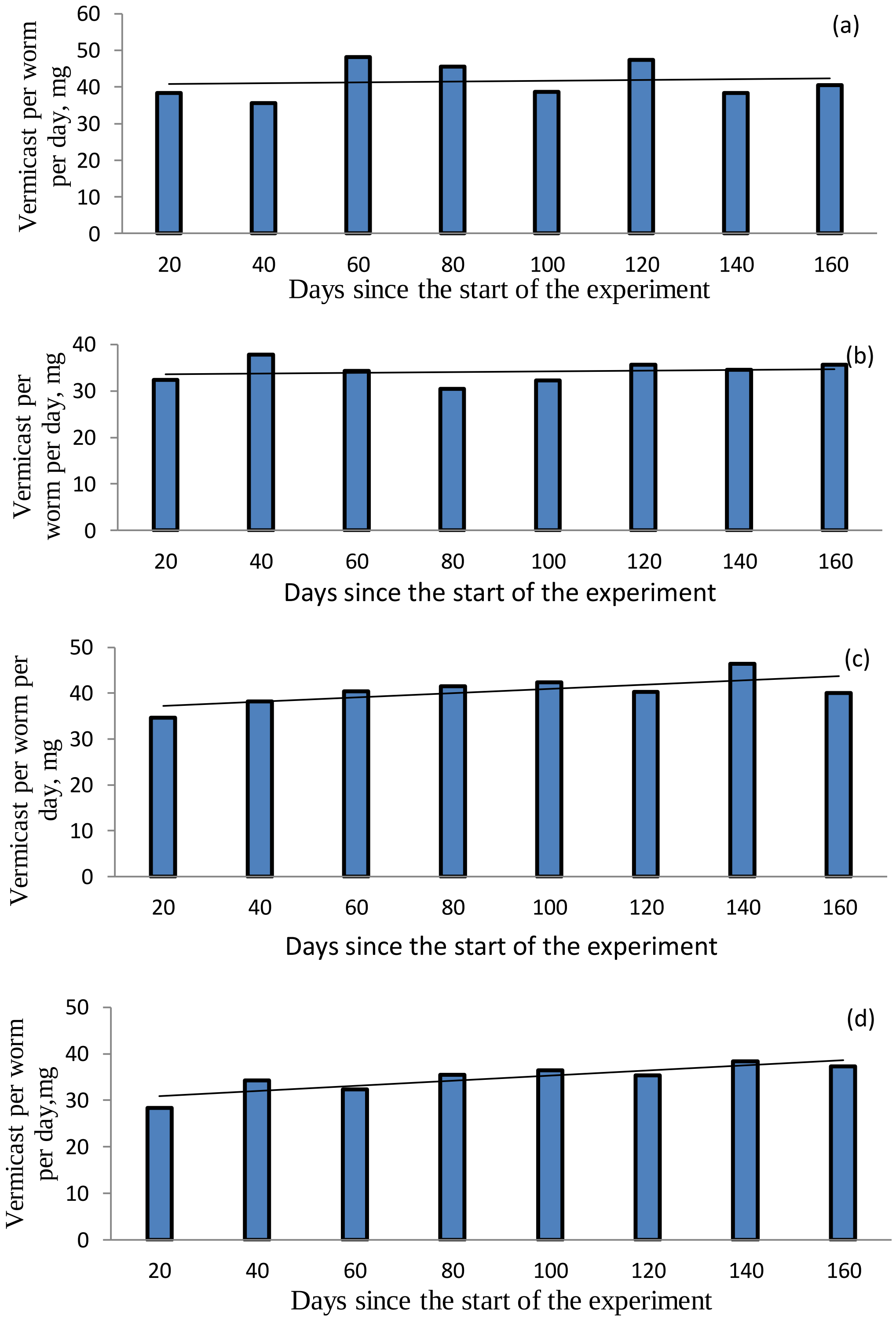

Figure 3.2: Vermicast generated by the second generation of earthworm born and grown in salvinia-fed vermireactors with four different earthworm species a) *E. andrei* b) *P. sansibaricus c) L. rubillus* and d) *D. willsi*

Table 3.17: Performance of the second and third generation of *D. willsi* born and grown in salvinia-fed vermireactors in comparison to the performance of manure-reared *D. willsi* with which the vermireactor were started

Number of days of reactor operation	*First generation**		*Second generation*		*Third generation*	
	Vermicast generated as a fraction of dry weight equivalent of feed mass %*	*Vermicast per worm , per day* (mg)*	*Vermicast generated as a fraction of dry weight equivalent of feed mass %*	*Vermicast per worm , per day (mg)*	*Vermicast generated as a fraction of dry weight equivalent of feed mass %*	*Vermicast per worm , per day (mg)*
20	9.6	19.5	14	28.4	18.7	37.8
40	12.1	24.5	17	34.3	19.8	40
60	13.4	27.2	16	32.4	17.6	35.7
80	15.8	32	17.5	35.5	19	38.4
100	15.4	31.2	18	36.5	20	40.4
120	17.2	34.8	17.5	35.4	18.9	38.3
140	15.5	31.3	19	38.4	22.3	45.2
160	15.1	30.6	18.4	37.3	19.1	38.7
Average ± SD	14.9±1.7	30.2±3.4	17.2±1.7	34.8±3.2	19.4±1.4	39.3±2.8

**The data of first 20 days have been excluded as it was a phase when the earthworm were acclimatizing with the feed and the reactor*

Table 3.18: Juveniles produced by the second and third generation of *D. willsi* born and grown in salvinia-fed vermireactors in comparison to the performance of manure-reared *D. willsi* with which the vermireactor were started

Number of days from start of the reactors	*Number of juvenile produced by first generation worms**			*Number of juveniles produced by second generation worms*			*Number of juveniles produced by third generation worms*		
	*Reactor 1**	*Reactor 2**	*Average ±SD**	*Reactor 1*	*Reactor 2*	*Average ±SD*	*Reactor 1*	*Reactor 2*	*Average ±SD*
20	3	2	2.5±0.7	5	4	4.5±0.7	7	5	6±1.4
40	3	5	4±1.4	5	6	5.5±0.7	8	8	8±0
60	3	6	4.5±2.1	4	5	4.5±0.7	9	6	7.5±2.1
80	4	6	5±1.4	6	6	6±0	7	6	6.5±0.7
100	6	3	4.5±2.1	6	5	5.5±0.7	6	8	7±1.4
120	6	4	5±1.4	6	8	7±1.4	7	9	8±1.4
140	5	5	5±0	5	6	5.5±0.7	6	10	8±2.8
160	6	5	5.5±0.7	6	7	6.5±0.7	8	7	7.5±0.7
Average ± SD	4.7±1.4	4.8±1.1	4.8±0.5	5.4±0.7	5.9±1.2	5.6±0.9	7.3±1	7.4±1.7	7.3±0.8

**The data of first 20 days have been excluded as it was a phase when the earthworm were acclimatizing with the feed and the reactor*

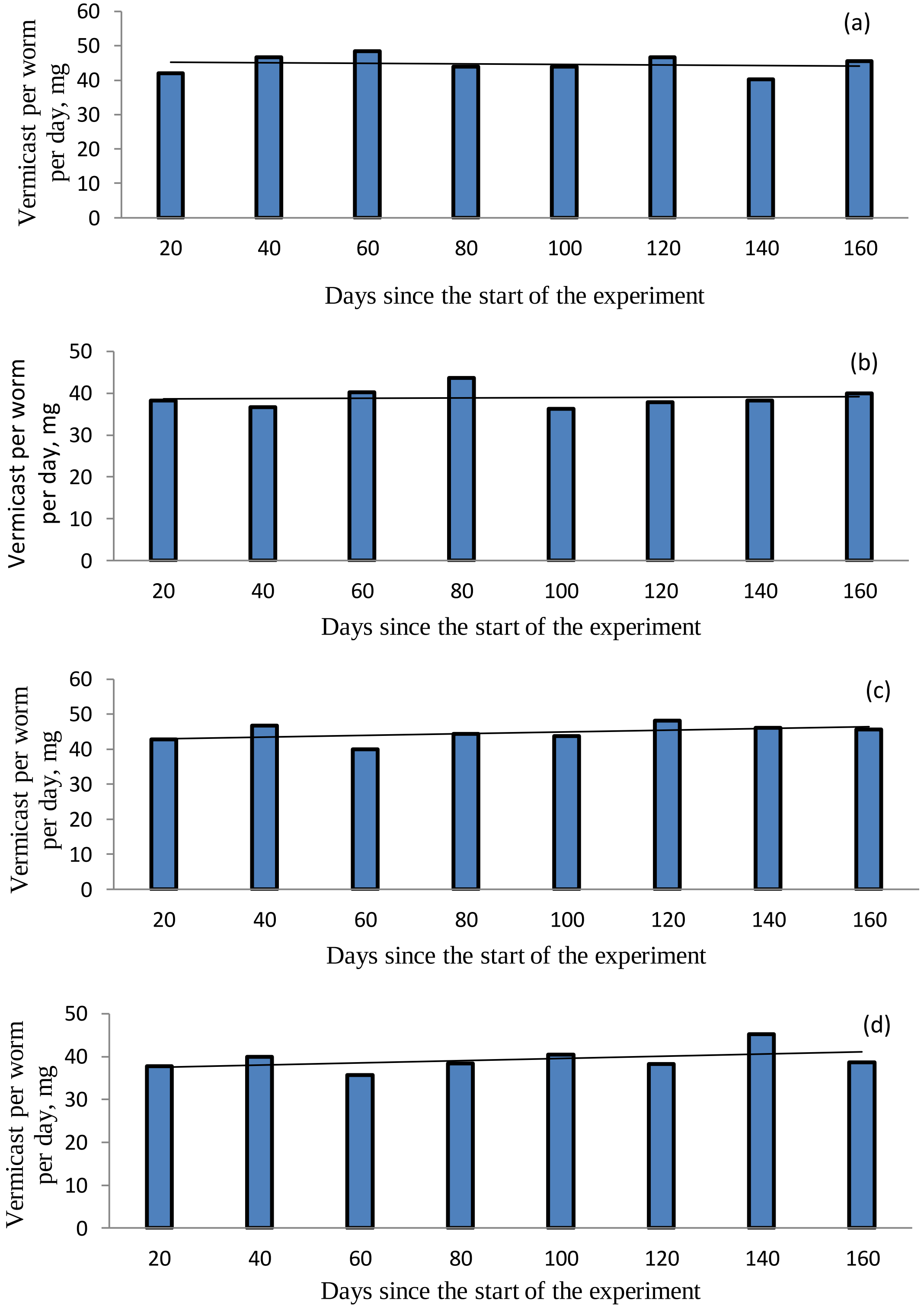

Figure 3.3: Vermicast generated by the third generation of earthworm born and grown in salvinia-fed vermireactors with four different earthworm species a) *E. andrei* b) *P. sansibaricus* c) *L.rubillus* and d) *D. willsi*

The same pattern was demonstrated by *D. willsi*. In terms of vermiconversion efficiency as well as fecundity, clear trend of third generation > second generation > first generation was seen (Table 3.17 – 3.19). Whereas there was a clearly rising trend in vermicast productions in the reactors run with pioneers (first generation), as seen in Figure 3.1d), the trends in reactors run with the second or the third generation were much flatter (Figures 3.2d and 3.3d). The relative efficiencies of the four species of the earthworms in vermicomposting salvinia may be seen in Figure 3.4. The relative felicity in the production of juveniles and cocoons is reflected in Figures 3.5 and 3.6. The figures show *E.andrei* to be the most suitable of the four species, in terms of efficiency in vermicast production as well as reproductive ability, followed by *L.rubellus*.

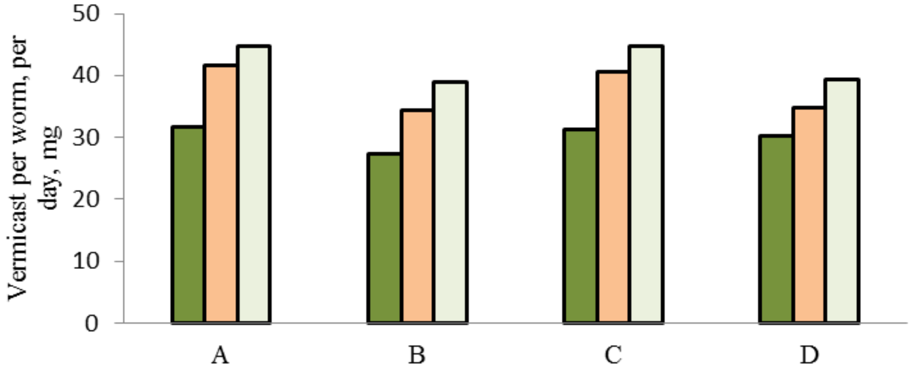

Figure 3.4: Relative efficiency of three generations of earthworms in vermicomposting salvinia A: *E.andrei;* B: *P.sansibaricus* C: *L.rubillus D*: *D.willsi.* 🟩First generation 🟧; Second generation⬜ ; Third generation

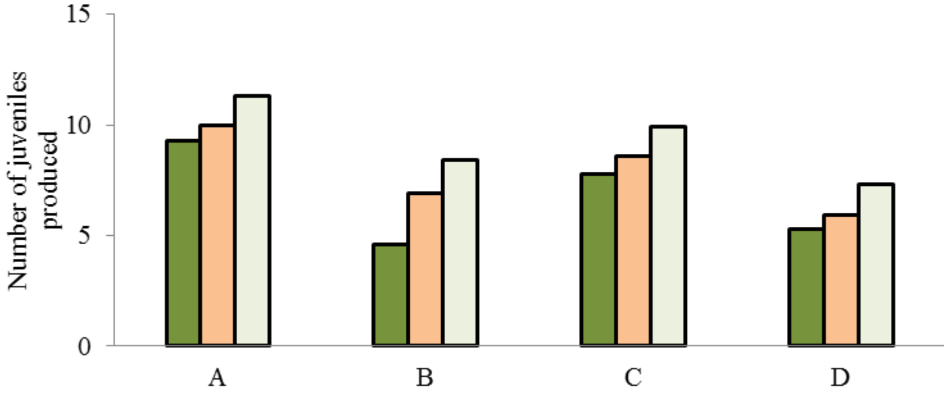

Figure 3.5: Juveniles produced by the second and third generation of earthworm born and grown in salvinia-fed vermireactors in comparison to the output of manure-reared pioneers. A: *E.andrei;* B: *P.sansibaricus* C: *L.rubillus D*: *D.willsi*.🟩 First generation;🟧 Second generation;⬜ Third generation

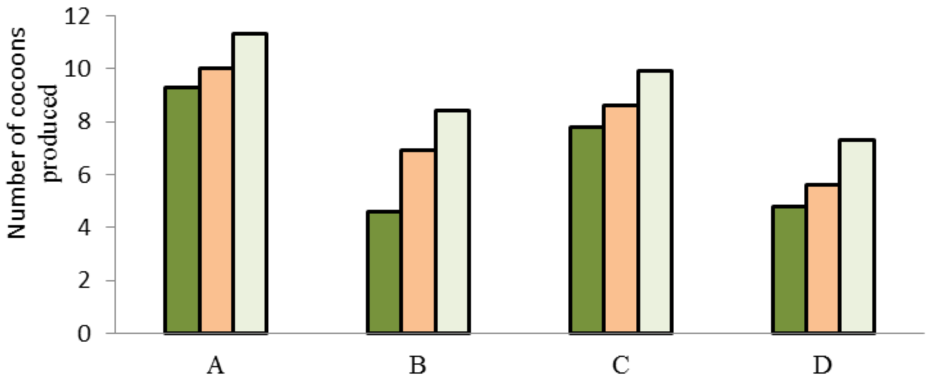

Figure3.6: Cocoons produced by the second and third generation of earthworm born and grown in salvinia-fed vermireactors in comparison to the output of manure-reared pioneers. A: *E.andrei;* B: *P.sansibaricu*s C: *L.rubillus D*: *D.willsi.*🟩 First generation;🟧 Second generation;⬜Third generation

Table 3.19: Cocoons produced by the second and third generation of *D. willsi* born and grown in salvinia-fed vermireactors in comparison to the performance of manure-reared *D. willsi* with which the vermireactor were started

Number of days from start of the reactors	*Number of Cocoons produced by first generation worms**			*Number of Cocoons produced by second generation worms*			*Number of Cocoons produced by third generation worms*		
	*Reactor 1**	*Reactor 2**	*Average ±SD**	*Reactor 1*	*Reactor 2*	*Average ±SD*	*Reactor 1*	*Reactor 2*	*Average ±SD*
20	2	2	2±0	4	5	4.5±0.7	5	5	5±0
40	5	5	5±0	7	6	6.5±0.7	7	6	7.2±0.7
60	4	6	5±1.4	8	4	6±2.8	5	7	7.4±1.4
80	4	5	4.5±0.7	4	5	4.5±0.7	6	5	6.2±0.7
100	5	6	5.5±0.7	6	7	6.5±0.7	5	7	7.4±1.4
120	6	4	5±1.4	5	8	6.5±2.1	7	8	8.2±0.7
140	7	5	6±1.4	8	6	7±1.4	8	6	8.4±1.4
160	4	8	6±2.8	6	5	5.5±0.7	6	8	8.4±1.4
Average ± SD	5±1.1	5.6±1.3	5.3±0.5	6±1.6	5.8±1.3	5.9±1	6.1±1.1	6.5±1.2	7.2±1.2

**The data of first 20 days have been excluded as it was a phase when the earthworm were acclimatizing with the feed and the reactor*

3.4. Summary

The impacts of the two weeds studied by the author ─ salvinia (*Salvinia molesta),* and ipomoea *(Ipomoea carnea)* ─ are then discussed. It is shown that despite concerted efforts, made all over the world, to eradicate or control these weeds have at best achieved only temporary and partial success. Rather than being contained, both weeds are invading ever new territories and colonizing ever larger tracts of land/water. Numerous attempts made in the past to utilize these weeds are reviewed. It is shown that of all the utilization options only vermicomposting is capable of handling the enormous quantities of biomass that is generated by these weeds.

In the next step the chapter reviews the state-of-the-art of phytomass vermicomposting and brings out the reasons why conventional vermireactors, which have been very successful in vermicomposting animal manure, have been unsuccessful in vermicomposting phytomass in an economically viable manner. It describes the paradigm of ‘high-rate vermicomposting’, recently developed by the author’s mentor, with which the author has succeeded in directly, rapidly, and sustainably vermicomposting salvinia and ipomoea as later described in this thesis.

Four species of earthworm ─ *E.andrei, P.sansibaricus, L.rubillus* and *D. willsi* ─ were explored in direct vermicomposting of salvinia. Whole plants of the weed were utilized in ‘high-rate vermireactors’ without any pre-composting, manure supplementation or any other form of pre-treatment. All experiments were carried out without interruption for 160 days.

Three series of studies were done. The first series utilized for vermicomposting of salvinia adult earthworms which had been born in cow-dung fed cultures and had grown to adulthood in them. The second series utilized earthworms born and raised in salvinia-fed cultures. Their next generation was then used for the third series of experiments. The objective was to see whether the second and the third generations display increasing adaptation to, and comfort with, the salvinia feed.

It was seen that:-

i) For each of the four species of earthworms studies by us, successive generations can be raised with salvinia as the sole feed.

ii) The animals of all the four species when grown on salvinia were as healthy and reproductive as the ones grown on animal manure were.

iii) Successive generations got increasingly acclimatized to salvinia and displayed increasing efficiency in vermicomposting salvinia.

iv) The reproductive ability of all the four species in salvinia-fed reactors increased as they produced their second and the third generation in it.

References

1. “Abbasi, S.A., Nayeem-Shah, M., Abbasi, T., 2014. Vermicomposting of phytomass: Limitations of the past approaches and the emerging directions, J. Clean. Prod. 93, 103-114

2. Abbasi, T., Gajalakshmi, S., Abbasi, S.A., 2009. Towards modeling and design of vermicomposting systems: mechanisms of composting/vermicomposting and their implications. Ind. J.Biotechnol. 8, 177-182.

3. Abbasi, T., Gajalakshmi, S., Abbasi, S.A., 2009. Towards modeling and design of vermicomposting systems: mechanisms of composting/vermicomposting and their implications. Ind. J. Biotechnol. 8, 177-182.

4. Abbasi, S.A., Nayeem-Shah, M., Abbasi. T., 2015. Vermicomposting of phytomass: limitations of the past approaches and the emerging directions. J. Clean. Prod.93, 103-114

5. Abbasi, T., Tauseef, S.M., Abbasi, S. A., 2011. Global Warming and the Role of Wetlands Lap Lambart Academic, Germany; ISBN 978-3846556009. xiv+264 pages.

6. Abbasi, T., Tauseef, S.M., Abbasi, S. A., 2011. Global Warming and the Role of Wetlands Lap Lambart Academic, Germany; ISBN 978-3846556009. xiv+264 pages

7. Banupriya, D., 2017. Towards development of mechanizable high-rate vermicomposting systems for coverting biodegradable solid waste into organic fertilizers, Pondicherry University thesis.

8. Edwards, C.A., Norman, Q. A., Sherman, R., 2011. Vermiculture Technology, Earthworms, Organic Waste and Environmental Management. CRC Press, 17-19.

9. Gajalakshmi, S., Abbasi, S.A., 2004. Earthworms and vermicomposting, Indian. J. Biotechnol. 3, 486-494.

10. Ganesh, P.S., Gajalakshmi, S., Abbasi. S.A., 2009. Vermicomposting of the leaf litter of acacia (*Acacia auriculiformis*): possible roles of reactor geometry, polyphenols, and lignin. Bioresour. Technol, 100, 1819–1827

11. Hussain, N., Abbasi, T., Abbasi, S. A., 2016a. Vermiremediation of an invasive and pernicious weed salvinia (*Salvinia molesta*). Ecol. Eng, 91, 432-440.

12. Kumar, M.R., Tauseef, S.M., Abbasi, T., Abbasi, S.A., 2015. Control of amphibious weed ipomoea (*Ipomoea carnea*) by utilizing it for the extraction of volatile fatty acids as energy precursors. Int. J. Aadv. Res. 6 (1), 73-78.

13. Patnaik, P., 2017. Towards a clean technology for the total utilization of the invasive xerophyte prosopis (*prosopis juliflora*, swartz, dc.), Pondicherry University thesis..

14. Tauseef, S. M., Abbasi, T., Banupriya, G., Banupriya, D., Abbasi S. A., 2013b. A new machine for clean and rapid separation of vermicast, earthworms and undigested substrate in vermicomposting systems, Comp. Sci. communicated.

15. Tauseef, S. M., Premalatha, M., Abbasi, T., Abbasi, S. A., 2013. Methane capture from livestock manure, J. Environ. Manag, 117, 187-207."

Chapter 4

Direct vermicomposting of ipomoea and the influence on it of three generations of earthworms

The studies described in the previous chapter were extended to ipomoea. The weed was directly vermicomposted, without any pre-composting, manure supplementation, or any other pre-treatment. Four species of earthworms were, separately, studied for the purpose.

The first series of experiments had adult earthworms which had been born in cow-dung fed cultures and had grown to adulthood in them. The second series utilized earthworms born and raised in ipomoea-fed cultures. Their next generation was then used for the third series of experiments. The objective was to see whether the second and the third generations display similarly increasing adaptation to, and comfort with, the ipomoea feed as was witnessed in case of salvinia.

4.1 Introduction

As was shown in Chapter 2, the reliance of the existing phytomass vermicomposting processes on cow-dung supplementation, besides their slowness, have been two of the prime reasons why phytomass vermicomposting has not come to be in vogue as animal manure vermicomposting has been. It was also brought out that the 'high-rate vermicomposting' paradigm introduced by S. A. Abbasi and coworkers (Abbasi *et al.*, 2009; 2014; 2011; 2015; Tauseef *et al.*, 2013 a, b) has the potential to remedy the lacunae.

In the preceding chapter we had demonstrated that salvinia can be directly and efficiently vermicomposted by utilizing the ‘high-rate vermicomposting’ paradigm. The reactors were operated without interruption for 160 days in each experiment which showed the robustness and long-term sustainability of the process. Another major feature of the study was to assess the performance of three successive generations of earthworms and to explore whether there was adaptive response and increasing liking of the unitary salvinia feed by the earthworm. We have now carried out similar experiments on ipomoea to see whether this weed can also be as gainfully vermicomposted, and whether earthworms born and raised on ipomoea as the sole feed become increasingly efficient in vermicomposting ipomoea.

4.2 Materials and method

Whereas in case of the vermicomposting of salvinia, reported in the previous chapter, whole plants of the free-floating weed were used in vermicomposting, in case of ipomoea only the leaves and soft parts of the stem were utilized. The rest of the ipomoea biomass is woody and is resistant to composting or vermicomposting. It can be used as fuel wood after sun-drying.

The leaves were obtained from the ipomoea stands available in and around the campus Pondicherry University. They were rinsed to remove muck and fed to the reactors. No chopping, mincing, or any other pretreatment was done.

The types of vermireactors employed and the rest of the designing and the execution of the experiments was identical to the one described in Section 3.2.

4.3 Results and discussion

4.3.1 Vermicomposting of ipomoea by the pioneers

The pioneers, who had grown to adulthood on cow-dung feed, took about 2 months to acclimatize to the ipomoea feed. They produced negligible vermicast during the first 20 days and performed progressively better till their output reached near average levels in another 40 days.

The apparent fraction of ipomoea vermicomposted ranged 8.5 — 10.1% in 20-day pulses (solid retention times or SRTs). This (Table 4.1) appears much less than the 13.5 — 15.6% range achieved with salvinia at the corresponding SRT (Table 3.1) but is not really so.

Table 4.1: Screening of four different earthworm species in pulse-fed reactors maintained with 2 kg ipomoea per pulse and 50 adult earthworms

Number of days from start of the reactors	*E.andrei*		*P.sansibaricus*		*L.rubillus*		*D.willsi*	
	Vermicast generated as a fraction of dry weight equivalent of feed mass %	*Vermicast per worm , per day (mg)*	*Vermicast generated as a fraction of dry weight equivalent of feed mass %*	*Vermicast per worm , per day (mg)*	*Vermicast generated as a fraction of dry weight equivalent of feed mass %*	*Vermicast per worm , per day (mg)*	*Vermicast generated as a fraction of dry weight equivalent of feed mass %*	*Vermicast per worm , per day (mg)*
0-20	7.2	24.8	4.4	15.3	6.5	22.6	5.3	18.3
21-40	8.2	28.5	5.3	18.3	8	27.8	6.8	23.7
41-60	8.9	30.7	8.2	28.3	9.7	33.6	8.3	28.8
61-80	10.2	35.2	9.4	32.6	10.3	35.6	9.5	32.8
81-100	11.1	38.4	9.3	31.9	10.1	35	9.7	33.6
101-120	10.5	36.3	8.9	30.8	11	37.7	10.2	35.2
121-140	11	38.2	9.4	32.5	10	34.5	8.9	30.4
141-160	10.8	37.4	8.9	31	10.1	35	10.3	35.7
Average ± SD	10.1±1.1	34.9±3.9	8.5±1.5	29.3±5.1	9.9±0.9	34.2±3.1	9.1±1.2	31.4±4.2

**The reading of first 20 days have been excluded as it was a phase when the earthworm were acclimatizing with the feed and the reactor*

This is because the dry weight of 2 kg of ipomoea leaves (346 g) is much greater than the dry weight, (202.2 g), of the corresponding fresh mass of salvinia. Hence 2 kg of fresh ipomoea contains 68% more substantive feed then 2 kg of fresh salvinia (the rest is only water). When the vermicomposting is quantified in terms of mass of vermicast generated per adult earthworm and per day, this factor gets eliminated. It is then seen that the extent of vermicast generated per earthworm per day from salvinia has a range (27.3 — 31.6 mg; Table 3.1) which is quite close to the range of vermicast output (29.3 — 34.9 mg; Table 4.1) achieved with ipomoea. Moreover, as explained earlier in Section 3.3.1, 50 ± 10% of organic carbon contained in any feed is either converted to worm zoomass or is lost as CO_2 (due to respiration by earthworms and microorganisms present in the feed) in the course of vermicomposting. Hence the figures of 8.5 – 10.1% reflect conversion of about twice as much feed as the vermicast produced. In other words, the *effective* conversion of feed to vermicast per 20 days in reactors with 25 earthworms per kg of ipomoea is in the range 17-20.2%. But in all the reactors, there is a rising trend in vermicast production with time (Figure 4.1). This means that vermicast output per pulse is set to rise. Secondly had we not been removing the juveniles and cocoons from the modules, they would be utilizing substantial parts of the feed.

The combination of both these factors are likely to have caused much more than 17-20 % utilization of salvinia per 20 days and the actual vermicast yield would have approached its theoretical maximum (of 50 ± 10%) in about 40 days. This rate is several times faster than the 90-120 days that are taken by conventional vermireactors.

Equally importantly, this rate has been achieved without any pre-composting, cow-dung supplementation, or even any pre-treatment of the ipomoea feed.

E. andrei produced most vermicast per unit time of all the four species, followed very closely by *L. rubellus*. *D.willsi* came next while *P.sansibaricus* registered the least vermicomposting efficiency of all. The difference in vermicast output between *E.andrei* and *L. rubellus* was not statistically significant ($p > 0.5$), but that between *L. rubellus* and *D.willsi* and between *D.willsi* and *P.sansibaricus* was ($p \leq 0.3$).

The number of juveniles and cocoons produced by the four species (Tables 4.2 and 4.3) followed the trend: *E.andrei* > *L. rubellus* > *P.sansibaricus* ~ *D.willsi* which was similar to the trend in vermicast production but with the exception that the difference between *P.sansibaricus* and *D.willsi* was not statistically significant (Tables 4.4 and 4.5).

The implication of these findings is that over longer term operation of vermireactors in which juveniles and cocoons are not removed, the reactors operated with *E.andrei* or *L. rubellus* will attain advantage over reactors operated with *P.sansibaricus* or *D.willsi* because the former will create many more agents that will feed upon ipomoea and vermicompost it than the later.

Table 4.2: Juveniles produced by four different earthworm species in pulse-fed reactors maintained on 500 g ipomoea per pulse and 10 adult earthworms

Number of days from start of the reactors	*E. andrei*			*P. sansibaricus*			*L. rubillus*			*D. willsi*		
	Reactor 1	*Reactor 2*	*Average ±SD*	*Reactor 1*	*Reactor 2*	*Average ±SD*	*Reactor 1*	*Reactor 2*	*Average ±SD*	*Reactor 1*	*Reactor 2*	*Average ±SD*
0-20	6	9	7.5±2.1	0	0	0±0	4	6	5±1.4	0	0	0±0
21-40	7	12	9.5±3.5	3	2	2.5±0.7	6	7	6.5±0.7	3	4	3.5±0.7
41-60	8	9	8.5±0.7	6	3	4.5±1.4	9	7	8±1.4	5	3	4±2.1
61-80	8	11	9.5±2.1	6	2	4±1.4	7	9	8±1.4	3	5	4±2.8
81-100	10	9	9.5±0.7	4	5	4.5±0.7	8	7	7.5±0.7	3	4	3.5±0.7
101-120	9	8	8.5±0.7	4	4	4±1.4	7	8	7.5±0.7	3	5	4±0
121-140	7	12	9.5±3.5	3	6	4.5±0.7	9	7	8±1.4	4	5	4.5±2.1
141-160	10	9	9.5±0.7	5	4	4.5±2.1	6	9	7.5±2.1	6	3	4.5±0.7
Average ± SD	8.4±1.3	10±1.6	9.2±0.5	4.4±1.2	3.7±1	4.1±0.7	7.4±1.3	7.7±0.9	7.6±0.5	3.8±1.2	4.1±0.9	4±1

**The reading of first 20 days have been excluded as it was a phase when the earthworm were acclimatizing with the feed and the reactor*

4.3.2 Performance of the second and the third generation of earthworms compared to the pioneers (first generation)

The performance of the second and the third generations of *E.andrei*, born and grown in ipomoea-fed vermireactors in comparison to the performance of manure-reared first (pioneer) generation in vermicomposting ipomoea is summarized in Table 4.6. There is a huge jump in the average rate of vermicast production by the second generation of *E.andrei* compared to the first: of the order of 26%. The third generation has still higher (by about 4 %) vermicomposting efficiency, which is not as dramatically different from the second generation as the second generation's is from the first, yet statistically significant at > 95% confidence level. It is also seen that whereas the pioneers took time to acclimatize with ipomoea feed, as reflected in negligible feeding in the first 20 days, the second and the third generation earthworms did not require any priming and began generating near-average vermicast from the outset.

Table 4.3: Cocoons produced by four different earthworm species in pulse-fed reactors maintained on 500 g ipomoea per pulse and 10 adult earthworms

Number of days from start of the reactors	*E. andrei*			*P. sansibaricus*			*L. rubillus*			*D. willsi*		
	Reactor 1	*Reactor 2*	*Average ±SD*	*Reactor 1*	*Reactor 2*	*Average ±SD*	*Reactor 1*	*Reactor 2*	*Average ±SD*	*Reactor 1*	*Reactor 2*	*Average ±SD*
0-20	3	2	2.5±0.7	0	2	2±2.8	2	2	2±0	0	4	2±1.4
21-40	5	4	4.5±0.7	3	2	2.5±0.7	2	3	2.5±0.7	2	3	2.5±0.7
41-60	3	6	4.5±2.1	3	3	3±1.4	4	3	3.5±0.7	4	2	3±0
61-80	6	5	5.5±0.7	4	3	3.5±0	4	5	4.5±0.7	4	4	4±0.7
81-100	6	4	5±1.4	3	6	4.5±0.7	4	4	4±0	4	3	3.5±2.1
101-120	9	5	7±2.8	2	6	4±2.1	6	3	4.5±2.1	5	2	3.5±2.8
121-140	5	8	6.5±2.1	7	2	4.5±1.4	4	4	4±0	3	5	4±3.5
141-160	8	6	7±1.4	3	5	4±2.1	6	3	4.5±2.1	3	6	4.5±1.4
Average ± SD	6.1±2	5.7±1.4	5.7±1.1	3.5±1.6	3.8±1.8	3.7±0.7	4.3±1.4	3.5±0.8	3.9±0.7	3.6±1	3.5±1.5	3.6±0.7

Table 4.4: Test of significance in the difference of juvenile production by four species of earthworms fed on ipomoea

Species	*Nature of the change in vermicast generation*	*Enhancement in vermicast production*
E.andrei in comparison to *L.rubillus*	increase	99%
L.rubillus in comparison to *P.sansibaricus*	increase	99%
P.sansibaricus in comparison to *D.willsi*	increase	70%

Table 4.5: Test of significance in the difference of cocoon production by four species of earthworms fed on ipomoea

Species	*Nature of the change in vermicast generation*	*Enhancement in vermicast production*
E.andrei in comparison to *L.rubillus*	increase	99%
L.rubillus in comparison to *P.sansibaricus*	increase	70%
P.sansibaricus in comparison to *D.willsi*	increase	60%

Table 4.6: Performance of the second and third generation of *E.andrei* born and grown in ipomoea-fed vermireactors in comparison to the performance of manure-reared *E.andrei* with which the vermireactor were started

Number of days of reactor operation	*First generation**		*Second generation*		*Third generation*	
	Vermicast generated as a fraction of dry weight equivalent of feed mass %*	*Vermicast per worm , per day* (mg)*	*Vermicast generated as a fraction of dry weight equivalent of feed mass %*	*Vermicast per worm , per day (mg)*	*Vermicast generated as a fraction of dry weight equivalent of feed mass %*	*Vermicast per worm , per day (mg)*
20	7.2	24.8	10.6	36.6	16.1	55.7
40	8.2	28.5	13.2	45.7	12.3	42.5
60	8.9	30.7	11.6	40.3	12.7	43.9
80	10.2	35.2	12.2	42.3	12.2	42.2
100	11.1	38.4	13.7	47.6	12.5	43.4
120	10.5	36.3	13.9	48.2	14.4	50
140	11	38.2	14	48.4	16.8	58.2
160	10.8	37.4	12.3	42.7	10.9	37.8
Average ± SD	10.1±1	34.9±3.9	12.7±1.1	43.9±4.5	13.5±1.9	45.5±5.6

**The data of first 20 days have been excluded as it was a phase when the earthworm were acclimatizing with the feed and the reactor*

The third generation of *E.andrei* produced larger number of juveniles (Table 4.7) and cocoons (Table 4.8) than the second generation and the second generation did so better than the first generation. The differences were significant at ≥ 95% confidence level (Tables 4.9 and 4.10).

Table 4.7: Juveniles produced by the second and third generation of *E.andrei* born and grown in ipomoea-fed vermireactors in comparison to the performance of manure-reared *E.andrei* with which the vermireactor were started

Number of days from start of the reactors	*Number of Juveniles produced by first generation worm**			*Number of Juveniles produced by second generation worms*			*Number of Juveniles produced by third generation worm*		
	*Reactor 1**	*Reactor 2**	*Average ±SD**	*Reactor 1*	*Reactor 2*	*Average ±SD*	*Reactor 1*	*Reactor 2*	*Average ±SD*
20	6	9	7.5±2.1	8	9	8.5±0.7	8	11	9.5±2.1
40	7	12	9.5±3.5	13	8	10.5±3.5	12	10	11±1.4
60	8	9	8.5±0.7	9	12	10.5±2.1	9	12	10.5±2.1
80	8	11	9.5±2.1	8	11	9.5±2.1	13	7	10±4.2
100	10	9	9.5±0.7	12	9	10.5±2.1	10	14	12±2.8
120	9	8	8.5±0.7	13	7	10±4.5	14	11	12.5±2.1
140	7	12	9.5±3.5	7	12	9.5±3.5	12	10	11±1.4
160	10	9	9.5±0.7	9	11	10±1.4	9	11	10±1.4
Average ± SD	8.4±1.3	10±1.6	9.2±0.5	9.9±2.4	9.9±1.9	9.9±0.7	10.9±2.2	10.7±2	10.8±1

**The data of first 20 days have been excluded as it was a phase when the earthworm were acclimatizing with the feed and the reactor*

Table 4.10: Test of significance in the difference of cocoon production by three generations of earthworms fed on ipomoea

Earthworm species	*Nature of the change in juveniles production in the second generation compared to the first*	*Confidence level(%) at which the difference was significant*	*Nature of the change in juveniles production in the third generation compared to the second*	*Confidence level(%) at which the difference was significant*
E.andrei	increase	95%	increase	96%
L.rubillus	increase	98%	increase	90%
D.willsi	increase	95%	increase	97%
P.sansibaricus	increase	99%	increase	99%

It can be surmised that:

i) Successive generations of *E.andrei* can be raised with ipomoea as the sole feed.

ii) Successive generations get increasingly acclimatized to ipomoea and display increasing efficiency in vermicomposting it.

iii) The reproductive ability of *E.andrei* in ipomoea-fed reactors increases as it produces its second and the third generation in it.

Table 4.8: Cocoons produced by the second and third generation of *E.andrei* born and grown in ipomoea-fed vermireactors in comparison to the performance of manure-reared *E.andrei* with which the vermireactor were started

Number of days from start of the reactors	*Number of Cocoons produced by first generation worms**			*Number of Cocoons produced by second generation worms*			*Number of Cocoons produced by third generation worms*		
	*Reactor 1**	*Reactor 2**	*Average ±SD**	*Reactor 1*	*Reactor 2*	*Average ±SD*	*Reactor 1*	*Reactor 2*	*Average ±SD*
20	3	2	2.5±0.7	3	6	4.5±2.1	9	7	8±1.4
40	5	4	4.5±0.7	6	4	5±1.4	8	9	8.5±0.7
60	3	6	4.5±2.1	7	9	8±1.4	5	9	7±2.8
80	6	5	5.5±0.7	8	6	7±1.4	10	7	8.5±2.1
100	6	4	5±1.4	9	7	8±1.4	8	6	7±1.4
120	9	5	7±2.8	6	9	7.5±2.1	9	8	8.5±0.7
140	5	8	6.5±2.1	7	9	8±1.4	7	10	8.5±2.1
160	8	6	7±1.4	8	7	7.5±0.7	9	7	8±1.4
Average ± SD	6.1±2	5.7±1.4	5.7±1.1	6.7±1.7	7.1±1.7	6.9±1.3	8.1±1.5	7.9±1.3	8±0.6

**The data of first 20 days have been excluded as it was a phase when the earthworm were acclimatizing with the feed and the reactor*

Table 4.9: Test of significance in the difference of juvenile production by three generations of earthworms fed on ipomoea

Earthworm species	*Nature of the change in cocoons production in the second generation compared to the first*	*Confidence level(%) at which the difference was significant*	*Nature of the change in cocoons production in the third generation compared to the second*	*Confidence level(%) at which the difference was significant*
E.andrei	Increase	99%	increase	98%
L.rubillus	Increase	96%	increase	94%
D.willsi	Increase	93%	increase	97%
P.sansibaricus	Increase	86%	increase	99%

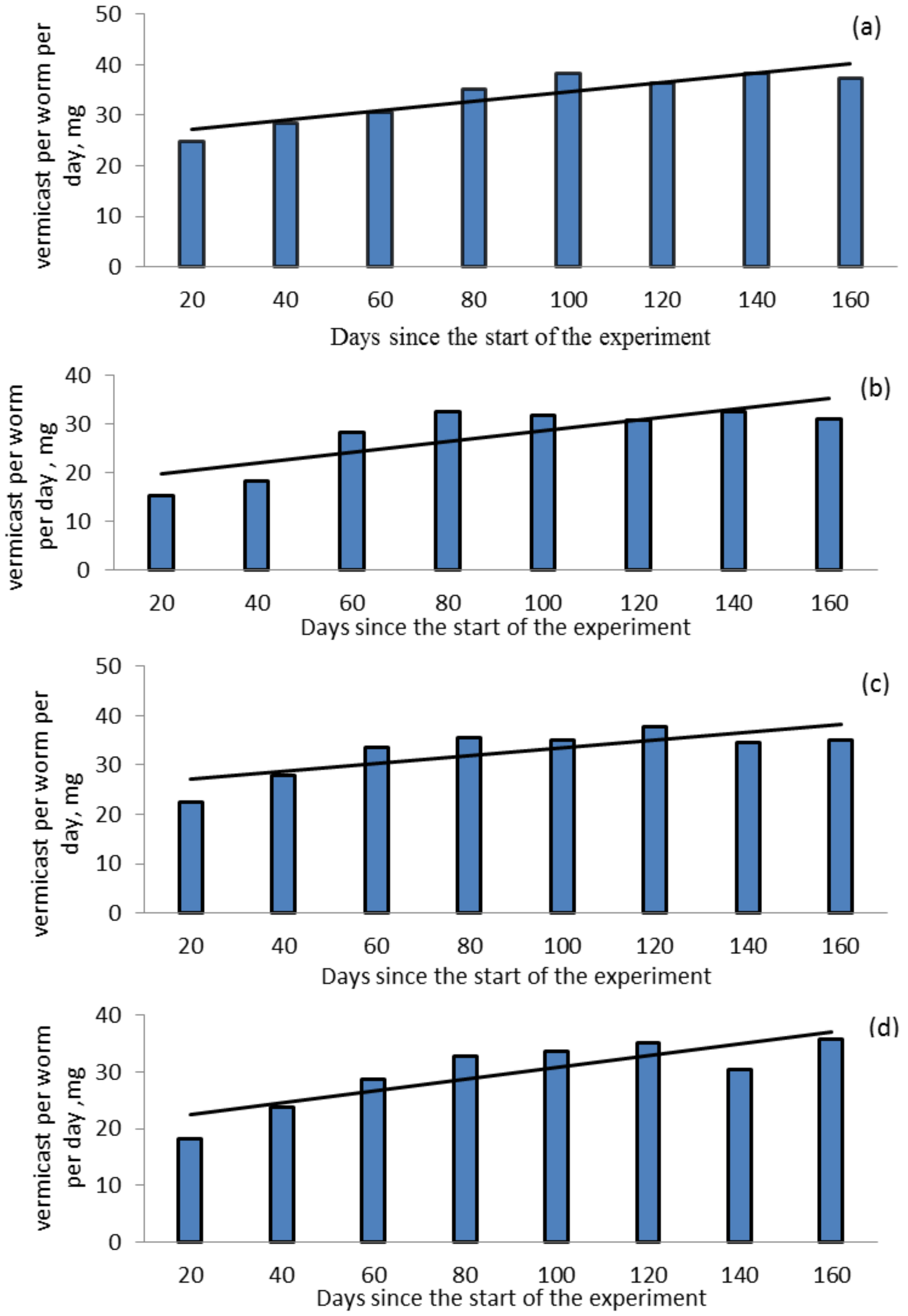

Figure 4.1: Vermicast generated in pulse-fed, semi-continuous reactors operated with a) *E.andrei* b) *P.sansibaricus* c) *L.rubillus* and d) *D.willsi* earthworms and fed with fresh ipomoea. Trend lines are also shown.

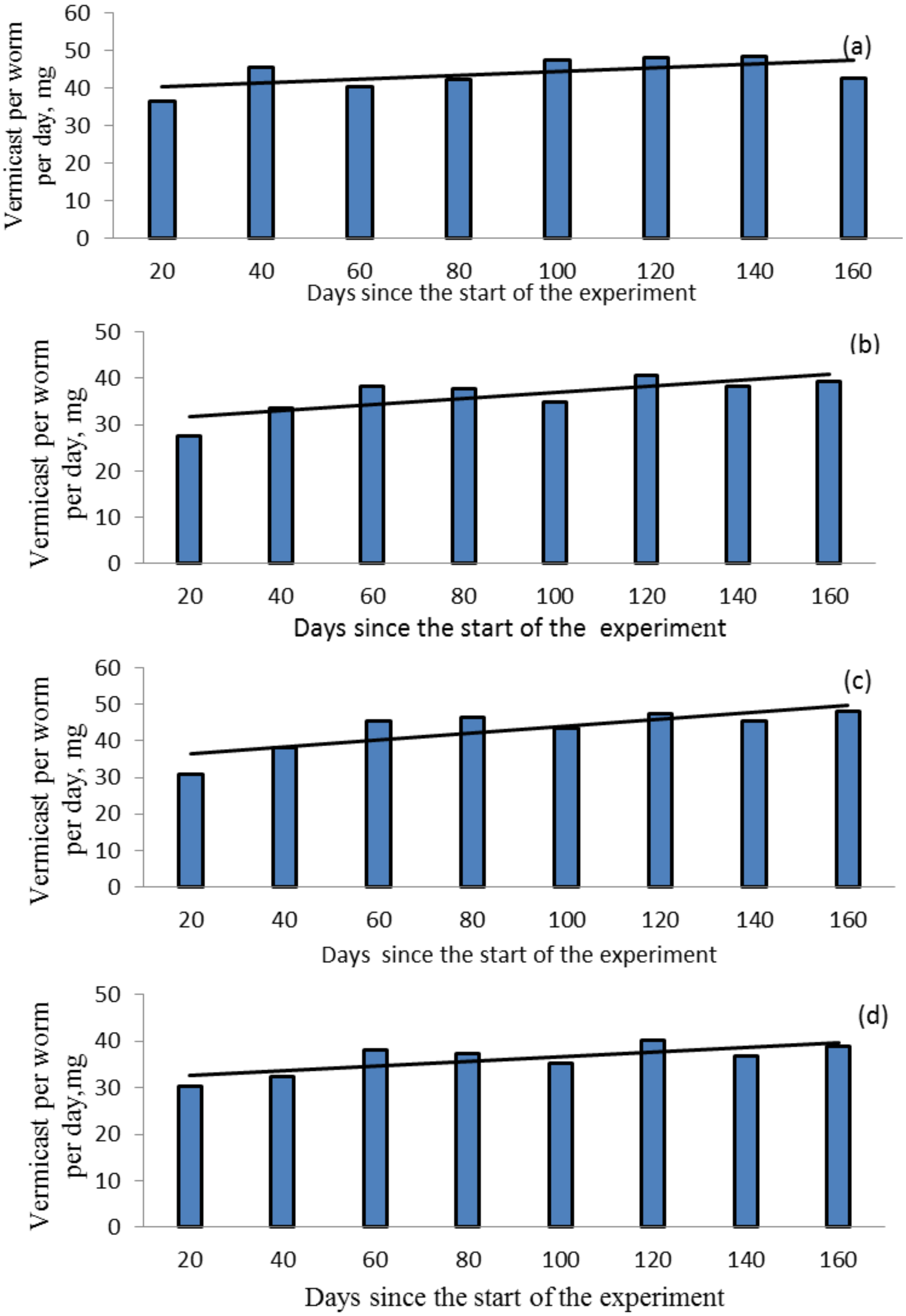

Figure 4.2: Vermicast generated by the second generation of earthworm born and grown in ipomoea-fed vermireactors with four different earthworm species a) *E.andrei* b) *P.sansibaricus c) L.rubillus* and d) *D.willsi*

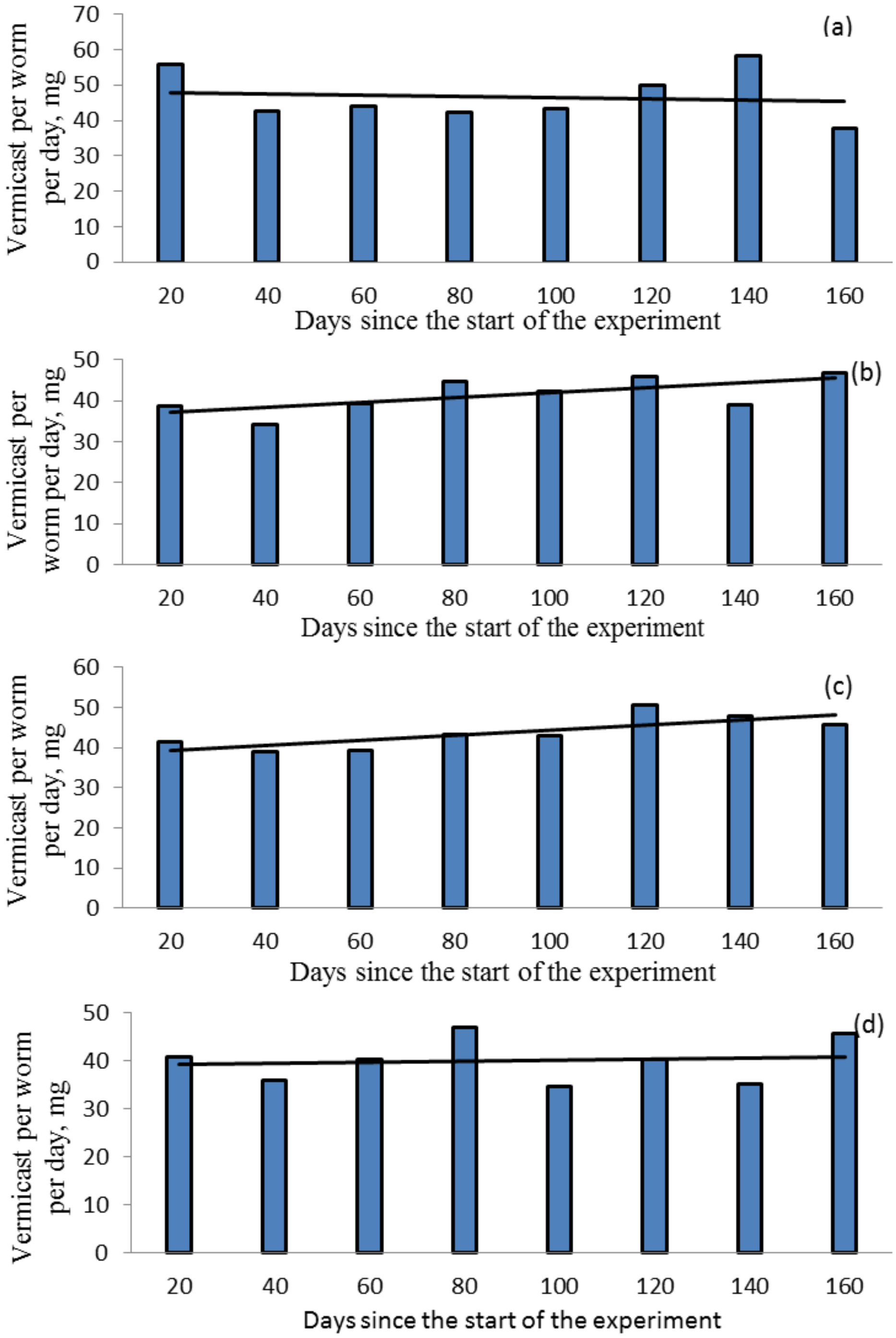

Figure 4.3: Vermicast generated by the third generation of earthworm born and grown in Ipomoea-fed vermireactors with four different earthworm species a) *E.andrei* b) *P.sansibaricus* c) *L.rubillus* and d) *D.willsi*

The trend line of vermicast production from ipomoea by the pioneer (first generation) of *E.andrei* has a clearly rising slope (Figure 4.1a), indicating that increasing adaptation to ipomoea feed by the earthworms is occurring who had been reared to adulated on cow-dung. In contrast the slope pertaining to the second generation has only a mild rise (Figure 4.2a). The trend line pertaining to the third generation is almost flat (Figure 4.3a). These patterns indicate that vermicomposting efficiency had almost peaked by the third generation and higher generations would perform similar to the third generation.

In case of *P.sansibaricus*, the second generation produced, on an average, about 24% more vermicast per unit time than the first generation (Table 4.11). The third generation exceeded it by another 14%. There was a long acclimatization period before the pioneers (first generation) began feeding upon ipomoea to their capacity, but no such priming was seen to be required by the second and the third generation. Further, as in the case of *E.andrei*, there was a sharp upward slope in the trend line of vermicast production by the pioneers (Figure 4.1b). The slope was less sharp in case of the second generation (Figure 4.2b) and still less sharp in case of the third generation (Figure 4.3b).

Table 4.11: Performance of the second and third generation of *P.sansibaricus* born and grown in ipomoea-fed vermireactors in comparison to the performance of manure-reared *P.sansibaricus* with which the vermireactor were started

Number of days of reactor operation	*First generation**		*Second generation*		*Third generation*	
	*Vermicast generated *as a fraction of dry weight equivalent of feed mass %*	*Vermicast per worm , per day* (mg)*	*Vermicast generated as a fraction of dry weight equivalent of feed mass %*	*Vermicast per worm , per day (mg)*	*Vermicast generated as a fraction of dry weight equivalent of feed mass %*	*Vermicast per worm , per day (mg)*
20	4.4	15.3	7.9	27.5	11.2	38.7
40	5.3	18.3	9.7	33.7	9.9	34.2
60	8.2	28.3	11	38.2	11.3	39.2
80	9.4	32.6	10.9	37.8	12.9	44.8
100	9.3	31.9	10	34.8	12.2	42.2
120	8.9	30.8	11.8	40.7	13.2	45.8
140	9.4	32.5	11	38.2	11.3	39.1
160	8.9	31	11.4	39.4	13.5	46.8
Average ± SD	8.5±1.5	29.3±5.1	10.5±1.2	36.3±4.2	11.9±1.2	41.3±4.3

**The data of first 20 days have been excluded as it was a phase when the earthworm were acclimatizing with the feed and the reactor*

The average number of juveniles and cocoons (Tables 4.12 and 4.13) that were produced by *P.sansibaricus* followed the order third generation > second generation > first generation. The differences were significant at 99% confidence level in all but one data pair (Tables 4.9 and 4.10).

The second generation of *L.rubellus* produced about 27% greater vermicast from ipomoea per worm per day than its pioneer (first) generation. But further improvement by the next generation was only marginal (Table 4.14). The trend of third generation being superior to the second and the second being superior to the first was manifest in the production of juveniles (Table 4.15) and cocoons (Table 4.16) as well.

Table 4.12: Juveniles produced by the second and third generation of *P.sansibaricus* born and grown in ipomoea-fed vermireactors in comparison to the performance of manure-reared *P.sansibaricus* with which the vermireactor were started

Number of days from start of the reactors	*Number of Juveniles produced by first generation worms**			*Number of Juveniles produced by second generation worms*			*Number of Juveniles produced by third generation worm*		
	*Reactor 1**	*Reactor 2**	*Average ±SD**	*Reactor 1*	*Reactor 2*	*Average ±SD*	*Reactor 1*	*Reactor 2*	*Average ±SD*
20	0	0	0±0	5	3	4±1.4	7	6	6.5±0.7
40	3	2	2.5±0.7	6	4	5±1.4	8	7	7.5±0.1
60	6	3	4.5±2.1	5	6	5.5±0.7	8	6	7±1.4
80	6	2	4±2.8	5	6	5.5±0.7	5	8	6.5±2.1
100	4	5	4.5±0.7	6	5	5.5±0.7	8	6	7±1.4
120	4	4	4±0	6	5	5.5±0.7	6	8	7±1.4
140	3	6	4.5±2.1	4	6	5±1.4	6	7	6.5±0.7
160	5	4	4.5±0.7	5	6	5.5±0.7	9	5	7±2.8
Average ± SD	4.4±1.2	3.7±1.1	4.1±0.7	5.2±0.7	5.1±1.1	5.2±0.5	7.1±1.3	6.6±1.1	6.9±0.3

**The data of first 20 days have been excluded as it was a phase when the earthworm were acclimatizing with the feed and the reactor*

Table 4.13: Cocoons produced the second and third generation of *P.sansibaricus* born and grown in ipomoea-fed vermireactors in comparison to the performance of manure-reared *P.sansibaricus* with which the vermireactor were started

Number of days from start of the reactors	*Number of Cocoons produced by first generation worms**			*Number of Cocoons produced by second generation worms*			*Number of Cocoons produced by third generation worms*		
	*Reactor 1**	*Reactor 2**	*Average ±SD **	*Reactor 1*	*Reactor 2*	*Average ±SD*	*Reactor 1*	*Reactor 2*	*Average ±SD*
20	0	2	2±1.4	2	4	3±1.4	6	5	5±0.7
40	3	2	2.5±0.7	5	3	4±1.4	5	5	5±0
60	3	3	3±2.1	5	4	4.5±0.7	5	6	5.5±0.7
80	4	3	3.5±2.8	3	5	4±1.4	4	6	5±1.4
100	3	6	4.5±1.4	6	4	5±1.4	6	5	5.5±0.7
120	2	6	4±1.4	3	6	4.5±2.1	5	5	5±0
140	7	2	4.5±1.4	3	5	4±1.4	4	6	5.5±1.4
160	3	5	4±1.4	6	3	4.5±2.1	5	5	5±0
Average ± SD	3.5±1.6	3.8±1.8	3.7±0.7	4.1±1.4	4.2±1	4.2±0.5	4.7±0.7	5.7±0.7	5.1±0.2

**The data of first 20 days have been excluded as it was a phase when the earthworm were acclimatizing with the feed and the reactor*

Table 4.14: Performance of the second and third generation of *L.rubillus* born and grown in ipomoea-fed vermireactors in comparison to the performance of manure-reared *L.rubillus* with which the vermireactor were started

Number of days of reactor operation	*First generation**		*Second generation*		*Third generation*	
	Vermicast generated as a fraction of dry weight equivalent of feed mass %*	*Vermicast per worm , per day* (mg)*	*Vermicast generated as a fraction of dry weight equivalent of feed mass %*	*Vermicast per worm , per day (mg)*	*Vermicast generated as a fraction of dry weight equivalent of feed mass %*	*Vermicast per worm , per day (mg)*
20	6.5	22.6	8.9	30.9	12	41.41
40	8	27.8	11.1	38.3	11.3	39
60	9.7	33.6	13.1	45.5	11.3	39.2
80	10.3	35.6	13.5	46.6	12.4	43.1
100	10.1	35	12.5	43.4	12.4	43
120	11	37.7	13.7	47.5	14.6	50.7
140	10	34.5	13.2	45.6	13.8	47.8
160	10.1	35	13.9	48.2	13.2	45.6
Average ± SD	9.9±0.9	34.2±3.7	12.5±1.7	43.3±3.3	12.6±1.2	43.7±4.1

**The data of first 20 days have been excluded as it was a phase when the earthworm were acclimatizing with the feed and the reactor*

Table 4.15: Juveniles produced by the second and third generation of *L.rubillus* born and grown in ipomoea-fed vermireactors in comparison to the performance of manure-reared *L.rubillus* with which the vermireactor were started

Number of days from start of the reactors	*Juveniles, number produced by First generation **			*Juveniles, number produced by Second generation*			*Juveniles, number produced by Third generation*		
	*Reactor 1**	*Reactor 2**	*Average ±SD**	*Reactor 1*	*Reactor 2*	*Average ±SD*	*Reactor 1*	*Reactor 2*	*Average ±SD*
20	4	6	5±1.4	6	5	5.5±0.7	4	7	5.5±2.1
40	6	7	6.5±0.7	6	9	7.5±2.1	12	6	9±4.2
60	9	7	8±1.4	9	8	8.5±0.7	10	11	10.5±0.7
80	7	9	8±1.4	10	9	9.5±0.7	9	12	10.5±2.1
100	8	7	7.5±0.7	11	10	10±0.7	12	10	11±1.4
120	7	8	7.5±0.7	12	9	10.5±2.1	10	10	10±0
140	9	7	8±1.4	9	10	9.5±0.7	10	14	12±2.8
160	6	9	7.5±2.1	10	10	10±0	12	9	10.5±2.1
Average ± SD	7.4±1.3	7.7±0.9	7.6±0.5	9.1±2.2	8.7±1.7	8.9±1.7	9.9±2.6	9.9±2.6	9.9±2

**The data of first 20 days have been excluded as it was a phase when the earthworm were acclimatizing with the feed and the reactor*

Table 4.16: Cocoons produced by the second and third generation of *L.rubillus* born and grown in ipomoea-fed vermireactors in comparison to the performance of manure-reared *L.rubillus* with which the vermireactor were started

Number of days from start of the reactors	*Number of Cocoons produced by first generation worms**			*Number of Cocoons produced by second generation worms*			*Number of Cocoons produced by third generation worms*		
	*Reactor 1**	*Reactor 2**	*Average ±SD **	*Reactor 1*	*Reactor 2*	*Average ±SD*	*Reactor 1*	*Reactor 2*	*Average ±SD*
20	2	2	2±0	2	7	4.5±3.5	2	5	3.5±2.1
40	2	3	2.5±0.7	6	2	4±2.8	6	5	5.5±0.7
60	4	3	3.5±0.7	3	6	4.5±2.1	7	3	5±2.8
80	4	5	4.5±0.7	6	4	5±1.4	5	4	4.5±0.7
100	4	4	4±0	4	5	4.5±0.7	5	5	5±0
120	6	3	4.5±2.1	3	7	5±2.8	5	7	6±1.4
140	4	4	4±0	4	6	5±1.4	6	7	6.5±0.7
160	6	3	4.5±2.1	4	5	4.5±0.7	6	6	6±0
Average ± SD	4.3±1.4	3.5±0.8	3.9±0.7	4±1.3	5.2±1.6	4.6±0.3	5.2±1.4	5.2±1.3	5.2±0.9

**The data of first 20 days have been excluded as it was a phase when the earthworm were acclimatizing with the feed and the reactor*

The same patterns were demonstrated by *D.willsi*. In terms of vermiconversion efficiency as well as fecundity, clear trend of third generation > second generation > first generation was seen (Tables 4.17 – 4-19). Whereas there was a clearly rising trend in vermicast productions in the reactors run with pioneers (first generation), as seen in Figure 4.1d), the trends in reactors run with the second or the third generation were much flatter (Figures 4.2d and 4.3d).

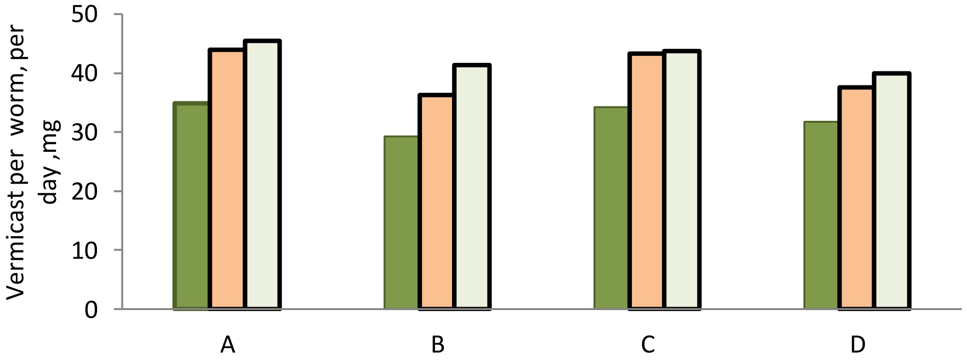

Figure 4.4: Relative efficiency of three generations of earthworms in vermicomposting ipomoea A: *E.andrei;* B: *P.sansibaricus* C: *L.rubillus* D: *D.willsi.*🟩First generation; 🟧 Second generation;⬜Third generation

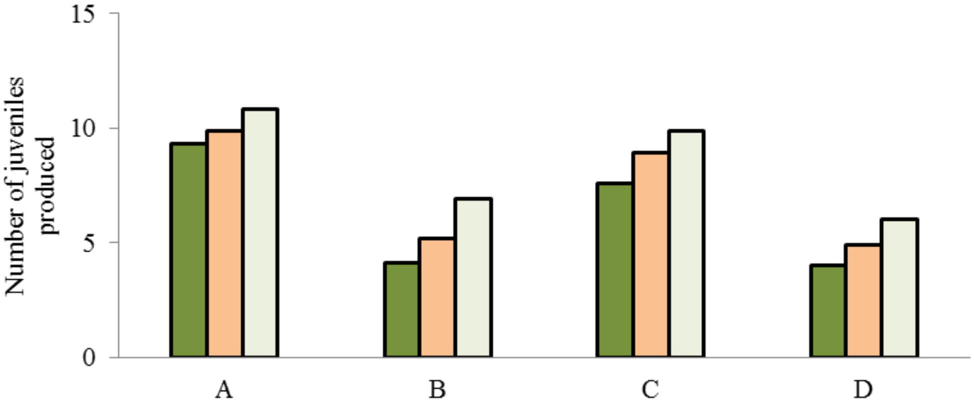

Figure 4.5: Juveniles produced by the second and third generation of earthworm born and grown in ipomoea-fed vermireactors in comparison to the output of manure-reared pioneers. A: *E.andrei;* B: *P.sansibaricus* C: *L.rubillus* *D*: *D.willsi.* 🟩 First generation; 🟧 Second generation; ⬜Third generation

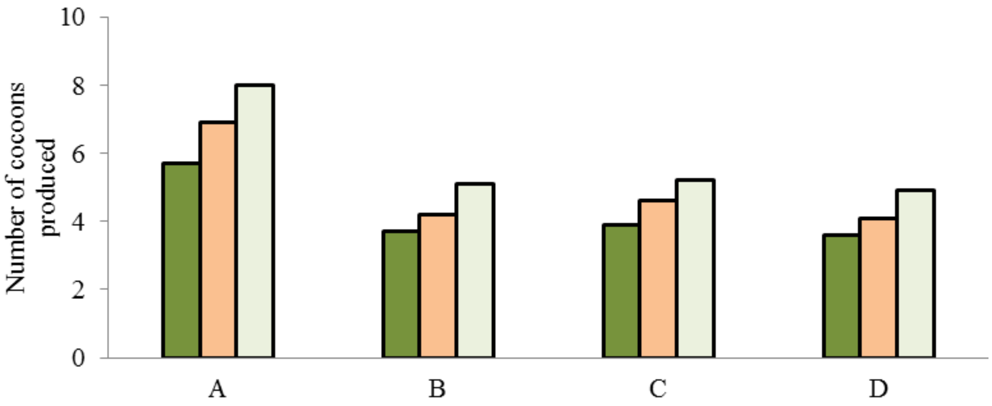

Figure 4.6: Cocoons produced by the second and third generation of earthworm born and grown in ipomoea-fed vermireactors in comparison to the output of manure-reared pioneers. A: *E.andrei;* B: *P.sansibaricus* C: *L.rubillus D*: *D.willsi.* 🟩 First generation;🟧Second generation; ⬜Third generation

Table 4.17: Performance of the second and third generation of *D.willsi* born and grown in ipomoea-fed vermireactors in comparison to the performance of manure-reared *D.willsi* with which the vermireactor were started

Number of days of reactor operation	*First generation**		*Second generation*		*Third generation*	
	*Vermicast generated *as a fraction of dry weight equivalent of feed mass %*	*Vermicast per worm , per day* (mg)*	*Vermicast generated as a fraction of dry weight equivalent of feed mass %*	*Vermicast per worm , per day (mg)*	*Vermicast generated as a fraction of dry weight equivalent of feed mass %*	*Vermicast per worm , per day (mg)*
20	5.3	18.3	8.7	30.2	11.8	40.8
40	6.8	23.7	9.4	32.4	10.4	36
60	8.3	28.8	11	38.2	11.6	40.3
80	9.5	32.8	10.7	37.2	13.6	47
100	9.7	33.6	10.2	35.2	10	34.7
120	10.2	35.2	11.6	40.1	11.7	40.4
140	8.9	30.4	10.2	36.7	10.2	35.3
160	10.3	35.7	11.2	38.8	13.2	45.8
Average ± SD	9.1±1.2	31.7±3.6	10.4±1	37.6±3.2	11.±1.3	40±4.6

**The data of first 20 days have been excluded as it was a phase when the earthworm were acclimatizing with the feed and the reactor*

Table 4.18: Juveniles produced by the second and third generation of *D.willsi* born and grown in ipomoea-fed vermireactors in comparison to the performance of manure-reared *D.willsi* with which the vermireactor were started

Number of days from start of the reactors	*Number of Juveniles produced by first generation worms**			*Number of Juveniles produced by second generation worms*			*Number of Juveniles produced by third generation worm*		
	*Reactor 1**	*Reactor 2**	*Average ± SD **	*Reactor 1*	*Reactor 2*	*Average ±SD*	*Reactor 1*	*Reactor 2*	*Average ±SD*
20	0	0	0±0	3	1	2±1.4	5	6	5.5±0.7
40	3	4	3.5±0.7	5	3	4±1.4	4	6	5±1.4
60	5	3	4±1.4	4	6	5±1.4	7	6	6.5±0.7
80	3	5	4±1.4	6	5	5.5±0.7	5	7	6±1.4
100	3	4	3.5±0.7	7	6	6.5±0.7	6	6	6±0
120	3	5	4±1.4	4	7	5.5±2.1	5	7	6±1.4
140	4	5	4.5±0.7	3	9	6±4.2	8	6	7±1.4
160	6	3	4.5±2.1	4	6	5±1.4	7	5	6±1.4
Average ± SD	3.8±1.2	4.1±0.9	4±1	4.5±1.3	5.4±2.4	4.9±1.4	5.9±1.3	6.1±0.6	6±0.6

**The data of first 20 days have been excluded as it was a phase when the earthworm were acclimatizing with the feed and the reactor*

Table 4.19: Cocoons produced by the second and third generation of *D.willsi* born and grown in ipomoea-fed vermireactors in comparison to the performance of manure-reared *D.willsi* with which the vermireactor were started

Number of days from start of the reactors	*Number of Cocoons produced by first generation worms**			*Number of Cocoons produced by second generation worms*			*Number of Cocoons produced by third generation worms*		
	*Reactor 1**	*Reactor 2**	*Average ±SD**	*Reactor 1*	*Reactor 2*	*Average ±SD*	*Reactor 1*	*Reactor 2*	*Average ±SD*
20	0	4	2±2.8	5	2	3.5±2.1	5	4	4.5±0.7
40	2	3	2.5±0.7	3	4	3.5±0.7	4	4	4±0
60	4	2	3±1.4	2	6	4±2.8	6	3	4.5±2.1
80	4	4	4±0	5	4	4.5±0.7	8	4	6±2.8
100	4	3	3.5±0.7	2	6	4±2.8	4	6	5±1.4
120	5	2	3.5±2.1	3	7	5±2.8	5	7	6±1.4
140	3	5	4±1.4	4	5	4.5±0.7	5	3	5±1.4
160	3	6	4.5±2.1	5	3	4±1.4	6	4	5±1.4
Average ± SD	3.6±1	3.5±1.5	3.6±0.7	3.4±1.8	4.8±1.5	4.1±0.5	5.4±1.3	4.4±1.4	4.9±0.8

**The data of first 20 days have been excluded as it was a phase when the earthworm were acclimatizing with the feed and the reactor*

The relative efficiencies of the four species of the earthworms in vermicomposting ipomoea may be seen in Figure 4.4. The relative felicity in the production of juveniles and cocoons is reflected in Figures 4.5 and 4.6. The figures show *E.andrei* to be the most suitable of the four species, in terms of efficiency in vermicast production as well as reproductive ability, followed by *L.rubellus*.

4.3.3 Effect of nature of substrate on fecundity

The performance of the four species of earthworm in salvinia-fed vermireactors relative to ipomoea-fed vermireactors is shown in Figures 4.7 – 4.9. In all cases except in case of the third generation of *L.rubellus*, more ipomoea was vermicomposted per worm than salvinia (Figure 4.7), even though the difference was only marginal. But the trend was not only reverse in the matter of juvenile and cocoon production (Figures 4.8 and 4.9) but there was a very pronounced difference between extent of reproduction in salvinia-fed reactors and ipomoea-fed reactors.

These are interesting findings and need further exploration. Since successive generations have shown increasing fecundity, it is possible that after fourth, fifth, or higher generation the difference in fecundity in respect of the two weeds may vanish. If it doesn’t happen it will mean that some ingredients in ipomoea suppress earthworm fecundity. The practical implication of it will be that in long-term continuous operation salvinia-fed vermireactor will score over ipomoea-fed vermireactors because the former will generate larger number of animals per unit time to process the feed.

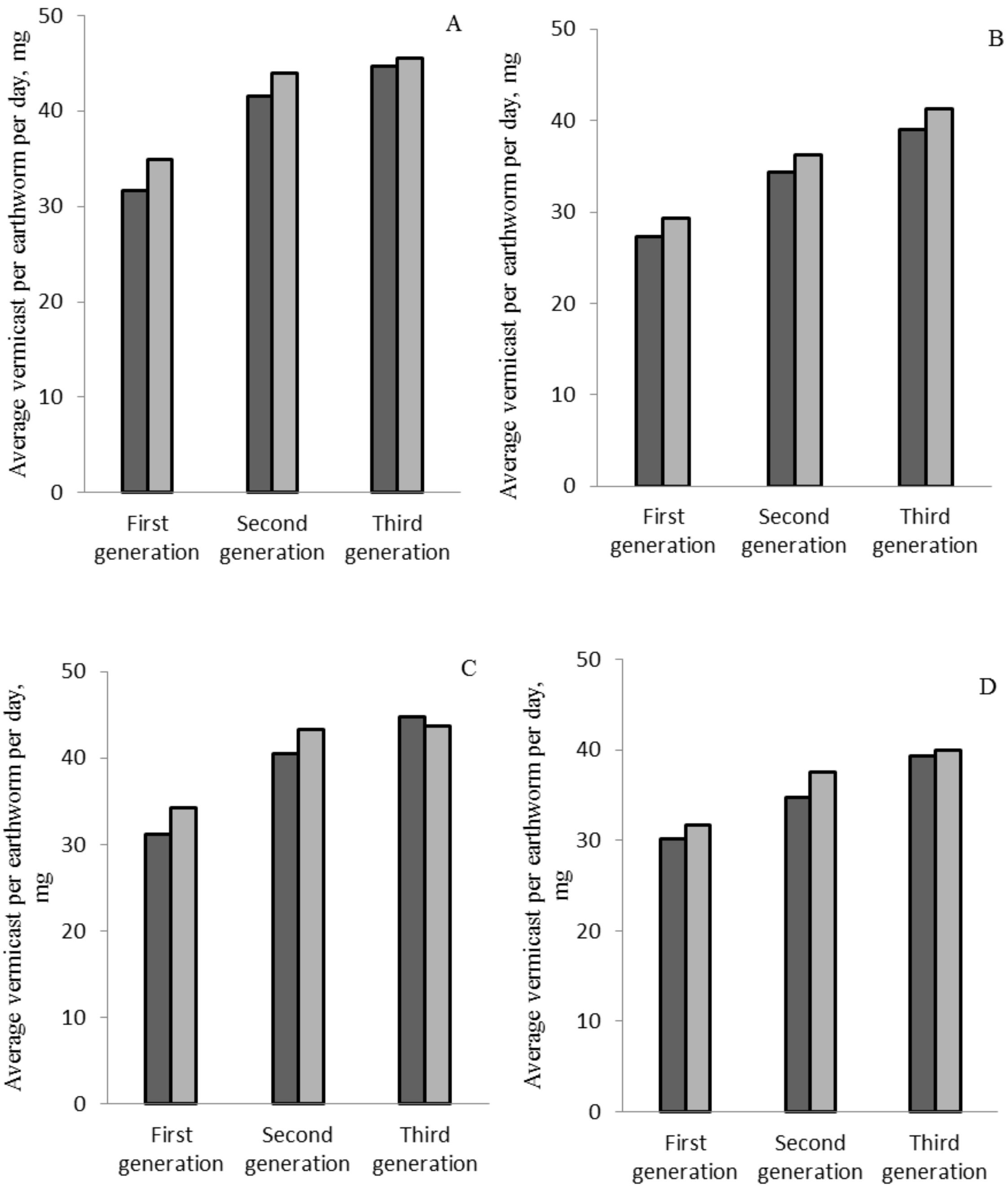

Figure 4.7: Average vermicast (per earthworm per day, mg) generated by A: *E.andrei*; B: *P.sansibaricus*; C: *L.rubillus*; D: *D.willsi* from ■ salvinia □ and ipomoea

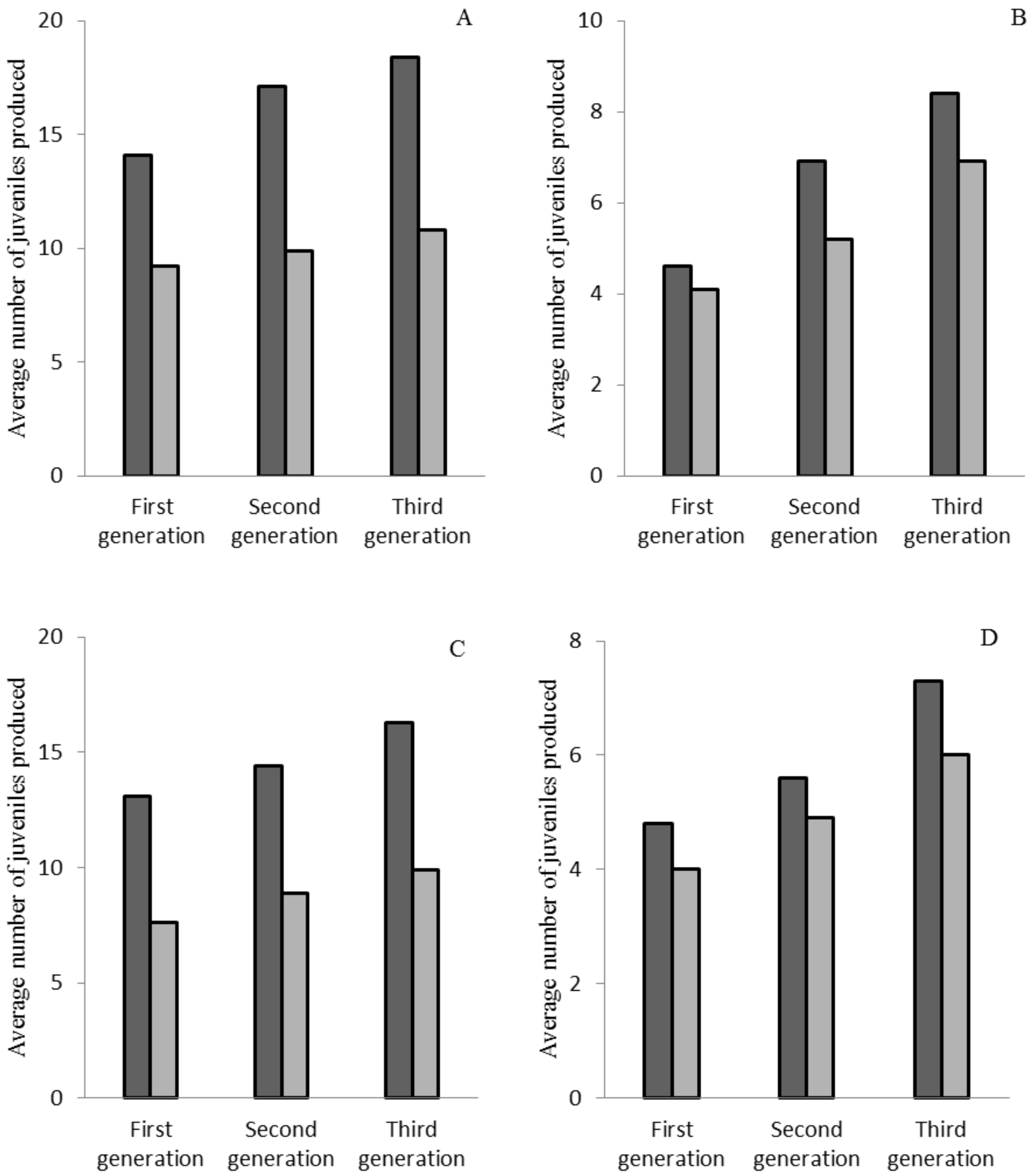

Figure 4.8: Average number of juveniles produced by A: *E.andrei*; B: *P. sansibaricus*; C: *L.rubillus*; D: *D.willsi* from ■ salvinia and □ ipomoea

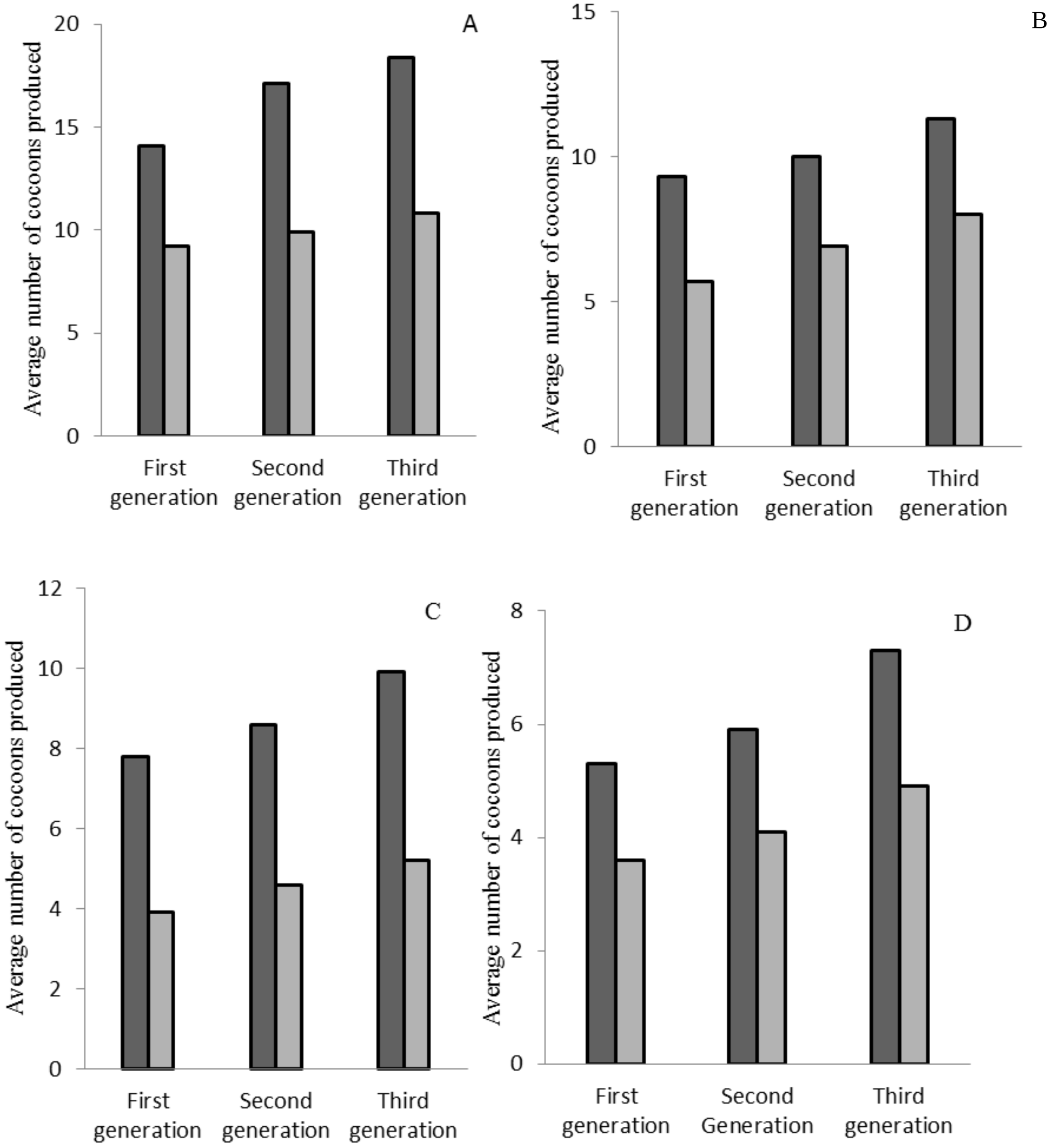

Figure 4.9: Average number of cocoons produced by A: *E.andrei*; B: *P.sansibaricus*; C: *L.rubillus*; D: *D.willsi* from ■ salvinia and □ ipomoea

4.4 Summary

The possibilities explored for generating vermicompost from salvinia, described in the previous Chapter were extended to ipomoea.

Four species of earthworm – (*E.andrei, P.sansibaricus, L.rubillus* and *D. willsi)* were explored for the direct vermicomposting of ipomoea. The succulent parts of the weed were utilized in 'high-rate vermireactors' without any pre-composting, manure supplementation or any other form of pre-treatment. All vermireactors were operated uninterruptedly for 160 days.

In the first series of experiments vermicomposting of ipomoea was studied with adult earthworms which had been born in cow-dung fed cultures and had grown to adulthood in them. The second series utilized earthworms born and raised in ipomoea-fed cultures. Their next generation was then used for the third series of experiments. These studies enabled us to see whether the second and the third generations display increasing adaptation to the ipomoea feed.

The studies revealed that:-

i) For each of the four species of earthworms studies by us, successive generations can be raised with ipomoea as the sole feed.

ii) The individuals of all the four species when grown on ipomoea as the sole feed were as healthy and reproductive as the ones grown on animal manure were.

iii) Successive generations got increasingly acclimatized to ipomoea and displayed increasing efficiency in vermicomposting ipomoea.

iv) The reproductive ability of all the four species in ipomoea-fed reactors increased as they produced their second and the third generation in it.

A comparison between the results of the experiments on vermicomposting of salvinia with the findings on ipomoea revealed an interesting trend. Even as the quantities of the two weeds converted to vermicompost per unit time by each adult earthworm of a given species were quite similar, the extent of reproduction achieved in ipomoea by all the four species was much lesser than that which was achieved in ipomoea. It indicated that either the adaption to ipomoea *vis a vis* reproduction is slow and may take several generations to peak, or that some chemicals in ipomoea suppress earthworm fecundity.

References

1. "Abbasi, S.A., Nayeem-Shah, M., Abbasi, T., 2014. Vermicomposting of phytomass: Limitations of the past approaches and the emerging directions, J. Clean. Prod. 93, 103-114

2. Abbasi, T., Gajalakshmi, S., Abbasi, S.A., 2009. Towards modeling and design of vermicomposting systems: mechanisms of composting/vermicomposting and their implications. Ind. J. Biotechnol. 8, 177-182.

3. Abbasi, T., Tauseef, S.M., Abbasi, S. A., 2011, Global Warming and The Role of Wetlands Lap Lambart Academic, Germany; ISBN 978-3846556009. xiv+264 pages.

4. Abbasi, S.A., Nayeem-Shah,M., Abbasi, T., 2015. Vermicomposting of phytomass: limitations of the past approaches and the emerging directions. J. Clean. Prod. 93, 103-114.

5. Tauseef, S. M., Abbasi, T., Banupriya, G., Banupriya, D., Abbasi S. A., 2013b. A new machine for clean and rapid separation of vermicast, earthworms and undigested substrate in vermicomposting systems, Comp. Sci. communicated.

6. Tauseef, S. M., Premalatha, M., Abbasi, T., Abbasi, S. A., 2013. Methane capture from livestock manure, J. Environ. Manag, 117, 187-207."

Chapter 5

Assessing the transformations that occur as salvinia is converted to its vermicompost

5.1 Introduction

In chapters 3, we had presented studies on the direct vermicomposting of salvinia using four species of earthworm: *Eisenia andrei, Perionyx sansibaricus, Lumbricus rubellus,* and *Drawida willsi*. It was followed by studies on the rate of vermicompost output and extent of reproduction achieved by the second and the third generation of earthworms ─ born and grown in weed-fed reactors ─ in comparison to the first generation animals which had been raised to adulthood on cowdung before being introduced into weed-fed vermireactors. All the earthworm species tested were highly successful in vermicomposting the salvinia biomass without any need of pre-composting, animal manure supplementation, or any other form of pre-treatment.

We now present studies carried out to see what transformations occur when salvinia is converted to vermicompost. It was also aimed to see whether the nature or the extent of the transformations are common across different earthworm species or very form species to species. The studies were supported by UV-visible spectrophotometry, Fourier-transform infrared (FTIR) spectrometry, thermogravimetry, differential scanning calorimetery, scanning electron microscopy.

5.2 Experimental

5.2.1 General

All chemicals were of analytical reagent grade, unless otherwise specified. Alkali-resistant borosilicate glassware and deionized, double distilled, water were employed throughout. Salvinia vermicomposts of each of the four earthworm species ─ *Eisenia andrei, Perionyx sansibaricus, Lumbricus rubellus,* and *Drawida willsi* ─ were generated as reported in Chapter 3.

5.2.2 C/N ratio

The samples were analyzed for carbon and nitrogen using auto-analyzer of vario EL cube model.

5.2.3 FTIR Spectrometry

Samples of salvinia and its vermicompost were oven dried, finely grounded, and then mixed with spectroscopic grade potassium bromide (KBr) powder. The mixtures were then thoroughly homogenized using a mortar and pestle, followed by pellet formation at a pressure of about 1MPa. The FTIR spectra were recorded over 4000 – 400 cm^{-1} at a frequency of 0.5 cm s^{-1} on a Nicolet iS50 FTIR spectrometer.

5.2.4 Thermal analysis

Thermo gravimetric analysis (TGA) and differential scanning calorimetry (DSC) were performed in a simultaneous thermal analyzer of model SDT Q600 V20.9 Build 20. Samples (50 mg each) were dried, manually grinded, and sieved to 0.2 mm size before being loaded in the TG. The thermogravity was explored in the temperature range 30 ^{o}C ─ 1,000 ^{o}C under nitrogen atmosphere at a heating rate of 10 °C/min, and a manometric pressure of 101 kPa.

For differential scanning calorimetry (DSC) oven dried samples (10-20 mg) were loaded in aluminium DSC pans in the nitrogen atmosphere. A temperature range of 30 ^{o}C ─

1,000 ^{o}C under a reduced nitrogen atmosphere at a temperature gradient of 5 °C/min was explored.

5.2.5 Scanning electron microscopy

Samples were sputtered with gold followed by recording the surface morphology using Hitachi, S-3400N electron microscope.

5.3 Results and discussion

The C:N ratio of the salvinia biomass and its vermicomposts are represented in Figure 5.1. In comparison to salvinia, which has a C:N ratio of 42.2, its vermicomposts had almost 3 times lesser CN ratio, in the range 14-14.8. The extent of reduction in the C:N ratio followed the order *P.sansibaricus* (14) > *D.willsi* (14.2) > *E.andrei* (14.3) > *L.rubillus* (14.8). But the very narrow range within which the C:N ratios of the four species agree with each other indicates that all the four earthworm species achieved almost similar success in drastically reducing the C:N ratio of salvinia.

A C:N ratio below 20 is considered an indicator of stabilized compost and is recommended for application in different soils, while a C:N ratio less than 15 is deemed 'highly desirable' for agronomic purposes (Deka *et al*., 2011; Hussain *et al*., 2016 a). The drastic, nearly 3-fold reduction in the C:N ratio of salvinia in the course of its vermicomposting reflects a very high degree of stabilization and indicates the suitability of the vermicompost as a nitrogen-rich organic manure (Hussain *et al*., 2016 b).

The fall of C:N ratio is primarily due to the loss of the organic carbon contained in salvinia through earthworms mediated aerobic biodegradation. The microganisms and earthworms convert the organic carbon partly into their biomass and the rest into CO_2, which then gets exhaled. There are also, possibly less pronounced, contribution to the fall in the CN ratio due to the addition of nitrogen-rich earthworm mucus into the vermicast (Ravindran *et al*., 2013).

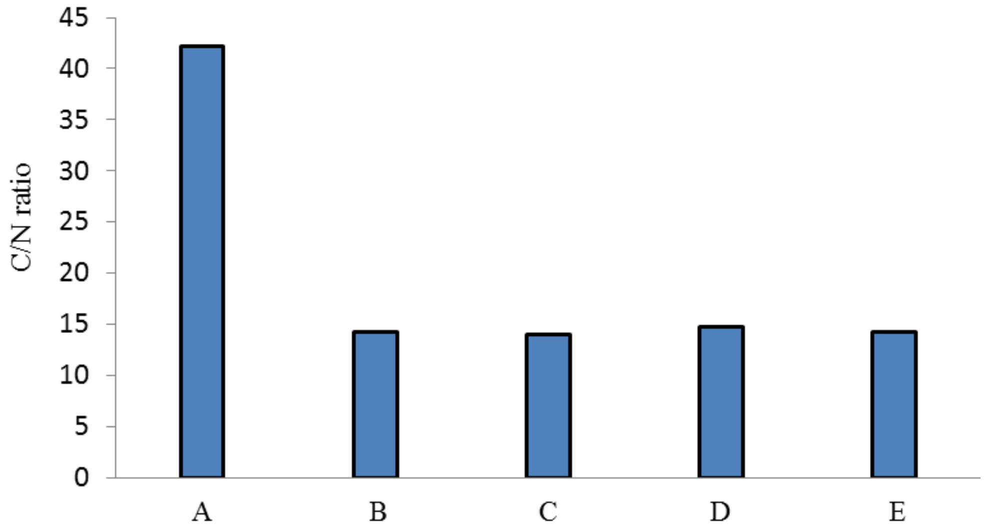

Figure 5.1: C: N ratios of A: salvinia plant, and of the vermicomposts derived from B: *E.andrei* C: *P.sansibaricus*; D: *L.rubillus* and E: *D.willsi*

5.3.1 FTIR Spectrometry

The FTIR spectrum of salvinia is presented in Figure 5.2. There is broad band between 3000 and 3500 cm^{-1}, depicting the strong O-H bond, due to the presence of organic acids, phenols and alcohols present in salvinia (Hussain *et al*., 2015, 2016). Next to it there is a peak at 2921 cm^{-1} due to aliphatic C-H stretching which is assigned to fatty acids and lipids (Xu *et al*., 2012; Teh *et al*., 2014), followed by a peak at 1738 cm^{-1} due to –COOH stretch of carboxylic acid and stretching vibrations of esters in the pectin of ligninous origin (Grube *et al*., 2006). The next prominent peak is at 1634 cm^{-1} caused by aromatic C=C vibrations (Mochochoko *et al*., 2013). Further down there is a peak around 1520 cm^{-1}, due to skeletal vibrations of the cellulosic or lignocellulosic materials (Bykov, 2008; Boeriu *et al*., 2004; Klein *et al*., 2010; Jun *et al*., 2014). It has been reported that salvinia contains exceptionally high phenol levels which makes it allelopathic, while the lignin content make it hardy and difficult to degrade (Hussain *et al*., 2016; 2017).

The peaks at 1445 and 1249 cm^{-1} may be due to the -OCH_3 stretching of lignin and C-O stretching of phenols (Hussain *et al*., 2015).Further up, a peak at 1021 cm^{-1} may be corresponding to the C-O stretch of polysaccharides, cellulose or hemicellulose (Ravindran *et al*., 2013).

In comparison the vermicompost of salvinia derived from all the four species of earthworms has remarkable changes in their FTIR spectra (Figures 5.3-5.6). The peaks in the 3100-3600 cm^{-1} range show a significant reduction in the phenolic and alcoholic content as salvinia undergoes vermicomposting, thereby reducing the chemicals that were responsible for the allelopathy of salvinia. In comparison to salvinia which had 92.5 % transmittance at the 3419 cm^{-1} peak, the vermicasts of the four species of earthworms had transmittance in 96 ─ 97% range at 3391 ─ 3417cm^{-1}. The reduction in the peak followed the order *D.willsi* > *L.rubillus* > *P.sansibaricus* > *E.andrei* but, given the very close range within which the transmittance varied, it can be said that all the four species were able to degrade phenols to a similar extent. The peaks at 2900-2800 cm^{-1} range are much shallower, while the peak at 1738 cm^{-1} was absent in all the vermicomposts, depicting the degradation of aliphatic compounds and the lignin content of salvinia.

There is a shift in the peaks at 1634 cm^{-1} for all the vermicomposts towards higher frequencies, with increasing intensity as well. In comparison to salvinia which had 95 % transmittance at the 1634 cm^{-1} peak, the vermicasts of the four species of earthworms had 91 ─ 95.5 % transmittance in the 1648 ─ 1655 cm^{-1} range. The increase in the intensity of the peak followed the order *P.sansibaricus*> *E.andrei*> *L.rubillus* > *D.willsi*. This may be due to the transition of the polymer structure from crystalline to amorphous and breakdown of lignin in the course of vermicomposting (Hussain *et al*., 2017). Barring the vermicompost derived from *D. willsi* there is a sharp peak emerging around 1385 cm^{-1} in the FTIR of vermicomposts of the other three species, which may be due to the N-O stretching, indicating an enhancement in the nitrogenous compounds present in the vermicompost. Whereas vermicomposting has evidently caused degradation of lignocellulose, lignin, and carbohydrates contained in salvinia, there is formation of polysaccharides as indicated by an increase in the intensities of the peaks in the 1000–1100 cm^{-1} range and shifting of the peaks towards higher frequencies. In comparison to salvinia which had 95 % transmittance at the 1021 cm^{-1} peak, the vermicasts of the four species of earthworms had transmittance in 87.5 ─ 95% range at 1033 ─ 1035 cm^{-1}.The intensity of the peak increased in the order *P.sansibaricus*> *L.rubillus*> *E.andrei* > *D.willsi*.

From the forgoing it can be seen that all the species of earthworm caused a degradation of chemicals in salvinia that are responsible for its antagonistic nature, and introduced plant and soil friendly features to it. The extent of this impact across the four species was similar in some of the aspects and varied in some other.

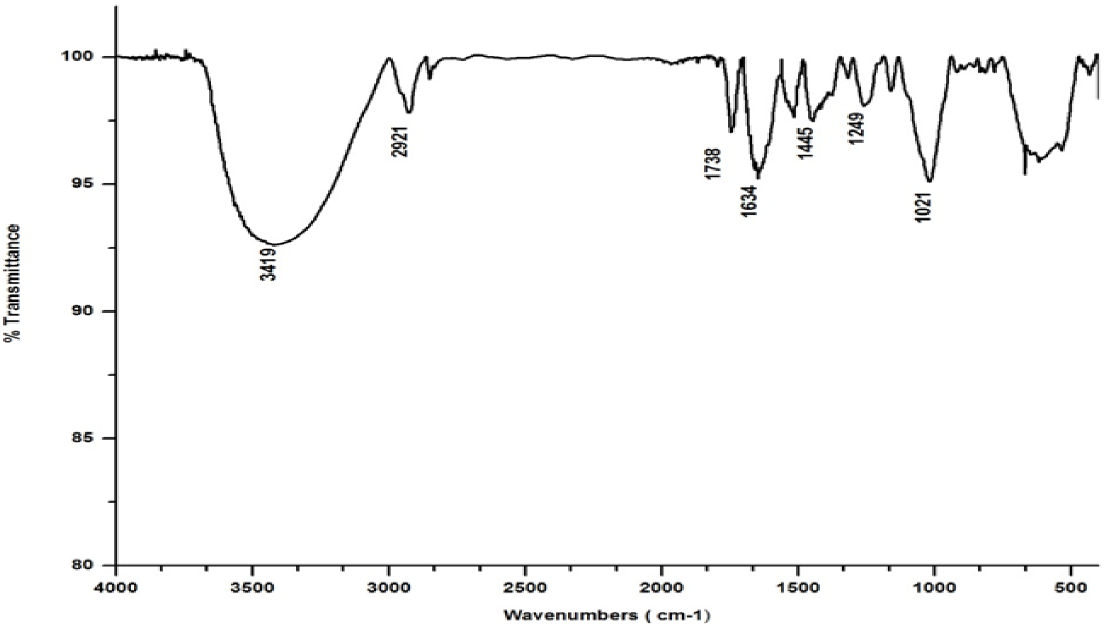

Figure 5.2: FT-IR spectra of salvinia leaves

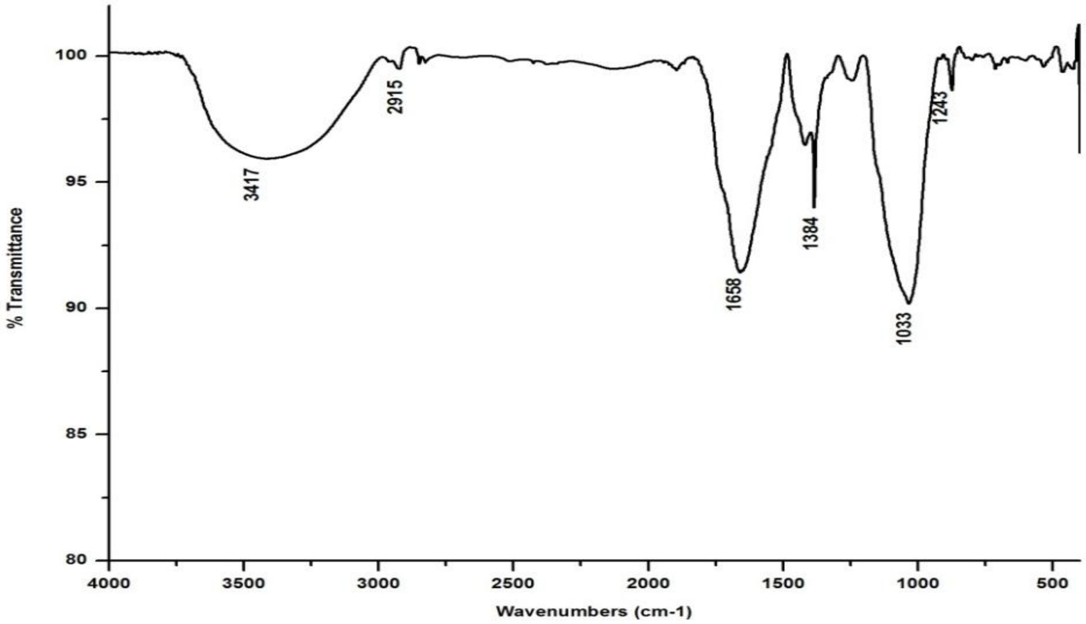

Figure 5.3: FT-IR spectra of vermicompost generated by *E.andrei*

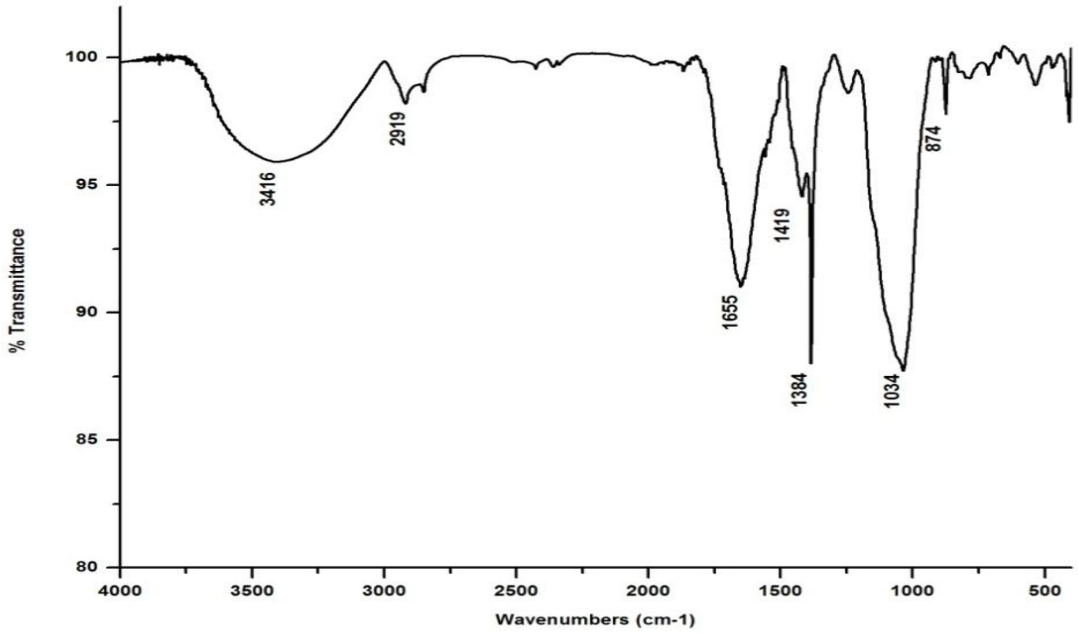

Figure 5.4: FT-IR spectra of vermicompost generated by *P.sansibaricus*

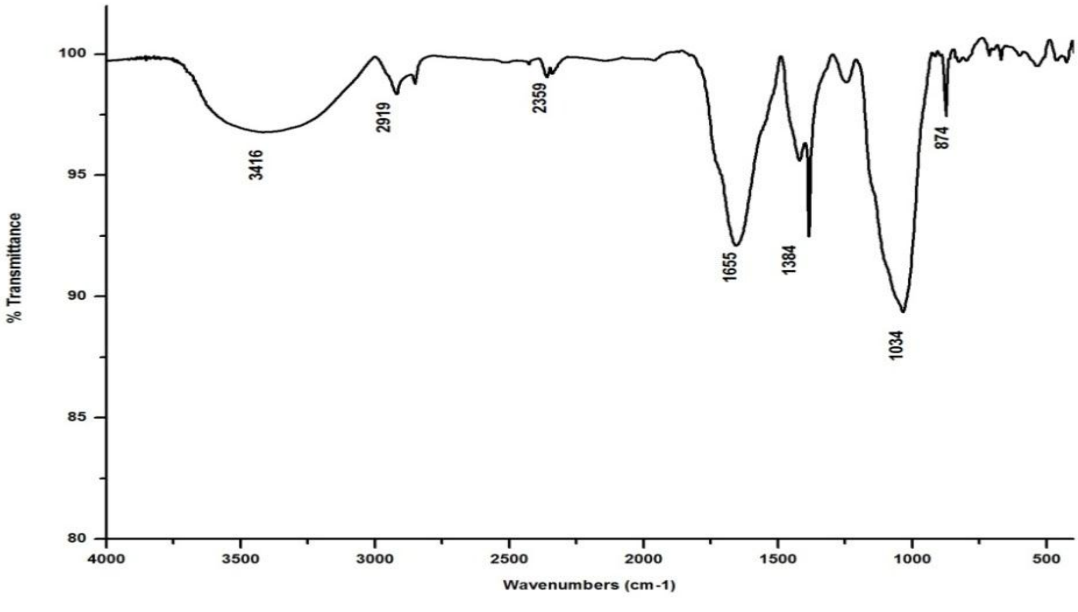

Figure 5.5: FT-IR spectra of vermicompost generated by *L.rubillus*

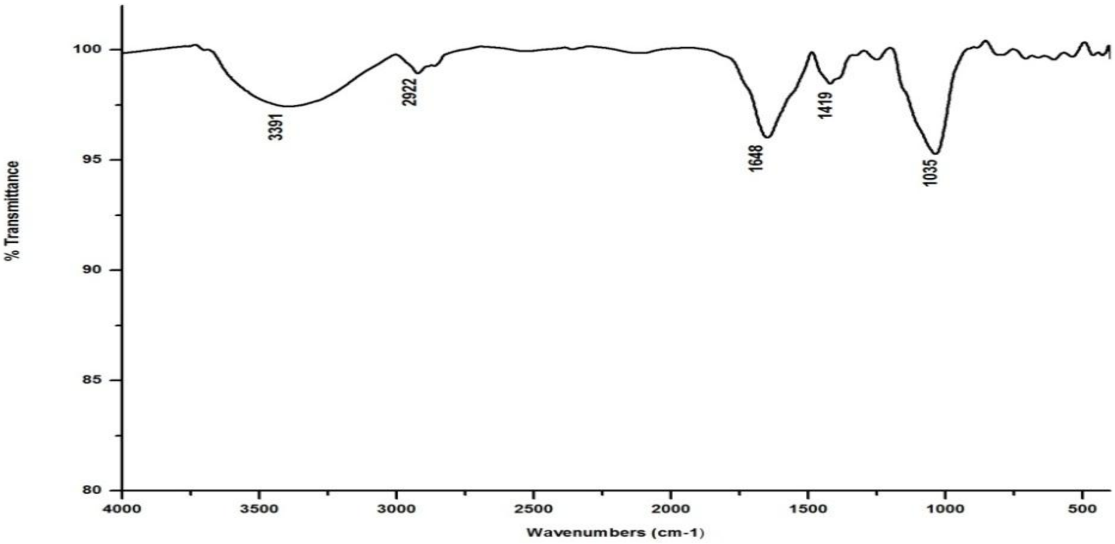

Figure 5.6: FT-IR spectra of vermicompost generated by *D.willsi*

5.3.2 Thermogravimetric analysis

Thermogravimetric (TG) curves of salvinia and its vermicomposts are presented in figures 5.7 -5.11. For salvinia, a mass loss of 93.1 % was recorded, while for the vermicomposts of *E. andrei*, *P. sansibaricus*, *L. rubillus* and *D. willsi* the mass loss was 72 %, 70 %, 71.5 % and 72.3 %, respectively. The TG curves showed that dehydration occurred during 60-150°C and decomposition occurred during 200-800°C in both salvinia and its vermicomposts. But in the vermicomposts the extent of mass loss was lesser than that which occurred in the parent substrate. This is reflective of the mineralization of the organic matter that had occurred as a result of vermicomposting (Deka *et al.*, 2011; Ravindran *et al.*, 2013; Hussain *et al.*, 2016).

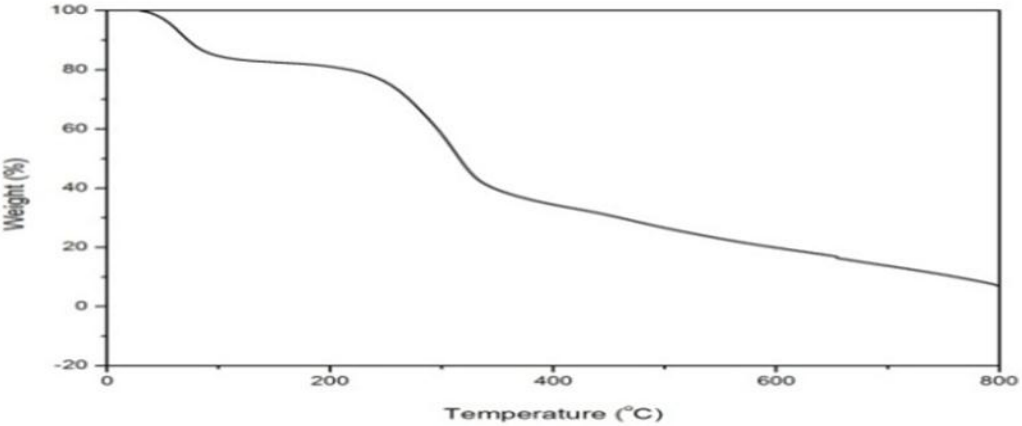

Figure 5.7: TG curve of salvinia plant

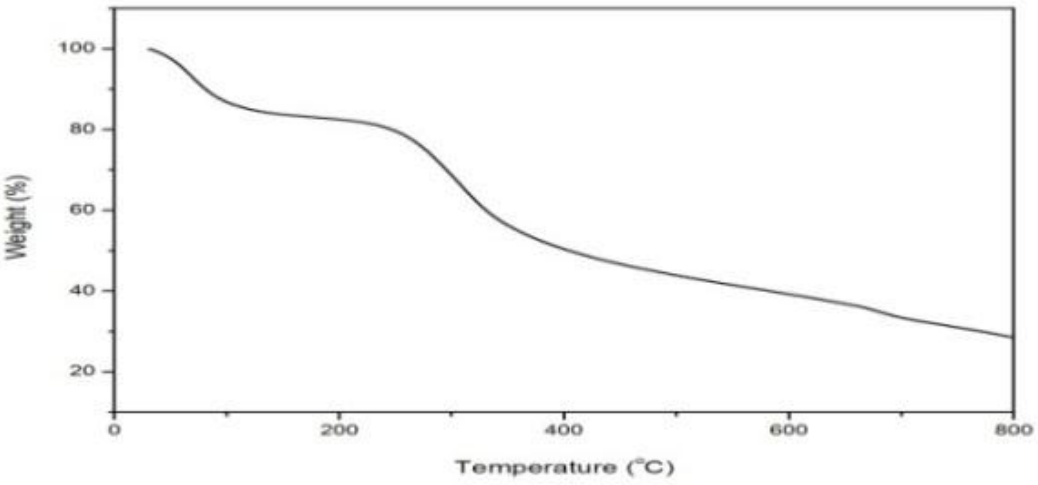

Figure 5.8: TG curve of vermicompost generated by *E.andrei*

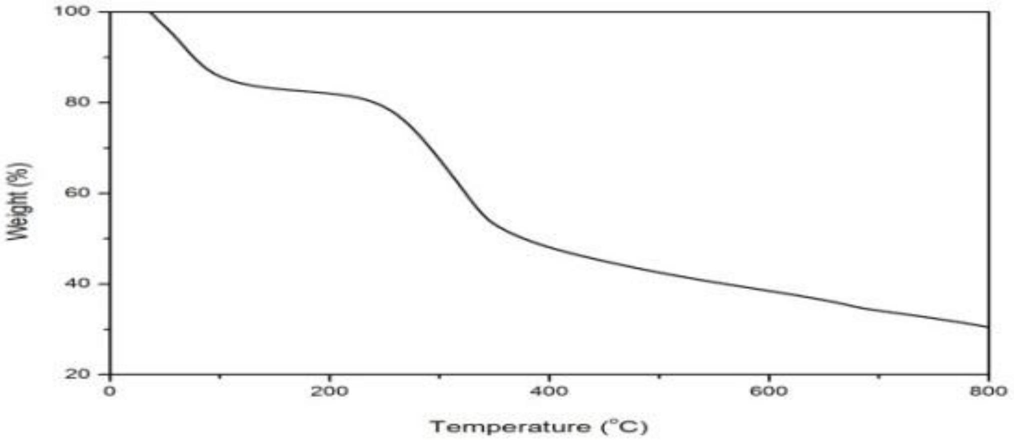

Figure 5.9: TG curve of vermicompost generated by *P.sansibaricus*

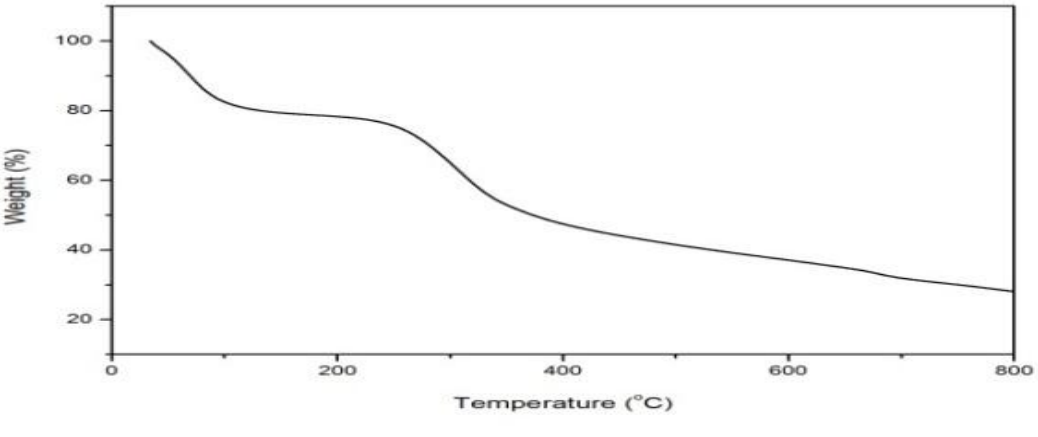

Figure 5.10: TG curve of vermicompost generated by *L.rubillus*

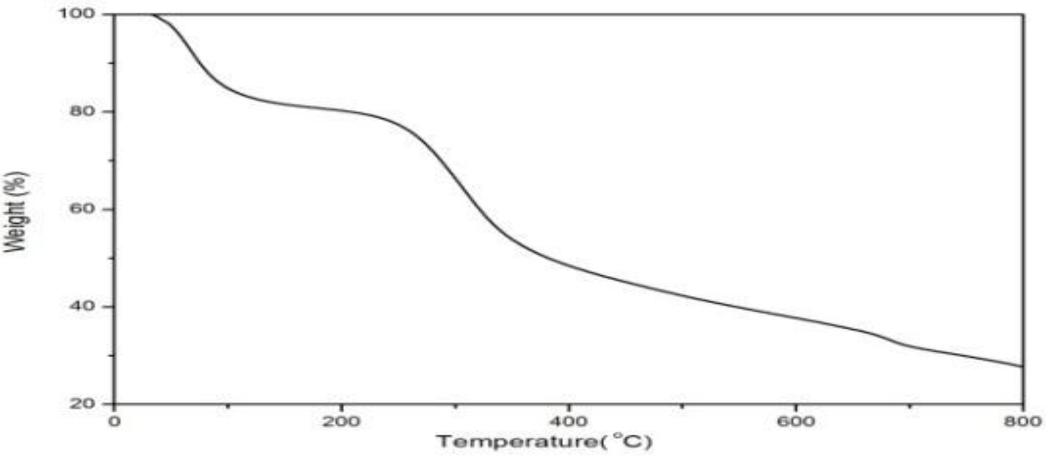

Figure 5.11: TG curve of vermicompost generated by *D.willsi*

5.3.3 Differential scanning calorimetry (DSC)

The DSC curve of salvinia has two exothermic peaks (figure 5.12). The first of these peaks occurs in the 300-350 ^{o}C range and the second one at 450-500 ^{o}C. The first peak may be due to the presence of carbohydrates and cellulose while second peak may be due to lignin and phenols.

In contrast both the exothermic peaks in the vermicomposts are seen to be shallower (Figures 5.13-5.16), The reduction in the exothermic peak follows the trend *E.andrei* > *P. sansibaricus* > *L. rubillus* > *D. willsi*. The results indicate that in the course of salvinia's vermicomposting there is a breakdown of simple carbohydrates, aliphatic compounds and aromatic compounds like lignin and phenols present in it (Fernandez *et al*., 2012; Ravindran *et al*., 2013; ElOuaqoudi *et al.*, 2014; Hussain *et al.*, 2016).

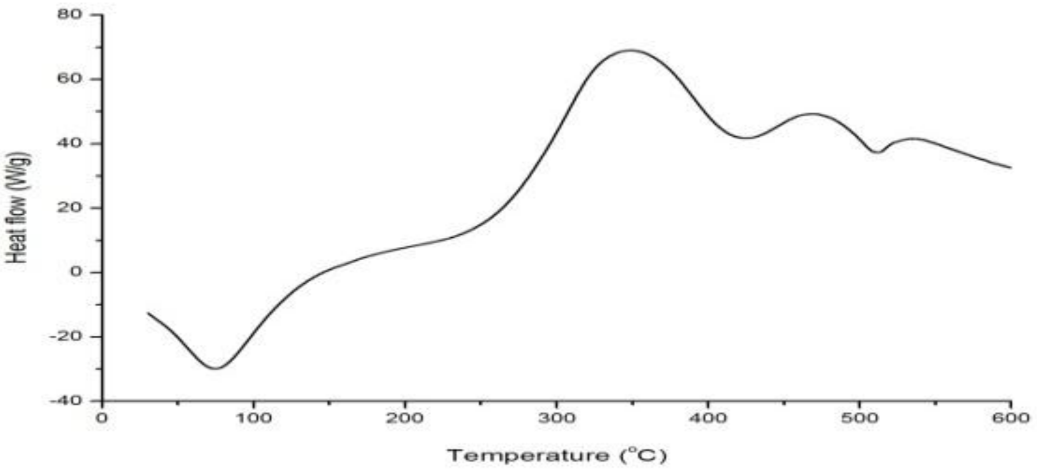

Figure 5.12: DSC curve of salvinia leaves

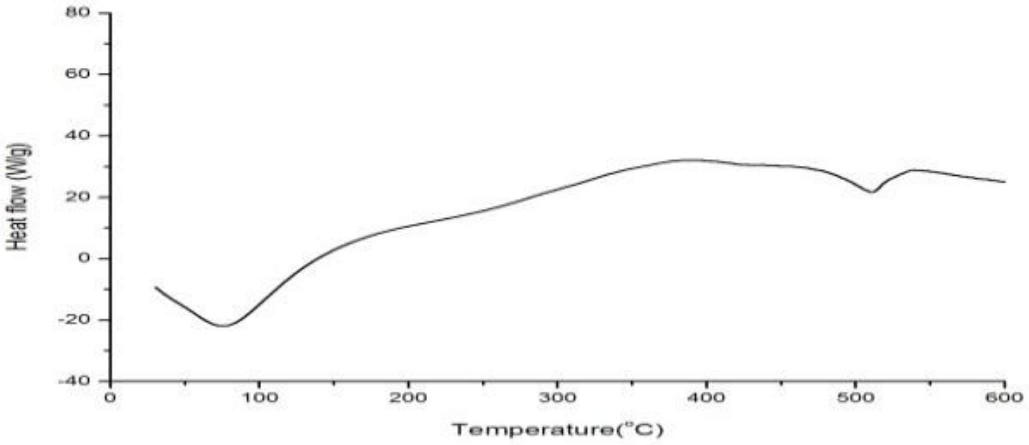

Figure 5.13: DSC curve of vermicompost generated by *E.andrei*

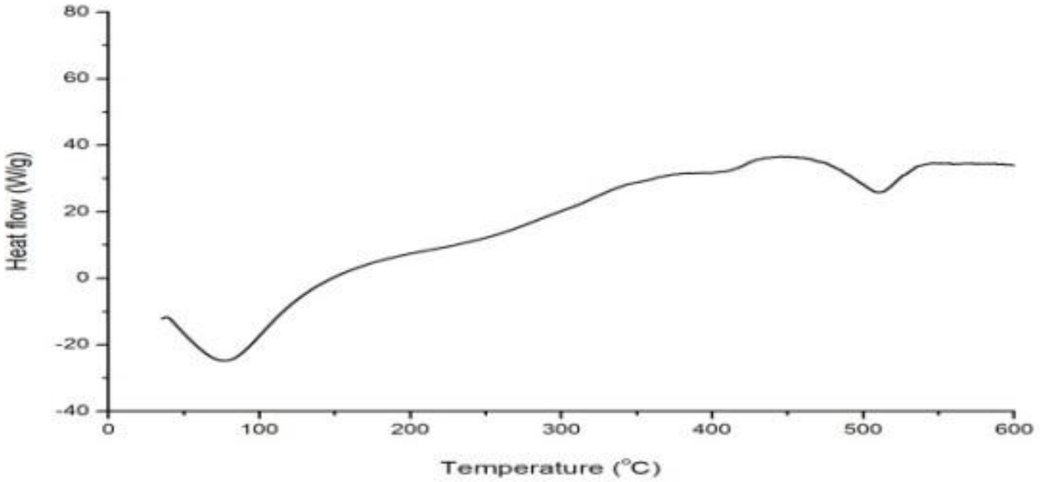

Figure 5.14: DSC curve of vermicompost generated by *P.sansibaricus*

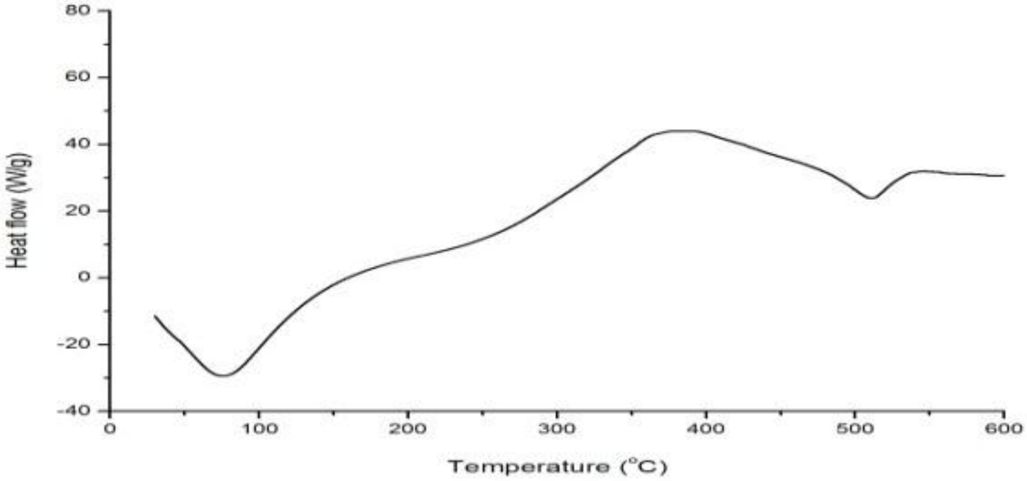

Figure 5.15: DSC curve of vermicompost generated by *L.rubillus*

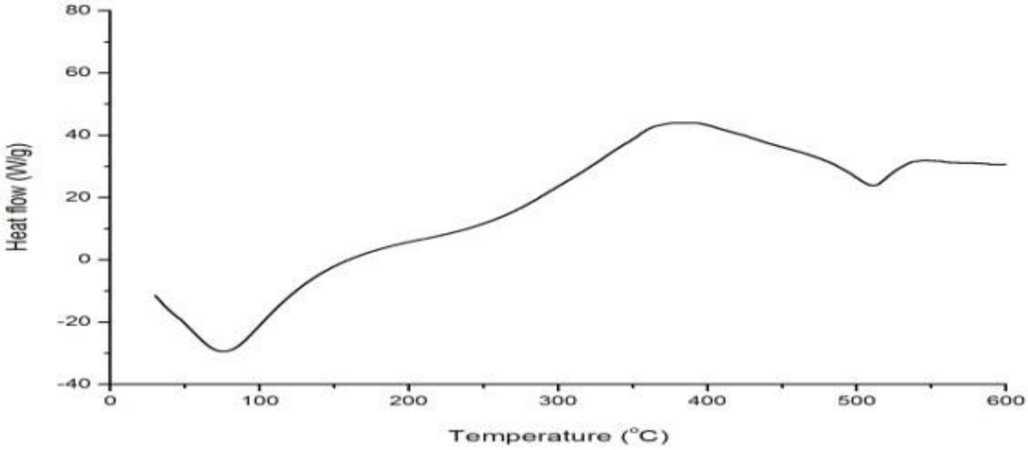

Figure 5.16: DSC curve of vermicompost generated by *D.willsi*

5.3.4 Scanning electron microscopy

Scanning electron micrographs of salvinia biomass indicate its robust and relatively contiguous structures — seemingly bound to lignin-containing fibers (5.16). In contrast, the micrographs of vermicomposts derived from all the four species of earthworms reflect disaggregated and withered material (Figures 5.17- 5.21).

When earthworms feed on the salvinia biomass, they grind the latter with their gizzard, (Ali *et al.,* 2015; Hussain *et al*., 2016c); there is further disaggregation as the microbial fauna acts on the substrate during the course of its passage through the earthworm gut (Atiyeh *et al.*, 2000; Edward *et al*., 2011; Hussain *et al*., 2016d). This facilitates progressive degradation of the salvinia biomass which is reflected in the SEM micrographs.

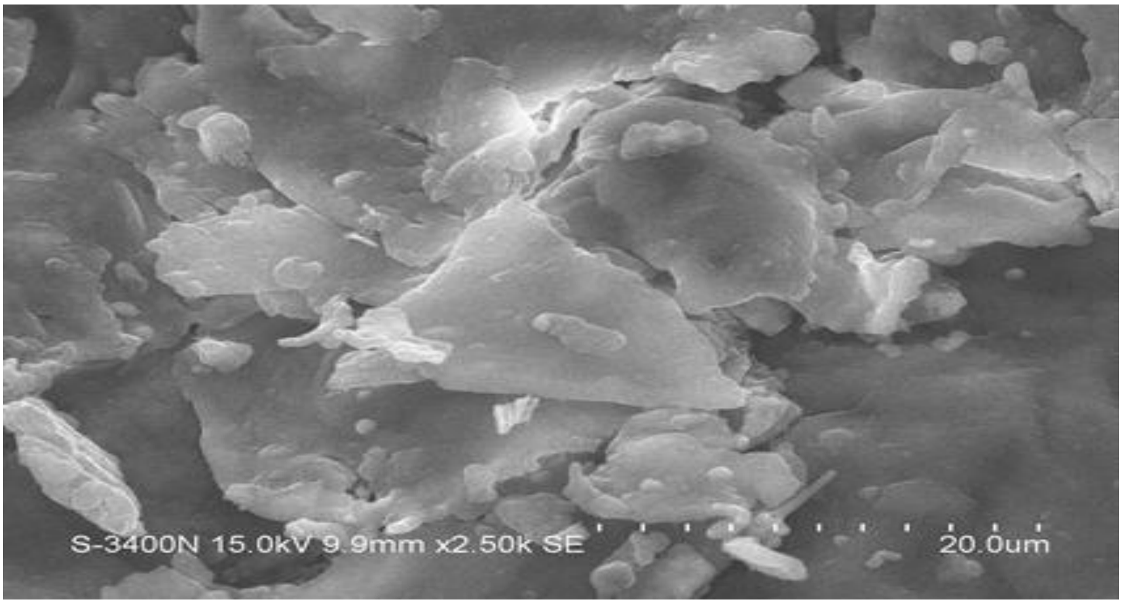

Figure 5.17: SEM images of salvinia leaves

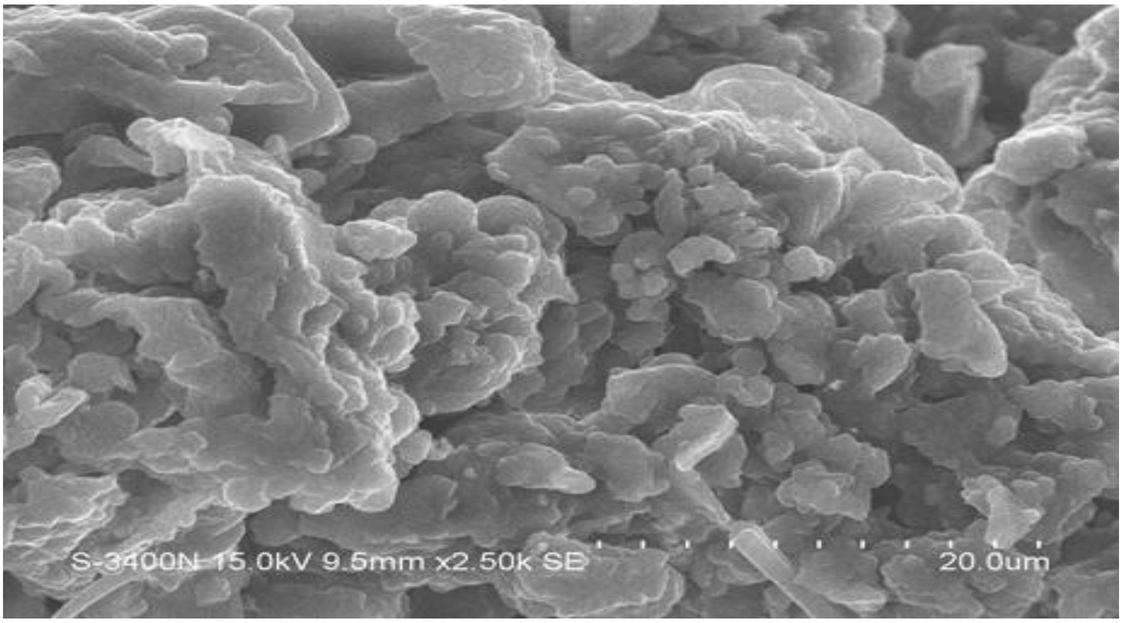

Figure 5.18: SEM images of vermicompost generated by *E.andrei*

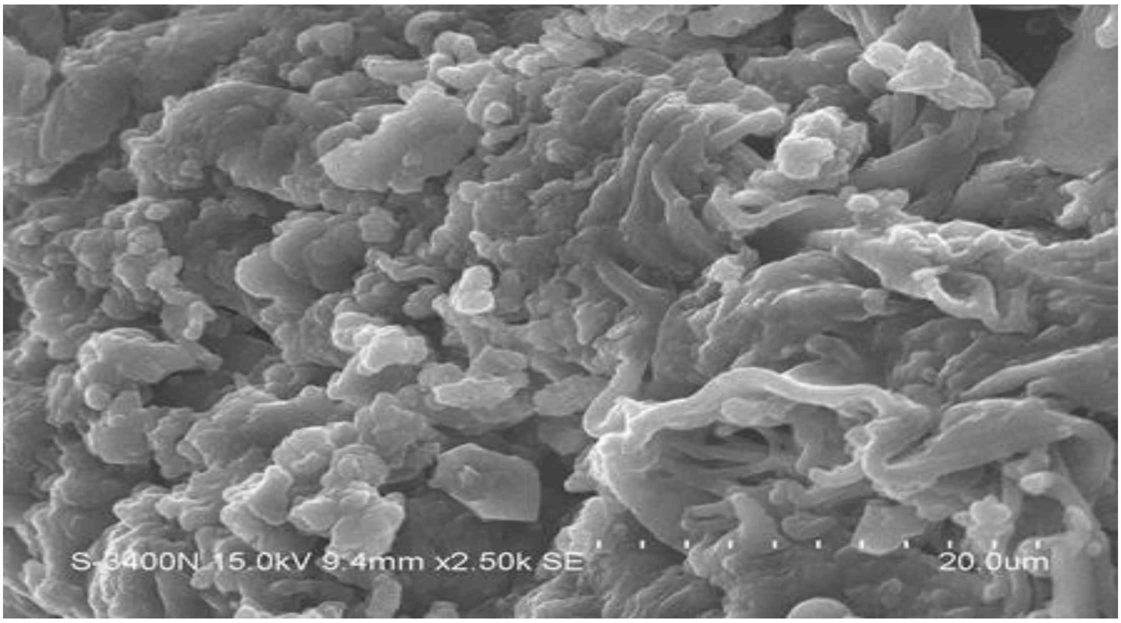

Figure 5.19: SEM images of vermicompost generated by *P.sansibaricus*

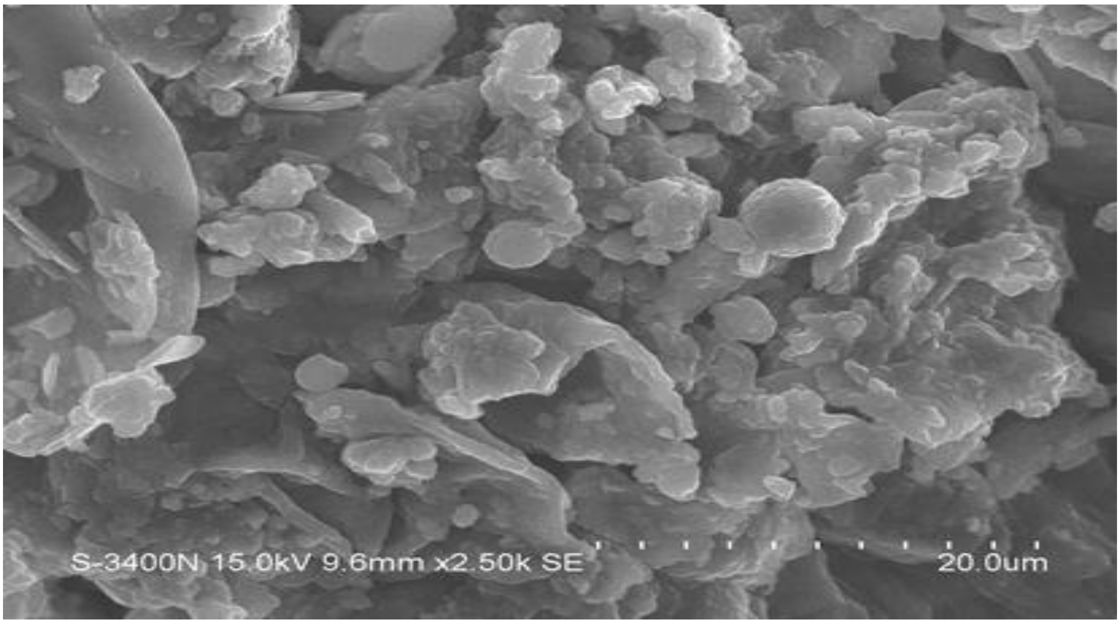

Figure 5.20: SEM images of vermicompost generated by *L.rubillus*

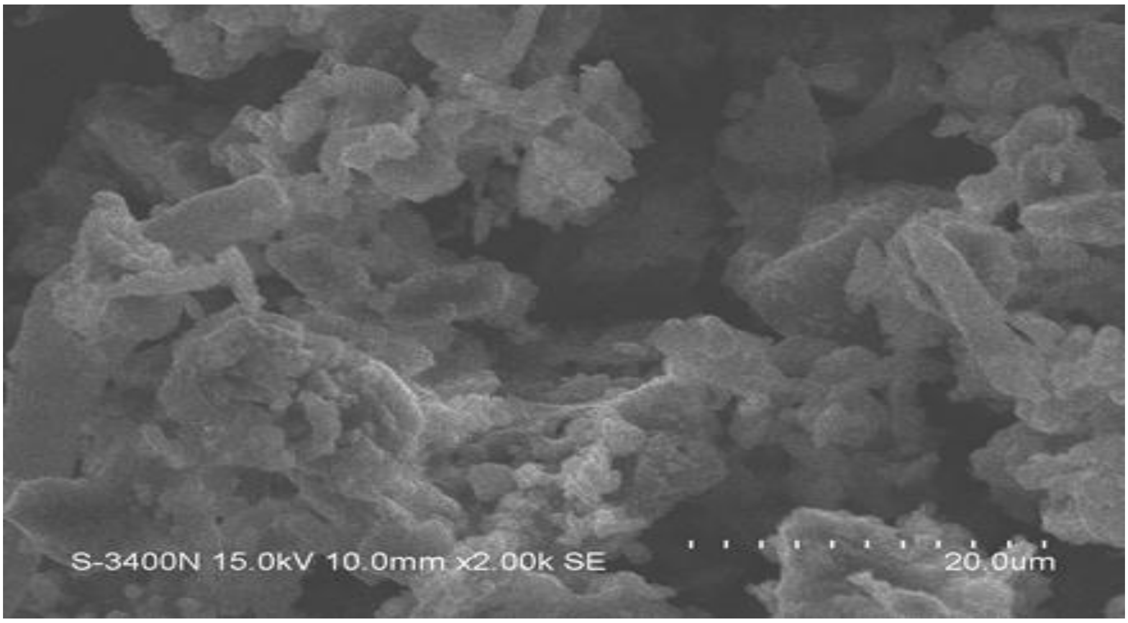

Figure 5.21: SEM images of vermicompost generated by *D.willsi*

5.4 Summary

This chapter has presented studies which were carried out to see what transformations occur when salvinia is converted to vermicompost. It was also aimed to see whether the nature or the extent of the transformations are common across different earthworm species or very form species to species. The studies were supported by UV-visible spectrophotometry, Fourier-transform infrared (FTIR) spectrometry, thermogravimetry, differential scanning calorimetery, and scanning electron microscopy.

It was seen that the C:N ratio of the salvinia vermicomposts derived from four species of earthworms was lesser by almost 3 orders of magnitude than the C:N ratio of salvinia. Further, vermicomposting had caused a reduction in the phenol and lignin content of salvinia. TGA and DSC indicated net mineralization when salvinia was turned into vermicompost as well as breakdown of simpler compounds like carbohydrates and complex aromatic compounds like lignin. SEM micrographs confirmed that the parent substrate is extensively fragmented and withered in the course of its vermicomposting.

References

1. “Ali Usman, Nida Sajid, Azeem Khalid, Luqman Riaz, Muhammad Muaz Rabbani, Jabir Hussain Syed, and Riffat Naseem Malik., 2015. A review on vermicomposting of organic wastes, Environ. Prog. Sustain. Energy, 34, 1050-1062
2. Atiyeh, R. M., Arancon, N., Edwards, C. A. and Metzger, J. D., 2000. Influence of earthworm-processed pig manure on the growth and yield of greenhouse tomatoes, Bioresour. Technol, 75, 175-180.

3. Boeriu, C.,Bravo, D.,Gosselink, R .,van Dam, J., 2004. Characterization of structure-dependent functional properties of lignin with infrared spectroscopy Ind. Crops Prod., 20 205-218

4. Bykov, Ivan. 2008. Characterization of natural and technical lignins using FRIR spectroscopy Master thesis, Lulea University of technol.,

5. Deka,H., Deka,S., Baruah,C.K., Das, J,. Hoque, S., Sanna, N.S., 2011.Vermicomposting of distillation waste of citronella plant (*Cymbopogon winterianus* Jowitt.) employing *Eudrilus eugeniae.* Bioresour. Tecbnol., 102 , 6944-6950

6. Edwards, C. A., Norman, Q. A., Sherman, R., 2011. Vermiculture Technology, Earthworms, Organic Waste and Environmental Management, CRC press, 17-19.

7. El Ouaqoudi, F.Z., El Fels, L., Winterton, P., Lemée, L., Amblès, A., Hafidi, M., 2014. Study of humic acids during composting of lingocellulose waste by infrared spectroscopic and thermogravimetric/thermal differential analysis. Compost Sci. Util. 22, 188–198.

8. Fernandez, J. M., Plaza, C., Polo, A., Plante, A.F., 2012. Use of thermal analysis techniques (TG-DSC) for the characterization of diverse organic municipal waste streams to predict biological stability prior to land application, Waste Manage, 32, 158–164.

9. Grubea, T.M., Linb, J.G., Leeb, P.H., Kokorevicha,S.,2006. Evaluation of sewage sludge-based compost by FT-IR spectroscopy. Geodenma 130,324-333

10. Hussain, N., Abbasi, T., Abbasi, S. A., 2016a. Vermiremediation of an invasive and pernicious weed salvinia (*Salvinia molesta*). Ecol. Eng, 91, 432-440.

11. Hussain, N., Abbasi, T., Abbasi, S. A., 2017. Toxic and allelopathic ipomoea yields plant-friendly organic fertilizer. J. Clean. Prod, 148, 826-835.

12. Hussain, N., Abbasi, T., Abbasi, S. A., 2016c.Vermicomposting-mediated conversion of the toxic and allelopathic weed ipomoea into a potent fertilizer, Process Saf. Environ. Prot, 103, 97-106.

13. Hussain, N., Abbasi, T., Abbasi, S.A., 2016b. Transformation of toxic and allelopathic lantana into a benign organic fertilizer through vermicomposting. Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy, 163,162-169.

14. Hussain, N., Abbasi, T., Abbasi, S.A., 2016d. Transformation of the pernicious and toxic weed parthenium into an organic fertilizer by vermicomposting. Int. J. Environ.Studies, 73 (5), 731-745.

15. Hussain, N., Abbasi, T.,and Abbasi, S. A., 2015. Vermicomposting eliminates the toxicity of Lantana (*Lantana camara*) and turns it into a plant friendly organic fertilizer, J. of Hazard. Mater.298, 46-57.

16. Jun, Hu, Shen, Dekui, Wu, Shiliang, Zhang, Huiyan, Xiao, Rui., 2014. Effect of temperature on structure evolution in char from hydrothermal degradation of lignin. J. Anal. Appl. Pyrolysis, 106, 118-124

17. Klein, A.P., Beach, E.S., Emerson, J.W., Zimmerman J.B., 2010. Accelerated solvent extraction of lignin from *Aleurites moluccana* (Candlenut) nutshells J. Agric. Food Chem. 58 , 10045-10048

18. Mochochoko, T., Oluwafemi, O.S.,Jumbam, D.N., Songca, S.P., 2013. Green synthesis of silver nanoparticles using cellulose extracted from an aquatic weed; Water hyacinth Carbohydr. Polym, 98, 290-294

19. Ravindran, B.,Sravani, R., Mandal, A.B., Contreras-Ramos, S.M.,Sekaran G.,2013. Instrumental evidence for biodegradation of tannery waste during vermicomposting process using *Eudrilus eugeniae* .J. Therm. Anal. Calorim., 111 , 1675-1684

20. Teh, C.Y., Wu, T.Y., Juan. J.C., 2014. Potential use of rice starch in coagulation-flocculation process of agro-industrial wastewater: treatment performance and flocs characterization .Ecol. Eng., 71, 509-519

21. Xu, Rensheng, Ye, Yang Zhao, Weimin., 2012. Introduction to Natural Products Chemistry CRC press, Washington, New York.”

Chapter 6

Assessing the transformations that occur as ipomoea is converted to its vermicompost

6.1 Introduction

In chapters 4, we had presented studies on the direct vermicomposting of ipomoea using four species of earthworm: *Eisenia andrei, Perionyx sansibaricus, Lumbricus rubellus,* and *Drawida willsi*. It was followed by studies on the rate of vermicompost output and extent of reproduction achieved by the second and third generation of earthworm ─born and raised to adulthood on cowdung before being introduced into weed- fed vermireactors. The earthworm species tested were highly successful in vermicomposting ipomoea without any need of pre- composting, animal manure supplementation, or any other form of pre-treatment.

We now present studies carried out to see what transformations occur when ipomoea is converted to vermicompost. It was also aimed to see whether the nature or the extent of the transformations are common across different earthworm species or very form species to species. The studies were supported by UV-visible and Fourier-transform infrared spectrometry, thermogravimetric and differential scanning calorimetry, and scanning electron microscopy.

6.2 Experimental

The methodology followed in this study is essentially same as reported in chapter 5. In essence the characteristics of the substrate (ipomoea) and of its vermicomposts generated from the four earthworm species were studied. FTIR spectra were recorded using Nicolet iS50 FT-IR spectrometer, Thermo gravimetric analysis (TGA) and differential scanning calorimetry (DSC) were performed with SDT Q600 V20.9 Build 20 model. The scanning electron micrographic (SEM) images were obtained with a Hitachi S-3400N scanning electron microscope. Carbon and nitrogen analysis were performed with auto-analyzer of vario EL Cube model.

6.3 Results and discussion

The C: N ratio of the ipomoea and its vermicomposts are represented in figure 6.1. In comparison to ipomoea, which has a C:N ratio of 28.2, its vermicomposts had almost 2 times lesser C:N ratio, in the range 15.2 – 16.7. The extent of reduction in the C:N ratio followed the order *E.andrei* (15.2) > *L.rubillus* (15.7) >, *P.sansibaricus* (15.8) > *D.willsi* (16.7). But the very narrow range within which the C:N ratios of the four species agree with each other indicates that all the four earthworm species achieved almost similar success in drastically reducing the C:N ratio of ipomoea. These findings are similar to the ones achieved in case of salvinia (Chapter 5). Interestingly salvinia had a much higher C:N ratio (42.2) than ipomoea (28.2) but upon vermicomposting both were reduced to similar levels ─ 14 to 14.8 in case of salvinia vermicompost and 15.2 to 16.7 in case of ipomoea vermicompost.

As stated in Section 5.3, a C: N ratio below 20 is considered an indicator of stabilized compost and is recommended for application in different soils, while a C: N ratio less than 15 is deemed ‘highly desirable’ for agronomic purposes (Deka *et al.*, 2011; Hussain *et al.*, 2016a). The drastic reduction in the C: N ratio of ipomoea to a level close to 15 in the course of its vermicomposting reflects a very high degree of stabilization occurring in the process of vermicomposting and indicates the suitability of the vermicompost as a nitrogen-rich organic manure (Hussain *et al.*, 2016 b).

The primary reasons for the precipitous fall in the C: N ratio of ipomoea is the loss of the organic carbon, contained in ipomoea, that occurs through earthworm−mediated aerobic biodegradation. The microorganisms and earthworms convert the organic carbon partly into their biomass and the rest into CO_2, which then gets exhaled. There is also, possibly less pronounced, contribution to the fall in the C: N ratio due to the addition of nitrogen-rich earthworm mucus into the vermicast (Ravindran *et al.*, 2013).

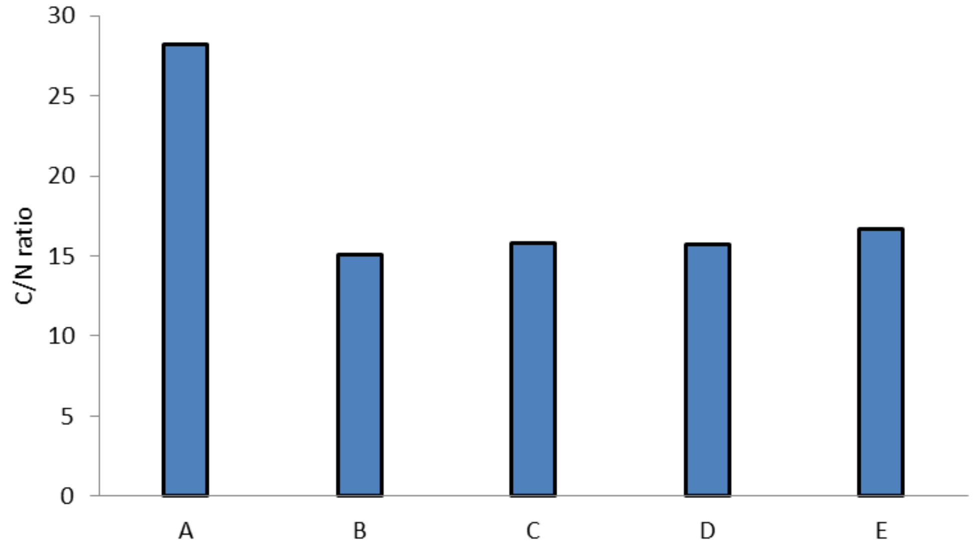

Figure 6.1 C:N ratios of A:Ipomoea leaves, and of the vermicomposts of B:*E.andrei*; C:*P.sansibaricus*; D: *L.rubillus* and E: *D.willsi*

5.3.2 FTIR Spectrometry

FT-IR spectrum of ipomoea is presented in figure 6.2. There is broad band between 3000 and 3500 cm^{-1}, depicting the strong O-H bond, due to the presence of alkaloids, phenols and alcohols present in ipomoea (Hussain *et al.*, 2017, 2017). As elucidated by Hussain *et al.*, (2017) the toxicity and allelopathicity of ipomoea is prominently due to the alkaloids and the phenolic and alcoholic compounds present in it. Next to it there is a peak at 2926 cm^{-1} due to aliphatic C-H stretching which is assigned to fatty acids and lipids (Xu *et al.*, 2012; Teh *et al.*, 2014), followed by a peak at 1746 cm^{-1} due to –COOH stretch of carboxylic acid and stretching vibrations of esters in the pectin of ligneous origin (Mochochoko *et al.*, 2013). The next prominent peak at 1647 cm^{-1} is caused by the aromatic C=C vibrations of the cellulosic or lignocellulosic materials (Bykov, 2008; Boeriu *et al.*, 2004; Klein *et al.*, 2010; Jun *et al.*, 2014).

The peaks at 1446 and 1259 cm^{-1} may be due to the -OCH_3 stretching of lignin and C-O stretching of phenols (Hussain *et al.*, 2015). Subsequently, a peak seen at 1018 cm^{-1} may be corresponding to the C-O stretch of polysaccharides, cellulose or hemicellulose (Ravindran *et al.*, 2013).

In comparison the FTIR spectra of the vermicomposts of ipomoea derived from all the four species of earthworms show significant molecular rearrangements wherein some of the biomolecules of the parent substrate are either reduced in concentration or eliminated (Figure 6.3-6.6). There is a significant reduction in the heights of peaks in the 3100-3600 cm^{-1} range, reflecting substantial reduction in the phenolic and alcoholic content occurring in the course of ipomoea’s vermicomposting. This reduces the concentrations of chemicals that were responsible for the allelopathy of ipomoea. In comparison to ipomoea which had 88.5 % transmittance at the 3420 cm^{-1} peak, the vermicasts of the four species of earthworms had transmittance in 95.5 ─ 97% range at 3388 ─ 3418 cm^{-1}.The reduction in the peak followed the order *P.sansibaricus* > *D.willsi* > *L.rubillus* > *E.andrei.* However, given the very close range within which the transmittance varied, it can be said that all the four species were able to degrade phenols to a similar extent. The peaks at 2900-2800 cm^{-1} range are much shallower, while the peak at 1764 cm^{-1} was absent in all the vermicomposts, reflecting the degradation of aliphatic compounds and the lignin content of ipomoea.

There is a shift in the peak at 1647 cm^{-1} for all the vermicomposts towards higher frequencies, with concomitant decrease in the intensity of the peak. This may be due to the degradation of carboxylic acid and lignin derivatives and the shifting of peaks is likely to have been caused by an increase in the formation of more oxidized, polycondensed, aromatic structures (humic acids), and formation of new polymers as a result of the breakdown of lignocellulosic material (Proniewicz *et al.,* 2001; Amir *et al.*, 2004). These transformations are likely to have been caused by the action of highly diverse enzymes and microflora that are known to be present in the earthworm gut (Edwards *et al.*, 2011; Huanga *et al.*, 2006). Earthworms tend to comminute the substances they ingest, with their gizzard. This causes a multi-fold increase in the surface area of the ingested substrate and facilitates colonization of microorganisms in and around the fragmented substrate particles. This, in turn, accelerates the oxidization, decomposition and stabilization of the organic matter.

From the forgoing it can be seen that all the species of earthworm caused a degradation of chemicals in ipomoea that are responsible for its antagonistic nature, and turned some of them into soil friendly chemicals. The extent of this impact across the four species was similar in some of the aspects and varied in some other.

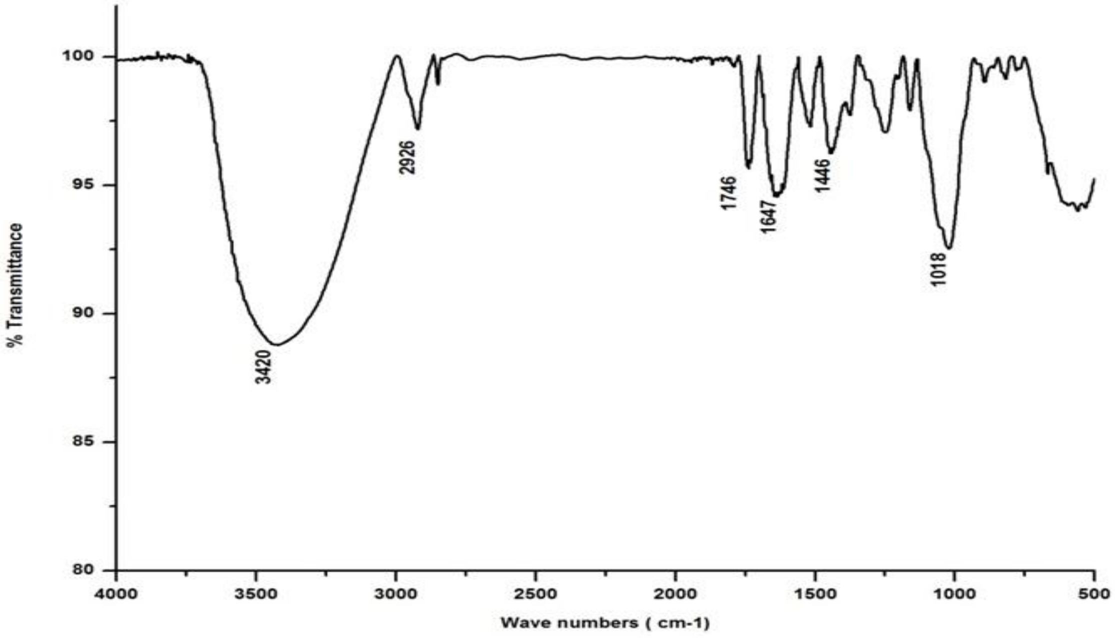

Figure 6.2. FT-IR spectra of ipomoea leaves

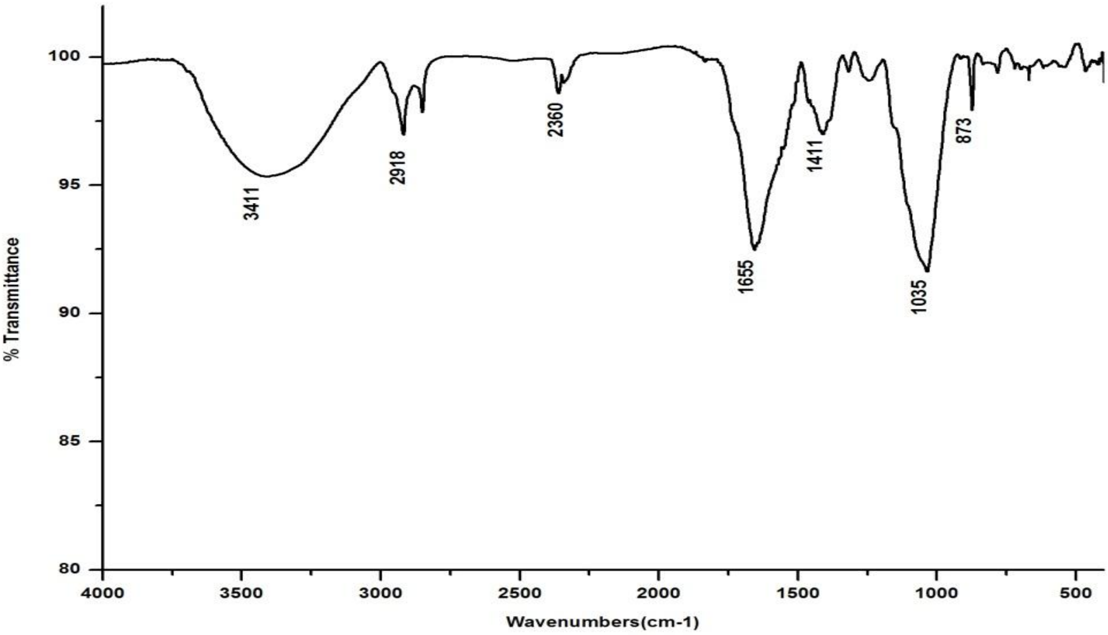

Figure 6.3.FT-IR spectra of vermicompost generated by *E.andrei*

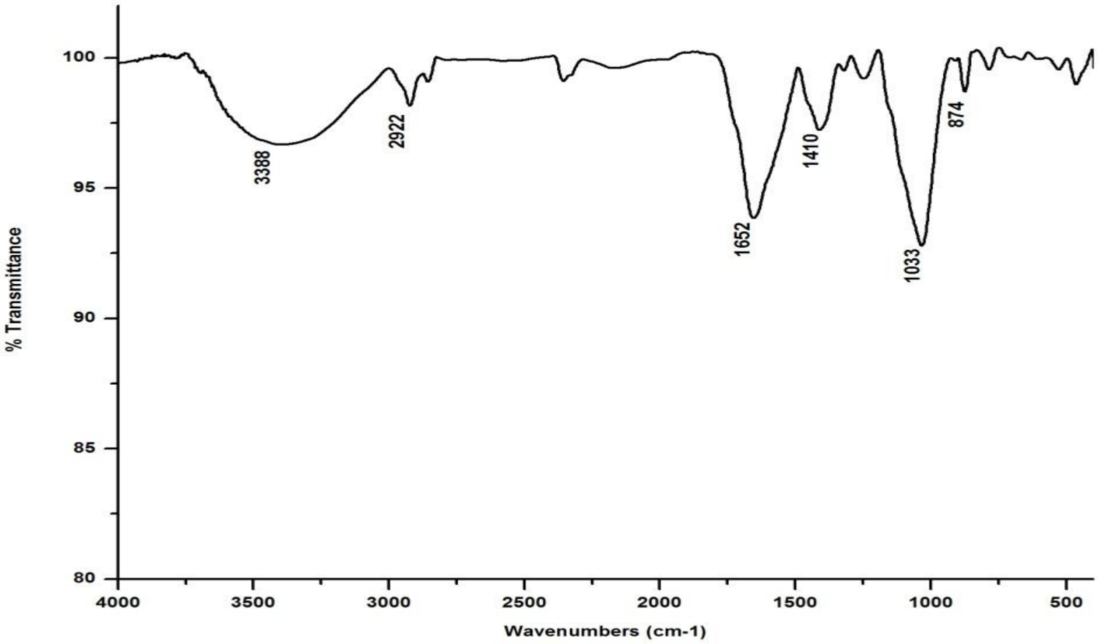

Figure 6.4. FT-IR spectra of vermicompost generated by *P.sansibaricus*

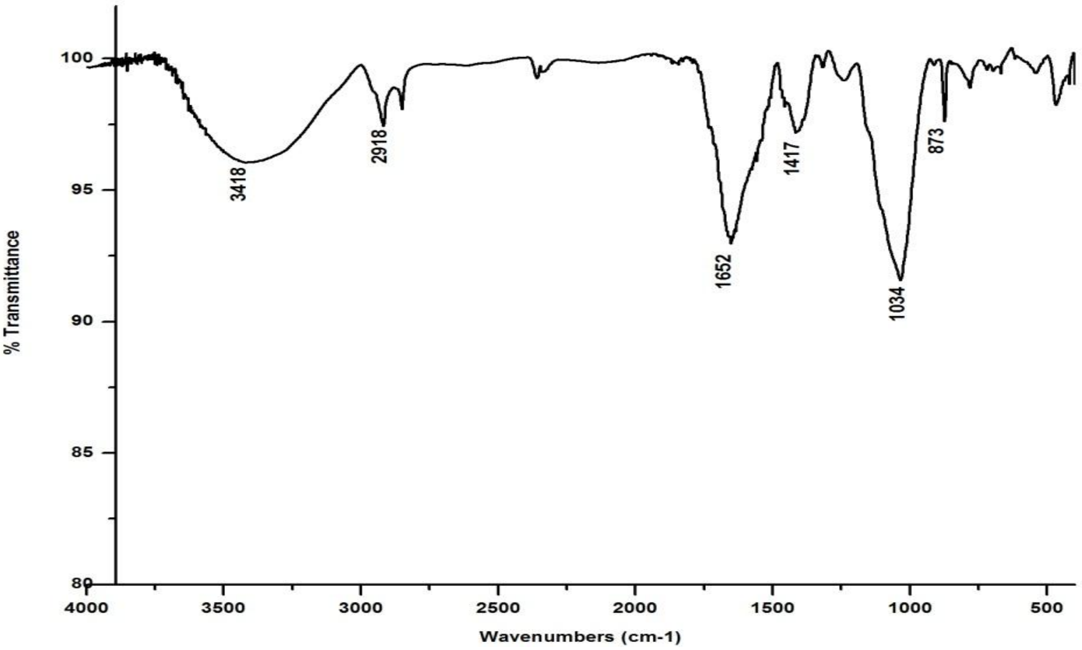

Figure 6.5. FT-IR spectra of vermicompost generated by *L.rubillus*

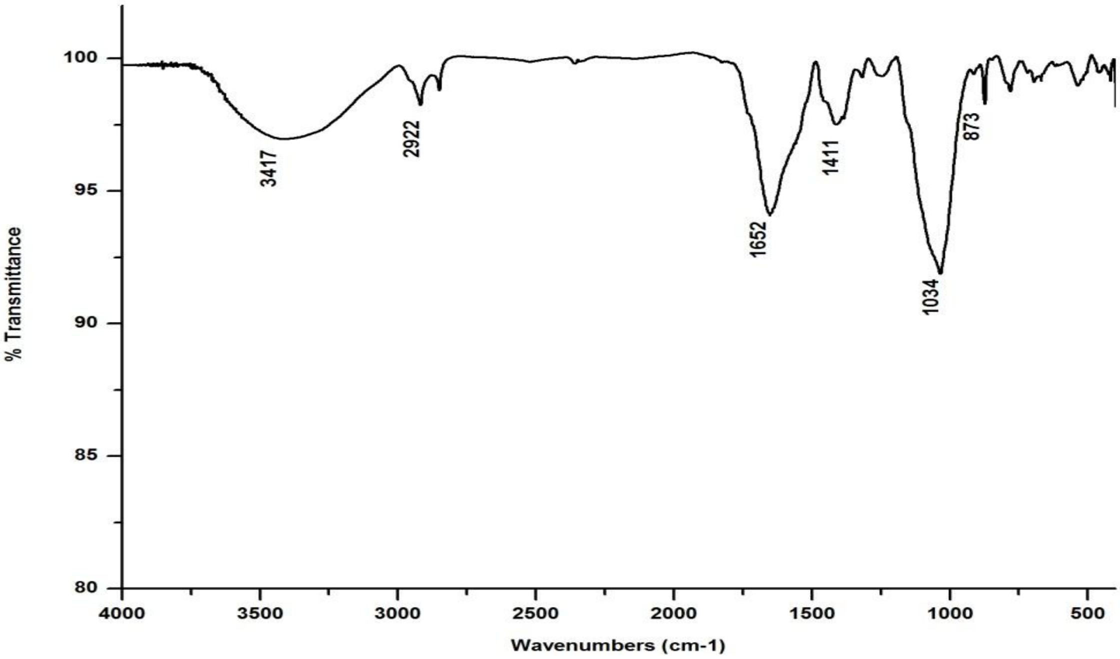

Figure 6.6. FT-IR spectra of vermicompost generated by *D.willsi*

6.3.2 Thermogravimetric analysis

Thermogravimetric (TG) curves of ipomoea and its vermicomposts are presented in figures 6.7-6.11. Ipomoea had a mass loss of 90 % while the vermicomposts of *E. andrei*

P. sansibaricus, *L. rubillus* and *D.willsi* had the mass losses of 78.7 %, 63.3 %, 61.6 %, and 55.1%, respectively. Dehydration is seen to have occurred during 60-150°C and decomposition during 200-800°C in both ipomoea and its vermicomposts. But in the vermicomposts the extent of mass loss was lesser than that which occurred in the parent substrate. This is reflective of the mineralization of the organic matter that occurred as a result of vermicomposting (Deka *et al*., 2011; Ravindran *et al*., 2013; Hussain *et al*., 2016).

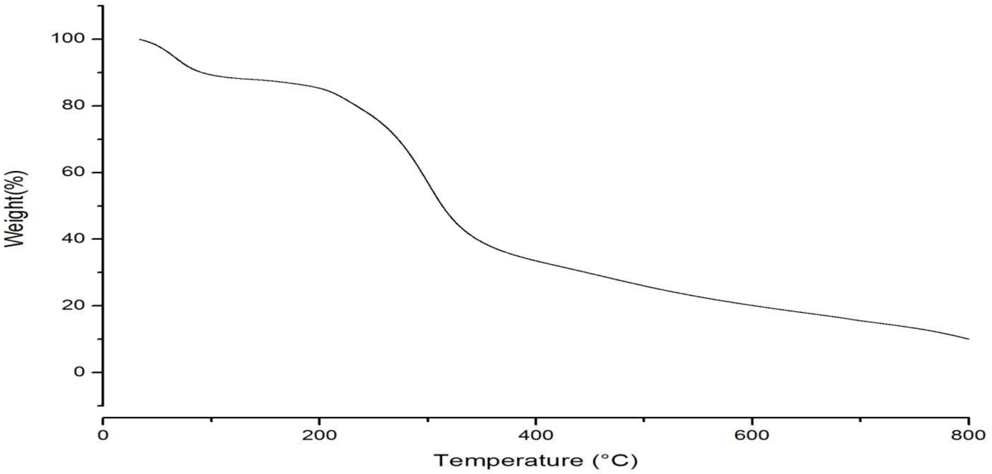

Figure 6.7: TG curve of Ipomoea leaves

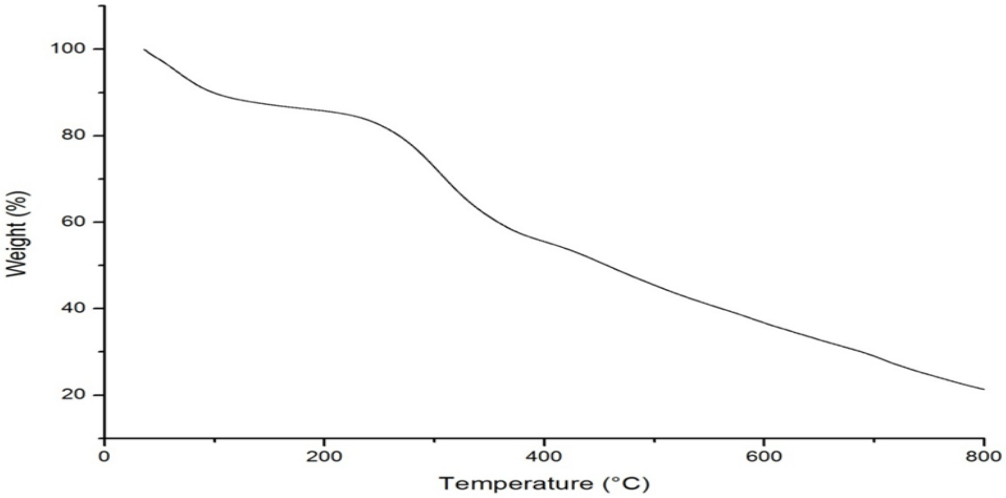

Figure 6.8: TG curve of vermicompost generated by *E.andrei*

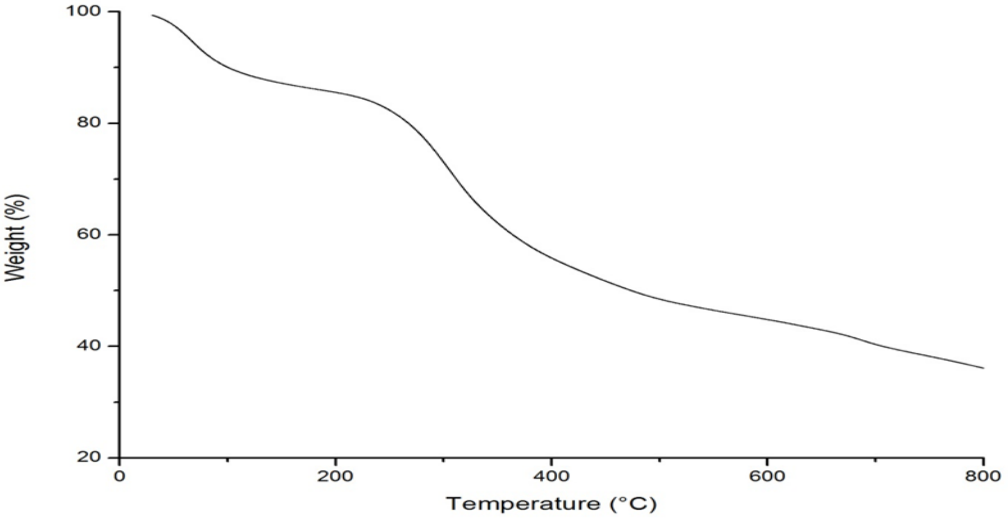

Figure 6.9: TG curve of vermicompost generated by *P.sansibaricus*

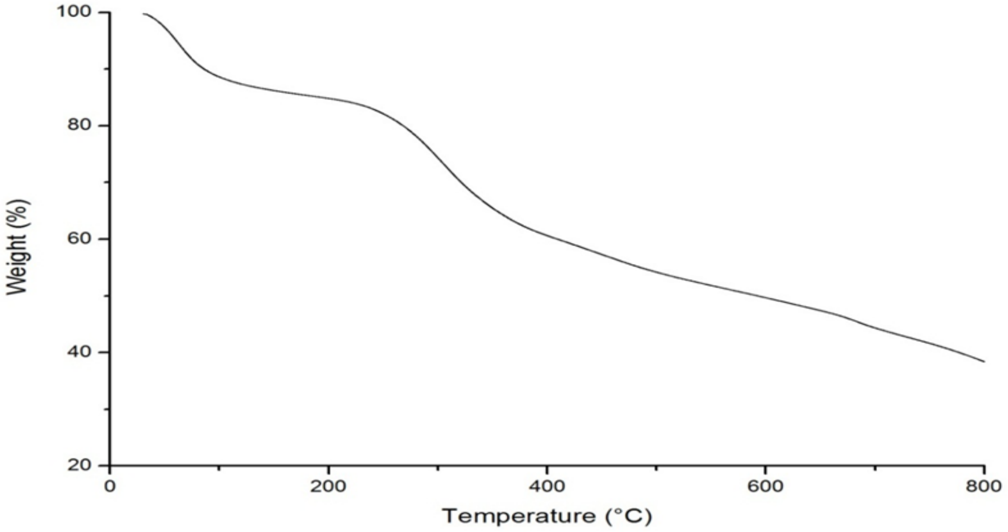

Figure 6.10: TG curve of vermicompost generated by *L.rubillus*

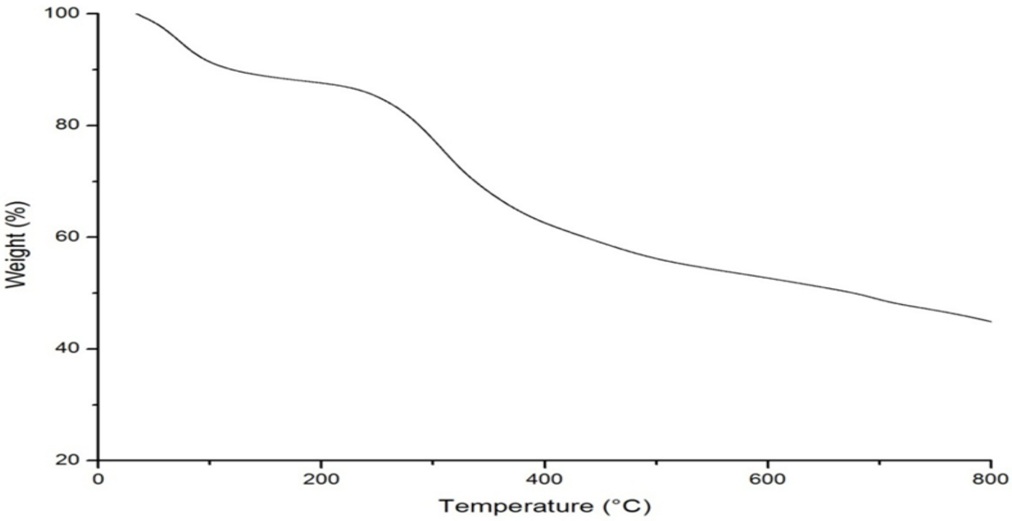

Figure 6.11: TG curve of vermicompost generated by *D.willsi*

6.3.3 Differential scanning calorimetry (DSC)

As was seen in case of salvinia (Section 5.3.3), DSC curve of ipomoea has two exothermic peaks (Figure 6.12). The first of these occurs in the 300-350 ^{o}C range and the second one at 450-500 ^{o}C. The first peak may be due to the presence of carbohydrate and cellulose while the second peak may be due to lignin and phenols.

Vermicomposts, too, have two exothermic peaks in each of their DSC curve (Figures 6.13-6.16) but the peaks are much more shallow. The reduction in the exothermic peak follows the trend, *P. sansibaricus*> *L. rubillus*> *E.andrei*> *D. willsi*. The results indicate that in the course of ipomoea's vermicomposting there is a breakdown of simpler aliphatic compounds as well as lignin and phenols present in it (Fernandez *et al*., 2012; Ravindran *et al*., 2013; El Ouaqoudi *et al*., 2014; Hussain *et al.,* 2016).

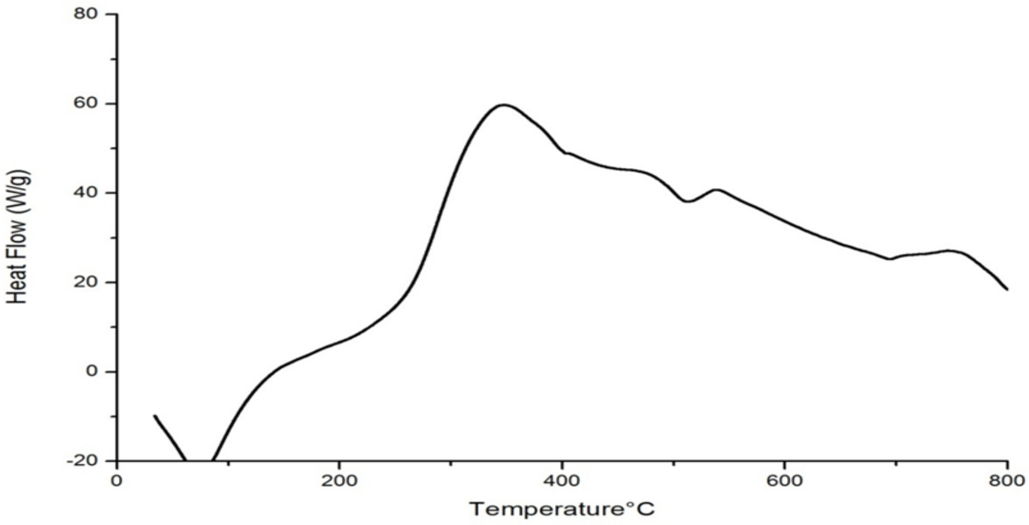

Figure 6.12. DSC curve of ipomoea plant

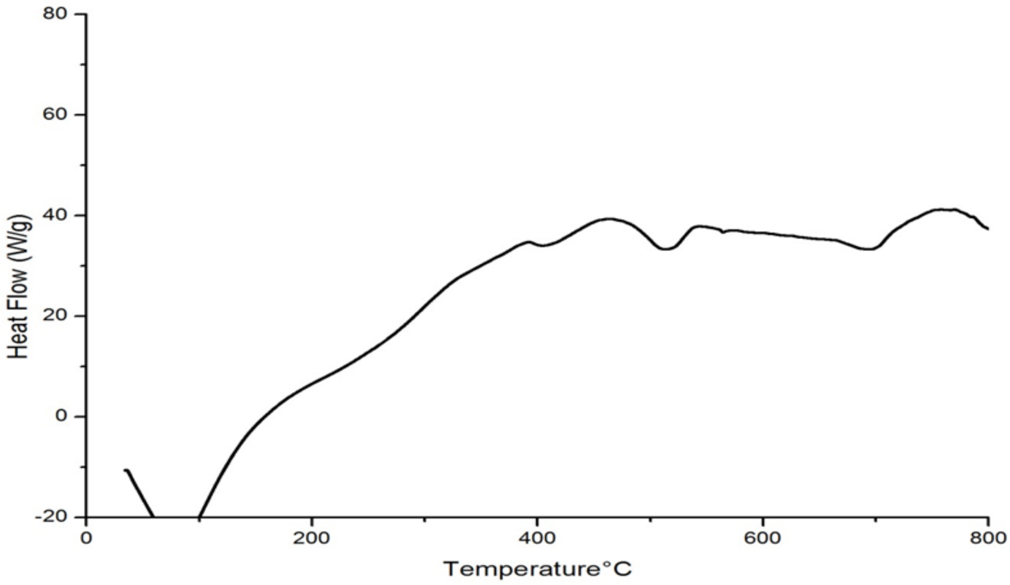

Figure 6.13. DSC curve of vermicompost generated by *E.andrei*

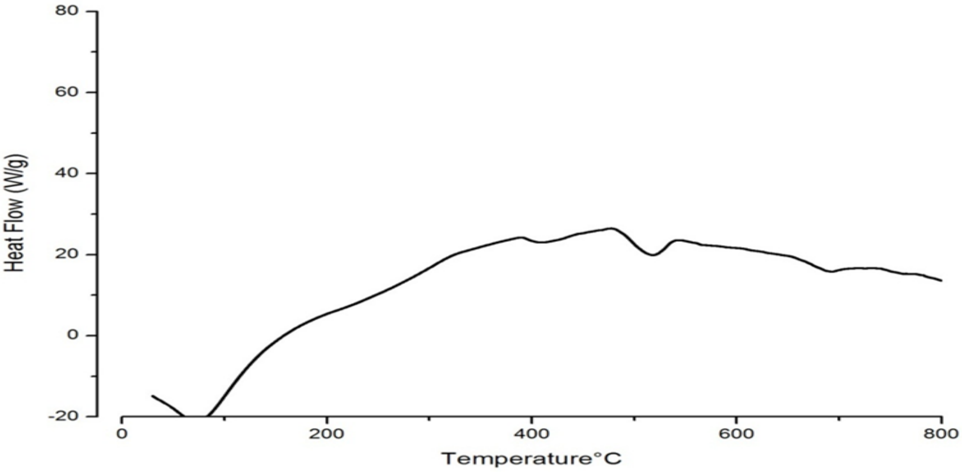

Figure 6.14. DSC curve of vermicompost generated by *P.sansibaricus*

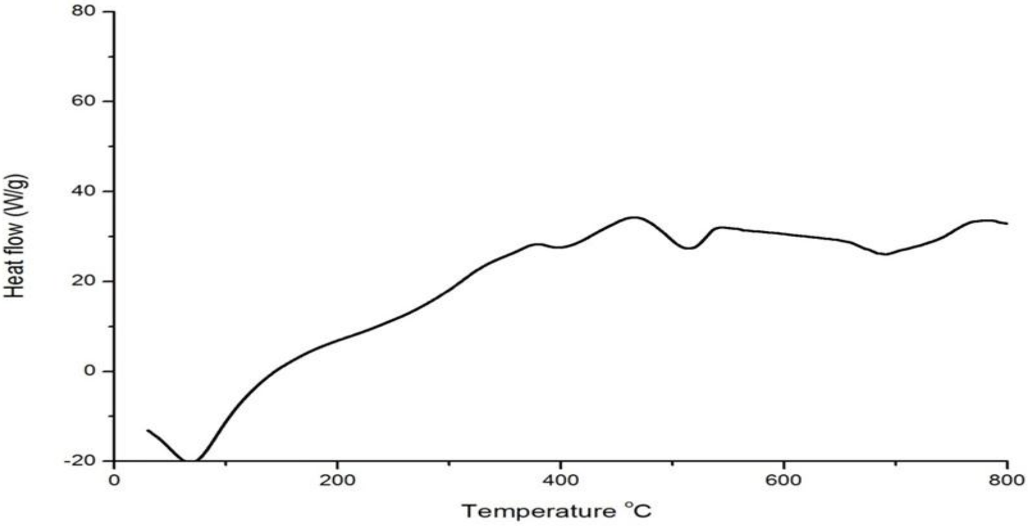

Figure 6.15. DSC curve of vermicompost generated by *L.rubillus*

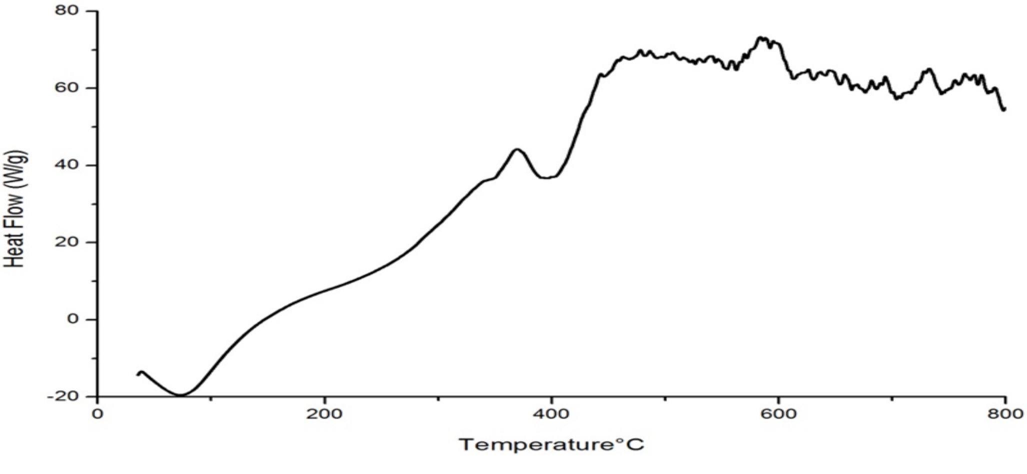

Figure 6.16. DSC curve of vermicompost generated by *D.willsi*

6.3.4 Scanning electron microscopy

As was seen in case of salvinia (Section 5.3.4), the scanning electron micrographs of ipomoea reflect the weed's robust and relatively contiguous structures — seemingly bound to lignin-containing fibers (Figure 6.17). In contrast, the micrographs of vermicomposts derived from all the four species of earthworms reflect disaggregation and withering (Figures 6.18- 6.21).

When earthworms feed on the ipomoea biomass, they grind the latter with their gizzard, (Ali *et al.,* 2015; Hussain *et al.*, 2016c); there is further disaggregation as the microbial fauna acts on the substrate during the course of its passage through the earthworm gut (Atiyeh *et al.,* 2000; Edward *et al.*, 2011; Hussain *et al.*, 2016d). This facilitates progressive degradation of ipomoea which is reflected in the SEM micrographs.

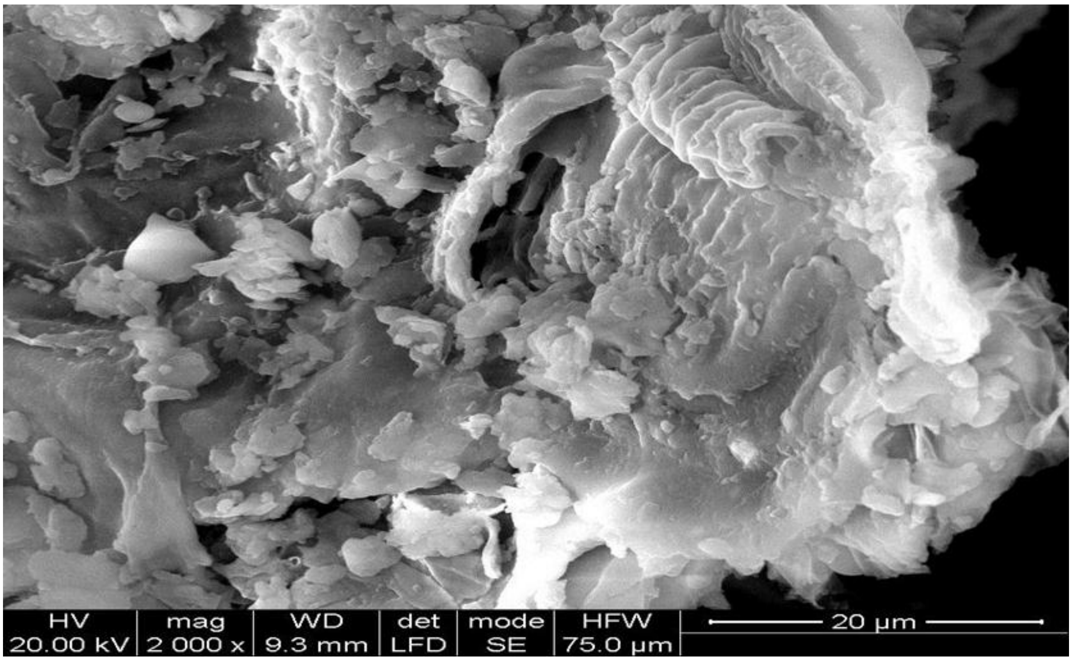

Figure 6.17. SEM images of Ipomoea leaves

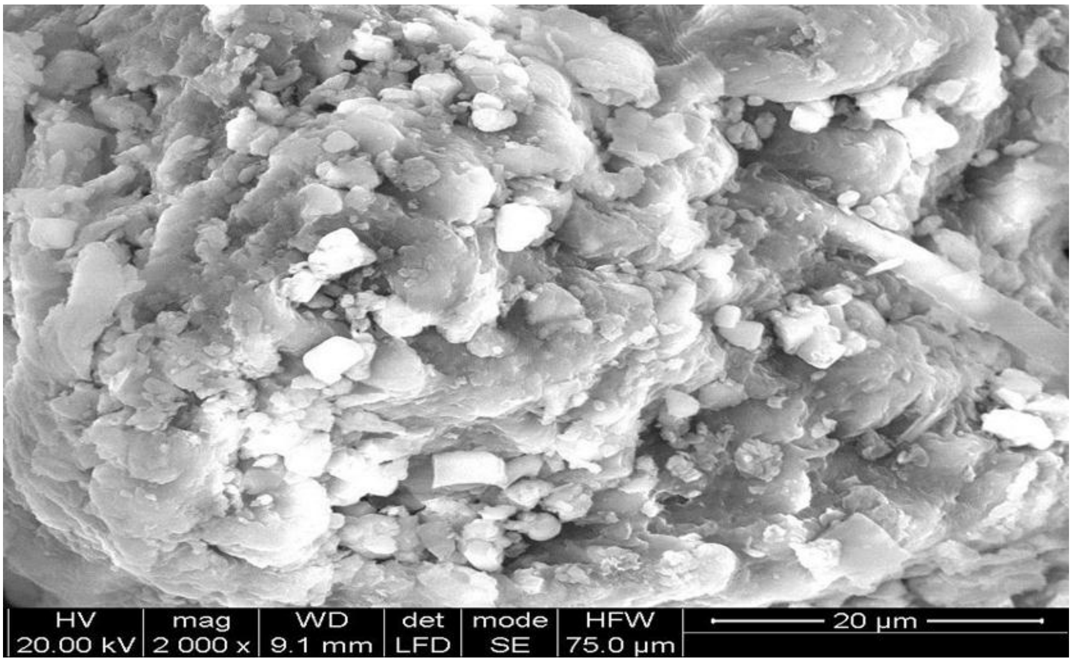

Figure 6.18. SEM images of vermicompost generated by *E.andrei*

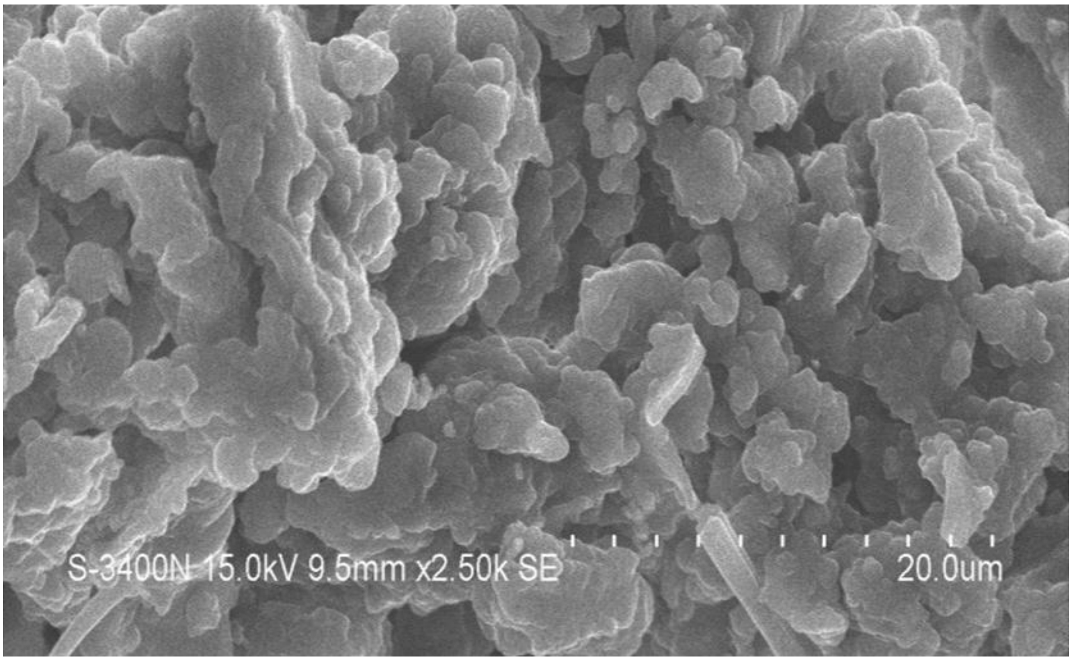

Figure 6.19. SEM images of vermicompost generated by *P.sansibaricus*

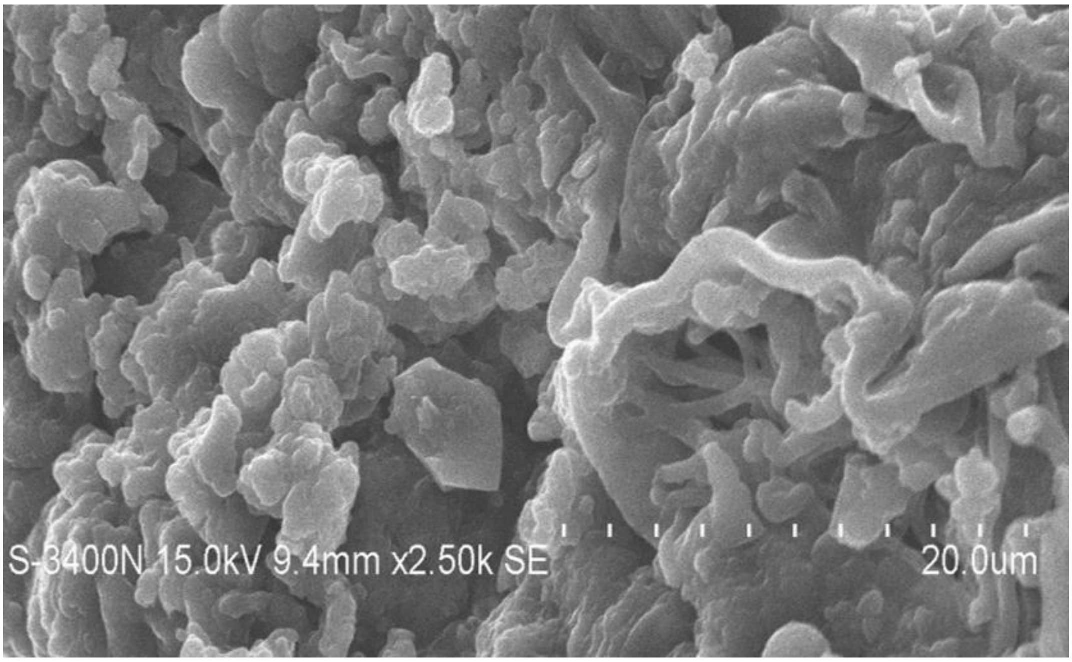

Figure 6.20. SEM images of vermicompost generated by *L.rubillus*

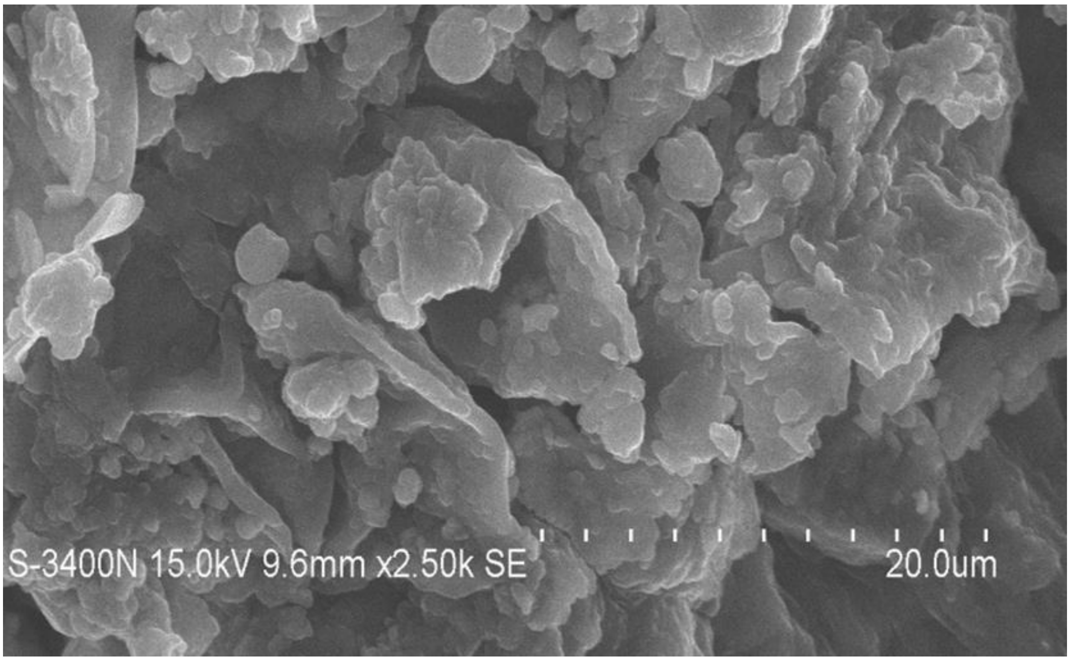

Figure 6.21. SEM images of vermicompost generated by *D.willsi*

6.4 Summary

An assessment of the transformations that occur, when ipomoea is converted to vermicompost, was carried out. The vermicasts of all the four species were studied in relation to the parent substrate (ipomoea), aimed to see whether the nature or the extent of the transformations are common across different earthworm species or very form species to species. UV-visible spectrophotometry, Fourier-transform infrared (FTIR) spectrometry, thermogravimetry, differential scanning calorimetery, and scanning electron microscopy were employed for the purpose.

The studied revealed that the C: N ratio of the ipomoea vermicomposts derived from four species of earthworms was lesser by almost 2 orders of magnitude than the C: N ratio of ipomoea. It had values close to 15 similar to the C: N ratios of the salvinia vermicomposts. There was significant mineralization, as revealed by TGA and DSC, when ipomoea was turned into its vermicompost. FTIR showed that vermicomposting significantly reduced the concentration of those biomolecules which are known to be responsible for the allelopathy and mammalian toxicity of ipomoea. There was also a breakdown of simpler compounds like carbohydrates as well as complex aromatic compounds like lignin. SEM micrographs showed that the parent substrate gets extensively fragmented and withered in the course of its vermicomposting, evidently contributing to its easier biodegradation and greater bioavailability of nutrients contained in it.

References

1. “Amir, S., Jouraiphy, A., Meddich, A., El Gharous, M., Winterton, P., Hafidi, M., 2010. Structural study of humic acids during composting of activated sludge-green waste: elemental analysis, FTIR and 13 C NMR. J. Hazard. Mat, 177(1), 524-529.

2. Atiyeh, R. M., Arancon, N., Edwards, C. A., Metzger, J. D., 2000. Influence of earthworm-processed pig manure on the growth and yield of greenhouse tomatoes, Bioresour. Technol, 75(3), 175-180.

3. Boeriu, C., Bravo, D., Gosselink, R.,van Dam ,J., 2004. Characterization of structure-dependent functional properties of lignin with infrared spectroscopy Ind. Crop. Prod., 20 , 205-218

4. Bykov, Ivan., 2008. Characterization of natural and technical lignins using FRIR spectroscopy Master thesis, Lulea University of technol.,

5. Deka, H., Deka, S., Baruah,C.K., Das, J., Hoque, S., Sanna, N.S., 2011.Vermicomposting of distillation waste of citronella plant (*Cymbopogon winterianus* Jowitt.) employing *Eudrilus eugeniae.* Bioresour. Tecbnol., 102 , 6944-6950

6. Edwards, C. A., Norman, Q. A., Sherman, R., 2011. Vermiculture Technology, Earthworms, Organic Waste and Environmental Management, CRC press, 17-19.

7. El Ouaqoudi, F.Z., El Fels, L., Winterton, P., Lemée, L., Amblès, A., Hafidi, M., 2014. Study of humic acids during composting of lingocellulose waste by infrared spectroscopic and thermogravimetric/thermal differential analysis. Compost Sci. Util. 22, 188–198.

8. Fernandez, J. M., Plaza, C., Polo, A., Plante, A.F., 2012. Use of thermal analysis techniques (TG-DSC) for the characterization of diverse organic municipal waste streams to predict biological stability prior to land application, Waste Manage, 32, 158–164.

9. Huanga, G. F., Wua, Q. T., Wongb, J. W. C., Nagarb, B. B., 2006. Transformation of organic matter during co-composting of pig manure with sawdust, Bioresour. Technol, 97, 1834–1842.

10. Hussain, N., Abbasi, T., Abbasi, S. A., 2017. Toxic and allelopathic ipomoea yields plant-friendly organic fertilizer. J. Clean. Prod, 148, 826-835.

11. Hussain, N., Abbasi, T., Abbasi, S. A., 2016c.Vermicomposting-mediated conversion of the toxic and allelopathic weed ipomoea into a potent fertilizer, Process Saf. Environ. Prot, 103, 97-106.

12. Hussain, N., Abbasi, T., Abbasi, S. A., 2016a. Vermiremediation of an invasive and pernicious weed salvinia (*Salvinia molesta*). Ecol. Eng, 91, 432-440.

13. Hussain, N., Abbasi, T., Abbasi, S.A., 2016b. Transformation of toxic and allelopathic lantana into a benign organic fertilizer through vermicomposting. Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy, 163, 162-169.

14. Hussain, N., Abbasi, T., Abbasi, S.A., 2016d. Transformation of the pernicious and toxic weed parthenium into an organic fertilizer by vermicomposting. Int. J. Environ. Studies, 73 (5), 731-745.

15. Hussain, N., Abbasi, T., Abbasi, S. A., 2015. Vermicomposting eliminates the toxicity of Lantana (*Lantana camara*) and turns it into a plant friendly organic fertilizer, J. Hazard. Mater. 46-57.

16. Hu, J., Shen, D., Wu, S., Zhang, H. and Xiao, R., 2014. Effect of temperature on structure evolution in char from hydrothermal degradation of lignin. J. Anal. Appl. Pyrolysis, 106, pp. 118-124

17. Klein, A.P., Beach, E.S., Emerson, J.W., Zimmerman J.B., 2010. Accelerated solvent extraction of lignin from *Aleurites moluccana* (Candlenut) nutshells J. Agric. Food Chem., 58 ,10045-10048

18. Mochochoko, T., Oluwafemi, O.S., Jumbam, D.N., Songca, S.P., 2013. Green synthesis of silver nanoparticles using cellulose extracted from an aquatic weed; *Wate hyacinth* Carbohydr. Polym, 98 290-294

19. Proniewicz Leonard M., Paluszkiewicz Czeslawa, Weselucha-Birczynska Aleksandra, MaJcherczyk Halina, Baranski Andrzej, Konieczna Anna., 2001. FT-IR and FT-Raman study of hydrothermally degraded cellulose, Journal of Molecular Structure, 596, 163-169.

20. Ravindran, B., Sravani, R., Mandal, A.B., Contreras-Ramos, S.M., Sekaran G., 2013. Instrumental evidence for biodegradation of tannery waste during

vermicomposting process using *Eudrilus eugeniae* .J. Therm. Anal. Calorim., 111, 1675-1684

21. Teh, C.Y., Wu, T.Y., Juan. J.C., 2014. Potential use of rice starch in coagulation-flocculation process of agro-industrial wastewater: treatment performance and flocs characterization .Ecol. Eng., 71, 509-519

22. Xu, Rensheng, Ye, Yang Zhao, Weimin., 2012. Introduction to Natural Products Chemistry CRC press, Washington, New York."

Chapter 7

Summary and conclusion

The First Chapter sets the context of the present thesis, briefly expousing why the work described in the thesis was attempted.

Chapter 2 presents a brief overview of the way huge quantities of phytomass that are generated by invasive plants all over the world and the harm it causes to biodiversity and other aspects of environmental health. The manner in which it contributes to global warming is also brought out.

The impacts of the two weeds studied by the author ─ salvinia (*Salvinia molesta),* and ipomoea *(Ipomoea carnea)* ─ are then discussed. It is shown that despite concerted efforts, made all over the world, to eradicate or control these weeds have at best achieved only temporary and partial success. Rather than being contained, both weeds are invading ever new territories and colonizing ever larger tracts of land/water. Numerous attempts made in the past to utilize these weeds are reviewed. It is shown that of all the utilization options only vermicomposting is capable of handling the enormous quantities of biomass that is generated by these weeds.

In the next step the chapter reviews the state-of-the-art of phytomass vermicomposting and brings out the reasons why conventional vermireactors, which have been very successful in vermicomposting animal manure, have been unsuccessful in vermicomposting phytomass in an economically viable manner. It describes the paradigm of ‘high-rate vermicomposting’, recently developed by the author’s mentor, with which the author has succeeded in directly, rapidly, and sustainably vermicomposting salvinia and ipomoea as later described in this thesis.

The exploration of four species of earthworm ─ *E.andrei, P.sansibaricus, L.rubillus* and *D. willsi* ─ in direct vermicomposting of salvinia is describe in **Chapter 3**. Whole plants of the weed were utilized in ‘high-rate vermireactors’ without any pre-composting, manure supplementation or any other form of pre-treatment. All experiments were carried out without interruption for 160 days.

Three series of studies were done. The first series utilized for vermicomposting of salvinia adult earthworms which had been born in cow-dung fed cultures and had grown to adulthood in them. The second series utilized earthworms born and raised in salvinia-fed cultures. Their next generation was then used for the third series of experiments. The objective was to see whether the second and the third generations display increasing adaptation to, and comfort with, the salvinia feed. It was seen that:

i) For each of the four species of earthworms studies by us, successive generations can be raised with salvinia as the sole feed.

ii) The animals of all the four species when grown on salvinia were as healthy and reproductive as the ones grown on animal manure were.

iii) Successive generations got increasingly acclimatized to salvinia and displayed increasing efficiency in vermicomposting salvinia.

iv) The reproductive ability of all the four species in salvinia-fed reactors increased as they produced their second and the third generation in it.

The extention of the possibilities explored for generating vermicompost from salvinia, described in the previous Chapter, to ipomoea is the theme of **Chapter 4**.

Four species of earthworm — (*E.andrei, P.sansibaricus, L.rubillus* and *D.willsi)* were explored for the direct vermicomposting of ipomoea. The succulent parts of the weed were utilized in 'high-rate vermireactors' without any pre-composting, manure supplementation or any other form of pre-treatment. All vermireactor were operated uninterruptedly for 160 days.

In the first series of experiments vermicomposting of ipomoea was studied with adult earthworms which had been born in cow-dung fed cultures and had grown to adulthood in them. The second series utilized earthworms born and raised in ipomoea-fed cultures. Their next generation was then used for the third series of experiments. These studies enabled us to see whether the second and the third generations display increasing adaptation to the ipomoea feed. The studies reveal that:

i) For each of the four species of earthworms studies by us, successive generations can be raised with ipomoea as the sole feed.

ii) The individuals of all the four species when grown on ipomoea as the sole feed were as healthy and reproductive as the ones grown on animal manure were.

iii) Successive generations got increasingly acclimatized to ipomoea and displayed increasing efficiency in vermicomposting ipomoea.

iv) The reproductive ability of all the four species in ipomoea-fed reactors increased as they produced their second and the third generation in it.

A comparison between the results of the experiments on vermicomposting of salvinia with the findings on ipomoea revealed an interesting trend. Even as the quantities of the two weeds converted to vermicompost per unit time by each adult earthworm of a given species were quite similar, the extent of reproduction achieved in ipomoea by all the four species was much lesser than that which was achieved in ipomoea. It indicated that either the adaption to ipomoea *vis a vis* reproduction is slow and may take several generations to peak, or that some chemicals in ipomoea suppress earthworm fecundity.

Chapter 5 presents studies which were carried out to see what transformations occur when salvinia is converted to vermicompost. It was also aimed to see whether the nature or the extent of the transformations are common across different earthworm species or very form species to species. The studies were supported by UV-visible spectrophotometry, Fourier-transform infrared (FTIR) spectrometry, thermogravimetry, differential scanning calorimetery, and scanning electron microscopy.

It was seen that the C:N ratio of the salvinia vermicomposts derived from four species of earthworms was lesser by almost 3 orders of magnitude than the C:N ratio of salvinia. Further, vermicomposting had caused a reduction in the phenol and lignin content of salvinia. TGA and DSC indicated net mineralization when salvinia was turned into vermicompost as well as breakdown of simpler compounds like carbohydrates and complex aromatic compounds like lignin. SEM micrographs confirmed that the parent substrate is extensively fragmented and withered in the course of its vermicomposting.

An assessment of the transformations that occur, when ipomoea is converted to vermicompost, is described in **Chapter 6.** The vermicasts of all the four earthworm species were studied in relation to the parent substrate (ipomoea), aimed to see whether the nature or the extent of the transformations are common across different earthworm species or very form species to species. UV-visible spectrophotometry, Fourier-transform infrared (FTIR) spectrometry, thermogravimetry, differential scanning calorimetery, and scanning electron microscopy were employed for the purpose.

It is seen that the C:N ratio of the ipomoea vermicomposts derived from four species of earthworms was lesser by almost 2 orders of magnitude than the C:N ratio of ipomoea. It had values close to 15 similar to the C:N ratios of the salvinia vermicomposts. There was significant mineralization, as revealed by TGA and DSC, when ipomoea was turned into its vermicompost. FTIR showed that vermicomposting significantly reduced the concentration of those biomolecules which are known to be responsible for the allelopathy and mammalian toxicity of ipomoea. There was also a breakdown of simpler compounds like carbohydrates as well as complex aromatic compounds like lignin. SEM micrographs showed that the parent substrate gets extensively fragmented and withered in the course of its vermicomposting, evidently contributing to its easier biodegradation and greater bioavailability of nutrients contained in it.

All-in–all, the thesis establishes the feasibility of vermicomposting two of the dreaded weeds – salvinia and ipomoea – in an inexpensive, rapid, and sustainable manner. It is also shown that the weeds aquire the attributes of a plant friendly organic fertilizer when they are converted into vermicast.