

FEASIBILITY OF VERMICOMPOSTING WATER CLOVER (*MARSILEA QUADRIFOLIA*) EMPLOYING THREE EPIGEIC EARTHWORM SPECIES

¹S.G. Antony Godson, and ²S. Gajalakshmi

¹Assistant Professor, ²Associate Professor

¹PG and Research Department of Biotechnology,

¹Hindusthan Arts and Science College, Nava India, Coimbatore-641028, Tamil Nadu, India,
605 014, India

Abstract: This paper presents the study conducted on the feasibility of vermicomposting the plant-water clover, *Marsilea quadrifolia*, in two forms— fresh and soaked— by three-epigeic species of earthworm, *Eudrilus eugeniae* Kinberg, *Eisenia fetida* Savigny, and *Perionyx excavatus* Perrier. The reactors were operated in a semi-continuous mode, and the performance was assessed by quantifying the vermicast output, growth, and reproduction of the earthworms over a period of 150 days. The experiments were run in duplicate. In all reactors, vermicomposition rate was higher in reactors with the soaked form of feed than the fresh form of the feed. The highest vermicast output was recorded with *E. fetida* followed by reactors with *E. eugeniae* and *P. excavatus*: The zoomass of *E. fetida*, *P. excavatus* increased over time only in the soaked form of feed; *E. fetida* gained zoomass than *P. excavatus* while, *E. eugeniae* did not show any improvement. The reactors' performance regarding vermicast production and zoomass gain amongst different earthworm species did not have a significant difference as per one-way ANOVA. In all the reactors, earthworm mortality occurred, which subdued reactor performance dramatically. Therefore, to improve water clover-fed vermireactor efficiency, the feed was pre-conditioned by soaking for 24 to 48 hours before vermicomposting.

IndexTerms: Semi-continuous, *E. fetida*, reactors, ANOVA, Vermicast output

I. INTRODUCTION

Marsilea quadrifolia L. commonly called as water clover and four-leaf clover is a macrophyte and amphibious non-flowering plant that belongs to Marsileaceae family. It is indigenous to Europe and Asia and is reported as a fast-growing and invasive non-indigenous creeper (Johnson, 1993; Serviss and Peck, 2008; Thiébaud, 2007). Water clover reproduces by both asexual and sexual method; due to its high reproduction behavior, denser growth pattern, and extreme environmental plasticity that leads to the displacement of native aquatic plants and subsequently result in becoming mono-species communities (Hulina, 1998). Besides the natural attributes of water clover affecting the local mollusc communities, the freely branched rhizome result in the indefinite growth. Sporocarp is another way of reproduction; which lie dormant for decades and at wet conditions male and female spores cross-fertilize and begin their population. Sporocarps can spread long distance with high rate of dispersal by flooding to the downstream from existing populations (Invasive-plant-fact-sheet, 2015). When the weed is not controlled, no matter what does the capability of physical/mechanical, chemical method but will perish other vegetation. However, the possible extent of the weed eradication by chemicals like herbicides might work well on the target species, but the adverse effect on non-target species leads to drastic environmental degradation. Water clover has been used as an edible plant as well as a source of medicine. It can also be used for phyto-sequestration due to its high bioaccumulation ability of heavy metals (Ahmad et al., 2010). Nevertheless, the outcome of population expansion outweighs benefits of use. For instance, it decreases recreational and aesthetic values, declines the ecological integrity, thereby increasing the research expenses for finding a complete solution for its total disposal and money spent towards its eradication by existing methods. In recent years, the use of phytomass as a sole substrate in vermicomposting without any pre-treatment or supplementation has been established (Makhija et al., 2011). The direct vermicomposting is a simple and easy method, which is capable of resolving the environmental and economic concerns over the existing conventional vermicomposting system (Nayeem-Shah et al., 2015).

In this paper, studies conducted on the use of earthworms *E. eugeniae*, *E. fetida*, and *P. excavatus* in generating vermicast from the plant water clover is reported. The most important aspect of the present work is that vermicomposting of water clover has been undertaken without the blend of any cow manure.

Choice of the earthworm species

The epigeic species, *E. eugeniae*, has been used in Europe, and North America for vermicomposting of animal manure due to its appetite, high growth rate, and higher frequency of reproduction (Gajalakshmi et al., 2001; Antony and Gajalakshmi, 2018 (2)). *E. fetida* is another epigeic species, which is peregrine and ubiquitous also, which can stand an extensive range of temperature and moisture (Domínguez, 2004). The third epigeic species of earthworm considered by us, *P. excavatus*— is endemic in India (Ismail, 1997). However, it is prevalent in various other areas all around the world. The use of these earthworm species in other countries has also grown, but its commercial application so far has been confined to the vermicomposting of animal manure (AshokKumar, 1994; Manna et al., 1997; Ismail, 1998). Despite that, these earthworm species are being utilized in laboratory

studies to vermicompost plant phytomass like *Cymbopogon winterianus*, *Parthenium hysterophorus* by blending with animal manure (Deka et al., 2011; Adarsh et al., 2011; Yadav and Garg, 2011). These earthworm species have also been successfully utilized to vermicompost phytomass like *Eichhornia crassipes*, *Azadirachta indica*, *Mangifera indica*, and *Ipomoea carnea*, without any supplementation of animal manure (Gajalakshmi et al., 2002 and 2005; Makhija et al., 2011; Gajalakshmi and Abbasi, 2004; Gaur and Singh, 1995; Antony and Gajalakshmi, 2018 (1)).

II. METHODOLOGY

A four-litre plastic container was employed as the vermireactors (diameter 25 cm, depth 8.2 cm). Vermibed was a 3 mm thick double layered moistened jute cloth. Water clover plant was gathered manually from Pondicherry University campus and adjacent regions; the collected plant was shredded and washed with tap water to ensure the materials were free of any adhered particles. A total of twelve reactors were operated. Each reactor was charged with 150 g dry weight equivalent of water clover. In one set of reactors, the plant was utilized without any pre-treatment (fresh form), and in another set of reactors, it was pre-soaked for 6 hours (soaked form) before being charged. Each reactor was started with the introduction of 20 healthy adult individuals of chosen species. The earthworms were picked up randomly from the cultures maintained with cow dung. The substrate was sprayed with enough quantity of water every day to keep up optimum moisture and was kept covered with wet gunny bag and nylon mesh. If any earthworms died during reactor operation, they were replaced with healthy mature earthworms. The vermireactors were operated in the semi-continuous mode of operation. Every 15 days once, the vermicompost and the earthworms were quantified. The reactor content was sieved (2mm-size sieve) to harvest vermicast out of the unutilized substrate. Subsequently, the dry weight of vermicast was determined using oven drying the sample at 105°C. Since the weight of substrate and vermicompost is happened to be varying from time to time and place to place, the method of quantification for all measurement in this study is based on dry weight, except the weight of earthworms, which were weighed as live weight following the water washing to remove adhering material off the earthworm and kept them for few minutes on tissue paper to wipe off excess water (Gajalakshmi et al., 2002 and 2005; Antony et al., 2015). In every run, the juveniles and cocoons were removed and segregated to the main culture, and the reactor operation was resumed after weighing with the same number of earthworms with which reactors were commenced. The reactors were restarted with the quantity of new fresh substrates equivalent to the dry weight of vermicast harvested in the previous run. All the quantifications and measurements were done once in 15 days. To present the vermicast produced from same earthworms and number, the earthworms with which the reactor was started were only used throughout the reactor operation.

Statistical analysis

One-way ANOVA and Tukey's test (Carver and Nash, 2012) at the 0.05 confidence level was used to evaluate the significant difference in the performance of the reactors with different substrate forms, and different earthworm species involved in vermicast production.

III. RESULTS AND DISCUSSION

Vermicast output in reactors with *E. fetida*, *E. eugeniae*, and *P. excavatus* in fresh and soaked forms of the feed, as a function of time, is presented in Figure 3.1. In the first two runs, the vermicast recovery was low, showing an acclimatization period. It depicts that the earthworms utilized in the experiments, which had been raised on cow dung, had taken some time to adapt to the new feed, water clover. The vermicast output started developing gradually from third run onwards. There was no consistency in vermicast production from the beginning of the reactor operation, which was due to the wide range of mortality of earthworms, which, in turn, reduced reactor performance drastically. The mortality occurred whenever the feed was added intermittently—the fresh feed was not tolerable by the earthworm species that led them to die. The non-preference of the feed by the earthworms and subsequent mortality may be due to the chemical nature of the plant—the plant is reported to have an ability to retain the rich quantity of heavy metals by the bioaccumulation process (Ahmad et al., 2010). Moreover, the structural component of water clover, which is mainly of polymers—cellulose, hemicellulose, lignin—and the presence of polyphenol, which is mainly degraded by cellulolytic fungi and bacteria, could also be a limiting factor for earthworms in decomposing water clover during vermicomposting process (Rosa et al., 2011). The reproducibility of reactors run in duplicate is presented in Table 3.1. Though the reactor was heterogeneous, the rate of reproducibility of the results in different runs is considered as good. It may be seen that only in a single case (7th run, fresh form of the feed with *E. eugeniae*) the duplicates agreed to within ± 9.1 mg. In all other instances, the duplicates were agreeing within ± 8.8 mg. The vermicast output per animal, per day, is reported in Table 3.2. The earthworm species, which generated more vermicast per earthworm per day from water clover was *E. fetida* followed by *E. eugeniae* and *P. excavatus*. In all instances, soaked form of water clover was vermicomverted significantly higher than the fresh form of the feed. The former form of the feed being pulpier than the latter might have been easier for the earthworm to ingest. One-way ANOVA was carried to analyze the statistical difference between the vermicast output generated from reactors with different earthworm species, and the forms of the feed. Statistically, significant difference was observed between fresh and soaked forms of the feed— $F(1, 118) = 10.101$, $P = .002$, whereas among the different earthworm species, there were no significant difference -- $F(2, 117) = 2.195$, $P = .116$ -- except with the pair *E. fetida* and *P. excavatus* ($P = 0.040$), in vermicast production.

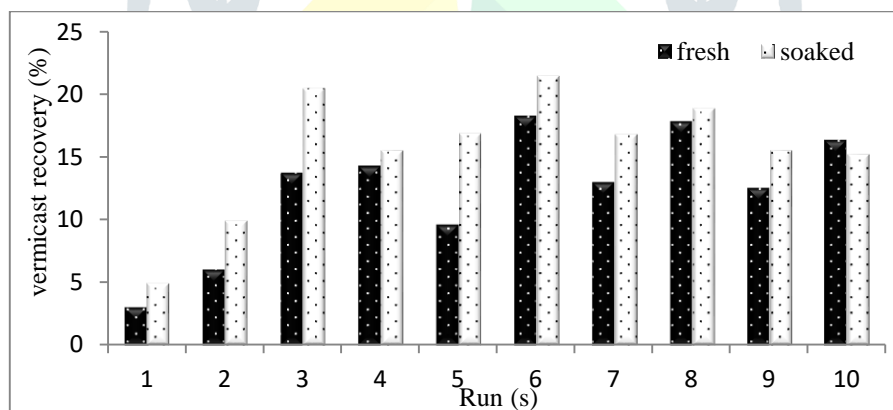
Table 3.1 Vermicast output as % of feed mass, in reactors charged with fresh or soaked forms of water clover

Days	Vermicast output %, (\pm SD)					
	<i>E. fetida</i>		<i>E. eugeniae</i>		<i>P. excavatus</i>	
	Fresh	Soaked	Fresh	Soaked	Fresh	Soaked

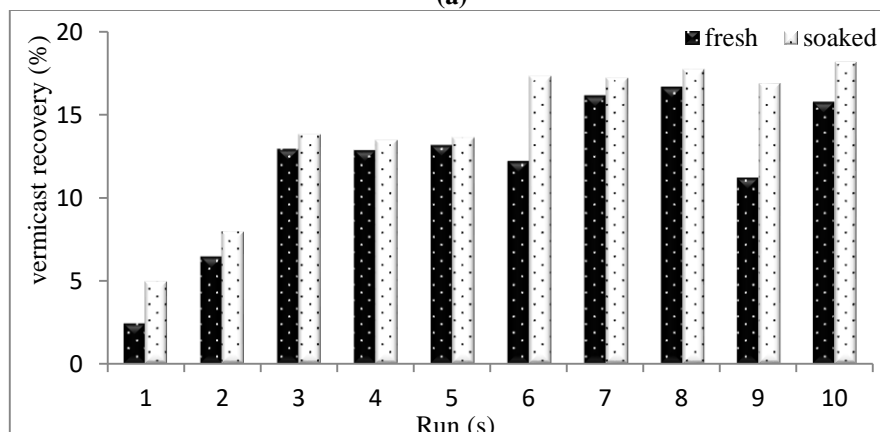
15	3±1.6	4.9±0.1	2.4±3	5±0.8	1.5±1.1	3.0±1.3
30	6.0±2.2	9.9±3.2	6.5±1.7	8±2	6.7±1.2	8.3±2.6
45	13.7±3	20.5±0.8	12.9±2.4	13.8±5.2	12.7±3.1	15±2.6
60	14.2±8.1	15.5±6.4	12.8±5.1	13.5±8.4	10.5±4.2	16.5±4.1
75	9.6±2.8	16.9±6.5	13.2±8.1	13.6±8.8	9.1±0.7	15.9±1.6
90	18.2±3.1	21.5±1.8	12.2±1.4	17.3±2.4	12.1±0.7	15.1±6.5
105	13.0±2.1	16.8±2	16.2±9.1	17.2±6.7	10±6.4	14.2±7.3
120	17.8±3.3	18.9±3.2	16.7±2.6	17.8±5.2	8.5±0.1	14.2±3.9
135	12.5±2.3	15.5±5.6	11.2±5	16.9±5.3	13.6±1.2	15.7±3.1
150	16.3±8.1	15.2±6.9	15.8±2.4	18.2±3.1	8.7±3.9	17.0±2.1

Table 3.2 Average vermicast output (mg, d⁻¹, worm⁻¹), in reactors charged with fresh or soaked forms of water clover

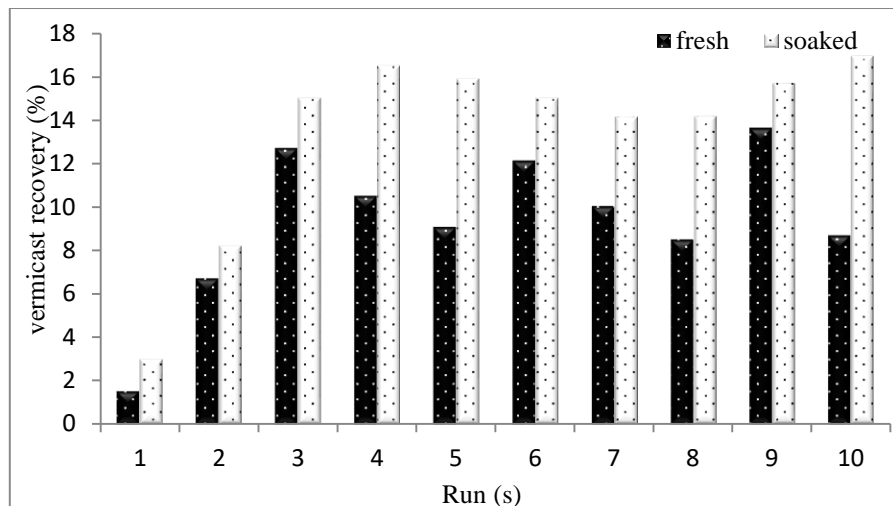
Days	Vermicast output, mg day ⁻¹ , worm ⁻¹					
	<i>E. fetida</i>		<i>E. eugeniae</i>		<i>P. excavatus</i>	
	Fresh	Soaked	Fresh	Soaked	Fresh	Soaked
15	5.1	8.4	4.1	8.4	2.6	5.1
30	10.2	16.9	11	13.5	11.4	14.1
45	23.3	34.8	22	23.5	21.6	25.6
60	24.2	26.4	21.8	22.9	17.9	28.1
75	16.3	28.7	22.4	23.2	15.4	27.1
90	31	36.5	20.7	29.4	20.6	25.6
105	22	28.6	27.5	29.3	17	24.1
120	30.2	32.1	28.3	30.2	14.4	24.1
135	21.2	26.4	19	28.7	23.2	26.7
150	27.7	25.9	26.8	30.9	14.7	28.9
Average	21.1	26.5	20.4	24	15.9	22.9



(a)



(b)



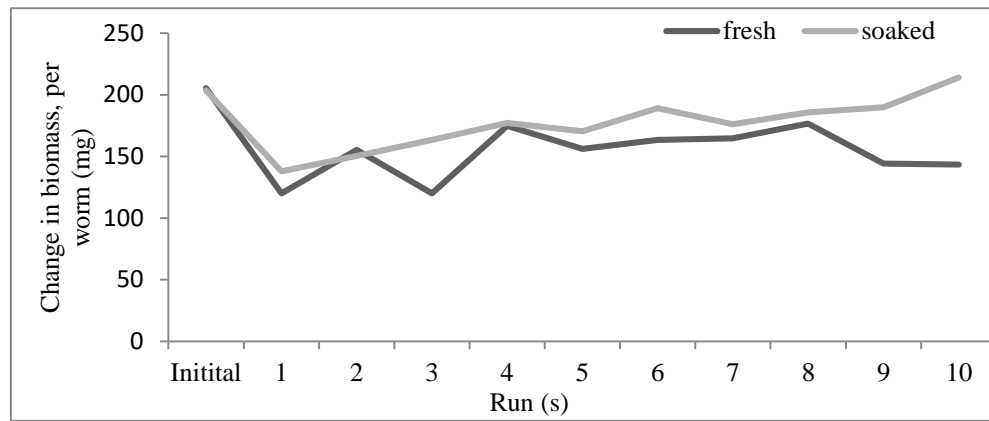
(c)

Figure 3.1 Vermicast output as % of feed mass, in reactors with (a) *E. fetida*, (b) *E. eugeniae*, and (c) *P. excavatus* charged with fresh and soaked forms of water clover

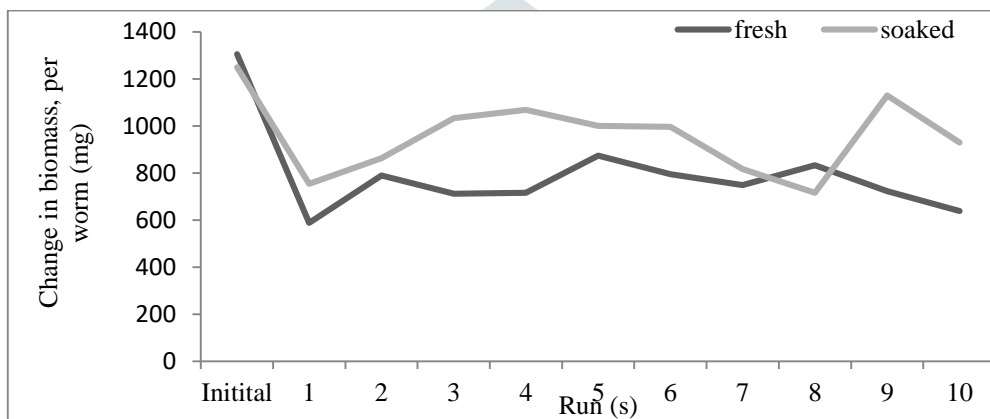
The earthworm zoomass has shown slight growth despite the mortality. In the case of the fresh form of feed in reactors with all the three-earthworm species, there was zoomass gain. While, in the case of a soaked form of feed, the zoomass gain was somewhat noticeable with *E. fetida* and *P. excavatus* but with *E. eugeniae*, there was no zoomass gain in both fresh and soaked forms of feed (Table 3.3; Figure 3.2). The vermicast recovery is set to go on increasing till the earthworms attained the peak of vermiconversion. Thenceforth, the fluctuation in vermicast output was observed (Figure 3.1), as the number of earthworms that were used in the reactors had demonstrated potency in their active age. The vermicast production and zoomass gain are positively correlated. Reproduction also occurred—cocoon were produced in all the reactors.

Table 3.3 Average zoomass (mg, per earthworm) recorded, in reactors charged with fresh or soaked forms of water clover

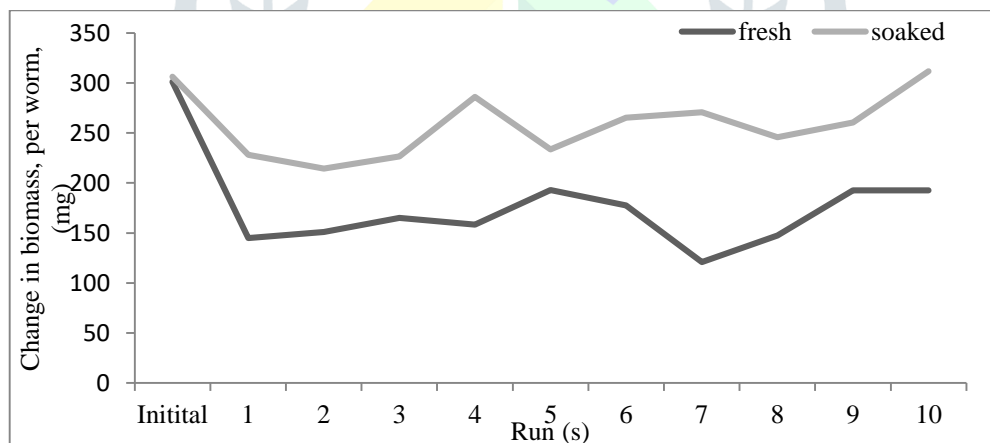
Runs	Zoomass, worm ⁻¹ , (mg)					
	<i>E. fetida</i>		<i>E. eugeniae</i>		<i>P. excavatus</i>	
	Fresh	Soaked	Fresh	Soaked	Fresh	Soaked
Initial	205.5	203.8	1305.5	1249.3	301.3	306.3
1	120.0	137.9	588.1	753.5	144.8	228.3
2	155.5	150.4	789.3	863.3	150.9	214.3
3	120.0	163.4	712.3	1032.5	165.2	226.6
4	174.8	177.3	715.2	1068.3	158.2	286.2
5	156.1	170.5	873.4	1000.5	193.0	233.5
6	163.5	189.1	795.2	995.9	177.6	265.3
7	164.8	176.2	748.3	816.2	120.9	270.7
8	176.6	185.8	832.8	715.2	147.4	245.9
9	144.3	189.8	723.1	1128.9	192.6	260.6
10	143.5	214.1	638.4	929.6	192.5	311.6
Total zoomass gain or loss (per worm)	-62.0	10.4	-667.1	-319.7	-108.8	5.3



(a)



(b)



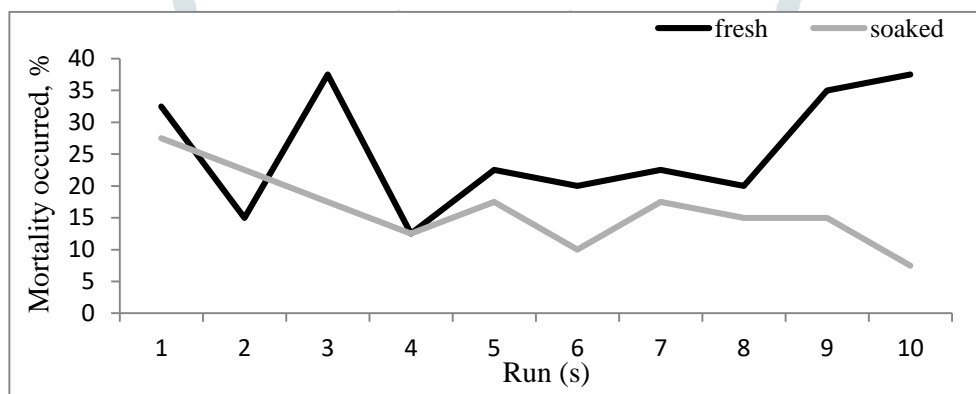
(c)

Figure 3.2 Change in zoomass (per worm), in reactors with (a) *E. fetida*, (b) *E. eugeniae*, and (c) *P. excavatus*, charged with fresh and soaked forms of water clover

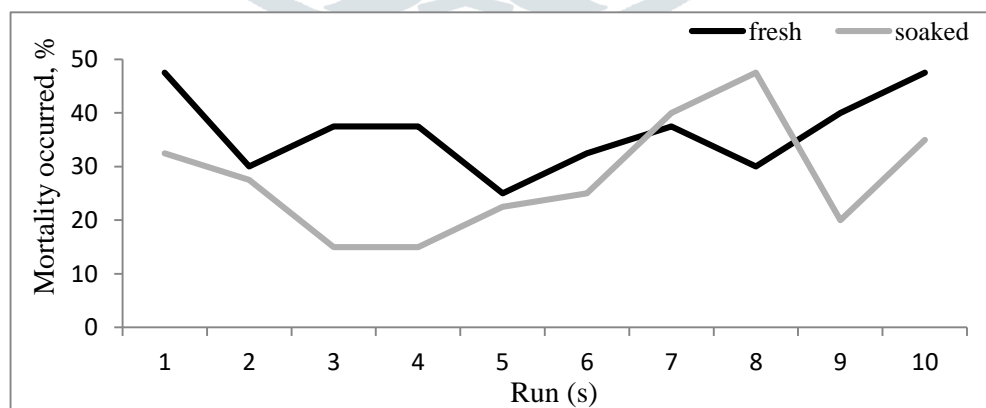
There was no particular trend in mortality in all the reactors, which was a major setback in reactor operation (Table 3.4). However, the vermicast output indicated that the efficiency of earthworm could be improved further if the feed is processed suitably (Figure 3.3). These observations confirm that the possibility of vermicomposting water clover can be successfully explored by three epigeic species, *E. fetida*, *E. eugeniae*, and *P. excavatus* in both fresh and soaked forms by operating reactors in the semi-continuous mode.

Table 3.4 Mortality (%), in reactors charged with fresh or soaked forms of water clover

Runs	Mortality, % (mean)					
	<i>E. fetida</i>		<i>E. eugeniae</i>		<i>P. excavatus</i>	
	Fresh	Soaked	Fresh	Soaked	Fresh	Soaked
1	32.5	27.5	47.5	32.5	42.5	20
2	15	22.5	30	27.5	42.5	27.5
3	37.5	17.5	37.5	15	37.5	27.5
4	12.5	12.5	37.5	15	42.5	12.5
5	22.5	17.5	25	22.5	30	30
6	20	10	32.5	25	35	22.5
7	22.5	17.5	37.5	40	57.5	22.5
8	20	15	30	47.5	50	32.5
9	35	15	40	20	35	32.5
10	37.5	7.5	47.5	35	37.5	22.5
Average	25.5	16.3	36.5	28	41	25



(a)



(b)

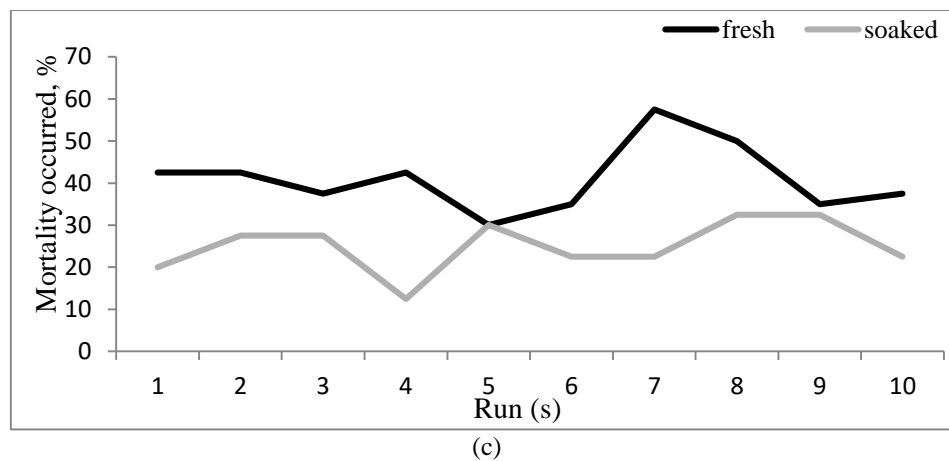


Figure 3.3 Mortality, in reactors with (a) *E. fetida*, (b) *E. eugeniae*, and (c) *P. excavatus*, charged with fresh and soaked forms of water clover

To improve water clover fed vermireactors efficiency, the feed may be pre-conditioned by soaking it for 24 to 48 hours. The other option may be that the reactors if operated in batch mode with the dried form of water clover may be efficient to be utilized in vermicomposting. Earthworms ingest organic waste in different degrees of decomposition, mixed with living microorganisms, microflora, fauna, and nematodes, and their dead remains. To a greater or lesser extent, many species prefer to consume mineral soil fractions and appear to prefer organic wastes mixed with cow manure supplementation than pure organic wastes. However, supplementation of cattle dung may add to the overall cost of the reactor operation in addition to the fact that procuring cow dung is also a tedious task as it is not found commonly as in earlier times.

The leaf in this experiment is a pure organic material, and the earthworms do not prefer them directly as feed during the initial phase, as indicated in many instances, due to its non-decomposed (or less decomposed) state (Doube *et al.*, 1997). Moreover, the non-preference of earthworms to the water-clover-feed is due to biopolymers, and polyphenolic compounds, as they need a suitable processing before being subjected into vermicomposting (Rosa *et al.*, 2011). Recycling agricultural, industrial or urban organic wastes can be possible by vermicomposting. However, to make them acceptable to earthworms, the feed/substrate may need some pre-treatment or pre-processing before subjecting them to vermicomposting; pre-composting, washing, mixing the organic matter or macerating are such preliminary treatments (Edwards *et al.*, 2011). Moreover, the limiting parameters such as temperature and seasonal changes tend to affect both earthworms' growth, and waste stabilization drastically; for the best vermireactor efficiency, degradable organic wastes should be pre-treated/pre-conditioned/pre-composted to have them acceptable for earthworms (Edwards, 1988). The finding in the present study that the earthworms survived and generated vermicast without cow dung is significant.

IV. SUMMARY AND CONCLUSION

All the three epigeic species of earthworms tested by us – *E. fetida*, *E. eugeniae*, and *P. excavatus*, grew and reproduced in vermireactors fed with fresh and soaked forms of water clover as the feed, over a five-month span.

Regarding the vermiconversion efficiency of the feed (as observed from the mass of vermicast generated in 15 days of a given feed rate), the soaked form of water clover was vermiconverted faster ($p=.002$) than the fresh form of the feed. The vermicast produced was higher in reactors with *E. fetida*, followed by *E. eugeniae*, and *P. excavatus*. The average vermicast recovery per animal per day was 26.5 mg, 24 mg, and 22.9 mg in the soaked form against 21.1 mg, 20.4 mg, and 15.9 mg achieved by the fresh form of the feed with *E. fetida*, *E. eugeniae*, and *P. excavatus* respectively. The zoomass of *E. fetida* and *P. excavatus* increased over time only in the soaked form of feed; *E. fetida* gained zoomass than *P. excavatus* while, *E. eugeniae* did not show any improvement. The per worm zoomass gain in *E. fetida* was 10.4 mg; in the case of *P. excavatus*, it was 5.3 mg with soaked forms of the feed. Cocoons were recorded in all the reactors. The number of cocoons formed by 20 individuals of *E. fetida*, *E. eugeniae*, and *P. excavatus* was 4, 6, 7.5 and 6, 4.5, 8 in fresh and soaked forms of the feed respectively.

V. REFERENCES

- [1] Adarsh, P.V., Jaswinder, S., Shahid, H.W., and Salwinder, S.D. 2011. Vermicomposting of tannery sludge mixed with cattle dung into valuable manure using earthworm *Eisenia fetida* (Savigny). *Bioresour. Technol.* 102, 7941-7945
- [2] Ahmad, A., Ghufuran, R., and Zularisam, A.W. 2010. Phytosequestration of Metals in Selected Plants Growing on a Contaminated Okhla Industrial Areas, Okhla, New Delhi, India. *Water, Air, and Soil Pollution*, 217(1-4): 255-266.
- [3] Antony, G. S.G., Gajalakshmi, S. Abbasi, S.A. 2015. Sustainable vermicomposting of *Mimosa pudica* in two different forms by employing three epigeic earthworm species, *International Journal of Environmental Science and Engineering Research (IJESER)*, Vol 6(1):1-6.

- [4] Antony, G. S.G., Gajalakshmi, S. 2018 (1). Exploring the Possibility of Utilizing Castor Bean (*Ricinus Communis*) Plant Leaves in Vermicomposting with three Epigeic Earthworm Species. *Journal of Emerging Technologies and Innovative Research (JETIR)*, vol 5(12). 501-507.
- [5] Antony, G. S.G., Gajalakshmi, S. 2018 (2). Feasibility of vermicomposting coral vine (*Antigonon leptopus*) Employing three epigeic earthworm species. *International Journal of Zoology and Applied Biosciences*, vol 3(6). 454-461.
- [6] Ashok-Kumar, C. (1994). State of the Art Report on Vermiculture in India, Council for Advancement of Peoples Action and Rural Technology (CAPART), New Delhi. pp. 60.
- [7] Carver R., and Nash, j., 2012. *Doing Data Analysis with SPSS: Version 18.0*, fifth ed. USA.
- [8] Deka, H., Deka, S., Baruah, C.K., Das, J., Hosque, S., Sarma, H., and Sarma, N.S. 2011. Vermicomposting potentiality of *Perionyx excavates* for recycling of waste biomass of java citronella- An aromatic oil yielding plant. *Bioresour. Technol.* 102, 11212-11217.
- [9] Domínguez, J., 2004. State-of-the-Art and New Perspectives on Vermicomposting Research. (Eds.), *Earthworm Ecology* (2nd ed) by Clive A. Edwards, *CRC Press*, Boca Raton, pp. 401-424.
- [10] Doube, B.M., Williams, P.M.L., Willmott, P.J. 1997. The influence of two species of earthworms (*Aporrectodea trapezoids* and *Aporrectodea rosea*) on the growth of wheat, barley and faba beans in three soil types in the greenhouse. *Soil Biol. Biochem.* 29, 503–509.
- [11] Edwards, C. A., Norman, Q. A., and Sherman, R., 2011. *Vermiculture Technology, Earthworms, Organic Waste and Environmental Management*, CRC Press. 17-19.
- [12] Edwards, C.A., and Burrows, I. 1988 The potential of earthworms composts as plant growth media. In: Edward CA, Neuhauser EF (eds). *Earthworms in waste and environmental management*. SPB Academic Publishing, The Hague; ISBN 90-5103-017-7, pp 21–32
- [13] Gajalakshmi, S., and Abbasi, S. A. 2004. Earthworms and vermicomposting, *Indian J Biotechnol*, 3,486-494.
- [14] Gajalakshmi, S., Ramasamy, E. V., and Abbasi, S.A. 2001. Towards maximizing output from vermireactors fed with cow dung spiked paper waste. *Bioresource Technology*. 79:67-72.
- [15] Gajalakshmi, S., Ramasamy, E.V., and Abbasi, S.A. 2002. High-rate composting–vermicomposting of water hyacinth (*Eichhornia crassipes*, Solms), *Bioresource Technology*, 83:235–239.
- [16] Gajalakshmi, S., Ramasamy, E.V., and Abbasi, S.A. 2005. Composting–vermicomposting of leaf litter ensuing from the trees of mango (*Mangifera indica*). *Bioresource Technology* 96: 1057–1061.
- [17] Gaur, A.C. and Singh, G. 1995. Recycling of rural and urban wastes through conventional composting and vermicomposting. In: Tandon, H.L.S. (Ed.). *Recycling of Crop, Animal, Human and Industrial Waste in Agriculture. Fertilizer Development and Consultation Organization*, New Delhi, India, pp: 31-49
- [18] Hulina, N. 1998 Rare, endangered or vulnerable plants and neophytes in a drainage system in Croatia, *Nat. Croat.*, 7(4): 279–289.
- [19] Invasive-plant-fact-sheet, European water Clover- *Marsilea quadrifolia*. [http:// www.illinois wild flowers .info /grasses/plants /water_clover.html](http://www.illinoiswildflowers.info/grasses/plants/water_clover.html) (accessed on August 2015)
- [20] Ismail, S., A. 1997. *Vermicology: Biology of Earthworms*. Orient Longman, India, 92p.
- [21] Ismail, S.A. 1998. The contribution of soil fauna especially the earthworms to soil fertility. I n: *Proceedings of the Workshop on Organic Farming*, Institute of Research in Soil Biology and Biotechnology, The New College, Chennai, pp. 9
- [22] Johnson, D.M. 1993, *Marsileaceae* Mirbel. In: *Flora of North America North of Mexico*. Volume 2. Pteridophytes and Gymnosperms, Oxford University Press, New York, p. 331-335.
- [23] Makhija M, Gajalakshmi S, and Abbasi SA. 2011. Screening of four species of earthworms for sustainable vermicomposting of *Ipomoea carnea*. In: Suresh MX, Jayanthi C, Balasankar K, Jason UB, editors. *Proceedings of the international conference on green technology and environmental conservation*, Dec 15–17. Chennai: Institute of Electrical and Electronics Engineers; p. 42–46.
- [24] Manna, M.C., Singh, M., Kundu, S., Tripathi, A.K., and Takkar, P.N.1997. Growth and reproduction of the vermicomposting earth- worm *Perionyx excavatus* as influenced by food materials. *Biology and Fertility of Solids*. 24 (1), 129-132.
- [25] Nayeem-Shah, M., Gajalakshmi, S., and Abbasi, S.A. (2015) Direct, Rapid and Sustainable Vermicomposting of the Leaf Litter of Neem (*Azadirachta indica*). *Appl Biochem Biotechnol* 175:792–801
- [26] Rosa E. Q., C., Jorge, L., F., M. (2011). Plant cell wall degrading and remodeling proteins: current perspectives. *Biotecnología Aplicada*. 28:205-215.
- [27] Serviss, B. E., and Peck, J. H. 2008, New and noteworthy records of several non-native vascular plant species in Arkansas, *J. Bot. Res. Inst. Texas*,2(1): 637-641
- [28] Thiébaud, G. 2007, Invasion success of non-indigenous aquatic and semi-aquatic plants in their native and introduced ranges. A comparison between their invasiveness in North America and France, *Biological Invasion*, 9:1-12.
- [29] Yadav, A., and Garg, V.K. 2011. Industrial wastes and sludge management by vermicomposting. *Environmental Science Biotechnology* 10, 243-276.