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Vermiremediation of an invasive and pernicious weed salvinia (*Salvinia molesta*)

Naseer Hussain, Tasneem Abbasi [1], S.A. Abbasi *

Centre for Pollution Control & Environmental Engineering, Pondicherry University, Puducherry 605 014, India

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ABSTRACT

In evidently the first study of its kind, vermicompost derived solely from salvinia (*Salvinia molesta* Mitchell), arguably the world's most invasive and dominant aquatic weed, has been assessed for its nature and composition. Salvinia is known to contain exceptionally high concentrations of polyphenols, and has unusually ligneous leaves—the two attributes that make it strongly allelopathic and hardy, enabling it to elbow out other vegetation. The present study, supported by UV–vis and FTIR spectrometry, thermo-gravimetric and differential thermal analysis, and GS-MS analysis, reveals that upon vermicomposting salvinia loses its allelopathy. It happens because chemicals responsible for the alleopathy of salvinia are destroyed, transforming the weed into a potent organic fertilizer. Much of the salvinia's lignin is also biodegraded. Scanning electron microscopy shows marked disaggregation of the material in the vermi-compost as compared to well-formed matrix of salvinia leaves. The findings open up the possibility of utilizing billions of tonnes of salvinia biomass – which not only goes to waste at present but is also a cause of serious harm to the environment – in making organic fertilizer.

1. Introduction

Salvinia (*Salvinia molesta* Mitchell) is a free-floating aquatic fern, characterized by its slender stem, floating leaves, and a root-like structure. In the reckoning of a few authors (Thomas and Room, 1986; Abbasi and Nipaney, 1986, 1993; Luque et al., 2014), it is the world's most rapidly colonizing of all weeds. In terms of rate of coverage of water-spreads (Coetzee et al., 2011), as well as net primary productivity (Abbasi and Nipaney, 1995), it is atleast as malevolent, if not more, as water hyacinth—which is widely believed to be the world's worst weed.

When salvinia invades any water-body it soon covers it from bank-to-bank, elbowing out all other free floating aquatic plants and cutting the sunlight off from the benthic vegetation. This restricts the photosynthetic and aerial replenishment of oxygen in the water lying beneath the salvinia carpet, playing havoc with the water's physico-chemical quality. This, in turn, devastates the biodiversity of the invaded wetland by letting none but a few very hardy species survive. Other impacts swiftly follow: loss of fisheries, stagnation of water which facilitates mosquito breeding, and prevention of water-based transport. A salvinia invasion of a water body eventually kills the water body (Ganeshkumar et al., 2014).

Salvinia has resisted all the attempts of control by physical, chemical or biological means (Julien and Griffiths, 1998; Costa-Pierce and Doyle, 1997; King and McIntosh Fitzsimmons, 2004). A few attempts to utilize salvinia biomass for waste management and effluent treatment (Choudhary et al., 2008), and as a source of forage (King and McIntosh Fitzsimmons, 2004; Moozhiyil and Pallauf, 1986a), biogas (Abbasi and Nipaney, 1984, 1985, 1991; Abbasi et al., 1990, 1992a, 1992b), and drugs (Chantiratikul et al., 2009), have been met with equally feeble success. This has left the ever-increasing biomass of salvinia unutilized. It appears that only if processes can be developed for profitably utilizing salvinia, it may not just counterbalance the expenses of mechanically removing salvinia but also exercise some control over its spread.

Among the options available to utilize biowaste is vermiconposting—its defining attributes being low energy requirements, low pollutant emissions, and potentially low cost (Abbasi et al., 2015). It has been known from the experience with vermicomposts derived from animal manure that vermicomposts have nutrients in more bio-available form, with increased humic acid content, and greater cation exchange capacity compared to their parent materials (Gilot, 1997; Atiyeh et al., 2002; Gajalakshmi and Abbasi, 2008; Edwards et al., 2011). Additionally, hormones, enzymes, and pest repellents are believed to be added to the

* Corresponding author.
E-mail address: prof.s.a.abbasi@gmail.com (S.A. Abbasi).
[1] Concurrently Visiting Associate Professor, Department of Fire protection Engineering, Worcester Polytechnic Institute, Worcester, MA 01609, USA.

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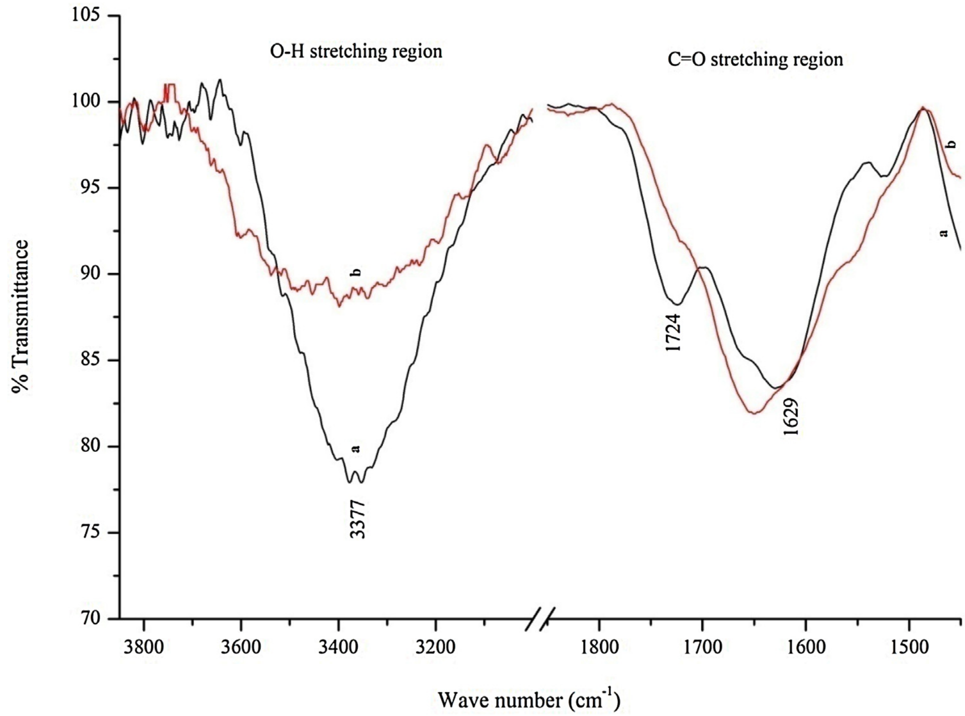

Fig. 1. FT-IR spectra of a) salvinia leaves and b) vermicompost, derived from salvinia leaves.

substrate as it passes through the earthworm gut and gets converted to vermicast (Edwards et al., 2011; Tomati and Galli, 1995; Doube et al., 1997; Abbasi and Ramasamy, 2001). But no past attempt in vermicomposting salvinia or any other phytomass by conventional vermireactors has shown the potential of large-scale utilization (Nayeem-Shah et al., 2014; Abbasi et al., 2015). Indeed the essentiality to pre-compost the phytomass and blend it with animal manure in the reported processes makes those processes inefficient and uneconomical. Moreover, the necessity of adding large quantities of animal manure further limits the viability of the reported methods because animal manure is a prized commodity with several competing uses (Nayeem-Shah et al., 2015).

Recently the authors have managed to overcome these hurdles by employing the concept of high−rate vermicomposting and associated technology developed earlier by S. A. Abbasi and co-workers (Gajalakshmi et al., 2002, 2005; Abbasi et al., 2009, 2011; Tauseef et al., 2013, 2014). With it direct and efficient vermicomposting of salvinia has become possible (Ganeshkumar et al., 2014). Novel, continuously operable, vermireactor systems have also been designed and patented to make way for large-scale vermicomposting (Tauseef et al., 2013).

But before any step is taken towards the detailed engineering of the salvinia vermicomposting process it is essential to establish that salvinia's vermicompost is safe for the soil and the plants and is supportive of plant growth. This question is particularly important for three reasons:

i) Salvinia contains polyphenols in concentrations of the order of 0.4–0.7% (Chantiratikul et al., 2009). This is several times higher than the threshold polyphenol concentration of 0.1% above which a plant becomes allelopathic (Rizvi and Rizvi, 1992). Given the manner in which salvinia explosively grows in water bodies while eliminating other species of plants, it appears certain that it is allelopathic.
ii) Salvinia leaves contain as much as 13.7% by mass of lignin (Moozhiyil and Pallauf, 1986b; Leterme et al., 2010) in contrast to the other major aquatic weed water hyacinth which contains 3.8–5.3% (Fileto-Pérez et al., 2013), and the terrestrial weed lantana which contains 6.30–11% (Kumar et al., 2009; Kwabiah et al., 2011). As lignin is very difficult to biodegrade–it almost totally resists composting and anaerobic digestion–salvinia vermicompost may be high in lignin and not ideal for the soil.
iii) Salvinia propagates vegetatively and even a tiny piece of its leaf can lead to massive salvinia infestation in a water-body. Hence it is necessary to know whether any vestige of undigested salvinia can come in the vermicast to create the risk of salvinia infestation through agricultural run-off in areas which are otherwise free from salvinia.

The present paper reports extensive investigations supported by UV–vis and FTIR spectrometry, thermogravimetric and differential thermal analysis, GS-MS analysis, and scanning electron microscopy to identify the changes that occur when salvinia is converted into vermicompost.

2. Experimental

2.1. General

All chemicals used were analytical reagent grade, and alkali-resistant borosilicate glass was employed throughout. Deionized, double distilled water was used for all analytical work.

Fresh whole plants of salvinia, which are about 1-ft long if measured from tip to toe, were collected from the ponds situated near the Pondicherry University campus. After gently wiping off adhering water the leaves were used directly for vermicomposting employing the epigeic earthworm *Eisenia fetida* in pulse-fed, high-rate, vermireactors in a manner detailed earlier (Ganeshkumar et al., 2014; Nayeem-Shah et al., 2015). No pre-composting or any manure supplementation was done. The vermicast, which was periodically harvested in each pulse, was obtained as a clear and precisely quantifiable product of the vermireactors. The quantities of the substrate and the vermicast were recorded on the basis of dry

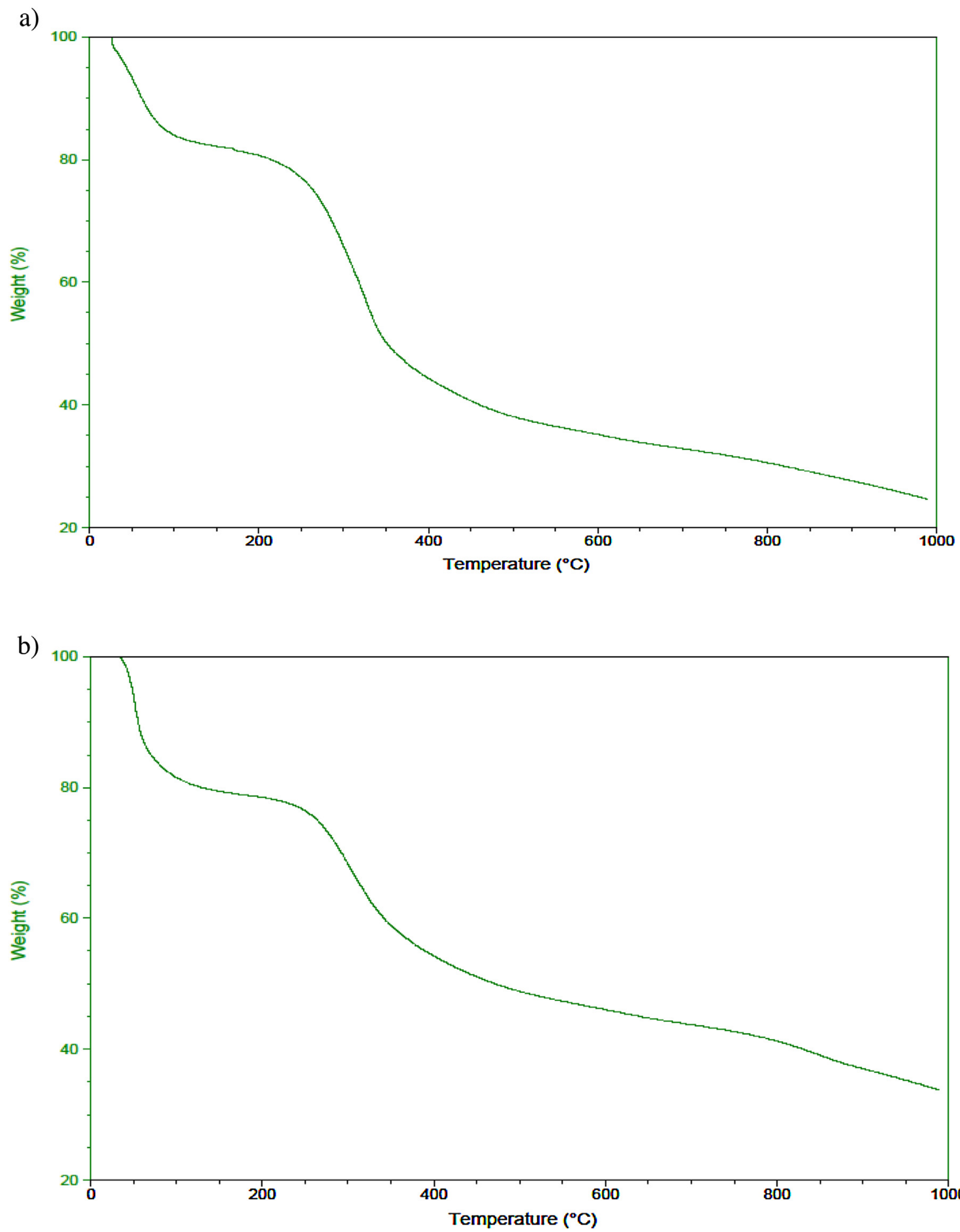

Fig. 2. TG curve of a) salvinia and b) vermicompost, derived from salvinia.

weights obtained by oven drying the samples to constant weight at 105 °C.

2.2. C:N ratio

Carbon and nitrogen were determined by the dichromate redox (Heanes, 1984) and kjeldahl (Kandeler, 1993) methods respectively, using Kel Plus semi-automated digesters.

2.3. UV–vis spectroscopy

The degree of humification occurring as a result of vermicomposting was determined spectrophotometrically with the method of Zbytniewski and Buszewski (Zbytniewski and Buszewski, 2005). In essence it involved taking 1 g each of the initial substrate and the vermicompost in separate 250-mL polyethylene flasks and extraction with 50 ml of 0.5 M NaOH by shaking for 2 h. The contents of each flask were left undisturbed overnight and then centrifuged at 3000 rpm for 25 min. The absorbance (A) of each supernatant

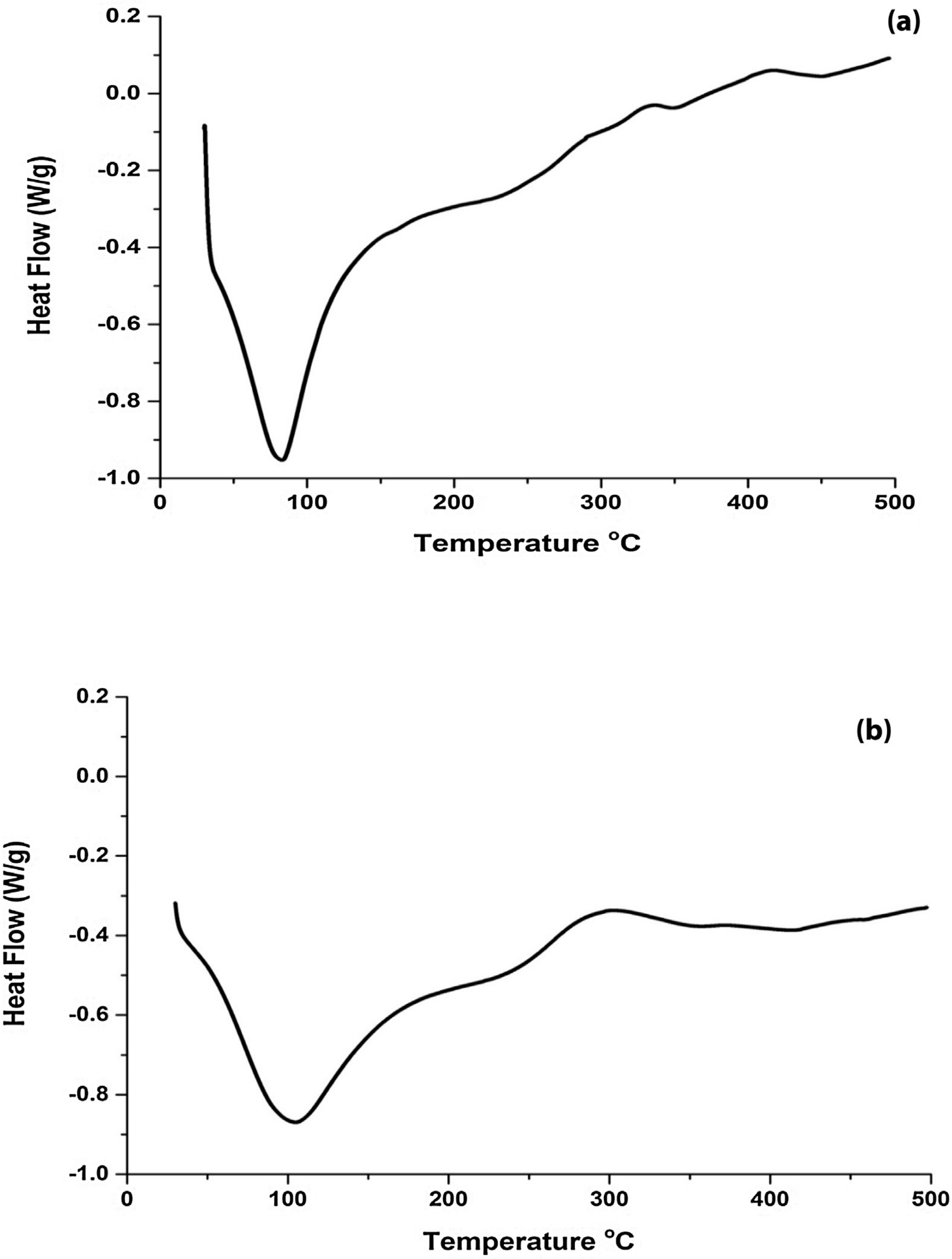

Fig. 3. DSC curve of a) salvinia and b) vermicompost, derived from salvinia.

was measured at 472 nm (A_{472}) and 664 nm (A_{664}). Of these, the absorbance at 472 nm reflects the organic material at the beginning of humification and the absorbance at 664 nm is indicative of strongly humified material (Gieguzynska et al., 1998; Ravindran et al., 2013). The degree of humification–often called the 'humification index' ($Q_{4/6}$)–was calculated by the ratio of A_{472} and A_{664}.

2.4. FTIR spectrometry

The samples were oven dried, finely ground, mixed thoroughly with KBr (spectroscopic grade), homogenized in an agate mortar, and pelletized at a pressure of about 1 MPa. The FT-IR spectra were recorded at a frequency of 0.5 cm/s on a Nicolet iS50 FT-IR spectrometer.

2.5. Thermal analysis

Thermogravimetric analysis (TGA) was performed with a TG analyzer, model SDT Q600 V20.9 Build 20 after the samples (20 mg) were ground in an agate mortar and sieved to ≤2 mm size. The temperature range of 30 °C–1000 °C was explored in a nitrogen atmosphere at a heating rate of 10 °C/min while the manometric pressure was maintained at 101 kPa.

For differential scanning calorimetric (DSC) analysis, the samples (10–20 mg) were loaded in aluminium DSC pan and gravimetric analyses were done under a nitrogen atmosphere, from 0 to 500 °C using a temperature gradient of 5 °C/min. Scans were routinely recorded by duplicates using DSC Q20 V24.2 Build 107.

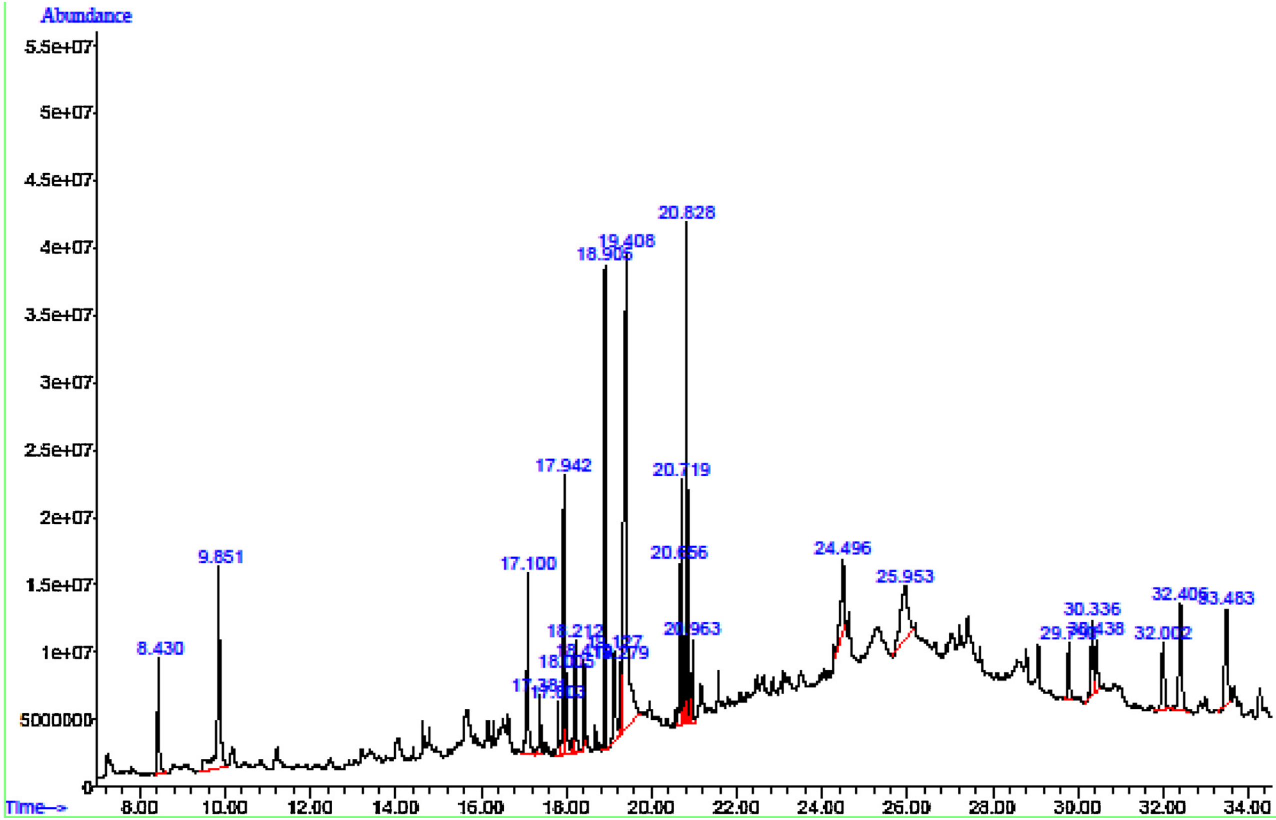

Fig. 4. Spectrum obtained from the GC–MS of salvinia.

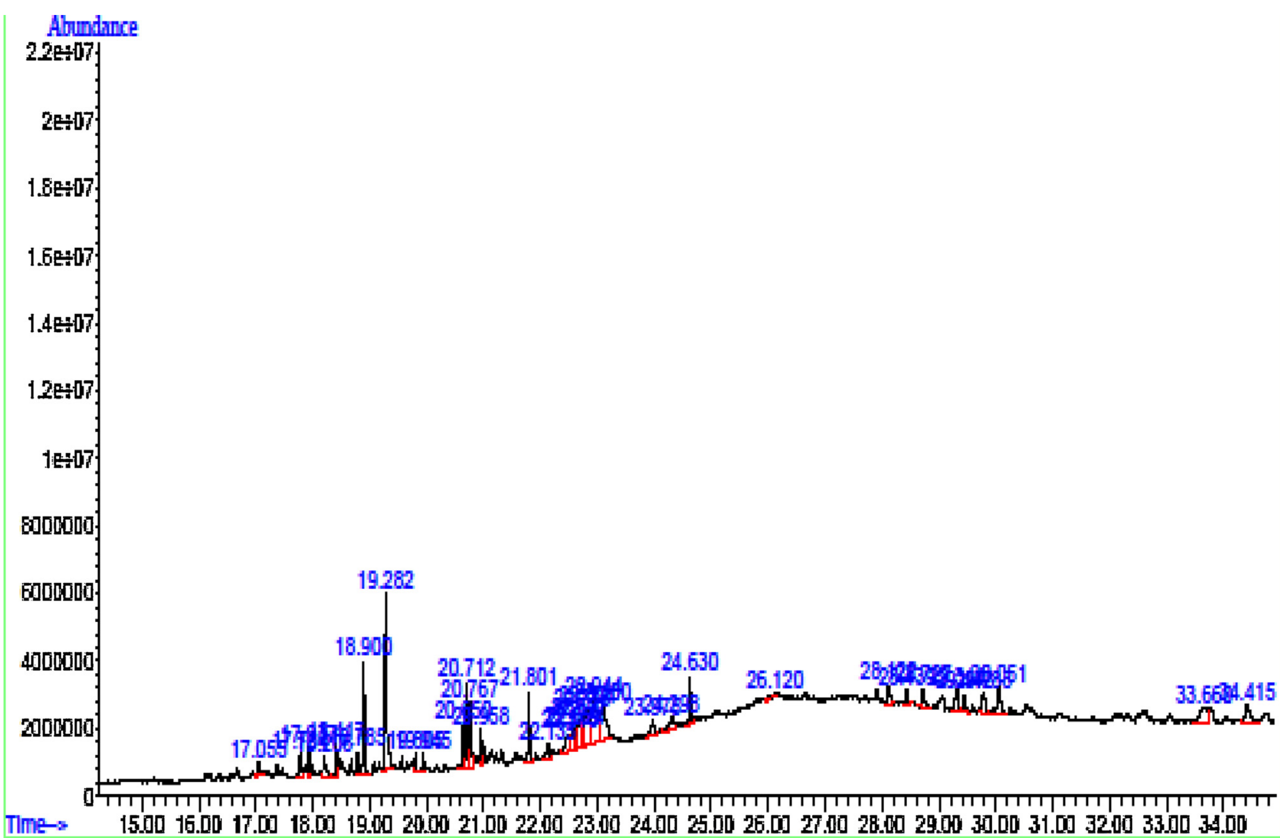

Fig. 5. Spectrum obtained from the GC–MS of salvinia vermicompost.

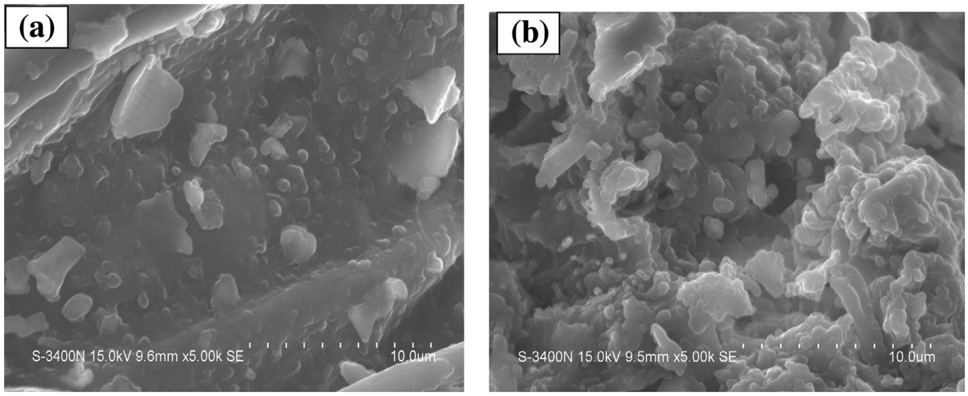

Fig. 6. SEM images of a) salvinia and b) vermicompost, derived from salvinia.

Table 1
The C/N ratio and the humification index values of salvinia and its vermicompost.

Parameter	Plant material	Vermicompost
C/N ratio	53.9 ± 2.9	9.35 ± 0.10
Humification index ($Q_{4/6}$) value	8.72 ± 0.65	1.49 ± 0.12

2.6. GC–MS analysis

The substrate and the vermicompost samples (5 g) were separately mixed with 100 ml of methanol–water (9:1) (v/v), mixtures in 250-mL sterilized conical flasks. They were equilibrated overnight and filtered through Whatman no. 42 (Maidstone) filters. Each extract was evaporated to dryness and the residue was collected, quantified and dissolved in spectroscopic grade methanol. From it, 1 μl samples were injected into the gas chromatograph part of the GC–MS system Agilent 7890A GC interfaced with Agilent 5975C MS. Compounds in the samples were identified by comparing the peaks obtained from the samples with the National Institute of Standards and Technology (NIST) library database (Martinez-Balmori et al., 2013).

2.7. Scanning electron microscopy (SEM)

Powders of dried salvinia leaves and vermicompost were sputtered with gold and their surface morphology was recorded using Hitachi, S–3400 N electron microscope.

3. Results and discussion

3.1. C:N ratio

The C/N ratio of salvinia (Table 1) was reduced sharply (from 53.9 to 9.35), when it got vermicomposted. A C/N ratio below 20 is indicative of significant stabilization of biomass to a level of acceptable maturity as a fertilizer, while a C/N ratio of 15 or less is deemed highly desirable for agronomic use of the compost (Morais and Queda, 2003; Deka et al., 2011). The over 5-fold fall in C/N ratio achieved during vermicomposting of salvinia reflects very high degree of stabilization and indicates the transformation of the weed into a nitrogen-rich substrate of potentially high fertilizer value. The transformation is principally due to the loss of carbon content in salvinia through worm-mediated aerobic biodegradation. Some of the organic nitrogen was also perhaps mineralized by the earthworms and added to the vermicompost in the form of mucus and excreta, further boosting the presence of N relative to C.

3.2. UV–vis spectroscopy

The results (Table 1) reveal that the humification index ($Q_{4/6}$) values of salvinia (8.72) were reduced sharply (to 1.49) as it got vermicomposted. A humification index value less than 5 is indicative of high level of organic material humification (Zbytniewski and Buszewski, 2005; Ravindran et al., 2013). The fall in the humification index achieved in the vermicomposting of salvinia reflects the weed's transformation into a highly humified and stabilized product. In the course of vermicomposting the earthworms fragment the substrate into finer particles with their gizzards. There is subsequent colonization of microorganisms in and around the fragmented substrate (Edwards, 1998; Arancon et al., 2005a). The process accelerates the oxidization, decomposition, and stabilization of the organic matter, and turns it into stable humus (Albanell et al., 1988; Orozco et al., 1996; Masciandaro et al., 2010). This is reflected in the humification index.

3.3. FT-IR spectroscopy

The FT-IR spectra of the substrate and its vermicompost (Fig. 1), show a broad band a 3377 cm^{-1} due to O-H stretching vibrations which are caused by the presence of alcoholic and phenolic hydroxyl groups involved in hydrogen bonds (Ravindran et al., 2013; Hussain et al., 2015). There is another peak at 1724 cm^{-1} which corresponds to the C=O stretching vibration of lignin (Bykov, 2008; Derkacheva and Sukhov, 2008; Xu Rensheng et al., 2012; Teh et al., 2014). The next prominent peak is at 1629 cm^{-1} caused by aromatic C=C vibrations (Mochochoko et al., 2013). Further down there is a peak at 1523 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).

In comparison, the FT-IR spectra of vermicompost shows a marked flattening of the 3398 cm^{-1} peak indicating that a drastic reduction in the phenolic content has accompanied the conversion of salvinia to vermicompost. The peaks at 1724 and 1523 cm^{-1} are absent, reflecting the degradation of lignocellulose, lignin, and carbohydrates contained in salvinia, in the course of vermicomposting.

3.4. Thermogravimetric analysis (TGA)

Thermogravimetric analysis of the substrate and its vermicompost (Fig. 2) indicates dehydration in the temperature range 60–150 °C and decomposition in the range 200–800 °C in both the substrate and its vermicompost (Ravindran et al., 2013). A maximum mass loss of 75.4% occurred in the substrate, while the maximum mass loss in the vermicompost was only 66.25%, indicating net mineralization having occurred in the vermicompost (Deka et al., 2011).

Table 2
List of compounds identified in the methanolic extract of salvinia leaves.

# peak	Retention time	Compound name	Peak Area (%)
1	8.429	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	2.60
2	9.848	4-Mercaptophenol	7.36
3	17.098	Tetradecanoic acid	4.31
4	17.381	2-Benzothiazolamine, 4,6-dimethyl-	1.17
5	17.804	Pentadecanoic acid	1.00
6	17.945	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, (1.alpha.,2.beta.,5.alpha.)	4.36
7	18.005	2-Pentadecanone, 6,10,14-trimethyl	1.61
8	18.213	Pentadecanoic acid	2.25
9	18.421	Phytol, acetate	1.31
10	18.904	Hexadecanoic acid, methyl ester	6.73
11	19.126	cis-9-Hexadecenoic acid	3.21
12	19.275	Dibutyl phthalate	1.02
13	19.409	n-Hexadecanoic acid	23.37
14	20.657	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	2.38
15	20.716	11-Octadecenoic acid, methyl ester	3.47
16	20.828	Phytol	7.93
17	20.961	Methyl stearate	1.28
18	24.497	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	2.79
19	25.953	2,2,4,7-Tetramethyl-3,6,9-trioxa-2 −silatridecane	5.53
20	29.786	Rishitin	1.53
21	30.336	Vitamin E	2.20
22	30.440	26-Nor-5-cholesten-3.beta.-ol-25-one	1.58
23	32.000	Campesterol	2.59
24	32.409	Stigmasterol	4.46
25	33.486	gamma.-Sitosterol	3.95

Table 3
List of compounds identified in the methanolic extract of salvinia vermicompost.

S. No	Retention time	Compounds identified	Peak area (%)
1	17.054	Tetradecanoic acid	1.30
2	17.782	Pentadecanoic acid	1.75
3	17.938	Phytol	1.48
4	18.205	Butanoic acid, 3-methyl-, 3,7-dimethyl-6-octenyl ester	1.63
5	18.421	1,4-Eicosadiene	1.77
6	18.785	Methyl 11-hexadecenoate	1.21
7	18.903	Hexadecanoic acid, methyl ester	5.55
8	19.282	*n*-Hexadecanoic acid	11.30
9	19.802	cis-10-Heptadecenoic acid, methyl ester	0.78
10	19.943	Tridecane	0.88
11	20.649	9,12-Octadecadienoic acid, methyl ester, (E,E)-	2.44
12	20.716	6-Octadecenoic acid, methyl ester, (Z)-	3.79
13	20.768	9-Octadecenoic acid, methyl ester, (E)-	3.28
14	20.961	Heptadecanoic acid, 14-methyl-, methyl ester	1.66
15	21.801	Cyclopropanedecanoic acid, 2-hexyl−, methyl ester	3.47
16	22.135	Cyclopropaneoctanal, 2-octyl-	1.34
17	22.521	Ethanamine, 2-phenoxy-	2.66
18	22.573	2,2-Dimethylpropanoic acid, 2,6-dimethylnon-1-en-3-yn-5-yl ester	1.34
19	22.625	1-Hydroxypyrene	1.27
20	22.699	Pyrene, hexadecahydro-	2.59
21	22.737	1H-Pyrazole-4-carboxylic acid, 5-amino-3-(2-fluorophenyl amino)-, amide	2.39
22	22.826	Pyrene, hexadecahydro-	4.78
23	22.945	1-Hydroxypyrene	8.93
24	23.101	Pyrene, hexadecahydro-	5.82
25	23.977	2-Pyridinamine, *N*-(4,5-dihydro-5-methyl-2-thiazolyl)-3-methyl-	1.75
26	24.297	Cyclopropaneoctanal, 2-octyl-	2.08
27	24.631	Bis(2-ethylhexyl) phthalate	2.54
28	26.124	2-Ethylacridine	0.57
29	28.122	N-Methyl-1-adamantaneacetamide	1.55
30	28.442	1,2-Bis(trimethylsilyl) benzene	0.89
31	28.724	Arsenous acid, tris(trimethylsilyl) ester	1.52
32	29.311	1,2-Bis(trimethylsilyl) benzene	2.19
33	29.445	1,2-Bis(trimethylsilyl) benzene	1.71
34	29.779	t-Butyldiphenyl(prop-2-ynyloxy) silane	2.19
35	30.054	1,2-Bis(trimethylsilyl) benzene	3.42
36	33.657	2-Ethylacridine	3.25
37	34.414	1,2-Bis(trimethylsilyl) benzene	2.93

3.5. Differential scanning calorimetery (DSC)

Differential scanning calorimetery of salvinia (Fig. 3a) yielded an endothermic peak with a maximum temperature at 100–200 °C, representative of dehydration reactions (Dell'Abate et al., 2000). There were two distinct exothermic peaks. Of these, the first peak in the range 250–350 °C could be due to the burning of simpler carbohydrates, lipids, amino acids and other aromatics (Ravindran et al., 2013). The second peak in the range 400–500 °C is possibly due to the dissociation of more complex aromatic structures of larger molecular weights, such as lignin and polyphenols, compared to the simpler compounds which dissociated earlier (Melis and Paola, 2004).

In comparison the first exothermic peak of the vermicompost (Fig. 3b) is much shallower and is seen to have shifted towards lower temperature, evidently due to molecular rearrangements, possibly by the degradation of carbohydrates, aliphatic compounds and other aromatics (Gomez et al., 2007; Fernandez et al., 2012; El Ouaqoudi et al., 2014). The peak in the range 430–500 °C, which was characteristic of highly complex aromatic compounds in the substrate, is absent in the vermicompost. It shows that the vermicomposting process has significantly degraded the highly complex aromatic compounds present in salvinia.

3.6. GC–MS analysis

The GC–MS spectra of the substrate and the vermicompost are presented in Figs. 4 and 5 respectively. There were 25 peaks in the former, as catalogued in Table 2. The maximum peak area was shown by *n*-hexadecanoic acid (23.37%), followed by phytol (7.93%); 4-mercaptophenol (7.36%); hexadecanoic acid, methyl ester (6.73%); 2,2,4,7-tetramethyl-3,6,9-trioxa-2–silatridecane (5.53%); stigmasterol (4.46%); bicycle [3.1.1] heptane, 2,6,6-trimethyl- (4.36%), and Tetradecanoic acid (4.31%). The other compounds had shown a peak area less than 4%.

In contrast, the GC–MS spectrum of vermicompost possesses higher number of peaks (37) in comparison to the substrate peaks (Table 3). The largest peak is of *n*-hexadecanoic acid (11.30%), followed by 1-hydroxypyrene (8.93%); pyrene, hexadecahydro- (5.82%); hexadecanoic acid, methyl ester (5.55%); Pyrene, hexadecahydro- (4.78%); 6-octadecenoic acid, methyl ester, (Z)- (3.79%); cyclopropanedecanoic acid, 2-hexyl-, methyl ester (3.47%); 1,2-bis(trimethylsilyl) benzene (3.42%); 9-octadecenoic acid, methyl ester, (E)-(3.28%); and 2-ethylacridine (3.25%). The other compounds had shown a peak area less than 3%.

The vermicompost is seen to contain higher number of fatty acids, alcohols, alkanes, alkenes, and nitrogenous compounds than the parent substrate even as the peak areas are generally lower than the parent substrate. This indicates that significant fragmentation, bio-oxidation and molecular rearrangements have occurred during the course of vermicomposting. Earthworms are known to digest long chains of polysaccharides, enhancing microbial colonization. Simultaneously the structure of cellulose, hemicellulose and lignin changes, leading to formation of new polysaccharides and humins.

3.7. Scanning electron microscopy

The micrographs of salvinia (Fig. 6a) are indicative of robust and relatively contiguous structures—apparently bound to lignin-containing fibers. In contrast the micrographs of vermicompost reflect disaggregated material (Fig. 6b). When ingesting a substrate, earthworms grind it with their gizzard (Usman et al., 2015); there is further disaggregation as enzymes and several species of bacteria act on the substrate during the course of its passage through the earthworm gut (Ranganathan and Vinotha, 1998; Edwards and Bohlen, 1996; Atiyeh et al., 2000; Arancon et al., 2005b). This facilitates progressive degradation of the substrate which is reflected in the SEM images.

4. Summary and conclusion

Extensive investigations, supported by UV–vis and FTIR spectrometry, thermogravimetric and differential thermal analysis, GS-MS analysis, and scanning electron microscopy have revealed that following major transformations occur when the weed salvinia (*S. molesta*) is vermicomposted by the earthworm *E. fetida*:

(a) A drastic reduction in the carbon-to-nitrogen ratio from 53.9 to 9.35, bringing this ratio within the range that is considered ideal for agronomic usage.
(b) A sharp reduction in the humification index–from 8.72 to 1.49–indicative of substantial mineralization of organic matter.
(c) Near complete degradation of phenols and sesquiterpene lactones which are responsible for the allelopathic impacts of salvinia.
(d) A shift from relatively more complex and larger biomolecules to simpler compounds, leading to less intense exothermic peaks in the differential scanning calorimetry.
(e) A transformation of 25 constituents, with very unevenly distributed concentrations, to 37 constituents of much less varied concentrations.
(f) A shift towards more withered and brittle particle surfaces.

Due to these transformations the allelopathy and other forms of toxicity of salvinia is totally destroyed, making it as good an organic fertilizer as animal manure-based vermicompost is known to be.

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