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Management of Lead Phytoremediated Aquatic Ferns (*Salvinia cucullata* Seg. and *Salvinia molesta* D. Mitch) Through Composting and Vermicomposting

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ABSTRACT

Water is the elixir of life and a universal solvent that carries varietal wastes. This contaminated water with toxic and undesirable heavy metals is a serious environmental problem which may be solved with phytoremediation. In the present investigation, the aquatic fern *Salvinia cucullata* Seg. and *Salvinia molesta* D. Mitch were treated with 50 ppm concentrations of Pb(NO₃)₂ solutions, which was below their lethal concentrations value. A comparative study was undertaken both in composting and vermicomposting by utilizing these treated aquatic ferns to determine the effect upon the quality of soil. All the composting and vermicomposting units enhance soil organic carbon [(1.95% - *S. molesta* vermicompost), (1.65% - Treated *S. molesta* vermicompost), (1.46% - *S. molesta* compost), (1.34% - *S. cucullata* vermicompost)] and NPK content. Between the two sets of treatments, phytoremediated aquatic fern amendments showed less enzymatic activities than the naturally grown aquatic fern amendment treatments in both normal composting and vermicomposting processes. The vermicompost made of treated aquatic ferns showed EC (electrical conductivity) and Pb (lead) within the permissible limit and was found little higher [(128.48 mg/kg - Treated *S. cucullata* compost), (97.13 mg/kg - treated *S. molesta* compost) in composting unit. However, the results of the present investigation indicated that the conversion of aquatic fern biomass into compost or vermicompost can be recommended as an effective and eco-friendly technology for not only to overcome their disposal problem but also can be used as fertilizer for sustainable production in agriculture.

1. Introduction

Aquatic macrophytes have shown to be one of the candidates in the aquatic system for pollutant uptake and biological indicators of heavy metal [1]. Aquatic ferns in particular exhibit exorbitant potential to remove various contaminants including heavy metals, organic compounds, and radionuclides from the environment [2-5]. Among various fern genera, *Salvinia*, a free-floating aquatic fern holds a distinct position because of several advantages including high productivity and tolerance to a wide range of temperatures [2]. Several species of *Salvinia* including *S. cucullata*, *S. molesta*, *S. herzogii*, *S. minima*, *S. natans*, *S. rotundifolia* show potential to remove various contaminants including heavy metals from wastewaters [2, 5-8]. Moreover, use of *Salvinia* in this manner still leaves the problem of its disposal unsolved.

In view of the problem of waste disposal as well as environmental pollution, an investigation on the use of phytoremediated biomass as compost or vermicompost has been carried out to find out its nutritional value and the enhanced soil C: N ratio. The utilization of such compost in crop field or agricultural fields is also being recommended since huge harvested phytoremediated biomass can be used as organic manure or bio-fertilizer without any harmful effect on the environment.

Vermicomposting is one of the method through which organic manure and other agricultural organic waste can be recycled for maintenance of soil organic matter and for sustaining soil productivity [9, 10]. It is an accelerated process of bio-oxidation and stabilization of organic waste involving interactions between earthworms and microorganisms [11]. Vermicomposting, with *Salvinia molesta* showed an efficient vermicompost production [12, 13]. Vermicomposting of aquatic weeds like water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*) and cattail (*Typha angustata*) using *Perionyx excavatus* recorded significantly higher macronutrient values as compared to normal composts [14].

In the present investigation an attempt was made to study the conversion of the *Salvinia cucullata* and *Salvinia molesta* biomass into a valuable vermicompost for managing its disposal problem as well as saving the environment from pollution. The experiments were aimed to know the variation in macronutrient and microbial activities in the compost and vermicompost produced through *Salvinia cucullata* and *Salvinia molesta* worked with *Perionyx excavatus*. It is expected that through composting and vermicomposting process the huge harvested biomass of phytoremediation can be used as organic manure or bio-fertilizer for sustainable agricultural practices.

2. Experimental Methods

2.1 Preparation of Vermicompost Unit

Epigeic species, *Perionyx excavatus* (Perrier, 1872) was obtained from National vermiculture unit (Office of the Soil Chemist), Balia, Balasore, Odisha and maintained in a rearing box by feeding cow-dung for further studies. The fresh aquatic ferns (*Salvinia cucullata* and *Salvinia molesta*) were washed with tap water and 10 g of each plant was exposed to 50 ppm lead nitrate solutions for 15 days, each having three replicates. It was found that almost 90-95% of metal was accumulated by these aquatic ferns. Then the equal amount of both fresh and phytoremediated aquatic fern of *Salvinia* were chopped into small pieces and were fed to the earthworms during vermicomposting.

2.2 Preparation of Vermibed

Vermicomposting units were prepared on clay pots, sized 12 inch height and 9 inch width. The clay pots were filled with some pebbles, sand and then followed by dried cow-dung up to 1/4th of pot height for providing shelter to earthworms and for maintaining moisture. Then the aquatic ferns were mixed with 30 days old cow-dung and some garden soil at 1:2:1 ratio and filled in the pots up to top individually with uniform biomass weight. Simultaneously, only cow-dung and soil in equal proportion were also filled in pots as control. Moisture was adjusted to 50-60% and 25

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numbers of both adult and juvenile earthworms (*Perionyx excavatus*) from rearing box were transferred to each vermicopots and covered with jute gunny sheets, and kept under complete shade. Moisture was maintained regularly by spraying water.

2.3 Experimental Site and Design

The pot experiment was laid out in randomized block design with three replicates at the Botanical garden of Utkal University, Bhubaneswar, Odisha. Altogether there were 27 pots, three replicates in each for control (T), *Salvinia cucullata* vermicompost (S. cu V), *Salvinia cucullata* compost (S.cu C), treated *Salvinia cucullata* vermicompost (T S. cu V), treated *Salvinia cucullata* compost (T S. cu C), *Salvinia molesta* vermicompost (S. m V), *Salvinia molesta* compost (S. m C), treated *Salvinia molesta* vermicompost (T S. m V), treated *Salvinia molesta* compost (T S. m C).

2.4 Analysis of Physico-Chemical Properties

The formation of vermicasting was observed after 15 days from the date of inoculation of the earthworms. The number of days for 100% conversion of filled feed material into vermicastings was recorded. The vermicastings were harvested, and stored for further studies. The harvested vermicomposts were analyzed for physical and chemical properties such as pH, electrical conductivity, soil moisture, organic carbon, nitrogen, phosphorous, potassium and lead at Department of Botany, Utkal University, Vani Vihar, Bhubaneswar, Odisha. Chemical parameters like pH and electrical conductivity (EC) were determined by using digital pH and conductivity meters. Organic carbon (OC) was estimated by following the procedure of Walkley and Black [15] rapid titration method. Nitrogen content was measured in terms of nitrite by titrimetric method. Phosphorous content in form of phosphate was measured by spectrophotometric method and potassium by flame photometer [16]. Pb was estimated by atomic absorption spectrophotometer (AAS). Soil respiration (CO₂ evolution) was measured by alkali absorption method [17, 18]. Soil cellulase activity (indicates of microbial population) was assayed by dinitrosalicylic acid method [19].

All the experiments were done in triplicates and the data were analyzed statistically and standard errors of mean (SEM) was calculated.

3. Results and Discussion

3.1 Utilization of Pb Treated Aquatic Ferns in Composting and Vermicomposting

Vermicomposting, with its reliance on worms including micro- or macro-organisms, to do the bulk of the work, was a relatively fast way process to make compost than in normal composting. Vermicomposting is a relatively quick process requiring less time to establish. Both in composting and vermicomposting the lead treated *Salvinia* plant materials took more time than normal *Salvinia* plants for the composting process (Table 1). This may be due to the toxic effect of lead on the earthworm and micro-organisms involved in the composting process.

Table 1 Time taken for completion of composting and vermicomposting process

S.No.	Treatments	Time taken for normal composting (in days)	Time taken for vermicomposting (in days)
T ₁	Control (no plant biomass amendment)	71±0.01	60±0.005
T ₂	<i>Salvinia cucullata</i> amendment	105±0.01	83±0.01
T ₃	Pb treated <i>Salvinia cucullata</i> amendment	110±0.03	87±0.005
T ₄	<i>Salvinia molesta</i> amendment	115±0.02	85±0.02
T ₅	Pb treated <i>Salvinia molesta</i> amendment	120±0.02	89±0.01

Values are 3 replicates of ±SEM

3.2 Soil Moisture

It is a physical parameter which is very important in plant nutrient uptake. In the present study (Tables 2 and 3) a higher percentage of soil moisture content was recorded in vermicompost (23 to 11.5%) as compared to normal compost (10.6 to 8.4%). This result indicated that vermicompost added increased porosity to soil and high water holding capacity.

Table 2 Soil moisture content in normal compost after 30 days of incubation

S. No.	Treatments	Fresh weight (in g)	Dry weight (in g)	Difference (in g)	Moisture content (%)
T ₁	Control (no plant biomass amendment)	5	4.47	0.53	10.6
T ₂	<i>Salvinia cucullata</i> amendment	5	4.57	0.43	8.6
T ₃	Pb treated <i>Salvinia cucullata</i> amendment	5	4.58	0.42	8.4
T ₄	<i>Salvinia molesta</i> amendment	5	4.56	0.44	8.8
T ₅	Pb treated <i>Salvinia molesta</i> amendment	5	4.57	0.43	8.6

Table 3 Soil moisture content in vermicompost after 30 days of incubation

S. No.	Treatments	Fresh weight (in g)	Dry weight (in g)	Difference (in g)	Moisture content (%)
T ₁	Control (no plant biomass amendment)	5	3.85	1.15	23
T ₂	<i>Salvinia cucullata</i> amendment	5	4.28	0.71	14.3
T ₃	Pb treated <i>Salvinia cucullata</i> amendment	5	4.42	0.57	11.5
T ₄	<i>Salvinia molesta</i> amendment	5	3.84	1.16	23.20
T ₅	Pb treated <i>Salvinia molesta</i> amendment	5	3.92	1.07	21.52

3.3 Soil Enzymes Activity

Soil enzymes are good indicators of soil quality since they are closely related to organic matter, physical characteristics, microbial activities and biomass in the soil as well as provide early information about changes in soil quality, and are assessed more rapidly.

3.3.1 Cellulose Activity

The results also revealed that normal compost and vermicompost underwent changes in microbial numbers and enzymatic activities. When the cellulase enzyme profiles were compared vermicompost registered greater enzyme activity (0.500 mg/g-S.cu, 0.351 mg/g-TS.cu, 0.481 mg/g-S.m, 0.543 mg/g-TS.m) than normal compost (0.397 mg/g-S.cu, 0.143 mg/g-TS.cu, 0.339 mg/g-S.m, 0.401 mg/g-TS.m) irrespective of period of incubation. Between the two sets of treatments, phytoremediated plant amendments showed less enzymatic activities than the naturally grown plant amendment treatments in both normal composting and vermicomposting processes found both in *S.cucullata* and *S. molesta* (Tables 4 and 5). The lower enzymatic activities of normal composting units might be attributed to the slow and incomplete stabilization of organic matter. More enzyme activities in vermicompost as compared to normal compost lead to quicker decomposition process by the presence of earthworms and aerobic heterotrophic microbial population. Haritha [20] in their study reported that the enzyme activities were more abundant in vermicompost than in normal compost leading to the conversion of agricultural waste into value added product. Lower enzymatic activities in the phytoremediated plant amended treatments of compost and vermicompost might be due to the toxic effect of the phytoaccumulated plant amendment.

Table 4 Soil enzyme activity (cellulase) on 15th day of incubation

S.No.	Treatments	Cellulase content (mg/g soil)	
		Normal composting	Vermicomposting
T ₁	C	0.154±0.03	0.165±0.07
T ₂	S.cu	0.198±0.05	0.250±0.02
T ₃	TS.cu	0.071±0.03	0.175±0.01
T ₄	S.m	0.219±0.01	0.290±0.05
T ₅	TS.m	0.200±0.01	0.271±0.01

Values are 3 replicates of ±SEM; Where, C-control, S.cu-*Salvinia cucullata*, TS.cu-treated *Salvinia cucullata*, S.m-*Salvinia molesta*, TS.m-treated *Salvinia molesta*.

Table 5 Soil enzyme activity (cellulase) on 30th day of incubation

Sl.No.	Treatments	Cellulase content (mg/g soil)	
		Normal composting	Vermicomposting
T1	C	0.308±0.02	0.331±0.01
T2	S.cu	0.397±0.04	0.500±0.03
T3	TS.cu	0.143±0.01	0.351±0.01
T4	S.m	0.339±0.02	0.481±0.05
T5	TS.m	0.401±0.02	0.543±0.03

Values are 3 replicates of ±SEM; Where, C-control, S.cu-*Salvinia cucullata*, TS.cu-treated *Salvinia cucullata*, S.m-*Salvinia molesta*, TS.m-treated *Salvinia molesta*.

3.4 Soil Respiration

Soil basal respiration, as measured by carbon dioxide production, is a direct indicator of microbial activities and indirectly reflects the availability of organic material [21, 22]. During the present investigation, the vermicompost units recorded more CO₂ evolution than normal compost units (Tables 6 and 7). Further, it was observed that increased CO₂ evolution was possible by increasing the period of incubation in both the compost units. The findings of the present investigation corroborates with the results obtained by Marinari [23] who found that addition of organic amendments stimulated soil basal respiration. This finding can be interpreted as a synergistic effect of soil and amendment of micro-organisms or to the stimulation of microbial growth by organic substrates in the amendments. Most of the carbon supplied by these amendments consists of partially decomposed material, easily degradable for use as energy and nutrient sources for soil microorganisms. Vermicompost reflected an increased soil microbial respiration as compared to normal compost. Earlier report also supported this findings [24].

Table 6 Soil CO₂ evolution on 15th day of incubation in both composting and vermicomposting

Sl. No.	Treatments	Soil CO ₂ evolution(mg/m ² /h ⁻¹)	
		Normal composting	Vermicomposting
T ₁	C	0.041±0.01	0.058±0.05
T ₂	S.cu	0.27±0.03	0.44±0.01
T ₃	TS.cu	0.073±0.07	0.090±0.01
T ₄	S.m	0.35±0.01	0.50±0.02
T ₅	TS.m	0.10±0.03	0.27±0.02

Values are 3 replicates of ±SEM; Where, C-control, S.cu-*Salvinia cucullata*, TS.cu-treated *Salvinia cucullata*, S.m-*Salvinia molesta*, TS.m-treated *Salvinia molesta*.

Table 7 Soil CO₂ evolution on 30th day of inoculation in both composting and vermicomposting

Sl. No.	Treatments	Soil CO ₂ evolution(mg/m ² /h ⁻¹)	
		Normal Composting	Vermicomposting
T ₁	C	0.073±0.03	0.15±0.03
T ₂	S.cu	0.55±0.01	0.76±0.01
T ₃	TS.cu	0.18±0.01	0.33±0.05
T ₄	S.m	0.70±0.01	0.88±0.02
T ₅	TS.m	0.36±0.03	0.58±0.03

Values are 3 replicates of ±SEM; Where, C-control, S.cu-*Salvinia cucullata*, TS.cu-treated *Salvinia cucullata*, S.m-*Salvinia molesta*, TS.m-treated *Salvinia molesta*.

3.5 Physico-Chemical Changes during Composting and Vermicomposting

The effect of compost and vermicompost on their physical and chemical properties is presented in the Tables 8 and 9. A significant difference was noticed in each physical and chemical property of the prepared aquatic weed amended compost and vermicompost. The pH value in composting units ranged 8.03 to 8.41 whereas in vermicomposting units it was 8.13 to 8.33. The highest amount of EC was recorded in control (2.04±0.06 ds/m) whereas in all the vermicompost treatments EC value had been recorded lower than the compost units. The percentage of organic carbon was found highest in S.mV (1.95±0.02) followed by T S.mV (1.65±0.08) and S.cuV (1.34±0.02) as compared to control (0.91±0.06). In composting units the organic carbon percentage was found highest in S.mC (1.46±0.01) followed by, S.cuC (1.18±0.01) compared with control (0.56±0.01). Mulongoy and Bedoret [25] reported that there was significantly higher amount of organic carbon and total nitrogen in vermicomposting soil than normal compost. In the present investigation, the major macronutrients NPK values were found more in vermicompost than in normal compost irrespective of *Salvinia* species amended. This result also indicated that earthworms increased the amount of extractable nitrogen by feeding on aquatic weeds biomass. Reddy and Okhura [26] reported that rice straw vermicompost possessed higher concentration of total nitrogen than normal compost.

Phosphorous is an essential nutrient element for all organisms and is added in agricultural fields in large quantities to obtain enhanced crop yield. The level of phosphorous available to plant also depends on its mobilization. When phosphorus is added to the soil as fertilizer it becomes an integral part of the phosphorous cycle [27]. Pradhan and Gupta [28] described the high amount of phosphorous found in paper mill sludge compost and vermicompost. In the present study except control both in compost and vermicompost, the phosphorous content was significantly increased. Increased phosphorous content in the aquatic ferns amended compost and vermicompost further indicated its usefulness as a fertilizer. Hence, this type of compost and vermicompost can be used as a phosphorus supplement in phosphorus deficient soil. Like nitrogen and phosphorous, potassium is also an essential nutrient element for

organisms and it is taken up by crops in large quantities. When the available potassium in the soil is below than the required amount, fertilizers and organic manures are added for enhanced crop yield. During the present study the potassium content in composting units were recorded as T (201.73±0.897), S.cuC (279.34±0.95), TS.cuC (310±0.45), S.mC (234.42±0.32) and TS.mC (275.54±0.51) kg/ha respectively (Table 8). Similarly the results of the vermicompost units (Table 9) with respect to K content were recorded as T (201.73±0.89), S.cuV (339.23±0.39), TS.cuV (328±1), S.mV (296.18±0.92), TS.mV (305.24±0.95) kg/ha which seems to be little higher than normal compost.

Table 8 Physical and chemical properties of different composting units

Parameters	T	S.cuC	TS.cuC	S.mC	TS.mC
pH	8.03±0.05	8.3±0.09	8.41±0.01	8.12±0.03	8.22±0.07
Electrical conductivity (ds/m)	2.04±0.06	1.83±0.06	1.96±0.01	1.60±0.02	1.71±0.01
Organic carbon (%)	0.56±0.01	1.18±0.01	1.03±0.01	1.46±0.01	1.18±0.05
Nitrogen (kg/ha)	167±1.09	503.3±0.13	447.3±0.03	540.3±0.42	518.2±1.35
Phosphorous (kg/ha)	38.3±0.38	171.8±0.25	183.2±0.42	203±0.46	155.4±0.48
Potassium (kg/ha)	194.1±0.08	279.3±0.95	310±0.45	234.4±0.32	275.5±0.51
Lead (mg/kg)	21.16±0.07	11.19±0.03	128.48±0.03	8.51±0.04	97.13±0.07

Data represents mean value of three determinations ± SEM; Where, T-control, S.cuC-*Salvinia cucullata* compost, TS.cuC- treated *Salvinia cucullata* compost, S.mC- *Salvinia molesta* compost, TS.mC-treated *Salvinia molesta* compost.

Earthworms have the ability to tolerate many kinds of chemical contaminants including heavy metals [29, 30]. This tolerance may be a positive attribute for assessing bioaccumulation or toxicity studies of severely contaminated sites. Earthworms are known to accumulate metals from the soil efficiently as observed by various authors [31-35]. The maximum permissible limit of the heavy metal- Pb in soil is 100 mg/kg according to CPCB (Central Pollution Control Board). In the present study it was observed that all the Pb treated aquatic ferns-vermicomposting units were within the permissible limit except TS.cuC (128.48±0.039 mg/kg). The lower concentration of Pb in the vermicompost added soil may be correlated with the ingestion of the metal Pb by the earthworm (*Perionyx excavatus*) thereby changing their ionic state in their gut through physiological action and render them in bio-available form for plants when excreted out.

Table 9 Physical and chemical properties of different vermicompost units

Parameters	T	S.cuV	TS.cuV	S.mV	TS.mV
pH	8.33±0.02	8.24±0.02	8.32±0.01	8.22±0.015	8.13±0.01
Electrical conductivity (ds/m)	2.03±0.02	1.78±0.03	1.84±0.05	1.62±0.01	1.66±0.03
Organic carbon (%)	0.91±0.06	1.34±0.02	1.07±0.06	1.95±0.02	1.65±0.08
Nitrogen (kg/ha)	211±2.30	528.9±1.02	489.6±0.26	615.5±0.72	575.8±2.7
Phosphorous (kg/ha)	25.4±1.46	197.7±0.64	190.5±0.32	210.3±0.55	193.3±0.88
Potassium (kg/ha)	201.7±0.8	339.2±0.39	328.1±1.01	296.1±0.92	305.2±0.95
Lead (mg/kg)	13.26±0.1	9.99±0.01	48.87±0.59	9.08±0.04	89.43±0.29

Data represents mean value of three determinations ± SEM; Where, T-control, S.cuV- *Salvinia cucullata* vermicompost, TS.cuV- treated *Salvinia cucullata* vermicompost, S.mV- *Salvinia molesta* vermicompost, TS.mV-treated *Salvinia molesta* vermicompost.

4. Conclusion

The present experiment indicated that good quality vermicompost can be easily prepared from lead phytoremediated *Salvinia* species and thus the disposal problem of phytoremediated aquatic weeds can be managed by their large scale utilization in making vermicompost, as useful organic manure. Earthworms in general are highly resistant to many chemical contaminants, organic and inorganic pollutants in soil and usually bio-accumulate them in their tissues without being affected. Earthworms can bio-accumulate high concentrations of metals including heavy metals lead in their tissues without affecting their physiology. In both the species of

Salvinia the vermicomposting recorded a significantly higher value than conventional composting. This vermibiotechnology is a positive approach for maximum utilization of heavy metals phyto remediated aquatic weeds. An additional benefit of this novel biological treatment system is to recover absorbed nutrients as a result of vermicomposting of the harvested plant materials. Since vermicomposting of aquatic weeds could optimize the soil C: N ratio and other soil metabolic activities, further extensive work can be taken up by soil scientists for enhancement of soil fertility. This technology can be transferred to the farmers through training programme and awareness campaign.

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