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Short communication

Vermistabilization of seaweeds using an indigenous earthworm species, *Perionyx excavatus* (Perrier)

Ramachandran Ananthavalli[a], Venkatasamy Ramadas[b], James Arockia John Paul[a], Balan Karunai Selvi[c], Natchimuthu Karmegam[d,*]

[a] Department of Zoology, Arumugam Pillai Seethai Ammal College, Tiruppattur 630 211, Tamil Nadu, India
[b] Department of Zoology, Raja Doraisingam Government Arts College, Sivagangai 630 561, Tamil Nadu, India
[c] Department of Botany, V. V. Vanniaperumal College for Women (Autonomous), Virudhunagar 626 001, Tamil Nadu, India
[d] Department of Botany, Government Arts College (Autonomous), Salem 636 007, Tamil Nadu, India

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ABSTRACT

The seaweeds, *Gracilaria edulis, Turbinaria ornata* and *Ulva reticulata* collected from Mandapam East Coast region of Tamil Nadu, India has been subjected to vermicomposting in combination with cowdung (CD) in 1:1 combination for 60 days under laboratory conditions. The percentage decomposition was ranged between 83.70 and 87.00 in the vermicompost of seaweed + CD combinations while it was 30.71 and 35.12% in worm-unworked compost where the decomposition rate of substrates with earthworms is significantly ($P < 0.05$) higher than the substrates without earthworms after 60 days. The physico-chemical characteristics of the vermicompost showed increased levels of electrical conductivity, NPK, while organic carbon, C:N ratio and organic matter content showed reduction in all seaweed + CD substrates. A higher amount of NPK of 1.38, 0.69 and 2.41% respectively in the vermicompost of *Turbinaria ornata* + CD was recorded with the respective percentage increase of 50.00, 68.29, and 34.64 over compost, and the values were significantly higher ($P < 0.05$) than in the initial substrate as well as the worm-unworked compost of *Turbinaria ornata* + CD. The activity of earthworms during vermicomposting of seaweed substrates significantly increased the total microbial population.

1. Introduction

Vermicomposting is a viable and eco-friendly technology where the organic materials are converted into vermicompost through the joint action of earthworms and microorganisms (Domínguez, 2018). Vermicomposting is given much attention now-a-days, due to the environmental concern, on the one hand organic waste recycling and on the other hand, nutrient rich vermicompost production for sustainable agriculture (Sharma and Garg, 2018a). The epigeic earthworms are suitable candidates for organic materials conversion into vermicompost where, *Eisenia fetida, Eudrilus eugeniae* and *Perionyx excavatus* are used world-wide for vermicomposting as these earthworms thrive in most environmental conditions excepting deserts and colder parts of the world. *Perionyx excavatus* an Asiatic indigenous earthworm species is considered to be the one of the best suited epigeic earthworms for vermicomposting in countries like India (Kale and Karmegam, 2010). A variety of organic waste materials in combination with bulking materials like cowdung have been successfully vermicomposted by using the earthworm, *Perionyx excavatus* (Pattnaik and Vikram Reddy, 2010; Deka et al., 2011; Parthasarathi et al., 2016; Sudharsan Varma et al., 2016; Yuvaraj et al., 2018a), and there are many organic substrates that can be used for vermiconversion into vermicompost are un-noticed. One such big resource is seaweeds which are plentily available in coastal areas. Already, the plentiful seaweed resources are utilized directly as fertilizer or used for compost and liquid fertilizer production for agricultural use (Illera-Vives et al., 2015; Mohanty et al., 2013; Zhang and Sun, 2017; Rani Juneius et al., 2018). These studies reiterate that the seaweeds can be used for compost or fertilizer production for better crop production and sustainable soil health. However, the utilization of these seaweeds as substrates for vermicompost production is very limited, and there is a need to standardize the method of vermicomposting seaweeds in view of their availability and nutrient contents. In consideration of the above facts, the present study has been undertaken to utilize the seaweeds available in Mandabam and Thondi Coastal region, Ramanathapuram District, Tamil Nadu for vermicomposting in combination with cowdung using the earthworm, *Perionyx excavatus*.

* Corresponding author.
E-mail address: kanishkarmegam@gmail.com (N. Karmegam).

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2. Materials and methods

2.1. Collection of seaweeds, cowdung and earthworms

The seaweeds abundantly found in the Mandabam and Thondi Coastal region, Ramanathapuram District, Tamil Nadu were collected based on the preliminary survey. Herbarium was prepared for each alga and the portion of same alga was preserved in 5% formalin in seawater as voucher specimens which were used for identification (Umamaheswara Rao, 1970, 1987; Krishnamurthi and Joshi, 1970; Oza and Zaidi, 2001). Three different seaweeds, *Gracilaria edulis* (red alga), *Turbinaria ornata* (brown alga), and *Ulva reticulata* (green alga) were identified and the voucher specimens were deposited in the Department of Zoology, Arumugam Pillai Seethai Ammal College, Tiruppattur, Tamil Nadu. The samples were thoroughly washed with sea water and placed in plastic bag and brought to the laboratory. The samples were washed four times with tap water to remove sand and excessive salt.

The cowdung (CD) was collected from nearby cattle sheds in fresh form and allowed to stabilize for one week and used for this study. The stabilization of CD was done to make it acceptable to the earthworms. The seaweeds were pre-decomposed in rectangular draining cement tanks of 75 cm × 60 cm × 45 cm size by sprinkling water, regular mixing and turning of the substrates for 15 days. Native earthworm species, *Perionyx excavatus* was obtained from the Vermiculture Unit of Biology Department, Gandhigram Rural Institute, Gandhigram, Tamil Nadu and was mass multiplied in vermiculture tanks using standard feed, cowdung, and used for vermicomposting studies.

2.2. Vermicomposting of seaweeds

Based on the previous studies reported by Karmegam and Daniel (2009a, b) and John Paul et al. (2011) on vermicomposting of different substrates, and on the preliminary studies with seaweeds, the ratio of organic substrate mix, i.e., 1:1 (50%:50%) proportion on dry weight basis was used in the present study. The pre-decomposed seaweeds were mixed with CD in 1:1 ratio, transferred to compost beds and moistened to hold 60–70% moisture content. For each experimental set, a control set was also maintained without earthworms. All the vermicomposting studies were carried out for 60 days using *Perionyx excavatus* in three replicates under controlled conditions. The physico-chemical characteristics of initial vermibed substrates, composts (worm-unworked) and vermicomposts were analysed as per standard procedures given below and the results were statistically interpreted.

2.3. Percentage decomposition of seaweed substrates

The percentage decomposition of vermibed substrates based on particle size reduction was calculated as follows (Goswami and Kalita, 2000):

$$\text{Percentage decomposition} = \frac{\text{A}-\text{B}}{\text{A}} \times 100$$

where, A = Total weight of organic substrate in the vermibed; B = Weight of decomposed material (sieved through material)

2.4. Physico-chemical analysis of seaweed substrates, compost and vermicompost

The initial substrates and the final substrates i.e., worm-worked (substrates introduced with worms, vermicompost) and the worm-unworked substrates (without earthworms, compost) were analysed for various physico-chemical parameters using standard procedures. pH was determined by a digital pH meter, electrical conductivity (EC) by Elico conductivity meter using 1:10 (w/v) suspension. Total organic carbon (TOC) was measured using the method of Walkley and Black (1934). Total Kjeldhal nitrogen (TKN) was determined after digesting the sample with concentrated H_2SO_4 and concentrated $HClO_4$ (9: 1, v/v) (Tandon, 1993). Total phosphorus (TP) was analysed using colorimetric method with molybdenum in sulphuric acid (Tandon, 1993). Total potassium (TK), calcium (Ca), sodium (Na), magnesium (Mg), copper (Cu), cadmium (Cd), lead (Pb), and zinc (Zn) were analysed by the method described by Tandon (1993), and manganese (Mn) by the method of Chopra and Kanwar (1991). The organic matter content (OMC) was calculated using standard procedure. The ratio of the percentage of carbon to that of nitrogen (i.e., C/N ratio) was calculated by dividing the percentage of carbon estimated for the sample with the percentage of nitrogen estimated for the same sample. The percentage increase/decrease of various physico-chemical parameters in the vermicompost over the initial and worm un-worked substrates (compost) were calculated as per the method of Ramalingam and Ranganathan (2001) and as adopted by Karmegam and Daniel (2009a), and the results were compared statistically (ANOVA).

2.5. Microbiological analysis

The total colony forming units (CFU) of bacteria, fungi and actinomycetes in the vermibed materials at the beginning of the experiment (initial) and at the end of the experiment (worm-worked and worm-unworked substrates) were enumerated using standard plate count method (Subba Rao, 1995). The total microbial population from 0th day onwards up to 60 days, once in 15 days was carried out using the same procedure. The Petri plates with 30–300 colonies were selected for enumeration of bacterial population. The bacterial, fungal and actinomycetes population was expressed as colony forming units (CFU) per gram of the sample. One gram of each sample was taken in a sterile conical flask containing nine ml of distilled water and shaken in a vortex mixer for 30 min. From this stock, various dilutions were prepared from 10^{-1} to 10^{-7} with sterile distilled water. One ml of the diluted sample was poured into Petri plates containing nutrient agar media, Martin's Rose Bengal agar media and Kenknight's media respectively for bacteria, fungi and actinomycetes. Three replicates were maintained for each observation.

2.6. Growth and reproduction of the earthworms

For growth and reproduction studies of earthworms, CD was used as standard rearing medium which served as control. Another batch of the same experimental set up was also maintained to observe the total biomass and the rate of reproduction of the worms in terms of their number. Separate set of experiments was maintained so as to un-necessarily disturb the vermicomposting process. To find out the biomass, the worms were handsorted, counted, washed with water, blotted on Whatman no. 1 filter paper and then weighed in digital balance. Afterwards the worms were immediately introduced into the respective vermibeds. This was done once in 15 days from the start of the experiment up to 60 days. The number of juveniles and the number of cocoons produced were recorded for each experimental set-up till the termination of the study. The growth rate of the worms was calculated as per the method of Mazantseva (1982).

2.7. Statistical analysis

The data were expressed as mean ± standard error (SEM). The physico-chemical characteristics of initial compost bed substrates, composts (worm-unworked) and vermicomposts (worm-worked) were statistically interpreted that includes students'*t*' test and ANOVA. The statistical significance of various treatments was evaluated by one-way analysis of variance (ANOVA) using SPSS version 18.0 (SPSS Inc., Chicago, USA). When there was a significant difference, Tukey's honestly significantly different (HSD) multiple comparison tests were performed at $P < 0.05$ significance level. Two-way ANOVA was used to find out the effect of different seaweed substrates on percentage increase/decrease of different parameters.

Table 1
Physico-chemical characteristics of initial and final seaweed + cowdung (1:1) combinations with and without earthworms (vermicompost and compost) for 60 days.

Parameters	*Gracilaria edulis* + CD			*Turbinaria ornata* + CD			*Ulva reticulata* + CD		
	Initial	Compost	VC	Initial	Compost	VC	Initial	Compost	VC
Decomposition (%)	–	30.71 ± 2.50	85.00 ± 4.35^{s}	–	35.12 ± 3.10	87.00 ± 5.91^{s}	–	32.33 ± 2.65	83.70 ± 5.42^{s}
pH	8.50 ± 0.49	8.11 ± 0.47	7.38 ± 0.43$^{N, n}$	8.53 ± 0.49	7.96 ± 0.46	7.14 ± 0.4$^{N, n}$	8.411 ± 0.49	8.09 ± 0.47	7.33 ± 0.43$^{N, n}$
EC (dS/m)	1.01 ± 0.08	1.31 ± 0.10	1.97 ± 0.15$^{S, s}$	1.11 ± 0.08	1.35 ± 0.10	2.11 ± 0.16$^{S, s}$	1.23 ± 0.09	1.37 ± 0.10	1.99 ± 0.15$^{S, s}$
TOC (%)	30.02 ± 1.50	26.01 ± 1.30	22.00 ± 1.10$^{N, s}$	31.22 ± 1.56	27.20 ± 1.36	22.02 ± 1.10$^{N, s}$	31.06 ± 1.55	28.05 ± 1.54	21.93 ± 1.10$^{S, s}$
TKN (%)	0.77 ± 0.05	0.83 ± 0.05	1.01 ± 0.06$^{S, s}$	0.82 ± 0.05	0.92 ± 0.06	1.38 ± 0.08$^{S, s}$	0.78 ± 0.05	0.98 ± 0.06	1.21 ± 0.07$^{S, s}$
TP (%)	0.342 ± 0.01	0.422 ± 0.03	0.622 ± 0.02$^{S, s}$	0.36 ± 0.01	0.41 ± 0.02	0.69 ± 0.03$^{S, s}$	0.313 ± 0.01	0.394 ± 0.02	0.583 ± 0.02$^{S, s}$
TK (%)	1.431 ± 0.09	1.541 ± 0.09	2.311 ± 0.14$^{S, s}$	1.52 ± 0.09	1.79 ± 0.11	2.41 ± 0.14$^{S, s}$	1.231 ± 0.07	1.33 ± 0.08	2.10 ± 0.13$^{S, s}$
Mg (%)	1.31 ± 0.10	1.49 ± 0.12	1.829 ± 0.15$^{S, s}$	1.32 ± 0.11	1.41 ± 0.11	1.832 ± 0.15$^{S, s}$	1.21 ± 0.10	1.32 ± 0.11	1.683 ± 0.13$^{S, s}$
Mn (%)	0.408 ± 0.02	0.495 ± 0.03	0.691 ± 0.04$^{S, s}$	0.411 ± 0.02	0.491 ± 0.03	0.693 ± 0.04$^{S, s}$	0.391 ± 0.02	0.43 ± 0.03	0.662 ± 0.04$^{S, s}$
Ca (%)	0.322 ± 0.03	0.450 ± 0.04	0.591 ± 0.05$^{S, s}$	0.372 ± 0.03	0.502 ± 0.04	0.621 ± 0.05$^{S, s}$	0.283 ± 0.02	0.375 ± 0.03	0.528 ± 0.04$^{S, s}$
C/N	39.00 ± 1.64	31.30 ± 1.31	21.70 ± 0.91$^{S, s}$	38.00 ± 1.60	29.50 ± 1.24	16.00 ± 0.67$^{S, s}$	39.82 ± 1.67	28.6 ± 1.20	18.12 ± 0.76$^{S, s}$
OMC (%)	51.6 ± 2.79	44.70 ± 2.41	37.84 ± 2.04$^{N, s}$	53.60 ± 2.89	46.78 ± 2.53	37.80 ± 2.04$^{S, s}$	53.42 ± 2.88	48.20 ± 2.60	37.71 ± 2.04$^{S, s}$
Na (%)	0.43 ± 0.04	0.41 ± 0.03	0.36 ± 0.03$^{N, n}$	0.61 ± 0.05	0.57 ± 0.05	0.48 ± 0.04$^{N, n}$	0.49 ± 0.04	0.43 ± 0.04	0.38 ± 0.03$^{S, s}$
Cu (ppm)	1.53 ± 0.10	1.59 ± 0.10	1.63 ± 0.11$^{N, n}$	1.58 ± 0.10	1.64 ± 0.11	1.71 ± 0.11$^{N, n}$	1.13 ± 0.06	1.15 ± 0.07	1.33 ± 0.09$^{N, n}$
Cd (ppm)	0.079 ± 0.01	0.071 ± 0.01	0.068 ± 0.01$^{N, n}$	0.073 ± 0.01	0.071 ± 0.01	0.064 ± 0.01$^{N, n}$	0.421 ± 0.04	0.401 ± 0.04	0.391 ± 0.04$^{N, n}$
Pb (ppm)	0.257 ± 0.02	0.25 ± 0.02	0.243 ± 0.02$^{N, n}$	0.24 ± 0.02	0.233 ± 0.02	0.213 ± 0.02$^{N, n}$	1.136 ± 0.10	1.131 ± 0.10	0.117 ± 0.01$^{N, n}$
Zn (ppm)	0.683 ± 0.05	0.634 ± 0.05	0.621 ± 0.05$^{N, n}$	0.623 ± 0.05	0.571 ± 0.05	0.423 ± 0.03$^{S, s}$	0.11 ± 0.01	0.10 ± 0.01	0.09 ± 0.01$^{N, n}$
Two-way ANOVA[#]									
Source of variation		***SS***	***df***	***MS***	***F***	***P-value***	***F crit***	***Significance***	
Between parameters		39878.68	15	2658.579	40.09953	0.001	2.014804	Highly significant	
Between substrates		59.7803	2	29.89015	0.450835	0.641	3.31583	Not significant	
Error		1988.985	30	66.2995					
Total		41927.45	47						

CD – Cowdung; VC – Vermicompost; Values are mean of three replicates ± SEM; The superscripts ‘S and N’ (vermicompost over initial substrates) and ‘s and n’ (vermicompost over worm-unworked compost) indicate statistically significant difference at $P < 0.05$ and not significant by ‘t’ test respectively, based on the percentage increase/decrease.

[#] Two-way ANOVA results showing the effect of vermicomposting (60 days) on percentage increase/decrease of physico-chemical characteristics of worm worked vermicompost over worm un-worked compost with respect to different substrates.

3. Results and discussion

3.1. Percentage decomposition of seaweed + CD substrates

The percentage decomposition of seaweeds + CD (1:1) composition, based on particle size reduction, after 60 days of vermicomposting with *Perionyx excavatus* showed higher rates than in the worm-unworked compost (Table 1). A maximum of 87% decomposition was recorded in the vermicompost of *Turbinaria ornata* + CD followed by the vermicomposts of *Gracilaria edulis* + CD (85.0%) and *Ulva reticulata* + CD (83.7%) where the values were statistically significantly higher than the values recorded for the worm-unworked composts of all the seaweed + CD combinations ($P < 0.05$). The decomposition rate of seaweed + CD composts ranged from 30.71 to 35.12% only; whereas, it was 83.7–87.0% in seaweed + CD vermicomposts. This clearly shows that the earthworm, *Perionyx excavatus* is well adapted to the feed mixture and decomposed the seaweed + CD combinations efficiently. The mechanical action of earthworms results in the fragmentation of organic substrates while the consumption by the earthworm and digestion in the gut rapidly degrade along with microorganisms in the gut and in the organic materials. Other studies with different organic substrates using epigeic earthworms are also in support of the present study findings. The decomposition rate of leaf litters, weed, pressmud, municipal solid wastes, etc. reported in the earlier studies are coinciding with the present study (Karmegam and Daniel, 2009a, b; Prakash and Karmegam, 2010; John Paul et al., 2011) who reported more than 70% decomposition rate with the earthworms, *Lampito mauritii* and *Perionyx ceylanensis*. Nevertheless, the decomposition of organic waste materials is dependent on the bulking materials like cowdung added along with organic substrates (Sharma and Garg, 2018a).

3.2. Physico-chemical characteristics of vermicompost

The pH of the initial seaweed substrates was towards alkalinity, ranged from 8.41 to 8.53 which showed reduction towards neutrality in composts (pH range: 7.96–8.11) and in vermicomposts (pH range: 7.14–7.96) of seaweed + CD substrates after 60 days. A maximum pH reduction of 7.14 was found in *Turbinaria ornata* + CD vermicompost followed by pH of 7.33 in *Ulva reticulata* + CD vermicompost and 7.38 in *Gracilaria edulis* + CD vermicompost (Table 1). The percentage of reduction of pH ranged between 9.1% and 16.3% in vermicompost over initial substrates and composts of seaweed + CD substrates (Fig. 1A, B and C). A maximum of 16.3% and 10.3% reduction was found in *Turbinaria ornata* + CD vermicompost over initial values and worm-unworked compost (Fig. 1B). However, the decrease in pH in vermicompost over initial substrates and worm-unworked composts did not differ statistically ($P > 0.05$). The change in pH towards neutrality/acidity during vermicomposting is probably due to the production of organic acids, ammonia and the release of carbon dioxide during the progression of decomposition of organic materials assisted by microorganisms and earthworms (Zhang et al., 2015; Ramnarain et al., 2018; Yuvaraj et al., 2018a).

The EC in seaweed + CD vermicomposts showed increase when compared with initial substrates and final worm-unworked composts. The range of EC in initial substrates was 1.01–1.23 dS/m which increased to the range of 1.97–2.11 dS/m in vermicomposts of seaweed substrates after 60 days of earthworm activity. The increase of EC in vermicomposts of seaweed + CD substrates over initial levels was 61.79–95.05% with a significant difference at $P < 0.05$. A higher percentage increase of EC was found in *Turbinaria ornata* + CD vermicompost (56.30%) over the compost of the same substrate followed by *Gracilaria edulis* + CD (50.38%) and *Ulva reticulata* + CD (45.26%) (Fig. 1). The results of the present study are in line with the studies by Shrimal and Khwairakpam (2010), Karmegam et al. (2012) and Yuvaraj et al. (2018b). The freely available minerals and ions generated during ingestion and excretion by the earthworms are the dependent factors for the raise in the level of EC (Garg et al., 2006; Singh and Suthar, 2012). Furthermore, the increase in EC during vermicomposting is mainly due to the release of soluble salts including phosphate and nitrate (He et al., 2016; Yuvaraj et al., 2018a).

The TOC, OMC and C/N in both composts and vermicomposts of seaweed + CD substrates were found to be decreased from initial levels. However, the decrease of TOC, OMC and C/N in worm-unworked compost was lower than that of the decrease observed in vermicomposts. A lower TOC of 21.93% was found in *Ulva reticulata* + CD vermicompost closely followed by *Gracilaria edulis* + CD vermicompost (22.0%) and *Turbinaria ornata* + CD vermicompost (22.02%). This shows that the vermicomposting activity is more or less equal in seaweed substrates. The percentage decrease of TOC in vermicompost over initial substrates of all seaweed + CD combinations was significant ($P < 0.05$) when compared with initial values. Whereas, the percentage decrease of TOC in vermicomposts over respective composts prepared with and without the earthworm *Perionyx excavatus* for 60 days was not significant ($P > 0.05$) excepting the vermicompost of *Ulva reticulata* + CD which showed statistically significant decrease (21.82%) over the compost of the same substrate. The percent decrease of TOC in vermicompost over initial substrates ranged from 26.72 to 29.97% whereas it was 15.42 to 21.82% over respective composts. Similar trend was also observed for OMC as decrease of TOC plays a major role in OMC reduction. The C/N, an important compost parameter also showed reduction in all final composts and vermicomposts of seaweed substrates. The lowest C/N of 16.0 was recorded in the vermicompost of *Turbinaria ornata* + CD followed by the vermicompost produced from *Ulva reticulata* + CD (18.12) and *Gracilaria edulis* + CD (21.70) (Table 1), and the respective percentage decrease was 45.76, 36.64 and 30.67% over the composts (Fig. 1A, B and C). The homogenization, fragmentation and mineralization of organic materials by the combined action of earthworms and microorganisms are the key factors for the reduction of TOC, C/N and OMC (Singh and Suthar, 2012; Parthasarathi et al., 2016; Lim and Wu, 2016; Suthar et al., 2017). Notably, TOC and C/N are the most traditional indicators of compost maturity (Malafaia et al., 2015).

The TKN, TP and TK contents in vermicomposts ranged from 1.01 to 1.38%, 0.583 to 0.69% and 2.10 to 2.41% respectively, and these values were significantly higher than that of initial substrates and worm-unworked composts ($P < 0.05$). A higher percentage increase of TKN was found in vermicompost of *Turbinaria ornata* + CD (50.0%) over the compost of the same substrates while it was 68.29% for TP in *Turbinaria ornata* + CD vermicompost and 57.89% for TK in *Ulva reticulata* + CD vermicompost respectively over their composts (Fig. 1). The percentage increase of TKN, TP and TK in all seaweed + CD vermicomposts were significantly higher ($P < 0.05$) over their respective values in initial substrates and composts. Similar results were also observed for Mg, Mn and Ca. Na in vermicomposts showed non-significant decrease percentage which ranged between 11.63% and 15.79% over composts. The Cu, Cd and Pb levels in vermicompost showed non-significant decrease (Fig. 1). Excepting the vermicompost of *Turbinaria ornata* + CD that showed significant decrease ($P < 0.05$) over compost, while all the other vermicomposts showed non-significant reduction of Zn. The variation in different physico-chemical parameters between three different seaweed substrates as compared with Two-way ANOVA showed that the difference in changes between parameters was highly significant ($P < 0.001$) whereas, it was not significant between the substrates ($P < 0.641$) (Table 1) implying that vermiconversion of seaweed substrates by the earthworm *Perionyx excavatus* is consistent. The increase of macronutrients such as TKN, TP and TK along with other nutrients implies that the vermicomposting process enriches the substrates with nutrients by mineralization. The studies conducted by other researchers also fall in line with the present study. Soobhany et al. (2015) opined that the nutrient levels in vermicompost depend on the type and amount of organic waste materials used as substrate. While vermicomposting silver oak and bamboo leaf litter waste solids spiked

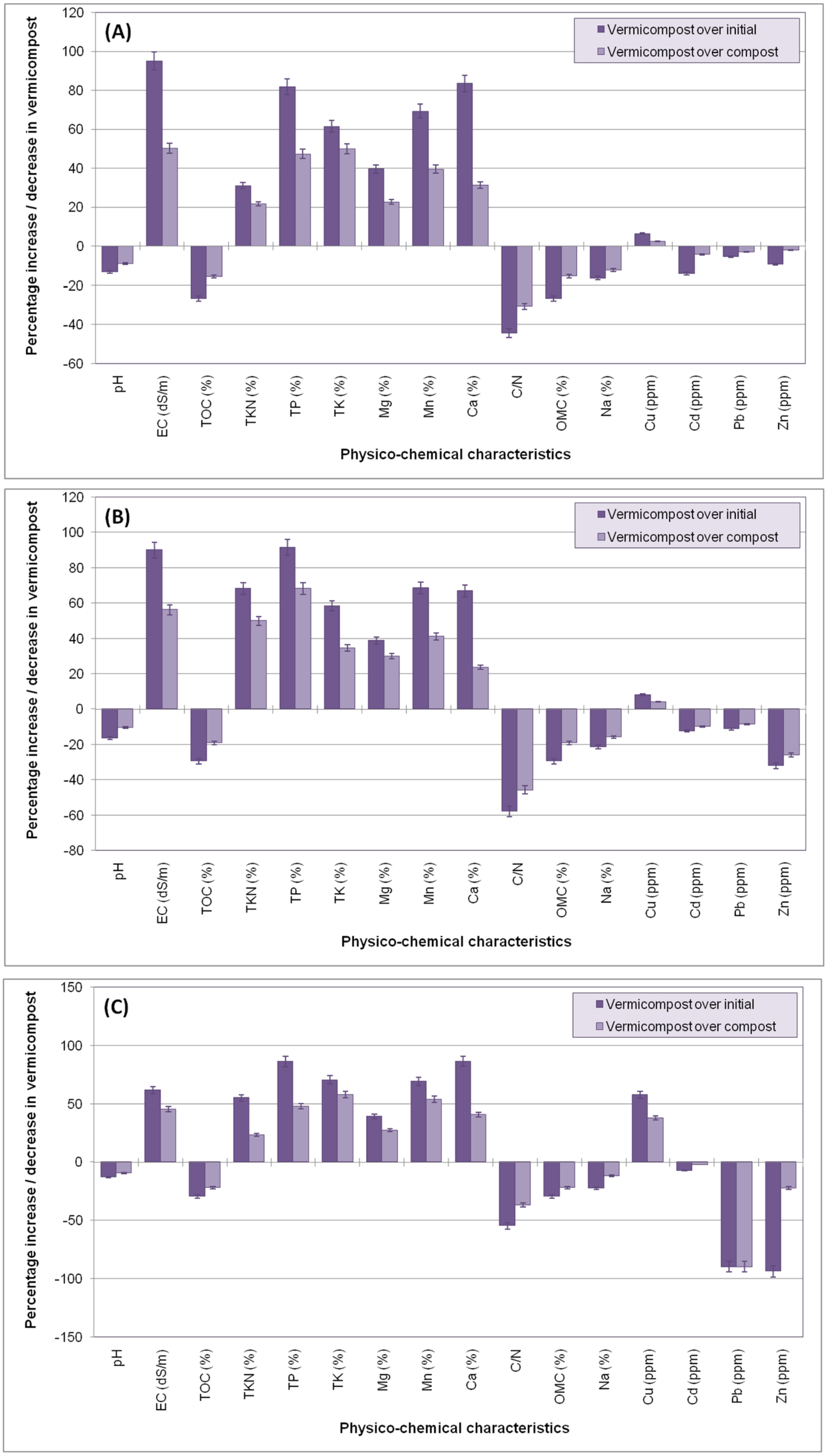

Fig. 1. Percentage increase/decrease of physico-chemical characteristics of vermicompost over initial substrate and worm un-worked compost (60 days). (A) *Gracilaria edulis* + CD, (B) *Turbinaria ornata* + CD, and (C) *Ulva reticulata* + CD. Values are mean of three replicates. Error bars indicate ± SEM.

with CD, Suthar and Gairola (2014) observed significant increase in TKN, TP, Ca, and $N–NO_3^-$ contents and concluded that mineralization rate was higher in the vermibeds with leaf litter and CD mixtures. Sudharsan Varma et al. (2016) highlighted increment of the nutrients (Na, TK, Ca, Mg, TKN) and available phosphorous during the degradation of water hyacinth with the earthworms. It is evident that the reduction of organic carbon associated with the liberation of carbon dioxide mainly by the respiratory activity of earthworms and microorganisms, the addition of nitrogen through the activity of earthworms, and humification and mineralization processes (Pramanik et al., 2016; Suthar et al., 2017; Sharma and Garg, 2018b; Huang and Xia, 2018). Additionally, the augmentation of TP and its mineralization during vermistabilization of organic substrates is responsibly due to the phosphatase and phytase activity either in the gut of earthworms or by the microorganisms in the vermicompost (Parthasarathi et al., 2016; Huang et al., 2017; Ghosh et al., 2018). Nonetheless, in the current study, the Cd, Pb and Zn contents showed decrease in all worm-worked seaweed and cowdung substrates and the difference is insignificant statistically. These kinds of reductions have already been reported in vermicomposting systems by Prakash and Karmegam (2010), John Paul et al. (2011) and Wang et al. (2017).

3.3. Total microbial population in vermicomposts

An increasing trend of bacterial, fungal and actinomycetes population was observed in compost and vermicompost of three different seaweed + CD substrates from initial levels (Fig. 2A, B and C). The increase in total microbial population in vermicomposts prepared from seaweed + CD combinations with *Perionyx excavatus* was significantly higher than that of initial substrates and composts ($P < 0.05$). The initial bacterial population range of 39–40 CFU × $10^7 g^{-1}$ was found to be increased to 53–56 CFU × $10^7 g^{-1}$ in compost and 144–146 CFU × $10^7 g^{-1}$ in vermicompost of seaweed + CD substrates (Fig. 2A). A maximum of 146 CFU × $10^7 g^{-1}$ of bacteria was recorded in the vermicompost of *Gracilaria edulis* + CD combination.

Initially, the average of total fungal population in seaweeds + CD combinations was 32.67 ± 4.10 CFU × 10^4 g^{-1} which increased in folds towards the end of the composting, i.e., on 60th day. The average fungal population of 53.67 ± 3.53 CFU × 10^4 g^{-1} and 91.67 ± 6.33 CFU × 10^4 g^{-1} was recorded in compost and vermicompost of seaweed + CD combinations respectively after 60 days (Fig. 2B). Among the seaweed + CD composts, *Gracilaria edulis* + CD combination showed higher total fungal population (56 CFU × 10^4 g^{-1}) than the composts of rest of the seaweed + CD combinations and the least fungal population (50 CFU × 10^4 g^{-1}) was observed in *Ulva reticulata* + CD compost. A maximum of 98 CFU × 10^4 g^{-1} was observed in *Gracilaria edulis* + CD vermicompost, whereas the compost of the same substrate after 60 days showed a fungal population of 56 CFU × 10^4 g^{-1} followed by *Turbinaria ornata* + CD (94 CFU × 10^4 g^{-1}) and *Ulva reticulata* + CD (83 CFU × 10^4 g^{-1}) (Fig. 2B). The difference in total fungal population between initial and compost, and compost and vermicompost of all seaweed + CD combinations was statistically significant ($P < 0.05$).

The average of total actinomycetes population in initial seaweed + CD combinations was 46.0 CFU × 10^1 g^{-1} which increased significantly towards the end of the composting, i.e., on 60th day. The average actinomycetes population of 54.6 CFU × 10^1 g^{-1} and 145.3 CFU × 10^1 g^{-1} was recorded in compost and vermicompost of seaweed + CD combinations respectively after 60 days. The least actinomycetes population (51 CFU × 10^1 g^{-1}) was observed in *Turbinaria ornata* + CD compost and the higher number was found in *Ulva reticulata* + CD (57 CFU × 10^1 g^{-1}) (Fig. 2C). A maximum of 152 CFU × 10^1 g^{-1} actinomycetes population was observed in *Turbinaria ornata* + CD vermicompost followed by *Gracilaria edulis* + CD (143 CFU × 10^4 g^{-1}) and *Ulva reticulata* + CD (141 CFU × 10^1 g^{-1}) (Fig. 2C). Overall, the total bacterial, fungal and actinomycetes

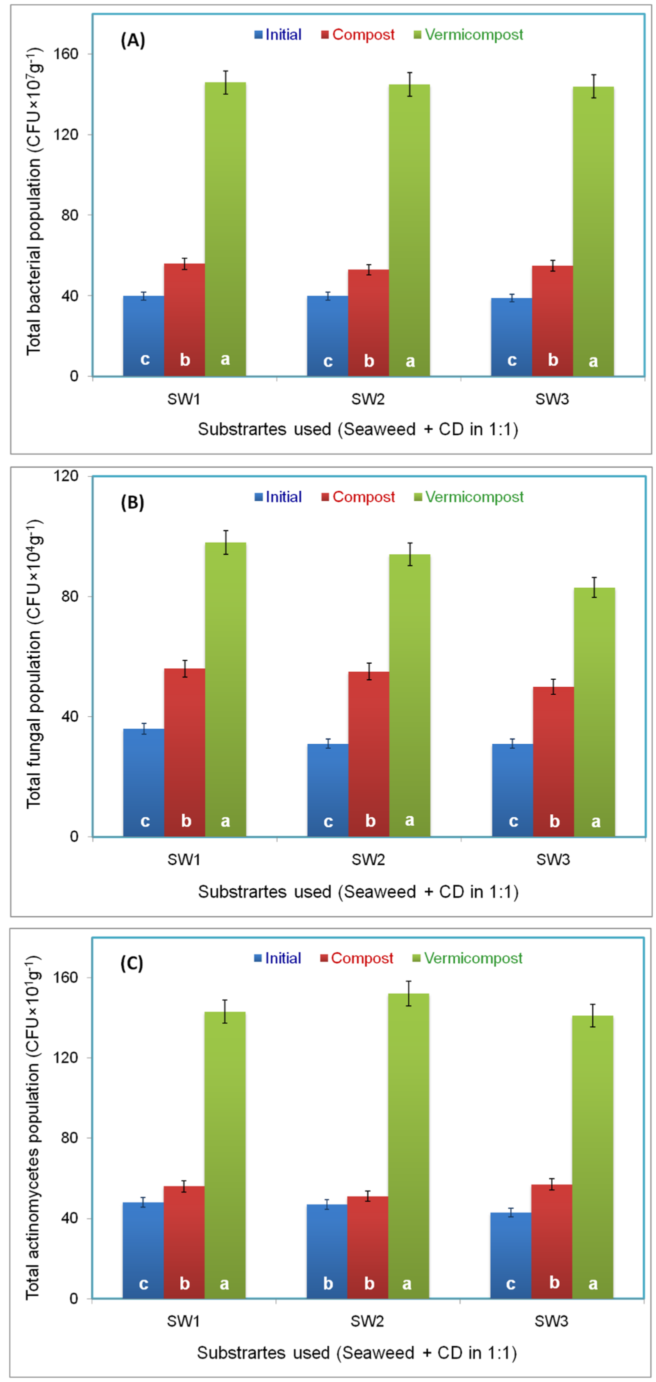

Fig. 2. Total bacterial (A), fungal (B) and actinomycetes (C) population in initial substrates and composts prepared with and without *Perionyx excavatus* using different seaweed + CD (1:1) combinations. Values are mean of three replicates. Error bars indicate ± SEM. The difference in mean values between the vermibed materials (bars) followed by the same letter are not significant at 5% level ($P < 0.05$) by Tukey's honestly significantly different (HSD) multiple comparison test. Initial – before vermicomposting (0th day); Compost – without earthworms, i.e., worm un-worked compost after 60 days; Vermicompost – with earthworms, i.e., worm worked vermicompost. SW1- *Gracilaria edulis* + CD, SW2 – *Turbinaria ornata* + CD, and SW3 – *Ulva reticulata* + CD.

population in vermicompost of seaweed + CD prepared using *Perionyx excavatus* showed significantly higher numbers compared to the composts prepared without *Perionyx excavatus*. There are several instances, the researchers draw attention to the increased level of microbial population in the vermicompost as to that of present study. The

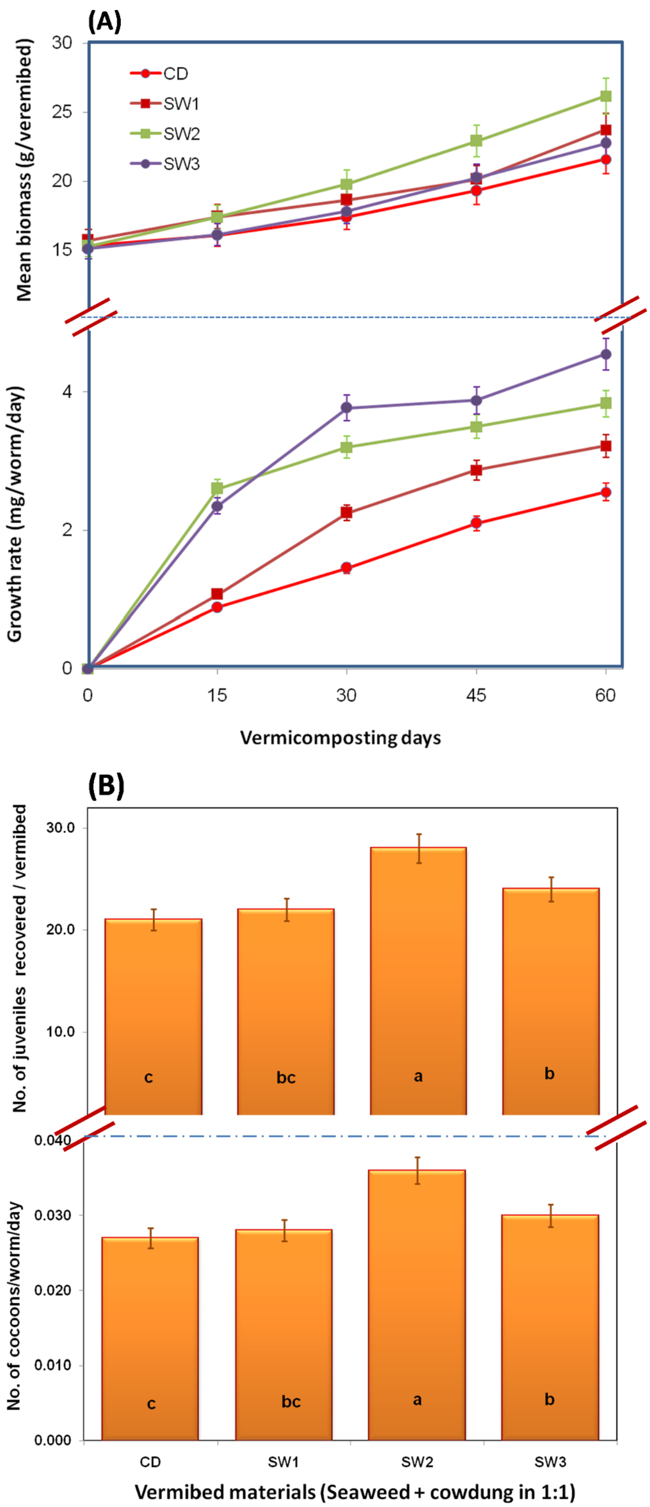

Fig. 3. Growth and reproduction of the earthworm, *Perionyx excavatus* during the seaweed + CD (1:1) vermicomposting (60 days): (A) Mean biomass/vermibed and growth rate, (B) Cocoon production rate/day and number of juveniles recovered/vermibed; Values are mean of three replicates. Error bars indicate ± SEM. The difference in mean values between the vermibed materials (bars) followed by the same letter are not significant at 5% level ($P < 0.05$) by Tukey's honestly significantly different (HSD) multiple comparison test. SW1- *Gracilaria edulis* + CD, SW2 – *Turbinaria ornata* + CD, and SW3 – *Ulva reticulata* + CD.

vermicomposted leaf litter CD mixtures vermicomposted with *Eisenia fetida* (Suthar and Gairola, 2014), sugar cane trash, leaf litter, pressmud, municipal solid waste with CD using *Perionyx ceylanensis* (John Paul et al., 2011; Karmegam et al., 2012), paper mill sludge with CD using *Perionyx excavatus* (Yuvaraj et al., 2018a), and poultry litter with CD and leaf litter using *Drawida sulcata* (Yuvaraj et al., 2018b), all these showed enhanced bacterial, fungal and actinomycetes which are well supportive to the present study.

3.4. Growth and reproduction of Perionyx excavatus

The growth and reproduction of *Perionyx excavatus* measured in terms of biomass and number of earthworms, cocoon production rate and growth rate in separate experimental set-up containing seaweed + CD (1:1) substrate combinations using cowdung alone as a standard culture medium (control), showed that the earthworm, *Perionyx excavatus* was well adopted to the seaweed substrates with comparatively good growth and reproduction to that of cowdung (Fig. 3A and B). The mean biomass of the earthworms (g/vermibed) was found to be increasing with the progression of composting days, i.e., 15th, 30th, 45th and 60th day. The mean biomass of 15.31, 15.30, 15.72 and 15.12 g/vermibed was introduced at the start of vermicomposting (0th day, initial) in CD, *Turbinaria ornata* + CD, *Gracilaria edulis* + CD and *Ulva reticulata* + CD combinations respectively, with an average of 15.36 ± 0.33 g/vermibed. A maximum biomass recovery of 26.16 g/vermibed was recorded in *Turbinaria ornata* + CD substrates (Fig. 3A). Likewise, the individual biomass of the earthworms recovered from different substrates after 60 days was 0.288, 0.348, 0.316, and 0.303 g/worm in CD, *Turbinaria ornata* + CD, *Gracilaria edulis* + CD, and *Ulva reticulata* + CD combinations respectively. The individual biomass of the earthworms recovered from seaweed + cowdung combinations was higher than that of the control i.e., CD. The two way ANOVA results showed that the increase of earthworm biomass with reference to the number of days and between seaweed + cowdung substrates was highly significantly different at 0.1% level.

The growth rate of the earthworm, *Perionyx excavatus* cultured in CD measured during regular intervals of 15 days, i.e., on 15, 30, 45 and 60 days showed 0.88, 1.45, 2.10 and 2.55 mg/worm/day respectively, where the values were found to be lower than that of the worms cultured in seaweed + CD substrates (Fig. 3A). The growth rate of the earthworms showed progressively increasing trend in all the substrates. The increase of growth rate significantly differed between vermibed substrates ($P < 0.05$). The cocoon production rate of the earthworms cultured in vermibed substrates, enumerated after 60 days, ranged from 0.027 to 0.036 cocoon/worm/day. A maximum of 0.036 cocoon/worm/day was observed in *Turbinaria ornata* + CD followed by *Ulva reticulata* + CD (0.030 cocoon/worm/day) > *Gracilaria edulis* + CD (0.028 cocoon/worm/day) > CD (0.027 cocoon/worm/day) (Fig. 3B). All the seaweed + CD substrates showed higher cocoon production rate than the standard diet, CD indicating the higher preference of seaweed + CD combinations. The difference in cocoon production rate of the earthworms in *Turbinaria ornata* + CD substrates was significantly different from CD at $P < 0.05$. The number of juvenile worms recovered from different vermibed substrates after 60 days showed variation between substrates. The number of juveniles recovered in seaweed substrates ranged between 22 and 28 worms/vermibed. The higher number of juveniles was recorded *Turbinaria ornata* + CD (28 worms/vermibed) which was significantly higher than CD, *Gracilaria edulis* + CD and *Ulva reticulata* + CD substrates at 5% significance level (Fig. 3B). Even though all seaweed + CD substrates were preferred by *Perionyx excavatus*, higher tendency was found towards *Turbinaria ornata* + CD substrates with reference to mean biomass, cocoon production and number of juveniles in the present study. The acceptability of organic substrate mixture by the earthworms is a good sign of efficient vermicomposting system. The findings of the present study also suggests that *Perionyx excavatus* is well adapted to the seaweed + CD substrates as can be seen from the results on growth rate, biomass of the worms, cocoon production rate and number of juvenile worms in the vermibeds. Similarly, various organic substrates amended with cowdung and other bulking materials used for vermicomposting with epigeic earthworms are in support of the present study (Gunadi et al., 2002; Suthar, 2009; Karmegam and Daniel, 2009a; Parthasarathi et al., 2016; Sudharsan Varma et al., 2016).

4. Conclusion

The 15 days pre-decomposed seaweeds, *Gracilaria edulis*, *Turbinaria ornata* and *Ulva reticulata* in combination with CD in 1:1 combination resulted in the production of nutritionally and microbiologically rich vermicompost using the epigeic earthworm, *Perionyx excavatus* for 60 days. The TOC, C/N and OMC showed significant reduction while pH, Cd, Pb and Zn showed lower decrease during vermistabilization of seaweed + CD substrates. Also, the important macronutrients, TKN, TP and TK along with EC showed significant increase which showed the mineralization of vermibed substrates during vermicomposting. In addition, the total microbial population increase in vermicompost favours the further decomposition of substrates. The higher growth and reproduction of the earthworm, *Perionyx excavatus* in seaweed + CD in comparison with CD alone substantiates the food preference of the earthworm resulting in the efficient conversion of seaweed substrates in to vermicompost. *Perionyx excavatus* can be employed for the production of vermicompost from seaweeds in combination with CD. Further, it is suggested that the seaweed biomass can also be utilized employing widely used earthworm species, so as to apply this technology in other parts of the world for vermicompost production. Also, there is a scope to utilize seaweeds along with biofertilizers or nutrient rich organic materials to produce enriched seaweed vermicompost for effective agricultural use.

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References

Chopra, S.L., Kanwar, L., 1991. Analytical Agricultural Chemistry, 4th Edn. Kalyani Publishers, New Delhi.
Deka, H., Deka, S., Baruah, C.K., Das, J., Hoque, S., Sarma, H., Sarma, N.S., 2011. Vermicomposting potentiality of *Perionyx excavatus* for recycling of waste biomass of Java citronella – An aromatic oil yielding plant. Bioresour. Technol. 102, 11212–11217. https://doi.org/10.1016/j.biortech.2011.09.102.
Domínguez, J., 2018. Earthworms and vermicomposting, in: Earthworms – The Ecological Engineers of the Soil, IntechOpen, pp.63-77. http://dx.doi.org/10.5772/intechopen.76088.
Garg, P., Gupta, A., Satya, S., 2006. Vermicomposting of different types of waste using *Eisenia foetida*: a comparative study. Bioresour. Technol. 97 (3), 391–395. https://doi.org/10.1016/j.biortech.2005.03.009.
Ghosh, S., Goswami, A.J., Ghosh, G.K., Pramanik, P., 2018. Quantifying the relative role of phytase and phosphatase enzymes in phosphorus mineralization during vermicomposting of fibrous tea factory waste. Ecol. Eng. 116, 97–103. https://doi.org/10.1016/j.ecoleng.2018.03.001.
Goswami, B., Kalita, M.C., 2000. Efficiency of some indigenous earthworm species of Assam and its characterization through vermitechnology. Ind. J. Environ. Ecoplan 3, 351–354.
Gunadi, B., Blount, C., Edward, C.A., 2002. The growth and fecundity of *Eisenia fetida* (Savigny) in cattle solids pre-composted for different periods. Pedobiologia 46, 15–23.
He, X., Zhang, Y., Shen, M., Zeng, G., Zhou, M., Li, M., 2016. Effect of vermicomposting on concentration and speciation of heavy metals in sewage sludge with additive materials. Bioresour. Technol. 218, 867–873. https://doi.org/10.1016/j.biortech.2016.07.045.
Huang, K., Xia, H., 2018. Role of earthworms' mucus in vermicomposting system: biodegradation tests based on humification and microbial activity. Sci. Total Environ. 610, 703–708. https://doi.org/10.1016/j.scitotenv.2017.08.104.
Huang, K., Xia, H., Cui, G., Li, F., 2017. Effects of earthworms on nitrification and ammonia oxidizers in vermicomposting systems for recycling of fruit and vegetable wastes. Sci. Total Environ. 578, 337–345. https://doi.org/10.1016/j.scitotenv.2016.10.172.
Illera-Vives, M., Labandeira, S.S., Brito, L.M., López-Fabal, A., López-Mosquera, M.E., 2015. Evaluation of compost from seaweed and fish waste as a fertilizer for horticultural use. Sci. Hort. 186, 101–107. https://doi.org/10.1016/j.scienta.2015.02.008.
John Paul, J.A., Karmegam, N., Daniel, T., 2011. Municipal solid waste (MSW) vermicomposting with an epigeic earthworm, *Perionyx ceylanensis* Mich. Bioresour. Technol. 102, 6769–6773. https://doi.org/10.1016/j.biortech.2011.03.089.
Kale, R.D., Karmegam, N., 2010. The role of earthworms in tropics with emphasis on Indian ecosystems. Appl. Environ. Soil Sci. 16. https://doi.org/10.1155/2010/414356. Article ID 414356.
Karmegam, N., Daniel, T., 2009a. Growth, reproductive biology and life cycle of the vermicomposting earthworm, *Perionyx ceylanensis* Mich. (Oligochaeta: Megascolecidae). Bioresour. Technol. 100 (20), 4790–4796. https://doi.org/10.1016/j.biortech.2009.05.004.
Karmegam, N., Daniel, T., 2009b. Investigating efficiency of *Lampito mauritii* (Kinberg) and *Perionyx ceylanensis* Michaelsen for vermicomposting of different types of organic substrates. Environmentalist 29, 287–300. https://doi.org/10.1007/s10669-008-9195-z.
Karmegam, N., Karthikeyan, V., Ambika, D., 2012. Vermicomposting of sugarcane trash and leaf litter in combination with pressmud using the earthworm, *Perionyx ceylanensis*. In: Vermitechnology III (Ed.: Karmegam, N.). Dyn. Soil Dyn Plant. 6 (SI-1), 57–64.
Krishnamurthi, V., Joshi, H.V., 1970. A Check List of Indian Marine Algae. Central Salt and Marine Chemical Research Institute, Bhavanagar, Gujarat, India, pp. 36.
Lim, S.L., Wu, T.Y., 2016. Characterization of matured vermicompost derived from valorization of palm oil mill byproduct. J. Agric. Food. Chem. 64, 761–769. https://doi.org/10.1021/acs.jafc.6b00531.
Malafaia, G., da Costa Estrela, D., Guimarães, A.T.B., de Araújo, F.G., Leandro, W.M., de Lima Rodrigues, A.S., 2015. Vermicomposting of different types of tanning sludge (liming and primary) mixed with cattle dung. Ecol. Eng. 85, 301–306. https://doi.org/10.1016/j.ecoleng.2015.10.010.
Mazantseva, G.P., 1982. Growth pattern in the earthworm *Nocodrilus caliginosus* (Oligochaeta: Lumbricidae) during the first year of life. Pedobiologia. 23, 272–276.
Mohanty, D., Adhikary, S.P., Chattopadhyay, G.N., 2013. Seaweed liquid fertilizer (SLF) and its role in agriculture productivity. Ecoscan. III, 147–155.
Oza, R.M., Zaidi, S.H., 2001. A Revised Checklist of Indian Marine Algae. Central Salt & Marine Chemicals Research Institute, Gijubhai Badheka Marg, Bhavnagar, Gujarat, India.
Parthasarathi, K., Balamurugan, M., Prashija, K.V., Jayanthi, L., Basha, S.A., 2016. Potential of *Perionyx excavatus* (Perrier) in lignocellulosic solid waste management and quality vermifertilizer production for soil health. Int. J. Recycl. Org. Waste Agricult. 5, 65–86. https://doi.org/10.1007/s40093-016-0118-6.
Pattnaik, S., Vikram Reddy, M., 2010. Nutrient status of vermicompost of urban green waste processed by three earthworm species—*Eisenia fetida*, *Eudrilus eugeniae* and *Perionyx excavatus*. Appl. Environ. Soil Sci. 13. https://doi.org/10.1155/2010/967526. Article ID 967526.
Prakash, M., Karmegam, N., 2010. Vermistabilization of pressmud using *Perionyx ceylanensis* Mich. Bioresour. Technol. 101, 8464–8468. https://doi.org/10.1016/j.biortech.2010.06.002.
Pramanik, P., Safique, S., Jahan, A., Bhagat, R.M., 2016. Effect of vermicomposting on treated hard stem leftover wastes from pruning of tea plantation: a novel approach. Ecol. Eng. 97, 410–415. https://doi.org/10.1016/j.ecoleng.2016.10.041.
Ramalingam, R., Ranganathan, L.S., 2001. Vermicomposting of pressmud boosts nutrient quality of compost. Ecol. Environ. Conserv. 7 (3), 297–299.
Ramnarain, Y.I., Ansari, A.A., Ori, L., 2018. Vermicomposting of different organic materials using the epigeic earthworm *Eisenia foetida*. Int. J. Recyc. Organic Waste Agric. 1–14. https://doi.org/10.1007/s40093-018-0225-7.
Rani Juneius, C.E., Sundari, M., Eswaralakshmi, R., Elumalai, S., 2018. Seaweed liquid fertilizers: A novel strategy for the biofortification of vegetables and crops. In: Patra, J., Das, G., Shin, H.S. (Eds.), Microbial Biotechnology. Springer, Singapore. https://doi.org/10.1007/978-981-10-7140-9_5.
Sharma, K., Garg, V.K., 2018a. Vermicomposting: A green technology for organic waste management. In: Waste to Wealth. Springer, Singapore, pp. 199–235. https://doi.org/10.1007/978-981-10-7431-8_10.
Sharma, K., Garg, V.K., 2018b. Comparative analysis of vermicompost quality produced from rice straw and paper waste employing earthworm *Eisenia fetida* (Sav.). Bioresour. Technol. 250, 708–715. https://doi.org/10.1016/j.biortech.2017.11.101.
Shrimal, S., Khwairakpam, M., 2010. Effect of C/N ratio on vermicomposting of vegetable waste, in: Karmegam, N. (Ed.), Vermitechnology II. Dyn. Soil Dyn. Plant. 4 (SI-1), 123–126.
Singh, D., Suthar, S., 2012. Vermicomposting of herbal pharmaceutical industry solid wastes. Ecol. Eng. 39, 1–6. https://doi.org/10.1016/j.ecoleng.2011.10.015.
Soobhany, N., Mohee, R., Garg, V.K., 2015. Experimental process monitoring and potential of *Eudrilus eugeniae* in the vermicomposting of organic solid waste in Mauritius. Ecol. Eng. 84, 149–158. https://doi.org/10.1016/j.ecoleng.2015.08.003.
Subba Rao, N.S., 1995. Soil Micro-organisms and Plant Growth. Oxford and IBH Publishing Co. Pvt. Ltd. (3rd Edn.), New Delhi.

Sudharsan Varma, V., Kalamdhad, A.S., Khwairkpam, M., 2016. Feasibility of *Eudrilus eugeniae* and *Perionyx excavatus* in vermicomposting of water hyacinth. Ecol. Eng. 94, 127–135. https://doi.org/10.1016/j.ecoleng.2016.05.058.

Suthar, S., 2009. Vermicomposting of vegetable-market solid waste using *Eisenia fetida*: Impact of bulking material on earthworm growth and decomposition rate. Ecol. Eng. 35 (5), 914–920. https://doi.org/10.1016/j.ecoleng.2008.12.019.

Suthar, S., Gairola, S., 2014. Nutrient recovery from urban forest leaf litter waste solids using *Eisenia fetida*. Ecol. Eng. 71, 660–666. https://doi.org/10.1016/j.ecoleng.2014.08.010.

Suthar, S., Pandey, B., Gusain, R., Gaur, R.Z., Kumar, K., 2017. Nutrient changes and biodynamics of *Eisenia fetida* during vermicomposting of water lettuce (*Pistia* sp.) biomass: A noxious weed of aquatic system. Environ. Sci. Poll. Res. 24, 199–207. https://doi.org/10.1007/s11356-016-7770-2.

Tandon, H.Z., 1993. Methods of Analysis of Soils, Plant, Water and Fertilizers. Fertilizer Development and Consultation Organization, New Delhi.

Umamaheswara Rao, M., 1970. An annotated list of the marine algae of Visakhapatnam (India). J. Linn. Soc. (Botany) 63, 23–45.

Umamaheswara Rao, M., 1987. Key for identification of economically important seaweeds. Bull. Cent. Mar. Fish. Res. Inst. 41, 19–25.

Walkley, A., Black, I.A., 1934. An examination of the Degtjareff method for determining soil organic matter and prepared modification of the chronic acid titration method. Soil Sci. 34, 29–38.

Wang, D., Ai, J., Shen, F., Yang, G., Zhang, Y., Deng, S., Zhang, J., Zeng, Y., Song, C., 2017. Improving anaerobic digestion of easy-acidification substrates by promoting buffering capacity using biochar derived from vermicompost. Bioresour. Technol. 227, 286–296. https://doi.org/10.1016/j.biortech.2016.12.060.

Yuvaraj, A., Thangaraj, R., Maheswaran, R., 2018a. Decomposition of poultry litter through vermicomposting using earthworm *Drawida sulcata* and its effect on plant growth. Int. J. Environ. Sci. Technol. https://doi.org/10.1007/s13762-018-2083-2.

Yuvaraj, A., Karmegam, N., Thangaraj, R., 2018b. Vermistabilization of paper mill sludge by an epigeic earthworm *Perionyx excavatus*: mitigation strategies for sustainable environmental management. Ecol. Eng. 120, 187–197. https://doi.org/10.1016/j.ecoleng.2018.06.008.

Zhang, J., Müller, C., Cai, Z., 2015. Heterotrophic nitrification of organic N and its contribution to nitrous oxide emissions in soils. Soil Biol. Biochem. 84, 199–209. https://doi.org/10.1016/j.soilbio.2015.02.028.

Zhang, L., Sun, X., 2017. Addition of seaweed and bentonite accelerates the two-stage composting of green waste. Bioresour. Technol. 243, 154–162. https://doi.org/10.1016/j.biortech.2017.06.099.