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Seaweeds as bioresources for vermicompost production using the earthworm, *Perionyx excavatus* (Perrier)

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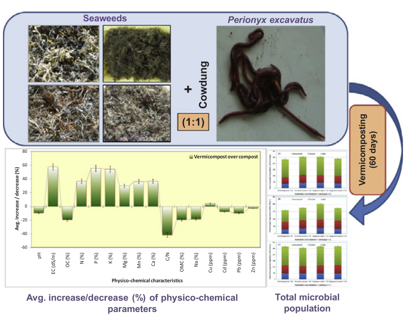
GRAPHICAL ABSTRACT



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ABSTRACT

Fifteen days pre-decomposed seaweeds, *Halimeda gracilis, Gracilaria corticata, Sargassum wightii* and *Sargassum swartzii* spiked with cowdung (1:1) were vermicomposted using *Perionyx excavatus* for 60 days. The pH in the vermicompost showed insignificant reduction while electrical conductivity showed significant enhancement ($P < 0.05$). The reduction of organic carbon in vermicomposts ranged from −37.78 to −50.97% over worm-unworked composts. Total NPK contents showed significant increment (26.72–78.17%) in vermicompost over worm-unworked composts. The difference in percentage increase/decrease between physicochemical parameters was statistically significant ($P < 0.001$) and the same pattern was found between substrates. The total microbial population in vermicomposts was significantly higher than that of initial and composts of all seaweed + cowdung combinations ($P < 0.001$). The growth and reproduction of *Perionyx excavatus* in seaweed + cowdung combinations showed equivalent or higher rates when compared with cowdung signifying that *Perionyx excavatus* is well suited to convert seaweed and cowdung combinations into nutrient rich vermicompost.

1. Introduction

Seaweeds or macroalgae are the abundant space occupiers and primary producers in the marine ecosystems with great ecological and economic significance (Egan et al., 2013; Ba-akdah et al., 2016). The drift-seaweed washed up on beaches has been used for centuries as

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natural fertilizer in many coastal regions throughout the world (McHugh, 2003). Seaweed improves soil structure by providing trace elements and growth activators (Blunden, 1991; Verkleij, 1992). Seaweeds are the enormous sources for various economically important value added products, including compost. Seaweed fertilizer contains lots of ready to use micro-nutrients which can be readily absorbed by the plants without any further chemical decomposition. Seaweeds have the necessary micro-elements and nutrients which on absorption increases the nourishment of plants (Pramanick et al., 2013). Studies clearly indicate that the seaweeds are good bioresources to be used as substrates for organic compost, and liquid fertilizer production (Rani Juneius et al., 2018; Sembera et al., 2018). Moreover, these studies have mainly focused on liquid fertilizer or compost production from seaweeds and their effects on crops and soil fertility.

Vermicomposting is a green technology for the production of valuable vermicompost from different kinds of organic substrates with various earthworm species including *Perionyx excavatus* (Suthar et al., 2017; Parthasarathi et al., 2016; Sharma and Garg, 2018a). Compost and liquid fertilizer production from seaweeds are well documented. The seaweed biomass has been infrequently utilized for vermiconversion or vermiculture (Lakshmi and Ebenezer, 2010a,b; Fantonalgo and Salubre, 2019). The study by Lakshmi and Ebenezer (2010a) used the seaweed *Kappaphycus alvarezii* along with vegetable wastes for vermiculturing of *Eisenia foetida*, and Lakshmi and Ebenezer (2010b) used 45 days anaerobically digested sap of *Kappaphycus alvarezii* for enriched vermicompost production with *Azolla pinnata*; while, the study by Fantonalgo and Salubre (2019) utilized finely ground and 15 days fermented seaweed, *Sargassum* sp. in combination with cowdung (3:1 ratio), primarily for vermicast production employing the earthworm, *Eudrilus eugeniae*, and the physico-chemical and microbiological changes during the process of seaweed vermicomposting, and in vermicompost, earthworm growth rate, biomass gain, cocoon production rate and number recovered are not well established. Nonetheless, the utilization of seaweeds as a substrate resource for vermicomposting using *Perionyx excavatus* is thus far unavailable. Hence the present study has been aimed to utilize the seaweeds, *Halimeda gracilis, Gracilaria corticata, Sargassum wightii* and *Sargassum swartzii* which are abundantly found in Mandapam East Coast region of Tamil Nadu, India for vermicompost production using the earthworm, *Perionyx excavatus*.

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, India were collected based on 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). Four different seaweeds, *Halimeda gracilis* (green alga), *Gracilaria corticata* (red alga), *Sargassum wightii* (brown alga) and *Sargassum swartzii* (brown alga) were identified and the voucher specimens were deposited in the Department of Zoology, Arumugam Pillai Seethai Ammal College, Tirupattur, 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 running 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 in this study. The stabilization of CD was done to make it acceptable to earthworms. The organic substrates, i.e., the seaweeds were subjected to initial decomposition 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, for predecomposition to effect. 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, CD, and used for vermicomposting studies.

2.2. Vermicomposting of seaweeds

For preliminary trials on standardization of seaweeds and CD ratio, 1:3, 2:2, 3:1 and 4:0 (25, 50, 75 and 100% of seaweed) were used. Between 25 and 50% of 15 days pre-decomposed seaweeds incorporation, no mortality of earthworms was observed with significantly different increase in physico-chemical parameters of vermicompost over initial and worm-unworked substrates. At the same time, between 75 and 100% seaweed incorporation, 50–70% mortality of earthworms with insignificant and least changes in physico-chemical parameters was observed. The use of 1:1 ratio was also reported to be appropriate for efficient vermiconversion of organic wastes with CD by previous workers (Yuvaraj et al., 2018), hence CD and seaweeds in 1:1 ratio (50%: 50%) was used. Accordingly, the pre-decomposed seaweed substrates 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 and vermicomposts were analysed.

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

The initial substrates and the final worm-worked substrates (introduced with earthworms, vermicompost) and the worm-unworked substrates (without earthworms, compost) were analysed for the physico-chemical parameters using standard procedures as detailed below. 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), total phosphorus (TP) and total potassium (TP) were analysed as per the method described by Tandon (1993). 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 (Ramalingam and Ranganathan, 2001).

2.4. 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 adopting standard plate count method (Subba Rao, 1995). The culture media, nutrient agar, Martin's Rose Bengal agar and Kenknight's were respectively used for bacteria, fungi and actinomycetes. The total microbial population from 0th day onwards up to 60 days, once in 15 days was carried out using the same procedure. Three replicates were maintained for each observation.

2.5. 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 hand-sorted, 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).

2.6. Statistical analysis

The data were expressed as mean ± standard error (SEM). The physico-chemical characteristics of initial vermibed substrates, composts (worm-unworked) and vermicomposts 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 by fixing the significance at level $P < 0.05$. Two-way ANOVA was used to find out the effect of different seaweed substrates on percentage increase / decrease and total microbial population.

3. Results and discussion

3.1. Physico-chemical characteristics

The initial levels of physico-chemical parameters in pre-decomposed seaweed + CD combinations are shown in Table 1. Initially the pH showed alkaline rage of 8.47–8.52 and the EC ranged between 1.05 and 1.30 dS/m. The TKN content was 0.76 ± 0.05, 0.86 ± 0.05, 0.80 ± 0.05 and 0.84 ± 0.05% respectively in *Halimeda gracilis, Gracilaria corticata, Sargassum wightii* and *Sargassum swartzii* spiked with CD (1:1). The C/N ratio ranged from 36.40 to 40.00 with an OMC range between 51.70 and 55.24. The other parameters, TP, TK, Mg, Mn, Ca, Na, Cu, Cd, Pb and Zn showed variation among the seaweed + CD combinations.

The seaweed + CD combinations with and without *Perionyx excavatus* after 60 days showed variation with respect to each parameter (Table 2). The EC recorded in the vermicompost recovered from *Halimeda gracilis* + CD (1:1) combination was 2.06 ± 0.15 dS/m which was 96.19% and 54.89% higher than that of the initial and worm un-worked compost. The total nitrogen in *Halimeda gracilis* + CD (1:1) vermicompost was 1.42 ± 0.09% which was significantly ($P < 0.05$) higher than the initial (0.76 ± 0.05%) and worm un-worked compost (0.91 ± 0.05%) (Table 2). The total potassium and phosphorus levels were also significantly higher ($P < 0.05$) in *Halimeda gracilis* + CD vermicompost than in *Halimeda gracilis* + CD compost (Total K and P: compost- 1.59 ± 0.10% and 0.398 ± 0.02%; vermicompost- 2.11 ± 0.13% and 0.591 ± 0.02% respectively). The Mg, Mn and Ca contents were significantly higher in the vermicompost of *Halimeda gracilis* + CD than in the worm un-worked compost. The copper content in vermicompost was also higher than that of initial and worm un-worked compost and the percentage increase was statistically not significant at 5% level (Table 2). The C/N ratio of vermicompost showed decrease (14.71 ± 0.62) over compost of *Halimeda gracilis* + CD combination (30.00 ± 1.26) and percentage decrease in vermicompost over compost was 50.97 which was highly significant ($P < 0.001$). Percentage decrease of TOC and OMC recorded for the vermicompost of *Halimeda gracilis* + CD (1:1) were 21.43 and 21.30 respectively over the control, i.e., worm un-worked compost and values were significantly different at 5% level. The pH, Cu, Cd, Pb and Zn values in vermicompost of *Halimeda gracilis* + CD combination showed statistically insignificant ($P > 0.05$) decrease when compared with the initial substrate and worm un-worked compost. The initial pH of 8.47 ± 0.49 showed reduction in worm un-worked compost (pH-7.98 ± 0.46) and in vermicompost (pH-7.36 ± 0.43) and the percentage decrease of pH in vermicompost over initial substrate (−13.11%) and worm un-worked compost (−7.77) was not statistically significant ($P > 0.05$) (Table 2). The percentage decrease of total TOC, C/N and OMC in vermicompost was significantly lower than in the initial substrate.

The EC recorded in the vermicompost recovered from *Gracilaria corticata* + CD (1:1) combination was 2.20 ± 0.17 dS/m which was 86.44% and 58.27% higher than that of initial substrate and worm un-worked compost respectively. The total nitrogen in *Gracilaria corticata* + CD (1:1) vermicompost was 1.46 ± 0.09% which was significantly higher than the initial (0.86 ± 0.05%; $P < 0.001$) and worm un-worked compost (1.11 ± 0.07%; $P < 0.05$) (Table 2). The total potassium and phosphorus levels were also significantly higher ($P < 0.001$) in *Gracilaria corticata* + CD vermicompost than in *Gracilaria corticata* + CD compost (Total K in compost: 1.74 ± 0.10%; vermicompost: 2.97 ± 0.18%; Total P in compost: 0.44 ± 0.03%; vermicompost: 0.73 ± 0.03%). The Mg, Mn and Ca contents were significantly higher in the vermicompost of *Gracilaria corticata* + CD than in the worm un-worked compost. The copper content in vermicompost was also higher than that of the initial and worm un-worked compost and the percentage increase was statistically not significant at 5% level (Table 2). The C/N ratio of vermicompost showed decrease (16.20 ± 0.68) compared to the compost of *Gracilaria corticata* + CD combination (26.40 ± 1.11) and percent decrease in vermicompost over compost was 38.64 which was highly significant ($P < 0.01$). Percentage decrease of TOC and OMC recorded for the vermicompost of *Gracilaria corticata* + CD (1:1) were 24.33 and 24.35 respectively over the initial levels, and the values were significantly different at 5% level. The pH, Na, Cd, Pb and Zn values in vermicompost of *Gracilaria corticata* + CD combination showed statistically insignificant ($P > 0.05$) decrease when compared with the initial substrate and worm un-worked compost. The initial pH of 8.51 ± 0.49 showed reduction in worm un-worked compost (pH-7.91 ± 0.46) and in vermicompost (pH-7.18 ± 0.42) and the percentage decrease of pH in vermicompost over initial substrate (−15.63%) and worm un-worked compost (−9.23) was not statistically significant ($P > 0.05$) (Table 2).

The physico-chemical characteristics of *Sargassum wightii* + CD (1:1) and *Sargassum swartzii* + CD (1:1) composts prepared with and without the earthworm *Perionyx excavatus* showed increase in the parameters, EC, TKN, TP, TK, Mg, Mn, Ca and Cu when compared with the initial levels; whereas, the pH, TOC, C/N, OMC, Na, Cd, Pb and Zn

Table 1
Initial physico-chemical characteristics of predecomposed seaweed + CD (1:1) combinations. CD = cowdung. Values are mean ± SEM.

Parameters	*Halimeda gracilis* + CD	*Gracilaria corticata* + CD	*Sargassum wightii* + CD	*Sargassum swartzii* + CD
pH	8.47 ± 0.49	8.51 ± 0.49	8.52 ± 0.49	8.49 ± 0.49
EC (dS/m)	1.05 ± 0.08	1.18 ± 0.09	1.30 ± 0.10	1.21 ± 0.09
TOC (%)	30.11 ± 1.51	31.32 ± 1.57	31.12 ± 1.56	31.33 ± 1.57
TKN (%)	0.76 ± 0.05	0.86 ± 0.05	0.80 ± 0.05	0.84 ± 0.05
TP (%)	0.321 ± 0.01	0.390 ± 0.02	0.42 ± 0.02	0.322 ± 0.01
TK (%)	1.29 ± 0.08	1.63 ± 0.10	1.72 ± 0.10	1.311 ± 0.08
Mg (%)	1.21 ± 0.10	1.38 ± 0.11	1.41 ± 0.11	1.26 ± 0.10
Mn (%)	0.401 ± 0.02	0.401 ± 0.02	0.423 ± 0.03	0.417 ± 0.03
Ca (%)	0.309 ± 0.02	0.391 ± 0.03	0.412 ± 0.03	0.311 ± 0.02
C/N	40.00 ± 1.68	36.40 ± 1.53	38.00 ± 1.60	37.29 ± 1.57
OMC (%)	51.70 ± 2.79	53.80 ± 2.91	55.24 ± 2.98	53.8 ± 2.91
Na (%)	0.53 ± 0.04	0.64 ± 0.05	0.67 ± 0.05	0.59 ± 0.05
Cu (ppm)	0.71 ± 0.05	0.62 ± 0.04	1.51 ± 0.10	0.613 ± 0.04
Cd (ppm)	0.054 ± 0.01	0.012 ± 0.00	0.62 ± 0.06	0.018 ± 0.001
Pb (ppm)	0.141 ± 0.01	0.16 ± 0.01	0.261 ± 0.02	0.134 ± 0.01
Zn (ppm)	0.621 ± 0.05	0.531 ± 0.04	0.692 ± 0.06	0.302 ± 0.02

Table 2
Physico-chemical characteristics of seaweed + CD (1:1) composts and vermicomposts (60 days).

Parameters	*Halimeda gracilis* + CD		*Gracilaria corticata* + CD		*Sargassum wightii* + CD		*Sargassum swartzii* + CD	
	Compost	VC	Compost	VC	Compost	VC	Compost	VC
pH	7.98 ± 0.46	7.36 ± 0.43^{NS}	7.91 ± 0.46	7.18 ± 0.42^{NS}	8.01 ± 0.46	7.12 ± 0.41^{NS}	8.01 ± 0.46	7.17 ± 0.42^{NS}
EC (dS/m)	1.33 ± 0.10	2.06 ± 0.15***	1.39 ± 0.10	2.20 ± 0.17***	1.40 ± 0.11	2.30 ± 0.17**	1.31 ± 0.10	2.01 ± 0.15***
TOC (%)	27.72 ± 1.39	21.78 ± 1.09*	29.32 ± 1.61	23.70 ± 1.19^{NS}	27.00 ± 1.49	23.00 ± 1.15^{NS}	28.2 ± 1.55	21.82 ± 1.09*
TKN (%)	0.91 ± 0.05	1.42 ± 0.09***	1.11 ± 0.07	1.46 ± 0.09*	1.20 ± 0.07	1.60 ± 0.10*	1.16 ± 0.07	1.47 ± 0.09*
TP (%)	0.398 ± 0.02	0.591 ± 0.02**	0.44 ± 0.03	0.73 ± 0.03***	0.52 ± 0.03	0.81 ± 0.03**	0.399 ± 0.02	0.610 ± 0.02***
TK (%)	1.59 ± 0.10	2.11 ± 0.13*	1.74 ± 0.10	2.97 ± 0.18***	1.97 ± 0.12	3.510 ± 0.21***	1.62 ± 0.10	2.21 ± 0.13**
Mg (%)	1.33 ± 0.11	1.721 ± 0.14*	1.42 ± 0.11	1.921 ± 0.15**	1.62 ± 0.13	2.10 ± 0.17*	1.37 ± 0.11	1.723 ± 0.14*
Mn (%)	0.51 ± 0.03	0.673 ± 0.04*	0.523 ± 0.03	0.701 ± 0.04*	0.52 ± 0.03	0.724 ± 0.04**	0.492 ± 0.03	0.687 ± 0.04**
Ca (%)	0.395 ± 0.03	0.601 ± 0.05***	0.434 ± 0.03	0.597 ± 0.05**	0.493 ± 0.04	0.633 ± 0.05*	0.394 ± 0.03	0.509 ± 0.04*
C/N	30.00 ± 1.26	14.71 ± 0.62***	26.40 ± 1.11	16.20 ± 0.68**	22.50 ± 0.95	14.00 ± 0.59**	24.30 ± 1.02	14.80 ± 0.62**
OMC (%)	47.60 ± 2.57	37.46 ± 2.02*	50.40 ± 2.72	40.70 ± 2.20^{NS}	46.40 ± 2.51	39.56 ± 2.14^{NS}	48.50 ± 2.62	37.49 ± 2.02*
Na (%)	0.49 ± 0.04	0.38 ± 0.03*	0.60 ± 0.05	0.51 ± 0.04^{NS}	0.63 ± 0.05	0.54 ± 0.04^{NS}	0.53 ± 0.04	0.41 ± 0.03*
Cu (ppm)	0.79 ± 0.05	0.82 ± 0.05 ^{NS}	0.69 ± 0.04	0.72 ± 0.05^{NS}	1.57 ± 0.10	1.68 ± 0.11^{NS}	0.697 ± 0.05	0.713 ± 0.05^{NS}
Cd (ppm)	0.052 ± 0.01	0.047 ± 0.00 ^{NS}	0.011 ± 0.00	0.011 ± 0.00^{NS}	0.59 ± 0.01	0.53 ± 0.01^{NS}	0.017 ± 0.002	0.015 ± 0.00^{NS}
Pb (ppm)	0.137 ± 0.01	0.123 ± 0.01 ^{NS}	0.15 ± 0.01	0.13 ± 0.01^{NS}	0.258 ± 0.02	0.233 ± 0.02^{NS}	0.129 ± 0.01	0.121 ± 0.01^{NS}
Zn (ppm)	0.591 ± 0.05	0.563 ± 0.05 ^{NS}	0.530 ± 0.04	0.52 ± 0.04^{NS}	0.631 ± 0.05	0.622 ± 0.05^{NS}	0.29 ± 0.02	0.28 ± 0.02^{NS}

CD – Cowdung; VC – Vermicompost; Values are mean of three replicates ± SE; *, **, *** and NS indicates statistically significant difference at $P < 0.05$, $P < 0.01$, $P < 0.001$ and not significant by 't' test respectively based on the percentage increase/decrease.

contents showed decrease from the initial values (Table 2). The pH was 8.10 in the *Sargassum wightii* + CD compost which showed non-significant decrease (pH: 7.12) in *Sargassum wightii* + CD vermicompost; whereas, it was 8.01 and 7.17 respectively in *Sargassum swartzii* + CD compost and vermicompost. The EC in vermicomposts of *Sargassum wightii* + CD and *Sargassum swartzii* + CD were 2.30 ± 0.17 and 2.01 ± 0.15 dS/m where the values were significantly higher when compared to the respective composts. The levels of TKN, TP, TK, Mg, Mn, Ca were significantly higher in vermicomposts of both *Sargassum wightii* and *Sargassum swartzii* CD combinations than in respective composts; while C/N showed significant reduction ($P < 0.01$) over compost. The reduction in TOC and OMC in the vermicompost of *Sargassum wightii* + CD was not statistically significant. The increase in Cu and reduction in Cd, Pb and Zn in vermicomposts of *Sargassum wightii* and *Sargassum swartzii* CD combinations over the worm-unworked composts was statistically insignificant ($P > 0.05$). The percent decrease of C/N in vermicompost of *Sargassum wightii* + CD over initial substrate (−63.16%) and worm un-worked compost (−37.78%) was significant at 0.1% and 5% levels respectively. The increment was 100% and 104.07% respectively in TKN and TK contents of *Sargassum wightii* + CD vermicompost over initial substrate combination.

The EC recorded in the vermicompost recovered from *Sargassum swartzii* + CD (1:1) combination was 2.01 ± 0.15 dS/m which was 66.12% and 53.44% higher than that of initial substrate and worm un-worked compost respectively. The Mg, Mn and Ca contents were significantly higher in the vermicompost of *Sargassum swartzii* + CD than in the worm un-worked compost. The copper content in vermicompost was also higher than that of initial and worm un-worked compost and the percentage increase was statistically not significant at 5% level. A maximum percentage decrease in vermicompost of *Sargassum swartzii* + CD over initial substrate was recorded for C/N (-60.31%) and the same trend was observed in the vermicompost over worm un-worked compost (−39.09%) where, both the values were significantly different from initial values at $P < 0.001$ and $P < 0.01$ respectively (Table 2).

The difference in percentage increase/decrease between physico-chemical parameters was statistically significant ($P < 0.001$) and the variation in increase/decrease of these parameters between different seaweed combinations did not differ significantly ($P > 0.05$) by two-way ANOVA (Table 3).

Comparatively, the compost beds with earthworms showed higher degree of increase/decrease of physico-chemical parameters than initial seaweed + CD substrates and worm-unworked composts. The pH showed decrease in vermicomposts of all the seaweed + CD combinations than in worm un-worked composts after 60 days of composting, which ranged from −7.77 to −11.11%, i.e., towards slightly basic to nearly neutral and the similar results have been reported by Yuvaraj et al. (2018) and Sharma and Garg (2018b) which might probably due to the release of carbon dioxide, ammonia as well as different organic acids by the degradation of organic wastes (John Paul et al., 2011; Zhang et al., 2015; Villar et al., 2016).

The EC showed 45.26–64.29% increment in vermicompost over compost with a maximum increase of 64.29% in *Sargassum wightii* + CD combination followed by the order: *Gracilaria corticata* + CD (58.27%) > *Halimeda gracilis* + CD (54.89%) > *Sargassum swartzii* + CD (53.44%). Similar reports have also been reported during vermicomposting of various wastes and the increment of EC could be due to the purging of inorganic ions and the release of soluble salts like phosphate and nitrate (Lazcano et al., 2008; He et al., 2016; Yuvaraj et al., 2018).

As can be seen from the results of the present study, TOC reduced at higher rates in the substrates introduced with earthworms which in turn favoured the decrease of C/N ratio and OMC in vermicompost over compost and initial materials. Furthermore, the combined action of earthworms along with the microorganisms resulted in the homogenization, fragmentation and mineralization of organic materials which in turn significantly reduced the TOC, OMC and C/N ratio (Lim and Wu, 2016; Suthar et al., 2017; Sharma and Garg, 2018b).

The TKN, TP and TK contents showed pronounced increment in vermicompost of all the seaweed combinations than in the compost. In the present study, the Mg, Mn and Ca contents showed maximum of 35.28 and 52.15% increase in the vermicomposts of *Gracilaria corticata* + CD and *Halimeda gracilis* + CD combinations respectively. The increase of Cu content in the vermicomposts of the seaweeds combination ranged from 2.30 to 15.65% (Table 2). The increase in these nutrients might probably due to the reduction of organic carbon related to the release of carbon dioxide by respiration and the addition of nitrogen by the activity of earthworms. Sharma and Garg (2018b) reported increased TKN, TP and TK contents in vermicompost of rice straw and paper waste amended with CD employing earthworm *Eisenia fetida* and analogous reports have also been made during vermicomposting of different organic substrates by Gupta and Garg (2008), Prakash and Karmegam (2010), Fu et al. (2015), Suthar et al. (2017) and Yuvaraj et al. (2018). Moreover, increased level of TP during vermicomposting is due to earthworm-gut derived phosphatase activity and also increased microbial activity in the cast (Parthasarathi et al.,

Table 3
Two-way ANOVA table 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.

Source of Variation	SS	df	MS	F	P-value	Significance level
Between parameters	61485.840	15	4099.056	65.785	< 0.001	0.1%
Between substrates	408.556	3	136.185	2.186	> 0.100	Not significant
Error	2803.945	45	62.310			
Total	64698.34	63				

2016; Huang et al., 2017). More convincingly, it has been reported that the earthworms' mucus facilitated the mineralization and humification of organic substrates and microbial activity (Huang and Xia, 2018). Supportively, Ghosh et al. (2018) recently reported that phosphatase and phytase enzymes are responsible for phosphorus mineralization and phosphatase enzyme influenced phosphorus mineralization in first 50 days of vermicomposting. However, the Cd, Pb and Zn contents showed decrease in all worm-worked seaweed and CD substrates and the difference is insignificant statistically. The results of the study by Wang et al. (2017) showed that the heavy metals like Cd, Pb and Cr showed significant reduction by the action of *Eisenia fetida*. The reduction or increase of micro and macro elements may be due to the increased utilization of these elements from the ingested organic materials by the worms and microbes for their growth and reproduction. These types of changes were already reported by Parthasarathi and Ranganathan (2000), Prakash and Karmegam (2010) and John Paul et al. (2011).

3.2. Total microbial population

The total bacterial population in the compost and vermicompost was increased from the initial levels in all the seaweed + CD combinations. The initial bacterial population range of 36–43 CFU × 10^7 g^{-1} was found to be increased to 53–65 CFU × 10^7 g^{-1} in compost and 143–151 CFU × 10^7 g^{-1} in vermicompost. A maximum of 151 CFU × 10^7 g^{-1} of bacteria was recorded in the vermicompost of *Sargassum wightii* + CD combination followed by the vermicompost of *Gracilaria corticata* + CD (146 CFU × 10^7 g^{-1}) > *Halimeda gracilis* + CD (145 CFU × 10^7 g^{-1}) > *Sargassum swartzii* + CD (143 CFU × 10^7 g^{-1}). Initially, the average of total fungal population in seaweeds + CD combinations was 33.75 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 54.25 CFU × 10^4 g^{-1} and 90.05 CFU × 10^4 g^{-1} was recorded in compost and vermicompost of seaweed + CD combinations respectively after 60 days.

The compost of *Sargassum swartzii* + CD combination showed a maximum total actinomycetes population of 68 CFU × 10^1 g^{-1} when compared with the composts of remaining seaweed + CD combinations. The compost of *Halimeda gracilis* + CD showed 63 CFU × 10^1 g^{-1} which was sequentially followed by the composts of *Sargassum wightii* + CD (61 CFU × 10^1 g^{-1}) and *Gracilaria corticata* + CD (58 CFU × 10^1 g^{-1}). A maximum of 156 CFU × 10^1 g^{-1} actinomycetes population was observed in *Sargassum wightii* + CD vermicompost, whereas the compost of the same substrate after 60 days showed a population of 61 CFU × 10^1 g^{-1} only. Next to *Sargassum wightii* + CD vermicompost, the highest total actinomycetes population was found in the vermicompost of *Halimeda gracilis* + CD (155 CFU × 10^1 g^{-1}) followed by *Sargassum swartzii* + CD (154 CFU × 10^1 g^{-1}) and *Gracilaria corticata* + CD (148 CFU × 10^1 g^{-1}).

In the current study, the difference in total bacterial, fungal and actinomycetes population between samples, i.e., initial and compost, and compost and vermicompost of all seaweed + CD combinations was highly significant ($P < 0.001$). The variation in total microbial populations between different substrates was significant ($P < 0.05$) excepting for total actinomycetes population (Table 4). The introduction of the earthworm, *Perionyx excavatus* in this study has contributed for the increase of the microflora in the organic matter which is essential for composting, soil fertility and soil health. The existence of symbiotic relationship between earthworms and microorganisms and also the presence of increased number of microorganisms in the vermicompost has been reported by some workers (Parthasarathi et al., 2007; Singh et al., 2015; Chen et al., 2018). The increase in the total microbial population during the vermicomposting process designates that the decomposition and mineralization process is assisted by these organisms together with the fragmentation and degradation activities of earthworms.

3.3. Growth and reproduction of earthworms

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, 14.72, 15.47, 15.20 and 15.21 g/vermibed was introduced at the start of vermicomposting (0th day, initial) in CD (control), *Halimeda gracilis* + CD, *Gracilaria corticata* + CD, *Sargassum wightii* + CD and *Sargassum swartzii* + CD combinations respectively, with an average of 15.18 ± 0.27 g/vermibed. *Sargassum wightii* + CD substrate showed a maximum biomass recovery of 29.31 g/vermibed on 60th day of vermicomposting which was closely followed by *Gracilaria corticata* + CD (27.40 g/vermibed) (Fig. 1). The total biomass of *Perionyx excavatus* in seaweed + CD combination was higher than the control. In CD culture medium, the worms showed the biomass of 15.31, 16.10, 17.40, 19.30 and 21.60 g/vermibed on 0, 15, 30, 45 and 60 day respectively. The individual biomass of the earthworms recovered from different substrates after 60 days was 0.288, 0.309, 0.365, 0.310 and 0.312 g/worm in CD, *Halimeda gracilis* + CD, *Gracilaria corticata* + CD, *Sargassum wightii* + CD and *Sargassum swartzii* + CD combinations respectively (Fig. 2). The individual biomass of the earthworms recovered from different vermibed substrates did not differ significantly from control substrate, at $P < 0.05$, excepting *Gracilaria corticata* + CD substrates which showed significant difference that could be attributed to the feeding preference of the earthworms, and nutrient contents in the substrate mix.

The growth rate of the earthworm, *Perionyx excavatus* cultured in the standard diet, 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. 3). The growth rate of the earthworms showed a progressively increasing trend. The growth rate of *Perionyx excavatus* in *Sargassum wightii* + CD showed maximum (4.55 mg/worm/day) on 60th day which gradually increased from 2.35, 3.77 and 3.88 mg/worm/day respectively on 15th, 30th and 45th days of growth.

The cocoon production rate of the earthworms cultured in vermibed substrates, enumerated after 60 days, ranged from 0.027 to 0.041 cocoon/worm/day. A maximum of 0.041 cocoon/worm/day was observed in *Gracilaria corticata* + CD substrate followed by *Sargassum wightii* + CD (0.037 cocoon/worm/day) > *Halimeda gracilis* + CD (0.032 cocoon/worm/day) > *Sargassum swartzii* + CD (0.031 cocoon/worm/day) > CD (0.027 cocoon/worm/day). All the seaweed + CD substrates showed higher cocoon production rate than the standard

Table 4
Two-way ANOVA table showing the effect of vermicomposting (60 days) of different substrates by the earthworm *Perionyx excavatus* on total microbial population.

Source of Variation	SS	df	MS	F	P-value	Significance level
Total bacterial population						
Between substrates	114	3	38	7.728814	< 0.02	5%
Between samples*	25896.5	2	12948.25	2633.542	< 0.001	0.1%
Error	29.5	6	4.916667			
Total	26,040	11				
Total fungal population						
Between substrates	321.6667	3	107.2222	4.381385	< 0.05	5%
Between samples*	6606.5	2	3303.25	134.9796	< 0.001	0.1%
Error	146.8333	6	24.47222			
Total	7075	11				
Total actinomycetes population						
Between substrates	36.66667	3	12.22222	1.148825	> 0.05	Not significant
Between samples*	25636.17	2	12818.08	1204.833	< 0.001	0.1%
Error	63.83333	6	10.63889			
Total	25736.67	11				

* Initial, compost and vermicompost.

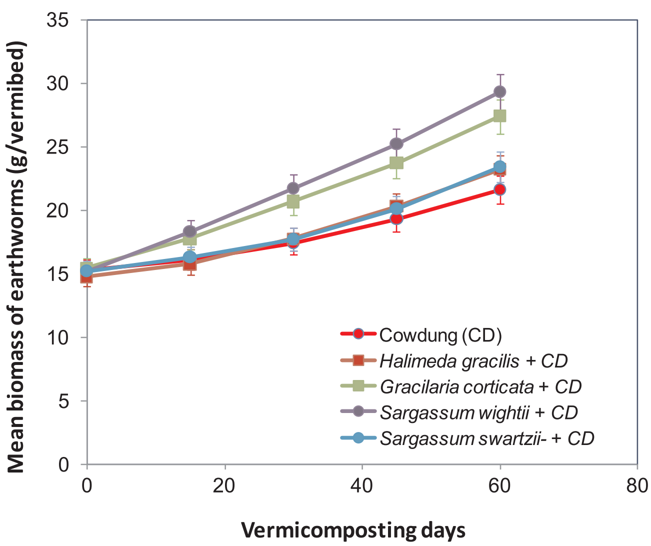

Fig. 1. Mean biomass (g) of the earthworm, *Perionyx excavatus* during seaweed + cowdung (1:1) vermicomposting (60 days). Values are mean of three replicates. Error bars indicate ± SEM.

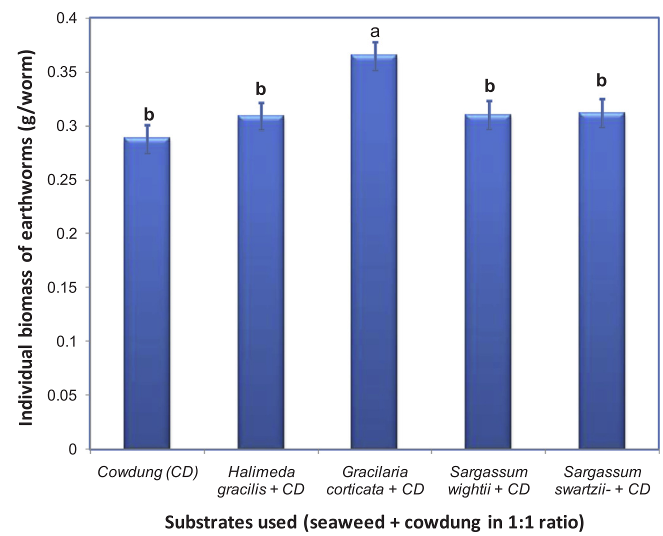

Fig. 2. Individual biomass (g) of the earthworm, *Perionyx excavatus* recovered from the vermibeds of seaweed + cowdung (1:1) combinations (60 days). Values are mean of three replicates. Error bars indicate ± SEM. The difference in mean values between the vermibed materials (bars) followed by the same letter are not significant at 5% level ($P < 0.05$) by Tukey's multiple comparison test.

diet, CD. The difference in cocoon production rate of the earthworms in seaweed + CD substrates was significantly different from CD at $P < 0.05$ (Fig. 4).

The number of juvenile worms recovered from different vermibed substrates after 60 days showed variation between substrates. The number of juveniles recovered in *Halimeda gracilis* + CD, *Gracilaria corticata* + CD and CD alone (control) was 21 worms/vermibed with statistically insignificant difference ($P > 0.05$). The higher number of juveniles was recorded in *Sargassum wightii* + CD with a maximum of 33 worms/vermibed followed by *Sargassum swartzii* + CD (28 worms/vermibed) and these values were significantly higher than control substrate, *Halimeda gracilis* + CD and *Gracilaria corticata* + CD, at 5% significance level (Fig. 5). The growth rate of *Perionyx excavatus* in *Sargassum wightii* + CD showed maximum (4.55 mg/worm/day) on 60th day. The increase of growth rate significantly differed between vermibed substrates ($P < 0.001$) and between number of days ($P < 0.001$). In addition, the cocoon production rate of the earthworms reared in vermibed substrates after 60 days ranged from 0.027 to 0.041 cocoon/worm/day with a maximum of 0.041 cocoon/worm/day in *Gracilaria corticata* + CD substrate. Likewise, the number of juvenile worms recovered from different vermibed substrates also showed variation between substrates.

Conspicuously, the acceptability of feed materials by the earthworms is very essential for maximum bioconversion of organic inputs in vermicomposting systems. Several studies have revealed that the dietary intake and environmental conditions greatly influence worm growth and reproduction. In three forms of diet (dung of cattle, horse and 1:1 mixture of cattle and horse) used by Gunadi et al. (2002) showed that the growth of *Eisenia fetida* was almost similar, but the biomass in cattle manure (565.7 ± 15.3 mg) was significantly more than horse manure (494.9 ± 22.8 mg) and 1:1 mixture (470.3 ± 22.0 mg). Consistently, cattle dung favoured biomass, growth and cocoon production. Worms survived up to 92, 68 and 66 weeks in cattle manure, 1:1 mixed manure and horse manure respectively. In the present study also CD added with seaweeds as bulking agent might have played a major role in the acceptability of the seaweed substrates by the earthworms. It was reported that there was a consistent trend for fewer cocoons to be produced with increasing times of pre-composting, but there was no clear pattern of effect of pre-composting on the number of earthworm hatchlings produced in case of *Eisenia fetida* (Gunadi et al., 2002). Deolalikar et al. (2005) reported that the bioaccumulation of any metal was not observed in the body of

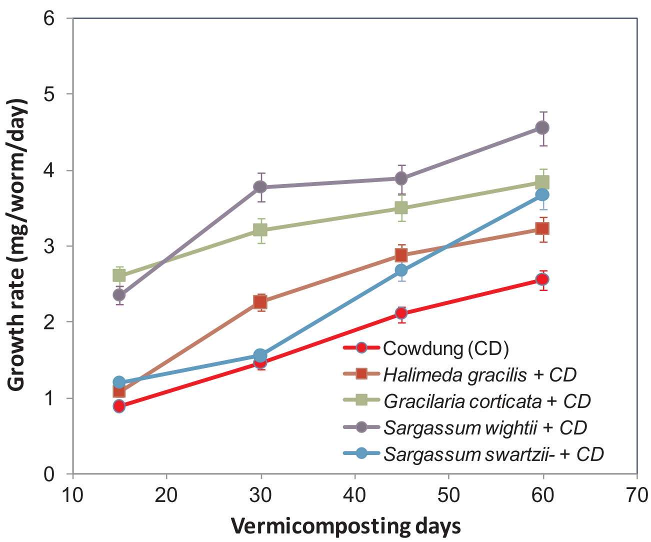

Fig. 3. Growth rate of the earthworm, *Perionyx excavatus* during the vermicomposting of seaweed + cowdung (1:1) combinations (60 days). Values are mean of three replicates. Error bars indicate ± SEM.

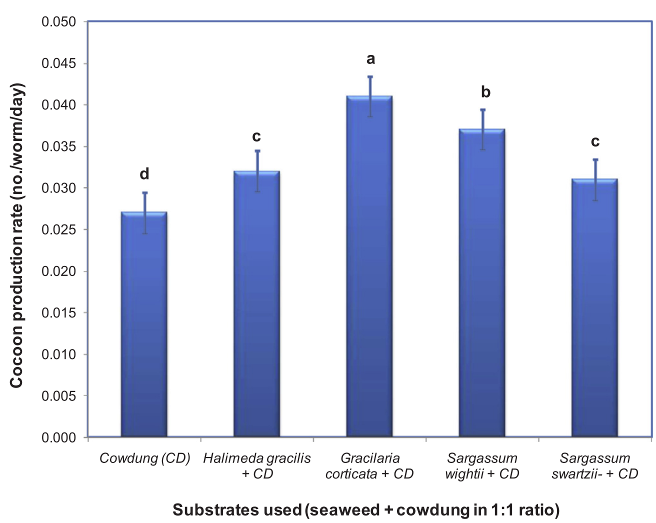

Fig. 4. Cocoon production rate of the earthworm, *Perionyx excavatus* reared in vermibeds of seaweed + cowdung (1:1) substrates (60 days). Values are mean of three replicates. Error bars indicate ± SEM. The difference in mean values between the vermibed materials (bars) followed by the same letter are not significant at 5% level ($P < 0.05$) by Tukey's multiple comparison test.

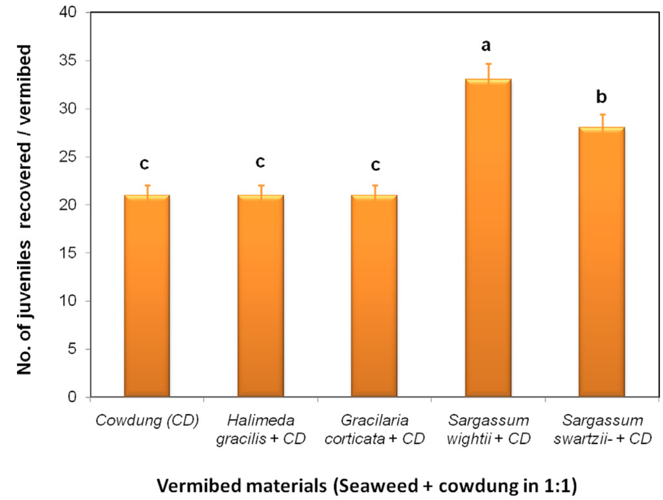

Fig. 5. Number of juvenile earthworms recovered/vermibed containing seaweed + cowdung (1:1) substrates. Values are mean of three replicates. Error bars indicate ± SEM. The difference in mean values between the vermibed materials (bars) followed by the same letter are not significant at 5% level ($P < 0.05$) by Tukey's multiple comparison test.

the earthworm, hence after vermicomposting, there is no harm to fishes to utilize earthworm biomass as their feed. *Perionyx sansibaricus* showed maximum biomass production, growth rate (mg day^{-1}), mean cocoon numbers, and reproduction rate (cocoon worm^{-1}) in vegetable waste + leaf litter. The increased level of plant metabolites in end product (vermicompost) and growth patterns of *Perionyx sansibaricus* in different organic waste resources demonstrated the candidature of this species for wastes recycle operations at low-input basis (Suthar, 2007). Cocoon production rate by the earthworm, *Perionyx ceylanensis* was found between 0.85 and 0.94 cocoons/worm/day and the hatching success between 74.67 and 82.67% (Karmegam and Daniel, 2009).

Chaudhuri and Bhattacharjee (2002) reported that CD, a natural food of *Perionyx excavatus*, was marginally better than the mixture with kitchen waste with regard to the rate of biomass increase and reproduction. Thus, the combination of CD with seaweeds might have played a major role in the growth and reproduction of *Perionyx excavatus* in the present study. In addition to the vermicompost characteristics, 2:2 (ultimately 1:1) combination of cashew leaf litter with various animal dungs, CD, sheep dung and horse dung substrates well supported the growth and reproduction of predominantly available indigenous epigeic earthworm, *Perionyx excavatus* (Parthasarathi et al., 2016). The growth and reproductive performance of *Perionyx excavatus* and other vermicomposting earthworms in different organic substrates as discussed in the above studies clearly demonstrate that efficient vermicomposting with nutrient rich vermicompost production requires good growth and reproduction of the earthworms. In the present study, despite the fact that variations occur in growth and reproduction of *Perionyx excavatus* in different seaweed + CD substrates, the results are very close when compared with the standard diet, CD. The worms showed comparably good growth and reproduction in seaweed substrates which has been well supported with the physico-chemical characteristics and fertilizer value of vermicompost. Presently about 22,000 tonnes of seaweeds are harvested annually in India with a mere 2.5% of the potential harvest of 870,000 tonnes (Anon, 2003). The addition of fermented seaweed *Kappaphycus alvarezii* sap and the biofertilizer, *Azolla pinnata* resulted in the production of enriched vermicompost (Lakshmi and Ebenezer, 2010b). There is a prospective scope to produce nutrient rich vermicompost from the large quantities of seaweed biomass availability through vermistabilization. The scope can also be extended depending on the requirement of the crop plants by further enrichment with effective beneficial microbial consortium for novel and enriched seaweed fertilizer production.

4. Conclusion

Vermicomposting of four different seaweeds, *Halimeda gracilis, Gracilaria corticata, Sargassum wightii* and *Sargassum swartzii* in combination with CD in 1:1 ratio using *Perionyx excavatus* resulted in the production of vermicompost with enhanced nutrients and microbial populations than in the worm-unworked compost. In addition, the TOC and C/N reduced greatly in the vermicompost of seaweed + CD substrates than the initial substrates and worm-unworked composts. The nutrient status, growth and reproduction of *Perionyx excavatus* in seaweed + CD combinations in the present study clearly showed that 15 days pre-decomposed seaweeds spiked with CD (1:1) can be used as bioresources for the production of vermicompost.

Declarations of interest

None.

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