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Feasibility of vermicomposting in biostabilization of sludge from a distillery industry

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ARTICLE INFO

Article history:
Received 2 October 2007
Received in revised form
28 January 2008
Accepted 3 February 2008
Available online 3 March 2008

Keywords:
Distillery sludge
Industrial waste
Vermicomposting
Metals
Cocoon
Sludge stabilization
Perionyx excavatus

ABSTRACT

The feasibility of vermicomposting technology to stabilize the distillery industry sludge mixed with a bulking agent (cow dung) in different proportions *viz.* 20% (T_1), 40% (T_2), 60% (T_3) and 80% (T_4), was tested using composting earthworm *Perionyx excavatus* for 90 days. The vermitreated sludge was evaluated for different physico-chemical parameters and all vermibeds expressed a significant decrease in pH (10.5–19.5%) organic C contents (12.8–27.2%), and an increase in total N (128.8–151.9%), available P (19.5–78.3%) as well as exchangeable K (95.4–182.5%), Ca (45.9–115.6%), and Mg contents (13.2–58.6%). Data suggested that inoculated earthworms could maximize the decomposition and mineralization rate, if sludge is used with appropriate bulking material for earthworm feed. Vermicomposting also caused significant reduction in total concentration of metals: Zn (15.1–39.6%), Fe (5.2–29.8%), Mn (2.6–36.5%) and Cu (8.6–39.6%) in sludge. Bioconcentration factors (BCFs) for metals in different treatments were also calculated and the greater values of BCFs indicate the capability of earthworms to accumulate a considerable amount of metals in their tissues from substrate. The reproduction biology of *P. excavatus* in different treatments was also monitoring during experimentation and they showed the maximum rate of biomass gain, growth (mg weight worm^{-1} week^{-1}) and cocoon production rate in T_2, while least values of these parameters were in T_4 treatment. The feasibility of earthworms to mitigate the metal toxicity and to enhance the nutrient profile in sludge might be useful in sustainable land restoration practices at low-input basis.

1. Introduction

Agriculture, food processing, pulp and paper, or any plant-based industry produces massive quantities of liquid, gaseous or solid wastes. Disposal and environmental friendly management of these industrial wastes has become a serious global problem. The traditional disposal methods such as open dumping and/or land filling practices of these materials are not only increasingly expensive, but impractical as open space becomes limited. Contamination of ground water, soils, as well as, food resources are some of the problems which have resulted from land filling practices of dumped waste materials. Therefore, there is an instant need of safe technology to manage such noxious industrial waste; the technologies must be ecologically sound, economically viable and socially acceptable. However, some agro-industrial sludges contain a considerable amount of plant nutrients, which can be reused for food production and land restoration practices. Thus, individually and cumulatively agro-industrial waste products seem to have the potential to afford eco-friendly, cost-effective and socially acceptable and sustainable bio-resources for increasing

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doi:10.1016/j.scitotenv.2008.02.005

agriculture productivity through multifaceted mechanisms and biotechnological applications.

Distillery is an important sub-unit of sugar production industry. Estimates show that organic waste produced from the distillery industry includes effluent of molasses 350,000 l/day; yeast sludge 20,000 l/day and spent malt grain wash 120,000 l/day. This huge quantity of effluent enters lagoons where it is aerated to reduce the Biological Oxygen Demand (BOD) and afterward the effluent is used for land irrigation. The solid particles settle in the lagoons to form a sludge that can be used as biofertilizer because of its nutritive value. However, prior to application the waste must be processed properly using appropriate composting method. Earlier, studies have revealed that vermicomposting could be an appropriate technology to transfer energy rich organic wastes to value-added products, i.e., vermicompost (Kale, 1998). Earthworm accelerates the transformation of organic waste material into more stabilized forms by aeration and bioturvation, by their excreta and qualitative or quantitative influence upon the telluric microflora (Vinceslas-Akpa and Loquest, 1997; Suthar, 2007a). Vermicomposting is stabilization of organic material involving the joint action of earthworms and microorganisms. However, compared to thermal composting, vermicomposting with earthworms often produces a product with a lower mass, lower processing time, humus content, phytotoxicity is less likely, more N is released, fertilizer value is usually greater, and an additional product (earthworms), which can have other uses is produced (Lorimor et al., 2001). Therefore, vermicomposting seems to be more appropriate and an efficient technology to convert industrial waste to a valuable community resources at low-input basis.

The objective of this study was, to test the feasibility vermicomposting to stabilize the distillery sludge mixed with cow dung in different ratios using earthworm *Perionyx excavatus* (Perrier). The level of metals in end product was also monitored so that the value of vermicompost as environmentally safe product can be determined.

2. Materials and methods

2.1. Earthworm and distillery sludge

Composting earthworms i.e. *Perionyx excavatus* (Perrier) were obtained from stock culture maintained in laboratory. Stock earthworms were cultured, in the laboratory, on partially decomposed cow dung mixed with leaf litter of *Mangifera indica*. To avoid the possibility of earlier exposure of earthworms to any contaminant, the second generations of composting earthworm was used for vermicomposting experimentation. Distillery sludge from the lagoons was obtained from the distillery unit of Sri Ganganagar Sugar mill Co-operatives Ltd., Sri Ganganagar, India. The dark brown coloured distillery sludge was collected in large-sized plastic circular containers and brought to the laboratory. The chemical characteristics of sludge are reported in Table 1. Collected distillery sludge with dry matter contents of about 46% was treated aerobically, in small aeration tank of 50 l capacity prepared by adding mechanically driven small aeration device, for about 15 days. Cow dung was used as amendment material during this study. The fresh cow dung used in experiment was obtained from a local cowshed. The chemical characteristics of cow dung are recorded in Table 1.

Table 1 – Chemical characteristics of distillery sludge and cow dung used in experiment

Parameters	Distillery sludge	Cow dung
pH	7.23±0.11	8.52±0.13
C_{org} (g kg^{-1})	264.65±6.99	285.99±5.99
N_{tot} (g kg^{-1})	4.78±0.21	20.84±1.31
P_{avail} (g kg^{-1})	40.77±2.10	5.72±0.23
K_{exch} (g kg^{-1})	8.56±0.16	4.93±0.12
Ca_{exch} (g kg^{-1})	89.36±3.27	12.71±0.24
Mg (mg kg^{-1})	345.96±10.48	261.17±4.94
Zn (mg kg^{-1})	454.86±14.21	297.20±1.37
Fe (mg kg^{-1})	578.12±21.04	282.95±2.76
Mn (mg kg^{-1})	441.25±11.71	198.49±2.57
Cu (mg kg^{-1})	54.97±3.80	32.26±1.94

Table 2 – Composition of treatments

Treatment	Treatment description	Distillery sludge (g)	Cow dung (g)
T_1	DS[a] (20%)[c]+CD[b] (80%)	100	400
T_2	DS (40%)+CD (60%)	200	300
T_3	DS (60%)+CD (40%)	300	200
T_4	DS (80%)+CD (20%)	400	100

[a] Distillery sludge (DS).
[b] Cow dung (CD).
[c] The figures in parenthesis indicates the percent content in the initial substrate material.

2.2. Treatment design

Aerobically treated distillery sludge was slightly dried in air and mixed with cow dung in different ratios to give different combinations. Cow dung was air dried and mixed (on volume basis) with distillery sludge in different ratios (Table 2). The experimental containers were prepared as per method described by Suthar (2007b,c). Experimental beddings were kept in triplicate for each treatment, and the control treatment had the same setup without earthworm. Twenty 5-week old clitellated, *P. excavatus* (having individual live weight in the ranges 301 and 310 mg) were collected from the stock culture and released into different plastic pot containers containing 750 g (on dry weight basis) of substrate material. The moisture level of substrates was maintained around 70–73%, throughout the study period by periodic sprinkling of adequate quantity of tap water. To prevent moisture loss, the experimental pots were covered with paddy straw. Containers were placed in a humid and dark room with a temperature of 28.5±0.4 °C (mean of the temperature recorded during the experiment duration±SEm). The important chemical parameters of substrate were measured in all the treatments before the introduction of earthworms and after each 15-day interval, up to 90 days. Some biological parameters of composting earthworm (biomass change, cocoon production, total mortality etc.) were studied in vermicomposting subsystem by following the method described by Suthar (2007b,c).

2.3. Chemical analysis of substrate and ready vermicompost

The pH was measured using digital pH meter (Systronic made) in 1/10 (w/v) aqueous solution (deionized water). Organic carbon was determined by the partially-oxidation method (Walkley and Black, 1934). Total nitrogen was measured by micro Kjeldahl method (Jackson, 1973). C:N ratio was calculated from the measured value of C and N. Extractable phosphorous was determined by following Olson's sodium bicarbonate extraction method (Olsen et al., 2002). Exchangeable elements (K, Ca and Mg) were determined after extracting the sample using ammonium acetate extractable method (Simard, 1993); analyzed by Perkin-Elmer model 3110 double beam atomic absorption spectrophotometer (AAS). To estimate the total metal contents (Cu, Fe, Zn, and Pb) in substrate, ready vermicompost, compost and earthworm tissues, 1 g dry sample was heated with 10 ml concentrated HNO_3, starting at 80 °C (Pedersen and van Gestel, 2001). The samples were than dried at 135 °C, redissolved in 40 ml 0.1 M HNO_3, centrifuged and filtered (Kamitani and Kaneko, 2007). The filtrate was used for metals using double beam atomic absorption spectrophotometer (Perkin-Elmer-model 3110).

2.4. Analysis of earthworms for metal contents

The clitellated specimens of earthworms were separated from each treatment containers and were washed in deionized distilled water. To avoid contaminations disposable gloves washed with HCl 1 N were worn during earthworm handling. To clear the ingested soil material from alimentary canal the worms were placed in sterilized glass petri dishes with one filter paper (Whatman No. 1) and a few drops of distilled water to maintain them moist. They were kept in the dark for 3 days and filter paper was changed daily. The earthworms of approximately equal weight were separated and subsequently killed through deep freezing. The alimentary contents of worms were removed by dissection. They were then oven dried (48 h at 70 °C) to constant weight. The dried earthworm tissues samples were tested for metal contents by following same method as described for soil metal contents.

2.5. Bioconcentration factor

Bioconcentration factors (BCF) for earthworm *P. excavatus* were estimated for metals in earthworm tissues and substrate materials using method as described by Mountouris et al. (2002). The bioconcentration factor (BCF) is defined as $BCF = C_{biota}/C_{substrate}$, where C_{biota} and $C_{substrate}$ were the total concentrations in taxa (earthworm) and substrate (used for vermicomposting experiment), respectively in mg/kg^{-1}.

2.6. Statistical analysis

One-way ANOVA was used to analyze the significant difference between treatments. Tukey's *t*-test was also performed to identify the homogeneous type of the bedding material in respect to different parameters used in this study. A paired-sample *t*-test was performed between control (compost without worms) and experiment (vermicompost with earthworms) for different chemical parameters.

3. Results and discussion

3.1. Dynamics of nutrients during vermicomposting process

As presented in Table 4, pH in all treatments was lower in vermicomposted material relative to their initial values (Table 3). Vermicomposting significantly lowered the pH of substrates at end, and the maximum reduction was in T_3 (−19.5% than initial), whereas T_4 (−10.5%) showed the lowest reduction for pH. The difference between composted and vermicomposted material for pH level was significant (*t*-test: $P<0.01$, for all treatments). The shift in pH during the study could be due to microbial decomposition during the process of vermicomposting. Elvira et al. (1998) concluded that production of CO_2 and organic acids by microbial decomposition during vermicomposting lowers the pH of substrate. Organic C was lower in the final product, i.e. vermicompost, when compared to the initial level in the treatments. The organic C loss (percent lower than initial) was in the order: T_3 (27.2%)>T_2 (21.1%)>T_4 (12.8%)>T_4 (9.5%) (Table 4). As compared to control, the vermicomposted material showed more organic C loss (*t*-test: $P<0.01$, for all treatments) from substrate. Earthworms fragment and homogenize the ingested material through muscular action of their foregut, which results in an increasing surface area for microbial action. Whereas, microorganisms biochemically degrade and provide some extra-cellular enzymes required for organic waste decomposition within the worm's gut. This biological mutuality results in C loss from substrates through respiration. However, the differences in C-loss patterns, between the treatments, were related to the proportion of amendment material in vermibed. The maximum C mineralization as observed in T_3 was possibly related to the suitable microbial conditions in this treatment than other treatments studied. A tend of N increase was in all vermibeds, at the end. The vermicomposted material showed comparatively more N content than composted material (*t*-test: $P<0.01$, for all treatments). The maximum N increase was in T_2 (+151.4% of the initial amount) followed by T_3 (150.6%), T_3 (141.2%) and T_1

Table 3 – Chemical characteristics of initial substrate material used in different treatments (mean ± SEm, $n=3$)

Treatment [a]	pH	C_{org}	N_{tot}	P_{avail}	K_{exch}	Ca_{exch}	Mg_{exch}	C:N ratio
T_1	8.0±0.11	275.8±6.70	5.9±0.15	13.9±0.06	8.39±0.18	33.6±0.43	28.1±0.30	46.5±0.65
T_2	7.8±0.05	285.5±6.60	7.0±0.21	21.2±0.33	8.53±0.14	38.4±0.22	30.5±0.33	41.0±0.64
T_3	7.7±0.02	282.2±3.64	8.1±0.30	25.2±0.35	8.67±0.11	41.5±0.58	39.0±0.48	34.8±1.50
T_4	7.6±0.04	282.8±6.74	8.5±0.13	26.1±0.27	8.75±0.05	45.5±0.51	40.3±0.13	33.3±1.23

[a] For treatment compositions see Table 2. All values in g kg^{-1} except to Mg (mg kg^{-1}) and pH.

Table 4 – Chemical composition of composted and vermicomposted distillery sludge (mean ± SEm, n = 3) at the end of experiment

Treatment[a]	pH		C_{org}		N_{tot}		C:N ratio	
	Compost	Vermicompost	Compost	Vermicompost	Compost	Vermicompost	Compost	Vermicompost
T_1	7.6±0.03	6.7±0.30	266.4±2.8	249.7±4.8	6.39±0.09	13.5±0.30	41.7±0.86	19.5±0.38
T_2	7.4±0.03	6.5±0.43	274.7±4.5	225.4±2.6	7.14±0.10	17.6±0.47	38.5±0.48	14.9±0.66
T_3	7.3±0.07	6.3±0.12	264.1±3.8	206.0±3.1	8.47±0.23	20.3±0.35	31.2±1.3	10.1±0.31
T_4	7.2±0.05	6.8±0.38	274.7±3.7	247.1±1.6	9.99±0.30	20.5±0.95	27.5±1.1	12.1±0.46
Treatment[a]	P_{avail}		K_{exch}		Ca_{exch}		Mg_{exch}	
	Compost	Vermicompost	Compost	Vermicompost	Compost	Vermicompost	Compost	Vermicompost
T_1	14.0±0.05	29.6±0.52	8.63±0.18	20.4±1.5	33.8±0.20	68.8±1.4	28.3±0.16	39.0±0.81
T_2	21.0±0.25	37.8±0.66	8.68±0.14	24.1±1.3	38.5±0.67	82.8±1.2	30.7±0.19	46.0±0.97
T_3	26.3±0.30	43.6±1.5	8.82±0.54	20.9±0.67	41.70±0.48	66.0±0.71	39.5±0.36	55.2±0.90
T_4	26.5±0.31	31.2±1.5	8.89±0.05	17.1±0.68	45.82±0.21	66.4±1.0	40.7±0.19	45.6±1.48

[a] For treatment compositions see Table 2. All values in g kg^{-1} except to Mg (mg kg^{-1}) and pH.

(128.8%). It is suggested that in addition to releasing N from compost material, earthworms also enhance nitrogen levels by adding their excretory products, mucus, body fluid, enzymes etc. to the substrate. Suthar (2006) suggested that decaying tissues of dead worms also add a significant amount of N to vermicomposting sub-system. In general, nitrogen enrichment pattern and mineralization activities mainly depend upon the total amount of N in the initial waste material (e.g., sludge) and on the earthworm activity in the waste decomposition sub-system (Kale, 1998; Suthar, 2007b). It is also suggested that the differences for N content in end product (vermicompost) could be related to the availability of metals in vermibeds, which directly affects the N mineralization rate. Recently, Suthar (2008) demonstrated a significant impact of high metal contents on mineralization rate during vermicomposting of sewage sludge.

The available P content was significantly higher in vermicompost than composted material (t-test: $P<0.01$, for all treatments). The maximum increase was in T_1 (91.4%) followed by T_2 (78.3%), T_3 (73.0%) and T_4 (19.5%) (Table 4). Studies have revealed that during vermicomposting the release of available P content from organic waste is performed partly by earthworm gut phosphatases, and further release of P might be attributed to the P-solubilizing microorganisms present in worm casts. In this study, the P mineralization rate decreased with increasing proportion of distillery sludge in vermibeds. It indicates the adverse impact of greater concentrations of distillery sludge on microbial enzymes related to P mineralization process in vermicomposting sub-system. Observed order of P mineralization rate: $T_1>T_2>T_3>T_4$ supports this hypothesis. Potassium content in end material was higher than the initial contents. Statistically, vermicomposted material showed significant difference for exchangeable K, Ca and Mg contents than composted (without worms) material (t-test: $P<0.01$, for all treatments). The pattern of K increase was recorded in the order: T_2 (182.5%)>T_1 (143.2%)>T_3 (137.6%)>T_4 (95.4%). Similarly, T_2 also showed the maximum increase (than initial level) for Ca and Mg (182.5 and 115.6%, respectively), whereas T_4 showed the lowest increase for both chemicals (95.4 and 45.9%, respectively). The mineralization rate during the vermicomposting process was in the order: $T_2>T_1>T_3>T_4$. However, when organic waste passes through the gut of worm the nutrients converted from unavailable forms to available forms, which consequently enrich the worm cast with higher quality plant nutrient. The similar pattern of calcium enhancement is well documented in the available literature (Hartensein and Hartenstein, 1981; Gupta and Garg, 2007; Suthar, 2007c). The C:N ratios of substrate material reflect the organic waste mineralization and stabilization during the process of decomposition. The loss of carbon as carbon dioxide through microbial respiration and simultaneous add of nitrogen by worms in the form of mucus and nitrogenous excretory material lowered the C:N ratio of the substrate. In this study the maximum reduction in C:N ratio was in T_3 vermibed (71.3% of initial value) followed by T_4 (63.7%)≈T_2 (63.7%) and T_1 (58.1%). The difference between composted and vermicomposted material was significant (t-test: $P<0.01$, for all treatments). Studies have revealed that C:N ratio, which is one of the most widely used

Table 5 – Metals in initial and final material (vermicompost) in different treatments (mean ± SEm, $n=3$)

Treatment[a]	Zn-total		Fe-total		Mn-total		Cu-total	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
T_1	288.1±6.2	174.1±2.0	332.5±4.1	233.5±3.9	235.8±4.5	160.3±2.8	35.1±2.8	21.2±0.5
T_2	335.7±5.3	241.1±5.3	405.4±4.6	291.1±2.3	262.5±3.1	166.7±4.0	38.4±1.8	28.8±1.2
T_3	368.2±7.2	249.2±6.3	458.0±5.8	373.2±6.2	295.8±5.3	238.7±3.6	41.1±2.8	29.8±0.9
T_4	430.0±6.2	365.3±2.9	512.2±7.7	485.8±3.6	431.9±3.9	420.5±2.8	44.0±2.7	40.2±0.6
ANOVA								
F		317.6		655.7		1420.7		124.8
P		<0.001		<0.001		<0.0001		<0.001

[a] For treatment compositions see Table 2. All values in mg kg^{-1}.

indicator of compost maturation, decrease sharply during vermicomposting process (Kale, 1998; Gupta and Garg, 2007; Suthar, 2008).

3.2. *Metal contents in end product (vermicompost)*

The total metal contents in vermicomposted material are shown in Table 5. Vermicomposting process caused a significant reduction in total metal contents than initial materials. The reduction in total contents of Zn, Fe, Mn and Cu ranged between 15.1 to 39.6%, 5.2 to 29.8%, 2.6 to 36.5%, and 8.6 to 39.6%, respectively. The reduction in metal content (as compared to initial level), for all the four treatment studied, was in the order: 28.7% (Zn)>25.2% (Cu)>22.6% (Mn)>20.4% (Fe). The greater reduction in metal content was in treatments, which showed the better decomposition as well as earthworm growth activities e.g. T_1 and T_2 treatments. Therefore, it is suggested that metal loss was related to the earthworm activity in the waste decomposition sub-system. The previous studied have revealed that earthworm can accumulate heavy metals in their tissues if reared in contaminated soils for long durations (Hartensein and Hartenstein, 1981; Gupta et al., 2005; Suthar et al., 2008). Data suggested that vermicomposting could be an appropriate technology for metal remediation from noxious industrial wastes. The ready vermicompost was not only rich in plant nutrient, but also have minimum risk of environmental contamination due to lower metal concentrations.

3.3. *Bioaccumulation of metals by earthworms*

The composting earthworms showed the greater concentration of metals in their tissues (Table 6). The concentration of total Zn, Fe, Mn and Cu in earthworm tissues ranged between 52.7 to 93.7 mg kg^{-1}, 31.0 to 106.6 mg kg^{-1}, 20.3 to 45.7 mg kg^{-1}, and 9.4 to 18.0 mg kg^{-1}, respectively. Although, earthworms collected from different treatments showed a considerable difference for tissues metal concentrations, but difference was not statistically significant for Zn (ANOVA, $P=0.277$) and Cu contents (ANOVA, $P=0.322$). Similarly, T_1, T_2 and T_4 did not show any significant difference in respect to tissues-Fe contents (ANOVA/Tukey's *t*-test; $P=0.095$). The observed difference for tissues metal contents could be related to the feeding or feeding rate or/and amount of metals in ingesting materials by inoculated earthworms. Some earlier studies have revealed that earthworm can accumulate a considerable amount of metals in their tissues if reared for long periods in contaminated soil/substrates (Lukkari et al., 2006; Suthar et al., 2008).

Recently, two basic approaches: the bioconcentration factors (BCFs) or bioaccumulation factors (BAFs) has been used widely to quantify the bioaccumulation of environmental pollutants in aquatic and terrestrial biota with assumption that organism achieve a chemical equilibrium with respect to a particular media or route of exposure (Mountouris et al., 2002; Hsu et al., 2006). In this study we recorded the bioconcentration factors (BCFs) in the ranges: 0.23–0.46 (Zn), 0.22–0.33 (Fe), 0.19–0.25 (Mn) and 0.41–0.47 (Cu) (Table 7). BCFs did not show any significant difference among treatments for total Zn (ANOVA/Tukey's *t*-test; $P=0.057$), Mn (ANOVA/Tukey's *t*-test; $P=0.055$) and Cu (ANOVA/Tukey's *t*-test; $P=0.814$), except in Fe (ANOVA: $F=6.49$; $P=0.290$) contents. The range of bioaccumulation factor was higher for Zn and Cu and after calculating the average of BAFs, the metals can be arranged in the order: Cu (0.45)>Zn (0.35)>Fe (0.27)>Mn (0.22). However, the greater metal concentrations in composting earthworms did not affect the waste

Table 6 – Metal contents in earthworm tissues (mean ± SEm, $n=3$) collected from different treatments at end

Treatment[a]	Zn-total	Fe-total	Mn-total	Cu-total
T_1	107.0±17.2 a	83.7±4.7 a	49.7±3.9 a	16.3±1.5 a
T_2	128.3±13.6 a	113.3±6.1 a	66.0±3.1 ab	18.0±1.5 a
T_3	122.3±1.8 a	150.3±11.4 b	68.0±6.0 ab	16.7±1.5 a
T_4	97.0±6.7 a	111.7±6.7 a	80.3±2.4 b	20.3±1.8 a

Mean value followed by different letters is statistically different (ANOVA; Tukey's *t*-test, $P<0.05$).
[a] For treatment compositions see Table 2. All values in mg kg^{-1}.

Table 7 – Bioconcentration factors (BCF) of the metals in *P. excavatus* species

Treatment[a]	Zn	Fe	Mn	Cu
T_1	0.37±0.08 a	0.25±0.01 ab	0.21±0.02 a	0.47±0.06 a
T_2	0.46±0.09 a	0.28±0.01 ab	0.25±0.01 a	0.47±0.04 a
T_3	0.33±0.01 a	0.33±0.03 b	0.23±0.02 a	0.41±0.05 a
T_4	0.23±0.02 a	0.22±0.02 a	0.19±0.01 a	0.44±0.05 a
ANOVA				
F	3.34	6.49	3.53	0.362
P	0.077	0.016	0.068	0.782

[a] For treatment compositions see Table 2.

Table 8 – Growth of *P. excavatus* (mean ± SEm, *n* = 3) in different treatments

Treatment[a]	Initial individual weight (mg)	Maximum individual weight achieved (mg)	Maximum live weight achieved	Maximum Growth rate (mg wt. worm^{-1} week^{-1})	Individual live weight at end (mg)
T_1	313.7 ± 1.80b	573.0 ± 8.95b	After 7th week	37.1 ± 1.46b	537.2 ± 9.19b
T_2	310.3 ± 0.88d	617.4 ± 3.70c	After 7th week	43.9 ± 0.50c	627.4 ± 0.88d
T_3	311.7 ± 1.76c	600.4 ± 10.6bc	After 8th week	37.9 ± 2.52bc	583.4 ± 11.10c
T_4	313.7 ± 0.88a	481.9 ± 4.0a	After 10th week	16.8 ± 0.49a	476.5 ± 4.05a
ANOVA					
F	1.32	65.47		61.95	50.03
P	=0.335	<0.001		<0.001	<0.001

Mean value followed by different letters is statistically different (ANOVA; Tukey's *t*-test, $P<0.05$).
[a] For treatment compositions see Table 2.

minimizing efficiencies of composting earthworms in decomposing sub-system. This hypothesis is supported by an excellent rate of mineralization in first three treatments *viz.* T_1, T_2 and T_3, which showed the greater BCFs values. According to Hopkin (1989) the earthworms have specific capacity to regulate metals, particularly trace metals, such as Cu and Zn, in their bodies and accumulation and regulation mechanisms could be species specific. The difference in BCFs for different metals studied could be due to different metabolic requirements of earthworms for metals (Lukkari et al., 2006). Hsu et al. (2006) have reported the higher bioaccumulation factors for earthworms collected from contaminated soils, which further support the hypothesis, that earthworm could be an important source to examine the metal contents in the soil/substrate (Suthar et al., 2008).

3.4. *Earthworm biology during vermicomposting process*

P. excavatus showed a significant difference for growth and reproduction performances: mean individual live weight at end ($F=50.02$, $P=0.001$), individual biomass gain ($F=46.90$, $P<0.001$), maximum individual growth rate (mg worm^{-1} week^{-1}) ($F=61.95$, $P<0.001$), total cocoon numbers ($F=41.69$, $P<0.001$) and mean reproduction rate (cocoon worm^{-1}) ($F=37.17$, $P<0.001$) among different treatments. The maximum and minimum mean individual live weight for *P. excavatus* was 617.4 ± 3.7 mg in T_2 treatment and 481.9 ± 4.5 mg in T_4 treatment, respectively (Table 8). Similarly, earthworm obtained the maximum biomass gain in T_2 (317.0 ± 10.2 mg) and was significantly higher than other treatments ($P<0.001$, for all). The maximum weight gain was followed by weight loss by the time of termination of the experiment. Suthar (2007b,c) and Gupta and Garg (2007) have reported the similar trend of weight loss in composting earthworms after a certain period in vermibeds. They attributed this weight loss to conversion of most of the used substrate to vermicompost, which cannot further support their growth. The maximum growth rate (mg wt. worm^{-1} week^{-1}) was recorded in T_1 (43.9 ± 0.50) followed by T_3 (37.9 ± 2.52), T_1 (37.1 ± 1.46) and T_4 (16.8 ± 0.49) ($P>0.001$, for all). However, there was a consistent pattern of deceasing worm growth rate in respect to increased sludge concentrations in treatments. It is suggested here that distillery sludge caused biological suppress on composting earthworm, if applied at higher rates in vermibeds, possibly due to more availability of growth retarding substances (e.g. salt and metals) for earthworms. The earthworm showed significant differences among treatments for total cocoon production rate (Table 9). *P. excavatus* produced cocoons in the ranges of 62.7 ± 3.18 (T_2)–14.0 ± 2.65 (T_4). Mean numbers of cocoon as well as cocoon worm^{-1} rate was in the order: $T_2>T_3>T_1>T_4$. The greater reproduction rate was registered in T_2 treatment (3.30 ± 0.17 cocoon worm^{-1}) that was significantly different than T_3 (2.77 ± 0.14 cocoon worm^{-1}), T_1 (2.0 ± 0.20 cocoon worm^{-1}), and T_4 (1.0 ± 0.13 cocoon worm^{-1}) ($P<0.05$) treatments. The cocoon production rate among different treatments could be attributed to the quantity of amendment material i.e. cow dung in substrate. The factors relating to the growth of earthworms may also be considered in terms of physiochemical and nutrient characteristics of waste feed stocks. It is suggested that organic waste palatability for earthworms is directly related to the chemical nature of the organic waste, which consequently affects the cocoon production rate in earthworms. The composting earthworms showed a statistically different pattern of earthworm mortality among different treatments (ANOVA: $F=20.1$, $P<0.01$). The maximal total earthworm mortality (% of initial population) was recorded in T_4 treatment (31.7 ± 4.43%), while T_1 and T_2 both showed the lowest earthworm mortality (6.7 ± 1.67%) for this study (Table 9). However, difference among first three treatments e.g. T_1, T_2 and T_3 ($P=0.344$) in respect to total worm mortality was not statistically significant (Table 9).

Table 9 – Cocoon numbers, reproduction rate and total earthworm mortality in different treatments (mean ± SEm, *n* = 3)

Treatment[a]	Nos. of cocoons produced during experimentation	Cocoon production rate (cocoon worm^{-1})	Total earthworm mortality during experiment (%)
T_1	37.7 ± 4.33b	2.01 ± 0.20b	6.7 ± 1.67a
T_2	62.7 ± 3.18 c	3.30 ± 0.17c	6.7 ± 1.67 a
T_3	48.0 ± 2.08b	2.77 ± 0.14c	13.3 ± 1.65a
T_4	14.0 ± 2.65 a	1.00 ± 0.13a	31.7 ± 1.40b
ANOVA			
F	41.70	37.17	20.10
P	<0.001	<0.001	<0.001

Mean value followed by different letters is statistically different (ANOVA; Tukey's *t*-test, $P<0.05$).
[a] For treatment compositions see Table 2.

The mortality in treatments containing higher proportion of distillery sludge could be related to the unfavourable micro-environment in vermibeds. More availability of toxic chemicals, due to higher proportions of distillery sludge in last two treatments (T_3 and T_4), possibly caused mortality at higher rate in composting earthworms.

4. Conclusions

Distillery sludge should not be treated as waste material because it has great agronomic potential. This study suggests that bio-stabilization of distillery sludge using earthworm could be a potential technology to convert noxious industrial by-product into nutrient rich biofertilizer. The vermicomposting process showed a demonstrable impact on total metal concentration of sludge. The higher values of bioconcentration factors (BCFs) for different metals indicate that earthworm can accumulate a considerable amount of metals in their tissues. Earthworm biomass production and reproduction performance was excellent in bedding those contained lower proportions of distillery sludge e.g. T_1 and T_2, which suggests that industrial sludge can retard the potentials of composting earthworms if applied at higher rate in vermibeds. This study indicates that distillery sludge could be utilized as an efficient soil conditioner for sustainable land practices, after processing by composting earthworms.

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