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Bioremediation of aerobically treated distillery sludge mixed with cow dung by using an epigeic earthworm *Eisenia fetida*

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Abstract The potential of the epigeic earthworm *Eisenia fetida* to stabilize sludge (generated from a distillation unit of the sugar industry) mixed with cow dung, in different proportions i.e. 20% (T_1), 40% (T_2), 60% (T_3) and 80% (T_4) has been studied under laboratory conditions for 90 days. The ready vermicompost was evaluated for its' different physico-chemical parameters using standard methods. At the end of experiment, all vermibeds expressed a significant decrease in pH (7.8–19.2%) organic C (8.5–25.8%) content, and an increase in total N (130.4–170.7%), available P (22.2–120.8%), exchangeable K (104.9–159.5%), exchangeable Ca (49.1–118.1%), and exchangeable Mg (13.6–51.2%) content. Overall, earthworms could maximize decomposition and mineralization efficiency in bedding with lower proportions of distillery sludge. DTPA extractable metal reduction in substrate was recorded between the ranges of 12.5–38.8% for Zn, 5.9–30.4% for Fe, 4.7–38.2% for Mn and 4.5–42.1% for Cu. Maximum values for the mean individual live weight (809.69 ± 20.09 mg) and maximum individual growth rate (mg wt. $\text{worm}^{-1}\ \text{day}^{-1}$) (5.81 ± 0.18) of earthworms was noted in T_1 treatment, whereas cocoon numbers (69.0 ± 7.94) and individual reproduction rate (cocoon $\text{worm}^{-1}\ \text{day}^{-1}$) (0.046 ± 0.002) was highest in T_2 treatment. Earthworm mortality tended to increase with increasing proportion of distillery sludge, and maximum mortality in *E. fetida* was recorded for the T_4 (45.0 ± 5.0) treatment. Results indicate that vermicomposting might be useful for managing the energy and nutrient rich distillery sludge on a low-input basis. Products of this process can be used for sustainable land restoration practices. The feasibility of worms to mitigate the toxicity of metals also reduces the possibility of soil contamination, which has been reported in earlier studies during direct field application of industrial wastes.

Keywords Vermicomposting · *Eisenia fetida* · Distillery sludge · Cocoon · Cow dung · Biomass production · Earthworm mortality

1 Introduction

The acute energy crisis and environmental degradation due to growth of industries has become a serious global problem. Industries produce huge quantities of liquid, gaseous or solid wastes, these waste products contribute to environmental pollution. The conventional disposal system for waste consists of open dumping or land filling of waste material. This practice is not sustainable due to leaching of certain toxic chemicals and metals from dumped wastes. 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. There is a need for a safe technology to manage these noxious industrial wastes; the technologies must be ecologically sound, economically viable and socially acceptable.

Some agro-industrial wastes contain a great amount of plant nutrient which can be utilized in food production and land restoration practices. In other words, these wastes could be utilized efficiently as a soil conditioner for sustainable soil fertility management programs. Presently there is a common concept to use the waste as a resource. Industrial byproducts require close scrutiny to determine their potential utilization. Possible uses for wastes include

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soil amendment as a fertilizer or conditioner, energy recovery (heat, liquid fuels, electricity), and production of chemicals (volatile organic acid, ammonia products, alcohols) (Westerman and Bicudo 2005). 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 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 liter/day; yeast sludge 20,000 liter/day and spent malt grain wash 120,000 liter/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 a composting method. Earlier, studies revealed that vermicomposting could be an appropriate technology to transfer energy rich organic wastes in to value-added products, i.e., vermicompost (Kale 1998; Elvira et al. 1998; Suthar 2006). Sennapa et al. (1995) demonstrated that *Eudrilus eugeniae* can breakdown the waste generated from distilleries when mixed with other potting materials. They recorded appreciable results during vermicomposting of distillery sludge mixed with pressmud, water hyacinth, plant litter, and cow dung in different proportions. The treatment consisting of three parts of distillery sludge and cow dung along with 1 part of press mud, water hyacinth and plant litter showed the maximum earthworm biomass production as compare to the others. Distillery sludge contains a high concentration of essential plant metabolites e.g. NPK and micro nutrients. Due to excellent nutritive value it, can be used as soil conditioner after processing through appropriate biotechnological devices.

In this study we examine to the suitability of a composting earthworm species i.e. *Eisenia fetida* (Savigny) for recycling distillery sludge mixed with a bulking agent (cow dung) in different ratios, to produce a value-added product (vermicompost). Vermicompost could be than used for sustainable land restoration practices.

2 Materials and methods

2.1 Earthworm and distillery sludge collection

Originally composting earthworms i.e. *Eisenia fetida* (Savigny) of different age groups were obtained from vermicomposting unit of a Cowshed: Shree Gaushala, Sri Ganganagar, India. Stock earthworms were cultured, in the laboratory, on partially decomposed cow dung mixed with leaf litter of *Mangifera indica.* 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 liter capacity prepared by adding mechanically driven small aeration device, for about 15 days. The fresh cow dung used in experiment was obtained from a local cowshed. The chemical characteristics of cow dung are recorded in Table 1.

2.2 Treatment design

Aerobically treated distillery sludge was slightly dried in air and mixed with cow dung in different ratios. Cow dung was air dried and mixed (on volume basis) with distillery sludge in different ratio to give four different treatments (Table 2). Plastic circular containers of appropriate size (28 cm diameter and 30 cm in depth) with pierced lid for aeration were used for vermicomposting experiments. Experimental beddings were kept in triplicate for each treatment, and the control treatment had the same setup without earthworm. All beddings were kept for one week prior to experimentation for thermal stabilization and initiation of microbial degradation of the substrate material. Twenty 4-week old clitellate, *E. fetida* (having individual live weight ≈300 mg) were collected from the stock culture and released into different plastic pot containers containing 750 g (on dry weight basis) of substrate

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_{exch} (g kg^{-1})	34.60 ± 10.48	26.12 ± 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})	243.25 ± 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

material. The moisture level of substrates was maintained around 65–70%, 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 29.4 ± 0.5°C (mean of the temperature recorded during the experiment duration ± SEm). Chemical parameters (organic C, total N, available P, exchangeable K, exchangeable Ca, exchangeable Mg, C:N ratio and metals) were measured for all the treatments before the introduction of earthworms and after each 15 day interval, up to 90 days, using standard methods.

Biochemical changes in substrate material as well as biomass and cocoon production in earthworms in each experimental container were measured at 15, 30, 45, 60, 75 and 90th day. Earthworms and cocoons, produced during the experiment, were separated from the substrate material by hand sorting, after which worms were washed in tap water to remove adhering material from their body, and subsequently weighed. Weight was recorded and the earthworms were returned to the appropriate containers. Separated cocoons were counted and introduced in separate bedding containing the same material in which their parents were reared. Data for biomass, cocoon numbers, and other growth parameters of the progeny produced i.e. growth rate (mg day^{-1}), maximum weight achieved and reproduction rate (cocoon worm^{-1} day^{-1}) were collected and recorded for the different treatments.

2.3 Chemical analysis

Homogenized sub samples of substrate material (10 g dry weight basis) were collected at day 0 (initial) and at each 15-day interval from each experimental container for chemical analysis. 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 Kjeldhal method (Jackson 1975). 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. 1954). 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). The heavy metals i.e. Cu, Fe, Zn and Mn were determined by means of AAS by following DTPA extraction method.

2.4 Statistical analysis

One-way ANOVA was used to determine the significant differences among treatments. Tukey's test was performed to identify where these differences occurred with respect to their different chemical and biological parameters (earthworm weight gain, individual growth rate, total cocoons produced during experiment, reproduction rate, and total mortality).

3 Results and discussion

3.1 Physico-chemical changes during vermicomposting process

As presented in Table 3, pH in all treatments was lower in all treatments (T_1–T_4) relative to their initial values. Maximum reduction was noted in T_3 whereas, T_4 showed lower reduction for the same. The observed differences between the pH at the start and end were significant ($P = 0.001$) for each treatment. 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. Similarly, Ndegwa et al. (2000) pointed out that a shift in pH might be related to the mineralization of the nitrogen and phosphorous into nitrites/nitrates and orthophosphates and bioconversion of the organic material into intermediate species of the organic acids. 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 recorded in the order: T_3 (25.8%) > T_2 (19.8%) > T_1 (8.6%) > T_4 (8.5%) (Table 3). However, statistically the differences between T_1 and T_4, for final C concentration, was not significant ($P = 0.668$). In general, the vermicomposting process refers

Table 3 Chemical characteristics of initial and final (vermicompost) materials in different treatments (mean ± SD, $n = 3$)

Treatment*	PH		C_{org}		N_{tot}		P_{avail}	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
T_1	8.0 ± 0.11	6.75 ± 0.03 c	275.82 ± 6.70	252.11 ± 5.28 c	5.93 ± 0.15	13.78 ± 0.52 a	13.87 ± 0.06	30.63 ± 3.13 a
T_2	7.77 ± 0.05	6.46 ± 0.05 b	285.49 ± 6.60	229.11 ± 9.54 b	6.97 ± 0.21	17.75 ± 1.06 b	21.18 ± 0.33	37.66 ± 2.09 b
T_3	7.80 ± 0.02	6.30 ± 0.02 a	282.24 ± 3.64	209.51 ± 5.68 a	8.11 ± 0.30	21.95 ± 2.59 c	25.24 ± 0.35	43.11 ± 2.22 b
T_4	7.60 ± 0.04	7.01 ± 0.06 d	282.76 ± 6.74	258.74 ± 6.74c	8.50 ± 0.13	19.58 ± 0.88 bc	26.13 ± 0.27	31.92 ± 2.13 a
	K_{exch}		Ca_{exch}		Mg_{exch}		C:N $_{ratio}$	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
T_1	8.39 ± 0.18	21.64 ± 3.13	33.56 ± 0.43	69.18 ± 3.28	28.14 ± 0.30	39.16 ± 3.09	46.45 ± 0.65	18.30 ± 0.54
T_2	8.53 ± 0.14	22.14 ± 2.96	38.37 ± 0.22	83.70 ± 3.04	30.49 ± 0.33	46.10 ± 1.91	40.99 ± 0.64	12.95 ± 1.24
T_3	8.67 ± 0.11	22.30 ± 1.70	41.45 ± 0.58	70.13 ± 4.70	38.99 ± 0.48	54.86 ± 1.78	34.82 ± 1.50	9.61 ± 0.83
T_4	8.75 ± 0.05	17.93 ± 1.46	45.51 ± 0.51	67.86 ± 2.51	40.30 ± 0.13	45.78 ± 2.74	33.28 ± 1.23	13.22 ± 0.29

* For treatment compositions see Table 2

Mean values followed by different letters is statistically different (ANOVA, Tukey's *t*-test, $P < 0.05$)

to feeding of earthworms on organic matter and microbial degradation; therefore, both earthworm and microorganism plays an important role in vermicomposting system (Suthar 2007a). Earthworms fragment and homogenize the ingested material through muscular action of their foregut, which results in an increasing of surface area for microbial action. Whereas, microorganisms biochemically degrades and provide some extra-cellular enzymes required for organic waste decomposition within the worm's gut. However, this biological mutualism results in the loss of carbon from substrates in the form of CO_2 due to decomposition and mineralization of organic C. The results observed in this study are in consistent with Suthar (2006) who reported significant C loss from organic waste after inoculation of worms. However, the differences in C-loss patterns, between the treatments, were related to the proportion of amendment, as well as, the proportion of distillery sludge in the substrate. The maximum carbon mineralization as observed in T_3 was related to the suitability of microbial conditions in this treatments than that of other treatments. Total N content in all studied treatments was found higher in end product i.e. vermicompost. Maximum N increase was noted in T_3 (170.7% of the initial amount) followed by T_2 (154.7%), T_1 (132.3%) and T_2 (130.4%). Statistically no significant difference was observed, for total N content in end product, between T_2 and T_4 ($P = 0.401$), and T_4 and T_3 treatment ($P = 0.283$) (Table 3). Results indicate that earthworms accelerate microbial-mediated nitrogen transformation during the process of vermicomposting. 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. 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 2007a). Lowest N mineralization occurred in treatments that contained higher proportion of distillery sludge (e.g. T_4). Therefore, the differences in N content of end product (vermicompost) could be related to the increased availability of metals in this substrate, which could be influence the activity of the microbial communities responsible for N mineralization. However, detailed study is still required to support the hypothesis.

The data indicates that, after completion of vermicomposting, the amount of available P was found higher in end product than initial values. Maximum increase was recorded in T_1 (120.8 %) followed by T_2 (77.8 %), T_3 (70.8 %) and T_4 (22.2 %) (Table 3). When organic matter passes through the gut of earthworm, results in some amount of phosphorus is converted to more available form. The release of phosphorous in available form is performed partly by earthworm gut phosphatases, and further release of P might be attributed to the P-solubilizing microorganisms present in worm casts. Le Bayon and Binet (2006) reported earthworm-mediated phosphatase enhancement in soils. They concluded that earthworm were responsible for additional alkaline phosphatases, produced in the worm gut and excreted through cast deposition. In the present study, however, there was a consistent trend in that the P mineralization rate decreased with increasing proportion of distillery sludge in the treatments. We conclude that higher concentrations the distillery sludge affects the production rate of microbial enzymes related to the P mineralization in composting sub-system.

Potassium content in the vermicomposted material was higher than the initial content. The pattern of K increase was recorded in the order: T_2 (159.5%) > T_1 (157.9%) > T_3 (157.2%) > T_4 (104.9%). However, there were no significant differences among treatments ($P = 0.202$). Delgado et al. (1995) reported higher potassium content in vermicompost produced from sewage sludge by red worms. According to Barois and Lavelle (1986) the earthworm primes its symbiotic gut micro flora with secreted mucus and water to increase degradation of ingested organic matter and the release of metabolites. Studies by Suthar (2006, in press) revealed that vermicomposting of organic residues significantly enhanced the concentration of exchangeable potassium in substrates. Vermicomposting accelerates the mineralization of plant metabolites and subsequently enriches the end product with more available forms of soil nutrients. Exchangeable Ca and Mg contents were also higher in the vermicompost than in the initial substrate. T_2 showed maximum increase for Ca and Mg both (118.1 and 51.2%, respectively) whereas, T_4 showed lowest increase for nutrients (49.1 and 13.6%, respectively) (Table 3). In composted material, Ca and Mg content increased in the order: $T_1 > T_2 > T_3 > T_4$. However, when organic waste passes through the gut of worm the nutrients can be converted from unavailable forms to available forms, which consequently enrich the worm casts with higher quality plant metabolites. However, the data are controversial. Garg and Kaushik (2005) found a significant increase in Ca and Mg content in substrate material, after the completion of vermicomposting process. Hartenstein and Hartenstein (1981) however reported a 11.5% and 7.1% loss in the amount of exchangeable Ca and Mg, respectively, in sewage sludge to with earthworms. Similarly Mitchell (1997) reported a decrease in the amount of Ca and Mg in feed-lot cattle manure after the manure was inoculated with *E. fetida*. However, the loss as reported for Ca and Mg in previous studies could be due to leaching of the more soluble forms of the metabolites.

Vermicomposted material showed a reduction in DTPA extractable metal content after completion of the experiment. The reductions ranged between 12.5 and 38.8% for zinc (Zn), 5.9 and 30.4% for iron (Fe), 4.7 and 38.2% for manganese (Mn) and between 4.5 and 42.1% for copper (Cu) (Table 4). The highest metal reduction was noted in treatments that showed maximum decomposition as well as earthworm growth activities (T_1 and T_2 treatments). Therefore, it is suggested that metal loss was related to the earthworm activity in the waste decomposition system. Some of the metals accumulated in body tissues and some was likely lost through leaching. Previous studies have revealed that earthworms can accumulate heavy metals in their tissues during the process of vermicomposting (Hartenstein and Hartenstein 1981; Graff 1982; Garg and Kaushik 2005; Gupta et al. 2005). Garg and Kaushik (2005) reported a considerable loss in heavy metal contents from solid textile mill sludge mixed with poultry droppings. They attributed the heavy metal loss from substrate to accumulation by earthworm body tissues. Gupta et al. (2005) studied the vermicomposting of fly ash by mixing it with cow dung in different ratios and reported 30–50% loss in heavy metal content in different combination, at the end. They reported that heavy metals bioaccumulated in earthworm tissues. The data reported here suggest that earthworms can efficiently reduce the metal content in substrate.

3.2 Earthworm biomass, cocoon production and mortality during vermicomposting

Earthworm biomass gain and reproduction performances in different studied treatments were also evaluated. Statistically earthworm showed significant difference for biomass production and reproduction potential: mean individual live weight at end ($F = 161.45$, $P = 0.001$), individual biomass gain ($F = 146.10$, $P < 0.001$), maximum individual growth rate (mg day^{-1}) ($F = 264.93$, $P < 0.001$), total cocoon numbers ($F = 25.02$, $P < 0.001$) and mean reproduction rate (cocoon worm^{-1} day^{-1}) ($F = 31.20$, $P < 0.001$) among different studied treatments. *E. fetida* showed maximum and minimum mean individual live weight at end on T_1 (782.39 ± 20.88 mg) and T_4 (537.23 ± 15.92 mg), respectively (Table 5). However, at the end, live individual weight was observed in following order: $T_1 > T_2 > T_3 > T_4$. Earthworm showed its peak biomass after 60 day in T_1 and T_2 and after 75 days in T_3 and T_4 treatments (Fig. 1, Table 4). The maximum weight gain was followed by weight loss by the time of termination of the experiment (Fig. 1). Neuhauser et al. (1988) have reported similar trend in weight loss in *E. fetida* for longer exposure duration in treated sludge.

Table 4 Heavy metals (DTPA extractable) in initial and final (vermicompost) material in different treatments (mean ± SD, n = 3)

Treatment*	Zn		Fe		Mn		Cu	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
T_1	288.14 ± 6.22	176.26 ± 4.02	332.45 ± 4.12	231.35 ± 4.85	235.82 ± 4.50	155.61 ± 3.59	35.06 ± 2.76	20.29 ± 1.51
T_2	335.69 ± 5.29	233.57 ± 3.22	405.37 ± 4.56	285.83 ± 4.26	262.50 ± 3.06	162.26 ± 2.41	38.36 ± 1.80	26.84 ± 2.49
T_3	368.23 ± 7.19	245.99 ± 5.19	457.91 ± 5.83	376.98 ± 3.99	295.77 ± 5.25	238.29 ± 2.59	41.09 ± 2.81	30.54 ± 1.74
T_4	430.01 ± 6.24	376.40 ± 4.46	512.19 ± 7.68	482.07 ± 3.97	431.93 ± 3.91	411.76 ± 3.01	44.02 ± 2.73	42.02 ± 3.07

* For treatment compositions see Table 2

Table 5 Growth of *E. fetida* (mean ± SD, n = 3) in different beddings

Treatment*	Mean initial individual weight (mg)	Maximum individual weight achieved (mg)	Maximum individual weight gained	Net individual weight gained (mg)	Growth rate (mg wt. worm^{-1} day^{-1})	Mean individual weight at end (90 days)	Net biomass production individual at the end (mg day^{-1})
T_1	460.89 ± 9.58 a	809.69 ± 20.09 c	After 60 days	348.80 ± 10.51 c	5.81 ± 0.18 c	782.39 ± 20.88 d	3.57 ± 0.13 d
T_2	472.36 ± 6.90 a	718.36 ± 5.91 b	After 60 days	245.80 ± 4.97 b	4.10 ± 0.07 b	710.85 ± 16.37 c	2.65 ± 0.25 c
T_3	456.31 ± 8.25 a	588.20 ± 10.75 a	After 75 days	131.89 ± 19.0 a	1.75 ± 0.25 a	580.78 ± 20.99 b	1.38 ± 0.10 b
T_4	469.61 ± 2.62 a	576.35 ± 20.23 a	After 75 days	106.74 ± 22.85 a	1.42 ± 0.31 a	537.23 ± 15.92 a	0.75 ± 0.21 a

* For treatment compositions see Table 2

Mean value followed by different letters is statistically different (ANOVA; Tukey's t-test, $P < 0.05$)

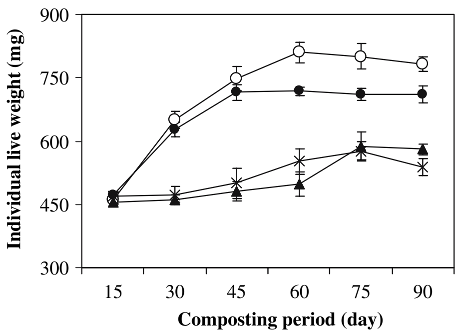

Fig. 1 Earthworm growth patterns during vermicomposting of distillery sludge mixed with cow dung (○ T_1,● T_2, ▲ T_3, × T_4)

This was correlated with conversion of most of the used substrate to vermicompost, which cannot further support their growth. The maximum growth rates of individual earthworms, in different treatments were calculated by taking the maximum weight gain achieved by *E. fetida*, subtracting the initial mean individual weight, and dividing the weight increase, by the number of days needed to reach the maximum weight (Edwards et al. 1998). Maximum growth rate (mg wt. worm^{-1} day^{-1}) was recorded in T_1 (5.81 ± 0.18) followed by T_2 (4.10 ± 0.07), T_3 (1.73 ± 0.25) and T_4 (1.42 ± 0.31) ($F = 264.93$, $P > 0.001$, for all). However, there was a consistent pattern in worm growth rate with a decrease observed with increasing sludge concentrations in treatments. It is concluded that distillery sludge caused biological suppress at their higher contents, in composting earthworms, due to higher concentration of salt and metals in vermibeds. However, metal binding by humic substance is a control on metal behaviour and availability in soil/manures (Stevenson 1982). Therefore, in this study, the better performance of earthworms in beddings those contained more proportion of cow dung, was due to more availability of organic matter, which protected the metal intake by worms and ultimately resists the possible toxicity by metals.

Earthworm showed drastic differences among different treatments for cocoon numbers produced during the experimental duration (Table 6). Cocoons numbers were found in the ranges of 69.0 ± 7.94 (T_2)–19.0 ± 9.57 (T_4). Mean total numbers of cocoon as well as cocoon worm^{-1} rate was recorded in the order: $T_2 > T_1 > T_3 > T_4$. The highest cocoon production potential (reproduction rate) was registered in T_2 treatment (0.046 ± 0.002, cocoon worm^{-1} day^{-1}) that was significantly different than T_1 (0.031 ± 0.005 cocoon worm^{-1} day^{-1}), T_3 (0.022 ± 0.003 cocoon worm^{-1} day^{-1}), and T_4 (0.019 ± 0.005 cocoon worm^{-1} day^{-1}) ($P < 0.05$). The cocoon production rate among different treatments could be attributed to the quality of the substrate. Edwards et al. (1998) concluded that the important difference between the

Table 6 Cocoon numbers, reproduction rate and total earthworm mortality in different treatments (mean ± SD, n = 3)

Treatment*	Nos. of Cocoons produced during experimentation	Cocoon production rate (cocoon worm^{-1})	Reproduction rate (cocoons worm^{-1} day^{-1})	Total earthworm mortality during experiment (%)
T_1	48.0 ± 7.55 b	2.76 ± 0.30 b	0.031 ± 0.005 b	10.5 ± 5.0 a
T_2	69.0 ± 7.94 c	4.14 ± 0.24 c	0.046 ± 0.002 c	13.3 ± 5.77 a
T_3	36.0 ± 7.81 ab	1.99 ± 0.33 ab	0.022 ± 0.003 ab	19.0 ± 5.57 a
T_4	19.0 ± 9.57 a	1.72 ± 0.44 a	0.019 ± 0.005 a	45.0 ± 5.0 b

* For treatment compositions see Table 2

Mean value followed by different letters is statistically different (ANOVA; Tukey's *t*-test, $P < 0.05$)

rates of cocoon production in the two organic wastes must be related to the quality of the waste material, which is one of the important factors in determining onset of cocoon production. Suthar (2007b) summarized that the factors relating to the growth of earthworms may also be considered in terms of physiochemical and nutrient characteristics of waste feed stocks. Thus organic waste palatability for earthworms is directly related to the chemical nature of the organic waste that consequently affects reproduction performance of the earthworm. Although, in present study, cocoon production numbers as well as reproduction rate decreased with increasing concentration of distillery sludge. Increasing proportion of sludge tends to check the cocoon production rate in worms, possibly due to higher availability of growth retarding compounds (i.e. metals, higher salt concentration, grease etc.).

Earthworm showed drastic differences in population mortality between studied combinations of the distillery sludge and cow dung. The maximal total earthworm mortality (45.0 ± 5.0%) was recorded in T_4 treatment, while T_1 bedding showed lowest earthworm mortality (10.5 ± 5.0%). However, there was no significant difference among first three treatments e.g. T_1, T_2 and T_3 ($P = 0.548$) for total worm mortality (Table 5). It is suggested that changing of the chemical environment around the earthworm or more availability of toxic chemicals, due to higher proportions of distillery sludge in last two treatments (T_3 and T_4), caused mortality in composting earthworms. Garg and Kaushik (2005) found that *E. fetida* showed more population mortality in beddings those contained fewer amounts of organic supplements in textile mill sludge vermibeds. Present study confirms and extends the hypothesis that more availability of industrial sludge in substrate material leads to increased earthworm mortality, which can be minimized by applying a sufficient toxicity minimizing or bulking agents like cow dung or plant residues (Suthar, 2007a).

4 Conclusions

Industrial materials such as distillery sludge should not be treated as waste material but as a valuable resource. Distillery sludge has a high nutritive value and might be used as fertilizer as well as soil amendment on a low-input basis. Vermicomposting of distillery sludge, after mixing it with a suitable bulking agent (cow dung) changed in the availability of plant metabolites. However, in both T_2 and T_3 treatment maximum increase in NPK as well as decrease in pH and organic C content was recorded in the vermicomposted material. Metals can be accumulated in various worm tissues to reduce of metal level in during the vermicomposting process and soil. The metal contamination is a major problem during direct field application of such sludge. Earthworm biomass production and reproduction performance was found excellent in bedding those contained lower proportions of distillery sludge i.e. T_1 and T_2. It is suggested that at higher concentrations, sludge drastically affects the decomposition efficiency of composting earthworms. Results indicate that distillery sludge mixed with cow dung could be utilized as an efficient soil conditioner for sustainable land restoration practices, at low-input basis, after processed by epigeic earthworms.

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