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ORIGINAL ARTICLE

Biomanagement of municipal sludge using epigenic earthworms *Eudrilus eugeniae* and *Eisenia fetida*

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ABSTRACT

Municipal solid waste and its stern pollution are an alarming problem that needs immediate attention. Efforts were made to explore the potential of epigenic earthworms *Eudrilus eugeniae* and *Eisenia fetida* in biological management of municipal sludge collected from various parts of study area using vermitechnology. Observed data suggest considerable decrease in toxic metals compared with the initial levels, the concentrations of heavy metals including Zn, Fe, Cu, Pb, Mn, Cr, Ni and Co has decreased noticeably in *E. eugeniae* treated municipal sludge. Similar effect was also observed in *E. fetida* as well, when compared to *E. fetida* and *E. eugeniae* latter was found to more effective in reducing the metal toxicity of the sludge; in addition, the earthworm enriches the sludge with various nutrients essential for plant and microbial growth. The soil and heavy metal dynamics shows a significant decrease, which draws a conclusion to exploit vermitechnology in biomanagement of municipal sludge using selected earthworm species.

Key words: *Soil dynamics, Heavy metals, Compost worms, Municipal waste*

Introduction

Municipal solid waste (MSW) is becoming a serious problem of concern. Disposal and effective management of municipal solids are posing a great challenge to India and other developing nations [9]. Uday Bhawalkar report indicates that India alone produces about 750 million tonnes of organic wastes every year. The management of MSW is an upcoming global challenge due to lack of sophisticated technology to process and eliminate volumous MSW generated daily in metropolitan cities. Anecdotal disposal causes detrimental impact on all components of the environmental milieu and health hazards. Although major portion of these wastes are cast-off in agriculture, not very efficient and essential nutrients go in vain. Organic wastes from cities and agricultural processing often dumped cause stern pollution. Although deprived in nitrogen, phosphorus, potassium and other requisite plant nutrients, organic wastes comprise 50% biocarbon, which feeds the soil processes that make the nutrients available to plants. Therefore, organic wastes are too valuable to be wasted [2,21,20].

Earthworms play a major role in the organic litter transit from the surface to inner layer of the soil; they pulverize the organic matter with soil particles and depart their castings throughout the soil profile. Burrowing activities of earthworms increase the water holding capacity along with providing optimum aerobic growth conditions for bacteria and plant roots [31]. Auspiciously, bacteria and plant roots survive

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under identical environmental conditions. Earthworms augment the growth of potent soil bacterial agents for decomposting organic wastes [6].

Earthworm browses selectively on soil pathogens and microbes. Hence, feeding is sufficient for earthworm maintenance. Unavailability of feed and moisture for a period drives them to go underground and hibernate. Under favourable conditions, they wake up as fully functional; thus, exploitation these beneficial qualities of earthworm in biomanagement gains importance. As municipal sludge serves as a reservoir for biocarbon [29], it is apposite to use earthworm in biomanagement of municipal wastes.

Organic waste vermicomposting by different species has been in wide practice. This include cattle dung [5,12,15], horse waste, pig waste, turkey waste [5], poultry droppings [10], mango leaves [28], water hyacinth [8], and paper waste [8]. Efforts have made in this study to stabilize the municipal sludge under laboratory conditions using submerged potential of epigenic earthworms *Eisenia fetida* and *Eudrilus eugeniae*. The composting potential of worm has also been evaluated upon municipal sludge treatment in laboratory scale. The changes in chemical properties of the substrate are measured at the end and are shown to produce promising results.

Materials and methods

Collection and processing of worms

The earthworm *E. eugeniae* and *E. fetida* were bought from a vermiculture farm in Vallum, Tanjore (10° 48' 0" North, 79° 9' 0" East), Tamil Nadu, India. The earthworms were reared in garden soil and garden waste vermibed of dimension 4 × 2 × 4/4 (length × breadth × height) sufficient for 2,000 to 3,000 worms with controlled moisture 35%–45% and temperature 26°C–28°C. Nylon net was used to cover the bed to prevent the entry of predators. Adequate watering was done daily to maintain optimum moisture conditions in the bed.

Cowdung (CD) procured from the Srinivasa cowshed, Tiruchirapalli, India, served as feed. The main characteristics of CD are total solids, 562 g/kg; pH (1:10 ratio) 7.81; total organic carbon (TOC), 481 g/kg; total nitrogen (TN), 5.8 g/kg; total phosphorus (TP), 6.4 g/kg and C:N ratio, 82.93. After acclimatization, for a period of 15 days, the worms selected for experiment were maintained under proper condition. The earthworms were hand-sorted, and the species were identified on naked morphological observations.

Preparation of pre-compost substrate

The municipal sludge (MS) was collected from six collection points, where the domestic waste along with industry and agro wastes has been dumped in and around Tiruchirappalli district, Tamil Nadu, India. The sludge was prepared by mixing municipal waste, soil and cowdung in combinatorial composition through which the optimum growth concentration and the lethal concentration (LC50) of MS were determined. The main characteristics of MS are total solids, 784 g/kg; pH (1:10 ratio) 9.1; TOC, 338 g/kg; TN, 4.66 g/kg and C: N ratio, 72.53.

Experimental design

Nine containers (30 cm diameter, 18 cm depth) were filled with feed mixture (on dry weight basis) containing different percentages of MS, CD and/or soil (Table 1). These mixtures turned over manually every 24 h for 15 days in order to eliminate volatile toxic substances. After 15 days, all containers were maintained at controlled environmental temperature (26–28°C), with ten earthworms in each container. The moisture content of the mixture in each container was maintained at 40%–45% throughout the study period by periodic sprinkling of adequate quantities of water.

Determination of Lethal concentration (LC50)

LC50 is the concentration of the substance, which kills 50% of the sample population during the test period [24], or it is the concentration of a poison, lethal to one half of the population [1]. Ten healthy worms in the soil medium are exposed to varying concentrations of municipal sludge *viz.* 50, 60, 70, 80 and 100. Mortality is recorded for each of the concentration for an exposure period of 72 hours.

Castings

Castings were collected at the optimum sludge concentration processed as described above, and *E. eugeniae* and *E. fetida* were introduced into the sludge at a concentration of MS, CD, and Soil in 3:1:1 (M6) and 2:2:1(M9), respectively (Table 1), under controlled conditions of temperature, humidity and moisture Temperature and moisture were maintained by adequate watering.

Heavy metal analyses

All the samples used on dry weight basis for chemical analysis are obtained by oven drying the known quantities of material at 110°C. All the chemicals used were of analytical reagent (AR) grade supplied by Merk, Mumbai, India. Alkali-resistant borosilicate glass apparatus and double distilled water was used throughout the study for analytical work. All the results of the analyzed samples were averaged. The pH was determined using double distilled water suspension of each mixture in the ratio of 1:10 (w/v)., Heavy metals in the soil before and after the treatment of earthworms were analyzed by Soil Testing Institute, Govt. of Tamil Nadu, Tiruchirappalli, and the results were tabulated. The concentration of heavy metals, i.e. Cu, Fe, Zn, Mn and Pb, was determined using diethylene-triaminepentaacetic acid (DTPA) extraction method.

Soil nutrient analysis

The pH was measured using pH615, digital pH meter in 1/10 (w/v) aqueous solution. Total Organic carbon (TOC) was determined by the partial-oxidation method [30]. Total nitrogen (TN) was measured by micro Kjeldahl method [13]. C: N ratio was calculated from the measured value of C and N. Total extractable phosphorous (TP) was determined by using Olson's sodium bicarbonate extraction method [18]. K, Ca and Mg were determined after extraction of the sample using ammonium acetate extractable method [23], and the samples were analyzed by Perkin Elmer AA-6300, double beam atomic absorption spectrophotometer (AAS).

Statistical analysis of data

The data in this study were analyzed statistically, and all values are presented as Mean ± SD (Standard deviation).

Results and discussion

Physico-chemical changes during vermicomposting

The pH of the final cast was lower in all treatment samples of *E. eugeniae* when compared to their initial values (Table 2). The reduction in pH was from the range of 6.7 before treatment to 6.3 after treatment. The shift in pH during the study was attributed to the production of CO_2 and microbial decomposition of organic acids during vermicomposting. TOC was lower after treatment (136 ± 14) when compared to the initial level in the sludge (454 ± 5) (Table 2). This deprivation suggests the quick organic matter mineralization by earthworms. Dominguez [3] points out that vermicomposting process involves the active participation of earthworms and microbes. The earthworm homogenizes the ingested material through muscular action of its foregut, adds mucus and provides enzyme rich environment to the material taken up by the worms, thereby increasing the surface area for microbial action, while the microorganisms perform the biochemical degradation. The microbial action would be complete in the extracelluar enzymatic environment of earthworms. This biological mutuality is suggested to cause TOC loss in the form of CO_2 from the municipal sludge during the decomposition and mineralization of organic waste [25]. Total N (TN) was significantly higher in the cast when compared to municipal sludge (Table 2). It is suggested that along with N release from compost material, earthworms augment nitrogen levels by releasing mucus and by accumulating excretory products, body fluids and other biological fluids rich in nitrogen. Decaying tissues of dead worms is yet another factor for TN hike to a significant amount. After treatment, all samples showed higher concentrations of TP when compared to that of the municipal sludge (Table 3). The difference between control and experimental value was statistically significant. The increase in the concentration of TP after treatment may be due to the action of gut phosphatase enzymes of the earthworm. After ingestion, the bound phosphorus is subjected to the action of phosphatase enzymes in the gut, thereby releasing the available phosphorus and also enriches the soil nutrient.

The concentrations of Ca (TCa) and Mg (TMg) were raised significantly after the treatment (Table 3). This study agrees with previous a report that states a significant increase in the level of Ca and Mg after the completion of vermicomposting [26]. It is a known fact that earthworm plays a critical role in the conversion of plant metabolites into available forms that is further subjected to microbial disintegration and recycling allied with their casts [3]. Similar results were obtained on treating the MS with *E. foetida* on physico-chemical parameters (Table 4), and the same trend was followed with TP, TK, TCa and TMg (Table 5). *E. eugeniae* appears to be more prominent in bioconversion when compared to *E. foetida.*

Heavy metal dynamics

When compared with the initial levels, the concentrations of heavy metals *viz* Zn, Fe, Cu, Pb, Mn, Cr, Ni and Co has decreased markedly in *E. eugeniae*-treated MS (Fig. 1). Similar effect was also observed in *E. fetida*-treated MS (Fig. 2). In addition, when compared to *E. fetida* and

Table 1: Content of different waste in initial mixture

Mixture No	Municipal sludge (g)	Cow dung (g)	Soil (g)
M1	150 (100)	0	0
M2	120 (80)	30 (20)	0
M3	105 (70)	45 (30)	0
M4	90 (60)	60 (40)	0
M5	75 (50)	75 (50)	0
M6	90 (60)	30 (20)	30 (20)
M7	75 (50)	37.5 (25)	37.5 (25)
M8	45 (30)	52.5 (35)	52.5(35)
M9	60 (40)	60 (40)	30 (20)

The values in the parenthesis indicate the percentage content in the initial feed mixture

Table 2: Properties of Municipal Sludge of various collection points before and after treatment of *E. Eugeniae*

Sample	pH		TOC		TN		C:N	
	BT	AT	BT	AT	BT	AT	BT	AT
CP1	8.4 ± 0.2	6.6 ± 0.3	454 ± 5	220 ± 9	7.2 ± 0.3	14.2 ± 0.3	63.1 ± 0.2	15.4 ± 0.2
CP2	8.3 ± 0.3	6.7 ± 0.1	381 ± 4	176 ± 5	5.9 ± 0.4	11.8 ± 0.4	64.5 ± 0.2	14.9 ± 0.1
CP3	8.1 ± 0.5	6.4 ± 0.2	330 ± 2	178 ±11	4.7 ± 0.1	10.8 ± 0.3	70.2 ± 0.1	16.4 ± 0.3
CP4	8.2 ± 0.1	6.3 ± 0.1	289 ± 6	147 ±12	4.6 ± 0.3	10.6 ± 0.5	62.8 ± 0.3	13.8 ± 0.2
CP5	8.2 ± 0.1	6.7 ± 0.4	291 ± 8	136 ±14	3.7 ± 0.2	09.0 ± 0.3	78.6 ± 0.3	15.1 ± 0.8
CP6	8.1 ± 0.2	6.5 ± 0.2	370 ± 7	213 ± 8	3.0 ± 0.5	09.2 ± 0.2	123.3 ± 0.5	23.1 ± 0.6

CP- Collection point; BT - Before treatment; AT - After treatment

Table 3: Nutrient Dynamics of Municipal Sludge of various collection points before and after treatment of *E. Eugeniae*

Sample	TP		TK		TCa		Tmg	
	BT	AT	BT	AT	BT	AT	BT	AT
CP1	6.8 ± 0.3	10.4 ± 0.3	4.8 ± 0.2	07.2 ± 0.4	3.2 ± 0.1	4.0 ± 0.4	17.8 ± 0.2	19.2 ± 0.1
CP2	5.9 ± 0.2	06.7 ± 0.4	5.8 ± 0.1	06.3 ± 0.4	2.2 ± 0.3	2.8 ± 0.2	15.4 ± 0.1	16.1 ± 0.3
CP3	5.4 ± 0.5	06.6 ± 0.2	5.4 ± 0.5	06.8 ± 0.4	2.4 ± 0.2	2.8 ± 0.5	20.7 ± 0.2	21.6 ± 0.2
CP4	5.2 ± 0.2	06.0 ± 0.3	5.0 ± 0.3	06.6 ± 0.2	2.1 ± 0.4	2.5 ± 0.3	16.1 ± 0.4	18.5 ± 0.2
CP5	3.6 ± 0.4	04.0 ± 0.1	2.5 ± 0.4	03.9 ± 0.3	2.2 ± 0.1	2.7 ± 0.1	21.2 ± 0.3	21.9 ± 0.3
CP6	7.6 ± 0.3	17.0 ± 0.2	9.2 ± 0.2	16.4 ± 0.3	4.6 ± 0.2	5.0 ± 0.4	25.4 ± 0.2	27.3 ± 0.1

CP- Collection point; BT - Before treatment; AT - After treatment

Table 4: Properties of Municipal Sludge of various collection points before and after treatment of *E. fetida*

Sample	pH		TOC		TN		C:N	
	BT	AT	BT	AT	BT	AT	BT	AT
CP1	8.1 ± 0.1	6.2 ± 0.2	421 ± 3	212 ± 9	6.9 ± 0.3	13.9 ± 0.3	60.2 ± 0.3	16.4 ± 0.1
CP2	8.3 ± 0.1	6.3 ± 0.2	351 ± 4	143 ± 6	4.7 ± 0.4	11.1 ± 0.2	61.5 ± 0.2	15.1 ± 0.1
CP3	8.1 ± 0.2	6.0 ± 0.1	315 ± 2	151 ±11	3.8 ± 0.4	9.3 ± 0.1	69.2 ± 0.2	15.4 ± 0.3
CP4	8.0 ± 0.2	6.3 ± 0.1	237 ± 5	139 ±15	3.6 ± 0.3	11.6 ± 0.4	61.3 ± 0.1	16.8 ± 0.1
CP5	8.2 ± 0.1	6.1 ± 0.2	231 ± 9	130 ±8	3.3 ± 0.2	08.9 ± 0.1	72.1 ± 0.3	14.9 ± 0.2
CP6	8.0 ± 0.1	6.2 ± 0.1	343 ± 4	211 ± 13	3.0 ± 0.2	09.0 ± 0.2	112.3 ± 0.5	22.5 ± 0.2

CP- Collection point; BT - Before treatment; AT - After treatment

Table 5: Nutrient Dynamics of Municipal Sludge of various collection points before and after treatment of *E. fetida*

Sample	TP		TK		TCa		Tmg	
	BT	AT	BT	AT	BT	AT	BT	AT
CP1	5.4 ± 0.4	09.8± 0.2	4.1 ± 0.2	06.7 ± 0.2	2.9 ± 0.2	3.9 ± 0.3	17.5 ± 0.1	18.9 ± 0.2
CP2	6.3 ± 0.1	06.3 ± 0.4	5.2 ± 0.2	05.5 ± 0.3	2.0 ± 0.1	2.4 ± 0.3	14.4 ± 0.1	15.9 ± 0.2
CP3	45 ± 0.2	05.6 ± 0.1	4.8 ± 0.3	05.8 ± 0.3	2.0 ± 0.1	2.6 ± 0.4	19.4 ± 0.3	21.6 ± 0.1
CP4	4.6 ± 0.3	05.9 ± 0.2	4.9 ± 0.3	06.0 ± 0.1	2.1 ± 0.3	2.1 ± 0.5	15.9 ± 0.4	17.9 ± 0.1
CP5	2.9 ± 0.1	03.4 ± 0.2	3.0 ± 0.3	03.1 ± 0.2	2.2 ± 0.2	2.3 ± 0.2	20.1 ± 0.2	20.7 ± 0.2
CP6	6.4 ± 0.2	16.0 ± 0.3	9.0 ± 0.1	15.4 ± 0.2	3.6 ± 0.1	4.9 ± 0.3	25.1 ± 0.1	26.4 ± 0.3

CP- Collection point; BT - Before treatment; AT - After treatment

E. eugeniae was found to more effective in reducing the metal toxicity of the sludge. The decrease in the metal concentration was attributed to the accumulation of metals in the body of the earthworm. Suthar and Singh [27] reported considerable amounts of metals in tissues of earthworms inoculated in distillery sludge for long periods. They correlated the metal loss from substrate with the metal level in earthworm tissues. In this study, the difference in the metal loss patterns in MS may also be correlated with their observation. Earthworms could accumulate some amount of metals; however, further analysis of tissues of inoculated earthworms is needed to support the proposed hypothesis. In addition, the authors of this study hypothesise another possible factor for this bioconversion, which may be due to the microbial fauna and flora that intake metal

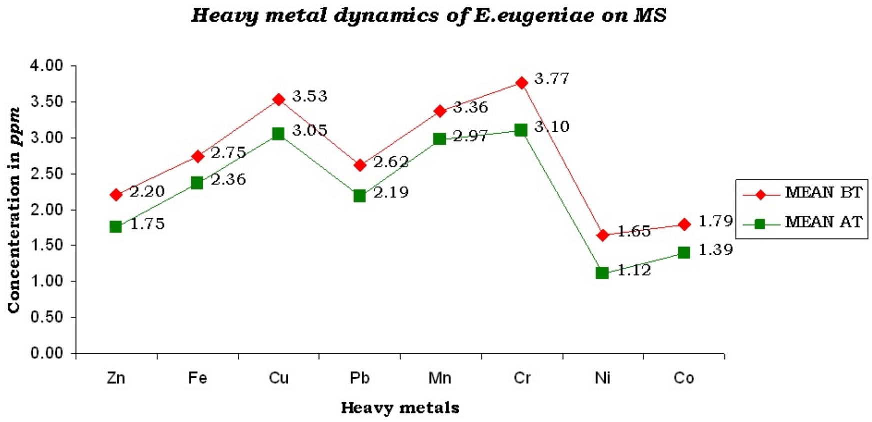

Fig. 1: Heavy metal concentration status of Municipal sludge (MS) before treatment (BT) and after treatment (AT).

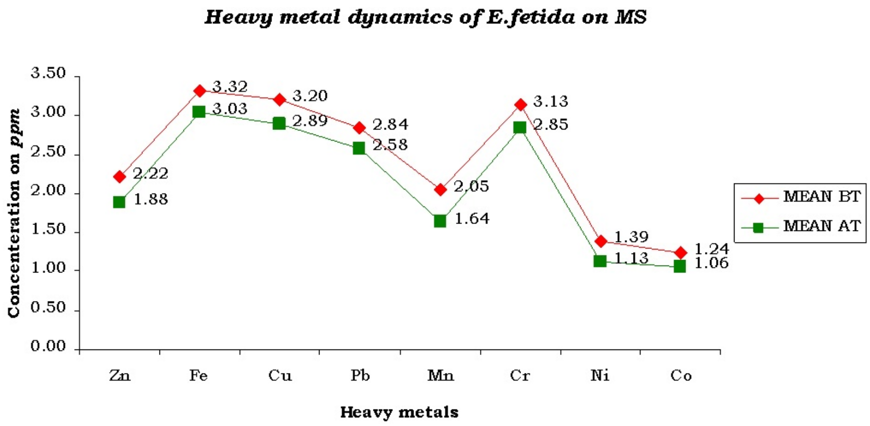

Fig. 2: Heavy metal concentration status of Municipal sludge (MS) before treatment (BT) and after treatment (AT).

ions as a vital factor for their enzymatic action. In addition, Siderophores are small, high-affinity iron-chelating compounds secreted by clause of aerates such as bacteria. Siderophores have the ability to chelate iron, thereby reducing the Fe^{3+} concentration in the sludge and adjacent environment [16.17]; microbes include *Pseudomonas aeruginosa* producing pyoverdine and *Yersinia pestis* producing yersiniabactin. Similarly, zinc is a common element of adenylate kinases from Gram-positive bacteria, which binds to a structural motif consisting of three or four cysteine residues, Cys-X2-Cys-X16-Cys-X2-Cys/Asp [19]. Various microorganisms, such as *Aspergillus niger*, *Gluconobacter spp.*, *Pseudomonas spp.* and acetic acid bacteria, generate acidic oxidation products of D-glucose [11]. Sugar acids, in particular 2-keto-D-gluconic acid, are able to solubilise phosphate minerals and phosphate rocks by chelation of the earth alkaline. Furthermore, 2-keto-D-gluconic acid plays a role in the reductive dissolution of metal oxides such as Cu, Pb, Mn, Cr, Ni and Co and other toxic metals in soils [22,14,4]. Along with this, numerous metal chelators are suspected to be present in microbial population. The presence of microbial fauna is sought to be a major factor rather than the accumulation of metals in the body tissues, as body tissue on accumulation may become toxic and disrupt the cellular functions. Hence, these toxic coumpounds get detoxified by various detoxification pathways, or their entry is prevented. The latter case is more predominant; therefore, it substantiates the note that the greater proportion of decrease observed in our study would not be possible unless otherwise microbes play a greater role. These findings further tempt us to characterize the microbial diversity and the microbial ability in vermicomposting. Inspite of these factors, it was believed that the earthworm provides the optimum conditions for the microbial actions; hence, their mutual participation is the success of biomanagement.

Conclusion

The vermicompost technology employed in this study reveals the enrichment of municipal sludge with nitrogen, phosphorus, potassium, calcium and magnesium; that it had low levels of metals. Moreover, *E. eugeniae* appears to participate effectively in biomanagement when compared to *E. fetida*. During vermicomposting process, some degree of metal toxicity was reduced possibly due to the bioaccumulation of some fractions of metals by the inhabiting earthworms, and the microbial population is coregulated by the earthworm's enzymatic milieu. The study provides a resonance source that vermicomposting can be a potential technology to convert the deleterious municipal wastes into nutrient rich, toxic-free value added materials and also provides a new dimensional approach to manage municipal waste biologically.

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