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Dynamics of Microbial Diversity, Micro- and Macronutrients during Tannery Sludge Treatment Using Epigeic Earthworms, *Eisenia fetida* and *Eudrilus eugeniae*

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ABSTRACT

Indiscriminate and uncontrolled discharge of metal-contaminated industrial effluents into the environment has become an issue of major concern in developing countries. The vermicomposting of organic material with earthworms minimizes environmental pollution and health hazards. Organic and inorganic minerals in earthworm casts are high and have positive effects; pollution can be solved using vermicomposting. Tannery effluent is one of the common industrial effluents containing poisonous heavy metals and toxic compounds such as zinc, copper, iron, manganese, chromium and others. This manuscript describes the improved nutrient availability and decreased toxic effect of tannery effluent, through vermicomposting, after treatment with two earthworms, *Eudrilus eugeniae* and *Eisenia fetida.* The latter were as beneficial as the former in terms of vermicomposting potential. A favorable environment for microbial diversity was achieved at an optimum sludge ratio of 1: 2: 2 and 2: 2: 1 when mixed with normal soil in its lethal concentration (55.5 and 54% (v/w), respectively for *E. eugeniae* and *E. fetida*). Thus, we recommend *E. eugeniae* and *E. fetida* in the agricultural use of soils contaminated with tannery sludge and tannery effluent.

Keywords: contaminated soil, soil dynamics, biomanagement, industrial effluent, vermicomposting
Abbreviations: LC_{50}, lethal concentration for 50% mortality; **ppm**, parts per million

INTRODUCTION

Industries and agriculture are two arms of a nation's economy and health. However, in recent years these two arms have been stained with industrial pollution (Rawat *et al.* 2009). Unprecedented growth of urbanization, industrialization, unplanned population, use, and abuse of the environment have resulted in an increased accumulation of solid waste materials (Kurucu and Chiristina 2008). India is one of the top 10 industrialized countries of the world today. Tanning in India has a long history, has been a traditional occupation for subdued groups of people, and currently employs more than 80,000 people mainly in three states, *viz.* Tamil Nadu, West Bengal, and Uttar Pradesh (Ravindran *et al*. 2008). Undesirable agents such as metal contaminants and organic pollutants from tannery effluent interact negatively with flora and fauna (Srivastava *et al.* 2005). In India, the tanning industry has a solid basis in the country's large livestock population. There are certain regulatory standards for effluents. Accordingly, the pH, total suspended solids, sulfides and chrome in tannery effluent have limits that should not be exceeded (Bosnic *et al.* 2000).

Vermicomposting, which uses earthworms to stabilize and transform organic wastes into valuable end-products, has been proposed as an alternative treatment technology for organic and inorganic wastes from agricultural, industrial, and municipal sources (Marsh *et al.* 2005). It is well established that earthworms can take up and accumulate heavy metals in their chloragogen cells (Huges *et al.* 1980; Beyar 1981). Chloragogen cells function similarly to liver cells in vertebrates, i.e. they store glycogen and neutralize toxins; this unique property allows earthworms to be exploited in both contaminated and uncontaminated environments (Renaud *et al.* 2007). Some species play a significant role in decomposing organic matter and mineral cycling (Lavelle *et al.* 2004; Tripathi and Bhardwaj 2004; Karmegam and Daniel 2009a).

Different species of earthworms have adapted to different environmental conditions such as temperature and moisture and to different soil types. These adaptations are responsible for a wide range of physiological differences between earthworm species. The earthworms, *Eudrilus eugeniae*, *Eisenia fetida*, *Perionyx excavatus*, *Perionyx ceylanensis*, *Perionyx sansibaricus*, *Lampito mauritii*, *Pheretima elongata* and some species of *Dichogaster* are used for vermicomposting in India and the list of earthworms with different potential, such as a short life cycle, efficient vermiconversion, adaptability to survive in local climatic conditions and voracious feeding of different organic substrates, is increasing (Julka *et al.* 2009; Karmegam and Daniel 2009a, 2009b; Suthar 2009). The most widely used worms are *Eudrilus eugeniae* and *Eisenia fetida*.

Vermitechnology can reduce the amount of organic wastes going to a landfill (Kumar 2005). There are thus environmental, economic and regulatory reasons for an increase in demand for vermicomposting. One area that is poised for development in the future is 'contract waste management' using vermiculture. Earthworms can consume and degrade all kinds of organic matter through enzymatic digestion, enrichment by nitrogen (N) excrements and transport of organic and inorganic materials (Laxshmi 2002). Mba (1978) found *E. eugeniae* and *E. fetida* capable of ingesting and excreting organic materials at a high rate. The intestine of earthworms harbours a wide range of microorganisms, enzymes and hormones allowing compost to be transformed into vermicasts within a short period of

Received: 7 September, 2009. Accepted: 6 October, 2010.

Original Research Paper

time; decomposition is thus rapid (Khambata and Bhat 1955; Sharma *et al.* 2005; Prakash *et al.* 2007).

This paper exploits vermitechnology as a way to enrich the nutrient content and detoxification of tannery sludge allowing the survival of earthworms.

MATERIALS AND METHODS

Chemicals and reagents

pH Indicators, Methyl Violet, crystal violet, safranin, Bromocresol purple, Kovac's reagent, Bromothymol blue, and Iodine solution were from Loba Chemie, Mumbai, MH, INDIA. Indole and Indole 3 Acetic acid were from SuvChem, Mumbai, MH, INDIA. Microbial medias *viz.* Tributyrin agar, Nutrient Agar, triple sugar iron agar, Voges Proskauer medium were obtained from Himedia Laboratories, Mumbai, MH, India Pvt. Ltd, All other chemicals used in this study was from Merk, Mumbai, MH, INDIA unless specified thereof.

Collection and processing of worms

The earthworms *Eudrilus eugeniae* and *Eisenia 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 in a 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 (26-28°C). Nylon net was used to cover the bed to prevent the entry of predators. Cowdung was used as feed. Adequate watering was done daily to maintain optimum moisture conditions in the bed. The earthworms were hand sorted and species were identified based on naked morphological observations (Nagavelamma 2004). After acclimatization for 15 days, clitellated adult worms were selected for the experiment.

Preparation of vermicompost

Tannery sludge was collected from the tannery industry near Sembattu, Tiruchirappalli (10° 45' 57" North, 78° 42' 53" East), Tamil Nadu, India. The sludge was prepared by mixing tannery effluent, soil and cow dung in the following ratios 1: 1: 1, 2: 1: 1, 3: 1: 1, 1: 2: 1, 2: 2: 1, 3: 2: 1, 1: 3: 1, 2: 3: 1, and 3: 3: 1. The above ratios were used to calculate the optimum growth concentration and lethal concentration (LC_{50}) of tannery effluent.

Determination of LC_{50}

LC_{50} is the concentration of a substance which kills 50% of test animals during a test period (Stephan 1977) or is the concentration of a poison, lethal to one half of the population (Alabaster and Lloyd 1982). Ten healthy worms in the soil medium were exposed to varying concentrations of tannery sludge mixed with different ratios of normal soil *viz.* 20, 40, 60, 80, and 100% (v/w). Mortality was recorded for each of the concentrations after an exposure period of 72 hrs.

Composting

Composts were collected at the optimum sludge concentration, as described above. *E. eugeniae* and *E. fetida* were introduced into the sludge at concentration of 1: 2: 2 and 2: 2: 1, respectively in controlled temperature (27±2°C), humidity (75-80%) and moisture (65-75%) conditions. Adequate watering was done to maintain the temperature.

Physico-chemical, micro- and macro-nutrient analyses

pH and electrical conductivity were measured by digital pH meter and conductivity bridge using 1:10 water solution. Micro- and macro-nutrients such as total calcium, sodium, magnesium sulphur, copper, zinc, iron, manganese and chromium were analyzed by the Soil Testing Institute, Govt. of Tamil Nadu, Tiruchirappalli using standard procedures.

Identification and biochemical characterization of microorganisms in the gut of earthworms

Earthworms were washed with sterile water to remove the surface microbial flora from their outer skin, sacrificed by freezing and their whole bodies were dissected. The gut region was separated into 10 segments (segments 1-3 runs from the mouth and ends with the esophagus; segments 4-7 include the heart, seminal receptacles, seminal vesicles, crop, gizzard and intestine whereas segments 8-10 include the septa, nephridia and anus). Bacteriological and biochemical analyses using these samples were carried out immediately.

Each gut region (1 g/ml) was placed in a sterilized test tube and serially diluted to obtain a pure culture of bacteria. Samples were screened with a routine microbial screening procedure. Biochemical analyses were performed to assess the microbial diversity in the fore-(segments 1-3), mid-(segments 4-7), and hindgut (segments 8-10) regions. Gram stain, Gekatub liquefaction, starch hydrolysis, lipid hydrolysis, lactose, dextrose, sucrose, H_2S production, nitrate reduction, indole production, methyl red test, Voges Proskauer test, citrate utilization test, urease (EC 3.5.1.5) catalase (EC 1.11.1.6) and oxidase (EC 1.1.3.4) activities were performed according to Castenholz *et al.* (2001) and Cappuccino and Sherman (1992).

1. Gram staining

The bacterial culture smear on a glass slide was covered with few drops of gentian violet (a mixture of methyl violet and crystal violet). After a minute of exposure to the staining solution, the slide was washed in water, then treated with few drops of Gram's iodine and allowed to act for a minute. The slide was again washed in water and then decolorized in absolute ethyl alcohol for 30 seconds and washed in water without any delay. The decolourized smear was finally treated with few drops of safranin, washed in water; excess water was removed using a blotting paper, dried in air and observed under microscope. Those bacteria that remain violet were ascertained as Gram positive and those that hold on pink/red were ascertained as Gram negative.

2. Starch hydrolysis

The bacterial isolates were streaked on starch agar culture plates and incubated for 24-48 hrs at 37°C. Iodine solution was poured over each streak to completely wet the entire surface of the plate and the plates were gently rotated and observed for clear zone. If the area immediately adjacent to the growth was clear, it is considered as starch hydrolysis positive.

3. Gelatin liquefaction

Isolated bacteria were grown overnight in Gelatin medium supplemented to contain (wt/vol) 12% gelatin, 0.0001% hemin, 0.2% maltose, 0.2% cellobiose, 0.1% glycerol, and 0.1% fructose. Cultures where the medium failed to solidify when refrigerated were scored as positive for gelatin liquefaction.

4. Starch hydrolysis

For this, starch agar supplemented with the following components, peptone 0.05%, KCl 0.01% (w/v), $MgSO_4 \cdot 7H_2O$ 0.05% (w/v), $(NH_4)_2SO_4$ 0.01% (w/v), NaH_2PO_4 0.01% (Srivastava and Baruah 1986) and 2% starch along with 0.8% agar was used. Colonies of the isolated strain were transferred by replica plating on to starch agar plates and incubated at 25 ± 2 or 37°C for 48 h. Plates with bacterial colonies were then flooded with Gram's iodine reagent (0.01 M I_2-KI solution). If a strain was amylolytic then it started hydrolyzing the starch present in the surrounding and in the zone degradation there was no blue colour formation. Selection was done as per colonies with and without clear and transparent zone as amylase producing (Amy+) and amylase non-producing (Amy-) strain, respectively.

5. Lipolytic activity

Lipids are high molecular components, that processing large amount of energy. The degradation such as by triglyceride accomplished by extracellular hydrolytic enzyme called lipases that cleave the ester bonds in their moles by the addition and H_2O to form the building blocks glycerol some of the microorganisms have the ability the hydrolyse lipids. Tributyrin agar is used to demonstrate the hydrolytic activity of exo enzyme lipase. Triglyceride tributyrin forms an emulsion when disperse in the agar producing an opaque medium for observing exo enzymatic activity. Tributyrin medium was prepared with 0.5% peptone, 0.3% yeast extract, 2% agar, and 1% tributyrin. The media were sterilized by autoclaving at 131°C for 15 min. Lipase-positive microorganisms were detected by their ability to hydrolyze the lipid substrates to produce free fatty acids. Colonies of lipolytic microorganism's tributyrin medium surrounded by zones against a turbid background of emulsified, unhydrolyzed lipid.

6. Skim milk medium

Commercially prepared sterile evaporated skim milk, concentrated two-fold, was used. A 30% skim milk-agar medium was prepared by adding 15 ml of the skim milk to 85 ml of sterile Nutrient Agar held at 42°C, it was then poured into Petri dishes. Proteolytic activity was demonstrated by a clearing zone in the medium surrounding the bacterial growth.

7. H_2S production

Some bacteria liberate sulphur from sulphur containing compounds. The sulphur is final acceptor leading to formation of hydrogen sulphide which can be detected in the triple sugar iron agar (TSI) medium. TSI medium was prepared, dispensed in tubes and sterilized. Later slants were prepared such that 1 inch butt was got. Single colony was picked up and stabbed down the centre of the agar butt and then streaked on the surface of slant. Then the tubes were incubated overnight at 37°C. After incubation black colour of butt indicates positive for H_2S production.

8. Sugar fermentation

Organisms utilize various sugars for their growth and carry out sugar fermentation. Sugar fermentation can be visualized by the colour change the indicator Bromocresol purple because of acid production. Gas production is visualized by bubble formation inside the Durham's tube. 90 ml of peptone water was prepared. One g of sugar was weighed and dissolved in 10 ml of distilled water. This 10 ml of sugar solution was added to 90 ml of prepared peptone water. Then, 0.1 ml of Bromocresol purple indicator dye was added. The sugar solution was dispensed in tubes. Durham's tubes were placed and the sterilized. Later cultures were inoculated and incubated overnight at 37°C.

9. Indole test

Some bacteria contain enzyme tryptophanase, which acts upon the tryptophan present in the peptone water and convert it in to indole and indole-3acetic acid. This indole reacts with the aldehydes in Kovac's reagent to form a red colored ring. Peptone broth was prepared and distributed 2 ml in sterile tubes, then autoclaved at 121°C for 15 min at 15 lbs pressure. The culture was inoculated and incubated overnight at 37°C. After incubation, few drops of Kovac's reagent was added which formed a red coloured ring at the top which is considered as positive for this test.

10. Methyl red test

Organism belonging to the family Enterobacteriaceae ferment glucose to produce mixed acids such as acetic, lactic, succinic and formic acids. As a result of which the final pH of the medium drops to less than 4.5, which is detected by pH indicators like methyl red (MR). MR-VP (Voges Proskauer) broth was prepared and dispensed 2 ml in sterile tubes, later on it was autoclaved. The culture was inoculated and incubated overnight at 37°C. A few drops of methyl red reagent were added, which resulted in the formation of a bright red colour.

11. Voges Proskauer test

Some Enterobacteriaceae produce 2,3-butylene glycol and acetoin by the fermentation of glucose and other carbohydrates (Celińska and Grajek 2009). These products are more natural in nature and any drop in the pH is noticed. The end products are detected by the addition of VP reagents. MR-VP broth was prepared and dispensed 2 ml in sterile tubes. Later on it was autoclaved. The culture was inoculated and incubated over night at 37°C. The 0.6 ml of 5% α-naphthol and 0.2 ml of 40% potassium hydroxide was added. The colour change was noticed.

12. Citrate utilization test

Certain organisms utilize citrate as a sole source of carbon, producing acetate and other alkaline carbonates bringing about a change in the colour of indicator dye Bromothymol blue from its original green colour to blue. Citrate agar was prepared, dispensed in tubes and then sterilized. Later on slants was prepared. The organism was inoculated and incubated overnight at 37°C. Colour change from green to blue gives positive result.

13. TSI agar

Some bacteria liberate sulphur from sulphur containing compounds. The sulphur is final acceptor leading to formation of hydrogen sulphide which can be detected in the TSI medium. TSI medium was prepared, dispensed in tubes and sterilized. Later slants were prepared such that 2.5 cm butt is got. Single colony was picked up and stabbed down the centre of the agar butt and then streaked on the surface of slant. Later the colony was incubated overnight at 37°C.

14. Oxidase test

Some organism possesses the enzyme oxidase that forms a part of the electron transport system. The enzyme oxidase oxidizes the reagent *N,N*-tetra methyl paraphenylene diamine dihydrochloride to a coloured product indophenol. This is visualized, when the organism is rubbed over a disc containing this reagent, was changes to purple colour. The oxidase disc was placed over a clean slide. Small portion of the bacterial culture was inoculated over the disc. Quick development of purple color indicates a positive result.

15. Catalase test

Some organism possesses the enzyme catalase. It breaks down the hydrogen peroxide to water and oxygen. This is visualized by the evolution of bubbles produced when drop of culture is placed over a drop of hydrogen peroxide. On a clean slide a drop of culture was placed. The drop of hydrogen peroxide was placed over the culture. Quick evolution of bubbles indicates a positive result.

16. Urease test

The urea agar slant was inoculated with a heavy inoculum and incubated at 35°C for 18-24 hrs. The change of colour from yellow to red was recorded as positive.

Statistical analysis

All data represent the means of three replicates. A 2-tailed students *t*-test was performed to determine significant differences between the number of earthworms and the nutrient status of soil in their respective units following mean separation by ANOVA at $P = 0.05$. Data was analyzed with SPSS v.11.1 software.

RESULTS

The LC_{50} was determined over a wide range of doses of tannery sludge (20-100%) (**Fig. 1**). Sludge was mortal between

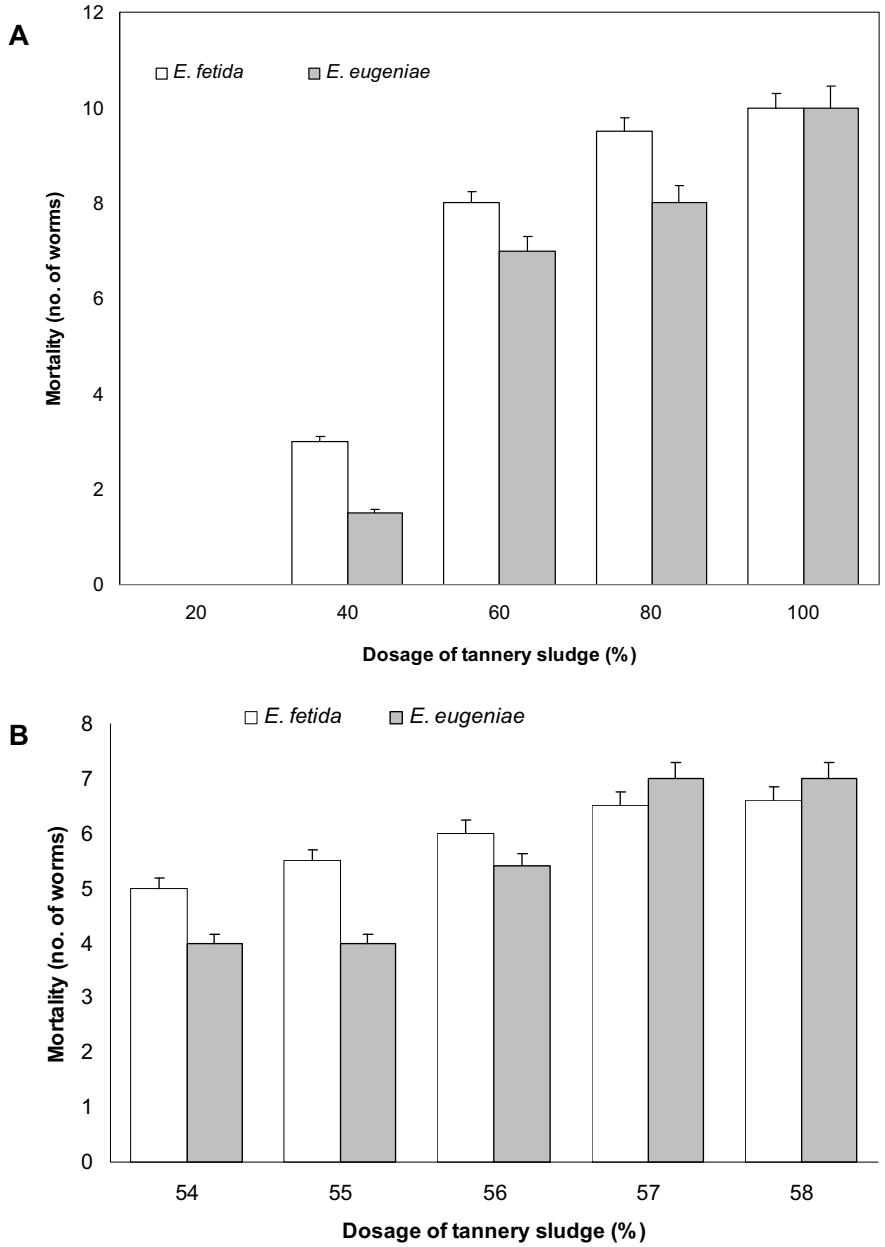

Fig. 1 (**A**) LC_{50} wide range value of *E. fetida* and *E. eugeniae*. (**B**) LC_{50} narrow range value of *E. fetida* and *E. eugeniae*.

55 and 60%, with higher mortality rates at higher doses i.e. 80 and 100%. At 60% tannery sludge, the mortality threshold, mortality rate was 54% for *E. fetida* and 55.5% for *E. eugeniae*.

Macro- and micronutrient analysis

The decrease in pH of the soil was not significant from 8.20 to 7.69 when analysed with student's *t*-test. Although, it slightly varies with control pH, 8.07 (**Fig. 2**), it was assumed not to have much influence over the soil character as both the control and treatment soil showed alkalinity. Hence, the microbial flora will not be disturbed in this variance.

As anticipated the electrical conductivity of the soil is improved from 1.80 dSm^{-1} in pretreatment to 2.0 dSm^{-1} after treatment (**Fig. 2**), soil electrical conductivity is an outstanding parameter in precision agriculture. Precision agriculture is an outgrowth of technological developments, such as the soil electrical conductivity measurement, which facilitate a spatial understanding of soil–water–plant relationships (Corwin and Lesch 2003). The future of precision agriculture rests on the reliability, reproducibility, and understanding of these technologies. This shows a good conversion of manure.

Similarly, the organic carbon significantly decreased from 8.81 before treatment to 6.29 after treatment; on the other hand the organic matter also significant decreased from 15.07 to 10.81. The total nitrogen, phosphorus and potassium increased in their respective percentages from 1.19, 0.40, 2.01% to 1.39, 0.49 and 2.56%, respectively (**Fig. 2**).

Treatment of *E. eugeniae* on sludge and consequent soil macronutrient analysis showed significant change in the soil pH from 8.26 to 7.14, yet, similar to the case of *E. fetida* the electrical conductivity of the soil is improved (2.4 dSm^{-1} to 2.8 dSm^{-1}). Similarly, the organic carbon decreased significantly ($P < 0.05$) from 8.50 to 5.28. The organic matter decreased from 14.60 to 9.31%, and the total nitrogen, phosphorus and potassium were increased from 1.60, 0.53, 1.62% to 1.79, 0.75, 2.64%, respectively (**Fig. 3**).

Micronutrient analysis of treated sludge showed a severe decrease in toxic compounds such as zinc, copper, iron, manganese, chromium, measured 2.71, 1.20, 18.21, 0.41, 0.74 parts per million (ppm) in pretreated sludge to 2.55, 1.09, 15.27, 1.23, 0.58 ppm in post treatment sludge reflecting the potential of *E. fetida* in detoxification. The other major nutrient constituting inorganic compound such as sodium, calcium, magnesium, and sulphur showed sig-

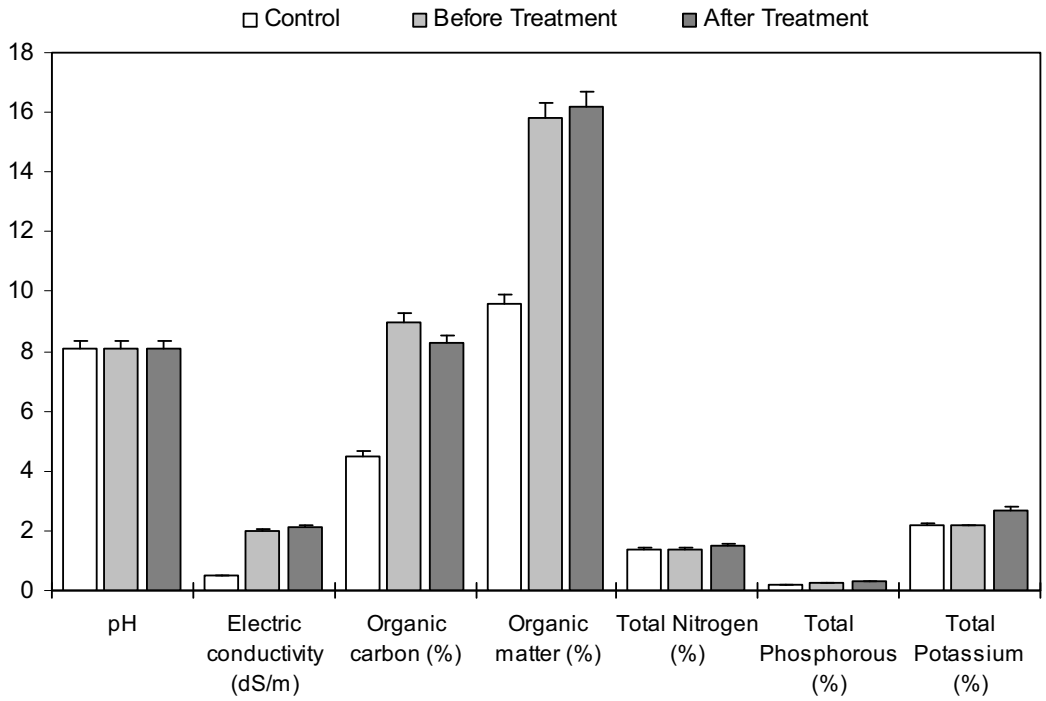

Fig. 2 Macronutrient analysis of tannery sludge treated before and after treatment of *E. fetida*. *Sludge (soil + tannery sludge + cowdung) in a 2: 2: 1 ratio.

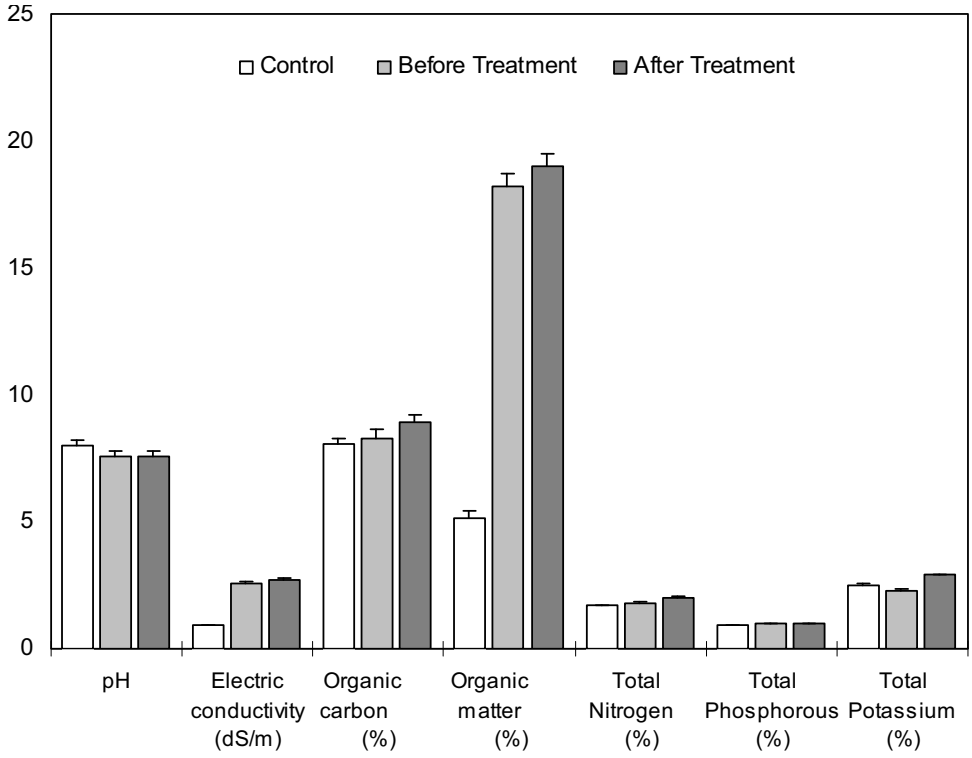

Fig. 3 Macronutrient analysis of tannery sludge treated before and after treatment of *E. eugeniae*. * Sludge (soil + tannery sludge + cowdung) in a 1: 2: 2 ratio.

nificant increase in their value in their respective percentage from 0.41, 3.29, 2.42, 0.28% to 0.55, 4.19, 2.88, 0.36%, respectively, contributing the nutritional enrichment of the soil (**Fig. 4**).

Similar observations were recorded with *E. eugeniae* (**Fig. 5**). Major nutrient constituting inorganic compounds such as sodium, calcium, magnesium, and sulphur showed significant increase in their value in their respective percentages from 0.40, 3.58, 2.02 and 0.61 to 0.59, 4.81, 2.61 and 0.85. The toxic compounds such as zinc, copper, iron, manganese, and chromium were decreased significantly from 2.69, 1.32, 18.52, 2.82, and 0.72 ppm to 1.68, 1.22, 16.25, 2.64, and 0.62 ppm.

Enumeration of microbial population

Aseptically dissected foregut, midgut and hindgut segments showed a great variation in the microbial diversity. From biochemical test and characterization study it was observed that many new species have been introduced into the gut region of *E. eugeniae* and *E. fetida* including various pathogens like *Salmonella*, *Shigella* and faecal coliforms. In addition to these bacteria *Bacillus*, *Streptococcus*, *Pseudomonas*, *Staphylococcus aureus*, *Micrococcus*, *Escherichia coli*

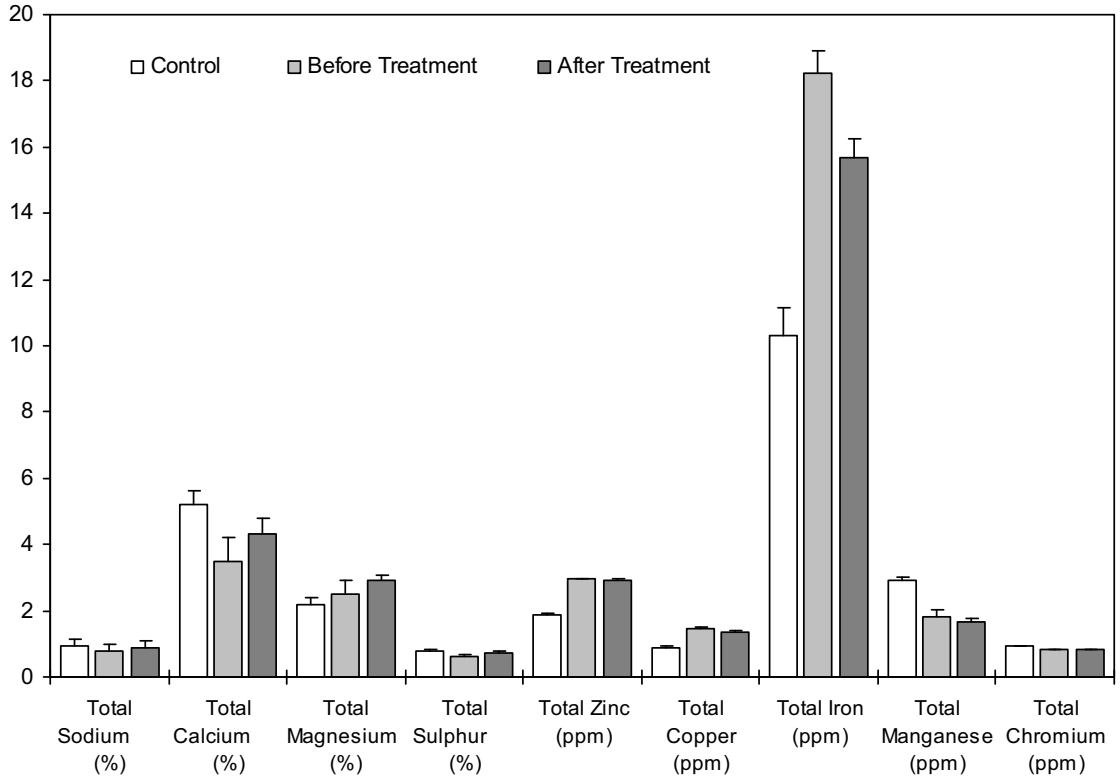

Fig. 4 Micronutrient analysis of tannery sludge treated before and after treatment of *E. fetida.* * Sludge (soil + tannery sludge + cowdung) in a 2: 2: 1 ratio.

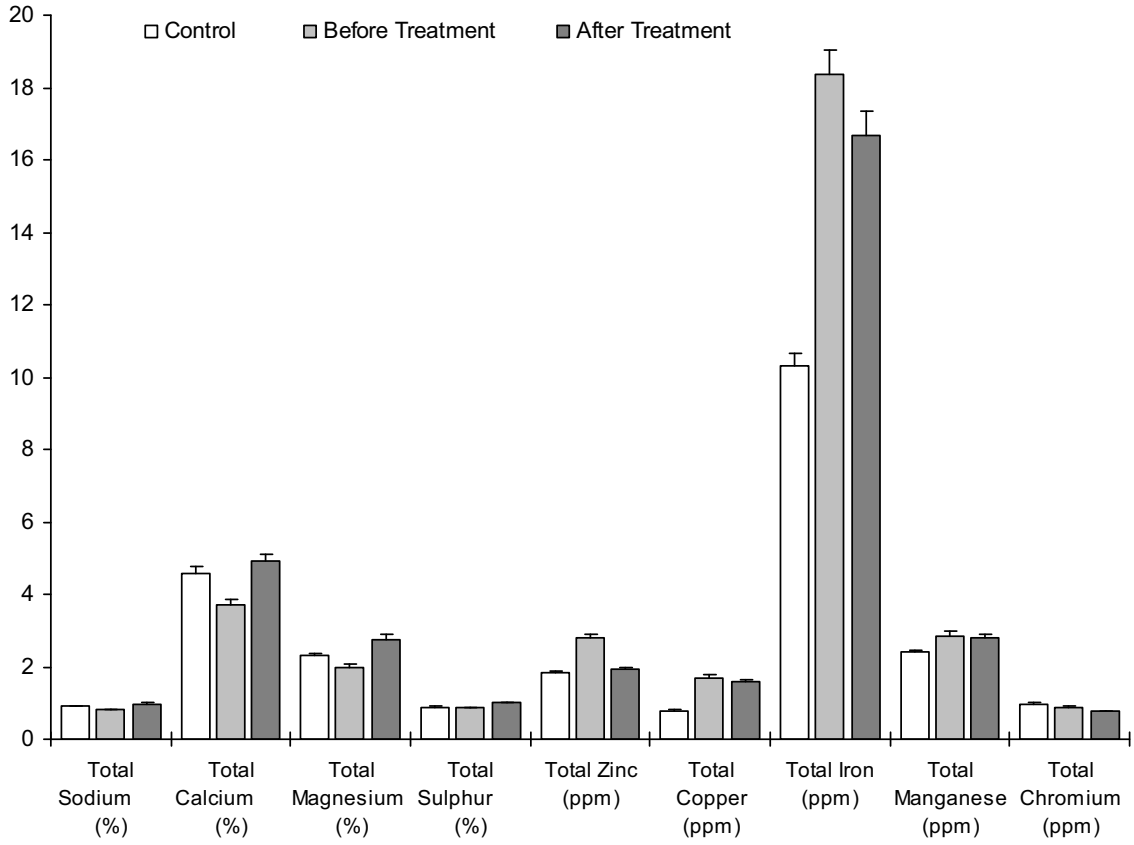

Fig. 5 Micronutrient analysis of tannery sludge treated before and after treatment of *E. eugeniae.* * Sludge (soil + tannery sludge + cowdung) in a 1: 2: 2 ratio.

were found in the tannery sludge treated worm gut after two weeks (**Table 1**).

DISCUSSION

The tannery industry discharges different types of waste into the environment in the form of liquid effluents containing organic matter in the form of chromium, sulphide,

Table 1 Microbial analysis in the gut region of earthworms.

Earthworm species used	Gram Stain	Gelatin liquefaction	Starch hydrolysis	Lipid hydrolysis	Lactose	Dextrose	Sucrose	H_2S Production	Nitrate reduction	Indole production	MR test	VP test	Citrate use	Urease activity	Catalase activity	Oxidase activity	
E. eugeniae Foregut	Coccus +	-	-	-	A	A	A	-	-	-	+	-	-	-	-	-	*Streptococcus intermidius*
"	Rod +	+	+	+	-	A	A	-	+	-	-	±	-	-	-	+	*Bacillus stearothermophilus*
"	Rod +	+	+	+	-	A	A	-	+	-	-	±	-	-	-	+	*Bacillus psychrophilus*
"	Rod +	+	+	+	-	A	A	-	+	-	-	±	-	-	-	+	*Bacillus cereus*
"	Rod +	+	+	+	-	A	A	-	+	-	-	±	-	-	-	+	*Bacillus cereus*
Hindgut	Rod +	+	+	+	-	A	A	-	+	-	-	±	-	-	-	+	*Bacillus polymyxa*
"	Rod +	+	+	+	-	A	A	-	+	-	-	±	-	-	-	+	*Proteus vulgaris*
E. fetida Foregut	Coccus +	-	-	-	A	A	A	-	-	-	+	-	-	-	-	-	*Streptococcus pyogenes*
"	Cocci +	+	-	-	-	-	-	-	±	-	-	-	+	+	-	-	*Streptococcus faecalis*
"	Rod -	-	-	-	AG	AG	A ±	-	+	+	+	-	-	-	+	-	*Escherichia coli*
"	Cocci +	+	-	-	-	-	-	-	±	-	-	-	+	+	-	-	*Micrococcus luteus*
"	Rod -	-	-	-	AG	AG	AG ±	-	+	-	-	+	+	-	+	-	*Enterobacter aerogenes*
Midgut	Rod -	-	-	-	AG	AG	AG	-	+	-	-	±	+	+	+	-	*Klebsilella pneumoniae*
"	Rod+	+	+	+	-	A	A	-	+	-	-	±	-	-	-	+	*Bacillus cereus*
Hindgut	Rod -	-	-	-	AG	AG	A ±	-	+	+	+	-	-	-	+	-	*Escherichia coli*
"	Rod -	+	-	+	-	-	-	-	+	-	-	-	+	-	+	+	*Pseudomonas aeruginosa*
"	Rod +	+	+	+	-	A	A	-	+	-	-	±	-	-	-	+	*Bacillus cereus*
"	Coccus +	+	-	+	A	A	A	-	+	-	+	±	-	-	+	-	*Staphylococcus aureus*

AG: acid gas; A: acid ("+": Positive; "-": Negative); MR test – methyl red test; VP test – Voges Proskauer test

ammonium and other salts; pollution becomes acute when tanneries are concentrated in clusters in arid and semi-arid areas. In the present study, two earthworm species, *E. eugeniae* and *E. fetida*, were exposed to tannery sludge at five different concentrations to asses whether they could be used to find a solution for this problem. Tannery sludge treated with both earthworms showed improved nutrient availability than the control (**Figs. 2-5**).

The present finding displays the composition of vermicompost of tannery sludge obtained by complying *E. fetida* and the contents of essential nutrients, in particular N, P, Ca, and Mg suggest considerable fertilizing worth of the analyzed material which does not derivate much from the amounts of the components determined (**Figs. 2-5**). This substantiates the work on *E. fetida* treatment evaluated by examining differences in bioaccumulation factors between amended and non-amended soils (Ownby *et al*. 2005). The different earthworm species *E. fetida* and *E. eugeniae* in individual and in combinations were utilized to compare the suitability of worm species for composting of sewage sludge as well as the quality of the product. The sewage sludge without blending can be directly converted into good quality fertilizer (Khwairakpam and Bhargava 2009).

Chromium (Cr) contamination of soils from the common practice of land-based disposal of tannery wastes under the assumption that the dominant species in the tannery waste would be the thermodynamically stable Cr (III) species (Secer *et al*. 2009). It now appears that despite the thermodynamic stability of Cr (III), the presence of certain naturally occurring minerals, especially Mn oxides, can enhance oxidation of Cr (III) to Cr (VI) in the soil environment (Avudainayagam *et al*. 2003). Decontamination of Cr-contaminated soils along with manganese (Mn), the thermostabilizing factor is attributed to *E. eugeniae* and *E. fetida* (Secer *et al*. 2009). Zn bioavailability to earthworms in either repository soil adding to Cr and Mn, the Zn toxicity is also decreased (Ownby *et al*. 2005).

Tested samples of vermicompost contained definitely smaller quantities of potassium in comparison to a standard organic fertilizer that is farmyard manure. Such a feature, however, can be determined by a small amount of potassium, which is being removed from the sludge in the treatment procedure with wastewaters. The main task of the earthworms in vermicomposting is to transform the plant mass, or its derivatives, to an amorphous state in their digestive systems. However, the effect of employing earthworms for composting raw sewage sludge is not particularly effective; its importance cannot be denied in the initial sewage composted together with plant mass or other organic components. The method facilitates the effective change of compost properties and enables to obtain useful manure. Another interesting finding of the present study is the change in the diversity of the bacterial flora of earthworms midgut treated with tannery sludge. This could be due to the entry of bacteria into the digestive systems from the tannery sludge and their association with the gut may be due to their environmental adaptive mechanism of the bacterial flora to the gut for nutrients.

ACKNOWLEDGEMENTS

The study was supported by Rajiv Gandhi National Research Fellowship Scheme (RGNFS), University Grants Commission (UGC), Government of India, New Delhi. The authors thank all the faculties of PG and Research Department of Zoology, EVR College of Arts and Science, Tiruchirapalli-620 023, Tamil Nadu, India, for constant support and suggestions during this study.

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