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Original article

Sustainable biotreatment of textile dye effluent water by using earthworms through vermifiltration

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A B S T R A C T

Objective: A research was conducted to explore the reuse possibilities of the textile dye effluent propagated from the textile dye industry. The current study reports vermiculture based effluent treatment method through the chief intention of interconversion of contaminated effluent water into ecologically harmless water.
Methods: Hence a new and typical technology, vermifiltration of effluent using wastewater eating earthworms has been evolved.
Results: Outcome recommended that vermifilter revealed maximal chemical oxygen demand (126 mg L^{-1}), Biological oxygen demand (56 mg L^{-1}), Total suspended solids (181 mg L^{-1}) and Total dissolved solids (298 mg L^{-1}) decreased by 85–89%, 76–80%, 73–77% and 71–76% by the result of vermifiltration. Bioavailability of some heavy metallic compounds such as Ni, Cd, Cu, Zn, Pb, and Cr brought down considerably during vermifiltration. Vermifilter and control metal accumulations ranged from 0.2 ppm to 2.34 ppm and from 0.10 ppm to 2.98 ppm respectively. The SEM illustration reveal that the morphology of the vermicast of control and vermifiltration which is differing significantly. When the concentration of textile dye sludge increases it also increases the destruction of tissues constantly. Histological of the gut domain exposed moderate harm in 50% dilution.
Conclusions: Therefore, these studies concluded that vermifiltration can be used as an eco-friendly technique which can be considered the same as a paradigm for textile industries effluents remedial development.

1. Introduction

Recently, the requisite for textile products has elevated extremely and which results in the increase in number of the textile industry and gradually also increases the wastewater in India. The district which is famous for textile industries is Coimbatore and it is also mentioned as Manchester of South India. In world's map, Tiruppur district surged with Noyyal river is familiar for their high exporting garments and pilling up as textile cities at the riverbanks. More than six lakh people get employment and exports about □25 billion a month by textile industry. Tirppur district alone releases the textile effluent of $10x10^4 m^3 day^{-1}$ (Prabha et al., 2017; Ranganathan et al., 2007) through their 10,000 dyeing,

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bleaching, and textile processing industries. It may be vulnerable to the human health and environment as it contains highly concentrated heavy metals, organic pollutants, pathogens (Xing et al., 2011) and a more amount of active effluent is obtained wastewater purification. Water is greatly consumed by the textile industries which is use for the dye process (Singh et al., 2019).

For example, to produce 1 kg of textile material 100 L of water is used and it also gives out more amount of waste. Every colour dye does not hitch on the fabric. Every year 280,000 tons of dyes are let out because of as high as 50% for reactive dyes. It results in dreadful adulteration of water resources which gets these contaminated effluent dyes (Sadiq et al., 2013). In the usage of water and chemicals during different transforming steps textile industries plays a major role. The materials which are high in colouration, temperature, pH, turbidity, total dissolved solids (TDS), total suspended solids (TSS), chemical oxygen demand (COD), biological oxygen demand (BOD) and other toxic chemicals are released as wastewater when they become resting materials. To know the efficacy in eliminating different sludge and its positive consequences vermifiltration (VF) has considered widely (Samal et al., 2017). Earthworms are used organically in the polluted water in this technique which is first promoted by Jose Toha professor (Wang et al., 2010). In Brisbane, the dairy and brewery industries wastewater contain significant levels TSS and BOD. Both the effluent samples were treated with earthworms resulted an effective removal of very high BOD (99%) and TSS (98%) levels. In Brisbane and Australia, the oxley wastewater treatment plant sludge treated with VF for 1–2 hrs at hydraulic retention time (HRT) resulted significant removal of BOD (99%) and COD (50%) respectively (Sinha et al., 2008). An another research was made in VF by Soto and Toha (2008) reported the 99% removal of BOD, 95% of TSS, 96% of VSS, 89% of N, and 70% of Pin the municipal waste collected from 1000 inhabitants and dumped into the pilot plant. The separated microorganisms antibacterial activity revealed the presence of particular microbes such as *E.coli*, *Alcaligenes*, *Bacillus*, *Klebsiella* and *Enterobacter* that intercept the development of other microbes present in VF definitely due to the liberation of antimicrobial factors (Arora et al., 2014; Huang et al., 2021). Exclusion of suspended solids, COD, N, and P of domestic wastewater and an integrated technology including VF for better nutrient removal vermifiltration plays a vital role (Kumar et al., 2014). The intention of this study is (i) To convert the textile dye effluent (TDE) into a usable form. (ii) To find the effectiveness of *Eudrilus eugeniae* earthworm in the waste interconversion process. (iii) To reduce the pH, BOD, COD, TSS, and TDS. (iv) To evaluate the damage developed in earthworms' tissues and also to analyse the heavy metals in tissues after the experiment. (v) To remove the unpleasant odour.

2. Materials and methods

2.1. Experimental animal and sample collection

Eudrilus eugeniae earthworms were cultivated in the open ground and the levels of textile dye effluents (TDE) were were subjected to different dilutions prior to the experiment. Physicochemical characteristics of effluents from TDE were tested prior to the commencement of the experiment. For each sample's triplicates were done. Figs. 1 and 2 were depicts the methodological flow-chart and schematic diagram of vermifilter for present study.

2.2. Vermifilter (VF) and Non-Vermifilter (NVF) process

The non-vermifilter reactors used in this experiment LxBxH measurements were 36×36×36 cm respectively. Reactor has a filtration unit in upper portion and a collecting unit in lower portion. Sand, garden soil and gravel were added to the filtration unit as the following procedures. At first, 10–20 mm sized gravel and then 2–4 mm sized gravel were filled into the reactor as 7 cm height to each gravel type. As a third layer, 1–2 mm granules sized sand and for the fourth layer garden soil were filled into reactor as 7 cm height of each layer respectively. Vermifilter reactors also has the similar setup along with the earthworms.

2.3. Experimental setup

Near to the VF reactors in a raised platform, a 10*L* sized plastic drums along with taps were assembled. The drums were filled with 6L of dye effluents. VF reactors and the plastic drum tap were connected with rubber tube (0.5 in.) with a hole. As a dripping system, the wastewater from drum was added to the vermibed surface by drop by drop due to the gravity. In VF bed, through the three layers the wastewater was diffused and finally reached the soil where the earthworms (*Eudrilus eugeniae*) were inhabited. In this experiment, soil bed contains 1 kg of earthworms (1000–1500) were introduced which represented $1–1.5\times10^3$ worms per (36×36×36 cm) of soil. At the end, the gravel and sandy layer collected from lower portion of the reactors kit. For the analysis VF and NVF reactors efficiency, 75%, 50%, and 25% textile dye effluents diluted with distilled water was used in triplicates. In VF beds, for all the experimental samples the HRT (8-10hrs) were kept evenly.

2.4. Physico-chemical parameters

From the VF and NVF collection units, filtered water was collected and analysed for various parameters such as pH, TSS, TDS, BOD, and COD. Based on the standard protocols, the parameters were tested (APHA, 1999). Wastewater and filtered water triplicate samples were analysed ($p < 0.05$) and their average results were considered.

2.5. Soil stratum & wormcast-heavy metal analysis

After careful removing of surface sewage, the topsoil (7 cm) samples (vermibed) were collected on regular intervals (7 days) in various sampling points and one composite were assorted from the same depth. Successively, from the substrates worms were collected and used for heavy metal analysis such as Copper (Cu), Nickel (Ni), Chromium (Cr), Lead (Pb), Zinc (Zn), and Cadmium (Cd) using Atomic Absorption Spectrophotometer.

2.6. Morphology of wormcast

Three experimental (TDE) groups such as VF-75%, VF-50%, VF-25% and Control group vermibeds from various treatment sockets were carefully sieved and their cast were collected separately. For the making of one composite sample, different samples collected similar deepness were mixed together. Collected cast samples by sieving (<2 mm) and then freeze-dried. Finally cast surface morphology was studied by using SEM.

2.7. Histological studies

At the end of the experiment, collected earthworms were maintained (24hrs, at 20 °C) in the petridishes set with moist filter paper for the excreta collection. At the end, the worms gut regions were fixed for the standard histological procedures reported by Humason (1979). Experimental and control group earthworms gut regions were dissected. Samples were blotted for the removal of mucus and finally washed with PBS. Immediately the samples were fixed in Bouin's fluid and processed furtherly. For light micro-

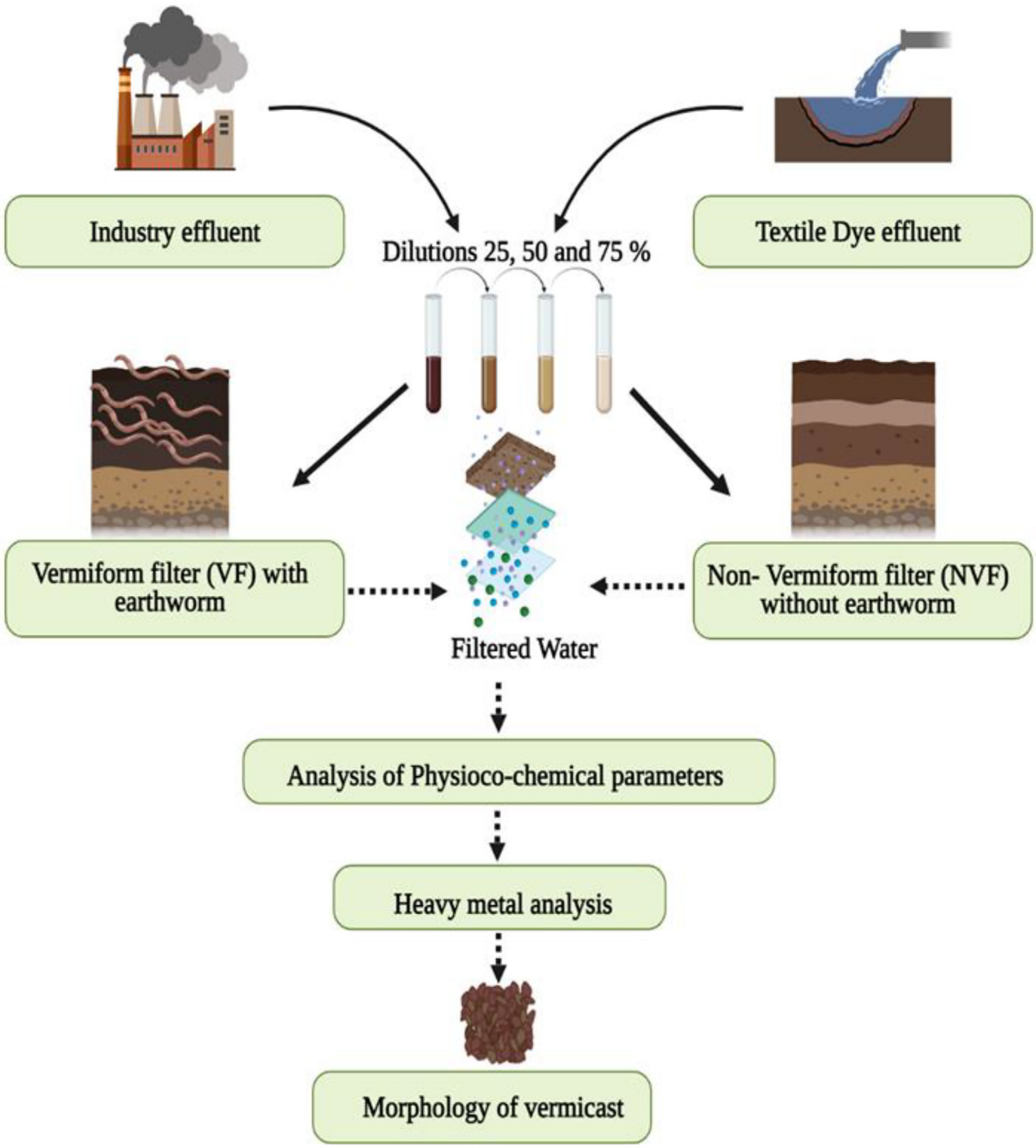

Fig. 1. Methodological flow-chart of study.

scopic observations, earthworms were anaesthetised with chloroform.

2.8. Statistical analysis

Results were statistically analysed by using SPSS (16.0 version) software. Mean differences and their significance ($P < 0.05$) between control and experimental groups were analysed by Homogenous subset with Duncan's post hoc testing.

3. Results

3.1. Physico-chemical composition

Textile Dye Effluents (TDE) and diluted wastewater average pH was measured as 10.50 and 8.93 in 75%, 8.32 in 50%, and 8.22 in 25%. At the end of the experimental duration the TDE pH values were measured as 8.16 in NVF 75%, 7.74 in NVF 50%, 7.62 in NVF 25% while for vermifilter samples pH identified as 7.30 in VF 75%, 7.19 in VF 50%, and 7.03 in VF 25% reactors (Table 1).

BOD parameter considered as the essential factor for the aquatic biota impact by pollutants. Raw TDE's BOD was measured as 1933 mg/L whereas BOD levels were measured as 1364.66 mg/L in 75%, 893.67 mg/L in 50%, and 408 mg/L in 25% respectively. For the reduction of BOD, in vermifilter the treated TDE various dilution were 266.66 mg/L in VF 75%, 144 mg/L in VF 50%, and 56.66 mg/L in VF 25% while for Non-vermifilter it was 376.66 mg/L in NVF 75%, 236 mg/L in NVF 50%, and 105 mg/L in NVF 25% respectively (Table 1).

Filters have difference in VF and NVF by variation of COD due to their increased organic compounds accumulation in the sewage water which acts as the principal indicator of COD. In VF and NVF units the COD of effluent is low as contrasted to the influent. The COD of the raw TDE was 5800 mg/L. The COD of diluted

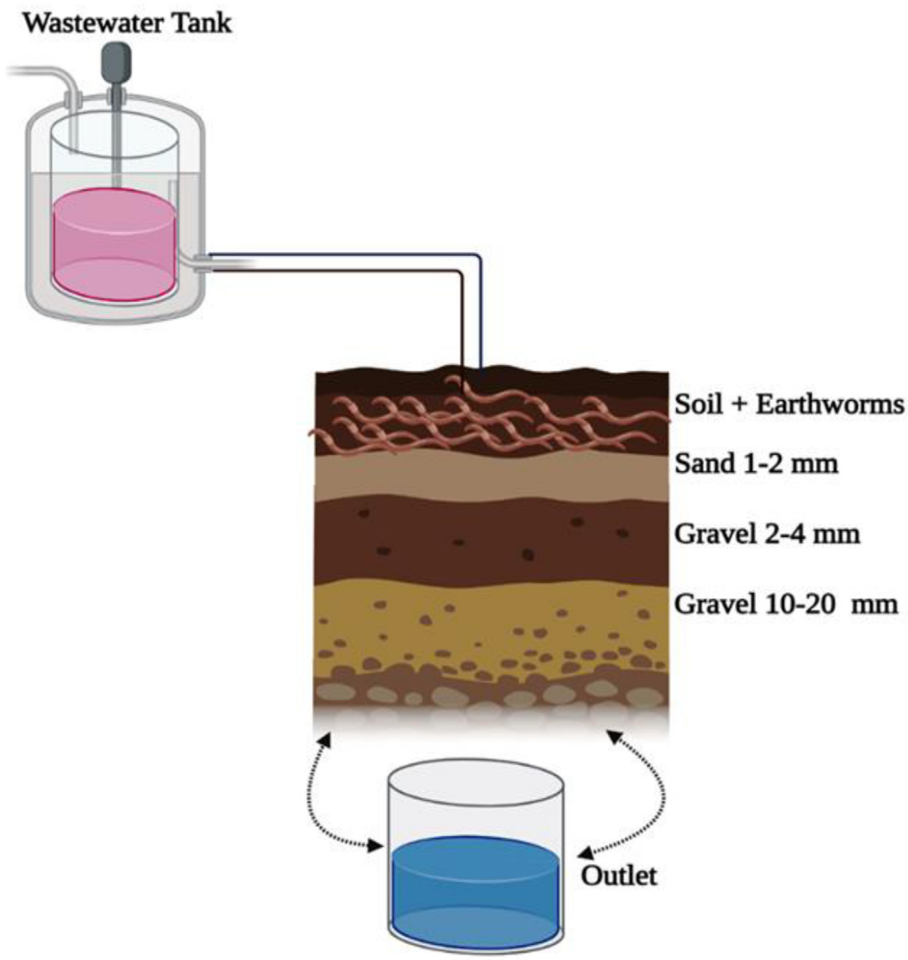

Fig. 2. Schematic diagram of Vermifilter.

Table 1
Physico-chemical parameters of various concentrations of untreated, NVF and VF treated textile dye effluents.

Physico- Chemical Parameters	National Environmental Quality Standard	TD effluent before treatment	Various Conc. of TD effluent	Diluted TD effluent	TD Effluent treated with NVF	TD Effluent treated with VF
pH	6–9	10.50	75%	8.93 ± 3.05[c]	8.16 ± 1.52[c]	7.30 ± 1.52[c]
			50%	8.32 ± 3.05[b]	7.74 ± 3.05[b]	7.19 ± 1.52[b]
			25%	8.22 ± 1.52[a]	7.62 ± 3.00[a]	7.03 ± 2.51[a]
TSS (mg/l)	50–150	6800	75%	6600.33 ± 7.50[c]	2100.00 ± 6.00[c]	589.00 ± 8.18[c]
			50%	6205.33 ± 14.74[b]	2106.00 ± 5.29[b]	401.00 ± 4.58[b]
			25%	3330.00 ± 7.02[a]	1007.33 ± 3.05[a]	181.00 ± 3.00[a]
TDS (mg/l)	3500	7600	75%	7556.00 ± 8.00[c]	3024.00 ± 4.00[c]	746.66 ± 4.16[b]
			50%	7247.33 ± 5.00[b]	2566.00 ± 2.00[b]	401.00 ± 4.58[b]
			25%	6005.00 ± 5.00[a]	2104.00 ± 4.00[b]	298.00 ± 2.00[a]
BOD_5 (mg/l)	80–250	1933	75%	1364.66 ± 9.86[c]	376.66 ± 3.05[c]	266.66 ± 3.05[c]
			50%	893.66 ± 4.04[b]	236.00 ± 4.00[b]	144.00 ± 4.00[b]
			25%	408.00 ± 5.29[a]	105.00 ± 3.00[a]	56.66 ± 4.16[a]
COD (mg/l)	150–400	5800	75%	4085.33 ± 5.03[c]	1196.66 ± 4.16[c]	556.66 ± 4.16[c]
			50%	2676.66 ± 4.16[b]	730.00 ± 10.00[b]	144.66 ± 2.51[b]
			25%	1216.33 ± 14.36[a]	293.33 ± 3.5[a]	126.00 ± 5.29[a]

Mean value in triplicates ± Standard deviation (SD) with significant difference at $P < 0.05$.
Identical lower-case superscripts denote similar values horizontally.
TD-textile dye, NVF-non-vermifilter, VF-vermifilter.

wastewater was 4085 mg/L in 75%, 2676.66 mg/L in 50%, and 1216.33 mg/L in 25%. COD removal at various concentrations for VF was 556.66 mg/L in 75% VF, 144.66 mg/L in 50% VF and 126.00 mg/L in 25% VF whereas for NVF reactors levels were observed as 1196.66 mg/L in 75%, 730.00 mg/L in 50% and 293.33 mg/L in 25% (Table.1).

The raw TDE results showed 6800 mg/L and 7600 mg/L for TSS and TDS respectively. Whereas the diluted TDEs TSS levels were 6600.33 mg/L in 75%, 6205.33 mg/L in 50% and 3330 mg/L in 25% groups. TDS of the diluted TDE was 7556 mg/L in 75%, 7247.33 mg/L in 50%, 6005.00 mg/L in 25% groups. The maximum TSS & TDS removal of the treated TDE different dilution in VF unit was observed by 589 mg/L in 75% VF, 401 mg/L in 50% VF, 181 mg/L in 25% VF and 746.66 mg/L in 75% VF, 401 mg/L in 50% VF, 298.66 mg/L in 25% VF, while in NVF unit minimum removal was recorded as 2100 mg/L in 75%, 2106 mg/L in 50%, 1007.33 mg/L in 25% NVF reactors and 3024 mg/L in 75%, 2566 mg/L in 50%, 2104 mg/L in 25% NVF reactors at the end of the run (Table.1).

3.2. Heavy metal dynamics in bioreactors

Heavy metal analysis of treated wormcast shows a severe increase in toxic compounds such as Pb, Cr, Cu, Cd, Zn and Ni (Fig. 3). Among the earthworm species, *E. eugeniae* was found to be more effective in reducing the metal toxicity of the sludge (Suthar, 2009). While significantly increased levels of Zn, Pb and Cr were observed due to the utilization of organic compounds whereas heavy metals cannot be utilized by the worms.

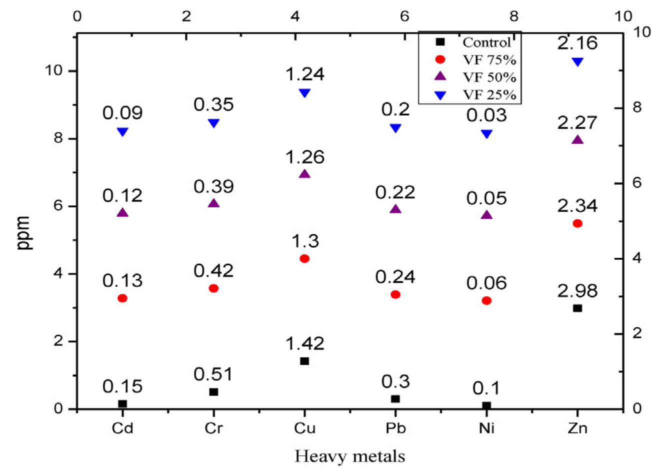

Fig. 3. Concentration of heavy metals in the treated earthworm cast.

3.3. Analysis of the surface structure of wormcast

In the physicochemical structure of the vermifiltration system (VF 75%, 50%, 25%) and control after column tests determined using SEM, were exposing the changes and their intensity (Fig. 4). During adsorption and precipitation process, soil or wormcast a greater number of organic contaminants were removed and which were played as wastewater filter media.

3.4. Histological studies of gut regions of the earthworm E. Eugeniae

The longitudinal muscles, circular muscles, epidermis and cuticle were clearly on the control. Epithelial cells derived cuticle layer forms the body wall. Next to the epidermal layer, circular and longitudinal muscles were surrounded which were responsible for the contraction and relaxation of body from their prostomium to anal region.

VF-75% worms' skin numerous regions showed the slightly damaged cuticle along with severely damaged epidermis. In numerous regions, the circular muscles were teared, similarly numerous gaps and cracks were observed in longitudinal muscles.

The cuticle and epidermis tissue structures were altered. The circular and longitudinal muscles were deformed (VF-50%) whereas the damages (VF-25%) observed in few regions of the circular muscles. The cuticle and epidermis and longitudinal muscles were also unaffected (Fig. 5).

4. Discussion

With this background, the present works try to support that the vermfiltration is believed to be very good novel technology. The pH differences between NVF and VF reactors were related to earthworm facilitated prompting organic compounds mineralization in wastewater. Similar results were reported that there was an increase in pH after VF processes (Xing et al., 2005). In NVF reactors, the TDE treated without vermiculture, the reduction BOD levels was between 72 and 74% whereas TDE treated with earthworms showed reduced BOD levels between 80 and 85%. While comparing the BOD and COD reduction studies, earthworm treated samples showed decreased COD reduction than BOD reduction. This condition was occurred due to the interconversion of organic compounds which increased the chemical substances (Tomar and Suthar, 2011). Due to microbial degradation, the reduction of the NVF treatment was significant.

The earthworms in the VF treatment reduced COD loads by about 86–89%, while in NVF treatment it was 70–75%. Under a pilot-scale project Sinha and his colleagues (2007) tested the dairy industries effluents by vermifiltration process. After treatment, 80–90% of the COD levels were reduced whereas their average reduction was 45%, and the reduced rate was distinctly higher in tested reactors than control reactors. It was noted by Sinha et al., (2008) and his associates in the urban wastewater.

In biofiltration unit, the microbial and geological system responsible for the reduction of COD where as earthworm releases significant levels of enzymes. Earthworms gut regions were flourished with microflora. Released enzymes and microflora flourishes the vermifiltration system and also helps to reduce the COD levels (Suthar, 2010). Ghatnekar et al., (2010) reported the conversion of gelatin industry effluents into less toxic chemical compounds by vermifiltration system which showed significant reduction of COD (90%) in the effluents.

The vermifiltration process of wastewater shows that earthworms had the capacity to remove solid fractions. Significantly reduced TSS and TDS were observed in TDE for both NVF and VF units. Removal process was significant in VF unit than NVF units. In VF units, the TSS and TDS were removed in high amounts from the TDE as 91–94% and 90–95%, whereas the NVF units showed 66–69% and 59–64% decreased in TDE. At the end of the process, treated effluents used for NVF and VF units showed significantly reduced TSS and TDS levels. Various authors reported about the effectiveness of a vermifiltration system. 90–92% and 90–95% of TSS and TDS were removed from the effluents due to the earthworm consumption (Sinha et al., 2007). Finally, the heavy metals were not utilized by worm which resulted as increased concentrations of heavy metals in the samples. When compared to control group, the VF unit process of organic utilization is high which resulted in the high concentrations of heavy metals. Kannadasan et al., (2013) reported the decreased levels of heavy metals due to the emergence of leachate from metallic cations by earthworm degradation process. The enhanced sludge resolving properties not only exclude the quantity of sludge that is needed to be removed, while the expenditures and ecosystem impacts due to additional sludge products (Karmegam et al., 2021) is very important.

In toxicological studies, the branch of toxicokinetics deals with the utilization, magnification and removal of heavy metals by the earthworm organs (Lee et al., 2008). Only a few studies were reported about the tissue damages developed by the textile dye effluents. In earthworms, intestinal chloragogen tissues were severely affected due to the intoxication of Cd (Morgan and Morgan, 1998). Similarly, Honeycutt et al., (1995) reported that Cd also absorbed through epidermal layers and finally diffused into the body coelom.

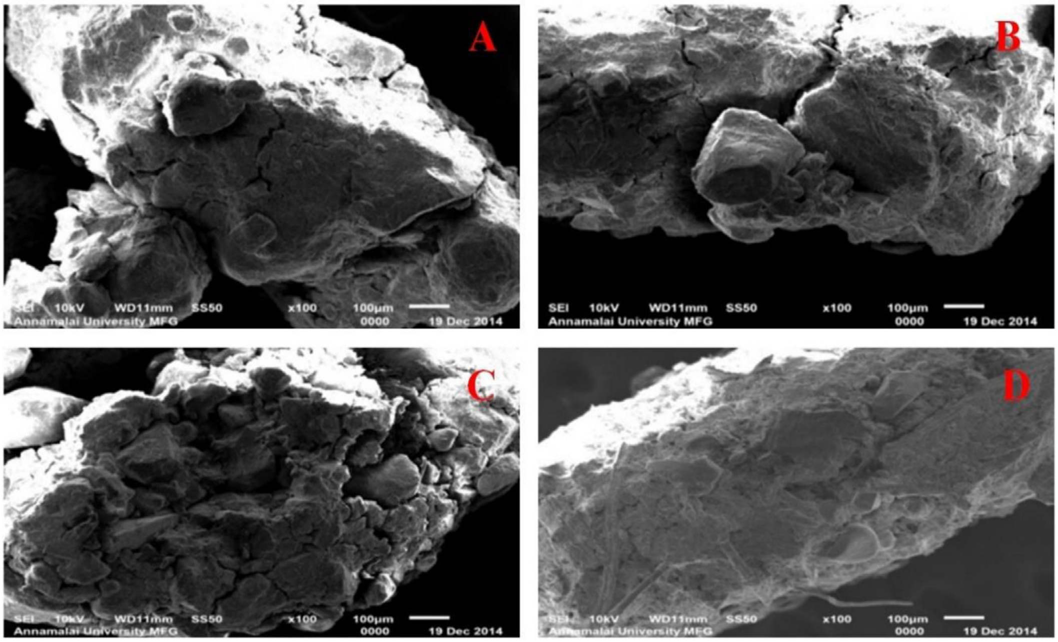

Fig. 4. SEM images of after (a) VF-75%, (b) VF-50, (c) VF-25%, and (d) Control sample of earthworm cast (100 μm).

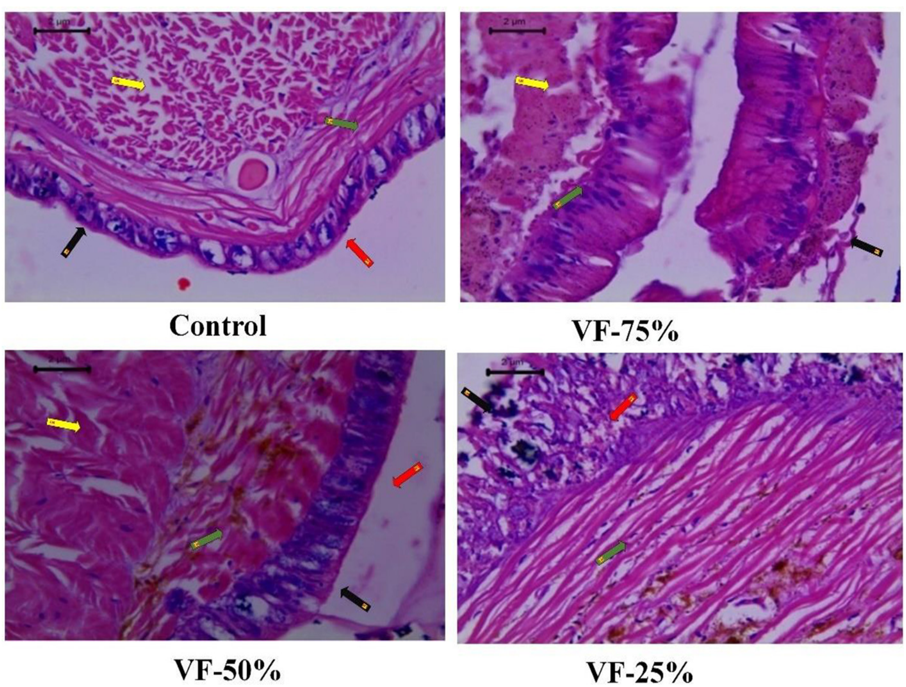

Fig. 5. Histological studies of cross section of the gut regions of the earthworm *E. eugeniae* (yellow arrow- Longitudinal Muscle, green arrow – Circular Muscle, red arrow – Epidermis, black arrow- Cuticle) (40x).

5. Conclusions

For the safe removal of sludge especially for the secured landfills, vermifiltration is considered as a prominent treatment than conservative methods. Vermifiltration is considered a sustainable and alternative technology for wastewater treatment process. Vermifiltration significantly reduces the COD, BOD, TDS, and TSS and neutralization of pH. Earthworms were actively converting unstable compounds into stable compounds during heavy metal chemical speciation. The treated textile dye effluents can be used for irrigation purposes. These studies concluded that vermifiltration process acts as commercial success for the reduction of enormous volume of sewage water. Simultaneously, the fortification of this technique conveniently used in agriculture and near industrial circumstances.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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