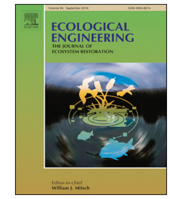
Ecological Engineering 120 (2018) 187–197

Contents lists available at ScienceDirect

Ecological Engineering

journal homepage: www.elsevier.com/locate/ecoleng



Vermistabilization of paper mill sludge by an epigeic earthworm *Perionyx excavatus*: Mitigation strategies for sustainable environmental management

Ananthanarayanan Yuvaraj[a], Natchimuthu Karmegam[b], Ramasundaram Thangaraj[a,*]

[a] *Vermitechnology and Ecotoxicology Laboratory, Department of Zoology, School of Life Sciences, Periyar University, Salem 636 011, Tamil Nadu, India*
[b] *Department of Botany, Government Arts College, Salem 636 007, Tamil Nadu, India*

A R T I C L E I N F O

Keywords:
Hazardous waste
Vermitechnology
Perionyx excavatus
Bioconcentration factor
Histopathological changes

A B S T R A C T

Abstract: The present study demonstrates the vermistabilization of paper mill wastewater sludge (PMS) spiked with cow dung (CD) employing indigenous epigeic earthworm *Perionyx excavatus* Perrier. A total of six treatments were prepared along with a positive control (PC) and negative control (NC). Twenty earthworms were released into each treatment including PC and NC without earthworms. The different proportions viz., T1 (CD – 100%), T2 (PMS: CD – 1:3), T3 (PMS:CD – 1:2), T4 (PMS:CD – 1:1), T5 (PMS:CD – 3:1), T6 (PMS:CD – 2:1), PC (PMS – 100%) and NC (PMS – 100%) and changes in chemical parameters and microbial properties were recorded during the course of 60 days. Vermistabilization caused a significant decrease in the level of heavy metals: Cd (2.9–27.8%), Cu (0.22–42.3%), Pb (1.3–56.3%) and Cr (0.8–46.2%). The bioconcentration factor (BCFs) was also calculated and great amount of heavy metals accumulated in their body (mg kg^{-1}) that ranged from 0.31 ± 0.003–0.45 ± 0.007 for Cd, 0.12 ± 0.005–0.24 ± 0.003 for Cu, 0.15 ± 0.005–0.31 ± 0.006 for Pb and 0.29 ± 0.007–0.56 ± 0.001 mg kg^{-1} for Cr, accumulation of heavy metals are in the order: Cr > Cd > Pb > Cu. The physicochemical parameters of earthworm treated substrate such as electrical conductivity, total nitrogen, total phosphorus and total potassium were significantly increased; whereas, pH, total organic carbon, C:N ratio (carbon: nitrogen) and C:P ratio (carbon: phosphorus) were reduced after 60 days of vermistabilization. The vermistabilized materials also had a higher population of bacteria (98.90 ± 0.30 CFU × 106 g^{-1}), fungi (43.75 ± 0.55 CFU × 103 g^{-1}) and actinomycetes (67.65 ± 0.45 CFU × 105 g^{-1}) than initial mixtures. Moreover, several histopathological changes were observed in earthworm tissues viz., disintegration of cells, irregular surface of epidermis, cellular debris, irregular cellular compartmentation, and oval-shaped nucleus. Higher level of histopathological abnormalities was recorded in PC (PMS-100%) while none were detected in lower concentrations of PMS. Furthermore, the study concludes that the paper mill sludge in a mixture of cow dung (1:1 ratio) can be a useful proposition for utilizing this hazardous waste through the adoption of vermitechnology.

1. Introduction

The paper manufacturing industry is one of the most important industries of the Indian economy and approximately 625 paper mills are found in India (Chandra et al., 2011), which produce about 10.92 million tons of paper per year (Deeba et al., 2016). Paper mills, during the paper manufacturing process, utilize a large number of different chemicals such as sodium hydroxide (NaOH), sodium sulfide (Na_2S) as well as sulfurous acid (H_2SO_3) and the end process releases colorful effluent with highly toxic chemical compounds (Pokhrel and Viraraghavan, 2004). During the paper production process, one ton of paper produces about 60 cubic meters of effluent. This effluent after treatment can generate a large amount of sludge about 40–50 kg dry sludge in one ton of paper produced (Bajpai, 2015). The paper mill sludge (PMS) contains toxic heavy metals such as chromium (Cr), cadmium (Cd), nickel (Ni) and lead (Pb) (Chandra and Rachna, 2012), which create serious environmental pollution after landfilling (Singh and Kalamdhad, 2013). Generally, heavy metals are accumulated on top of the soil (Liénard et al., 2014), which generate chronic toxicological issues in living organisms (Li et al., 2009). Therefore, unsafe removal of PMS sludge and the presence of heavy metals could affect the soil and beneficial microorganisms resulting in the declining level of plant production (Liu et al., 2005) and ultimately affecting food chain (Perlatti et al., 2014).

As there is an urgent requirement for remediation of toxic heavy metal from PMS, vermistabilization, an eco-friendly technology is an

* Corresponding author.
E-mail address: thangaraj@periyaruniversity.ac.in (R. Thangaraj).

https://doi.org/10.1016/j.ecoleng.2018.06.008
Received 3 January 2018; Received in revised form 19 May 2018; Accepted 5 June 2018
0925-8574/

appropriate method to decrease the level of hazardous materials from the sludge (Negi and Suthar, 2013). Earthworms are also known as "ecosystem engineers" because of their influence on the soil physical, chemical and biological properties (Blouin et al., 2013). Additionally, earthworms can alter the soil pollutants and reduce the level of soil heavy metals (Becquer et al., 2005; Maity et al., 2008). Recent reports have confirmed the stabilization of industrial sludge/wastes through earthworms such as paper mill sludge (Kaur et al., 2010; Negi and Suthar, 2013), fermented tannery (Ravindran et al., 2016), municipal solid waste (Soobhany et al., 2015), food industry waste (Garg et al., 2012), distillery industry (Suthar and Singh, 2008), leather processing industry (Ravindran et al., 2008), milk processing industry (Suthar et al., 2012), etc.

The earthworms can alter the soil structure and enhance the rate of decomposition (Lemtiri et al., 2014; Fahey et al., 2013), stimulate the soil nutrients and also affect heavy metal cycles are well documented by Sizmur et al. (2011) and Richardson et al. (2015). Moreover, most of the ecotoxicology experimentations have been associated with earthworms because it can alter the level of pollutants in metal contaminated environments (OECD, 1984, 2004; Dickinson, 2000). Spurgeon et al. (1994) reported that worms can survive in heavy metal polluted soils and metal accumulation in their internal body is well documented (Nahmani et al., 2007; Suthar et al., 2014). During the vermistabilization process, certain factors can stimulate the earthworm survival rate such as pH (5–8), moisture content (40–55%) and C/N ratio (carbon/nitrogen) (Lim et al., 2016). Furthermore, different amendment materials like cow dung (Lim et al., 2016) and plant waste materials (Lim et al., 2011), have been known to enhance the earthworm survival rate and improvement of the remediation process. According to recent literature, earthworm treated wastes show a reduction in the concentration of heavy metals (Azizi et al., 2013; Singh and Kalamdhad, 2013; Fernández-Gómez et al., 2013; Wang et al., 2013). Recently, Usmani et al. (2017) investigated the management of coal fly ash through vermicomposting employing three earthworm species for 90 days and at the end of the experiment, heavy metals such as Cr, Ni, and Cu reduced by 58.82%, 71.94%, and 51.67%, respectively.

Previews researchers have reported the composting of PMS (Marche et al., 2003; Hazarika et al., 2017; Gea et al., 2005; Stoica et al., 2009; Graydon et al., 1999; Gregory et al., 1999; Abdul et al., 1997) but the biological properties are not well addressed. Recently, Hazarika et al. (2017) concluded that rotary drum composting of PMS for 20 days resulted in heavy metal (Cu, Fe, Zn) reduction that ranged from 0.8 to 13.4%. The main disadvantage of the composting method is that several factors may affect the rate of composting such as aeration, moisture content as well as the nature of sludge are well documented by several researchers (Beaudin et al., 1999; Jiang et al., 2011; Kazemi et al., 2016). On the other hand, quite a few authors have reported interesting results on vermicomposting of PMS (Elvira et al., 1998; Kaur et al., 2010; Sonowal et al., 2013; Negi and Suthar, 2013) but bio-concentration factor and earthworm histopathological studies were unreported. Keeping this in view, the present work was carried out to vermistabilize PMS using an indigenous epigeic earthworm species *Perionyx excavatus*. These earthworms can tolerate a wide range of changes in pH, temperature as well as substrate moisture content and are able to remediate several hazardous materials (Bhattacharjee and Chaudhuri, 2002).

2. Materials and methods

2.1. Earthworms, paper mill sludge, and cow dung

The indigenous earthworm *P. excavatus* (Indian blue worm) was used for vermistabilization of PMS. These worms were collected from an organic agricultural farm in Salem District, Tamil Nadu, India and then brought to our laboratory for identification. Furthermore, earthworms were cultured in circular plastic containers (0.43 m diameter; 0.34 m height), with feeding materials like partially decomposed *Tectona grandis* leaf litter and garden soil (3:1 ratio). The second generation of *P. excavatus* was used for experimentations to eliminate any contamination from first generation earthworms. Furthermore, earthworm parameters such as changes in biomass, cocoon production, reproductive and mortality rate and chemical characteristics along with biological properties were analyzed during the vermistabilization process.

Fresh PMS was obtained from wastewater treatment plant of a paper mill (Balaji Paper and Boards Pvt. Ltd.) located at Pallipalayam, Erode, Tamil Nadu, India. The PMS having 60–70% moisture content was collected in round shaped plastic containers (23.4-liter capacity) and excess level of brown colored wastewater was removed. The collected PMS was air-dried, separately spreading over a polythene sheet (5.2-meter length × 4.3-meter breath) for 4 days. Furthermore, air dried PMS samples were pre-composted for three weeks prior to the vermistabilization process. The main physicochemical characteristics of the PMS sludge are: pH: 8.30 ± 0.55; electrical conductivity: 0.44 ± 0.02 (dsm^{-1}); organic carbon: 28.85 ± 0.45 (%); total nitrogen: 0.20 ± 0.01 (%); total phosphorus: 0.23 ± 0.20 (%); total potassium: 0.05 ± 0.05 (%); C:N ratio (carbon: nitrogen): 144 ± 0.40.

Cow dung (CD) was procured from Rani Cowshed, Karuppur, Salem, Tamil Nadu, India. The CD was partially dried and used for further experimentations and the main physicochemical characteristics of CD are: pH: 8.4 ± 0.20; electrical conductivity: 0.18 ± 0.05 (dsm^{-1}); organic carbon: 5.3 ± 0.25 (%); total nitrogen: 0.3 ± 0.01 (%); total potassium: 0.03 ± 0.05 (%); total phosphorus: 0.01 ± 0.07 (%); C:N ratio (carbon: nitrogen): 17.6 ± 0.01.

2.2. Preparation of experimental set-up

For laboratory trial, totally eight circular containers (43 cm diameter; 34 cm height) were taken for the experimentation and marked as T1, T2, T3, T4, T5, T6, positive control (PC) and negative control (NC). For experimentation, one kilogram (dry weight basis) of PMS was mixed with CD in different ratios as presented in Table 1 and each treatment including PC and NC were maintained with three replicates. Twenty mature *P. excavatus* was collected from stock culture and released into the respective treatments including PC while NC was maintained without earthworms. Furthermore, 0.8 kg (dry weight basis) of *Tectona grandis* leaf litter was added. The temperature and moisture content in treatments were maintained at 301 ± 1 K and 72 ± 2% respectively. The experimental containers were kept in dark room. Moreover, changes in the physic[oc]hemical characteristics of the treatments were recorded regularly at 0, 20, 40, and 60 days. The collected samples were oven dried at 333.1 K and kept in decontaminated plastic airtight containers for further analysis. The biological parameters such as earthworm weight, cocoon production, mortality rate

Table 1
Composition of treatments.

Treatments	Composition	*Perionyx excavatus* (n)
T1	[a]CD – 100%	20
T2	[b]PMS:CD – 1:3	20
T3	PMS:CD – 1:2	20
T4	PMS:CD – 1:1	20
T5	PMS:CD – 3:1	20
T6	PMS:CD – 2:1	20
[c]PC	PMS – 100%	20
[d]NC	PMS – 100%	without earthworms

[a] CD – cow dung.
[b] PMS – paper mill sludge.
[c] PC – positive control.
[d] NC – negative control.