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Pathogen reduction in septic tank sludge through vermicomposting using *Eisenia fetida*

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ABSTRACT

This study evaluated the potential of earthworms (*Eisenia fetida*) to remove pathogens from the sludge from septic tanks. Three earthworm population densities, equivalent to 1, 2, and 2.5 kg m^{-2}, were tested for pathogen removal from sludge. The experimental phase lasted 60 days, starting from the initial earthworm inoculation. After 60 days, it was found that earthworms reduced concentrations of fecal coliforms, *Salmonella* spp., and helminth ova to permissible levels (<1000 MPN/g, <3 MPN/g, and <1 viable ova/g on a dry weight basis, respectively) in accordance with Official Mexican Standard of environmental protection (NOM-004-SEMARNAT-2002) (SEMARNAT, 2002). Thus, sludge treatment with earthworms generated Class A biosolids, useful for forest, agricultural, and soil improvement.

1. Introduction

In Mexico, wastewater treatment has become critically important in the last few years. The residual sludge produced represents only 1–2% of the volume of treated wastewater, however, its treatment is a highly complex and costly activity. It typically requires between 20% and 60% of the wastewater treatment plant's total operating costs. Challenges associated with handling wastewater sludge need to be addressed because of its negative impact on the environment and the resulting damage to human health. Frequently, some sludge is undertaken outside the treatment plant boundaries (Sperling and Andreoli, 2007). Other types of sludge that must be considered in wastewater treatment plants are those generated in on-site sanitation systems (e.g., septic tanks) (Barrios, 2007).

Sewage sludge (biosolids) is mainly discharged into landfills, but it is sometimes discharged into clandestine garbage dumps or natural gullies. This negatively affects the health of soil, surface water, groundwater, flora, fauna, and humans associated with the site (Martinez, 1996). Future challenges in sludge management include increasing knowledge on the characteristics of sludge, especially microbial content, in order to provide suitable treatments. In addition, methods for turning sludge into valuable products should be researched, primarily for agricultural applications (Barrios, 2007).

In the Yucatan Peninsula in Mexico, the municipal sewage system covers less than 8.2% of the populated area. The use of septic tanks in this region is quite common; they account for a critical part of the sewage infrastructure and are an important source of sewage sludge generation. When considering septic tanks as part of the main wastewater treatment system, 63% of the populated region is covered by sewage infrastructure (CNA, 2003).

A sludge management plan must consider several factors, including financial, social, environmental, technical, political, and public health issues; but overall, it must be sustainable (Barrios, 2007). In recent years, alternative biological methods for treating sewage sludge from water purification plants have received a lot of attention due to being considered effective and economical. One of the main biological approaches to sludge management is vermicomposting (Banu et al., 2001; Cardoso and Ramirez, 2002; Sherman-Huntoon, 2000).

Vermicomposting is the biological inactivation of pathogenic organisms in sludge using earthworms (Teixeira, 2007). It is an efficient and economical (Banu et al., 2001) procedure that produces

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compost, with value added agriculture (Teixeira, 2007). However, to establish the vermicomposting procedure, it is necessary to perform an analysis of the population dynamics of earthworms in different organic substrata that are found in a specific region, since the chemical and physical characteristics of the substrata have an effect on the population dynamics of these annelids (Santamaria and Ferrera-Cerrato, 2002).

In this study, three population densities (1.0, 2.0, and 2.5 kg m^{-2}) of the earthworm *Eisenia fetida* were tested for contaminant reduction (fecal coliform, *Salmonella* spp. and helminth ova) in septic tank sewage sludge. The techniques that were used are those established by Official Mexican Standards of environmental protection (NOM-004-SEMARNAT-2002) (SEMARNAT, 2002). It was observed that all three earthworm population densities provided favorable results in sludge vermicomposting, since the resulting product was classified as Class A biosolids based on pathogen and parasite content, and was considered safe for urban use (forest, agricultural, and soil improvement) and for handing during application.

2. Methodology

2.1. Fieldwork

The experimental fieldwork for this study took place in the earthworm-breeding module of the Instituto Tecnologico de China (IT China), in Campeche, Mexico (19°46′ N and 90°30′ W). Approximate elevation was 24 m above sea level (INEGI, 2007).

Septic sludge was obtained from a company (Servicio Palma) in Campeche, the capital city of the state of Campeche. The company offered wastewater cleaning services and discharge devices (septic tanks, drains, and wells). The sludge was transported in 200 L plastic cylinders and then placed in 1300 L containers.

Earthworm inoculations were performed in experimental units after an acclimation process to assure their survival in sludge. Approximately 1 kg of raw sludge (RS) was placed in a plastic container, and 20 juvenile and adult *E. fetida* earthworms were added. Surviving earthworms were assessed after 24 h. This activity was performed every other day for 20 days and then daily thereafter. Earthworm inoculations were performed when there was 100% survival. Temperature and pH (smart check; Orion) were also assessed during this period.

The experimental phase began with earthworm inoculations and lasted for 60 days. Pre-composted sludge (PCS) (approximately 10 kg) was placed in plastic containers (51.2 × 33.9 × 28.3 cm) that were perforated at the bottom to allow liquid drainage. *E. fetida* earthworms (juvenile and adult) were added to the containers at three population densities (with triplicate experiments per density): 1 kg m^{-2} (Ferruzzi, 1986), 2 kg m^{-2} (Crites and Tchobanoglous, 2000), and 2.5 kg m^{-2} (Cardoso and Ramirez, 2002). Two containers were used as controls without earthworms. Earthworms were taken from the IT China stock. Experimental units were kept covered with a screen mesh and placed in the Institute's earthworm-breeding module, which had 90% shade mesh. Temperature (daily), moisture, and pH (every other day) were assessed in the sludge from the containers using portable measuring devices, including a common mercury thermometer, made of glass, a soil moisture meter (Cole-Parmer), and a pH pocket meter (Orion), respectively.

Earthworms from each experimental unit were extracted and weighed on an electronic balance to assess survival. Individual average weight was calculated based on the weight of 100 earthworms from each experimental unit. A vermicompost sample was taken from each experimental unit (1 kg) for cocoon counting and then subsamples of each sample (100 g) were analyzed more closely with a magnifying glass. Vermicompost from each box was turned over manually prior to sampling to ensure homogeneity.

2.2. Laboratory work

Microbiological and chemical analyses were performed on RS, PCS (prior to earthworm inoculations), vermicomposted sludge (VCS), and controls (day 60). The microbiological characterization consisted of the analysis of fecal coliform (MPN procedure), *Salmonella* spp. (MPN procedure), and helminth ova (quantification of viable helminth ova), according to the techniques indicated in Official Mexican Standard NOM-004-SEMARNAT-2002 (SEMARNAT, 2002) for sludge and biosolids. The chemical characterization consisted of the analysis of total solids (TS) and total volatile solids (TVS), according to the techniques set forth in the Mexican Standard for water quality (NMX-AA-034-SCFI-2001), and organic matter (OM) determination was based on the Mexican Standard of environmental protection NMX-AA-021-1985 (SECOFI, 1985).

A sample was taken from the stock of earthworms that were used for the inoculation of the experimental units, to determine initial chemical composition (bromatological analysis). This was compared to a similar analysis of earthworms harvested from the different experimental units (day 60). The analytical procedures used are described in Official Methods of Analysis (AOAC, 1994).

2.3. Mexican Official Standard of environmental protection NOM-004-SEMARNAT-2002

Mexican Official Standard NOM-004-SEMARNAT-2002 classifies biosolids as Class A, B, or C, depending on their content of heavy metals, pathogens and parasites, and their potential for exploitation (forest, agricultural, and soil improvement potential) (SEMARNAT, 2002). According to Mexican Official Standard NOM-004-SEMARNAT-2002 and to the origin of the sludge used in the current investigation, solids were classified depending on pathogen and parasite content.

2.4. Statistical analysis

A multiple-sample comparison was performed to identify whether there were any significant differences among the means of initial and final individual weight and biomass, and a multiple range test at the 95% confidence level (significance was defined as $P < 0.05$) was used to determine which means were significantly different from one another.

3. Results and discussion

3.1. Pre-composting

In the present study, a 30-day pre-composting period was necessary for the earthworms to adapt to sewage sludge and show 100% survival after 24 h. Garg et al. (2006) pointed out that pre-composting is essential to prevent earthworm mortality during vermicomposting, because during this period, the release of volatile gases may be potentially toxic to the worms. Dominguez and Edwards (2004) indicated that earthworms are very sensitive to ammonia and cannot survive in organic waste that contains high levels of this cation (e.g., fresh cattle waste).

Garg et al. (2006) found that a 2-week pre-composting period was sufficient for several types of manure. Santamaria and Ferrera-Cerrato (2002) reported that a 30-day pre-composting period was necessary before inoculation of *Eisenia andrei* into bovine