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Vermiremediation: Analysis of contaminated diesel in soil using *Eisenia fetida* and ZnO nanoparticles with cow dung

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Cow dung is a valuable source of manure to improve soil quality. This study aims to enhance the soil quality by decreasing diesel contamination in the soil through the vermicomposting of cow dung and nanoparticles of ZnO. Using a powder made from cow dung, zinc ions may be easily converted into ZnO nanoparticles. To increase the quality of soil, *Eisenia fetida* and nanoparticles of ZnO mixed with cow dung was used. These nanoparticles were characterized by FT-IR, SEM, and TEM. The diesel impure soils were examined for 70 days by gas chromatography. Observations showed that the soil samples without the earthworms had a higher concentration of diesel than the earthworm present in the soil. In this method, diesel (4.5 ml) was reduced by almost 50% after 70 days. Diesel concentrations were significantly higher during sampling time than they were later in soil contaminated with *E. fetida*.

KEYWORDS

diesel, *E. fetida*, ZnO (NPs), SEM, TEM, cow dung

Introduction

Soil contamination is caused by a wide range of human activities, including industrial and agricultural wastes. Vermiremediation, which uses an earthworm called *Eisenia fetida*, was successful in purifying diesel-contaminated soil (Behnaz et al., 2020). Abiotic and biotic components of the soil interact mechanically and biochemically with *Eisenia fetida* to promote plant growth and productivity (Dada et al., 2015). Burrowing earthworms ingests soil particles so that they are mechanically broken down, and this provides them with increased surface area for biotic activities. Earthworm burrows facilitate water movement, enhance soil aeration, and facilitate the movement of nutrients and oxygen. Also, earthworms increase the soil quality (Sinha et al., 2008). They create favorable conditions for bacteria and improve soil aeration by stimulating and accelerating the microbial activity (Singleton et al., 2003). A large number of biodegraded microorganisms are found in the guts of earthworms. They are reduced into the soil as vermicast by the worms (Dabke, 2013). Vermi transformation involves the decomposition of degradable organic contaminants such as pesticides and herbicides using enzymes (such as peroxidases) and microorganisms (such as bacteria and fungi) found in the alimentary canal of earthworms (Shi et al., 2020). The use of various treatment methods has been proposed for oily sludge in recent years, including land farming, incineration, stabilization/ solidification, solvent extraction, ultrasonication, pyrolysis, chemical treatment, and bioremediation (Ping et al., 2012; Teresa et al., 2012; Leandro et al., 2020). Earthworms have the ability to survive harsh chemical conditions, which makes them ideal for purifying hydrocarbon-based aromatic contaminants (Luc et al., 2011; Jacobo et al., 2014). During vermicomposting, cow dung is commonly used, owing to its effective contribution to worm growth (Xin et al., 2016). As such, metal nanoparticles (MgO, NiO, CuO, ZnO, TiO_2, etc.) and their metal oxides (Ag, Au, Pt, Cu, Zn, etc.) become the most feasible solution, and physically, they differ from each other such as their big outside area-to-volume ratio, controlled morphology (consistent and homogenous), and small sizes (Hirpara and Gajera, 2020). ZnO-based nanoparticles have gained popularity in the synthesis and production process due to their decreased toxicity (Veeramani et al., 2013). Due to the high energy and toxic chemical requirements, neither of these methods is easily scaleable. Because harmful chemicals can be utilized in the synthesis process and because they persist on the nanoparticle surface despite repeated washings, this technique's biocompatibility is compromised for a number of reasons, one of which is that it can be used to treat diseases (Khan et al., 2018; Shahid et al., 2019). Worldwide demand for petroleum products is still high, both for use as fuel and as lubricants in machinery. Because crude oil is so prevalent and widely used, soil degradation brought on by crude oil derivatives is a serious ecological issue. Gasoline hydrocarbons can disrupt water relationships and gas exchange in plants, inhibit germination, and promote chlorophyll degradation (Bona et al., 2011). Alkyl, ethylene, naphthenic, and aromatic hydrocarbons are the most hazardous petroleum xenobiotics (Gray et al., 1994). Organic pollutants persistent in the atmosphere are polycyclic aromatic hydrocarbons, which decrease plant growth (Jain et al., 2011). Additionally, many earthworms can help clean up polluted soils from various oils like engine oil (Murugan et al., 2022). In soils, petroleum hydrocarbons are toxic to plants and microorganisms. Mixtures of petroleum products are commonly falling in the soils of mechanic workshops in developing countries, and diverse types of petroleum products can penetrate into the soil at the same time or at different times. Based on the literature report, previous research work was expensive and took more time to remove diesel from the soil. The aim of this work was to remove 50% diesel from soils using cow manure and ZnO nanoparticles in a quick and inexpensive process.

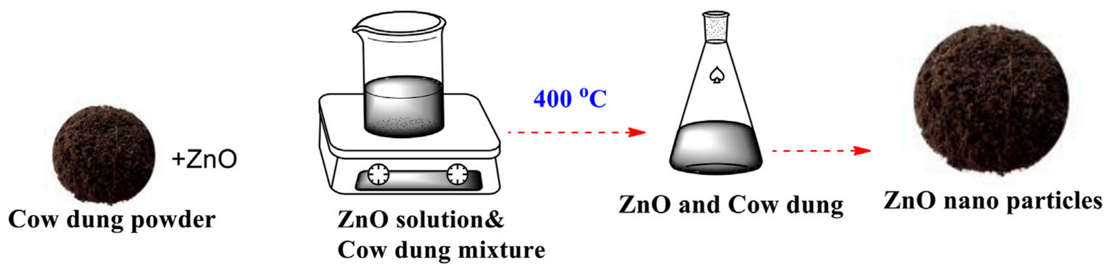
2 Materials and methods

2.1 Sources of materials

Zinc nitrate was purchased from Sigma-Aldrich. The earthworms used in this experiment weighed around 0.89*0.81 g each. Merck *eicosane* is the supplier of gasoline and diesel fuel. FT-IR (Shimadzu 8201pc, Japan 4,000–1,000 cm-1) spectra were captured using the KBr disc approach. Gas chromatography–mass spectroscopy was performed using a PerkinElmer Clarus SQ8 GC-MS model (EI, United States). Scanning electron microscopy and transmission electron microscopy were used to confirm the morphology of ZnO nanoparticles. A scanning electron microscope (SEM) typeVP-1450 (LEO, Co., Germany) was utilized for the SEM study. LEO 912 AB (TEM) equipment was utilized for the study. Red soil (local soil name) cow manure was purchased from a local market.

2.2 Experimental design

For the experiment, 100 g of fine-sieved previously identified red soil (local soil, South India) was added to a 500-ml beaker. The soils were selected for this study, according to the Indian naming system (Pitchaikkaran et al., 2022) and the USDA key to taxonomy (Staff, 2010). The 4500-ppm (4.5 ml) diesel was added to the dried soil. The cow dung was crushed to remove large lumps, air-dried, and stored in a dry area. *Eicosane* soil–diesel mixture, cow dung, and the ZnO nanoparticles were stirred following the procedures (Schwartz et al., 2012). For every group of beakers, one is with earthworms and another one



SCHEME 1
Synthesis of ZnO nanoparticles using cow dung

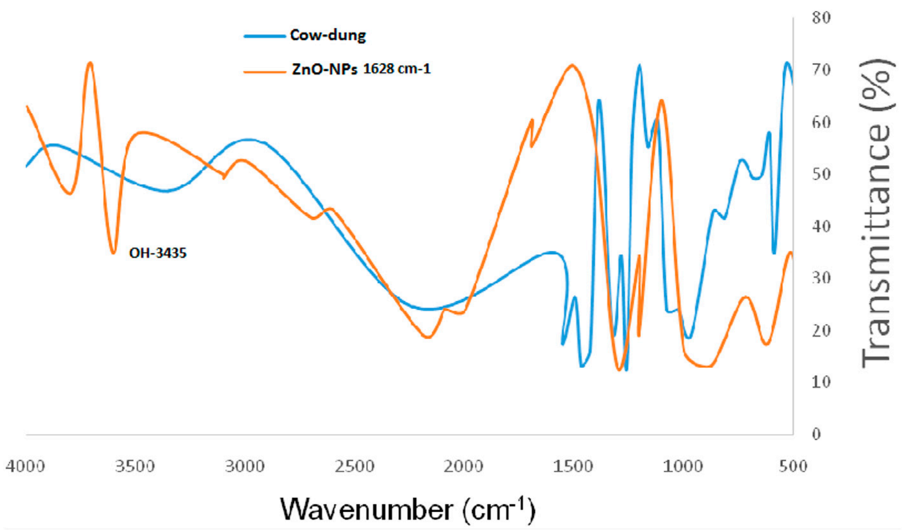


FIGURE 1
FT-IR spectrum of the cow dung–ZnO nanocomposite.

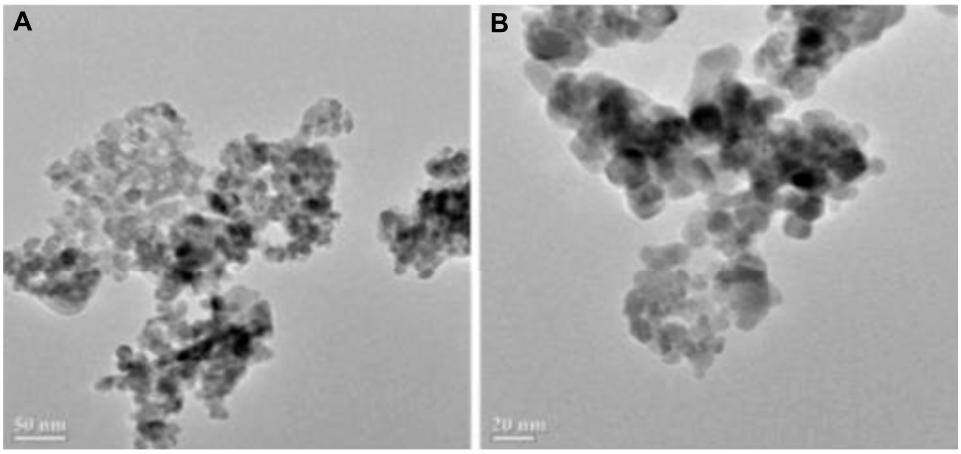


FIGURE 2
TEM image of ZnO nanoparticles.

without earthworms. In the control experiment, there were no earthworms. Each beaker of earthworm soil was weighed, and 15 earthworms were added in 48 h on top of the soil in each beaker (Barkley et al., 2011).

At room temperature, both the experimental and control beakers were incubated for 5–70 days. A gauze lid was placed on the beakers, and they were left at room temperature. The soil's total petroleum hydrocarbon content was extracted using a standard literature method (Contreras-Ramos et al., 2006). A PerkinElmer GC-MS Clarus SQ-18 model instrument was used to determine the amounts of petroleum hydrocarbon in the soil samples.

2.2.1 Preparation of ZnO with the cow dung extract

The extract for the reduction of ZnO ions into nanoparticles (ZnO) was made by combining 60 g of cow dung. After that, the mixture was boiled for 60 min. The extract was cooled to room temperature before being filtered onto a filter paper. To be used in future research, the extract was kept in the refrigerator (Ponnusamy et al., 2021).

2.2.2 Preparation of zinc nanoparticles

A stirrer heater was used to boil 60 ml of water and dried cow dung (10 g) to 70–80°C for the synthesis of nanoparticles.

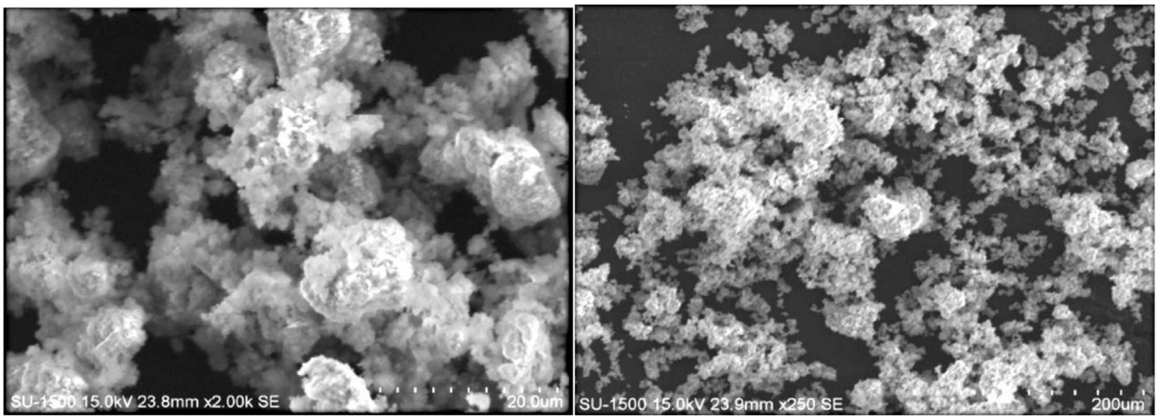


FIGURE 3
SEM image of the ZnO nanoparticle.

FIGURE 4
Soil stabilization using *Eisenia fetida*.

As the temperature reached 70°C, 10 g of zinc nitrate was applied to the solution. This mixture is then reduced to a deep yellow paste by boiling it. This paste was then deposited in a ceramic crucible and heated for 3 h at 350°C in an air-heated furnace. For characterization purposes, a light-yellow powder was obtained and carefully collected and packed. To obtain a finer nature for characterization, the substance was mashed in a mortar pestle. Scheme 1 depicts the synthesis of ZnO nanoparticles.

3 Results and discussion

3.1 Characterization of ZnO nanoparticles: FT-IR spectra

According to the IR spectra (Figure 1), peaks at 3272cm^{-1} and 1628cm^{-1} 3,445 attributed to ZnO and OH functional groups, respectively. It was concluded that the extract was responsible for removing metal ions and for capping the nanoparticles because bioactive molecules were present in the extract.

3.2 Characterization of ZnO nanoparticles

3.2.1 Transmission electron microscopy

To learn more about the size and morphology of the ZnO nanoparticles, a TEM study was performed. TEM images of ZnO nanoparticles at different magnifications are shown in Figures 2A,B. The average particle size of ZnO nanoparticles can be seen in the TEM images to be in the range of 16–27 nm.

3.2.2 Scanning electron microscopy

Zinc particles and zinc aggregates can be seen in the scan of SEM. The SEM image revealed a spherical-shaped nanoparticle with a diameter of 200 nm. Figure 3 shows the formation of aggregated molecules in the 12 m range. Figure 4 indicates soil stabilization with cow dung ZnO nanoparticles.

3.2.3 Table 1 and GC-MS (with and without earthworms)

Cow dung and ZnO nanoparticles were prepared by a slight modification of the experimental procedure (Mupondi, 2010; Ponnusamy et al., 2021). The eicosane, the earthworms, and the soil–petroleum mixture were prepared by following the literature

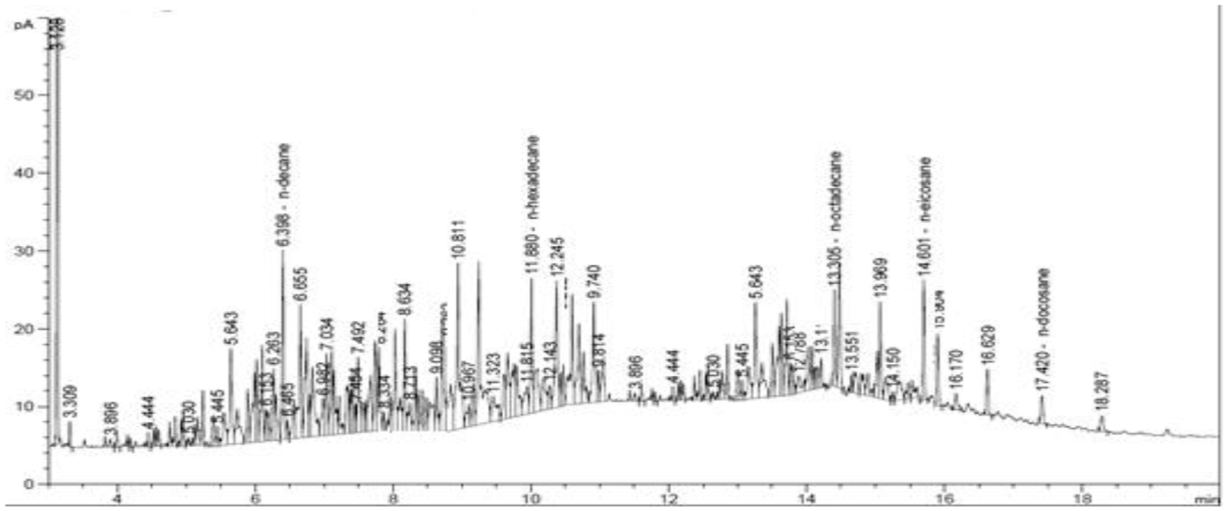


FIGURE 5
Diesel GCMS (without earthworms).

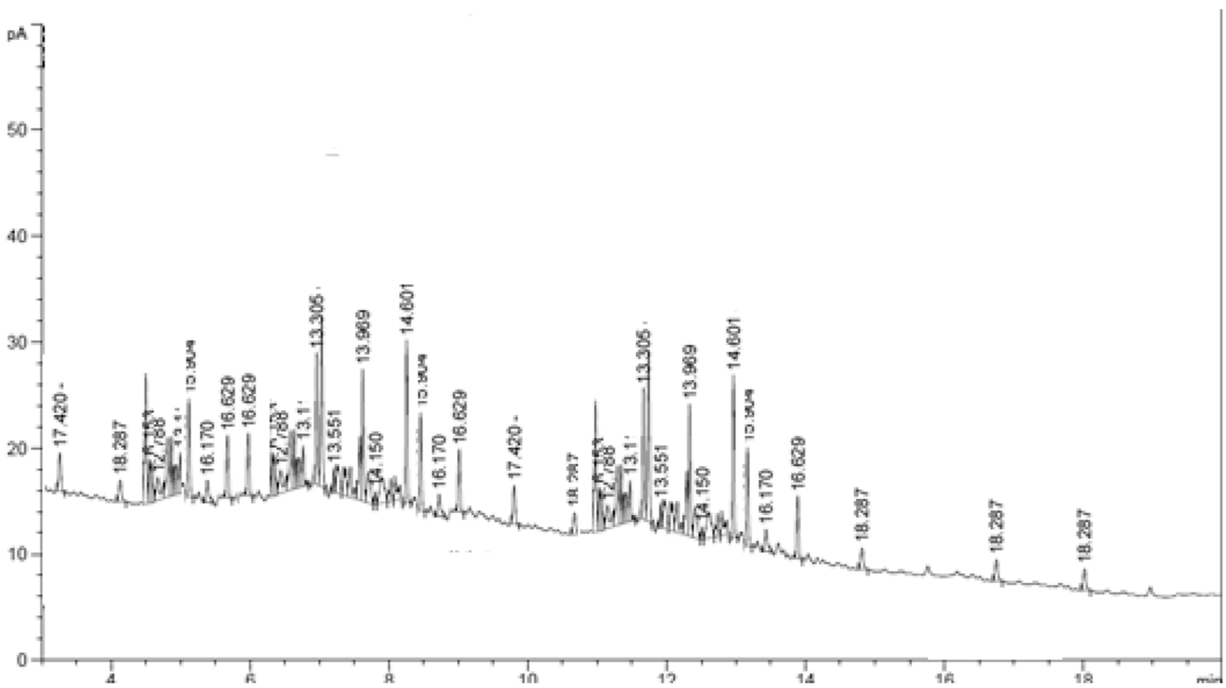


FIGURE 6
Diesel GCMS (with the earthworm *E. fetida*).

method (Mohan et al., 2011). Mature *E. fetida* earthworms had been purchased from a local market.

In diesel-contaminated soil, the addition of *E. fetida* and ZnO nanoparticles increased the minimum soil quality during 70 days. Such vermiremediation processes increase the soil nature and reduce the diesel contamination in soil naturally (Isabela et al., 2022).

Many methods can be used to achieve vermicomposting in practice. A contaminated field or farmland may be seeded directly with earthworms. In addition to earthworms, cow dung, poultry droppings, or formulated supplements can be added to the soil as nutrient media (Hickman and Reid, 2008). During a period of 60 days, *Eisenia fetida* and *Lumbricus terrestris* were used to

TABLE 1 Effect of the *E.fetida* earthworm, cow dung, and ZnO nanoparticles on the diesel-contaminated soil.

S.No	Soil name	Contamination of hydrocarbon in soil	Standard concentration value in ppm	After treatment of *E. fetida* and nanoparticles value in ppm
01	Red soil	Diesel	4,500	2,500

remediate soils polluted with petroleum hydrocarbons, measured as total petroleum hydrocarbons. There was a significant reduction in soil TPH by the end of the experiment (Almutairi, 2019). The use of earthworms as a biomonitoring agent may not be appropriate when earthworms are being used to clean up contaminants. The adaptations that earthworms have developed physiologically and genetically may influence risk assessments in long-term contaminated sites (Spurgeon and Hopkin, 2000) (Ponnusamy et al., 2021). Organic carbon contents and soil toxicity (NPK) were reduced in vermiremediation treatment (Abdollahinejad et al., 2020; Vasilyeva et al., 2020; Ugochukwu et al., 2021). The FT-IR results indicate the ZnO nanoparticles present in cow dung. SEM analysis indicates the morphology of nanoparticles. The GC-MS shows diesel reduction with and without earthworms of *E. fetida*. It could be hypothesized that petroleum hydrocarbons in polluted soils incubated with an earthworm could have resulted from the degradation of diesel since they were absent in polluted soils not incubated with an earthworm. Figure 5 indicates without earthworm GC-MS, and Figure 6 shows the earthworm-treated diesel GC-MS. Based on the GC-MS result, the earthworms of *E. fetida* contained 50% diesel hydrocarbons, as was observed in the study. *E. fetida* may be capable of vermiremediating petroleum through degradation (vermidegradation).

This suggests that *E. fetida* contributes to the degradation of diesel in soil and that these hydrocarbons are possible degradation products. Incubation of soil with an earthworm for 70 days resulted in a higher concentration of degradation by-products, indicating that the longer the period the greater the degradation is. A single petroleum hydrocarbon was detected initially in the soil but was lost completely during the study Table 1.

4 Conclusion

Globally, soil is continuously subjected to contamination from diesel, petrol, and industrial wastes. We used the traditional method of vermiremediation to remove oil contamination in the soil. This research revealed some findings that are useful for the future application of vermiremediation to remove diesel oil from soil using *E.fetida* earthworms. The less toxic metals are used to prepare cow dung nanoparticles. The ZnO nanoparticles were characterized by FT-IR, SEM, and TEM. Based on the results of this study, it can be concluded that *E fetida* has the potential to facilitate the cleansing of diesel-contaminated soil. As 70 days would not be enough time to achieve full remediation, 50% diesel was removed from soils in a short period of time and at a low price. Such remediation can be extended to a longer period to achieve a higher level of soil remediation.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Author contributions

RS: Project administration and all aspects of this manuscript's chemistry and biology have been investigated. The manuscript was writing original draft preparation through the contributions of all authors. AI: All kinds of characterization. AA: Data collection. HAA: Chemical compounds characterization, and RG: Data analysis and English Corrections.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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