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# Bioremediation of contaminated lead soil by *Eudrilus Eugeniae* and synthesis of nanoparticles

[1] Gokul Prashanth V, [2] Dr. Lakshmi Prabha M

[1] IV B. Tech, Department of Biotechnology, Karunya University, Coimbatore Tamil Nadu, India.
[2] Asst. Professor (SG), Department of Biotechnology, Karunya University, Coimbatore Tamil Nadu, India.

**Abstract**

**Aim:** To explore the potential of earthworms namely *Eudrilus eugeniae* in bioremediation of lead contaminated soil and convert it into high quality soil which holds promise to play a significant role in cleaning the environment.

**Methods:** Collection of lead contaminated soil and the selection earthworm species *Eudrilus eugeniae* and treating of contaminated soil using earthworm three experimental groups were maintained. The vermi-treatment was done for 60 days and further analysis using Atomic absorption spectroscopy (AAS), Fourier Transform Infrared Spectroscopy (FTIR) and X-ray powder diffraction (XRD).

**Results:** The FTIR and XRD spectrum shows their detoxification ablities and the chemical structure and composition of the soil samples. The earthworm species *Eudrilus eugeniae* has the ability to synthesize nanoparticles. They can be used to synthesize designer nanoparticles at controlled conditions. There are several uses of hese creatures that are yet to be discovered. Further studies can prove much more uses of these earthworm species *Eudrilus eugeniae*.

**Conclusions:** The earthworm species *Eudrilus eugeniae* is found to be efficient in cleaning the environment. If they are able to survive in presence of toxic heavy metals. They are found to be good bioaccumulators of toxic heavy metals without any decrease in their population. They are able to accumulate 30% of their body weight with the toxic chemicals.

**Keywords:** Earthworm, Atomic absorption Spectroscopy, Fourier Transform Infrared Spectroscopy, X-ray Diffraction

## 1. Introduction

Biological entities and inorganic materials have been in constant touch with each other ever since inception of life on the earth. Due to this regular interaction, life could sustain on this planet with a well-organized deposit of minerals. Recently scientists become more and more interested in the interaction between inorganic molecules and biological species. A variety of inorganic nanoparticles with well-defined chemical composition, size and morphology have been synthesized by using living organisms and their applications in many cutting-edge technological areas have been expored

One of the major environmental problems worldwide is the heavy metal contamination of soil. Soil is the vital part of eco-system which nurtures the life on earth. Any toxic substance released to the soil enters the food chain, ultimatelly reaching to humans causing several health issues. Heavy metals cannot be degraded and hence the best way is to recycle and reuse. Vermitechnology has been proved to be a promising tool for bioremediation of heavy metal contaminants. Earthworms are found to the best detoxifiers and bioaccumulators. (Bhattacharya and Mukherjee, 2008) Earthworm's detoxification pathway could be manupuated for the extraction and synthesis of metal nanoparticles from heavy metal contaminated sites (Brunello Ceccanti, Grazia Masciandaro, Carlos Garcia, Cristina Macci, Serena Doni, 2006) [6]. Charles darwin called them friends of farmers and unheralded soldiers of mankind working day and night under the soil.

Heavy metal contamination of soil is one of the major environmental problems and results mainly from mining, smelting procedures and automobiles as well as natural activities. Chemical and metallurgical industries are the most important sources of heavy metal contamination in the environment (Brumelis *et al.* 1999) [5]. The main threats to human health from heavy metals are associated with exposure to lead, cadmium, mercury, and arsenic (Alvarez, Evans, Milham, Wilson, 2004) [1]. These metals have been extensively studied and their effects on human health regularly reviewed by international bodies such as the WHO.

Heavy metals have been used by humans for thousands of years. Although several adverse health effects of heavy metals have been known for long time, exposure to heavy metals continues and is even increasing in some parts of the world, in particular in less developed countries, though emissions have declined in most developed countries over the last 100 years. (Andrade, Mahler, 2002) Cadmium compounds are currently used in re-chargeable nickel-cadmium batteries. Cadmium emisions have increased dramatically during the $20^{th}$ century, cadmium-containing products are rarely re-cycled, but often dumped together with household waste (Baker, 1981) [2]. The adverse health effects of cadmium exposure may occur at lower exposure levels than previously anticipated, primarily exposed to mercury *via* food, fish being a major source of methyl mercury exposure and dental amalgam.

## 2. Materials and Methods

### Collection of lead contaminated soil

The heavy metal contaminated soil sample which is high lead concentration was collected from the highly polluted region of poosaripalayam lake, Coimbatore, Tamil Nadu. Surface soil sample (0-15cm) was collected from the region near the dyeing textile industries (De Vries, Romkens, Schutze, 2007). The sampling site was bear with no plantation and were all the effluent streams and solid wastes were accumulating.

**Selection of Earthworm species**
The earthworm species *Eudrilus eugeniae* was collected from the Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu.

**Treating of contaminated soil using earthworm**
Three experimental groups were maintained. 1 Kg of heavy metal contaminated soil was inoculated with earthworms of around 60-100 Nos. in an earthern pot ($S_1$) placed under shade with frequent moisturing of soil. Contaminated soil without inoculation of earthworms was maintained at same conditions for the control group (C). The vermi-treatment was done for 60 days.

**Sample preparation**
The soil sample was air-dried and slightly crushed after separating the earthworms. The earthworms were also washed with distilled water, dried and crushed into fine powder. Both soil sample and crushed earthworm's tissue were sieved through a 40µm wired mesh.
Sample groups are as follows:

- C – Control for heavy metal contaminated soil collected from Poosaripalayam lake region, Coimbatore
- $S_1$ – Soil sample obtained after the vermi-treatment of heavy metal contaminated soil sample
- $W_1$ – Tissue sample of *Eudrilus eugeniae* after treatment of heavy metal contaminated soil

**Extraction**
The fine particles obtained were immersed by 0.12M EDTA for 10min and then shaken for 1 hour for dispersion. After dispersed, the sample was wet-sieved to 0.011mm and mass of <0.011mm were separated through centrifugation at 12,000rpm for 20 minutes. The EDTA solution was discarded and the particles were resuspended in distilled water, vortexed well and centrifuged at 12,000 rpm for 20 minutes (De Vries, Romkens, Schutze, 2007). The washing process was repeated twice and the particles were subjected to various characterization steps.

**Atomic absorption spectroscopy (AAS)**
Atomic absorption spectoscopy (AAS) is a spectro-analytical procedure for the quantitative determination of chemical elements using the absorption of optical radiation (light) by free atoms in the gaseous state. In analytical chemistry the technique is used for determining the concentration of a particular element (the analyte) in a sample to be analyzed. AAS can be used to determine over 70 different elements in solution or directly in solid samples.
EDTA extacted solution of the sample filtered through Whatman No: 1 filter paper of particle size <11µm was analyzed for heavy metal element lead (Pb) using AAS.

**Fourier Transform Infrared Spectroscopy (FTIR)**
Infrared, or IR, spectroscopy is a type of vibrational technique where molecular vibrations are analyzed. It is used to obtain an infrared spectrum of absorption, emission photoconductivity or Raman scattering of a solid, liquid or gas. An FTIR spectrometer simultaneosly collects spectral data in a wide spectral data in a wide spectral range. This confers a significant advantage over a dispersive spectrometer which measures intensity over a narrow range of wavelengths at a time. Fourier tranform, a mathematical proces is used to convert the raw data into the actual spectrum.
In this study, FTIR data were collected for the fine powdered sample with particle size less than 11µm at a range of 500-4000$cm^{-1}$.

**X-ray powder diffraction (XRD)**
X-ray powder diffraction (XRD), is an instrumental technique that is used to identify minerals, as well as other crystalline material. XRD provides with a fast and reliable tool for routine mineral identifiaction. The samples were powdered and sieved through a No. 400 (40 µm) sieve. The x-ray source was a cu anode operating at 40 kv and 30 mA using cuKα radiation with a diffracted beam graphic-monochromator. Data were usually collected between 10 and 80 in 2 theta with a scanning time for 60 seconds. The data was coolected using 1,000 (deg) divergence slit and a 0.3000 mm receiving slit. The data were corrected for intensity aberrations with a diffractometer calibration curve for the Bragg-Brentano goniometric dimensions.

## 3. Results
**Elemental Analysis**
The elemental analysis was performed using Atomic absorption spectroscopy. The results of AAS elemental analysis is shown in table 1.

**Table 1:** Concentration of heavy metals in the sample

| Elements (mg/l) | c | $S_1$ | $W_1$ |
|---|---|---|---|
| **Lead** | 0.205 | 0.013 | 0.103 |

Table 1 shows the concentration of heavy metal element present in each test sample groups. The contaminated soil from poosaripalayam dyeing industrial area had a high concentration of heavy metals, which was far higher than the permissible limits of indian standards. After the vermi-treatment of 60 days, the levels of heavy metal have lowered considerably. The worm's tissue sample shows a very concentration of these heavy metal that have been accumulated from the soil. The table clearly shows the efficiency of *Eudrilus eugeniae* in bioaccumulation of heavy metals. There wasn't any decrease in the population of *Eudrilus eugeniae* grown in the contaminated soil and hence it is evident that they survive in adverse conditions.

**Fourier Transform Infrared Spectroscopy**

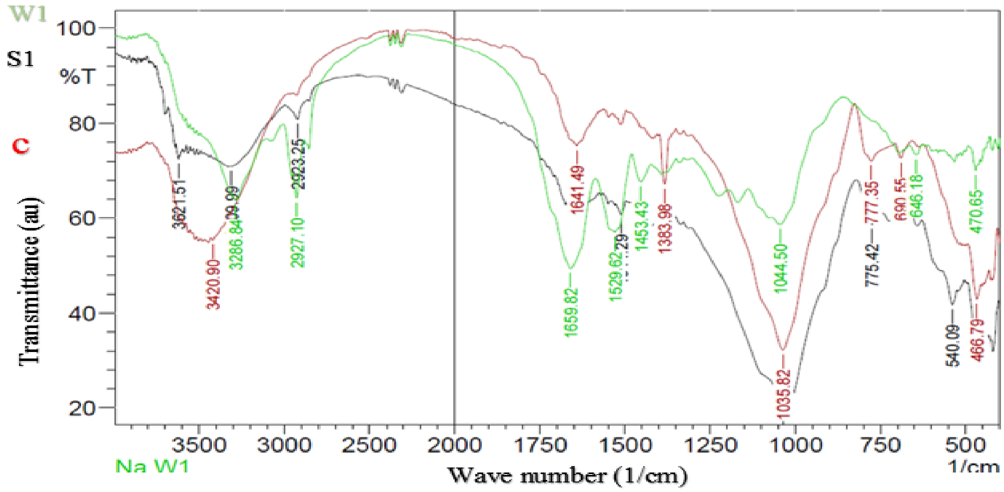


**Fig 1:** FTIR spectrum of control and vermi-treated soil

Figure 2 represents the FTIR spectrum of the three samples, namely, C, S, W, The bands at 3800-3600cm$^{-1}$ (kaolin) represents SiO-H stretches and the Si-O-Si stretch at 1030cm$^{-1}$(Smidt Meissl, 2007) in the control slightly shifted in the spectra of the treated samples. However, the vermi-treatment may have enhanced the dissolution of silicates, as these peaks decreased for the contaminated sample. However, the peaks do not overlap, revealing the persistence of these edges, even if they are less concentrated (Carballo, Gil, Gomez, Gonzalez-Andres, Moran, 2008) [7]. The Si-O-M bonds shows band in the range 550-800cm$^{-1}$. The band at 544.54 cm$^{-1}$ in the control and 552.32 in vermi-tissue represents the metal-oxygen vibrations, probably due to the presence of heavy metal complexes (Du *et al.* 2007). Whereas in the vermi-treated sample the band shifted to 528.98cm$^{-1}$ showing changes in metal-oxygen vibrations. Pb forms strong complexes with soil organic matter and can compete with most other metals for sorption sites (Strawn and Sparks, 2000). The strong peaks at 2358.94 cm$^{-1}$ in treated soil represents $CO_2$ streches that are characteristic of soil matter which is completely absent in vermi-tissue sample. There are two bands in vermi-tissue sample at 2923 cm$^{-1}$, attributed to the C-H stretch of aliphatic structures, and a band at 1649 cm$^{-1}$ refers to C=C (Carballo *et al.* 2008) that are absent in vermi-treated soil. In this particular case, the bands assigned at 1649 cm$^{-1}$ and 1396 cm$^{-1}$ may also be related to the interactions with the free water and the organic matter fractions (Haberhauer, Gerzabek, 1999).

**X-ray powder diffraction (XRD)**

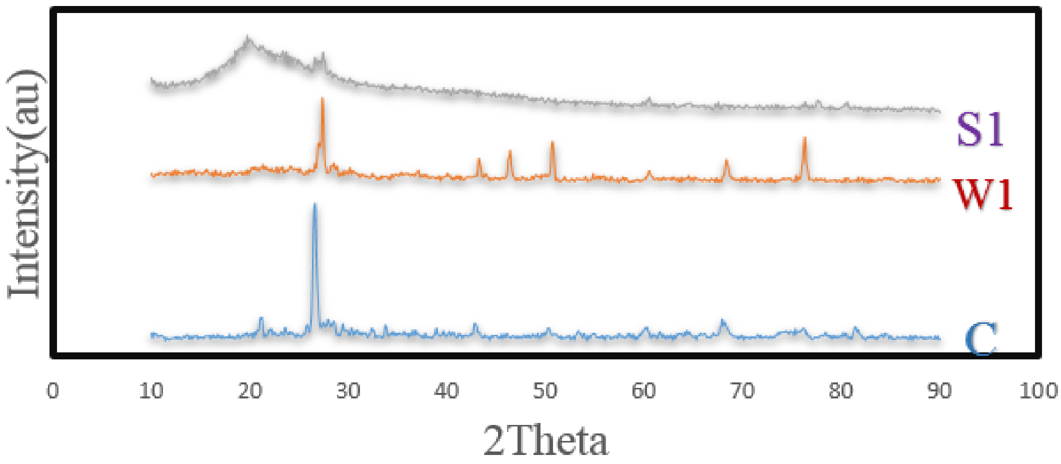


**Fig 2:** XRD spectrum of control and vermi-treated soil

The XRD spectrum clearly shows the ability of *Eudrilus eugeniae* in detoxification and accumulation. Mostly the metallic Pb and minerals of Pb compounds are present in the contaminated soil. The peaks formed in the control shows the presence of toxic heavy metal compounds of lead. The intense peaks corresponding to the 2-theta "Between" 20-30 are the minerals of lead. The predominant Pb species were metallic Pb, cerussite ($PbCO_3$), hydrocerussite ($2PbCO_3$-Pb $(OH)_2$), and litharge (PBO). Quartz ($SiO_2$) is represented by peak 'Q' which is present abundantly in soil. In the sample $W_1$, some of the peaks are shifted and others have disappeared. This is believed to be the change in chemical structure due to the detoxification action of *Eudrilus eugeniae*. The sample S shows the absence of strong peaks "Between" 20-30, showing the removal of toxic chemicals. The small fine peaks represent the soil minerals.

**4. Discussion**

The contaminated soil from Poosaripalayam textile industrial area had a high concentration of heavy metals, which far higher than the permissible limits of Indian standards, after the vermi-treatment of 60 days, the levels of heavy metals have lowered considerably. The worm's tissue sample shows a very high concentration of these heavy metals that have been accumulated from the soil. The table clearly shows the efficiency of *Eudrilus eugeniae* in bioaccumulation of heavy metals. There wasn't any decrease in the population of *Eudrilus eugeniae* in the contaminated soil and hence it is evident that they can survive in adverse conditions.

The XRD spectrum clearly shows the detoxification ability of *Eudrilus eugeniae*. The peaks formed in the control shows the presence of toxic heavy metal compounds of lead and nickel. The intense peaks corresponding to the 2theta values "Between" 20-30 are the minerals of lead (Crussite). Where in the sample W, some of the peaks are shifted and others have disappeared. This is believed to be due to the detoxification action of *Eudrilus eugeniae.* The sample S shows the absence of strong peaks "Between" 20-30, showing the removal of toxic chemicals. The small fine peaks represent the soil minerals.

The FTIR spectrum clearly shows the shifts in the peaks that is characteristic of the chemical changes happening during the detoxification process by the earthworms. Absence of an intense crust shows the removal of a specific functional group. The detoxification process is believed to be due to the combined action of enzymes and bacterial populations in earthworm's gut. The study shows the formation of heavy metal nano-particles from heavy metal contaminated soil through vermi-treatment using *Eudrilus eugeniae* which can be a very promising technique for cleaning the environment and utilizing the non-degradable heavy metal waste. Futher studies are required for a detailed understanding of the mechanism, so that more precise and structured nano-particle synthesis can be made possible.

**5. Conclusion**

The earthworm species *Eudrilus eugeniae* is found to be efficient in cleaning the environment. If they are able to survive in presence of toxic heavy metals.They are found to be good bioaccumulators of toxic heavy metals without any decrease in their population. They are able to accumulate 30% of their body weight with the toxic chemicals. They are specifically found to be tolerant to Pb as higher levels of Pb was dectected in their body. They can convert these heavy metals into non-bioavailable.

The FTIR and XRD spectrum shows their detoxification ablities and the chemical structure and composition of the soil samples. Several metal compounds and toxic functional groups previously present was removed by the worm action. These are believed to be due to the presence of seversl enzymes present in earthworm's gut. These enzymes can remove the readily reactive sites of toxiccompounds.

The earthworm species *Eudrilus eugeniae* has the ability to synthesize nanoparticles. Several enzymes and bacteria present in worm's gut contibute towards the nanoparticles synthesis. They can be used to synthesize designer nanoparticles at controlled conditions. They are low-cost, ecofridendly creatures which require less attention. There are several uses of hese creatures that are yet to be discovered. Further studies can prove much more uses of these earthworm species *Eudrilus eugeniae*.

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