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Original Article

Bioremediation of lead and nickel contaminated soil by *Eudrilus eugeniae* and the characterization of synthesized nanoparticles

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Abstract

Bionanotechnology has emerged for developing a green and ecofriendly technology for recycling the non-destructive waste materials and cleaning the environment .the present study focuses on the bioremediation of heavy metal contaminated soil and safe disposal of heavy metals and minerals with the help of earthworms , namely *Eudrilus eugeniae* as an ecofriendly technique. The characterization studies were conducted on these heavy metal nanoparticles in soil by Dynamic Light Scattering to determine the particle size; Atomic Absorption Spectroscopy to analyze the free elements present and X-ray Diffraction study to determine the toxic chemical compounds and minerals present in the soil. Bioremediation of lead and nickel contaminated study using *Eudrilus eugeniae* was found very efficient. The study shows that the lead and nickel levels have reduced to the safety standard levels. *Eudrilus eugeniae* were able to accumulate lead and nickel contaminants in their tissue and reduce them to nano-sized particles.

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 explored.[1]

One of the major environmental problems worldwide is the heavy metal contamination of soil. Soil is the vital part of ecosystem which nurtures the life on earth. Any toxic substance released to the soil enters the food chain, ultimately 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. Earthworm's detoxification pathway could be manipulated for the extraction and synthesis of metal nanoparticles from heavy metal contaminated sites. Charles Darwin called them "friends of farmers and unheralded soldiers of mankind working day and night under the soil" [2].

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. The main threats to human health from heavy metals are associated with exposure to lead, cadmium, mercury, and arsenic .These metals have been extensively studied and their effects on human health regularly reviewed by international bodies such as the WHO [4].

The general population is exposed to lead from air and food in roughly equal proportions. During the last century, lead emissions to ambient air have caused considerable pollution, mainly due to lead emissions from petrol. Children are particularly susceptible to lead exposure due to high gastrointestinal uptake and the permeable blood–brain barrier. Blood levels in children should be reduced below the levels so far considered acceptable, recent data indicating that there may be neurotoxic effects of lead at lower levels of exposure than previously anticipated. Although lead in petrol has dramatically decreased over the last decades, thereby reducing environmental exposure, phasing out any remaining uses of lead additives in motor fuels should be encouraged. The use of lead-based paints should be abandoned, and lead should not be used in food containers. In particular, the public should be aware of glazed food containers, which may leach lead into food. Exposure to arsenic is mainly via intake of food and drinking water, food being the most important source in most populations. Long-term exposure to arsenic in drinking-water is mainly related to increased risks of skin cancer, but also some other cancers, as well as other skin lesions such as hyperkeratosis and pigmentation changes. Occupational exposure to arsenic, primarily by inhalation, is causally associated with lung cancer. Clear exposure–response relationships and high risks have been observed.

Bioremediation is the enhancement of live soil organisms such as fungi, bacteria and plant to break down hydrocarbon and other organic contaminants. Bioremediation involves the transformation of complex or simple chemical compounds into non hazardous forms by biological agents. It is a relatively cheap and effective means of cleaning the environment and involves the application of organisms and nutrients such as inorganic or organic phosphate and nitrogen to the contaminated soil. The heavy metal content of soil is an important factor when bioremediation is considered for oil contamination cleanup. Due to their biological, chemical and physical actions, earthworms can be directly employed within bioremediation strategies to promote biodegradation of organic contaminants. Earthworms are 'unheralded soldiers of mankind' created by Mother Nature. Although the great visionary scientist Sir Charles Darwin indicated about them long back but very few biologists really realized that. Now it is being realized and revived all over the world and services of earthworms are being utilized with a technological approach. Earthworms can tolerate toxic chemicals in environment.

Several studies have found that earthworms effectively bio-accumulate or biodegrade several organic and inorganic chemicals including 'heavy metals', 'organochlorine pesticide' and micropollutants like 'polycyclic aromatic hydrocarbons' (PAHs) residues in the medium in which it inhabit. *Eudrilus eugeniae* is an earthworm species indigenous to Africa but extensively bred in the USA, Canada, Europe and Asia for the fish bait market, where it is commonly called the African night crawler. [4]

Nanoparticles, particles having one or more dimensions of the order of 100nm or less, have attracted great attention due to their unusual and fascinating properties, and applications advantageous over their bulk counterparts. There are a large number of physical , chemical, biological and hybrid methods available to synthesize different types of nanoparticles Although physical and chemical methods are more popular in the synthesis of nanoparticles, the use of toxic chemicals greatly limits their biomedical applications, in particular in clinical fields. Therefore, development of reliable, nontoxic and eco-friendly methods for synthesis of nanoparticles is of utmost importance to expand their biomedical applications. One of the options to achieve this goal is to use earthworms to synthesize nanoparticles. [5]

2. Materials and methods

2.1 Collection of heavy metal contaminated soil

The heavy metal contaminated soil sample which is high lead concentration was collected from the highly polluted region of Ukadam Lake, Coimbatore, Tamil Nadu. Surface soil sample (0-15cm) was collected from the region near the dyeing textile industries [6]. The sampling sites were bear with no plantation and the place where the effluent streams and solid wastes were accumulated.

2.2 Selection of Earthworm species

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

2.3 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 (S1) placed under shade with frequent moisturizing 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.

2.4 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
- V – Soil sample obtained after the vermi-treatment of heavy metal contaminated soil sample
- W – Tissue sample of *Eudrilus eugeniae* after treatment of heavy metal contaminated soil.

2.5 Extraction

The powdered samples of C , S and W were divided into four equal sets : 2 sets of powdered samples of C , S and W with fine sieved particles were immersed in 0.12M EDTA in 100ml of distilled water for 10min and then shaken for 1 hour for dispersion. After dispersed, the sample undergone through centrifugation at 12,000 rpm for 20 minutes. The supernatant was separated out and was given for analysis. (AAS and DLS).

A set of powdered sample of C, S and W were also given for analysis (XRD)

2.6 Estimation analysis

2.6.1 Atomic absorption spectroscopy (AAS)

Atomic absorption spectroscopy (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 extracted solution was analyzed for heavy metal element using AAS.

3.6.2 Dynamic Light Scattering (DLS)

Dynamic light scattering is a technique in physics that can be used to determine the size distribution profile of small particles in suspension or polymers in solution. When light hits small particles, the light scatters in all directions (Rayleigh scattering) as long as the particles are small compared to the wavelength (below 250 nm). Even if the light source is a laser, and thus is monochromatic and coherent, the scattering intensity fluctuates over time. This fluctuation is due to small molecules in solutions are undergoing Brownian motion, and so the distance between the scatterers in the solution is constantly changing with time. This scattered light then undergoes either constructive or destructive interference by the surrounding particles, and within this intensity fluctuation, information is contained about the time scale of movement of the scatterers. The dynamic information of the particles is derived from an autocorrelation of the intensity trace recorded during the experiment.

3.6.3 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 identification. 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 collected using 1,000 (deg) divergence slit and a 0.3000 mm receiving slit. The data were corrected for intensity aberrations with a diffract meter calibration curve for the Bragg-Brentano goniometric dimension

Figure 1: Metal contaminated soil

Figure 2: *Eudrilus eugeniae*

Figure 3: Worms are washed with distilled water after vermi-treatment

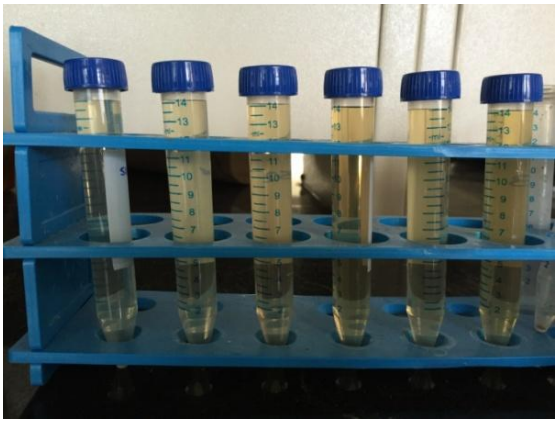


Figure 4: Samples dispersed in EDTA

3. Results

3.1 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 Nickel and Lead in the sample

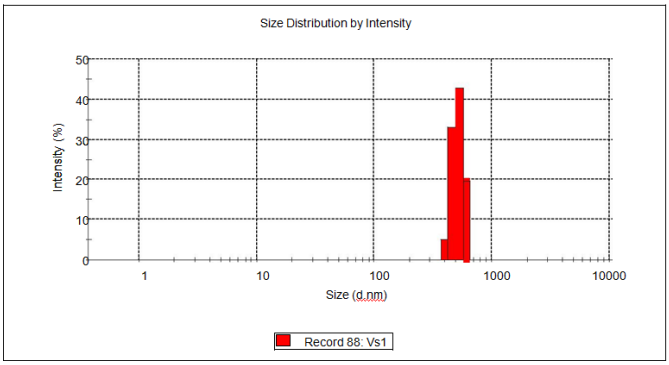
ELEMENTS (mg/g)	C	Vs	Ws
Nickel	0.502	0.263	0.261
Lead	0.82	0.41	0.33

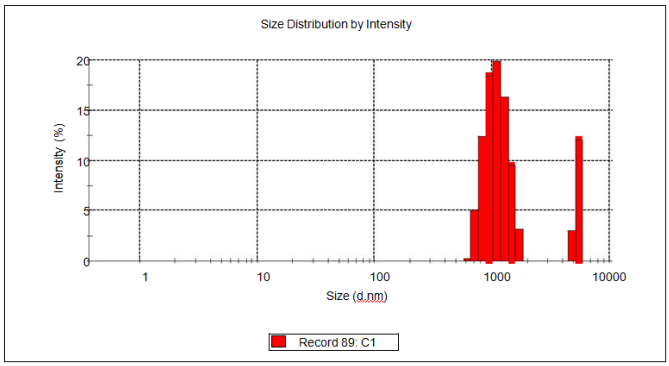
The elemental analysis was performed using Atomic absorption spectroscopy. The results of AAS elemental analysis is shown in table 1. Table 1 shows the concentration of heavy metal element present in each test sample groups. The contaminated soil from Ukadam lake area had a high concentration of heavy metals like lead and nickel which was far higher than the permissible limits of Indian standards. After the vermi-treatment of 60 days, the levels of nickel and lead have lowered considerably. The worm's tissue sample shows a very concentration of this heavy metal that has 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

3.2 Particle size analysis

Particle size distribution of the sample analysed by Dynamic Light Scattering (DLS) technique is as follows:

Figure 2: Particle size distribution of control and vermin-treated soil





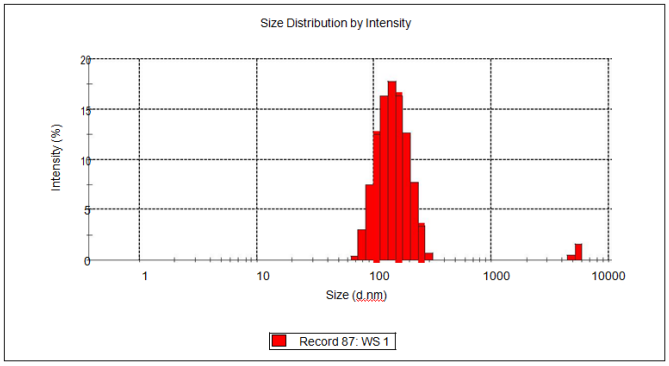
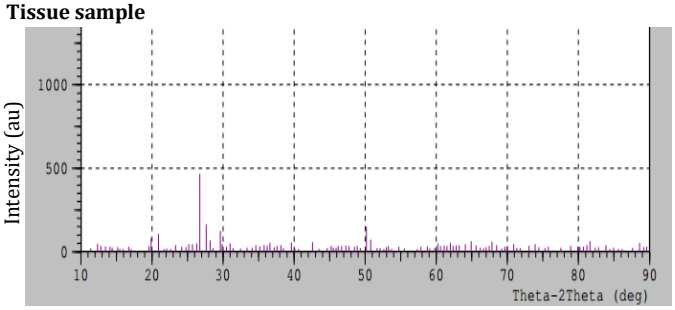


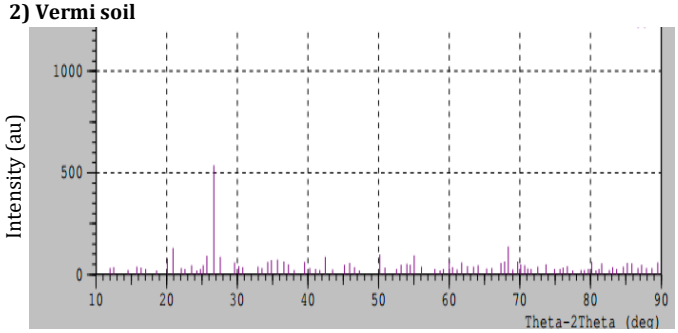
Figure 2 shows the particle size distribution in three test samples. Compared to Control(C) where the particle size is more than 1000nm, the size has reduced. In sample VS1 and WS1, the particle size range is below 1000nm. In worms' tissue sample the particle size is below 500nm.In vermin-treated soil sample the particle size is between 500-1000nm ranges. Hence the result shows the efficiency of *Eudrilus eugeniae* in reducing the particle size of metals.

3.3 X-ray diffraction (XRD).

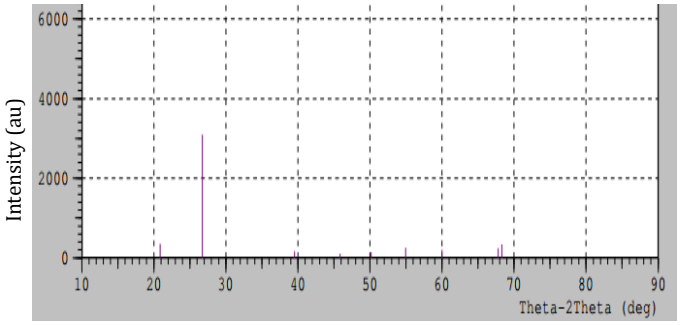
Tissue sample



2) Vermi soil



3) Metal soil



The XRD spectrum clearly shows the ability of *Eudrilus eugeniae* in detoxification and accumulation. Mostly the metallic Pb and Ni and minerals of Pb and Ni 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. In the sample W1, 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. [7]

4. Discussion

The contaminated soil from Ukkadam lake 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 peak 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 particle size distribution among the three samples C, S1 and W1 clearly indicated the earthworm's ability to synthesize nano-sized particles. Compared to the control(C) the particle size reduced considerably. Samples S1 and W1 ,the particle size range is below 1000nm.In worm tissue the particle size range is less than 500nm.the particle size has considerably reduced due to worm action. 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. Further 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 and Ni were detected in their body. They can convert these heavy metals into non-bioavailable.

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

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

References

[1]. Sinha, R.K., and Heart, S. Earthworms: The 'Unheralded Soldiers of Mankind' and 'Farmer's Friend' Working Day and Night Under the Soil: Reviving the Dreams of Sir Charles Darwin for Promoting Sustainable Agriculture. *American-Eurasian Journal of Agricultural and Environmental Sciences*, 2009; 5, pp. 1-55.

[2]. Brumelis G, Brown DH, Nikodemus O, Tjarve D." The monitoring and risk assessment of Zn deposition around a metal smelter in Latvia", *Environmental Monitoring and Assessment*, 1999; 58(2): 201-212.

[3]. Hirano, T., and Tamae, K. Earthworms and Soil Pollutants. *Sensors* (Basel), 2011; 11(12), pp. 11157-1167.

[4]. Adriano, D.C.(2001) "Trace elements in the terrestrial environment". Springer, New York

[5]. Liu J., Qiao S. Z., Hu Q. H., and Lu G. Q. "Magnetic nanocomposites with mesoporous structures: synthesis and applications," *Small*, 2011; 7(4): 425–443.

[6]. Davies, N.A., Hodson, M.E., and Black, S. "Changes in toxicity and bioavailability of lead in contaminated soils to the earthworm *Eisenia fetida* (Savigny 1826) after bone meal amendments to the soil". *Environmental Toxicology and Chemistry*, 2002; 21: 2685-2691.

[7]. Markman S., Guschina A. I., Barnsleya S., Buchanan L. K., Pascoe D. and Muller C. T. "Endocrine Disrupting Chemicals Accumulate in Earthworms Exposed to Sewage Effluents," Cardiff School of Biosciences, Cardiff University, Cardiff, *Journal of Chemosphere,* 2007; 70(1): 119-125.