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Earthworms and Microorganisms Interacting in the Detoxification of Metal Contaminants: A Study

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Abstract - Through direct intake or contact with contaminated sites, the food chain, drinking contaminated ground water, a reduction in food quality due to phyto-toxicity, and a lack of land suitable for agriculture, heavy metal contamination poses serious risks to humans and ecosystems. This leads to food insecurity. Therefore, every living cell must generally be resistant to heavy metals. We can now exploit the potential of biological diversity to clean up pollution, a process known as bioremediation, thanks to advancements in science and technology. The following study's main goal is to evaluate how endogenic earthworm Pontoscolexcorethrurus and its gut microbiota interact with heavy metal absorption from contaminated soil samples.The following study uses a variety of materials and techniques, including the collection of plant material, the isolation of bacteria from earthworm guts, the analysis of the concentration of heavy metals in soil samples using atomic absorption spectroscopy, as well as their characterization and amplifying metal resistant genes.The findings of the Gram staining test were used to categorize bacteria as Gram negative bacilli, Gram positive cocci, or Gram positive bacilli. Delftia and S. aureus bacteria were then cultured under cadmium stress to see if their growth patterns underwent any notable changes.The observed changes in the bacterial communities of the earthworms may therefore be used as a warning sign of potential soil pollution, it is hereby concluded. Bacillus cereus and Delftia sp. can be used to remediate soil that has been contaminated with various heavy metals because they can withstand higher concentrations of heavy metal.

Keywords - Pontoscolexcorethrurus, Phyto-toxicity, Heavy Metal Contamination, Atomic Absorption Spectroscopy

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INTRODUCTION

A significant environmental problem is soil pollution. Rapid industrialization has led to a significant increase in the amount of harmful substances in soil, such as heavy metals and organic pollutants. For a variety of toxicants that are released into the environment, soil serves as the main sink. Only a few of the countless factors that contribute to heavy metal contamination in soil include emissions and waste from various industries, fertilisers, coal combustion residues, sewage, pesticides, and mine tailings. Heavy metals are more susceptible to direct and accidental exposure due to their characteristics, such as their rapid generation rates from human-made activities and their inability to be kept in a single spot.The majority of metals have been shown to be durable and do not disintegrate as a result of microbial or chemical attack. In addition to reducing soil microbial activity and agricultural production, high levels of heavy metals also put human health at risk through the food chain (Mclaughlin et al, 1999).

The most common heavy metal contaminants found in soil are lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), copper (Cu), mercury (Hg), and nickel (Ni). Three of the most prevalent soil pollutants are cadmium (Cd), copper (Cu), and zinc (Zn). One of the top three heavy metal poisons is cadmium, which also serves no known biological function. It is known that cadmium affects a variety of bodily enzymes.It is believed that Cd has a deleterious effect on the enzymes necessary for protein reabsorption in kidney tubules, which results in renal damage that results in proteinuria. Through the direct destruction of bone or indirectly through renal failure, cd may also cause demineralization of the bone. Workplace exposure to airborne Cd in excess may harm lungs and increase the risk of lung cancer (Bernard,2008).

Copper is the third most prevalent metal in the world. Drinking water from copper pipes generally contains copper, as do chemicals used to stop the growth of algae.Airborne copper can come from suspended soil particles, combustion products, manufacturing or processing copper-containing goods, or mine tailings. The main sources of emission are agriculture, sludge from publicly owned treatment facilities, and solid waste from cities and industries. Although the body needs copper, too much of it can harm the liver, kidneys, stomach, and intestines in addition to causing anaemia. Long-term copper exposure can result in headaches, nausea, dizziness, vomiting, and diarrhoea in addition to nose, mouth, and eye irritation. Wilson's disease, which is characterised by hepatic cirrhosis, brain damage, renal failure, and copper buildup in the cornea, is brought on by persistent copper poisoning (Wuana and Okieimen, 2011).

Another necessary trace element is zinc. Zinc is relatively harmless in compared to other heavy metal ions. It serves as a component of biological enzymes and is essential for the creation of complexes like zinc fingers in DNA. Zinc exposure over an extended period of time prevents the body from absorbing copper. Zinc participates in cytotoxic processes and causes cell death in the brain. Free zinc ions in solution are particularly toxic to plants, invertebrates, and even fish.

Through direct intake or contact with contaminated sites, the food chain, drinking contaminated ground water, a reduction in food quality due to phyto-toxicity, and a lack of land suitable for agriculture, heavy metal contamination poses serious risks to humans and ecosystems. This leads to food insecurity. Therefore, every living cell must generally be resistant to heavy metals. We can now exploit the potential of biological diversity to clean up pollution, a process known as bioremediation, thanks to advancements in science and technology.

Earthworms are significant members of the terrestrial food chain. They are referred to be the "intestines of the earth" because they increase microbial activity, improve soil fertility, and decompose organic materials. Among other environmental toxins, pesticides, heavy metals, DDT, and PCBs can all have detrimental effects on earthworms. Earthworms have been used as biological indicators of environmental pollution. They are able to bioaccumulate a variety of chemical pollutants, including heavy metals and organic pollutants, in their tissues.Earthworms can absorb heavy metals by storing them in waste nodules generated within the body cavity, immobilising them in gut wall cells, or excreting them through calciferous glands. It has been shown that some populations of earthworms can endure heavy metal concentrations that are far higher than the levels known to have lethal effects (Stürzenbaumet et al., 1999).

The enormous volume of soil that earthworms consume spreads bacteria. Earthworms and microorganisms engage in a variety of complex interactions. Earthworms have a vast number of microorganisms in their stomachs. Degradative enzymes that break down organic molecules and fix nitrogen are produced by microbes for them (Kumar et al, 2010). It is not yet known, however, whether the bacteria that earthworms host contribute in any significant way to the detoxification process.

Soil Pollution by Heavy Metals and Bioremediation

One of the biggest risks to the environment and to people's health is heavy metal contamination of soil. Ba, Cu, Cr, Co, Ni, and Zn are heavy metals that are frequently found in soil (Krishna and Govil, 2007). Pesticides, fertilisers, organic and inorganic amendments, mining, rubbish, and sludge residues are just a few of the methods by which heavy metals can reach the soil (Capri and Trevisan, 2002). As geology and human activity have expanded, so have the types and concentrations of heavy metals in soil, causing significant environmental deterioration (Su et al., 2014). Heavy metals can linger in soil for a long time since they do not dissolve or disappear from it.

The majority of metals are needed in soil in trace levels for processes like plant uptake. Growing environmental heavy metal emissions pose a major threat to all living things. Heavy metal-polluted soils cause plants to perform, produce, and grow more slowly (Chibuike and Obiora, 2014). By interfering with the activities of soil fauna, heavy metal pollution can change the qualitative and quantitative functioning of soil ecosystems. It can also result in heavy metal transmission to soil fauna predators, which can contaminate the terrestrial food chain. By influencing abiotic parameters like temperature and humidity, metal contamination of soil directly affects the number of microorganisms that are present. A boost in the variety of thermophilic organisms is possible when pollution levels are reduced since it causes the environment to warm up (Grzés, 2009).Upon examination of the effects of Cd, Cu, Zn, and Pb on soil microorganisms and microbially mediated soil activities, the relative toxicity of the metals decreased in the following order: Cd > Cu > Zn >Pb (Baath, 1989). Both necessary and non-essential metals can damage cell membranes, alter the specificity of an enzyme, impair cellular function, and even damage DNA at large levels. They have been linked to kidney and liver damage, birth defects, cancer, skin sores, retardation leading to disabilities, and a number of other health problems (Järup, 2003).

New and cost-effective solutions must be created in order to purge contaminated environments, make them safe for human habitation and consumption, and protect the health of the ecosystems that sustain life. We can now use the potential of biological diversity for pollution cleanup, a process known as bioremediation, thanks to advancements in science and technology.

Bioremediation has an impact on the toxicity, solubility, and absorption properties of metals (Malik, 2004). In bioremediation, a way of removal from the solid material is offered by the solubilization of heavy metal contaminants. In some microbial species, heavy metal ions are reduced by enzymes; the reduced form of the heavy metal ions is very insoluble and precipitates out of solution. By using autotrophic and heterotrophic leaching, other bacteria can mobilise heavy metals, causing volatilization and the breakdown of insoluble metal complexes (Malik, 2004). Due to its high bioavailability and propensity for bioaccumulation in soil, heavy metal pollution has clear ecotoxicological effects. Therefore, it is thought crucial to comprehend the workings of these heavy metal trafficking pathways inside terrestrial invertebrates like the earthworm.

Effect of Heavy Metals on Earthworms

In contrast to many vertebrates, which are exposed indirectly through the food chain, soil invertebrates, such earthworms, have direct contact with soil pore water or food exposure (Kammenga et al., 2000). The earthworm's skin is a primary channel for pollution absorption since it is very water permeable (Jajer et al., 2003; Vijver et al., 2005). Due to their extensive soil consumption and ongoing exposure to poisons through their digestive system, earthworms (Morgan et al., 2004). Metals build up in the stomach and posterior alimentary canal of earthworms that reside in heavy metal-contaminated environments (Andre et al., 2009).

Earthworms are a crucial aspect of soil function and are essential in the chemical element conversion process. Insecticides, heavy metals, and lipophilic organic micropollutants including polycyclic aromatic hydrocarbons (PAH) and polychlorinated biphenyls (PCB) may all be removed from soil by earthworms (Contreras-Ramos et. al, 2006). Earthworms may bio-accumulate significant quantities of metals, especially heavy metals, in their tissues without harming their physiology, according to Hartenstein et al. (1981), even when the metals are virtually non-bioavailable. According to studies, heavy metals including cadmium (Cd), mercury (Hg), lead (Pb), copper (Cu), manganese (Mn), calcium (Ca), iron (Fe), and zinc (Fe) may be absorbed and bioaccumulated by earthworms (Zn).

They could take in and gather unusually large amounts of zinc (Zn) and cadmium (Cd). Earthworm delicate and non-calcareous tissues may readily absorb cadmium due to its high mobility. No mortality or weight loss was reported in the study of uranium's effects on earthworms, however cytotoxic and genetic effects were noticed at low natural uranium concentrations (Giovanetti et al., 2010). Earthworms exposed to cadmium have been demonstrated to undergo physiological alterations, such as bioaccumulation (Stürzenbaum et al., 2004), a little weight loss (Burgos et al., 2005), and the development of oxidative DNA damage (Nakashima et al,2008). It was shown that heavy metals had a stronger effect on Eiseniafetida life cycle traits including cocoon production and hatching rate than survival or weight change (Nahmani et al.2007).

Earthworm Interaction with Microbes

In addition to a throat, oesophagus, and gizzard, earthworms also contain an anterior intestine that secretes enzymes and a posterior intestine that absorbs nutrients. During digestion, the number of bacteria can rise up to 1000-fold. Furlong et al. (2002) found that a number of soil microorganisms, including Firmicutes and Pseudomonas species, reproduce as they pass through the digestive system of the L. rubellus. It has been suggested that microorganisms provide food for earthworms. In order to transform organic materials into water-soluble aggregates, earthworms and microbes collaborate.

Studies show that earthworms contain substantially more bacteria and actinomycetes overall than the surrounding soil. However, additional information suggests that the population increased from the front to the back of the earthworm gut (Wan Wong, 2004;). For their nutritional needs, earthworms rely significantly on microorganisms; by breaking down and contaminating decaying organic waste, they promote microbial activity. The organisation of the soil bacterial communities is significantly influenced by earthworms. Earthworms were shown to reduce the amount of Pseudomonas sp. that was injected, and consequently the genetic potential for Atrazine degradation in normal soil (Kersante et al., 2006).This investigation aims to examine the relationship between the gut microbiota of the endogeic earthworm *Pontoscolexcorethrurus* and the absorption of heavy metals from contaminated soil samples. Due to the fact that they live and feed in the mineral soil layers, endogeic earthworms were chosen for the study. As a result of their regular contact with contaminated soil, these earthworms are adversely impacted by the presence of metal pollutants in the soil. This study will contribute to the description of the bacteria in earthworm stomachs involved in the absorption and detoxification of heavy metal pollutants. The effectiveness of soil bioremediation might potentially be increased by the use of this information in field studies.

METHODOLOGY

- **Collection and management of *Pontoscolexcorethrurus* earthworms under semi-natural soil conditions:** During the monsoon season, earthworms (*Pontoscolexcorethrurus*) were hand-sorted from garden soil. Each box was filled with one kilogramme of dirt and six earthworms will be introduced. The weight of the earthworms will be recorded before they are placed in the boxes. A thin coating of humus was applied to the soil. For proper aeration, holes will be punched in the boxes.
- **Heavy metal exposure of *Pontoscolexcorethrurus* earthworms to**

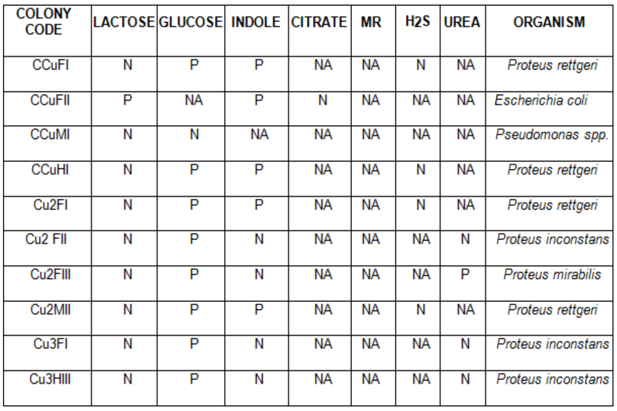
Cadmium, Copper, and Zinc: Six uniformly sized earthworms will be weighed and exposed to escalating quantities of cadmium, copper, and zinc. Metal salts will be used to introduce the metals. The salts will be weighed and pulverised before being mixed well into the soil before the earthworms are inserted.

- **Isolation of bacteria from the guts of earthworms exposed to heavy metal treatment:** Earthworms that survive the heavy metal treatment for 15 days will be isolated to study the gastrointestinal microflora.
- **Identification of bacteria isolated from the guts of heavy metal-treated *Pontoscolexcorethrurus* earthworms:** Bacterial colonies will be classified based on physical criteria such as size, colour, border and shape, surface, elevation, optical nature, and texture. Using the 16Sr RNA sequencing approach, the numerous microorganisms that were preferentially accumulated in the gut of metals (Cd, Cu, and Zn) treated earthworms will be discovered.
- **Analysis of heavy metal concentrations in soil samples using Atomic Absorption Spectroscopy (AAS):** Atomic Absorption Spectroscopic tests on soil samples utilised for development of *Pontoscolexcorethrurus* earthworms were used to examine their potential to bio-accumulate heavy metals in the soil. This research allowed us to determine the amount of metals in the soil before and after the growth of *Pontoscolexcorethrurus* earthworms.
- **Characterization of bacteria preferentially accumulated in the stomach of *Pontoscolexcorethrurus* earthworms:** The bacteria reported to be preferentially aggregated in the guts of earthworms treated to cadmium and copper treatments was studied further. The isolated bacterial cultures' multi metal tolerance will be studied using an Agar Well Diffusion test. The antibiotic sensitivity of the isolated bacterial species will be determined using the agar well diffusion technique. The resistance of soil microorganisms to herbicides was investigated using the agar well diffusion technique.
- **Amplification of metal-resistant genes seen in the chromosomes of bacteria isolated from metal-treated *Pontoscolexcorethrurus* earthworms:** The genes for heavy metal resistance (cadmium, zinc, and copper) were located in these microorganisms using the NCBI website. A BLAST search was run to see if these genes are present in the bacterium. The correct primers and conditions for performing Polymerase chain reaction (PCR) to detect the presence of metal resistance genes in these bacteria was also be determined. Primer3's primer design tool was used to select primers for the genes to be amplified by PCR.

RESULTS

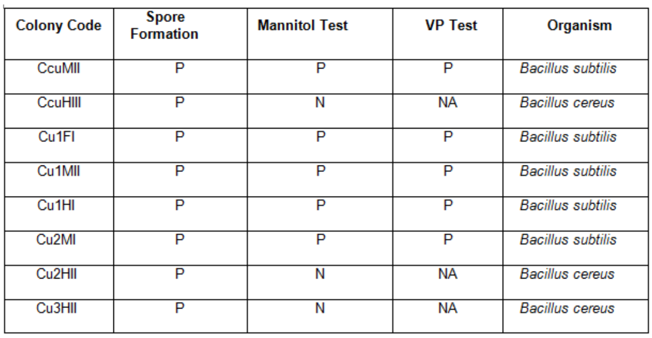
- The bacteria were classified as Gram negative bacilli, Gram positive cocci, or Gram positive bacilli based on the findings of the Gram staining test. Based on the nature of the bacteria's Gram features, several biochemical tests were performed on them. Tables 1,2, and 3 present the findings of the biochemical characterization of the bacterial colonies.
- Using the 16Sr RNA sequencing approach, the bacteria that had gathered in the stomach of *Pontoscolexcorethrurus* earthworms had been discovered.
- *Delftia sp. and Staphylococcus aureus* were the microbes that were recovered from the cadmium-treated earthworms' guts. By observing the growth patterns of these isolated microorganisms in media supplemented with various amounts of cadmium salt, the tolerance of these microorganisms to cadmium was examined in vitro (Table 4).

Table 1: Biochemical tests performed on Gram negative bacilli isolated from gut homogenate of earthworms under Cu stress



COLONY CODE	LACTOSE	GLUCOSE	INDOLE	CITRATE	MR	H2S	UREA	ORGANISM
CCuFI	N	P	P	NA	NA	N	NA	*Proteus rettgeri*
CCuFII	P	NA	P	N	NA	NA	NA	*Escherichia coli*
CCuMI	N	N	NA	NA	NA	NA	NA	*Pseudomonas spp.*
CCuHI	N	P	P	NA	NA	N	NA	*Proteus rettgeri*
Cu2FI	N	P	P	NA	NA	N	NA	*Proteus rettgeri*
Cu2 FII	N	P	N	NA	NA	NA	N	*Proteus inconstans*
Cu2FIII	N	P	N	NA	NA	NA	P	*Proteus mirabilis*
Cu2MII	N	P	P	NA	NA	N	NA	*Proteus rettgeri*
Cu3FI	N	P	N	NA	NA	NA	N	*Proteus inconstans*
Cu3HIII	N	P	N	NA	NA	NA	N	*Proteus inconstans*

Table 2: Biochemical tests performed on Gram positive bacilli isolated from gut homogenate of earthworms under Cu stress



Colony Code	Spore Formation	Mannitol Test	VP Test	Organism
CcuMII	P	P	P	*Bacillus subtilis*
CcuHIII	P	N	NA	*Bacillus cereus*
Cu1FI	P	P	P	*Bacillus subtilis*
Cu1MII	P	P	P	*Bacillus subtilis*
Cu1HI	P	P	P	*Bacillus subtilis*
Cu2MI	P	P	P	*Bacillus subtilis*
Cu2HII	P	N	NA	*Bacillus cereus*
Cu3HII	P	N	NA	*Bacillus cereus*

Table 3: Biochemical tests performed on Gram positive cocci isolated from gut homogenate of earthworms under Cu stress

COLONY CODE	CATALASE TEST	ORGANISM
Cu1FII	N	*Streptococcus* spp.
Cu2HI	N	*Streptococcus* spp.
CcuHII	P	*Staphylococcus*
Cu1MI	P	*Staphylococcus*
Cu1HII	P	*Staphylococcus*
Cu2MIII	P	*Staphylococcus*
Cu3FI	P	*Staphylococcus*
Cu3FII	P	*Staphylococcus*
Cu3HI	P	*Staphylococcus*

Table 4: Concentrations of the Cadmium salt used to study the tolerance of selected bacteria using growth patterns

Flask ID	Conc. of Cadmium salt (mg/L)
Blank	NA
Control	0
A	0.5
B	1.0
C	2.0
D	4.0
E	8.0

- *Delftia and S. aureus* bacteria were cultured under cadmium stress, and any notable alterations in their development patterns were looked for (Figure1).

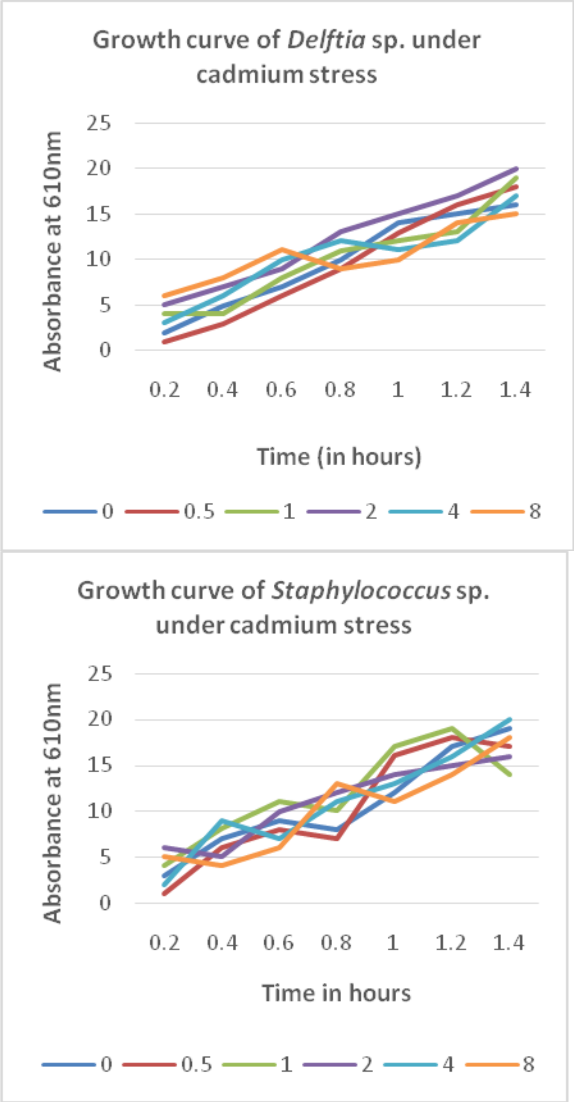


Figure 1: Growth patterns of *Delftia sp. and Staphylococcus aureus* in the presence of cadmium salts

CONCLUSION

The main objective of the subsequent study was to assess the relationship between heavy metal absorption from contaminated soil samples and the gut microbiota of the endogenic earthworm Pontoscolex*corethrurus*. The study that follows makes use of a range of resources and methods, including the collection of plant material, the isolation of bacteria from earthworm guts, the analysis of the amount of heavy metals in soil samples using atomic absorption spectroscopy, as well as their characterization and amplifying metal resistant genes. Bacteria were classified as Gram negative bacilli, Gram positive cocci, or Gram positive bacilli based on the results of the Gram staining test. Then, bacteria from *Delftia and S. aureus* were cultivated under cadmium stress to observe if their growth patterns changed noticeably.The study came to the conclusion that the earthworms' bacterial communities had undergone changes, which could be used as a sign of potential soil pollution. Because they can withstand higher concentrations of heavy metal, *Bacillus cereus and Delftia sp*. can be used to remediate soil that has been contaminated with different heavy metals.

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