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# A Study on Earthworm and Microbe in the Detoxification of Metal Pollutants

**Pawar Balasaheb Ramrao[1]*, Dr. Munshi Lal Patel[2]**

[1] PhD Student, Kalinga University Raipur

[2] Assistant Professor, PhD Guide, Kalinga University Raipur

***Abstract - Pollution of the soil is a serious issue for the planet. Soil pollution from organic contaminants and heavy metals, among others, has increased dramatically as industrialisation has progressed rapidly. A variety of pollutants released into the environment are absorbed by the soil. Sources of heavy metals in soil range from industrial emissions and wastes to fertilizers and coal combustion residues to sewage and pesticides to mine tailings. Heavy metals have a greater possibility for direct and accidental exposure due to traits such as fast production rates through human activities and their inability to be kept in a localized location. In this paper researcher study the earthworm and microbe in the detoxification of metal pollutants.***

***Keywords - Pollution, heavy metals, microbe, earthworm.***

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## INTRODUCTION

Earthworms' eating, casting, and burrowing considerably improve the soil's physical, chemical, and biological qualities. The fundamental physi- cal characteristics of soil are affected differently by earthworms of the epigeic, endogeic, and anecic lifestyles. Epigeic plants like Lumbricusrubellus, Eisenia fetida, or Dendrodrilusrubidus thrive in the humus zone of soil. They derive most of their sustenance from the organic stuff that grows on top of the mineral soil, although they will ingest the occasional particle of soil. Epigeic species are those that live in the soil, rather than in the air. They dig their burrows into the organic material layer, or between 0 and 2.5 centimeters deep into mineral soil, where they feast mostly on elements rich in bacteria. [1]

Epigeics tend to be little, reddish-brown worms that are shorter than 7.5 cm. The largest and longest Anecic species, which are all reddish brown in color and range in length from 12.5 to 20.0 cm, are Lumbricusterrestris, Aporrectodea longa, and Dendrobaenaplatyura. They live in burrows, consume decaying plant matter from the soil's surface, then defecate and urinate via an entrance. The top 50 cm of soil is ideal for the Endogeic Species, which live off the organic stuff there. Differentiating them from epigeic and anecic species are their intensely pink heads and gray bodies. Adults of endogeic species could be anywhere from 3 centimeters to 12.5 centimeters in length.[2]

In general, earthworms may digest 60% of their body mass in soil or organic matter, and they excrete their feces in the form of pellets known as earthworm castings, which they deposit in the soil near their burrows. The majority of worm poop falls into four distinct categories. The first is spherical and is often made by larger earthworm species like anecics and endogeics. Endogeics and anecics may also create the second kind, however this one lacks structure. Finally, these organisms also drop round pellets on the ground. Smaller species of earthworms, such as epigeics, tiny endogeics, and diverse anecics, create the fourth kind, which looks granular or pellet-like. When it comes to the impact of their excrement on soil structure, earthworm species may be rather picky. [3-4]

Granular castings are on the other side of the spectrum from the preceding three categories, which are all bigger, denser, and more compact. There are some chemical and biological similarities between earthworm faeces and the organic matter that these worms eat. Both the earthworm's digestive microbes and the worm's own mechanical grinding mechanism help to create this quality. When compared to the organic material that earthworms consume, the cast of earthworms has a higher concentration of nutrients, a lower C:N ratio, more stable microbial characteristics, and higher extracellular enzyme activity. Earthworm castings are thus commonly utilized as a natural fertilizer.[5]

Earthworms largely alter soil properties via their eating, casting, and burrowing habits. As a result of the mineral soil OR organic material they consume, earthworms may alter the features of the soil in their castings, which they collect close to the soil's surface. Due to the high levels of carbon, nitrogen, & water, the organic components in worm castings are rapidly mineralized. Soil aggregates build more steadily as a consequence. Stronger contact between soil particles is promoted by the presence of fungal hyphae as well as other microbial compounds in faeces, which is a major contributor to the creation of stable soil aggregates. Earthworms' gallery-building activities, which increase macropores in soil, are species- and environment-specific. These galleries may be as little as 1 mm or as large as 10 mm in diameter, depending on the earthworm's ecological classification. [6]

Species that are epigeic tend to be tiny and occupy the top few centimeters of soil. They tunnel vertically or horizontally for a short distance through the first few centimeters of soil. Endogeics are organisms that tunnel underground constantly, creating a system of interconnected passages. These animals have a habit of defecating within their galleries, which might prevent water from reaching the ground below. Vertical galleries dug by anecic earthworms may extend as far as 2 meters underground. These galleries have a more stable water flow and greater bulk soil density around them. When earthworms poop, the readily decomposable organic matter is mixed with the mineral soil below, creating aeration.[7]

## MATERIAL AND METHODS

Pontoscolexcorethrurus were hand-caught by digging them out of the garden soil during the wet season. Two weeks were spent keeping them healthy in large, ventilated containers (32x29x27cm). Soil and shredded, dried leaves were mixed together in a 3:2 ratio to make humus. The humus was utilized to provide the earthworms with the nutrition they need while being kept in the lab. Earthworms had their digestive systems cleansed by letting them eat wet filter paper in a sealed cage for 48 hours. This helped ensure that the worms all started off with the same set of bacteria and other microorganisms.

After that, we put the worms in a series of identical containers (21x18x9cm). Boxes were given one kilogram of dirt and six earthworms. Before placing earthworms in the containers, their individual weights were recorded. A little layer of humus has settled over the soil. The boxes have vent holes cut in them to provide proper ventilation.

From this research, we know that the soil in which earthworms thrive often contains unhealthy levels of copper, and zinc. On day zero, we measured the weight of six earthworms of identical size, and then on days one through six, we exposed them to copper, and zinc at varying concentrations. As chloride salts, the metal were first introduced. In order to get the soil ready, the salts were applied as a powder but then mixed in with the earthworms. In terms of heavy metal concentrations, we kept triplicates of everything. As a baseline, we analysed non-enhanced soil. A little coating of humus was spread over the dirt in each of the pots. The perforations in the boxes made it possible for air to circulate within. For 15 days, we kept earthworms in water, monitoring their weight and death rate at the 3, 7, 10, and 15-day marks. After the earthworms had recovered from the metal treatment, the bacteria and fungi in their digestive systems were analysed.

To investigate the sequence, we employed the NCBI blast pattern matching programme. A phylogenetic analysis was performed after a multiple sequence alignment to determine the degree of similarity between our sequence and the sequence obtained from the blast results. The results show that the bacteria acquires its resistance to the toxic substances from genes located on its chromosomes.

## RESULTS

**Table 1: Pontoscolexcorethrurus was subjected to various copper chloride concentrations.**

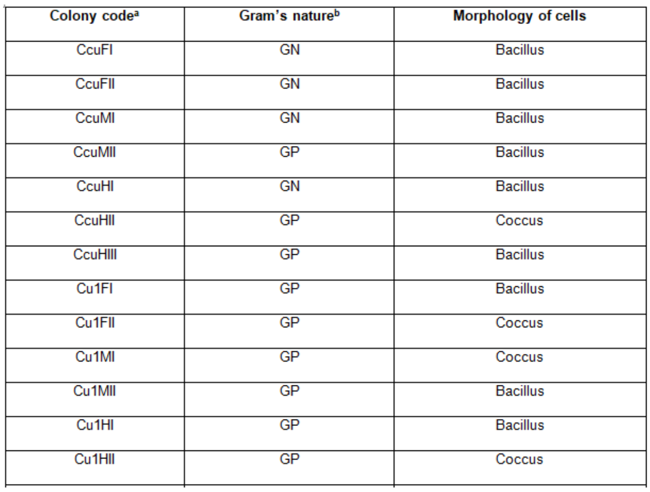
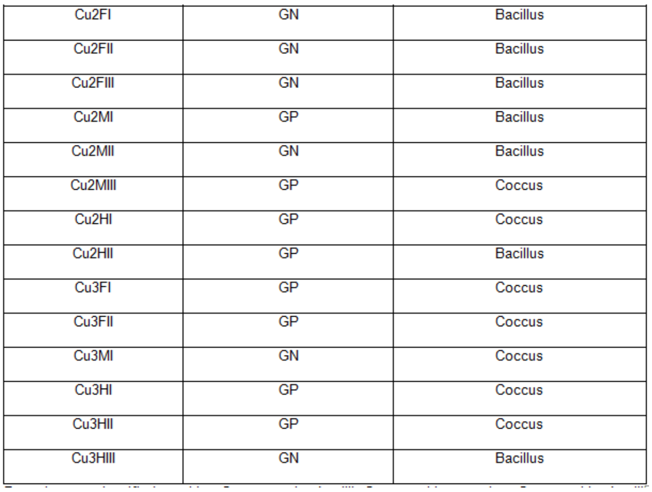
| Concentration of salt $CuCl_2$(mg/kg dry wt. of soil) | Labelonboxes |
|---|---|
| 0 | CcuA,CcuB,CcuC |
| 100 | Cu1A,Cu1B,Cu1C |
| 200 | Cu2A,Cu2B,Cu2C |
| 400 | Cu3A,Cu3B,Cu3C |
| 800 | Cu4A,Cu4B,Cu4C |
| 1600 | Cu5A,Cu5B,Cu5C |
| 3200 | Cu6A,Cu6B,Cu6C |

In the long run, earthworms who were fed Copper lost weight. At the end of the 15-day period, no earthworms had survived in soil containing 800mg/kg or more of copper /kg of dry weight, while earthworms exposed to more than 1600mg/kg of metal /kg of dry weight died within three days.

**Table 2: Pontoscolexcorethrus was subjected to various zinc chloride concentrations.**

| Concentration of salt $ZnCl_2$ | Label |
|---|---|
| 0 | CZn |
| 3200 | Zn4 |
| 100 | Zn1 |
| 1600 | Zn3 |
| 6400 | Zn5 |
| 400 | Zn2 |
| 13200 | Zn6 |

### Table 3: Gram staining of microorganisms recovered from copper-treated earthworms.

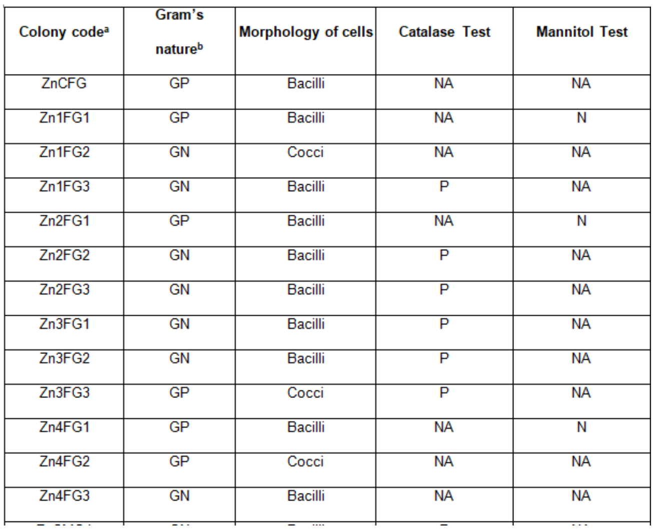



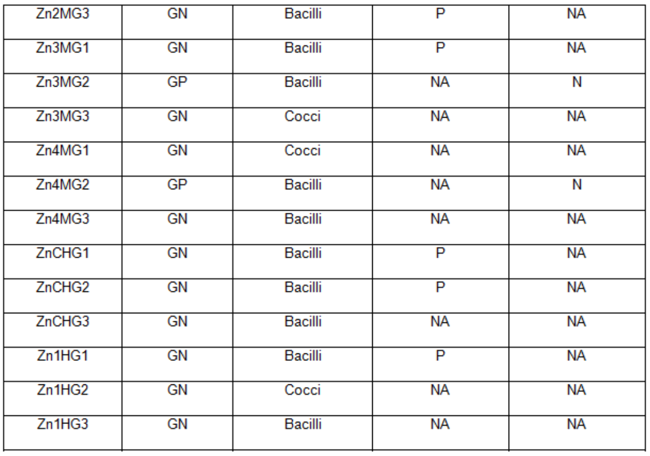
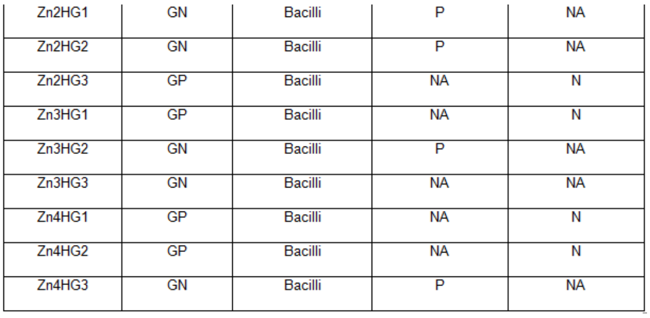
| Colony code[a] | Gram's nature[b] | Morphology of cells |
|---|---|---|
| CcuFI | GN | Bacillus |
| CcuFII | GN | Bacillus |
| CcuMI | GN | Bacillus |
| CcuMII | GP | Bacillus |
| CcuHI | GN | Bacillus |
| CcuHII | GP | Coccus |
| CcuHIII | GP | Bacillus |
| Cu1FI | GP | Bacillus |
| Cu1FII | GP | Coccus |
| Cu1MI | GP | Coccus |
| Cu1MII | GP | Bacillus |
| Cu1HI | GP | Bacillus |
| Cu1HII | GP | Coccus |
| Cu2FI | GN | Bacillus |
| Cu2FII | GN | Bacillus |
| Cu2FIII | GN | Bacillus |
| Cu2MI | GP | Bacillus |
| Cu2MII | GN | Bacillus |
| Cu2MIII | GP | Coccus |
| Cu2HI | GP | Coccus |
| Cu2HII | GP | Bacillus |
| Cu3FI | GP | Coccus |
| Cu3FII | GP | Coccus |
| Cu3MI | GN | Coccus |
| Cu3HI | GP | Coccus |
| Cu3HII | GP | Coccus |
| Cu3HIII | GN | Bacillus |

Bacteria were classified as either Gram-negative bacilli, Gram-positive cocci, or Gram-positive bacilli based on the findings of a Gram's staining test. Based on their Gram nature, the bacteria were subjected to a variety of biochemical assays. Tables provide the outcomes of biochemical analysis of the bacterial colonies. Isolated bacterial cultures were identified based on their biochemical properties.

In order to determine whether bacterial species were enriched or preferentially accumulated in response to copper treatment, the isolates from bacteria under copper stress were compared to those from untreated controls. The gut bacteria of copper-treated earthworms showed a preferential accumulation of Staphylococcus aureus and Bacillus cereus.

### Table 4 : Microorganisms recovered from zinc-treated earthworms were subjected to biochemical testing.



| Colony code[a] | Gram's nature[b] | Morphology of cells | Catalase Test | Mannitol Test |
|---|---|---|---|---|
| ZnCFG | GP | Bacilli | NA | NA |
| Zn1FG1 | GP | Bacilli | NA | N |
| Zn1FG2 | GN | Cocci | NA | NA |
| Zn1FG3 | GN | Bacilli | P | NA |
| Zn2FG1 | GP | Bacilli | NA | N |
| Zn2FG2 | GN | Bacilli | P | NA |
| Zn2FG3 | GN | Bacilli | P | NA |
| Zn3FG1 | GN | Bacilli | P | NA |
| Zn3FG2 | GN | Bacilli | P | NA |
| Zn3FG3 | GP | Cocci | P | NA |
| Zn4FG1 | GP | Bacilli | NA | N |
| Zn4FG2 | GP | Cocci | NA | NA |
| Zn4FG3 | GN | Bacilli | NA | NA |
| ZnCMG1 | GN | Bacilli | P | NA |
| ZnCMG2 | GN | Bacilli | NA | NA |
| ZnCMG3 | GN | Bacilli | NA | NA |
| Zn1MG1 | GN | Bacilli | P | NA |
| Zn1MG2 | GN | Bacilli | P | NA |
| Zn1MG3 | GN | Cocci | NA | NA |
| Zn2MG1 | GN | Cocci | NA | NA |
| Zn2MG2 | GP | Cocci | NA | NA |
| Zn2MG3 | GN | Bacilli | P | NA |
| Zn3MG1 | GN | Bacilli | P | NA |
| Zn3MG2 | GP | Bacilli | NA | N |
| Zn3MG3 | GN | Cocci | NA | NA |
| Zn4MG1 | GN | Cocci | NA | NA |
| Zn4MG2 | GP | Bacilli | NA | N |
| Zn4MG3 | GN | Bacilli | NA | NA |
| ZnCHG1 | GN | Bacilli | P | NA |
| ZnCHG2 | GN | Bacilli | P | NA |
| ZnCHG3 | GN | Bacilli | NA | NA |
| Zn1HG1 | GN | Bacilli | P | NA |
| Zn1HG2 | GN | Cocci | NA | NA |
| Zn1HG3 | GN | Bacilli | NA | NA |
| Zn2HG1 | GN | Bacilli | P | NA |
| Zn2HG2 | GN | Bacilli | P | NA |
| Zn2HG3 | GP | Bacilli | NA | N |
| Zn3HG1 | GP | Bacilli | NA | N |
| Zn3HG2 | GN | Bacilli | P | NA |
| Zn3HG3 | GN | Bacilli | NA | NA |
| Zn4HG1 | GP | Bacilli | NA | N |
| Zn4HG2 | GP | Bacilli | NA | N |
| Zn4HG3 | GN | Bacilli | P | NA |

Exposed earthworms had a higher concentration of a certain bacterium than the control group did. The increased metal tolerance of earthworms may be due to the higher concentration of helpful microorganisms in their guts under unfavourable circumstances. Furthermore, this suggests that bio-remediation with earthworms and microorganisms is a viable option for cleaning up heavy metal pollution. Even while earthworms are present in the soil, they may have never interacted with the organisms that build up there. It's possible that the earthworms' tolerance to the metal was acquired when they consumed prey species that had already been exposed to it.

Using the NCBI website, it was possible to identify the genes in microorganisms' chromosomes that give resistance to heavy metals like copper, and zinc.

**Table 5: Possible Metal resistance genes in the chromosome of specifically harbored bacteria**

| **Microorganisms** | **Copperresistantgenes** | **Zincresistantgenes** |
|---|---|---|
| *Staphylococcussp.* | - | **rep** in plasmidpC194[gi187729629;NC_010626.1] |
| *Delftiasp.* | **copA**[gi160895450;NC_010002.1] | - |
| *Bacilluscereus* | **AH621**[gi238801471;NZ_CM000719.1 | **ZntR**[gi-238801487;NZ_CM000735.1] |
| *Aeromonashydrophila* | **AHA_2962**[gi-117617447;NC_008570.1] | **ZntR**[gi-117617447;NC_008570.1] |

The abbreviations for these proteins are ZntR (Zinc responsive transcription regulator), AHA (copper/silver resistance periplasmic protein), and Rep (replicon initiation protein). PCR reactions were performed on the genes ZntR, AHA 2962.

**Genes were amplified using primers made with the help of the Primer3-primer design tool.**

There was no amplification in the other bacteria tested (Staphylococcus, Delftia, and Bacillus). It might be because these resistance genes are present in other bacterial strains of the same species. Potentially existent but unamplified metal resistance genes might be the subject of future research. The genes bcere0007 rs08915 (for copper resistance in Bacillus cereus) have been reported to confer resistance to copper, respectively, in a variety of Staphylococcus and Bacillus species.

## CONCLUSION

We looked examined the microbiota of Pontoscolexcorethrurus earthworms to see how heavy metals like copper, and zinc affected them. Earthworms were killed in as little as three days at soil concentrations over 80 mg/kg. If soil contains more than 1600 mg of cu per kilogramme of dry weight, all earthworms would die within three days. Soil with as high as 1600 mg of zinc per kilogramme of dry weight did not affect their health. Indicators of soil pollution may be gleaned by tracking changes in the bacterial communities of grubs over time. Due to their increased resistance to heavy metals, bacteria such as Bacillus cereus and Delftia sp. might be utilised to clean polluted ground. Before these bacteria may be used for bioremediation, further study into their tolerance of toxic substances and the mechanism by which they accept them is necessary. It will shed light on, and aid in understanding, the wide variety of strategies that may be used to enhance the organism's bioremediation capabilities.

## REFERENCES

1. Timmermans,M.J.,Ellers,J.,Roelofs,D.andVan Straalen,N.M.(2019).Metallothionein mRNA expression and cadmium tolerance in metal-stressed and reference populations of the springtail Orchesellacincta. Ecotoxicology.14: 727-739.

2. Tripathi, G. and Bhardwaj, P. (2020). Comparative studies on biomass production lifecycles and composting efficiency of Eisenia fetida (Savigny) and Lampito mauritii(Kinberg). Biores . Technol. 92, 275–283.

3. Umminger,B.L.(2019).Physiologicalstudiesonsupercooledfish Fundulusheteroclitus. Carbohydrate metabolism and survival at sub zero temperature. J. Expt . Zool. 17: 159-74.

4. Van Straalen, N.M. and Timmermans, M.J.T.N.(2021).Genetic variation in toxicant stressed populations: an evaluation of the genetic erosion hypothesis. Human and Ecological Risk Assessment.8: 983-1002.

5. Venter, J. M. And Reinecke, A. J. (2017). The life cycle of the compost worm Eisenia fetida(Oligochaeta). South African J.Zool.23: 161-165.

6. Nagavallemma, K.P., Wani, S.P., Stephane, Lacroix, Padmaja, V.V., Vineela, C.,BabuRao,M.andSahrawat,K.L.(2017).Vermicomposting:Recyclingwastesintovaluableorganicfertilizer.GlobalThemeonAgrecosystemsReport no. 8. Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics. 20.

7. Nakasaki, K., Sasaki, M.,Shoda, M. and Kubota,H.(2019).Characteristics of Mesoplilic bacteria isolated during thermophilic composting of sewage sludge. Appl. Environ. Microbiol. 49: 42-45.

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**Corresponding Author**

**Pawar Balasaheb Ramrao***

PhD Student, Kalinga University Raipur