# Analysis of Heavy Metal, Density of Earthworm and Microbial Activities in Industrial Area Sewage Soil Using Jeevamrutha and Vermicompost

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**ABSTRACT**

This study aims to determine the quality of agricultural soil by using amendments like Jeevamrutha, Vermicompost, Earthworm (*Eudriluseugeniae)* and their combination *Eudriluseugeniae*is added along with Jeevamrutha, Vermicompost and their combined nature in 8 composting pits respectively and decomposition done for Two months. It was found that heavy metal concentration decreased in the post-treated Industrial area sewage soil with amendments such as Jeevamruatha, Vermicompost and their combined nature along with Earthworm (*Eudriluseugeniae*) compared with pre-treated Industrial area sewage soil. High concentration of heavy metals found in *Eudriluseugeniae*earthworm body before subjecting it to decomposition, whereas the post treated Earthworms (*Eudriluseugeniae)* showed less amount of Bioaccumulation of heavy metals in earthworm body.The post treated industrial area sewage soil showed decreased earthworm density compared to pre-treated soil. Among the the post-treated soil earthworm density is slightly increased in industrial area sewage soil treated with Vermicompost. It was observed that Microbial activity (colony count) increased in the post treated industrial area sewage soil, by Addition of amendments such as Jeevamrutha, Vermicompost, and combination of both show better growth of microbial colony in soil.

Industrial area sewage earthworms identified where they belonged to Megascolecidae family. High concentration of heavy metals found in that IAS soil *earthworm* body (Megascolecidae family) indicating the toxicity and pollution caused by the industry to soil.

**Keywords:** Jeevamrutha, Vermicomposting, Earthworms, Industrial Area Soil, Agriculture Area Soil, Heavy Metals.

## INTRODUCTION

Vermicomposting is an eco-friendly and cost-effective waste management technology in which waste is processed by the privilege of both earthworms and their associated microorganisms. It is a significant way of enhancing soil fertility and plant growth. Vermicompost associated bacteria and other microbes enhance soil fertility by direct mechanisms like nitrogen fixation, phosphate solubilization, production of growth regulators, plant hormones, enzymes for promoting plant growth and essential process, signal molecules and by indirect mechanisms like protection of plants from biotic and abiotic stress. Thus, vermicompost application enriches soil fertility by amplifying the bacterial diversity, enriching and activating the beneficial microbes for the betterment of agriculture (Immanuel J *et al.,)*

Earthworms are one of the keystone species of soil fertility and health. Earthworms are also termed as a farmer's friend, ploughman, biological indicator and soil engineer. Earthworms increase the soil's physiochemical properties through organic matter degradation, stimulate microbial activity, soil aggregation, water holding capacity and improve soil porosity, which paves the way for increasing infiltration and reduction in soil erosion. It also has a multifunctional role in plant growth through decomposition of organic matter into nutritional rich product either by itself and/or mutualistic association with other beneficial microbes, as soil burrows improve the root penetration especially in compacted soil and reduces the soil borne pathogens (Tahat et al. 2020). A plethora of references reveals the capacity of earthworms in conversion of infertile barren land into fertile land and a few reports stated an importance of earthworms' metabolites. Earthworms play an important role in sustainable agriculture system which remains unexplored. To reduce the impact of modern agricultural practices, development of eco-friendly sustainable farming systems will be one of the great choices.

Chemical fertilizers are widely used to increase crop yields; nevertheless, excessive use can harden the soil, lower fertility, intensify insecticides, contaminate air and water, and endanger human health and the environment. Their continuous use can also deplete essential soil nutrients and minerals that are naturally found in fertile soil. Chemical fertilizers will boost a plant's productivity, but plants will not be healthy and strong as plants are grown in such conditions and do not have sufficient time intervals to mature and develop into healthy root growth, strong stems, or nutritious fruits and vegetables. They will also have a lesser chance of surviving since they are more susceptible to pests and diseases due to a lack of a strong immune system and enough resistance to these causes.By adopting ecological and sustainable farming practices we can reverse the declining trend in global productivity and as well as environmental protection (Bulluck and Ristaino,2002)

Jeevamrit is a traditional fermented liquid organic concoction commonly used as soil microbial enhancer in natural farming. It is a rich source of Bacteria, fungi, actinomycetes and also contains other beneficial microorganisms. It is claimed that the application of jeevamrit stimulates crop growth and repels some of the insect-pests.It is prepared by using simple on-farm inputs like cow dung, urine, pulse flour, jaggery, water and microbial rich healthy soil. The sugars present in Jaggery acts as ready source of energy for growing microbes, while pulse flour (besan) acts as a nitrogen source in the formulation. Cow dung and urine provide nitrogen and other essential nutrients for growing microorganisms.

Soil, Cow urine and dung provide the culture of beneficial microbes in the jeevamrit. During last few years,there has been an increasing interest on jeevamrit and other liquid organic formulations for use in organic and natural farming. The scientific characterization and validation of jeevamrit is under progress (Kumar et al., 2021).

Manjunatha*et al*. reported that the use of Jeevamrutha treated organics, improves the physico-chemical and biological properties of soil, besides improving the efficiency of applied farmyard manure. They also confirmed that the potential of Jeevamrutha is to supply materials and to act as food support for beneficial microbes'. In view of the above,the current study focused on determining the influence of microbial inoculants such Jeevamrutha and vermicomposting.

Heavy metal pollution of soil remains a severe problem due to its various harmful effects on the health of soil-dwelling organisms as well as humans (Rayetal., 2019;Masindiand Muedi,2018). Both natural

and anthropogenic sources discharge heavy metals into the environment. Natural sources include erosion, weathering of metalrich rocks, bacterial activity and volcanic eruptions, while anthropogenic sources include waste disposal, fossil fuel combustion, smelting, industrial activities and agricultural practices such as the use of metal-containing fertilizers and pesticides
(Engwaetal., 2019; Alengebawyetal., 2021). They accumulate in soft tissues and become toxic if not metabolized in the body (Masindiand Muedi,2018). So, depending on the concentration and duration of exposure, all heavy metals can be potentially toxic to living beings (Alietal., 2019).
Heavy metal contamination of soil has a wide range of negative impacts on soil-dwelling organisms which can be either acute or chronic. Earthworms are important components of most temperate, terrestrial ecosystems in terms of both biomass and activity. They have positive impact on soil's physical, chemical and biological properties (Bartlettetal, 2010; Shietal., 2017). They have close contact with the soil, consume high amounts of soil and have less external barriers to the soil solution.
Therefore, earthworms have been widely used in soil eco-toxicological investigations (Sivakumar, 2015). They consume organic matter, break it down and mix it with soil to produce water stable aggregates (Hiranoand Tamae, 2011). Prolonged exposure of earthworms to heavy metals can negatively affect their survivability, growth, sexual development, cocoon production, behavior and density (Andreetal., 2010). Heavy metals like Cd, Cu, Hg, Zn and Pb are mostly bioaccumulated in earthworm's tissues and have adverse effects, making them suitable biological indicators for monitoring soil pollution from heavy metals (Zhangetal., 2009; Uwizeyimanaetal.,2017).

**Materials and Methods:**

Soil sample collected in "Antharasanahalli Industrial area , Tumakuru. Tumakuru is a city located in the southern part of Indian state of Karnataka .Antharasanahalli is a locality in Tumkur city in Karnataka state India. It belongs to Bangalore division. Antharasanahalli, Tumkur pin code is 572106 and postal head office is Northern Extension. Shanthi Nagar, Arakere, Tuda layout ,Chickpete,Harpet circle are the nearby localities of Tumkur. Distance between Tumkur and Antharasanahalli is 7kms an extent of 208 acres has been acquired by KIABD .It is an industrial area and is situated towards Ammanikere road to Sira road .It is home to various numbers of industries, Santoor soap industry -Wipro ,film caps rubber and Oil industry .It is including manufacturing, textiles, and engineering. The presence of industrial units in Antharasanahalli contributes to the economic development of the region.

**Procedure of soil sample collection:**

Material required:-Spade or auger (screw or tube or post hole type), Hand hoe, Core sampler, Sampling bags, Plastic tray or bucket .
We Divided the field into different homogeneous units, removed the surface litter at the sampling spot. Dig the plough depth of 15cm and collected at least 10 to 15 samples from each sampling unit and placed in a tray. Made a 'V' shaped cut to a depth of 15cm remove thick slices of soil from top to bottom of exposed face of the 'V'-shaped cut and place in a clean container and collected the sample in a clean polythene bag. We labelled the bag as industrial sewage soil sample.

**Method Of Preparation of Jeevamrutha (Liquid Form):**

Prepared Jeevamrutha by taking 200 litres of water with 10 kg of Indian/desi cow dung and 10 litre of desi cow urine to above liquid formulation, added 2 kg of Jaggery, 2kg of gram flour and one handful of soil. All the ingredients were mixed in a plastic drum and the drum was kept in a cool place and away from the sunlight for about 10 days. During this period the content was mixed thoroughly with a wooden stick for every 24 hours. The fresh preparation of Jeevamrutha was moderate green in colour with mild foul odour. As the storage period progressed, the preparation become darker in colour with a strong foul odour.

**Method of Vermicomposting**:

We arranged Vermicompost unit (Polybag) in a cool, moist, and shady site and collected cow dung, cow urine, various plant material, sheep and goat excreta. At the bottom of the Polybag we prepared fine bedding by adding above materials. We left for 25 - 30 days for partial decomposition of waste materials. After decomposition the bedding materials goes down in the Polybag. After a fine decomposition of about 30-35 days we released a "*Eudrilus eugeniae*" worm (2kg) on the upper layer of the bed. Water should be sprinkled immediately after release of worms and maintain a 60-70% of moisture. Bed should be kept moist by sprinkling of water, cow dung, cow urine and bed should be turned once after 20-25 days to maintain aeration and proper decomposition. We got vermicompost ready in 70-80 days.

**Method of Preparation of 8 sample pits:**

Collected the earthworm (*Eudrilus eugeniae)* at vermicompost production unit KVK(krishi vigyan Kendra) hirehalli,Tumakuru and industrial sewage soil from Industrial area Antharasanahalli. Taken 8 polythene bags and added 10 kg of Industrial sewage soil to these polythene bags and named as S2A and S2B(Industrial area sewage soil control);; S4A and S4B(Industrial area sewage soil+Vermicompost); S6A and S6B(Industrial area sewage soil+Jeevamrutha); S8A and S8B (Industrial area sewage soil+Jeevamrutha+ Vermicompost). To S4A and S4B polythene bags added 500g of vermicompost and to S8A and S8B added 250 grams of vermicompost and 100 ml of Jeevamrutha to each bag. To above all bags released 14 grams of earthworm (*Eudrilus eugeniae*) aged about 45 days old, and kept these samples for about two months in shady place. After 15 days of pit preparation organic matter of about 200g is added to all the pits (i.e. 2 % of ratio of total soil present) and frequent water sprinkling is done in order to maintain the moisture in pits.

**Heavy metal analysis of Industrial area seawge soil:-** heavy metals in the soil is analyzed by DTPA extraction method and using Atomic absorption spectrophotometer instrument.

**Heavy metal analysis of Earthworm(*Eudrilus eugeniae*):-:**

Collected Earthworms from industrial sewage area of about 5g and allowed it for degutting process for about 1 day (24 hours) and microwave the earthworms at 60 degrees Celsius for about 24hours. After a fine dry, grind the earthworms into a fine powdered with a help of pestle and mortar. Then we took 0.25 grams of the earthworm powder and added concentrated $HNO_3$ and $H_2O_2$ in a ratio of 5:1 into Teflon digestion vessel and prepared the above solution for digestion process.

**Microbial Analysis of different Samples:**

To isolate the bacteria of different samples, it needs the following materials:

Samples to be analyzed, Nutrient Agar media, Sterilized petri plates, Sterilized test tube and test tube stand, Pipettes, Spirit lamp, Transparent tape, Disinfectant, Cotton plug, Paper towels, measuring cylinder, Slide, needle, Incubator for maintaining the appropriate temperature, Laminar air flow, Microscope for observing bacterial colonies.

**To prepare nutrient media for bacterial culture, we followed these steps:**

The nutrient media for bacteria culture is usually made up of a combination of ingredients such as peptone, beef extract, agar (for solid media), and various salts and sugars. These components provide the necessary nutrients for bacterial growth.

We weigh 28 grams of nutrient Agar and dissolve it in a 1 liter of distilled water. We heated the mixture while stirring until it completely dissolves. Sterilize the media by autoclaving at high pressure and temperature. After sterilization, we allow the media to cool down to a suitable temperature before use. Then we poured the Agar media into sterilized Petri dishes and spread them uniformly. We allow the media to solidify completely before use.

**To sterilize the apparatus using UV radiation, we followed these steps:**

We cleaned the surfaces of the UV sterilization chamber to remove any visible debris or contaminants. We placed the apparatus in a UV sterilization chamber or cabinet. Then we closed the chamber and turn on the UV lamp and set the timer. We allowed the UV radiation to expose the apparatus for about 15-30 minutes. After the sterilization process is complete, we carefully diluted the samples through serial dilution.

**Serial dilution is a technique used to gradually reduce the concentration of a bacterial sample.**

We took 3 test tubes i.e., $10^{-1}$,$10^{-2}$,$10^{-3}$ and we added 10 ml of distilled water for 1st($10^{-1}$ ) test tube and add 9ml for other 2 test tube ($10^{-2}$,$10^{-3}$).For $10^{-1}$ test-tube we added 1gram of soil sample. Shake for about 2-3 minutes and took 1ml from $10^{-1}$ and added to $10^{-2}$ so its dilution decreases then we took 1ml from $10^{-2}$ and added to $10^{-3}$ test tube.This creates a 1:10 dilution, where the concentration of bacteria is now 1/10th of the original.

**Simple method to isolate bacteria from a soil sample:**

We transferred 1ml of $10^{-3}$ test tube sample to sterilized Petri plate which contains nutrient medium. Incubate the plates at an appropriate temperature (around 35°C) for a specific period (e.g., 24-48 hours). After incubation, we observe the plates for bacterial growth. Individual colonies have appeared as distinct spots on the agar surface. We counted the bacterial colonies by using plate count method.

**Data Analysis:**

Data has been analyzed by using one–way ANOVA and standard deviation.The values of all parameters of different soil samples agriculture, industrial and their combined samples are tabulated and analysed by statistical method using mean, standard deviation, single ANOVA using excel version 2013 and further the comparison of values of all parameters of different soil sample and combined samples have been calculating significance values and represented by plotting graph.

## RESULTS AND DISCUSSION

### Analysis of Heavy metals in Industrial area sewage soil

**Table 1:- Analysis of Heavy metals of Industrial area sewage soil samples with different amendments.**

| Parameters | Industrial area soil | Industrialarea soil+Jeevamrutha | Industrial area soil+ Vermicompost | Industrialarea soil+Jeevamrutha+ vermicompost | P-Value |
|---|---|---|---|---|---|
| **Cd(ppm)** | 00 ± 00 | 00± 00 | 00± 00 | 0.01 ± 0.014142 | 0.4769 |
| **Pb(ppm)** | 2.75 ± 0.042426 | 2.585± 0.0494975 | 2.42 ± 00 | 2.72 ± 0.084853 | 0.01098 |

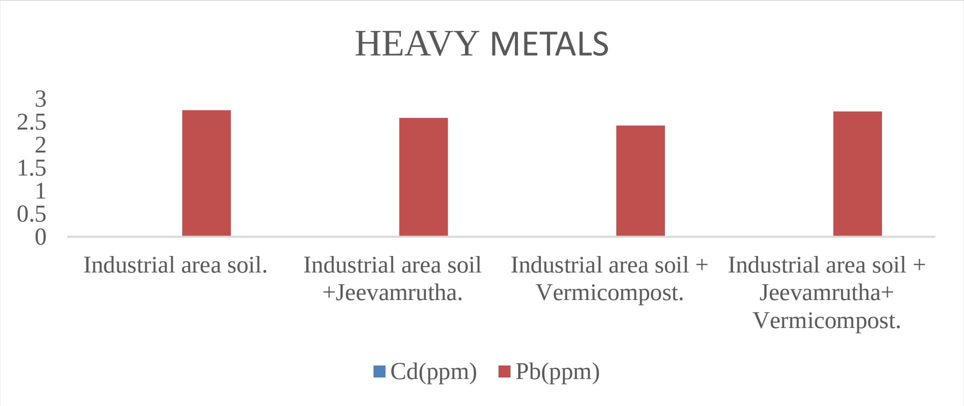


**Figure 1: Graphical representation of Heavy metals of Industrial area sewage soil samples with different amendments.**

In the pre-treated soil, the concentration of heavy metals such as lead and cadmium was present in high concentration that is Pb-18.07ppm, Cd-0.22 ppm. Then the soil is treated with amendments like Jeevamrutha, Vermicompost and their combined nature along with Earthworm (*Eudriluseugeniae*). After completing 2 months of decomposition the concentration of heavy metals was drastically decreased comparing to the post-treated soil.

Comparison among post treated Industrial area sewage soil samples, the variations in Cd values between control and other soil samples were found to be not significant(0±0). The variations in Pb values between control and other soil samples was found to be significant .The mean Pb value of control Industrial area sewage soil was found to be 2.75±0.042426, the mean Pb value of IAS+JA was found to be 2.585±0.0494975and the mean Pb value of IAS+VC was found to be 2.42±0.0 while the mean Pb value of IAS+JA + VC was found to be 2.72±0.084853. Among the post treated soil, Heavy metal concentration was high in IAS soil control comparatively to other 3 samples ( Ag+JA , Ag+VC , Ag+JA+VC ) results are congruence. P. Govil. et al., (2001) who have observed that soil in the Industrial development area significantly contaminated, showing two to three times higher level of toxic elements then normal. Many heavy metals such as Cd, As, Ni etc are present above the normal distribution of soil. After completing two months of decomposition the heavy metals (Pb) drastically decreased in Soil samples such as IAS+JA , IAS+VC , IAS+JA+VC .our results are in congruence with following research papers.

Earthworm ingests large amount of substrate and therefore exposed to heavy metals through their skin and intestine. They concentrate heavy metals in their body from the substrate ( Morgan and Morgan, 1999, Leonard *et al*., 2001) due this reason Vermicomposting can be used in the toxic metal removal and breakdown of complex chemical to non-toxic forms (Jain *et al*., 2004). Patnaik and M.Vikram Reddy (2011) The conclude that earthworm*Eudriluseugeniae* uptake more metals from urban waste , they also revealed that accumulation of heavy metals in worm's tissue. According to (Suthar *et al*.,2008) Decline in the content of heavy metals As, Pb, Cd in the Vermicompost may be due to the fact that earthworms accumulate heavy metals in the cells of their cholorogogenous tissue (yellow tissue) and there is a variation in the binding of metal with the ligands of these tissue that leads to a variable bioaccumulation of various metals in the tissue. According to ( Xin He. *et al*., 2016) The total amount of 9 heavy metals was substantially lowered during sewage sludge Vermicomposting. Residual fraction of heavy metals were elevated due to forming of organo metal substance. P. Singh .*et al*., (2020) reported that heavy metals are very dangerous for almost all living organisms, they found that addition of JA can suppress the damaging effect of heavy metals on pollen grains. the epithelial layer of gut absorbs available forms of metals during the transiting of wastes through it as the bioaccumulation of a high concentration of metals in earthworm tissues is well documented (Suthar and Singh, 2009; Suthar *et al*., 2008). In addition, earthworms seemed to decrease the mobility and bioavailability of heavy metals during vermicomposting through two major types of cellular adaptation to toxicity of metals: one involves binding of metals to nuclear proteins and the formation of inclusion nuclear bodies; the second type is a cytoplasmic process involving synthesis of a specific metal binding protein, metallothionein within the chloragogenous tissue (Cherian and Nordberg, 1983). Several studies indicated that vermicomposting could significantly reduce the heavy metals, extracted by diethylene triamine pentacetic acid (DTPA) (Song *et al*., 2014). Hee*et al*., 2009 reported that aerobic composting could decrease the heavy metals belonging to exchangeable and carbonate fraction, while increasing the axidizable and residual forms of heavy metals, their studies matching with our results of Cd and Pb.

**Analysis of Heavy metal content in Earthworms body (*Eudrilus eugeniae*) subjected to IAS soil.**

**Table 2 :-Comparision of heavy metal concentration in the earthworms body(*Eudrilus eugeniae*) with four different amendments.**

| Sample (Earthworms) | Lead (ppm) | Cadmium (ppm) |
|---|---|---|
| ***Eudrilus eugeniae* (pre)** | 132.1 | 3.0 |
| **IAS control EWMS(post)** | 0.00 | 2.97 |
| **IAS+JA EWMS(Post)** | 0.00 | 2.72 |
| **IAS + VC EWMS(post)** | 0.00 | 5.94 |
| **IAS+JA+VC EWMS(post)** | 0.00 | 0.00 |

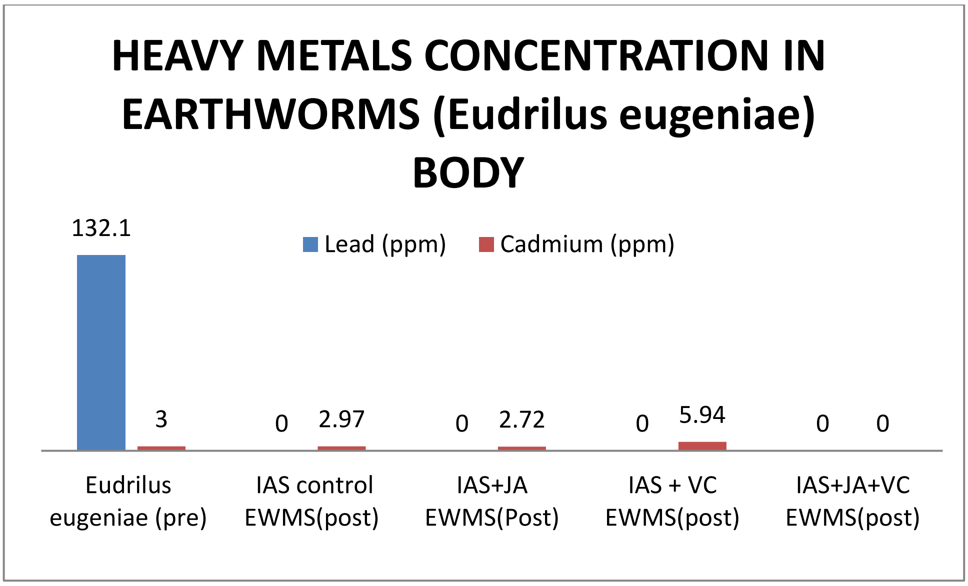


**Figure2:- Graphical representation of Comparision of heavy metal concentration in the earthworms body (*Eudrilus eugeniae*) with four different amendments.**

*Eudrilus eugeniae*sp was used for decomposition. Heavy metal concentration was high in the earthworms body before subjecting it to decomposition that is Lead-132.1 ppm and cadmium-3.0 ppm. then earthworms were subjected to different amendments such as Jeevamrutha , Vermicompost, and its combination where it was observed that heavy metals concentration in earthworms body was drastically decreased after completing the decomposition. In control IAS soil earthworms contained Lead-0.0 , Cadmium-2.97 , IAS soil+JA earthworms contained Lead- 0.0 , cadmium-2.72, IAS soil+VC earthworms contained Lead-0.0 , Cadmium-5.94, IAS soil+JA + VC earthworms contained Lead- 0.0 , cadmium-0.00.

It was observed that decreased concentration of lead and cadmium in earthworms body(*Eudrilus eugeniae)*.That may be because of microorganisms present in our soil sample which converted them into harmless. According to Jyothi B., *et al*., (2014) microbial cells can convert metal ions from one state to another hence reduce their harmfulness. The uptake of heavy metals by microorganisms occurs via bioaccumulation which is an active process and adsorption. Several microorganisms have been used to clean up heavy metals contaminated environment (Kim I. H *et al*., 2015).

Since heavy metals are persistent in the environment, they enter organisms from the environment and accumulate in them (Alietal., 2019). Earthworms accumulate heavy metals present in soil mostly through direct dermal contact and ingestion (Wijaywardenaetal., 2017). The heavy metals accumulation by earthworms is affected by various factors such as earthworm genera, soil physiochemical properties, contamination level and other environmental conditions such as soil moisture andtemperature (Xiaoetal., 2022). The intestinal region specifically the chloragogenous

tissue that coats the coelomic surface of the intestine was reported to accumulate high concentration of heavy metals (Sturzenbaumetal., 2004). Mostly longitudinal and circular muscles of earthworm's gut are involved in heavy metal accumulation (Kilic,2011). Levequeetal.(2013) studied Pb localization in earthworm tissues and found that Pb was primarily localized in longitudinal muscles and gut wall of exposed worms.

Heavy metal pollution not only negatively affects earthworms but also poses a threatto the well being of human beings and other living organisms throughbiomagnification via the food chain (Duoetal., 2019). Earthworms are a significant part of diet of many predator species (birds, moles and shrews) representing a path for the movement of pollutants through food webs (SpurgeonandHopkin, 1996;Langdonetal., 2003; Kumaretal., 2022). Pollutants taken up by earthworms may
accelerate the transfer of chemicals into the food web due to bio-accumulation and biomagnifications processes (Shoreetal., 2014). Bioaccumulation of heavy metals in the food chain may not have a substantial impact on earthworms, but it can cause serious harm at higher trophic levels. Thus earthworm toxicological studies can be utilized for establishment of safe environmental toxicant levels (Sivakumar,2015).

**Identification Of Industrial Area Sewage Earthworms.**

Collection**:-** Industrial area sewage earthworms were collected manually at Antharasanahalli by digging, where the found in 15 cm depth .

**Figure 2: Collected Earth Worms.**

After their collection they were degutted for 24 hours and stored in formalin diluted in water with ration 1:10.

Examination:**-**Earthworms was examined under **LABOMED CZM6 Stereozoom microscope**. Where the observed Earthworms were photographed using Redmi **company phone,Model-M2001J2I ,Camera-108MP rear camera.**

An official government website - https://earthwormsofindia.com/identify-wizard.php?parent=12 was used to identify the family of IAS earthworms.

Description:-Body was cylindrical,medium size(7.1cm length and 3mm-Width),segments 116-145,weight 0.6-2g. Body uniformly grayish brown except darkish clitellum.

**Dorsal pores arranged linearly were observed which confirmed that the earthworms belonged to family Megascolecidae**

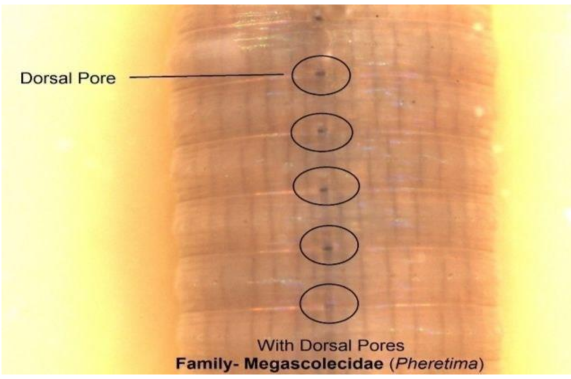


**Figure 3: Dorsal pores observed in IAS soil Earthworms.**

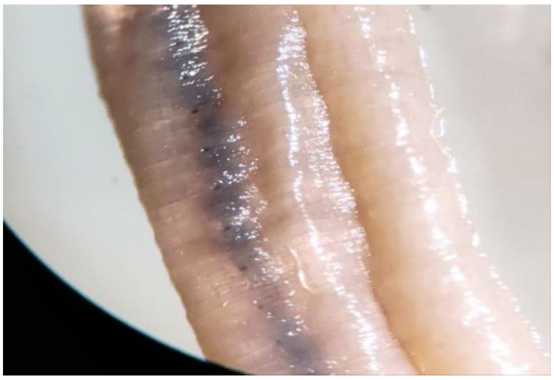

**Figure 4: Key Identification Character Found in official website of India.**

**Analysis of Heavy metal content in Earthworms body (Megascolecidae Family)found in Industrial area sewage soil.**

**Table 3:-Heavy metal concentration in the earthworms body(Megascolecidae) found in IAS soil.**

| Sample (Earthworms) | Lead(ppm) | Cadmium(ppm) |
|---|---|---|
| **IAS EWMS(Megascolecidae)** | 135.1 | 5.2 |

While collecting the Industrial area sewage soil, some earthworms were also collected from that area which further identified as they belonged to Megascolecidae family. Then these earthworms were subjected to biochemical analysis in order to find the concentration of heavy metals in its body. After the analysis earthworms body contained lead-135.1ppm and cadmium-5.2ppm.

our results are in congruence with Ruiz *et al.*, 2009; Lukkari et al 2006, 2004 Industrial, mining, and agricultural activities significantly contaminate soils with heavy metals. This is increasingly becoming a major environmental problem and is damaging ecosystems near emission sources. Most heavy metals eventually enter surface soil layers, where they bind to organic matter and reduce the mobility and bioavailability of useful trace elements in those parts of an ecosystem where essential biological processes, such as nutrient decomposition and mineralization, occur. Earthworms can absorb heavy metals from contaminated soils, which simulates the actions of key elements in the body and causes diseases.(GulzinatSeribekkyzy*et al.*,2022) Earthworms are important soil organisms, contributing to many beneficial ecological services that can be harmed by heavy metal contamination. Because of their permeable skin and constant contact with soil through their digestive tract, they are extremely sensitive to soil contamination. Heavy metals get bioaccumulated in their bodies and cause major health problems. In this review, we describe the findings of various studies on the toxicity of heavy metals to

earthworms, mechanisms of toxicity, detoxification responses and consequences on the environment. Heavy metals have deleterious impact on earthworms at all levels of organization, including inhibiting enzyme activities, causing DNA damage, reducing survival, growth and cocoon production, altering behavior and reducing total earthworm community diversity and biomass. These negative effects of heavy metals on earthworms can have disproportionate adverse consequences for community stability as well as serious ecological consequences for the entire terrestrial ecosystem. So, heavy metal pollution of soil poses a serious threat to earthworms, reducing their activity and posing a risk to the environment and human health(Renuyadav*et al.*, 2023) They are recognized as important biological indicators of soil pollution because they give useful information about environmental risks and there is a correlation between the concentration of certain pollutants in the soil and their body (Nannonietal., 2011;Levequeetal, 2013). Eiseniafetida (Savigny, 1826) is
the most widely used species in ecotoxicological investigations and may be used as a valuable bio-indicator for monitoring soil contamination (Hiranoand Tamae,2011). Ecotoxicological research on earthworms is particularly needed as they are important indicators for food production and environmental services. More precisely, heavy metals deposited into the soil can bioaccumulate and cause physiological changes in earthworms, which can have further negative effects on the environment and ultimately human health. The significance of earthworms in ecosystem functioning has led to several studies describing heavy metal toxicity in earthworms, but there is no comprehensive study that summarizes all aspects of heavy metal toxicity in earthworms (RenuYadav*et al.*, 2023)This review highlighted the toxic effects of heavy metals on earthworms and its further implications on the environment. Earthworms are a keystone species that make up the majority of soil fauna. Heavy metal contamination of soil is a serious threat to earthworms which reduces their activity and consequently poses a risk to the environment and human health. Negative effects on keystone species can have disproportionately adverse consequences for community stability as well as serious ecological consequences for the entire terrestrial ecosystem. Through food chaintransmission and bioaccumulation, heavy metals have direct and indirect effects onanimal and human health. Therefore, it is critical to protect earthworms againstpollution in order to retain biodiversity and sustain soil ecological functions. They canalso serve as sentinel species to determine how serious the impacts of soil pollutionare; for example, if earthworm populations are harmed, it is a sign that contaminationlevels are dangerous to the ecosystem. Due to the complexity of the soil environment and the specificity of heavy metals, theecological risk of heavy metal toxicity to earthworms requires further research.Although all the reported studies increased the knowledge about the heavy metals toxicity to earthworms, there are knowledge gaps about several points. The further studies are needed to better understand (i) the mechanism of absorption,bioaccumulation and distribution of heavy metals, (ii) the factors affecting themetabolic pathways in metal-contaminated earthworms and (iii) the detoxification reactions of earthworms. Future research should also focus on pinpointing molecular triggers that lead to decreased fertility or undesirable consequences such asgenotoxicity and epigenetic effects in future generations. There is need to find somenew techniques and sustainable ways to efficiently overcome the heavy metalpollution, so that soil organisms can be protected from heavy metal toxicity. Asignificant challenge for the future is also to identify a sustainable system to optimizethe soil faunal diversity with biomass and their impacts on soil quality(RenuYadav*et al.*, 2023).

**Analysis of Microbial activity in Industrial area sewage soil**

**Table 4: Microbial activity in different soil sample.**

| Samples | Trials 1 | Trial 2 | Mean | Standard Deviation |
|---|---|---|---|---|
| **IAS soil+ EWMS** | 95.57 | 22.14 | 58.855 | 51.9228509 |
| **IAS soil+ VC+ EWMS** | 45.28 | 26.85 | 36.065 | 13.031978 |
| **IAS soil +VC + JA + EWMS** | 23.57 | 9.57 | 16.57 | 9.89949494 |
| **JA** | 8.71 | | | |
| **VC** | 12.85 | | | |
| **IAS soil** | 15 | | | |

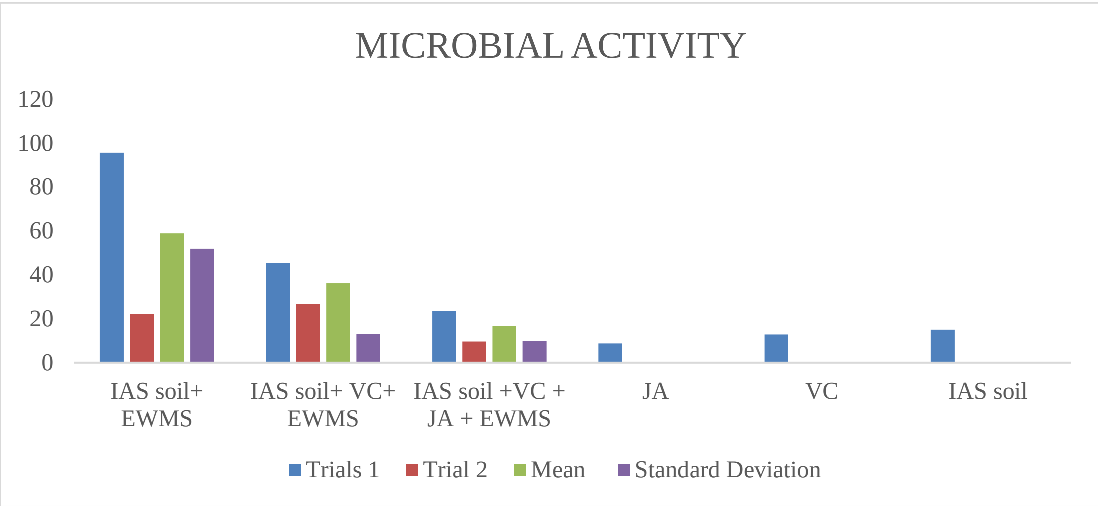


**Figure 5: Graphical representation of microbial activity Industrial area sewage soil samples with different amendments.**

Work was confined only up to counting Bacterial colonies. Varying numbers of colonies was observed. Microorganisms play definite and important role in soil fertility; they convert complex molecular compounds into ionic forms. Soil organic matter holds more than 95% of soil nitrogen, 5-60% of total phosphorous and about 30% of soil sulphur. Availability of these nutrients is condition to the decomposition of organic matter by microorganisms. Lack of microorganisms may result in accumulation of organic matter which affects the soil by clogging the soil texture (Dr G. S. Sidhu).

It was observed that Microbial activity (colony count) in the pre-treated soil is less compared to the post -treated soil. Addition of amendments such as Jeevamrutha, Vermicompost, and combination of both show better growth of microbial colony in soil. kulkarni*et al*.,2019 noticed that higher colony forming units (CFU) in Jeevamrutha were recorded between 9$^{th}$ to 12$^{th}$ days after preparation. In the preparations , higher number of bacterial CFUs *viz Azatobacter* sp., *Bacillus*sp.,*Beijerinckia*sp.,*Chromatium*sp., *Chromobacterium*sp.,*Pseudomonas*sp.,*Rhodomicrobium*sp.,*Serretia* sp., *Xanthomons*sp., were recorded.

According to Immanuel *et al.*,2022 Earthworms increase microbial activity significantly, so they are considered to play a major role in enhancing the production of plant growth regulators. Thus, vermicomposting amplifies the diversity and population of beneficial microbial communities which improves the physicochemical and biological properties of agricultural soil.Earthworms movements help improve soil porosity and aeration, thus supporting microbial growth and reproduction (Jia Cao *et al.*, 2015)

Jeevamrutham is the rich source of the beneficial micro-organism such as nitrogen fixing and phosphate solublizing bacteria From the study if wästound that leevamrutham is efticiently used between 9 to 12$^{th}$ days of preparation.Addition of such organic liquid manure would help to improve efficient microbial consortia thereby increasing NPK content and plant growth promoting factors. The use of Jeevamrutham is the best alternative to chemical fertilizer and our bioenhancer could be potent source to improve soil fertility,crop productivity and quality(kulkarni S.S *et al.*, 2019) .Vanaja *et al*. stated that Jeevamrutha is a plant growth-promoting substance containing beneficial microorganisms that provides the necessary nutritional requirement for growth and yield of a crop.Microorganisms are well activated in soil following the addition of Jeevamrutha which also maintains soil productivity.

Vermicomposting is a decomposition process involving interactions between earthworms and microorganisms. In this process the microorganisms are responsible for the biochemical degradation of organic matter whereas, earthworms act as crucial drivers of the process by fragmenting and conditioning the substrate, increasing suraceara for microbiological activity and altering its biologicalactivitydramatically(santebennurujayappaveeresh*et al*.,2013)

In the recent past, with the adverse impacts of intensive agriculture, natural farming has gained popularity among the farmers. Use of fermented organic concoctions in place of synthetic fertilizers and other agrochemicals under natural farming contain live microorganisms to feed both the soil and plants. Along with this use of organic concoctions also promotes proliferation of earthworms and diversified soil microorganisms which further helps in maintaining long-term soil fertility(samleshkumari 2022 *et al.,)*

**Industrial Area Sewage Soil Earth Worm(*Eudrilus eugeniae*) Density after subjecting it to decomposition.**

**Table 5: Comparison of earthworm density of Industrial area sewage soil samples with four different amendment.**

| TREATMENT | TRIAL 1 | TRIAL 2 | MEAN | STANDARD DEVIATION | STANDARD ERROR |
|---|---|---|---|---|---|
| Control IAS soil EWMS | 7.84 | 8.69 | 8.265 | 0.601040764 | 0.425 |
| IAS soil EWMS+JA | 9.88 | 8.9 | 9.39 | 0.692964646 | 0.49 |
| IAS soil EWMS+VA | 10.3 | 14.42 | 12.36 | 2.913279938 | 2.06 |
| IAS EWMS+VC+JA | 0.21 | 7.9 | 4.055 | 5.437651147 | 3.845 |

$$\text{Growth rate} = \frac{\text{Final density} - \text{Initial density}}{\text{Initial density}}$$

**Table 6: Comparison of growth rate of Industrial area sewage soil samples with four different amendments.**

| TREATMENT | INITIAL DENSITY | FINAL DENSITY | GROWTH RATE |
|---|---|---|---|
| Control IAS soil EWMS | 14±0 | 8.265±0.601040764 | -0.409642857 |
| IAS soil EWMS+JA | 14±0 | 9.39±0.692964646 | -0.329285714 |
| IAS soil EWMS+VA | 14±0 | 12.36±2.913279938 | -0.117142857 |
| IAS EWMS+VC+JA | 14±0 | 4.055±5.437651147 | -0.710357143 |

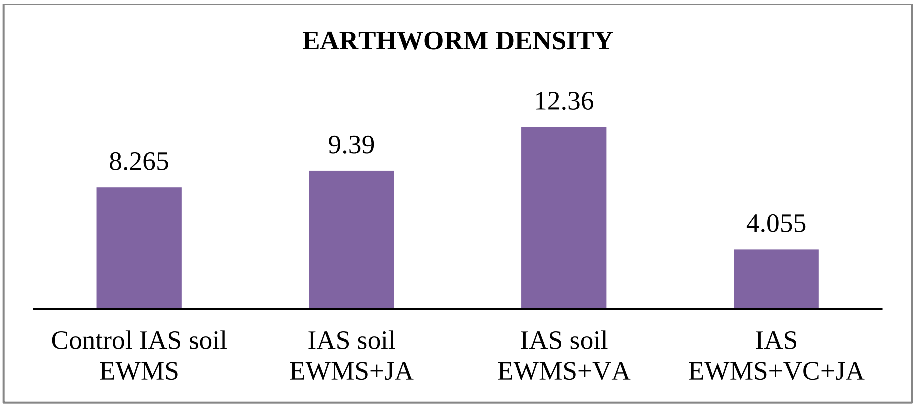


**Figure 6: Graphical representation ofComparison of growth rate of Industrial area sewage soil samples with four different amendments.**

From our work we observed that earthworm density is decreased in all the post treated samples that is Industrial area sewage soil sample (8.265±0.601040764), Industrial area sewage soil+ Jeevamrutha (9.39±0.692964646),Industrial area sewage soil+ Vermicompost(12.36±2.913279938), Industrial area sewage soil+ vermicompost +Jeevamrutha (4.055±5.437651147) compared with the initial earthworm density i.e. (14±0). According to XiaomingZou and Grizelle Gonzalez the decrease in earthworm density may result from changes in the chemistry of organic inputs, rather than in soil properties and litter quantity. Sartaj Ahmad Bhat.*et al.*, increasing percentages of wastes significantly affected the number and weight of worms. The earthworm population density with total soil C and N and plant available N tending to increase and bulk density tending to decrease with greater time since disturbance (K.M Smetak.*et al.*,).Nath and Chaudhuri reported that food sources with a significant amount of easily metabolizable organic matter facilitate growth and reproduction in the earthworm.As in our post samples the OC, EC, N, K, is increased whereas P, Ca, S and also heavy metals like Cd, Pb decreased. Where all these factors shows decline in earthworm density with respect to pre and post samples
When compare within the post samples the IAS soil treated with Vermicompost has more worm density than compare with other three samples. In these sample pH, P, K, Ca, Mg is more and OC is lessthan compare with other two post samples. Earthworm density and activity are influenced by factors such as soil structure and texture (Nuutinen*et al.*, 1998, Baker and Whitby, 2003), soil moisture and temperature (Berry and Jordan, 2001), food quantity and quality (Lee 1985, Edwards and Bohlen, 1996, curry, 2004) Vermicompost also add organic carbon to the soil and encourage microbial activity in the soil, thus

helping in improving soil health (Arivazhagan, Kandasamy, and Maniram 2019; Kushwah et al. 2020; Rajanna et al. 2012).

## CONCLUSITION

After completing two months of decomposition,It is observed that heavy metal(Cd,Pb) concentration decreased in the post-treated Industrial area sewage soil with amendments such as Jeevamruatha, Vermicompost and their combined nature along with Earthworm (*Eudriluseugeniae*) compared with pre-treated Industrial area sewage soil. High concentration of heavy metals (Cd,Pb) found in *Eudrilus eugeniae* earthworm body before subjecting it to decomposition, whereas the post treated Earthworms(*Eudriluseugeniae)* showed less amount of Bioaccumulation of heavy metals in earthworm body.The post treated industrial area sewage soil showed decreased earthworm density compared to pre-treated soil. Among the the post-treated soil earthworm density is slightly increased in industrial area sewage soil treated with Vermicompost. It was observed that Microbial activity (colony count) increased in the post treated industrial area sewage soil, by Addition of amendments such as Jeevamrutha, Vermicompost, and combination of both show better growth of microbial colony in soil.

Industrial area sewage earthworms identified where they belonged to Megascolecidae family. High concentration of heavy metals found in that IAS soil earthworm body (Megascolecidae family) indicating the toxicity and pollution caused by the industry to soil.

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