

Effects of Silver Coated Paper Dishes on Growth, Reproduction, Nutrient Content and Bioaccumulation of Earthworm Species, *Eudriluseugeniaekinberg*, 1867.

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ABSTRACT : In hotels, restaurants, birthday parties, marriage ceremony etc. Silver coated thin polythene paper dishes are commonly used. Afterwards they are thrown along with food waste near road side or in the dumping ground. Silver has antibacterial property which may hinder the process of bacterial decomposition. Therefore, efforts are made to find out possible way of decomposition of Silver coated thin polythene paper dishes and food waste along with soil, cow dung earthworm and microbes. Also effect of Ag⁺⁺ on earthworm for 60 days of experiment. The results revealed that silver coated paper dishes do not much affecting the growth and mortality of worms but they decrease the rate of reproduction. Significant increase in macronutrients viz., N, P, and K is observed in all the composting beds as compare to control. The maximum NPK is recorded in composting E₅ bed with biocatalyst, *Aspergillusniger* and earthworms.

In bioaccumulation experiment earthworms are very well adapted for accumulation of silver in their body. Composting bed with biocatalyst (mixture of decomposing bacteria), *A.niger* and earthworm, *Eudriluseugeniae* was found to be polythene degradation potential. Significant decrease in weight of thin polythene was observed in E₅ as compare to E₁, E₂, E₃ and E₄ composting beds

KEYWORDS: Earthworm, Biocatalyst, *Aspergillusniger*, Nutrients, Bioaccumulation, Polythene degradation, etc.

I. INTRODUCTION

Silver is a transition metal element whose medicinal use as antimicrobial agent has been documented since 1000 B.C. It is used as a health additive in traditional Chinese and Ayurvedic medicine et al. [1]. Nowadays, the burgeoning nano-technology industry is quickly producing nano-material that is being incorporated into consumer product. A list of more than 500 consumer products that claim to include some form of engineered nano-particles.[2]. Of these, about 20% contain silver nano-particle e.g. socks, paints, bandages and food containers etc are incorporate with nanosilver (n-Ag) to exploit its antimicrobial properties.et al. [3].

Annually around 3 lac kg of silver waste enters in ecosystem worldwide. It is expected that 20 tones of biocidal silver will enter terrestrial ecosystem and 20 tons of it will be discarded into European water ways by 2020 A.D. Silver is a known toxin and nanosilver has enhanced human and environmental toxicity. The USEPA (United State of Environmental Pollution Agency) views silver in surface waters as a "Priority pollutant", and after mercury it is the most toxic metal to aquatic organism. It is also toxic to important soil organisms and micro-organisms in sewage treatment plants [4].

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Earthworms are useful to clean up the soil from various pollutants like heavy metals [5], and are able to accumulate heavy metal in their tissues from the surrounding soil and might be reduces toxicity et al. [6]. Adverse effect of Ag-nano particles and Ag-ions on reproduction, growth and mortality on *E. foetida*. They also reported that Silver can readily bioaccumulate in aquatic organisms. Depending on age, size, sex, health and metabolism; the gastropods and arthropods can also bioaccumulate silver in their bodies et al. [7].

Silver has some potential toxic effects on beneficial soil microorganisms, which play a major role in nitrogen fixation microbes like ammonifying bacteria and chemolithotrophic bacteria in soil communities. These organisms deliver many crucial nutrients which are most essential in soil formation [8]. These soil bacteria can play key role in N₂ fixing and the breakdown of organic matter. These bacteria also form symbiotic relationship with legumes plants, which provide a major source of fixed nitrogen for individual as well as other plants. Silver disturbs denitrification processes leading to ecosystem disruption. Environmental denitrification is important because excess nitrates can reduce plant productivity, which results in eutrophication in water bodies' viz., rivers, lakes and marine ecosystem and acts as drinking water pollutants [9].

The treatment centers are typically not properly designed to remove nanoparticles. Therefore, sewage sludge may contain silver NPs. In many countries; sewage sludge is dried and applied as a fertilizer e.g. in Germany, every year about 2 million tones of dry sewage sludge is used as a fertilizer et al. [10].

Bacteria are the smallest and most abundant microbes in the soil. A gram of soil may contain billions of bacteria. Most live in the top cover of soil up to 10 cm depth where organic matter or leaf litter is present. Among soil bacteria, *Bacillus subtilis* and *Pseudomonas fluorescens* are acts as the decomposers. Whereas *Rhizobium*, *Azotobacter*, *Agrobacterium* and *Azospirillum* are N₂ fixing bacteria et al. [11]. A fungi species, *Aspergillus niger* has been reported to show biodegradation in plastics and polythene [12]. During the last 2-3 decades, plastic materials have gained widespread use as they have been increasingly used in food, clothing, shelter, transportation, construction, medicine, etc. They are resistant to biodegradation. A general estimate of worldwide plastic waste generation is annually about more than 55 million tons et al. [13]. Kathiresan¹⁴ (2003) has reported that the high diversity of microorganisms in mangrove soil is capable of degrading plastics, at a slower rate.

Currently scanty information is available regarding the effect of silver on growth, reproduction and bioaccumulation of earthworm. Similarly effect of microbial inoculation and *Aspergillus niger* (fungi) on decomposition and degradation of thin polythene of silver coated dishes. Hence, in present study our aim is to find out the effect on growth, nutrient content, bioaccumulation of silver by earthworm and determining the ability of *A. niger* to degrade the thin polythene

II. MATERIAL AND METHODS

Earthworm species, *Eudriluseugeniae* was obtained from State Government agriculture nursery, Sakri, Dist-Dhule (M. S.). In the laboratory, the stock culture was maintained in the plastic container using partially decomposed bio - waste and cow dung as a growth medium. Only healthy adult worm having well developed clitellum were used for the vermicomposting experiment. The silver coated paper dishes were purchased from local market. The natural soil and a month old cow dung (CD) were collected from agriculture field and cow shed respectively.

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Experimental set up

The experiment was performed in small plastic tough having 2 Kg capacities. Each tough contain about 1 kg biowaste including soil. The details of groups were shown in table 1.

Table - 1

Sr. No.	Groups	Composting beds
1	Control	Soil 40% + CD 30% + food waste 20% + 10% Paper
2	Expt 1	Soil 40% + CD 30% + food waste 20% + 10% SCD
3	Expt 2	Soil 40% + CD 30% + food waste 20% + 10% SCD + 2gm Biocatalyst
4	Expt 3	Soil 40% + CD 30% + food waste 20% + 10% SCD + <i>A. niger</i>
5	Expt 4	Soil 40% + CD 30% + food waste 20% + 10% SCD + 20 worms
6	Expt 5	Soil 40% + CD 30% + food waste 20% + 10% SCD + Biocatalyst + <i>A. niger</i> + 20 worms

CD = Cow dung, SCD =Silver coated dishes

All the material in each composting bed was mix thoroughly with water and kept for 60 days. To maintain humidity (75 ± 5 %) water was sprinkled at the interval of 3 to 4 days and the bed was covered by gunny bags to prevent evaporation and protect the worms from predators. Small holes were drilled at the bottom of each tough for good drainage. All the beds were kept under shed to avoid the direct sunlight

Worms counting and cast analysis

At the end of experiment period of 60 days, bed were removed from tough and it was sun dried for 1-2 days by making heaps, all worms going at the bottom. On third day the worms were separated and counted. The results are shown in table - 2. The percent increase in the number of earthworms was determined by the formula [15].given below;

$$\% \text{ increase} = \frac{\text{Earthworm counted} - \text{worms introduced}}{\text{Earthworms counted}} \times 100$$

Similarly the worm cast was collected and subjected for nutrient analysis at Shetjamin laboratory, SatanaDist-Nasik (M.S.). The total nitrogen (N %) was estimated by Kjeldahl digestion and distillation method [16]. Diacid of sample was carried out using concentrated HNO₃ and HClO₄ mixed in 9:4 ratios. The digested extracts were used to determine total K⁺ using Flame photometer [17]. Whereas phosphorus (P) and silver bioaccumulation from soil and earthworm were measured by using atomic adsorption spectrophotometer i.e. AAS [18]. The results were calculated and analyzed statistically and data is presented in the table 2.

III. RESULT AND DISCUSSION

Earthworm counting:Growth, reproduction, nutrient content and bioaccumulation of silver etc are presented in table-2. From this table it was noticed that, the maximum number of worm's i.e 72.22 % was counted in the vermibed E₅, while in the vermibed E₄, the worm number was 65.5 % only. Thus, silver coated dishes along with soil, cow dung and food waste are not significantly affect on growth as well as mortality of earthworms but decrease the reproduction rate. Similarly, worms along with soil and CD shown significant increase in worms; this might be due to microbial inoculation and there must be mutualistic association between earthworms and microbes. Our results are corroborated with early work done by [18,19,20].The combination of three tier systems reduces the time of composting, gives quality biofertilizer and good growth of worms. Suthar and Singh [21].revealedthat when organic waste passesthrough gut of worms some quantity of organic minerals may converted into more exchangeable form. Vermibed containing soil, cow dung and grasses appeared ideal substrate for the growth of earthworm *E. foetida*et al. [22]

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Nutrient content: The data of nutrient content of composting beds are also presented in table 2. In all the composting beds except bed E₁, significant increase in NPK was observed as compare to control, while it was decreased in E₁ bed. This decreased amount might be due to silver coated dishes. No much variation seen in composting bed E₂ and E₃. Maximum NPK seen in the E₅bed containing worms, biocatalyst and *Aspergillusniger*. This might be due to the bioaccumulation of Ag⁺⁺ by earthworm and enhanced microbial growth. Supporting activity of worms, biocatalyst and degradation of thin polythene by *A. niger* probably gives maximum NPK content after 60 days.

Ag bioaccumulation: Earthworm species, *E. eugeniae* found to be good for bioaccumulation of silver in their body. Control bed without silver coated dishes therefore shown Ag⁺⁺ content below detectable level (BDL). In the first experimental bed (E₁) with SCD, the amount of Ag⁺⁺ where found to be maximum (i.e. 0.0202 ppm) than other experimental beds viz., E₂, E₃, E₄and E₅. Reduction in Ag contents in the soil of E₄ and E₅ bed and slightlyincreased or bioaccumulation in earthworm probably indicate role of microbial inoculation. We obtained similar results with previous researchers like Vasanthiet al. [23].

Biodegradation of thin plastic: 100 gm of silver coated thin polythene paper dishes were cut into smaller pieces or strips. They were mixed in the composting bed for 60 days. At the end of the experiment, the strips were taken out and washed properly with double distilled water followed by 70% ethanol. The strips were dried and weighed. The final weight loss were calculated and compared.

The experimental bed E₃shows reduction in weight of thin polythene as compare to E₁E₂and E₄. This might be due to the inoculation of *A. niger*. Similarly maximum weight loss observed in E₅ composting bed; this might be due to the inoculation of *A. niger*, biocatalyst and worms. Probably the worms might be facilitating the growth of microbes.

Our results are corroborated with *A. niger*attach to the surface of the plastic strips and start growing using the polymer as their only carbon source, degrading them into molecular weight monomer and dimmers. *A. niger* formed a fungal mat over the plastic strips and revealed a properly to engulf them Mukharjee and Chatterjee, [24]. They also reported that the fungi, *Aspergillusniger* degrade the low density polythene(up to 5.8%) within a month.

Table-2: Growth, reproduction, nutrient content and bioaccumulation of Ag⁺⁺

Vermibed Group	Worms count			Nutrient Content (%)			Ag % Bioaccumulation		Plastic biodegradation In gms	
	Introduced	Counted	% incr. ease	N%	P%	K%	Soil	Worm	Introduced	Undigested
Control - Soil 40% + CD 30% + food waste 20% + 10% paper	-	-	-	0.45	0.027	369	BDL < 1 ppm	-	100	00
E1 - Soil 40% + CD 30% + food waste 20% + 10% SCD	-	-	-	0.40 (-12.5)*	0.021 (-28/57)**	340 (-8.52)*	0.0202	-	100	38.1
E2 - Soil 40% + CD 30% + food waste 20% + 10% SCD + 2gm Biocatalyst	-	-	-	0.67 (32.83)**	0.038 (28.95)**	509 (27.50)**	0.0186	-	100	28.3
E3 - Soil 40% + CD 30% + food waste 20% + 10% SCD + <i>A. niger</i>	-	-	-	0.68 (33.82)***	0.035 (22.86)**	440 (16.14)**	0.0192	-	100	27.2
E4 - Soil 40% + CD 30% + food waste 20% + 10% SCD + 20 worms	20	58 ± 4	65 ± 5	0.85 (47.05)***	0.040 (32.50)**	700 (47.28)***	0.0119	0.0067	100	21.2
E5 - Soil 40% + CD 30% + food waste 20% + 10% SCD + Biocatalyst + <i>A. niger</i> + 20 worms	20	72 ± 3	71 ± 2	0.89 (49.44)***	0.050 (46.00)***	800 (53.87)***	0.0112	0.0078	100	16.6

Significant value: *P<0.05, **P<0.01, *** P<0.001. Values in the parenthesis are % change over control. CD (Mean ± SEM, n=4)

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IV. CONCLUSION

- Silver coated dishes not affect on growth and mortality of worms but slight decrease in reproduction.
- Silver coated dishes if composted along with soil, cowdung, biocatalyst, *A. niger* and earthworms gives quality biofertilizer.
- The earthworm *E. eugeniae* was found to good for bioaccumulation of Ag⁺⁺ and can reduce the soil toxicity.
- *Aspergillusniger* has potential to degrade the thin polythene.
- It is feasible technology for hotel / restaurant and lawn owners.

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