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Eisenia fetida: A Bioremedial Worm for Sanitary Napkins and Diapers

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Abstract

Sanitary napkins and baby diapers are widely used around the world by menstruating women and babies. Once used, they are either dumped in landfills or incinerated which releases harmful gases such as carbon monoxide into the environment. Such material contains plastic, cotton, and synthetic substances which take hundreds of years to bio-degrade under normal conditions, hence polluting the earth's surface. The present study aims to use the earthworm species *Eisenia fetida* to fasten the process of biodegradation.

Layers of fresh compost (which included waste materials, cow dung slurry, and water, were allowed to decompose for 15 days), cow dung, *Eisenia fetida*, fine compost, hay, used and unused sanitary napkins and diapers, and water were used to set up the experimental setting for degradation. The weights of the napkins and diapers initially and weekly were observed which ultimately decreased. Used diapers were set up for four to five weeks, and their weight decreased from 103g to 17.3g once every seven days, bacteria were isolated from vermicompost and the earthworms' gut by cultivating them on nutrient agar plates. Gram staining was used to further define the bacterial colonies. Gram-positive cocci, bacilli, Gram-negative rods, cocci, and bacilli containing spores and other variety in the bacterial population was seen. According to the results of the current study, vermiremediation is a very efficient, environmentally benign, and zero-waste approach for the disposal of sanitary napkins that can be used widely to reduce environmental contamination caused by them.

Keywords: Biodegradation, bacteria, Eisenia fetida.

1. Introduction

Sanitary napkins and baby diapers are usually made up of cotton, plastic, cellulose, fluff pulp, polyethylene, adhesives, superabsorbent, and release paper. These take hundreds of years to decompose in landfills[1]. The plastic present in them contaminates the soil and may reach underground water bodies. With many disease-causing bacteria, the problems arise due to improper disposal of hygiene products. According to recent statistics, every year around 12.3 billion used sanitary pads and 20 billion pieces of used diapers are dumped into landfills in India alone. The current study aims to use earthworm species *Eisenia fetida* in degrading sanitary napkins and baby diapers by vermiremediation[2].

Eisenia fetida is a species of earthworm that is used both domestically and industrially. These worms are usually 3.5-13cm in length and 0.3-0.5cm in diameter with a flexible body. They are usually reddish brown in color and can vary in gradients. Upon multiplication, they help to quicken the process of biodegradation process efficiently by breaking down decaying natural remains and this is used as high-



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quality organic compost, rich in nutrient matters like nitrogen, carbon, and sulphur. Earthworms are members of the Clitellata subclass within the larger phylum Annelida and are non-microscopic, but rather readily observable organisms. These oligochaete annelids play a crucial role in soil ecosystems worldwide.

Vermicompost is the product of the decomposition process by worms in the presence of several natural ingredients like vegetable peels, fruit peels, cow dung, hay, dry leaf,etc. After the preparation of fresh compost, the worms are added to it and let for decomposition. After a period of 45 days fully formed compost is obtained. This product is used for the rearing of earthworms[3].

Bacterial isolation also helps in the degradation of some of the important bacteria present in Grampositive and Gram-negative species. These help in faster decomposition by enzyme activity. This processaims to use earthworms and bacteria in the process of biodegradation by mimicking natural environments [4].

2. Materials and Methods

Eisenia fetida is a species of earthworm widely found in India. It is commonly known as red wiggler worm or manure worm and is used in the process of vermicomposting[5]. These worms were collected from the Biocentre in Hulimavu, Bengaluru.

Firstly, compost was freshly prepared by adding kitchen waste such as vegetable and fruit peels, hay, wastepaper, dry leaves, and water. These materials were put into a big bin along with cow dung slurry to facilitate the formation of compost. This setup was kept in a shady place and left to decay for a period of 15 days with stirring in between once every 2 days. After the incubation period, a semi-degraded compost was formed and used for further experimental setup. In the current study, sanitary napkins and baby diapers are treated in vermicompost with *Eisenia fetida*. The pads and diapers were procured from Jan aushadhi Kendra. The sanitary pads consisted of cotton, layers of polyethylene, adhesives, synthetic fibers, etc. Similarly, the diapers consist of cellulose, polyethylene, and a super absorbent polymer, as well as tapes, elastics, and other adhesive materials, which take a long time to biodegrade[6, 7].

The morphological study of *Eisenia fetida* was carried out using a wax dissection box by crude method. The photographs of the earthworms were taken and morphologically analyzed. The worm was reddish brown in appearance and was 2-4cm in length, around 3-4mm in width. Next was the experimental setup where a total of six rectangular pots of size 1ft were used, out of which two were used for sanitary napkins, two for diapers, and two for control. Layers of freshly prepared compost, hay, and decomposed compost, along with cow dung slurry were added to the pots. In between these layers used and unused sanitary napkins and diapers were placed. Upon the samples 50- 60g of earthworm were added to each pot, these samples were later covered by compost, hay, and perforated tiles to maintain humid conditions for the earthworms to grow. The controls consisted of only decomposed compost with unused sanitary napkins and diapers, mimicking the conditions in a landfill. These were kept for an incubation period of 4 weeks with weekly checks for the parameters of weight analysis of napkins and diapers along with analysis of microbial diversity in earthworm gut and vermicompost. The weight analysis was done once every 7 days, where the napkins and diapers were carefully removed from the pots and kept for 4 hours of direct sunlight as a part of drying, later the weights were recorded in grams on a weighing machine and compared with the control.



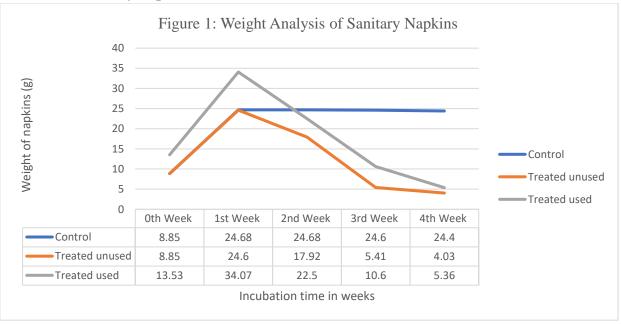
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The examination for microbial activity and diversity included the preparation of 50ml Nutrient agar (NA) for bacterial examination and 50ml Martins Rose Bengal agar (MRBA) for fungal examination, which was poured into two petri plates each for solidification after autoclaving at 121° for 20 mins.

The microbial diversity was checked in earthworm gut and the compost present in pots. This was done once in a week. Earthworms were isolated in a wax dissection box and dissected at the gut region using a sharp sterile blade and forceps. Little amounts of cotton and compost were found inside the gut. With a sterile loop, the material inside the gut was taken and streaked onto the plates, incubating them for 48 hours. Similarly, small amounts of compost were taken from pots and subjected to the process of serial dilution. Serial dilution is a technique that is used to obtain a precise measurement of bacterial or other microbe population in soil samples. 1g of sample was taken, mixed with 10 ml of saline prepared, and then serially diluted further. Later, 1ml of 10⁻³ dilution was spread plated onto NA and MRBA plates. The plates(NA) were incubated in an incubator at 37° for 48 hrs and the MRBA plates were incubated at room temperature. After the incubation period, the plates were checked for bacterial and fungal growth.

3. Results and Discussion

The setup for biodegradation consisted of controls for napkins and diapers (without earthworms), used and unused pads, and diapers. These were initially weighed, and the weights were individually checked in grams every seven days. A sudden increase in weight is seen in 1st week due to the absorption of water present in the compost by the pad and diaper.



Degradation of sanitary napkins

The above graph(Fig1) represents the weekly degradation of control, used, and unused sanitary napkins. The initial weights of control, unused, and used pads are 8.85g, 8.85g, and 13.53g.

The weights in 1st week have increased due to the absorption of water by the cotton in the sanitary pads. In 2nd week, the control weight remains the same as in 1st week, 24.68g.The treated unused napkin was reduced from 24.6g to 17.92g and the treated used napkin was reduced from 34.07 g to 22.5g. In 3rd week, the control weight was reduced to 24.6g. The treated unused napkin reduces from 17.92g to 5.41g

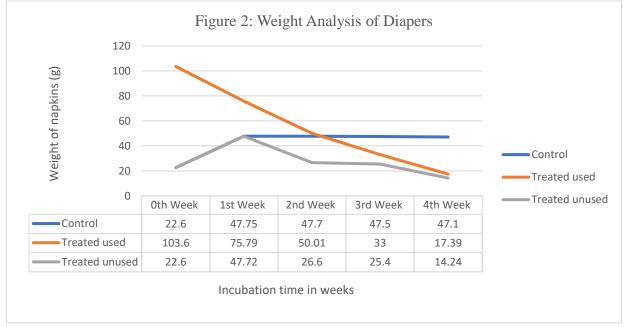


and the treated used napkin reduces from 22.5g to 10.6g. In the final week of incubation, the control napkin weight was reduced to 24.4g. The treated unused napkin was reduced to 4.03g and the treated used napkin was reduced to 5.36g.

The rate of degradation of used and unused sanitary napkins are 84% and 85% respectively, whereas the rate of degradation of control is 1.1%.

The formula for calculating the rate of degradation = (Initial weight- final weight $\times 100$) \div Initial weight.

Degradation of baby diapers



The above graph (Fig 2)represents the weekly degradation of control, used, and unused baby diapers. The initial weights of control, unused, and used diapers are 22.6 g, 103.6g, and 22.6g.

The weights in 1st week have increased due to the absorption of water by the gel in the diapers. In 2nd week, the control weight remains almost the same as in 1st week,47.7g. The treated unused diapers were reduced from 75.79g to 50.01g,and the treated used diapers were reduced from 47.72g to 26.6g. In 3rd week, the control weight was reduced to 47.5g. The treated unused diapers were reduced from 50.01g to 33g and the treated used diapers were reduced from 26.6 g to 25.4g. In the final week of incubation, the control diaper weight is reduced to 24.4g. The treated unused reduces to 17. 39g and the treated used diaper reduces to 14.24 g.

The rate of degradation of used and unused diapers is 77% and 70% respectively, whereas the rate of degradation of control is 2.1%.

Bacteria	Earthworm				Vermicompost			
	1st	2nd	3rd	4th	1st	2nd	3rd	4th
	week	week	week	week	week	week	week	Week
Gram-positive Cocci	+	+	-	-	-	-	-	-
in clusters (GPCC)								
Gram positive Bacilli	-	+	+	+	+	+	-	+

Figure 3: Table Representing the Bacterial Diversity.



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(GPB)								
Gram-positive short rods (GPSR)	-	-	-	-	+	+	+	-
Gram positive Cocci (GPC)	-	-	-	-	-	-	+	+
Gram negative Cocci (GNC)	+	-	-	-	+	-	-	-
Gram negative Bacilli (GNB)	-	+	+	+	-	+	-	+
Gram-negative rods with spores (GNSRS)	+	+	-	-	-	-	-	-
Gram-negative Diplococcus (GND)	-	-	_	-	-	+	-	-

The biodegradation of treated unused and used pads and diapers has been influenced by the action of the earthworm species *Eisenia fetida* in the vermicompost. Along with the earthworms, the bacterial flora present in the environment of the earthworms' gut and the vermicompost have played an important role in determining the rate of degradation of given material by feeding on them[8].

The above table represents the different types of bacteria present at different times during this experiment. It was observed that GNB AND GPB were majorly seen in the process, which may have contributed more than other types of bacteria.

The weight of used sanitary napkins was 34.07g in 1st week, and the weight of unused napkins was 24.6g, which has been reduced to 5.36g and 4.03g, respectively in a period of 4 weeks whereas the control is reduced from 24.68g to 24.4g. Hence the rate of degradation of used and unused sanitary napkins is 84% and 85% respectively, whereas the rate of degradation of control is 1.1%. Similarly, diapers have the rate of degradation of used and unused diapers are 77% and 70% respectively, whereas the rate of degradation of control is 2.1%.

In about four weeks, just a 0.28g and 0.35g weight difference was noticed when comparing the control weights in napkins and diapers, which do not include any earthworms in their setup. With these numbers, it can be assumed safe to mention that the earthworms and bacteria feeding on the raw materials have played a significant part in the degrading of sanitary napkins and diapers along with an increased number of bacterial colonies each week helping in the degradation process[9].

4. Conclusion

The results obtained from this study indicate that *Eisenia fetida* has played a major role in degrading sanitary napkins and diapers by vermicomposting which is a cost-effective zero waste and eco-friendly waste management system. The waste generated after this process can be used as manure for plants as it contains earthworms and compost which help in increasing the fertility of soil. The earthworms which help in degrading sanitary napkins and diapers by feeding on them, convert the fed material into a nutrient-rich substance that is called compost or manure[10]. The bacterial isolates were also found in this process which may have also contributed to degradation. It can be concluded by stating that earthworms and bacteria have played a major role in accelerating the process of degradation which can be used on small and large scale to decontaminate the land and avoid polluting the environment.



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Figure 4: Preparation of fresh compost



Figure 5: Earthworms collected from Biocentre



Figure 6: Initial weight of napkins and diaper



Figure 7: Setup for Vermiremediation



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Figure 8: Dissection of earthworm

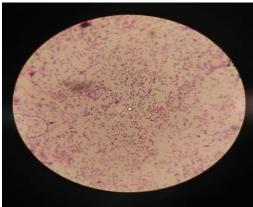


Figure 9: Analysis of Bacterial colonies





Figure 10: Result after 4th week



5. Acknowledgement

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6. References

- 1. Austrian, K., Kangwana, B., Muthengi, E. *et al.*, "Effects of sanitary pad distribution and reproductive health education on upper primary school attendance and reproductive health knowledge and attitudes in Kenya: a cluster randomized controlled trial", *Reprod Health* **18**, 179 (2021).
- 2. Czarnecka, Elżbieta & Walczak, Maciej & Kumar, Gopalakrishnan & Piechota, Grzegorz & Nowaczyk, Jacek, "Degradation of biodegradable diapers as an element of circular economy in waste containing various plastics", Journal of Cleaner Production, (2022).
- Espinosa-Valdemar, Rosa & Turpin-Marion, Sylvie & Delfín-Alcalá, Irma & Vazquez, Alethia., "Disposable diapers biodegradation by the fungus Pleurotus ostreatus", Waste management (New York, N.Y.), (2011). 31. 1683-8. 10.1016/j.wasman.2011.03.007.
- 4. Ferronato, N.; Nova Pinedo, M.L.; Torretta, V., "Assessment of Used Baby Diapers Composting in Bolivia", *Sustainability* **2020**, *12*, 5055.
- 5. lżbieta Czarnecka, Maciej Walczak, Gopalakrishnan Kumar, Grzegorz Piechota, Jacek Nowaczyk, "Degradation of biodegradable diapers as an element circular economy in waste containing various plastics", Journal of Cleaner Production, Volume 377, 2022, 134426.
- 6. Kim, Hyunseong, "A Study on the Utilization of the Earthworms Eisenia fetida and Eisenia andrei for the Disposal of Polymers", International Journal of Environmental Science and Development, (2016).
- 7. Kumari S, Gautam K, Seth M, Anbumani S, Manickam N., "Bioremediation of polycyclic aromatic hydrocarbons in crude oil by bacterial consortium in soil amended with Eisenia fetida and rhamnolipid", Environ Sci Pollut Res Int. 2023 Jul;30(34):82517-82531.
- 8. Mengistu, T., Gebrekidan, H., Kibret, K. *et al.*, "Comparative effectiveness of different composting methods on the stabilization, maturation, and sanitization of municipal organic solid wastes and dried fecal sludge mixtures", *Environ Syst Res* **6**, 5 (2018).



- 9. Park CJ, Barakat R, Ulanov A, Li Z, Lin PC, Chiu K, Zhou S, Perez P, Lee J, Flaws J, Ko CJ., "Sanitary pads and diapers contain higher phthalate contents than those in common commercial plastic products", Reprod Toxicol. 2019 Mar;84:114-121.
- 10. Vavouraki, A.I.; Kornaros, M.,"Vermi-Conversion of Anaerobic Sludges by *Eisenia fetida* Earthworms", *Fermentation* **2023**, *9*, 512.