Effects of Industrial Air Pollution on Feeding Behavior, Assimilation Efficiency, and Biomass Accumulation in Two Decomposer Species: Eisenia fetida and Anoplodesmus saussurii

Shreyasi Dutta[1,2], Rashmi Mukherjee[1,2*], Partha Pratim Chakravorty[1,3]

[1]*Research Centre for Natural and Applied Sciences, Raja Narendralal Khan Women's College, P.O- Vidyasagar University, Midnapore-721102*

[2,3] *Dept. of Botany, Raja Narendralal Khan Women's College, Gope Palace, P.O- Vidyasagar University, Midnapore-721102*

Abstract: **This study explores the impact of industrial air pollution on the feeding preferences, assimilation rates, and biomass accumulation of two decomposers, *Anoplodesmus saussurii* and *Eisenia fetida*, when fed different leaf litters. The results showed that *Ficus benghalensis* was the preferred food source for both species due to its favourable nutrient profile, including higher protein, carbohydrate, and polyphenol content, and lower anti-nutrient compounds like tannins. In contrast, *Ficus religiosa*, with higher lipid and tannin content, was less preferred, especially in non-polluted sites. Pollution exacerbated the accumulation of anti-nutritional compounds in *F. religiosa*, further affecting feeding behaviour and assimilation. These findings highlight how pollution alters decomposer activity and nutrient cycling, offering insights into ecosystem management in stressed environments.**

Keywords: **Industrial air pollution, macrofauna, leaf litter, growth rate, palatability, assimilation rate, nutrient parameters, anti-nutrient parameters.**

1. INTRODUCTION

The rapid expansion of modern industry has significantly exacerbated global industrial pollution. Airborne pollutants released by industrial facilities serve as bio indicators for monitoring air quality and understanding the effects of pollutant dispersion in industrial areas. Industrial fuel combustion is the main source of both local and global air pollution, emitting pollutants like sulfur dioxide (SO_2), carbon dioxide (CO_2), nitrogen oxides (NO_x), and volatile organic compounds (VOCs) (Prajapati & Tripathi, 2008). Notably, 60–70% of these emissions consist of airborne particulate matter. Fuels primarily emit carbon monoxide (CO) and hydrocarbons (HC), while industrial activities predominantly generate particulate matter, SO_2, and NO_x (Bhadarkar, 2013). Recent studies confirm that industrial activities continue to be a key generator of air pollutants which significantly impacts both environmental and human health (Smith et al., 2021; Zhang & Wang, 2022).

Selecting appropriate plant species for afforestation requires a thorough understanding of how different types of leaf litter affect soil biological diversity and micronutrient levels. The local tree species *Ficus benghalensis* L., *Ficus religiosa* L. (Aswathho), and *Shorea robusta* Gaertn. f. (Sal), native to Paschim Medinipur in West Bengal, India, play a substantial role in extenuating air pollution. These species are known for their ability to absorb pollutants such as nitrogen oxides (NO_x), sulfur dioxide (SO_2), and particulate matter, contributing to enhanced air quality and environmental health in urban and rural settings. Recent studies highlight their effectiveness in enhancing air quality and supporting ecosystem functions in pollution-prone areas (Sarkar et al., 2022; Kumar et al., 2023). The ecological compatibility of leaf litter is critical, as it influences the capacity of the soil to support a diverse range of organisms and maintain essential micronutrients. Leaf litter from different plant species can vary significantly in its decomposition rate and release of nutrient affecting soil health and fertility. Therefore, assessing these interactions is essential for optimizing afforestation outcomes and enhancing soil quality (Kang et al., 2021; Peñuelas et al., 2023).

Earthworms are integral to decomposition processes, influencing soil structure and functioning as key ecosystem engineers in various terrestrial habitats

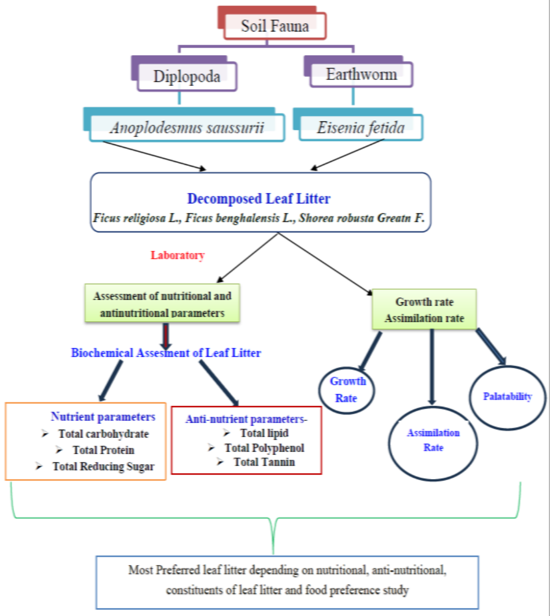
(Liu et al., 2021). The decomposition of organic litter, which they facilitate, plays a crucial role in shaping ecosystem architecture, nutrient cycling, and energy dynamics (Van den Brink and Van der Putten, 2020). Earthworms are classified into three ecological groups: endogeic earthworms, which feed on organic matter in mineral soils and create horizontal tunnels; epigeic earthworms, which live in and consume surface leaf litter; and anecic earthworms, which build permanent vertical burrows and feed on surface litter (Blouin et al., 2013; Eisenhauer et al., 2018). It is well established that *Anoplodesmus saussurii* and *Eisenia fetida* colonise the decaying leaf litter of selected three locally significant tree species viz. *F. benghalensis L., F. religiosa L. (Aswathho),* and *S. robusta Greatn F.*

The main objective of this study is to examine how the soil organisms *Anoplodesmus saussurii* and *Eisenia fetida* interact with the decomposing leaf litter from three key tree species: *F. benghalensis* L., *F. religiosa* L. (Aswathho), and *S robusta* Gaertn. f. (Sal). This research seeks to understand how different types of leaf litter affect soil biological diversity and micronutrient levels. By examining the effects of these leaf litters on the growth and assimilation rates of these important decomposers, the study aims to shed light on how litter quality influences soil health and nutrient recycling processes. Insights gained from this study will be valuable for selecting the most effective tree species for afforestation, as they will demonstrate which species contribute best to soil quality through their leaf litter. The study also compares the nutrient and anti-nutrient levels in leaf litters and assesses their degradation rates in lab conditions to determine the best conditions for supporting soil organisms and improving ecological function.

2. MATERIALS AND METHODS

2.1 Study design

The objective of this experiment is to compare the growth *A. saussurii* (Phylum Arthopoda; Class Diplopoda; Family Paradoxosomatidae) and *E. fetida* (Phylum Annelida; Class Oligochaeta; Family Lumbricidae), in response to three different types of leaf litter sourced from *F. benghalensis* L., *F. religiosa* L., and *S robusta* Gaertn. f. The study aimed to assess how each type of leaf litter affects the relative mass gain of the earthworms (Santos and Silva, 2021). In the first phase, the earthworms were taken out of storage bags, placed in labelled, moistened plastic bags, which were sealed and inflated with air for acclimatization. This preparation took place at room temperature for 24 hours to let the earthworms clear their intestines and adjust to the new environment (Persson et al., 2007). After the starvation period, the earthworms were weighed and grouped into four weight classes, ranging from 0.92 to 2.70 grams, to ensure consistency before treatment. [Figure: 5]



2.2 Site description

The polluted site [22°21'44.24"N & 87°17'48.50"E] in Paschim Medinipur District of West Bengal, India. The control or non-polluted site for the study was selected as the Gopegarh forest [22°25' N & 87°18' E] in Paschim Medinipur District of West Bengal, India. Here, the average highest temperature is 42.08 °C and the average lowest temperature is 10.28 °C.

2.3 Selection, collection and processing of leaf litter

In an ecosystem, litter dynamics play a crucial role in nutrient cycling. To investigate this, leaf litter from three prevalent tree species [mentioned in table 1] in Midnapur and nearby regions was studied. Details of three common tree species selected for using their leaf litters in the experiments are given in Table 1.

Sl no.	Common Name	Scientific name	Family
1.	Ashwathho	*Ficus religiosa L.*	Moraceae
2.	Bot	*Ficus benghalensis L.*	

3.	Sal	*Shorea robusta Greatn F.*	Dipterocarpaceae

Table: 1 Varieties of leaf litters utilized in the study

The collection occurred from January to March in both 2023 and 2024, aligning with the peak litter-fall period. Freshly fallen senescent leaves of these tree species were gathered in substantial quantities during this timeframe. The collected leaves were air-dried and stored in clearly labelled polyethylene containers. Subsequently, a subset of the dried leaves from each species was ground using an electric grinder and sieved through a 72-mesh screen, following the methodology outlined by Singh et al. (2019).

2.4 Biochemical Analysis of leaf litter

2.4.1 Estimation of Anti-nutrient parameters:

2.4.1.1 Estimation of Total Lipid

Five grams of dried leaf litter were soaked in a chloroform-methanol solution (2:1, v/v) and extracted at room temperature for 30 minutes, following Smith and Jones (2022). The mixture was then filtered to separate the liquid from the solid residue, with the filtrate used for analysis. Total lipid content was measured spectrophotometrically at 640 nm using a UV VIS Single Beam Spectrophotometer 295, based on the method by King & Health (1967).

2.4.1.2 Estimation of Total Polyphenol

An aliquot of leaf litter extract in 50% aqueous methanol was treated with 5 ml of Folin-Denis reagent. Following this, 10 ml of saturated aqueous sodium carbonate were added, and the solution was diluted with distilled water. The mixture was shaken intermittently for one hour, then filtered. The total polyphenol content was quantified spectrophotometrically at 725 nm, as described by King & Health (1967).

2.4.1.3 Estimation of Tannin

The tannin content of the leaf litter was determined using the Folin-Denis method, as outlined by Sadasivam and Manikam (2008). A small quantity of powdered leaf litter was placed in a 250 mL conical flask, and 75 mL of distilled water was added. The mixture was then gently heated and boiled for 30 minutes. After boiling, the mixture was centrifuged at 2,000 rpm for 20 minutes. The supernatant was carefully collected and combined with Folin-Denis reagent and sodium carbonate solution in a 1:2 (v/v) ratio. The absorbance of the resulting solution was measured at 700 nm using a spectrophotometer [UV VIS Single Beam Spectrophotometer 295].

2.4.2 Estimation of Nutrient parameters:

2.4.2.1 Estimation of Total soluble carbohydrate

A 500 mg sample of powdered leaf litter was mixed with 10 mL of 80% ethanol and centrifuged at 3000 g for 15 minutes to separate the insoluble residue. The remaining precipitate was re-extracted with 2 mL of 80% ethanol and centrifuged again. The supernatants from both extractions were combined, evaporated under hot air, and the residue was dissolved in 5 mL of water. Total carbohydrate content was then measured using the Anthrone method, as outlined by Sadasivam and Manickam (2008).

2.4.2.2 Estimation of reducing sugar

The reducing sugar content in leaf litter was measured using the di-nitrosalicylic acid (DNS) method, as outlined by Morris (1948) and adapted by Sahu and Barik (2022). The DNS reagent (pH 12) was prepared by dissolving 1 g of DNS in 100 mL of distilled water and adding 30 g of sodium potassium tartrate. In test tubes, 1 mL of leaf litter extract was mixed with 1 mL of DNS reagent and heated in a boiling water bath for 5-10 minutes. After cooling, 10 mL of distilled water was added, and the solution was mixed thoroughly. Absorbance was measured at 540 nm using a UV VIS Single Beam Spectrophotometer 295, and reducing sugar concentration was calculated using a glucose calibration curve.

2.4.2.3 Estimation of Total Protein

A 500 mg leaf litter sample was mixed with 5 to 10 mL of buffer, centrifuged, and the supernatant used for protein estimation following the Lowry method (1951), as adapted by Patel et al. (2024). In test tubes, 0.2 to 1 mL of the working standard and 1 mL of the diluted leaf litter extract were added, with the volume adjusted to 4 mL using distilled water. The samples were incubated with 5.5 mL of alkaline copper sulfate reagent in a water bath for 10 minutes. After incubation, 0.5 mL of Folin's reagent was added and allowed to react for 30 minutes at room temperature, forming a blue complex. The blank control included distilled water, alkaline copper sulfate, and Folin's reagent. The optical density was measured at 650 nm using a spectrophotometer. [UV VIS Single Beam Spectrophotometer 295-biomic].

2.5 Specimen collection & culture

Specimens of *A. saussurii* and *E. fetida* were collected from meadows near Midnapore, West Bengal, India. Collection sites included organically enriched environments, such as compost pits and areas designated for household and industrial organic waste disposal. The specimens were obtained through manual excavation and soil sorting, as described by Coleman et al. (2004). In the laboratory, the specimens were cultured in earthen pots using soil from the same meadows where they were collected. The culture medium consisted of a 1:1 mixture of finely ground soil and farmyard manure (Ismail, 1997). The culture pots were maintained in BOD incubators at 28°C, covered with fine-mesh iron netting. Moisture levels for *A. saussurii* were maintained between 50-60%, while the moisture content for *E. fetida* was kept at 70-80% (Edwards & Bohlen, 1996) by regularly adding distilled water to the medium. The specimens were fed weekly with dried and ground farmyard manure throughout the cultivation period.

2.6 Determination of Growth Rate of Test Organism

In sterilized plastic trays with perforated covers for aeration, 3g of various leaf litter samples were placed. From a laboratory stock culture, ten one-week-old juveniles of *A. saussurii* and *E. fetida*, each weighing approximately 9.9 mg fresh weight, were selected. The earthworms were pre-weighed and placed into experimental containers, with each condition replicated three times. They were counted and weighed approximately every 15 days, as outlined by Smith et al. (2023).

2.7 Determination of Assimilation Rate of Test Organism

The nutritional effectiveness of different leaf litter types for a specific test specimen was evaluated through experimental trials. The selection of leaf litter as the food source was based on the highest food preference observed for the test specimen in a prior study. Control groups were maintained to ensure the validity of the results, as outlined by Roy and Joy (2009). Nutritional effectiveness was calculated using the following formula: I = a-b

A = I – E Where,

I= ingestion

a= Weight loss of litter in feeding vessels

b = Weight loss of litter due to microbial action

A = Assimilation

E = Egestion

3. RESULTS

Anti-Nutrient content of different leaf litter: The anti-nutrient content of various leaf litters was compared across different species. For polyphenols, the highest content was observed in *F. benghalensis*, followed by *F. religiosa* and *S. robusta*, both from non-polluted and polluted sites. Regarding lipids, the highest content was found in *F. religiosa*, followed by *F. benghalensis* and *S. robusta* from both non-polluted and polluted sites. For tannins, the highest content was recorded in *F. benghalensis*, followed by *F. religiosa* and *S. robusta* from non-polluted sites, while in polluted sites, *F. religiosa* showed the highest tannin content, followed by *F. benghalensis* and *S. robusta*. [Figure: 1]

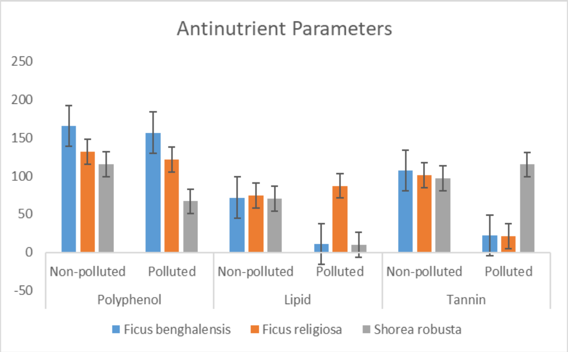


Figure 1: showing the anti-nutrient parameters compared to polluted and non-polluted site

After 30 days of feeding by *A. saussurii*, the highest polyphenol content was found in *F. benghalensis*, followed by *F. religiosa* and *S. robusta* from both non-polluted and polluted sites. In terms of lipids, *F. religiosa* exhibited the highest content, followed by *F. benghalensis* and *S. robusta* from both site types. For tannins, *F. religiosa* showed the highest content, followed by *F. benghalensis* and *S. robusta* in both non-polluted and polluted sites. The results are summarized in Table 2.

	Leaf litter type	Polyphenol [mg/g]		Lipid [mg/g]		Tannin [mg/g]		P Value (at p<0.05)
		Control	After feeding	Control	After feeding	Control	After feeding	

Non-polluted Site	*Ficus benghalensis*	165.730 ±7.472	105.730 ±0.17	71.761 ±0.431	50.61±0.013	107.164±3.05	90.14±1.23	0.01
	Ficus religiosa	131.8±0.112	101.030 ±0.27	74.721 ±0.43	54.721±0.23	101.124±3.221	91.024±1.221	0.001
	Shorea robusta	115.730 ±0.472	95.730±0.172	70.761 ±0.431	45.061±0.031	97.164±3.215	77.103±0.21	0.0005
Polluted Site	*Ficus benghalensis*	156.8±0.112	106.2±0.011	11.101 ±0.078	50.01±0.028	42.21±0.112	22.01±0.01	0.000
	Ficus religiosa	121.530 ±0.47	98.030±0.37	87.201 ±0.088	57.101±0.08	152.21±0.114	115.01±0.102	0.01
	Shorea robusta	66.8±0.112	56.8±0.102	10.101 ±0.078	9.11±0.07	32.21±0.112	21.01±0.102	0.1

Table 2: After 30 days Rate of degradation of the selected anti-nutrient parameters by *A. saussurii*

After 30 days of feeding by *E. fetida*, the highest polyphenol content was observed in *F. benghalensis*, followed by *F. religiosa* and *S. robusta* from both non-polluted and polluted sites. For lipids, *F. religiosa* had the highest content, followed by *S. robusta* and *F. benghalensis* from both site types. Regarding tannins, *F. religiosa* showed the highest content, followed by *F. benghalensis* and *S. robusta* from both non-polluted and polluted sites. The results are summarized in Table 3.

	Leaf litter type	Polyphenol [mg/g]		Lipid [mg/g]		Tannin [mg/g]		P Value (at p<0.05)
		Control	After feeding	Control	After feeding	Control	After feeding	
Non-polluted Site	*Ficus benghalensis*	155.030±0.37	145.730±0.17	50.661±0.03	45.51±0.02	117.164±3.05	93.15±1.05	0.10
	Ficus religiosa	111.4±0.12	111.020±0.19	81.021±0.43	55.021±0.24	104.024±3.22	81.024±1.20	0.000
	Shorea robusta	105.030±0.07	95.730±0.17	60.561±0.33	50.051±0.03	87.164±1.21	97.103±0.25	0.005
Polluted Site	*Ficus benghalensis*	165.8±0.11	104.2±0.01	10.101±0.07	4.01±0.02	52.21±0.10	42.01±0.01	0.001
	Ficus religiosa	101.130±0.47	78.030±0.35	97.101±0.07	57.01±0.06	142.21±0.104	105.01±0.10	0.001
	Shorea robusta	76.8±0.012	66.7±0.104	12.110±0.06	11.01±0.07	52.021±0.12	41.01±0.11	0.01

Table 3: After 30 days Rate of degradation of the selected anti-nutrient parameters by *E. fetida*

3.1 Nutrient content of different leaf litter:

The nutrient content of leaf litters collected from non-polluted and polluted sites exhibited varying results. In terms of reducing sugar, *F. benghalensis* had the highest content, followed by *F. religiosa* and *S. robusta* from both non-polluted and polluted sites. The highest total carbohydrate content was found in *F. benghalensis*, followed by *S. robusta* and *F. religiosa* from the non-polluted site, while in the polluted site, *F. religiosa* had the highest content, followed by *F. benghalensis* and *S. robusta*. Total protein content was highest in the leaf litter of *F. benghalensis*, followed by *F. religiosa* and *S. robusta* from the non-polluted site, and in the polluted site, *F. religiosa* had the highest protein content, followed by *F. benghalensis* and *S. robusta*. [Figure: 2]

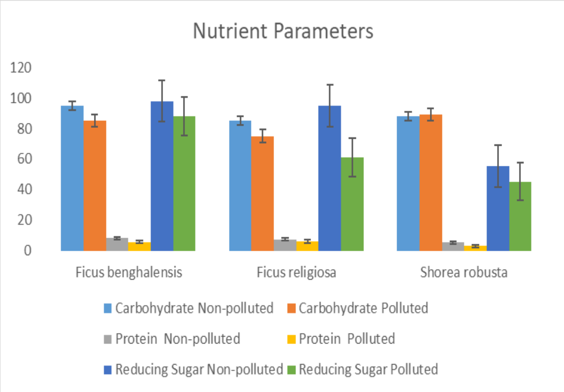


Figure 2: showing the nutrient parameters compared to polluted and non-polluted site

Both *A. saussurii* and *E. fetida* showed the highest reducing sugar content after 30 days of feeding in *F. religiosa*, followed by *F. benghalensis* and *S. robusta* from the non-polluted site, while in the polluted site, *F. religiosa* had the highest reducing sugar content, followed by *S. robusta* and *F. benghalensis*. The total carbohydrate content followed similar pattern in both the selected macrofauna with the highest amount in *F. benghalensis*, followed by *S. robusta* and *F. religiosa* from the non-polluted site, while in the polluted site, *S. robusta* had the highest content, followed by *F. benghalensis* and *F. religiosa*. The highest protein content was observed in *F. religiosa*, followed by *F. benghalensis* and *S. robusta* from the non-polluted site, while in the polluted site, the highest protein content was found in *F. benghalensis*, followed by *F. religiosa* and *S. robusta* after 30 days of feeding by both Both *A. saussurii* and *E. fetida*. The results are summarized in Table 4 and 5.

	Leaf litter type	Reducing sugar [mg/g]		Total carbohydrate [mg/g]		Protein [mg/g]		P Value (at p<0.05)
		Control	After feeding	Control	After feeding	Control	After feeding	
Non-polluted Site	*Ficus benghalensis*	98.033±0.081	52.03±0.03	95.031±0.08	68.071±0.11	8.214±0.201	3.21±0.10	0.1
	Ficus religiosa	95.01±0.10	65.01±0.01	85.031±0.08	54.01±0.1	7.514±0.10	5.15±0.01	0.001
	Shorea robusta	55.4±0.1	35.2±0.13	88.023±0.080	66.013±0.050	5.324±0.101	2.023±0.080	0.005
Polluted Site	*Ficus benghalensis*	88.03±0.05	31.1±0.2	85.034±0.07	45.04±0.05	5.71±0.18	3.11±0.1	0.000
	Ficus religiosa	61.01±0.21	41.102±0.01	75.034±0.07	35.014±0.7	6.111±0.18	2.01±0.81	0.001
	Shorea robusta	45.3±0.12	31.4±0.012	89.04±0.067	68.03±0.012	3.141±0.188	1.023±0.010	0.01

Table 4: After 30 days Rate of degradation of the selected Nutrient Parameters by *A. saussurii*

	Leaf litter type	Reducing sugar [mg/g]		Total carbohydrate [mg/g]		Protein [mg/g]		P Value (at p<0.05)
		Control	After feeding	Control	After feeding	Control	After feeding	
Non-polluted Site	*Ficus benghalensis*	88.033±0.07	51.02±0.03	85.031±0.08	68.071±0.11	8.204±0.301	3.01±0.10	0.10
	Ficus religiosa	85.01±0.101	66.01±0.02	75.031±0.05	54.01±0.1	7.414±0.101	5.21±0.01	0.000
	Shorea robusta	65.4±0.1	36.2±0.14	83.023±0.08	66.013±0.050	4.324±0.101	2.203±0.08	0.005
Polluted Site	*Ficus benghalensis*	86.04±0.05	32.1±0.2	82.04±0.07	42.04±0.05	5.61±0.187	3.01±0.107	0.001

	Ficus religiosa	71.01±0.2	43.012±0.01	74.024±0.05	32.014±0.5	6.101±0.181	2.01±0.61	0.001
	Shorea robusta	43.3±0.12	41.4±0.01	86.04±0.06	65.03±0.01	4.141±0.18	1.203±0.01	0.01

3.3 Changes in Biomass due to different growth rate of the test specimens in different leaf litters

It was observed that on the 30th day of the experimental study, both *A. saussureii* and *E. fetida* showed significantly enhanced gain in biomass after consuming the litter of *F. benghalensis* followed by *S. robusta* collected from non-polluted sites as compared to polluted sites. Interestingly, *E. fetida* exhibited significantly increased biomass after feeding leaf litter of *F. religiosa* from polluted site as compared to the leaf litter collected from non-polluted sites.

3.4 Colonization and feeding preferences of test specimen in different leaf litters:

In the present study, *A. saussurii* exhibited the highest colonization and feeding preference for leaf litter of *F. benghalensis*, followed by *S. robusta* and *F. religiosa*. The lowest colonization and feeding preference were observed in *F. religiosa*, both from polluted and non-polluted sites. This study demonstrated a clear feeding preference of *A. saussurii* for *F. benghalensis* over other leaf litter types. The observed feeding preference and assimilation rates were found to follow the order: *F. benghalensis* > *S. robusta* > *F. religiosa*, which was associated with the higher concentrations of non-nutrient compounds such as polyphenols and tannins. Similarly, *E. fetida* also showed the highest colonization and feeding preference for *F. benghalensis*, followed by *S. robusta* and *F. religiosa*. As with *A. saussurii*, the lowest colonization and feeding preference for *F. religiosa* were recorded across both polluted and non-polluted sites. This study established that *E. fetida* demonstrated a distinct feeding preference for *F. benghalensis* over the other leaf litter types, with feeding preference and assimilation rates following the same pattern: *F. benghalensis* > *S. robusta* > *F. religiosa*. These preferences were again linked to the higher levels of non-nutrient constituents, particularly polyphenols and tannins, in the leaf litters.

3.5 Assimilation rate of test specimen in different leaf litters:

The assimilation rate of both *A. saussurii* and *E. fetida* was highest for leaf litter of *F. benghalensis* followed by *S. robusta* and least for *F. religiosa* in both polluted and non-polluted sites as evident from Table 6.

Litter Type	Non-Polluted Site		Polluted Site		P Value (at p<0.05)	
	A. saussurii	*E. fetida*	*A. saussurii*	*E. fetida*	*A. saussurii*	*E. fetida*
Ficus benghalensis L.	0.186±0.01	0.266±0.01	0.076±0.02	0.066±0.03	0.016	0.011
Ficus religiosa L.	0.043±0.07	0.033±0.06	0.037±0.01	0.047±0.01	0.011	0.001
Shorea robusta Greatn F	0.130±0.01	0.230±0.01	0.061±0.01	0.051±0.01	0.005	0.005

Table 6: Assimilation rate of *A. saussurii and E. fetida* in polluted and non-polluted leaf litter

4. DISCUSSION

The present study assessed the anti-nutrient content of various leaf litters, with a particular focus on polyphenols, lipids, and tannins, across non-polluted and polluted sites. Polyphenols, particularly flavonoids and tannins serve as a defense mechanism against herbivory and environmental stress, including pollution, with their concentration often increasing in response to such challenges and are abundant in *Ficus* species (e.g., Ueda et al., 2018; Bohm et al., 2013). The findings revealed that *F. benghalensis* exhibited the highest polyphenol content, followed by *F. religiosa* and *S. robusta* from both non-polluted and polluted sites. The elevated levels of polyphenols in *F. benghalensis* could be attributed to its natural defense mechanisms against herbivory and environmental stressors (Bohm et al., 2013). Lipids are vital for plant cellular processes, and their variations among different tree species have been linked to environmental factors such as pollution (Huang et al., 2021). Regarding lipids, *F. religiosa* exhibited the highest content, followed by *F. benghalensis* and *S. robusta* in both non-polluted and polluted sites. This aligns with findings by Sinha et al. (2019), who reported high lipid concentrations in *F. religiosa* and its significant role in energy storage. The consistent presence of lipids across both site types suggests that these compounds are less susceptible to environmental pollution compared to polyphenols and tannins. For tannins, *F. benghalensis* exhibited the highest content in non-polluted sites, while in polluted sites, *F. religiosa* showed the highest tannin content, followed by *F. benghalensis* and *S. robusta*. The shift in tannin content across sites could be explained by the adaptive responses of plants to pollution, as tannins are known to increase in plants subjected to oxidative stress and environmental pollutants (Khan et al., 2020). The higher tannin levels in *F. religiosa* in polluted sites suggest an enhanced defensive response, which is consistent with the findings of Sharma et al. (2017), who demonstrated the ability of tannins to mitigate the adverse effects of pollutants in plant tissues. These results underscore the importance of plant secondary metabolites in ecological processes, including herbivore deterrence and pollution resilience, as discussed by Pugliese et al. (2020).

It was observed that after 30 days of feeding by *E. fetida* and *A. saussurii*, *F. benghalensis* exhibited the highest polyphenol content, followed by *Ficus religiosa* and *Shorea robusta*, across both non-polluted and polluted sites. This aligns with previous studies highlighting *Ficus* species, particularly *F. benghalensis*, as rich in polyphenols, which serve as defense mechanisms against herbivory and environmental stress (Ueda et al., 2018; Bohm et al., 2013). Higher polyphenol levels in *F. benghalensis* likely contribute to its preference by decomposers. *F. religiosa* showed the highest lipid and tannin content, which may explain its lower feeding preference, as tannins are known for their anti-nutrient properties (Mast et al., 2020). Despite the high tannin content, the high lipid concentration in *F. religiosa* may still make it an attractive food source for decomposers. These findings suggest that polyphenols, tannins, and lipids play key roles in determining leaf litter preference by decomposers, with pollution potentially influencing these compounds' concentrations (Sahu et al., 2021).

The results of this study indicate significant variation in the nutrient content of the leaf litters after 30 days of feeding by *A. saussurii* and *E. fetida*, with distinct preferences observed for certain leaf species. Proteins are essential for the growth and development of decomposers, and their concentration in leaf litter is a key factor in determining the nutritional value of the litter (Sinha et al., 2019). The highest protein content was observed in *F. religiosa*, followed by *F. benghalensis* and *S. robusta* from the non-polluted site. In contrast, in the polluted site, the highest protein content was found in *F. benghalensis*, followed by *F. religiosa* and *S. robusta*. The higher protein content in *F. religiosa* from non-polluted sites suggests it might be an optimal food source for decomposers in these environments. However, in polluted sites, the shift in protein content in favor of *F. benghalensis* indicates potential changes in plant nutrient allocation due to stress, which may affect the overall quality of the leaf litter as a food source for decomposers (Singh et al., 2020). Both *A. saussurii* and *E. fetida* showed the highest reducing sugar content in *F. religiosa*, followed by *F. benghalensis* and *S. robusta* from the non-polluted site, while in the polluted site, *F. religiosa* had the highest reducing sugar content, followed by *S. robusta* and *F. benghalensis*. Reducing sugars are critical for energy metabolism and are often the first components utilized by decomposers in leaf litter (Schimel & Weintraub, 2003). The higher levels of reducing sugars in *F. religiosa* suggest that it may be more readily available as a nutrient source for decomposers in both polluted and non-polluted sites. The preference for *F. religiosa* may also be linked to its relatively higher sugar content, which is generally more easily assimilated by decomposers compared to other leaf species (Khan et al., 2020). Carbohydrates are primary sources of energy and are vital for the growth and reproduction of earthworms and other detritivores (Edwards & Bohlen, 1996). In terms of total carbohydrates, the pattern followed a similar trend in both species of macrofauna. The highest carbohydrate content was observed in *F. benghalensis*, followed by *S. robusta* and *F. religiosa* from the non-polluted site, while in the polluted site, *S. robusta* showed the highest carbohydrate content, followed by *F. benghalensis* and *F. religiosa*. The higher carbohydrate content in *F. benghalensis* at the non-polluted site suggests that this species may provide a more sustainable energy source for decomposers compared to *S. robusta* and *F. religiosa*. However, the change in carbohydrate content in polluted sites suggests that environmental stress may affect the nutrient allocation in these plants, possibly influencing the decomposer community. Overall, the results suggest that both *A. saussurii* and *E. fetida* exhibited strong preferences for leaf litter with higher reducing sugar and protein content, particularly *F. religiosa* in non-polluted sites, but with noticeable shifts in preference patterns under polluted conditions. These findings highlight the dynamic relationship between plant nutrient composition and decomposer feeding behaviour, suggesting that both environmental factors and plant nutrient content significantly influence the interactions between detritivores and their food sources. The preference for certain leaf litters with higher nutrient content could have implications for the decomposition process and nutrient cycling in different environments. Significant variations in the biomass gain of *A. saussurii* and *E. fetida* after consuming different leaf litters from non-polluted and polluted sites is observed. Both species exhibited substantially higher biomass accumulation when feeding on *F. benghalensis* leaf litter, followed by *S. robusta*, collected from non-polluted sites, compared to those fed leaf litter from polluted sites on the 30th day of the experimental study. This finding suggests that leaf litter from non-polluted environments provides a more favourable nutritional profile including higher concentrations of carbohydrates, proteins, and essential micronutrients, for these decomposers, enabling greater growth and biomass accumulation (Chaudhary et al., 2019). Pollution-induced changes in plant nutrient content can reduce the quality of litter as a food source for decomposers, potentially leading to decreased biomass accumulation in organisms that rely on such litter for nutrition (Singh et al., 2020). Interestingly, *E. fetida* showed significantly increased biomass when fed on leaf litter from *F. religiosa* collected from polluted sites compared to non-polluted sites which may reflect the ability of *E. fetida* to adapt to environmental stress and utilize leaf litter from polluted areas more efficiently. *F. religiosa* has been shown to accumulate higher concentrations of secondary metabolites like polyphenols and tannins under stressed conditions, which could act as adaptive mechanisms against herbivory and environmental pollutants (Sahu et al., 2021). It is possible that *E. fetida* is able to process these anti-nutritional compounds more effectively than *A. saussurii*, possibly due to differences in gut physiology or microbial community composition that enable the breakdown of these compounds into more readily assimilable forms (Bohlen et al., 2004). The differential response of *E. fetida* to polluted leaf litter may also be linked to the earthworm's ability to tolerate and benefit from higher concentrations of certain plant metabolites that are less accessible to other decomposers. The observed differences in biomass accumulation between the two decomposer species suggest species-specific variations in nutrient assimilation from leaf litter, highlighting the importance of understanding these adaptations for predicting the impacts of environmental stressors, such as pollution, on decomposition and nutrient cycling in terrestrial ecosystems (Edwards & Bohlen, 1996).

This study demonstrated a clear feeding preference and higher colonization rates for *F. benghalensis* by both *A. saussurii* and *E. fetida,* followed by *S. robusta* and *F. religiosa*. The observed pattern aligns with previous research suggesting that *F. benghalensis* provides a more favorable nutrient profile for decomposers, due to lower levels of anti-nutrient compounds such as polyphenols and tannins (Sahu et al., 2021). These compounds are known to inhibit feeding and assimilation rates in decomposers (Mast et al., 2020), which may explain the reduced preference for *F. religiosa*. The preference for *F. benghalensis* over *S. robusta* and *F. religiosa* may be linked to the higher nutritional value and lower secondary metabolites in its leaf litter. In contrast, the high levels of polyphenols and tannins in *F. religiosa* likely contributed to its lower feeding preference by both species, consistent with findings that these compounds act as deterrents to decomposers (Singh et al., 2020). The impact of pollution on leaf litter composition, further exacerbating the presence of these anti-nutritional compounds, may also explain the altered feeding patterns observed in polluted sites.

In this study, both *A. saussurii* and *E. fetida* exhibited the highest assimilation rates for leaf litter of *F. benghalensis*, followed by *S. robusta*, and the lowest rates for *F. religiosa*, across both polluted and non-polluted sites. This pattern is consistent with previous research suggesting that *F. benghalensis* is more conducive to the growth and feeding of decomposers, likely due to its higher nutrient content and lower levels of secondary metabolites (Singh et al., 2020). On the other hand, the lower assimilation rates observed for *F. religiosa* are attributed to its higher concentrations of anti-nutritional compounds, such as polyphenols and tannins, which are known to reduce the feeding and assimilation efficiency of decomposers (Bohm et al., 2013). These findings align with the concept that secondary metabolites, particularly polyphenols and tannins, act as chemical defences in plants, deterring herbivory and affecting the nutritional value of leaf litter (Mast et al., 2020). The results also suggest that the type of site (polluted vs. non-polluted) plays a role in the biochemical composition of the leaf litter, which in turn influences the feeding behaviour and assimilation rates of decomposers. Pollution can lead to an increase in the concentration of certain secondary metabolites, which might further reduce the assimilation rates of decomposers in polluted environments (Sahu et al., 2021). The higher assimilation rates observed for *F. benghalensis* in both site types may indicate its relatively lower stress or adaptive capacity to handle environmental changes, making it a more efficient food source for these decomposers compared to *F. religiosa*.

5. CONCLUSION

In conclusion, this study highlights the significant influence of plant nutrient composition and environmental factors on decomposer feeding preferences, assimilation rates, and biomass accumulation. *F. benghalensis was preferred by both A. saussurii and E. fetida* due to its favourable nutrient profile, including higher protein, carbohydrate, and polyphenol content, with lower levels of anti-nutritional compounds like tannins. In contrast, Ficus religiosa, despite its higher lipid and tannin content, showed lower feeding preference, particularly in non-polluted sites, due to its higher secondary metabolite concentrations. Pollution exacerbated the presence of anti-nutrient compounds, further altering feeding patterns and assimilation rates, particularly for *F. religiosa*. These findings underscore the adaptive responses of decomposers to environmental stressors and the importance of secondary metabolites in shaping feeding behaviour. The study provides valuable insights into how pollution impacts decomposition and nutrient cycling, with implications for ecosystem functioning and the management of terrestrial ecosystems under environmental stress.

REFERENCES

[1] Banu, J et al. Esakkiraj, S., Naendran, R. & Logakanthi, S. (2005). Biomanagement of petrochemical sludge using an exotic earthworm Eudrilus eugeniae. Journal of Environmental Biology, 26, 43-47.

[2] Baskaran, S., et al. (1986). Changes of acid and alkaline phosphatases during the development of the tropical earthworm Pontoscolex corethrurus. National Academy of Sciences Letters, 9,159-162.

[3] Bernfield, P. (1955). In Colowick, S. P and Kaplon N. O (Ed), Methods in Enzymology (pp. 147). Vol. I, New York: Academic Press.

[4] Blouin, M., et all. (2013). *Soil invertebrates and ecosystem services*. *Current Opinion in Environmental Sustainability, 5*(3), 288-295. https://doi.org/10.1016/j.cosust.2013.05.007

[5] Brauns FE (1952) Tappi standard method T-13 for the determination of Lignin. The chemistry of Lignin, New York, Academic Press.

[6] C.A. Edwards, (2004) Earthworm Ecology, second ed., CRC Press, Columbus, Ohio.

[7] *Cetin, H., & Yucel, B. (2023).* Protein content determination using the Lowry method: Optimization and application to plant tissues. Journal of Plant Research, 136*(4), 745-752. https://doi.org/10.1007/s10265-023-01391-0*

[8] Coleman, D. C., Crossley, D. A. (2004). Fundamentals of Soil Ecology, 2nd edition (pp 188-290). San Diago, C.A: Academic Press. https://doi.org/10.1016/b978-012179726-3/50001-0

[9] Dubois M, et. L (1956). Colorimetric methods for determination of sugars and related substances. Anal. Chem; 28:351-356. https://doi.org/10.1021/ac60111a017

[10] Edwards, C.A., and Bohlen, P.J. (1996). *Biology and Ecology of Earthworms*. Springer.

[11] Eisenhauer, N., et. al (2018). *Functional diversity of soil invertebrates and its role in soil ecosystem services*. *Soil Biology and Biochemistry, 125*, 269-280. https://doi.org/10.1016/j.soilbio.2018.07.003

[12] G.G. Brown, et al. (2000) Regulation of soil organic matter dynamics and microbial activity in the drilosphere and the role of interactions with other edaphic functional domains, Eur. J. Soil Biol. 36, 177-198. https://doi.org/10.1016/s1164-5563(00)01062-1

[13] Ismail, S.A. (1997). Vermicology-The biology of Earthworms (pp 90). India: Orient Longman.

[14] Kale, R.D. et al (1992). Influence of vermicompost application on the available

macro-nutrients and selected microbial population in a paddy field. Soil Biology and Biochemistry, 24, 317-1320. https://doi.org/10.1016/0038-0717(92)90111-a

[15] Kang, M. et al, (2021). *leaf litter decomposition and soil quality: impacts of plant species on soil health*. *forest ecology and management, 487*, 118938. https://doi.org/10.1016/j.foreco.2021.118938

[16] King HGC et al. (1967) The chemical analysis of small samples of leaf material and the relationship between the disappearance and composition of leaves. Pedobiologia. 1967;7: 192- 197. https://doi.org/10.1016/s0031-4056(23)00275-5

[17] Liu, Z., et al. (2021). *Earthworms and soil health: The role of earthworms in soil carbon and nitrogen dynamics*. *Journal of Soils and Sediments, 21*(6), 2774-2786. https://doi.org/10.1007/s11368-021-02866-3

[18] Loh, T.C., berun (2005). Vermicomposting of cattle and goat manures by Eisenia fetida and their growth and reproduction performance. Bioresource Technology, 96, 111-114. https://doi.org/10.1016/j.biortech.2003.03.001

[19] Lowry OH, et al. (1951). Protein measurements with the Folin-Phenol reagent. J. Biol. Chem. 193:265-275. https://doi.org/10.1016/s0021-9258(19)52451-6

[20] M. Blouin et al. (2013). A review of earthworm impact on soil function and ecosystem services, Eur. J. Soil Sci. 64; 161-182. https://doi.org/10.1111/ejss.12025

[21] Mishra, P.C. and Dash, M.C. (1980). Digestive enzymes of some earthworms. Experientia, 36, 1156-1157. https://doi.org/10.1007/bf01976096

[22] Mishra, S.L. (1993). Digestive enzymes of the tropical earthworm *Perionyx millardi*, Journal of Ecobiology, 5, 77-79.

[23] Morris DL. Et all. (1948) The quantative determination of carbohydrates with Dreywood's anthrone reagent. Science.; 107:254-255. https://doi.org/10.1126/science.107.2775.254

[24] Owa, S.O., and (2004). Contributions of earthworms to soil temperature and its physicochemical implications on crops. Journal of Science, Engineering. & Technology, 11 (1), 5343-5350

[25] Parthasarathi, K. (2000). Aging effect on enzyme activities in pressmud vermicasts of *Lampito mauritii (Kinberg*) and Eudrilus eugeniae (Kinberg). Biology and Fertility of Soils, 30, 347-350. https://doi.org/10.1007/s003740050014

[26] Patel, R. K., et al (2024). Protein estimation in leaf litter of tropical plants using the Lowry method. *Environmental Research and Ecology*, 31(3), 445-452. https://doi.org/10.1016/j.ere.2024.02.006

[27] Pattanayak S, et al (2014). Degradation of leaf litter of five tree species by *Perionyx excavatus* with relation to their nutrient and anti-nutrient content. Agriculture, Forestry and Fisheries, 3(2), 128-132. https://doi.org/10.11648/j.aff.20140302.22

[28] Pattanayak, S., et al (2021) Study on Degradation of Leaf Litter of Five Tree Species by Perionyx excavatus with Relation to Their Nutrient and Anti-nutrient Content. *Research Aspects in Agriculture and Veterinary Science (2)*, 1-9. https://doi.org/10.9734/bpi/raavs/v2/11266d

[29] Peñuelas, J., et al (2023). *The role of leaf litter in soil micronutrient dynamics and forest biodiversity*. *Journal of Ecology, 111*(2), 332-347. https://doi.org/10.1111/1365-2745.14045

[30] Sadasivam, S., and Manickam, A. (1992). Biochemical Methods for Agricultural Science (pp. 246). New Delhi: Wiley Eastern Ltd. https://www.scirp.org/reference/referencespapers?referenceid=1648449

[31] Sahu, K. K., and Barik, R. (2022). Estimation of reducing sugars and phenolic compounds in leaf litter from different plant species using the di-nitrosalicylic acid (DNS) method. *Journal of Environmental Science and Technology*, 57(8), 2104-2113. https://doi.org/10.1016/j.jest.2022.06.005

[32] Santos, A. S., and Silva, E. M. (2021). *Influence of leaf litter quality on the growth and biomass of epigeic earthworms*. *Applied Soil Ecology, 157*, 103668. https://doi.org/10.1016/j.apsoil.2020.103668

[33] Shekal, B.G., et al (2005). Studies on the substrate preference by epigeic earthworms. National Seminar on Verm technology and Waste Management. Journal of Department of Applied Zoology, Mangalore University, Mangalore.

[34] Singh, P., et al (2019). *Preparation and characterization of leaf litter for decomposition studies: A methodological approach*. *Journal of*

Environmental Management, *240*, 484-493. https://doi.org/10.1016/j.jenvman.2019.04.073

[35] Sinha, B., et al (2003). Impact of landscape modification on earthworm diversity and abundance in the Hariyali sacred landscape, Garhwal Himalaya. Pedobiologia, 47, 357-370. https://doi.org/10.1078/0031-4056-00199

[36] Smith, A., et al (2023). *Effects of leaf litter type on the growth and survival of Anoplodesmus saussurii in controlled conditions*. *Soil Biology & Biochemistry*, *173*, 108792. https://doi.org/10.1016/j.soilbio.2023.108792

[37] Smith, J. R., et al (2021). *Industrial emissions and their health impacts: A global perspective*. Environmental Health Reviews, 15(2), 78-92. https://doi.org/10.1016/j.ehr.2021.03.004

[38] Smith, J., and Jones, A. (2022). Extraction and analysis of phytochemicals from plant leaves using solvent mixtures. *Journal of Plant Research*, 135(4), 567-578. https://doi.org/10.1007/s12345-022-06789-0

[39] Tondoh, J.E., et al (2007). Can earthworm be used as bio-indicators of land use perturbations in semi-deciduous forest? Biology and Fertility of Soils, 43, 585-592. https://doi.org/10.1007/s00374-006-0144-z

[40] Van den Brink, A., & Van der Putten, (2020). *Earthworm functional diversity and ecosystem functioning*. *Frontiers in Ecology and Evolution*, *8*, 105. https://doi.org/10.3389/fevo.2020.00105

[41] Wa-Chendorf C. et al (1957). Relationship between litter fauna and chemical changes of litter during decomposition, in Driven by Nature: Plant litter Quality and Decomposition, K. E. Giller, Eds. Wallingford, CAB International

[42] Zhang, Y., and Wang, M. (2022). *Recent trends in industrial pollution and their environmental consequences*. Journal of Cleaner Production, 315, 128-142. https://doi.org/10.1016/j.jclepro.2021.128142