

Xenobiotic Bioconcentration in Terrestrial Oligochaetes to Evaluate Soil Quality

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ABSTRACT:

Excessive use of pesticides, heavy metals, and detergents may have negative effects on non-target organisms in soil and water and may seriously harm ecosystems. A total of 181 earthworm specimens from various habitats were gathered from three separate locations, namely the Anthikkad and Chalakudi locality in the Thrissur district and the Meenangadi locality in Wayanad. We were able to identify four different earthworm species which belonged to two distinct families and four different genera. Thereafter from this study it was clear that the Density of different species of earthworm (*P. sansibaricus* and *L. mauritii*) collected from study areas are same. Pb was found to be undetectable in earthworm samples from study sites out of the four pollutants evaluated. Earthworm samples from Anthikkad and Meenangadi sites had the greatest levels of the heavy element Zn. Of the 32 pesticides tested, 4 organochlorine pesticides and 4 organophosphorus pesticides were found to be at in samples collected from two different districts. By statistical analysis it was concluded that soil samples from all the sites (As, Cs & Ms) were homogeneous. It is not essential to be exposed to a large concentration of xenobiotics for the bodily tissues to become toxic; by extending the exposure period, the concentration can rise to dangerous levels. One more sensitive and early-warning biomarker of ecosystem health is the observation of earthworm immunological capability.

Keywords:

Megascolecidae, Glossoscolecidae, Organochlorine pesticides, Organophosphorus pesticides, lead (Pb), cadmium (Cd)

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INTRODUCTION

Due to the widespread use of agropesticides, soil pollution is a problem in many nations throughout the world. The specific xenobiotic concentration present and its persistence define the impact of agrochemicals on the community or its constituent populations. Heavy metals, pesticides, detergents, etc. used in excess can have an adverse effect on non-target organisms in soil and water that may seriously harm ecosystems. Some of the chemicals which are used in great quantities in our country are those which have been banned long time back by many foreign countries. For that reason, it is important to properly assess xenobiotic risks to the soil community, and such an assessment should be part of a system of sustainable agriculture.

Organic matter breakdown and nutrient cycling in terrestrial ecosystems are crucially influenced by soil organisms including invertebrates and microbes. Ecological clusters of soil organisms within the network were critical in maintaining soil ecosystem multifunctionality (Yi-Fei et al., 2022). For the assessment of anthropogenic risk among terrestrial invertebrates, earthworms are the highest priority sentinel creatures (Šrut, 2022). In the soil compartment, earthworms and plants have frequently been employed as models for biomarker studies because of how quickly they adapt to changes in the ecosystem (Atli, Alptekin, Tükel, and Canli, 2006) and also significantly improve soil fertility. After being digested by earthworms, the soil goes through a variety of changes in the worm's gut. Several workers have examined how various pesticides affect the bodies of earthworms (Migliani and Bisht, 2019; Kaka, Opute and Maboeta, 2021). A consistent approach was used in some recent research to examine the impact of pesticides on soil invertebrates in tropical environments (Aja, Jaya, Vijayakumaran, and Reynolds, 2012).

Perionyx sansibaricus (*P. sansibaricus*) showed maximum biomass production. *Lampito mauritii* (*L. mauritii*) is commonly found in Kerala and Tamil Nadu, India. It has a long and cylindrical narrow body which is bilaterally symmetrical. *L. mauritii* is 80 to 210 mm in

length with a diameter of 3.5 – 5 mm, and is light brown in colour, with purplish tinge at the anterior end. The role of earthworm *Lampito mauritii* (Kinberg) in amending lead and zinc treated soil is already established (Maity, Padhy and Chaudhury, 2008). In Indian soils *L. mauritii*, *P. sansibaricus*, *P. excavatus*, *Dichogaster bolau*, *Amyntas morrisi*, and *Drawida willsi* are very common and their composting potential is well established (Singh, 1997, Gajalakshmi, Ramasamy and Abbasi (2002), Tripathi and Bhardwaj, 2004).

Earthworms can gather and concentrate huge amounts of inorganic and organic contaminants (Guhra, Stolze, Schweizer and Totsche, 2020) including bioconcentration of heavy metals, pesticides (Katagi and Ose, 2015) and are easy to handle, have a wide geographic distribution, and have effective detoxifying systems for their survival (Sanchez-Hernandez, Narváez, Cares, Sabat and Naidu, 2023). In addition to being a prominent food source for birds, other animals, and invertebrates, earthworms are crucial in the amplification of soil contaminants. Many xenobiotics can penetrate soils and ground water, transfer through food webs, bioaccumulate and negatively impact biota, especially soil microbes (Gall, Boyd, and Rajakaruna, 2015). According to earlier studies, earthworms use sophisticated chemosensory perception to locate and move towards food sources as well as to locate and avoid potentially dangerous surroundings (Hirano and Tamae, 2011).

Heavy metal exposure and environmental contamination are becoming increasingly problematic on a global scale. The epidermis of earthworms is an important pathway for pollutant uptake, according to several studies (Sanchez-Hernandez, 2006; Turgay, Kizilkaya, Namlı and Camci Cetin, 2011). The presence of toxicity in the bodily tissues does not require exposure to large concentrations; instead, toxic concentrations can be reached by extending the exposure period. In order to quickly conduct agrochemicals during downpours, earthworm burrows can act as preferential flow pathways. The current effort aims to evaluate the effects of pollutants on the population density

and diversity of earthworms gathered from certain flood-affected areas where earthworms have died in mass. The study also emphasises the buildup of pollutants including heavy metals in the body of field collected earthworms by assessing Bio Concentration Factor and also tried to analyze the selected pesticide concentration namely, organochlorine (OC) and organophosphorus (OP), in the earthworm body.

MATERIALS AND METHODS

Description of Location and sample

Two sites selected for the study include, Anthikkad (A) and Chalakudi (C) from Thrissur District and one site from Meenangadi (M) from Wayanad District. Anthikkad is abundant with Koal wetland (10.4531 N and 76.1146 E) mainly emphasis in paddy cultivation and Chalakudi (10.3070 N and 76.3341 E) accommodating Chalakudy river well known for its fish diversity. Meenangadi (11.6596 N and 76.1726 S) in Wayanad has been designated as a carbon neutral panchayat that promotes zero-carbon development and is considered one of Kerala's climate change hotspots. The earthworms can be gathered from these two districts including variety of ecosystems, coconut plantations, banana plantations, vegetative sites, residential areas, and coal fields. The taxonomic keys were

used to identify adult earthworms. For analysis, adult clitellated earthworms weighing 300–600 mg on average were chosen. The collected worms may be stored in 10% formaldehyde solution, and updated taxonomic keys must be used for morphological features-based taxonomic identification. The essential field information, including habitat description, location, date of collection, and the numbers of organisms collected, were recorded. All species may be serially numbered. Each sample collected should be assigned a code. All the sample specimens are placed in the Zoology Museum of Sree Krishna College, Guruvayur and Sree Kerala Varma College, Thrissur.

Earthworms are gathered using the traditional approach of hand-digging and separating them for population research. The worms are collected, and an estimate of the earthworms per square meter at each location is made (Table 1). The test samples were given to the Soil Science Department of the Kerala Forest Research Institute at Peechi, Thrissur for heavy metal analysis and the Central Instrumentation Laboratory, Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur for pesticide concentration analysis (Organochlorine: OC and Organophosphorus: OP).

Table 1: Showing sample collection details four different locations

Sl. No.		Sample details		
		Code	Nature of land Wet lands	Description of collection sites
Thrissur	1	TAS ₁₉	Flood affected	Nearby sites Wetland of site Anthikkad
	2	TAS ₁₉	Flood affected	Nearby residential areas of Anthikkad
	3	CTPS ₈₁	Flood affected	Thachuda Parambu, Paddy field, Banana and Coconut Plantations
	4	CPPS ₂₄	Flood affected	Puthu Parambu Padam, Paddy Field
Wayanad	5	WMS ₂₀	Flood Affected	Cultivated land, Near lake area Wayanad
	6	WMS ₁₈	Flood Affected	Paddy fields Wayanad

TAS- Sample collected from Anthikkad, CTPS- Sample collected from Chalakudy, WMS- Sample collected from Wayanad

Experimental protocol

Analysis of Pesticides

32 Pesticides under two broad categories are yet to be analyzed include Organo Chlorine (OC) pesticide: The concentration of 18 different Organo chlorine (OC) pesticides selected for analysis include Hexachlorobenzene, α -Lindane, γ Lindane, Heptachlor, Heptachlor epoxide, Trans chlordane, Aldrin, BHC, cis chloridane, endosulphan-1, endosulphan-2, Endrin, Endosulfan sulfate etc. and 14 different Organo phosphorus (OP) pesticides selected include Diazinon, Isazophos, Chlorpyrifos, Chlorpyrifos- methyl, Phosmet, Azinphos, Pyrazaphos etc.

Analysis of heavy metals

For the analysis five heavy metals lead, Cadmium, Copper, Nickel and Zinc are selected. These may be analyzed by using AAS method.

Calculation of Bio Concentration factor

Bioconcentration Factor (BCF) for earthworms collected from different sites (samples from field) were estimated for metals in earthworm tissues and substrate materials (soils) using the method described by Mountouris, J. E., Norey, C.G., Morgan, A. J. and J. Kay, (2002). The BCF is a quotient calculated as follows: $BCF = C_{biota}/C_{substrate}$, where C_{biota} and $C_{substrate}$ are the total concentrations (in mg/kg) in taxa (earthworm) and substrate (soil),

respectively. All the data obtained were statistically analyzed.

RESULTS AND DISCUSSION

A total of 181 earthworm specimens were gathered from various habitats in the study area representing two different districts (Thrissur and Wayanad), including coconut plantations, residential areas, banana plantations, vegetation sites, and vegetation sites. We were able to distinguish six different earthworm species from two separate families which belongs to four different genera as a consequence of this investigation (Table 2). There are 33 species in the family Glossoscolidae, which represents the single genus *Pontoscolex*, and 148 species in the family *Megascolecidae*, which represent the three genera *Megascolex*, *Perionyx*, and *Lampito*. The recognized worms are *Pontoscolex corethrurus* (*Pontoscolex*), *Megascolex cochiniensis* and *Megascolex konkanensis* (*Megascolex*), *Perionyx excavates* and *Perionyx sansibaricus* (*Perionyx*) and *Lampito mauritii* (*Lampito*). *Lampito mauritii* represents the species with the highest population density of the four species studied, was discovered at all of the collection sites (Table 2). *Perionyx sansibaricus* (*P. sansibaricus*) and *Lampito mauritii* (*L. mauritii*) are the peregrines especially abundant in Kerala - Tamilnadu region, hence here we are testing the equality of proportion of them from selected study area.

Table 2: Diversity and Density of different species of earthworm collected from study areas

Family	Name of the species	Number
Megascolecidae	<i>M. kokanensis</i>	13
	<i>M. cochiniensis</i>	9
	<i>P. sansibaricus</i>	45
	<i>P. excavates</i>	25
	<i>L. mauritii</i>	56
Glossoscolidae	<i>P. corethrurus</i>	33

H_0 : The proportion of Density of different species of earthworm (*P. sansibaricus* and *L. mauritii*) collected from study areas are same and

H_1 : The proportion of Density of different species of earthworm (*P. sansibaricus* and *L.*

mauritii) collected from study areas are not same.

$$Z = \frac{(p_1 - p_2)}{\sqrt{(PQ \frac{(n_1+n_2)}{(n_1n_2)})}} \text{ follows } N(0,1)$$

$Z = 1.6598$ and $Z_{\alpha} = 1.96$ at $\alpha = 0.05$, p value $= 0.0970$

Accept H_0 and conclude that the proportion of Density of different species of earthworm (*P. sansibaricus* and *L. mauritii*) collected from study areas is same

Heavy metal Analysis

The concentrations of Pb, Cd, Cu, Ni and Zn were noticed in earthworm and soil samples. The concentrations of heavy metals in the tissues

of earthworms and soil samples are depicted in Table 3. However, while Pb concentrations are highest in soil samples from two sites (Anthikad and Meenangadi), which was found to be below detectable levels in earthworm samples collected from these sites. Apart from lead, the amount of heavy metals analyzed was comparatively low in both earthworm and soil sample collected from Chalakudy. Presence of the heavy metal, Zn and Ni are found in all the sample sites.

Table 3: Heavy metal analysis of samples (Soil and earthworm) collected from flood affected areas

S. No.	Metal Analysed	A_E	A_S	CE	CS	M_E	M_S
1	Pb	BDL*	62.5	0.57	56	BDL*	70
2	Cd	11	1.22	BDL*	0.98	10.52	1.18
3	Cu	37	1.01	BDL*	0.86	BDL*	0.78
4	Ni	6	0.5	2.01	0.5	1.05	0.43
5	Zn	132.9	4.3	0.007	BDL	61.9	2.16

*BDL- Below Detectable Level, A_E -Earthworm sample Anthikad, A_S - Soil sample Anthikad, C_E - earthworm sample Chalakudy, C_S - Soil sample Chalakudy, M_E - Earthworm sample Meenangadi, M_S - Soil sample Meenangadi

Then testing the homogeneity of Heavy metal presence of Soil samples collected from flood affected areas (A_S , C_S & M_S).

H_0 : All A_S , C_S & M_S are homogeneous, against

H_0 : All A_S , C_S & M_S are not homogeneous.

From ANOVA it was clear that for $\alpha = 0.05$, p value = **0.982248** hence accept our Null Hypothesis and conclude that all A_S , C_S & M_S are homogeneous (Table 4).

Table 4: ANOVA for testing homogeneity between or within groups

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	27.54537	2	13.77269	0.017939	0.982248	3.885294
Within Groups	9213.164	12	767.7637			
Total	9240.709	14				

ss-sum of squares, df-degrees of freedom MS-mean sum of squares, F- calculated test statistics, F crit- From table with $\alpha = 0.05$ and d.f (12,2)

Bio Concentration Factor (BCF)

To calculate the BCF, the metal content of both the soil sample and the earthworm tissue must be known. Just 20 samples (soil and earthworm samples) out of the 30 employed for the detection of metal concentrations (10 soil samples and 10 earthworm samples) could give

both of these information. The concentration of Pb in earthworms gathered from Anthikad and Meenangadi, as well as the concentration of Cu in samples from Meenangadi, concentration of Cd, Cu and Zn from samples collected from Chalakudy were below detection levels (Figure 1). So BCF values of these samples could not be

calculated. BCF values for Cd in E1 (BCF of Anthikad) and E3 (BCF of Meenangadi) were found to be 9.01 and 8.91 respectively. The

unusual rise of BCF for Cu was observed in E1 (36.63) and that for Zn was 30.9 (Figure 1).

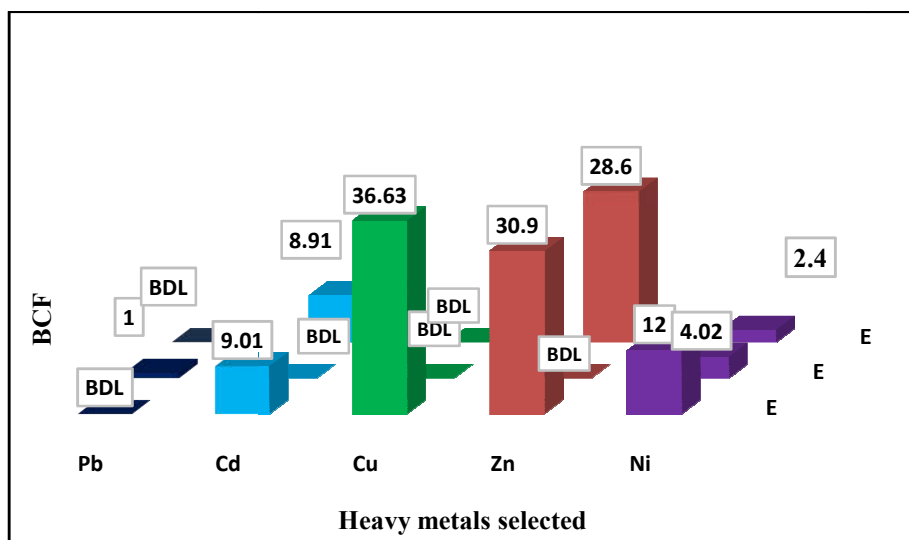


Figure 1: BCF of selected heavy metals in sample from three sample sites

BCF- Bio Concentration Factor, E1- BCF of samples collected from Anthikad; E2- BCF of samples collected from Chalakkudy; E3- BCF of samples collected from Meenangadi; BDL- Below Detectable limit

Pesticide analysis

The concentrations of pesticides in the tissues of earthworms and soils collected from flood affected area were found to be depicted in Table 5. Of the 32 pesticides tested 2 Organochlorine pesticides, Endosulfan-1 and op- DDT) and 1 Organophosphorous (Pyrazaphos) in earthworm samples from Anthikad, Endosulphan 1, Pyridafenthion, Pyrazaphos soil samples from Anthikad, Cis-Chlordane,

Endosulfan-1,op- DDT, pp- DDT (Organo chlorine pesticides), Pyridafenthion, Phoslone, Pyrazaphos (Organo phosphorous pesticides) from Chalakkudy, pp-DDT in earthworm samples from Meenangadi and op- DDT, Endrin, pp-DDT, Pirimiphos- ethyl, Phosmet were found to be in detectable levels in soil samples from Meenangadi.

Table 5: GCMS Analysis for Pesticide residues

SL No	Chemical constitution/ Name of the Pesticide	Calculated amount (ppb*)					
		A _E	A _S	C _E	C _S	M _E	M _S
I	Organochlorine Pesticides						
1	Hexachlorobenzene	-	-	-	-	-	-
2	α- Lindane	-	-	-	-	-	-
3	γ-Lindane	-	-	-	-	-	-
4	β-Lindane	-	-	-	-	-	-
5	Hepatochlor	-	-	-	-	-	-

6	Aldrin	-	-	-	-	-	-
7	Hepatochlor epoxide	-	-	-	-	-	-
8	Trans- Chlordane	-	-	-	-	-	-
9	Cis- Chlordane	-	-	0.492	0.399	-	-
10	pp- DDE (Di chloro diphenyl dichloro ethylene)	-	-	-	-	-	-
11	Endosulfan-I	4.56	4.56	3.54	-	-	-
12	Deildrin	-	-	-	-	-	-
13	op- DDT(Di chloro diphenyl dichloro ethylene)	6.13	-	3.765	-	-	6.12
14	Endrin	-	-	-	-	-	4.78
15	op- DDD (Di chloro diphenyl dichloro ethane)	-	-	-	-	-	-
16	Endosulfan-II	-	-	-	-	-	-
17	pp- DDT(Di chloro diphenyl dichloro ethane)	-	-	0.744	0.744	5.21	5.21
18	Endosulfan Sulfate	-	-	-	-	-	-
II	Organo Phosphorous Pesticides						
19	Diazinon	-	-	-	-	-	-
20	Isazophos	-	-	-	-	-	-
21	Chlorpyrifos- methyl	-	-	-	-	-	-
22	Fenitrothion	-	-	-	-	-	-
23	Chlorpyrifos	-	-	-	-	-	-
24	Primiphos- ethyl	-	-	-	-	-	0.84
25	Quinalphos	-	-	-	-	-	-
26	Pyridafenthion	-	5.2	8.654	-	-	-
27	Pirimiphos- ethyl	-	-	-	-	-	-
28	Phosmet	-	-	-	-	-	7.34
29	Phoslone	-	-	6.557	-	-	-
30	Azinphos- methyl	-	-	-	-	-	-
31	Pyrazaphos	9.86	10.0	6.811	-	-	-
32	Azinphos- ethyl	-	-	-	-	-	-

A_E-Earthworm sample Anthikad, A_S - Soil sample Anthikad, C_E- Earthworm sample from Chalakudy, C_S- Soil sample from Chalakudy, M_E - Earthworm sample Meenangadi, M_S - Soil sample Meenangadi;*Units of measurement: ppb (parts per billion); '-' - Not Detected

Earthworms are the best indicators of both direct and indirect anthropogenic changes in soil, according to numerous studies (Fusaro, Gavinelli, Lazzarini and Paoletti, 2018; Al-Maliki, Al-Taey, and Al-Mammori, 2021). According to several researchers, morpho-physiological reactions to toxicants in worms include coiling and curling of the worms, mucus secretion, raising of the body and extrusion of coelomic fluid, glandular swelling, segmental

constriction and white banding, and a decrease in digging activity (Aja, Jaya, Vijayakumaran, and Reynolds, 2014). The earthworms are well suited to serve as accumulation indicators for the presence of bioavailable chemicals in the soil (Xiao et al., 2022). Earthworms can signal soil quality by analyzing the uptake of pollutants directly from the soil through the highly vascularized surface of the epidermal layer.

From the analysis it was clear that the Density of different species of earthworm (*P. sansibaricus* and *L. mauritii*) collected from study areas are same. The earthworms found in the current study are native peregrines from the Megascolecidae family represents 81% of total worms collected. In concordance with our study, DeSilva, Pathiratne, vanStraalen, and vanGestel, (2010) reported 60% of earthworms belonged to the Family Megascolecidae in a pesticide polluted site. Several reports state that after flooding, the top soil is replaced by the new layer that doesn't work like a sponge. In concordance with our study, Gosh (2021) noted that members of the family Megascolecidae seem to be fit for such climate change. Several species of Megascolecid earthworms are able to survive in inundated soils but there are large differences between species in response to flooding conditions. By ANOVA it was concluded that all soil samples namely, As, Cs & Ms are homogeneous. The earthworms are well suited to serve as accumulation indicators for the presence of bioavailable chemicals in the soil. This is because earthworms reside in soil and are more or less in constant contact with some portion of the soil. This is achieved by the uptake of contaminants directly from the soil through the highly vascularised surface of the epidermal layer of the earthworm.

From the present study, Bio Concentration Factor values were observed to be higher in Anthikad. According to Hsu, Selvaraj, and Agoramoorthy (2006), earthworms obtained from polluted soils had a high bioaccumulation factor, supporting the conclusion that they could be a valuable source for determining the metal levels of the soil. The higher value of the metal bioconcentration factor indicated a potential danger of contamination entering higher food chains (Suthar and Singh, 2008). Zinc showed significant BCF values in two sites (Anthikad and Meenangadi) in the current study, indicating a strong potential for transference along the trophic chain. The following list of BCF values is based on this study. Cu is followed by Zn, Ni, Cd, and then Pb. Copper is detected in earthworms from Anthikad in substantial amounts in the current investigation. Although copper is a recognized essential metal, soil organisms can easily access it through

bioavailability, which has a harmful effect. Although it was previously believed that metals in organic wastes were not poisonous to earthworms, Cu toxicity is now thought to be the most likely cause of the low density of earthworms in places with heavy contamination. Heavy metal released by traffic activities on the road sides, industrial purposes etc. are considered to be important sources of soil pollution. Examining the level of heavy metal concentration in the soil and the earthworm tissue used as bio indicator of heavy metal pollution.

There are several reports available for the impact of pesticides on the population of earthworms. DeSilva et al. (2010) stated that after 3 months of treatment, the effects of the pesticide chlorpyrifos were not discernible in earthworm tissues, which is consistent with our results. Espinoza-Navarro, Ponce-LaRosa and Bustos-Obregón (2017) observed on earth worm exposed to malathion, diazinon and methamidophos show that these compounds alter the external morphology the earthworm, *E. foetida*. During the course of application of pesticides on crops, pesticides are deposited in the soil due to drift and run off, which in turn affect the population of earthworms. In the present study reported various pesticides like Endosulfan-1, op- DDT, pp- DDT, Cis-Chlordane and Pyridafenthion, Phosalone, Pyrazaphos from samples collected from different study sites. Most of the studies, endosulfan showed a gradual decrease in weight of earthworms when they exposed to different concentrations this pesticide (Farrukh and Ali, 2011). The evidence of high Carbofuran residues in soil and water due to intensive use of carbamate pesticides and its impact on biological system have been indicated by both field and laboratory studies from many parts of the world (Nofyan, Lamin, Patriot, and Kanedi (2017). It is important to generate greater bioethics concern in governmental authorities as well as in the population that continues marketing and indiscriminately applying these high toxicity pesticides and their residues, using them in popular vegetable products with high sales or consumption rates, thus altering the environment and public health (Kavvalakis and Tsatsakis, 2012). The fact that earthworms

consume a significant portion of the decomposed litter, manure, and other organic waste placed on soil and contribute to its transformation into rich topsoil makes them particularly suitable as bioindicators of soil toxicity (Al-Maliki et al., 2021).

CONCLUSION

The relevance of study in the context of safeguarding human health as well as that of other terrestrial vertebrates that feed on earthworms and the health of natural settings. The study focuses on how pollution affects the population density and diversity of earthworms that were gathered from various flood-affected areas of Thrissur and Wayanad district. It is not essential to be exposed to a large concentration of xenobiotics for the bodily tissues to become toxic; by extending the exposure period, the concentration can rise to dangerous levels. The present work focused on examining the level of heavy metal concentration in the soil and the earthworm tissue which can be used as bio indicator of heavy metal pollution. Several pesticides and toxicants are undoubtedly detrimental to earthworm development and reproduction, even at low concentrations. However, indiscriminate and excessive use of these xenobiotics may result in a greater buildup of these in the soil, which cannot be regarded as safe for the fauna ultimately lead to serious risk to human health. It is crucial to increase public and governmental awareness of ethical issues in order to stop the marketing and indiscriminate use of these very harmful pesticides and their residues.

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