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ASSESSMENT OF ACUTE TOXICITY AND FECUNDITY EFFICIENCY OF *Eudrilus eugeniae* EARTHWORMS EXPOSED TO TEXTILE DYE EFFLUENT

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ABSTRACT

The focus of this study was to investigate the acute toxicity, growth and survival responses of earthworm species *Eudrilus eugeniae*, exposed to textile dye effluent. The textile dye effluent samples were collected from Erode, Tamil Nadu (India) and analysed for physicochemical parameters and heavy metal content. The effluent samples were used in subsequent toxicity and biochemical tests on *E. eugeniae*. Garden soil was used in comprehensive study on the behaviour of *E. eugeniae*. The natural soil test method was used to expose the epigeic earthworm species *E. eugeniae* to varying concentrations (5 to 25 mL kg^{-1}) of textile effluent for 7 and 14 days. The results revealed that the LC_{50} values of textile effluent were 26.55 and 6.586 mL kg^{-1} after 7 and 14 days, respectively. The lethal effects of textile effluent were assessed by exposing earthworms to 5 concentrations (5, 10, 15, 20, and 25 mL kg^{-1}) of LC_{50} of textile effluent for 28 days to record fecundity and growth rates. Maximum growth of earthworms was observed in control group (10.12 cm length and 6.04 g weight) while effluent-treated earthworms showed reduced growth with increased effluent concentration affecting the growth more severely. A lower number of cocoons and young ones (31.4 cocoons and 107.4 young ones) were observed in high concentration (25 mL kg^{-1}) of textile effluent in comparison to the control group (65.6 cocoons and 306.4 young ones). The study revealed that textile effluents cause potential health risk to *E. eugeniae* if exposed to high concentrations.

Keywords: Bioindicators, ecotoxicology, *Eudrilus eugeniae*, growth & fecundity sub-lethal effects, textile dye effluent

INTRODUCTION

The textile industry globally generates around 1 trillion dollars as revenue, contributes to about 7% of total world exports and employs approximately 35 million workers worldwide (Desore and Narula, 2018). The textile dyeing industry is responsible for colouring fabrics and textiles to create a wide range of colours and designs. Dyes can be applied to textile products by various methods, including immersion, spraying and printing. However, the dyeing process can be resource-intensive and has the potential to cause pollution and harm the environment. Textile industry is one of the major sectors that consume a large amount of chromium based dyes (Paul *et al*., 2020). Therefore, greater emphasis has to be laid on the use of non-biodegradable organic compounds, especially textile dyes (Orts *et al*., 2018). The dye effluents high in colouration, temperature, pH, turbidity, total dissolved solids (TDS), total suspended solids (TSS), chemical oxygen demand (COD), biological oxygen demand (BOD) and other toxic chemicals are released as waste. In the usage of water and chemicals during different transforming steps textile industries plays a major role in contamination of aquatic bodies (Hassan and Carr, 2018; Kannadasan *et al*., 2021).

India is the 2nd largest manufacturer and exporter of textile products in the world (Ministry of Textile, Govt. of India, Annual Report, 2018-19). Coimbatore district is famous for textile industries and it is also considered as the Manchester of South India. Erode, located on the banks of Kaveri river in Tamil Nadu is known as "Loom city" or "Textile city" due to its vibrant handloom and power loom industry. The textile industry in Erode includes several modern and mechanized textile processing mills in the medium and large-scale sectors, as well as around 30 semi-mechanized processing units and approximately 1200 manually operated tiny sector units. These units are primarily engaged in dyeing of yarn and cloth and printing on fabrics, providing employment to approximately 18,000 people. The textile industry in Erode alone plays a vital role in the economic development of the region and has contributed significantly to India's textile exports. However, like other textile centers, it also faces challenges related to environmental pollution and sustainability, which require ongoing attention and efforts to address.

Soil acts as a basic source of agricultural growth and influences almost all parts of the life cycle (Emadodin *et al.,* 2020). The excessive concentration of pollutants in soil consequently reduces fertility and quality of soil and ingress in food web. Studies have indicated that some benzidine-based dyes are the main cause of cancer in experimental animals as these are converted to benzidine by metabolic processes after entering into animals (Mohan and Shukla, 2022). Evidences reveal that benzidine-based dyes are probable human carcinogens. Harmful dyes cause death to the soil microorganisms, which affect agricultural productivity. Azo dyes, in particular, are highly poisonous to the ecosystem. The dyes are soluble organic compounds (Mahapatra, 2016), especially those classified as reactive, direct, basic and acids.

The dyes can stabilize in soil colloid within a few weeks and are retained in soils for long term (Imran *et al*., 2015). Soil fauna especially the earthworms play a vital role in maintaining soil health and ecosystem functioning. Earthworms are considered ecosystem engineers due to their ability to modify soil structure/deleterious chemicals, enhance nutrient cycling and promote plant growth (Parihar *et al.*, 2019). However, exposure to pollutants such as dye effluent can have adverse effects on the health and survival of soil fauna (Furlong *et al*., 2017). Studies have shown that exposure to dye effluent can lead to changes in soil pH, organic matter content, and microorganism populations, which can affect the survival and reproduction of earthworms. In particular, the earthworm *E. eugeniae* has been shown to be sensitive to dye effluent exposure. The present study was aimed to assess growth, reproduction, survival and acute toxicity of earthworm species *Eudrilus eugeniae*, exposed to textile dye effluent.

MATERIALS AND METHODS

Physicochemical characteristics of textile dye effluent

Some physicochemical characteristics of textile dye effluent samples like temperature, pH, turbidity, total hardness, colour, odour, dissolved oxygen (DO), BOD, COD and conductivity were analysed as per the standard procedures outlined by the American Public Health Association (APHA, 2012).

Physicochemical characteristics of garden soil

The garden soil samples were analysed for some physical and chemical soil quality parameter like pH, electrical conductivity (EC), organic carbon (OC), available nitrogen (N), phosphorus (P), and potassium (K) (Dahama, 2002; Daji, 1998; Gaibe *et al*., 1976; Hausenbuiller, 1978; Hudson, 1994; Jackson, 1973; Johnston, 1986). The heavy metal analysis was done by using AAS atomic absorption spectrophotometer (A Shimadzu AA-6800, Shimadzu Corp., Kyoto, Japan) (APHA, 2005).

Maintenance of E. eugeniae

The epigeic earthworm *E. eugeniae* were obtained from Tamil Nadu Agricultural University, Coimbatore, India, transported with their mother culture and moist soil to the laboratory. The earthworms were acclimatized for 15 days in a rearing tank (95 × 55 × 75 cm^3) containing a 10 cm layer of uncontaminated cow dung mixed with garden soil in 1:1 ratio. A thin layer of leaves and dried moist grass was added to the tank to provide shade and maintain moisture levels.

Treatment and their maintenance

Organic waste (cow dung) served as a medium of growth for the worms in the sieved garden soil and cow dung mixed in 1:1 ratio (Bhattacharjee and Chaudhuri, 2002). The mixture was allowed to dry under sunlight for 10 h. The experiment was run in plastic trays and the mixtures were manually turned after every 24 h for 3 days, so as to eliminate possible volatile toxic gases toxic to the earthworms. The total weight of each tray was kept at 0.5 kg. After mixing subsequent amount of water, the worms were allowed to be contained in suitable perforated plastic containers with the soil.

Acute toxicity tests

Acute toxicity tests as per ISO guideline were conducted with adult, laboratory bred *E. eugenia*. The earthworms (10 for each group) were introduced to 5 different series of concentrations of textile effluents (5, 20, 30, 40, and 50 mL kg^{-1}) were used. Each concentration was tested with replicates using ten animals per replication and LC_{50} was assessed. In each group of concentration, equal numbers of earthworms were used. The effect of different textile effluent concentrations (LC_{50} and LC_{90}) was measured (OECD, 2004). Viability of worms was recorded after 7 and 14th days. Care was taken to rear the worms with > 80% moisture in immediate surroundings maintained by automated water sprinkler. The trays were covered with a jute mat to prevent birds from picking the earthworms.

Sub-lethal toxicity testing method

The reproduction test with *E. eugeniae* was aimed to assess the effects of soil contaminants at sublethal concentrations (0, 5, 10, 15, 20 and 25 mL kg^{-1}) on the reproductive capacity of earthworms. Endpoints included reproductive parameters (cocoon production $adult^{-1}$ $week^{-1}$, juveniles hatching $adult^{-1}$ $week^{-1}$ and cocoon viability) and weight change in adults. During experiment, mature adult worms were exposed to above sublethal concentrations of textile effluent in garden soil. The soil was homogenized and air-dried soil before sieving; and adding to the test chamber; and brought to the required moisture content. Ten acclimatized individuals were added to each vessel containing 0.5 kg dry soil with cow dung. Growth effects and mortality rate were determined after 4 weeks and effects on reproduction assessed after 8 weeks of exposure. This test was standardized as per ISO (2008) which is recognized by the Organization for Economic Cooperation and Development (OECD) and ISO.

Data analysis

The LC_{50} was calculated using Karber method (Dede and Kaglo, 2001), and 95% confidence limits were computed using computer program USEPA, version 1.5. All the data were presented as mean ± SD and statistical significances among treatments were calculated by using of one-way analysis of variance and covariance; and least significant difference test using SPSS (version 16.0).

RESULTS AND DISCUSSION

Physicochemical properties of textile dye effluent

The physicochemical characteristics (temperature, pH, turbidity, total hardness, colour, DO, BOD, COD and conductivity) of textile dye effluent water were compared with the standard permissible limits for the safe discharge of trade effluents into natural water bodies (Table 1). The observed temperature of effluent was 39.33 ± 0.17˚C. During collection, temperature of effluent was higher than the normal discharge values. Discharge of these kind of effluents in water bodies cause direct loss to aquatic ecosystem. These effluents result in a wide variety of changes in aquatic plant communities, including changes in species composition, standing crop, net production and a loss of floral and faunal diversity (Leena and Selvaraj, 2019). Apart from this the elevated temperature typically decreases the level of dissolved oxygen of water. This can harm fishes, amphibians and other aquatic organisms. In this study the pH was slightly alkaline (9.55 ± 0.10) which was above the permissible range of TNPCB (6.0-8.0) and WHO (6.0-9.5). Increased in pH may be due to the presence of various salts and other detergents, excessive use of sodium chloride, carbonate, hydrogen peroxide and sodium hydroxide during the bleaching process (Kaur *et al.*, 2010). Higher pH values along with heavy metals enhance the toxicity.

Table 1: Physicochemical properties of textile dye effluent collected from Erode district, Tamil Nadu, India (2022)

Parameters	Values	Standard values	
		TNPCB & YOU (2020)	WHO (2006)
Temperature (°C)	39.33 ± 0.17	-	-
pH	9.55 ± 0.10	6-8	6.0-9.5
Turbidity (NTU)	38.26 ± 0.12	30	-
Total hardness (mg L^{-1})	846.94 ± 0.75	-	500
Colour	Grey/brown colour	-	-
Odour	Pungent	-	-
DO (mg L^{-1})	1.58 ± 0.22	4	-
BOD (mg L^{-1})	172.14 ± 0.90	30	6
COD (mg L^{-1})	340.16 ± 0.85	250	10
Conductivity (µS cm^{-1})	1833.23 ± 2.77	-	1400

Each value is Mean ±SD of four replicates. The values are statistically significant at 0.5% with DMRT

The turbidity of the textile effluent was 38.26 ± 0.12 NTU were significantly higher than the standard permissible limit of TNPCB (30 NTU). The high concentration of suspended and dissolved chemical impurities in the textile effluent matter causes more turbidity. High turbidity levels can also reduce oxygen levels in the water, leading to the death of fish and other aquatic organisms that rely on oxygen for respiration.

The effluent was grey/brown in colour with pungent odour and this may possibly due to the textile dyeing and bleaching process. The colour of effluent sample was found unacceptable to be discharged into the natural water bodies. This agreed with the previous findings regarding textile effluents Faryal and Hameed (2005) wherein the effluents from textile industries were highly coloured and had a foul smell. Additionally, the absorption of light by dyes can lead to an increase in water temperature, further impacting aquatic plant growth and reducing the overall oxygen levels in the water. This can hinder the ability of aquatic organisms, such as fish, to see and locate their prey, reducing their survival and growth rates (Nagarajan and Megharaj, 2019).

Total hardness was 846.94 ± 0.75 mg L^{-1} and it exceeded the discharge limits of TNPCB and WHO. The total hardness increases when textile dye effluent mixes with water bodies due to the presence of dissolved nutrients such as carbonates, sulphates, fluorides, sodium, and nitrates. These can combine and lead to high levels of total hardness in the water. This is because textile dye effluent often contains various ions, including calcium and magnesium, which can contribute to water hardness. When this effluent mixes with a water body, it can raise the concentration of these ions, thus increasing the overall hardness of the water (Kalyani and Srinivasan, 2013).

The DO content in dye effluent was very low (1.58 ± 0.22 mg L^{-1}) than the permissible limits of TNPCB and WHO. The BOD was higher (172.14 ± 0.90 mg L^{-1}) than the tolerance limit fixed by TNPCB (250 mg L^{-1}). The high values of BOD showed that the effluent was highly oxygen demanding waste that caused the depletion of DO. DO is the fundamental requirement for aquatic and land animals. The COD was found to be 340.16 ± 0.85 mg L^{-1} which was higher than the tolerance limit fixed by TNPCB (250 mg L^{-1}). Addition of textile effluent can cause a decline in DO levels in water bodies. This is because textile effluent contains high levels of organic matter, which can serve as a food source for the microbes in water. As these microorganisms consume organic matter, they also consume in the process, leading to a decline in DO levels. This can have negative impacts on aquatic life that require oxygen to survive. Temperature and DO have an inverse relationship in water. As water temperature increases, the solubility of oxygen decreases, resulting in lower DO levels. Conversely, as water temperature decreases, the solubility of oxygen increases, resulting in higher DO levels. This relationship is important in aquatic ecosystems as changes in temperature can have significant impacts on DO levels and therefore affect aquatic life (Wetzel and Likens, 2000).

Electrical conductivity (EC) reveals the ability of a substance to conduct electrons and it is highly influenced by the presence of ionic salts and other dissolved solids (Lukman *et al*., 2013). The conductivity of textile effluent water was 1833.23 ± 2.77 µS cm^{-1} which was higher than WHO permitted limit (1400 µS cm^{-1}). The selected textile effluents had higher concentration of EC, so were considered unfit for agriculture practices. Textile dye effluents contain various dissolved ions such as sodium, chloride, sulphate, and other salts, which increase the overall conductivity of water. As a result, the EC value of water can be used as an indicator of water pollution due to textile dye effluent discharge. The addition of excess lime (calcium oxide) during initial (preliminary) treatment process was responsible for high pH value (Basker *et al*., 2006). Elevated pH (7.85-12.85) and EC values (21.4- 95.5 ds m^{-1}) have been reported by Abd El-Rahim *et al*. (2010).

Heavy metals assessment in textile dye effluent

The heavy metal analysis of textile effluent revealed that it contained high concentration of heavy metal than the permissible limits (WHO, 2011). Cr, Cd, As, Pb and Ni are high toxic substances for humans and other living organisms and these were high in test textile effluent. In addition, the effluents are released without treatment from textile processing industries like colouring, printing and dying process. Yaghoubian *et al*. (2019) have reported that cadmium content above the permitted levels are highly toxic, which causes acute symptoms like nausea & respiratory disorders, and exposure to high concentrations can lead to loss of consciousness in humans. High As levels in effluents are due to the massive use of surfactants and leaching agents in fabric processing (Khandegar *et al*., 2013). Heavy metals tend to accumulate in tissues of aquatic organisms and move to food chain, so cause bioaccumulation and health problems (Zhang, 2016). Pb is a known cumulative poison causing hypertension, tiredness and several neurological disorders. Textile effluents are highly abundant in Pb salts such as PbO_2 which have adverse impact on kidney function (Chandra *et al.,* 2017). High Cr cause liver and kidney disorders, whereas low Cr content acts as trace element in lipid and protein metabolism and also maintains glucose tolerance factor (Boateng *et al.,* 2015). Cu toxicity to aquatic life is highly dependent on the alkalinity of aquatic ecosystem, and Cu is highly toxic at low alkalinity (Khoddami *et al*., 2016).

Table 2: Heavy metals concentration in textile dye effluent

Heavy metals (mg L^{-1})	Concentration detected (mg L^{-1})	Standard WHO values (mg L^{-1})
Iron (Fe)	53.14 ± 0.08	0.3
Copper (Cu)	4.50 ± 0.11	1.0
Manganese (Mn)	5.45 ± 0.03	1.0
Zinc (Zn)	4.65 ± 0.20	5.0
Nickel (Ni)	1.38 ± 0.02	0.02
Cobalt (Co)	0.14 ± 0.00	0.1
Lead (Pb)	0.30 ± 0.08	0.01
Aluminium (Al)	0.46 ± 0.03	1.0
Vanadium (V)	0.18 ± 0.00	0.1
Chromium (Cr)	2.24 ± 0.03	0.05
Molybdenum(Mo)	0.76 ± 0.02	0.1
Magnesium (Mg)	72.51 ± 0.54	50
Arsenic (As)	0.44 ± 0.03	0.01
Cadmium (Cd)	0.76 ± 0.02	0.01

Each value is mean ± SD of 4 replicates. The values are significant at 0.5% with DMRT

Physiochemical properties of garden soil

The physicochemical properties of soil revealed that its colour was medium brown with clean and pleasant smell. The pH affects soil nutrient and much microbial activity. The pH range of 6.8 to 8.0 has been recommended optimum for plants growth. In present study, the soil pH was 7.36 and the above 7.5 value of pH shows basic nature with no significant differences. The electric conductivity of soil was 1.15 µs cm^{-1}. The soil temperature, total organic carbon, and available N, P and K are given in Table 3. These values are favourable for normal life functions of biota that ensure proper organic matter decomposition, increased nitrogen mineralization, uptake of soluble substances, and metabolism. We observed the soil temperature of 23.61 ± 0.98, total organic carbon 10.1%, available N 0.15 mg kg^{-1}, available P 0.56 mg kg^{-1} and available K 0.96 mg kg^{-1}. Soil physico-chemical properties greatly impact the bioavailable and mobile heavy metals in soil (Minnikova *et al*., 2017). Proper nutrition is critical for maintaining healthy earthworm populations in culture, and it is essential to provide a suitable habitat with adequate nutrient availability. Earthworms require macronutrients such as nitrogen, phosphorus, and potassium for their proper growth and reproduction.

Table 3: Some physiochemical characteristics of garden soil

Properties	Quantity
Temperature (°C)	23.61 ± 0.98
pH	7.36 ± 0.04
Colour	Medium brown
Odour	Clean and pleasant
Electric conductivity (µS)	105
Total organic carbon (%)	10.1
Available nitrogen (mg kg^{-1})	0.15
Available phosphorus (mg kg^{-1})	0.56
Available potassium (mg kg^{-1})	0.96

Each value is Mean ±SD of four replicates. The values are statistically significant at 0.5% with DMRT

Heavy metals analysis of soil

Heavy metals are a group of elements with metallic properties that include transition metals, metalloids, lanthanides, and actinides (Singh *et al*., 2011). Some of them are essential cofactors for different enzymes, while others are non-essential (Theron *et al*., 2012). The first group includes the trace elements Co, Cu, Fe, Mn, Mo, Se, and Zn, whose concentrations are closely regulated by interactions with binding proteins since they represent potential risks to cell function (Theron *et al.,* 2012). The second group comprises As, Cd, Pb, Hg, Po, W, and V; non-essential metals that constitute potent toxins and enter the cells/tissues thanks to their physicochemical properties, like ionic charge (Duce and Bush, 2010; Johri *et al.,* 2010). The latter deserve special attention since they do not have any essential function in living organisms, are highly toxic at low exposure levels, and are considered as main threat to the life forms (Atobatele and Olutona, 2015). As per Environmental Protection Agency (EPA), As, Cd, Pb, and Hg, are among the most toxic metals in environment (Goyer, 2004). In present study the heavy metals present in soil in high concentration (mg kg^{-1}) were iron (125.83 ± 1.59), manganese (21.42 ± 0.32), zinc (6.44 ± 0.27), nickel (8.29 ± 0.26), aluminium (7.33 ± 0.11) and magnesium (185.79 ± 1.36). The cadmium (0.063 ± 0.003) and arsenic (0.003 ± 0.002) and lead (7.54 ± 0.18) were in very small amounts (Table 4). So the soil samples showed more fertility and less toxicity.

Table 4: Heavy metal analysis of garden soil

Metals	Content (mg kg^{-1})
Iron (Fe)	125.83 ± 1.59
Copper (Cu)	0.032 ± 0.005
Manganese (Mn)	21.42 ± 0.32
Zinc (Zn)	6.44 ± 0.27
Nickel (Ni)	8.29 ± 0.26
Cobalt (Co)	0.035 ± 0.004
Lead (Pb)	7.54 ± 0.18
Aluminum (Al)	7.33 ± 0.11
Vanadium (V)	0.046 ± 0.003
Chromium (Cr)	5.56 ± 0.35
Molybdenum (Mo)	0.052 ± 0.005
Magnesium (Mg)	185.79 ± 1.36
Arsenic (As)	0.003 ± 0.002
Cadmium (Cd)	0.063 ± 0.003

Each value is mean±SD of four replicates. The values are statistically significant at 0.5% with DMRT

Acute toxicity determination (LC_{50}) of textile effluent

The earthworms were exposed to 5 concentrations of textile effluent (5, 10, 15, 20, and 25 mL kg^{-1}) to assess LC_{50}, LC_{90} and for reproduction test. During experimentation, the earthworms in control soil were healthy and normal without any mortality. *E. eugenia* were treated with textile dye effluent and the percent mortality calculated over a period of 14 days. The LC_{50} values were 26.550 and 6.586 mL

kg^{-1} after 7 and 14 days, respectively. The observed LC_{90} values of textile effluent treated *E. eugeniae* following 7 and 14 day's exposure was found to be 65.810 and 55.744 mL kg^{-1}, respectively (Table 5). Earthworms are considered as an ideal model for studying the soil contaminants. The mortality of earthworms exposed to textile dye effluent occurs due to the toxicity of the chemicals present in the effluent. When earthworms are exposed to high concentrations of chemicals, it can lead to physiological stress and damage to their internal organs, which can ultimately lead to death.

Table 5: Acute toxicity assessment, LC_{50} and LC_{90} of earthworm after the treatment of textile dye effluent

Duration (days)	Concentration (mL kg^{-1})					95% confidence limit		Regression equation	χ^2 (df = 4)
	10	20	30	40	50	LC_{50}	LC_{90}		
7	3.2 ± 0.83[a]	4.0 ± 0.70[b]	5.2 ± 0.83[c]	6.6 ± 0.54[d]	8.0 ± 0.70[e]	26.55	65.81	$Y = -0.867 + 0.033x$	0.098*ns*
14	5.6 ± 0.54[a]	6.0 ± 1.41[b]	7.4 ± 0.89[c]	8.0 ± 0.70[d]	8.8 ± 0.44[e]	6.58	55.74	$Y = -0.172 + 0.026x$	0.100*ns*

Each value is mean ±SD of four replicates. The values are statistically significant at 0.5% with DMRT

In soil ecosystem, toxicants cause synergistic or antagonistic adverse impact on the health of earthworms (Tiwari *et al.,* 2019). The presence of excess heavy metals in soil leads to increased worm mortality (Mostafaii *et al.,* 2016). Cu and Cd were found toxic to earthworms at 1.5 and 0.1 g kg^{-1} waste, respectively. The studies suggested that earthworms are susceptible to the free form of heavy metals. Among heavy metals, Cd is considered the most toxic, followed by Cu and As (Sunilkumar *et al*., 2008). Cd is a well-known metal showing most adverse effects on soil organisms, including earthworms (Dziubanek *et al*., 2017). The toxicity of a chemical to earthworms depends on the feeding strategies adopted by the species of earthworms and the availability of pollutant (Schon *et al*., 2017). While Cd seemed to be highly toxic to earthworms, other heavy metals such as Cu, Ni, and Zn reportedly are more toxic (Elyamine *et al*., 2018). The pattern of mortality suggests that the effects of pollutants on earthworms were as a result of absorption and/or uptake of chemicals across the worm's body, through either body wall or by ingestion. In present study, dye effluent at concentrations of 26.550 and 6.586 mL kg^{-1} caused a 50% mortality in *E. eugenia* within 7 and 14 days, respectively.

Growth efficiency of E. eugeniae after exposure to textile effluent

The growth was measured in term of weight and length of earthworm. Significant difference in body weight and length were observed after treatment with different concentration of textile dye effluent as compared to the control after 28 days of experiment (Table 6 and 7). The maximum growth was observed in control (10.12 cm length and 6.04 g weight), while the effluent treated earthworm showed less growth as compared to the control. When the effluent concentration was increased the growth in earthworms was affected. When earthworms were continuously subjected to heavy metal treatment, they signalize the patterns of retarded growth in all the treatments. The result was supported by Kaur and Hundal (2016) who indicated significant decrease ($p>0.05$) in body weight when earthworms were reared in contaminated soil. Less growth of worms exposed to heavy metals has also previously been reported (Lapinski and Rosciszewska, 2008; Miguel *et al.,* 2012; Bilialis *et al.,* 2013) which may be attributed to the avoidance behaviour and low feeding activity on metal contaminated substrate. Gomez *et al.* (2009) reported a loss in body weight due to the reduced feeding in worms. Similar discontinuous patterns of body weight were observed in worms reared in Pb, Cd, and Ni contaminated soil (Kaur and Sangha, 2014). In present study, the reduction in growth rate was possibly due to the depression of food intake and utilization of major proportion of absorbed food energy in metabolism. If food intake or assimilation is reduced, there will be less energy and protein available for growth, so the rate declines. However, heavy metal exposure induced certain physiological changes that may be attributed to biomass reduction, as worm feeding in treated and control soils was reported to be similar by Kreutzweiser *et al*. (2008) and Urmila *et al.* (2019).

Table 6: Biomass and length of earthworms after the treatment of textile dye effluent

Days	Effluent concentration (mL kg^{-1})					
	Control	5	10	15	20	25
	Mean weight of earthworm (g)					
1	2.42 ± 0.25^{a}	2.66 ± 0.25^{b}	2.52 ± 0.41^{c}	2.42 ± 0.35^{ad}	2.44 ± 0.20^{ade}	2.32 ± 0.19^{f}
7	3.08 ± 0.23^{a}	2.84 ± 0.21^{b}	2.72 ± 0.08^{c}	2.58 ± 0.22^{d}	2.42 ± 0.22^{e}	2.22 ± 0.13^{f}
14	4.12 ± 0.76^{a}	3.62 ± 0.19^{b}	3.52 ± 0.23^{c}	3.36 ± 0.15^{d}	3.22 ± 0.19^{e}	3.08 ± 0.08^{f}
28	6.04 ± 0.53^{a}	5.06 ± 0.65^{b}	4.46 ± 0.11^{c}	4.18 ± 0.16^{d}	4.02 ± 0.19^{e}	3.92 ± 0.13^{f}
	Mean length per earthworm (cm)					
1	6.62 ± 0.20^{a}	6.68 ± 0.16^{ab}	6.78 ± 0.08^{c}	6.42 ± 0.38^{d}	6.54 ± 0.48^{e}	6.38 ± 0.34^{fd}
7	7.50 ± 0.22^{a}	7.60 ± 0.27^{b}	7.46 ± 0.34^{ac}	7.28 ± 0.19^{d}	6.98 ± 0.10^{e}	6.72 ± 0.47^{f}
14	8.54 ± 0.25^{a}	8.80 ± 0.26^{b}	7.80 ± 0.22^{c}	7.70 ± 0.23^{d}	7.20 ± 0.27^{e}	6.94 ± 0.15^{f}
28	10.12 ± 0.31^{a}	9.98 ± 0.30^{b}	9.36 ± 0.36^{c}	9.22 ± 0.27^{d}	8.38 ± 0.47^{e}	7.48 ± 0.31^{f}

Each value is Mean ±SD of four replicates. The values are statistically significant at 0.5% with DMRT

Effect of textile effluent on fecundity of earthworm

The number of cocoons and young ones produced were observed after exposure of *E. eugeniae* to textile dye effluent. A lower number of cocoons and young ones (31.4 cocoons and 107.4 young ones) were observed in high textile effluent concentration (25 mL kg^{-1}) when compared to the control group (65.6 cocoons and 306.4 young ones) [Table 7]. A significant decline in the total number of cocoon production was reported in all the treatments of textile dye effluent. Lower reproduction rates in heavy metal-presented textile effluent exposed worms may be due to the detention in copulation (Novais *et al.*, 2011), deleterious gamete development (Rongquan and Canyang, 2009), egregious embryo development (Kumar *et al*., 2008), lesser hatchling success and slow growth due to stress (Sivakumar *et al*., 2009; Urmila *et al.*, 2019).

Table 7: Fecundity potential of earthworm after the treatment of textile dye effluent

Fecundity	Mean fecundity $earthworm^{-1}$					
	Dye effluent concentration (mL kg^{-1})					
	Control	5	10	15	20	25
Cocoons (No.)	65.6 ± 2.30^{a}	59.8 ± 2.86^{b}	52.8 ± 2.28^{c}	47.6 ± 2.30^{d}	39.8 ± 1.48^{e}	31.4 ± 1.67^{f}
Young ones (No.)	306.4 ± 3.91^{a}	240.0 ± 3.80^{b}	195.2 ± 3.11^{c}	151.8 ± 3.96^{d}	134.2 ± 3.34^{e}	107.4 ± 2.60^{f}

Each value is mean ±SD of 4 replicates. The values are statistically significant at 0.5% with DMRT

Conclusion: Earthworm toxicity studies are crucial in determining the safety threshold for chemicals in environment. Heavy metals in textile dye effluent can significantly impact earthworm growth, reproduction, and survival. The studies suggested that the effluent can cause mortality and affect the growth and reproduction rates. Earthworms play crucial role in maintaining soil health and ecosystem balance, so making it essential to regulate and monitor the discharge of textile dye effluent to prevent any harmful effect. The study emphasizes the importance of earthworm toxicity studies in providing a safety threshold for chemicals and extending basic mortality tests to include chronic or growth and reproduction tests.

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