SPECIES SPECIFIC CHEMICAL RESPONSE OF EUDRILUS EUGENIAE AND NOTOSCOLEX KAYANKULAMENSIS WITH REFERENCE TO UREA AND DETERGENT

Project Submitted to the University of Kerala in Partial Fulfillment of the Requirements for the Degree of Bachelor of Science

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CERTIFICATE

This is to certify that this project entitled **'Species Specific Chemical Response of *Eudrilus eugeniae* and *Notoscolex kayankulamensis* with reference to Urea and Detergent'** is an authentic record of the work carried out by ~~Mr~~/Miss ...HRIDYA SAJITHAKUMARY PILLAI...

B.Sc. Zoology (VI semester) student under my supervision and guidance and that no part of this report has been submitted earlier for any other degree or diploma.

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DECLARATION

I ………………………………………………………………………………………………… do hereby declare that this project entitled **'Species Specific Chemical Response of *Eudrilus eugeniae* and *Notoscolex kayankulamensis* with reference to Urea and Detergent '** is the bonafide work carried out by me under the supervision and guidance of Dr. Reeja Jose, Assistant Professor, Department of Zoology, Bishop Moore College, Mavelikara for the partial fulfillment of the requirements for the degree of Bachelor of Science and that no part of this project work has been submitted earlier for award by any other degree, diploma or recognition of any university.

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INTRODUCTION

1.1 SOIL

The weathered and fragmented outer layer of the earth's terrestrial surface forms the soil. The rocks disintegrate and decompose by various physical, chemical and biological means; by the action of various microscopic plants and animals and result in the formation of soil. The physical process includes various forces of nature such as expansion and contraction of rocks caused by continuous heating and cooling, stresses caused by freezing, forces of water, penetration of roots, and by external forces of particles by either wind, water or ice. Various chemical process like hydration, oxidation, reduction, solution, dissolution, continuous precipitation, removal of components by leaching or volatization and various physicochemical exchange reactions tend to decompose original minerals in the parent rock. Beyond the initial weathering process, this soil undergoes various physicochemical and biochemical process that enhance the organic matter present in the soil and causes the formation of secondary minerals in the soil.

The typical development of a soil and its profile, called paedogenesis. This process begins with the physical disintegration or weathering of the exposed rock (Hillel, 1982). Soil profile shows various horizontal layers called horizons of varying thickness, properties and particle size. Gradual accumulation of organic matter near the surface layer brings about the development of the first Horizon called A Horizon. This layer is formed by the stabilizationof a greater or a lesser degree of organic matter cementation where the chemical weathering processes like hydration, oxidation, reduction, dissolution precipitation and seeping may bring about the formation of clay and this clay thus formed tend to migrate along with several other materials and minerals such as the soluble solvents from the A Horizon and accumulates in the intermediate zone called as the B Horizon which is situated in between the A Horizon and parent rock material, C Horizon. Important aspects of soil formation and profile development are the twin processes of eluviation and illuviation (washing out and washing in respectively) wherein clay and other substances emigrate from the overlying eluvial A Horizon and accumulate in the underlying illuvial B Horizon which therefore the first from a Horizon in composition and structure. (Hillel, 1982). The withered parent material accumulated with calcium carbonate, calcium phosphate and incompletely withered irregular fragments of rock matter forms the mineral horizon C Horizon. This Horizon has less biological activity and with water content, often called subsoil. The layer below is impermeable and is composed mainly of parental rocks or bedrocks from which the soil is formed. This Horizon even lacks the presence of roots of plants. This is the final Horizon,R Horizon.

Based on the various particle size and the porosity between the adjacent particles, the soil is differentiated mainly into four types, sandy soil, silt clayey soil and loamy soil.

Sandy Soil: The soil is dry light weight and gritty and does not hold water well. Sandy soil is generally acidic and lacks a lot of minerals and nutrients. During hot summer months, the soil warms up and drains out water quickly as it does not hold water well. The particle size of course sand ranges from 2 – 4.75mm, medium sand ranges from 0.425 – 2 mm and fine sand ranges from 0.075 – 0.425 mm.(R).

Silt: Silt soil is packed with nutrients as it originates from river sediments. The particles are medium-sized and therefore retain water quite well but drainage can become a problem. An issue with silt soil is that it is easily compacted. Silt is a non-plastic or low plasticity material due to its fineness. Due to its fineness, when wet it becomes a smooth mud that you can form easily into balls or other shapes in your hand and when silt soil is very wet, it blends seamlessly with water to form fine, runny puddles of mud.

Clay: Clay particles are the finest of all the soil particles, measuring fewer than 0.002 mm in size. It consists of microscopic and sub-microscopic particles. Clay is a fine-grained cohesive soil.

Loamy Soil**:** these are the most fertile soils with a high humus content and a moderate particle size and adequate aeration. The way the other particles combine in the soil makes the loam.

1.2 SOIL ORGANISMS

Soil biology is the study of microbial, faunal activity and ecology in soil. Organisms that spend a significant portion of their life cycle within a soil profile or at the soil-litter interface are termed as soil organisms. Organisms such as archaea, bacteria, actinomycetes, fungi, algae, protozoa, and a wide variety of fauna including springtails, mites, nematodes, earthworms, ants, insects etc. are some examples of soil organisms. Soil organisms play a major role in the formation of soil. The action of these organisms are important for maintaining healthy soils. They can change the physical organisation of soil by creating burrows, can add nutrients to the soil through the breakdown of dead leaves, and can help to control the population of other soil organisms.

Factors affecting soil organisms at a regional special scale.

- Soil pH
- Total phosphorus
- Climate
- Distance

Importance of soil microorganisms in agriculture: Soil microbes and other macro-organisms are essential for decomposing organic matter and recycling old plant material. Some soil bacteria and fungi form relationships with plant roots that provide important nutrients like nitrogen or phosphorus.

1.2.1 EARTHWORM

Earthworm are ubiquitous macroscopic organisms that inhibit almost all soils. These belongs to the class Oligochaete of phylum Annelida. There are 1,800 species of terrestrial worms. Earthworms occur in virtually all soils of the world in which the moisture and organic content are sufficient to sustain them.

The body of the earthworm is segmented which looks like many little rings joined or fused together. The earthworm is made of about 100-150 segments. The segmented body parts provide important structural functions. Segmentation can help the earthworm move. Each segment or section has muscles and bristles called setae. The bristles or setae help anchor and control the worm when moving through soil. The bristles hold a section of the worm firmly into the ground while the other part of the body protrudes forward. The earthworm uses segments to either contract or relax independently to cause the body to lengthen in one area or contract in other areas. Segmentation helps the worm to be flexible and strong in its movement. If each segment moved together without being independent, the earthworm would be stationary.

Earthworms are hermaphroditic; i.e., functional reproductive organs of both sexes occur in the same individual. The eggs of one individual, however, are fertilized by the sperm of another individual. During mating two earthworms are bound together by a sticky mucus while each transfers sperm to the other. The worms separate and form cocoons; the cocoon moves forward, picking up eggs at the 14th segment; at the 9th and 10th segments it picks up the sperm deposited by the other earthworm. The cocoon slides over the head, and fertilization takes place. Within 24 hours after the worm's mate, the cocoon is deposited in the soil. Miniature earthworms usually emerge from the cocoon after two to four weeks. They become sexually mature in 60 to 90 days and attain full growth in about one year. Earthworms usually remain near the soil surface, but they are known to tunnel as deep as 2 m during periods of dryness or in winter.

Their food consists of decaying plants and other organisms. They also ingest large amounts of soil, sand, and tiny pebbles. It has been estimated that an earthworm ingests and discards its own weight in food and soil every day**.**

Earthworms provide food for a large variety of birds and other animals. Indirectly they provide food for humans by assisting plant growth. Earthworms aerate the soil, promote drainage, and draw organic material into their burrow. This last service accelerates the decomposition of organic matter and produces more nutritive materials for growing plants.

1.2.1.a Types of earthworms based on their ecology

There are mainly three types of earthworms are epigeic, endogenic and anecic. Epigeic worms, which are also known as surface dwellers because they live above soil level, endogenic worms, which live below ground, and anecic worms, which live below soil level but explore at and above soil level to find sources of food.

Epigeic earthworms: These worms do not build burrows, and instead reside amongst decaying organic matter on the soil surface. These are also sometimes called compost earthworms, or surface-dwelling earthworms, as they live on the surface of the soil amongst piles of leaves or compost heaps. They feed on decaying plant matter, leaf litter, and dung. They have a dark colouring that enables them to live above ground more safely, camouflaging themselves in piles of leaves or topsoil. Their dark pigmentation also helps to protect them from UV rays. They have strong muscles for their size, which enables them to move faster than other types of worms, which is important as living above ground they are most at risk from predators. These worms are important in composting and are known to rapidly consume and excrete composting material to help it break down more quickly. They are also able to reproduce very quickly, increasing the population of worms within the compost. They are small in size, usually ranging from between less than an inch up to seven inches in length.

Endogenic earthworms: These worms burrow within the top layers of soil and rarely come up to the surface, preferring instead to literally live within the earth. They are most commonly found in the uppermost layers of soil where they create semi-permanent, horizontal burrows or under rocks and logs, though some will burrow deep into the soil. They typically only make an appearance on the ground surface in instances of heavy rain, as the extra moisture prevents them from drying out. These worms are fairly small and generally measure between one and twelve inches. They tend to be very pale or translucent and colourless, and they have weaker muscles than epigeic worms, which means they move more slowly. They help to mix minerals and air within the soil and help with aeration as they eat the soil itself.

Anecic earthworms: These worms are those that burrow vertically in the mineral layers of soil, creating permanent burrows as deep as six feet below surface level. Their burrow systems are quite extensive and can be as big as one inch in diameter. These worms collect food from above ground in the form of organic matter such as fallen leaves and drag them back underground to their burrows. They are also known to eat soil and some litter. These worms encompass some of the most common types of earthworms, worms used for fishing bait, and night crawlers. They have very weak muscles and are the slowest moving of all types of worms, as they do not have any need to move quickly. They have some pigmentation but are often a milky colour, especially native worms, as they predominantly reside underground. They can drastically range in size, anywhere from one inch to a huge sixty inches in length.

1.2.1.b Life cycle of an earthworm

The earthworm life cycle, like many others, starts with an egg. Within the egg, a young earthworm develops until it is ready to hatch. The egg is encased in an egg casing called a cocoon. The number of eggs within one cocoon can vary between species, but most species have just one.

1) Cocoons tend to be 'lemon' shaped, but the specific shape varies between species. The amount of time that they take to hatch is also very variable and can vary by species, but also by environmental conditions. For example, for some species the cocoons may hatch quicker in warmer conditions than in cooler conditions and other species may 'wait out' undesirable drought conditions within the soil as the cocoon stage.
2) Hatchlings look just like mini-earthworms, they're just smaller and paler. As the hatchling feeds and grows it will gain the same colour as an adult earthworm.

3) Juvenile earthworms look very much like the adults but are missing the saddle (or clitellum)
4) Adults (or sexually mature) earthworms can be easily recognised through the presence of the saddle. Earthworms are hermaphrodite organisms, meaning that each earthworm has both male and female sexual reproduction organs.
5) Sexual reproduction involves two earthworms. The two earthworms produce a slime tube and grip onto each other using the tubercula pubertatis (located on the saddle). The slime tube provides the right environment for the two earthworms to exchange sperm, with each earthworm storing the sperm of its' partner for use later. Because both earthworms are performing the function of both a male and female during sexual reproduction, they are known as simultaneous hermaphrodites. Following this sperm exchange the earthworms separate. Asexual reproduction can also be undertaken by some species of earthworm. This involves a single earthworm producing young from unfertilised eggs and is known as parthenogenesis.
6) A mucus sheath is formed around the clitellum and is moved along the earthworm until it comes off the head end. Along this journey it picks up the egg(s) and the sperm of the earthworm that was mated with. This mucus sheath forms the cocoon and fertilisation of the egg occurs within the cocoon.

1.2.1.c <u>Contribution of earthworms in enhancing fertility of soil</u>

Earthworms mix the different layers of soil and bind the soil with organic matter. Due to this mixture the organic matter gets dispersed in the soil and also allows plants to access the nutrients they retain and enhance the soil's fertility. Earth worms provide optimum condition for the growth and development of plants by maintaining the soil pH. Earthworms also helps to mix the humus with the minerals in the soil, humus contains many useful nutrients for healthy soil. They redistribute organic materials within the soil, increase soil penetrability and under certain conditions, influence ion transport in soil. Root distribution may be modified and microbial activity increased by their burrowing and feeding activities. They influence the supply of nutrients in several ways. Not only is earthworm tissue and cast material enriched in certain nutrients, relative to the soil matrix but ingestion of organic material increases the rate of cycling (Sayers and Springiest, 1984)

- **<u>Vermicomposting</u>**

Vermicomposting or worm composting is a simple biotechnological process for converting biodegradable waste into organic manure with the help of earthworms. It is mesophilic and bio-oxidative process. The raw materials required for vermicomposting are worm bin, composting worms, bedding and organic wastes. The end product of vermicomposting is called vermicompost. Vermicompost is an organic fertilizer that enhance soil fertility physically, chemically and biologically. Physically, vermicomposting-treated soil has better aeration, porosity, bulk density and water retention. Chemical properties like pH, electrical conductivity and organic matter content are also improved for better crop yield. Vermicompost is consisted of a number of nutrients in the form that can be readily taken up by the plants. Vermicompost also contains plant growth regulators and plant growth hormones.

Preparation of Vermicomposting:

1. A container with suitable dimensions is chosen and a worm bed. This worm bed may contain any of the following like old papers, sugar cane trash, paddy husk, and coir waste. A thin layer of soil is spread over this mixture and the humidity is maintained at 40-45%.
2. A mix of organic waste, slurry from a biogas plant and cattle dung is spread over the bed and it is kept for half digestion for a period of two weeks. During this time, the temperature of the bedding will rise to 50-55 °C.
3. After the temperature is cooled down to 30 degrees, the earthworms are introduced. Around 500 earthworms are introduced for 100 kg of organic material.
4. The bed is covered with straw and jute clothes to protect the worm. The temperature is maintained at 20-30°C and the moisture content is kept at 45-50%. (pH: 6.5-7)
5. The compost will be ready in around 60 days and after it is ready the worms are separated by spreading the vermicompost on a plastic sheet in a heap under sunlight. As earthworms are sensitive to sunlight, they will move to the bottom of the heap and the top layer of the compost can be removed.

Phases of Vermicomposting: There are two major phases of vermicomposting namely active phase and maturation phase. Active phase duration is depended on the species of earth worm. It is mainly depended on the gut-associated processes (GAPs). The decaying organic matter and microorganisms undergo many modifications when they transit through the earthworm's intestine is called gut-associated processes. Maturation phase is characterized by the displacement of the earthworms towards the layers of nondecomposed organic waste, during which microorganisms alone take over control of the decomposition of earthworm's cast. In this stage of the process the mixture should stays at room temperature. A series of secondary reactions are produced that triggers condensation and polymerization of the humus takes place in this stage. At the end of this stage compost is obtained.

Methods of vermicomposting: Different types of vermicomposting methods from small scale domestic to large scale commercial are present that are depended upon the amount of organic waste to be generated as output. Some of the major methods of vermicomposting systems include Domestic Vermicomposting Systems, Worm Compost Heap, Tanks Above the Ground, Windrows, Static Pile Windrows, Top-fed Windrows, Stacked Bin System, Fully Automated Flow-Through Reactors etc.

- **Commonly used worms in vermicomposting**

All earthworms are not suitable for vermicomposting. Only those that live on the soil surface and thrive on organic wastes are suitable. About 3627 species of earthworms have been identified in the world. Out of it only 3 to 5 per cent species are capable of vermicomposting. Some of the species that are extensively used in vermicomposting are *Eisenia andrei, Eiesnia foetida, Dendrobaena veneta, Perionyx excavatus, Eudrilus eugeniae.*

Eisenia andrei is epigenic, i.e. it prefers to live in compost or leaf litter rather than mineral soils. It can be distinguished from *E. fetida* as it is darker in colour, and the characteristic stripes are less pronounced. *Eisenia fetida* commonly called red wigglers are reddish-brown in color, they have small rings around their body. These worms live in rotting vegetation, compost, and manure. They are epigean and rarely found in soil. *Dendrobaena veneta*, this species is large earthworm that can also survive in soil with potential for use in vermiculture, although it is not very prolific, it grows rapidly (Edwards, 2004). *Perionyx excavatus* is a commercially produced earthworm. Some of the popular names for this species are composting worms, blues, or Indian blues. This species is marketed for its ability to create fine worm castings at a higher rate. *Eudrilus eugeniae*, also called the African Nightcrawler, is an earthworm species native to tropical west Africa and now widespread in warm regions under vermicompost.

1.3 Environmental pollution – Impact on life with reference to earthworm

Earthworms are the most abundant invertebrates in the soils of temperate regions and are extremely important for soil formation (Edwards, 2004). Earthworms participate in nutrient cycling in terrestrial ecosystems and in the formation of the soil profile from the physical, chemical and microbial perspectives (Bartlett *et al.*, 2010). They improve its structure by increasing the macroporosity, which affects aeration, water dynamics and organic and inorganic matter breakdown (Wen *et al.*, 2006). Earthworms are permanently in close contact with soil particles and microorganisms present in the soil via both a highly permeable skin and an alimentary tract (Jagger *et al.*, 2003; Drake and Horn, 2007). Therefore, they are significantly affected by the pollutants that reach the soil system and are thus well suited for the monitoring of soil contamination.

Two epigeic species, i.e., *Eisenia fetida* and *Eisenia andrei*, have been used for many years to monitor ecotoxicity. There are two sets of guidelines, i.e., those from the Organization for Economic Cooperation and Development (OECD) and those from the International Organization for Standardization (ISO), for the assessment of the ecological risk of contaminated soil, the determination of the acute toxicity of chemicals on earthworms (OECD, 1984; ISO, 1993), and the effect on their reproduction (ISO, 1998; OECD, 2004). They are able to take up chemicals from pore water through their skin and via soil ingestion.

The presence of contaminants in the soil disturbs major physiological functions of earthworms, such as survival, nutrition, immunity, growth, and reproduction, and these effects depend on the matrix, exposure time, and the types and doses of the pollutants in the environment. Biomarkers detect the effects of contamination at an early stage before sub lethal effects, such as inhibition of growth and reproduction, become apparent (Kammenga *et al.*, 2000).

Reactions to pollution may be monitored on various levels, the whole-body level (viability, weight loss, reduction of reproduction, and escape reaction) the organ and tissue level (histopathological changes), the cellular level (decrease in the physiological conditions of the cells) and the molecular level (the up and down regulation of the expression levels of genes that are sensitive to the environmental changes, transcriptome profiling) (Owen *et al.*, 2008; Asensio *et al.*, 2013; Roubalova *et al.*, 2014; Sanchez-Hernandez *et al.*, 2014).

Similarly, to other invertebrates, earthworms rely on natural nonspecific innate immunity for defence and lack anticipatory, specific and lymphocyte-based immune mechanisms. Additionally, the natural barriers of earthworms represent the first line of protection against the invasion of microorganisms. The first nonspecific protective barrier in earthworms is the skin, which consists of the epidermis and a thin cuticle that covers the entire body. The exposure of the earthworms *Lumbricus rubellus* and *Lumbricus variegatus* to C- 60 fullerene nanoparticles has been described to result in cuticle damage with underlying pathologies of the epidermis and muscles (Van Der Ploeg *et al.,* 2013). Furthermore, the exposure of *E. fetida* to sub-lethal concentrations of 1,2,4-trichlorobenzene results in ultrastructure alterations of the cuticle and skin, and the reduction of mucus production by secretory cells. At higher concentrations, mucus production disappears, and the cuticle is loosened and weakened (Wu *et al.,* 2012). Exposure of the earthworm *E. fetida* to soil containing tetraethyl lead (TEL) and lead oxide (a gasoline additive) causes ruptures of the cuticle and skin, extrusion of the coelomic fluid and inflexible metameric segmentation (Venkateswara Rao *et al.,* 2003). The coelomic fluid of earthworms contains different typesof cells that are generally termed coelomocytes. Coelomocytes have been described to respond to a wide range of pollutants and therefore are often used in soil ecotoxicology assessment.

At the cellular level, two immune system-related parameters have been used as sensitive sub lethal endpoints in assessment of the toxicity of pollutants in earthworms: phagocytosis and NK-like cell activity. Phagocytosis represents an important defence mechanism that begins with the recognition of non-self, which is followed by the engulfment and destruction of phagocytosed particles. The inhibition of phagocytosis in earthworms that are exposed to various metals and organic substances, such as polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-p- dioxins/dibenzofurans (PCDDs/Fs), has been described (Ville *et al.,* 1995; Fugere *et al.,* 1996; Fournier *et al.,* 2000; Belmeskine *et al.,* 2012). The NK-like cell activity has been demonstrated to be suppressed by polyaromatic hydrophobic hydrocarbons (PAHs) (Patel *et al.,* 2007), PCBs (Suzuki *et al.,* 1995), and PCDDs/Fs (Belmeskine *et al.,* 2012). At the subcellular level, the lysosomal membrane stability system has been identified as a specific target of the toxic effects of contaminants.

Earthworms are one of the most abundant terrestrial invertebrates in the temperate regions and important “ecosystem engineers.” They are well suited to use in monitoring potential contamination or other soil impacts because of the constant contact between their permeable skin and the surrounding soil, which makes them sensitive to changes in the chemical and physical soil properties (Roubalova *et al.,* 2015). The presence of manufactured nano-objects (MNOs) in various consumer or their (future large-scale) use as nano agrochemical have increased with the rapid development of nanotechnology and therefore, concerns associated with its possible ecotoxicological effects are also arising. MNOs are releasing along the product life cycle, consequently accumulating in soils and other environmental matrices, and potentially leading to adverse effects on soil biota and theirassociated processes.

Earthworms are an ecologically significant group of organisms and playan important role in soil remediation, as well as acting as a potential vector for trophic transferof MNOs through the food chain. Various adverse effects on the different earthworm life stages have been reported, including reduction in growth rate, changes in biochemical and molecular markers, reproduction and survival rate (Roubalova *et al.*, 2015).

1.4 OBJECTIVES:

Now a days the usage of fertilizers like urea are at a hike for getting higher agricultural yields, also the usage of detergents in households is unexceptional. Earthworms are the friends of farmers and great benefactors of soil and agriculture.

- To detect the effect of urea and detergent on *Eudrilus eugeniae* and *Notoscolex kayankulamensis*, two species of earthworms.
- To observe the morphological and physiological changes taking place in their bodies in response to the chemicals exposed.
- To compare the duration taken by each species to respond to the particular chemicals.
- To determine the variation shown by each species to selected chemicals.

REVIEW OF LITERATURE

Gestel (1992) on application of benomyl to *Aporrectidea longa* and *A.rosea* in the field settings were compared with the results obtained from the application of benomyl in the laboratory settings and the effects were observed in the reproduction rates. Results of field studies on the earthworm toxicity of pesticides are in agreement with those of laboratory studies when a homogeneous distribution of the pesticide dosage over the top 2.5-cm soil layer is chosen as a starting point. Cikutovic, 1993 observed that cocoon production was found to be the most sensitive parameter for paraquat, fentin, benomyl, phenmedipham, carbaryl,copper oxychloride, dieldrin.

Gupta and Saxena, 2003 studied the effects of carbaryl, an N-methyl carbamate insecticide, on the reproductive profiles of the earthworm, *Metaphire posthuma* and found sperm head abnormalities even at the lowest test concentration of 0.125 mg/kg. Wavy head abnormalities were observed at 0.125 mg/kg carbaryl, whereas at 0.25 mg/kg and 0.5 mg/kg, the sperm heads became amorphous and the head nucleus was turned into granules deposited within the wavy head.

Espinoza-Navarro and Bustos-Obregon, 2005 treated *E. fetida* with organophosphate insecticide malathion and observed that malathion decreased the spermatic viability in spermathecal, changing the cell proliferation patterns and modifying the DNA structure of the spermatogonia. Malathion had a noticeable effect on the sperm count and its metabolites affected the sperm quality. Zhou, 2006, discussed about the toxic effects of acetochlor and methamidophos in *E. fetida* in the region of northeast china and reported that the weight of the earthworms was a more sensitive index compared to the mortality rates indicating the toxic effects of acetochlor and methamidophos.

Celine Pelosi (2014) accesses the sensitivity of *E. fetida and E. andrei* to pesticides. Their analysis showed the effects of pesticides in all levels of their body organisation like disruption of enzymatic activities, increased individual mortality rates, decreased fecundity and fecundity rates, changes in individual behaviour such as feeding rates and overall decrease in community biomass anddensity.

Long *et al.,* 2017 conducted studies showing the effect of urea on epigeic earthworm species (*E. foetida*) in clay soil. The results were observed as adverse effects to the chemical properties of soil. Mortality of Earthworms were seen as the end point. Changes in the weight of the adult earthworms and significant effects in the reproduction of the earthworms were also observed. Dash and Mohapatra, 2018 experimented the toxic effects of urea on *E. fetida* by simple contact filter paper testing method and found out deleterious effects of urea on earthworm such as lesions, inflammations, neural retention and defoliation and separation of body parts leading to death.

Sunish *et al.,* 2019 studied the effects of detergent on earthworms for a period of 60 days set up in three trays with a gradual increase in the addition concentrations of detergent. The changes in the earthworms were observed in terms of decrease in weight length and diameter with the increase in concentrations of detergent.

Rishikesh and Tiwari (2019) determined the acute toxicity of three formula grade pesticides namely, triazophos (an organophosphate, OP), deltamethrin (a pyrethroid) and combined pesticide (triazophos + deltamethrin) was determined in earthworm, *Eudrilus eugeniae*. They were exposed to different concentrations of these pesticides for 48 h by paper contact toxicity method. Morphological alterations such as coiling, clitellar swelling, mucus release and bleeding followed by body segmentation were observed in exposed earthworm. Acetylcholinesterase (AChE) activity assayed in different regions of body segment exhibited a significant ($p < 0.05$) decrease in its activity particularly in the pre-clitellar region as compared to other regions. The altered behavioural responses in pesticides exposed earthworms would have been due to decline in AChE activity of the nervous system.

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MATERIALS AND METHODS

3.1 METHOD

A simple method of naturalistic observation was adopted to study the changes and reactions of earthworms to urea and detergent. A comparative study between two species of earthworms were adopted.

3.2 MATERIALS

3.2.1 Earthworms

Earthworms are highly liable to changes in ecological factors, particularly those essential to the soil, and earthworm performance can, therefore, reflect soil contamination. Two species of earthworms were studied on a basis of comparative analysis.

The earthworms used were *Eudrilus eugeniae* and *Notoscolex kayankulamensis.*

The oligochaete *E. eugeniae*, one of the favourite worm species for composting and it is also the Organization for Economic Co-operation and Development (OECD)-recommended earthworm test species. Adult earthworms each weighing 200mg on an average with well-developed clitella were obtained from the Vermiculture unit, Central Plantation Crops Research Institute, Kayamkulam Alappuzha dist.

The *Notoscolex kayankulamensis* were collected simply by digging the soil. Adult with well-developed clitella were collected. Native species were collected because they will be more in number as compared to exotic species and the adaptations will be different. The native species were obtained from Krishnapuram, Kayamkulam, Kerala.

3.2.2 Soil

Loamy soil was taken for culturing the earthworms, as it is moderately aerated and water is retained to an efficient level. Hence the soil organisms can move about freely and which in turn helps to increase the soil fertility and nutrient cycling.

Typical loam soil will consist of roughly 50% soil solids (a combination of sand, silt, and clay) and 50% pore spaces and water. Atmospheric gases (most notably, oxygen and carbon dioxide) also occupy pore spaces and can move passively through the soil profile, depending on surface conditions. The size and distribution of pore spaces will depend on the size and shape of the mineral particles, as well as the activity of microorganisms. A loamy soil being the combination of both sandy and clayey soil along with silt has a 50% pore space which is nearly half the volume of solid composition of the soil.

Earthworm populations are usually highest in places with moist, loamy soil. They often aren't present in sandy soils because these soils dry out very quickly and force worms to either go deeper into the soil where they could die or enter diapause (hibernation) until conditions improve. They also have a tendency to be low in organic matter that serves as a food source for earthworms. While clayey soils can reduce the aeration and the movements due to closely arranged soil particles. As they can highly retain water for a longer period of time, this also affects the earthworm population.

3.3 CHEMICALS USED

3.3.1 Urea

Urea, also known as carbamide, is an organic compound with chemical formula $CO(NH_2)_2$.

This amide has two amino groups ($–NH_2$) joined by a carbonyl functional group (–C(=O)-). It is thus the simplest amide of carbamic acid. It is a colorless, odorless solid, highly soluble in water.

3.3.2 Detergent

Detergents are surfactants that can hold soil and foreign particles in suspension when diluted. These amphithetic molecules contain a polar or charged hydrophilic group at the end of a long lipophilic hydrocarbon tail.

They are cleaning products manufactured from synthetic chemical compounds. Detergents figure in an extensive array of industrial and home cleaning applications, including laundry and freshwater detergents.

3.4 EXPERIMENTAL SETUP AND METHODS OF APPLICATION OF CHEMICALS

3.4.1 Experiment with Urea

Eight plastic pots were taken as the container bin to carry out the experiment. Each of the eight pots were filled with 500-gram loamy soil. Water was added to the pot to moisturize the soil upto 75-90% for the survival of the earthworms.

The eight pots were divided into two sets of four pots each. The pots were also provided with fallen leaves for nutrition. The pots were protected from the contamination of pests and predators from the external environment and the earthworms were allowed to feed and undergo ecological acquisition for ten days before the application of urea. The pots were labelled as I, II, III, IV and 1, 2, 3, 4. 200 milligram of urea was added to pot labelled I and 1. 400 milligram of urea was added to pot labelled II and 2. And 800 milligram of urea was added to pot labelled III and 3. The pot labelled IV and 4 were taken as the controls. The urea was powdered and mixed with the soil to spread it completely with soil to study the proper results. Five earthworms of species *Eudrilus eugeniae* were added into each pots labelled I, II, III, IV. And five earthworms of the *Notoscolex kayankulamensis* were added into each pots labelled 1, 2, 3, and 4 so as to do a comparative study.

3.4.2 Experiment with Detergent

Similar method was done with detergent. Eight plastic pots were taken as the container bin to carry out the experiment. Each of the eight pots were filled with 500-gram loamy soil. Water was added to the pot to moisturise the soil upto 75-90% for the survival of the earthworms.

The eight pots were divided into two sets of four pots each. The pots were also provided with fallen leaves for nutrition. The pots were protected from the contamination of pests and predators from the external environment and the earthworms were allowed to feed and undergo ecological acquisition for ten days before the application of detergent. The pots were labelled as I, II, III, IV and 1, 2, 3, 4. 200 milligram of detergent was added to pot labelled I and 1. 400 milligram of detergent was added to pot labelled II and 2. And 800 milligram of detergent was added to pot labelled III and 3. This was observed for seven days and after that same quantity of detergents were added to the respective pots on a daily basis. The pot labelled IV and 4 were taken as the controls. The detergent was mixed with the soil to spread it completely with soil to study the proper results. Five earthworms of species *Eudrilus eugeniea* were added into each pots labelled I, II, III, IV. And five earthworms of the *Notoscolex kayankulamensis* were added into each pots labelled 1, 2, 3, and 4 so as to do a comparative study.

Experimental Setup

POT WITH 500G SOIL

POT WITH SOIL, LEAVES AND EARTHWORMS

Pots containing *Eudrilus eugeniae* exposed to various concentrations of Chemicals

Pots containing *Notoscolex kayankulamensis exposed* to various concentrations of Chemicals

RESULTS

4.1. EXPERIMENT WITH UREA (*Chart 4.1*)

4.1.1 In the case of *Eudrilus eugeniae*

Earthworms in the pot III carrying 800 mg of urea died completely after 9 hours of application of urea. Earthworms in the pot II carrying 400 mg of urea died completely after 26 hours of application. Earthworms in the pot I carrying 200 mg of urea died completely after 52 hours of application (*Table 4.1).*

It was bit difficult to observe the results due its less thickness. It was observed that the earthworms were becoming threadlike and their mucus coating was dissolved in the soil. The body was detached into numerous pieces at various points and gets mixed with the culture medium making it difficult even to find it out *(Fig. 4.1.1 a and b).*

4.1.2 In the case of *Notoscolex kayankulemensis*

Pot 3 carrying the highest amount of urea i.e. 800 mg shown the results at the earliest. Earthworms in the pot 3 died completely after 4 hours of application of urea. Earthworms in the pot 2 carrying 400 mg of urea died completely after 7 hours of application. Earthworms in the pot 1 carrying 200 mg of urea died completely after 26 hours of application *(Table 4.1).*

It was observed that the clitella and the region near to the tail were infected and inflammated. The clitella were filled with blood and soil like particles that together form a dark almost black coloured substance. As the time proceeds, the skin at many regions was rupturing and the body tissue and fluids were oozing out of it which eventually led to the death of the earthworm and disintegration of its whole body *(Fig 4.1.2 a, b and c).*

4.2 EXPERIMENT WITH DETERGENT *(Chart 4.2)*

200 mg, 400 mg, 800 mg of detergent were added in pot I, II, III and pot 1, 2, 3 respectively for seven days. It doesn't show any characteristic reactions except in pot III and 3 marked with very less amount of decreased activity in the earthworms. So, we added the corresponding amount of detergent in the respective pots on a daily basis and observed the earthworms after every 12 hours. The results were as follows;

4.2.1 In the case of *Eudrilus eugeniea*

Earthworms in the pot III carrying 800 mg of detergent died completely after 3 days of application of detergent. Earthworms in the pot II carrying 400 mg of detergent died completely after 3.5 days of application. Earthworms in the pot I carrying 200 mg of detergent died completely after 4 days of application *(Table 4.2).*

It was bit difficult to observe the results due its less thickness. It was observed that the earthworms were becoming thread-like. The body was detached into numerous pieces at various points and gets mixed with the culture medium making it difficult even to find it out. It was also observed that the activity of earthworms decreased as hours or days proceeds. The mucus layer was completely collapsed and dissolved in the soil. It was noted that thickness of the earthworms was reducing which is an indication of weight loss. (*Fig 4.2.1a).*

4.2.2 In the case of *Notoscolex kayankulemensis*

Pot 3 carrying the highest amount of detergent i.e. 800 mg shown the results at the earliest. Earthworms in the pot 3 died completely after 3.5 days of application of detergent. Earthworms in the pot 2 carrying 400 mg of detergent died completely after 4 days of application. Earthworms in the pot 1 carrying 200 mg of detergent died completely after 4.5 days of application *(Table 4.2).*

These earthworms were larger in diameter and length because of which it can be observed easily. It was observed that the detergents caused their large body to decrease in diameter and their body was getting elongated making cuts at some points in their body. The mucus layer was dissolved in the soil and the body was getting detached as time proceeds. The overall body weight was reduced *(Fig 4.2.2a).*

Table of contents based on the experiment with urea

Species of earthworm used	Survival rate of earthworms in various concentration of Urea		
	200 mg	400 mg	800 mg
Eudrilus eugeniae	52 hours	26 hours	9 hours
Notoscolex kayankulemensis	26 hours	7 hours	4 hours

Table 4. 1 **shows the effect of urea on selected earthworm species**

Table of contents based on the experiment with detergent

Species of earthworm used	Survival rate of earthworms in various concentration of Detergent		
	200 mg	400 mg	800 mg
Eudrilus eugeniae	4 days	3.5 days	3 days
Notoscolex kayankulemensis	4.5 days	4 days	3.5 days

Table 4. 2 **shows the effect of detergent on selected earthworm species**

FIGURES

Fig 4.1.1a *Fig 4.1.1b*

Figures 4.1.1a* and *4.1.1b* shows the effect of urea on *Eudrilus eugeniae

Fig 4.1.2a *Fig 4.1.2b*

Figure 4.1.2c

Figures 4.1.2a, 4.1.2b* and *4.1.2c* shows the effect of urea on *Notoscolex kayankulemensis

Fig 4.2.1a

Figure 4.2.1a* shows the effect of detergent on *Eudrilus eugeniae

Fig 4.2.2a

Figure 4.2.2a* shows the effect of detergent on *Notoscolex kayankulemensis

CHARTS

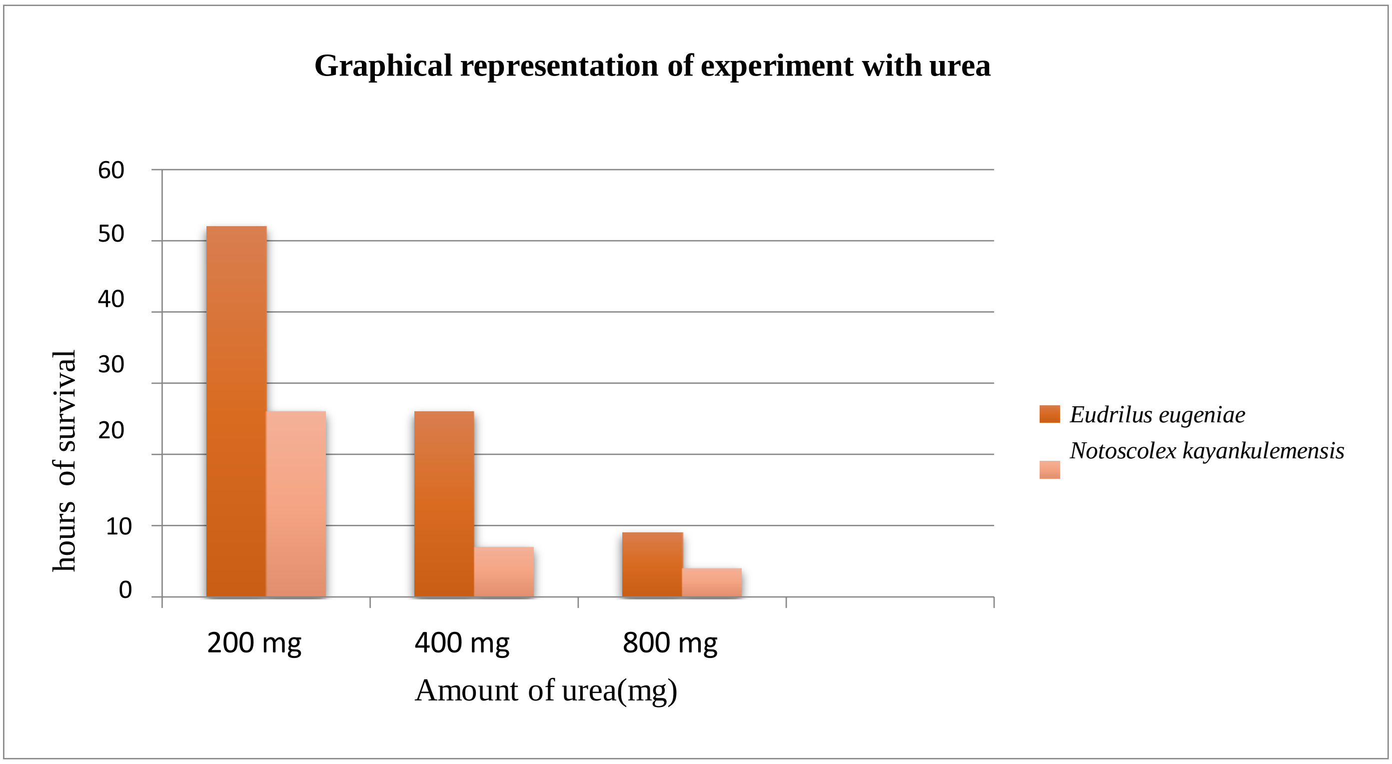

Chart 4.1 **Graph shows the effect of urea on selected earthworm species**

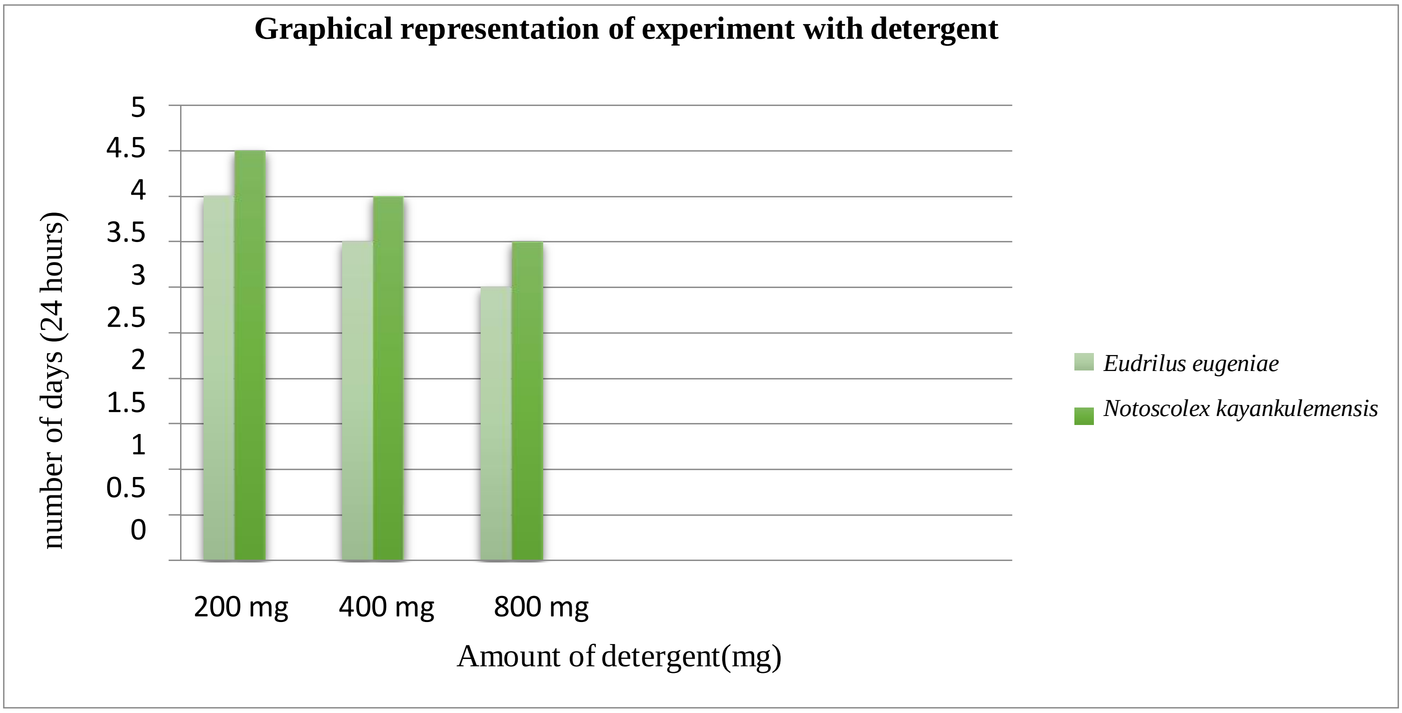

Chart 4.2 **Graph shows the effect of detergent on selected earthworm species**

DISCUSSION

Earthworms are considered as a major macro fauna of soil. They are present abundantly in healthy agricultural soils. They are regarded as the biological markers of soil quality and help to improve the physical, chemical and biological qualities of the soil. Earthworms are known to increase the soil porosity by tunnelling through the soils and helps to improve the soil aeration, as a result of which the plant roots can penetrate easily and water absorption increases. The carbon, nitrogen, phosphorous quantities of the soil are maintained due to their activity and the casts play an important part in soil formation.

Intensive usage of fertilizers by farmers alongside for improving the soil quality and fertility and increased crop production has known to affect the quality of the soil adversely. Urea being such a fertiliser is used in large amounts to increase the nitrogen levels of the soil. Urea has a high potential for acidification of soil. When urea is applied to soil it undergoes chemical reaction with water to form Ammonium, which then gets converted into nitrate by bacteria. This process releases hydrogen ions, which can lower the soil pH and makes it more acidic. With the increased soil acidity, the soil becomes unsuitable for the micro-faunal and macrofaunal organisms of the soil. High levels of nitrate in the soil can be toxic to earthworms.

Detergents are a common household product and is used at a greater quantity on a daily basis. They contain a polar or charged hydrophilic group at the end of a long lipophilic hydrocarbon tail. Releasing of these detergents in the soil can have far reaching impacts. They can enter into soil and water bodies from different sources. Among the different contaminates, these as an important pollutant has serious risk to natural ecosystems. With varying concentrations of the solutions and the frequency of usage, the effects are more or less depended.

In the present study,"Species Specific Chemical Response of *Eudrilus eugeniae* and *Notoscolex kayankulamensis* with reference to Urea and Detergent", the effects of varying concentrations of urea and detergent on direct applications to two species of earthworms were observed. Mortality of the earthworms were taken as the end point.

On the application of urea in concentrations of 200mg, 400mg, and 800mg to the two species, *Eudrilus eugeniae* and *Notoscolex kayankulamensis,* proved varying results. *Eudrilus eugeniae* had a higher resistance and the worms carrying 800mg of urea completely died after 9hrs of application and 400mg and 200mg died at 26 and 52hrs respectively. While *Notoscolex kayankulamensis* died at an earlier rate of 4hrs,7hrs and 26hrs respectively.

With the addition of detergents of the same quantities, it was observed that not much characteristic reaction was seen in the earthworms expect at the highest level of 800mg. The earthworm showed very much decreased activity and hence the quantity of the detergent was increased on a daily basis and they observed after every 12hrs.

It was observed that the native species which is *Notoscolex kayankulamensis* showed a higher resistance towards detergent and highest concentration carrying earthworms died only after 3.5 days of application of detergent. The earthworms in 400mg and 200 mg died after 4 and 4.5 days respectively. *Eudrilus eugeniae* died after a period of 3 days(800mg), 3.5 days (400mg) and 4 days (200mg).

In relation to the other studies mentioned, the earthworms exposed to related conditions showed similar characteristics in their bodies. The bodies of the earthworms in this treatment were ruptured and the live worms were weakened. They were not very active and took a long time to burrow into the soil. The experiment also showed that, both affected the reproduction of the adult earthworms and the mortality of the juveniles. After the juveniles were exposed to urea there was a decrease in their number (Dash and Mohapatra, 2018; Sunish *et al.*, 2019).

Urea can initially be very beneficial to the earthworm population; but the long-term effects are negative. At a high concentration, the effects of urea on earthworms are clearly observed by high mortality, negative effects on reproduction as well as the changes in the activity of the earthworms in the soil. It was observed that urea affects the juveniles at any dose. The mortality of juveniles is proportional to the concentration of urea. The growth rate of juveniles was also affected by the exposure to urea.

Recommendations

- In this study, by observing the various factors and effects we suggest that the concentration of urea should be lowered below the toxicity rates and alternative methods for improving the soil quality must be adopted which also promotes the living conditions of the earthworm populations.
- Though detergents are used in a daily basis and it is an unavoidable material in every household, there dilutions and concentrations must be maintained in such a level that the faunal population of the soil are not affected. It is also advisable to avoid the direct disposal of such solution without proper treatment.

CONCLUSION

Understanding the role of fauna on soil physical properties is important in developing sustainable agricultural managements. The extensive use of new chemical methods affects the faunal populations greatly decreasing the quality of the soil. This in turn also affects the species diversity of a particular area.

In this study, we mainly observed and experimented using earthworms on the basis of urea and detergent, both being extensively used in today's world for easy life. It was seen that both have an adverse effect on the life cycle of the earthworm which can in-turn affect the ecosystem and thereby disrupting the delicate balance of the food chain.

We studied the effect of urea and detergent at varying levels in two species of earthworm namely, *Eudrilus eugeniae* and *Notoscolex kayankulamensis*. The results were astonishing. It was easier to observe the results of *Notoscloex kayankulamensis* as its size was larger and difficult to observe the results of *Eudrilus eugeniae* as it was very thin. The experiment with urea shown that *Eudrilus eugeniae* died completely after 9, 26, 52 hours with the application of 800, 400, 200 mg of urea respectively and *Notoscolex kayankulamensis* died completely after 4, 7, 26 hours with the application of 800, 400, 200 mg of urea respectively. The experiment with detergent shown that *Eudrilus eugeniae* died completely after 3, 3.5, 4 days with the application of 800, 400, 200 mg of detergent respectively and *Notoscolex kayankulamensis* died completely after 3.5, 4, 4.5 days with the application of 800, 400, 200 mg of detergent respectively. It was observed that the clitella of the earthworms were highly affected, enlarged and filled with dark coloured substance mixed with blood in the case of urea. The body was getting elongated marked by the weight loss with the application of detergent. Both species of earthworms were detached into smaller pieces and mixed with soil, with the application of urea and detergent.

When we look into the experiment with the aspect of a comparative study, we observed that the *Eudrilus eugeniae* was more resistant towards urea on comparison with *Notoscolex kayankulamensis*, whereas *Notoscolex kayankulamensis* was more resistant towards detergent when compared with *Eudrilus eugeniae.*

Apart from the cause of urea and detergent in earthworms, these higher concentrations of chemicals used are biomagnified and finally reaches the human bodies which in turn accumulates and causes various chronic diseases and disabilities.

Therefore, we suggest through this study to use a lower concentration, below the prescribed toxicity levels, of chemicals for a sustainable and healthy lifecycle of ecosystem. The more and better we shift to bio fertilizers and organic practices, the more we support the micro and macro faunal populations of the soil.

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