PESTICIDES AS A CURSE TO NON-TARGET SOIL MACROFAUNA: AN EXAMPLE OF HISTOLOGICAL ALTERATIONS IN EARTHWORM

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Abstract:

Earthworms, called as "nature's ploughman" or "friend of farmers", form major soil macro-fauna. They perform variety of essential ecological function like decomposition of organic matter, nutrient recycling, increasing the porosity, aeration and water holding capacity etc. To meet the nutrient requirement of expanding population, there is an extensive utilization of agrochemicals like pesticides and inorganic fertilisers. Unregulated and overdose of these agrochemicals affect beneficial soil fauna like earthworms. Two pesticides, chlorpyrifos and glyphosate, were studied with respect to their impact on integument of compost worm, *Eisenia foetida*. Exposure of *Eisenia foetida* to these pesticides significantly altered the integument rendering the earthworms susceptible to weight loss and detrimental behavioural alterations.

Keywords: *Eisenia foetida*, chlorpyrifos, glyphosate, integument.

Introduction:

The earthworms called "ecosystem engineers of nature" are continually exposed to pesticides and contaminants in soil due to their abundance in agricultural soils. They constitute about 80% of soil macro-fauna. Therefore they are usually selected for eco-toxicological studies (Rault, Mazzia and Capowiez, 2007). Earthworms are involved in evolution and conservation of natural fertility of soil since their arrival on mother earth some 600 million years ago.

They are found in all soil types when physical parameters like food, moisture and temperature are optimum (Kale, 1993). They are involved in nutrient recycling, soil aeration and improving soil structure, breakdown of organic matter etc (Edwards and Bohlen, 1996). Earthworms act as biological indicators of soil. Since vermicasts are rich in plant nutrients, soils loaded with casts require less agrochemical like pesticides and inorganic fertilisers (Dash and Senapati, 1986).

Soil is a major reservoir of microbes which meet the food requirement of earthworms. As a result varies symbiotic relations like mutualism, parasitism and commensalism is established between earthworms and microbes. The gut of earthworms and vermicompost bed provide an ideal environment for the growth, development and distribution of microbes (Lavelle, 1988). Microbes also provide C and N required for the growth and reproduction of earthworms (Edward and Bohlen, 1996). Earthworms are selective about the microbes they eat (Dash and Senapati, 1986).

There is extensive utilization of agrochemicals like pesticides and chemical fertilisers throughout the world to feed the expanding human population. In this context, it is imperative to study the impact of these agrochemicals on soil macro and micro fauna and flora.

Chlorpyrifos is an organophosphate pesticide which acts by inhibiting the cholinesterase enzyme. If the organisms are exposed to same pesticide for multiple times through same route, it results in synergistic effect (Bardin *et al.*, 1994). Chlorpyrifos is commonly used to control insect pests due to its broad spectral effect and low half life.

Glyphosate is a non-selective herbicide that acts by inhibiting an enzyme 5-enolpyruvylshikimic acid-3-phosphate synthase (ESPS) of shikimic acid pathway for synthesis of aromatic amino acid. Glyphosate is relatively non-toxic to animals since ESPS is absent in animals.

Certain organisms which are intolerant to agrochemicals undergo physiological, morphological or behavioural adaptations to survive and reproduce. These alterations could be used to study effect of chemicals on such organisms. In India and other developing countries, agrochemicals are not regulated, resulting in their use much higher than optimised dose affecting the beneficial soil fauna like earthworms.

In view of these, present study was designed to study the effect of two pesticides, chlorpyrifos and glyphosate histology of body wall of earthworm, *Eisenia foetida*. According to the principle of "structure reveals function", histological changes after exposure to pesticides were studied to assess toxicity conditions as a biological marker (Srivastava, Yadav and Trivedi, 2008).

Materials and methods:

The earthworm, *Eisenia foetida*, was purchased from Goseva Anusandhan Kendra, Mahal, Nagpur and acclimated to laboratory conditions for a week under optimum conditions (25°C temperature and 30-40% relative humidity). About ten clitellated earthworms were selected and exposed to sub-lethal concentrations (1-5 mg/Kg of soil) of two pesticides, glyphosate and chlorpyrifos. Chlorpyrifos was uniformly hand mixed with soil while glyphosate was dissolved in water and sprinkled before addition of earthworms. Control was maintained without adding any pesticide for 56 days. Earthworms were pre-weighed and inoculated into the test set. All treatments were used in triplicate. The conditions during experiment were maintained at optimum level of 25°C and 30-40 % moisture. The humidity was maintained at 30-40% by sprinkling dechlorinated water.

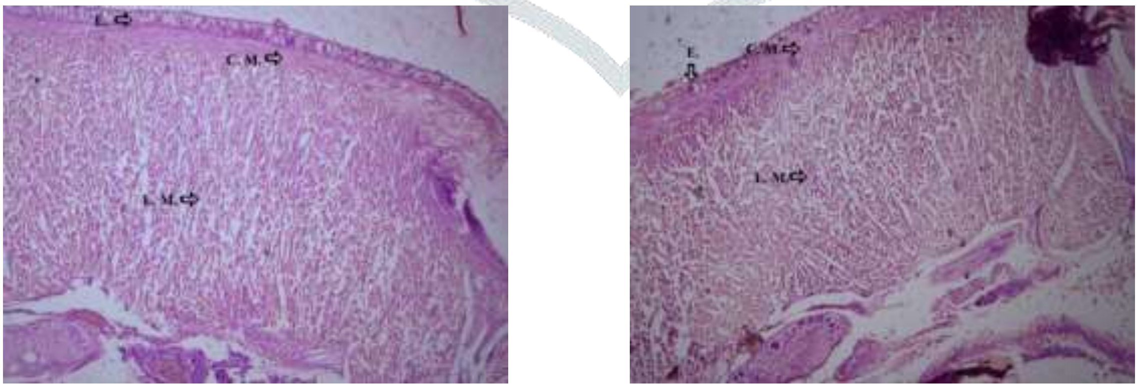
The biomass of earthworms was monitored every 7th day for a total period of 56 days. The average biomass values of earthworms were recorded during study period. After a study period of 56 days, two earthworms were sampled for histological studies.

Sections were taken from the post clitellar region of earthworms for preparation of histological slides. Sections were blotted free of mucus and preserved immediately in Bouins fixative to avoid putrefaction. The slides were prepared according to standard HE staining techniques. The photographs were taken under fluorescent microscope with camera attachment.

Results and discussion:

The body wall of earthworm is divided into three distinct layers; cuticle, single layered epidermal epithelium and bilayered muscle (circular and longitudinal). The cuticle is the thin, pervious, flexible and outermost covering supported by epidermis. A single layer of epidermis consists of four types of cells. The circular muscles form thin layer beneath epidermis to make earthworm long and slender while much thicker longitudinal muscle layer makes worm short and stout.

The gut of earthworms is lined with visceral peritoneum consisting of large chloragogen cells. Chloragogen cells form a chloragogenous layer which is involved in phagocytosis, excretion, metabolism (an equivalent of vertebrate liver), synthesis and storage of glycogen and fat.



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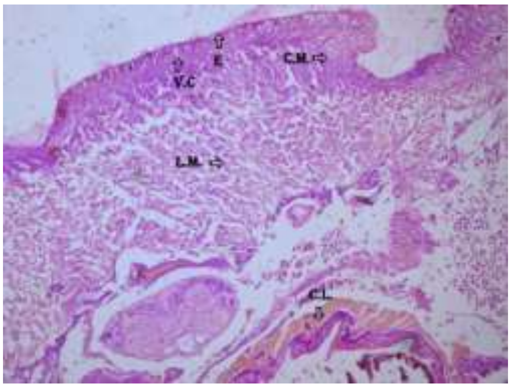

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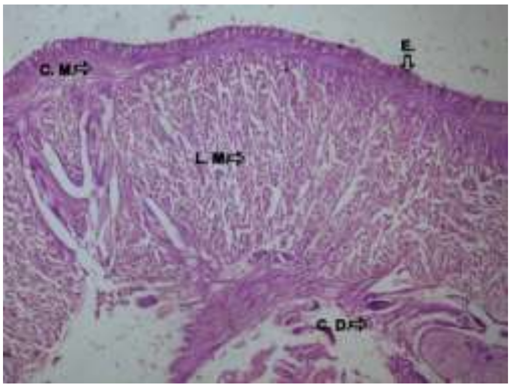

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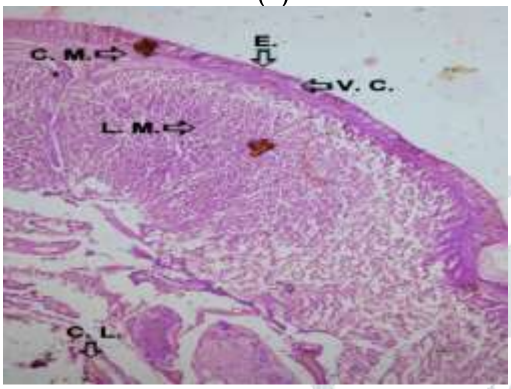

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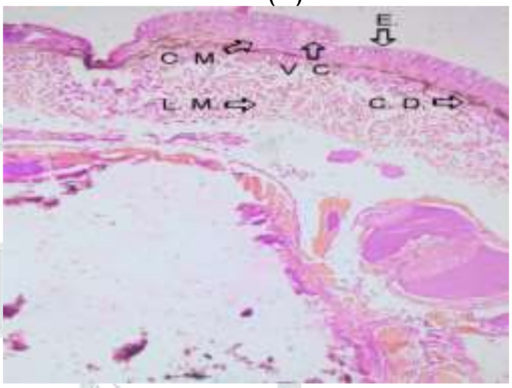
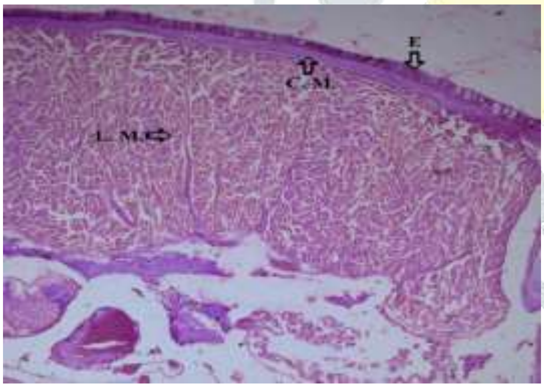


(f)

Histological changes in the body wall of *Eisenia foetida* exposed to chlorpyrifos for 56 days. (a): Control-showing intact epidermis and muscle layers. (b), (c), (d), (e) and (f): progressive degeneration of epidermis and muscle layers following exposure to 1 mg, 2 mg, 3 mg, 4 mg and 5 mg/kg soil of chlorpyrifos. There was a progressive vacuolization and formation of pyknotic nuclei in epidermis, loss of integrity of circular and longitudinal muscles, appearance of cell debris.



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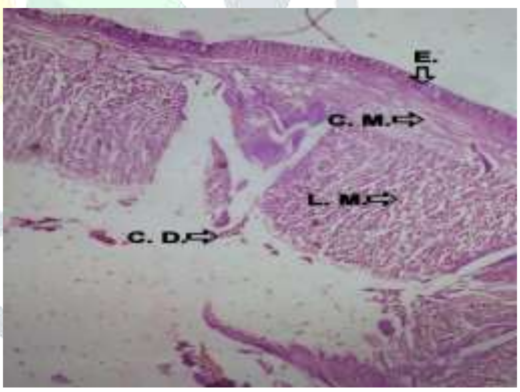

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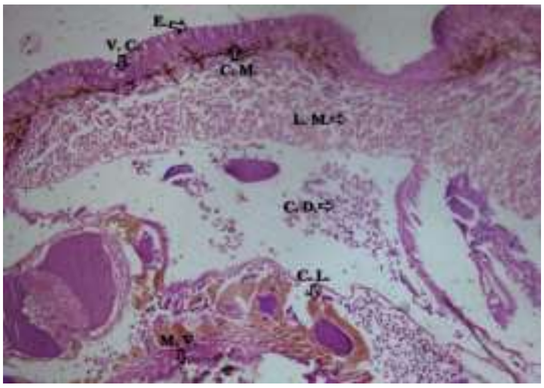

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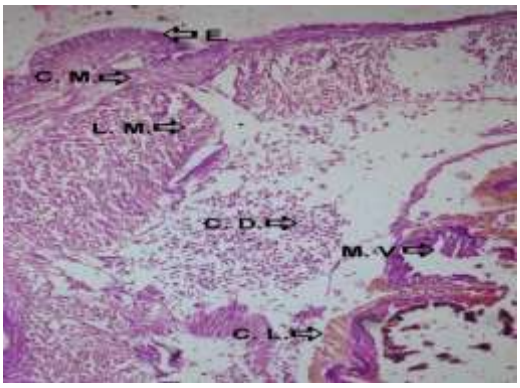

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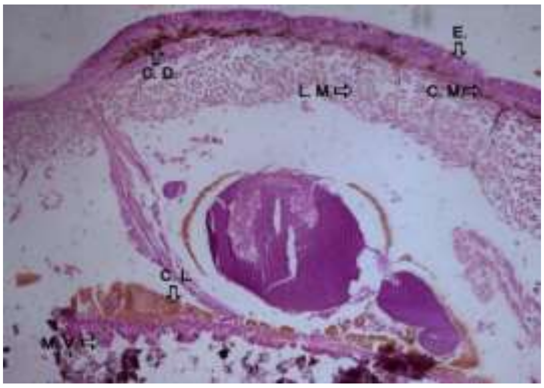

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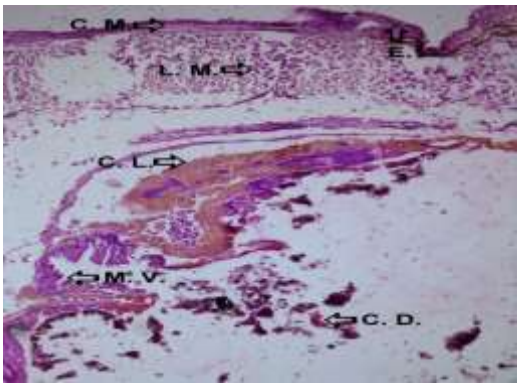

(f)

Histological changes in the body wall of *Eisenia foetida* exposed to glyphosate for 56 days. (a): Control-showing intact epidermis and muscle layers. (b), (c), (d), (e) and (f): progressive degeneration of epidermis and muscle layers following exposure to 1 mg, 2 mg, 3 mg, 4 mg and 5 mg/kg soil of glyphosate. There was a progressive vacuolization and formation of pyknotic nuclei in epidermis, loss of integrity of circular and longitudinal muscles, appearance of cell debris.

The effect of chlorpyrifos and glyphosate on body wall of *Eisenia foetida* was shown in fig. no-1 and fig. no-2 respectively. The impact of agrochemicals like pesticides on earthworms is limited and needs to be updated (Castellanos and Hernandez, 2007). These pesticides enter into the body of worm through dermal exposure or ingestion of pesticide contaminated litter (Vijver *et al*., 2003). The body wall of earthworms not only acts as primary barrier to environmental contaminants but transport ions (Clauss, 2001).

The toxicity of chlorpyrifos and glyphosate to earthworm was found to increase with increase in concentration or exposure time. Venkateswar Rao, Surya Pavan and Madhavendra (2003b) demonstrated that chlorpyrifos was more toxic to *Eisenia foetida* while Kumar and Singh (2017) included that the phorate was histologically less toxic to *Lampito mauritti* and *Metaphire posthuma*.

Youn (2005) noticed anatomical symptoms like fragmentation, swelling, loss of mucus layer and body wall rupture in *Peryonyx excavatus* and *Eisenia andrei* exposed to MTBE (methyl tetra-butyl ether) and imidacloprid due to microscopic damage. Progressive changes such as hypertrophy and hyperplasia of body wall after exposure to toxicants serve as general defence mechanism by increasing the distance between toxicant and general viscera (Baynes and Hodgson, 2004; Poleksic *et al.*, 2010). Long term exposure to pesticides, chlorpyrifos and glyphosate showed progressive atrophy, necrosis and erosion of body structures leading to generalised thinning of body wall. An increase in the concentration of pesticides, glyphosate and chlorpyrifos, was followed by subsequent increase in the damage to cuticular wall of *Eisenia foetida*. Extensive damage to musculature of earthworm might impact their burrowing and migratory capabilities.

Polypheretima elongata exposed to textile dyes similarly showed autolysis of posterior region (Ramaswami and Subbram, 1992). After exposure to chlorpyrifos and glyphosate, integument undergoes marked changes like loss of compactness of epithelial lining (Kavitha and Anandhan, 2018) rupture of cuticle, distortion of muscle layer, vacuolization of epidermal cells. Amaral et al. (2006) noticed prominent alterations in earthworm body parts after exposure to OP pesticides and discussed the role of earthworms as biological indicators of soil pollution.

The change in the gut and integument of earthworm exposed to atrozine was also observed by Oluah *et al.* (2016). The adverse impact of malathion on the structural integrity and body wall of *Eisenia foetida* was also demonstrated by Bansiwal and Ray (2010). An increase in muscular disintegration of *Eisenia foetida* was seen with increase in butachlor concentration (Gobi and Ganusekaran, 2010).

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