Chapter IX

HISTOLOGICAL STUDIES OF EARTHWORMS ON THE TREATMENT OF INDUSTRIAL SLUDGE

OVERVIEW

Earthworms have been widely used in the breakdown of a wide range of industrial and organic waste including tannery, distillery sludge and poultry, municipal waste. The metabolic systems of earthworms transforming these waste into organic form as vermicompost and accumulate some heavy metals in their tissues, which cause damage or change in their organization and function of the same. Wide literature survey shows, only very few authors have undertaken research to assess the effect of vermicompost obtained from tannery and distillery sludge and their impact on histology of the animal (Filipek-Mazur et al., 2000). Therefore, the investigations aimed to study the effect of tannery and distillery sludge on the histology of the earthworms. Experiments were conducted in their direction and the change in the cell structure of the animal was explained with evidences of the cross section of the various regions of earthworms.

INTRODUCTION

Earthworms are regarded as one of the most suitable animals for testing the toxicity of chemicals in soils and have been adopted as standard organisms for eco toxicological testing. Acute and chronic toxicity tests have been used traditionally to asses the toxicity of contaminants, with mortality and changes in biomass, reproduction rates and behavioral responses representing endpoints. The uptake, accumulation, and elimination properties of metals by earthworm are the major part of toxicology, which is called toxicokinetics (Lee et al., 2008). However, limited works were carried out to assess the damages to tissues due to the effect of tannery and distillery sludge. So, the experiments were carried out to expose the changes to tissues with various regions and conditions.
MATERIALS AND METHODS

The histology of gut of earthworm was studied adopting the routine paraffin method (Humason, 1979). Gut of earthworm, dissected out from the control and experimental animals, were blotted free of mucus, washed thoroughly in physiological saline, cut into pieces of desired size and fixed in Bouins fluid fixative immediately after autopsy. Fixation was carried out at room temperature for 24 hr, after which the tissues were transferred to 70% alcohol. Several changes of 70% alcohol were given until the yellow colour disappeared from the tissues. The tissues were then dehydrated by passing through ascending grades of alcohol, cleared in xylene, infiltrated with molten paraffin, and finally embedded in paraffin wax (58°C MP).

Tissue section of 5-μm thick transverse and longitudinal sections were obtained using a rotary microtome (Leica, Germany). The sections, thus obtained, were stained in Harris hematoxyline and eosin, dehydrated using alcohol, cleared in xylene and mounted using dihydroxy phthalate xylol (DPX). The stained slides were observed in a CarlZeiss (Germany) Axio-2 Plus research microscope (Chapter IV).

RESULTS AND DISCUSSION

The body wall consists of cuticle secreted by the epithelial cells, which underlines it. The epithelium has 4 types of cells. Most of the cells are tall columnar and epithelial cells, but between them are many mucus-secreting cells usually filled with granules (or) globules. In certain regions of the skin, sensory cells present some of which are photoreceptors, have a reticular cytoplasm containing a clear body, which seem to act as a lens. There found are short cells abundant on the prostomium and the first and last 2 (or) 3 segments others are small clusters of very tall, thin cells with minute sensory hairs. Under the epidermis is a layer of circular muscles and below this is much thicker layer of longitudinal muscles. The circular muscles contract to make the worm longer and thinner, the longitudinal to become short and stout. The pigment in the body wall is present in amoeboid cells in very young worms, but later only crowded granules can be found. Like hemoglobin and chlorophyll, this pigment, with a spectroscope, gives the absorption bands, which indicate that it is a porphyrin.

The capacious coelom is lined by a peritoneal epithelium. It has two parts, the parietal peritoneum, which is a thin layer of flattened epithelial cells pressed against the
inner surface of the body wall, and the visceral peritoneum, which is pressed against the viscera, chiefly the intestine. While in a mammal, the visceral peritoneum is also of thin flattened cells, in earthworms it is composed of the very large chloragogen cells. The “brown bodies” often found with in the coelom of earthworms, especially in the hinder segments, are massed aggregations of these phagocytic cells and contain encysted nematodes, worm out setate, protozoan cysts and large amount of pigment granules form choragogen cells. In the coelomic fluid rounded cells called eleocytes present. These cells are bright yellow coloured and always filled with minute lipid droplets. The adults of *E. eugeniae*, *E. fetida* and *P. excavatus* have a distinct swelling called clitellum. It is located about one third of the way down the earthworm. It produces most of the materials secreted to form earthworm cocoon. The clitellum forms a band that can be flared, non-flared, saddle shaped (or) annular. It is generally found between the segments 14-17. The cuticle, epidermis, the circular muscles and longitudinal muscles are clearly on the left side. The ventral nerve cord, the dorsal and ventral blood vessel are clearly seen. The folding of the intestine the typhlosole, the intestinal epithelium is definite. A large bright yellow tissue, which contains number of eleocytes with numerous fat droplets, is also seen. At the centre of the section is the intestine. The intestine is lined with ciliated epithelium. A significant structure, the typhlosole is present in the intestine. This is a fold of the dorsal wall, which projects into the intestine and performs several important functions; first, it slow down the passage of food so that, it may be acted upon for longer time by the digestive fluids; second, it increases the surface area for the secretion of digestive enzymes and their, it increase the area for absorption of the products of digestion. Typhlosole hangs down into the lumen of the intestine. The peritoneum covering the intestine and portions of the major blood vessels is modified in earthworm to form chloragogen layer. Waste materials accumulate in the chloragogen cells, which eventually separate from the intestinal wall and float freely in the coelomic fluid. Some of these detached cells are ingested by the amoebocytes of the coelom, which interns migrate later to the superficial tissues, disintegrate and deposit their contents in the form of a protective pigment. Other chloragogen cells are swept into the ciliated funnel of the nephritic and thence of the outside. Chloragogen cells store glycogen, which is then distributed when there, cells break loose and disintegrate.
Histological studies of foregut, midgut and hindgut regions of the earthworm’s *E. eugeniae, E. fetida* and *P. excavatus* treated with tannery sludge

**Foregut**

Histology of the tested fore gut reveals that the cuticle is untouched with the damage. Not much spoilage in the area of circular muscles but in some part of the longitudinal muscle can see the signs of injury. Detachment of peritoneum from longitudinal muscles can also be seen in some of the areas. The lumen of the intestine is not much harmed and the epithelial lining. The ventral nerve cord is damaged to a smaller extend. The dorsal and ventral blood vessel ruined largely. In brief, the 45% of the distillery sludge does not affect the tissues of earthworm much.

**Midgut**

In mid gut region of the cuticle and the epidermis are intact. The circular and longitudinal muscles are also unaffected. The peritoneum shows the signs of disconnect at several places. The typhlosole and the nephridiopore are clearly visible. The chlorachogen cells crumble to a considerable extent. The ventral nerve cord is not much damaged where as the dorsal blood vessel and ventral blood vessel have shown the signs of damage. The nephridia are feebly visible. Generally, the tissue of the test individuals were not much affected in this concentrations.

**Hindgut**

The cuticle of the earthworm hind gut region; under it, the epidermis is faintly visible. Light destruction is visible in some parts of circular muscles. However, in the longitudinal muscles more damage and fissures are seen in few areas. The peritoneum covering the intestine is injured largely. The intestinal epithelial lining, the lumen of the intestine are also damaged. Greater degree of injury to the dorsal and ventral blood vessel is observed. Ventral nerve cord is damaged beyond recognition. In short, the acute concentration of distillery sludge has a telling effect on the histology of the earthworms *E. eugeniae E. fetida* and *P. excavatus*. 
Histological studies of foregut, midgut and hindgut regions of the earthworm’s E. eugeniae, E. fetida and P. excavatus treated with distillery sludge

Foregut

The cuticle is well distinct except breaks in one or two places. The epidermis is lightly damaged. The circular and longitudinal muscles are well formed and show no marks of damage. The ventral nerve cord and the ventral blood vessels are very much unaffected. Apart from regular cells, there is a large bright yellow body is serving to be noticed which could be an eleocyte. Normally the eleocyte are filled with lipid droplets. The peritoneum is damaged in one or two places. The intestine wall and the epithelial lining of the intestine are damaged in several places. The nephridiopore is visible distinctly. The 45% distillery sludge has slightly affected the histology of earthworm.

Midgut

The cuticle is damaged in some areas. The epidermis has eroded completely in few areas. The circular muscle and longitudinal muscle are well developed in several places. The peritoneum is damaged in most part of the section. The ventral nerve cord and ventral blood vessels are visible clearly. The chloragogen cells are spread throughout the coelom. Accumulations of some foreign bodies are also seen in chloragogen cells. The intestine wall is severely damaged. In general, the effect of 56% concentration of distillery waste is much more visible than in other concentrations.

Hindgut

The cuticle is slightly damaged in several areas. The epidermis is also damaged largely. Even the circular muscles have shown the signs of damage in several areas. The longitudinal muscle has shown the signs of crakes in major parts of the regions. There is no signs of peritoneum wall at all. The intestinal epithelium is injured beyond recognition. The chlorocogen cells have damaged. Signs of accumulation of foreign bodies and vacuolization of coelom are observed. The ventral nerve cord is faintly visible, where as the dorsal and ventral blood vessel and the nephridia are seen faintly. In short, the clitteral region of 70% distillery sludge exposed earthworms is severally affected. Coelom of earthworms, especially in the hinder segments, are massed aggregations of these phagocytic cells and contain encysted nematodes, worn out
setate, protozoan cysts and large amount of pigment granules form choragogen cells. In the coelomic fluid rounded cells called eleocytes present. These cells are bright yellow colored and always filled with minute lipoid droplets.

The feasibility of using earthworms for industrial waste management is dependent on the fundamental knowledge of basic parameters governing the survival and growth of earthworm species. The present set of investigation is aimed in this direction and the results are encouraging. Researchers reported that the passage of organics through the earthworm’s gut significantly alters the physical structure of the material. Large particle are broken down into numerous smaller particles, with a resultant enormous increase in surface area. Soil pollution bio-indicators are essential to establish environmental standards. Earthworms and hypogenous micro arthropods were chosen to test ecological risks derived from soil amendment with Tannery and distillery sludge. The objective of culturing earthworms in tannery and distillery sludge revealed that the earthworm could live in the tannery and distillery waste. Investigation by other researchers in other waste products also have yielded encouraging results Gajalakshmi et al., (2001) reported the feasibility of vermi-composting as a viable process for the gainful utilization of industrial sludge in a environmentally clean manner. Pulp and paper industries, which releases waste water to about 330 m²/tonne of paper/day. This effluent contains toxic elements which inhibit the growth of the soil micro flora (Sastry et al., 1977; Oblisami and Palanisami, 1991; Sandana, 1995). The chemical immobilization is a relatively inexpensive in situ remediation method that reduce soil contaminant solubility, but the ability of this remediation treatment to reduce heavy metal bioavailability and eco-toxicity to soil invertebrates has not been evaluated Conder et al., (2001). Domínguez, (1997) reported that earthworms can dispose off about 35% to 55% of bio-available heavy metals by accumulating them in their tissues. In the present investigation the *Eudrilus eugeniae*, *E.fetida* and *P.excavatus* was subjected to various concentrations of tannery and distillery sludge. It has been observed that the sludge is toxic to earthworms observed only miner damages. The paradox of the industrial sludge having huge organic load and increased mortality could be solved when the miner damage is assessed histological studies. The histology of earthworm, which is subjected to various concentrations of tannery and distillery, show that increase in the concentration of sludge gradually increases the damages of the tissues. The soft peritoneal membrane lining intestinal epithelium is more affected. The
brown bodies in the coelom in the experimental groups are chloragogen cells. Chloragogen cells are the sites of accumulation of waste products is already been well established. The presences of yellow bodies, elecocytes where the fat droplets are accumulated like adipose tissues in higher organism are also the sites of accumulation of toxicants from the industrial sludge. The results and the discussions of the present investigation reconfirm the belief that there is no such thing as industrial wastes only wasted organics. Earthworm can be used to dispose off all sorts of industrial waste including tannery and distillery waste. The processing of waste by earthworm would help to reduce a major environmental problem, the mounting solid wastes.
Fig 9.1 Histological studies of foregut, midgut and hindgut regions of the earthworms *E. eugeniae*, *E. fetida* and *P. excavatus* reared in control soil (10x)

C - Cuticle  
E - Epeidermis  
Cm - Circular muscle  
Lm - Longitudinal muscle  
Cg - Chloragogen cells  
Ec - Epithelial cells  
Dbv - Dorsal blood vessel  
Vbv - Ventral blood vessel  
N - Nebhrostome  
Ni - Nephridium  
S - Setae  
L - Luman  
Pg - Pigment  
Co - Coelom

Fg - Foregut  
Mg - Midgut  
Hg - Hindgut

EE - *E. eugeniae*, EF - *E. fetida*, PE - *P. excavatus*
Fig: 9.2 Histological studies of foregut, midgut and hindgut regions of the earthworms *E. eugeniae*, *E. fetida* and *P. excavatus* treated with tannery sludge (10x)

\[ \text{C - Cuticle} \quad \text{Vbv - Ventral blood vessel} \]
\[ \text{E - Epeidermis} \quad \text{N - Nebhrostone} \]
\[ \text{Cm - Circular muscle} \quad \text{Ni - Nephridium} \]
\[ \text{Lm - Longitudional muscle} \quad \text{S - Setae} \]
\[ \text{Cg - Chloragogen cells} \quad \text{L - Luman} \]
\[ \text{Ec - Epithelial cells} \quad \text{Pg - Pigment} \]
\[ \text{Dbv - Dorsal blood vessel} \quad \text{Co - Coelom} \]

Fg - Foregut  Mg - Midgut  Hg - Hindgut

EE - *E. eugeniae*, EF - *E. fetida*, PE - *P. excavatus*
Fig: 9.3 Histological studies of foregut, midgut and hindgut regions of the earthworms *E. eugeniae*, *E. fetida* and *P. excavatus* treated with distillery sludge (10x)

EE - *E. eugeniae*, EF - *E. fetida*, PE - *P. excavatus"