ISSN: 0976-3031

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 9, Issue, 4(I), pp. 26154-26158, April, 2018

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

EFFECT OF TANERY SLUDGE ON GUT REGION OF EARTHWORM, *EISENIA FOETIDA*

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DOI: http://dx.doi.org/10.24327/ijrsr.2018.0904.2003

ARTICLE INFO

Article History:

Received 10th January, 2018
Received in revised form 20th February, 2018
Accepted 8th March, 2018
Published online 28th April, 2018

Key Words:

Earthworm, histology, heavy metal, tannery sludge, vermicompost,

ABSTRACT

The histological effect of chromium on the body tissues of the earthworm, *Eiseniafoetida* was determined by light microscopy. The earthworms were exposed to crushed tannery sludge up to 30 days in different concentrations. Histology of the foregut of *E. foetida* showed changes in circular muscles, a detachment of the peritoneum from longitudinal muscles in the foregut. In some areas of the midgut, the cuticle, the intestinal wall was found damaged with complete eradication of peritoneum. In hindgut, the damage was observed in epidermis, circular muscles, longitudinal muscle showed signs of cracks in major parts of the region. Further, the intestinal epithelium was injured beyond recognition.

INTRODUCTION

Earthworms are ubiquitous and abundant in nature. They form a major biota component in all vermicomposting studies and they have been used by most of the substrate conversion studies ranging from sludge to solid wastes (Weeks *et al.* 2004; Loureiro and Nogueira 2005). In soils, earthworms constitute 60–80% of the animal biomass and play a critical ecological role (Siekierska and Urbańska-Jasik 2002). It is also easy to determine earthworm life cycle, its growth, reproduction, biochemical and histological responses (Booth *et al.* 2000; Venkateswara Rao *et al.* 2003a). Earthworms are soil-dwelling organisms involved in the process of soil formation and organic matter decomposition hence, plays an important role in a functionality of soil and in assessing the terrestrial ecotoxicological risk (VenkateswaraRao and Kavitha 2004; Reinecke and Reinecke2007; Zhou *et al.* 2007; Olayinka 2011). Hence they are prone to various toxicological components amongst which heavy metals are ingredients in most of the sludges and solid wastes that come out as an end product or waste (Cortef *et al.* 1999).

Tanning in India has a long history. Tannery sludge contains undesirable agents such as organic and inorganic toxic compounds (Borkowska *et al.*, 1996) which negatively interact with the flora and fauna (Srivastav *et al.*, 2005). Particular attention should be paid to the content of heavy metals whose presence in sewage sludge and later in soil poses a hazard for plants, animals, and people (Kabata-Pendias and Pendias 1993). Heavy metals affect the growth, morphology, and metabolism of microorganisms in the soil because the denaturation of the integrity of cell membrane takes place. Contaminated soils have become a primordial problem since they lead to groundwater contamination and biomagnifications of chemical compounds through food webs and sometimes affect human health (Lemtiria *et al.* 2016). Vermicomposting uses earthworm to stabilize and transform organic wastes into valuable end products and has been proposed as an alternative treatment technology for organic and inorganic wastes from agricultural, industrial and municipal sources (Marsh *et al.*, 2005). The earthworm can accumulate heavy metals in their chloragogen cells (Huges *et al.*, 1980; Beyar 1981).

Metals that are ingested by earthworms during a feed and through dermal uptake are known to cause the deleterious effect on various physiological functions of earthworms. Some metals even prove fatal while the rest of the metals have residual toxicity. The study of heavy metals effects remain largely undefined and a bulk of the work is directed towards acute toxicity studies in relation to population, reproduction pattern, and behavioral aspects of earthworm species. Harstenstein *et al.* (1981) have reported accumulation of heavy metals in earthworm tissues and on the growth of earthworms. Chromium is one of the potential toxic element exists in several

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oxidation states, discharged from the industrial plants. There are few studies related to the deleterious effects of stressors or toxicological compounds/ elements such as chromium on earthworms (Arillo and Melodia 1991; Sheppard *et al.* 1992; Sheppard and Evenden 1992; Labrot *et al.* 1999; Manerikar*et al.* 2008).

Eiseniafoetida earthworm is low cost, easy to culture and easy to standardization of toxicological examinations thus considered as appropriate biomonitors to decide the environmental risk of tannery sludge as heavy metals, chemicals and pesticidal contaminated soils (Lanno *et al*. 2004; Xiao *et al*. 2006, Castellanos and Hernandez 2007).In the acute and sub-chronic ecotoxicological study, the exposed earthworm to soil provides information about pollutant bioavailability and its toxic effects (Lukkari *et al*. 2004, 2005; Giambi *et al*. 2007). The histological analysis may signal towards the damaging effects of any organisms such as earthworm resulting from a toxic exposure. The objective of the present study was to the examined toxicity of tannery sludge via histological analysis on body tissues of earthworm, *Eugenia foetida*.

MATERIALS AND METHODS

Sample collection

The earthworm *Eisenia foetida* of approximate 0.4-0.6 g weight and 5.5-6 cm length was collected from Aditya Pharma School, Moradabad, from the moist surface soil and were kept in a plastic tub. The solid tannery sludge was collected from 'Leather Technology Park', Banthar, Unnao, Utter Pradesh, India, and vermicompost were procured from Biotech Park, Kursi Road, Sector-G, Jankipuram, Lucknow, Uttar Pradesh.

Experimental setup

The crushed tannery sludge and compost were mixed in a total volume of 3 kg in different ratios to prepare the concentration of 10%, 20%, 30%, 40%, 50% and vermicompost was kept as control. The mixture and control were kept in clean plastic tubs in three replications (R_1, R_2, and R_3).The acclimatized earthworm, *E.foetida* was kept in the all experimental and control tubs. The moisture content of the mixture was maintained at 70-80% throughout the experimental period.

Histological analysis

After 30 days, the earthworm was taken out from the tray and washed with running water to remove the soil from the body and cut into foregut (FG), midgut (MG) and hindgut (HG). After cutting, the body parts of earthworm fixed in Bouin's fluid for 24 hours, were washed in running tap water for a few minutes and stored in 70% alcohol for further tissue wax preparations using routine standard laboratory procedures. The body parts were then processed using step by step dehydration with different grades of alcohol, then cleared using xylene.

The cleaned tissues were infiltrated with molten paraffin wax at 55-58^{0}C and appropriate sizes of the tissue blocks were prepared. The serial sections of 5-7 microns of foregut, midgut, and hindgut were incised in a rotary microtome (Weswox) using a fine blade. After deparaffination and downgrading in alcohols, the sections of the foregut, midgut, and hindgut were stained with the Double-staining procedure of hematoxylin followed by Eosin. The detailed microscopic observations were done in stereo research microscope with an attached camera.

RESULTS AND DISCUSSION

The tannery sludge contained many inorganic and organic waste which influence the histopathology of an earthworm. The present study was aimed to study the effect of tannery sludge on the earthworm *Eisenia foetida* by histological assessment as a tool. The present investigation of histology of earthworm in the foregut region revealed that the cuticle was undamaged. There was not much spoilage in the circular muscles area but there was some sign of injury in some part of the longitudinal muscle. However, a detachment of peritoneum in the longitudinal muscles and loosening of longitudinal muscle were observed in the treated groups with tannery effluents. The lumen, the intestinal membrane and epithelial lining of intestine were not much harm. The dorsal and central blood vessel was largely affected. Elangotation of microcilli was also observed (Fig. 1).

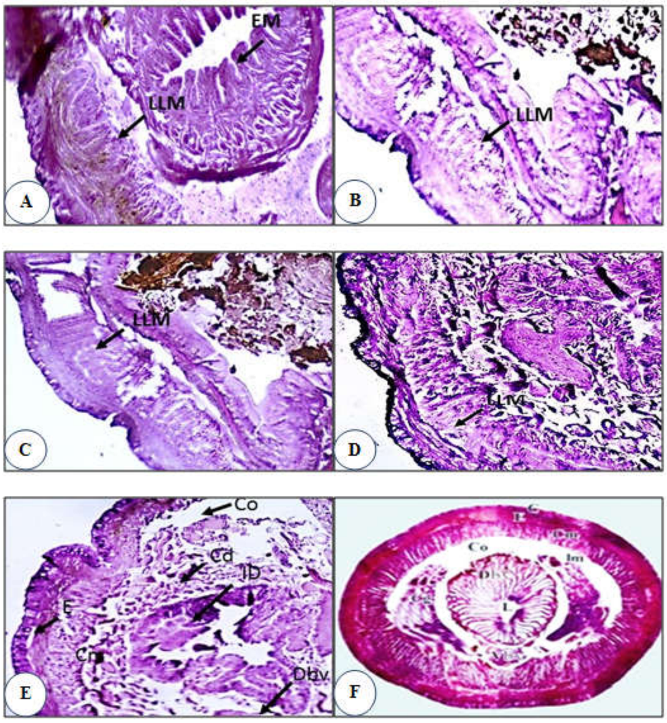

Fig 1 Histological sections of foregut region of earthworm *Eisenia fetida* exposed to different concentrations of tannery sludge for 30 days. A: 10% tannery sludge, B: 20% tannery sludge, C: 30% tannery sludge, D: 40% tannery sludge, E: 50% tannery sludge , F: control. Abbreviations shows- C: Cuticle, Co: Coelom, Llm: Loosening of longitudinal muscle, L: Lumen, Lm:longitudinal muscle, Cm: Circular muscle, Cg: Chloragogen cells, E: Epidermis, Dbv: Dorsal blood vessel, Vbv: ventral blood vessel, S: Setae, Im: Intestine membrane, Em: epithelial membrane.

All these changes were parallel with the different concentration of tannery effluents from 10% to 50%. These were increased with the increment of the concentrations.

In the midgut region, the peritoneum and intestinal membrane were got severely damaged, and dilation of blood vessels 50% concentration was also observed (Fig. 2). While cuticle and epidermis of midgut were intact in a lower concentration of tannery sludge. Typhlosole was clearly visible. The chorachogen cells were crumbled and spread throughout the coelom. Further, the longitudinal muscle showed symptoms of cracks in major parts of the region, dilation of blood vessel, loosening of longitudinal muscles at higher concentration (30%, 40%, and 50% concentration) but no sign of damage was observed in the peritoneal wall, whereas, the intestinal epithelium was found to be injured beyond recognition.

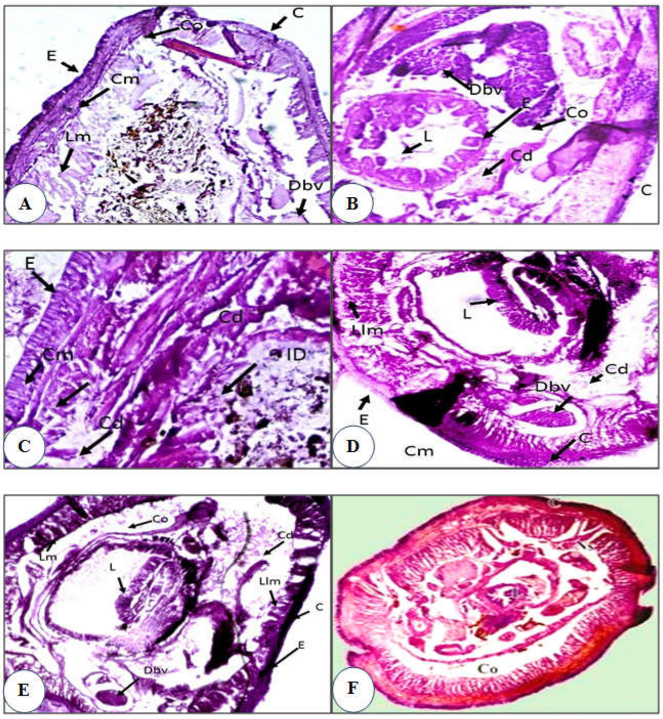

Fig 2 Histological sections of midgut region of earthworm *Eisenia fetida* exposed with different concentrations of tannery sludge. A: 10% tannery sludge, B: 20% tannery sludge, C: 30% tannery sludge, D: 40% tannery sludge, E: 50% tannery sludge , F:control. Abbreviations shows- C: Cuticle, Co: Coelom, Llm: Loosening of longitudinal muscle, L: Lumen, Lm:longitudinal muscle, Cm: Circular muscle, Ns: Nephrostome, Cg: Chloragogencells, E: Epidermis, Dbv: Dorsal blood vessel, Ty:Typhosole,S: Setae, Im: Intestine membrane.

In hindgut region, the cuticle was slightly damage and epidermis was largely damaged. Light destruction was visible in some parts of circular muscles in lower concentration (10% and 20% concentration of tannery sludge). However, at higher concentration (40% and 50% concentration), the longitudinal muscles were highly damage and peritoneum covering the intestine were injured largely. The intestinal lumen and epithelial lining were damaged beyond recognition. The greater degree of injury to the dorsal and ventral blood vessel and chlorocogen cells were observed.

There is various research reported accumulation of heavy metals and pesticidein earthworm tissues and on the growth of earthworms (Sundarapriya *et al.* 2008; Oluah *et al.* 2010; Sharma and Satyanarayan 2011). The chemical immobilization is relatively inexpensive in situ remediation method that reduces 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 under *et al*., (2001). Dominguez, (1997) reported that earthworms can dispose of about 35% to 55% of bio-available heavy metals by accumulating them in their tissues. In the present investigation, *E. fetida* was subjected to various concentrations (10%, 20%, 30%, 40% and 50%) of tannery sludge and it was observed that though the sludge was toxic, it cause damage in the structure of the foregut, midgut, and hindgut of the earthworm. The histological analysis showed that increase in tannery sludge concentration gradually increased the extent of damage to the gut tissues. Since one of the major exposure routes of earthworm was through dermal contact, which has been caused by the exposure to heavy metals available in the soil solution (Vijver *et al.* 2005; Hobbelen *et al.* 2006).

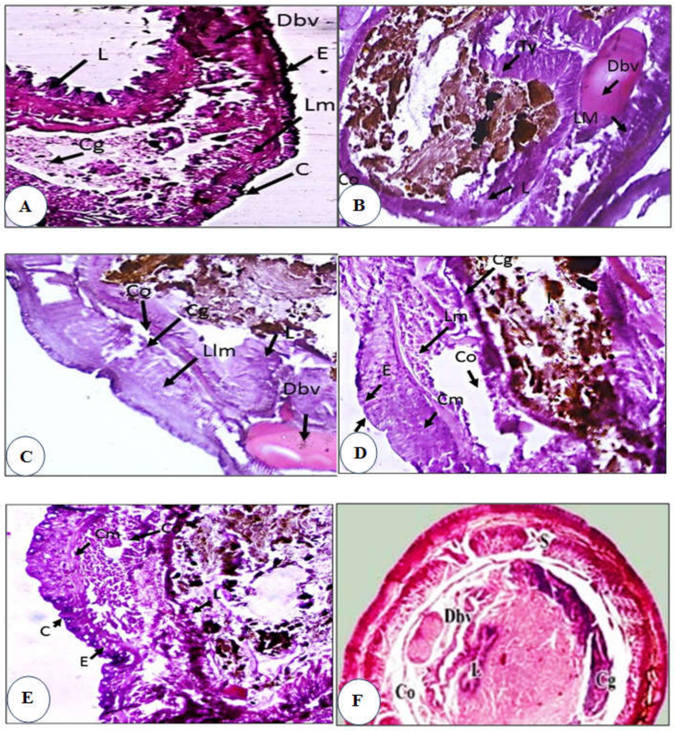

Fig 3 Histological sections of hindgut region of earthworm *Eisenia fetida* exposed with different concentrations of tannery sludge. A: 10% tannery sludge, B: 20% tannery sludge, C: 30% tannery sludge, D: 40% tannery sludge, E: 50% tannery sludge , F: control. Abbreviations shows- C: Cuticle, Co: Coelom, Llm: Loosening of longitudinal muscle, L: Lumen, Lm:longitudinal muscle, Cm: Circular muscle, Ns: Nephrostome, Cg: Chloragogencells, E: Epidermis, Dbv: Dorsal blood vessel, Ty: Typhosole, S: Setae, Im: Intestine membrane.

The result showed that soft peritoneal membrane and circular and longitudinal muscle was more affected. The same report was observed by Kiliç (2011), who observed that metals were found to cause damage and to be accumulated mainly in the circular and longitudinal muscles of earthworms exposed to polluted soils. The chlorogogen cells, brown bodies presented in the coelom and separating the absorptive epithelium from coleom were the main site of accumulation of tannery sludge (Morgan *et al.* 2002; Giovanetti *et al.* 2010). As indicated by Morgan *et al*. (2002), morphological changes in the earthworm chloragogenous tissue are a method for handling of bigger quantities of metals. The disposal of these contaminants might be accomplished through the expulsion of entire chloragocytes (Cancio *et al.*1995), which empower earthworm to endure high concentrations of metals in the soil, at any rate to a specific point (Stürzenbaum *et al.* 1998; Langdon *et al.* 1999, 2001).

CONCLUSION

The population explosion, urbanization, industrial development leads to pollution of air, water, and land which in turn leads to many imbalances in the ecosystem. Sludge or waste from tannery causes skin diseases, pulmonary diseases, sometimes cancer, kidney damage, oral lichen, in human being hence, the present work was carried out to study the histological changes caused by the exposure of tannery sludge on earthworm

(*Eisenia fetida*). Histological studies revealed, the effect of tannery sludge on the tissues of FG, MG and HG region. The increase in the concentration of sludge gradually increased the damage of the tissues in the FG, MG, and HG of an earthworm. Hence, an earthworm can be a potential bioaccumulation of heavy metals at a low level.

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How to cite this article:

Venkatesh Kumar Ramappa *et al*.2018, Effect of Tanery Sludge on Gut Region of Earthworm, Eisenia Foetida. *Int J Recent Sci Res.* 9(4), pp. 26154-26158. DOI: http://dx.doi.org/10.24327/ijrsr.2018.0904.2003

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