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Assessment of histological alterations induced by heavy metal exposure on earthworms

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Abstract
The experiment was conducted to determine the effect of heavy metals *viz.* arsenic and chromium on the vital tissue of tropical earthworm *Eudrilus eugeniae i.e.* intestine. The earthworms were exposed to 24h with varying concentrations. Chromium was found to cause more deleterious effect, damaging the cellular portion of the intestinal region. In general, earthworms can be used as biomarkers in toxicity studies related to heavy metals at cellular levels and the observed trends could be taken as an early warning to the imminent threats of heavy metal pollution to epigeic earthworm species.

Keywords: earthworms, heavy metal, histology, toxicity

Introduction
Earthworms are soil dwelling organisms involved in the process of organic matter decomposition which results in increasing the soil fertility [1]. The earthworm species, *E. eugeniae* is indigenous to Africa but has also been bred in the USA, Canada and Asia which is mainly used for vermicomposting in tropical and sub-tropical countries [2]. In industries heavy metals are used enormously all over the world and their adverse effects have been known for a long time. Although many precautions have been taken to limit the exposure of heavy metals to humans, the adverse effects exerted on animals have been generally neglected. Earthworms ingest the metals through feed or by dermal uptake which are known to negatively affect the physiological functions [3]. Arsenic is a widely distributed metalloid having significant risk factors for cancer when exposed to contaminated environment [4]. Chromium (Cr) is a heavy metal that exists in both trivalent and hexavalent forms; the former is needed in minute quantities for glucose and lipid metabolism in humans and animals [5]. Evidence from literature suggest that histological effects of heavy metals remain largely undefined and bulk of the work is directed towards acute toxicity studies in relation to population, reproduction pattern, and behavioral aspects of earthworm species. Histological changes in the chloragogen tissue of the earthworm *Eisenia fetida* was studied after administration of sublethal concentrations of different fluorides [6]. It has been reported that out of five metals the intestinal region was mostly affected by chromium followed by cadmium, copper, lead and zinc [7]. The aim of the study was to determine histological changes in intestinal region of earthworm after administration of varying concentrations of heavy metals.

Material and Methods
The adult earthworms' *E. eugeniae* were collected by hand sorting method and washed with distilled water and allowed to depurate the gut contents and then exposed to the various concentration of heavy metals (Table 1). The histology of earthworm's intestine was studied by adopting the routine paraffin method [8]. Post clitellar segments of earthworm were dissected from the control and treated earthworms. These segments were blotted to make them mucus free. Thereafter, they were washed thoroughly in physiological saline (0.9%, pH 5.5). Then the segments were cut into pieces of desired size and fixed in Bouins fluid fixative immediately after autopsy. Fixation was carried out at room temperature (28ºC ±2) for 24 h, after which the tissues were transferred to alcohol (70%). The tissues were transferred to alcohol (70%) repeatedly 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). Transverse tissue sections of 5-µm thickness were obtained using a rotary microtome.

The sections, thus obtained, were stained in Harris hematoxylene and eosin followed by dehydrating them in alcohol. The tissues were then cleared in xylene and mounted using dihydroxy phthalate xylol (DPX). The stained slides were observed in a microscope.

Table 1: Description of treatments given to the test earthworm species along with control

S. No	Treatment	Description
1	Control	Control
2	T1	Arsenic 1.04 %
3	T2	Arsenic 2.09 %
4	T3	Arsenic 3.13%
5	T4	Chromium 0.06%
6	T5	Chromium 0.13 %
7	T6	Chromium0.19%

Results and Discussion

Several previous reports have suggested the histological alterations in earthworms tissues exposed to heavy metals. In *Eisenia andrei* exposed to soil contaminated with a collection of metals (not including Cr) and radionuclides represents the loss of cellular integrity and the fusion of and reduction in cell margins [9]. Considering the fact that heavy metals are toxic, histopathological studies were undertaken on intestine of earthworm *E. eugeniae* which has been exposed to heavy metals like arsenic and chromium. Control trials depict overall arrangement of the intestinal region with clear epidermis and cuticle (Fig. 1), middle circular and longitudinal layers are intact. The inner lumen is well defined with multifold intestinal villi and typhlosole region normal (Fig. 2, 3). Fig. 4 shows transverse section of intestinal region of earthworm treated with 24h of arsenic exposure. The utter cellular detachment of chloragogenous tissue can be seen with fused villi. Fig. 26 shows transverse section of intestinal region of earthworm treated with 24h of chromium metal. Fusion of villi is severe and necrotic changes were evident alongwith increased cellularity in mucosa indicating severe toxicity due to chromium metal. *Lumbricus terrestris* inhabiting volcanic soils mainly containing Zn and Cd represents the reduction in radial thickness of the intestinal epithelium [10]. In contrast, an enlargement of the epithelial cell lining has been reported in *L. terrestris* in the Porsuk River Basin, Turkey, polluted with a combination of heavy metals which included Cd, Cr, Cu, Pd and Ni [11].

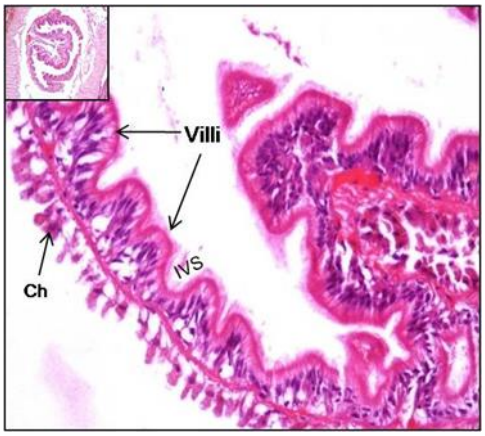

Fig 1: Transverse section of the intestinal region of *E. eugeniae* during 24h exposure to distilled water

Fig 2: Transverse section of the intestinal region of *E. eugeniae* during 24h exposure to arsenic metal 1.04%

Fig 3: Transverse section of the intestinal region of *E. eugeniae* during 24h exposure to arsenic metal 2.09%

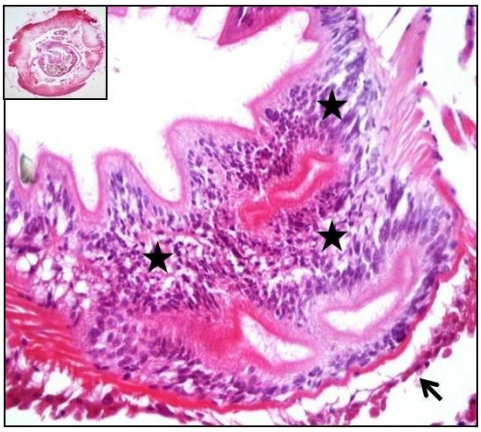

Fig 4: Transverse section of the intestinal region of *E. eugeniae* during 24h exposure to arsenic metal 3.13%

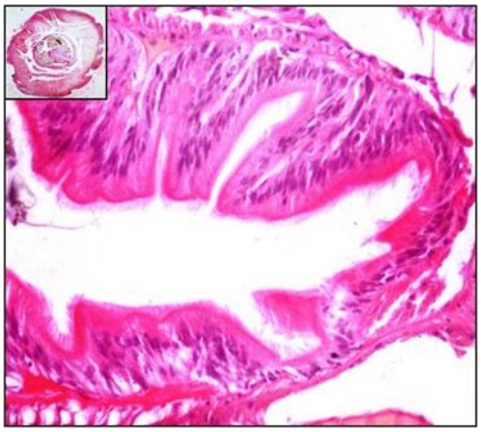

Fig 5: Transverse section of the intestinal region of *E. eugeniae* during 24h exposure to distilled water

Fig 6: Transverse section of the intestinal region of *E. eugeniae* during 24h exposure to chromium metal 0.06%

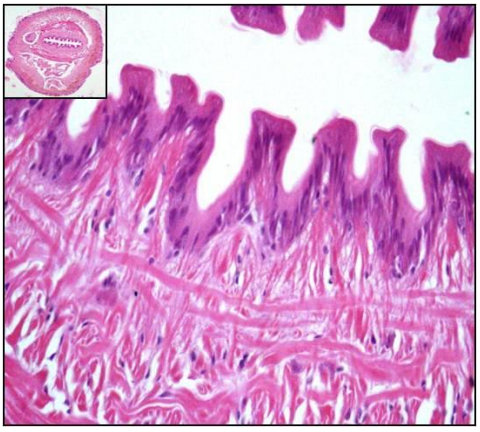

Fig 7: Transverse section of the intestinal region of *E. eugeniae* during 24h exposure to chromium metal 0.13%

Fig 8: Transverse section of the intestinal region of *E. eugeniae* during 24h exposure to chromium metal 0.19%

Conclusion

In general the study shows that the heavy metals had adverse effects on non-target organisms particularly the earthworms that plays crucial role in improvising the physico-chemical properties of soil. However, the histological damage observed in the present study, even at the lowest level of heavy metals inferred that chromium and arsenic are toxic metal for worms and former was found to cause more deleterious effect in the intestinal regions.

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