Pesticides are known to produce morphological, anatomical and physiological changes in the vital organs of non targeted animals like earthworms which are considered as biomarkers of soil system. Phorate a common organophosphate pesticide used in different agro ecosystems for the pest control also have adverse effect on the earthworms. The toxicity on the prostomial region was studied by 1/3rd of the LC50 value of phorate for the duration of 30 days and histopathological observations were recorded. The experimental worms were exposed to the calculated sub lethal dose of phorate for 30 days and histopathological studies of prostomial region were performed using routine paraffin method (Humason, 1979). The microscopic observations of histopathological manifestations of exposed *Metaphire posthuma* to phorate showed in the damage of prostomial epithelial tissues, necrosis of glandular cells and partial damage with cloudy swelling of longitudinal muscles, damage in circular and longitudinal muscles. It may be concluded that toxic effect of phorate may change the structural integrity of the tissues that might be applied in the environmental risk assessment. Further, it may be add that imbalanced uses of pesticide in agricultural land may disturb sustainability of the biotic and abiotic parameters of the soil, which are solely responsible for the crop yield.

**Key words:** Earthworm, Phorate, Histopathology, *Metaphire posthuma*.
OECD Guideline, 207 (OECD, 1984). The 14 days artificial soil toxicity test of phorate, LC$_{50}$ value 20.5mg/kg was calculated by probit analysis method of Finney (Finney, 1971), a sub lethal dose (1/3rd of the LC$_{50}$ value) was selected to assess the effect of phorate on the endogeic Indian earthworm species Metaphire posthuma. For the experimental media preparation, 03 plastic troughs each filled with 1 kg of soil substrate designated as C (Control) and T (Treatment). The control was mixed only with water. For treatment, the sub lethal concentration of phorate was added and mixed with soil substrate using 300 ml of water to ensure homogenous mixture and required moisture. 10 clitellated Metaphire posthuma were introduced into each experimental media. The troughs were covered by nylon net. It was maintained at the room temperature 22 ±2°C with 30-40% moisture. The duration of study was 30 days, during which media were watered regularly to avoid dryness. The experiment was repeated five times. The histology of the prostomial part of Metaphire posthuma was studied adopting the routine paraffin method of (Humason, 1979). All the reagents used in the present study were of analytical grade and used without any further purification. The control and experimental earthworms were taken out, blotted free of mucus, washed thoroughly in physiological saline, cut in to pieces of desired size and fixed in bouins fluid 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 tissue were then dehydrated by passing through ascending grades of alcohol, cleaned in xylene, infiltrated with molten paraffin, and finally embedded in paraffin wax (58 ˚C MP). Tissue section of 5 micro meter thick section were obtained, were stained in hematoxylene and eosin, dehydroxy phthalate xylol (DPX). The stained slides were observed under the olmupus research microscope (CKX-41).

**ANIMAL ETHICS**

The experiment was accomplished under the approval of Ministry of Environment, Forest & Climate Change (Animal Welfare Division), Committee for the Purpose of Control and supervision of Experiments on Animals (CPCSEA), Government of India, vide letter no. F.No.25/34/2014-CPCSEA. The animals were treated and handled humanly in accordance with the Constitution of Institutional Animal Ethics Committee (IAEC) and OECD Guideline, 207 (OECD, 1984).

**RESULTS AND DISCUSSION**

In the present work the toxicity of phorate, its effect on the prostomial part and body wall tissues of the earthworm, *M. posthuma* was studied for control and experimental earthworms after 30 days. The study showed progressive signs and symptoms of toxicity such as coiling, curling and excessive mucus secretion with sluggish movement. In the histological sections of the prostomial part and body wall tissues, damage in the epithelial tissues, necrosis of glandular cells and partial damage with cloudy swelling of longitudinal muscles, damage in circular and longitudinal muscles and loss of structural integrity in the longitudinal muscles, internal and ectodermal tissue erosion leading to total damage of the body wall were observed. The results of the study suggested that the toxic effects of phorate are mediated through its effect on the structural integrity of the tissues that might be applied in environmental risk assessment.

Primarily, these toxicants passing through the skin throughout the body wall. Previous studies suggested that earthworm skin has direct contact to the contaminated soils and is considered as a significant route to uptake of toxicants by several other earlier workers (Saxe, et.al., 2001, Kumar and Singh, 2017, Kumar and Singh, 2017, Jager et.al., 2003 and Vijver et.al., 2003). Epidermis and cuticle represent a primary barrier that protects earthworm’s body from the environment and are also responsible for the transport of ions, thus allowing/blocking xenobiotics to enter the body (Clauss, 2001). The current investigations on the earthworm *Metaphire posthuma* have revealed that the contact toxicity of phorate through its integument was increased exposure time of the given toxicant. It is evident from the results that phorate can be rated as highly toxic to these earthworms after 30 days of exposure. The present toxicant is comparatively several folds less toxic to *Eisenia fetida* in comparison to the earlier studies on other organophosphate (OP) insecticides (Venkateswara, et. al., 2003b, Venkateswara and Kavita, 2004, Sameena and Ayesha, 2015). However work of Zang et.al., (2000) revealed that these worms were highly sensitive to Chloronicotinoid insecticide, Imidacloprid with an LC$_{50}$ of 0.1 and 0.034µg cm$^{-2}$ after 24h and 48h of exposure, respectively. Rajashree, et.al., (2014) also concluded that the effect of organophosphate pesticide methyl parathion and phorate are very toxic and act as neurotransmitter to the soil earthworm *Eudrilus eugeniae*. Amrita and Preeti
Fig. 1: Regression equation to determine LC$_{50}$ of phorate for *Metaphire posthuma*.

Fig. 2: Earthworm *Metaphire posthuma* in experimental condition.

Fig. 3: Earthworm *Metaphire posthuma*, showing intact body wall, circular and longitudinal muscles under controlled conditions.

Fig. 4: Cloudy swelling of longitudinal muscles, damage in circular and longitudinal muscles and loss of structural integrity *Metaphire posthuma* under experimental conditions.

(2016) reported reduction of spermatic follicles after dimethoate toxicity in adult earthworm *Eudichogaster kinneari*.

Gobi et al. (2004) have found the glandular cell enlargement and vacuolization in the intestine of the earthworm *Perionyx sansbaricus* exposed to sub lethal concentration of herbicide butachlor. An extreme (2 fold) nuclear swellings has been reported in *E. fetida* exposed to herbicides (Fischer and Molnar, 1992 and Morowati, 2000) has reported that *Pheretima elongate* exposed to herbicide glyphosate loss of epithelial cell structure in intestine, lacking regeneration of the cells.
and total loss of chromatin from first week to the third week of exposure and a marked regeneration of the cells in the fourth week of exposure. Bansiwali and Rai (2010) observed that sublethal dose of organophosphate insecticide Malathion has induced marked pathological changes in the body wall such as ruptured cuticle, with distortion of the shape of longitudinal muscle cells.

The histopathological evaluation revealed that the cuticular membrane and ectodermal layers were completely disintegrated and profusion of glandular epithelium given protection to the muscle layers of the body wall. It is evident from earlier reports that the morphological and histological changes were prominent when earthworms were exposed to different toxic metals and other OP pesticides (Amaral, et al., 2006). Earthworms ingest large amounts of soil and are therefore elective non-target organisms exposed to pesticides through their external and internal surfaces. These findings indicate that tested organophosphate compound phorate have a potential to cause significant histopathological changes and act as a destructive element to the earthworm Metaphire posthuma (Kumar and Singh, 2017, Kumar and Singh, 2017). Thus morphological or anatomical changes in these organisms are one of the suitable indicators for monitoring the effectiveness of soil pollutants.

CONCLUSION

The result of the study suggested that the toxic effects of phorate are mediated through its effect on the morphological and structural integrity of the tissues. It further showed that the pesticide had adverse effect on non target organisms particularly the earthworms that are critical in evaluation of soil fertility. This study indicates the importance of earthworm's histopathological bioassay as an indicator of environmental pollution.

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