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Histopathological alterations in earthworm (*Eudrilus eugeniae*) ventral nerve cord caused by Diammonium phosphate

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Abstract
Mostly human beings look into the benefits of newly synthesized chemicals, but keeping aside the benefits, the research should be focused on the impact of these chemicals on the living system. In terrestrial ecosystem, earthworms are considered as the ecosystem engineers because they play many important roles to maintain quality of soil. In present study an experiment was conducted to study histopathological alterations of ventral nerve cord of earthworm *Eudrilus eugeniae*. For the study earthworms were exposed to sub lethal dose of DAP for different time period and it was found that as the time period increases the histopathological alterations also showed more damage in ventral verve cord of earthworm. So inorganic fertilizer should be used in ecological safe limit.

Keywords: earthworm, fertilizers, DAP, ventral nerve cord, histopathological alterations

1. Introduction
Agriculture is considered as the backbone of India which totally depends upon quality of soil. In last few decades, green revolution has promoted the use of fertilizers to increase the quality of soil. Fertilizers are indeed essential for the healthy growth of plants but are they harmful for the soil and environment. In soil ecosystem earthworms form the major part of macro fauna, also called as ecosystem engineer [1, 2]. Earthworm plays many important roles, such as biomanipulator [3] and a potential contributor in organic waste disposal or vermicomposting [4, 5, 6]. The *Eudrilus eugeniae* a species of earthworm commonly used for wormicomposting in tropical and subtropical countries [7]. It is also utilized as protein source in animal feed. *Eudrilus eugeniae* is indigenous to Africa but also bred in Spain, UK, USA, Canada, Europe and Asia but there have been little research is done on these clitellar earthworm from the Indian subcontinent. So in this study a widely used fertilizer DAP (Diammonium Phosphate) was selected for the experimental work. Ten healthy gut evacuated earthworms were exposed to sub lethal dose of DAP for different time period. On the other hand a control group was also set with only water soil and same number of earthworms.

2. Materials and Methods
2.1 Study area selection
Bhusawal is biggest Taluka of Jalgaon district located on the bank of Tapti River and has an average elevation of 209 meters.

2.2 Earthworms
Earthworm (*Eudrilus eugeniae*) were purchased from wormicompost unit of Nasik and cultured in wormicomposting unit of the Smt. P. K. Kotecha Mahila Mahavidyalaya, Bhusawal.

2.3 Experimental set up
Earthworms were acclimatized in laboratory for one month. At the time of experiment worms were washed with sterile water to avoid dermal infection and ten gut evacuated earthworms were exposed to sub lethal dose of DAP (189.22mg/kg) for 24, 48, 72 and 96 hours at 30 ± 2°C in the soil containing 10% organic matter. Moisture was maintained by sprinkling the water. A control was also set only with water and soil. The troughs were covered with wet muslin cloth to avoid loss of moisture and crawling out of the earthworms from trough. After exposure earthworms were placed in petriplate containing moist filter paper for 6 hours to devoid the gut content. Then the worms cleaned again externally with 70% ethanol, dissected and proceeded for preparation of histological slides including fixation, processing-(Dehydration clearing and infiltration), embedding, sectioning, mounting of sections, and staining.

3. Result and Discussion
Acute exposure of DAP to *Eudrilus eugeniae* revealed that there was no significant changes were found at 24 hours treatment. Neuroglia and neuropiles loose the shape and rigidity at 48 hours exposure. Damage in three nerve fibers, one median and two lateral nerve fibers also found in 72 hours treatment. At 96 hours exposure shrinkage was prominent with necrotic and degenerative changes.
Very few researchers worked on the histopathology of ventral nerve cord. Histological study of ventral nerve card revealed presence of three giant fibers one dorsal and two lateral fibers. Present results are supported by researcher [8]. They worked on

P. darnleiensis and noted a nerve cord with giant fibers. These fibres have key role in the worm's rapid responses in all oligochaetes [9, 10].

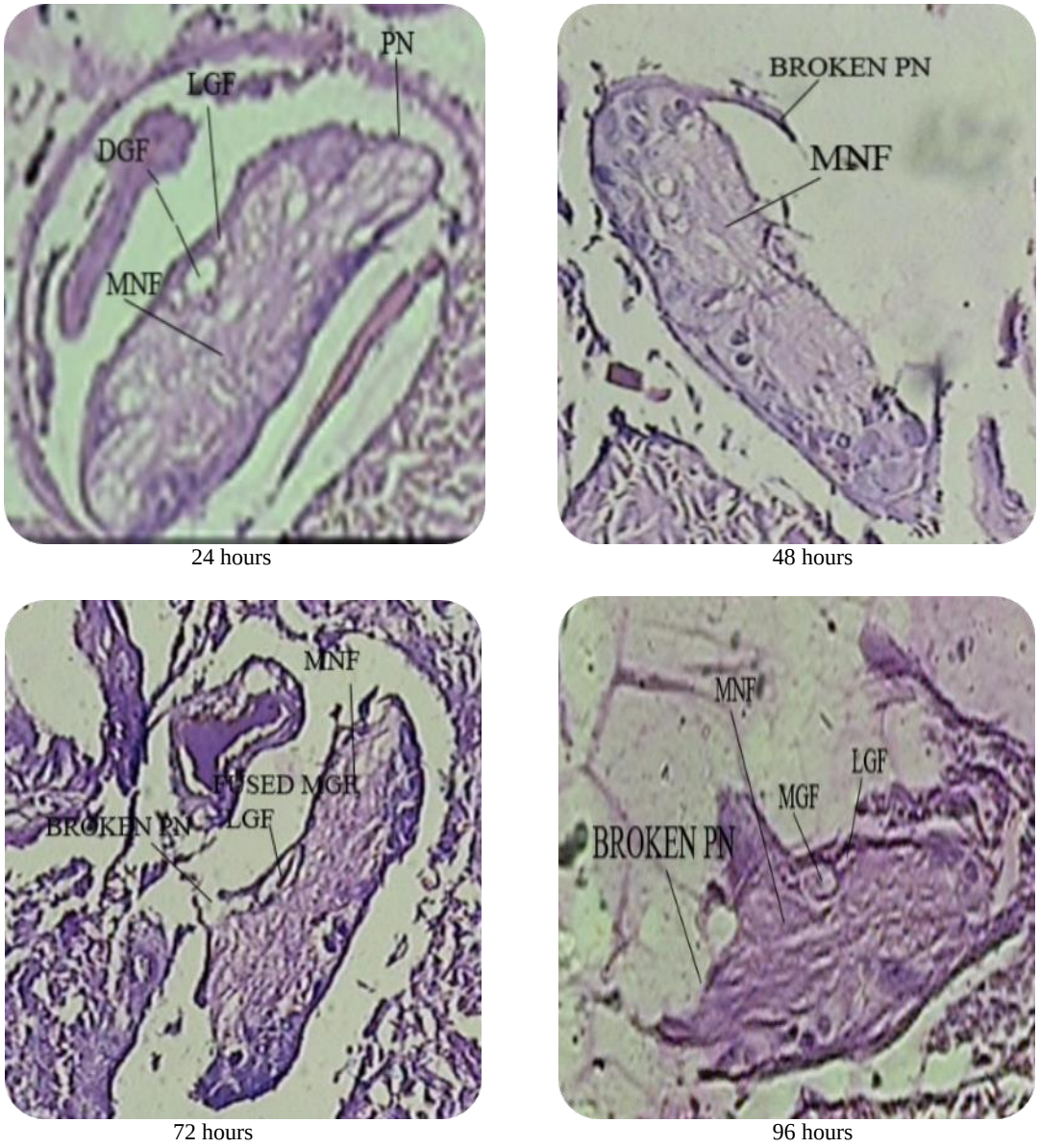

Fig 1: Histological study of ventral nerve cord of Eudrilus eugeniae (100X) treated with Diammoninum Phosphate (189.22mg/kg).

Conclusion

In India the role of chemicals is well recognized and established for the success in agriculture sector. India is the second biggest consumer of fertilizer in the world next only to China. Histopathological alterations in earthworm due to DAP fertilizer can be considered as a marker of toxicant exposure. The results of the present investigation clearly demonstrate that treatment of inorganic fertilizer DAP are very harmful to *Eudrilus eugeniae*. It is advised that the use of chemical fertilizer DAP should be within ecologically safe limit.

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