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Histopathological alterations in body wall and alimentary canal of the earthworm as biomarker of copper oxychloride exposure

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Copper oxychloride is a broad-spectrum fungicide applied to the foliage of a wide variety of fruits and vegetables. [1]. It is sprayed directly on crops and may contaminate soils. Since its general toxicity is considered to be low, there are only a few studies about the toxicity of this substance on soil fauna [2]. However it has been shown to reduce populations of some earthworm species in field trials [3].

Earthworms are continuously exposed to soil chemicals through their alimentary surfaces and skins. Because of the capacity to accumulate and concentrate large quantities of inorganic and organic pollutants, they are widely recognized as suitable organisms for biomonitoring the effects of heavy metals and other chemicals in contaminated soils. Histopathological alterations in earthworms have been reported as valuable markers of heavy metal toxicity in previous studies which may signal a prior or ongoing damaging effect on these organisms [4,5,6].

Hence, the present study focused on the histopathological responses in body wall and alimentary canal of earthworm (*Aporrectodea caliginosa*) resulted from copper oxychloride exposure. Samples consisted of control and experimental group animals which were exposed to different doses of copper oxychloride (35, 100, 350 mg/kg artificial soil) during 2,7 and 14 days of periods. Healthy worms weighing between 300-400 mg each were left on filter paper in petri dishes for 24 h in order to reduce the soil content in their gut. Worms were cut into 0.5 cm pieces and were fixed in paraformaldehyde(4%) in phosphate buffer pH 7.2. They were dehydrated in a graded series of ethanol and treated with a mixture of LR White and ethanol (2:1) (v:v) for 1 h at RT. Samples were then embedded in LR White Resin, sectioned at 700 nm thickness, stained with toluidin blue and observed under a Leica DM 750 bright field microscope. Significant changes were observed at the doses of 100 and 350 mg/kg and after the periods of 7 and 14 days with the increasing intensity of lesions depending on exposure time. These lesions were generally characterized by enlargement and torsion of epithelial cell lining, hyperplasia of glandular cells, increased mucus production and deformation of muscular structure. Intestinal epithelium and chloragogenous cells lost their intact nature. Necrosis was also observed at the dose of 350 mg/kg after 14 days in epidermal and intestinal tissues of animals. The study showed that copper oxychloride exposure may result in a crucial toxicity in bioindicator organism *Aporrectodea caliginosa* depending on the dose and duration of exposure leading to significant deformation of tissues in vital parts of their body.

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