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BIOACCUMULATION AND HISTOLOGICAL STUDIES IN ZnO NANOPARTICLES EXPOSED *EUDRILUS EUGENIAE*

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ABSTRACT: Metallic nanoparticles are highly involved in various products and released into the environment by either accidently or intentionally. This study focused about the bioaccumulation and toxic effect of ZnO nanoparticles in Earthworm *Eudrilus eugeniae.* ZnO nanoparticles exposed earthworm tissues showed accumulation. Increased concentration of nanoparticles observed as increased concentration of accumulation. Bodywall and gut tissues showed altered morphology due to the exposure and assimilation of ZnO nanoparticle. This study proved that low concentration of ZnO nanoparticles affects the earthworm tissues.

KEYWORDS: Bioaccumulation, ZnO, Histology, LC50.

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1.INTRODUCTION

Metal oxide NPs are manufactured in large scale for both industrial and household purposes. Some authors reported increasing application of the NPs leads to environmental toxicity [1]. Increased usage of nanoparticles leads to the purpose of developing the characterization of nanotoxicity of the particles to the biological organisms. Nanotoxicology helps to understand the size-specific behaviour and impact of nanoparticles on organisms and the environment [2]. Zinc oxide (ZnO) nanoparticles are mostly used as a UV light scattering, as an additive in cosmetics such as sunscreens, toothpastes and beauty products [3] and also widely used in rubber manufacturing process, for production of solar cells, LCDs, pigments (as a whitener), chemical fibers,electronics and textiles [4, 5]. It is an essential ingredient in almost all types ofantifouling paints [6]. For the past two decades, the bulk ZnO has been increasingly replaced by ZnO NPs because of their enhanced antibacterial properties

[7]. Nanoparticles are released into the environment from their products through normal usage and then wastewater streams and become a threat to ecosystems [8,9]. Depending on local practices, varying proportions of sewage sludge are disposed of in landfills, incinerated, or applied to agricultural lands as biosolids. Usually 60-80% of sewage sludge is applied to the land [10]. Terrestrial eco-systems are expected to be an ultimate sink for a large portion of NPs [11] which raises concerns about their potential for ecological effects, entry into food webs, and ultimately human exposure from consumption of contaminated agricultural products [12]. Another Direct absorption, through their external body by the process called dermal absorption [13, 14].Soil represents a major recipient of nanoparticles entering the environment. Engineered nanoparticles may enter soil via wastewater treatment or the effluent from industrialization processes and many other anthropogenic activities which inhibit the organisms in the terrestrial ecosystems [15]. Earthworms constitute 60-80% of the soil biomass[16]. They are widely contributed in standard toxicity tests [17] and appeared to be suitable biomonitoring organisms, particularly for their strong interaction and permanent direct contact with soil. The uptake, accumulation, and elimination properties of metals by earthworm are the major part of toxicological studies [18].Histology is the most useful tool for determining the influence of agricultural pesticides, industrial pollutants, organic wastes etc., at tissue level of an organism as it provides useful information concerned with the growth, damage and disorganization of tissues. The intestine is the first line of defense against chemical insults through the oral route [19]. Earthworms directly influence the persistence of pollutants in soil by metabolizing a parent compound in their gut [20, 21], by transporting pollutants to depth and increasing the soil bound fraction in soil or by absorbing pollutant residues in their tissues.

2. MATERIALS AND METHODS

2.1 Preparation of Vermibeds and earthworm collection

The experimental beds were prepared with organic waste majorly cow dung in rectangular plastic tubs (of 17x17"x51" size) in triplicates. The ZnO nanoparticles 0.25gm/Kg, 0.5gm/Kg, 0.75gm/Kg and 1gm/Kg were mixed in the tubs respectively. Kale [22] recommended minimum of five earthworms required for converting one kg of organic wastes (10kg, n=50). Pre-weighed breeders of the selected nightcrawler species of the earthworm, *Eudrilus eugeniae* were introduced manually in each tub. Controls were maintained for separately without ZnO nanoparticles. The experimental tubs were placed indoor in the laboratory to avoid direct sunlight (to prevent water evaporation), rain (to prevent excess moisture) and to protect the worms from predators. Water was sprinkled on alternate days to maintain the optimal moisture (50-55%) of the beds for the growth of the worms. At the end of the 60 days, 10 mature earthworms were collected from each group.

2.2 Bioaccumulation studies

For estimation of Zn concentration in earthworm tissues (body wall and gut tissues) dissected, weighed and digested with 3 ml of conc. nitric acid for 24 hrs, evaporated and the residue dissolved in

4% nitric acid. Zn concentration was quantified by atomic emission spectrometry with inductively coupled plasma (ICPAES) [23]. For Zn quantification, Variant Liberty 110 spectrometer with 40.68MHz radio frequency generator and 0.75m Czerny-Turner monochromator, plasma flow 12Lmin^{-1}, V-Groove nebulizer, rotation pump 15rpm, 10sec integration time and automatic background is used. The results were statistically analyzed by One way ANOVA between the control and ZnO NPs treated groups.

2.3 Histological studies

Histology is the technique used to study complete architecture of the tissue patterns by means of examination and analysis of cell/tissue physiology & morphology at the microscopic level.

A. Fixation of Tissues in 10% Formaldehyde Solution:

The earthworm was cleanly washed with tap water to remove any dirty on its body. Sacrifice the earthworm in 10% Formaldehyde Solution for 5 min. Body wall and gut tissues were dissected as 3-5cm length. The tissues were then put in 50% Formaldehyde Solution in 60º C for 24 hours for fixation (Table 1).

B. Processing

After fixation, the tissue was washed with distilled water and put in 60% Isopropyl alcohol for 1 hour for dehydration. Similarly the steps were performed in 70%, 80%, 90% and 100 % (two times) each at incubation of 1 hour at 60ºC.After dehydration, the tissues were allowed for clearing the ethanol by put in xylene for 45 min.

C. Embedding of tissues in Paraffin wax

After clearing, the tissues were put in a wax 1 at 60º C for 2 hours then transfer the tissues to wax 2 for overnight and in wax 3 each for 2 hours. Using two L-shaped equipment a paraffin block was prepared and in that the processes tissue was embedded for sectioning.

Table 1: Steps Followed in Histology

Step	Temperature	Time
Fixation - 10% formaldehyde	50°C	24 hours
Dehydration- 60% isopropyl alcohol	50°C	1 hour
Dehydration- 70% isopropyl alcohol	50°C	1 hour
Dehydration- 80% isopropyl alcohol	50°C	1 hour
Dehydration- 90% isopropyl alcohol	50°C	Overnight
Dehydration- 100% isopropyl alcohol	50°C	1 hour
Dehydration- 100% isopropyl alcohol	50°C	1 hour
Clearing – Xylene	50°C	1 hour
Wax impregnation – Paraffin Wax 1	58-60°C	Overnight
Wax impregnation – Paraffin Wax 2	58-60°C	3 hours
Wax impregnation – Paraffin Wax 3	58-60°C	3 hours

D. Sectioning

Section paraffin blocks at desires thickness (usually 5-7µm) on a microtome and the ribbon was carefully transferred to an Egg albumin: Glycerol (50:50) coated slide overlaid with distilled water. Place the slide in 60º C for half an hour to melt the paraffin then deparaffinize slides in two changes of xylene for 5 minutes each. Transfer the slide to isopropyl alcohol for 2 minutes before washing the deparaffinize slides in water.

E. Staining

The deparaffinized slide washed in water was stained with haematoxylin (which stains nucleus) for 6-8 minutes and the excess stains were removed by water wash followed by acid alcohol treatment for 30 seconds. The nucleus stained slide was counter stained with eosin (which stains cytoplasm) for 30 seconds and the excess stains were removed by dipping in water and isopropyl alcohol. Then the slide was dipped in xylene and the slides were mounted by overlaid with DPX followed by careful plotting of the cover slip and used for microscopic examination.

3. RESULTS AND DISCUSSION

3.1 Bioaccumulation studies

Body wall and gut tissues analyzed for Zn concentration after treating with 60days. Selection of Body wall and Gut for bioaccumulation studies due to their continuous exposure to the ZnO nanoparticles from the soil and biomagnifying through the villi present in the lumen of the gut. Control group (Table 2) also showed presence of Zn concentrations as 4.02±0.55 and 4.09±0.74 in body wall and gut tissues due to their nutrient absorption nature from the soil. ZnO nanoparticles treated earthworm tissues showed high levels of Zn, among the tested two tissues, gut organ showed high Zn levels than body wall due to their intake and epidermal transfusion of the ZnO nanoparticles into the gut tissues. One way ANOVA results showed significant results between the control and ZnO NPs treated groups. The results suggested that the earthworms uptake and accumulate Zn content in their body tissue, even by increasing the dose and increasing the concentration (Figure 1).

Table 2: Zinc concentration in body wall and gut tissues of *E. eugeniae* after 60 days exposure of ZnO nanoparticles

Sr. No.	Groups	Zn Concentrations (µg/g)	
		Body wall	Gut
1	Control	4.02±0.55	4.09±0.74
2	0.25gm/kg	4.56±0.95	4.88±0.78
3	0.50gm/kg	5.44±0.84	6.02±0.69
4	0.75gm/kg	7.84±0.79	10.74±0.98
5	1.0gm/kg	9.98±0.56	12.45±1.12

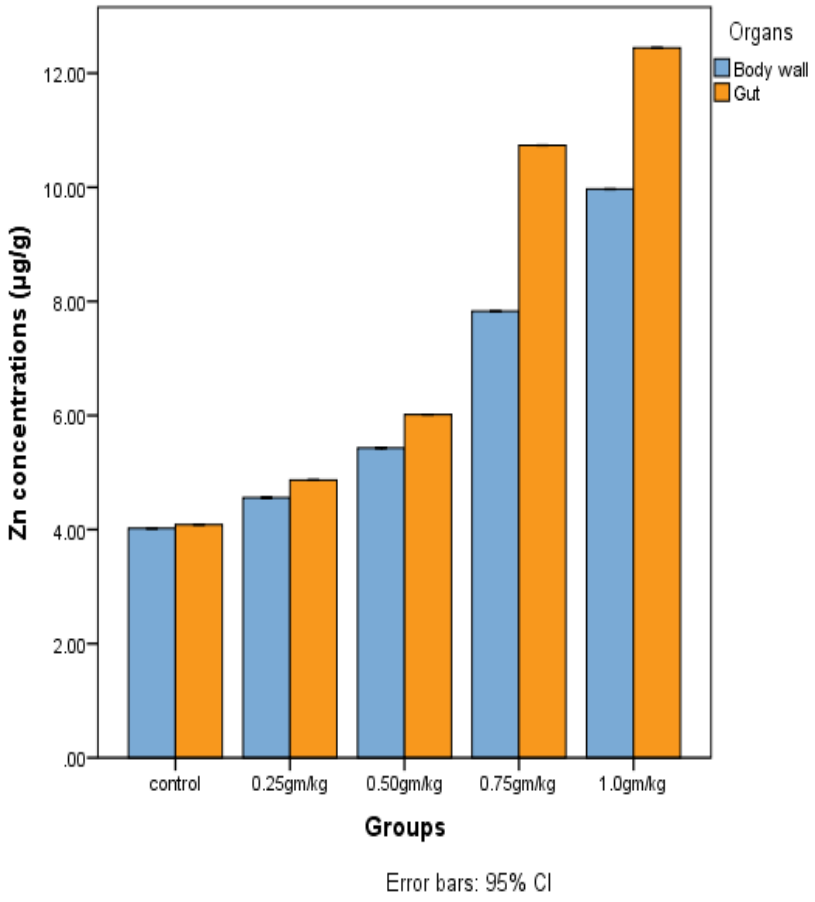

Figure 1: Zinc concentration in body wall and gut tissues of *E. eugeniae* after 60 days exposure of ZnO nanoparticles

3.2 Histological studies

Body wall of the control group earthworms (A) showed normal architecture of epidermal cells (EC) which is surrounded by circular muscles (CM) followed by longitudinal muscles (LM). Exposure of ZnO nanoparticles from the surrounding soil cause Fibrosis (F) in the circular and longitudinal muscles and also mucous proliferation (MP) in the circular muscle layer and lipid perforations in the cuticle in 0.25gm/kg, 0.5gm/kg groups. Body wall tissues were severely damaged in higher concentration (0.75gm/kg and 1.0gm/kg) of ZnO NPs exposed group. Diffused and loss of architecture of the circular membrane (DCM), Vacoulation (V) in the circular muscle layer, damaged cuticle layers (DCL) were observed (Figure 2).Control group earthworm Gut tissues showed properly arranged chlorogen (Ch) cells around villi (V) with proper inter villous space (IVS) involved in the absorption in the lumen (L). But in the ZnO NPs treated earthworm showed degenerated villi (DV) with disoriented lumen (DL) in 0.25gm/kg and 0.50gm/kg treated groups. Along with these disturbances, disoriented chlorogen cells (Dch) were observed in 0.75gm/kg and 1.0gm/kg ZnO NPs treated groups (Figure 3).

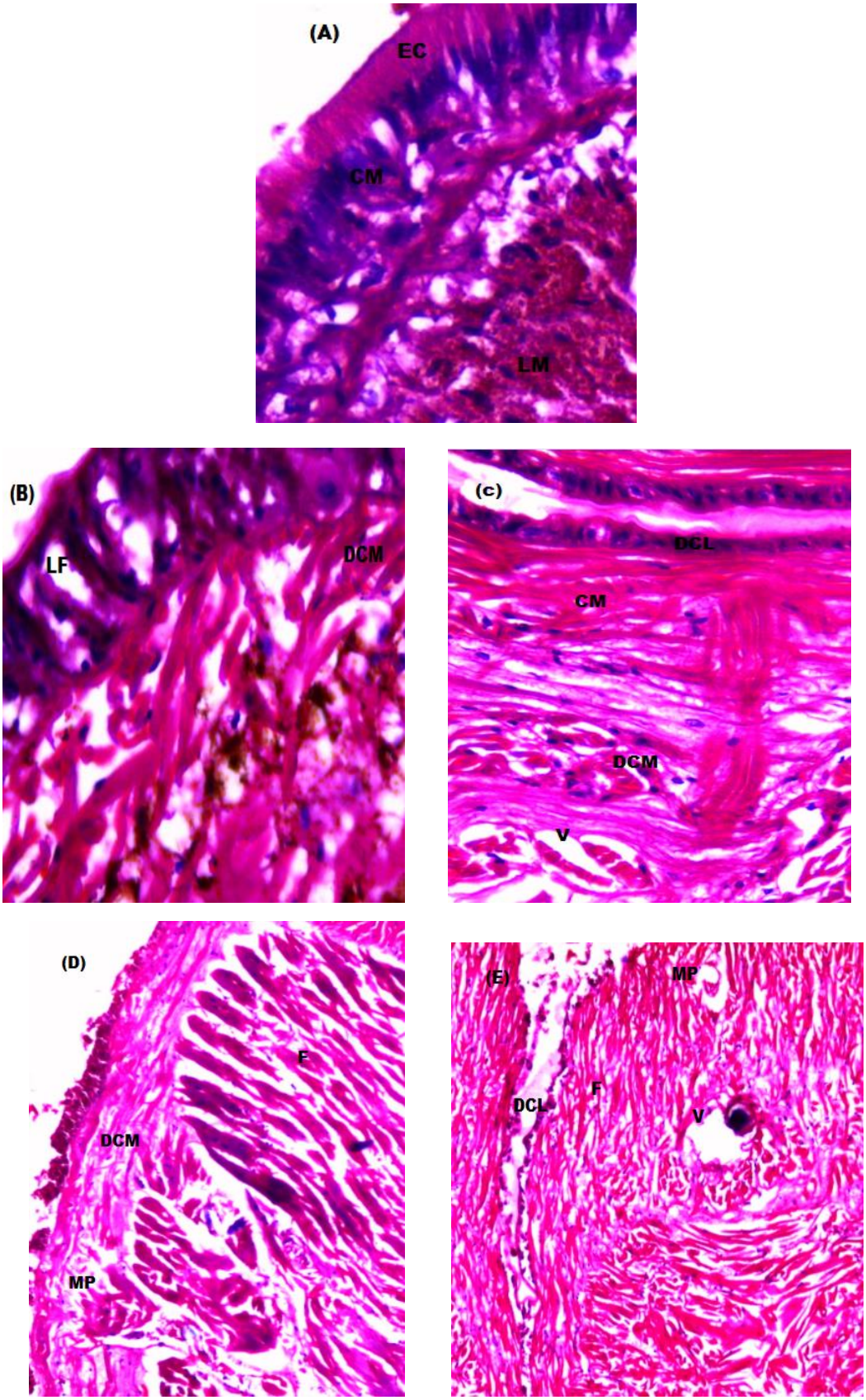

Figure 2: Body wall of *E. eugeniae* earthworm: Control (A) and ZnO nanoparticles (B-0.25gm/kg, C- 0.5gm/kg, D-0.75gm/kg, E-1.0gm/kg) exposed groups

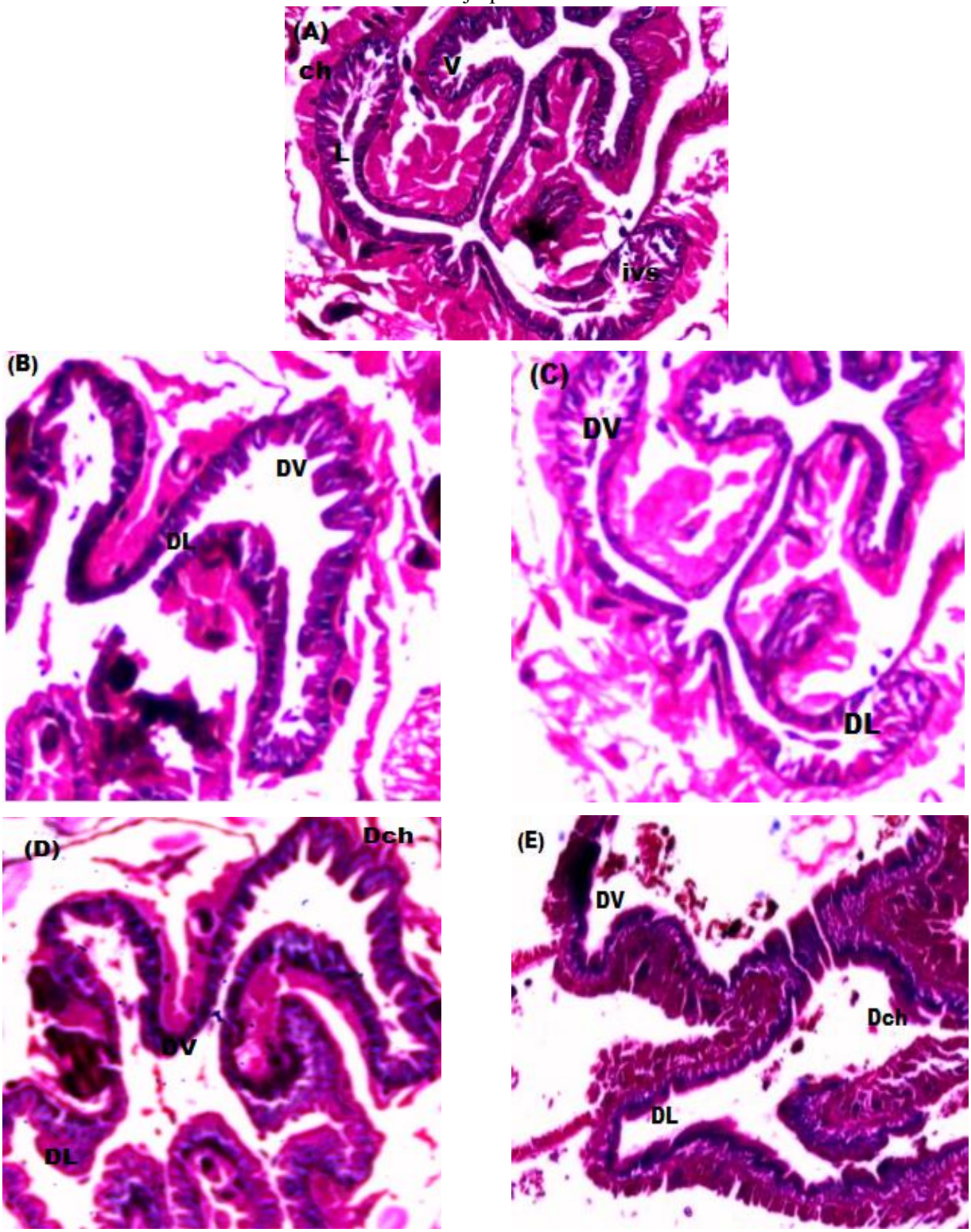

Figure 3: Gut of *E. eugeniae* earthworm: Control (A) and ZnO nanoparticles (B-0.25gm/kg, C- 0.5gm/kg, D-0.75gm/kg, E-1.0gm/kg) exposed groups

Exposure of CeO2 NPs damaged the body wall but with little or no injury to the gut or clitellum. Smaller metal or metal oxide particles sometimes show greater toxicity than larger ones [24, 25]. In the present study, effect of particles of various sizes and shapes were investigated, including a quite angular NM-212 CeO_2 (10–80 nm) particle, which could potentially mechanically damage epidermal tissues. The bioavailability of NPs increases with decrease in size of NPs. Gardea-Torresdey *et*

al.[26] reported that Au1± and Au3± can also be taken up from soil by plants and subsequently reduced to metal within the tissues. Control earthworms (*Lumbricus rubellus*) showed normal structure of the ectoderm, without epithelial lifting or necrosis. The underlying circular and longitudinal muscles were normal for all control earthworms, and pathologies such as fibrosis, hydropic change or atrophy were absent. Earthworms exposed to 15.4 mg C60/kg soil showed some loss of the cuticle with enlarged mucocytes and some hyperplasia of the epidermal cells. The gut epithelial cells were thin and showed more elongated nuclei in combination with mild fibrosis in the treated cells of the control earthworms. *Lumbricus rubellus* earthworms exposed to AgNO3 nanoparticles showed slight erosion of epithelial cells and some thickening of the circular muscle were noted in tissue anterior to the clitellum [27]. *Pheretima elongata* intestine wall showed epithelial layer, the circular and longitudinal muscular layer, and the peritoneum which is modified as a chloragogen layer [28]. Bansiwal and Rai [29] 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. Gobi *et al.* [30] were found the glandular cell enlargement and vacuolization in the intestine of the earthworm *Perionyx sansibaricus* exposed to sublethal concentration of herbicide butachlor. Bioaccumulation of Cu, Cd, Pb and Zn by earthworms is well documented and is thought to be in the chloragogenous tissue surrounding the posterior alimentary canal [31] basically involved in absorption chemical residues from the intestine. Chlorogen cell are essential for absorbing elements from the soil into the biosystem. In our histology studies, the chlorogen cells around the villi were severely damaged in higher concentration ZnO nanoparticles exposure.

4. CONCLUSION

The toxic effect of ZnO nanoparticles in Earthworm *Eudrilus eugeniae*were studied by bioaccumulation and histological observation of bodywall and gut tissues. ZnO nanoparticles exposure into the soil showed accumulation of nanoparticles in earthworm tissues. Bodywall and gut tissues showed altered morphology due to the exposure and assimilation of ZnO nanoparticle. This study proved that low concentration of ZnO nanoparticles affects the earthworm tissues.

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CONFLICT OF INTEREST

Authors have no any conflict of interest.

REFERENCES

1. Kahru A, Dubourguier HC, Blinova I, Ivask A, Kasemets K. Biotests and biosensors for ecotoxicology of metal oxide nano particles: A minireview. Sensors. 2008; 8: 5153-5170.

2. Ramesh R. Nanotoxicological evaluation of synthesized TiO2 and SiO2 nanoparticles on common cat fish *Mystus vittatus* (Bloch 1794). Ph.D. Thesis. Bharathidasan University, Tiruchirappalli. 2011.
3. Serpone N, Dondi D, Albini A. Inorganic and organic UV filters: their role and efficacy in sunscreens and suncare products. Inorg. Chim. Acta. 2007; 360: 794-802.
4. Dastjerdi, R. Montazer, M. A review on the application of inorganic nano-structured materials in the modification of textiles: focus on anti-microbial properties. Colloid Surf. B., 2010; 79(1): 5-18.
5. Song W, Zhang J, Guo J, Zhang J, Ding F, Li L, Sun Z. Role of the dissolved zinc ion and reactive oxygen species in cytotoxicity of ZnO nanoparticles. Toxicol. Lett. 2010; 199(3): 389-397.
6. IPPIC (International Paint and Printing Ink Council) http://www.ippic.org/site/assets/docs/Public.2012.
7. Padmavathy N, Vijayaraghavan R. Enhanced bioactivity of ZnO nanoparticles-an antimicrobial study. Sci. Technol. Adv. Mater. 2008; 9(3): 35-44.
8. Kiser MA, Westerhoff P, Benn T, Wang Y, Perez-Rivera J, Hristovski K. Titanium nanomaterial removal and release from wastewater treatment plants. Environ. Sci. Technol. 2009; 43(17): 6757-6763.
9. Mueller NC, Nowack B. Exposure modeling of engineered nanoparticles in the environment. Environ. Sci. Technol. 2008; 42(12): 4447-4453.
10. OECD. Financing water and Environment infrastructure for All: some key issues. OECD, Paris. 2004.
11. Unrine JM, Hunyadi SE, Tsyusko OV, Rao W, Shoults-Wilson WA, Bertsch PM. Evidence for bioavailability of Au nanoparticles from soil and biodistribution within earthworms (*Eisenia fetida*). Environ. Sci. Technol. 2010a; 44:8308-8313.
12. Gottschalk F, Sonderer T, Scholz RW, Nowack B. Modeled environmental concentrations of engineered NMs (TiO2, ZnO, Ag, CNT, Fullerenes) for different regions. Environ. Sci. Technol. 2009; 43(24): 9216-9222.
13. Van Gestel CAM, Koolhass KE. Water-extractability, free ion activity, and pH explain cadmium sorption and toxicity to *Folsomia candida* (Collembola) in seven soil-pH combinations. Environ. Toxicol. Chem. 2004; 23: 1822-1833.
14. Vijver MG, Vink JPM, Miermans CJH. Oral sealing using glue: a new method to distinguish between intestinal and dermal uptake of metals in earthworms. Soil Biol Biochem 2003; 35-45.
15. Schlich K, Terytze K, Kerstin HR. Effect of TiO_2 nanoparticles in the earthworm reproduction test. Environmen Sci. 2012; 24: 5-15.
16. Abdul Rida AMM. Les vers de terre et l'environnement La recherché. 1994; 25: 260-267.
17. Van Straalen WM, Salaun C, Veen WAG, Rijpkema, YS, Ho G, Boxem TJ. Validation of protein evaluation systems by means of milk production experiments with dairy cows. Netherlands J

Agri sci. 1994; 42(2): 89-104.

18. Lee BT, Shin KH, Kim J, Kim KW. Progress in Earthworm Ecotoxicology. Adv Environ Monitor 2008; 248-258.
19. Muthukaruppan G, Gunasekaran P. Effect of Butachlor Herbicide on Earthworm *Eisenia fetida*—Its Histological Perspicuity. Appl. Environ. Soil Sci. 2009; 1-5.
20. Gilman AP, Vardanis A. Carbofuran: comparative toxicity and metabolism in the worms *Lumbricus terrestris* and *Eisenia fetida*. J Agricul. Food Chem. 1974; 22(4): 625-628.
21. Stenersen J, Gilman A, Vardanis A. Carbofuran: its toxicity to and metabolism by earthworm (*Lumbricus terrestris*). J Agri. Food Chem. 1974; 22(2): 342-347.
22. Kale RD. Earthworms: Nature's gift for utilization of organic wastes. In Earthworm Ecology, ed. CA Edwards, CRC press, The Netherlands, 1998; 355-376.
23. Gheorghita H, Elena D, Dorel H. Research Regarding the Influence of Plant Management System and Shoot Removal on the Intensity of Physiological Processes at Cucumber Cultivated in Solariums. Agriculture and Agricultural Science Procedia 2015; 6: 139-144.
24. Manier N, Bado-Nilles A, Delalain P, Aguerre-Chariol O, Pandard P. Ecotoxicity of non-aged and aged CeO2 nanomaterials towards freshwater microalgae. Environ. Poll. 2013; 180: 63-70.
25. Rodea-Palomares I, Boltes K, Fernandez-Pinas F, Leganes F, GarciaCalvo E, Santiago J, Rosal R. Physicochemical characterization and ecotoxicological assessment of CeO_2 nanoparticles using two aquatic microorganisms. Toxicol. Sci. 2011; 119: 135-145.
26. Gardea-Torresdey JL, Peralta-Videa JR, de la Rosa G, Parsons JG. Phytoremediation of heavy metals and study of the metal coordination by X-ray absorption spectroscopy. Coordin. Chem. Rev. 2005; 249: 1797–1810.
27. Van Der Ploeg MJ, Handy RD, Heckmann LH, Van Der Hout A, Van Den Brink NW. C60 exposure induced tissue damage and gene expression alterations in the earthworm *Lumbricus rubellus*. Nanotoxicol. 2013; 7(4): 432-440.
28. Morowati M. Histochemical and histopathological study of the intestine of the earthworm (*Pheretima elongata*) exposed to a field dose of the herbicide glyphosate. The Environ. 2000; 20(2): 105-111.
29. Bansiwal K, Rai N, Kurawar R, Rathor DS. Morphological and Behavioural changes in Earthworms during Malathion intoxication and effect on soil Fertility. Elec. J Environ. Sci. 2010; 3: 39-43.
30. Gobi M, Suman J, Ganesan SV. Sublethal Toxicity of the Herbicide Butachlor on the Earthworm *Perionyx sansibaricus* and its histological changes (5ppm). J Soils Sed. 2004; 5(2): 82-88.
31. Li CWHM, Cui YB, Li DS, Chen J, Yang LY. Toxicological effects of TiO_2 and ZnO nanoparticles in soil on earthworm *Eisenia fetida*. Soil Bio. Biochem. 2010; 42: 586-591.