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ACUTE TOXICITY AND MORPHOMETRIC STUDIES IN ZnONPS EXPOSED *EUDRILUS EUGENIAE*

Abbas M, Meeramaideen M, Mohamed Shamsudin*

Department of Zoology, Jamal Mohamed College (Autonomous),

Tiruchirappalli, Tamil Nadu, India.

ABSTRACT: ZnO nanoparticles considered as the one of the most commonly used metal-based nanoparticles for various biological and industrial applications. *Eudrilus eugeniae* species shows faster growth rate and the second most widely used earthworm for vermicomposting activities and also used as the biological indicators for soil contamination. The acute toxicity of ZnO NPs to *Eudrilus eugeniae* earthworm observed between 1.80 to 2.00 gm/kg by using probit analysis. Four different concentrations (0.25gm/Kg, 0.5gm/Kg, 0.75gm/Kg and 1gm/Kg) were exposed to the acclimatized earthworms. At the end of 4th week, the average growth rate of treated groups showed significantly ($P<0.05$) decreased growth rate than control group. ZnO NPs treated earthworms showed reduced body size ($F=564.31$) and body length ($F=294.57$) which resulted significantly reduced body volume which resulted in decreased Cocoon and Hatching production rate in ZnO NPs treated groups. The exposure of ZnO NPs even in low concentration cause harm to the soil invertebrate (earthworm).

KEYWORDS: *Eudrilus eugeniae*, Growth rate, cocoon rate, LC50.

Corresponding Author: Dr. Mohamed Shamsudin*Ph.D.

Department of Zoology, Jamal Mohamed College (Autonomous), Tiruchirappalli, Tamil Nadu, India.Email Address: abbasakshii@gmail.com

1.INTRODUCTION

ZnO nanoparticles, is one of the most commonly used types of metal-based nanoparticles. In case of metal-based nanoparticles, like ZnO, TiO_2, Ag and CeO_2 toxicity will at least partly be due to the release of free metal ions [1] while effects may further be enhanced by the specific properties related to the small size and consequent high surface activity of the particles. Recently, some attempts have

been made to determine toxicity and bioaccumulation of ZnO nanoparticles in soil invertebrates [2-3].The influence of heavy metals in soils on earthworms and their bioaccumulation has been the subject of many studies for a long time [4-6] and used as the biological indicators of contamination due to their fairly consistent relationships between the concentrations of certain contaminants in earthworms and soils [7]. *Eudrilus eugeniae* species shows faster growth rate and is the second most widely used earthworm for vermicomposting. Among different species of earthworms various morphological measurements has been reported [8-10], whereas the variation in total body length was measured from the first to last segment [11]. *Eudrilus eugeniae* worms appear 'brown/red to dark violet' like animal flesh colour in general. The length of the body ranges from 32 - 140 mm with 145-196 segments and it gains upto 12mg/worm/day. A week after attainment of maturity the individuals commence lying cocoon, almost one cocoon/day or up to 4 cocoons/day for 46 days, depending upon the environmental conditions. Incubation period for the cocoons ranges from 16-17 days and upto hatchlings emerge from a single cocoon [12]. The acute toxicity (LC50) bioassay helps to assess the toxicity of the substances within short intervals and helps to define the safe levels threshold concentration by means of an application factor. In earthworm, chemical toxicity were evaluated by the mortality parameter [13-15] and their survival nature considered as less sensitive parameter towards the ecotoxicological concepts [16]. Xiao *et al.* [17] suggested that growth can be regarded as sensitive parameters to evaluate the chemical toxicity on earthworms. Zhou *et al.* [18] have reported that the weight of the earthworms was a more sensitive index compared to the mortality in indicating toxic effects. Zhou *et al.* [19] assessed and found the adverse effects of pollutant (8 weeks) on earthworm growth. Based on the degree of intoxication and time of exposure the weight factor acts as a valuable indicator of physiological stress [20-21], growth and reproduction are considered as the useful sub lethal criteria [22-23]. This study framed to observe the endpoints include reproductive parameters (cocoon production per adult per week, juveniles hatching per adult per week and cocoon viability) and weight change of adults. Growth effects and mortality are determined after four weeks and effects on reproduction are assessed after eight weeks of ZnO nanoparticles exposure.

2. MATERIALS AND METHODS

2.1. Experimental Organism

Eudrilus eugeniae also called as the "African night crawler", an epigeic exotic earthworm (Table 1). The earthworm *Eudrilus eugeniae* species are collected from Panikkam Patty, Tiruchirappalli district, Tamil Nadu and transported to the Environmental Research laboratory, Jamal Mohamed College (Tiruchirappalli) for acclimatization. The worms are acclimatized for 10 weeks before initiation of experiment. The adult earthworms are collected and separated into groups. Groups are maintained in plastic containers (45x30x15cm) contains 8kg of soil.

Table 1. Taxonomic Position of the experimental organism

Phylum :	Annelida
Class :	Oligochaeta
Order :	Neooligochaeta
Family :	Eudrilidae
Genus :	*Eudrilus*
Species :	*eugeniae*

2.2. Soil preparations

Experimental soil for worm rearing is prepared by mixing cow dung and degraded organic waste like dried leaves with 4.3% organic matter and pH 5. The soil is sieved through a 5mm sieve and transferred to plastic containers [24]. Humidity of the soil is maintained by water spraying at regular intervals. By Dry mix method,the prepared soil is mixed with various weights of ZnO nanoparticles, placed in a polypropylene tubs and left undisturbed for 24h in room temperature. The quantity of ZnO nanoparticles is depends on the experimental group concentration as 0.25gm/Kg, 0.5gm/Kg, 0.75gm/Kg and 1gm/Kg. After 24h, earthworms were introduced into the prepared tubs and observed. The unamended negative control soil with worms is also maintained.

2.3. Acute toxicity test

Adult earthworms were selected to determine the toxicity of the ZnO NPs. Procedures used were based on those described by Heimbach [25] as modified by Ahmed *et al.* [26]. Different concentrations of ZnO NPs were prepared in 100 mL of distilled water and mixed with 500 g of artificial soil. Thirty prewashed and ventilated mature earthworms were then introduced into each container and placed in an incubation chamber at a temperature of 23°C± 1°C, 70%–90% relative humidity, 12:12 photoperiod. All the containers were covered with pieces of smooth cotton cloth to prevent earthworms from escaping. After 28 days a mortality count of the earthworms was conducted by washing away the artificial soil, and toxicity was calculated with the usage of probit analysis.

2.4. Growth rate

Three different sublethal concentrations were selected to study the growth rate of preclitellate earthworms exposed to ZnO NPs for 28days. Earthworms (n=12) were removed from the artificial soil for every week and weighed in a physical balance at every fortnight interval with full gut [27] and returned to their respective containers. The growth rate of the treated and untreated worms was determined by measuring the weight at the beginning of incubation and weight after ZnONPs exposure. Controls without any nanoparticles were maintained separately. Body volume [8] and Growth Rate [28] were calculated using the equation:

$$\text{Body volume} = \pi L \left(\frac{1}{2} d\right)^2$$

$$\text{Growth rate} = \ln \frac{W_t}{W_0} \times 100$$

where L is the length , d is the body size; W_0 is the weight at the beginning of the experiment and Wt is the weight after t days of exposure.

2.5. Cocoon Production and Hatching rate

After the removal of the adult earthworms at 28th day, the containers were returned to the controlled temperature room for a further 28 days to allow juveniles to hatch from laid cocoons. The number of cocoons laid by the earthworm species in three different concentrations and control group were separated manually from the experimental tubs in every fortnight, counted and tabulated. Cocoons were taken and maintained separately in the petriplates containing three different concentrations of ZnO NPs, to find out the number of hatchlings per cocoon. Total number of hatchlings collected from each petriplate were counted and tabulated for statistical analysis.

2.6. Statistical Analysis

Lethal concentration of the ZnO NPs is determined by probit with regression. The data are expressed as mean±standard deviation. Differences between control and treatment groups are analyzed using one way ANOVA, with least significant differences. The ANOVA test carried out using SPSS (21 version) software.

3. RESULTS AND DISCUSSION

3.1. Acute toxicity test

ZnO NPs induce harmful effects to the earthworm and their levels are higher than 1.0g/kg in soil. *Eudrilus eugeniae* was treated with ZnO NPs and the observations showed increased lethality while increased ZnO NPs concentration. The observations were analyzed and recorded. By using probit analysis, the 50% of the earthworm mortality was observed between 1.80 to 2.00 gm/kg ZnO nanoparticles (Figure 1) in three replicates. Lahive *et al.* [29] reported Median lethal concentration (LC50) and effective concentration (EC50) values as 317.8 and 294.6 mg Ce/kg to the earthworm, *Eisenia fetida*. Hu *et al.* [3] presented data on the toxicity and bioaccumulation of Zn in the earthworm *Eisenia fetida* after 7 days exposure to ZnO nanoparticles in OECD artificial soil

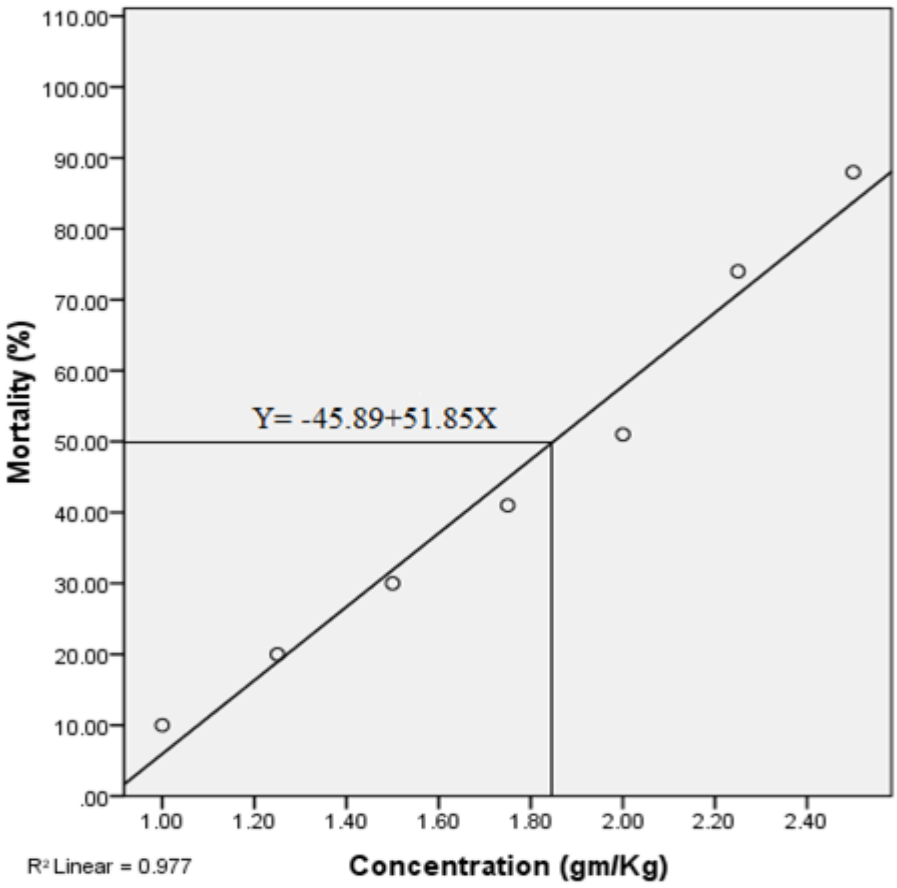

Figure 1:Probit analysis Graph showing LC50 of ZnO NPs in *Eudriluseugeniae*

3.2. Growth Rate

Control group earthworms showed significantly increased body weight and at the end of the experiment i.e. 4th week, the clitellum was completed formed in mature worms. Comparison of these results showed that weight gain by worms in control group was significantly higher than ZnO NPs treated groups (Table 1). Initial weight and final weight of the worms were measured. The average growth rate results showed significantly (F=1368) decreased growth rate when compared to the control group (Figure 2). Other studies on metal-based nanoparticles [30, 31, 2] trigger some concerns. The effects of CeO2 NPs to *Eisenia fetida*, is studied at 28th and 56th day for survival and reproduction parameters [29]. In *Eudrilus eugeniae,* total segments varied from 80 to 100 with thick cylindrical collar- clitellum between segments 13-20 [32].Body volume of the control and ZnO NPs treated earthworms were measured by their body size and body volume where recorded at the end of the experiment. ZnO NPs treated earthworms showed reduced body size (F=564.31) (Table 2) and body length (F=294.57) which resulted significantly reduced body volume (Figures 3& 4).The CuO and ZnO NPs effects on the overweight, mortality, accumulation and reproduction were measured at the end of 7th and 14th day two periods after exposure to NPs. The number of the earthworm egg decreased in both 7th& 14th days through increasing the NPs concentration. Reproduction of the earthworm was severely affected in ZnO than the CuO exposure in the seventh

day [33]. Exposure to AgNO3 to *Lumbricus rubellus* did not reduce weight gain, but a significant reduction in cocoon production down to 60% was observed, as compared with the unexposed control treatment [34].

Table 1. Average Growth rate of the *Eudrilus eugeniae* exposed to ZnO NPs

Groups	Initial weight (gm)	Final weight (gm)	Growth rate (gm/worm/day)
Control	0.871±0.03	3.917±0.055	111.43±1.99
0.25gm/kg	0.862±0.03	2.810±0.021	66.66±2.02
0.50gm/kg	0.870±0.02	2.305±0.380	52.74±1.86
0.75gm/kg	0.870±0.02	1.824±0.040	23.92±6.9
1gm/kg	0.874±0.02	1.351±0.043	20.23±0.53

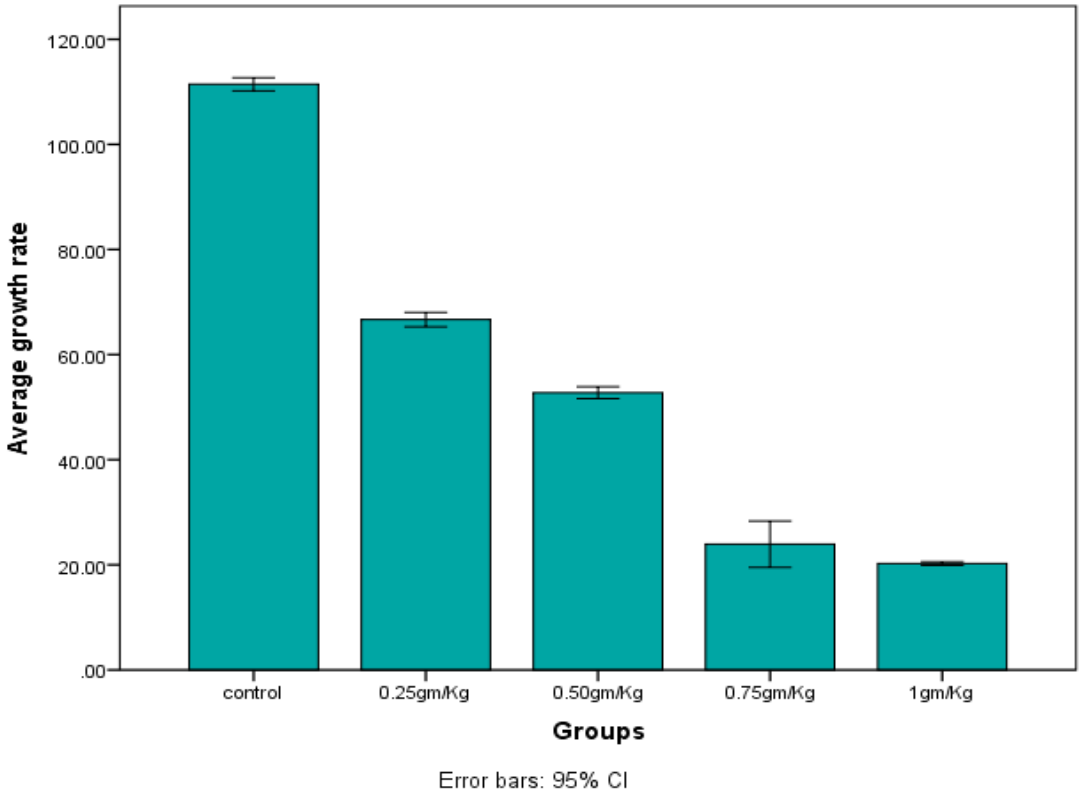

Figure 2:Average Growth rate of the *Eudrilus eugeniae* exposed to ZnO NPs

Table 2: Various parameters of *Eudrilus eugeniae* exposed to ZnO NPs

Groups	Body Size (mm)	Body length (cm)	Body volume (mm/worm)
Control	5.19±0.09	13.32±0.33	111.43±1.99
0.25gm/kg	4.50±0.09	12.70±0.36	66.66±2.02
0.50gm/kg	3.80±0.09	11.47±0.36	52.74±1.86
0.75gm/kg	3.36±0.12	10.50±0.25	23.92±6.9
1gm/kg	3.01±0.19	9.42±0.27	20.23±0.53

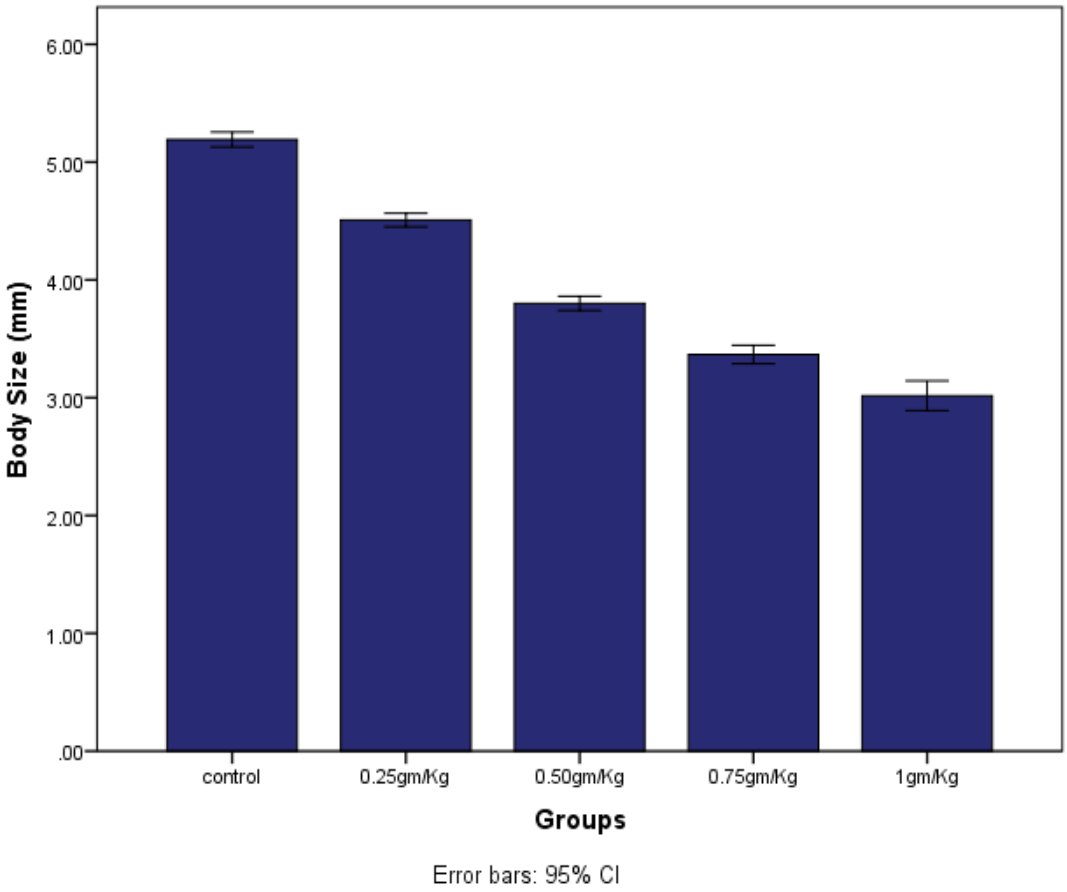

Figure 3: Body size of the *Eudrilus eugeniae* exposed to ZnO NPs

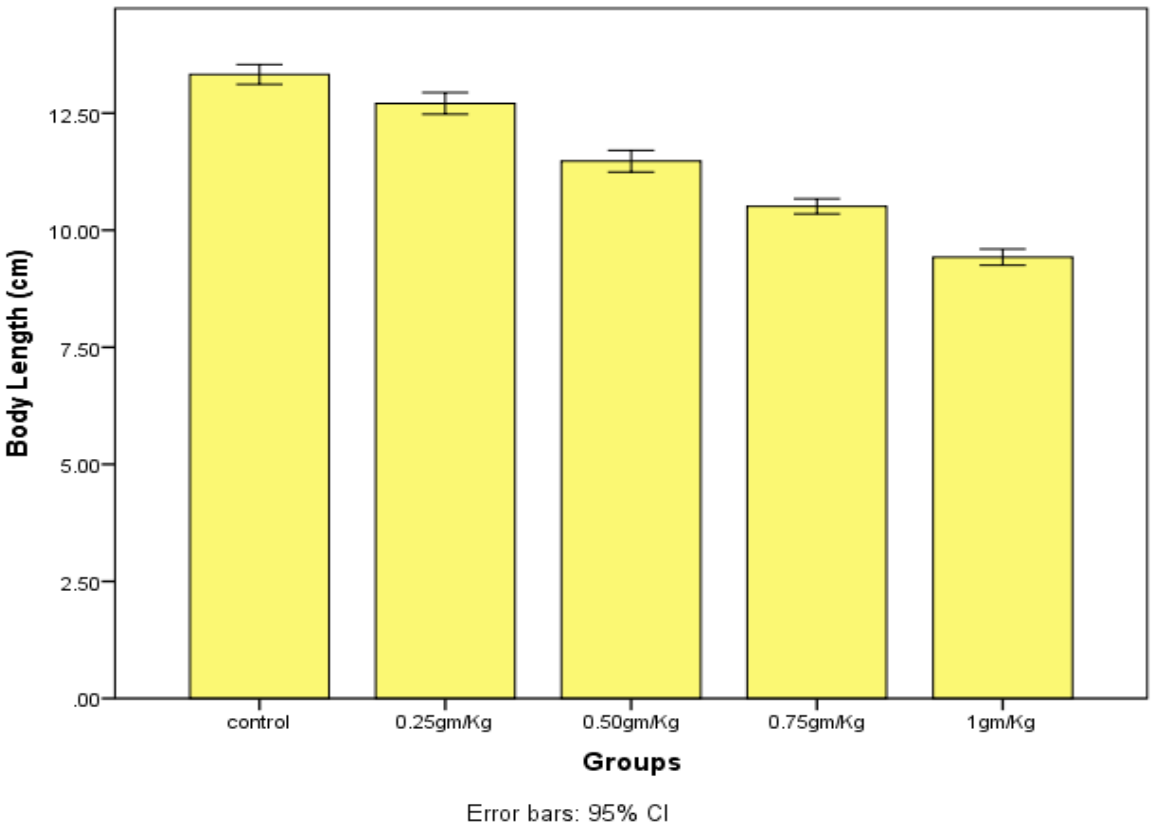

Figure 4: Body Length of the *Eudrilus eugeniae* exposed to ZnO NPs

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3.3. Cocoon and Hatching production rate

The premature worms started producing clitella after two weeks and after the third week, the cocoons were observed first time. In control, there was marked increase in biomass and cocoon production with time till the end of experiment. Control groups showed significantly increased cocoon production and hatching rate (Figures 5 & 6) whereas treated groups showed decreased cocoon production rate due to variations in their body mass and volume. At the end of the experiment, hatching rate were drastically decreased in ZnO NPs treated groups (Table 3). A Significantly decreased in reproduction were observed in *Eisenia fetida* exposed to AgNO3 (94.21 mg kg^{-1}) [35]. Reproduction declined by 50% when exposed to 750mg $Znkg^{-1}$ in *Eisenia veneta* [36]. Adult *Lumbricus rubellus* earthworms exposed to AgNP survived the 4week exposure and their weight gain was significantly reduced (down to 44%), compared with the control group, as was reproduction (down to 18%) [34].

Table 3: Cocoon and Hatching production rate in Control and ZnO NPs exposed *Eudrilus eugeniae*earthworms

Groups	Cocoon production	Hatching rate
Control	500.91±4.37	1502.75±13.13
0.25gm/kg	447.75±3.19	1343.25±9.58
0.50gm/kg	411.91±2.64	1235.75±7.93
0.75gm/kg	374.16±2.79	1122.50±8.37
1gm/kg	344.66±3.36	1034.00±10.09

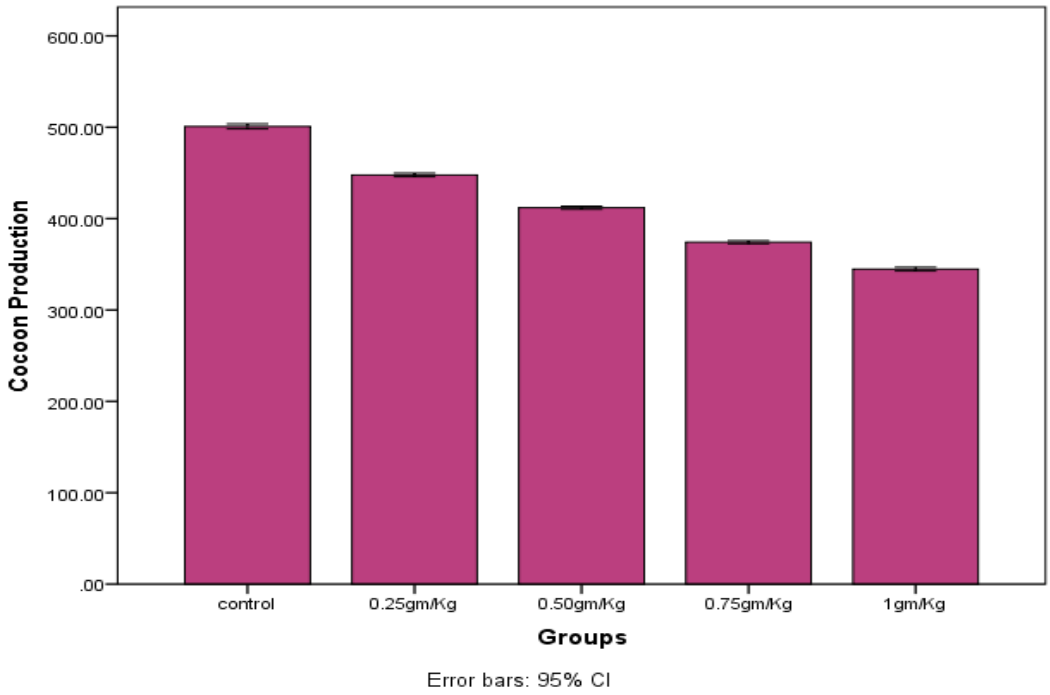

Figure 5: Cocoon production rate in Control and ZnO NPs exposed *Eudrilus eugeniae*earthworms

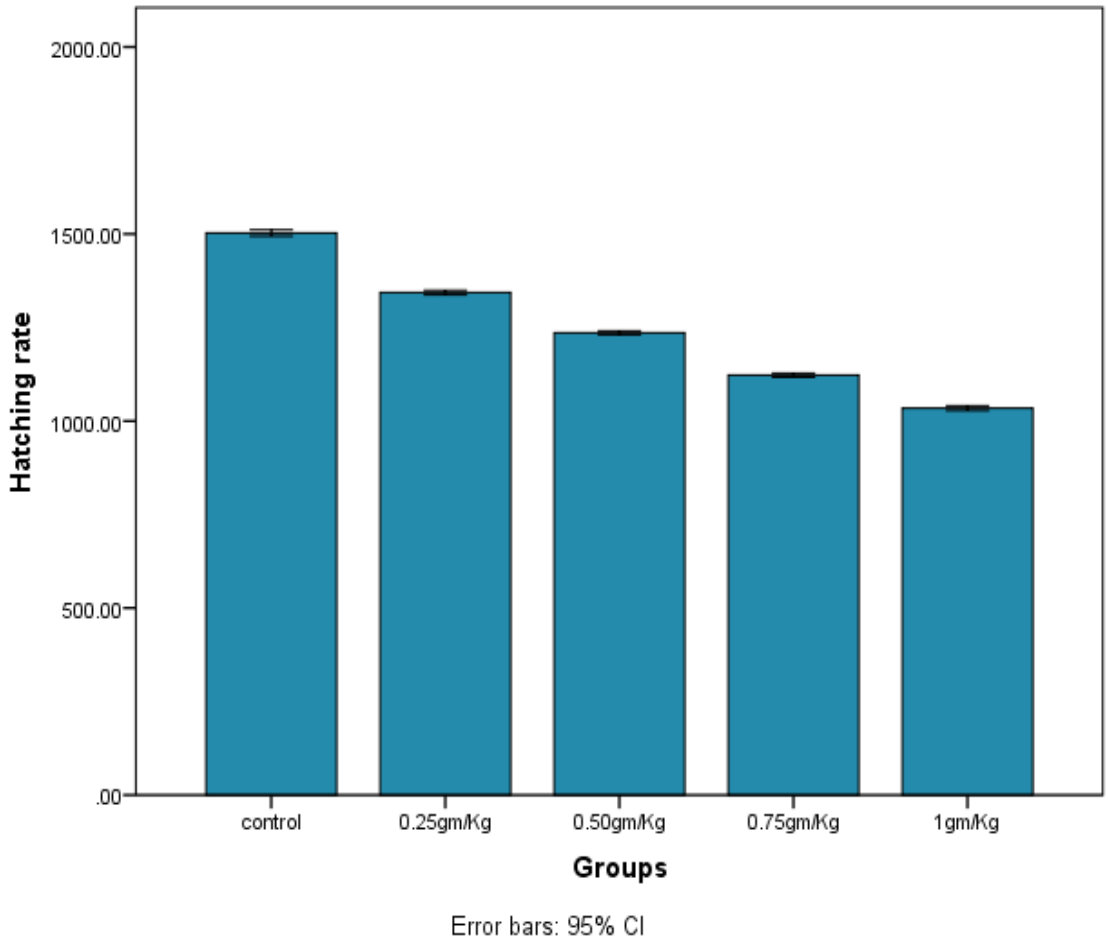

Figure 6: Hatching production rate in Control and ZnO NPs exposed *Eudrilus eugeniae*earthworms

Earthworm reproduction was affected following exposure to soils amended with 1000 mg/kg Ag, and Cu nanomaterials [37] and also with ≥ 3000 mg/kg Al_2O_3 [38]. Mean growth rate was 3.11 ± 0.28 mg/worm/day in *E. fetida*, The percentage of relative growth rate was 14.74 ± 1.39% in *E. fetida.* For *L. rubellus* earthworms, observations made at the organismal level (on survival, growth and cocoon production) have been used to demonstrate effects of heavy metal and pesticide exposure on growth and development of earthworm populations in the field, with various population models [39-41].Weight of the earthworms increased significantly in control worms however weight loss was recorded in pesticide-treated worms was time dependent during the 4-week test period [42].

4. CONCLUSION

In this study, the acute toxicity of ZnO NPs to *Eudrilus eugeniae* earthworm observed between 1.80 to 2.00 gm/kg. Average growth rate, body size, body length, body volume, Cocoon and Hatching production rate were significantly disturbed in the ZnO NPs (0.25gm/Kg, 0.5gm/Kg, 0.75gm/Kg and 1gm/Kg) exposed groups when compared to the control earthworm. The exposure of ZnO NPs even in low concentration cause harm to the soil invertebrate (earthworm).

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CONFLICT OF INTEREST

No

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