TOXICITY AND HISTOPATHOLOGICAL EFFECT OF ATRAZINE (HERBICIDE) ON THE EARTHWORM *Nsukkadrilus mbae* UNDER LABORATORY CONDITIONS

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ABSTRACT

The toxicity and histopathological effects of the herbicide Atrazine to the earthworm Nsukkadrilus mbae were studied under laboratory conditions. N. mbae were exposed to different concentrations of Atrazine (0.0, 0.4, 0.8, 3.0 and 9.0 mg/kg soil) for 96 hours and mortality was recorded every 24 hour. In addition, sections of the worm were made after 96 hours for microscopic examination. There was no mortality in the control group but in the experiments groups throughout the study. The mortality in the different treatment groups was significantly different (P > 0.05) and was concentration dependent. The LC_{50} of Atrazine after 24, 48, 72 and 96 hours were 8.60, 7.05, 7.37 and 7.23 respectively. The histopathological manifestations of exposing N. mbae to the herbicide included damage to the chloragogenous layer, damage of the epithelial tissues; glandular enlargement of the epithelial tissues, prominent vacoulations and pyknotic cells. The result of the study showed that both mortality and histopathology data could be used in environmental risk assessment of Atrazine.

Keywords: Toxicity, Mortality, Histopathology, Atrazine, Earthworm, *Nsukkadrilus mbae*

INTRODUCTION

Oligocheate worms are key invertebrate community of terrestrial ecosystem and soil fertility assessments are often related to their abundance. The need to produce more food for the ever increasing world population especially in the developing economies requires extensive use of agrochemicals such as pesticides and herbicides with its attendant effects on non-target soil fauna like the earthworms. Thus, the earthworms have been used as model animals for the study the effects of agrochemicals on soil fauna (Cock *et al.,* 1980; Gobi *et al.,* 2004). Herbicides have been reported to have adverse effect on the survival of earthworms (Van Gestrel and Van Dis, 988; Ribidoux *et al.,* 1999), growth and reproduction (Helling *et al.,* 2000; Zhou *et al.,* 2007; Corriela and Moreira, 2010).The herbicide acetochlor caused adverse effect on the sperm count and DNA in *Eisenia foetida* (Xiao *et al.,* 2006). Several other studies) have demonstrated the lethality of herbicides and pesticides to earthworm and their histopathological effects (Gupta and Sundaraman, 1988; Sorour and Larink, 2001; Lydy and Linck, 2003; Gobi *et al.,* 2004; Rombke *et al.,* 2007; Mosieh, 2009).

In Nigeria, Atrazine is a common herbicide used in farmlands and to date there seems to be dearth of information regarding its effect on earthworms. This study was undertaken with view to investigating the toxicity of Atrazine to Nsukka earthworm as well as its histopathological effects.

MATERIALS AND METHODS

Earthworm: Four hundred earthworms used in this study were collected from the Zoological Garden Wormry, University of Nigeria, Nsukka and acclimatized for seven days before the commencement of the study. After acclimatization, three hundred and ninety (390) individuals were randomly divided into five treatment groups of 45 worms. Each treatment was further randomized into three replicates containing 15 earthworms each. Earthworms in treatments A, B, C, D and E were exposed to 0.0, 0.4, 0.8, 3.0 and 9.0 mg Atrazine/kg soil, respectively. The A group served as the control experiment. All the soils were sieved using 0.3 mm fine mesh sieve, autoclaved at 100 ^{o}C for one hour and moistened to 85% moisture content.

Atrazine: Commercial preparations of (1-chloro-3-ethylamino-5-isopropylamino-2, 4, 6-triazine) containing 360g/l was used as the stock solution for the study. Using this working stock, sublethal concentrations of Atrazine (0.3, 0.8, 3.0 and 9.0mg/kg soil) were prepared.

Mortality: The mortality in the groups were recorded every 24 hour for a period of 96 hours. The earthworms were confirmed dead when they remained immobile and motionless when pricked or touched with an object. The percentage mortality was calculated using Abbott (1925) method for toxicity studies after correcting for natural (control) mortality using the formula: % mortality = [(TM – CM) ÷ (N – CM)] x 100, where TM = total mortality, CM = natural (control) mortality, N = number in the treatments.

Histopathology: At the end of the toxicity tests, live earthworms from each treatment including the control were taken and washed with distilled water. The earthworms were transferred into jars containing agar gel and left for another 96 hours to facilitate the removal of the sand content of the gut as agar is easily eaten by earthworm (Pokarzheyskii *et al.,* 2000; Gobi *et al.,* 2004). Thereafter they were cut into two and put in a specimen bottle and fixed with Bouin's fluid for 12 hours before subjecting it to histological procedures of embedding in paraffin wax, sectioning and staining with haematoxylin eosin for microscopic observation.

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Statistical Analysis: The LC_{50} was determined using probit analysis (Finney, 1971). The data obtained was analysed for differences between the treatment groups using one-way analysis of variance (ANOVA) at 95 % level of significance followed by F-LSD post-hoc test.

RESULTS

Mortality: The result of toxicity of Atrazine to *N. mbae* showed that there was no mortality in the control group throughout the study period. The mortality in the treatment groups differed significantly at each interval ($P > 0.05$) (Table 1). The percentage mortality in the group treated with 0.4 and 3.0 mg/kg soil did not vary ($P < 0.05$) after 24 hours (Table 1). The percentage mortality increased from 6.7 and 11.10% after 24 hours to 37.8 and 73.3% in the group treated with 0.4 and 8.0mg/kg soil respectively. Similarly, the percentage mortality increased from 6.7 and 17.8% to 77.8 and 80% in the groups treated with 3.0 and 9.0 mg/kg soil respectively (Table 1). The survival rate of *N. mbae* exposed to Atrazine varied significantly ($P > 0.05$) among treatment groups but was dose dependent with 100 % survival in the control group throughout the study period (Table 2).

The LC_{50} values were 8.60, 7.05 mg Atrazine / kg soil after 24 and 48 hours, respectively. The corresponding chi-square values after 24 and 48 hour are 3.526 and 17.595, respectively. The chi-square values at 72 and 96 hours intervals are 5.258 and 16.118, respectively (Table 3). The LC_{50} of Atrazine to earthworm at the tested concentrations were 8.60, 7.05, 7.35 and 7.25 after 24, 48, 72 and 96 hours, respectively. The regression plots (based on the probit analysis) of the effect of Atrazine on *N. mbae* at different intervals are presented (Figures 1 – 4).

Table 1: Mortality of *Nsukkadrilus mbae* exposed to soils contaminated with varied concentrations of Atrazine

Concentration (mg/kg soil)	Duration of Treatment (hours)			
	24	48	72	96
Control (0.0)	0 (0)	0(0)	0(0)	0(0)
0.4	3.0 (6.7)	8.0 (17.8)	16.0 (35.6)	17.0 (37.8)
0.8	5.0 (11.1)	9.0 (20.0)	21.0 (46.7)	34.0 (73.3)
3.0	3.0 (6.7)	11.0 (24.41)	19.0 (42.2)	35.0 (77.8)
9.0	8.0 (17.8)	28.0 (62.2)	31.0 (68.9)	36.0 (80.0)

Percentage mortality are presented in parenthesis

Table 2: Percentage survival of *Nsukkadrilus mbae* exposed to soils contaminated with varied concentrations of Atrazine

Concentration (mg/kg soil)	Duration of Treatment (hours)			
	24	48	72	96
Control (0.0)	100	100	100	100
0.4	93.3	82.2	64.4	62.2
0.8	88.9	80.0	53.3	26.7
3.0	93.3	75.6	57.8	22.2
9.0	82.2	37.8	31.1	20.0

Table 3: LC_{50} values, regression equation, 95% fudicial limits and chi-square values of *Nsukkadrilus mbae* exposed to soils contaminated with varied concentrations of Atrazine

Duration of Treatment (hours)	LC_{50}	Regression Equation	LFL	UFL	Chi-square (X^2)
24	8.60	Y = 4.4035x – 5.9079	5.94	21.10	3.526`
48	7.05	Y = 4.4035x + 5.3562	5.0	16.08	17.595
72	7.35	Y = 4.4035x + 8.3300	5.18	17.34	5.258
96	7.23	Y = 4.4035x + 3.8075	5.12	16.68	16.118

LFL = Lower Fudicial Limit; UFL = Upper Fudicial Limit

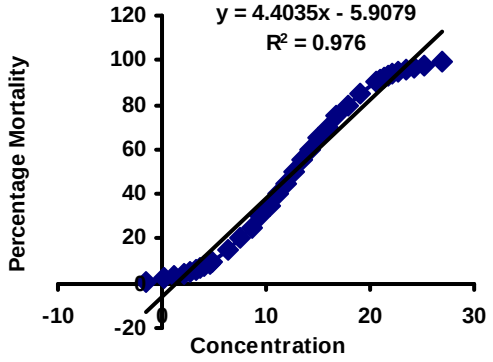

Figure 1: Probit plot of *Nsukkadrilus mbae* exposed to soils contaminated with varied concentrations of Atrazine for 24 hour

Histopathology: The effects of Atrazine on the histology of the earthworm *N. mbae* after 96 hours exposure are shown in Figures 5, 6, 7 and 8. In the control the chloragogenous layer (ch), the muscle (mc) and epithelium (ep) maintained their normal structure (Figure 5). The result showed that the herbicides affected the histology of *N. mbae.* When the earthworm was exposed to 0.4mg/kg soil, there was slight damage to the chloragogenous tissue (peritoneum) of *N. mbae* (Figure 6). Prominent histopathological effects included slight vacoulations of the epithelium especially the proximal areas. The effect of 0.8mg/kg Atrazine on the earthworm histology showed that the epithelial tissue was vacuolated following cytolysis (Figure 7). There were many pyknotic cells in the chloragogenous layer. It was observed that the muscle layer has lost its compactness. Most of the epithelial cells have lost their nuclei.

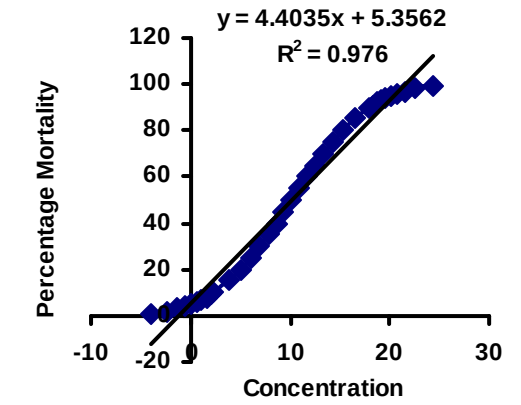

Figure 2: Probit plot of *Nsukkadrilus mbae* exposed to soils contaminated with varied concentrations of Atrazine for 48 hours

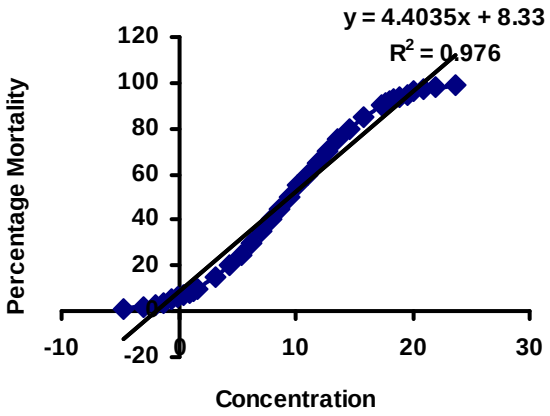

Figure 3: Probit plot of *Nsukkadrilus mbae* exposed to soils contaminated with varied concentrations of Atrazine for 72 hours

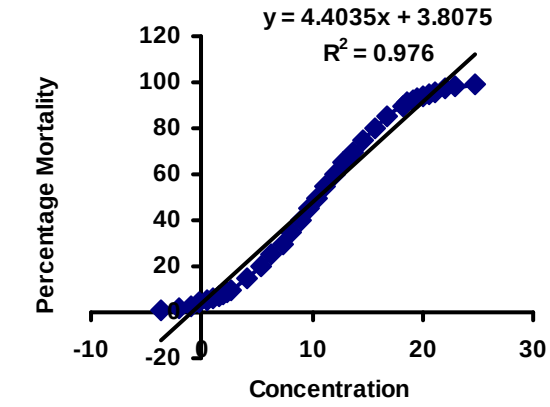

Figure 4: Probit plot of *Nsukkadrilus mbae* exposed to soils contaminated with varied concentrations of Atrazine for 96 hours

In the earthworm exposed to 3.0mg/kg soil of Atrazine, the chloragogenous layer was damaged (Figure 8). There are many pyknotic cells in both the chloragogenous and epithelial layers. The natural architecture of the muscles was disrupted. Besides being damaged, the epithelial tissues had prominently folds with glandular enlargements.

DISCUSSION

The result of this study showed that Atrazine had significant effect on *N. mbae.* Working with *Fenamiphos,* Caceres *et al.* (2010) reported that the LC_{50} of the herbicide to earthworm *Eisenia foetida* was 228 mg/kg soil while the LC_{50} of Benomyl (fungicide) to earthworm was 12.9 mg/kg soil (Rombke *et al.,* 2007). The 96 hour LC_{50} of Atrazine to earthworm in this study was found to be 7.23mg/kg soil. When the earthworm *E. foetida* was exposed to varying concentrations of acetochlor and methamidophos the LC_{50} values were 115 mg/kg soil and 29.5 mg/kg soil respectively (Qi-xing *et al.,* 2006).

The mortality of earthworm, *E. foetida* when treated with acetochlor and urea were 0.867% and 100% respectively (Xiao *et al.,* 2004). This is comparable with the result of this study in which the mortality of *N. mbae* ranged from 37.8 – 80 % when exposed to Atrazine. Similarly, Xiao *et al.* (2006) reported sublethal effect of acetochlor on the earthworm *E. foetida.* The result of this study demonstrated that Atrazine was toxic to *N. mbae.* This was in agreement with the work of Lydy and Linck (2003) that Atrazine was more toxic than Chlorpyrifos on soil organisms. However, Correia and Moreira (2010) reported that earthworms exposed to soils spiked with glyphosate were all alive throughout the study period.

The low survival rate of earthworm when treated with Atrazine in this study is in agreement with the report of Landrum *et al.* (2006) on the survival rate of the earthworm *E. foetida* exposed to Perchlorate. The observed histopathology in the form of destruction of the peritoneum (chloragogenous layer) and the epithelium agrees with the findings of Gobi *et al.* (2005) when the earthworm *Perionyx sansibaricus* was treated with Butachlor. Similarly, Gupta and Sundaraman (1988) had earlier reported damage to the chloragogenous

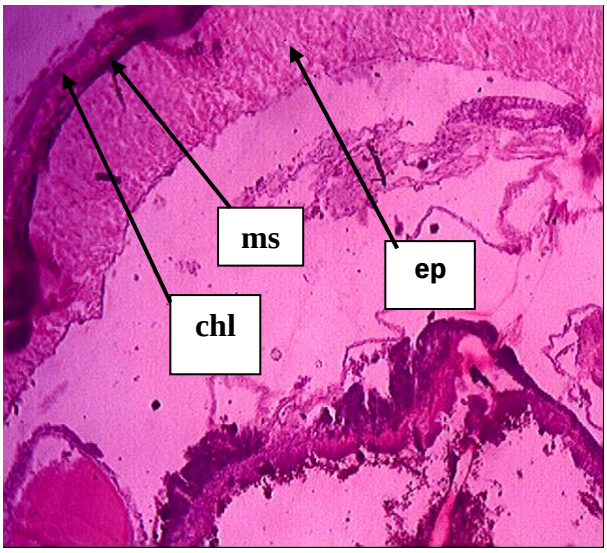

Figure 5: Control showing normal organisation of the chloragogenous and epithelial layers. Mag. X 200. (Ep = epithelium, ch = chloragogenous layer; ms = muscle layer)

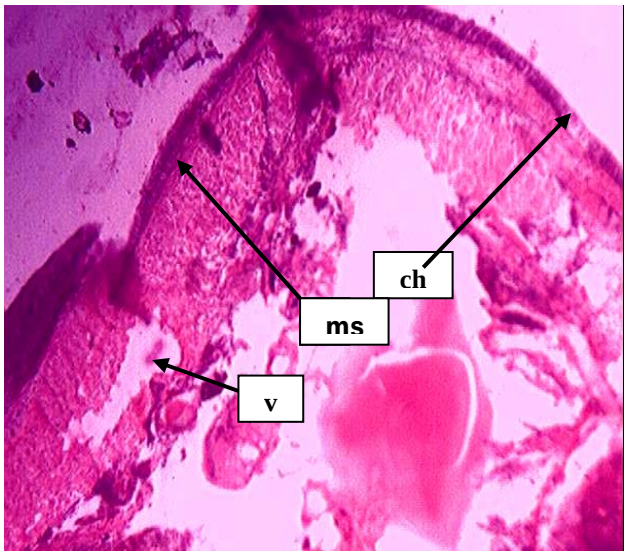

Figure 6: Section of the earthworm, *Nsukkadrilus mbae* exposed to 0.4mg Atrazine/kg soil for 96 h. Mag. X 200. (va= vacuolated epithelial tissue, ep =epithelium, ch = chloragogenous layer)

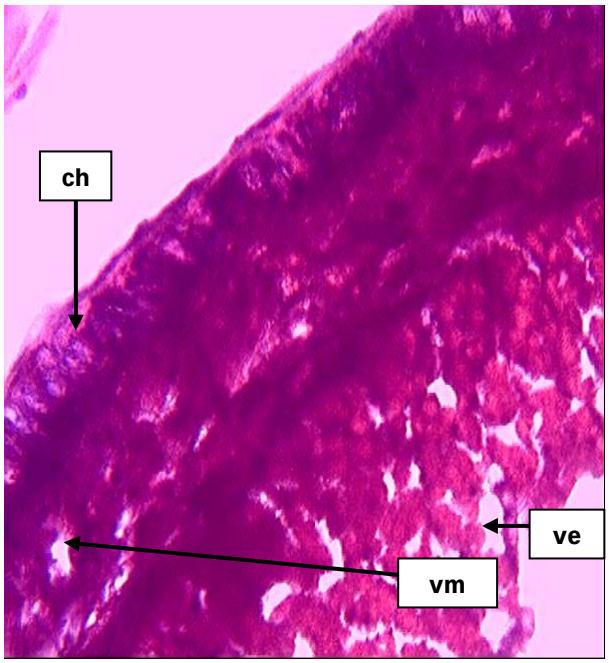

Figure 7: Section through the intestine of the *Nsukkadrilus mbae* exposed to 0.8mg Atrazine/ kg soil for 96 h. Mag. X 200. (vm= vacuolated muscle fibre, ve = vacuolated epithelium, ch = chloragogenous layer without nuclei)

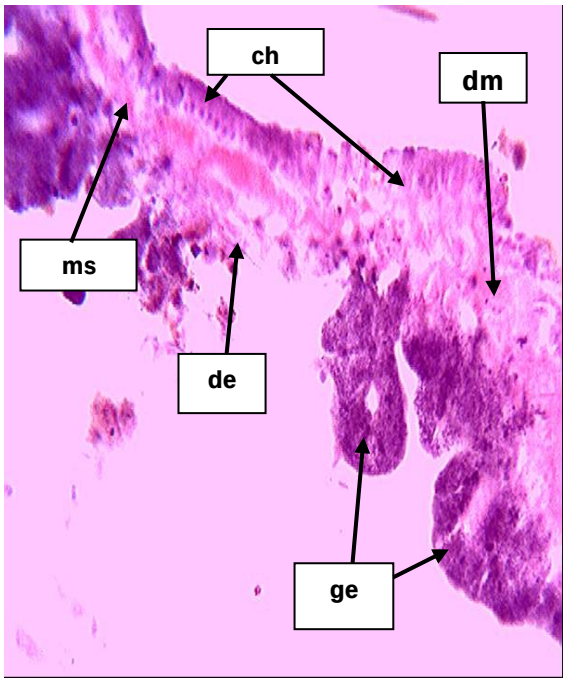

Figure 8: Section through the intestine of the *Nsukkadrilus mbae* exposed to 3 mg Atrazine/kg soil for 96 h. Mag. X 200. (ge = glandular epithelium, de= destroyed epithelium, ms = muscle separating from the chloragogenous layer, dm = destroyed muscle fibres, ch= chloragogenous layer)

layer cell in the earthworm *Pheretima posthumus* exposed to Carbaryl. Mohssen (2000) observed that Glyphosate caused damage to the epithelial tissues of *Pheretima elongate.*

In conclusion, the result of the study suggested that the toxic effects of Atrazine are mediated through its effect on the structural integrity of the tissues. Its effect on other biological indicators of stress and pollution (Gobi *et al.,* 2004; Xiao *et al.,* 2006) could play a major role in its lethal effect. It further shows that the herbicide had adverse effects on non-target organisms particularly the earthworms that are critical in evaluation of soil fertility.

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