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Assessment of Contamination and influence of herbicide (pendimethalin) on Fish ((*Tilapia nilotica*) by using Serum AST as indicator in White and Blue Nile State, Sudan

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ABSTRACT

This study was aimed to assess the contamination and effect of the herbicide (pendimethalin) on fish (*Tilapia nilotica*) in White and Blue Nile Stream by using serum Aspartate amino transferase (AST) as indicator for this study. It was carried out on December 2009. Water and fish samples were collected from four locations; location 1: Blue Nile Stream (Singa area), location 2: recycled – water (Kenana area), location 3: White Nile Stream (Kenana area) and location 4: drainage – water (Kenana area). Pendimethalin was extracted from both water and fish fat extracted edible tissues.. Compared to location 1 sudden increase in serum aspartate amino transferase (AST) was observed in location 2, 3 and 4. These finding are indicated White Nile State is more contaminated by pendimethalin due to more uses of this herbicide for control growth of the weed in Kenana area.

Key words: Pendimethalin, Pesticide, Herbicide, Skeletal muscle cells, Carcinogen.

Introduction

The monitoring of the fish for chemical contamination in the Kenana area is a critical activity for protecting human health because this area is important for sport fishing and other recreational activities. The term pesticides refer to a broad class of crop-protection chemicals. Herbicides are the most widely used chemicals in agriculture **(**National Academy of Sciences, 1993). Pesticides help or control hundreds of weed species, more than one million species of harmful insects and some 1,500 plant diseases **(**National Agricultural Chemicals Association, 1993). Pendimethalin is considered as moderately persistent herbicide that can give rise to long-lasting metabolites. It contains dinitroanilines, which reportedly it could result in the formation of carcinogenic nitrosamines (Environmental Protection Agency, 1997). Aspartate aminotransferase (AST) is an enzyme that found in high amounts in skeletal muscle cells (Berk and Korenblat, 2007). Pendimethalin is highly toxic to fish and aquatic invertebrates (Meister, 1992). The chemicals also have the ability to bioaccumulate and biomagnify, and can bioconcentrate upto 70,000 times their original concentrations (Ritter *et al.,* 2007). Pendimethalin widely used herbicide, has been classified as a group C possible human carcinogen by the U.S. Environmental Protection Agency (Hou *et al.,* 2006). The gill of the fish is the main organ for different functions, such as gas exchange, ion regulation and excretion of metabolic waste products (Wood, 1991). Its complexity and constant contact with the external environment make the gill to be the first target for waterborne pollutants (Mallatt, 1985). The pollutants are not only entering the organism through the gills, but also exert their primary toxic effects on the bronchial epithelium (Playle *et al.,* 1992) which in turn, may influence the general gill functions (Montero *et al.,* 2005). Pesticides may continue to poison non-target organisms in the environment and increase risk to humans (Centers for Disease Control and Prevention, 2007). By disruption its poison on the endocrine, reproductive, and immune systems and causes cancer; neurobehavioral disorders (Ritter *et al.,* 2007). The objectives of this study are assessed the pollution of water and fish with herbicide (Pendimethalin) by using serum aspartate amino transferase enzyme (AST) as indicator in White Nile Province (Kenana area, Sudan) and Blue Nile Province (Singa area).

Materials And Methods

2.1 Biological Experiment:

Water and fish of different sex, age (21 – 30 days) and weight (150 gm – 1.2 kg) was collected from four locations (Blue Nile Stream, Recycled – water, White Nile Stream and Drainage – water).. Blood sample were collected from fish heart and stored at 5 ^{o}C until analysis, blood was centrifuged at 30000 rpm for serum separation. Serum AST concentrations were measured, then pendimethalin was extracted from both water and fish samples separately according to following method

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2.2 Extraction of pendimethalin from water samples:

250 ml of representative sample was partitioned with 50, 50, 25 ml mixture of n-hexane: diethylether (9:1). The combined extracts were dried through anhydrous sodium sulphate and concentrated, then taken in 2 ml of n-hexane(Paula *et al.,* 2007).

2.3 Extraction of pendimethalin from fish tissues:

The 50 gm of the edible part of fish from each sample was homogenized. Then extraction of pendimethalin was performed by using 150 ml and 100 ml of acetonitrile. Samples were filtered and rinsed twice with 25 ml of the solvent. The combined extract was concentrated by using a rotary vacuum and evaporated over a hot water bath (less than 50^{o}C) to 50 ml. The liquid – liquid partitioning was taken as follows: the concentrated extract was taken in a 500 ml separator funnel, then diluted with 250 ml of 5% aqueous sodium chloride and partitioned into 150, 150 and 100 ml of n-hexane. The combined n-hexane layer was passed through anhydrous sodium sulphate and concentrated to near dryness and take in about 10 ml n-hexane (Paula *et al.,* 2007).

2.4 Measurement of pendimethalin concentration by HPLC:

A calibrated HPLC device was set for measurement of pendimethalin concentration as follows: Column: ODS (18), Flow rate: 1 ml / minute, Injection volume: 10 µL, Oven temp: 30 ^{o}C. Mobile phase: acetonitrile: water (80: 20) (Oblinger *et al.,* 1999) and (U.S. Environmental Protection Agency, 1992).

2.5 Measurement of AST concentration:

It was determined according to method described by (Murray, 1984**).** Working solution was prepared by adding 2 ml from reagent 1 (buffer, lactate dehydrogenase (LDH), malate dehydrogenase (MDH), L - aspartate, pH 7.8) and 500 µl from reagent 2 (substrate α-ketoglutarate). It was mixed and kept in 37^{o}C, 1 ml was taken from working solution, then 100 µl from serum was added, was mixed and incubated at 37^{o}C for 1 minute. Initial absorbance was read at 1 minute intervals, the difference between absorbance were calculated.

The average absorbance difference per minute: Δ A / minute × 1750 (factor) = U/ L.

A calibrated spectrophotometer (Awareness Technology, model No. 1904 plus, serial No. 1904-5252) was set for measurement of AST concentrations.

2.6 Histopathologcial parameters:

skeletal muscles and gills were collected in clean, sterilized urine containers (from the autopsied fish), labeled and cleaned with distilled water, preserved in 10% formalsaline.

Sequence of steps which were carried out according to method described by (Bancroft and Gamble, 2002) for preparation the slides: As follows

1. Suspected sites of liver were cut into small pieces and the kidney was also cut into 4 lobes, the cut organs were dehydrated in solutions of 30% alcohol for two hours, then solutions of 50% alcohol for two hours, finally 70% alcohol for two hours to attain the preservation level. 2. Continuation of dehydration: 70% alcohol (twice 1/4 an hour, 1/4 an hour), 90% alcohol / 2 hours, 95% alcohol / 2 hours, 100% alcohol / 1 hour, 100% alcohol / 1 hour. 3. Clearing: Xylene 1 / 3/4 an hour, Xylene 2 / 1/2 an hour or Chloroform overnight. 4. Impregnation: Wax 1 / 1 hour, Wax 2 / 1 hour. 5. Embedding: Tissue is embedded in cassette. 6. Section: Microtome was used. 7. Mounting on slides: Formaldehyde and gelatin were used. 8. Wax fixation and tissue elongation: Slides were put on oven at temperature < 45º C. 9. Wax removal: Xylene 1 / 2 minutes, Xylene 2 / 2 minutes, Absolute alcohol 1/ 2 minutes, Absolute alcohol 2 / 2 minutes, 90 % Alcohol / 2 minute, 70 % Alcohol / 2 minutes, Distilled water / 2 minutes. 10. Staining: Stain with iodine and haematoxillin for 10 minutes 11. Blueing: Wash under running tab water if overstrained dip quickly in acid alcohol (3 drops of HCl in 70% alcohol), then distilled water / 1/2 minute, Iodine / 1/2 minute, Distilled water / 1/2 minute, 70% alcohol / 1/2 minute, 90% alcohol / 1/2 minute, Absolute alcohol 1 / 1/2 minute, Absolute alcohol 2 / 1/2 minute, Xylene 1 / 1/2 minute and Xylene 2 / 1/2 minute. 12. Covering with Canada balsam/

2.7 Statistical Analysis:

Three samples were taken, analyzed and averaged. Data were assessed by using The Analysis of Variance (ANOVA) as described by Gomez and Gomez (1984).

Results and Discussion

3.1 Contamination of Fish and Water by Herbicide:

Table 1 indicated in Blue Nile Stream, the pendimethalin in tissues of fish and water is not found, but enzyme of AST is found in serum of the fish within normal range(10 -34 ppm) that reported by Berk and Korenblat (2007). These finding reveal that area of Blue Nile Stream is less contaminated with pendimethalin. While in White Nile Stream, the pendimethalin in water (269.6ppm ± 43.6) is higher than in tissues of fish (25.5ppm ± 4.03), in addition the concentration of serum AST (118.5 ppm ± 41.5) is high compared with those values (21.3 ppm ± 10.5) in Blue Nile Stream. These results explained that White Nile Stream is more polluted by pendimethalin than Blue Nile Stream. In Kenana area (White Nile Province), the pendimethalin in tissues of fish and water for the Recycle - water became higher compared with White Nile Stream, but lower compared with Drainage – water. In addition concentration of serum AST (471.1 ppm ± 42.9) is high in Drainage - water compared with those values (280 ppm ± 43.8) in Recycle - water in Kenana area. These findings are indicated that drainage – water and recycle - water in Kenana area are more contaminated with pendimethalin. Increase in AST associated with significantly increase of pendimethalin concentration in recycle and drainage – water in Kenana area and consequently an increase fish tissues due to bioaccumulation of pendimethalin.

Table1: shows concentration (ppm) of pendimethalin on fish, water and serum AST as indicator

Sample	Blue Nile stream	White Nile stream	Recycle - water in Kenana	Drainage - water in Kenana
Fish	0	25.5 (± 4.03)	42.8 (± 5.1)	68.6 (± 6.6)
Water	0	269.6 (± 43.6)	451.1 (± 43.6)	591.9 (± 46.5)
Serum AST	21.3 (± 10.5)	118.5 (± 41.5)	280 (± 43.8)	471.1 (± 42.9)

Mean is averaged of thirty replicates

3.2 Skeletal muscles and gills dissection:

Plate (a) indicated in location of Blue Nile, the skeletal muscle of fish is normal.

Plate (b) showed that in recycled – water, it is clearly observed that there is (**n**) muscle necrosis, with fragmentation of sarcoplasm in the right half compared with muscle on the left half.

Plate (c) observed that in White Nile Stream, it is clearly noted that there is (**n**) muscle necrosis, with calcified muscles (**ca**).

Plate (d) noted in drainage – water, there is (**n**) muscle necrosis, with calcified muscles (**ca**), and mononuclear cell infiltration (**m**). The finding is explained there is gradual muscle changes were observed in plates b, c and d compared plate a. Any diseases that affect on liver cells leads to increase AST levels and cause primary muscle changes (Berk and Korenblat, 2007). Janssen *et al.,* (1989) reported that skeletal muscle is known to contain an isozyme of AST that may be released into the blood stream following muscle necrosis.

Plate (a-1); indicated in location of Blue Nile, the gills of fish is shown nearly normal regular pattern of secondary filaments.

Plate (b-1): showed that in recycled – water, there are secondary lamellae which indicating the hypertrophy (**h**).

Plate (c-1): observed that in White Nile Stream, there is swelling (**s**), curling (**cu**) and necrosis (**n**) of secondary lamellae.

Plate (d-1) noted in drainage – water, there is thickening (**t**), clumping (**cl)**, and necrosis (**n**) of secondary lamellae. The findings are supported the results that obtained by Playle *et al.,* (1992) and Monteiro *et al.,*(2005) whose reported that pendimethalin not only enter the organism through the gills, but also exert its primary toxic effects on the branchial epithelium and may influence the general gill functions

Conclusion:

The herbicide pendimethalin, is water pollutant and causes toxicity to fish and other aquatic invertebrates. Toxicity can end up in humans through the food chain. We recommend that water used in agriculture and industry should be completely recycled before reaching rivers and other sources of human drinking water or fishery activities.

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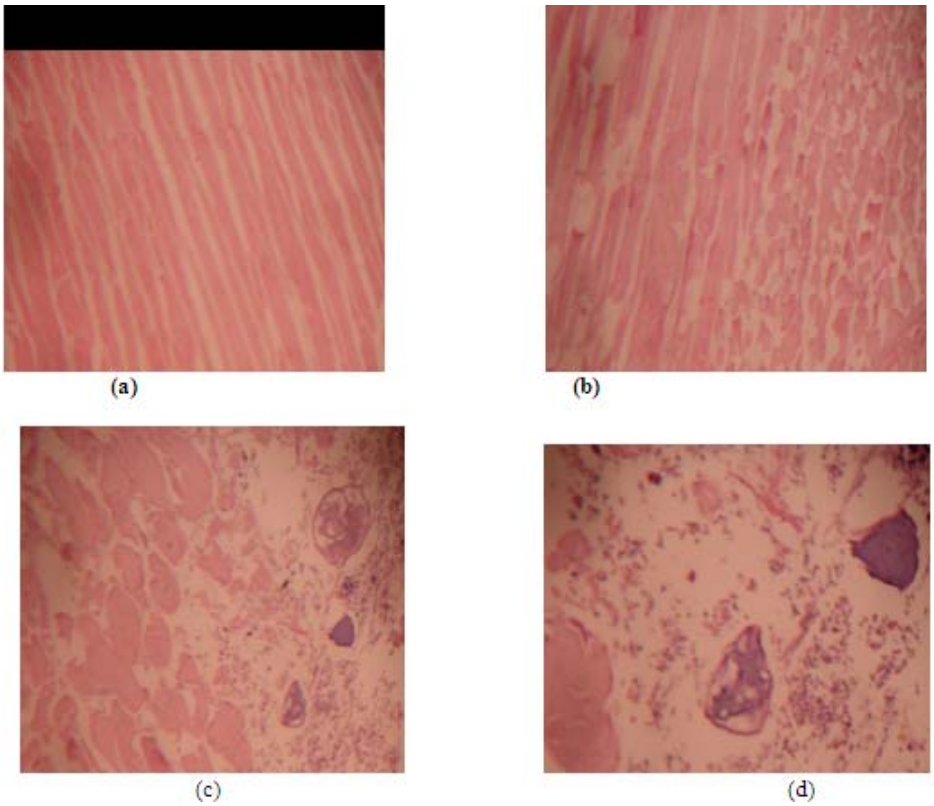
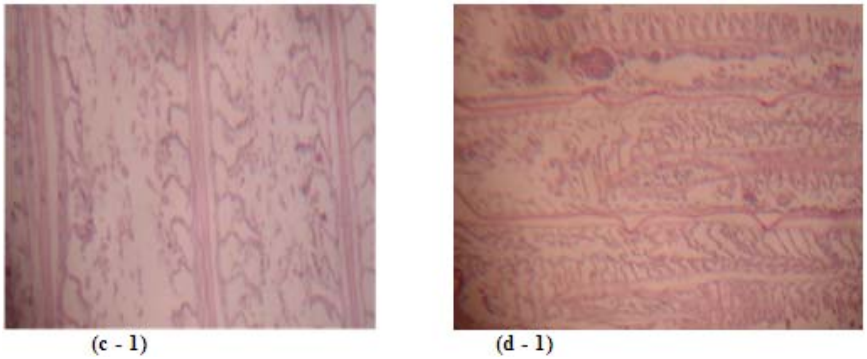


Plate
(a) Location 1 Blue Nile Stream, Normal fish skeletal muscle, (H&E × 250). Skeletal muscle cells
(b) Location 2: Recycled – water (Kenana area): (**n**) muscle necrosis, with fragmentation of sarcoplasm in the right half compared with muscle on the left half. (H&E × 100). Skeletal muscle cells
(c)Location 3: White Nile Stream : (**n**) muscle necrosis, with calcified muscles (**ca**). (H&E × 250). Skeletal muscle cells
(d) Location 4: drainage – water(Kenana): (**n**) muscle necrosis, with calcified muscles (**ca**), and mononuclear cell infiltration (**m**). (H&E × 400). Skeletal muscle cells



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(c)Location 3: White Nile Stream : (**n**) muscle necrosis, with calcified muscles (**ca**). (H&E × 250). Skeletal muscle cells
(d) Location 4: drainage – water(Kenana): (**n**) muscle necrosis, with calcified muscles (**ca**), and mononuclear cell infiltration (**m**). (H&E × 400). Skeletal muscle cells

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