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Naphthalene induced Biochemical changes in *Anabas testudineus*

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Key words: Acetylcholine esterase, Aggregation of blood cells, Protein, ATP, Experimental and Control set.

Abstract

Pollution of aquatic ecosystems can be ascertained through the organisms residing in it. Biochemical changes in fish reflect the type of degradation occurring in living systems under the influence of any toxicant. Naphthalene is easily absorbed and distributed inside the body and is metabolized mainly by liver. *Anabas testudineus* was used as toxicity test organism to analyze various forms of damage. Basic water quality indices were checked before designing the bioassay test. Lethal concentration Of Naphthalene which killed 50% of Test animals (LC_{50}) was determined after exposing the fish to varying concentrations for a period of 96 hours. The test specimen selected had average weight of 2-5gm and was procured from Central Institute of Freshwater Aquaculture (CIFA). After exposure to Naphthalene, the test organism showed major changes in the glycogen content, acetylcholine esterase, protein and adenosine triphosphate with increase in toxicant concentration. Constant decrease in the biochemical parameters indicates stress condition of *Anabas testudineus*.

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Introduction

Naphthalene is a non-polar, polycyclic aromatic hydrocarbon (PAH) with two benzene rings which occurs naturally as a component of coal tar and crude oil and is manufactured for use principally as a chemical intermediate .Our environment is getting polluted by the release of various PAH compounds. Naphthalene is found in wide range of products, including petroleum products, mothballs, wood preservatives, solvents, dyes and can be released to the aquatic environment through direct or indirect discharges. In aquatic environment Fishes are easily affected by different pollutants .Fishes have received maximum attention in ecotoxicology studies as they can be used as biomarkers for monitoring the health of fishes and aquatic environment (Zhang *et al.*2004).The present study was carried out to observe the damage done by Naphthalene as it is widely used by Human population in everyday life. *Anabas testudineus* was selected to carry out the 96 hr LC_{50} toxicology test. Naphthalene was selected as it is commonly used in everyday life in various forms. Changes caused by Naphthalene in aquatic animals particularly fishes can be used for extrapolation of the results to humans.

Materials and methods

Water quality parameters

Water quality parameters were tested using Standard methods by APHA.

Dissolved Oxygen (Winkler's method)

Water sample was collected in 250 ml stoppered bottles avoiding entrapping of air bubbles. The bottles were kept submerged in water. The stopper was removed and 2ml each of manganous sulphate (Winkler A) and alkaline iodide-azide solution(Winkler B) was dispensed one after the other keeping the pipettes well below the surface of Water.The stopper was replaced and contents were shaken by inverting the bottles. Dissolved oxygen present in water got precipitated in the form of yellowish brown precipitate. The precipitates were allowed to settle down. The precipitate was dissolved by adding 2ml of concentrated H_2 SO_4 and the contents were mixed thoroughly.50ml of fixed water sample was taken in a flask and titrated against sodium thio-sulphate solution till the colour changed to pale straw. This was followed by addition of 2 drops of starch solution (freshly prepared) and titrated further till the blue colour disappeared. The amount of titrant used was noted down.

Chloride

50ml of water sample was taken in a conical flask. To the water sample 5 drops of potassium chromate indicator was added to the sample. Sample developed yellow colour and the contents were titrated against 0.014N $AgNO_3$ until a brick red colour appears. The end point was noted down.

Total Phosphate and Orthophosphate

25ml of water sample was taken in a conical flask. A distilled water blank was run simultaneously. To 1ml ammonium molybdate solution, 3drops stannous chloride solution was added and the contents were shaken. Blue colour appeared and after 10 minutes reading was taken at 690nm. The value of O-PO_4 mgI-1 was deduced with the help of a standard curve.

25 ml of water sample was taken and dried in a 100ml conical flask. The sample was cooled and 1ml of perchloric acid was added, the flask was shaken gradually so that the residue got completely covered with the acid. The flask was gently heated on a hot plate till the residue was colourless .The temperature was increased to evaporate the perchloric acid. It was not allowed to dry up completely. The residue was allowed to cool and 100ml of distilled water followed by one drop of phenopthalein indicator was added. It was titrated against 8% NaOH solution until slight pink colour developed.

Test Organism

Fishes weighing 2-5 gm were brought from CIFA [Central Institute of Freshwater Aquaculture, Bhubaneswar] hatchery and acclimatized in Laboratory conditions for two weeks. Morphometric characterization of *Anabas testudineus* was done

before designing the dose. Naphthalene dose concentration was tested from 0.1 mg/l to 5.4 mg/l to obtain lethal concentration value. 20 fishes were taken for each concentration tested and the tests were carried out in triplicate to avoid any error. The fishes were given dry commercial feed having 45% protein.

Dose Concentration

The concentration range taken was 0.1 ,0.2, 0.3, 0.4, 0.5, 0.6, 0.7,0.8, 0.9,1.0.1.1,1.2, 1.3, 1.4, 1.5,1.6,1.7,1.8,1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3, 3.1, 3.2 , 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4 mg/l respectively. Estimation of acetylcholine esterase, protein, glycogen was carried out by Spectrophotometric method by Ellman *et al.*, 1961; Lowry *et al. (1951)*. Blood Smear preparation was done by differential staining method using field stain A and B along with Giemsa. Giemsa staining gave better results in comparison to field staining.

Results and discussion

LC_{50} - 5.4 mg/l of Naphthalene killed 50% of experimental animals. After deriving LC_{50} sublethal dose concentration was designed and the sets were labelled as experimental set 1, Set 2, Set 3 and Set 4 with one comparative Control set. Protein, glycogen, adenosine triphosphate, acetylcholine esterase activity decreased in all experimental animals compared to control fish. Blood cells showed aggregation and chain formation under the influence of Naphthalene toxicity. Aggregation of blood cells is bound to hamper oxygen carrying capacity and also affect the defense mechanism of aquatic animal. Naphthalene was found to be toxic to most of the cells and tissues and can even leach into ground water in greater quantities compared to other PAHs. Due to its release into to environment, higher levels of Naphthalene will be found in the ground water than other PAHs.

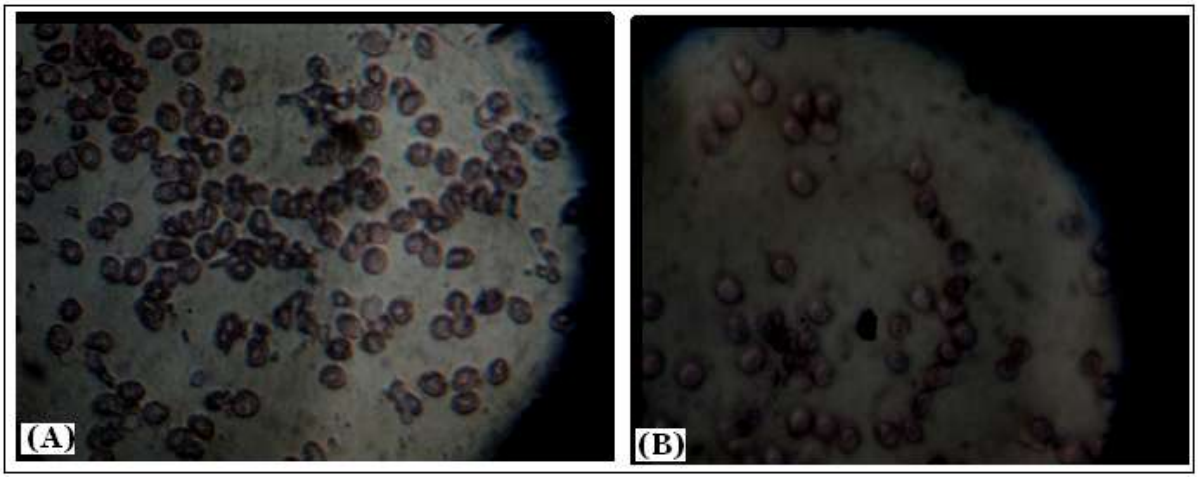

Plate 1. (A) Blood Smear of Control fish (B) Blood Smear of Experimental fish.

Protein concentration decreased from 4.284 mg in control animal to 3.97 mg in experimental animal. Zayaprgassarazan *et al.,* 1996 reported decrease in protein after exposure to lindane in *Anabas testudineus*. .Glycogen percentage also showed decrease in concentration from 110.25 mg to 105.14 mg indicating stress condition. Pattern of decrease from control to experimental in fishes indicates continuous transfer of glucose from tissues to the site of stress. Similar results in *T. mossambica* has been reported by Rao *et al.*, 1979; Kabir *et al.*, 1983 and Latha (2007) in *Anabas testudineus*. Adenosine triphosphate activity decreased from 1.48 units per milligram to 0.98 units. The brain acetyl cholinesterase activity showed a significant decrease in all the treated fishes compared to Control fish. Esterase enzyme catalyzes conversion of Acetycholine into acetic acid and choline. Decrease in esterase activity will reduce the flow of neurotransmitters towards the synapse, which in turn will lead to mis

co-ordination amongst neurons. Dose dependent decrease in glycogen has been reported by Tintos *et al.*, 2005 in rainbow trout (*Oncorhynchus mykiss*).Liver is the primary organ which receives polycyclic aromatic hydrocarbon and might be creating stress condition in fishes. Similar results have been reported involving increase in transamination activity in juvenile Sea Bass.

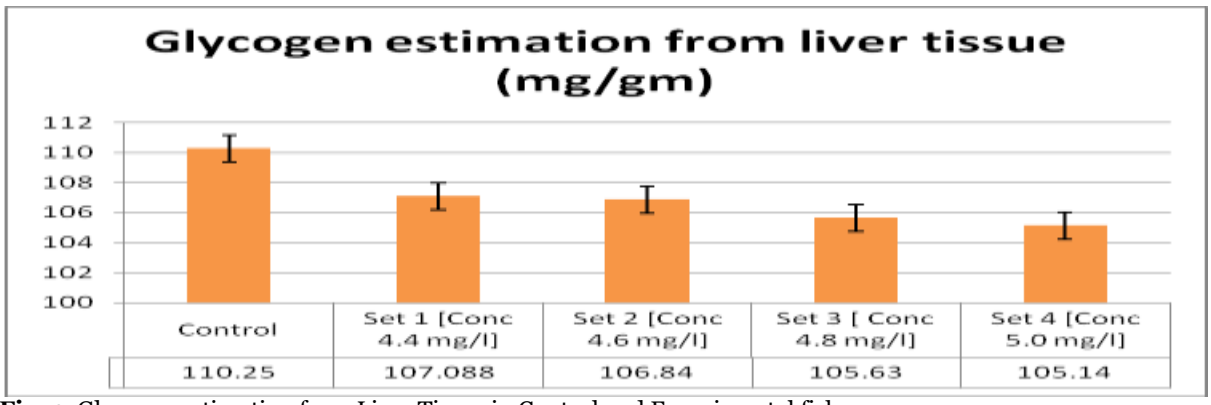

Fig. 1. Glycogen estimation from Liver Tissue in Control and Experimental fishes.

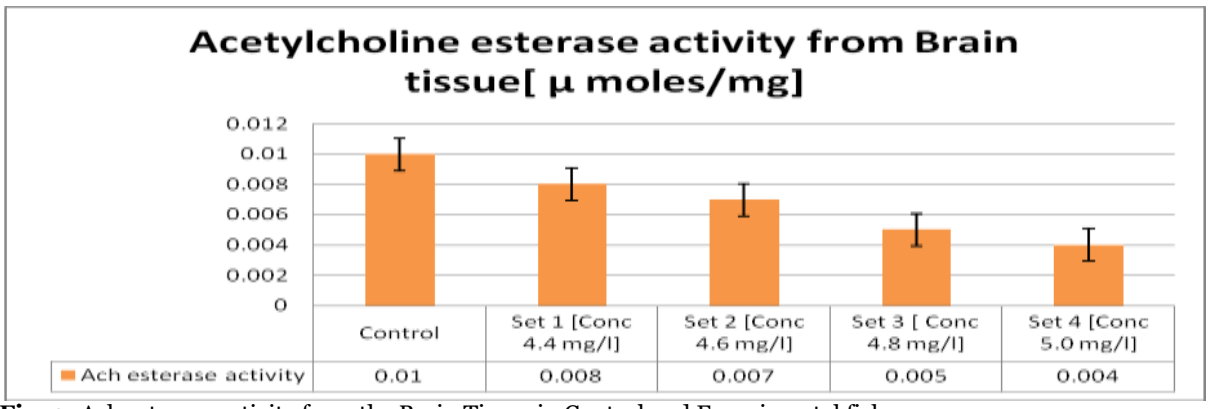

Fig. 2. Ach esterase activity from the Brain Tissue in Control and Experimental fishes.

Polycyclic aromatic hydrocarbons readily accumulate in aquatic food chain and are absorbed by fishes. PAH deplete liver glycogen concentration along with inducing other changes. Regular monitoring of aquatic organisms is required for better understanding of the toxicity of PAHs.

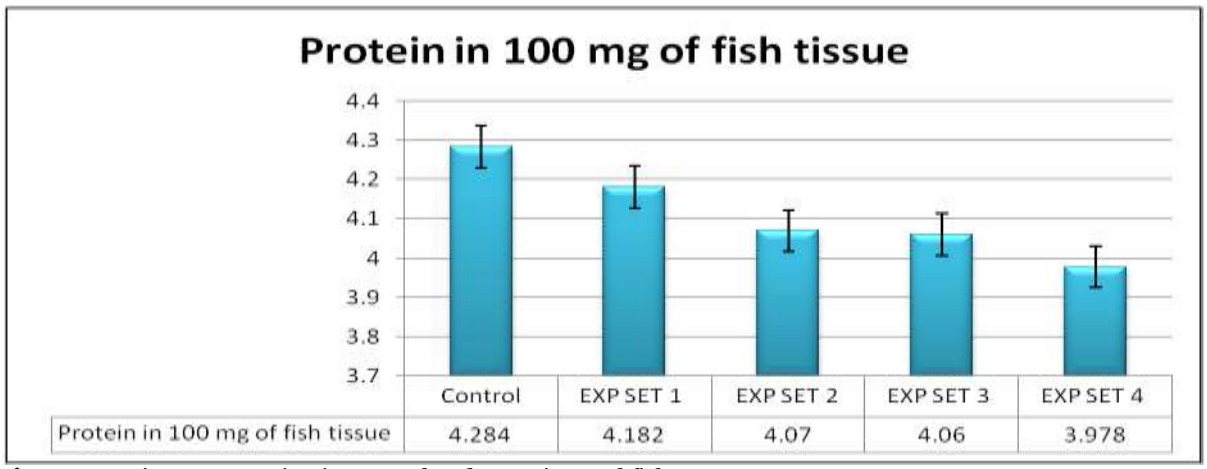

Fig. 3. Protein concentration in Control and Experimental fishes.

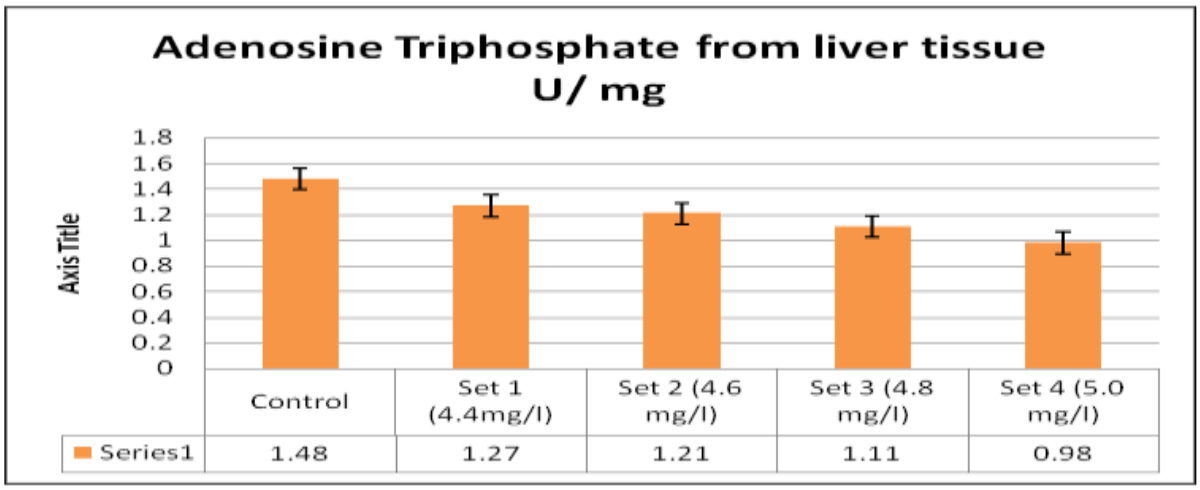

Fig. 4. Adenosine Triphosphate concentration in Control and Experimental fishes.

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