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IMPACT OF GLYPHOSATE ON INTERMEDIARY AND MITOCHONDRIAL METABOLISM ON A FRESHWATER FISH, *APLOCHEILUS LINEATUS* (VALENCIENNES)

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Abstract: Glyphosate is a systemic broad spectrum herbicide, with the potential of causing neurological problem, affecting reproductive system, causing DNA damage and cancer. This paper evaluates the impact of glyphosate on intermediary and mitochondrial metabolism in a freshwater fish, *Aplocheilus lineatus* Valenciennes. Exposure of fish to glyphosate significantly decreased the activities of NADH dehydrogenase, malate dehydrogenase, isocitrate dehydrogenase and cytochrome C oxidase and increased the activity of malic enzyme in mitochondrial metabolism. In intermediary metabolism, glyphosate decreased the activity of glucose 6 phosphatase, malate dehydrogenase and increased lactate dehydrogenase and malic enzyme activity significantly. Fragmentation of DNA was observed only after prolonged exposure of fish to glyphosate and electropherogram also revealed altered protein profile. These results are relevant in understanding the impact of glyphosate in alterations in the activity of intermediary and mitochondrial metabolic enzymes.

Keywords: Glyphosate, Cytochrome c oxidase, Glucose 6 Phosphatase, Lactate dehydrogenase, Malic enzyme, DNA fragmentation.

INTRODUCTION

Herbicides are used to kill or otherwise manage certain species of plants considered to be pests. The use of herbicides in agricultural practices has expanded sharply in the last two decades, both with regard to weed control as well as to crop desiccation (Francis *et al*., 1985; Newman, 1970).

Glyphosate, [N-(phosphonomethyl)glycine], is a broad spectrum herbicide primarily used in agricultural applications for the control of a great variety of annual, biennial and perennial grasses, sedges, broad leaved weeds and woody shrubs. (Ayoola, 2008). It is also used for the control of aquatic weed in fish ponds, lakes, canals and slow running water (Tsui and Chu, 2008). Glyphosate is very slightly to moderately toxic to the aquatic animals, but the commercial formulation, Roundup®, is considered more toxic due to the addition of the surfacant, Polyoxyethylene Amine (POEA) (Brausch and Smith, 2007). It reaches aquatic environment due to the proximities of the agricultural fields along the water bodies.

Fish have served as bio-indicators of environmental pollution and played significant roles in assessing potential risk associated with contamination in aquatic environment (Lakra and Nagpure, 2009). The use of fish as bio-indicators permit early detection of aquatic environmental problems (Van Der Oost *et al*., 2003). The fish species, *Aplocheilus lineatus* (Order Cyrinidontiformes; family Aplocheilidae) is hardy and a common fish in all the freshwater habitats in Kerala. This paper records the results on the impact of glyphosate on intermediary and mitochondrial metabolism. Intermediary metabolism, which describes all reactions concerned with the storage and generation of metabolic energy required for the biosynthesis of low-molecular weight compounds and energy storage compounds (Mathews and Van Holde, 1996). In the intermediary

metabolism pathway, the structure of each enzyme plays a crucial role in determining the specific properties of each reaction. The major enzymes in intermediary metabolism pathway investigated in the current research are glucose-6-phosphatase, cytosolic isocitrate dehydrogenase, cytosolic malate dehydrogenase and lactate dehydrogenase.

The most prominent roles of mitochondria are to produce the energy currency of the cell, ATP, through respiration, and to regulate cellular metabolism. The central sets of reactions involved in ATP production are collectively known as citric acid cycle or the Krebs cycle. The major enzymes in mitochondrial metabolism pathway investigated in the current research are isocitrate dehydrogenase, malate dehydrogenase, malic enzyme, NADH dehydrogenase and cytochrome c oxidase. Moreover, the effect of glyphosate on muscle protein profile and DNA fragmentation, if any, was also analysed.

MATERIALS AND METHODS

The fish, *Aplocheilus lineatus* used in the experiments was collected from local suppliers and the fishes were kept in large cement tanks containing dechlorinated tap water at an optimum temperature of 26 ± 2^{0}C natural photoperiod and fed with laboratory prepared fish feed daily. Two weeks prior to experiment, the fish were transferred to glass tanks. The laboratory acclimated fish were divided in to 4 groups of ten each in separate glass tanks. LC_{50} dose as per probit analysis was found to be 650µl/l. Hence a sublethal dose of 600µl/l glyphosate was selected for dosage exposure. The first group of fish served as control. Fish in groups II, III and IV were exposed to 600µl/l glyphosate of water for the period 5, 10 and 15 days respectively. Freshwater sample having the specified doses of glyphosate was replaced every three days. After the above said periods of exposure, muscles were taken from the body of the fish and kept frozen at -80^{0}C in an ultralow deep freezer for further analysis. Fresh samples of muscle tissue were used for DNA fragmentation assay and also for SDS-PAGE analysis.

Assay of intermediary metabolic enzymes

Chilled muscle was separately homogenized at 4^{0}C in 0.25ml SET buffer (pH 7.4) for the assay. Homogenates were centrifuged 2000rpm at 4^{0}C for 10min in a high-speed refrigerated centrifuge (Eppendorf, Germany). The supernatant collected was used as enzyme source. All these enzymes were assayed spectrophotometrically at room temperature (Perkin Elmer, USA).

Glucose 6 Phosphatase (D-Glucose-6-Phosphatase Phosphohydrolase): The activity was assayed by the method of Swanson (1955). The supernatant was collected and analyzed for inorganic phosphate by the method of Fiske and Subbarow (1925) and the absorbance was measured at 640nm in uv visible spectrophotometer. The specific activity of the enzyme was expressed as nano moles inorganic phosphate liberated/min/mg protein.

Lactate Dehydrogenase (LDH): Lactate dehydrogenase was assayed according to the method of King (1965). The specific activity of the enzyme was expressed as IU/min/mg protein.

Cytosolic Malic Enzyme (ME) $_l$-Malate: $NADP^+$ Oxidoreductase:The activity of ME was determined by the method of Ochoa (1955 a).

Cytosolic Isocitrate Dehydrogenase (ICDH) – (Isocitrate Dehydrogenase $NADP^+$): The enzyme was assayed by the method of Ochoa (1955 b).

Isolation of mitochondria and assay of enzymes: Isolation of mitochondria from muscle was carried out according to the method prescribed by Irving and Watson (1976). All the above enzymes were assayed spectrophotometrically (Perkin Elmer, USA) at room temperature.

Isocitrate Dehydrogenase (ICDH) – (Isocitrate Dehydrogenase NADP): The enzyme was assayed by the method of Ochoa (1955).

Malate Dehydrogenase (MDH) ($_l$-Malate: NAD^+ Oxidoreductase): The activity of MDH was determined by the method of Mehler et al. (1948).

Malate Dehydrogenase / Malic Enzyme (ME) ($_l$-Malate: $NADP^+$Oxidoreductase): The activity of ME was determined by the method of Ochoa (1955 a).

NADH Dehydrogenase: The activity of NADH dehydrogenase was assayed according to the method of Minakami *et al.* (1962).

Cytochrome C Oxidase: Cytochrome c oxidase activity was assayed by the method of Pearl et al, (1963).

Protein content for all enzymes was estimated using

the same tissue extract according to the protocol of Bradford, (1976).

SDS-PAGE

Electrophoretic analysis was performed according to the method of Laemmli (1970). The protein bands in gel were visualized and analyzed by Gel documentation (Labomed platform) using Gel doc apparatus (Gelstan, Medicare, Germany) and photographed.

Muscle DNA isolation and electrophoresis was carried out according to the method of Iwasa *et al* (1996) with some modifications. DNA fragmentation was measured by DNA spectrophotometric method (Wolozin *et al.*, 1996). Intact DNA was separated from fragmented DNA by centrifugal sedimentation followed by precipitation and quantification using DPA. To minimize formation of oxidative artifacts during isolation, 2, 2, 6, 6 – tetramethyl pipridinoxyl was added to all solutions and all procedures were performed on ice. The protocol includes the lysis of cells and the release of nuclear DNA, a centrifugation step with the generation of two fractions (intact and fragmented DNA, precipitation of DNA, hydrolysis and colorimetrical quantification upon staining with DPA, which binds to deoxyribose.

The percentage of fragmented DNA was calculated by,

% fragmented DNA = (S+T/ S+T+B) x 100 or (T/ T+B) x 100

Statistics

Data analysis was done by ANOVA. The differences in means were tested by using Duncan (1955) analysis. Significant level used was $P < 0.05$. All the statistical analyses were performed using the software SPSS 22.0 for Windows.

RESULTS AND DISCUSSION

The exposure of *A. lineatus* to 600µl/L affected the mitochondrial and intermediary metabolism. Glyphosate significantly reduced the activity of glucose 6 phosphatase, cytosolic isocitrate dehydrogenase and elevated cytosolic malic enzyme activity (Fig. 1). It also significantly increased the activity of lactate dehydrogenase (Fig. 2). In mitochondrial metabolism on exposure to glyphosate, the activity of cytochrome c oxidase and ICDH decreased and Malic enzyme activity significantly elevated (Fig .3). Also, MDH (Fig. 4) and NADH dehydrogenase (Fig. 5) found to be significantly decreased. Sublethal concentration of glyphosate caused decrease in protein content (Fig. 6) in the exposed fish as compared to that of control. DNA fragmentation (Fig. 7) was observed only after prolonged exposure of fish to glyphosate but this change was not detected in agarose gel electropherogram. The electropherogram of muscle tissue also revealed altered protein profile (Fig. 8; Table:1) *ie*., appearance of new band, increase and decrease in band volume.

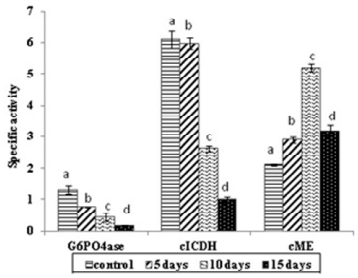


Fig. 1. Effect of glyphosate on G6PO4ase (Glucose 6 phosphatase) cICDH (Cytosolic Isocitrate dehydrogenase) and cME(Cytosolic malicenzyme)*

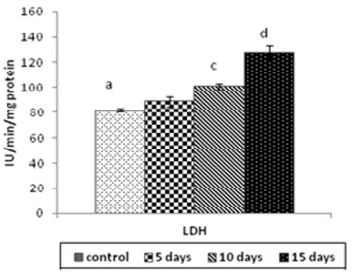


Fig. 2. Effect of glyphosate on lactate dehydrogenase (LDH)*

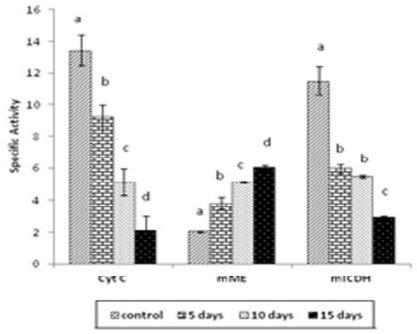


Fig. 3. Effect of glyphosate on Cyt C (Cytochrome C Oxidase) mME (Mitochondrial Malicenzyme) mICDH (Mitochondrial Isocitrate dehydrogenase) *****

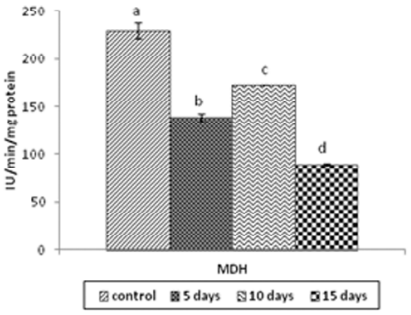


Fig. 4. Effect of glyphosate on malate dehydrogenase (MDH)*

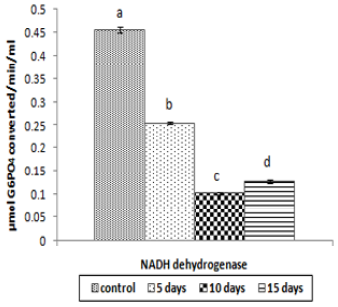


Fig 5. Effect of glyphosate on NADH Dehydrogenase*

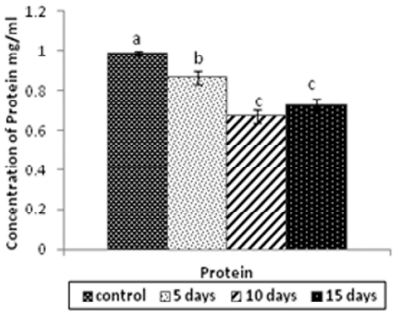
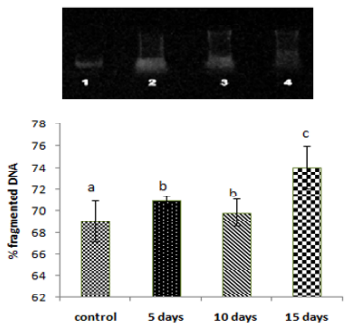


Fig 6. Effect of glyphosate on protein content in muscle tissue*



*Mean values of different superscript letters (a,b and c,d) were significantly different ($p<0.05$).

Fig 7. DNA fragmentation assay by Agarose Gel Electrophoresis method and effect of Glyphosate on DNA fragmentation in the muscle of *A. lineatus* by DPA method*

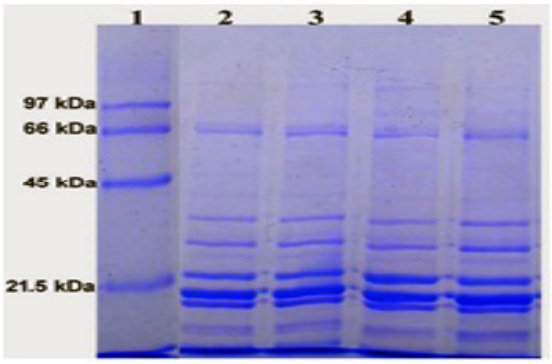


Fig. 8: Electropherogram of muscle tissue of *A. lineatus* exposed to glyphosate

Table 1: Gel documentation of electrophoresis of muscle protein of *A. lineatus* exposed to glyphosate

Band Name	Lane 1Marker)		Lane 2Control)		Lane 3 (5 days)		Lane 4 (1days)		Lane 5 (15 days)	
	MW (KDa)	BV	MW (KDa)	BV	MW (KDa)	BV	MW (KDa)	BV	MW (KDa)	BV
Band 1	97	1,19,115	70.37	1,25,721	71.57	1,35,171	94.64	61,285	73.29	1,42,900
Band 2	66	1,46,889	41.63	1,29,083	42.77	2,03,926	73.41	1,38,955	43.14	3,29,975
Band 3	45	1,50,525	35.87	2,89,563	37.04	3,70,577	42.77	2,58,782	37.1	4,46,253
Band 4	31	1,24,660	33.4	1,04,626	34.26	1,17,068	36.73	3,37,545	34.55	2,20,846
Band 5	21	1,11,975	29.52	5,02,173	30.59	5,83,248	34.26	80,120	30.38	7,38,109
Band 6			26.26	8,57,301	27.49	9,12,001	30.12	6,73,345	27.21	10,60,483
Band 7			24.92	4,91,593	26.04	6,74,010	27.26	9,78,074	25.91	4,62,135
Band 8			21.8	3,04,292	22.44	3,44,355	25.78	5,47,789	22.29	5,20,379
Band 9							22.29	3,76,053		

Red colour indicates increase in band volume. Green colour shows decrease in band volume. Blue colour indicate newly formed band.

When experimental fish is exposed to glyphosate, the activity of glucose 6 phosphatase decreased significantly. Glucose 6 phosphatase is responsible for contributing free glucose to the blood from liver glycogen pool and from other precursors of glucose-6-phosphate and catalyse a critical step in gluconeogenesis and plays an important role in regulation of glucose homeostasis (Nordlie *et al.*, 1993). Glucose 6 phosphatase is related to homeostatic regulation of blood glucose by glycogenolysis and glucogenolysis (Herman and Nordlie, 1972).

In the present study, the activity of lactate dehydrogenase increased significantly. Lactate dehydrogenase plays an important role in carbohydrate metabolism and catalyzes the inter conversion of lactate and pyruvate. The increase in the activity of LDH might be due to tissue damage (Kristoffersen *et al.*, 2008). The increase in LDH activity also suggested a significant increase in the conversion of pyruvate to lactic acid, thereby leading to the deficiency of pyruvate and increase in lactic acid.

Exposure to cypermethrin produced a significant increase in the activities of lactate dehydrogenase in Korean rock fish, *Sebastes schlegeli* (Jee *et al.*, 2005), *Rhamdia quelen* (Borges *et al.*, 2007) and *Labeo rohita* (Das and Mukherjee, 2003). Carbofuran exposure increased LDH activity in the muscle, brain and liver of catfish (Singh and Sharma, 1998). The lactate dehydrogenase activity increased significantly in red muscle of Nile Tilapia following cadmium exposure (Almeida *et al.*, 2001). All these studies are in accordance with our results. Hence LDH can be recognized as a potential marker for assessing the toxicity of a chemical.

The most common mitochondrial shuttle present in cells is the malate aspartate shuttle (MA). This shuttle involves four-enzymes, mitochondrial aspartate amino transferase ($mA_{sp}AG$), cytoplasmic aspartate aminotransferase, mitochondrial malate dehydrogenase (mMDH) and cytoplasmic malate dehydrogenase (cMDH). The MA shuttle transfers NADH across the inner mitochondria for subsequent oxidation and also regenerates the NAD^+ in the cytoplasm required for further cytoplasmic conversion of lactate to pyruvate or for Embden-Meyrhof pathway activity. In the absence of these shuttles, the NAD^+ pool within the cytoplasm quickly become exhausted and prevent the cell from using either lactate or glucose as an energy source (Benderdour *et al.*, 2004).

From this viewpoint, the effect of glyphosate on cytosolic and mitochondrial malic enzyme was investigated and the activities of both enzymes were found to increase significantly. In contrast to mammalian skeletal muscle mitochondria, the only substrate that crustacean and fish mitochondria

oxidize at a high rate is malate. The mitochondria isolated from muscle of fish exhibit a high activity of malic enzyme. Assuming that malic enzyme is responsible for the conversion of malate to pyruvate in animal muscle, it could be expected that the mitochondria which possess high activity of this enzyme should oxidize malate very rapidly when oxygen is available which would lead to rapid depletion of malate in cells. It had been recently reported that fish from lakes contaminated with heavy metals had higher activities of malic enzymes and glucose 6 phosphate dehydrogenase, the two enzymes in lipid metabolism. (Van Der Kraak *et al*., 2001). This study is in accordance with the present result.

In the present study, glyphosate exposure significantly reduced ICDH activity in muscle of *A. lineatus*. Isocitrate dehydrogenase catalyzes the reversible oxidation of isocitrate to oxalosuccinic acid, followed by decarboxylation, leading to the formation of á-ketoglutarate. The reduced activity of ICDH may influence energy and redox status. Maternal exposure to glyphosate caused functional abnormalities in the specific activity of these enzymes found inside cells – isocitrate dehydrogenase, malate dehydrogenase and glucose 6 – phosphate dehydrogenase in liver, heart and brain of pregnant rats and their foetuses (Daruich *et al*., 2001). All enzymes are involved in the generation of NADPH which has many essential roles in metabolism.

In the present investigation the oxidative enzyme, malate dehydrogenase showed a reduction in its activity, which indicates the suppression of oxidative metabolism in the fish exposed to glyphosate. As malate dehydrogenase is the oxidative enzyme involved in Krebs cycle, any disturbance in this enzyme activity will affect the Krebs cycle. Since this cycle represents a central oxidative pathway for carbohydrates, fats and amino acids, if there is any disturbance in this cycle, the whole metabolism is likely to be affected. In support of present investigation, several reports are available on decrease in the activity of MDH after exposure to different pesticides. Similar results were observed in *Tilapia mossambicus* exposed to malathion (Kabeer Ahmed *et al*., 1983) *Clarias batrachus* exposed to endosulfan (Srinivas ,1993) and in mice exposed to benzene hexachloride (Thakore *et al*., 1981).

It is known that the oxidative enzymes like MDH act as indicators of aerobic respiration, hence the inhibition of MDH indicates the prevalence of anaerobic conditions imposed by the stress factor of glyphosate toxicity. As MDH is the key enzymes in TCA cycle, with the inhibition of malate dehydrogenase, the metabolic pathway might have turned to anaerobic to meet the increased energy demands during the glyphosate exerted toxic stress. The decrease in MDH activity also indicates the impairment of oxidative metabolism in the mitochondria as a consequence of hypoxic conditions under pesticide exposure, most probably by disrupting the oxygen binding capacity of the respiratory pigment. The decrease may be also due to the disorganization of mitochondria, affecting enzymes of TCA cycle and decreased state of respiration (Shomessubra Bag *et al*., 1999).

The activity of NADH dehydrogenase decreased significantly in the muscle of *A. lineatus* after exposure to glyphosate. NADH dehydrogenase or Complex I is an electron carrier in the electron transport system in the inner mitochondrial membrane. It is an iron-sulfur-containing flavoprotein reversibly oxidizing NADH to NAD^+. Pesticides such as pyridaben, rotenone and fenpyroximate are found to decrease NADH dehydrogenase activity (Sherer *et al.*, 2007). Other chemicals such as thiazoles (Andreani *et al.*, 1995) pyrethroids and permethrin (Gassner et al., 1997) flunarizine and cinnarizine (Veitch and Hue, 1994) are inhibitors of mitochondrial NADH dehydrogenase. All these findings are in accordance with the present study.

In the present study, cytochrome c oxidase activity decreased significantly in the fishes after exposure to glyphosate. Cytochrome c oxidase is a multi subunit protein complex (complex IV), located exclusively in the cristae of the mitochondrial inner membrane. It catalyses the terminal oxidation reaction in the respiratory chain phosphorylation system responsible for most of the ATP synthesis during aerobic tissue metabolism (Tyler, 1992).

The significant inhibition of cytochrome c oxidase by glyphosate in the present study, suggested that

the oxidative metabolism of exposed fish might have been altered. Hence glyphosate can be considered as uncouplers of oxidative phosphorylation which result in reduced synthesis of ATP. The decrease in the cytochrome c oxidase activity synchronizes with the decrease in the activity of other mitochondrial enzymes such as ICDH, MDH and NADH dehydrogenase.

The activities of the respiratory enzymes succinate dehydrogenase, MDH and cytochrome c oxidase also decreased considerably under methyl parathion exposure (Ramakrishna *et al*., 2005). Accordingly in the present study, the activity of MDH and cytochrome c oxidase decreased significantly. These results suggest that glyphospate has a profound effect on the oxidative metabolism of the fish which results in low ATP turnover, possibly due to its influence on the respiratory center of the brain.

In the present study, glyphosate acts as uncouplers as its exposure significantly inhibited the activity of NADH dehydrogenase and cytochrome c oxidase. Hence, glyphosate acts as electron transport inhibitors.

Fish exposed to glyphosate showed decreased protein content compared to control. The reduction in protein content indicates that under stress conditions, the tissue proteins may undergo proteolysis, which may have resulted in the production of free aminoacids, which can be used in the tricarboxylic acid cycle for energy production. The tissue proteins are metabolized to produce glucose by the process of gluconeogenesis and it is utilized for energy production under stress conditions (Elumalai and Balasubramanian, 1999). Yadav *et al*., (2007) had reported that the animals exposed to chemicals obtain extra energy requirement from tissue protein. This might be the reason for the lesser protein content found in the present study.

In this study, when % fragmented DNA was quantified by DPA method, significant increase in % fragmented DNA was observed in the muscle of *A. lineatus* exposed to glyphosate only after 15 days of exposure. But on agarose gel electropherogram, no significant change was detected at any concentrations, in which all the bands appeared as single high molecular weight intact DNA. It has been demonstrated that pollutant exposure does lead to corresponding increase in DNA damage (Steinert, 1999).

Any changes to DNA may have long lasting effects but the self repairing capability of DNA may affect the precise interpretation of relevant bioassays (Connell *et al.*, 1999). According to Black *et al*., (1996), the presence of high molecular weight DNA may not necessarily be a result of the exposure to low impact areas but rather, due to the induction of DNA repair mechanisms after the exposure to a highly impacted area. It is assumed that at lower concentration of glyphosate, the induction of DNA repair mechanisms might have resulted in high molecular weight intact DNA, hence no change was observed on the agarose gel.

The electropherogram of muscle tissue revealed the presence of certain extra bands and also increase and decrease in band volume. These bands are assumed to be of stress proteins that are induced as a result of exposure to glyphosate. The changes in protein subunit band patterns may be due to change in the turn over (Synthesis/degradation) of various proteins. Such altered protein band profile was observed in both sexes of *Anabas testudineus* exposed to different doses of Bisphenol A (Aruna Devi, 2013). The effect of NaCl stress was investigated in terms of SDS-PAGE protein banding pattern and new bands appeared in response to salt stress (Vinay *et al*., 2009).Such disturbances in protein profile was also observed in Asian sea bass exposed to copper (Paruruckumani *et al*., 2015).

Changes in protein profile are regarded as important biomarkers of the metabolic potential of cells, as these plays the main role in regulating the activities of cells. Their ratios also provide significant information about the way in which, mechanism, these contents regulate the multifaceted activities of cells. Thus, the electrophoretic techniques are promising tools for identifying protein profile in response to stress and sublethal level of xenobiotics. Thus it is evident from the current study that glyphosate disrupted intermediate and mitochondrial metabolism in *A. lineatus*

CONCLUSIONS

Energy metabolism plays a key role when the animal is forced to expend more energy to mitigate toxic

stress. Toxicants are known to affect metabolism in fish. The results of present work showed the effect of glyphosate on mitochondrial metabolism and intermediary metabolism This study also showed that fish are excellent subjects for the study of various effects of contaminants present in water samples since they can metabolize, concentrate and waterborne pollutants. More testing is needed to fully understand the effects of glyphosate exposure to aquatic and terrestrial organisms and to determine the overall safety and use of glyphosate based herbicides with their respective surfactants.

REFERENCES

Almeida, J.A., Novelli, E.L.B., Dal Pai Silva, M and Alves Junior, R .2001. Environmental cadmium exposure and metabolic responses of Nile Tilapia, *Orechromis niloticus. Environ Pollut.*, 114 :169-175.

Andreani, A., Sambaldi, M., Leoni, A., Locatelli, A., Ghelli, A., Ratta, M., Benelli, B and Espost, M.D. 1995. Thienylimidazo [2,1-b] thiazoles as inhibitors of mitochondrial NADH dehydrogenase. *J Med Chem*., 38(7): 1090-1097.

Aruna Devi, C. 2013. Role of Bisphenol A as an endocrine disruptor in a fresh water fish, *Anabas testudineus*. PhD Thesis, University of Kerala.

Ayoola, S.O.2008. Toxicity of glyphosate herbicide on Nile tilapia *(Oreochromis niloticus*) juvenile. *Afr J Agric Res*., 3: 825-834.

Benderdour, M., Chauon, G., Comk, B., Ayoub, R., Becaundry, D., Foisy, S., de Blois, D and Des Rosier, C. 2004. Decreased cardiac mitochondrial $NADP^+$ isocitricdehydrogenase activity: a marker of oxidative stress in hypertrophy development. *Am J Physiol Heart Circ Physiol*., 287: 2122-2133.

Black, M.C., Ferrell, J.R., Horning, R.C and Artin, L.K. 1996. DNA strand breakage in freshwater mussels (*Anodonta drandis*) exposed to lead in the laboratory and field. *Environ Toxicol Chem*., 15(5): 802-808.

Borges, A., Scotti, L.V., Siqueira, D.R., Zanini, R., Amaral, F.D., Jurinitz, D.F and Wassermann, G.F.2007. Changes in hematological and serum biochemical values in jundiá, *Rhamdia quelen* due to sub-lethal toxicity of cypermethrin. *Chemosphere*, 69(6):920-6.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram

quantities of protein utilizing the principle of protein-dye binding. Anal Biochem.,72: 248-254.

Brausch, J.M and Smith, P.N.2007. Toxicity of three polyethoxylated tallowamine surfactant formulations to laboratory and field collected fairy shrimp *Thamnocephalus platyurus. Arch. Environ. Contam. Toxicol.,* 52(2): 217-221.

Connell, D., Lam, P., Richardson, B and Wu, R. 1999. *Introduction to Ecotoxicology*. Oxford Blackwell, NY.

Daruich, J., Zirulni, F and Gimenez, M.S .2001. Effect of the herbicide glyophosate on enzymatic activity in pregnant rats and their fetuses. *Envn. Res.*, 85: 226-231.

Das, B.K and Mukherjee, S.C. 2003. Toxicity of cypermethrin in *Labeo rohita* fingerlings: Biochemical enzymatic and hematological consequences. *Comp Biochem Physiol Toxicol Pharmacol* ., 137: 325-333.

Duncan, D.B. 1955. Multiple Range and Multiple F Tests. Biometrics, 11: 1-42.

Elumalai, M and Balasubramanian, M.P. 1999. Infuence of naphthalene on coterase activity during viellogenesis of marine edible crab, *Scylla serata*. *Bull. Envn. Contam. Toxicol*., 62: 743-748.

Francis, B.M., Lampman, R.L and Metcalf, R.L. 1985. Model ecosystem studies of environmental fate of five herbicides used in conservation tillage. *Arch. Envn. Contam. Toxicol.,* 4: 693-704.

Fiske, C.H and Subbarow, Y .1925. The colorimetric determination of phosphorus. Biol. Chem., 66: 375-400.

Gassner, B., Wuthrich, A., Scholtysik, G and Solioz, M .1997. The pyrethroids, permethrin and cyhalothrin are potent inhibitors of the mitochondrial complex I. *J. Pharmacol. Exp. Therap.*, 281 (2): 855-860.

Herman, H.L. and Nordlie, R.C. 1972. Hepatic carboryls phosphatise: Glucose phospho transferase distribution, comparative kinetics and responses to alloxan or mono heptulose- induced diabetes. *Arch. Biochem. Biophys*., 152:180-186.

Irving, D.O and Watson. K .1976. Mitochondrial enzymes of tropical fish. Comp. Biochem. Physiol., 54B: 81.

Iwasa, M., Maeno, Y., Inoue, H., Koyama, H and Matoba, R. 1996. Induction of apoptotic cell death in rat thymus and spleen after a bolus injection of methamphetamine. Int. J. Leg. Med., 10923- 28.

Jee, J.H., Masroor, F. and Kang, J.C. 2005. Responses of Cypermethrin-induced stress in haematological parameters of Korean rockfish, *Sebastes schlegeli* (Hilgendorf). *Aqua. Res.*, 36: 898–905.

Kabeer Ahamed. I., Sambasiva Rao, K.R.S and Ramanab Rao, K.V.B. 1983. Dehydrogenase system of the Teleost, *Tilapia mossambica* under augmented sublethal malathion. *Stern. J. Ani. Phsiol*., 30: 101-106.

King, J .1965. The dehydrogenases or oxidoreductases lactate dehydrogenase. In Practical clinic Enzymol London Van Nostrand D Company Ltd., 83-93.

Kristoffersen, P., Rask, A.M., Grundy, A.C., Frozen,

J., Kempenaar, C., Raisio, J., Schroeder, H., Spijker, J., Verschwele, A and Zarina, L. 2008. A review of pesticide policies and regulations for urbanamenity areas in seven European countries weed research. *Advances in Agronomy,* 118: 48(3).

Lakra, W.S and Nagpure, N.S . 2009. Genotoxicological studies in fish: a review. *Indian J. Anim. Sci.,* 79: 93-98.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of
bacteriophage T_4 .*Nature,* 227(259): 680-685.

Mathews, C.K and Van Holde, K.E. 1996. *Biochemistry.* Menlo Park, Ca: The Benjamin/Cummings publishing company.

Mehler, A.H., Kornberg, A., Grisolia, S and Ochao. 1948. Chemistry and methods of enzyme: Desmolases. J. Biochem., 174: 961-977.

Minakami, S., Ringleo, R.C and Singer, T.P. 1962. Respiratory chain linked dihydrodiphosphopyridine nucleotide hydrogenase assay of enzyme in soluble particulate and insoluble preparation. J. Biol., 237: 569-579.

Newman, J.F. 1970. Ecological implications of the use of paraquat and diquat as aquatic herbicides. *Chem. Ind. Lond.,* 45: 1424-1425.

Nordlie, R.C., Bode, A.M and Foster, J.D. 1993. Recent advances in hepatic glucose-6-phosphatase regulation and function. *Proc. Soc. Exp. Bio. Med.,* 203: 274-285.

Ochoa, S. 1955a. Malic Enzyme Methods. Enzymol., 66: 323-325.

Ochoa, S. 1955b. Isocitrate dehydrogenase methods. Enzymol., 66: 232-236.

Paruruckumani, P.S., Maharajan, A., Seema, P., Fazildheen, M., Ganapiriya, V and Kumarasamy, P .2015. Characterisation of protein profile in the Asian sea bass, *Lates calcarifer* (Bloch). *J Fish Aquat.,* 2(6): 409-415.

Pearl, W., Casarcao, F. and Zwedach, B.W. 1963. Micro determination of cytochrome oxidase in rat tissue by oxidation of N phenyl –p phenylene diamine. *J. Histochem. Cytochem.,* 102-104.

Ramakrishna, C.M., Kumaraguru, A.K and Balasubramanian, M.P. 2005. Impact of distillery effects on Carbohydrate metabolism of fresh water fish, *Cyprinus carpio*. *Ecotoxicol.,* 14: 693-707.

Singh, R.K and Sharma, B. 1998. Carbofuran induced biochemical changes in *clarias batrachus. Pest Sci.,* 53: 285-290.

Sherer, T.B., Richardson, J.R., Testa, C.M., Seo, B.B., Panov, A.V., Yagi, T., Matsuno-Yagi, A., Miller, G.W and Greenamyre, J.T. 2007. Mechanism of toxicity of pesticides acting at complex I: Relevance to environmental etiologies of Parkinson's disease. *J. Neurochem.,* 100(6): 1469-79.

Shomessubra Bag., Tasmim Vora., Runa Ghetak., Irani Nilufer., Dennes D'Mello Leon., Pouira James Pereira Christine Cutinho and Vaman Rao. 1999. A study of toxic effects of heavy metal contaminants from sludge-supplemented diets on male wistar rats. *Ecotoxicol Environ Safe.,* 42 :16-170 .

Srinivas, R. 1993. Impact of endosulfan on carbohydrate metabolism in the fresh water fish *Clarias batrachus* (Linn). PhD Thesis. Osmania University Hyderabad India.

Steinert, S.A. 1999. DNA damage as a bivalve biomarker. *Biomarkers,* 4(6) 492-496.

Swanson, M.A. 1955. Glucose-6-phosphatase from liver. Meth. Enzymol., 2: 541-543.

Thakore, N., Kashyap, B., Ani,l K., Suresh Lakkad., Bhadabhaic Bhatt, K., Devendra Aravinda Babu., Shrikanth Kashyap Chattterjee, K and Surath. 1981 .Sequential changes in ICDH and MDH in mice exposed to technical grade BHC and their possible relationship to liver tumors. *Pest Biochem. Physiol.,* 15: 262-266.

Tsui, M.T and Chu, L.M. 2008. Environmental fate and non-target impact of glyphosate-based herbicide (Roundup) in a subtropical wetland. *Chemosphere,* 71: 439-446.

Tyler, D.D. 1992. The mitochondrion in Health and disease. VCH Pub., New York, pp. 318-323.

Van Der kraak, G.J., Hontela, H and Montreal, Q.C.2001. Canadian network of toxicology centers (NTC). *Executive Summary Annual Research Symposium.,* 20-21.

Van Der Oost, R., Beyer, J and Vermeulen, N.P.E. 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: A review. *Envn. Toxicol. Pharmacol.,* 13: 57-149.

Veitch, K. and Hue, L. 1994. Flunarizine and cinnarizine inhibit mitochondrial complexes I and II: Possible implication for parkinsonism. *Mol. Pharmacol.,* 45 (1): 158-163.

Vinay, K., Varsha, S., Nikam, T.D., Narendra, J and Mahadeo, G.S. 2009. Anti oxidant enzyme activation and protein profiling under salt stress in indicalice genotypes differing in salt tolerance. *Arch Agro Soil Sci.,* 55(4): 379-394.

Wolozin, B., Iwasaki, K., Vito, P., Ganjei, J.K., Lacana, E and Sunderland, T. 1996. Participation of presenilin 2 in apoptosis enhanced basal activity confused by an Alzhieimer mutation. Science, 89: 433-438.

Yadav, A., Gopesh, A., Pandey, R.S., Rai, D.K and Sharma, B. 2007. Fertilizer industry effluent induced biochemical changes in fish water teleost *Channa straitus* (Bloch). *Bull. Environ. Contam. Toxicol.,* 79: 588-595.