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Protein profiling and molecular expression of Indian climbing perch on exposure to aquatic toxicants of Buckingham canal, Chennai, Tamil Nadu, India

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Abstract
Fish provide excellent material for the study of mutagenic or carcinogenic potential of water samples, since they can metabolize, concentrate and store water-borne pollutants. Biomarkers are able to detect oxidative stress and DNA fragmentation which constitute early warning indicators assessable prior to severe alteration in single organism and the community as a whole. Comet assay has critically important applications in the field of toxicology ranging from aging and clinical investigations to genetic toxicology and molecular epidemiology. In the present study, the Indian climbing perch, *Anabas testudineus* from Buckingham canal, Chennai, Tami Nadu, India was selected for the investigation. In the six tissues (brain, gill, intestine, kidney, liver and muscle) of *Anabas testudineus*, protein bands ranging from 17 to 250kDa were observed and variable numbers of proteins were visible in each tissue. DNA damage demonstrated that there was a significant difference ($p<0.05$) between the experimental fish (9.53 ±2.30) from Buckingham canal and the control fish (0.5 ±0.76), wherein there was approximately double the number of cells with DNA damage, indicating presence of genotoxic agents in the environment. The present study also revealed that, fish can be a potential bioindicator for environmental monitoring.

Keywords: Anabas testudineus, protein profile, DNA

1. Introduction
The term protein profiling refers to extraction, separation and characterization of different proteins expressed in an individual. Proteins can be separated from the tissues or body parts of an individual using several types of lysis buffers available. Selection of the buffer depends upon the type of organism and nature of the source or body part from where the proteins are to be separated. Electrophoresis of proteins is a useful tool for protein profiling and molecular weight determination. Protein profiles studies have been done on tissues of *Tilapia mossambicus* [1-3]. Such analysis of protein profiles of important tissues of the fishes may serve as markers to study the Piscean taxonomy and can also serve as baseline information for stress protein analysis as well as other pollutant related studies [4]. Fish provide excellent material for the study of mutagenic or carcinogenic potential of water samples, since they can metabolize, concentrate and store water-borne pollutants. Biomarkers are able to detect oxidative stress and DNA fragmentation which constitute early warning indicators assessable before more severe alteration in single organism and the community as a whole [5].
The comet assay is a rapid and very sensitive fluorescent microscopic method to assess DNA damage and repair in individual cells. This assay has critically important applications in the field of toxicology ranging from aging and clinical investigations to genetic toxicology and molecular epidemiology. Since the introduction of the comet assay in 1988, a number of advancements have greatly increased the flexibility and utility of this technique for detecting various forms of DNA damage and repair in virtually any eukaryotic cell [6]. Therefore, in the present study, *Anabas testudineus* from the Buckingham canal was selected for the investigation since it serves as an important biological indicator of water quality, which is affected by the global environmental degradation.

Also, because fish serve as an experimental model and hypotheses deduced from investigations on them, can be extrapolated to human system to a certain extent. Further, fishes form a major link in the food web of the aquatic ecosystem and their ability to produce a large number of offspring and their survival at high population densities, make them suitable for experimentation.

2. Materials and Methods

2.1. Study Area

Chennai the capital of Tamil Nadu is situated on the eastern coast of India, on 13 0 4' north latitude and 8 0 15' east longitude. There are three water ways that flows through the city, *viz*., Cooum River, Adyar River and Buckingham canal. The Buckingham canal is a man-made water canal linking the above mentioned two rivers. The canal extends from Nellore in Andhra Pradesh to Marakkanam near Puducherry. The length of this canal in Andhra Pradesh is 257km, and 163km is in Tamil Nadu. Approximately, 31km is within the city limits of Chennai (Figure 1). Within the city of Chennai, the canal is badly polluted from sewage and industrial effluents, and the silting up of the canal has left the water stagnant, creating an attractive habitat for mosquitoes and the water quality is considered to be highly toxic and completely non-potable. These three waterways are severely polluted in Chennai city, particularly the Buckingham canal by sewage, industrial wastes, storm water drainage and garbage, as a result of haphazard urbanization.

2.2. Test organism

Taxonomic position:

Kingdom	:	Animalia
Phylum	:	Chordata
Sub Phylum	:	Vertebrata
Class	:	Pisces
Order	:	Anabantiformes
Family	:	Anabantidae
Genus	:	*Anabas*
Species	:	*testudineus*

Anabas testudineus Bloch 1792, commonly called climbing perch is an extremely hardy, small, brown or dark greenish-brown fish, native to Southeast Asia. It is highly adapted to life in a seasonal tropical environment. It can tolerate very turbid and brackish water conditions; possesses an accessory air-breathing organ that enables it to survive out of water for several days; and uses its highly mobile sub operculum and strong fin spines to pull itself over land to move between bodies of water. The fish has the ability to aestivate during the dry season. Under extreme circumstances it's even able to aestivate for several weeks by burying itself into moist ground [7]. It inhabits the majority of drainage systems across its native range and has been recorded in many different habitat-types including swamps, marshes, lakes, canals, pools, small pits, rice paddies, puddles, tributaries and main river channels. Though primarily a lowland freshwater species, it also occurs in brackish coastal environments in some areas.

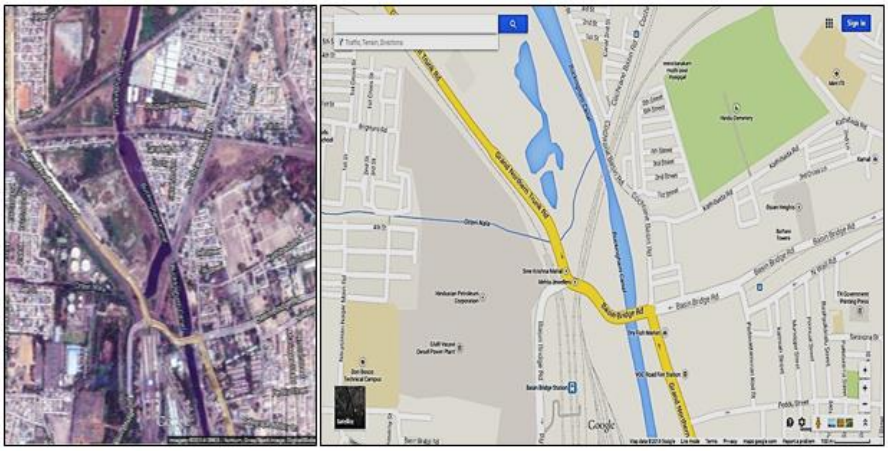

Fig 1: Study area – Buckingham canal

2.3. Molecular and protein profiling assay

The organs of the control and experimental fish, *Anabas testudineus*, *viz*., brain, gill, intestine, kidney, liver and muscle were removed and washed with 1x Phosphate Buffered Saline (PBS). After removal of 1x PBS solution, the cells were gently lysed, then transferred to a fresh eppendorf tube (1.5mL) and centrifuged at 3000rpm for 10 minutes at 4 ^{o}C. The pellet was collected and 500µL of 1x Radio Immuno Precipitation Assay (RIPA) buffer was added using a syringe, mixed gently and incubated at 4^{o}C for 10 minutes and followed again by centrifugation at 3000rpm for 10 minutes at 4^{o}C. The supernatant was then carefully transferred to a new eppendorf (2.0mL) with the required protein. The amount of protein to be quantified was done using a nano drop instrument and was estimated in µg/µL. For running, SDS PAGE the method of Laemmli [8] was adopted. Image J software was used to measure the protein profile (kDa).

2.4 Comet Assay

The comet assay also called 'Single Cell Gel Electrophoresis' is a rapid and very sensitive fluorescent microscopic method to assess DNA damage and repair in individual cells. This assay has critically important applications in fields of toxicology ranging from aging and clinical investigations to genetic toxicology and molecular epidemiology. The principle of the assay is based upon the ability of denatured, cleaved DNA fragments to migrate out of the cell under the influence of an electric field, whereas undamaged DNA migrates slower and remains within the confines of the nucleoid when a current is applied. Evaluation of the DNA "comet" tail shape and migration pattern allows for assessment of DNA damage. In this assay, cells are immobilized in a bed of low melting point agarose, followed by gentle cell lysis, treated with alkali to unwind and denature the DNA and hydrolyze sites of damage. The samples are then submitted to electrophoresis and stained with a fluorescent DNA intercalating dye. The

sample is then visualized by fluorescence microscopy.

3. Results

In the six tissues of *Anabas testudineus*, protein bands ranging from 17 to 250kDa were observed and variable numbers of proteins were visible in each tissue (Figure 2). In the brain of *Anabas testudineus*, protein bands of 55, 46, 28 and 21kDa were intensely expressed in affected fish. Protein bands of 95, 72 and 130 kDa of high molecular proteins were found to express less intensively in the affected fish. Gill exhibited bands of proteins of molecular weights and visible differences in the banding pattern in certain regions and 17, 21, 48 and 72kDa protein bands were intensely expressed. High molecular weight proteins detected were 72, 95 and 130kDa. Protein bands of low molecular weight detected were of 17 and 21kDa in the affected fish. Protein bands of 36, 26 and 11kDa were observed clearly in affected intestine tissue. Kidney tissue exhibited protein bands of 95, 53, 48, 22 and 22kDa which were highly expressed. Liver tissues from the affected fish revealed the presence of three highly expressed proteins of 95, 72, 53 and 28 kDa. Among the three protein bands one (95kDa) was highly expressed. Protein profiling of the muscle tissues showed protein bands of 75, 68 and 17kDa which were intensely expressed, of which two bands were of high molecular weight and one of low molecular weight. Further, the peak intensity of proteins of tissues of control and experimental *Anabas testudineus* are presented in Figure 3.

Isolated hepatic cells of *Anabas testudineus* (control and experimental) were subjected to comet assay. The levels of DNA damage in *Anabas testudineus* was shown as the tail moment, tail length and as the percentage of migrated DNA. An endpoint "tail moment" is defined as the product of the tail length and the fraction of DNA in the tail. DNA damage demonstrated that there was a significant difference ($p<0.05$) between the experimental fish (9.53 ±2.30) from Buckingham canal and the control fish (0.5 ±0.76), wherein there was approximately double the number of cells with DNA damage, indicating genotoxicity in the environment (Figure 4).

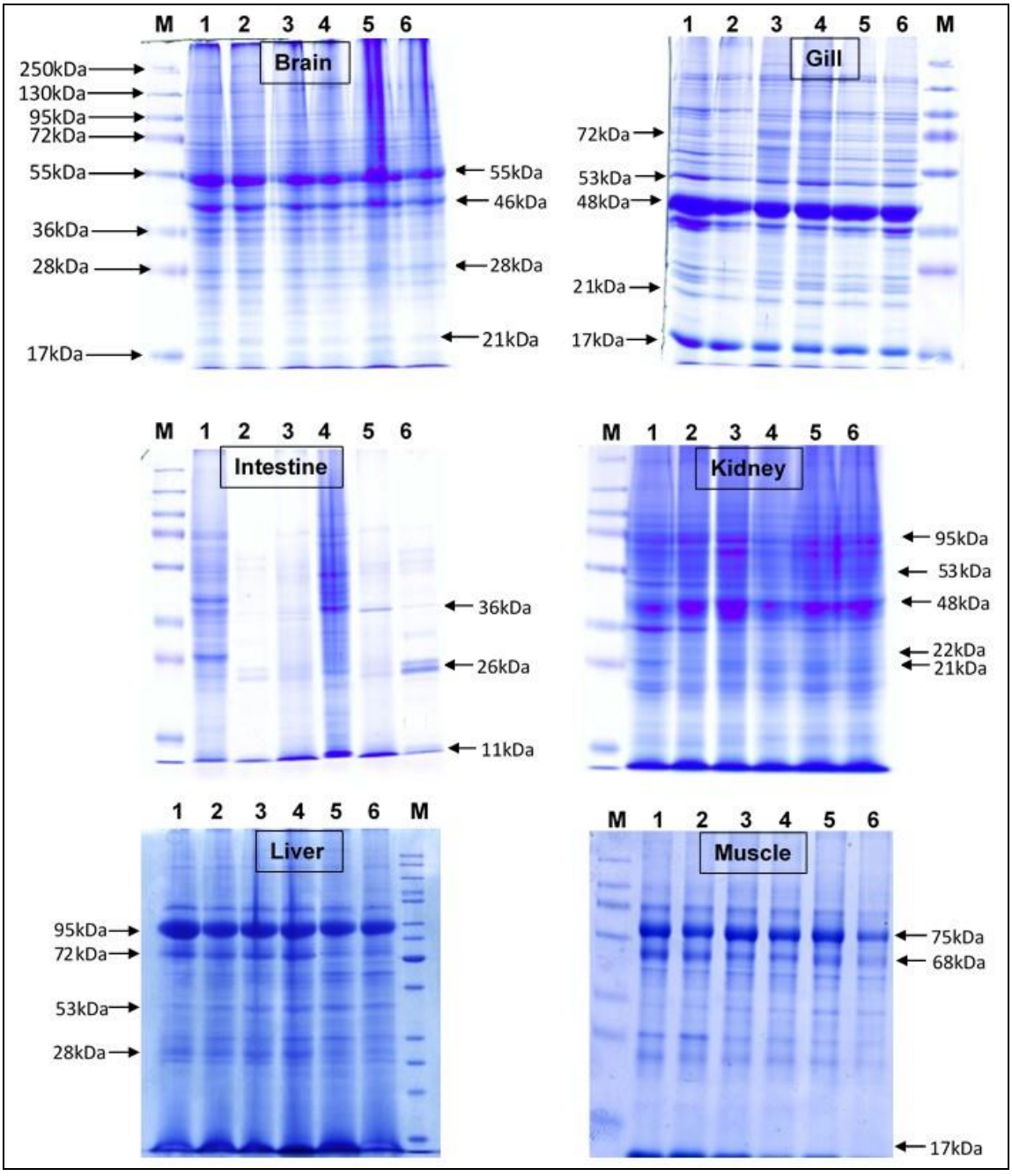

Fig 2: Electropherogram of the tissues of control and experimental *Anabas testudineus*. M: Protein ladder; 1: control; 2-6: experimental

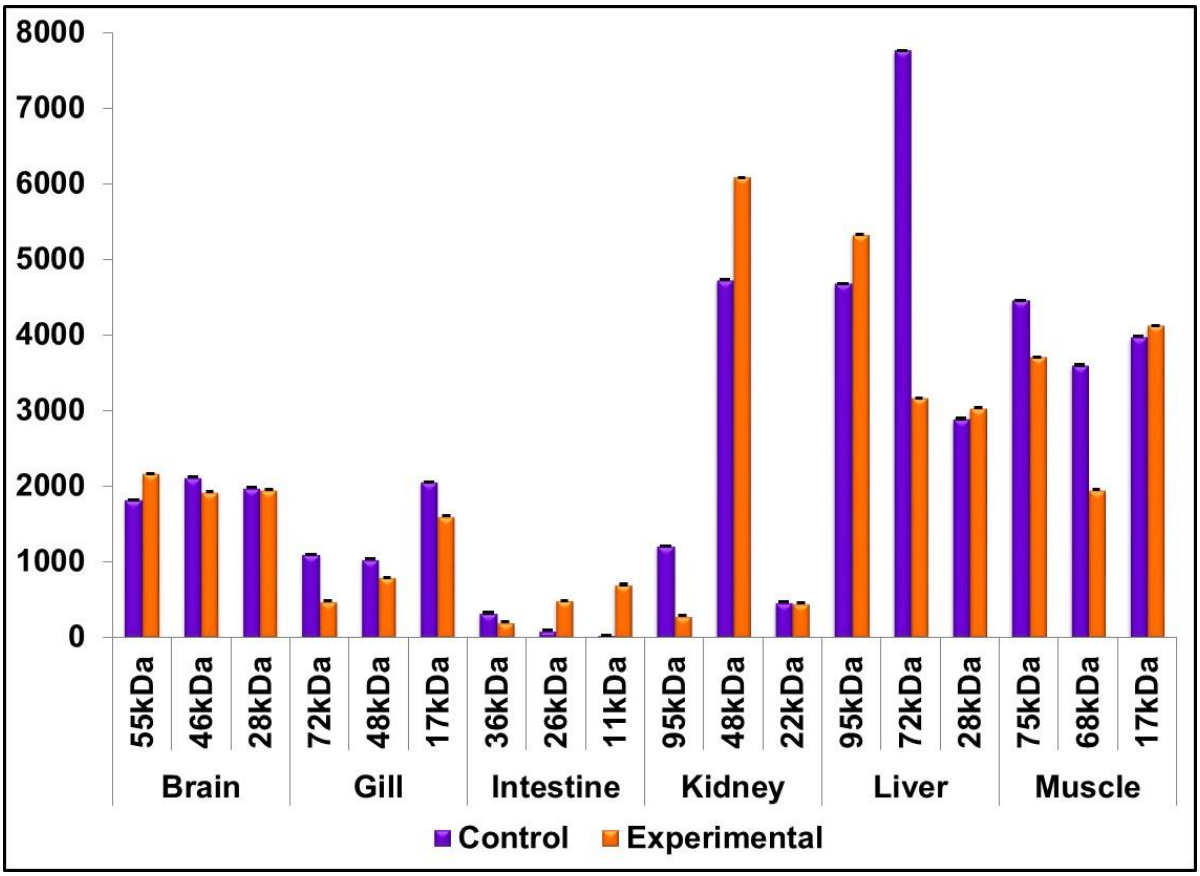

Fig 3: Peak intensity of proteins of tissues of control and experimental *Anabas testudineus*

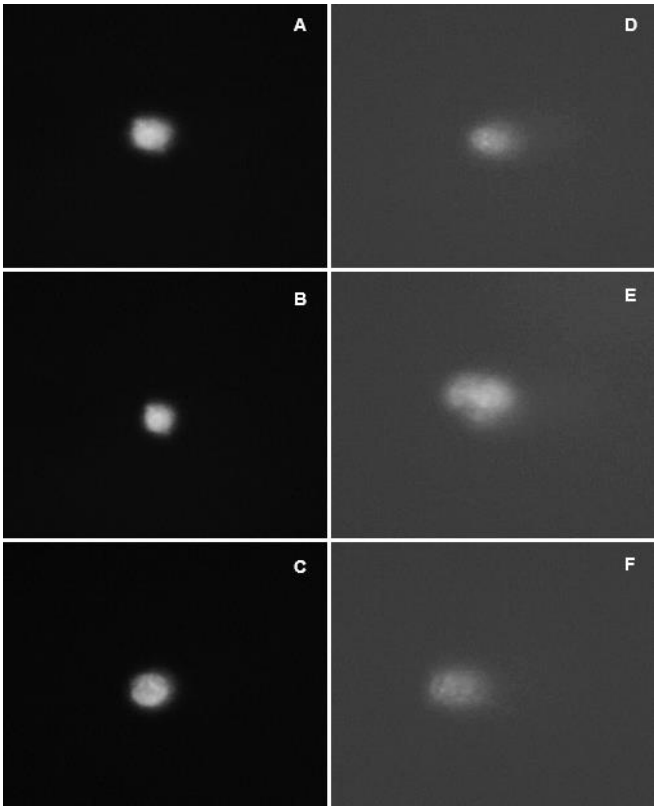

Fig 4: Comet assay of *Anabas testudineus*. A, B and C: Control; D, E and F: Experimental

4. Discussion

Electrophoretic analysis are employed to study the effects of aquatic pollutants on different tissues of the fish inhabiting different aquatic bodies [3, 4]. In the present study, there were two newly expressed protein band of p 21 and p 53kDa in the tissues of the experimental group which was not obvious in the control group. This indicates that expressions of these protein bands may be under the influence of stress due to toxic accumulation in the tissues of the affected *Anabas testudineus.* The p53 is a transcriptional activator and a tumor suppressor protein which controls several aspects of cell functions, *viz.*, cell- cycle progression, apoptosis and senescence. The p53-p21 pathway mediates telomere dependent replicative senescence which was sensitive to telomere attrition and also encompasses the DNA damage mechanism. Advancing ages well associated with increasing systemic levels of oxidative stress, with evidence provided by studies show an increase in markers of oxidative DNA damage [9]. Various cell growth regulatory proteins including p53, p21 and p16 exhibit altered activation states or are up regulated with senescence and are involved with cell cycle arrest and also represent a possible pathway to describe how pollution toxicity affects the fish. These responses to pollution environment are likely to have deleterious effects on blood cell development. Subsequently, the ATM' p53 pathway is activated in response to DNA damage and acts as a mediator of cell cycle arrest, apoptosis or cell death in fish. Finally after persistent damage, some cell begins to lose their normal structure and function, and then irreversible cell death occurs [1]. Thus, this technique may also prove to be helpful in the study of biochemical systematics of the fish diversity.

Testing for environmental pollutants is an ever growing concern. Various tests in organisms have been utilized for the detection and identification of toxic substances in the air, water and soil. Use of pollution indicative biomarkers in fish is an effective strategy for monitoring the aquatic environment and diagnosing adverse biological influences [10]. In the present study, comet assay in *Anabas testudineus* was

utilized to conduct an environmental assessment of aquatic pollutants of Buckingham canal, located in Chennai, Tamil Nadu, India. The results demonstrated that the samples had a significantly greater number of comets suggesting the presence of genotoxic agents in the aquatic environment and this assay provides a tool in the monitoring of water pollution and environmental risk assessment. Comet assay has been widely used in genetic toxicology and environmental biomonitoring especially in aquatic organisms [11] as a powerful tool for measuring the relationship between DNA damage and the exposure of aquatic organisms to genotoxic pollutants. Comet assay identifies repairable injuries or alkali label sites along with direct measurement of DNA strand breaking capacity of a tested agent [12].

Laboratory studies analyzing toxicity in fish tissues exposed to heavy metals can aid in understanding the impact of residual heavy metals on fish in the field [13]. The effect of a toxicant depends on several factors, *viz*., transport of metal across the membrane, the metabolic rate of the animal, physicochemical properties of the water and the seasonal changes in the taxonomic composition of different trophic levels [14]. The tissues for genotoxicity assays were selected on the basis of functional criterion where liver is the main xenobiotic metabolizing organ, gill the primary contact organ and whole blood the carrier of the pollutant [15]. The present study clearly indicated reduction in viable cell per cent by aquatic pollutants. Lower cell viability (per cent) at higher exposure concentrations may be due to increased oxidative cellular damage. Gill was the least affected organ among all the tissues. Lower cell viability in blood erythrocytes of liver may be due to their higher accumulation capacity. Lowest cell viability in liver was reported in juvenile trout (*Scophthalmus maximus*) exposed to cadmium contaminated sediments [16]. The present study is also in agreement with reduction of liver cell viability in *Channa punctatus* [17] and *Anabas testudineus* [18] treated with mercuric chloride and sodium arsenite, respectively. The genotoxicity detected in the group of the affected fish had significantly a higher level per cent of DNA damage and increased tail length than the control fish. Ganesan *et al.* [19] reported similar trend of DNA damage in their studies on *Oreochromis* exposed to aquatic pollutants and chromium toxicity. The results of the present investigation pertaining to DNA damage demonstrated that there was a significant difference between the fish from Buckingham canal and control fish, in which there was approximately double the number of cells with DNA damage, indicating genotoxicity in the environment. Sasaki *et al.* [20] suggested that, the determination of genotoxicity as a result of environmental contamination of water should be conducted with water as a whole and not specifically for each (contaminating) component, and that the comet assay would be a good test for this type of monitoring. The data on genotoxicity of *Anabas testudineus* from Buckingham canal demonstrated the poor quality of that environment, without determining the specific polluting components. Yabe and Oliveira [21] reported that an elevated concentration of heavy metals (lead and zinc) in the water of Igapo lake, were the possible agents, responsible for DNA damage and their studies are in consistent with the present study on *Anabas testudineus* from Buckingham canal.

There are various mechanisms reported for DNA damage. Increased tail DNA (per cent) at higher exposure concentrations indicate greater damage which may be due to increased activity of glutathione regenerated from glutathione disulfide. The decrease in tail DNA (per cent) indicated lower presence of damaged DNA at longer exposure. This may be due to various mechanisms like loss of damaged cells, or DNA repair, or toxicity of other contaminant that could prevent the enzymatic process of DNA damage [22] or gene activation of metabolizing enzymes like cytochrome P450 in various tissues that provides a defensive mechanism against the pollutants [23], or a combination of some of these. The tissue specific variation in the DNA damage may be the result of distinctive chemical mechanism of activation or detoxification of the test chemical. This difference may also be due to the variation in the accumulation capacity of tissues as the liver accumulates most toxicants which were found to be responsive to DNA damage. Significantly, higher DNA damage in liver tissue of *Anabas testudineus* was found in the present study which is in agreement with higher DNA fragmentation due to arsenic and mercury in liver cell of *Anabas testudineus* [17] and pollutants of Chromepet lake [19]. The liver of juvenile trout (*Scophthalmus maximus*) was found to be more sensitive than the gill, followed by the blood and spleen to cadmium contaminated sediment exposure [16]. Sivachandran *et al*. [24] also reported significant higher DNA damage in the liver and testicular tissues of fishes exposed to tannery effluents for a period of thirty days. The comet assay is non-destructive methodology, preserving the organism and ecosystem. The present study, utilized this assay as a biomarker of genotoxic potential in the water of Buckingham canal, which was found to be effective in revealing double DNA damage in the experimental *Anabas testudineus* when compared to the control. Based on these results, it can be suggested that *Anabas testudineus* is a good biomarker of genotoxicity.

5. Conclusion

The present study revealed that, aquatic pollutants/toxicants cause toxic and tissue specific genotoxic effects in *Anabas testudineus.* Pathological effects shown by toxicants are not uniform in the entire population but vary with the concentration of toxicants, age and health of the test organism which contribute to the severity of these symptoms. The present investigation suggested that *Anabas testudineus* is a good bioindicator of environmental genotoxicity, molecular and biochemical assays constituting a sensitive, rapid and an economical method for the detection of oxidative stress, and DNA damage, exhibting it as the biomarker for non-specific genotoxicity in *Anabas testudineus*. The present study also revealed that, fish can be a potential bioindicator for environmental monitoring. The induction of genotoxicity by aquatic toxicants/heavy metals at environmental concentration suggests great concern regarding potential genetic damage to aquatic organisms. However, further investigation is needed, including the standardization of the method and the measurement, before these assays can be used as the standard biomarker of aquatic environs.

6. References

1. Ganesan S, Sultana M. Impact of aquatic pollutants on the protein profile of the fish, *Tilapia mossambicus* from Chrompet lake, Chennai, India. Aquatic Biology. 2010; 24(1):151-156.
2. Gayathri V. Impact of PAH on the protein profile of the fish, *Tilapia mossambicus*. Ph.D. thesis, University of Madras, Chennai, Tamil Nadu, India, 2010.
3. Dheenadayalamurthy. Characterization and impact of

aquatic pollutants on the biochemical, histopathological and molecular changes in various tissues of a fish, *Channa punctatus* and *Oreochromis mossambicus*. Ph.D. thesis, University of Madras, Chennai, Tamil Nadu, India, 2012.
4. Suresh. Studies on the biochemical, cytogenotoxicity, histopathological and molecular changes in fish *Terapon jarbua* (Forsskal) from Ennore creek, Tamil Nadu. Ph.D. thesis, University of Madras, Chennai, Tamil Nadu, India, 2013.
5. Ateeq B, Farah MA, Ahmad W. Detection of DNA damage by alkaline single cell gel electrophoresis in 2,4-dichlorophenoxyacetic-acid and butachlor exposed erythrocytes of *Clarias batrachus*. Ecotoxicology and Environmental Safety. 2005; 62:348-354.
6. Van der Oost R, Beyer J, Vermeulen NPE. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environmental Toxicology and Pharmacology. 2003; 13:57-149.
7. Biswas, Shah MS. Taxonomic comparison of local and Thai Koi (*Anabas testudineus*, Bloch) from Khulna, Bangladesh. SAARC Journal of Agriculture. 2009; 7(1):19-28.
8. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 1970; 227:680-685.
9. Barcellos, HMH, Dix TA. Redox- mediated activation of latent transforming growth factor-1. Molecular Endocrinology. 1996; 10:1077-1083.
10. Silva KTU, Pathiratne A. *In vitro* and *in vivo* effects of cadmium on cholin esterases in Nile tilapia fingerlings: implications for biomonitoring aquatic pollution. Ecotoxicology. 2008; 17:725-731.
11. Lee RF, Steinert S. Use of the single cell gel electrophoresis/comet assay for detecting DNA damage in aquatic (marine and freshwater) animals. Mutation Research. 2003; 544:43-64.
12. Mughal A, Vikram A, Ramarao P, Jena GB. Micronucleus and comet assay in the peripheral blood of juvenile rat: establishment of assay feasibility, time of sampling and the induction of DNA damage. Mutation Research. 2010; 700:86-94.
13. Walker PA, Kille P, Hurley A, Bury NR, Hogstrand C. An *in vitro* method to assess toxicity of waterborne metals to fish. Toxicology and Applied Pharmacology. 2008; 230:67-77.
14. Chen CY, Pickhardt PC, Xu MQ, Folt CL. Mercury and arsenic bioaccumulation and eutrophication in Baiyangdian lake, China. Water, Air and Soil Pollution. 2008; 190:115-127.
15. Singh NP, Ogburn CE, Wolf NS, Belle GV, George MM. DNA double-strand breaks in mouse kidney cells with age. Journal Biogerontology. 2001; 2:261-270.
16. Kilemade MF, Hartl MGJ, Sheehan D, Mothersill C, Van Pelt FNAM, O'Halloran J *et al*. Genotoxicity of field collected inter-tidal sediments from Cork harbor, Ireland, to juvenile trout *Scophthalmus maximus* (L.) as measured by the comet assay. Environmental and Molecular Mutagenesis. 2004; 44:56-64.
17. Akter MS, Ahmed MK, Akhand AA, Ahsan N, Islam MM, Khan MS. Arsenic and mercury induce death of *Anabas testudineus* (Bloch) involving fragmentation of chromosomal DNA. Terrestrial and Aquatic Environmental Toxicology. 2009; 3:42-47.
18. Ahmad MK, Islam S, Rahman S, Haque MR, Islam MM. Heavy metals in water, sediment and some fish of Buriganga river, Bangladesh. International Journal of Environmental Research. 2010; 4:321-327.
19. Ganesan S, Rubabanjum KM, Sultana M. Detection of DNA damage in lymphocytes of *Oreochromis mossambicus* using comet assy. Aquatic Biology. 2010; 25(2):136-140.
20. Sasaki VF, Izumiyama F, Matsusaka N, Asano N, Saotome K, Sofuni T *et al*. Detection of genotoxicity of polluted sea water using shellfish and the alkaline single-cell gel electrophoresis (SCE) assay: a preliminary study. Mutation Research. 1997; 393:133-139.
21. Yabe MJS, Oliveira EO. Metais pesados em ´aguas superficiais como estrat´egia de caracterizac¸ ao de bacias hidrogr´aficas. Qu´ımica Nova. 1998; 21:551-556.
22. Rank J, Jensen K. Comet assay on gill cells and hemocytes from the blue mussel *Mytilus edulis*. Ecotoxicology and Environmental Safety. 2003; 54:323-329.
23. Wong CKC, Yeung HY, Woo PS, Wong MH. Specific expression of cytochrome P4501A1 gene in gill, intestine, and liver of tilapia exposed to coastal sediments. Aquatic Toxicology. 2001; 54:69-80.
24. Sivachandran R, Sultana M, Saravanan R, Malathy S. Tannery effluents induced DNA damage in liver and testicular tissues of fresh water fish, *Channa striatus*. Asian Journal of Science and Technology. 2014; 5(7):427-430.