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Eco-Genotoxicity Assessment in Vemband Lake Using Comet Assay on Fish, *Etroplussuratensis*

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Abstract

The Vembanad Lake, part of a massive coastal wetland ecosystem, located in the west coast of Kerala is an ecologically sensitive one. This Ramsar site forms a unique habitat for many biologically and economically important organisms. The lake is now seriously polluted by chemicals and other wastes from various sources. Pollution not only affects quantity but also the quality of aquatic organisms. This study assessed the in-situ genotoxicological effects of pollutants on tissues of the fish, *Etroplussuratensis* in the Vemband lake Kerala, employing comet assay. Samples were collected during post monsoon and pre monsoon period from the north part of the lake which surrounds mainly the Ernakulum district. Comet assay results demonstrated serious type III and type IV DNA damages in the *Etroplussuratensis* .Degree of damage varies in different seasons. Increased amount of DNA in the tail of Cometwas found in the pre-monsoon months compared to that of the post monsoon.Study reflects that fishes are seriously affected by the pollutedwater. Degradation of the lake will have far reaching ecological and economic effects.Bioaccumulation of chemicals in fishes will also affect human health when they are taken as food. Study emphasizes the need forthe conservation of such wetlands.

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Keywords: *Etroplussuratensis , Seasonal variations, Genotoxicity, Comet assay*

1.Introduction

The Vembanad wetland system lie in thetropical region between 09˚00’ -10˚40’N and 76˚00’-77˚30’E. It is identified as Ramasar site no 1214 for conservation purposes. It has a unique physiography, hydrology, land use, flora and fauna. VembanadBackwater Lake and adjoining Kol lands are the major components of the Vembanad wetland system. The Vembanad Lake is bordered by Ernakulam, Kottayam, Alappuzha and Thrissur districts of Kerala and is fed by nearly 10 rivers.The major commercial and economic activities in the lake include industries, agriculture, fisheries, lime shell mining, backwater tourism, etc. Overexploitation of resources and a range of interventions such as salinity barriers, flood water drains and industrial complexes in the drainage basins are found to have severe impact on the ecological status of the wetland leading to its degradation (Remain,etal. 2010). Ernakulam district, anindustrial hubof Kerala releases a considerable amount of chemical effluents to water body, posing a serious challenge. Since this area supports nearly half of the population of Kerala state, consequences of any ecological destruction to this wet land will have a far reaching effect.

Fishes play an inevitable role in the trophic food web and are the best pollution indicators of aquatic environment. Branded pearl spot binomially *Etroplussuratensis* is the state fish of Kerala, and forms a major edible fish food. Quality of the ecosystem determines the quantity and quality of fish resources. There are signs of decline in the Vemband fishery resources, evident in lesser numbers of species and decline of fishery production (Asha, etal.,2014). Pollution not only affects quantity but also the quality of aquatic organisms. This study assessed the in-situ genotoxicological effects of pollutants on tissues of the fish, Etroplussuratens is in the Vemband lake Kerala, using Comet Assay.Comet Assay is a simple and sensitive

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technique for evaluating in-vivo, in-vitro, and in-situ DNA damage in different tissues of fish such as gill, liver, kidney, and blood after exposure to different kinds of pollutants of aquatic environment (Dhawan, et al., 2009).

2.Materials and Methods

Study Area and Sampling

This study was performed in the northern region of Vemband Lake. Study site is extremely a brackish water area, bordering the Ernakulum district of Kerala state. Sampling was carried out from three randomly selected sites [R1 R2 R3] with the help of local fisher man. Collections were done in two phases of the hydrological cycle, one in the month of December 2019 (post monsoon) and others during the months January to March 2020 (pre monsoon). Samples were transported to laboratory along with the water taken from the site in separate containers. In lab the liver was dissected out and named L1 (December) L2 (January) L3(February), L4 (March) for the comet assay studies.

Method

Most reliable technique for screening the Genotoxicity is the alkaline single cell gel electrophoresis assay (SCG or SCGE assay), commonly known as comet assay, which is an important biomarker of genotoxicity in fish (Mitchelmore andChipman, 1998). The comet assay under alkaline condition can identify DNA damage of different kinds such as single-strand breakage, alkaline labile sites, and DNA cross-links that are induced by chemicals (Tice et al., 2000). Significant advantages include its sensitivity for detecting low levels of DNA damage, the requirement for only small numbers of cells per sample, its ease of application and low cost, and the short time needed to perform the assay (Liao et al. 2009).

The samples (Liver 1, Liver 2, Liver 3 and Liver 4) were minced into small pieces with a sterile scalpel. The tissue samples were washed with calcium and magnesium free balanced salt solution. Dispase(0.6-2.4U/mL in calcium and magnesium free balanced salt solution) was added to the samples and incubated at 370C for 20 minutes. It was then spun at 1000rpm and the supernatant was used for the study.

Fully frosted microscope slides were pre-coated with 1ml of 0.75% normal melting point agarose (NMA Invitrogen, USA) and stored at 4ºC. This layer was removed before use and 120µl of 0.75% NMA was pipetted into the slides, which were then covered with cover slips. Samples were mixed with 10µl of low melting point agarose (Novex, Invitrogen) in 1:1 ratio and pipetted over the first layer of agarose. NMA (80µl) was used as a final protective layer. After each step the slides were incubated at 4ºC for 10 min. to allow agarose to set.

Slides were placed in cold lysing solution containing12.5 µM NaCl,100 mm Na2EDTA, 10mm Tri Base pH 10 and 1% SDS to which 10% DMSO and 1% Triton X 100 were added immediately prior to use. After lysis slides were placed in electrophoresis buffer (300 mm NaOH and Na2EDTA PH13) for 20min to allow unwinding of DNA. Electrophoresis was conducted in the same buffer by applying an electric current of 0.8V/cm (300mA) for 20minutes using an electrical supply (Power case, Life Technologies).Finally, slides were washed in neutralization buffer (0.4µL Tris, pH7.5) three times for 5mins each, dried and stained with 50 µl ethidium bromide (20µg/ml). The slides were photographed using Inverted epifluroscent microscope Olympus CKX41 attached with Opitka Pro5 CCD camera. Comets were scored using Tritek comet scoring software and correlated statistically.

Liver tissue was selected of study. Liver tissues shows higher genotoxic effects, because they are the detoxifying organsand are constantly exposed to the pollutants of water that may cause extensive DNA damage in them.

Results and Discussions

Comet assay is used to check the effect of the chemicals on the integrity of DNA content (Bolognesi and Cirillo, 2014). DNA damage is calculated as the DNA tail area/whole DNA area (%) and the comet tail length (from the center of DNA head to the end of the DNA tail) in cells. The bigger the DNA tail area (%) or the longer the DNA tail length, the more significant the damage. Based on the presence of DNA in tail and the intensity of the tail types of damage they can be divided into five groups.The classes of DNA damage type 0 to type IV were given by Anderson et al. (1994). The amount of DNA migrated as comet tail of various

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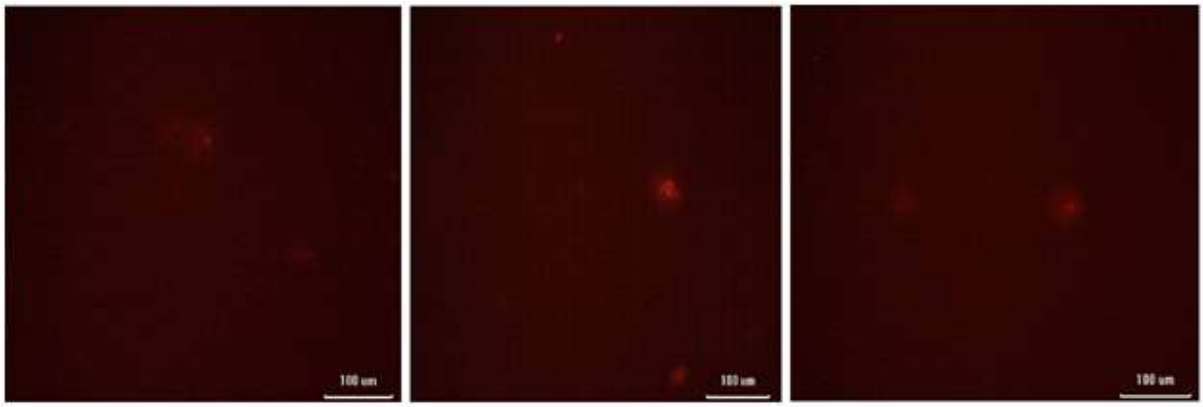

Figure 1, Type 0 DNA damage.Liver tissue (Sample L1) analyzed by Comet Score TM indicating non-significant DNA damages.

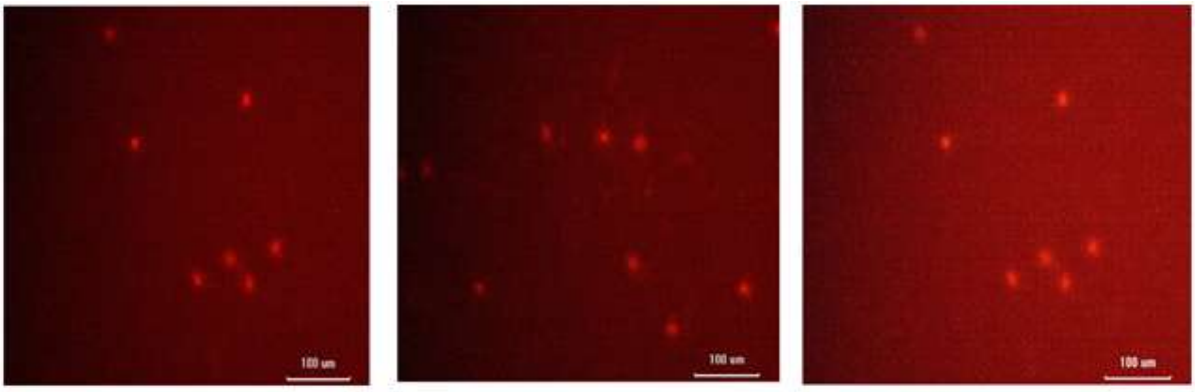

Figure 2, Type 1 DNA damage. Liver tissue (Sample L2) analyzed by Comet Score TM indicating significant DNA damages

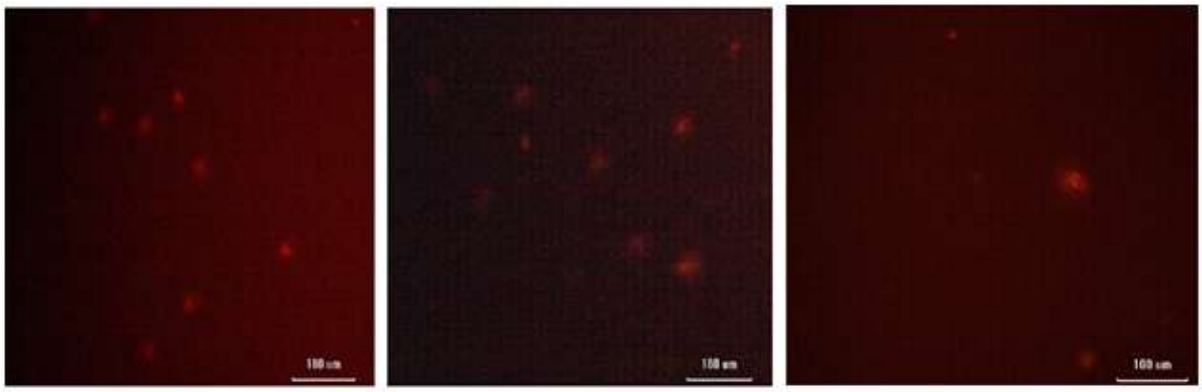

Figure 3, Type 1&2 DNA damage. Liver tissue (Sample L3) analyzed by Comet Score TM indicating significant DNA damages

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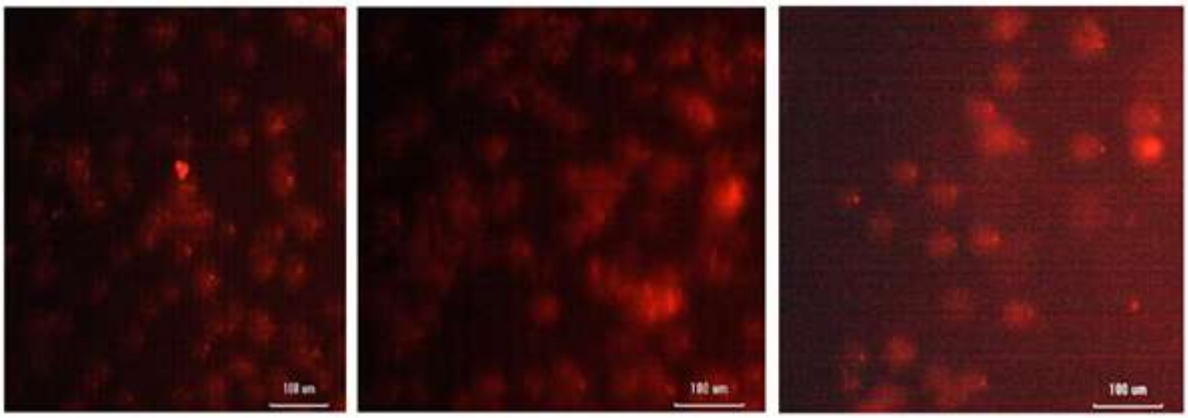
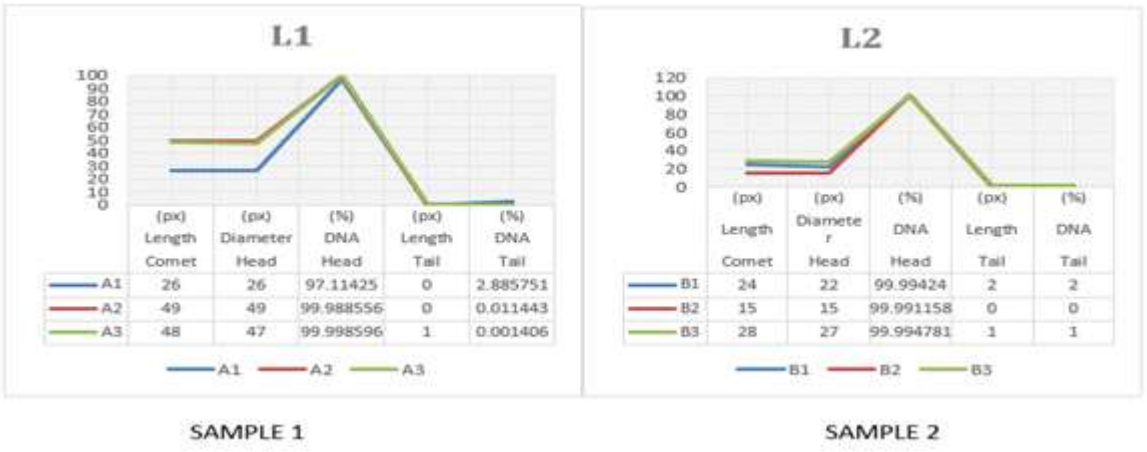


Figure 4, Type III &IV DNA damages. Liver tissue (Sample L4) analyzed by Comet Score TM indicating very significant DNA damages.



SAMPLE 1 SAMPLE 2

Graph 1Liver tissue (Sample L1&L2), showingLength of thecomet, Diameter of head, Percentage of DNA found in thehead, Length of the comet Tail &Percentage of DNA found in the Comet Tail analyzed by Comet Score.

SAMPLE 3 SAMPLE 4

Graph2(Liver tissue (Sample L3&L4) showing Length of the comet, Diameter of head, Percentage of DNA found in the head, Length of the comet Tail &Percentage of DNA found in the Comet Tail analyzed by Comet Score

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lengths was known as DNA damage. The first one being the cells with intact nucleus showing no DNA damage type 0. The second one with Mild DNA damage with little or negligible amount of comet tail called as the type I and type II. Type III is the next ones showing high DNA damage with a pronounced tail of comet. The fifth class showing much amount of DNA in tail is the type IV. Time dependent increase in tail DNA was found. Large comet tails of DNA were seen the sample plate IV.

In the present study, degree of damage varies from month to month. Liver sample (L1) collected in December showed with Comet length(px) 26,49 and 48 respectively, and 2.88 %,0.011%and 0.001% DNA in the tail (Figure 1, Graph 1 sample 1) implying negligible amount of DNA in the comet tail.Sample (L2) collected during the month January showed type 1DNA damage (Figure 2). Comet lengths of (px) 24, 15 and 28 was observed respectively with 2%and 1% ofDNA in the tail (Graph 1, SampleL2). Type 1 and 2 DNA damage was observed in sample (L3) collected in February (figure 3), with comet length(px) 18,35and 26 respectively and 4.45%,3.93%and 53.9% of DNA in the tail (Graph 2, Sample 3).Sample (L4) collected in march indicated significant DNA damages -type III and IV (Figure 4) with comet length (px) 36,44 and 37 respectively and with 32.12%,14.04% and 29.98% of DNA in the tail(Graph 4, Sample 4).
Integrity of nucleus is very essential for the normal functioning,as Nucleic Acid of the nucleus controls all the activities of cell. The development and growth of the fishes depend upon the stable content of DNA which serves as important biochemical parameter(Buckley, 1980). Active protein synthesis and cell growth are dependent on the DNA content (Tasneem and Yasmeen 2018).Whenever an organism is exposed to highly potent chemicalsdissolved in water, its nucleic acid content changes.It leads to several changes in DNA which may be due to the increased activity of the enzyme DNase function. Another important reason for DNA damage may be the inhibition of enzymes that replicate or repair DNA (Guilherme,et al., 2012). The intactness of the DNA is the important part of the normal cellular process. The changes in DNA may be due to the physiochemical interaction of the chemicals with the cellular DNA and produces a number of primary changes such as single strand breaks, double-strand breaks, DNA protein crosslink, and damage to purine and pyrimidine bases (Van Loon, et al., 1991).However, whenever the DNA breaks are formed the damage rectifying mechanism operates in the body, but in the present study higher frequency of type IV &V DNA damages, (Figure 3&Figure 4, Graph 2, Sample 3&4)might be due to the presence of these chemicals in constant and higher level in waterbody that disrupt the functioning of rectifying machinery. Differences in DNA damage recovery may be attributed to the rate of metabolite removal/detoxification or DNA repair capacityof different species. (Avelyno, etal,2018).

The literature clearly indicated that untreated industrial sludge and municipal discharge, runoff from agricultural fields, water holding pesticides and chemical including heavy metals leads to severe contamination of VembanadLake (K.N.Remani et al .2010,Asha et al 2014). Heavy metals have the ability to bind to phosphates and other organic molecules like DNA heterocyclic base residues of DNA leading to alterations in the primary and secondary structures and finally to mutations(Nagarani, et al.2012).Pollutants affect the DNA directly by causing DNA strand breaks or indirectly by producing reactive oxygen species(ROS) which then damages the DNA by forming adducts or causing lesions (O'Brien 1988). DNA repair systems which try to heal the damaged DNA can do so only up to a certain threshold, beyond which DNA damage can persist, in the form of lesions or unrepaired DNA, leading to mutagenesis and carcinogenesis (Moustacchi, 2000). Many pesticides have been proven to induce changes in DNA (Massimo,et al., 2000; Vrhovae andZeljezic, 2000) and structural changes at the level of chromosomes (Mathur, 1988). Acharya et al. (2005) found that there was a significant decline in the DNA content in Labeorohita on exposure to ammonia. There was a significant decrease in the DNA content of liver of zebra fish, Daniorerio, on exposure to cypermethrin (Ansari and Kumar, 1988). Studies on the effect of monocrotophos on Cyprinuscarpiopointed out that pesticide leads to several changes in DNA which may be due to the increased activity of the enzyme DNAase function (Maruthanayagam and Sharmila ,2004).

Results indicated elevated levels of genotoxic damage in the sample collected from pre monsoon

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seasons compared to that of post monsoon seasons.Sample collected in December month showed type I&0 DNA damages with negligible amount of the DNA in the tail while sample collected in March showed maximum DNA in the tail. There is a higher possibility for the chemicals to be accumulated in the water bodies during non-rainy season, because there is no flow of water, and the water is more or less stagnant, and the evaporation rate is higher in these months. Eco-toxic study on Vembanad lake proved that the chemical in the water effected the fishes badly, especially during pre-monsoon season. It is a clear indication of adverse effects of pollutants on fish health.

Conclusion

Quantity and quality of fishes depend on the quality of water bodies. Present eco-toxic study on *Etroplussuratensis* in Vembanad Lake reflects that fishes are seriously and adversely affected in their natural habitat. Serious DNA damage is an indicator of bad water quality. Results also indicated elevated levels of genotoxic damage in pre monsoon seasons compared to that of post monsoon season. Degradation of the lake will have far reaching ecological and economic effects. Bioaccumulation of chemicals in fishes will also affect human health when they are taken as food. Dumping of waste from industries, cities and leaching of fertilisers and pesticides from agriculture land should be cheeked to reduce toxic pollution of Vembanad welands.

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