Fish ecogenotoxicology: a potential tool for monitoring genetic toxicity

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Abstract

Ecogenotoxicology is a potential tool that works upon the techniques of genetic toxicology as well as to analyze the prominent adverse effects of environmental toxicants due to genotoxic agents on the ecological health of the ecosystem. On the other hand, in human toxicological studies, ecogenotoxicology analyzes the impact of genotoxicants on a given population and simultaneously implements the principles related to genetic toxicology in hazard assessment. Fishes have shown a potential to work as an effective genetic model in the field of aquatic pollution evaluation of pollution in aquatic ecosystems. Several fish species found in contaminated areas were administrated in the experiments related to the aquatic environment, and encouraging evidence of environmental mutagens was recorded in the selected freshwater fish population. Several tests related to genotoxicity and their applications in environmental monitoring were reported worldwide in freshwater fishes. Thus, ecological genotoxicology is the key to detecting pollution in aquatic environments early. The present review is focused on the role of ecogenotoxicology in the field of environmental monitoring, as well as an emphasis on the establishment of fish as a model in the genotoxicity evaluation of environmental toxicants. For the critical screening and validation of genotoxins, environmental genotoxicology of the gene pool along with population should also be included to enhance the sensitivity and specificity of test protocols, as an early warning and to devise the monitoring strategies. Limitations and future prospects of ecogenotoxicology and recent advancements were critically reviewed along with suitable recommendations for future research.

Keywords: Freshwater fishes, Environmental assessment, Genetic material damage, Genotoxicity tests, Ecogenotoxicology.

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INTRODUCTION

Environmental pollution has become a major health hazard for humans and animals. Studies revealed that several toxicants from domestic wastes as well untreated or semi-treated effluents from industries and several chemicals such as pesticides, insecticides, and herbicides are being widely used in the agriculture field. Released toxicants hold a variety of chemicals, insecticides, cosmetic release, pesticides as well as several heavy metals. These toxicants have the capability of altering water quality indices, causing a direct adverse effect on the aquatic inhabitants (Devaux et al., 2021; Gartiser et al., 2020). Such alteration in water quality exhibits remarkable adverse effects on the aquatic

organisms in terms of DNA damage, protein oxidation, membrane damage, and mortality (Fig 1).

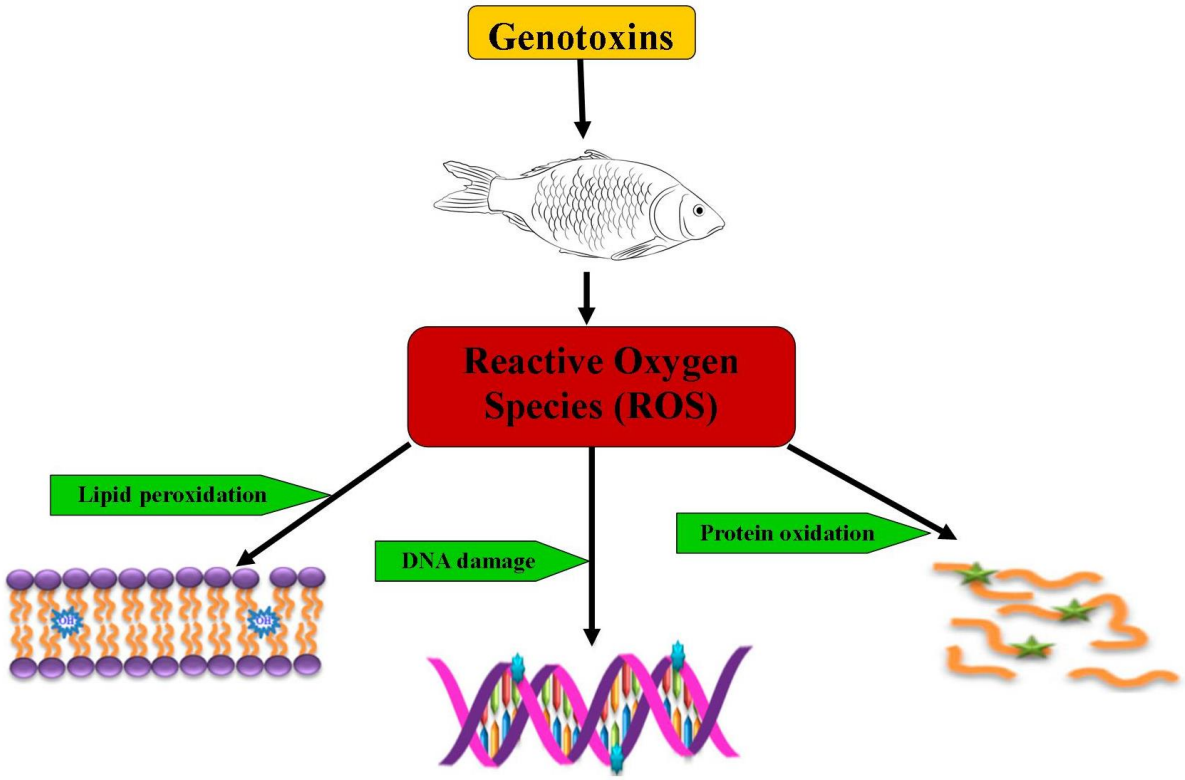

Fig.1. Graphical illustration of genotoxic pathway

Furthermore, these harmful toxicants or compounds such as heavy metals, insecticides, hydrocarbons, and pesticides are released in water bodies directly or indirectly. Moreover, when toxicants are released in water bodies at a higher concentration (above the permissible limit) results in heavy mortality of aquatic organisms such as fish, crabs, mussel and shell fish etc on other hands when toxicants being released at low concentration may cause bio accumulation of such toxicants and pollutants (Catteau et al., 2021; Milinkovitch et al., 2019). At a later stage, such bioaccumulation of toxicants may enter the food web and ultimately reaches the human body and cause severe effects.

Environmental pollution results in the genetic structural integrity of DNA. Ecogenotoxicology evaluates the interaction of DNA-damaging agents on the health of the organisms of the ecosystem. Since the ecosystem is dynamic it is not easy to evaluate the effect of genotoxicants, at the ecosystem level, where population and communities are studied because the observation observed may be due tothe initial events of exposure. Therefore interactions of organisms in the environment and their adaptation to environmental changes can be evaluated by studying changes at the DNA level to elucidate complex changes at the ecosystem level.Several studies exhibited the possible reason for this phenomenon. They concluded that several genotoxins had been reported in the aquatic ecosystem, resulting in adverse effects such as DNA damage and protein oxidation in fish, crabs, shell fish and mussels. Studies emphasized the need to understand the

seriousness of this environmental problem, which must be focused on and handled adequately to conserve aquatic biodiversity (Marchand et al., 2020; Kapour & Nagpure 2018).

A genotoxicagent holds the capacity to provoke any mutation or any other aspect of indicative impact, causing any mechanistically related mutations. Toxicants found in the environment may result in genetic alteration in specific populations in various ways, such as genetic drift, any mutation, and any form of genetic adaptation. These agents may be mutagenic as well as carcinogenic, with the holding capacity of alteration in the genome integrity and the credibility related to the biological expression. The field of genetic toxicology deals with any possible intervention between the genetic material of a cell and any agent which may cause damage to DNA and any potential relatable adverse impact on the organism's health.

Genetic toxicology or Ecogenotoxicology is a tool that deals with the applications and technology for genetic toxicology to analyze any possibilities of environmental toxicity in the shape of genotoxicity-causing agents on the status of the environment (Davico et al., 2020; Sinha 2019).

Impact of genotoxins on organisms

The presence of toxins may not necessarily be indicated as pollution. In fact human activities don't relentlessly conclude in the form of adverse effects on the health of organisms. Researchers have given a lot of effort to frame an effective strategy that emphasizes to decide that how and to what extent environmental toxicants and genotoxicants behave like a pollutant. In other words, it may be stated that if the levels of toxicants in the given ecosystem reach a specific threshold limit, it may result in severe effects on biodiversity. To extract the phenomenon may be mentioned that the concentration of toxicants or genotoxicants in both conditions (high or low concentration) extracting a concrete conclusion about the presence of the genotoxins or toxicants requires a thorough thought process with the approach of effect-oriented analyses. Environmentalist firmly believes that any implication or conclusion about the status of a healthy ecosystem must not follow a nuclear line of evidence simultaneously if any toxicant reported being hazards at any concentration must be thoroughly investigated (Paravani et al., 2018; Sumathi et al., 2018). Further, any substances that may impact the quality of the gene, DNA, and genome must be demarked as genotoxicants, as genotoxins must be investigated with specific concern owing to focus on any possible relationship with DNA damage, mutagenesis, and Oncogenesis.

Concisely, any possibility of DNA damage may elevate the likelihood of mutagenesis in case a DNA lesion is unrepaired or incorrectly repaired, resulting in a long-lasting or permanent alteration in the genome at the location of the lesion. Any damage results in mutations and may lead teratogenesis, specifically if targeting the germ line or taking place during embryogenesis, ultimately causing malformations. While on another side, any mutation taking place in proto-oncogenes may convert it into the active form of oncogenes. Furthermore, if the situation continues to increase in the form of expression may cause degeneration (anaplastic) or tumour (neoplastic) proliferative cells. For instance, any possible relationship among PAH metabolites results through cytochrome P450 (CYP) may exhibit metabolite formation and ultimately may give rise to DNA adducts (Santana et., 2020; White et al., 2015).

Every organism possesses a powerful mechanism capable of repairing DNA damage at any level with the help of two effective methodologies named base excision repair and nucleotide excision repair. Several environmental genotoxins and toxicants may

strive for pro-oncogenic impact by hampering DNA repair enzymes. Similar findings were reported in the case of heavy metals (Cu, Pb, Cd) where heavy metals adversely impacted for mamidopyrimidine DNA glycosylase (FPG). The exact pathway explaining the genotoxic impact of any toxic compounds is still under exploration, however, metabolites of arsenic, are believed to have an adverse effect on DNA strands, most probably due to the activities of (ROS) reactive oxygen species (Fig 2).On another side, several heavy metals results in ROS by hampering the electron transport chain, while some heavy metals (hydrogen peroxide) cause genotoxic ROS through the DNA oxidant hydroxyl radical.

So far, most of the attempts to identify the complexity of the reasons as well as the impact of DNA strand breakage is undoubtedly oversimplified. Clearly, it may be mentioned that the onset of DNA strand damage is inevitable due to the adverse effect of genotoxicants; meanwhile, in the involvement of other toxicants, the possibility of DNA damage may not be ruled out in the environment. Although, any possible critical impacts of genotoxicants, at the organism level or even at the level of population exhibit the requirement analyzing any possible DNA damage and to elaborate any possible linkage with environmental stress (Mahboob et al., 2022; Brack et al., 2017).

Assessments related to genetic hazards focus on the genetic material alterations in humans and freshwater organisms. The close interaction of DNA damage, mutation, and the onset of different cancer has been proved and exhibited by several scientific attempts. Multiple review attempts were made to exhibit the presence and potential of genotoxins released industries. Freshwater and marine fishes have shown a remarkable potential to serve as an effective genetic model in toxicology estimation in aqueous ecosystems. As supported by several studies, it may be stated that fish species reported in the contaminated ecosystem have instigated the hypothesis of hampered reproductive capacity re/ported and growth in the carcinogenic ecosystem (Salunke et al., 2022; Brack et al., 2018).

Evaluation of genetic alteration through ecogenotoxicology

The primary question is what would be the appropriate method to evaluate any possible hazard and risk related to genotoxic substances and by which method such alteration may be analyzed. In present conditions, it is challenging to exhibit any impact of environmental toxicants, which also include genotoxicants, especially at the stage of the ecosystem, due to the dense population and large communities are being observed (Bhaskaran et al., 2020; Vethaak et al., 2017). This problem may be solved with the help of several studies based on ecogenotoxicity. These studies emphasized the need for distinction among various pathways using the reagents being baled to reach the genetic structure of organisms. Further subsequent impacts may cover subsequent attempts to understand such a process.

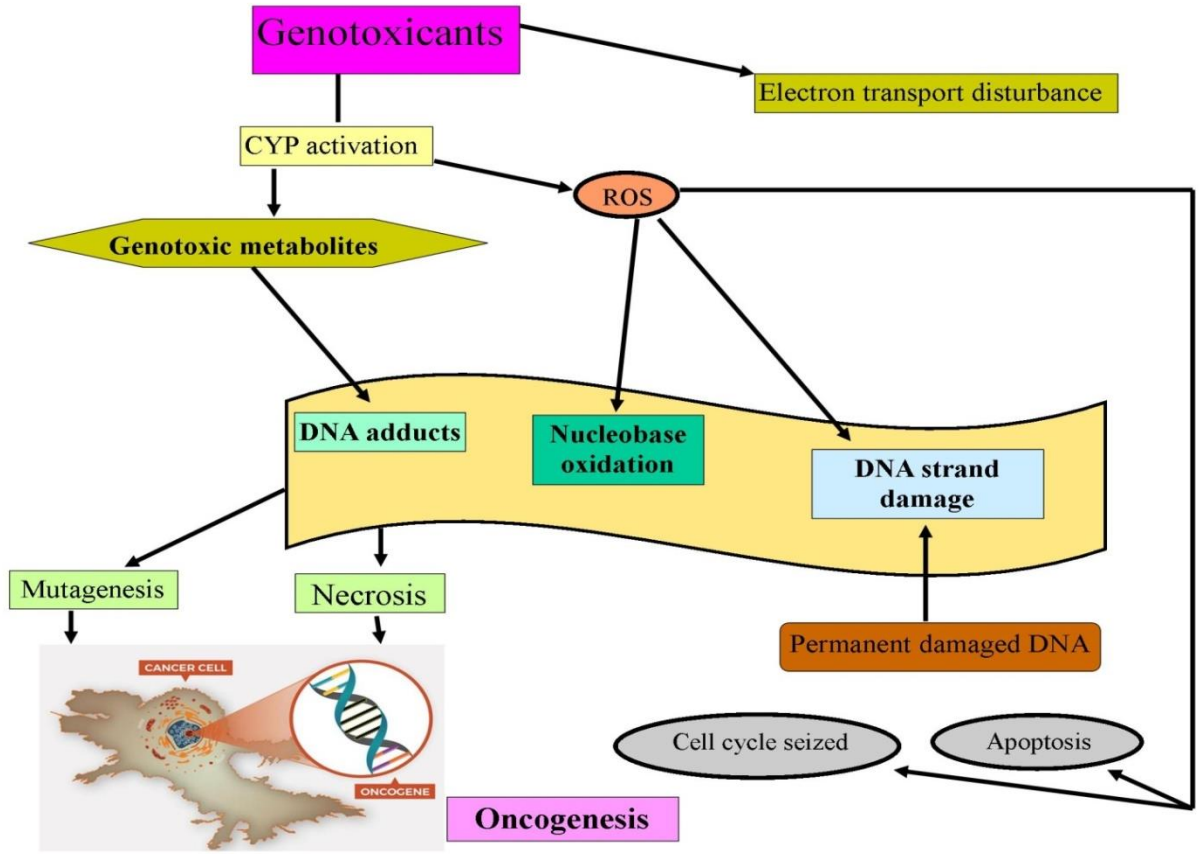

Fig.2. Graphical illustration of ROS pathway in DNA damage

The most effective method to rectify this issue is to observe the ecosystem in the form of dynamic interactions of stable matter and living organisms where the living material adapts according to the changes in the environment. Such phenomena are physiological and also possess a genetic basis. Therefore, based on previous studies, it may be stated that any changes at the genetic level may put some light to explain complex changes in ecosystem. The genetic structure of organism may correlate with genotoxicants in several methods and this information of the cellular machinery may provide some new possibilities to forecast and check toxicant induced damage to genome among exposed organism. Genotoxicants may change the integrity of genetic material and result mutations followed by subsequent genetic changes as well as non-mutagenic effects. As per the studies, genotoxic agents may directly affect the genetic material, where as affected organism may attempt to minimize the damage and attempt repair. Several attempts were reviewed to understand the movement of genotoxic stress within the somatic cell as well as the possible mechanism was also studied thoroughly. The exact cellular processes responsible for regulating such events in the DNA are complicated and less understood. DNA alteration by genotoxicity is reported differently in different organisms. Moreover, the class of genotoxic agent and its metabolite activity and its suppression in cell exhibits aberrant properties. Furthermore, developing a relationship between genotoxic agents within the environment and their removal in concerned generations of that organism is still difficult to analyze as individuals carrying adverse mutations are removed from the organisms due to an effective selection

against ill-fit organisms (Harvey et al., 2019; Ossana et al., 2016).

Involvement of fishes in Ecogenotoxicology

A particular class of genotoxins is chemicals responsible for DNA depletion in several fresh and marine animals, resulting in malignancies, retarded growth, disturbed development, lower embryo survival rate, and negative effect on larvae and adults development, at last, resulting in the shape of hampered production. As evidently proven by several attempts, genotoxicity reduces health as well as fitness in fish populations. Furthermore, it may transfer and affect human health through the food chain. So far, some major advancements have been reported in a few mammals. However, desired progress is still awaited, which may be capable of establishing the evaluation pattern to analyze potential hazards and risks originating from genotoxins in aquatic ecosystems, especially fishes (Mascini et al., 2020; Hani et al., 2018).

The reason behind the selection of fish as a model in the area of ecogenotoxicology could be that fishes are a highly sensitive indicator of limnology and may become potential bio-indicator against new toxicants entered into the aquatic environment. Freshwater fishes works as an effective genetic model to evaluate the pollution in the aquatic ecosystem. Furthermore, present information related to toxicants in the aqueous ecosystem has raised the utility of fishes as indicators for analyzing and monitoring carcinogens and mutagens. Scientifically, it has been established that water bodies are considered suitable repositories for releasing biological and other forms of waste. On the other hand, fishes serve a key role in the trophic web and in the bioaccumulation of toxicants (Table 1). The fish cells hold vital traits such as poikilothermic behavior and a lower rate of repair mechanism; they are susceptible to the induction of genetic damage. In fishes, the repair process of DNA is found to be slow. Therefore, fishes may exhibit remarkable possibilities to be used as a bio-monitoring indicator (Roex et al., 2019; Catteau et al., 2019).

Fishes have been used as a model in eukaryotic genotoxicity as well as mutagenicity analysis, and such practice makes them a potential organism in Comet assay, DNA repair process, test for Chromosomal aberration, Micronucleus assay, and sister chromatid exchange test. Therefore, further studies may focus on the utility of fish in genotoxicity detection resulting from aquatic toxicants in the DNA level of fishes. Such efforts would help develop effective strategies for ecosystem and fish fauna conservation by determining the safe level of pollutants in the aquatic environment.

Table 1. Tabular presentation exhibiting the adverse effect of toxicants on target organs

	Toxicant	**Test organism**	**LC50 value**	Affected organ	**Reference**
1	Acephate	*Pimephales promelas*	>1000 mg/L	shrinkage of liver cell mass	Brack et al., 2019
2	Alaclor	*Salmo gairdneri*	2.4 mg/L	cytoplasmic granularity	Brack et al., 2019
3	Akton	*Channel catfish*	400 µg/L	atrophy of cells	Brack et al., 2019
4	BHC	*Carassius auratus*	348 µg/L	Glomeruli in kidney	Brack et al., 2019
5	Carbaryl	*Salvelinus namaycush*	690 µg/L	change in haematocrit levels	Brack et al., 2019
6	Carbofuran	*Perca flavescens*	147 µg/L	atrophy of cells	Brack et al., 2019
7	DDT	*Salmo gairdneri*	8.7 µg/L	shrinkage of liver cell	Bhaskaran et al.,

				mass	2020
8	Endosulfan	*Ictalarus punctatus*	1.5 µg/L	cytoplasmic granularity	Bhaskaran et al., 2020
9	Diazinon	*Channa punctatus*	3.09 ppm	partial loss of liver plate	Bhaskaran et al., 2020
10	Diazinon	*Anabas testudineus*	6.55 ppm	change in haematocrit levels	Bhaskaran et al., 2020
11	Diazinon	*Barbodes gonionotus*	2.72 ppm	atrophy of cells	Bhaskaran et al., 2020
12	Elsan	*Channa punctatus*	0.43 ppm	pycnotic changes of cell nuclei	Bhaskaran et al., 2020
13	Permethrin	*Cyprinus carpio*	35 µg/L	Pre-maturation of ova	Jena et al., 2021
14	Biosal	*Cyprinus carpio*	4.21 mg/L	change in haematocrit levels	Jena et al., 2021
15	Cypermethrin	*Labeo rohita*	4.0µ/L	cytoplasmic granularity	Jena et al., 2021
16	Dimethoate	*Heteropneustes fossilis*	2.98mg/L	atrophy of cells	Jena et al., 2021
17	Methyl parathin	*Catla catla*	4.8ppm	Pre-maturation of ova	Jena et al., 2021
18	λCyhalothrin	*Danio rerio*	0.119µ/L	cytoplasmic granularity	Jena et al., 2021
19	Cypermethrin	*Colisa fasciatus*	0.02mg/L	Pre-maturation of ova	Jena et al., 2021
20	Metasystox	*Nemacheilus botia*	7.018 ppm	precipitated mass in gills	Cant et al., 2022
21	Malathion	*Labeo rohita*	15mg/L	shrinkage of liver cell mass	Cant et al., 2022
22	Rogor	*Puntius stigma*	7.1ppm	cytoplasmic granularity	Cant et al., 2022
23	Endosulfan	*Channa striatus*	0.0035ppm	cytoplasmic granularity	Cant et al., 2022
24	Malathion	*Heteropneustes fossilis*	0.98ppm	Gill shrinkage	Cant et al., 2022
25	Termifos	*Clarias gariepinus*	0.86 mg/L	precipitated mass in lamellae	Cant et al., 2022
26	Endosulfan	*Catla catla*	0.98 µg/L	change in haematocrit levels	Salunke et al., 2022
27	Endosulfan	*Cirrhinus mrigala*	1.06 µg/L	shrinkage of liver cell mass	Salunke et al., 2022
28	Endosulfan	*Labeo rohita*	2.15 µg/L	Pre-maturation of ova	Salunke et al., 2022
29	Dimethoate	*Labeo rohita*	24.55 µg/L	cytoplasmic granularity	Salunke et al., 2022

Significance of ecogenotoxicology in environmental analysis

Human toxicological studies focus on the individual involvement of organisms. On the other hand, ecogenotoxicology focuses on the adverse effects of genotoxicants related to the organism and related population. Several studies reported the onset of hepatic tumors in multiple freshwater species inhabited in the contaminated environment, whereas in some studies, neoplasms in fish were recorded as a result of wastewater effluents (Catteau et al., 2020, Kaur et al., 2018).

Several studies reported that the interaction with DNA resulting factors might appear in carcinogenic-DNA adducts, as reported in freshwater mussels and several freshwater fishes from exposed regions (Yazdani 2020; Hussain et al., 2018). Therefore, detection of adducts may raise the possibilities of bio-indicator establishment as well as it documentation. A similar methodology was administrated to examine DNA damage in beluga whales to determine any possible exposure to benzo pyrene (BaP) (a potential carcinogen) (Pandey et al., 2020; Gajski et al., 2019).

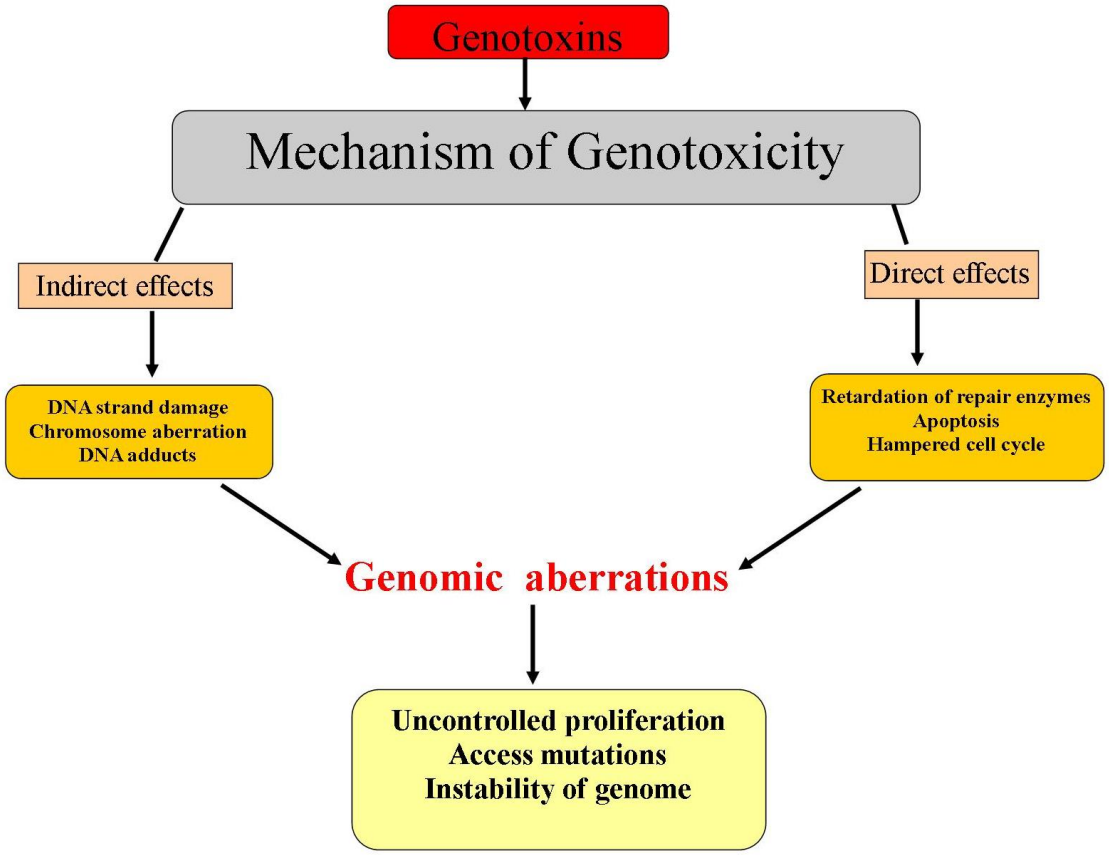

Fig 3. Genotoxic pathway resulting direct and indirect genomic aberrations

Environmental genotoxicity may be monitored with the help of the detection of DNA damage in an excessive form. On another side a similar approach was administrated on two species of turtles, *Chelydra serpentine, Trachemis scripta*, with the help of an alkaline DNA unwinding assay. Furthermore, the study was conducted on DNA strand break in sun fish, through the DNA alkaline unwinding method and establishment of biological marker related to

environmental genotoxicity. In freshwater streams, running water holds sediments containing metal, inorganic chemicals, and radio nuclides. These dissolved components may affect erythrocyte of the inhabitants with the help of erythrocyte micronucleus test on several species of fishes. The study of shellfish while assessing aquatic pollutants exhibited mutagenic effects in various water bodies. According to the present scenario, some major known toxicants in water bodies have gathered interest for the establishment of fishes in the form of indicators as carcinogens (Fig 3). Freshwater organisms have shown promising possibilities to be used as a carcinogenic indicator to assess and evaluate the water health of the water body (Crayton et al., 2020; Peixoto et al., 2017).

Different toxicants belonging to different categories as mutagenic, teratogenic, and carcinogenic may be easily assessed through the aquatic organisms as these organisms exhibit remarkable sensitivity against these toxicants even at very low concentrations. Fishes such as *Synodontis clarias* and *Tilapia nilotica* have shown promising prospects in the area of ecogenotoxicology by administrating micronucleus test and further its verification was performed through cytogenetic damage index as well as assessment of genotoxins in aquatic ecosystem (Jacquin et al., 2020; Farag 2018).

At the ground level, genetic ecotoxicology has shown potential outcomes due to the exposure to environmental genotoxicants in the form of the disease, reduction in reproductive success as well as genotypic diversity alterations by implementing checkpoints such as frequencies of gametes loss, mortality of early embryo due to lethal exposure of carcinogens, hampered development, and last but not the least any possible mutations making effects on the gene pool of exposed organisms.

Effects on exposed populations may be analyzed, where such population has been characterized ecologically, but on the other hand, related information regarding consequences of genotoxic effect on the genome is limited. The fundamental principle of estimating the adverse effects of genotoxins on genetic material is not entirely a new concept. Ecogenotoxicity may be evaluated and cross-checked through the evaluation of the current scenario and historical background of the organism with the help of molecular genetic techniques (Fanali et al., 2021; Halim et al., 2020). While studying heterozygous populations, studies emphasized the possibility that selected genotypes highly sensitive to genotoxic exposure may be established as bioindicators. Genotoxic exposures may behave as a selective drive through the elimination of susceptible genotypes. On the other hand, it may also reduce the number of generations that may be contributed in the future. Furthermore, similar reduction may be reported in the overall genetic alteration within the specific population or any possible change in genotypic frequencies (Bej et al., 2021; Ismail et al., 2018).

Involvement of ecogenotoxicology in the detection of environmental threats

Assessment of genetic hazards focuses on any possible alteration in the gene pool of aquatic organisms. It may include fishes or any other aquatic animal. A handful of review attempts were made to explain the role of ecogenotoxicology in environmental risk assessment but still regulatory authorities throughout the globe need a supportive database on the potential of genotoxic agents to produce genetic alterations (Cant et al., 2022; Paravani et al., 2019). As in the current situation, any pharmaceutical industry may not register any new drug formulation without furnishing mutagenicity analysis. In fact, under ecogenotoxicology, any possible impact of genotoxic agents on the populations and ecosystem is thoroughly examined. Mutagenicity testing of any genotoxic agent was administered on several organisms from

different populations related to any risk assessment of a particular genotoxic agent.

Preliminary tests of mutagenicity were performed on a battery (in-situ), and simultaneously with an in-vitro test of a carcinogen was also administrated based on a similar experimental design. The outcome of the study suggested the possible extrapolation to carcinogenic risk for humans by analyzing the prolonged exposure level, such level of risk was linearly extrapolated from the lowest possible effective dose. Assessment of ecological risk is directly related to a broader range of species on the contrary of human genotoxicology, where the target of the study is a population instead of a single individual (Anitha et al., 2019; Taju et al., 2017).Several test organisms were studied for carcinogenic tests and risk assessment. Initially, rats and mice were studied as extrapolation to human metabolism, making them suitable models for study. On the other hands, for the extrapolation with environmental conditions, carcinogenicity studies were administered on fish, crabs, and mussel to evaluate the ecological risk assessment protocol (Jena et al., 2021; Pandey et al 2018).

Utility of ecogenotoxicology model in ecological threat assessment

Several higher animals were treated by environmental toxicants ''in vivo,'' and different methods were administrated to assess the situation. Under the guidance of previous attempts, a few methods were selected by various researchers to establish the possible link between the carcinogenic agents and the genetic material of freshwater fishes, as well as the possibilities of bioindicators establishment were also explored. Some widely used methods are discussed below:

(i) Comet assay

This method was developed to assess any possible DNA damage caused by any toxicants(Fig 4).At the initial stage, this method was implemented to estimate the DNA damage in living eukaryotic cell. At a later stage, further advancements were administrated to improve the efficiency of the system, devising an electrophoretic microgel method in neutral conditions, and acridine orange was used to stain DNA. Furthermore, the alkaline process of the comet assay was designed (Hartman et al., 2018), and due to this advancement, the system developed a higher capacity of breakage sensitivity.

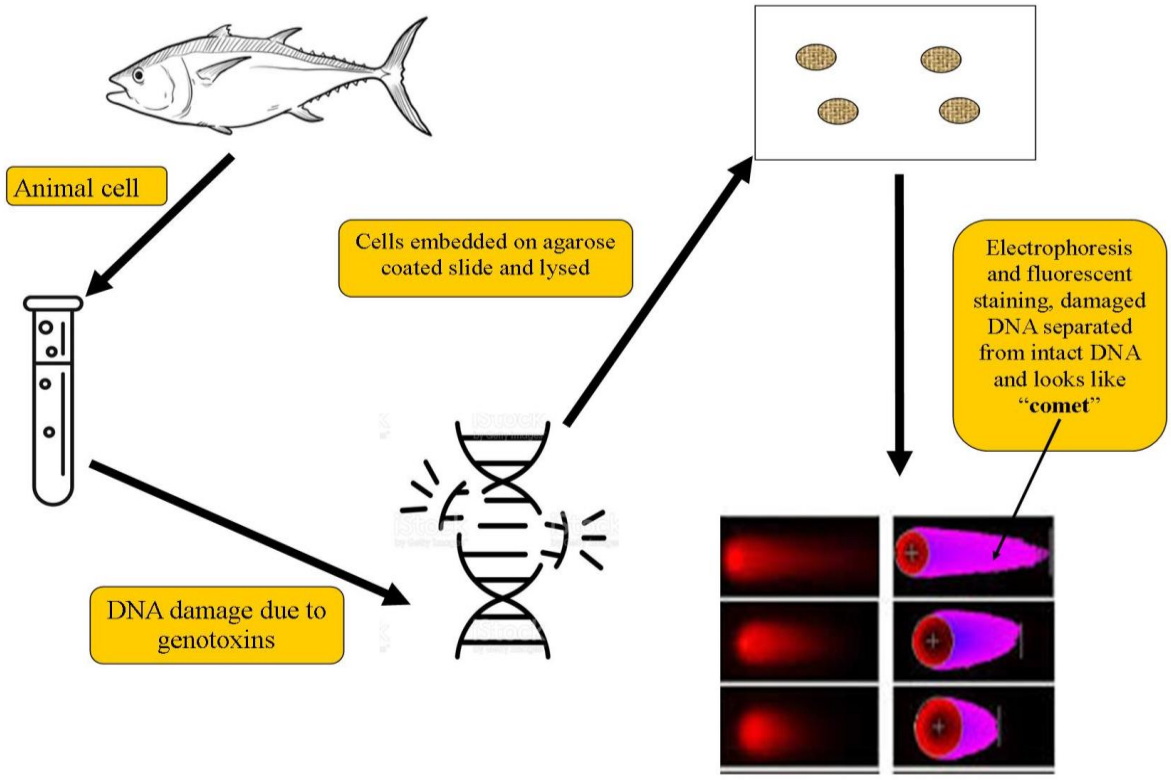

Fig 4. Graphical presentation of Comet assay

In this method, test tissue or cells were mixed with agarose (low melting) and were placed and lysed with the help of alkaline buffer and ionic detergents. Obtained and liberated DNA was resolved in an electrophoresis chamber and stained for further evaluation through fluorescence microscopy. During the observations, it was observed that cells with greater DNA damage exhibited rapid and higher movement from the nucleus to the anode (Catteau et al., 2022). The outcome in the form of comet-like structures was analyzed on the tail (length). Several review attempts are available related to the applicability of the comet assay in environmental toxicity assessment and its relevance to the wide range of freshwater organisms.

(ii) DNA unwinding assay (alkaline)

The value of DNA strand breakage concerning the total DNA content may be estimated by a particular assay named alkaline unwinding assay. The unwinding of DNA strands appears at the individual strand break. Therefore the double-stranded DNA content left after a specific period of alkaline treatment would be inversely proportional to the number of break strands. The reported ratio is presented in the form of 'F' value, and this recorded value is capable of measuring the relative double stranding capacity of a specific DNA. Similar in-situ studies were administered to detect the specific environment's genotoxic potential through the DNA alkaline unwinding assay, such as fish cells, juvenile fish stages, and crustaceans (Nikinmaa et al., 2020).

(iii) Chromosome structural aberration assay

This assay focuses on the chromosomal aberrations, including aberrations related to structure, for instance, fragments and different aberrations taking place due to direct DNA

breakage or the DNA synthesis inhibition. One of the promising features of this assay is that cytogenic impacts may be recorded in animals (in vivo) and in culture-developed cells (in vitro). In the routine process, cultured cells are exposed to the specific component and afterward exposed to Colcimide or Colchicine, a metaphase-arresting factor. Several workers performed similar cytogenic attempts on *Myxine gluttinosa* and several other freshwater fishes (Esmaeilbeigi et al., 2021).

(iv) Micronucleus assessment

Several fragments of a chromosome or a complete strand of chromosomes, those are not administrated in the nuclei of daughter cells and further reported in the cytoplasm, are known as micronuclei. Micronucleus assessment is a very effective and highly sensitive method 'in vivo' examination of genotoxic properties related to different factors. Ecological bio-analysis through micronucleus assays is normally performed ''in vivo'' under the exposure of related test organisms. After that, a microscopic analysis of different components such as erythrocytes and gill is performed. As previously recorded, chromosomes in the fish cells are normally small in size and found in large numbers, making fish a very effective tool for micronucleus assay (Mitkovska et al., 2020).

(v) SCE test

SCE analysis stands for Sister Chromatid Exchange test. It is an efficient tool to detect any possibility of reciprocal exchange of DNA segments among the chromatids of two sister chromatids belonging to a double chromosome (Bikham et al., 2018). So far insufficient database is available related to the molecular basis of SCE frequency in the effect of mutagenic agents. However, this method has still exhibited some promising outcomes as a model for genotoxicity in several organisms, such as freshwater mussels and fish cells (Raat et al., 2018).

Latest developments in ecogenotoxicity

Recently, some promising advancements were reported related to genotoxicology in freshwater organisms. In some investigations, attempts were made to establish zebra fish as the model for mutagens detection, as the plasmid holds rpSL gene as a mutational target gene. Furthermore, Rai *et al*. (2018) developed transgenic fish with a holding capacity of multiple copies of a bacteriophage lambda vector, which can harbor cII gene in the form of a mutational target. Similarly, in other freshwater fish studies recorded p53 tumor suppressor gene may be further examined as a possible biomarker for genotoxins in fishes (Dunn 2016; Saha et al., 2016).

During a study, a series of ponds (heavily contaminated) with the dominance of *Gambusia affinis* population were studied for multiple years and observed the inverse correlation between DNA damage and fecundity of fish (Everaarts & Sarkar 2019; Bej et al., 2017). Observed findings were implicated for higher-order environmental impacts, as well as a contaminant-induced selection of specific phenotypes.

Higher mutation tendency due to environmental toxicants may negatively affect the specific population and is still under debate among researchers. On the other hand, an increasing number of studies involving ecogenotoxicity are available, although identifying clear cause-effect relations is increasingly complicated. Some recent investigations have furnished the evidence that genetic diversity has elevated among the fish population holding the radionuclide-contaminated regions related to the sites. Such information has been supported by allozyme analysis and randomly amplified polymorphic DNA technique (RAPD).

Certain individuals exhibit particular banding patterns at toxicant affected site, exhibit higher fecundity rate and lower tendency of DNA

strand damage. Similar patterns were also recorded with allozyme study, especially at the site of nucleoside phosphorylase locus in the contaminated locations. Based on records, it may be stated that heterozygotes exhibited higher fecundity and lower DNA strand damage than homozygotes (Cajaraville et al., 2019; Sarkar et al., 2016).

Limitations in ecogenotoxicology

So far, genetic ecotoxicology would be addressing environmental genotoxicity as disease, reduction in reproductive success, and alteration in genotypic diversity with the help of several endpoints such as gametes loss tendency due to cell death, lethal mutations resulting in embryo death, retarded development, and cancer. However, at ground level, different methods may only assess the endpoints, such as gamete loss or teratogenic.

Most of the presently available genotoxicity tests were developed in late 80's. Still, researchers are looking for the exact site and mechanism of genotoxicity in various organisms and environments. Certain studies mentioned that the target site of toxic action might not be the similar target site of administrated toxin. In acute or sub-acute toxicity testing, multiple endpoints were reported to determine toxicity, but similar findings were not recorded in the case of genotoxicity assay. Moreover, in the case of various reagents still no details are available in terms of specific test system or protocols. In most of the guidelines, strong and effective recommendations are still wanting, and existing findings are incapable of establishing specific genotoxins causing damage to DNA strands. Furthermore, no accurate suggestions are available related to a threshold level of genotoxins compounds and their organ-specific impacts (Odeigah & Osaneyinpeju 2019; Patel et al., 2016). At last may be concluded that looking for a single testing system protocol for detection of specific genotoxins has still a long way to go.

Conclusion

As a concluding remark, it may be stated that environmental genotoxicology exhibits the promising potential for early identification and analysis of toxicants in aquatic ecosystems, especially in fish, crabs and mussels or any test animal. Fishes have been used as a strong genetic model for toxicant detection. Thorough investigations have highlighted the role of fishes and other aquatic organisms in understanding the process of mutagenesis and its adverse effect on the population's reproductive success. Different genotoxicity tests on various aquatic organisms were conducted, and their implications were analyzed. Based on such investigations may be stated that fish cells have a great potential to be established as a genotoxicity indicator due to certain traits such as, lower repair mechanism, xenobiotic metabolism and poikilo-thermic nature. As already mentioned that fishes hold a slower repair mechanism when compare to mammals, make them a strong sentinel organism for ecological bio-monitoring with the help of various tools and techniques such as comet assay, DNA repair synthesis, chromosomal aberration test and sister chromatid exchange assay. Screening of genotoxins should also include environmental genotoxicology of the gene pool along with population to enhance the sensitivity and specificity of test protocols.This should enable us to use the unique markers for cytogenetic damage to be used as a bio-indicator, as an early warning and to devise the monitoring strategies to determine the health of the ecosystem. Further, exhaustive attempts to improve these tools in detection of early genotoxicity will help formulating long term plans and strategies for aquatic environmental conservation.

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