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Toxicogenomics for water quality assessment

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ABSTRACT

Water streams receive a huge amount of wastes from different sectors including urban areas, industrial and agricultural activities. Hence, water bodies will be contaminated with complex, ill-defined mixtures of chemicals and most water organisms will be exposed, to varying degrees, to this contamination. In this context, chemical analysis of water is not sufficient to assess their toxic potential for wildlife and humans. This is because the bioavailability, the biological activities, and interactions between different environmental chemicals are not completely understood and considered when hazard assessments and predictions of possible ecotoxicological effects are made based on concentrations alone. Therefore, a simultaneous application of bioassays is a good complement to chemical analyses and a useful tool to establish the ecological effects to environment, as it provides the complete response of test organisms to all the compounds in the water..Among the methods available to assess the many possible toxic effects caused by the chemicals present in the environment, the analysis of DNA alterations in aquatic organisms has been shown to be a highly suitable method for evaluating the genotoxic contamination of environments, being able to detect toxicity at low concentrations of contaminants in a wide range of species. The pollution-induced genetic damage might cause adverse effects to species and affect the stability of ecosystems.

In this mini review we focused our attention on the relevance of different biological assay techniques on genotoxic potential and water quality assessment.

INTRODUCTION

Majority of aquatic systems are polluted due to admixture of domestic waste, industrial effluents, and many other pollutants that are adversely affecting the human health and the ecosystem of the water bodies. The aquatic environment constitutes the major part of our life so; its safety is related to human health safety.As a result of anthropogenic activities, water systems worldwide are subjected to thousands of pollutants. Nowadays, monitoring of important pollutants is a must; however dealing with all pollutant compounds would be practically impossible. Moreover, the definite effects of polluted environment on human health remain mostly unknown since compound toxicity data is often absent. With the release of increasing amounts of new chemicals into the aquatic environment, new monitoring strategies are requiredfor best assessment of water quality on human health. About 300 million tons of synthetic compounds are used annually in industrial and consumer products, and partially find their way to natural water [1]. Such contamination can become an increasing problem for aquatic environment [2, 3]. Many of such chemical compounds (such as hormones) raise concern, especially when the effects on various physiological endpoints are unknown. However, chemical analytical monitoring of all individual compounds would be practically impossible. Moreover, for majority of compounds the effects on biota remain unknown since toxicity data is often

absent. Therefore, sensitive in vitro bioassay tests should be applied [4]. There are some advantages to bioassay tests: (i) they can detect unknown compounds that trigger a specific biological effect and (ii) the effects of entire mixtures of compounds present in a sample can be determined. However there is a disadvantage of bioassays application their focus on a relatively narrow selection of physiological endpoints and the ecological / human relevance often remains unclear [5].

Toxicology may be defined as the study of stressors and their adverse effects on living organisms. One sub-discipline deals with hazard identification, mechanistic toxicology and risk assessment. Detailed understanding of action mechanisms of chemicals being assayed will improve the efficacy of tasks. The derivation of some mechanistic knowledge traditionally evolves from studying a few genes in order to implicate their function in mediation of toxicant effects. As a result of development of thousands of new compounds has to be accelerated [6].Using biological monitoring techniques by fish and other aquatic biota offers the possibility of checking water pollution with fast responses on low concentrations of direct acting toxicants [7, 8, 9].

The rapid development and evolution of genomic-[10], proteomic[11], and metabonomic- [12]based technologies has accelerated the application of gene expression for understanding chemical and other environmental stressors' effects on biological systems. All of the previous technologies lead to the development of a new field, "toxicogenomics", which proposes to apply global mRNA, protein and metabolic analysis related technologies to study the effects of chemical hazards on living organisms [6].

Comet assay and micronucleus (MN) test are widely applied in genotoxicity testing and biomonitoring. While comet assay permits to measure direct DNA-strand breaking capacity of a tested agent MN test allows estimating the induced amount of chromosome and/or genome mutations [13].

The following article provides a relatively comprehensive mini review upon potentiality of the micronucleus test and comet assay for assessment of DNA damage and accordingly it can be used as an informative platform in toxicogenomics studies of water pollution effect on living organisms.

Micronucleus

Among the tests for genotoxicity, the micronucleus test has been widely utilized in fish to determine exposure to water pollutants, in the environment as well as under experimental laboratory conditions **[**14]. Micronuclei are structures that contain chromosome fragments without centromeres (acentric fragments) and/or whole chromosomes that are unable to travel to the spindle poles during mitosis[15,16].The MN test is a very sensitive tool for DNA assessment at the chromosomal level as it can measure chromosome loss and breakage also [15]. Previously, metaphase analysis was used in analysis of numerical and structural chromosome aberrations; however it is time consuming and needs skilled persons. The MN test was used as simple screening technique and considered as an alternative to the chromosome aberration assay. By using MN technique, chromosome aberrations are detected indirectly via chromatin loss from the nucleus leading to MN in the cytoplasm of the cell [17, 18]. MN can be detected only in dividing cells. Adding to cell cultures cytochalasin-B, an inhibitor of the mito-tic spindle that prevents cytokinesis, permits to recog-nize cells that have completed one nuclear division by their binucleated appearance [19].

Water pollution was estimated in three tilapia species (*Oreochromisniloticus*, *Oreochromisaureus and Tilapia zilli*) and Clariasgariepinus by using micronucleus test. The test has been used successfully as a toxicogenomic technique. Micronucleus test does not depend on any karyotypic characteristics; it is simple, reliable and sensitive. Study results recommended using micronucleus test in the assessment of water pollution and aquatic mutagens [20]. Another study used MN assay in water pollution evaluation was done by Kumar, [21]. Fresh water fish *Channapunctatus* was used as a model for estimating water pollution. Study results recommend using MN in fish erythrocyte as a sensitive indicator for evaluation and assessment of aquatic pollution. Abdel-Gawad [22]used micronucleus test as a molecular biomarker to study the effect of aquatic contaminants on tilapia fish.

Comet assay

The single cell gel electrophoresis (SCGE) assay, commonly called the comet assay, is a genotoxicity test able to detect DNA damage induced by alkylating, intercalating and oxidizing agents **[**23]. The comet assay is a rapid and very sensitive fluorescent microscopy-based method for measuring DNA damage, protection and repair at the level of individual cells [24].In this assay cells are embedded in agarose, lysed and then electrophoresed. Negatively

charged broken DNA strands exit from the lysed cell under the electric field and form a comet shape with "head" and "tail". The amount of DNA in the tail, relative to the head, is proportional to the amount of strand breaks. The limit of the comet assay sensitivity is approximately 50 strand breaks per diploid mammalian cell. In order to achieve various objectives, different modifications of the comet assay have been developed. In its alkaline version, which is mainly used, DNA single-strand breaks, DNA double-strand breaks, alkali-labile sites, and single-strand breaks associated with incomplete excision repair sites results in increased DNA migration. In the neutral type the DNA molecule itself appeared as double stranded structure which enables uncovering of double stranded DNA breaks [25].In accordance with international guidelines for genotoxicity testing, comet assay is recommended for follow-up testing of positive in vitro experiments. It is particularly useful as a tool for the evaluation of local genotoxicity, especially for organs/cell types which cannot be easily evaluated with other traditional tests [16].

Comet assay is a very important technique for monitoring genotoxicity in aquatic environment.For this purpose, fishes were used as test organisms in which it is possible to detect DNA damage induced by direct mutagens and pro-mutagens in both fresh and salt water **[**26]. Comet assay was employed in the determination of the genotoxic potential of water resources such as rivers and lakes. Meanwhile, the comet assay has been proposed as a tool to monitor genotoxicity in ocean and continental waters, utilizing fish for the detection of DNA damage induced by direct-acting mutagens and pro-mutagens dissolved in the water as well as environmental analysis of water samples **[**27].

Abdel-Gawad*et al.*, [7]used comet assay for environmental assessment of pollution on aquatic insects and fish in River Nile, Egypt. The study results suggested that using genotoxicity tests as comet assay in aquatic biota provided adequate sensitivity to be used in monitoring water pollution.Both MN and comets appear by loss of DNA material from the nucleus in micronuclei and in comet tail, respectively. Therefore, both methods reflect secondary rather than primary effects of DNA damage[13].

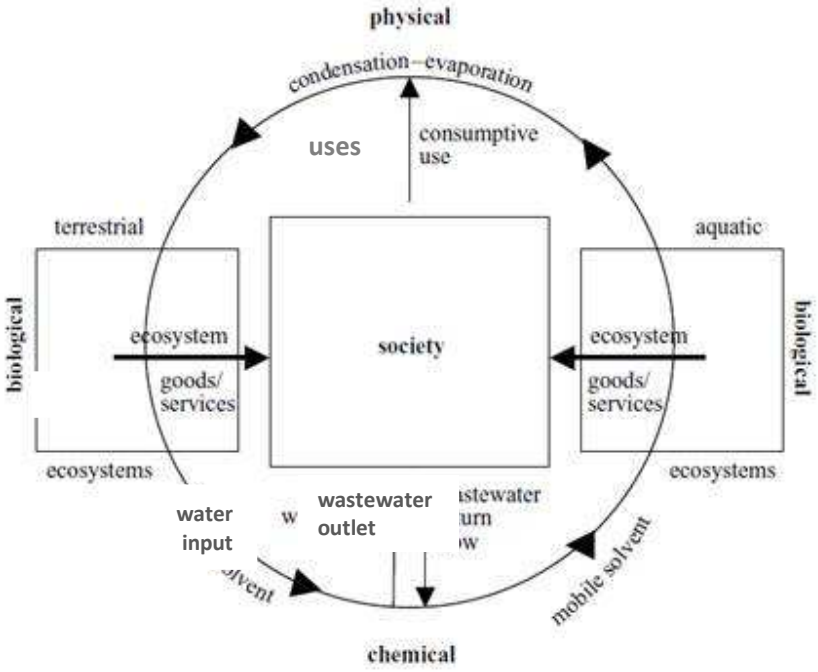

Figure 1: Water and wastewater cycle in the environment

Water quality assessments

One of the principal hygienic problems of all time, including the present, is the quality of the water, which should be made available to man in accordance with his physiological needs. No specification that can be applied to water for human consumption, with respect to physiological criteria for its chemical composition, can ignore the contribution made to the regular human intake of these components through food consumption and breathing of the ambient

atmosphere as shown in figure 1. The compositions of water, which will best maintain life in the flora and fauna of the earth is the subject of discussion. Environmental pollution and industrialization on a global scale have drawn attention to the vital need for developing new hygienically friendly purification technologies [28].The pollutants enter into the aquatic bodies through sewage and with the runoff from agricultural wastes. Many of these polluting agents contain a number of chemicals that are highly persistent and have mutagenic and/or clastogenic properties. Typical examples are the elevated cancer rates in fish exposed to polycyclic aromatic hydrocarbons (PAHs) and to copper mine wastes in surface water [29]. Polycyclic aromatic hydrocarbons (PAHs) and their nitratedderivatives (NPAHs) are ubiquitous organic pollutants in the environment showing carcinogenic and/or mutagenic health effects [30,31,32]. Several studies have shown that a wide range of chemical pollutants in aquatic ecosystem affect essential physiological functions in various aquatic organisms and causes adverse effects at cellular and molecular levels [33, 34].

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