MUTAGENIC EFFECT OF SOME ENVIRONMENTAL POLLUTANTS IN THE EGYPTIAN LAKES AS REVEALED BY SHORT TERM ASSAY

By

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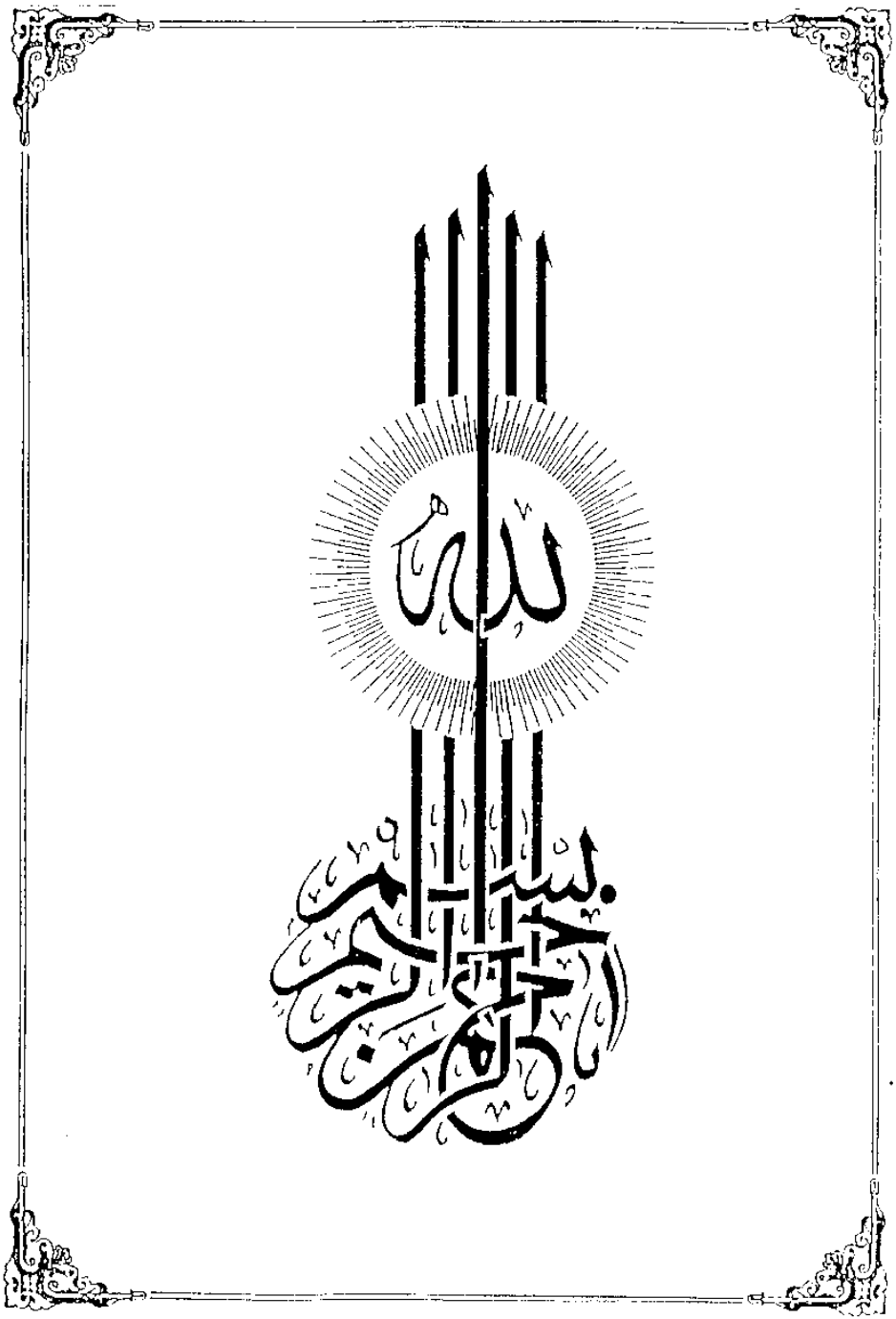

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Abstract

Mutagenic activity of some Egyptian lakes water, i.e., Qaroun, Brolus and Mariut were tested using different short term assays of mutagenicity. Different end points in two eukaryotic organisms (yeast *S. cerevisiae* and *D. melanogaster*) were examined. These end points could be categorized in two levels (genetic markers and protein level). The results obtained using the surface water showed that Brolus water has significant mutagenic response more than Qaroun and Mariut water.

Keywords: Water; Mutagenic activity; Drosophila; S. cerevisiae

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1. INTRODUCTION

Much concern has been focused in the last two decades on water-borne mutagenic and/or carcinogenic substances. Because water is a good solvent for a large number of chemicals, hundreds of chemicals (several classes) are usually present in water and others make a suspension with it. Many authors have reported the presence of mutagens in water. Always the rivers water is the main source of drinking water. The presence of mutagenic substances in rivers water suggests the possibility of health hazards to people in the future because of the high correlation between mutagenic and carcinogenic potentialities of many chemicals (Sugimura *et al.*, 1977). Therefore, more extended investigations on mutagenic activities of pollutants in rivers water are considered to be necessary.

On the other hand, drinking water which is usually supplied by rivers or lakes contains different amounts of natural and anthropogenic organic compounds from many sources (e.g. agriculture, industry, etc.). Many the compounds in finished (treated) water probably are formed by reactions of the constituents of the untreated water with chlorine during disinfection. Certain of these compounds (e.g., dichloromethane, chloroform) are known to be mutagenic and/or carcinogenic in experimental organisms at doses many thousands times greater than levels to which man is exposed through the water supply (Nestmann *et al.*, 1979).

In more recent studies by Vartiainen *et al.*, (1992), they concluded that, chlorinated drinking water in many countries contains a compound, (3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone) which seems to be responsible for one third to over one half of the mutagenicity of drinking water by using *Salmonella* strain TA100.

In Egypt, The cropping pattern and continuous use of land caused an

increase in pest populations which make the use of pesticides essential for crop production. On the other hand, the wide spread use and often the abuse (including application and waste) of agrochemicals and the dramatic waste of industrial and/or communal waste in water sources including lakes, led to heavily polluted areas which might contain a variety of carcinogenic and mutagenic compounds.

Qaroun, Brolus and Mariut are large lakes occuping different area in Egypt. Plenty of contaminants are drained in these lakes which different in quantity and quality. The obvious pollutions of the lakes are probably due to agricultural and industrial activities. Meanwhile, the growing urbanization of rural area around the lakes contributes to the sizable pollution scored in each lake. Fishing and salts productions are the main activities carried out in the lakes which give the struggle against pollution its national economic priority.

Moreover, trials have been already started to use some of the Egyptian lakes as phycofarms to increase algae production either in quantity and/or in quality. The importance of algae as one of the best sources of proteins and fine chemicals makes its improvement a highly promising field. In addition, algae play a sizable role in the recycling of heavy metals from brackish and salty water.

As a trial to find out more sensitive techniques to test the mutagenic effect of the crude water than the available techniques which use the extracts or unconcentrated water, the present study suggests two techniques in two eukaryotic organisms, yeast and *Drosophila* to be used in this respect.

The first technique depends on analysis of total protein in *S. cerevisiae*. As proteins are direct product of genes since the biosyntheses of different proteins is controlled by specific genetic codes. Therefor, mutants could affect proteins as gene(s) product(s) especially in the case of non-sense mutations and/or differential regulations (switching on and off). Polyacrylamide gel electrophoretic

techniques offer an excellent opportunity to study the sub-structure differences in proteins among different genotypes and for detecting protein alterations. Since the different electrophoretic bands represent transcriptional and subsequent translational events, it is possible to test the mutagenic effect of any substance by comparing of protein electrophoretic banding patterns between the treated and untreated yeast cells.

The second technique is the mutagenic effect on enzyme loci in *D. melanogaster*. The differences in electrophoretic mobility as detected by starch gel electrophoresis reflect the allelic variations of the enzymes. The changes in the gene frequencies between untreated and treated flies might be due to mutational events.

The present investigation was designed to study the comparative mutagenic potentiality of the surface water of three Egyptian lakes namely, Qaroun, Brolus and Mariut. Several methods have been employed to achieve this goal, they are:

1- The induction of mitotic gene conversion, reverse mutation and mitotic crossing over in *S. cerevisiae* strain D7.

2- The induction of aneuploidy and mutations other than monosomy in *S. cerevisiae* strain D61-M.

3- The induction of total protein variation in *S. cerevisiae* strain D7.

4- The induction of sex linked recessive lethal mutations in *D. melanogaster*.

5- The induction of enzyme loci mutations in *D. melanogaster*, as the frequencies of alleles controlling the production of eleven enzymes are subject to alterations. These enzymes are Est, Adh, Acph, Ao, Aph, Odh, 6-pgd, α-Gpdh, Mdh, Me and Pgm.

2. REVIEW OF LITRATURE

Recent surveys have revealed the widespread occurrence of trace levels of several classes of chemicals in water. Although many of these substances have probably harmful effects including mutagenic and/or carcinogenic activities, it is very difficult to identify every contaminant of water and it is also more difficult to test every known compound for its mutagenicity or carcinogenicity. On the other hand, because of the small quantities of contaminant material present in water, more sensitive and adequate test methods have been adopted in this respect.

Mutagenic response of contaminants (i.e, organochloride pesticides, polyaromatic hydrocarbons, organophosphorous pesticides and trialkyl-arylphosphate) of drinking water was observed by Williams *et al.*, 1982. They found dose-related increases in mutagenicity due to extracts from 11 of the drinking water supplies.

In Egypt, the contamination of the river Nile water with organic pesticides has been reviewed (Aly and Badawy, 1984 Badawy and El-Dib, 1984 Badawy and El-Dib, 1985). They reported that there are some polluted insecticides such as organo-chlorin and poly chlorinated biphenyls (PCBs) were found in the river Nile water, they also found residual levels of the studied compounds in the river samples followed the order: DDT> endrin> lindane> BHC. According to (Edwards, 1978) persistence of organo-chlorine insecticides followed the order: DDT> dieldrin> lindane> chlordane> heptachlor> aldrin. It's important to notice that the presence of trace quantities of organo-chlorne and PCBs in natural water may generate concern in regard to public health. Meanwhil, it has been reported that DDT, DDE and PCBs were able to induce the formation of hepatic enzymes in birds and mammals (El-Dib and Badawy, 1985)

2.1- Application of short-term mutagenicity test for the examination of water pollution.

2.1.1- Microbial mutagenicity assays

The *Salmonella* mutagenicity assay (Ames test) has been used extensively for evaluating the mutagenicity of water samples. Among the five tester strains, originally recommended by Ames and coworkers (1975) for routine screening purposes (i.e., TA1535, TA1537, TA1538, TA98, and TA100) TA98, and TA100 have been reported to be the most sensitive for detecting the mutagenicity of drinking water. These two strains are widely used in most *Salmonella* Ames tests. They are also very sensitive to mutagens in general (Meier J.R., 1988). Few studies on drinking water concentrates have been done with *Salmonella* strains (TA98, TA102 and TA104) whichwere recently recommended for mutagenicity screening (Maron and Ames, 1983).

Loper (1980) discussed in details some important issues about the data production and analysis which differ in several respects from recommendations made for testing pure chemicals. It is important to reemphasize on one recommendation in particular: namely the need to perform assays with multiple doses.

Results from such studies do not meet the most widely accepted criteria for the determination of mutagenicity in the Ames test which requires a dose-related increase response (De Serres and Shelpy, 1979; Maron and Ames, 1983). The interpretation of the findings can be very misleading without knowledge of the dose response characteristics, i.e., it is particularly meaningless to make comparisons among different water treatment processes without some indications that the responses observed are increasing with dose or not. For quantitative comparisons of data, assurance is needed that the responses at the selected dose

within the initial linear portion of the dose-response curve should be statistically modeled to estimate potentialities.

At the Water Research Center in England a two-step fluctuation procedure has been accepted in which the bacteria are treated with the test sample in liquid medium containing histidine and biotin for 16-18 hrs prior to incubation for 48 hrs in selective medium lacking histidine (Forster *et al.*, 1983). In a liquid suspension of fluctuation tests which detect reversion to histidine-independent, growth in the Ames *Salmonella* tester strains has also been used extensively for mutagenicity analysis of water samples (Forster and Wilson, 1981; Forster *et al.*, 1983; Wilcox and Horth, 1984; Wilcox and Denny, 1985; Monarca *et al.*, 1983, 1985a,b and Harrington *et al.*, 1983).

The fluctuation procedure has some possible advantages over the Ames plate test method in that (1) it is reportedly more sensitive for the detection of certain compounds than plate procedures; (Green *et al.*, 1976); (2) aqueous samples can be directly incorporated in the assay in volumes up to 95% of the total medium volume; and (3) stimulatory as well as inhibitory effects of the sample on the growth cells can be directly determined without additional plating step. Stimulatory effects of unconcentrated water samples on bacterial growth probably gave false positive mutagenicity results (Forster *et al.*, 1983; Herringtone *et al.*, 1983; and Monarca *et al.*, 1985 a). The main disadvantage of the fluctuation method is that it is a more tedious and time-consuming procedure than the Ames plate assay since it requires dispensing reagents into multiple tubes or multi-well plates (usually 48 or 96) for each dose sample, although this operation can be automated. The 2-step procedure requires an extra set of additions to selective medium for each well. Monarca *et al.*, (1985 a) have used a micro scale fluctuation test procedure (using microtiter plates) to test the mutagenic activity in water

concentrates, and have indicated that the sensitivity of this method permits examining smaller water volumes.

In addition to the numerous studies demonstrating mutagenic reversion to histidine independence in *S. typhimurium*, the ability of drinking water concentrates to induce the SOS Chromotest response, measure of DNA damage, in *E. coli* has been shown by one group using the SOS Chromotest (Bourbigot *et al.*, 1986).

2.1.2-Eukaryotic assay systems

The use of eukaryotic assay systems, and in particular mammalian cell assays, is often cited as being more relevant for the determination of the mutagenic activities of chemicals to humans. Certainly on theoretical grounds this is a reasonable assumption because of the substantial differences in cellular membranes and the organizational and functional aspects of DNA between eukaryotes and prokaryotes. However, the over all concordance between carcinogenic response in rat or mice and *in vitro* mutagenic and carcinogenic response appears to be similar for the *Salmonella* assay and any of three widely used system assays with mammalian cells (i.e., chromosomal aberrations or sister-chromatid exchange in chinese hamster ovary cells, mutations at the thymidine kinase locus in mouse lymphoma cells) Tennant *et al.*, (1987). This finding is based on results from only a small subset from the National Toxicology Program database (73 chemicals) and may change as the number of chemicals evaluated in the short-term tests increase. Nevertheless, at present little seem to be gained in terms of predicting the carcinogenicity of a chemical in rodents by screening chemicals with numerous short-term assays (John R. Meier,1988). Systematic comparison of responses of drinking water concentrates in different assay systems have not been made, but several workers have reported results for bacterial and eukaryotic assays on the same water sample. Therefore, in the brief surveys of the