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Assessing the environmental/human risk of potential genotoxicants in water samples from lacustrine ecosystems: The case of lakes in Western Greece

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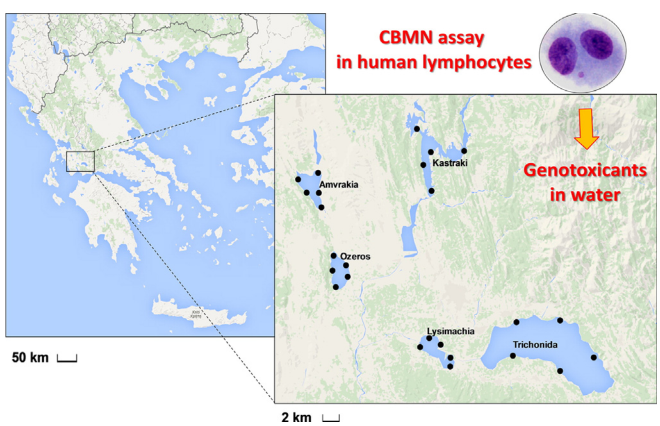
HIGHLIGHTS

- The water quality from 5 lakes in Western Greece were assessed.
- The CBMN assay was used for assessing the presence and human risk of genotoxicants.
- Water samples from the 5 lakes were genotoxic in cultured human lymphocytes.
- The presence of genotoxicants in the studied lakes could enhance their human risk.

GRAPHICAL ABSTRACT



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ABSTRACT

Lakes, representing major freshwater resources, play a crucial role for both humans and ecosystems. Based on the increasing international interest in the contamination of water resources by genotoxic compounds, the present study aimed to evaluate the genotoxic potential of surface water samples collected from the five (5) lakes (Amvrakia, Lysimachia, Ozeros, Trichonida, Kastraki) located in Aitoloakarnania regional unit (Western Greece). The genotoxic potential of surface water samples was evaluated by employing the Cytokinesis Block MicroNucleus (CBMN) assay in cultured human lymphocytes. In the former assay, lymphocytes were treated with 1, 2 and 5% (v/v) of surface water from each lake. Statistically significant differences (1.7 to 3.3 fold increase in MN frequencies vs. the control) were seen at the dose of 5% (v/v) in all studied lakes. At the dose of 2% (v/v) statistically significant differences (1.7 to 2.6 fold increase in MN frequencies vs. the control) were observed in all studied lakes except Trichonida lake. Finally, at the dose of 1% (v/v) statistically significant differences (2.3 and 2.5 fold increase in MN frequencies vs. the control) were observed in the Ozeros and Lysimachia lakes. The evaluation of the potential genotoxic effects and the analysis of the physicochemical parameters of lakes' surface water samples is a first step in our effort to evaluate the water quality, in terms of the presence and environmental/human risk of

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genotoxicants in the studied lake ecosystems. The present study showed for the first time the presence of genotoxic substances in surface waters of the studied lakes.

1. Introduction

Lacustrine systems, represent major freshwater resources, thus playing a crucial role for both humans and ecosystems. Given that the surface waters of lakes are commonly used as a source of drinking water as well as for agricultural and recreational activities around the world, their contamination is becoming a major problem worldwide due to their impairment and the concomitant enhancement of deleterious effects on fauna and flora as well as humans by way of food and water supplies (Ohe et al. 2004; EEA, 2011, 2015). In Europe, where >500,000 lakes have been reported, with the majority of them (about 80–90%) to be small (surface area between 0.01 and 0.1 km^2), while almost 16,000 lakes have a surface area exceeding 1 km^2 (Kristensen and Hansen, 1994), the European Environmental Agency (EEA, 2015) reported a serious contamination of their surface waters with a wide range of anthropogenic chemicals and hazardous substances, mainly derived from industrial, agricultural, domestic sources and municipal sewage treatment plants, as well as from surface runoff and atmospheric deposition (White and Rasmussen, 1998; Ohe et al., 2004; Konstantinou et al., 2006; Zegura et al., 2009).

Although the progressive reduction of hazardous substances, as well as the definition of environmental quality standards (EQSs) for priority substances, into surface waters is an immediate priority (WFD; Directive 2000/60/EC; Directive 2013/39/EU; reviewed by the Official Journal of the European Union, 2013), the physical-chemical analysis of water samples routinely performed for assessing the presence of hazardous substances or priority pollutants into aquatic ecosystems do not represent a reliable tool for assessing water quality. In fact, the existence of additive, synergistic or antagonistic effects of chemical substances, being present in mixtures in natural waters, are not always clear, since most of them are present at low or even hardly-determined levels in surface waters (Pollack et al., 2003), a fact that could underestimate the observed harmful effects of hazardous substances upon freshwater (including lakes) biota (Csillik et al., 2000; Isosaari et al., 2006; Quiroz et al., 2010; Charalampous et al., 2015). For instance, Helma et al. (1998) stated that the adverse effects of chemical substances, being present in complex environmental samples, could not rely on chemical characterization due to limitations in predicting their synergistic and antagonistic effects. In this context, the use of bioassays is highly recommended for integrating the additive, synergistic and antagonistic effects of contaminants (Zegura et al. 2009; Tsarpali et al. 2012; DeFu et al., 2015; Charalampous et al., 2015). Among them, in vitro mutagenicity/ genotoxicity assays, such as the Cytokinesis Block MicroNucleus (CBMN) assay, should also be included in water quality assessment in order to efficiently estimate the presence of genotoxic substances in surface waters (Pellacani et al., 2006; Charalampous et al., 2015; Wernersson et al., 2015), without necessarily previous physicochemical characterization of water samples (Jha, 1998; Ohe et al., 2004; Ginebreda et al., 2014).

Although many studies related with the investigation of both genotoxic and cytotoxic effects of surface water samples have been performed mostly in plant cells (Grant et al., 1992; Ma et al., 1995; Kong et al., 1998; Cotelle et al., 1999; Unyayar et al., 2006; Pellacani et al., 2006; Liu et al., 2013; Li et al., 2014), there are several limitations when extrapolated to human beings due to the obvious differences between animal and plant cells. In this context, the CBMN assay, representing a simple, rapid and sensitive genotoxicity screening method, could be used for assessing the risk of different types of chemical substances and their ability to cause genetic damage and carcinogenetic processes in humans (Bonassi et al., 2011; OECD, 2014). The CBMN assay detects the potential clastogenic and aneugenic activity of chemicals in cells that have undergone cell division after exposure to chemical currently tested (OECD, 2014).

Taking into consideration that (a) genotoxicants (mutagenic and carcinogenic compounds) in water can cause genetic damage by different mechanisms, (b) the use of in vitro assays is increasing for ethical reasons versus animal experimentation and (c) the use of the CBMN assay is highly recommended as an acceptable experimental tool, in order to evaluate the genotoxic effects of contaminants in freshwater samples (Connon et al., 2012; Charalampous et al., 2015; Wernersson et al., 2015; Han et al., 2016, Simonyan et al., 2016), the present study was assessed for the first time the genotoxic potential of surface water samples collected from the five (5) lakes located in Aitoloakarnania regional unit (Western Greece), in human cells (cultured human lymphocytes) by the use of the CBMN assay. The evaluation of the potential genotoxic effects as well as the analysis of the physicochemical parameters of lakes' surface water samples is a first step in our effort to evaluate the water quality, in terms of the presence and environmental/ human risk of genotoxicants in the studied lake ecosystems.

2. Materials and methods

2.1. Description of the study areas

The present study concerns five lacustrine ecosystems; four natural lakes (Amvrakia, Lysimachia, Ozeros, Trichonida) and one artificial (Kastraki Reservoir), located in Western Greece (Fig. 1). The main features of these fresh water environments are given in Table 1, while details are provided in the supporting material file (SM 2.1).

2.2. Water samples collection

Water samples were collected from 5 different sampling sites along each lake (depth 0–0.5 m; almost 5 L of water collected from each point source) in October 2014 (Fig. 1). The established sampling sites are considered representative stations for assessing water quality, being as close as possible to those previously reported in the literature for comparing our measurements (Overbeck et al., 1982; Koussouris et al., 1993; Psilovikos et al., 1995; Tafas et al., 1997; Doulka, 2010; Chalkia et al., 2012; Chalkia and Kehayias, 2013a, b; Thomatou et al., 2013a). All samples from each lake were pooled and immediately transfer to laboratory, filtered by a Whatmann GD/X filter (0.25 mm and 0.2 μm pore size) in order to remove dissolved particles (e.g. dust, parasites, bacteria, viruses) and further maintained in sterilized bottles at −80 °C for further analysis.

2.3. Physicochemical analysis of surface water samples

The estimation of pH, conductivity (Cond), salinity (Sal) and total dissolved solids (TDS) was performed in situ with the use of a HQ40 multi-meter (Hach Lange GmbH, Germany). The determination of chemical oxygen demand (COD, with the use of open reflux method), nitrates and nitrites (NO_3^--N and NO_2^--N), ammonia-nitrogen (NH_4^+-N), chlorides (Cl^-), total suspended solids (TSS) and volatile suspended solids (VSS), were measured according to Standard Methods for the Examination of Water and Wastewater (Clesceri et al., 1999). Biochemical oxygen demand (in terms of BOD_5) was measured with the use of the OxiTop® measuring system by the method given by WTW. Total nitrogen