WaBoLu-Hefte

WaBoLu
02
05
ISSN
0175-4211

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Umwelt
Bundes
Amt
Für Mensch und Umwelt

WaBoLu
02
05
ISSN
0175-4211

Current approaches to cyanotoxin risk assessment, risk management and regulations in different countries

Compiled and edited by

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Federal Environmental Agency

GREECE:
Cyanotoxin Risk Assessment, Risk Management and Regulation

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INTRODUCTION

Studies on phytoplankton in some Greek lakes (Vegoritis, Volvi, Mikri Prespa, Doirani and Kastoria) have shown that prolonged cyanobacterial blooms can occur, lasting up to 8 months, which are dominated by potentially toxic species (Moustaka-Gouni and Nikolaidis 1990; Moustaka-Gouni 1993; Tryfon and Moustaka-Gouni 1997; Temponeras et al. 2000; Vardaka et al. 2000). Generally, cyanobacterial water blooms in Mediterranean countries may be expected to be of extended or even continuous duration throughout the year, particularly in eutrophic freshwaters experiencing high temperatures and irradiance, stratification of the water column, high retention time of water and low zooplankton grazing pressure.

In Greece from 1987 to 2000 hepatotoxic cyanobacterial blooms were observed in 9 out of 33 freshwaters examined (Lanaras et al. 1989; Vardaka 2001; Gkelis et al. 2001a,b). Microcystins (MCYSTs) were detected by HPLC in 7 of these lakes and the total MCYST concentration per scum dry weight ranged from 42.2 to 2564 µg g^{-1} (Gkelis et al. 2005). Cyanobacterial genera (*Microcystis, Anabaena, Anabaenopsis, Aphanizomenon, Cylindrospermopsis*) with known toxin producing taxa were present in 31 freshwaters. The two most abundant toxin producing species encountered were *Microcystis aeruginosa* and *Anabaena flos–aquae* (Gkelis et al. 2001b). However, there have been no reported incidents of adverse health effects to humans or animals, although perhaps as a result of unawareness.

Several MCYST variants have been identified in the cyanobacterial blooms. The most abundant variants are MCYST–LR and MCYST–RR, while MCYST–LA, MCYST–YR and desmethylated derivatives of MCYST–LR and MCYST–RR have also been found (Gkelis et al. 2001a). In addition, other bioactive peptides (anabaenopeptins A and B) have been identified and quantified in some Greek lakes (Gkelis et al. 2005). There is no evidence to date for the occurrence of neurotoxic cyanobacterial blooms in Greece (Cook et al. 2004).

The greater awareness concerning cyanotoxins at a scientific level in Greece is not reflected at a national governmental level in terms of the instigation of monitoring programmes and legislation. The occurrence of cyanotoxins in Greek freshwaters may have serious consequences for drinking water resources, due to the reliance on surface waters for domestic drinking supplies. To date, low concentrations (<1 µg L^{-1}) of MCYSTs have been reported in drinking water sources (Gkelis et al. 2004). However, a persistent bloom of *Microcystis aeruginosa* (biovolume >10 µL L^{-1}) has been observed in 2003 in Polyphytos Reservoir (unpublished data). Despite the known presence of cyanotoxins at certain times of the year, waterbodies (lakes and reservoirs) used for recreation, drinking-water supplies, aquaculture, irrigation and wildlife refuges, are not monitored. Only at the academic and research level has the cyanotoxin risk for waterbody users been assessed and the need for management action considered.

RISK ASSESSMENT: Cyanobacterial biovolumes, microcystin concentrations, and bioaccumulation

Cyanotoxin risk has been assessed in Lake Kastoria after studying seasonal patterns of cyanobacterial and MCYST-LR occurrence (Vardaka 2001; Cook et al. 2004). Cyanobacterial biovolume was high (>11 µL L^{-1}) throughout the year (Fig. 1) and was in excess of Guidance Level 2 (10 µL L^{-1}) proposed by WHO for recreational waters and Alert Level 2 for drinking water (Bartram et al. 1999). From April to November, cyanobacterial biovolumes in some of the surface water samples exceeded Guidance Level 3, with the potential for acute cyanobacterial poisoning. Intracellular MCYST–LR concentrations (max 3186 µg L^{-1}) exceeded the WHO guideline for drinking water (1 µg L^{-1}) from September to November (Fig. 2) with a high risk of adverse health effects following ingestion or contact with lake water (Cook et al. 2004; Vardaka 2001). Based on toxicological data the involuntary ingestion of 2 mg MCYST–LR during swimming in such water could cause liver disease in a 10 kg child (Falconer et al. 1999).

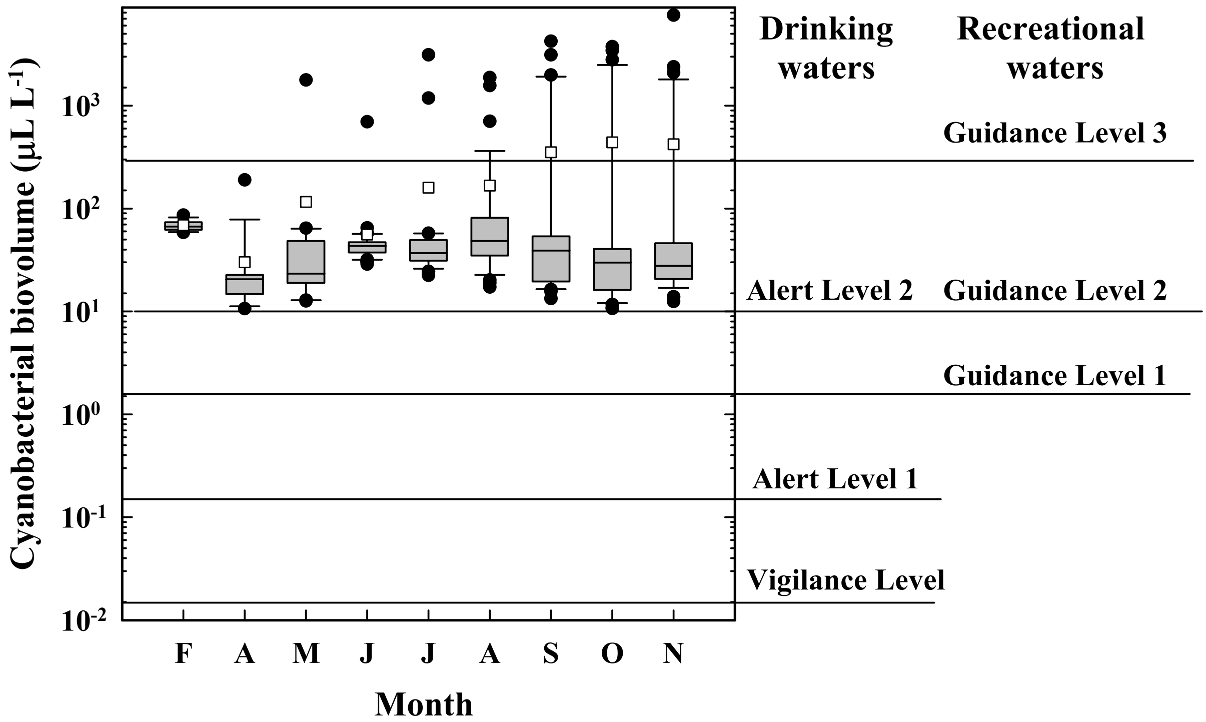

Fig. 1. Distribution of cyanobacterial biovolume values (volume of cyanobacteria per volume of lake water) represented by box and whisker plots, measured in Lake Kastoria from 1994 to 1997

(for experimental details see Vardaka 2001 and Cook et al. 2004) and Alert and Guidance levels proposed by the World Health Organization (Bartram et al. 1999) for drinking water supplies and recreational waters, respectively.

Outlying values (circles) and the mean value (squares) for each month independent of year are given. The total number of samples collected for a particular month ranges from 15 to 35. (Modified from Cook et al. 2004).

Low MCYST–LR concentrations (mean values <1 µg L^{-1}) with the exception of the individual samples indicated, are observed from February to June with no associated health risks (Fig. 2). In July and August MCYST–LR concentrations tend to increase, with about 14 % of the samples posing a moderate to high risk of adverse health effects. MCYST–LR concentrations are maximal in September, October and November, with 14 % of samples indicating the potential for a high risk of adverse health effects. From August to November MCYST–LR concentrations are higher than 2 µg L^{-1}, the threshold recommended by WHO for recreational waters above which, appropriate actions should be taken (Falconer et al. 1999). In L. Kastoria 41 % of the samples from the surface water (0-0.2 m) had MCYST–LR concentrations higher than 1 µg L^{-1}, the WHO provisional guideline value for drinking water.

MCYST–LR concentration was positively and significantly correlated with the total cyanobacterial biovolume (r^2=0.724, $P<0.05$, n=277) (Cook et al. 2004; Vardaka 2001). Therefore, cyanobacterial biovolume can be used as an indicator of the *in situ* MCYST–LR concentration in L. Kastoria. MCYST–LR concentrations >1 µg L^{-1} were observed when *Microcystis* species (mainly *Microcystis aeruginosa*) constituted >50 % (v/v) of the cyanobacterial biovolume (Cook et al. 2004; Vardaka 2001). *Microcystis* strains isolated from L. Kastoria had total MCYST concentrations per dry weight cyanobacterial cells, of 90 to 1200 µg g^{-1} and structural variants –LR, [D-Asp^3] –LR, –RR and –LA were detected (Gkelis et al. 2001a).

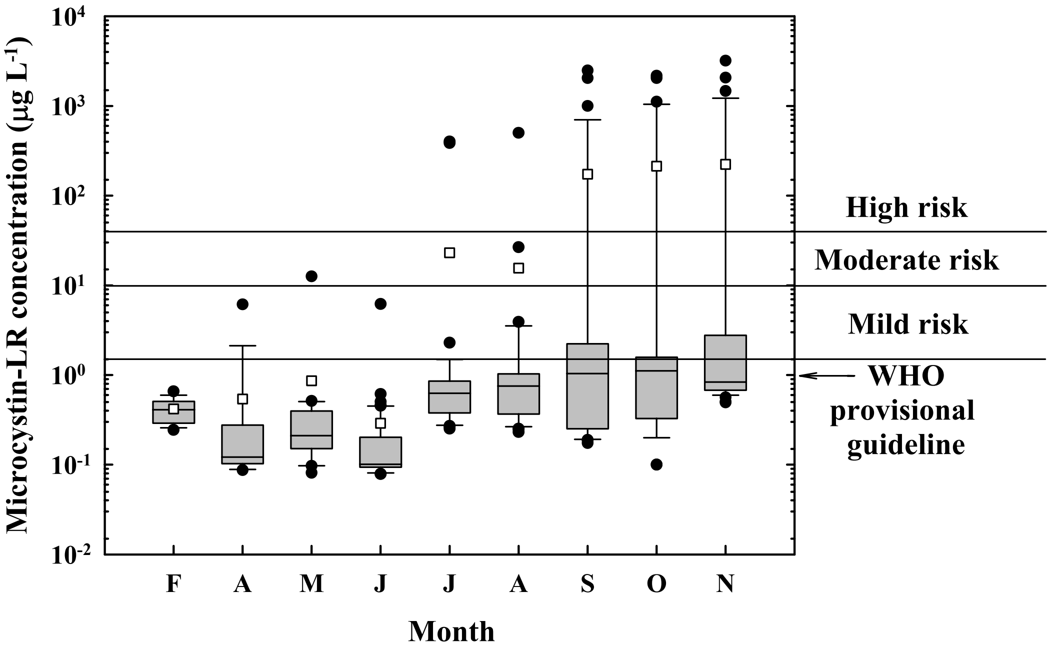

Fig. 2. Distribution of intracellular Microcystin–LR concentrations (MCYST–LR per volume of lake water) in Lake Kastoria from 1994 to 1997

(for experimental details see Vardaka 2001 and Cook et al. 2004) compared with guidelines for assessing adverse health risks in drinking-water (Falconer et al. 1999). Outlying values (circles) and the mean value (squares) for each month, independent of year, are given. The total number of samples collected for a particular month ranges from 15 to 35. (Modified from Cook et al. 2004).

Preliminary evidence indicates that microcystins are accumulated in some aquatic fauna, with average MCYST concentrations in fish and frog muscle of 225 and 125 ng per g, respectively, (Gkelis et al. 2002). A Tolerable Daily Intake (TDI) value for MCYSTs of 0.04 µg per kg body weight per day has been proposed by the WHO (Kuiper–Goodman et al. 1999). If an adult human (60 kg) was to consume 300 g of fish or frog from the lakes examined, the

Estimated Daily Intake of MCYSTs ingested would exceed the TDI on average by 28 and 15 times, respectively (Gkelis et al. 2002). Therefore, lake products targeted for human consumption should be monitored for MCYST content. A more comprehensive study of the cyanotoxin concentrations in lake fauna is necessary, in order to implement lake management policies for protecting human health and averting economic damage to lake-related industry.

The situation described for L. Kastoria is representative of other Greek lakes which also have high cyanobacterial biovolumes (Cook et al. 2004) and microcystin concentrations (Gkelis et al. 2005) in the summer and autumn. The combination of high cyanobacterial biovolumes and MCYST concentrations in water samples and the presence of MCYSTs in the food chain indicate elevated risks of acute toxicosis and adverse human health effects in several Greek lakes.

RISK MANAGEMENT

Inland water bodies are managed in terms of water usage, but are not managed yet with respect to their ecological water quality and cyanotoxin concentrations. Furthermore, there are no specific regulations concerning cyanotoxins. Subsequently, lakes experiencing cyanobacterial blooms may be used for recreational activities and athletic events, such as swimming, rowing and water-skiing and also for aquaculture. Lake products, such as fish, frogs and mussels are not monitored for cyanotoxins despite research evidence for the presence of microcystins in the tissues of lake fauna (Gkelis et al. 2002). In some instances local authorities are not willing to acknowledge the hazards that cyanotoxins present and resent the characterisation of a waterbody as potentially toxic. In the absence of a serious problem or national governmental intervention this attitude will probably continue to prevail.

The absence of cyanotoxin management policies for the lakes where cyanotoxins occur, presents a potential hazard for human health and wildlife concerning the consumption of lake products and water. The instigation of monitoring programs for the presence of MCYSTs in the waterbodies and the quality control of lake products are required. To date cyanotoxins have been monitored only for the duration of funded research programmes and these have not included the monitoring of commercial aquaculture products.

RESPONSIBILITY AND REGULATION

The legal situation concerning inland water administration is not always clear and several local administrative bodies and authorities are responsible, often with conflicting interests. None of these authorities alone has at present the resources for cyanotoxin work and the coordination between them has proven difficult.

Local Departments of the Ministry of Agricultural Development and Food are responsible for the safety of aquaculture products, produced in areas under their jurisdiction, targeted for human consumption. This Ministry also monitors the characteristics of the surface and ground waters which are used for irrigation and they acknowledge that there are problems in some lakes, for example, pollution and decreasing water levels (Greek Parliament 2001a). Environmental issues and ecological water quality are the responsibility of the Ministry for the Environment, Physical Planning and Public Works. Legally, this Ministry is not obliged to regularly monitor phytoplankton (including cyanobacteria) in waterbodies (Greek Parliament 2001b). The surface waters used for human consumption are of very good quality (Greek Parliament 2001b) in accordance with Directive 75/440/EEC. Currently, implementation of the Water Framework Directive (2000/60/EC) is underway, for the improvement of the ecological water quality of inland waters. Protected areas of the environment, including inland waters, are managed by newly created Protected Areas Management Bodies. The potability of drinking water is the responsibility of the Ministry of Health and Welfare.

The State Electricity Company is in control of water resources (lake water, reservoirs) used by hydroelectric power plants. The Athens Water Company draws drinking water from some surface water sources, such as the eutrophic Lake Yliki (phytoplankton biovolume >10 μg L^{-1}; Moustaka-Gouni, unpublished data), and phytoplankton abundance is monitored. There are no scientific publications to our knowledge on this monitoring. The presence of *Cylindrospermopsis raciborskii* in L. Yliki has been reported (see Cook et al. 2004) and also low concentrations (<0.1 μg L^{-1}) of MCYSTs (Gkelis et al. 2004). The Thessaloniki Water Company draws water from the River Aliakmonas which is linked to the outflow of freshwaters (e.g. Polyphytos Reservoir, Lake Kastoria) in which toxic cyanobacterial blooms (e.g. *Microcystis aeruginosa*) are known to occur. Currently cyanotoxins are not monitored by the company. Only a primitive test for the presence of algae is used. Low concentrations of MCYSTs (<1 μg L^{-1}) have been independently detected (Gkelis et al. 2004) in the Aliakmonas River. An increase in cyanotoxin concentrations in these water sources may cause adverse human health effects.

Municipalities and Local Councils have occasionally initiated phytoplankton studies in waterbodies under their jurisdiction. The General Secretariat of Research and Technology, Athens, and the EU have awarded research grants for the study of phytoplankton and cyanobacterial toxins, however these grants have been limited.

There is no central infrastructure for the study of cyanotoxins at present. However, the National Reference Laboratory for the monitoring of marine biotoxins (1993/383/EEC; amendment 1999/312/EC) has been established. Their work at present is focused on the monitoring of marine phytoplankton abundance and composition.

CONCLUDING REMARKS

To date there is no legislation in Greece concerning cyanotoxins. In addition, to the best of our knowledge, at a national government level the responsible Ministries have not taken issue with the cyanotoxin case. Presently in general, only the research community in Greece is aware of the seriousness of the cyanotoxin situation. An EU lead awareness campaign would be most beneficial for informing governmental and local authorities in Greece about global incidents, scientific and applied developments. In addition, educating young scientists, training students and informing the general public are also critical steps which should be taken.

The easiest and most cost effective approach, currently feasible, for cyanotoxin risk assessment in Greece, in the absence of national infrastructure and coordination, is the monitoring of cyanobacterial biovolume. Ideally, species identification would additionally indicate if known toxin producing cyanobacteria were present in the biomass, however this requires more highly skilled manpower. The correlation between total cyanobacterial biovolume and MCYST-LR concentration in L. Kastoria enables the prediction of cyanotoxin risk levels, even though this may underestimate the total cyanotoxin concentration. Such a method could be successfully implemented particularly for monitoring during periods of known high cyanotoxin risk. In a country with a relatively limited infrastructure the routine monitoring of samples for cyanotoxin requires the local implementation of quick, cost-effective methods. Additionally, the establishment of a national laboratory for cyanotoxins is necessary to make the Greek efforts more efficient and effective for the benefit of the public.

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