Cyanobacterial harmful algal bloom in the Delta of Axios River in the Northern Greece: Impacts and monitoring

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Abstract. Acyanobacterial harmful algal bloom (CyanoHAB) has occurred in May 2015 in the Delta of Axios River, in Thessaloniki, in the region of Central Macedonia, in Northern Greece. The bloom expanded to the coastline of Thermaikos Gulf near the areas of Chalastra and Kimina. Toxic *Anabaena* sp. was isolated from the water samples. Microcystin-RR(50ppb) was confirmed with immunoassay method for microcystins (Adda specific ELISA) and Liquid Chromatography- Mass Spectrometry (LC-MS).In this area monitoring of microcystins and its algal blooms are only experimentally applied by a satellite system. This research investigated the toxin and microcystin cells in the local coastal water and emphasize to the satellite remote sensing, as a precaution, to reduce and avoid impacts on animals' health, Public Health and ecosystems, after harmful algal blooms.

Keywords: cyanobacteria, microcystins, algalbloom, Thermaikos Gulf, satellite remote sensing.

1 Introduction

Cyanobacteria are ancient gram negative, prokaryotic, photosynthetic micro-organisms, which are distributed globally in fresh, brackish and marine waters and terrestrial environments Toxic species can be potentially hazardous for animals' and public health, especially during the "water bloom phenomenon", since they produce secondary metabolites, the cyanotoxins (Whitton and Potts, 2002). The problem of cyanobacterial harmful algal blooms (CyanoHABs) has risen the last decades (O'Neil *et al.,* 2012). Climate changes and human activities, such as the intensive use of fertilizers with nitrates and phosphates salts and the farm animals', industrial and urban wastes running off into the coastal waters, have significant role in the proliferation of harmful cyanobacterial blooms (Paerl and Huisman, 2009).

In Greece CyanoHABs have been reported mostly in lakes (Gelis *et al.,* 2006), but there in not enough data in marine environments. In late May 2015 during research sampling for toxic cyanobacteria in Axios River, it was found that there was a bloom

Proceedings of the 8th International Conference on Information and Communication Technologies in Agriculture, Food and Environment (HAICTA 2017), Chania, Greece, 21-24 September, 2017.

near the Delta of the river. It was decided to take water samples from the Delta and the surrounding area to search for the potential presence of cyanobacteria toxic species. Axios is the largest river in the region of Central Macedonia. Its annual runoff into Thermaikos Gulf comes up to 279m^3s^{-1}, enriching the shallow coastal zones (Nikolaidis *et al.,* 2006). The Delta of the river and its surrounding land are important wetland, protected by the Ramsar Convention (Jones *et al.,* 2012).Thermaikos is a semi-closed gulf located in the northwest of Aegean Sea, with intensive fishing, mussel farming and recreational activities (Koukaras, 2004). The expansion of toxic cyanobacterial blooms into the shallow coastal waters in Thermaikos Gulf, could affect the ecosystem, the fishing and farming activities and the public health. The scope of this research was to detect the presence of toxic cyanobacteria after blooming and to propose its monitoring systems, beyond the typical sampling and microbiological and chemical technics.

2 Materials and Methods

2.1 Field Sampling

In late May 2015 after expansion of a cyanobacterial blooming to the coastline of Thermaikos Gulf, surface water samples were collected from three sampling places, one near the Delta of Axios, one near Chalastra and one near Kimina (40^o 32′ 39.22′′Band 22^o 45′ 16.74′′E, 40^{o}30′ 12.60′′Band 22^{o}42′ 19.40′′E, 40^{o}31′ 13.94′′Band 22^{o}41′ 43.29′′E) (Figure 1). During each sampling temperature, salinity, dissolved oxygen and ph of the surface water were recorded by anYSI 556 handheld multiparameter instrument (YSI Incorporated, Ohio, USA).To investigate the presence of cyanobacteria500ml of surface water were aseptically sampled, hold in a sterile 500ml flask and transferred in the laboratory in insulated cold boxes. One hundred and fifty milliliters (150ml) were filtered through filters with 0.45μm pore diameter (PALL CORPORATION, Michigan) and 150ml were filtered through Whatman GF/C filters (Sigma–Aldrich, Germany). The second filter was kept frozen at -80^{o}C, until toxin detection.

2.2 Isolation and Identification of Cyanobacteria

Filtered water samples through filters with 0.45μm pore diameter, were incubated on Marine Agar and cyanobacteria growth was obtained according to Kalaitzidou *et al*., 2015.Cyanobacteria from colonies showing typical morphology were observed in an optical microscope (Olympus CH30), after Gram staining. The taxonomy system according to Anagnostidis and Komárek (1985) was used to classify cells showing typical morphology. Immobilecells that were long, filamentous with heterocysts were characterized as presumptive *Anabaena* sp. Bacterial cultures were further purified on Marine agar. DNA from bacteria of a single colony per plate was extracted and further analyzed with polymerase chain reaction, using specific primers for the amplification of the cyanobacterial 16S rRNA fragments (Forward primer CYA359F,

5′-GGG GAA TYT TCC GCA ATG GG-3′ and reverse primer CYA781R, 5′-GAC TAC TGG GGT ATC TAA TCC CAT T-3′ (Nübel et al., 1997)

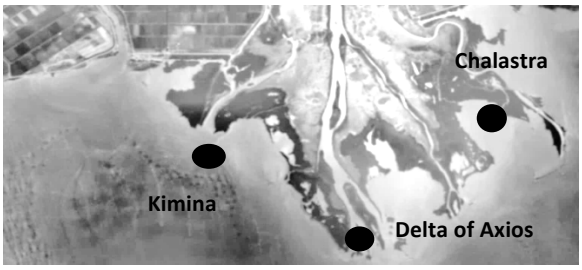

Fig. 1. Sampling places in the Delta of Axios River

2.3 Sample Analysis for Microcystins

On examination day the sampled water that was filtered through Whatman GF/C filters and then stocked at -80^{o}C, was defrost at 2^{o}C, dissected and extracted with methanol 75% (Merck, Germany). The extraction method was previously described by Kalaitzidou *et al.,* 2015. The extracts were first examined for microcystins with a commercial immunoassay method for microcystins (Adda specific) ELISA kit (Enzo Life Sciences Inc, USA). The detection limit was 0.10ppb microcystin-LR analogues. Samples with concentration over than 1ppb were considered positive and were further examined by Liquid Chromatography- Mass Spectrometry (LC-MS) (ACQUITY UPLC I-Class System, WATERS, USA).

3 Results and Discussion

The results of our research confirmed the presence of the freshwater toxic strain of *Anabaena* sp. only in one out of the three sampling places(Delta of Axios River). (figure 2)In this sampling place the LC-MS was positive for microcystin-RR (figure 3). The toxin concentration in water samples was 50ppb.

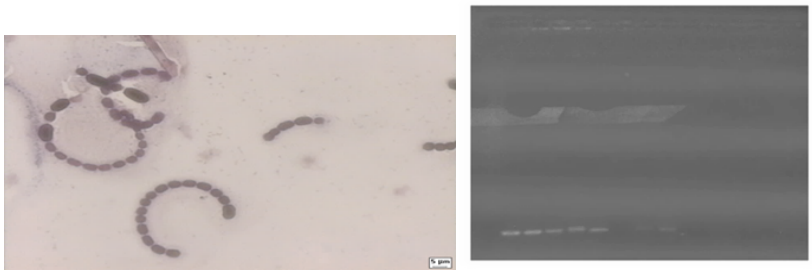

Fig. 2. *Anabaena* sp. cells and PCR detection of 16S rRNA of cyanobacteria.

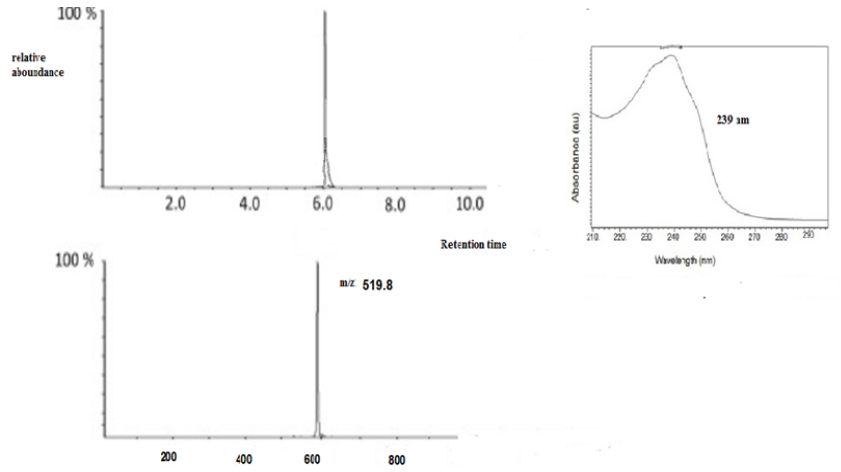

Fig. 3. LC/MS settings for microcystin-RR. The retention time was 6.08, precursor ion was 519.8, quantifier ion was 135.1 and the UV absorption spectrum was 239nm.

It is suggested that the harmful algal bloom existing this period in this place was favored by the climate conditions and the physicochemical parameters. Water temperature was 16^{o}C, salinity was 15psu and ph was 8.38. Moreover some fish and crustaceans mortality was observed in the area. The detection of *Anabaena* sp. in algal blooms have also been reported in the Baltic Sea, demonstrating that higher flow rates within estuaries of rivers and coastlines may cause expansion of the blooms to the marine environments (Engström-Öst, and Mikkonen, 2011). Discharges of freshwater into coastal marine environments inducing toxic cyanobacteria bloom have been described also in Adriatic Sea in Lake Occhito (De Pace et al., 2014). In the case of Adriatic Sea the expansion of the blooming affected the farming of the Mediterranean mussel *Mytilus galloprovincialis*, as microcystins were detected in these mussels in high concentration.

The impact of harmful cyanobacterial blooms is of great concern, since toxic species can kill fish, seabirds and marine mammals and enter the human food chain. Moreover nontoxic species cause economic losses due to the damage of the ecosystem, the reduction of the fisheries and water resources and the recreational activities(Anderson, 2009). These impacts led the United States Environmental Protection Agency (US EPA) to issue guideline for monitoring criteria for inland and marine waters using satellite remote sensing (Gibson*et al.,* 2000). Typical monitoring systems based on sampling water cost (Corrales and Maclean, 1995). Monitoring by satellite remote sensing can be the solution to transmit on time data and permit efficient alerts (Kutser *et al.,* 2006).

In Greece satellite monitoring systems are still experimental. In Thermaikos gulf chlorophyll-a (Chl-a), total suspended matter and sea surface temperature, were monitored by Medium Resolution Imaging Spectrometer (MERIS) and Moderate Resolution Imaging Spectroradiometer (MODIS) satellite systems. The results provided spatial and temporal patterns for the water quality parameters, showing that

the increased Chl-a concentration, especially during late spring and summer, may induce high algal concentration (Monachou*et al.,* 2014). In Ionian Sea an experimental program is implemented in cooperation between Greek and Albanian governments, with the code name "***SAIMON".*** The aim of the program is to monitor by "satellite real time monitoring" the eutrophication risk for the marine waters over the Greek-Albanian cross border area (http://saimon.getopendata.gr).

In conclusion typical monitoring strategies can detect but not predict cyanobacterial blooms. Satellite remote sensing is considered as a promising technique for tracking and detecting cyanobacterial harmful algal blooms. It can be implemented with traditional monitoring microbiological and chemical methods to reduce costs and working hours in the laboratories. It provides advantages, such as satisfactory spatial and temporal patterns, frequency and duration of exceeding nutrient criteria parameters, area mapping and collecting of useful data to control blooming and implement mitigation strategies.

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