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Biochemical responses of filamentous algae in different aquatic ecosystems in South East Turkey and associated water quality parameters

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ABSTRACT

To the best of our knowledge, any study about biochemical response of filamentous algae in the complex freshwater ecosystems has not been found in the literature. This study was designed to explore biochemical response of filamentous algae in different water bodies from May 2013 to October 2014, using multivariate approach in the South East of Turkey. Environmental variables were measured in situ: water temperature, oxygen concentration, saturation, conductivity, salinity, pH, redox potential, and total dissolved solid. Chemical variables of aqueous samples and biochemical compounds of filamentous algae were also measured. It was found that geographic position and anthropogenic activities had strong effect on physico-chemical variables of water bodies. Variation in environmental conditions caused change in algal biomass composition due to the different response of filamentous species, also indicated by FTIR analysis. Biochemical responses not only changed from species to species, but also varied for the same species at different sampling time and sampling stations. Multivariate analyses showed that heavy metals, nutrients, and water hardness were found as the important variables governing the temporal and spatial succession and biochemical compounds. Nutrients, especially nitrate, could stimulate pigment and total protein production, whereas high metal content had adverse effects. Amount of malondialdehyde (MDA), H_2O_2, total thiol groups, total phenolic compounds, proline, total carbohydrate, and metal bioaccumulation by filamentous algae could be closely related with heavy metals in the ecosystems. Significant increase in MDA, H_2O_2, total thiol group, total phenolic compounds, and proline productions by filamentous algae and chlorosis phenomenon seemed to be an important strategy for alleviating environmental factors-induced oxidative stress as biomarkers.

1. Introduction

Water pollution with anthropogenic activities has become one of the most important environmental problems concern impacting the biota especially in the freshwater ecosystems (Sanayei et al., 2009; Armah et al., 2010). Deterioration of environment is perceived to be deleterious or undesirable for biotic life, in this sense human activities have degraded watersheds, generating awareness and increase in the scientific development of biomonitoring programs to assess the status of aquatic systems (Directive, 2000; Hering et al., 2006). The management of water resources insist on precise and accurate tools to measure the biological integrity of aquatic ecosystems (Directive, 2000; Reynolds et al., 2002; Padisak et al., 2006; Cao et al., 2007; EC (European Communities), 2009).

Biomonitoring of aquatic ecosystems is a scientific technique for assessing environment, based on sampling and analysis of organism's biomass (Directive, 2000; Zhou et al., 2008; EC (European Communities), 2009). The organisms in different environmental conditions can produce some chemicals reflecting xenobiotic stress especially heavy metal stress. Amount of these compounds provide information about environmental chemicals from anthropogenic activities and assessing water quality. Monitoring relationship between organisms and corresponding environmental variables can directly offer data on the potential effects and actual integrated toxicities of pollutants, reflecting the corresponding deleterious degree in the environment.

Filamentous algae are primary producers and bioindicators in aquatic ecosystems (Bellinger and Sigee, 2010; Schneider and

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Lindstrøm, 2011; Schneider et al., 2013). They give information on the environmental trophic state and can be used to establish geographical and temporal variations in the bioavailability of contaminants through measurements of those contaminants in their biomass (Rainbow, 1995). Filamentous algae can take excessive nutrients such as nitrate and phosphorus to produce algal biomass, which accumulate undesired compounds like heavy metals and thus they can enhance the water quality of systems. Furthermore, algae give metabolic response to contaminants, which could point to important biomarkers. Especially, heavy metal stress can cause the disturbance of normal metabolism and biological function, and even death of organisms. With regard to these mentioned respects, filamentous algal species can be good biomonitors of aquatic ecosystems and are efficient in reporting measurable bioavailable concentrations of the contaminants. Use of filamentous algae as a biomarker has several advantages; they (*i*) can be determined on macroscopic and microscopic examination without cultivation; (*ii*) can reflect changes in environmental conditions over extended periods due to their prolonged presence at a particular site; (*iii*) can accumulate contaminants without being severely affected or killed by levels encountered in the water bodies; (*iv*) can be easily collected from environments and easily cultivated in the laboratory if required; (*v*) have enough abundance and widespread throughout the world's aquatic ecosystems for the repetitious sampling and comparison (Rainbow and Philips, 1993; Karez et al., 2004).

Biochemical responses of filamentous algae in different ecosystems conditions can be used to evaluate the ecological assessment of them. Determining biological responses to various environmental conditions, understanding relationships between stress factors and response variables, giving signals the exceedance of critical physiological thresholds or tolerance limits can provide important information about ecosystems for use in weight-of-evidence approach to ecological risk assessment (Van der Oost et al., 2003; Hutchinson et al., 2006). Survival of filamentous algae in aquatic environment contaminated with pollutants depends on its ability to generate and transit signals that adjust the metabolism. Therefore, searching of biochemical response with signal molecules as biomarker mediating stress tolerance is an important issue for understanding how algal species respond to pollutants. From that point, searching of biochemical components produced by filamentous algae in various freshwater ecosystems is the first attempt to investigate as more detail on the Earth. The aims of this study were (*i*) to investigate relationship between physico-chemical factors and biochemical compounds of filamentous algae in the South East of Gaziantep, using multivariate approach, (*ii*) to estimate ecological preferences of filamentous species for environmental variables by use of multivariable techniques, and (*iii*) to evaluate biochemical responses of filamentous algae and investigate changes in their biochemical compounds as biomarker potentials.

2. Materials and methods

Water and filamentous algal samples were taken from 10 different sampling sites in the South West of Gaziantep, Turkey (Fig. 1). Water bodies in this region are commonly used for irrigation purpose. Filamentous algae and water samples were taken from the sampling sites at May 2013, November 2013, May 2014, and October 2014. Environmental variables were measured in situ: water temperature, oxygen concentration, saturation, conductivity, salinity, pH, redox potential, and total dissolved solid (TDS), by use of YSI oxygen–temperature meter (YSI professional plus model, USA) from just beneath of the surface in the stations.

Filamentous algae biomass was harvested by deploying 1 m^2 quadrates at 0.1–0.25 m depth by five replicate quadrates. The percentage of attached algal coverage was measured in each quadrate. All filamentous biomass was harvested with scraping tools from the quadrates. Collected algal biomass was put into 1 L polyethylene bottle filled with sampling site water. Six degree scales [0 absent, 1 very rare (1–20%), 2 rare (21–40%), 3 frequent (41–60%), 4 abundant (61–80%), and 5 common (81–100%)] were used for the determination of relative abundance in covered quadrates.

All samples were stored in coolers with ice packs, pending transfer to the laboratory until determination of biochemical and chemical compounds. Algal assemblage was gently washed with tap water and characterized by use of microscope (Olympus BX53 model with DP73 model digital camera and Cell Sens Vers. 1.6 imaging software). Algal cell biovolumes were estimated from measurements of specific cell volume by approximating geometric shapes of cells (Rott, 1981; Hillebrand et al., 1999; Sun and Liu, 2003). Dimensions of the at least 20 cells were measured to calculate the mean volume of each taxon. The primary identification keys (Prescott, 1982; John et al., 2002; Sheath and Wehr, 2003) were consulted to assist algal identification.

Analyses of chemical variables (e.g.; $N–NH_4$, $N–NO_3$, $N–NO_2$, and $P–PO_4$) were carried out by standard methods (APHA et al., 1989). After filtration by Sartorius, chemical analyses of aqueous samples were done by use of Ion Chromatography (Thermo Scientific Dionex ICS-5000, HPIC system). Heavy metals were analyzed using inductively coupled plasma-optical emission spectrometry (ICP-OES, Perkin Elmer, Optima 2100 DV). Turbidity of water was measured nephelometrically and expressed in NTU.

2.1. Analyses of biochemical compounds

Pigment composition as total carotene, chlorophyll *a* and *b* of filamentous algae were measured by use of a spectrophotometer (UV/VIS Jenway 6305) at 470, 663, and 646 nm, respectively, using 80% acetone method (Wellburn, 1994).

Lipid peroxidation level in algal biomass was determined by measuring malondialdehyde (MDA) content based on the method of Zhou (2001). Amount of algal proline was determined by following a method proposed by Bates et al. (1973). Protein content was determined with the Folin–Ciocalteu method as described by Lowry et al. (1951). Total phenolic compound was determined by using the Folin–Ciocalteu method (Ratkevicius et al., 2003). Total carbohydrate was determined by following a method proposed by Dubois et al. (1956). Amount of starch was estimated by following of McCready et al. (1950). Hydrogen peroxide (H_2O_2) content was determined according to a method proposed by Sergiev et al. (1997). Thiol (–SH) group content in algal extract was estimated by following a method proposed by Ellman (1959).

2.2. Analysis of metal content of algae

In order to determine bioaccumulation of heavy metal content, dried algal sample was suspended in 10 ml of highly pure 14 M HNO_3 using microwave digestion. This mixture was heated by gradual increase of temperature from 80 to 200 °C. About 0.25 ml of 37% HCl was added into cooled solution for the mineralization. After then, residual solution was diluted with pure deionized water to 20 ml volume. Determination of heavy metal (Cu^{2+}, Cd^{2+}, Cr^{2+}, and Pb^{2+}) contents of filamentous biomasses were performed with ICP-OES.

2.3. FTIR-ATR analysis

A fourier transform infrared equipped with an attenuated total reflection spectrometer (Perkin-Elmer Spectrum 100 FTIR–ATR)

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