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Evaluating biochemical response of filamentous algae integrated with different water bodies

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ABSTRACT

The present study prompted the second attempts to evaluate biochemical responses of filamentous algae under different physico-chemical variables in various water bodies in Turkey. These variables were investigated by use of multivariate approach in the years of 2013 (May and November) and 2014 (May and October). Studied ecoregions had the different geographic position, climate, land-use, and anthropogenic activities, could strongly affect physico-chemical variables of water bodies, which caused to change or regulate in algal biomass composition due to the different response of filamentous species. Besides, biochemical responses of species changed at different sampling times and stations. Multivariate analyses indicated that temperature, heavy metals, and nutrient contents of aquatic systems were found to be major variables driving the spatial and temporal occurrence and biochemical contents of filamentous species. Total protein and pigment production by filamentous algae were high in water bodies having high nutrients, whereas they were low in high heavy metal contents. Amount of malondialdehyde (MDA), H_2O_2, total thiol group, total phenolic compounds, proline, total carbohydrate, and bioaccumulation of metals by filamentous algae were closely related with heavy metal contents of water bodies, indicated by the multivariate approach. Significant increase in aforementioned biochemical compounds with a distinct range of habitats and sensitive-tolerance to environmental conditions could make them highly valuable indicators.

1. Introduction

Deterioration of physico-chemical variables with anthropogenic activities strongly changes biota in the majority of freshwater ecosystems. Loading huge amount of effluents from industries, municipal and agricultural activities into streams and/or lakes has become one of the major environmental problems (Ducharne et al., 2007; Ato et al., 2010). Point and non-point source of pollution, associated with changing of land use patterns and practices, have resulted in huge impairments to water bodies. 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). The management of water resources insists on precise and accurate tools to measure the biological integrity of aquatic ecosystems (Directive, 2000; Cao et al., 2007).

Discharging of effluents containing heavy metals, dyes, salts, nutrients, and other chemical compounds into freshwater ecosystems can cause stress for aquatic biota. Stress conditions cause the generation of reactive oxygen species (ROS), mainly superoxide anion ($O_2^{\bullet -}$), singlet oxygen ($^1O_2$), hydroxyl radical ($OH^{\bullet}$), and hydrogen peroxide (H_2O_2) (Pinto et al., 2003). Some compounds like carotenoids, glutathione, phenolics, and proline are produced by cellular defense systems to get rid of ROS (Pinto et al., 2003; Branco et al., 2010; Volland et al., 2014). Membrane lipid peroxidation causes the formation of various breakdown products, such as MDA, related to the index of lipid peroxidation. This peroxidation can induce cell damage by changing membrane permeability creating similar conditions to water stress which promotes the synthesis of proline. Under the metal stress, proline can bind with heavy metal ions, may contribute to complete life cycle of the organism (Sinha and Saxena, 2006).

Biological monitoring of aquatic ecosystems based on field samples containing many species, all of which may contribute to the assessment of environment is a scientific technique (Zhou et al., 2008). Habitat preferences of algae are frequently useful in providing information on physico-chemical characteristics of the aquatic environment. Cultural eutrophication, acidification, and metal contamination can dominate filamentous green algae (Cattaneo et al., 1995).

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Human activity, development of industry and natural Earth processes lead to the release of numerous metals, metalloids and metallic nanoparticles that can act as stressors for algae growth and metabolism. In different environmental conditions, algae can produce some chemicals reflecting stress, especially heavy metal stress. When algal cells are exposed to metals stress, the formation of ROS such as OH, O_2^-, $^1O_2$ and H_2O_2 occurs and interacts with lipids, proteins, and nucleic acids, resulting in their degradation. Synthesis of antioxidant compounds or enzymes (superoxide dismutase, catalase) is also a defense mechanism against oxidative stress. Amount of these compounds provide information about environmental chemicals from anthropogenic activities and assessing water quality. Monitoring biochemical responses of algae against environmental changes can offer data on their potential biomarkers.

Environmental changes can cause to be an environmental stress factors which lead resulting loss or dominance of particular bioindicator species is preceded by biochemical and physiological changes in the algal community referred to as 'biomarkers.' Filamentous algae produce different biochemical responses for varying polluted aquatic systems and are bioindicators of ecosystem conditions (Bellinger and Sigee, 2010; Çelekli et al., 2016a). Algal assemblages and the amounts of biochemical compounds can give us information about biological responses to the water quality. Metabolic responses of algae to pollutants could point to specific biomarkers. Heavy metal stress can cause the disturbance of normal metabolism and biological function, inhibition of photosynthesis, reduction of cytochrome, cellular mutation, putrescence, and even death of algae. In recent years, use of macroalgae for assessing water quality has been increased, especially in rivers (Schneider et al., 2013; Çelekli et al., 2016a). Use of filamentous algae has several advantages for monitoring of water quality; they (*i*) can be easily collected from environments; (*ii*) have enough abundance and wide distribution for the repetitious sampling and comparison; (*iii*) can be determined on macroscopic and microscopic examination; (*iv*) can survive and accumulate under high levels of pollutants; (*v*) can reflect change in environmental conditions over extended periods due to their prolonged presence at a particular site (Karez et al., 2004; Bellinger and Sigee, 2010).

Biomarkers can be used to evaluate the ecological risk assessment as (*i*) characterization of toxicity mechanisms involved in biological responses, (*ii*) providing an information about biological responses for use in weight-of-evidence approach to ecological risk assessment, (*iii*) understanding relationships between stress and response, (*iv*) giving signals the exceedance of critical physiological thresholds or tolerance limits, and (*v*) monitoring changes in environmental health. In polluted aquatic ecosystems, survival of filamentous algae depends on its ability to generate and transit signals that adjust the metabolism (Çelekli et al., 2016a). To understand, how algal species respond to the pollutants, searching for signal molecules as biomarker mediating stress tolerance is an important issue. Searching of biochemical compounds produced by filamentous algae in various freshwater ecosystems in the South East of Gaziantep is the first attempt to investigate as more detail on the Earth by Çelekli et al. (2016a). The present study prompted the second attempts to evaluate biochemical responses of filamentous algae under different physico-chemical variables in various water bodies in the West of Gaziantep. This ecoregion has different geographic factors, climate, land-use, and anthropogenic activities strongly affect environmental factors, which could regulate filamentous species composition and their environmental optima. From that point, the aims of this study were (*i*) to explore biochemical response of filamentous algae with associated different aquatic ecosystems, (ii) to estimate ecological preferences of filamentous species for different environmental variables by use of multivariable techniques, (*iii*) to investigate relationship between physico-chemical factors and biochemical compounds of filamentous algae in the West of Gaziantep, using multivariate approach, and (iv) to evaluate changes in their biochemical compounds as biomarker potentials.

2. Materials and methods

2.1. Sampling

Filamentous algae and water samples were taken from the sampling sites between May 2013 and October 2014 from 21 different sampling sites in the West of Gaziantep, Turkey (Fig. 1). Sampling stations (different creeks with low water current and reservoirs), their altitudes and obtained filamentous algae at different sampling times were summarized in Table 1.

By deploying 1 m^2 quadrats at 0.1–0.25 m depth, filamentous algae biomass was estimated. Five replicate quadrates were harvested and the percentage of attached algal coverage was measured in each quadrate. Filamentous biomass, which was loosely attached on the surface, was harvested with scraping tools from the quadrates. Moreover, *Spirogyra* species were found to be loosely floating on the surface. 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 kept and transferred to the laboratory under chilled condition until the determination of biochemical and chemical compounds. After gentle washing with tap water, the algal assemblage was characterized by the use of a 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 (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) were consulted to assist algal identification.

2.2. Physicochemical analysis of water

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. Geographical data (elevation, altitude, and longitude) were read from a geographical positioning system (Table 1).

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, AWWA, WPCF, 1989). After filtration by Sartorius, chemical analyses of water samples were done by use of Ion Chromatography (Thermo Scientific Dionex ICS-5000, HPIC system). For cations analysis; IonPac CS12A analytical column with its capillary and guard columns were used. Operation conditions were maximum pressure 4000 psi for standard and microbore; 5000 psi for capillary, mobile phase with acidic eluents (100% acetonitrile); ion-exchange groups (grafted carboxylic acid and phosphoric acid); and functional group of medium hydrophilic.

The IonPac As19 analytic column in combination with the AG19 guard column was used for the analysis of inorganic anions. The selectivity of the IonPac AS19 guard plus analytic column set had been designed to retain fluoride well out of the water dip and to separate oxyhalides and the common anions using hydroxide gradients.

Heavy metals were analyzed using inductively coupled plasma-optical emission spectrometry (ICP-OES, Perkin Elmer, Optima 2100 DV). The turbidity of water was measured nephelometrically and expressed in NTU.

2.3. Biochemical analysis of filamentous algae

Pigment composition (total carotene, chlorophyll a and b), lipid peroxidation level, hydrogen peroxide, proline, thiol group, total protein, total phenolic compound, total carbohydrate and moisture