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Review

Euglenoid Flagellates in Ecotoxicological Research: Progress and Perspectives

Sreejith Kottuparambil

Red Sea Research Center (RSRC), King Abdullah University of Science and Technology (KAUST), Thuwal, 23955-6900, Saudi Arabia
sreejith.kottuparambil@kaust.edu.sa

Abstract: Euglenoids are a common component of primary producers in high acidic and organic shallow freshwater systems. They are free-living photosynthetic motile flagellates exhibiting rapid responses to various external stimuli including chemical pollution. The unique combination of diverse physiological processes of Euglena such as photosynthesis, movement, and orientation offers a range of sensitive criteria that respond to aquatic pollutants. This has led to the development of several acute and chronic assay methods to monitor the adverse impacts of potent toxicants in aquatic ecosystems. Euglenoids also reflect the consequences of physical stress factors such as UV radiation. This review summarizes the ecotoxicity studies using euglenoid flagellates as model species and their potential to contribute toward rapid screening of ecological impacts of pollution in the aquatic environment. Factors determining the reliability of *Euglena* tests are outlined.

Keywords: Euglena; toxicity; pollution; heavy metals; PAHs; pesticides; UV radiation; F_v/F_m

1. Introduction

Ecotoxicity tests using microalgae are advantageous to evaluate aquatic pollutants, in terms of their sensitivity to toxicants, cost-effectiveness, simplicity, and feasibility of the test methods. Microalgae are the fundamental organisms in aquatic ecosystems and due to their position as primary producers in the trophic chain and they form the foundation for energy and nutrients cycling. Microalgae are highly vulnerable to dissolved and suspended pollutants due to their ubiquitous occurrence, large surface-to-volume ratio, short life cycles, and high sensitivity (Pikula et al., 2019). They are the primary route of entry for a large number of toxic chemicals to the aquatic food web, and any impact on them will subsequently affect the upper trophic levels (Bouldin et al., 2008; Hu et al., 2015; Netto et al., 2012). The contamination of aquatic systems by natural and anthropogenic pollutants is a growing concern worldwide. Therefore, understanding the interaction of toxicants with microalgae is important to evaluate their potential adverse effects on various compartments in the natural environment. Microalgae are preferable toxicity test organisms due to their rapid cell division rates and ease of culturing (Stone et al., 2019). The contemporary research in this line focuses on developing algal biosensor-based early warning systems for effective water body management and preservation of ecological status (Gosset et al., 2019).

Euglenoid flagellates are predominantly freshwater unicellular protists, with evolutionary significance, as the intermediate species between animals and plants (Wolken, 2012). They are mainly photosynthetic eukaryotes, with chloroplast surrounded by three membranes probably acquired by secondary endosymbiosis of a green alga (Gibbs, 1978). However, in the absence of light, *Euglena* can switch from an autotrophic to a heterotrophic mode of nutrition, which can be reverted to autotrophy when light is available. Due to their unique movement characteristics, euglenoids are extensively used to investigate taxis movements and signal transduction in microorganisms (Streb et al., 2002). Moreover, biotechnological applications of these organisms have gained recent scientific

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attention, particularly, the bioactive secondary metabolites (Kottuparambil, 2020; Kottuparambil et al., 2019) and the use of biomass as a food supplement due to its high nutritional and health benefits (Symonds et al., 2018).

Euglenoid flagellates were recognized as a reliable ecotoxicological test species after the development of the automated early warning system, ECOTOX, which measures the motility, orientation, and shape of *Euglena* cells, as sensitive criteria to chemical pollution in water (Azizullah & Häder, 2018). The image analysis software of ECOTOX allows for real-time measurement of motility parameters of the flagellate population, which are statistically analyzed for their dose-dependence on toxicants, deriving insights into their harmful effects on the environment. In the last decade, this tool has been widely used to monitor various stressors on flagellate microalgae, ranging from UV radiation (Kottuparambil et al., 2012) to organic and inorganic pollutants (Azizullah et al., 2011b; Kottuparambil et al., 2014), in short-term and long-term tests. Moreover, the combined approach of ECOTOX and pulse amplitude modulated (PAM) chlorophyll-*a* fluorometry provided an efficient method to assess the ecotoxicology and ecophysiology of euglenoid flagellates (Kottuparambil et al., 2014; Kottuparambil et al., 2012).

The most widely used euglenoid organism in toxicity studies is the genus *Euglena* (*Euglena gracilis* and *E. agilis*). As a potential toxicity model organism, they possess critical advantages such as ubiquitous occurrence, easiness to culture in a variety of growth media, taxonomical significance, and possession of sensitive biological endpoint parameters. Their unique movement and orientation characteristics are ideal for understanding the biology of taxis movements in unicellular organisms. Moreover, due to their high tolerance to sewage and chemical pollutants, *Euglena* species are ideal for bioremediation of organic and inorganic contaminants in natural water bodies (Mahapatra et al., 2013; Mendoza-Cózatl et al., 2006). Their movement characteristics, growth, and photosynthetic parameters are recognized as reliable, sensitive, and reproducible biological parameters to assess the adverse impacts of a spectrum of aquatic contaminants. This review summarizes ecotoxicological studies involving euglenoids as model organisms, emphasizing the broad array of toxicants studied and the effectiveness of various test criteria analyzed.

The following sections illustrate the major classes of aquatic pollutants and stressors which are extensively studied adopting euglenoids as model organisms.

2. Heavy metals

Heavy metals are potent toxic contaminants in industrial and domestic discharges and are harmful both lethally and sublethally to aquatic organisms including microalgae (Sánchez, 2008). While some heavy metals are essential nutrients for microalgae, showing direct positive effects on the organisms at low doses, some others result in serious toxicological symptoms, including mortality (Boyd, 2010). Moreover, they persistently accumulate in the biota and result in long-term adverse effects. In euglenophytes, heavy metals-induced threats to cellular growth, photosynthesis, and orientation in the water column have been widely reported (Azizullah et al., 2013a). However, towards the highly toxic Mercury, *Euglena* sp. showed significant tolerance through accumulation and volatilization (Devars et al., 2000).

The movement in response to the light (phototaxis) and gravity (gravitaxis) by euglenoid flagellates is the strategy to orient themselves in a band of ideal light conditions. Motility and orientation are sensitive parameters to heavy metal stress in *Euglena*. Euglenoids respond to pollutant stress through reduction of motility and changes in morphology from elongated motile to round motile forms (Gupta & Agrawal, 2005). This response is dose-dependent, therefore, can help assess the water quality. Under copper, mercury, and cadmium exposures, phototaxis was most affected in aged cultures of *E. gracilis* while swimming velocity was most sensitive in younger cultures (Stallwitz & Hädert, 1993). However, cell shape and motility were less sensitive to copper, nickel, lead, and zinc after short-term exposure (Danilov & Ekelund, 2001). The direct effect of metals on the stretch-sensitive channels on the cell wall of *Euglena* influences their ability to orient in the gravity

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field (Tahedl & Häder, 1999). This mechanism results in quick responses in motility and velocity.

Heavy metals affect microalgae photosynthesis at various sites of photosystem I (PSI) and photosystem II (PSII), and the electron transport chain (Scordino et al., 2008). The photosynthesis measurement by PAM chlorophyll-*a* fluorometry is a feasible tool for metal toxicity assessments in microalgae, including *Euglena*. Photosynthesis in *Euglena* is the primary target of toxic heavy metals such as copper, chromium, and aluminum (Danilov & Ekelund, 2002; Rocchetta & Küpper, 2009). Significant reductions in the photosynthetic quantum yield (F_v/F_m) and electron transport rates (ETR) of *E. gracilis* have been reported after exposure to copper (Ahmed & Häder, 2010a). Likewise, hexavalent chromium induces lipid peroxidation and affects the levels of fatty acids, especially those related to chloroplast structures and photosynthetic activity in *E. gracilis* (Rocchetta et al., 2006). PAM fluorometry measures any impairment in quantum efficiency and photosynthetic electron transport as variation in the PSII chlorophyll-*a* fluorescence. PAM fluorometric microplate toxicity tests using *E. agilis* showed significant dose-dependent variation in the fluorescence emission, induced by heavy metals, for example, silver and mercury (Figure 1). The reduction in F_v/F_m indicates the reduced efficiency of light energy utilization under metal stress, which can be due to the damage to reaction center pigment molecules and or loss in chlorophyll content (Ahmed & Häder, 2010b). Chlorophyll-*a* fluorescence parameters were sensitive to metals such as copper, therefore, are the appropriate test criteria for acute and chronic toxicity assays (Ahmed & Häder, 2010a). Significant dose-response of *Euglena* photosynthesis was reported for copper, zinc, and lead (Danilov & Ekelund, 2001) while cadmium and nickel did not alter photosynthesis in chronic assays (Ahmed & Häder, 2010b). However, photosynthetic parameters of *E. gracilis* were less sensitive against copper and silver ions as compared to cell motility parameters (Strauch et al., 2016).

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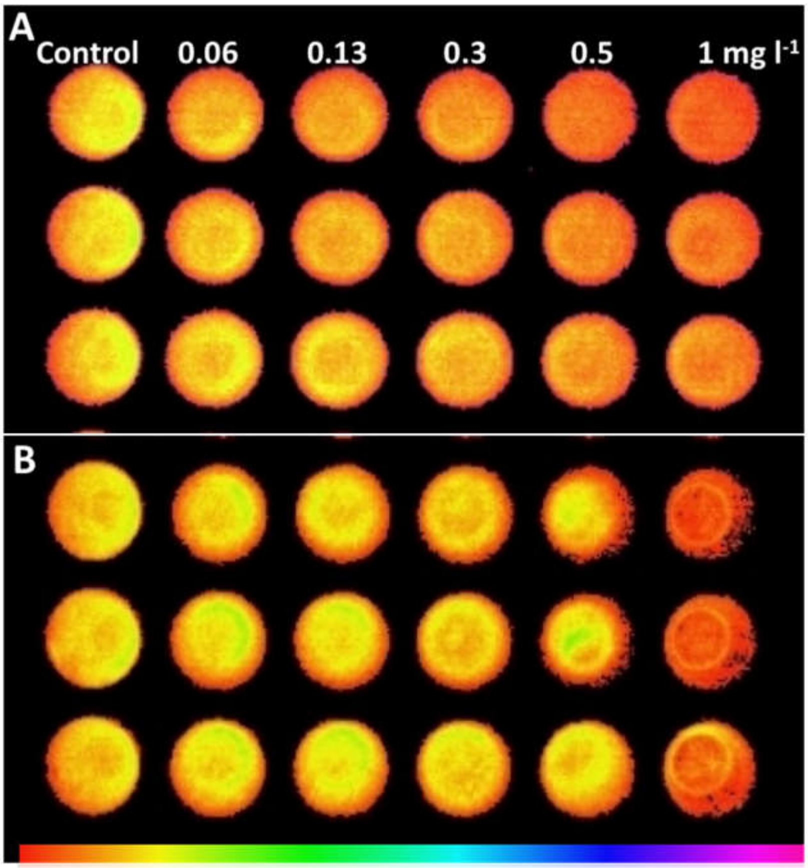

Figure 1. PAM fluorometry (Imaging PAM, Walz, Germany) false-color minimum fluorescence (F_0) image of *Euglena agilis* exposed to a gradient of model heavy metals; (A) Silver, and (B) Mercury in a 24 h microplate toxicity test. Respective concentrations of metals are shown. The false-color scale ranges from 0.000 (black) to 0.999 (purple).

Several heavy metals delay or inhibit cell proliferation rates in *Euglena* (Einicker-Lamas et al., 2002; Gajdosova & Reichrtova, 1996; Navarro et al., 1997). However, Albergoni et al. (Albergoni et al., 1980) reported that copper did not affect the growth rate of *E. gracilis* while cadmium did, accompanied by a significant intracellular accumulation of the latter. Similarly, the growth rate of *E. gracilis* at different aluminum concentrations did not show significant differences, under 15.0 mg L^{-1} Al (Danilov & Ekelund, 2002). Uptake and accumulation of heavy metals have been proposed as a tolerance mechanism of *Euglena*, which was significantly enhanced by metal pretreatment, as exemplified by the influence of mercury pretreatment on subsequent cadmium uptake and tolerance (Avilés et al., 2003). Euglenoids isolated from industrial effluent tanks showed high tolerance to chromium and lead, therefore, can be exploited for metal detoxification and environmental cleanup operations (Rehman, 2011). The euglenoid flagellate *Distigma proteus* removed 48%, 68%, 80%, and 90% cadmium from the medium after 2, 4, 6, and 8 days, respectively, proving the potential use of the species in bioremediation (Rehman et al., 2007). The metal tolerance in *Euglena* is greatly benefitted by the activation of metalloregulatory proteins (Avilés et al., 2003) and the increased antioxidant enzyme activities, such as alternative oxidase (AOX) (Castro-Guerrero et al., 2008). Taken all together, there are multiple target sites for heavy metals in euglenoids and, therefore, these organisms are efficient biological models for the study of metal poisoning and resistance mechanisms in eukaryotic cells (Einicker-Lamas et al., 2002).

A recent study also proposes *Euglena*-based toxicity assessments for rapid screening of arsenite (As-III), one of the most hazardous carcinogenic and genotoxic toxicants (Tahira et al., 2019). They showed that cell growth and photosynthetic pigments (chlorophyll- *a*, *b*, and total carotenoids) were significantly affected at higher concentrations of As-III, while slight stimulation was observed in motility, orientation, and cell compactness of the test flagellate.

3. Pesticides

Pesticides, including herbicides, insecticides, and fungicides are important components of agricultural systems worldwide, however, their extensive use in recent years has resulted in significant contamination and adverse impacts in soils, terrestrial and aquatic ecosystems, and toxic effects on humans and nonhuman biota (Carvalho, 2017). The first study on the effects of pesticides on a euglenoid microalga showed the inhibition of the growth and survival of *E. gracilis* by parathion, malathion, nabam, and vapam (Moore, 1970). Four chlorinated hydrocarbon pesticides (DDT, aldrin, chlordane, and dieldrin) and one organophosphate pesticide (diazinon) resulted in insignificant alteration in population density but reduced the amount of ATP in *E. elastica*, suggesting that the pesticides interfere with photophosphorylation in the light reaction of photosynthesis (Clegg & Koevenig, 1974). The herbicide glyphosate showed no effect on the motility of cells, but significantly altered the gravitactic orientation and velocity of the moving cells (Pettersson & Ekelund, 2006). The upward swimming and velocity of *E. gracilis* were sensitive criteria for herbicide impacts (Pettersson & Ekelund, 2006). ECOTOX analysis provided a rapid estimation of small changes in the number of cells swimming upwards (antigravitactic behavior) at higher concentrations of the organochlorine herbicide, atrazine (Netto et al., 2012). Herbicides target the cytoskeletal components involved in both the elongation and contraction of the *E. gracilis* cells, resulting in changes in movement behavior (Kawano et al., 2004). The ECOTOX analysis for herbicide toxicity was more sensitive, as the automated system is capable of detecting changes that cannot be detected through visual observation (Netto et al., 2012). Chlorophyll-*a* fluorescence and motility parameters of *E. gracilis* were significantly affected by the carbamate pesticide, carbofuran, and the organophosphorus pesticide malathion (Netto et al., 2012). Since the inhibitory effects of pesticides in short-term were stronger than in long-term experiments, ECOTOX-based movement analysis offers a rapid and sensitive method of acute assessment of pesticides in *Euglena*.

PAM fluorescence imaging is a rapid and sensitive tool for investigating PSII inhibiting biocides used in antifouling paints, such as diuron and Irgarol. The quick inhibition of photosynthetic performance of *E. agilis* was observed in a dose-dependent manner as shown in Figure 2. This observation also proves the efficacy of euglenoids in microplate toxicity assays for PSII inhibiting biocides. This method provides a valuable bioanalytical tool for rapid and inexpensive effects-based assessment, requiring less amount of the algal suspension. Moreover, a combination of real-time fluorescence and motility analysis is capable to provide ecologically significant data at environmentally relevant concentrations of the toxicants.

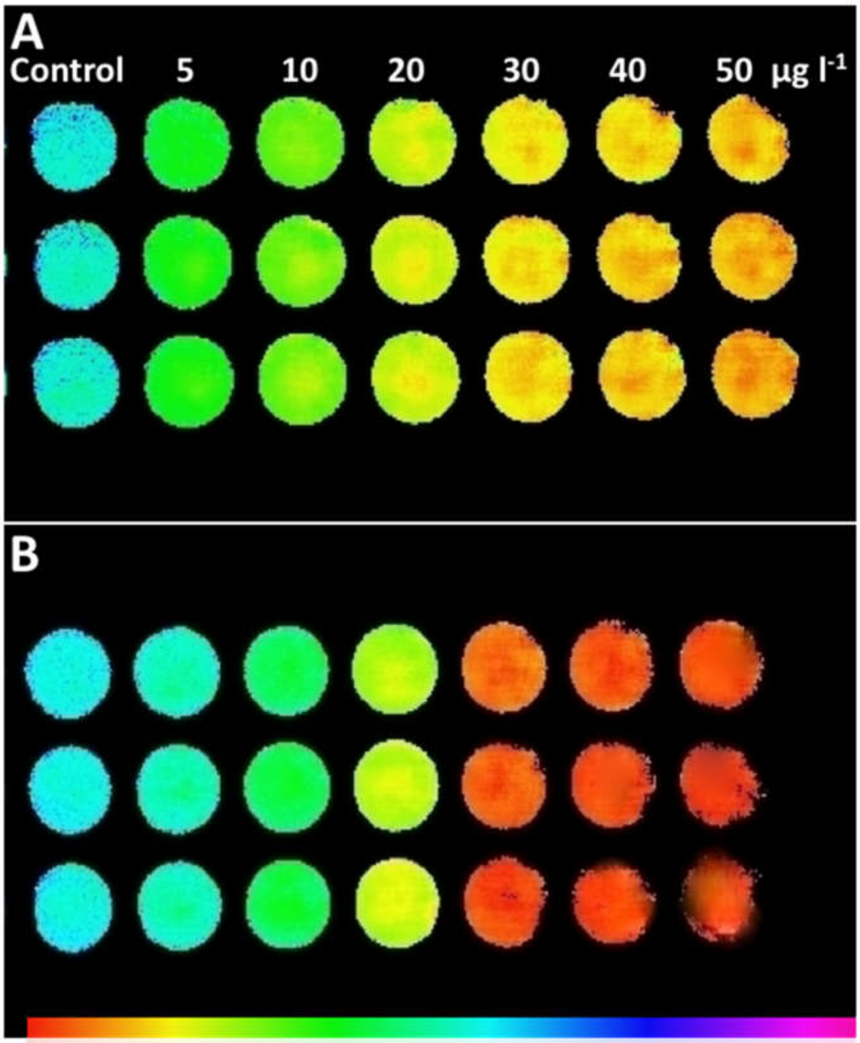

Figure 2. PAM fluorometry (Imaging PAM, Walz, Germany) false-color fluorescence image of maximum photosynthetic quantum yield (F_v/F_m) of *Euglena agilis* exposed to a gradient of model herbicides; (A) Diuron, and (B) Irgarol in a 1 h microplate toxicity test showing the dose-depended inhibition. Respective concentrations of herbicides are shown. The false-color scale ranges from 0.000 (black) to 0.999 (purple).

4. Effluents

Wastewater or effluents has been frequently reported to adversely affect aquatic organisms, but their effect on microalgae has been addressed rarely (Azizullah et al., 2015). Euglenoids are widely studied to develop rapid and sensitive effluent toxicity tests due to the prompt response of their critical parameters. For example, the motility parameters of *Euglena* respond rapidly to changes in the composition of their environment, which allows for the quick estimation of impacts of raw effluents in natural waters before getting diluted naturally. Toxic organic components in effluents may cause genotoxicity through DNA damage in *Euglena* (Li et al., 2009). Photosynthetic efficiency of *E. gracilis* was very sensitive to effluent toxicity and is promising for use in regular toxicity tests of wastewater from industries such as pulp and paper factories (Danilov & Ekelund, 1999). A feasible assay method for industrial wastewater samples from various locations was developed using F_v/F_m and ETR of *E. gracilis* (Azizullah et al., 2015). However, in acute tests, gravitactic orientation and cell compactness of *E. gracilis* were more sensitive to wastewater toxicity (Azizullah et al., 2012a). Moreover, the *Euglena* test method has been successfully used to assess the general water quality of the Indus river in Pakistan, revealing the adverse effects of urban effluents from the city of Dera Ismail Khan, situated on the west bank of the Indus river, on the physicochemical and ecological properties of the river water (Azizullah et al., 2014a). Euglenoid tests of wastewater samples revealed significant adverse effects on photosynthesis in algae and, hence, the overall primary productivity of aquatic ecosystems, although the extent of effects depends on the nature of the effluent source (Azizullah et al., 2015). However, chronic tests of at least 7 days are reported to provide more explanatory and significant results on wastewater toxicity (Danilov & Ekelund, 2001). Advanced microscopic ecotoxicological assessment using the freshwater flagellate, *Phacus brachykentron* (Pochm.) revealed that the presence of deformed cells and/or bulky intracellular paramylon bodies might be used as acute environmental bioindicators of harmful organic matter enrichment in water (Nannavecchia et al., 2014). The *Euglena* tests of effluents may show strong differences in the toxicity which depend on the dilution by precipitation and the composition of the municipal and industrial effluents (Häder, 2018).

5. Nanoparticles

Engineered nanoparticles (in the range between 1 and 100 nm in at least two dimensions) are extensively used in various fields and their release into aquatic ecosystems causes serious concerns. Several species of euglenoids are a potential source for *in vivo* and *in vitro* biosynthesis of nanomaterials such as silver nanoparticles (Li et al., 2014). However, metal nanoparticles are toxic to aquatic organisms, and investigating their effects on euglenoids may reveal their potential impacts on primary producers in freshwater ecosystems. Several studies have adopted *Euglena* as a model organism to elucidate the adverse effects of nanomaterials in microalgae, such as carbon (Hu et al., 2015), cadmium (Brayner et al., 2011), gold (Lee et al., 2015), silver (Li et al., 2015), and iron (Huang et al., 2017) nanoparticles. The emerging carbon nanomaterial graphene oxide affects euglenoid growth through shading effects and oxidative stress (Hu et al., 2015). Polonini et al. (Polonini et al., 2014) conducted a toxicity assessment for micro-and nanosized barium titanate, an emerging material for various biomedical applications, revealing statistically significant toxic effects on *E. gracilis* growth and viability after 24 hours, even at small concentrations (1 μg mL^{-1}).

Euglena quickly responds to metal nanoparticles by changing motility and cell shape, causing structural and functional changes and cell death (Noble & Lacerda, 2014). In addition, photosynthetic yield in *E. gracilis* responded in a dose-depended manner to acute exposure to silver nanoparticles (Li et al., 2015). Recently, an aggregation-induced emission luminogen (AIEgen)-based imaging technique and a traditional inductively coupled plasma mass spectrometry (ICP-MS) method has been used to suggest that silver ions released from silver nanoparticles extracellularly dominated the nanoparticle toxicity in *E. gracilis* (Zhang & Wang, 2019). The effective tolerance mechanism adopted by *Euglena* is the particle internalization by vesicles formed by the cells through the endocytosis process (Brayner et al., 2011). Smaller nanoparticles dissolve much faster, resulting in higher uptake rates by algae, and thereby exhibit greater toxicity toward the algae (Zhang & Wang, 2019). Euglenoids accumulate more nanoparticles than other microalgae and are transferred to high-level organisms via the food chain (Lee et al., 2015). The sensitivity of *Euglena* to nanomaterials was higher than other microorganisms, suggesting the potential of euglenoids in nanoparticle risk assessment (Dorobantu et al., 2015). Moreover, the motility parameters are ideal test criteria for nanoparticle toxicity in *Euglena*.

6. Organic toxic chemicals

A wide spectrum of organic compounds originated from industrial processes and domestic activities are harmful to aquatic organisms. Motility and photosynthesis parameters of euglenoids are effectively used to design ecotoxicity assessments of organic toxicants. Kottuparambil et al. (Kottuparambil et al., 2014) proposed a rapid phenol toxicity test method using motility and chlorophyll-*a* fluorescence measurements of *E. agilis*. Phenol significantly reduced F_v/F_m (Figure 3) and the maximum photosynthetic electron transport rate (rETRmax) after 1 h exposure. In addition, the motility and swimming velocity exhibited high phenol sensitivity. Nitrobenzene significantly decreased the chlorophyll content and the growth rate in *E. gracilis* (Zhu & Deng, 2006), whereas pentachlorophenol reduced the photosynthetic efficiency after 24 h of exposure (Danilov & Ekelund, 2001). 48 h benzene treatment resulted in a significant reduction of cell growth in a dose-dependent manner in *E. gracilis*, along with induction of morphological changes, formation of lipofuscin, and decreased chlorophyll content (Peng et al., 2013). Similarly, individual and mixed BTEX (benzene, toluene, ethylbenzene, and xylenes) reduced the growth rate, lowered chlorophyll production, and changed the cell shape of *E.* gracilis (Peng et al., 2015). The adverse effects of these compounds were due to direct non-specific toxic effects rather than the reactive metabolites by biotransformation (Peng et al., 2015). Chlorophyll-*a* fluorescence imaging has recognized chronic effects of anthracene, a 3-ringed polycyclic aromatic hydrocarbon (PAH) on photosynthetic performance of *E. agilis* (Kottuparambil & Park, 2019) with significant alteration in F_0 and the maximum fluorescence, F_m (Figure 4). These reports strongly suggest the potential application of euglenoids to monitor volatile toxic organic compounds in natural water samples and to predict the individual or mixed toxicities in aquatic ecosystems.

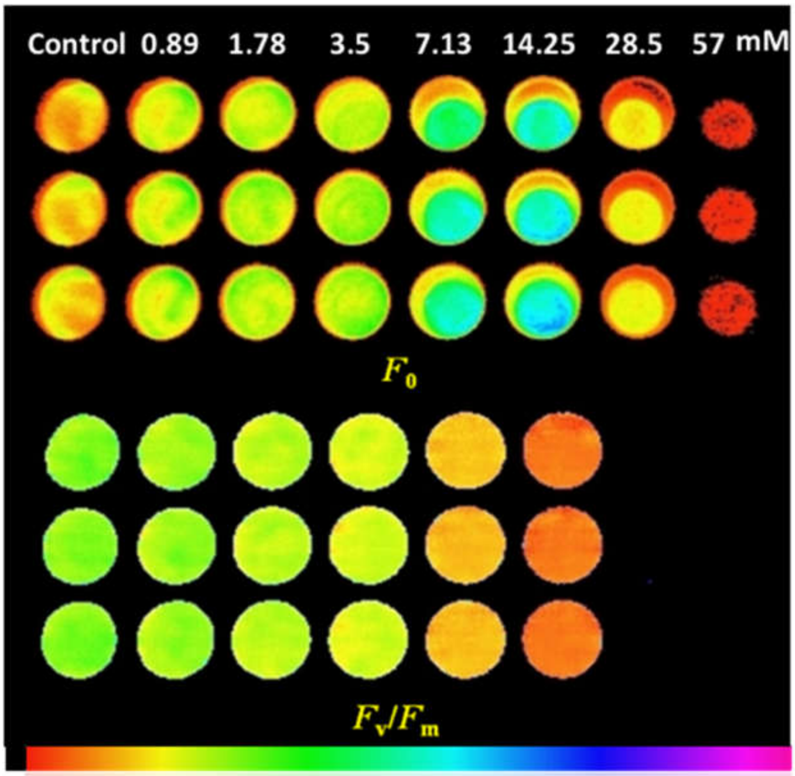

Figure 3. PAM fluorometry (Imaging PAM, Walz, Germany) false-color fluorescence image of *Euglena agilis* exposed to Phenol (1 h) showing dose-dependent effects on minimum fluorescence, F_0 (top) and maximum photosynthetic quantum yield, F_v/F_m (bottom). Absence of F_v/F_m signal at higher concentrations of phenol illustrates the absolute inhibition of photosynthesis. Respective phenol concentrations are shown. The false-color scale ranges from 0.000 (black) to 0.999 (purple).

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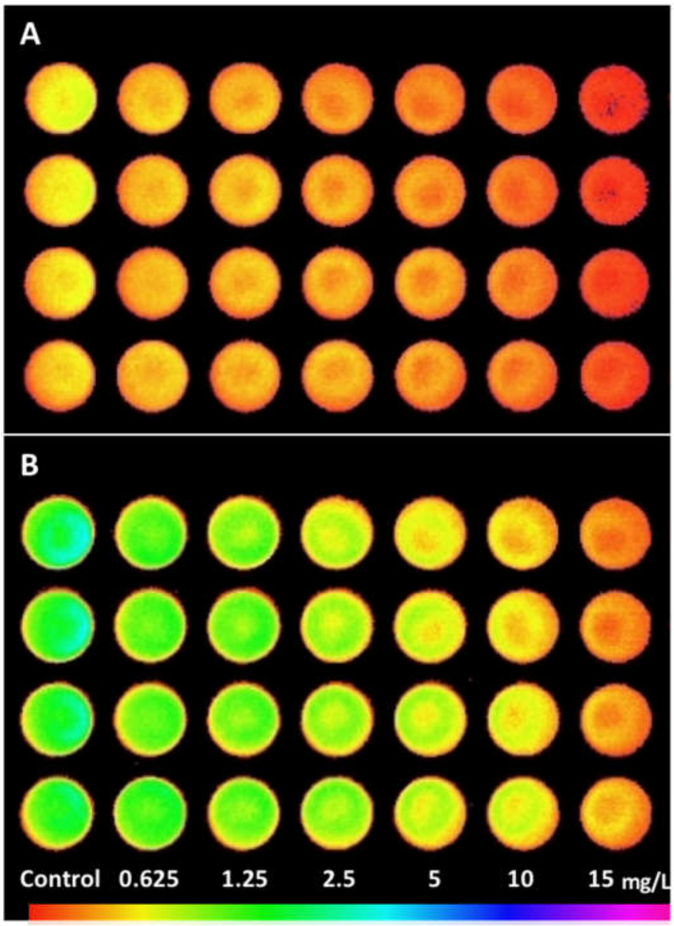

Figure 4. PAM fluorometry (Imaging PAM, Walz, Germany) false-color fluorescence image of *Euglena agilis* exposed to anthracene (96 h) showing dose-dependent effects on minimum fluorescence, F_0 (A) and maximum fluorescence, F_m (B). Reduction in fluorescence emission at higher anthracene doses indicates severe loss of pigments and/or inactivation of the PSII reaction centers. Respective concentrations of anthracene are shown. The false-color scale ranges from 0.000 (black) to 0.999 (purple).

8. Other aquatic pollutants

Several studies have explored euglenoid responses to assess the ecological impacts of common chemical pollutants in aquatic ecosystems. Among them, liquid detergents are very common in freshwater bodies, released by domestic usage and urban run-off. Motility parameters of *E. gracilis* were found to be sensitive to common liquid detergents (Azizullah et al., 2014b; Azizullah et al., 2012b). Azizullah et al. (Azizullah et al., 2014b; Azizullah et al., 2013b) recorded the maximum adverse effects of dishwasher detergent on motility, velocity, cell shape, and gravitaxis of *E. gracilis* after 1 h of exposure. Likewise, in chronic toxicity tests (7 days), motility, velocity, and photosynthesis of *E. gracilis* were affected by the commercial laundry detergent Arial, at a concentration above 1 mg l^{-1} and significant growth inhibition was observed at 1 mg l^{-1} (Azizullah et al., 2012b). Another study proposed photosynthesis and pigments in *Euglena* as sensitive endpoints for acute and chronic detergent toxicity tests (Azizullah et al., 2014b).

Wood ash, created by burning of various biofuel products and used in forestry as a resource of nutrients for depleted mineral soils and restoration of acidified soils and surface waters, is ultimately a serious aquatic pollutant. Photosynthetic parameters of *E. gracilis* were insensitive up to 12.5 g l^{-1} wood ash over 7 days (Ekelund & Andreas Aronsson, 2007), whereas motility and growth parameters were significantly altered at higher doses of raw wood ash (10–25 g l^{-1}), mainly due to the high alkalinity (Andreas & Ekelund, 2005). Although motility parameters responded rapidly to wood ash toxicity, *Euglena* was more

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tolerant to wood ash solutions than other phytoplankton such as *Chlamydomonas reinhardtii* (Ekelund & Andreas Aronsson, 2007).

Fertilizers are common contaminants in freshwater bodies but are not considered to be toxic agents in conventional ecotoxicology. Azizullah et al. (Azizullah et al., 2011a) designed a rapid screening test of fertilizers diammonium phosphate (DAP) and urea, based on motility, velocity, cell shape, and gravitaxis of *E. gracilis*. DAP was more toxic than urea with 24 h EC_{50} (motility) values of 1.38 g l^{-1} and 16.95 g l^{-1} for DAP and urea, respectively. Motility and photosynthesis of *E. gracilis* were found to be sensitive to potassium sorbate, a widely used antioxidant and antimicrobial preservative on short-term exposures (Engel et al., 2015). Another study has investigated the potential ecogenotoxicities of 4, 4′′-dibromodiphenyl ether (BDE-15), one of polybrominated diphenyl ethers (PBDEs) by detecting its effects on growth, pigment contents, antioxidant enzyme activities, and DNA damage in microalgae *E. gracilis*, reporting the mutagenic effects (Zhang et al., 2010).

A study on the toxicity assessment of suspended particles on *E. gracili*s revealed the higher toxicity of nanoscale particles than common-scale ones in the natural size range present in the Gonghu Bay of Taihu Lake in China, providing evidence for the effectiveness of ecological restoration to reduce the toxicity of suspended particles, especially the toxicity of nanoscale ones (Xiao et al., 2018). Likewise, polystyrene microplastics exerted chronic toxicity effects on *E. gracilis* indicated by inhibition of growth, motility, and photosynthetic efficiency, primarily due to internalization of the particles in cytoplasm and organelles as individual particles or aggregates (Sun et al., 2021). Moreover, the interactive effect of microplastics and potentially toxic elements aggravates intracellular oxidative stress, growth inhibition, and upregulation of redox-related genes in *E. gracilis* (Li et al., 2022; Liao et al., 2020).

9. Solar ultraviolet (UV) radiation

The ecological consequences of the increase in solar UV radiation have gained considerable attention in recent years since UV (UV-A and UV-B) alters the production and community structure of primary producers in aquatic ecosystems (Häder et al., 2011). Solar UV radiation is also known to impair the motility and swimming velocity of photosynthetic flagellates which would limit their capacity to adapt to the surrounding light. Euglenoid flagellates have been used as biological dosimeters in monitoring the impacts of harmful UV radiation on aquatic organisms. The high energy of UV induces photoinhibition and reduced cell compactness and motility in *Euglena* (Figure 5), accompanied by abrupt shifts in taxis movement patterns (Figure 5) induced by intracellular reactive oxygen species (ROS) formation (Kottuparambil et al., 2012). Excess radiation energy provokes a change in the gravitaxis signal from negative to positive in euglenoids, suggesting that UV reverses gravitaxis of the cells so that they move downward in the water column (Richter et al., 2007). Photosynthetic oxygen production, pigmentation, and protein composition were also found to be seriously impaired (Gerber & Häder, 1992). Photo-orientation, chlorophyll integrity, and cellular motility was severely affected by UV-B radiation in *E. sanguinea* (Gerber & Häder, 1994). Euglenoids have developed effective protection mechanisms to avoid or reduce the damage induced by UV radiation, such as the production of pigments and the repair mechanisms in hours of darkness and during daylight. In *E. sanguinea*, artificial UV-B irradiation induced intracellular astaxanthin diester accumulation which suggests that the carotenoid is a photoprotective pigment in euglenoids (Gerber & Häder, 1994). Moreover, reactive oxygen species (ROS) play a significant role (Figure 6) in the response of *E. agilis* to UV-B radiation (Kottuparambil et al., 2012). *E. agilis* exposed to excessive UV-B lost its plant-like features, investing all its stored energy into the movement rather than into sustaining its photosynthetic machinery. This is an effective recovery mechanism as it allows the population to find the most appropriate niche where the organism may regain its photosynthetic capacity for survival (Kottuparambil et al., 2012). These studies underpin that euglenoid flagellates are ideal organisms for studying the physiological consequences of harmful UV radiation in primary producers of aquatic ecosystems.

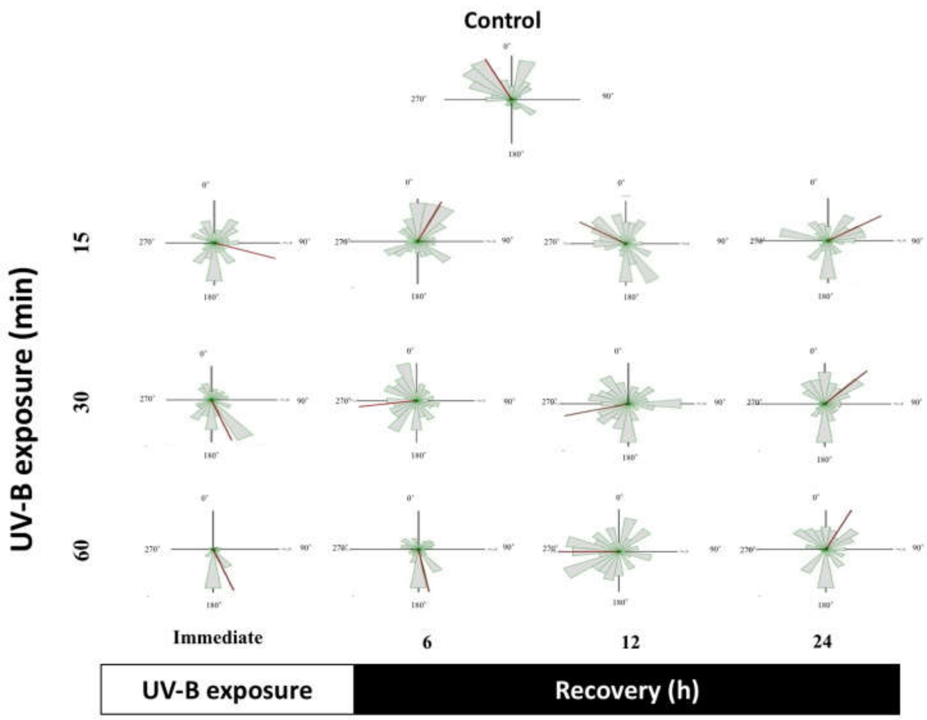

Figure 5. Circular histograms showing a shift in the directional movement of *Euglena agilis* on exposure to UV-B radiation and subsequent recovery.

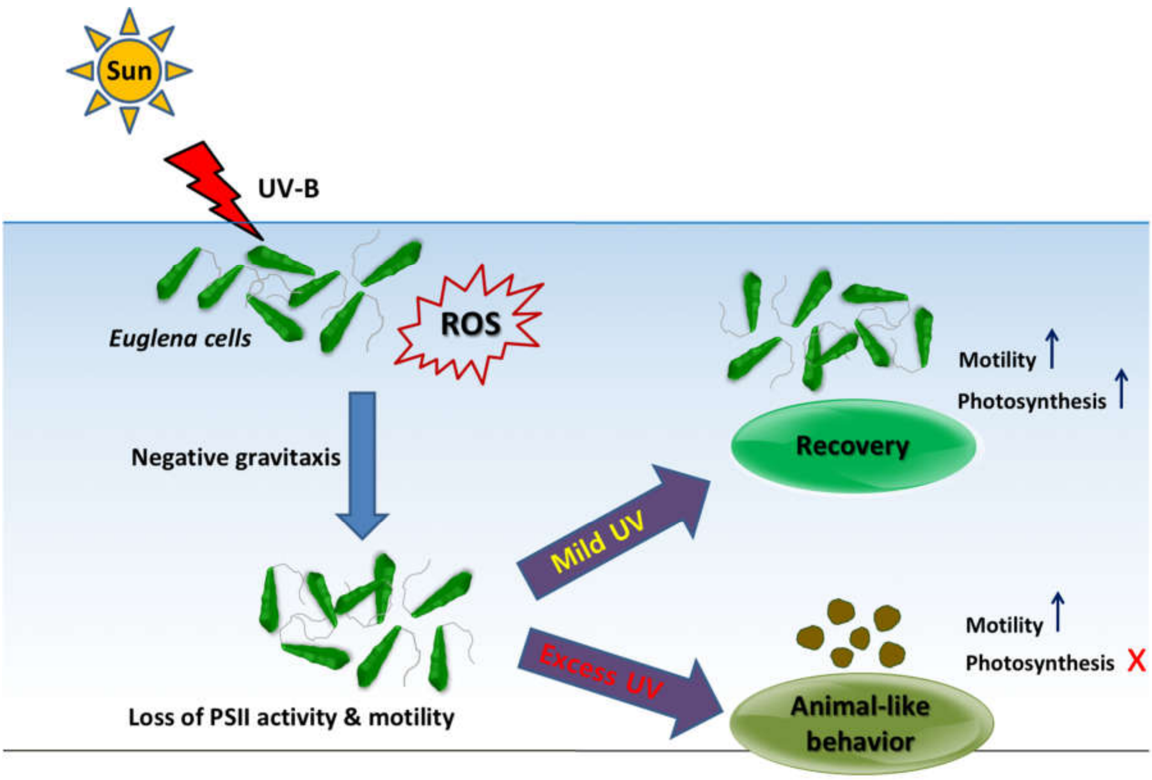

Figure 6. Schematic representation of overall UV impacts and stress responses in *Euglena*.

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10. Conclusions

Euglenoids are a group of predominantly free-living unicellular microorganisms that mostly live in eutrophic shallow freshwater bodies where various pollutants are potentially threatening the microalgal communities. The vital physiological features of Euglenoids such as photosynthesis, movement, and orientation are good indicators of external stimuli from physicochemical and biological stressors such as toxic chemicals and UV radiation. The ECOTOX system provided a breakthrough for the euglenoid test for water and effluent quality assessments.

Automated image analysis systems are highly desirable for application in water quality test settings. However, the reliability of the *Euglena*-based tests depends on the careful selection of appropriate endpoint parameters since a wide variability of response exists among various parameters to different stressors. The motility parameters are also affected by several abiotic factors, therefore proper control of the test conditions must be achieved to assure the unbiased analyses of the responses during the tests. Another critical aspect is the duration of the test which additionally influences the significant output of the assays. The selection of proper exposure time should be done considering the nature of the toxicant, available toxicity mechanism, its persistence in the environment, and the adaptability of the test criterion to the toxic effects. The rapid, robust, and consistent results of the *Euglena* test are valuable for risk assessment of aquatic contaminants; however, information on the origin and chemical characterization of the toxicants are extremely essential to draw relevant conclusions and recommendations based on the tests.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

UV, ultraviolet; PSI, photosystem I; PSII, photosystem II; PAM, pulse amplitude modulation; F_v/F_m, maximum quantum efficiency of photosynthesis; F_0, minimum fluorescence; F_m, minimum fluorescence; ETR, electron transport rate; ROS, reactive oxygen species; AOX, alternative oxidase; BTEX, benzene, toluene, ethylbenzene, and xylenes; PAH, polycyclic aromatic hydrocarbon; DAP, diammonium phosphate; BDE-15, 4, 4′′-dibromodiphenyl ether; PBDE, polybrominated diphenyl ethers.

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