AACL BIOFLUX
Aquaculture, Aquarium, Conservation & Legislation
International Journal of the Bioflux Society

Effects of hydrogen peroxide on *Compsopogon caeruleus* (Rhodophycophyta) and two superior plants

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Abstract. Hydrogen peroxide was investigated as a potential algaecide for the filamentous epiphytic alga, *Compsopogon caeruleus* (Balbis ex C.Agardh). The goal was to determine if hydrogen peroxide could be used to eliminate *C. caeruleus*, without affecting two other aquatic plants, *Ceratopteris thalictroides*, and *Hygrophila rosanervis*. A zebrafish (*Danio rerio*) was also exposed during the treatment, to observe the effects of hydrogen peroxide on fish. Concentrations between 1mM and 6 mM l^{-1} were tested for their ability to induce bleaching or tissue disintegration in plant and algal tissues. 1 mM l^{-1} hydrogen peroxide had no major effect on the alga, or on the plants. The 3 mM solution induced partial bleaching in *C. thalictroides* and damaged significantly de filamentous alga. The 6 mM solution killed completely the alga and damaged significantly the *C. thalictroides*. *H. rosanervis* suffered minor lesions during the treatment. *D. rerio* wasn't affected by the mentioned concentrations of hydrogen peroxide.
Key Words: hydrogen peroxide, *Compsopogon, Ceratopteris, Hygrophila*, algaecide, bleaching, tissue degradation.

Kivonat. A hidrogén-peroxid algaölő hatása volt a kutatás tárgya, az anyag algaölő képességeit vizsgálva egy fonalas epifita algán, a *Compsopogon coeruleus*-on. A cél a hidrogén-peroxid azon tulajdonságának a vizsgálata volt, hogy képes-e elpusztítani a fonalas algát, anélkül, hogy ártana két másik vízi növénynek, a *Ceratopteris thalictroides*-nek és a *Hygrophila rosanervis*-nek. Egy vörös zebrán (*Danio rerio*) szintén a hidrogén peroxid, a halakra kifejtett hatása került vizsgálat alá. 1 mM és 6 mM l^{-1} peroxid tartalmú oldatokban a növényi és algaszövetek károsodát figyeltük. 1 mM l^{-1} hidrogén-peroxid nem károsította jelentősen a fonalas algát és nem befolyásolta a vízi növényeket. A 3 mM-os oldat enyhén károsította a *C. thalictroidest*, az algát pedig jelentősen. A 6 mM-os oldat teljesen elpusztította az algát, de a *C. thalictroidest* is jelentősen károsította. A *H. rosanervis* enyhén sérült a kezelés alatt. A vörös zebrán nem jelentkeztek stressz jelei az említett koncentrációk hatására.
Kulcsszavak: hidrogén-peroxid, *Compsopogon, Ceratopteris, Hygrophila*, algaölők, kifehéredés, szövetlebomlás.

Introduction. Hydrogen peroxide (H_2O_2) has been recognised as an important non-toxic herbicide in aquaculture in the late seventies (Quimby 1981; Stratford et al 1984), altough the first experimental results showed its corrosive effects on plant cells in the 1880's (Landsborough 1927). Hydrogen peroxide is a natural byproduct of photosynthesis and several metabolic processes (chloroplastic, mitochondrial, and plasma membrane-linked electron transport systems) produce reactive oxigen species such as H_2O_2. It has a crucial role in plant metabolism and cell signaling, and is being produced by metabolic processes induced by changes in temperature, photoactive radiation and pathogenes (Zhang et al 2001). Hydrogen peroxide can be produced enzymatically, in the dark, by cell-surface enzymes, in the case of photosynthetic phytoplancton (Palenik et al 1987), or it is generated photosynthetically, in photochemical reactions. In both cases, reactive oxigen species can accumulate and this may result in significant damage to cell structures. This damaging effect may affect the cell wall, the photosynthetic organelles, fatty acids, aminoacids and DNA. H_2O_2 is a powerful oxidizer and cand be used as an algicide or herbicide to eliminate unwanted algal and plant species in aquaculture (Bud 2010). The succesful use of hydrogen peroxide as a herbicide relies on the coordonation of the appropriate dosage

(concentration) and the duration of the treatment by taking into account the possible destabilizing agents like light, dissolved organic matter, dissolved metals or other possible catalizers. Unicellular, colonial, filamentous algae and aquatic macrophytes have different resistance to hydrogen peroxide. Unicellular and colonial alge are the most vulnerable to H_2O_2. In the case of filamentous algae and other macrophytes a higher dose and a longer exposure time is necessary (Quimby 1981; Stratford et al 1984). Quimby measured the short-term toxicity (exposure time up to 60 minutes) of H_2O_2 and observed that plants show 30% damage after 1 week. Treatments with higher concentrations of the solution had better resuls, but hydrogen peroxide decomposed rapidly under continuous illumination. Stratford found a direct relation between the concentration of the solution, the breakdown of hyrogen peroxide, photon flux density and damage in plant tissues. Hydrogen peroxide decomposes rapidly under light and has a more intense effect this way, but exposure time decreases with rapid decomposition. The goal of this study is to observe the effects of high concentrations of hydrogen peroxid (1mM to 6 mM l^{-1}) on two aquatic plants and a filamentous alga. Oxidative damage to algal and plant tissues did not involve photosynthesis, because the plants were not illuminated. The correlation between the cell structure and the resistence to oxidative effects and bleaching was examined.

Material and Method. Two aquatic plant species (*Hygrophila rosanervis* and *Ceratopteris thalictroides*) and a filamentous epiphytic alga (*Compsopogon caeruleus)* were selected to perform the experiment with the main goal to compare the cellular structures after the treatment with hydrogen peroxide, to determine the grade of resistence of the selected plants versus the filamentous alga. *C. caeruleus* is a fast-growing, non-parasitic alga, present in most aquariums with favorable illumination and nutrition. Alga-eating fish, like *Ottocinclus affinis* and *Crosochelius siamensis* are feeding poorly on this alga, so there is little chance to eliminate it by biological control. *H. rosanervis* and *C. thalictroides* are fast-growing aquatic plants with low demands, preferred in aquascaping. The plants were purhased from a local pet shop and planted in a 100 liter aquarium, illuminated with four 25 watts T5 fluorescent tubes (Osram 965, with a color temperature of 6500K). Nitrate levels were kept at 10 mg l^{-1} with KNO3 for a month. After a month, *C. caeruleus* was blooming in the aquarium. Two aquatic plants with healthy stems and leafs and an aproximately same amount of filamentous alga was selected and positioned in a 3 liter glass aquarium, filled with tapwater mixed with water filtered with a reversed osmosis filter (Aquatic Nature Osmobox 150). This mixture was necessary to avoid metallic impurities wich could influence the breakdown of H_2O_2. Three aquariums were selected this way. A zebrafish (*Danio rerio*) was introduced in each aquarium to observe the effects of the oxidative treatment on fish. The experiment was performed in room temperature. The samples were not illuminated to avoid the destabilization of the hydrogen peroxide. The breakdown of the H_2O_2 during the oxidation process was monitored by measuring the level of dissolved oxigen with a Hanna Hi 9828 multimeter. The Hydrogen peroxide was purchased from a local store in a 3% aqueous solution and was added to the samples in the form of ice cubes as described by Quimby (1981). 1mM l^{-1} (2.37 ml solution per ice cube) H_2O_2 was dissolved in the first aqiuarium, 3 mM (7.11 ml), respectively 6mM (14.22 ml) was added into the second and third aquarium. Canges induced by the oxidation process in cells and tissues were evaluated and photohraphed with a Muller Biosphere-t brightfield microscope. Measurements and samples for microscopic examination were taken in regular intervals (Figure 1) Tissues and cells were evaluated by visual scoring (0 to 10, were 10 is dead) taking into account the scale of bleaching (number of chloroplasts), cell wall integrity and dissue degradation.

Results and Discussion. Concentrations of H_2O_2 ranging from 1 mM to 6mM were eliberated in each of the three aquariums after the melting of the ice cubes. Bubbles of oxigen appeared immediately on the bottom of the aquarium, indicating that the porocess of decompositon has begun: $H_2O_2 \rightarrow H_2O + O_2$. The level of dissolved oxygen

has started to rise immediately. The levels of DO indicated the changes in the decomposition process (see Figure 1).

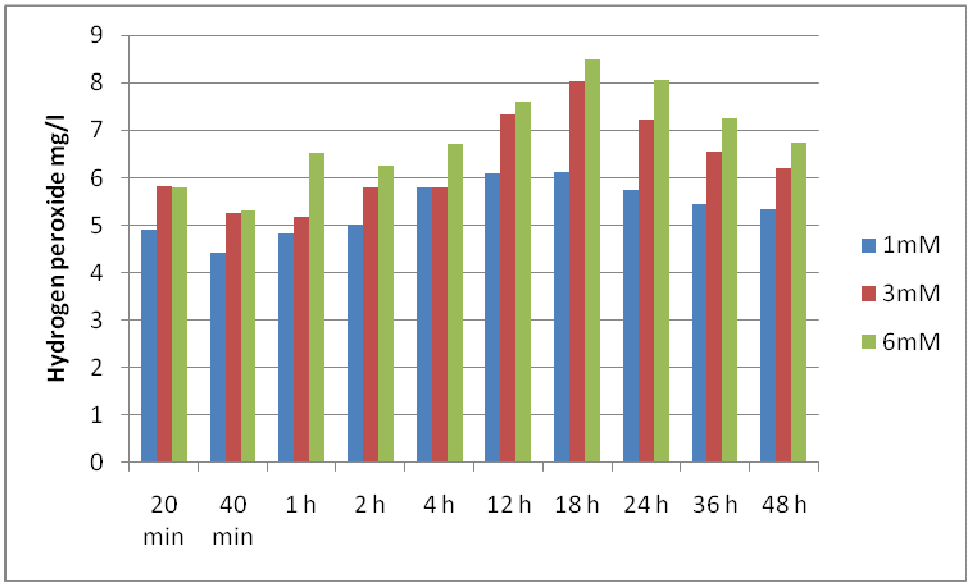

Figure 1. Dissolved oxigen levels measured during the decomposition process of H_2O_2

Plant and algal samples were taken from each aquarium in the same time with the measurments. The samples were examined microscopically at 80x, 200x and 800x magnifications.

Table 1

Eeffects of H_2O_2 at various concentrations and exposure times on *C. thalictroides*, *H. rosanervis* and *C. caeruleus* [injury scale: 0 (no injury) to 10 (dead)]

Species	*Concentration of H_2O_2*	*Exposure time to H_2O_2*	*Injury to plants and alge*	*Remarks*
H. rosanervis	1 mM	4 h	0	
C.thalictroides	3 mM	1 h	0	
C. caeruleus	3 mM	1 h	1	
C. caeruleus	6 mM	4 h	2	
C. thalictroides	6 m M	4 h	1	
C. caeruleus	6 mM	12 h	5	
H. rosanervis	3 mM	12 h	1	
C. thalcitroides	6 mM	12 h	3	
C. caeruleus	3 mM	12 h	3	
C. thalictroides	3 mM	18 h	3	
C. caeruleus	3 mM	18 h	4	
C. caeruleus	6 mM	18 h	6	
C. thalictroides	6 mM	18 h	6	
C. thalictroides	6 mM	24 h	7	
C. caeruleus	6 mM	24 h	7	
C. caeruleus	1 mM	36 h	2-4	
C. caeruleus	3 mM	36 h	6-8	
H. rosanervis	6 mM	36 h	1-2	
C. caeruleus	6 mM	36 h	9-10	
C. caeruleus	6 mM	48 h	10	Desintegration of the filaments
C. thalictroides	6 mM	48 h	8-9	
C. thalictroides	3 mM	48 h	3-4	
C. caeruleus	3 mM	48 h	7-8	

Altough the oxidation process was visible (oxigen bubbles) in both aquariums treated with 1mM and 3mM, plant and algal tissues showed little or no oxidative damage in the first 4 hours, and the damage was minimal in the first 12 hours. Bleaching appeard first in algal tissues, at 3mM, after 4 hours of exposure, but no further bleaching was observed in te first 12 hours. Small white spots (indicating chlorophyll loss) in *C. thalictroides* tissues also appeared after 4 hours. Partial bleaching in algae and small white spots in plant tissue were scored visually between 1 and 3 points. *H. rosanervis* has not been damaged in the first 24 hours of treatment by the 1mM and 3mM concentrations and showed little damage during the whole treatment. Treated with 1mM H_2O_2, *C. caeruleus* bleached partially after 24 hours, but it has not been scored with more than 4 damage points and it wasn't killed during the 48 hours of treatment. Plants in the aquarium treated with 3 mM solution survived the treatment, altough *C. thalictroides* has bleached partially (Figure 2).

The filamentous alga has bleached significantly, but hasn't died during the treatment (Table 1, Figure 3). Further damage probably would have been observed after 48 hours, but the lack of illumination and nutrition probably would have altered the results.

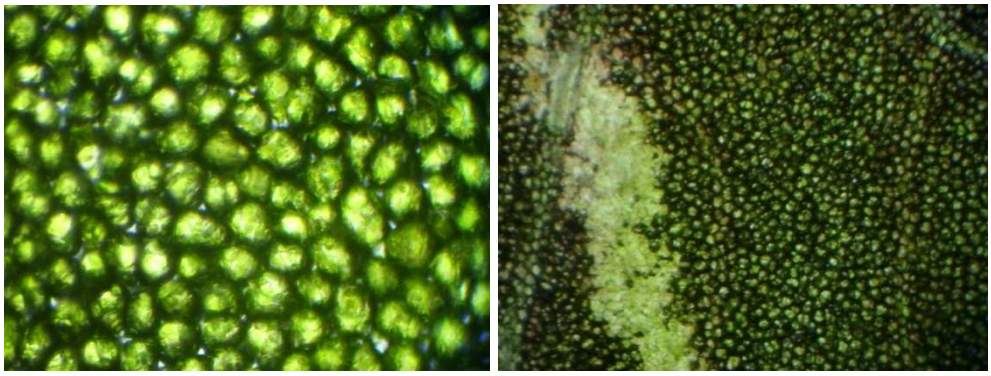

Figure 2. Healthy and damaged plant tissue

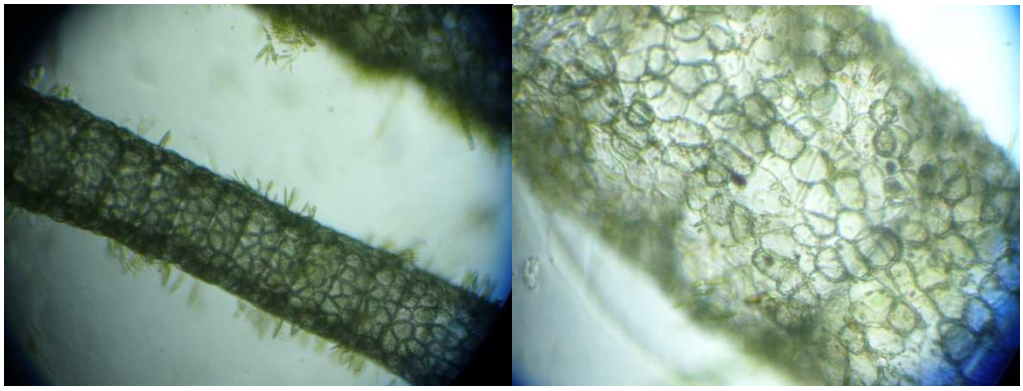

Figure 3. Healthy and partially bleached algal filament

In the case of the aquarium treated with 6mM H_2O_2, *C. caeruleus* has shown signs of bleaching after 4 hours and has lost aproximately half of its chlorophyll content after 12 hours and most of it after 24 hours. Filaments started to disintegrate after 36 hours and the alga was considered dead after 48 hours. *H. rosanervis* was damaged, but not significantly, mostly at the edge of the leafs and survived the treatment. *C. thalictroides* has bleached partially after 12 hours and the damage was significant after 24 hours (Table 1). After 36 hours holes, visible with the naked eye, appeared mostly on the older leafs. Younger leafs showed less damage, but only the stem remained unafected, while some of the leafs has disintegrated (Figure 4). *Danio rerio* has survived in all three aquariums and showed no signs of stress during the experiment.

Figure 4. Completely bleached, partially disintegrated algal filaments and partially bleached *Ceratopteris* plant leaf

Conclusions. Hydrogen peroxide can be used as an algaecide in aquariums. In the absence of continuous illumination, the breakdown of H_2O_2 is slower and does not influence photosynthesis. Without illumitation, higher concentrations can be used with better overall results, in a shorter period of time, without affecting the health of fish.

A 1mM solution is not enough to cause damage in plant and algal cells, thus cannot be used as an algaecide. A 3 mM l^{-1} concentration can be used to control algae without affecting plants and fish, altough the mortality wont be 100% after a 48 hours of treatment. The 6mM solution killed all the filamentous algae during the treatment, but it has also affected negatively the *C. thalictroides*. A 6mM solution can be a succesful algaecide in aquariums but it will kill plants with smaller and thinner leafs. The resistence of *C. caeruleus* to H_2O_2, compared with *C. thalictroides* is high. The alga cannot be eliminated with H_2O_2 without affecting this plant. *H. rosanervis* will resist to concentrations high enoug to eliminate this filamentous alga. Concentrations up to 6 mM l^{-1} may be enough to kill most filamentous alge, bout the treshold value may differ with different algal species (Momeu & Péterfi 2007). Altough H_2O_2 was not toxic to fish it cannot be taken for granted in the case of all aquarium inhabitants (Powell & Perry 1997). Snails and shrimps might have a low resistence to H_2O_2 and might die during the treatment (Adeyinka & Rim-Rukek 1999). Further researches about the effect of this substance on invertebrates would provide valuable data. Outdoor aplications of sthis substance as an algicide or as a herbicide are possible, but several factors must be taken into account wich may alter the results of the treatment: light, dissolved organic matter, phytoplankton might alter the breakdown process of H_2O_2, and several monocellular or invertebrate organisms may die during the treatment.

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Received: 05 November 2010. Accepted: 27 November 2010. Published online: 28 November 2010.
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How to cite this article:
Vass L., Bud I., 2010 Effects of hydrogen peroxide on *Compsopogon caeruleus* (Rhodophycophyta) and two superior plants. AACL Bioflux **3**(5):367-372.