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# CHLOROPHYLL RESPONSE OF AQUATIC MOSS *FONTINALIS ANTIPYRETICA* HEDW. TO CU, CD AND PB CONTAMINATION *EX SITU*

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**Abstract.** Aquatic moss *Fontinalis antipyretica* Hedw. pigments ratio (chlorophyll *a* and *b*) was investigated under controlled conditions to study chlorophyll response to the effects of copper, cadmium and lead. The species was selected both due to its proven role as a biomonitor and morphological characteristics of its leaves. It was established that the moss has significant capability to maintain moderate chlorophyll *a* and *b* levels exposed at increasing heavy metals (Cu, Cd and Pb) concentrations. The chlorophyll (*a* and *b*) content is a reliable parameter and its small but persistent decreasing usually reflects stress and could be associated with marked effects on photosynthesis and long-term survival.

**Keywords:** chlorophyll, *Fontinalis antipyretica*, heavy metals

## INTRODUCTION

Aquatic bryophytes play an important role in the freshwater ecosystems since they are primary producers, support periphyton and provide a refuge, and occasionally food, for macroinvertebrates, amphibian, and fish (BOWDEN, 1999). Despite the above their physiological ecology is an object of single researches.

Freshwater ecosystems pollution reflects negatively aquatic biota. Photosynthetic organisms may be exposed to simultaneous heavy metals stresses, which would cause severe damage affecting basic metabolic processes. As a response to contaminations bryophytes immediately react on metabolic and physiological level since they lack roots and other similar systems (TYLER, 1990; YURUKOVA *et al.*, 1996).

Aquatic moss *Fontinalis antipyretica* is resistant to contamination and is suitable for monitoring heavy metals and toxic elements in the environment. As a proper object the species has been investigated in a number of studies: DIETZ, 1972;

EMPAIN, 1977; SAY *et al.*, 1981; WEHR *et al.*, 1983; BRUNS *et al.*, 1995; YURUKOVA *et al.*, 1997; etc. The moss has been also, on occasion, an object of laboratory experiments (CENCI, 2000; VAZQUEZ *et al.*, 2000).

*Fontinalis antipyretica* in contrast to other bryophytes could assimilate bicarbonates as a C source for photosynthesis which explains its presence in alkali water environment (PEÑUELAS, 1985a). The distance through which $CO_2$ diffusion is accomplishing from water to chloroplasts is small for the fineness and monolayer leaves, and as a result even low $CO_2$ concentration could stimulate considerably photosynthetic level. Notwithstanding lower photosynthetic activity is established in bryophytes in comparison with freshwater algae (PEÑUELAS, 1985b). The same author (1985b) estimates slight increase of the *Fontinalis antipyretica* chlorophyll *a*/chlorophyll *b* ratio parallel to other bryophyte species since the moss is adapted to shaded habitats and is fully submerged. At the same time the specimens from upper *Fontinalis* tuffs light exhibited to a greater extent do not show morphological differences with specimens shaded from upper layers (BIEHLE *et al.*, 1998).

*Fontinalis antipyretica* appears to be the most tolerant to contamination among 6 aquatic bryophytes studied for pigment content changes towards pollution (PEÑUELAS, 1984b). Mean chlorophyll *a* level of 0,302±0.05 % d.w. is measured at the moss natural sites without replacing, chlorophyll *b* 0.143±0.01 % d.w. respectively. Similar research was accomplished by LÓPEZ & CARBALLEIRA (1989) in the range of 32 rivers (NW Spain) with five aquatic bryophytes. *Fontinalis antipyretica* show a great stability to contamination and capability to bear chlorophyll loss up to 43%. The observed recovery following the initial stress after transportation at anthropogenic influenced conditions is approved as an indication of certain adaptation (LÓPEZ *et al.*, 1994).

BRUNS *et al.* (1997) also applied chlorophyll level as a parameter towards *Fontinalis antipyretica* application as biomonitor of heavy metals.

Copper, cadmium and lead are important and widespread heavy metals released into the aquatic ecosystems by diverse sources. Cadmium is relatively mobile in freshwater ecosystems and can be accumulated by freshwater biota. The general effects of Cu, Cd and Pb toxicity in higher plants include chlorosis and reduced growth. More specifically, Cd interacts with water balance, damages the photosynthetic apparatus, lowers chlorophyll and carotenoid content, affects the activity of several enzymes through replacement of other metal ions (SANIT`A DI TOPPI & GABBRIELLI, 1999).

*Ex situ* experiment was done in order to provide information about potential effects that Cu, Cd and Pb may have on the aquatic moss species. In the current study enhanced levels of Cu, Cd and Pb were used with respect to those usually found in nature, to observe clear physiological effects that would otherwise be difficult to detect. The observed data in the current study are part of integrated research on morphology, physiology and accumulation of heavy metals in aquatic mosses and their medium.

**MATERIAL AND METHODS**

In the present investigation, the species was assembled from the upstream of the Maritsa River (1900 m a.s.l.), unpolluted area in the Rila Mountain. The control plants were collected together with water. Tufts of *Fontinalis antipyretica* were washed in stream to remove sediments and attached particles.

The experiment of 8 days was conducted in a scientific laboratory at the Faculty of Biology, University of Sofia “Kliment Ohridski”. Plants were placed into containers with capacity of 5 liters and exposed in solutions of copper and cadmium with increasing concentrations: 0.1; 0.5 and 1.5 mg/l Cu and 0.5; 1.5 and 3.5 mg/l Cd, and in 1.5 mg/l Pb respectively. Temperature and light conditions were moderate. Moss samples weighing 0.1 g were collected for analysis after 24, 48, 72 и 96, 192 hours. Simultaneously after the $96^{th}$ hour specimens were transferred to non contaminated water for 96 hours and analyzed with purpose to test their ability for recovery.

Each sample consisted terminal 3 cm tips with the purpose of eliminating the epiphytic organisms’ production (STREAM BRYOPHYTE GROUP, 1999). Determination of chlorophyll content followed SHLYK (1968). Spectrophotometric analysis of photosynthetic pigments was performed after extraction with 90% acetone (SPECOL 10 absorption spectrophotometer) at the Faculty of Chemistry, University of Plovdiv “Paisii Hilendarski”.

**RESULTS AND DISCUSSION**

In spite of the long residence time in the solutions, at the end of the experiment the specimens were considerably non affected, showing a former green color and with single dead strands.

The obtained 384 data results could be summarized as follows.

In copper treated specimens were no significant differences between chlorophyll *a* levels corresponding to the exposed hours, except for the first two Cu concentrations where was observed a decrease up to 2 times at the 48-hour. Despite variables measured, chlorophyll *a* in all tested copper solutions at the final 192-hour had similar levels to the background (1.60 mg/g w.w.).

Chlorophyll *a* maintained its control value in Cd solution with lowest concentration and showed increasing at the end of the so called recovering period (i.e. -96-hour). Middle cadmium concentration diminished chlorophyll *a* ratio 2 times at the 48-hour. The pigment reached 2.00 mg/g w.w. during the 96-hour. Analogically with identical chlorophyll *a* levels measured at the 192-hour in the three Cu solutions, in the Cd-treated moss pigment level at the same hour was close to the background.

It could be summarized that the most crucial test period both in Cu and Cd solutions was 48-hour, whereas chlorophyll *a* increasing started at the 72-hour, and background chlorophyll *a* level was reached thereafter at 96-hour.

In lead solution moss tissues illustrated slight differences in chlorophyll *a* level during the study, most similar with the chlorophyll *a* reaction in the first Cd solution.

There were significant differences in the chlorophyll *a* levels between the three metal regimes during the exposure period. Maximum pigment concentration of 2.10 mg/g w.w. was read at the -96-hour in the moss leaves treated with Cd1 solution, since minimum concentration (0.86 mg/g w.w.) was measured in the tissues tested in medium Cu and Cd concentrations at the 48-hour.

Chlorophyll *b* measured in the same samples from the above test solutions followed the chlorophyll *a* course during the exposure periods. However more marked differences between highest and lowest concentrations were observed at the copper exposed specimens (over 6 times).

Table 1 shows the overall effects of the solutions on the pigment ratios during the experiment.

**Table 1.** *Effects of Cu, Cd and Pb solutions on the chlorophyll a and b of the aquatic moss Fontinalis antipyretica - overall statistics (Surfer 8.0 applied).*

| | 24 hour | | 48 hour | | 72 hour | | 96 hour | | 192 hour | | -96 hour | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **chl a** | **chl b** | **chl a** | **chl b** | **chl a** | **chl b** | **chl a** | **chl b** | **chl a** | **chl b** | **chl a** | **chl b** |
| Minimum, mg/g w.w. | 1.00 | 0.64 | 0.86 | 0.36 | 0.97 | 0.70 | 1.60 | 1.10 | 1.20 | 0.62 | 1.00 | 0.67 |
| Maximum, mg/g w.w. | 1.90 | 2.00 | 1.60 | 1.10 | 1.80 | 1.30 | 1.98 | 2.30 | 1.80 | 1.30 | 2.10 | 2.40 |
| Median | 1.50 | 0.99 | 1.20 | 0.99 | 1.20 | 0.94 | 1.80 | 1.60 | 1.50 | 1.00 | 1.70 | 1.50 |
| First quartile | 1.18 | 0.77 | 0.95 | 0.60 | 1.10 | 0.89 | 1.65 | 1.23 | 1.33 | 0.88 | 1.35 | 0.98 |
| Third quartile | 1.75 | 1.52 | 1.53 | 1.08 | 1.70 | 1.15 | 1.80 | 2.07 | 1.68 | 1.18 | 1.92 | 2.25 |
| Standard error | 0.13 | 0.19 | 0.12 | 0.11 | 0.13 | 0.08 | 0.05 | 0.18 | 0.08 | 0.09 | 0.14 | 0.26 |
| Variance | 0.11 | 0.26 | 0.09 | 0.09 | 0.12 | 0.04 | 0.02 | 0.23 | 0.05 | 0.05 | 0.15 | 0.48 |
| Average deviation | 0.26 | 0.41 | 0.23 | 0.26 | 0.28 | 0.15 | 0.10 | 0.40 | 0.17 | 0.17 | 0.30 | 0.59 |
| Standard deviation | 0.34 | 0.51 | 0.30 | 0.30 | 0.34 | 0.20 | 0.13 | 0.47 | 0.22 | 0.23 | 0.38 | 0.70 |

Chlorophyll *a* levels are in the range 1.80 – 1.20 mg/g w.w. at the 192-hour in moss specimens from solutions with highest concentrations of the selected heavy metals. Comparison to the background chlorophyll *a* (1.60 mg/g w.w.) and to the lowest chlorophyll *a* level measured in the same species *in situ* (0.80 mg/g w.w., 7 km before the town of Plovdiv) suggests that Maritsa River middle flow is suffering from larger copper, cadmium and lead pollution than the cited heavy metal levels read in the river water samples (for example up to 0.006 mg/dm$^3$ Cu, 7 km before the town of Plovdiv, whereas Pb and Cd are under the detection limit of 0.001 and 0.0002 mg/dm$^3$ respectively). The same element has maximum concentration of 59 mg/kg d.w. analyzed in corresponding moss samples.

The ratio chlorophyll *a*/chlorophyll *b* in moss treated with Cu solutions varied from 0.8 to 2.4 and from 0.6 to 1.7 for Cd solutions respectively, both with mean ratio value 1.3. Similar mean chlorophyll *a*/*b* ratio had affected with Pb moss– 1.5, whereas narrower margin between maximum and minimum values (1.2 – 1.9) was obtained. Relative pigment ratios are reported from *in situ* researches of *Fontinalis antipyretica* in Bulgaria and Spain (YURUKOVA & GECHEVA, 2004; Peñuelas, 1984b). Pigment ratio maximum value established at 48-hour in moss treated with Cu2 solution corresponds with the same ratio value cited from GIMENO & PUCHE (1999) during transplantation of *Fontinalis hypnoides* in polluted sites.

Chlorophyll rates showed to be most affected at the middle both Cu and Cd concentration notwithstanding *Fontinalis* marked sensibility towards Cu and stability to cadmium (GLIME, 2003). On the basis that Cd influenced photosynthesis globally by chlorophyll degradation (OTERO *et al.*, 2006) the obtained results suggests that copper has similar outcome.

Results showed chlorophyll loss up to 47% (Cu2 solution at 48-hour) which corresponds with established about 43% chlorophyll losses in the same species by LÓPEZ & CARBALLEIRA (1989). Approximately 25% is the pigment loss in *Fontinalis antipyretica* at its nature sites between the upstream of the Maritsa River and industrial and urban area of Plovdiv district (YURUKOVA & GECHEVA, 2004).

This seems to prove that *F. antipyretica* is tolerant species to Cu, Cd and Pb pollution and has the ability to bear a serious chlorophyll losses under stress.

## CONCLUSION

Under the experimental conditions used in the present study, the heavy metals were more stressing during the first 48 hours of exposure, since the tested elements had more slight effects on chlorophyll levels at the end of the experiment.

The moss showed sensitivity to Cu, Cd and Pb exposure but did not display long-term significant reduction in chlorophyll *a* and *b* levels.

Taking into account that the tested heavy metals could operate additively and through different mechanisms on the physiological parameters, the observed chlorophyll increasing after a certain period could be related to an enhanced protection against stress which launch is probably due to the time factor mainly.

Measuring the chlorophyll (*a* and *b*) content as a parameter indicating stress in aquatic moss *Fontinalis antipyretica* establishes the method is low-cost, rapid and effective. Decreasing chlorophyll concentrations usually reflects increasing stress and small but persistent effects on pigment ratio could be associated with marked effects on photosynthesis and the long-term survival. The results of the current study suggest that a regulatory framework incorporating the physiological parameter as a biomarker of the specific reaction towards anthropogenic pressure on aquatic ecosystems could be adopted. Thirsdy

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# СЪДЪРЖАНИЕ НА ХЛОРОФИЛ ВЪВ ВОДНИЯ МЪХ *FONTINALIS ANTIPYRETICA* HEDW. ПРИ НАТОВАРВАНЕ С CU, CD И PB *EX SITU*

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**(Резюме)**

Проследено е нивото на хлорофил (хлорофил *a* и *b*, съотношение хлорофил *a*/хлорофил *b*) във водния мъх *Fontinalis antipyretica* Hedw. при контролирани условия с цел да се проучат потенциални ефекти на Cu, Cd и Pb. Видът е избран за обект на настоящото изследване поради доказаната му роля на биомонитор и морфологичните особености на листата му.

Установено е, че водният мъх има сравнително големи възможности да поддържа умерени нива на хлорофил *a* и *b* в условия на нарастващи концентрации на тежки метали (Cu, Cd и Pb). Съдържанието на хлорофил (*a* и *b*) е икономичен, бърз и ефективен параметър. Малки, но постоянни промени могат да се асоциират с явни ефекти спрямо фотосинтезата и оцеляването в дългосрочен план.