# DECREASE OF THE CHLOROPHYLL CONTENT ASSOCIATED WITH HEAVY METAL ACCUMULATION IN *FONTINALIS ANTIPYRETICA* HEDW.

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***ABSTRACT***

*Because of morphological and physiological properties, mosses are suitable species for the uptake of heavy metals. Fontinalis antipyretica Hedw. is a pollutant-sensitive indicator in fresh water. This aquatic moss is as an indicator organism to optimize the indication of heavy metal loads at biochemical levels.* F. antipyretica *was studied for its ability to accumulate heavy metals in the present study, each sample were irrigated with heavy metal solutions (0,02 M $CuCl_2$, $ZnCl_2$ and $Pb(NO_3)_2$). At the end of the investigation, the negative effects of heavy metals on the chlorophyll content were determined. The chlorophyll concentration of F. antipyretica was decreased. This significant decrease of chlorophyll concentration due to the heavy metal exposure. Samples were collected from Ayazma Stream, Ida Mountain (Çanakkale, Turkey) in July 2017.*

***Key words:*** *Fontinalis antipyretica, heavy and alkaline metals, chlorophyll, Turkey.*

## 1. INTRODUCTION

Bryophyte is a traditional name used to refer to all land plants that do not have true vascular tissue and are therefore called "**non-vascular**" plants. Their soft tissue makes fossil records bleak but the oldest evidence that has so far been found can be dated back to almost 500 million years ago.

Mosses; are cryptogamic organisms that can grow in almost all terrestrial ecosystems and have the ability to multiply even in extreme environmental conditions because they can withstand long standing droughts. They accumulate heavy metals in their tissues due to their high surface, volume ratios, roots, transmission bundles, and simple structural features without cuticular structures. Because of these properties, dissolved gases in the atmosphere during the long period of time show an elemental composition with respect to the particle-like substance and metal ions (Brown, 1984; Conti and Cecchetti, 2001). As a result, they are the ideal plants for the accumulation of metals, the collection of all seasons of the year, and the accumulation of heavy metals due to their wide geographical distribution (Tonguç, 1995). Many species of mosses are preferred as biomonitors because of their high absorption in the atmosphere (Steinnes and Berg, 1995). Thanks to these properties, many species of bouillonite are used for environmental pollution studies. It has been determined that the species of *Marchantia polymorpha, Bryum argenteum, Dicranella heteromalla* and *Pottia turuncata* are cadmium, zinc and copper, and *Pleuruchaete squarrosa* and *Timmiella barbuloides* are deposited in lead and copper at certain rates (Briggs, 1972; Nash, 1972; Kurt, 2012). Bryophytes are known as efficient accumulator of heavy metals because of their following properties:

1. They lack true root system and depend largely on atmospheric deposition for their requirements of mineral elements.
2. They usually lack continuous cuticle layer and thus their tissues are easily permeable to water and minerals, including the gaseous pollutants in the atmosphere and the metal ions.
3. Their tissues have numerous negatively charged groups. Their cell walls possess high exchange capacity and even their dead tissues have capacity to bind ions.
4. They generally obtain mineral nutrition from wet and dry deposition of particles and soluble salts. However, in certain bryophytes, uptake of metals from substrate occurs, mainly with rising capillary water. Such bryophyte species are less suitable for the monitoring of heavy metals.

*Fontinalis antipyretica* appears to be the most tolerant to contamination among other aquatic bryophytes studied for pigment content changes towards pollution (Peñuelas, 1985). 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%. Bruns *et al*. (1997), also applied chlorophyll level as a parameter towards *Fontinalis antipyretica* application as biomonitor of heavy metals. In a study, *Fontinalis antipyretica* showed sensitivity to Cu, Cd and Pb exposure but did not display long-term significant reduction in chlorophyll a and b levels (Gecheva and Yurukova, 2004).

In the present study, using a moss species, *Fontinalis antipyretica* Hedw., as environmental bioindicator by analyzing metal accumulation in different metal solution for determination of chlorophyll content collected in Ida Mountain (Kazdagı), (Çanakkale, Turkey) was studied.

## 2. MATERIALS and METHODS

### 2.1. Collection and Sample Preparation

*Fontinalis antipyretica* (Figure 1) collected from Ayazma Stream (Ida Mountain) (39° 49´ 51.7980 and 26° 2´ 51.9072) in July 2017 (Çanakkale, Turkey) (Figure 2, 3). Ida Mountain (Kazdagi) National Park is in the district of Edremit, in Balikesir province and this National Park stretches from East to West along the north coast of the Gulf of Edremit at the northern extremity of the Aegean. Collected mosses were cleaned carefully by removing small stones, soil, dead remains. These samples were kept in natural daylight for about 3-7 days at room temperature (25$^{o}$C-30$^{o}$C) to dry naturally.

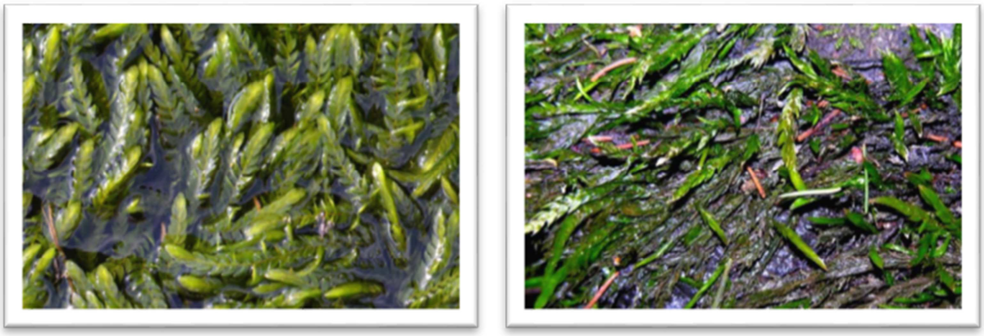

**Figure 1.** *Fontinalis antipyretica* Hedw.

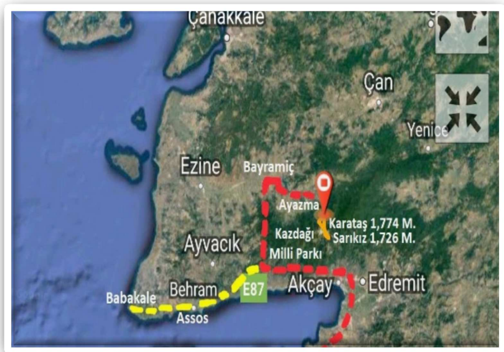


**Figure 2.** Sampling sites

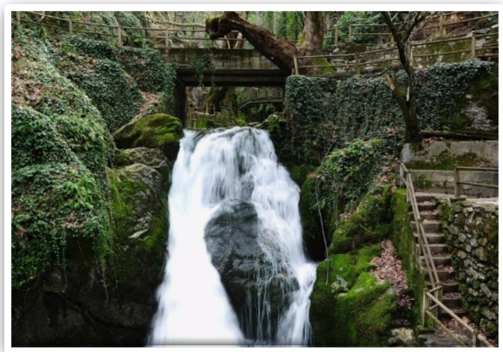

**Figure 3.** Ayazma Stream in Çanakkale

### 2.2. Treatment of Samples

For experimental analyses, the dried samples were converted into flour by a grinder. Moss samples (0.5 g) were immersed in 250 ml 0.02 M metal solutions for 72 hours. These metal solutions were 0.02 M $CuCl_2$, $ZnCl_2$ and $Pb(NO_3)_2$. 0.5 g samples of each species were immersed deionized water for 72 hours for control (Figure 4). The treated moss samples were allowed to dry naturally at room temperature for chlorophyll estimation.

**Figure 4.** Treatment of samples


**Figure 5.** Jenway U.V. spectrophotometry

The concentrations of chlorophyll of the samples were determined according to the method of Arnon (1949), 0.1 g samples were homogenized with 80 % of 15 ml (v/v) acetone for 15 minutes. Moss samples were filtered from Whatman No 1 filter paper. The absorbance values obtained from extraction were determined by Jenway U.V. Spectrophotometry (Figure 5). Total chlorophyll was determined at 652 nm, chlorophyll-a was determined at 663 nm and chlorophyll-b was determined at 645 nm.

## 3. RESULTS and DISCUSSION

Chlorophyll content is an extremely important parameter in estimating the plant production level. It is one of the most important molecule that absorbs sunlight and uses its energy to synthesize carbohydrates from $CO_2$ and water. This process is known as photosynthesis and is the basis for sustaining the life processes of all plants. Bryophytes can assimilate during very low light regime. Light saturation levels for many bryophytes have been found around 20 % of full sunlight for a wide range of bryophytes. All bryophytes have pigments, chlorophylls *a* and *b*, xanthophyll and carotene and they store starch as energy saver molecule in plastids.

One of the methods used to investigate the effects of stress factors on plants is to determine photosynthetic pigment contents. Heavy metals and other heavy metal groups used in our study have also been shown to adversely affect the photosynthesis mechanisms of plants (Foy et al., 1978, Kastori et al., 1992). Damage to photosynthetic pathways or direct photosynthesis, disruption of the enzymes involved in photosynthesis, and membrane viability are evidence of the indirect effects of metals (Heath, 1994).

As can be seen from Table 1, for control groups chlorophyll concentrations were found (chlorophyll-*a*/chlorophyll-*b*) 0.38/0.18 mg/g. The concentrations of chlorophyll-*a* and chlorophyll-*b* decreased after uptake from metal solutions. When the samples of *Fontinalis antipyretica* were compared with those of the control, photosynthetic pigment contents were higher in $CuCl_2$ treated samples; chlorophyll *a* showed lowest concentration. On the other hand, chlorophyll *b* value was lowest in Pb $(NO_3)_2$. The total chlorophyll values of all three solutions were lower than that of the control group, whereas chlorophyll b value was increased in Pb $(NO3)_2$

**Table 1.** The results of determined values for chlorophyll content of *Fontinalis antipyretica* before and after treated at 0.02 M $CuCl_2$, $ZnCl_2$ and Pb $(NO_3)_2$ (mg/g).

| Pigment | Control group | $CuCl_2$ | $ZnCl_2$ | Pb $(NO3)_2$ |
|---|---|---|---|---|
| Chlorophyll a | 0.380 | 0.037 | 0.187 | 0.129 |
| Chlorophyll *b* | 0.180 | 0.078 | 0.172 | 0.022 |
| Total Chlorophyll | 0.560 | 0.115 | 0.359 | 0.151 |
| Chlorophyll a/b | 2.110 | 0.470 | 1.080 | 5.860 |

When the samples of *Fontinalis antipyretica* were compared with control group after treated with $CuCl_2$, the amount of chlorophyll *a* decreased at the ratio of 34 % and the amount of chlorophyll *b* decreased at the ratio of 10 %.

As a result of the study, moss samples after treated with $ZnCl_2$, the amount of chlorophyll - *a* decreased at the ratio of 19 % and the amount of chlorophyll - *b* decreased at the ratio of 1 %, and treated with Pb $(NO_3)_2$, the amount of chlorophyll - *a* decreased at the ratio of 26 % and the amount of chlorophyll - *b* decreased at the ratio of 16 % (Figure 6).

Chlorophyll a/b amount of Cu applied *Fontinalis antipyretica* decreased by 22 % changes in chlorophyll a / b ratio were determined to be influenced by the conditions between Photosystem I and II and there were differences in this respect. It is known that the metal effect decreases the chlorophyll a / b ratio, and the structural deterioration of the stroma and granular lamellae (Chettri et al., 1998). The reduction in total chlorophyll content is a metal-specific response to photosynthesis inhibition and chlorophyll degradation (Bazzas et al., 1974).

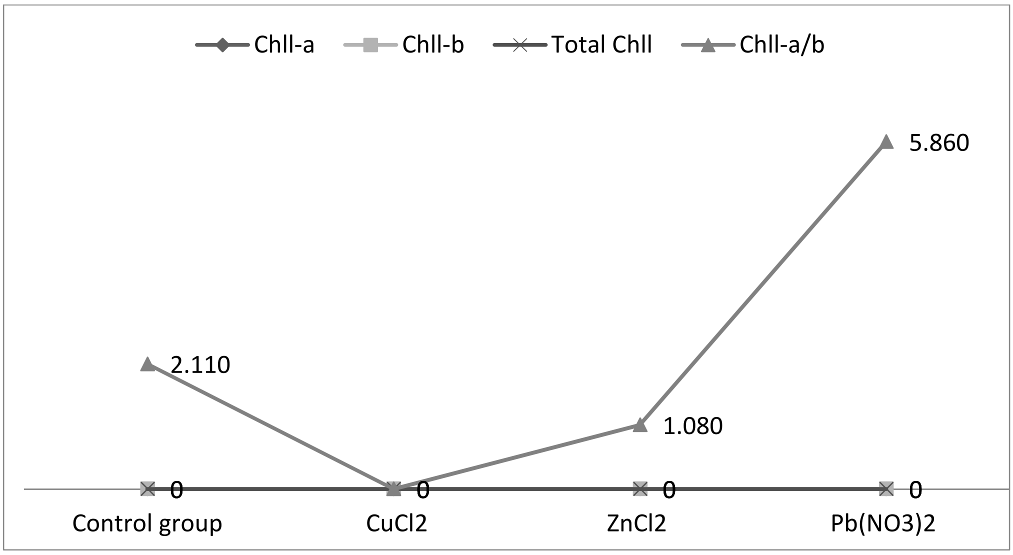


**Figure 6.** Chlorophyll content of *F. antipyretica* decreases with metal accumulation

There are studies indicating that chlorophyll a, chlorophyll b and total chlorophyll content are reduced in heavy metal pollution (Chettri et al., 1998; Öncel et al., 2000; Monni et al., 2001).

Chlorophyll content of plants decreases with stress conditions. These conditions can be air pollution and heavy metal toxicity. Significant decreases in chlorophyll-*a*, chlorophyll-*b*, total chlorophyll, and the chlorophyll-*a* to -*b* ratio with an increase in Cu accumulation reflects the inhibitory effect of this metal on pigment biosynthesis, which may be a metal specific action (Panda & Choudhury, 2005). Patsikka et al., 2002, indicated that Cu can block the photosynthetic transport chain.

Toxic effects of heavy metals such as Cu, Pb, and Zn are apparent in a wide range of plant cellular activities that take place in all plant groups, such as protein synthesis, photosynthesis, respiration, membrane structure and mineral nutrition (Azeez & Banerjee, 1986).

Chlorophyll rates indicated to be most affected in the middle both Cu and Cd concentration notwithstanding *Fontinalis* marked sensibility towards Cu and stability to cadmium (Glime, 2003). Yurukova and Gecheva (2004), in their work almost 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. 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 loss under stress.

Several works are present in literature in which moss was used as bioindicator, with particular concern to metals (Gerdol and Cenci, 1999). Other researchers proposed the use of moss in the removal of heavy metals from wastewaters (Al Asheh and Duvnjak, 1999). Decreases in photosynthetic pigments, carotenoids have been recorded in laboratory studies (Krupa et. al. 1996, Wozny and Krzeslowska, 1993).

Mosses have been proven suitable for the uptake of some heavy metals and also for environmental biomonitoring because of their morphologic and physiologic properties (Sawidis et al.2008).

## 4. CONCLUSION

One of the methods used to investigate the effects of stress factors on plants is to determine photosynthetic pigment contents. Heavy metals groups used in the present study also have negative effects on the photosynthesis mechanisms of plants.

The results obtained in this study are similar to previous studies. Heavy metal accumulation showed a significant inhibitory effect on chlorophyll-a, chlorophyll b, and total chlorophyll in bryophytes.

As a result, 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.

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