MORPHOGENETIC AND BIOCHEMICAL CHANGES OF THE GAMETOPHYTE AND SPOROPHYTE, INDUCED BY LEAD, IN CASE OF SOME SPECIES OF FERN

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Abstract. The aim of this study is to determine the influence of lead on the species: *Athyrium filix-femina* (L.) Roth, *Dryopteris filix-mas* (L.) Schott and *D. affinis* (Lowe) Fraser-Jenk. In order to reach this issue, we proposed the following objectives: the monitoring of the gametophyte differentiation on the soil that was contaminated with different lead concentrations and the estimation of the physiological status of the 3 species of fern in the initial development status (gametophyte and young sporophyte) through the analysis of the photosynthetic pigments and the determination of the antioxidant potential through the analysis of polyphenols. The variants tested were V_1=0.1 g Pb^{2+}, V_2=0.2 g Pb^{2+}, V_3=0.5 g Pb^{2+}, V_4=1 g $Pb^{2+}$and Control. Of the three species considered in the study, we have noticed, for the *Athyrium filix-femina*, the most advanced differentiation of the gametophyte. Low Pb concentrations: V_1 and V_2 stimulate the pigments content at *A. filix-femina*, respectively at *Dryopteris filix-mas*, and, of the pigments, the carotenoids have a protective role. A protective role against the stress produced by the heavy metal action is also possessed by the polyphenols, which usually tent to grow compared to the control, but without noticing significant differences.

Keywords: ferns, pigments, polyphenols, lead.

Rezumat. Modificări morfogenetice și biochimice ale gametofitului și sporofitului, induse de plumb, la unele specii de ferigi. Scopul acestei lucrări a fost de a determina influența Pb asupra speciilor: *Athyrium filix-femina* (L.) Roth, *Dryopteris filix-mas* L. Schott și *D. affinis* (Lowe) Fraser-Jenk. Pentru a putea realiza acest aspect ne-am propus următoarele obiective: monitorizarea diferențierii gametofitului pe solul contaminat cu diferite concentrații de Pb și estimarea condiției fiziologice a celor trei specii de ferigi aflate în stadii inițiale de dezvoltare (gametofit și sporofit tânăr) prin analiza pigmenților asimilatori și determinarea potențialului antioxidant prin analiza polifenolilor. Variantele experimentale testate au fost V_1=0.1 g Pb^{2+}, V_2=0.2 g Pb^{2+}, V_3=0.5 g Pb^{2+}, V_4=1 g Pb^{2+} și Martor. Dintre cele 3 specii luate în studiu, la *Athyrium filix-femina* s-a observat cea mai avansată diferențiere. Concentrațiile mici de Pb: V_1 și V_2 stimulează conținutul de pigmenți la *A. filix-femina* respectiv la *Dryopteris filix-mas*, iar dintre pigmenți, carotenoizii au rol de protecție. Rol de protecție împotriva stresului provocat de acțiunea metalelor grele au și polifenolii care au, de regulă, tendință ascendentă comparativ cu martorul, însă fără a exista diferențe semnifcative.

Cuvinte cheie: ferigi, pigmenți, polifenoli, plumb.

INTRODUCTION

The irrational exploitation and the human activities influence the quality of the atmosphere, the hydrosphere and the lithosphere, leading to their degradation. The pollution of the soil with heavy metals has a high significance, due to their persistency in the environment, the bioaccumulation at the level of the trophic chain and the effects on the human health (LIU et al., 2010; WUANA & OKIEIMEN, 2011). Some fern species have the ability to hyperaccumulate metals, so they play an important role in phytoremediation (RATHINASABAPATHI, 2011). The gametophyte of *Asplenium scolopendrium*, *A. trichomanes-ramosum*, *Cystopteris fragilis*, *Polypodium vulgare* cultivated *in vitro* on lead-containing medium have the capacity to bioaccumulate the metal (SOARE et al., 2015).

Lead is one of the most abundant heavy metals (TIWARI et al., 2013), which is featured by low solubility and high linking capacity at colloidal level. Thus, the residence time in soil is high, so that it could influence, on a large period of time, plant growth and metabolism (BHATTI et al., 2013).

VELCHEVA et al. (2012) consider that the gathering of objective information on the phytotoxicity in the environment is made considering the growth and the functional parameters. The reduction of the plant productivity is the result of the contamination of ecosystems with heavy metals, which act on the photosynthetic pigments and, implicitly, on the photosynthesis, reducing its efficiency (GRUCA-KROLIKOWSKA & WACLAWSKI, 2006). The pigment content provides information on the physiologic status of the plant: it ensures the optimum for the absorption of light (WAHID & GHAZANFAR, 2006) and directly influences the primary production (CURRAN et al., 1990).

The antioxidant mechanisms of the plants, based on carotenoids, enzymes (catalase, peroxidase, etc.), polyphenols, compensate the stress that is produced by the action of heavy metals. Polyphenols represent a large and diverse group of secondary metabolites of plants, which influence important processes: the germination of seeds, the growth of plants and the regulation of enzymatic activity (KAROLEWSKY & GIERTYCH, 1994). Also, they have a protective role against the reactive species of oxygen, the UV light, pathogenic factors, parasites and predators (MOJZER et al., 2016).

The aim of this study is to determine the influence of lead on the species of *Athyrium filix-femina* (Linnaeus) Roth (1799), *Dryopteris filix-mas* (Linnaeus) Schott (1834) and *D. affinis* (Lowe) Fraser-Jenkins (1979). In order to reach this issue, we proposed the following objectives: the monitoring of the gametophyte differentiation on the soil that was contaminated with different lead concentrations and the estimation of the physiological status of the three species of fern in the initial development status (gametophyte and young sporophyte) through the analysis of the photosynthetic pigments and the determination of the antioxidant potential through the analysis of polyphenols. The study provides new data on the morphogenesis and biochemical behavior of the gametophyte, as well as the young sporophytes in some native fern species in the presence of lead.

MATERIALS AND METHODS

The initial biologic material was represented by spores collected from *Athyrium filix-femina* (Linnaeus) Roth (1799), *Dryopteris filix-mas* (Linnaeus) Schott (1834) and *D. affinis* (Lowe) Fraser-Jenkins (1979). The spores of the three species were collected during August and September 2016 from samples in the Vâlsan Valley: North 45^{o}20', East 0,24^{o}43' (Argeș County, Romania). Subsequently to the collecting process, they have been kept in the refrigerator (4^{o}C) for 2 months. The soil has been sterilized at 105°C in the oven for two and a half hours. The biological material and the soil used in the experiment did not contain Pb before the experiment was initiated, the quantitative determination of the metal was achieved by inductively coupled plasma atomic emission spectrometry (ICP-AES) (SOARE et al., 2015). For each variant, we used 200g of soil, which was soaked in Knop solution: $Ca(NO_3)_2$:1.00 $g \cdot L^{-1}$; $MgSO_4$: 0.25 $g \cdot L^{-1}$; KH_2PO_4: 0.25$g \cdot L^{-1}$; KNO_3: 0.25$g \cdot L^{-1}$. We dissolved, in Knop solution, the lead acetate, in different quantities, in order to reach some progressive concentrations: V_1=0.1 g Pb^{2+}, V_2=0.2 g Pb^{2+}, V_3=0.5 g Pb^{2+}, V_4=1 g Pb^{2+}. After a homogenous mixing, the soil was uniformly distributed in Petri boxes (50mg/box). To start the experiment, the spores were dispersed on the whole surface of the soil in the box, and the boxes were put in the growing room (16h of lighting, 8h of darkness, constant humidity, temperature 25°C in the day and 15°C at night) and periodically watered with distilled water. Periodical observations on the gametophyte and sporophyte differentiation were made using the OPTIKA B275 microscope and using the OPTIKA SZR stereomicroscope. For the biological material reached after 4 months from the beginning of the experiment, we determined the content of photosynthetic pigments (the spectro-photometric method), as well as of the polyphenols (ORȚAN et al., 2015). The statistical interpretation of the data was made using the SPPS software (Version 16 for Windows). The values are the means of 3 repetitions ± standard deviation; a, b, c, d - Duncan test results: the comparisons were made between Control and $V_{1-4.}$

RESULTS AND DISCUSSION

The differentiation of the gametophyte is the most advanced in the *A. filix-femina* species: after one month, the chordate prothallia, with reproductive organs (antheridia with viable anterosoids and archegonia) (Table 1), and, during the second month, the low size sporophyte is differentiated (Table 2). In case of *D. affinis*, after the first month, the chordateprothallium stage only emerged in the control and in case of the variants with low concentrations of Pb^{2+} (V_{1-2}). In the other cases, we can notice the following stages: young prothallia, blade and prothallium filament, stages that are characteristic for the *D. filix-mas* species also, irrespective of the variant.

Table 1. The gametophyte development after 1 month.

VARIANTS	*Athyrium filix-femina (Aff)*	*Dryopteris filix-mas (Dfm)*	*Dryopteris affinis (Da)*
	One month		
Control/C	chordate prothalia, antheridia, archegonia	young chordate prothalia	chordate prothalia
V_1Pb	chordate prothalia, antheridia	prothallium blade and filament	chordate prothalia
V_2Pb	chordate prothalia, antheridia	prothallium blade and filament	chordate prothalia
V_3Pb	chordate prothalia, antheridia, archegonia,	young prothalia	prothallium blade and filament
V_4Pb	young chordate prothalia, antheridia,	prothallium blade and filament	young prothalia

Table 2. The gametophyte developmentafter 2 months

VARIANTS	*Athyrium filix-femina*	*Dryopteris filix-mas*	*Dryopteris affinis*
	2 months		
Control/C	sporophyte & gametophyte	sporophyte & gametophyte	chordateprothalia
V_1Pb	sporophyte & gametophyte	gametophyte (antheridia, archegonia)	sporophyte & gametophyte
V_2Pb	sporophyte & gametophyte	gametophyte with archegonia	sporophyte & gametophyte
V_3Pb	sporophyte & gametophyte	sporophyte & gametophyte	sporophyte & gametophyte
V_4Pb	sporophyte & gametophyte	sporophyte & gametophyte	sporophyte & gametophyte

Two months after the beginning of the experiment, the differences between the variants started to progressively reduce: next to the gametophyte, usually represented by the chordate prothallia, the sporophyte emerges, with exceptions (*D. affinis* control and V_{1-2} *D. filix-mas*), and, after 4 months, the sporophyte stage emerges at all the variants, being predominant (Figs. 1-5).

The determination of the photosynthetic pigments. 4 months after the beginning of the experiment, we determined the content of pigments: chlorophyll *a*, chlorophyll *b* and carotenoids. According to Figs. 6, 7, 8 for the chlorophyll, we can notice, in most of the cases, a growing trend; thus, for species *A. filix-femina* at V_1Pb and V_3Pb, the growth reaches 22, respectively 14% compared to the M variant; for *D. filix-mas*, the determined values of chlorophyll are usually between 0.179 (V_3Pb) and 0.240 (V_4Pb).

Figure 1. *Aff*, *C*-4 months.

Figure 2. *Aff*, V_4Pb-4 months.

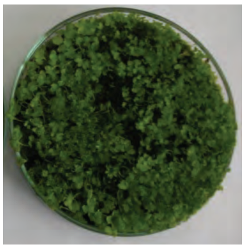

Figure 3. *Dfm*, M-4 months.

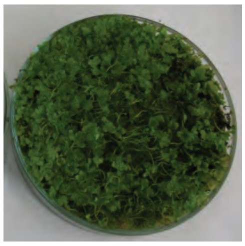

Figure 4. *Dfm*, V_3Pb-4 months.

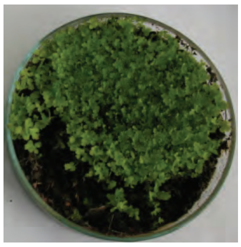

Figure 5. *Da*, V_3Pb-4 months (originals).

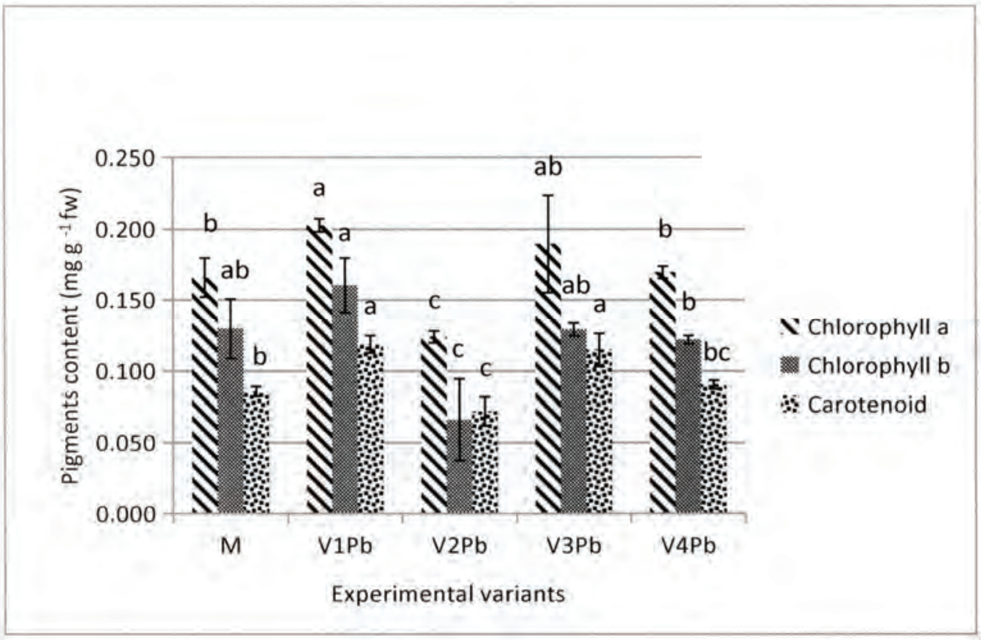

Figure 6. Content of pigments in *Athyrium filix-femina*. (The values are the means of 3 repetitions ± standard deviation; a, b, c, d - Duncan test results: the comparisons were made between Control and V_{1-4}).

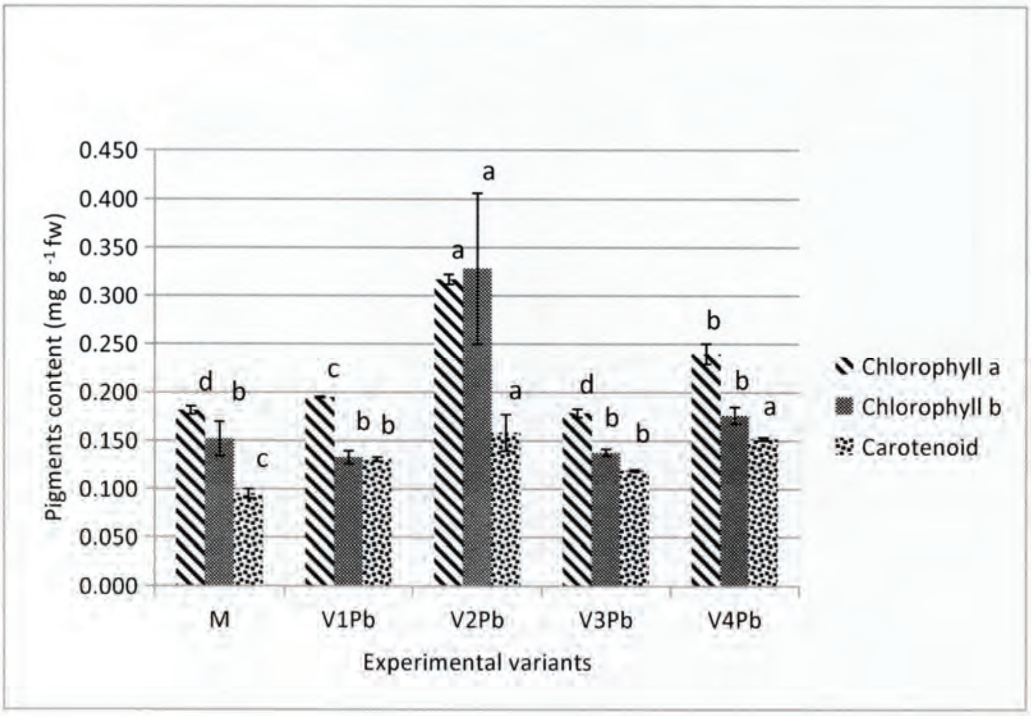

Figure 7. Content of pigments in *Dryopteris filix-mas*. (The values are the means of 3 repetitions ± standard deviation; a, b, c, d - Duncan test results: the comparisons were made between Control and V_{1-4}).

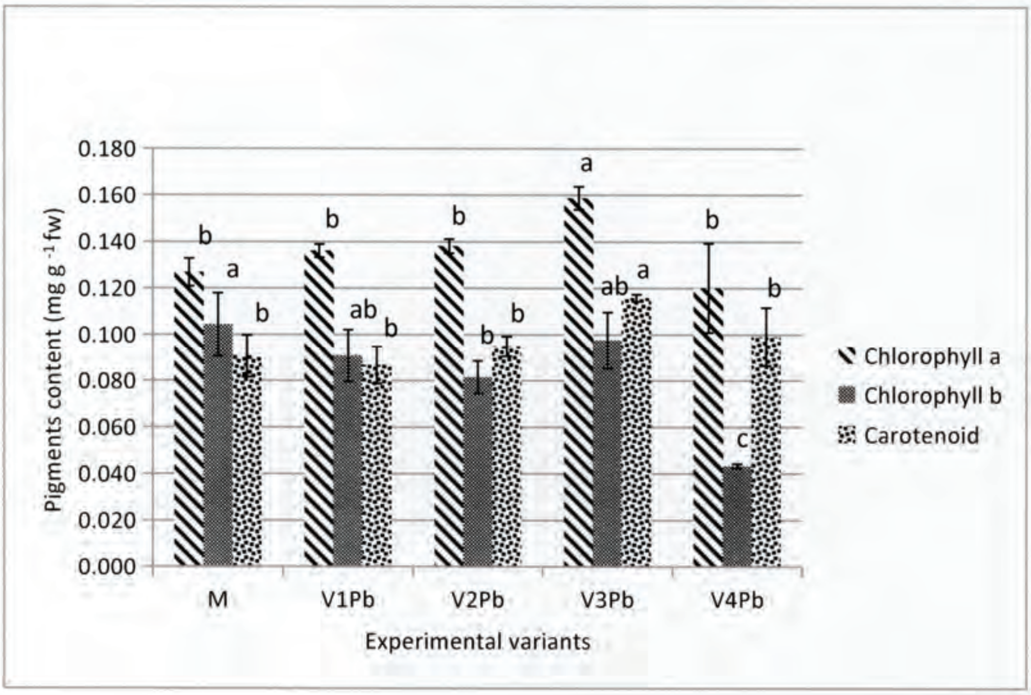

Figure 8. Content of pigments in *Dryopteris affinis*. (The values are the means of 3 repetitions ± standard deviation; a, b, c, d - Duncan test results: the comparisons were made between Control and V_{1-4}).

In the case of the 3rd species, *D. affinis*, for chlorophyll *a*, the single significant growth is seen at V_3Pb. LIU et al. (2010) noticed that both the high and the low concentrations of Pb (100, 500, 2000 mg/ kg^{-1} of dry soil) stimulated the chlorophyll synthesis in the incipient stages of wheat growth. A growth was also noticed, for *Medicago sativa*, in the total photosynthetic pigments content (chlorophyll *a* and *b*), for the variant of 50 ppm Pb compared to the control (OLTEANU et al., 2008). Of the 12 studied species, SEDZIK et al. (2015) decided that in case of the rye, wheat and sunflower there are no significant differences between the total content of chlorophyll of the control and the variant with 1mN Pb, and for the *Nicotiana tabacum* (ALKHATIB et al., 2012) the effect of the $Pb(NO_3)_2$ found in various concentrations (5, 10, 25, 50, 100, 300, 500 µM) on the chlorophyll content was insignificant. In the V_2Pb variant, we notice contrary results in case of the two species: for *A. filix-femina*, we registered the lowest chlorophyll concentration, with a growth of 16% compared to the control, as for the *D. filix-mas*, we reached the highest value, of 0.317 (a growth of 74% compared to M).

The same trends were also noticed for chlorophyll *b* and carotenoids, reaching low concentrations for *A. filix-femina* and high concentrations for the *D. filix-mas*. Except the V_2Pb variant, on the first two species, there are no significant differences between the chlorophyll *b* content of the Pb variants and the control. In case of *D. affinis*, at the highest concentration of Pb^{2+} (V_4Pb), we confirm the result of VODNIK et al. (1999): chlorophyll *b* is much more sensitive than chlorophyll *a* at the treatment with Pb, the reduction being significant in our case. For the *Brassica pekinensis*, the Pb with a concentration of 4 mmol/kg^{-1} has stimulated the chlorophyll *b* content, but it did not influence the quantity of chlorophyll *a* (XIONG et al., 2006), and in case of two species of moss tree of the *Thuidium* genes (*T. delicatulum* and *T. sparsifolium*), we noticed an insignificant reduction of the chlorophyll *a*, *b* and total chlorophyll reduction after the exposure to $Pb(NO_3)_2$ (SHAKYA et al., 2008).

The carotenoids display a growing trend for *D. filis-max*, with significant growths for all the Pb variants. The significant growing trend emerges at the *A. filix-femina* in only two cases of V_1Pb and V_3Pb, and in case of *D. affinis* at V_3Pb. BHATTI et al. (2013) noticed a significant increase in the carotenoids content for the two types of *Triticum aestivum* subsequently to the exposure at concentrations of 0, 40 and 60 ppm Pb. For *Daphne jasmine*, the Pb nitrate treatment stimulated the carotenoid and chlorophyll synthesis (WISZNIEWSKA et al., 2015). The growth of the carotenoids content, as a result of the action of heavy metals, has the role of protecting the plant from ROS (BHATTI et al., 2013; SCHWARZAUR-ROCKETT et al., 2013); the *Arabidopsis thaliana* species especially uses carotenoids to compensate the stress produced by Pb (BAEK et al., 2012). For the *Pluchea sagitallis* (ROSSATO et al., 2012) and *Asplenium scolopendrium* (DRĂGHICEANU et al., 2016) species, Pb did not influence the quantity of carotenoids.

Total polyphenol content. 4 months after the beginning of the experiment, we determined the total content of polyphenols (Fig. 9) and we noticed the following: for *A. filix-femina* we noticed a growing trend for the V_1 and V_3 variants; the growth reaches 10% for the V_3Pb and nearly 49% from the control to V_1Pb (significant growth compared to the control). For *D. filix-mas*, the total polyphenol content is usually between 30.6% (V_3Pb) and 42.75% (V_4Pb). The exception in the species also emerges at V_2Pb (also met in pigments) where we notice a half-life of the value reached in the control. On the second species of *Dryopteris*, the highest growth is 15% for V_1Pb, and for the V_3Pb, we reached 8% lower value reported to the control. According to SCHWARZAUR-ROCKETT et al. (2013), the growth of the phenolic compounds content represents a defense mechanism against the stress produced by the Pb. At the concentration of 900 mg Pb kg^{-1}, the growth of the phenolic content was significant for wheat (PAZOKI, 2015) and for *Vigna radiata*, Pb (50 ppm, 120 ppm) led to the reduction of the polyphenolic content (NAJAFI & JAMEI, 2014). HARANGOZO et al. (2014) noticed a low decrease in the content of polyphenols for flax for the variant of 1092 mg kg^{-1}Pb, but without overpassing the value of the control.

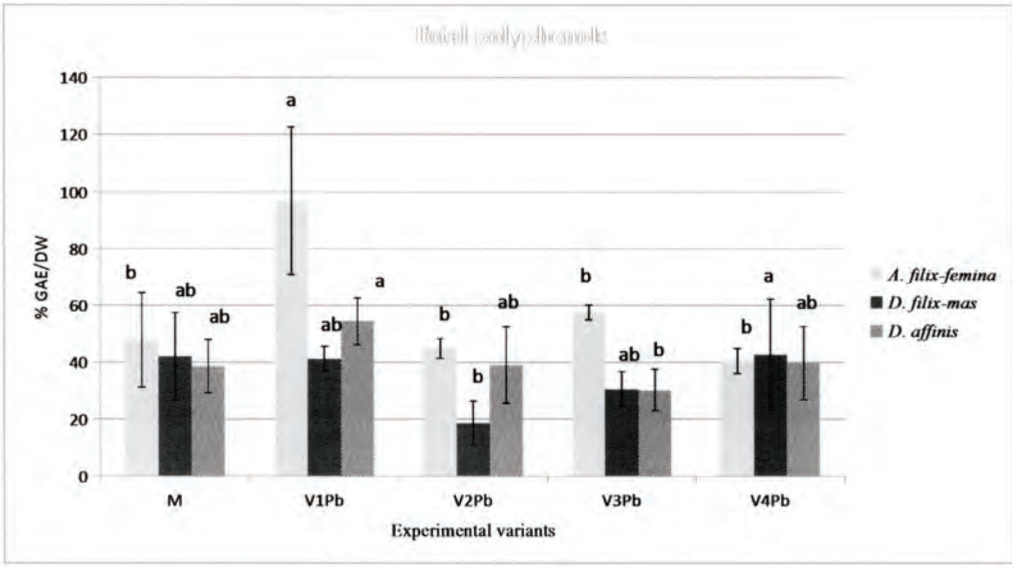

Figure 9. Total polyphenol content after 4 months (Gallic Acid Equivalents/Dry Weight).

CONCLUSIONS

Of the three species considered in the study, we noticed, for the *Athyrium filix-femina*, the most advanced differentiation of the gametophyte, one month after the beginning of the experiment (chordate prothallium with antheridia and archegonia). Differences are progressively reduced during the four months so that it emerges at all species next to gametophyte and sporophyte. Low Pb concentrations: V_1 (0.1 g Pb^{2+}) and V_2 (0.2 g Pb^{2+}) stimulate the pigments content at *Athyrium fillix-femina*, respectively at *Dryopteris filix-mas*, and, of the pigments, the carotenoids have a protective role. A protective role against the stress produced by the action of heavy metals is also possessed by the polyphenols, which usually tent to grow compared to the control, but without noticing significant differences.

ACKNOWLEDGMENT

The authors thank the following researchers for their contribution to the work (quantitative determination of the Pb): Irina Fierăscu, Radu Caludiu Fierăscu (The National Institute for Research & Development in Chemistry and Petrochemistry, ICECHIM, Bucharest).

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Received: March 31, 2017
Accepted: July 7, 2017