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THE EFFECTS OF NICKEL ON THE MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF FERNS

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INTRODUCTION

"Nickel (Ni) is an essential microelement for plants and some animal species, but not for humans" (Gad, 2014). In plants, Ni has an important role in many physiological processes including seed germination, vegetative and reproductive growth, photosynthesis, and nitrogen metabolism (Shahzad et al., 2018).

Also called the "allergen of the year" in 2008, Ni is a ubiquitous metal that is used in many modern technology applications (Duda-Chodak and Blaszczyk, 2008). Because of its increase in industrial use, the phytotoxicity caused by Ni excess has become very common; it causes alteration of mineral nutrition (absorption, transport, and distribution), photosynthesis, respiration, water balance and inhibition of growth (Matraszek et al., 2017).

The main mechanisms by which Ni is taken up by plants is passive diffusion (for soluble Ni compounds) and active transport (for chelated compounds) (Ahmad and Ashraf, 2011). Soluble compounds (chlorides or nitrates) have greater mobility than insoluble compounds (oxides and sulphides) and the concentration of Ni in plants and other soil organisms is generally closer to the soluble forms of Ni in the soil (Nie et al., 2015). Ni mobility also depends on the texture, composition and mineralogical structure of soil (Kabata-Pendias, 2001).

The content of Ni in the soil varies greatly and has been estimated in the range of 3-1000 ppm (Iyaka, 2011). Soils with high concentration of Fe and Co contain large amounts of Ni (Harasim and Filipek, 2015). Ni availability in the soil varies with pH (De Macedo et al., 2016) so that a decrease in pH increases the solubility and mobility of Ni (Iyaka, 2011; Harasim and Filipek, 2015).

The effects of Ni excess on plants appear at the morphological, physiological and biochemical levels and may be due to direct action of the metal or its tendency to compete with other cations (Mg^{2+}, Ca^{2+}, Fe^{2+}, and Zn^{2+}) (Sengar et al., 2008).

Heavy metal toxicity on organisms can be evaluated using acute and chronic toxicity tests. In 2009, Catalá et al. published the first acute phytotoxicity test – TTC test, based on fern spores, a test that was optimized for different species taking into account the characteristics of the spores (presence /absence of chlorophyll, cell wall structure, etc.).

The use of pteridophyte spores and gametophytes for the toxicity test shows a lot of advantages: spores are available throughout the year; they can be easily collected in large quantities and preserved in the laboratory; germination tests are not expensive and they require regular laboratory equipment; gametophytes are grown on simple nutrient media and the results obtained are relevant for superior plants (Singh and Devi, 1989; Catalá et al., 2009; Rodriguez-Gil et al., 2010; Catalá et al., 2011; Marugán et al., 2012; Soare et al., 2013)

The aim of this study was to evaluate the morphological and biochemical changes induced by nickel in the gametophyte and sporophyte of three fern species: *Athyrium filix-femina* (Linnaeus) Roth (1799), *Dryopteris filix-mas* (Linnaeus) Schott (1834) and *D. affinis* (Lowe) Fraser-Jenkins (1979).

MATERIAL AND METHOD

The biological material was represented by spores of *Athyrium filix-femina (Aff)*, *Dryopteris filix-mas (Dfm)* and *Dryopteris affinis (Da)* collected from different mature individuals to ensure genetic diversity and kept in the refrigerator (4^oC) till the experiment (Soare and Aldoiu, 2010). The biological material was obtained from the Vâlsan Valley, Arge County, Romania (North $45^o20'$, East $0.24^o43'$).

The spores were uniformly distributed on the surface of sterilized forest soil with different Ni content: Control = 0 g Ni^{2+}, V_1= 0.1 g Ni^{2+}, V_2 = 0.2 g Ni^{2+}, V_3 = 0.5 g Ni^{2+}, V_4 = 1 g Ni^{2+}. The variants were kept in the growing room and periodically watered with distilled water for 4 months. The temperature values were 25°C during the day and 15°C at night and the humidity and lighting

conditions were controlled (16 hours of light and 8 hours of darkness). During this period we made observations on the development of gametophytes and sporophytes.

After four months we determined the content of photosynthetic pigments and total polyphenols. The determination of photosynthetic pigments (chlorophyll a and b) and that of carotenoid pigments were performed spectrophotometrically using the Holm (Holm, 1954) formulae. The results obtained were expressed in mg g^{-1} fresh weight (fw).

The total polyphenol content was determined through the spectrophotometric method, using Folin-Ciocâlteu reagent (Merck), by measuring absorbance at 765 nm (Or□an et al. 2015). The results obtained were expressed in % gallic acid equivalents (GAE)/dry weight (dw) (ISO 14502-1:2005(E)).

The statistical interpretation was performed using SPSS (version 16 for Windows). We calculated the mean value and the standard deviation. We also performed the comparisons between the mean values using the Duncan test. Periodical observations on gametophyte and sporophyte differentiation were made using the OPTIKA B275 microscope with an A630 Canon Power Shoot camera and using the OPTIKA SZR stereomicroscope.

RESULTS AND DISCUSSIONS

Gametophyte and sporophyte differentiation. After the first month of exposure, the most advanced stage of gametophyte differentiation was chordate prothalia, which was preponderantly observed in *D. affinis* (Fig. 1). In *A. filix-femina,* besides chordate prothalia, antheridia and archaegonia were observed in Control. Also, in *Athyrium* in V_4Ni the gametophyte had reached the chordate prothalia stage while in the V_{1-3}Ni variants prothallium blade and antheridia were noticed (Fig. 2) (Table 1).

In *D. filix-mas,* the stage of the gametophyte for Control and V_1Ni (Fig. 4) was chordate prothalia. In V_3Ni prothallium filaments and germinated spores were observed (Fig. 3).

Except for Control and the variants with high Ni concentrations of *D. affinis* (V_3 *Aff* - Fig. 5 and *Dfm*, V_4 *Dfm* – Fig. 6), sporophytes and gametophytes were observed after 2 months of exposure in all variants (Fig. 7) (Tab. 2).

Four months after the start of the experiment, the differences were significantly reduced, and the sporophyte appeared in all variants (Fig. 8 - 12).

Table 1. The gametophyte development after 1 month

Variants	*Athyrium filix-femina (Aff)*	*Dryopteris filix-mas (Dfm)*	*Dryopteris affinis (Da)*
Control	chordate prothalia, antheridia, archaegonia	young chordate prothalia	chordate prothalia
V_1Ni	prothalium blade, antheridia	young chordate prothalia	young chordate prothalia
V_2Ni	prothalium blade, young prothalia, antheridia,	young prothalia and prothalium filaments	chordate prothalia
V_3Ni	prothalium blade, young prothalia, antheridia	prothalium filaments, germinated spores	chordate prothalia
V_4Ni	young chordate prothalia, antheridia	prothalium blade, young prothalia	young prothalia

Table 2. The gametophyte development after 2 months

Variants	*Athyrium filix-femina (Aff)*	*Dryopteris filix-mas (Dfm)*	*Dryopteris affinis (Da)*
Control	$G_{n+}S_{2n}$	$G_{n+}S_{2n}$	chordate prothalia
V_1Ni	$G_{n+}S_{2n}$	$G_{n+}S_{2n}$	$G_{n+}S_{2n}$
V_2Ni	$G_{n+}S_{2n}$	$G_{n+}S_{2n}$	$G_{n+}S_{2n}$
V_3Ni	gametophyte (chordate prothalia, antheridia, archaegonia)	gametophyte (chordate prothalia, antheridia, archaegonia)	$G_{n+}S_{2n}$
V_4Ni	$G_{n+}S_{2n}$	gametophyte (chordate prothalia, antheridia, archaegonia)	$G_{n+}S_{2n}$

Abbreviations: sporophyte S_{2n}, gametophyte: G_n

Fig. 1. *Da* V_2Ni 1month (x40)

Fig. 2. *Aff* V_2Ni 1 month (x100)

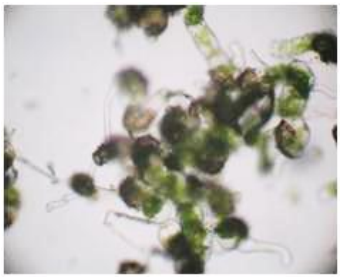

Fig. 3. *Dfm* V_3Ni 1 month (x100)

Fig. 4. *Dfm* V_1Ni 1 month (x10)

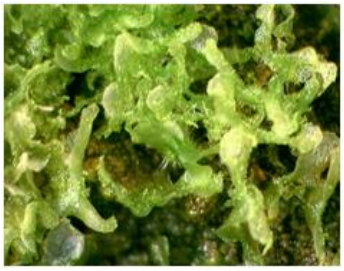

Fig. 5. *Aff* V_3Ni 2 months (x10)

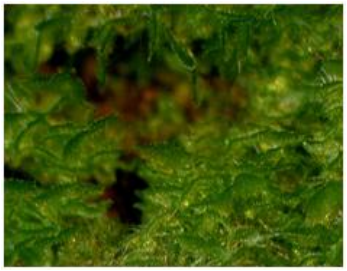

Fig. 6. *Dfm* V_3Ni 2 months (x10)

Fig. 7. *Da* V_3Ni 2 months (x10)

Fig. 8. *Aff* V_2Ni 4 months

Fig. 9. *Dfm* V_2Ni 4 months

Fig. 10. *Da* M 4 months

Fig. 11. *Da* V_2Ni 4 months

Fig. 12. *Da* V_1Ni 4 months

Heavy metals, such as nickel, influence spore germination, gametophyte and sporophyte differentiation in pteridophytes, biomass, growth process, etc. Thus, megaspore germination, sporophyte formation and length growth of the first root and of the leaf in the fern *Regnellidium diphyllum* were significantly affected by the presence of nickel (Kieling-Rubio et al., 2012). An important biomass decrease in the fern *Azolla filiculoides* was recorded when exposed to 4 mg L^{-1} Ni for 15 days (Khosravi et al., 2005).

The content of photosynthetic pigments. The only significant increase of chlorophyll *a* (17%) in *Athyrium filix-femina* was observed in V_2Ni while the increases of carotenoid content were in the range of 13-27% and were significant compared to the Control ($p<0.05$) (Fig. 13).

In *Dryopteris filix-mas* there were no significant differences between the chlorophyll content from Control and V_1, but for the other variants downward trends for both chlorophyll (a and b) and carotenoid content were observed (Fig. 14).

As regards the chlorophylls, the opposite was observed in *D. affinis*: chlorophyll *a* presented an upward trend while the content of chlorophyll *b* decreased (Fig. 15). The content of chlorophyll *a* in Control was smaller than that determined in Ni variants of this species; the highest increase of 25% for chlorophyll *a* was recorded in V_1. Chlorophyll *b* is more sensitive to Ni than chlorophyll *a* in *Dryopteris affinis* and *Athyrium filix-femina*.

Small concentrations of Ni can stimulate chlorophyll synthesis. Plant species that present tolerance to heavy metals in juvenile stages can produce tolerant adults. The research in this field can be used to establish potential crops on contaminated fields. Stimulation of the amounts of chlorophyll and carotenoids at low concentration (hormesis) of Cu, Cr, Ni, and Cd was also observed by Juknys et al. (2009). In *Triticum aestivum* the increase of Ni concentration in the soil has led to a decrease of the total chlorophyll by 6-70% due to the disturbance of Mg assimilation. Furthermore, the content of carotenoids decreased (Shafeeq et al., 2012). The content of assimilatory pigments of *Groenlandia densa* decreased only in the variants with high concentration of Ni (Yilmaz and Parlak, 2011). In *Pleurochaete squarrosa* the carotenoid content decreased with 30%, 22% and 24% due to Ni, Pb and Cu exposure while in *Timmiella barbuloides* only Ni affected the carotenoid content producing a 21% increase of the value compared to Control (Aydoğan et al., 2017). According to Campanharo et al. (2010) Ni influences pigment accumulation in plants by changing the ratio/ proportion between chlorophylls or between chlorophyll and carotenoids. Thus, carotenoids are more sensitive than chlorophyll and chlorophyll *b* is more susceptible to Ni than chlorophyll *a*.

Polyphenol content. The highest value obtained for the polyphenol content was determined in *Athyrium filix-femina* 83.12% G.A.E in V1. This variant presented a 35% increase compared to Control. Another increase for this species was observed in V_3 where the value determined was 21% higher than Control. The only significant increase in polyphenol content in *D. filix-mas* was determined in V_4Ni and it was 26% higher than control. Regarding the content of polyphenols in *D. affinis*, the value obtained for the variants with Ni did not show significant differences compared to the Control. They were included in the range 34.44% - 46.13% (Fig. 16). The content of polyphenols in *Thymus vulgaris* decreased as the concentration of Ni in soil increased (Kulbat and Leszczyńska, 2015). After Ni exposure the polyphenol content in *Triticum aestivm* increased both in roots and shoots (Pandolfini et al., 1992). Ni exposure did not change the total content of soluble phenols from leaf rosette in *Matricaria chamomilla* (Kováčik et al., 2009). However, in *Pisum sativum* the content of phenols increased in all Ni variants (Singh et al., 2009). After Ni exposure, the content of photosynthetic pigments and polyphenols increased significantly compared to Control (Rajaei and Mohamadi, 2015).

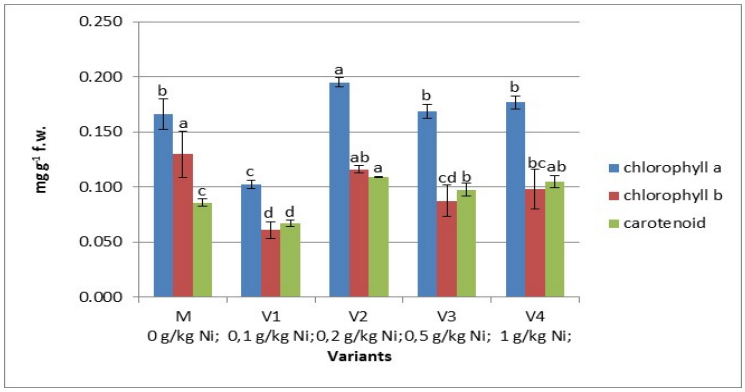

Fig. 13. Content of pigments in *A. filix-femina* (a, b, c, d – Duncan's test results, comparisons made between Control and V_{1-4})

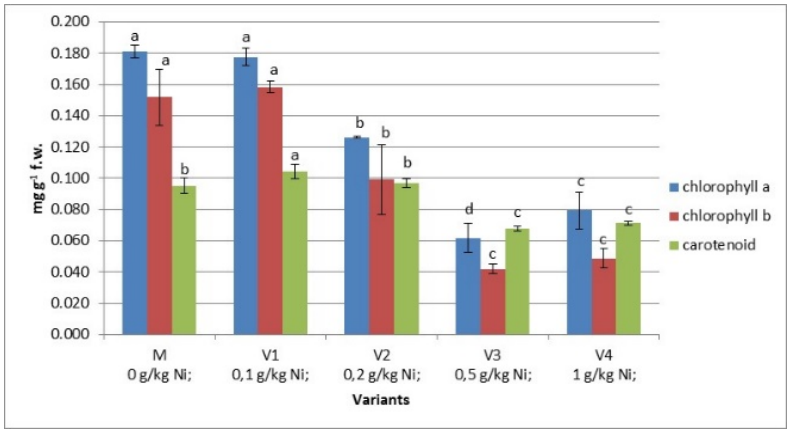

Fig. 14. Content of pigments in *D. filix-mas* (a,b,c,d -Duncan test results, the comparations were made between Control and V_{1-4})

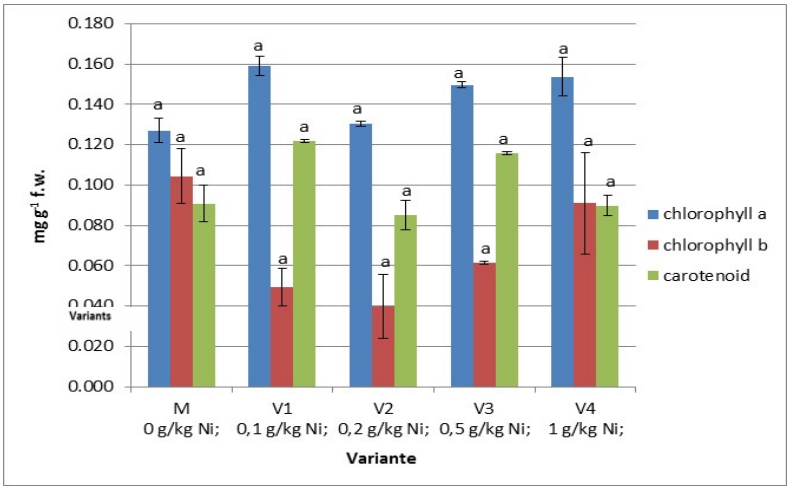

Fig. 15. Content of pigments in *D. affinis* (a,b,c,d -Duncan test results, the comparations were made between Control and V_{1-4})

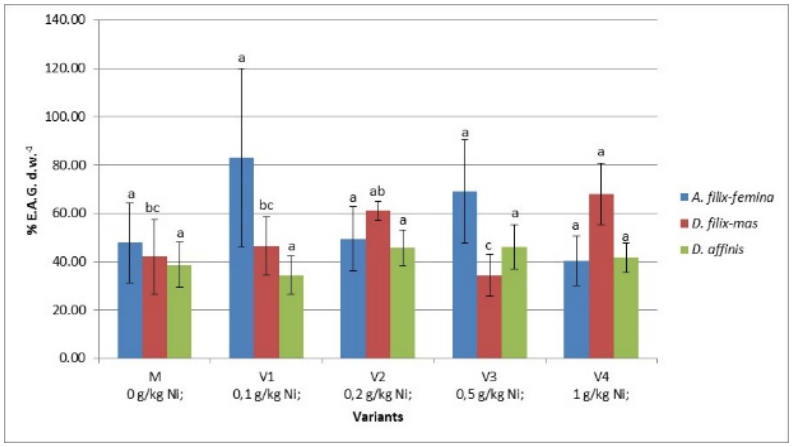

Fig. 16. Total polyphenol content after 4 months of exposure (Gallic Acid Equivalents /dry weight)

CONCLUSIONS

Under the Ni influence differences between the tested species as well as between the variants of the same species regarding gametophyte and sporophyte development were recorded. The Ni effect on the content of photosynthetic pigments varied depending on species. Small concentrations of Ni stimulated the content of chlorophyll *a* in *D. filix-mas* (V_1) and *A. filix-femina* (V_2). Chlorophyll *b* is more sensitive to Ni than chlorophyll *a*. Generally, the polyphenol content tended to increase in the variants with Ni compared to the Control confirming their protective role against heavy metal stress.

ABSTRACT

The aim of this study was to evaluate the morphological and biochemical changes induced by nickel in the gametophye and sporophyte of *Athyrium filix-femina* (L.) Roth, *Dryopteris filix-mas* L. Schott and *Dryopteris affinis* (Lowe) Fraser-Jenk. The spores were uniformly distributed on the surface of sterilized soil with different Ni content: Control = 0 g Ni^{2+}, V_1 = 0.1 g Ni^{2+}, V_2 = 0.2 g Ni^{2+}, V_3 = 0.5 g Ni^{2+}, and V_4 = 1 g Ni^{2+}. The variants were kept in the growing room and periodically watered with distilled water for 4 months. During this period we made observations on the development of gametophytes and sporophytes. After that we determined the content of photosynthetic pigments and total polyphenols. The effect of Ni on the content of pigments varied depending on species. In *Dryopteris filix-mas* there were no significant differences between the chlorophyll content from Control and V1, but for the other variants we observed a downward trend for both chlorophyll (a and b) and carotenoid content. Chlorophyll b is more sensitive to Ni than chlorophyll a in *Dryopteris affinis* and *Athyrium filix-femina*. In *Athyrium filix-femina*, the highest content of polyphenols was obtained in the first variant. Generally, the polyphenol content tended to increase in the variants with Ni compared to the Control, confirming their protective role against heavy metal stress.

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