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STRESS RESPONSE TO NICKEL IN *ASPLENIUM SCOLOPENDRIUM* L. AND *DRYOPTERIS FILIX-MAS* (L.) SCHOTT.

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Abstract
The aim of this study was to determine the physiological response and the defensive potential in species Asplenium scolopendrium L. and Dryopteris filix-mas (L.) Schott under the action of nickel. The following experimental variants were tested: 0, 250, 500, 1000, 1500 mg Ni kg^{-1}soil. One month, and three months respectively, after the initiation of the experiment, the amount of assimilating pigments was determined. One month after the initiation of the experiment, there were no significant differences between the variants with Ni and the control as far as the content of chlorophyll (a and b) and carotenoids was concerned. The results obtained three months after the inception of the experiment indicate that, at low concentrations, Ni stimulates the synthesis of chlorophyll. In the same period there occurred antioxidant mechanisms: increase in the amount of carotenoids and increased activity of catalase. In the species Dryopteris filix-mas, the variant with 1.000 mg Ni kg^{-1}soil, the amount of chlorophyll was significantly reduced, and the catalase activity was 3 times higher than that obtained in the control group.

Keywords: nickel, ferns, pigments, catalase;

1. INTRODUCTION

Nickel is one of the ubiquitous metals, and represents about 3% of the Earth's composition, being the fifth chemical element in terms of abundance, after Fe, O_2, Mg and Si (Bhalerao et al., 2015). Up to the present about 1% of the Ni resources were identified (ca. 130 million tons of Ni), as laterites and sulphide deposits (United States Geological Survey).

Metallic Ni and its alloys are included in Group 2B – possibly carcinogenic to humans – according to the classification made by the International Agency for Research on Cancer, and in the classification made by the Agency for Toxic Substances and Disease Registry, it ranks 57 in the Priority List of Hazardous Substances.

Gerendás et al. (1999) believes that Ni is a "candidate to be included on the list of the 13 essential minerals". The inclusion is made after the fulfillment of three conditions: its absence is not likely to deny the achievement of all the development stages of the life cycle of a plant, its function should not be specific, so that it cannot be replaced, it must be directly involved in the structural processes or the metabolism of the plant (Grusak, 2001).

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Brown et al. (1987) demonstrated that Ni is essential for the development of the species *Hordeum vulgare*, a result that was subsequently extrapolated to superior plants. Similarly, Ni is a co-factor for ureasis, and has an important role in metabolism N. Being a microelement, Ni is essential in low concentrations (0.05-10 mg kg^{-1} dry weight) (Nieminen et al., 2007), and when such values are exceeded, there occurs the toxic effect due to its interference with the ions of other essential metals, or through induction of oxidative stress (Chen et al., 2009). The level of toxicity varies by species, genotype, stage of development, cultivation conditions, etc., and must be correlated with the dose and exposure time. (Kovácik et al., 2009). As a rule, the Ni concentrations in plants reflect the concentrations of the metal in the soil (Kabata-Pendias, 2001). The toxicity of Ni conduces to: chlorosis, necrosis, wilting, affecting growth, photosynthesis and metabolism, reducing the amount of pigments and transpiration rate, inhibiting the activity of Mg and Fe ions.
The aim of this study was to determine the physiological response and the defensive potential of two species of fern, *Asplenium scolopendrium* and *Dryopteris filix-mas*, to the action Ni, found in soil in different concentrations. The species studied belong to the same order, i.e. *Polypodiales*. *Asplenium scolpendrium* is a circumpolar neutron-basiphilic species, which can be encountered in Europe; *Dryopteris filix-mas* has a much larger area of distribution (it is cosmopolitan), being encountered in the hilly and mountainous region in Europe, Asia, America and in Madagascar; this species tolerates large pH variations. Both species are mesothermophilic, preferring soils with moderate to high humidity.

2. MATERIALS AND METHODS

Dryopteris and *Asplenium* mature specimens were collected in Vâlsan Valley (August 2015). After acclimation, the plants were transplanted into pots with plant soil, where $NiSO_4$ was added at various concentrations, thus obtaining the following experimental variants 0, 250, 500, 1000, 1500 mg kg^{-1}sol Ni. During three months, the plants were maintained in greenhouse conditions, being watered regularly with 200 ml of distilled water. At regular intervals, the pots were moved between them in order to ensure uniform conditions; three samples with 4-5 mature leaves were used for each concentration. The physiological response of the two species was assessed by determining the amount of assimilating pigments: chlorophyll a, chlorophyll b, and the carotenoids in an acetone extract, by means of a spectrophotometer and Holm's formulas (Holm, 1954). Catalase activity was determined by titration with $KMnO_4$. The results obtained were statistically analyzed using SPSS with (version 16 for Windows).

3. RESULTS AND DISCUSSIONS

The content of chlorophyll is one of the most important factors ensuring the performance of photosynthesis under optimal conditions (Jiang and Hung, 2001).
As shown in Figure 1 and 2, Ni influence on the amount of pigment, after one month of exposure, was not significant compared with the control. A slight increase was found in the concentration of chlorophyll a in the variant with 250 mg Ni kg^{-1} soil, in *Asplenium scolopendrium*. Low levels of Ni stimulate the synthesis of photosynthetic pigments (Singh and Pandey, 2011). Similar results were obtained by Kováčik et al. (2009) in *Matricaria chamomilla*, 10 days after the inception of the experiment with Ni: there were no significant differences in the concentration of pigments, and the ratio of chlorophyll a/b, and the optical density remained unchanged.
Three months into the experiment, there appeared significant changes in pigments (Figure 3). In *Asplenium scolopendrium*, for the lowest concentration of Ni in the soil, a further increase was observed in the chlorophyll concentration; the result obtained in this variant (1.535 mg g^{-1} fw) had the highest value determined in the experiment. The increase in the pigment content can be

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considered an ability that the plant has to tolerate, or compensate the stress caused by low concentrations of Ni (Doganlar et al., 2012). In the other variants of the same species, the values were close to the value obtained in the control.

In the case of chlorophyll b, in this species, the only significant difference was recorded by comparing the value obtained for the variant with the lowest Ni concentration (250 mg kg^{-1} soil) – where there has been a slight tendency to increase the amount of chlorophyll b – with the value for the variant with the highest Ni concentration (1500 mg kg^{-1} soil).

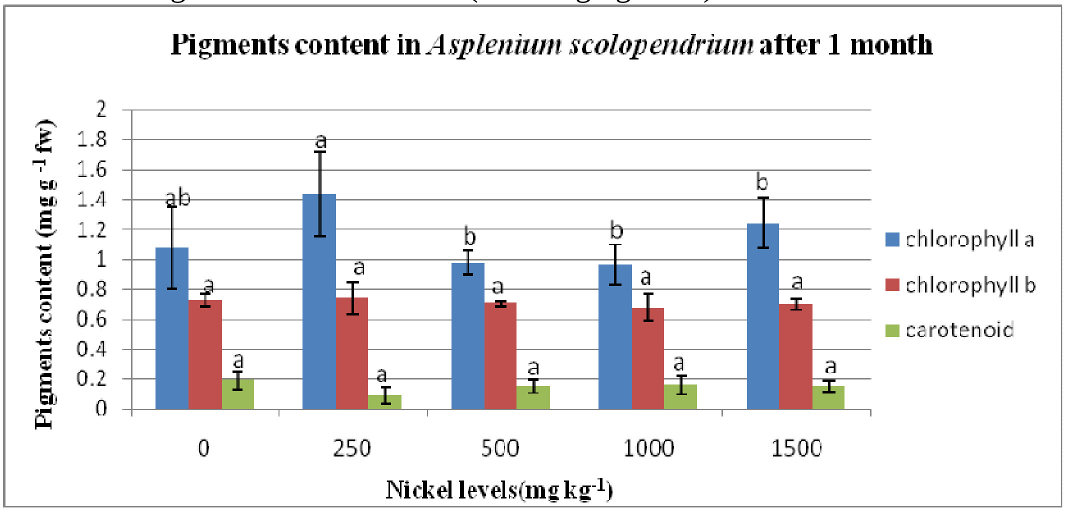

Figure 1. Pigments content in Asplenium scolopendrium after 1 month of exposure

In the experiment conducted by Bazihizina et al. (2015), in the species *Psidium guajava*, after 2 months of exposure, the total chlorophyll content showed no significant differences for the Ni variants compared to the control. Instead, the concentration of carotenoids increased with the increase in the Ni concentration, and the highest increase was in the variant with 3000 µM Ni^{2+}, where almost a doubling was recorded.

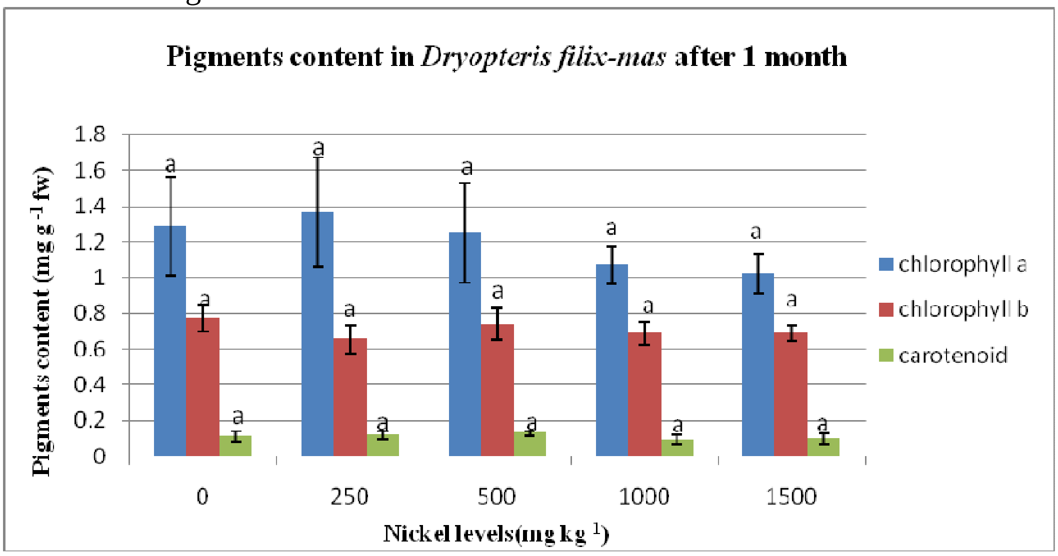

Figure 2. Pigments content in Dryopteris filix-mas after 1 month of exposure

In the carotenoids, the situation found in both species was similar: thus, in all Ni variants an increase was observed in the concentration of pigments with antioxidant properties; however, the increase was significant only at the lowest concentration of Ni in the soil. The increase or decrease

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of the content of carotenoids is influenced by the type of metal, as they are nonenzymatic antioxidants, with a role in fighting oxidative stress (Singh and Tewari, 2003).

In the *Zygophyllum xanthoxylon* seedlings exposed to various concentrations of Ni (0, 50, 150, 450, 900 mg kg^{-1}) no chlorosis symptoms were observed. There was an increase in the content of pigments (chlorophyll a and b, carotenoids) at low concentrations of Ni (50 mg kg^{-1}). With the increasing concentration of metal, a decrease was observed in the concentration of pigments, more specifically the content of chlorophyll and carotenoids decreased to the value obtained in the control; the chlorophyll b decrease was progressive, and the value obtained for the highest concentration of Ni in soil was higher than that obtained in control (Lu et al., 2010).

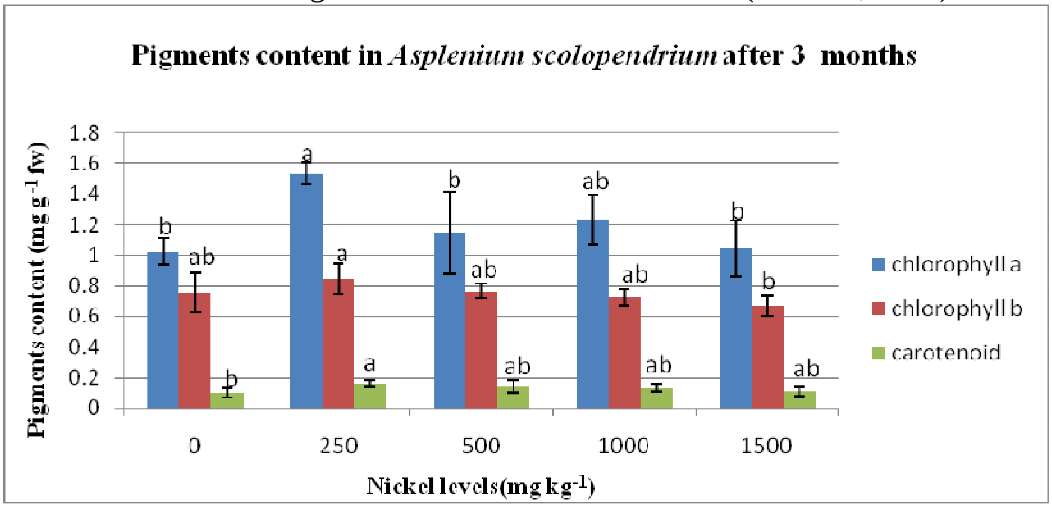

Figure 3. Pigments content in Asplenium scolopendrium after 3 month of exposure

The situation encountered in the *Dryopteris filix-mas* species is similar to those presented previously (Fig. 4): the small concentrations of Ni (250, 500 mg kg^{-1} soil) lead to an increased amount of chlorophyll a (the values are in the range of 1.07-1.12) and chlorophyll b (increases of 10 and 12% compared to control). At a concentration of 1000 mg kg^{-1} soil a significant reduction was found in the amount of chlorophyll a compared with the control; the decrease in chlorophyll b was gradual, so that the differences are not significant. The decrease in the content of photosynthetic pigments due to the stress produced by Ni has been reported in *Vigna mungo* (Dubey and Pandey, 2011), in two varieties of *Raphanus sativus* (Latif, 2010), and also in wheat leaves (Gajewska and Sklodowska, 2007), and wheat seedlings (Gajewska et al., 2006). Due to exposure to the action of heavy metals, the activity of antioxidant enzymes can change (increasing or decreasing), or it can remain constant (Jiang and Hung, 2001; Zhang et al., 2007). Yan et al. (2008) found that treatment with Ni increased catalase activity in plants. A situation encountered in *Raphanus sativus* (Latif, 2010), in *Zea mays* seeds (Ghasemi et al., 2012), in *Catharanthus roseus* (Arefifard et al., 2014), and in *Helianthus annuus* (Hassanpour Esfahani and Rezeyatmand, 2015).

Table 1. Influence of Ni on catalase activity in the two species of ferns 3 months after the inception of the experiment (ml $KMnO_4$ g^{-1} s.p.)

Species / *Variants*	0 mg kg^{-1}Ni	250 mg kg^{-1} Ni	500 mg kg^{-1} Ni	1000 mg kg^{-1} Ni	1500 mg kg^{-1} Ni
Asplenium scolopendrium	7.39±0.2^b	6.78±0.58^b	7.01±0.02^b	7.10±0.35^b	8.28±0.16^a
Dryopteris filix-mas	5.67±0.17^b	12.95±2.94^a	14.89±2.70^a	15.52±4.19^a	11.56±1.64^a

Legend: the values are averages of 3 replicates ± standard deviation; a, b, c, d – the results obtained from the Duncan test: the comparisons were made between Control and V_{1-3} for each metal

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If in the species *Asplenium scolopendrium* significant differences were observed only at the highest concentration of Ni in the soil, compared to the control, in *Dryopteris filix-mas* the increase in catalase activity was observed in all experimental variants. The largest increase occurred in the Ni concentration of 1000 mg kg^{-1} soil, where almost a tripling of activity was recorded.

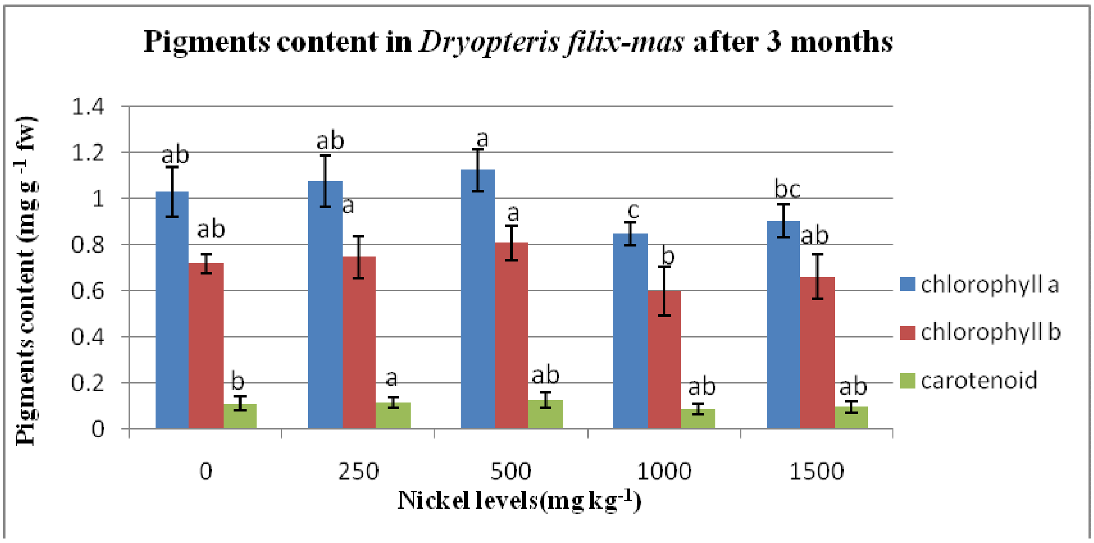

Figure 4. Pigments content in Dryopteris filix-mas after 3 month of exposure

Baccouch et al. (1998) observed that catalase activity in the shoots of *Zea mays* increased after an interval of 4 days of exposure to $NiCl_2$. For *Nasturtium officinale* the maximum amount of catalase was obtained in the fifth day after exposure at a concentration of 5 mg L^{-1} Ni (Duman and Ozturk, 2010), and in *Lemna gibba* – after 72 hours, at a concentration of 4 mg L^{-1} Ni (Doganlar et al., 2012).
Catalase activity is correlated with the level of Ni in the environment (Ghasemi et al. 2012), and is influenced by the duration of the exposure (Baccouch et al, 1998). Catalase is one of the most important factors of eliminating the H_2O_2 resulting from oxidative stress caused by exposure to the action of Ni. Increased activity of catalase represents a defense mechanism of the plant against the stress caused by this metal.

4. CONCLUSIONS

In conclusion, the two species show tolerance to the action of Ni, throughout the experiment no symptoms of toxicity were observed. Low concentrations of Ni boost the amount of chlorophyll a. Increased levels of carotenoids and increased activity of catalase were intended to compensate oxidative stress resulting from exposure to the action of Ni. According to the results, the species *Dryopteris filix-mas* presents a lower tolerance to Ni compared to *Asplenium scolopendrium.*

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