

Original Research Article

Arabidopsis thaliana* reaction in response to cadmium and *Trichoderma

fungi additive into the soil

ABSTRACT

The aim of this study was to determine the effect of the substrate inoculation (silty soil) using *Trichoderma* genus fungi on cadmium phyto-availability and process of photosynthesis disturbances at *Arabidopsis thaliana*. The two-factor experiment was established, in which the 1st variable factor was the presence or absence of *Trichoderma* genus fungi in the substrate, while the 2nd order factor was the growing amounts of cadmium in the soil. During the vegetation, measurements of chlorophyll fluorescence parameters were carried out. After the experiment complete, the cadmium content was determined in aboveground parts of *Arabidopsis thaliana*.

The cadmium doses incorporated in the silty soil (2, 10, 30 mg Cd/kg DM soil) did not cause any significant disturbances within the photosynthetic system of *Arabidopsis thaliana*. Only Fv/Fm parameter values showed some deterioration in the photochemical efficiency of PSII. Cadmium content in the aboveground parts of plants increased along with the soil contamination by this metal, both with and without addition of *Trichoderma* genus fungi. However, plants growing on soils with lower doses of cadmium (2 and 10 mg Cd/kg) with *Trichoderma* genus fungi addition, contained less of the element than those with no fungi addition.

Keywords: *Arabidopsis thaliana*, chlorophyll fluorescence, *Trichoderma*, cadmium

INTRODUCTION

Plants of natural ecosystems and agro-ecosystems are subject to exposure to a number of adverse factors (cold, drought, heavy metals, etc.), referred to as environmental stress that disrupt physiological processes. The photosynthesis process is particularly vulnerable to the

26 environmental stress and changes in the photosynthetic system of plants can be illustrated by
 27 measuring the chlorophyll fluorescence parameters (Kalaji and Rutkowska 2004, Kuklová et
 28 al. 2015, Li et al. 2012).

29 One of the main factors negatively affecting the natural environment are heavy metals
 30 (Hejzman and al. 2014). Industry, energy production, and transport are the most significant
 31 sources of metals emitted into the atmosphere. Of these metals, cadmium is characterized by
 32 high toxicity and high capacity to bio-accumulate within plants (Kabata-Pendias 2000).
 33 Accumulation of cadmium in soils and plants is determined by the type of geological subsoil
 34 (Kaniuczak and Hajduk 1995, Gorlach and Gambuś 1996, Kabata-Pendias 2000), application
 35 of wastes for fertilizing and intensive mineral NPK nutrition (Kabata-Pendias 2000,
 36 Kaniuczak et al. 2011). Dust emitted into the atmosphere as a result of natural processes and
 37 human activities is also the source of cadmium.

38 Changes in soil properties through liming (Gorlach and Gambuś 1996, Jansson 2002,
 39 Bolan et al. 2003, Kaniuczak et al. 2011), fertilization using phosphorus compounds or
 40 enrichment in organic matter also reduce the phyto-availability of heavy metals (Gorlach and
 41 Gambuś 1996, Spiak et al. 2000, Leszczyńska and Kwiatkowska-Malina 2013). Another way
 42 to reduce the bio-accumulation of ballast metals and negative effects of their influence on
 43 plants is the use of mycorrhizal inoculation. In recent years, *Trichoderma* genus fungi invoke
 44 a lot of interest in the world of science. These microorganisms are among the most studied
 45 and applied means for biological plant protection. Its mechanism of action is based on the
 46 colonization of roots, the support for plant growth, and the protection against stress factors
 47 such as pathogenic organisms (Benitez 2004, Dłużniewska 2008).

48 The aim of this study was to determine the effect of the subsoil inoculation with
 49 *Trichoderma* genus fungi on cadmium phyto-availability and process of photosynthesis at

50 *Arabidopsis thaliana* – thale cress – that is a model organism of a small size and high
51 resistance.

52 MATERIALS AND METHODS

53 **Experimental design.** The two-factor pot experiment was carried out in the
54 Laboratory of Plant Physiology, University of Rzeszow. The experiment was established by
55 means of a randomized split-plot method in 4 replications. The first variable factor was the
56 presence (+ T) or absence (-T) of *Trichoderma* genus fungi applied into the soil in the form of
57 a granular formulation Trianum-G in the quantity recommended by the manufacturer
58 (Koppert BV), i.e. the first application of 750 g/m³, while the second 375 g/m³ subsoil.
59 Another variable factor consisted of cadmium doses applied into the soil in amounts of 0, 2,
60 10, 30 mg Cd/kg DM soil. At the same time, they were the experimental objects: 0, A, B, C
61 (Table 1). Thale cress (*Arabidopsis thaliana*) was the test species.

62 **Plant material and growth conditions.** Slightly acidic soil of pH_{KCl} 5.57 (hydrolytic
63 acidity 4.5 cmol(+)/kg) and granulometric composition of silty soil (clay content 8%), was
64 used as a subsoil for experiment. The soils contained an average of 7.1 g/kg DM of organic
65 carbon, and 1.12 g/kg DM of nitrogen. Total content of cadmium was 0.703 mg/kg DM.

66 *Arabidopsis* plants were grown in pots containing 1 kg of soil previously dried and
67 sieved through a sieve with a diameter of 0.5 mm mesh.

68 Uniform basic nutrition was applied for all objects by incorporating water solutions of
69 the following salts into the subsoil: 0.126 g KNO₃, 0.118 g Ca(NO₃)₂, 0.123 g MgSO₄, and
70 0.085 g KH₂PO₄ per 1 kg of soil (Troufflard et al. 2010). Cadmium was incorporated in the
71 form of water solution of 3CdSO₄·8H₂O.

72 Growth density of the test species (*Arabidopsis thaliana*) was 3 plants per pot. Soil
73 moisture was kept stable at 40% in the first phase of experiment and 50% in the second phase.

74 Plants were grown for 46 days maintaining the temperature at the level of 22/18 °C, at
75 photoperiod 16/8 h (day/night).

76 **Chlorophyll fluorescence.** During plant vegetation period, disturbances of
77 photosynthesis process were monitored by measurements of chlorophyll fluorescence
78 parameters using fluorometer IMAGING-PAM in maxi version (Walz). Following parameters
79 were determined: F_0 – zero fluorescence of objects adapted to darkness ; F_M – maximum
80 fluorescence; F_V – variable fluorescence $F_V = F_M - F_0$; F_V/F_0 – maximum efficiency of water
81 splitting at the donor side of PSII and F_V/F_M – maximum photochemical efficiency of PSII.

82 All measurements were made in four replicates, while a single replicate consisted of 10
83 measurements.

84 **Chemical analysis.** After completion of the pot experiment, the plant material was
85 dried at 75 °C and grinded. Cadmium content in aboveground parts of *Arabidopsis thaliana*
86 was determined by means of atomic absorption spectrometry (AAS, using Hitachi Z-2000)
87 device after digestion in a microwave system in concentrated HNO_3 with addition 30% H_2O_2
88 in Berghoff apparatus (Buszewski et al. 2000).

89 Biological accumulation coefficients (BAC) for cadmium as a ratio of its content in
90 shoots to soil, was also calculated.

91 **Statistical analysis.** The experiment was established by means of a randomized split-
92 plot method in 4 replications. The results were statistically processed using bi-factor variance
93 analysis (ANOVA) and the Kruskal-Wallis test using Statistica 10 software.

94

95 **RESULTS AND DISCUSSION**

96 Detection and analysis of chlorophyll fluorescence parameters can be used as a precise
97 tool for studying the photosynthesis under stress conditions. Kalaji and Rutkowska (2004)
98 reported, that values of individual fluorescence parameters are interrelated and changing one

will affect the others. F_0 indicator provides information on the excitation energy losses during its transfer from the energy antenna to the PSII reaction center. Under the influence of stress factors F_0 usually increased (Kalaji and Rutkowska 2004).

Measured results of chlorophyll fluorescence parameters are illustrated in Figure 1. Values of parameter F_0 at plants growing on the subsoil without *Trichoderma* addition (-T) are within the range 0.161–0.174 AU, while at plants grown on the subsoil with *Trichoderma* (+T) – within the range 0.153–0.181 AU. Both in one (+T) and the other (-T) experimental variant, value of F_0 decreased along with the increase of cadmium concentration in the soil. Present experiment proved statistically significant ($p=0.01$) decrease in F_0 value along with the increase of cadmium content in the subsoil (bi-factorial ANOVA), while addition of *Trichoderma* genus fungi into the subsoil had no considerable effects on zero fluorescence value ($p=0.79$). Research performed by Balakhnin et al. (2005) revealed that applied cadmium doses did not affect the F_0 indicator, that remained at level comparable to the control object. In turn, Vassilev et al. (2004), when studying the cadmium impact on barley plants, recorded statistically significant decrease of zero fluorescence, which could result from lowering the contents of photosynthetic pigments in leaves.

Value of the maximum fluorescence indicator F_M depends on many factors, among others the type of saturating light and chlorophyll content in a tested tissue. In the case of plants exposed to stress factors, the F_M value decreases (Kalaji and Łoboda 2010). The F_M values achieved in the experiment vary from 0.672 to 0.690 AU for variant (-T) as well as from 0.647 to 0.621 AU for variant (+T). Addition of *Trichoderma* genus fungi resulted in the decrease of F_M parameter value at every level of cadmium content in the soil, statistically significantly ($p=0.04$) only at plants grown on the subsoil with the highest cadmium rate applied (30 mg/kg).

Variable fluorescence F_v is a parameter, low values of which prove a poor PSII activity and losses of excitation energy in the form of emitted heat. F_v value is reduced by the impact of environmental stress (high temp., frost, etc.), that cause damage to the thylakoids (Reigosa and Weiss 2001). Exposure of plants to cadmium ions usually makes the decrease in variable fluorescence F_v (Kalaji and Łoboda 2010) as well as F_v/F_0 values (Balakhnina et al. 2005, Li et al. 2012). In present experiment, F_v parameter oscillated from 0.498 to 0.529 AU at plants grown on the subsoil without *Trichoderma* and from 0.466 to 0.498 AU at plants under the influence of *Trichoderma* fungi. For every levels of cadmium concentration in the subsoil, F_v reached higher values for variant, to which Trianum G preparation was not added. Influence of increasing cadmium content in the subsoil on F_v appeared statistically insignificant ($p=0.51$), while presence of *Trichoderma* genus fungi caused statistically significant decrease in variable fluorescence value ($p=0.01$).

Parameter F_v/F_0 characterizes the maximum efficiency of water splitting at the donor side of PSII system. In the preset experiment, the F_v/F_0 parameter is in the range 2.85–3.28 AU (-T) and 2.60–3.24 AU (+T). The subsoil inoculation using *Trichoderma* genus fungi has no effect on F_v/F_0 parameter value. Parameter F_v/F_0 considerably increased due to increased amount of cadmium added to the soil (Kruskal-Wallis test $p=0.00$). Parameter F_v/F_0 is remarkably sensitive towards various stress factors (Pereira et al. 2000).

For majority of plants under no stress conditions and at the stage of full development, this parameter value equals to 0.83 (Angelini et al. 2001). In present experiment, the F_v/F_M parameter takes the values below 0.80 for plants at every level of cadmium dose applied to the soil, including the level 0 (control). The Kruskal-Wallis test also revealed that growing extent of soil contamination due to cadmium caused highly significant increase in F_v/F_M parameter ($p=0.00$), although all achieved results of F_v/F_M were below 0.80 level. The optimum value of F_v/F_M at plants not exposed to stress should range from 0.80 to 0.83. Decrease of this value

indicates worsening of the photochemical capacity of (Björkman and Demmig 1987). Li et al. (2012) and Liu et al. (2011) observed reducing the ratio F_v/F_m in conditions of cadmium intoxication.

The cadmium rates (0, 2, 10, 30 mg/kg DM soil) used for the silty soil and the application of *Trichoderma* genus fungi or their absence) differentiated cadmium contents in the aboveground parts of thale cress (*Arabidopsis thaliana*) (Fig. 2). Cadmium concentration in aboveground parts of *Arbidopsis thaliana* arose along with its content increase in the subsoil, and in variant (-T) it ranged from 0.50 to 48.03 mg/kg DM, whereas in variant (+T) – from 0.33 to 54.06 mg/kg DM (Fig. 2). It was also observed that content of this element in plants at objects 0, A, and B was lower in the presence of *Trichoderma* genus fungi by 34%, 16%, and 10%, respectively. No doubt, it was the result of the protective influence of *Trichoderma*, because mycorrhizal fungi protect plants from heavy metal penetration from the environment by means of secreting substances that bind metals, adsorption enormous amounts of metals on the wall surface, or detoxification within own cells (Wójcik and Tukendorf 1995).

Belleghem et al. (2007), in studies upon sub-cellular cadmium localization in roots and leaves of *Arabidopsis thaliana*, showed that the tolerance mechanism of thale cress consists in excluding cadmium from shoots (“excluder strategy”). This strategy includes blocking the metal in roots (acting as a biological barrier) and transport from leaves back to the roots. The root’s function as the barrier was confirmed among others by studies of Dauthieu et al. (2009), who observed that amount of cadmium being the subject of allocation from roots to shoots at *Arabidopsis thaliana* is decreased along with its concentration increase in medium. In present experiment, addition of *Trichoderma* fungi into the subsoil, at the highest dose of cadmium, caused a slight increase in the cadmium content in the aboveground parts of thale cress.

Biological Accumulation Coefficient (BAC) was calculated as ratio of heavy metal in shoots to that in soil (Cui et al. 2007). When applying cadmium at rates 2 and 10 mg/kg subsoil, the BAC coefficients reach lower values for variant (+T) as compared to (–T), and at dose 30 mg/kg, the numbers are adverse (Table 2). On soils contaminated to a lesser degree, *Trichoderma* genus fungi a small extend reduced the transport of cadmium from the subsoil to the aboveground parts of thale cress plants, while this type of ability was not observed on soils extremely polluted due to this element.

REFERENCES

- Angelini G., Ragni P., Esposito D., Giardi P., Pompili M.L., Moscardelli R., Giardi M.T. (2001): A device to study the effect of space radiation on photosynthetic organisms. *Physica Medica* XVII, Supplement 1
- Balakhnina T.I., Kosobryukhov A.A., Ivanov A.A., Kreslavskii V.D. (2005): The effect of cadmium on CO₂ exchange, variable fluorescence of chlorophyll and the level of antioxidant enzymes in pea leaves. *The Russian Journal of Plant Physiology*, 52: 15-20.
- Belleghem F., Cuypers A., Semane B., Smeets K., Vangronsveld J., Haen J., Valcke R. (2007): Subcellular localization of cadmium in roots and leaves of *Arabidopsis thaliana*. *New Phytologist*, 173: 495-508.
- Benitez T., Rincon A.M., Limón M.C., Codón A.E. (2004): Biocontrol mechanism of *Trichoderma* strains. *International Microbiology*, 7: 249-260.
- Björkman O., Demming B. (1987): Photon yield of O₂ evolution and chlorophyll fluorescence characteristic at 77K among vascular plants of diverse origins. *Planta*, 170: 489-504.
- Bolan N.S., Adriano D.C., Mani P.A., Duraisamy A. (2003): Immobilization and phytoavailability of cadmium in variable charge soils. II. Effect of lime addition. *Plant and Soil*, 251: 187-198.

- 197 Buszewski B., Jastrzębska A., Kowalkowski T., Górna-Binkul A. (2000): Monitoring of
198 selected heavy metals uptake by plants and soils in the area of Toruń, Poland. Polish
199 Journal of Environmental Studies, 9: 511-515.
- 200 Cui S., Zhou Q., Chao L. (2007): Potential hyper-accumulation of Pb, Zn, Cu and Cd in
201 enduring plants distributed in an old smelter, northeast China. Environmental Geology,
202 51: 1043-1048.
- 203 Dauthieu M., Deniax L., Nguyen C., Panfili F., Perrot F., Point-Gautier M. (2009): Cadmium
204 uptake and distribution in *Arabidopsis thaliana* exposed to low chronic concentration
205 depends on plant growth. Plant and Soil, 322: 239-249.
- 206 Dłużniewska J. (2008): The Effect of Foliar Fertilizers on the Development and Activity of
207 *Trichoderma* spp. Polish Journal of Environmental Studies, 17: 869-874.
- 208 Gorlach E., Gambuś F. (1996): Research into the possibility of limiting accumulation of
209 cadmium by plants from soils contaminated this metal. Soil Science Annual, 47: 31-39.
- 210 Hejzman M., Müllerová V., Vondráčková S., Száková J., Tlustoš P. (2014): Establishment
211 of *Bryum argenteum* and concentrations of elements in its biomass on soils contaminated
212 by As, Cd, Pb and Zn. Plant, Soil and Environment, 60: 489 – 495.
- 213 Kabata-Pendias A. (2000): Biogeochemistry of cadmium. Scientific Papers PAN "Humans
214 and the Environment", 26: 17-24. (in Polish)
- 215 Kalaji M.H., Łoboda T. (2010): Chlorophyll Fluorescence in the study of the physiological
216 condition of plants. Warsaw, SGGW. (in Polish)
- 217 Kalaji M.H., Rutkowska A. (2004) Reactions of the photosynthetic apparatus of maize
218 seedlings to salt stress. Advances of Agricultural Sciences Problem Issues, 496: 545-558.
219 (in Polish)
- 220 Kaniuczak J., Hajduk E. (1995): Cadmium and lead in some soils of south-east Poland.
221 Advances of Agricultural Sciences Problem Issues, 418: 241-246. (in Polish)

- 222 Kaniuczak J., Hajduk E., Właśniewski S. (2011): Effect of liming and mineral fertilization on
223 cadmium content in grain of spring barley (*Hordeum vulgare* L.) and winter wheat
224 (*Triticum aestivum* L.) cultivated on loessial soil. Journal of Elementology, 16: 535-542.
- 225 Kuklová M., Hniličková H., Kukla J., Hnilička F. (2015): Environmental impact of the Al
226 smelter on physiology and macronutrient contents in plants and Cambisols. Plant, Soil and
227 Environment, 61: 72-78.
- 228 Leszczyńska D., Kwiatkowska-Malina J. (2013): The influence of organic matter on yield and
229 quality of Winter wheat *Triticum aestivum* ssp. *Vulgare* (L.) cultivated on soils
230 contaminated with heavy metals. Ecological Chemistry and Engineering S, 20: 701-708.
- 231 Liu C., Guo J., Cui J., Lü T., Zhang X., Shi G. (2011): Effects of cadmium and salicylic acid
232 on growth, spectral reflectance and photosynthesis of castor bean seedlings. Plant and Soil,
233 344: 131-141.
- 234 Li X., Zhang L., Li Y., Ma L., Bu N., Ma C. (2012): Changes in photosynthesis , antioxidant
235 enzymes and lipid peroxidation in soybean seedlings exposed to UV-B radiation and/or
236 Cd. Plant and Soil, 352: 377-387.
- 237 Pereira W.E., Siqueira D.L., Martínez C.A., Puiatti M. (2000): Gas exchange and chlorophyll
238 fluorescence in four citrus rootstocks under aluminium stress. Journal of Plant Physiology,
239 157: 513-520.
- 240 Reigosa R.M.J., Weiss O. (2001): Fluorescence techniques. In: Reigosa R.M. (ed) Handbook
241 of Plant Ecophysiology Techniques., Dordrecht, the Netherlands, Acad. Publ.
- 242 Spiak Z., Radoła J., Romanowska M. (2000): Effect of phosphorus and nitrogen fertilization
243 on zinc uptake by the plants. Advances of Agricultural Sciences Problem Issues, 471: 521-
244 528. (in Polish)

Troufflard S., Mullen W., Larson T.R., Graham I.A., Crozier A., Amtmann A., Armengaud P. (2010): Potassium deficiency induces the biosynthesis of oxylipins and glucosinolates in *Arabidopsis thaliana*. BMC Plant Biology, 172: 1-13.

Vassilev A., Lidon F.C., Ramalho J.C., Matos M., Bareiro M.G. (2004): Shoot cadmium accumulation and photosynthetic performance of barley plants exposed to high cadmium treatments. Journal of Plant Nutrition, 27: 775-795.

Wójcik A., Tukendorf A. (1995): The strategy of avoiding stress resistance of plants to heavy metals. Botanical News 39: 33-40. (in Polish)

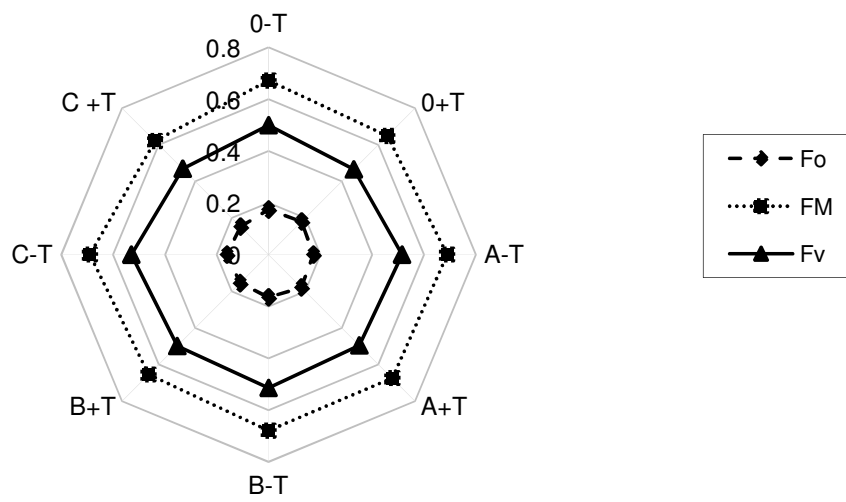
Table 1. The experimental scheme

Object	Soil with <i>Trichoderma</i> genus fungi addition (+T)	Soil without <i>Trichoderma</i> genus fungi addition (-T)
	Cadmium dose [mg/kg DM soil]	
Control 0	0	0
A	2	2
B	10	10
C	30	30

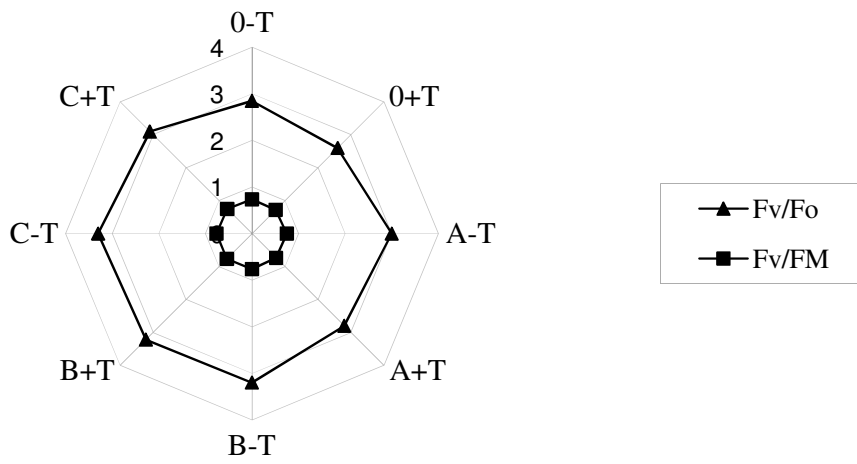
Table 2. Values of Biological Accumulation Coefficient (BAC) at *Arabidopsis thaliana* plants grown on silty soil contaminated with cadmium to various extents with (+T) or without presence (-T) of *Trichoderma* genus fungi

	Cadmium dose into the subsoil [mg/kg DM]/Object							
	0/A		2/B		10/C		30/D	
	-T	+T	-T	+T	-T	+T	-T	+T
BAC	0.71	0.47	2.48	2.09	1.80	1.61	1.60	1.80

Figure 1. Radar graphs of measured physiological traits in leaves of *Arabidopsis thaliana* depending on addition of *Trichoderma* fungi and cadmium doses



267



268

269

270

Figure 2. Cadmium content in the aboveground parts of *Arabidopsis thaliana* [mg/kg DM] depending on cadmium dose in the subsoil (-**T** – subsoil without *Trichoderma* addition; +**T** – subsoil with *Trichoderma* addition)

