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ORIGINAL PAPER

RESPONSE OF OAT (*AVENA SATIVA*) TO THE ADDITION CADMIUM TO SOIL INOCULATION WITH THE GENUS *TRICHODERMA* FUNGI

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Abstract

The aim of the experiment was to determine the effect of subsoil inoculation with fungi of the genus *Trichoderma* on the cadmium phytoavailability and process of photosynthesis of oat (*Avena sativa*). A two-factor pot experiment was established, in which the 1st variable factor was the presence or absence of *Trichoderma* fungi in the substrate, while the 2nd order factor was the increasing soil content of cadmium. During the plant growing season, disturbances in the photosynthesis process were monitored by measurements of the chlorophyll content and fluorescence parameters (on the first, third and flag leaf). The following parameters were determined: F_0 – zero fluorescence of objects adapted to darkness, F_M – maximum fluorescence, F_V – variable fluorescence, F_V/F_0 – maximum efficiency of water splitting at the donor side of PSII and F_V/F_M - maximum photochemical efficiency of PSII. After completion of the pot experiment, the yield of the plant and the content of cadmium in roots and aerial parts were determined. The results indicate that an increasing cadmium concentration in the soil did not influence the yield of roots and straw of oat, although it caused a significant decrease of grain yield. The cadmium concentration in roots and aerial parts of oat was increasing proportionally to the increasing amount of this metal in the soil, and the addition *Trichoderma* fungi did not influence significantly the yield and cadmium phytoavailability by roots and aerial parts of oat. The biggest changes in values of chlorophyll fluorescence parameters were noted on the first leaf. The addition of *Trichoderma* fungi to the subsoil had a positive influence on the content of chlorophyll in leaves.

Keywords: heavy metals, plants, chlorophyll fluorescence.

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INTRODUCTION

Heavy metals are among the main factors negatively affecting the natural environment (HEJCMAN et al. 2014). Industry, power generation and transport are the most significant sources of metals emitted into the atmosphere. Heavy metals, rather than being biodegraded, undergo biotransformation as a consequence of complex physico-chemical and biological processes which occur in soil and which can decrease toxicity of these elements to live organisms (BARAN, WIECZOREK 2015). Of these metals, cadmium is characterized by high toxicity and high capacity to bio-accumulate within plants (DA ROSA, CORRÊA et al. 2006, URAGUCHI et al. 2009, ABBAS et al. 2017, GUO et al. 2017). Accumulation of cadmium in soils and plants depends on the type of geological subsoil, application of waste for fertilization, and intensive mineral NPK nutrition. Dust emitted into the atmosphere as a result of natural processes and human activities is another source of cadmium. In consequence, soil pollution with heavy metals is a widespread problem and therefore solutions are searched for to remove these elements from the surface or to reduce their toxic influence on living organisms.

Changes in soil properties through liming, fertilization with phosphorus compounds or enrichment with organic matter also reduce the phyto-availability of heavy metals (LESZCZYŃSKA, KWIATKOWSKA-MALINA 2013). Addition of other cations to soil (for example Zn or Cu) can alleviate Cd toxicity by decreasing the Cd concentration in plant tissues owing to a possible antagonistic effect (MURTAZA et al. 2017). Another way to reduce the bioaccumulation of ballast metals and negative effects of their influence on plants is the use of mycorrhizal inoculation. For example, KONIECZNY, KOWALSKA (2017) described arbuscular mycorrhizal fungi (AMF), which live in symbiosis with the majority of plant species, as being able to reduce the Zn uptake by plants when this element is present in an increased concentration in the plant root zone. AMF could enhance the resistance of rice to Cd through alterations of the subcellular distribution and chemical forms of Cd in this plant (LI et al. 2016). In recent years, fungi which belong to the genus *Trichoderma* have gained much interest in the world of science. These microorganisms are among the most studied and applied biological plant protection measures (SONG et al. 2015). The mechanism of their action is based on the colonization of roots, supporting the plant growth and protecting against stress factors such as pathogenic organisms (DŁUŻNIEWSKA 2008). Studies indicate that *Trichoderma* increase Cd biosorption (BAZRAFSHAN et al. 2016) and may limit the Cd uptake by crop plants. SONG et al. (2015) indicate that *Trichoderma* inoculation increases plant biomass, but may also elevate the total Cd uptake by plants.

The aim of this research was to determine the effect of subsoil inoculation with fungi of the genus *Trichoderma* on the cadmium phytoavailability and process of photosynthesis of oat (*Avena sativa*).

MATERIAL AND METHODS

A two-factor pot experiment with 4 replications was designed according to the randomized split-plot method. The first variable factor was the presence (+T) or absence (-T) of *Trichoderma* fungi applied into soil as the granular formulation Trianum-G, in quantities recommended by the manufacturer (Koppert BV), i.e. the first application of 0.44 g kg^{-1}, and the second one of 0.22 g kg^{-1} DM soil. The other variable factor consisted of cadmium doses applied into soil in amounts of 0, 3, 5, 10, 20, 25 mg kg^{-1} DM soil (Table 1). The oat *(Avena Sativa)* was the test species.

Table 1

The design of the experiment

Object		Cadmium dose (mg kg^{-1} DM soil)
Subsoil without *Trichoderma* genus fungi addition (-T)	subsoil with *Trichoderma* genus fungi addition (+T)	
0 – T control	0 + T control	0
I – T	I + T	3
II – T	II + T	5
III – T	III + T	10
IV – T	IV + T	20
V – T	V + T	25

Slightly acidic soil of pH_{KCL} 5.57 (hydrolytic acidity 4.5 cmol(+) kg^{-1}) with the textural composition of silty soil (clay content 8%) was used as subsoil for the experiment. The soil contained an average of 7.1 g kg^{-1} DM of organic carbon and 1.12 g kg^{-1} DM of nitrogen. The total content of cadmium was 0.703 mg kg^{-1} DM. *Avena sativa* was grown in pots containing 2 kg of soil previously dried and sifted through a 0.5 mm mesh sieve. Four oat plants were grown in each pot.

Identical basic nutrition was applied to all pots, by incorporating water solutions of the following salts into the subsoil: 0.2 g N; 0.05 g P; 0.2 g K; 0.025 g Mg per 1 kg DM. Fertilizers were introduced in the form of water solutions of the following salts: NH_4NO_3, KH_2PO_4, KCl and $MgSO_4 \cdot 7H_2O$ Cadmium was incorporated in the form of water solution of $3CdSO_4 \cdot 8H_2O$. Soil moisture was kept stable at 40% in the first phase of experiment and 50% in the second phase. Plants were grown for 120 days, maintaining the temperature at 23/18°C and the photoperiod of 16/8 h (day/night).

During the plant growing period, disturbances in the photosynthesis process were monitored by measurements of chlorophyll fluorescence parameters using a WALTZ fluorometer. Measurements were made on the first, third and flag leaf. The following parameters were determined: F_0 – zero fluorescence of objects adapted to darkness; F_M – maximum fluorescence;

F_V – variable fluorescence $F_V = F_M - F_0$; F_V/F_0 – maximum efficiency of water splitting at the donor side of PSII and F_V/F_M – maximum photochemical efficiency of PSII.

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

After completion of the pot experiment, the plant material was dried at 75°C and ground. The cadmium content in roots and aerial parts of oat (straw and grain) was determined by atomic absorption spectrometry (AAS, using Hitachi Z-2000 Japan), after digestion in a microwave system in concentrated HNO_3 with addition 30% H_2O_2 in a Berghoff apparatus (Buszewski et al. 2000).

According Spiak and Wall (2000), apart from determination of significant differences in plant yields, it is also worth calculating the tolerance index (T_i). This parameter displays the ratio of yield obtained on soil polluted by metals to yield gathered on controlled soil and can achieve the following values: Ti < 1; Ti = 1; Ti > 1. The value Ti < 1 means curbing the growth or total death of plants; Ti = 1 indicates the lack of influence of an increased metal content on yield, whereas Ti > 1 indicates a positive effect of the metal on the growth and development of plants.

The results were statistically processed using bifactor variance analysis (ANOVA) and the Tuckey test in Statistica 10 software (StatSoft Inc.).

RESULTS AND DISCUSION

The results of the dry mass yield of oat indicate a lack of significant influence of the increasing cadmium amount in soil on the volume of yield (Figure 1). Aside the growing concentration of cadmium, another factor, i.e. the presence of (+T) or absence (-T) of *Trichoderma* fungi in the subsoil, was taken into consideration. Data in Figure 1 show that neither the increasing cadmium concentration in the subsoil nor the soil inoculation with *Trichoderma* fungi had a significant effect on the yield of roots, straw and grain of oat.

The results of the experiment enabled us to derive values of the index of tolerance Ti in the range from 0.85 to 1.21 (Table 2). Ti obtained a value higher than one when *Trichoderma* fungi had been added (except the V degree of cadmium pollution), whereas in the variant without *Trichoderma* the Ti value was less than 1.

The application of cadmium to subsoil led to distinct changes in the content of this metal in plants (Figure 2). The content of cadmium in roots and aerial parts of oat increased proportionally to the increase in its content in the subsoil. Most cadmium was accumulated in roots, slightly less in straw and the least in grain.

In most treatments the addition of *Trichoderma* fungi to the subsoil did

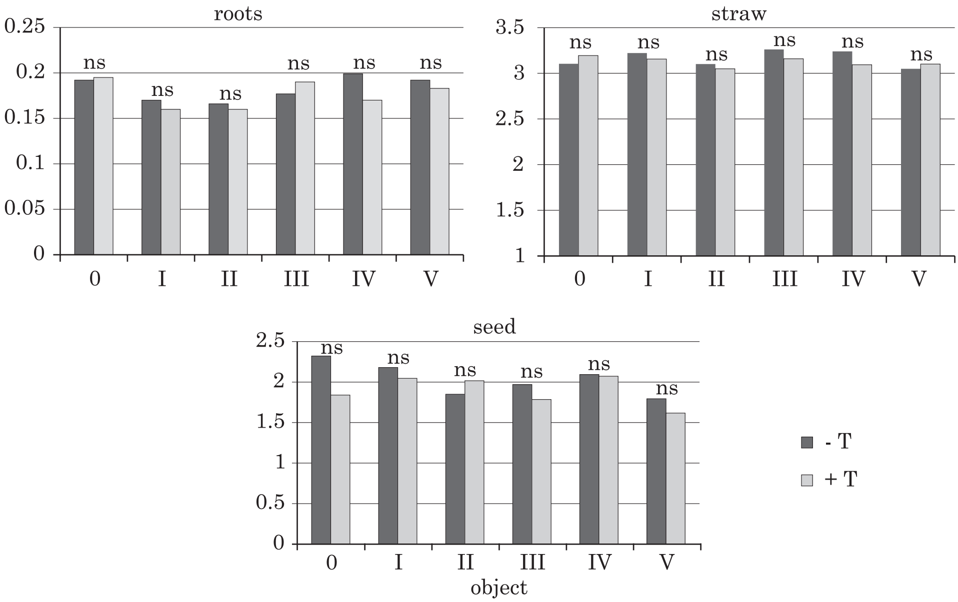


Fig.1. The yield of roots and aerial parts of oat (g pot^{-1}) grown on soil contaminated with cadmium in different doses, with (+T) or without (-T) *Trichoderma* genus fungi addition: ns – non-significant difference (ANOVA and Tuckey's test $p < 0.05$)

Table 2

Tolerance index (Ti) of test plants

Subsoil	Object				
	I	II	III	IV	V
	Ti				
Without *Trichoderma* (-T)	0.99	0.85	0.97	0.99	0.86
Relative error (%)	0.9	8.4	0.2	0.3	0.1
With *Trichoderma* (+T)	1.21	1.12	1.16	1.08	0.99
Relative error (%)	10.9	9.1	8.9	9.4	8.9

not significantly influence the cadmium phytoavailability to individual parts of the test plant. A significant decrease in the content of the metal caused by the addition of *Trichoderma* occurred only in the roots of plants growing in subsoil polluted by cadmium in the amounts of 20 and 25 mg kg^{-1} DM.

Cadmium is a heavy metal that can be particularly easily absorbed from the surface, and its content in soil affects its concentration in plants (GRUCA-KRÓLIKOWSKA, WACŁAWEK 2006). Values of the correlation coefficient (r) compiled in Table 3 numerically account for the relationship between the soil cadmium content and its accumulation in individual parts of the plant. The calculated r values point to a strong positive correlation between these parameters. The relationship is practically the same in soil with and without the addition of *Trichoderma* fungi.

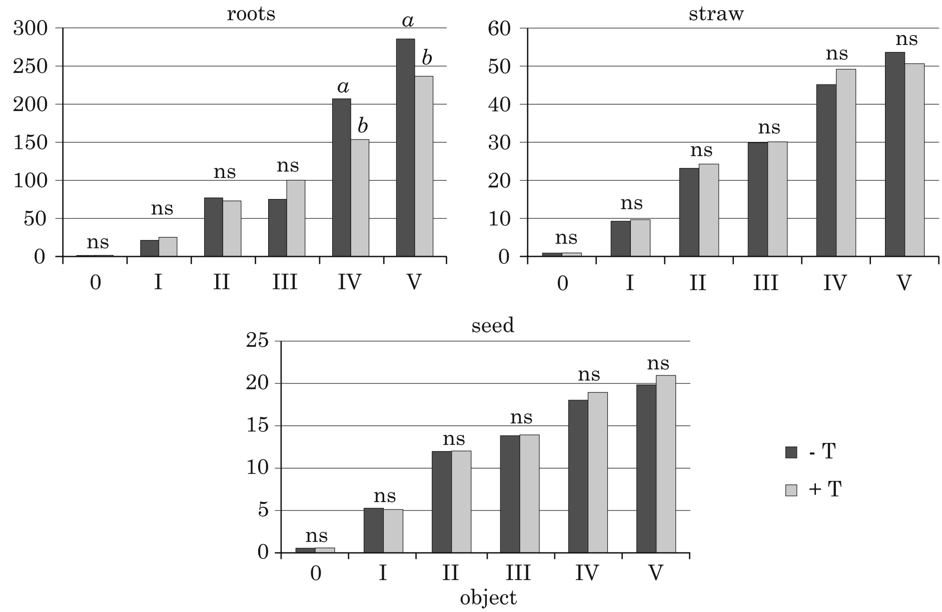


Fig. 2. Cadmium content in the roots and aerial parts of oat (μg g-1 DM) grown on soil contaminated with cadmium in different doeses, with (+T) or without (-T) *Trichoderma* genus fungi addition: *a*,*b* – significant difference, ns – non-significant difference (ANOVA and Tuckey's test $p < 0.05$)

Table 3

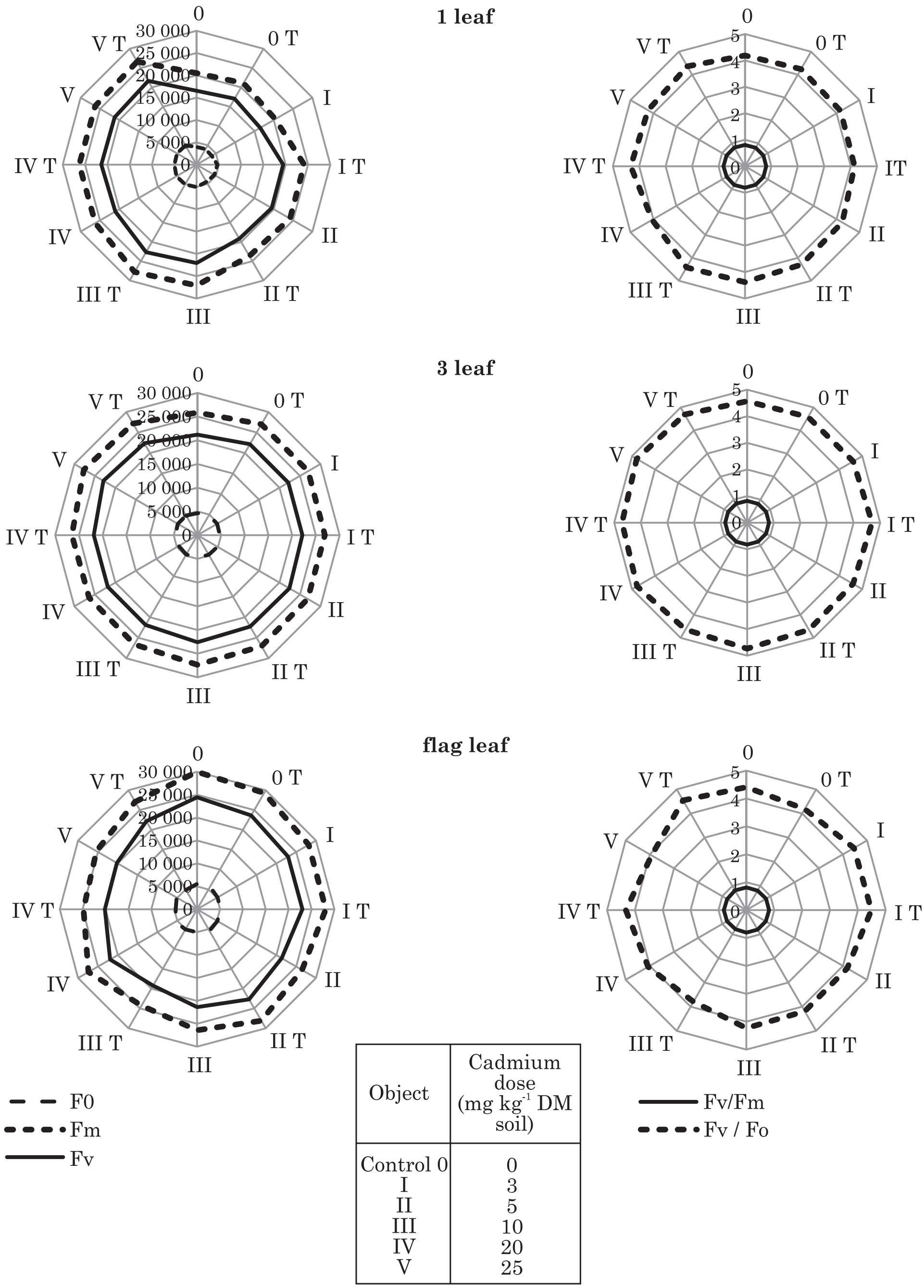
Simple correlation coefficients (r) between cadmium dose into the subsoil and its content in the roots and aerial parts of oat plants grown on subsoil without (–T) and with (+) *Trichoderma* genus fungi addition

	Cd – roots		Cd – straw		Cd – seed	
	–T	+T	–T	+T	–T	+T
Cadmium dose into the subsoil	0.95^{***}	0.98^{***}	1.00^{***}	0.99^{***}	0.98^{***}	0.99^{***}

r – significant at *** $p = 0.001$

The technique of measuring chlorophyll fluorescence parameters served in the reported research to assess the function of the photosynthetic apparatus in plants. A great advantage of the applied approach is that it does not cause long-lasting damage to plants and can be carried out during their growth (BAKER, ROSENQUIST 2004). Under optimal conditions for photosynthesis, solar energy is absorbed by chlorophyll and only a small portion of it is reemitted as fluorescence. If an anomaly in the PSII occurs, photosynthesis is restricted and fluorescent radiation increases. Figure 3 presents our results of measured chlorophyll fluorescence parameters.

According to BAKER and ROSENQUIST (2004), the F_0 parameter indicates the energy loss during its transport from energy antennae to the PSII.



Object	Cadmium dose (mg kg^{-1} DM soil)
Control 0	0
I	3
II	5
III	10
IV	20
V	25

Fig. 3. Radar graphs of measured physiological traits in leaf 1, 3 and flag of oat plants grown on soil contaminated with cadmium in different doses, with (T) or without *Trichoderma* genus fungi addition

A high value of this parameter informs us that the activation energy is transferred significantly more slowly between molecules of photosynthetic pigments. This deceleration occurs when a given plant is exposed to physiological stresses, for example salt stress, high or low temperature stress, stress due to the presence of heavy metals (Kalaji, Rutkowska 2004).

The value of zero fluorescence parameter F_0 measured on the first leaf was increasing with the growth in the cadmium concentration in surface. The Tuckey's test showed that there were statistically significant differences between plants growing in soil of the I and IV degrees of cadmium pollution. On the third and the flag leaf, the influence of higher cadmium doses in the soil on the value F_0 was unobserved. Regarding the presence or absence of *Trichoderma* fungi in subsoil, the differences in value F_0 were statistically non-significant.

Value of the maximum fluorescence F_M indicator depends on many factors, including the type of saturating light, its parameters and chlorophyll content in a tested tissue. The decrease of F_M values proves that the studied photosynthesising object is under stress, for not all acceptors of electrons in the PSII can be totally reduced (Michel-Lopez et al. 2016)

It has not been observed in the current study whether the decreasing values of F_M parameter on the first and third leaf coincided with an increasing intensity of the stress factor, which was cadmium. In general, a higher value of F_M was observed in plants growing in the presence of *Trichoderma* in the subsoil.

The variable fluorescence F_V is another parameter whose low values prove poor PSII activity and losses of excitation energy in the form of emitted heat. Fv value is reduced by the impact of environmental stress (high temp., frost, etc.), which cause damage to thylakoids. Exposure of plants to cadmium ions usually induces a decrease in variable fluorescence Fv as well as in Fv/F_0 values (Balakhnina et al. 2005, Li et al. 2012). In the plants we studied, lower values of Fv under a higher cadmium concentration in the subsoil were observed in the flag leaf, whereas different results were achieved on the first and third flag leaves. The addition of *Trichoderma* fungi to the subsoil did not cause significant changes of F_V values.

The maximal efficiency of water splitting at the donor side of the PSII system determined by the parameter F_V/F_0 under optimal condition is 3-4 (Kalaji, Guo 2008). The results show that the F_V/F_0 was slightly above 4 in most cases, in both plants growing with and without the *Trichoderma*.

The parameter F_V/F_0 characterizes the maximum efficiency of water splitting at the donor side of PSII system. F_V/F_0 can be used as a reliable indicator of the photochemical activity of the photosynthetic apparatus, because the maximum value of 0.83 appears at the stage of full development and under stress-free conditions (Basu et al. 1998). Our results indicate a decrease of the parameter F_V/F_M below the 0.83 value in all the experimental objects, and the subsoil inoculation with *Trichoderma* fungi had no effect on the F_V/F_M parameter.

The amount of photosynthetic dye contained in a given plant depends on its species and variety, and also on both environmental and anthropogenic factors (Kozłowski et al. 2001). In the plant objects studied, an increase in the chlorophyll content was observed in the consecutive leaves, that is parallel to the growth of a plant (Table 4). The addition of *Trichoderma* to the

Table 4

Index of chlorophyll content (SPAD) in separate leaves of oat

Subsoil	Object	1 leaf	3 leaf	Flag leaf
Without *Trichoderma* (–T)	0	2.92 ± 0.73*a*	10.09 ± 2.98*b*	16.76 ± 5.08*c*
	I	2.04 ± 0.18*a*	12.17 ± 1.19*b*	18.59 ± 6.51*c*
	II	1.86 ± 0.87*a*	6.49 ± 0.73*b*	9.71 ± 2.53*c*
	III	2.23 ± 0.40*a*	12.80 ± 1.65*b*	14.45 ± 2.19*b*
	IV	2.47 ± 0.45*a*	16.05 ± 3.56*b*	22.48 ± 3.13*c*
	V	2.19 ± 0.44*a*	12.90 ± 3.60*b*	20.33 ± 6.17*c*
With *Trichoderma* (+T)	0	2.42 ± 0.30*a*	16.17 ± 4.23*b*	19.65 ± 3.52*b*
	I	2.65 ± 0.76*a*	13.85 ± 2.29*b*	37.22 ± 10.23*c*
	II	2.96 ± 0.31*a*	16.22 ± 3.11*b*	29.44 ± 3.85*c*
	III	3.07 ± 1.09*a*	13.99 ± 2.24*b*	20.99 ± 5.05*c*
	IV	3.64 ± 0.41*a*	15.41 ± 2.21*b*	27.36 ± 7.40*c*
	V	3.36 ± 0.43*a*	15.02 ± 2.83*b*	27.27 ± 6.91*c*

Results are mean ± SD;
a,*b*,*c* – significant difference (ANOVA and Tuckey's test $p < 0.05$)

subsoil had influenced positively the amount of dye contained in leaves. On the other hand, it was not possible to demonstrate a clear and direct influence of the increasing cadmium content in soil on the amount of dye in leaves, as neither a stable decrease nor a consistent increase was observed.

Table 5 presents values of simple correlation coefficients (*r*), which express numerically the dependence between the cadmium content in soil and the values of chlorophyll fluorescence parameters determined in the first, third and flag leaf. The r values obtained indicate a strong positive correlation between the amounts of cadmium in both the soil and plant. This dependence is practically the same in soil with and without the addition of *Trichoderma* genus fungi. Also, r values indicate existing dependences (sometimes very strong) and often differ in the sign between the cadmium content in subsoil and the chlorophyll fluorescence in oat leaves. These values often differ in the sign for the flag and first leaf. Also, the r results indicate a weakening dependence between the cadmium amount in soil and the parameters F_M, F_V, F_V/F_M and Fv/F_0 in the third and flag leaf under the influence of the addition of *Trichoderma*. This implies that as a plant grows the defensive mechanisms, supported by the presence of *Trichoderma* in the subsoil, are intensified.

Table 5

Simple correlation coefficients (r) between cadmium dose in subsoil and parameters of fluorescence and content of chlorophyll (SPAD) in 1, 3 and flag leaf of oat cultivated on subsoil without (–T) and with (+T) *Trichoderma* genus fungi addition

Parameters of fluorescence and content of chlorophyll	1 leaf		3 leaf		Flag leaf	
	Cd_{soil} (–T)	Cd_{soil} (+T)	Cd_{soil} (–T)	Cd_{soil} (+T)	Cd_{soil} (–T)	Cd_{soil} (+T)
F_0	0.91***	0.81***	- 0.10	-0.53*	-0.30	-0.84***
F_M	0.88***	0.84***	0.58*	-0.17	-0.84***	-0.67**
F_V	0.86***	0.84***	0.69**	-0.04	-0.87***	-0.59*
F_V/F_M	-0.10	0.67**	0.93***	0.64*	-0.94***	0.16
F_V/F_0	-0.01	0.69**	0.95***	0.65**	-0.94***	0.19
SPAD	-0.29	0.93***	0.53*	-0.17	0.40	0.001

r – significant at * $p = 0.05$ ** $p = 0.01$; *** $p = 0.001$

CONCLUSIONS

1. The increasing cadmium concentrations in the subsoil (from 0 to 25 mg kg^{-1}) and addition of *Trichoderma* genus fungi did not influence the yield of roots, straw and grain of oat.

2. Cadmium concentrations in roots and aerial parts of oat increased proportionally to the growth in the content of this metal in the subsoil. The biggest amount of cadmium was accumulated in roots, slightly less in straw and the least in grain.

3. The addition of *Trichoderma* fungi to the subsoil in most object did not influence significantly the cadmium phytoavailability to individual parts of the test plant. There was a significant decrease in the content of the metal caused by the addition of *Trichoderma* only in the roots of plants growing in soil polluted by cadmium in doses of 20 and 25 mg kg^{-1} DM.

4. The biggest changes in values of fluorescence parameters were noted in the first leaf, suggesting that as the plant grows its defensive mechanisms were developed, thus alleviating the impact of the stressor.

5. No significant influence of *Trichoderma* fungi was determined with respect to the alleviation of disturbances in the course of photosynthesis in plants growing on soil polluted with cadmium.

6. As the plants grew, an increase in the chlorophyll content was observed. The addition of *Trichoderma* fungi to the subsoil had a positive influence on the amount of dye contained in leaves.

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