JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal
Published by Faculty of Biotechnology and Food Sciences

INFLUENCE OF FUNGI OF THE GENUS *TRICHODERMA* ON PHYTOAVAILABILITY CADMIUM AND PHYSIOLOGICAL CONDITION OF MAIZE (*ZEA MAYS*)

Magdalena Marchel[1], Paulina Duma[1], Alina Owsiak[2]*

Address(es): Dr Magdalena Marchel
[1]Department of Processing and Agricultural Commodities University of Rzeszow Faculty of Biology and Agriculture, ul. Zelwerowicza 4, 35-601 Rzeszow, Poland
[2]Department of Biochemistry and Cell Biology University of Rzeszow Faculty of Biology and Agriculture, ul. Zelwerowicza 4, 35-601 Rzeszow, Poland

*Corresponding author: magdarrak@gmail.com

doi: 10.15414/jmbfs.2015.4.special2.79-82

ARTICLE INFO

Received 5. 12. 2014
Revised 10. 12. 2014
Accepted 20. 1. 2015
Published 2. 2. 2015

Regular article

OPEN ACCESS

ABSTRACT

The aim of conducted research was to determine in what degree inoculation of soil by fungi *Trichoderma* modifies process of phytoavailability of cadmium through roots and above ground parts of maize (*Zea mays*) and how it affects cadmium activity as damaging factor of photosynthetic apparatus of plants. Outline of vase experiment covered 5 objects (control without addition of cadmium and 4 objects with increasing amount of cadmium in amount from 10 to 80 mg.kg^{-1} d.m. soil), and within each object two variants: soil without (-T) and with (+T) addition of fungi of *Trichoderma* (+T) in shape of granulated preparation of *Trianum–G* in amount of 750 g.m^{-3} soil. Increasing amount of cadmium in surface caused decrease of harvest size of roots and above ground parts of maize, but inoculation of surface by *Trichoderma* fungi softened those changes. Amount of cadmium in plants was increasing promotionally to increasing amount of that metal in soil, but the presence of fungi of *Trichoderma type* caused decrease of phytoavailability cadmium by above ground parts of maize. Decrease parameters of chlorophyll fluorescence F_0, F_M, Fv, Fv/F_M Fv/F_0 showed lack of disturbances in photosynthetic apparatus of maize despite decrease the amount of chlorophyll in leaves under the influence of inserted cadmium doses to soil.

Keywords: Maize, cadmium, *Trichoderma***,** chlorophyll fluorescences

INTRODUCTION

Cadmium is a heavy metal, which huge concentration in the environment results in absorption by plant organisms and causes effects of toxicity **(Czeczot *and* Skrzycki, 2010).** Possibilities of taking cadmium by plants, and including it to the trophic chain by that, are different and dependent on chemical shape of its compounds, and from its solubility in liquids at the same time **(Maciołek *and* Zielińska, 2013).** Cadmium phytoavailbility increases when reaction of soil is acid when content of organic material is low and in presence of fungus in surface **(Matkowski *et al.*, 2007).**
Maize is one of the oldest and commonly cultivated plants in the world. Flour, kasha and corn flakes are used for food. Flour made from corn does not have gluten in itself, what constitutes to huge advantage from the dietary point of view. Maize grain contains the highest content of starch and therefore is characterized as the most energetic grain of all cereals, but protein and minerals content is not as high as in other cereals **(Chňapek *et al.*, 2014).** Biological active compounds, included in grain of sweet corn have application in prevention of sight organ condition, cancer and cardiovascular diseases. Production of this crop is very important due to increased population, limited land, environmental and biotic stresses. Over the years, conventional breeding has been used as a tool for sustainable production of this crop. Nowadays, biotechnological tools can be helpful to enhance breeding and subsequently maize production **(Jakubeková *et al*., 2011)**.
Due to that the problem of soil pollution by heavy metals is quite common, very often solutions of their removal from the surface or decreasing toxic influence on living organisms are being tried to be found. One of the methods is bioremediation, in which microorganisms like fungus, bacteria are used in destruction and neutralization of dangerous substances **(Buczkowski *et al.*, 2002).**
Recently bigger interest is caused by the possibility of using strains of *Trichoderma* in cultivation of plants **(El-Katatny *and* Emam 2012; Vinale *et al.*, 2008).** Properties of supporting the growth of plants, protection against stressful conditions, especially against pathogens of plants are attributed to those fungi. Many positive features of those fungi, for example universality of existence in nature, quick growth in artificial cultivations results, that more often they are used in order to increase the harvest of cultivation and protection of plants with application of lower amount of pesticides **(Smolińska *and* Kowalska, 2008).**

The aim of conducted research was to determine in what degree of the surface inoculation of mycelium does *Trichoderma* modify processes of phyto-digestibility of cadmium through roots and above ground parts of sweet corn (*Zea mays*). Also it was sought to determining the influence of cadmium on damages of photosynthetic apart of plants and determining influence of fungi of the genus *Trichoderma* from a view of modifying factor on stressor's activity.

MATERIAL AND METHODS

Researches were conducted based on potted and lab experiments. Outline of potted experiment covered 5 objects (control without application of Cadmium and 4 objects with increasing cadmium dose in soil), and within each object two variants (Table 1):
1. Soil without application of fungi of the genus *Trichoderma* (-T)
2. Soil with application of fungi of the genus *Trichoderma* (+T) in shape of granulated preparation *Tranum-G* in amount preferred by the producer (Koppert B.V.) that is first application: 750 g.m^{-3} of soil, and second application: 375 g.m^{-3} of soil.
Tested plant was maize (*Zea mays*) stock of 3 plants in one vase. Lightly acid soil (pH_{KCL}=5.57) with granulometric composition of simple dust was used in the experiment. Identical primary fertilization: 0.2 g N; 0.05 g P; 0.2 g K and 0.025 g Mg on kg d.m. soil was used on every object. Fertilizers were applied in forms of water solutions of given salts: NH_4NO_3, KH_2PO_4, KCL and $MgSO_4{\cdot}7H_2O$. Cadmium was introduced to soil in form of salt water solution $3CdSO_4{\cdot}8H_2O$, simulating pollution of soil on four levels. Solutions of fertilizers and salts of cadmium were being mixed with soil for one week before sowing the seeds. Plants were cultivated through 72 days, maintaining temperature 23-25°C with photoperiod 16/8 h (day/night).

Table 1 Scheme of experiment

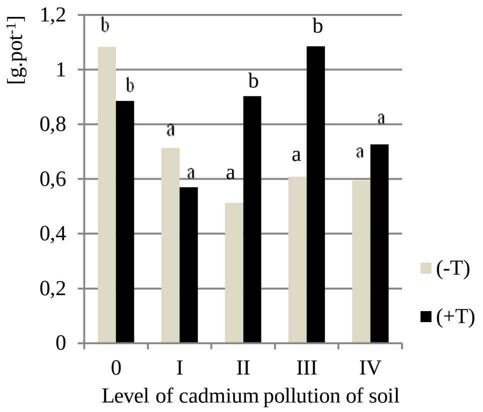
Treatment	Soil with addition of fungi of the genus *Trichoderma* (+T)	Soil without addition of fungi of the genus *Trichoderma* (-T)
	Cadmium dose [mg.kg^{-1} d.m. soil]	
Control 0	0	0
I	10	10
II	20	20
III	40	40
IV	80	80

During vegetation plants were watered by distilled water, maintaining soil humidity during first phase of experiment on level of 40%, and next on 50% of capillary water capacity. Disturbances in the process of photosynthesis were monitored through fluorescence measurement and content of chlorophyll (fluorometer IMAGING-PAM Walz company).
The following parameters were measured: F_0- fluorescence zero of objects adapted to darkness, F_M – maximal fluorescence, F_V – fluorescence changeable, F_V/F_0 – maximal effectiveness of water fission after donor side PSII and F_V/F_M – maximal photochemical effectiveness of PSII. After finishing potted experiment plant material was dried in temperature of 75°C, majority of harvest and cadmium amount in both roots and above ground parts were marked with application of spectrophotometer of atomic absorption ASA (Hitachi Z–2000). Roots and above ground parts were analyzed separately.
The significant differences between the treatments were analyzed by analysis of variance (ANOVA) and Tukey's test at $p < 0.05$ by using the Statistica program, version 10.

RESULTS AND DISCUSSION

Obtained results of maize yield indicate negative influence of cadmium in soil on majority of yield of growing plants without application of fungi of the genus *Trichoderma* (Figure 1).

ROOTS



ABOVE GROUND PARTS

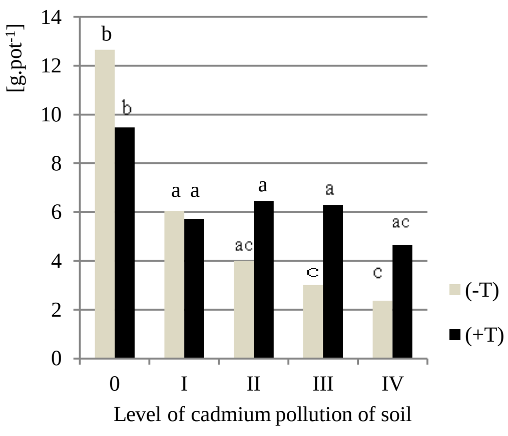


Figure 1 Yield of roots and above ground parts of maize [g.pot^{-1}] growing on soil with significant cadmium concentration without addition (-T) and with addition (+T) fungi of the genus of *Trichoderma*
Different letters show significant difference ($p< 0.05$).

Yield of roots was in difference between 0.51- 1.08 g.pot^{-1} (variant –T) and 0.57 – 1.09 g.pot^{-1} (variant +T). Improvement of size of root harvest of growing plants can be seen in presence of *Trichoderma* on soil of second and third pollution level in comparison with analogical but without protective role of fungus.
Yield of above ground parts of maize was at interval from 2.36 – 12.65 g.pot^{-1} (variant –T) and 4.64 – 9.47 g.pot^{-1} (variant +T). Inoculation of soil with fungi of the genus of *Trichoderma* caused growth of yield on soils with second, third and fourth level of cadmium pollution.
Cadmium application to soil had an influence on significant growth of content of that metal in plants (Figure 2). In roots of plants, growing without *Trichoderma* in surface the amount of cadmium was at interval between 1.02– 539.38 μg·g^{-1} whereas in roots of plants growing in presence of fungi was from 1.20 to 409.22 μg.g^{-1}. Differences in amount of cadmium in roots, caused by application of *Trichoderma* to soil, turned out to be vital statistically only in plants, growing in soil with the highest cadmium dose.
The amount of cadmium in above ground parts of maize reached presented values: 0.95 – 145.42 μg.g^{-1} (variant –T) and 0.98 – 123.93 μg.g^{-1} (variant +T). It was observed that above ground parts of growing plants with presence of fungi of the genus *Trichoderma* took less cadmium from polluted soils than analogical, deprived from protective activity of *Trichoderma*.
Kinetics of chlorophyll fluorescence is useful informative tool in studies of influence of environmental stresses on photosynthesis **(Kalaji *and* Łoboda, 2010)**. In optimal conditions for course of photosynthesis solar energy absorbed by chlorophyll is only in small part reemitted in shape of fluorescence. In case of anomaly in function of PSII the stop of photosynthesis takes place with the growth of fluorescent radiation.
Obtained results in parameters of chlorophyll fluorescence in maize leaves present figure. 3. Values of starting fluorescence F_0 did not change significantly with growth of cadmium dose in surface. Also from the point of view of presence and lack of fungi of the genus *Trichoderma* in surface differences in values F_0 were not significant statistically.
The value of maximal fluorescence F_M belongs to many factors, including type of sucking out light, its parameters and from content of photosynthetic dye in studied tissue. Decrease values of F_M indicates that studied photosynthetic object is under the influence of stress, for not all acceptors of electrons in PSII can be reduced totally **(Kalaji *and* Łoboda, 2010).**
Decrease of parameter values F_M is not observed in obtained results during the growth of the intensity of stress factor, which was cadmium. In general higher value of F_M was observed in plants, growing on soils highly polluted with cadmium in presence of fungi of the genus *Trichoderma*. Parameter F_V is a difference of values F_M and F_0. Values of F_V depends on maximal quantum productivity of PSII and is lower under influence of environmental stresses **(Kalaji *and* Łoboda, 2010)**. In studied plants were observed higher values of F_V in plants growing on soils with addition of fungi of *Trichoderma* type, whereas the stree factor, which was cadmium, did not cause essential changes in values of parameter F_V.
Maximal productivity of water splintering in donored side of PSII, determined by parameter F_V/F_0 in optimal conditions is 3-4 **(Kalaji *and* Guo, 2008)**. Obtained results indicate that values F_V/F_0 are included in range from 3-4 in plants, growing with and without presence of fungi of *Trichoderma* type.
Parameter F_V/F_M, determining potencial productivity of PSII can be used as a reliable indicator of photochemical activity of photosynthetic apparatus, because for the majority of plants in stage of full development and in stress free codition maximal value of that quotient is 0.83 **(Björkman *et* Demmig 1987; He *et al.*, 1996; Basu *et al.*, 1998).** Obtained result in the experiment indicate decrease of F_V/F_M parameter below values of 0.83 in all studied objects.

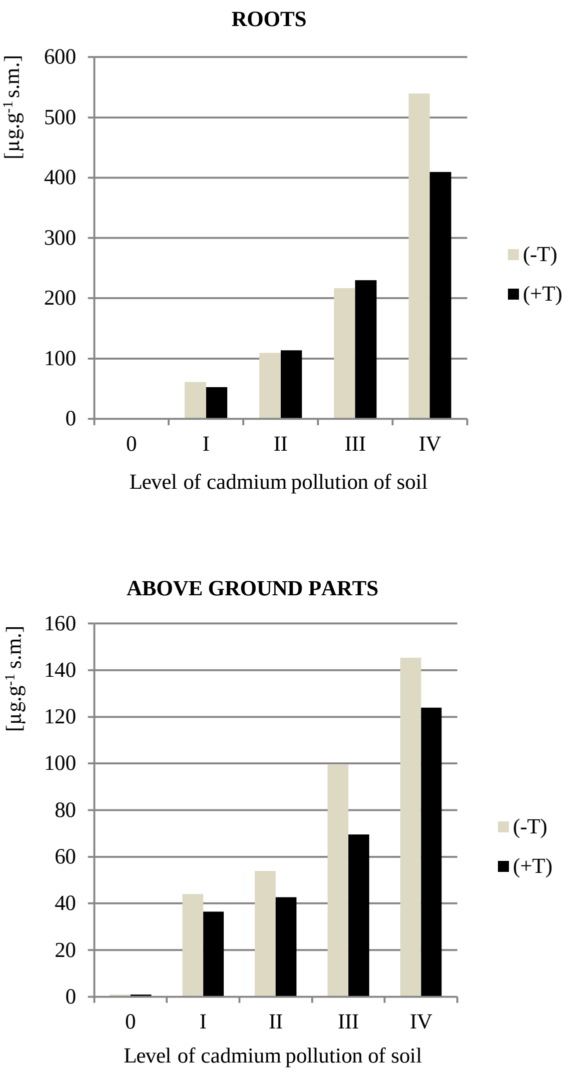


Figure 2 The cadmium amount in roots and above ground parts of maize, cultivated on soil with increasing cadmium concentration without addition (-T) and with addition (+T) fungi of the genus *Trichoderma*
Different letters show significant difference ($p< 0.05$).

The amount of photosynthetic dye in given plant depends on species and variety of studied plant, and also from both environmental and antropogenic factors **(Chen *and* Kreeb, 1990; Kozłowski *et al*., 2001; Krzesłowska, 2004)**. In studied objects it was observed not high decrease of indicator of chlorophyl content SPAD in leaves of growing plants on polluted with cadmium soils in comaprison to control (Table 2).

Table 2 Index of chlorophyl content [SPAD] in maize leaves cultivated on soil with increasing cadmium concentration (I–IV) without addition (-T) and with addition (+T) fungi of the genus *Trichoderma*

Treatment	(+T)	(-T)
Control 0	15.80	13.81
I	13.19	12.04
II	11.24	11.53
III	12.17	12.03
IV	12.05	12.02

Addition of fungi of the genus *Trichoderma* to soil did not have an influence on amount of chlorophyll in leaves of maize significiantly.

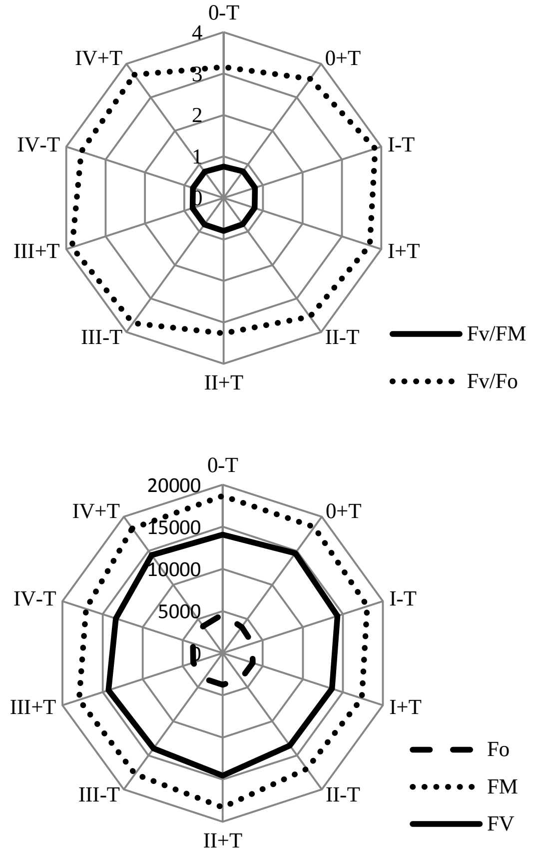


Figure 3 Radar charts of physiological changes of features in maize leaves cultivated on soil with increasing cadmium concentration without addition (-T) and with addition (+T) fungi of the genus *Trichoderma*

DISCUSSION

Tolerance index is a relation of the amount of yield, obtained on soil, polluted by metals, to yield, which was gathered from monitored soil and it can present the following values: Ti<1; Ti=1; Ti>1 **(Spiak *and* Wall, 2000).** Values Ti<1 means stop of plant growth or their total death; Ti=1 indicates lack of influence of metal on both growth and development of plants. Presented results of experiments allowed determining the tolerance index in division from 0.19 – 0.68 for yield of roots and from 0.47-1.23 for yield of above ground parts (Table 3). Ti took bigger values for plants growing with participation of fungi of the genus *Trichoderma* in comparison to the rest. Values of Ti>1 were stated in case of roots of plants, growing on soil with addition of *Trichoderma* on second and third level of cadmium pollution. The others objects values Ti<1, which indicates, that in this case cadmium was a factor, which limited growth of plants, and its action was in small degree eased by addition of fungi of the genus *Trichoderma*.

Table 3 Index tolerance of maize cultivated on soil with increasing cadmium concentration (I–IV) without addition (-T) and with addition (+T) fungi of the genus *Trichoderma*

Treatment	I	II	III	IV	I	II	III	IV
	Ti -roots				Ti - above ground parts			
(–T)	0.66	0.47	0.56	0.55	0.48	0.32	0.24	0.19
(+T)	0.64	1.02	1.23	0.82	0.60	0.68	0.66	0.49

Fungi of the genus *Trichoderma* are known from having ability of symbiosis with roots of plants - mycorrhiza. Effect of mycorrhizal fungi on taking and accumulation of cadmium depends from species of fungus, species of plant and metal concentration in ground. **Matkowski *et al*. (2007)** stated, that introduction of fungi *Trichoderma harzianum* to polluted by cadmium soil caused increased concentration of that element in above ground parts of sweet corn. According to those authors it was connected with increased amount available for plants forms Cd in soil. On the other hand, **Majewska *et al*. (2012)** in their researches on influence of fungi *Trichoderma koningii* on cadmium accumulation in biomass of grass (*Festuca ovina*) stated significant statistically decrease of content of that metal in roots and above ground parts of plants, growing on polluted by cadmium soil, grafted on spore from fungi of *Trichoderma* type.

In table 4 there were compared values of coefficients of straight corelation (r) mentioning in numbers relation between amount of cadmium in soil and its accumulation in roots and above ground parts of maize. Counted values r indicate strong, positive corelation between those parameters. That relationship is practically the same on soil with and without fungi of *Trichoderma* type.

Table 4 Simple correlation coefficients (r) between the amount of cadmium in soil, and its concentration in roots and above ground parts of maize, cultivated on soil without addition (-T) and with addition (+T) fungi of the genus *Trichoderma*

r	Cd – roots		Cd – above ground parts	
	(–T)	(+T)	(–T)	(+T)
Cd - soil	0.99***	0.99***	0.97***	0.98***

r significant et ***p=0.001

During conducted research technique of parameters measurement of chlorophyl fluorescence was used to assess function of photosynthetic apparatus of plants. Big advantage of that method is the fact, that it does not cause long-lasting damages of plants and it can be conducted during their growth **(Baker *and* Rosenquist, 2004).** Counted coefficients of correlation r between cadmium content in soil and values of individual parameters of chlorophyll fluorescence indicate presence of negative relations of F_0 F_M and F_V in case of plants growing without addition of fungi of the genus *Trichoderma* (Table 5). In other words, as the amount of cadmium in soil increases, the value of beginning, maximal and changeable fluorescence decreases. Also the amount of chlorophyll in leaves decreases. Addition to soil fungi of *Trichoderma* type weakened that relation and it caused change of its mark (besides values of SPAD).

Table 5 Simple correlation coefficients (r) between the amount of cadmium in soil and individual fluorescence parameters and chlorophyll amount in above ground parts of maize cultivated on soil without addition (-T) and with addition (+T) fungi of the genus *Trichoderma*

r	Cd - soil (–T)	Cd - soil (+T)
F_0	-0.55*	0.54*
F_M	-0.68**	0.17
F_V	-0.74**	0.06
F_V/F_M	0.34	-0.42
F_V/F_0	0.33	0.31
SPAD	-0.56*	-0.44

significant et *p=0.05 **p=0.01

CONCLUSION

1. Increasing amount of cadmium in soil (from 10 to 80 $mg.kg^{-1}$) caused decrease in yield size of both roots and above ground parts of maize. Addition of fungi of the genus *Trichoderma* to soil softened negative cadmium influence on yield of maize.
2. The amount of cadmium in roots and above ground parts of maize increased with growth of that metal in surface both in soils with and without addition of fungi of the genus *Trichoderma*. Significantly higher cadmium amounts accumulated in roots than in above ground parts.
3. Inoculation of soil by fungi of the genus *Trichoderma* had an influence on reduction of cadmium phytoavailbility by above ground parts of maize.
4. Values parameters of chlorophyll fluorescence F_0, F_M, Fv, Fv/F_M, Fv/F_0 indicate on lack of disturbances in photosynthetic apparatus of maize under the influence of cadmium doses in soil.
5. Inserting cadmium dose to soil caused decrease in amount of chlorophyll in maize leaves, whereas the presence of fungi of the genus *Trichoderma* did not have any influence on those changes.

REFERENCES

BAKER N.R., ROSENQUIST E. 2004. Aplication of chlorophyll fluorescence can improve crop production strategies an examination of future possibilities. *J. Exp. Bot.*, 55(403), 1607-1621. **http://dx.doi.org/10.1093/jxb/erh196**

BASU P.S., SHARMA A., SUKURMAN N.P. 1998. Changes in net photosynthetic rate and chlorophyll fluorescence in potato leaves induced by water stress. *Photosynthetica,* 35, 1, 13-19. **http://dx.doi.org/10.1023/A:1006801311105**

BJÖRKMAN O. DEMMIG B. 1987. Photon yield of O_2 evolution and chlorophyll fluorescence characteristic at 77K among vascular plants of diverse origins. *Planta,* 170, 489-504.

BUCZKOWKSI R., KONDZIELSKI I., SZYMAŃSKI T. 2002. Metody remediacji gleb zanieczyszczonych metalami ciężkimi. UMK Toruń. ISBN 83-231-1376-9.

CHEN T., KREEB H. K. 1990. Investigation of combined effects of Pb, NaCl and water deficit on *Zea mays L.* in; (ed.) Bohac J. Proceedings of the VI international conference – *Bioindicatores Deteriorisationis Regionis.* Institue of Ladnscape ecology CAS, 348-356.

CHŇAPEK M., TOMKA M., BALÁŽOVÁ Ž., GÁLOVÁ Z. 2014. Protein complex of wheat, buckwheat and maize in relation to celiac disease. *Journal of Microbiology, Biotechnology and Food Sciences*, 3, 88-92.

CZECZOT H., SKRZYCKI M. 2010. Kadm – pierwiastek całkowicie zbędny dla organizmu. *Postępy Hig. Med. Dośw*., 64, 38-49. ICID: 904693.

EL-KATATNY M.H, EMAM A.S. 2012. Control of postharvest tomato rot by spore suspension and antifungal metabolites of Trichoderma harzianum. *Journal of Microbiology, Biotechnology and Food Sciences*, 1(6), 1505-1528.

HE J., CHEE C.W., GOH C.J. 1996. Photoinhibition of *Heliconia* under natural tropical conditions: the importance of leaf orientation for light interception and leaf temperature. *Plant. Cell Environ*., 19, 1238-1248. **http://dx.doi.org/10.1111/j.1365-3040.1996.tb00002.x**

JAKUBEKOVÁ M., PRETʼOVÁ A., OBERT B. 2012. Somatic embryogenesis and plant regeneration from immature embryo callus of maize (*Zea mays L.*). *Journal of Microbiology, Biotechnology and Food Sciences*, 1(4), 478-487.

KALAJI M.H., GUO P. 2008. Chlorophyll Fluorescence: A Useful Tool In Barley Plant Breeding Programs. *Photochemistry Research Progress* (Sancheza i Gutierrez S.J. eds.), Nova Science publishers, Inc., 439-463.

KALAJI M.H., ŁOBODA T. 2010. Fluorescencja chlorofilu w badaniach stanu fizjologicznego roślin. SGGW Warszawa. ISBN 978-83-7583-119-1.

KOZŁOWSKI S., GOLIŃSKI P., GOLIŃSKA B. 2001. Barwniki chlorofilowe jako wskaźniki wartości użytkowej gatunków i odmian traw. *Zesz. Probl. Post. Nauk Rol*. 474, 215-223.

KRZESŁOWSKA M. 2004. Metale śladowe. Komórki roślinne w warunkach stresu, t. I, cz. II. Red. A. Woźny i K. Przybył. UAM, Poznań: 103-164.

MACIOŁEK H., ZIELIŃSKA A., DOMARECKI T. 2013. Oddziaływanie geobiologiczno-chemiczne kadmu i ołowiu na środowisko przyrodnicze. *Journal of Ecology and Health*, 17(2), 63-71.

MAJEWSKA M., KUREK E., LATA L., CZYŻO I. 2012. Akumulacja kadmu w biomasie trawy (*Festuca ovina*) rosnącej w glebie zanieczyszczonej tym metalem i szczepionej zarodnikami *Trichoderma koningii*. Proceeding of the work of Scientific Conference: *Odpady organiczne – problemy i sposoby zagospodarowania,* 20-22 IX 2012, Falenty.

MATKOWSKI K., PLĄSKOWSKA E., MOSZCZYŃSKA E., PATORCZYK-PYTLIK B., GEDIDA K., KULCZYCKI G. 2007. Badania zbiorowisk grzybów w glebie zanieczyszczonej kadmem nawożonej różnymi materiałami organicznymi. *Zesz. Probl. Post. Nauk Rol*. 520 (1), 345-351.

SMOLIŃSKA U., KOWALSKA B., 2008. Grzyby z rodzaju *Trichoderma* – szansa w ochronie roślin czy złudna nadzieja? *Nowości Warzywnicze.* 46, 39 – 50.

SPIAK Z., WALL Ł., 2000. Współzależność zawartości cynku w glebach i roślinach w warunkach polowych. *Zesz. Probl. Post. Nauk Roln*., 471, 145-152.

VINALE F., SIVASITHAMPARAM K., GHISALBERTI E.L., MARRA R., WOO S.L., LORITO M. 2008. *Trichoderma* –plant – patogen interactions. *Soil Biol. Biochem*, vol. 40, 1-10. **http://dx.doi.org/10.1016/j.soilbio.2007.07.002**