Journal of Elementology ISSN 1644-2296

Radkowski A., Radkowska I., Gala-Czekaj D., Bocianowski J. 2018.
Effect of multi-walled carbon nanotubes on the germination and growth characteristics of three fodder grasses in vitro and in chernozem soil.
J. Elem., 23(1): 21-33. DOI: 10.5601/jelem.2017.22.1.1402

ORIGINAL PAPER

EFFECT OF MULTI-WALLED CARBON NANOTUBES ON THE GERMINATION AND GROWTH CHARACTERISTICS OF THREE FODDER GRASSES *IN VITRO* AND IN CHERNOZEM SOIL*

Adam Radkowski[1], Iwona Radkowska[2], Dorota Gala-Czekaj[3], Jan Bocianowski[4]

[1]Department of Grassland Management
[3]Department of Agrotechnology and Agricultural Ecology
University of Agriculture in Krakow, Poland
[2]Department of Technology, Ecology and Economics of Animal Production
National Research Institute of Animal Production, Balice, Poland
[4]Department of Mathematical and Statistical Methods
Poznan University of Life Sciences, Poland

Abstract

The aim of the study was to investigate the effect of two types of multi-walled carbon nanotubes: raw MWCNTs and carboxylated MWCNTs, on the germination, growth and selected physiological properties of grasses: timothy grass, common meadow grass and meadow fescue. The response of these species to 0.1, 0.2, 0.3, 0.5 and 8.3 mg ml^{-1} MWCNTs and 0.15, 0.30, 0.45, 0.75 and 16.7 mg ml^{-1} carboxylated MWCNTs was evaluated in a laboratory experiment. Then, germination energy and germination ability of the grasses were assessed. The effect of administering 15 g pot^{-1} MWCNTs and 80 g pot^{-1} carboxylated MWCNTs was assessed in a two-year pot experiment. Other examined parameters included SPAD value, dry matter yield, root mass, and the content of macro- and microelements in the grasses. Both types of CNTs inhibited seed germination, and seeds of all grasses treated with the highest concentration of carboxylated MWCNTs did not germinate at all. Soil supplementation with carboxylated MWCNTs significantly decreased the dry matter yield of common meadow grass but improved this parameter in timothy grass and meadow fescue. Application of MWCNTs significantly reduced dry matter yield in all studied grasses. The lowest mean SPAD value was observed for MWCNT-treated common meadow grass, and the highest one appeared in meadow fescue with carboxylated MWCNTs. In most cases, the presence of raw nanotubes reduced root mass and the content of macro- and microelements, while carboxylated nanotubes increased root mass and the content of these elements.

Keywords: *Phleum pratense*, *Poa pratensis*, *Festuca pratensis*, MWCNTs, SPAD.

---

mgr inż. Dorota Gala-Czekaj, Department of Agrotechnology and Agricultural Ecology, University of Agriculture in Krakow, al. Mickiewicza 21, 31-120 Krakow, Poland, e-mail: d.gala@ur.krakow.pl

* This Research was financed by the Ministry of Science and Higher Education of the Republic of Poland.

INTRODUCTION

Carbon nanotubes (CNTs) are an interesting and promising engineered nanomaterial with unique mechanical, chemical, electrical and optical properties (Baughman et al. 2002, Roth, Carroll 2015), offering multiple possibilities of application in various industries (De Volder et al. 2013, Sweeney et al. 2015), pharmacy and medicine (He et al. 2013). Carboxylated nanotubes are produced by oxidation of MWCNTs and contain multiple carboxyl groups (Eitan et al. 2003). They have hydrophilic character, form stable gels in water and are not contaminated with amorphous carbon or metals (Lam et al. 2006).

CNTs can be released into the environment directly during the use of materials that contain them or as a waste from sewage treatment plants, waste incineration plants or landfills (Petersen et al. 2011). Toxicity of nanotubes depends on many factors, e.g. their surface area, solubility, shape, coatings or type of functional groups that form them (Scown et al. 2010). Ecotoxicity of CNTs depends on the type of ecosystem – aquatic ecosystems are more sensitive to contamination than terrestrial ones. According to Petersen et al. (2011) and Russier et al. (2011), environmental toxicity of CNTs is moderate, and slow biodegradation of this material is mediated by soil enzymes.

The presence of CNTs in the natural environment has been increasing due to their common use in various industries for production of industrial and consumer goods. Because of their prevalence, CNTs are expected to become a serious source of environmental pollution. This requires detailed research to determine potential benefits for the economy and risks to living organisms caused by these new materials, previously unknown to the environment (Jackson et al. 2013).

As reported in available studies, CNTs are not toxic to crop plants but their effect on plants is usually studied *in vitro* or hydroponic cultures. Plant response to the presence of CNTs in a substrate depended on the length of CNTs, their concentration, type and experimental conditions (Miralles et al. 2012, Smirnova et. al. 2012, Wang et al. 2012).

This paper describes the effects of multi-walled CNTs on the germination, growth and selected physiological parameters of timothy grass, meadow fescue and common meadow grass. The response of these species to the presence of CNTs in an artificial substrate was tested in laboratory conditions. Grass growth and development were studied in pots filled with chernozem during two growing seasons.

MATERIAL AND METHODS

Characteristics of nanotubes

MWCNTs were purchased from CNT Co., Ltd., 806 Mecarium Officetel, 593 Yeonsu 2-Dong Yeonsu-Gu, Incheon, Korea. Commercial characteristics of MWCNTs provided by the manufacturer are as follows: diameter 1-50 nm, length 1-25 µm, purity min. 95%, metal oxide max. 5%, bulk density 0.03-0.06 g cm^{-2}, bet 150-250 m^2 g^{-1}.

Plant material

The following species were used in both experiments: timothy grass (*Phleum pratense* L.) cv. Owacja, meadow fescue (*Festuca pratensis* Huds.) cv. Fantazja, and common meadow grass (*Poa pratensis* L.) cv. Struga. All the cultivars were bred by the Małopolska Plant Breeding Company (Kraków, Poland). The germination ability of seeds before sowing into pots was 98% for timothy grass, 92% for meadow fescue, and 82% for common meadow grass.

An *in vitro* experiment

A laboratory experiment was set up to investigate the response of meadow fescue, timothy grass and common meadow grass to raw MWCNTs and carboxylated MWCNTs added to the water used for watering the seeds. The seeds were placed in Petri dishes (100 seeds per dish) on a double layer of filter paper moistened with redistilled water, enriched with CNTs as necessary. Each experimental variant was repeated four times. MWCNTs concentrations were 0.1 (C1), 0.2 (C2), 0.3 (C3), 0.5 (C4), and 8.3 (C5) mg ml^{-1}, and for carboxylated MWCNTs they were 0.15 (C1), 0.30 (C2), 0.45 (C3), 0.75 (C4) and 16.7 (C5) mg ml^{-1}. The difference between concentrations of MWCNTs and carboxylated MWCNTs was due to a large difference in their bulk density. Raw MWCNTs have a very low bulk density, and an addition of even small amounts significantly increases the volume of a substrate. Moreover, the concentrations were chosen arbitrarily to provide the widest range possible.

Germination energy of meadow fescue and timothy grass was assessed after 7 days, and that of common meadow grass – after 14 days. Germination ability was estimated after 14, 10 and 28 days for meadow fescue, timothy grass, and common meadow grass, respectively, on a Jacobsen germination apparatus, according to PN-79/R-65950 standard (ISTA 2010).

A pot experiment

A two-year (2014-2015) pot experiment was conducted at the Plant Breeding Station in Polanowice near Kraków (N 50°20′82″, E 20°08′43″; 220 m a.s.l.) in a greenhouse under uncontrolled temperature and light conditions. During the pot experiment carried out from April to September, the temperature at night and during the day was 8-15°C and 22-32°C, respectively. Relative air humidity in the greenhouse was 55-60%.

5.02 dm^3 polyethylene pots were filled with topsoil collected down to the depth of 30 cm of loess-based degraded chernozem. Chemical properties of the soil were as follows: pH in KCl – 7.2, and absorbable P, K, and Mg at 57.0, 138.4, and 45.9 mg kg^{-1}, respectively. All pots were fertilized each year with NPK at 0.3 g N, 0.08 g P and 0.2 g K kg^{-1} of the soil in the form of NH_4NO_3, KH_2PO_4, and KCl, and the doses were adjusted to the plants' nutrient requirements. In the first year, mineral fertilizers were used two weeks before sowing the seeds, and their solutions were carefully mixed with the substrate. In the second year, the fertilizers were applied in early spring before the vegetative growth started.

The following experimental variants were established for each species: control without CNTs (4.7 kg of soil), soil enriched with carboxylated (COOH) MWCNTs (80 g pot^{-1}), soil with raw MWCNTs (15 g pot^{-1}). Concentrations of CNTs were chosen arbitrarily, so that they were the largest addition to the soil, while retaining its basic characteristics. The difference in concentrations between raw MWCNTs and carboxylated (COOH) MWCNTs was due to a large difference in their bulk density. In all experimental variants, CNTs were carefully mixed with the top layer of the soil down to about 10 cm, just before sowing the seeds.

The seeds (30 per pot) were sown on 27 March 2014 in three replications. During the growth, the plants were watered with redistilled water and the soil moisture was maintained at 60% of maximum water capacity.

Every year, the SPAD value in upper leaves was measured using a Minolta SPAD 502DL chlorophyll meter (Minolta, Osaka, Japan). Three measurements were performed – one before each harvest. The measurements were taken for each pot and included twenty fully developed leaves.

The grasses were harvested three times in both years of the experiment. The first crop was harvested at the heading stage, and the other ones – at 7-week intervals. The plants were cut with grass scissors at 5-6 cm over the soil. Grass from each pot was collected and, after drying in an oven at 75°C, its dry matter yield in g DM pot^{-1} was assessed.

Roots of grasses were washed in sieves under running water and then dried in an oven at a temperature not exceeding 60°C. Afterwards, absolute dry matter was determined at 105°C for 3 h until obtaining constant weight.

For chemical analysis, a random sample of plant material from each pot and each harvest was collected. Each sample was analyzed for the content of N, P, K, Ca, Mg, Na, Cu, Mn, Fe, and Zn. The nitrogen content was determined by elemental analysis (Dumas) according to the testing procedure MCMZ/PB-03. The potassium content was determined by atomic absorption spectrometry (AAS) with flame atomization according to MCMZ/PB-07.

The phosphorus content was determined by UV-Vis spectrometry according to MCMZ/PB-06. The content of calcium, magnesium and sodium was determined by AAS with flame atomization according to PN-EN 15505:2009 standard. The manganese content was determined by AAS with flame atomization according to MCMZ/PB-08. Zinc and iron content was determined by AAS with flame atomization according to PN-EN 14084:2004 standard. The copper content was determined by AAS with electrothermal atomization (ETAAS) according to PN-EN 14084:2004 standard.

Statistical analysis

Three-way analysis of variance (ANOVA) was carried out to determine the effects of year, species, CNT and the following interactions: year×species, year×CNT, species×CNT, and year×species×CNT, on dry matter yield. Two-way ANOVA was carried out to determine the effects of species, CNT as well as species×CNT interaction on the content of N, P, K, Ca, Mg, Na, Cu, Mn, Fe, Zn, SPAD and root weight. Three-way ANOVA was carried out to determine the effects of species, CNT, doses and the following interactions: species×CNT, species×dose, CNT×dose, species×CNT×dose on the germination energy and germination ability. The least significant differences (LSDs) *post-hoc* test was used to distinguish significant treatments for analyses with significant exposure. Coefficients of variation were also calculated to investigate changes in the content of individual elements.

RESULTS

Mean germination energy and germination ability were variable and depended on the species, type of CNTs and concentration of the applied suspension (Figure 1). The highest concentration of MWCNTs (8.3 mg ml^{-1}) decreased germination energy of common meadow-grass by 41%, of timothy-grass by 19%, and of meadow fescue by 10% as compared with control. In the case of timothy grass and meadow fescue, the other MWCNT concentrations did not cause differences in germination ability and germination energy or the differences were insignificant. Common meadow grass was the most sensitive species to MWCNTs – subsequent concentrations resulted in a gradual inhibition of germination. Different observations were made for the plants watered with water containing carboxylated (COOH) MWCNTs. In common meadow grass, a gradual decrease in both germination energy and germination ability was noticed until complete inhibition at the highest concentration of 16.7 mg ml^{-1}. Timothy grass was more resistant to COOH MWCNTs at 0.15, 0.30, 0.45 and 0.75 mg ml^{-1}, and the decrease in germination ability and germination energy was slower. Similarly as for common meadow grass, complete inhibition of germination was observed at the highest concentration of 16.7 mg ml^{-1}.

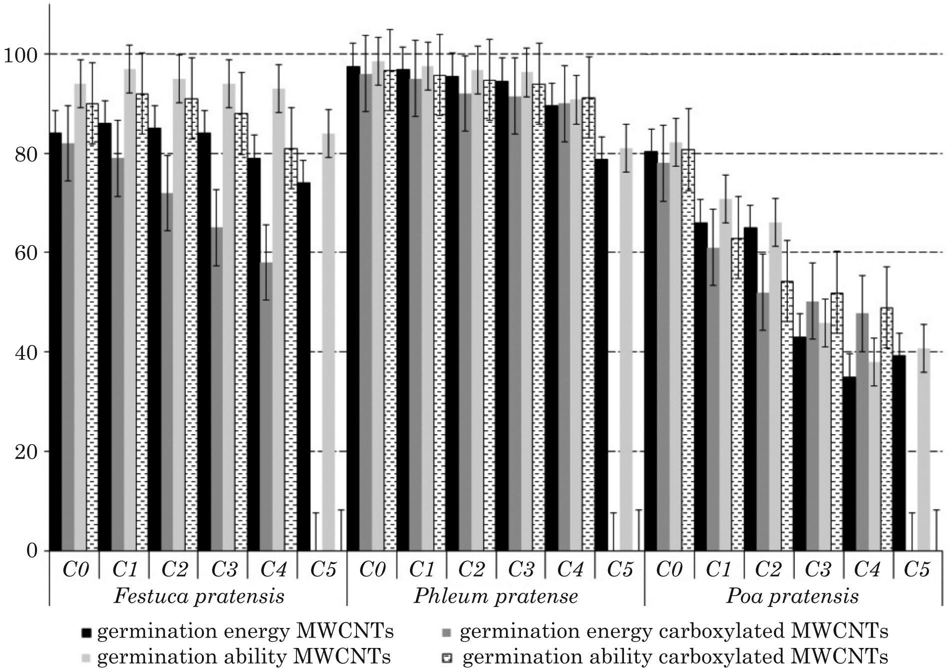

Fig. 1. Germination energy and germination ability of the investigated species of forage grasses depending on the concentration of MWCNTs and carboxylated MWCNTs (means taken from three series). Germination energy: $LSD_{0.05}$ species 0.501, dose 0.708, CNT 0.409, species×dose 1.227, species×CNT 0.708, dose×CNT 1.002, species×dose×CNT 1.735; Germination ability: $LSD_{0.05}$ species 0.4225, dose 0.5975, CNT 0.345, species×dose 1.0349, species×CNT 0.5975, dose×CNT 0.845, species×dose×CNT 1.4635

The SPAD value in the pot experiment varied depending on a variant, ranging from 7.69 to 20.78 (Table 1). The lowest mean SPAD (7.69) was observed for common meadow grass treated with raw MWCNTs, and the highest one (20.78) – for meadow fescue treated with carboxylated (COOH) MWCNT suspension. Addition of carboxylated (COOH) MWCNTs increased the SPAD value in all variants as compared with the control. Contrary to this, the presence of raw MWCNTs decreased SPAD in timothy grass and common meadow grass as compared with the control.

The mean dry matter yield in the pot experiment depended on a species and growing season and ranged from 31.7 to 46.3 g pot^{-1} in the control, from 33.4 to 60.7 g pot^{-1} in carboxylated MWCNT treated plants, and from 18.7 to 32.3 g pot^{-1} in raw MWCNT treated plants (Figure 2). Soil supplementation with carboxylated MWCNTs significantly decreased the dry matter yield of common meadow grass but significantly improved this parameter in meadow fescue and timothy grass. Contrary to this, soil supplementation with raw MWCNTs significantly reduced the dry matter yield in all investigated species.

Table 1

SPAD value in the studied forage grasses depending on the type of carbon nanotubes (means from the 2-year study)

Grass species	Nanotubes	SPAD value	Mean
Timothy grass	0	14.80 ±1.29	13.88 ±1.73
	MWCNTs	11.08 ±1.01	
	carboxylated MWCNTs	15.75 ±2.29	
Common meadow grass	0	10.43 ±1.29	10.61 ±0.97
	MWCNTs	7.69 ±0.59	
	carboxylated MWCNTs	13.72 ±1.39	
Meadow fescue	0	12.26 ±0.96	15.12 ±1.07
	MWCNTs	12.33 ±1.28	
	carboxylated MWCNTs	20.78 ±1.71	
Mean	0	12.50 ±1.33	-
	MWCNTs	10.37 ±1.15	
	carboxylated MWCNTs	16.75 ±1.81	
Coefficient of variation		28.51%	-
$LSD_{0.05}$	species: 1.16, CNT: 1.16, species×CNT: 2.02		

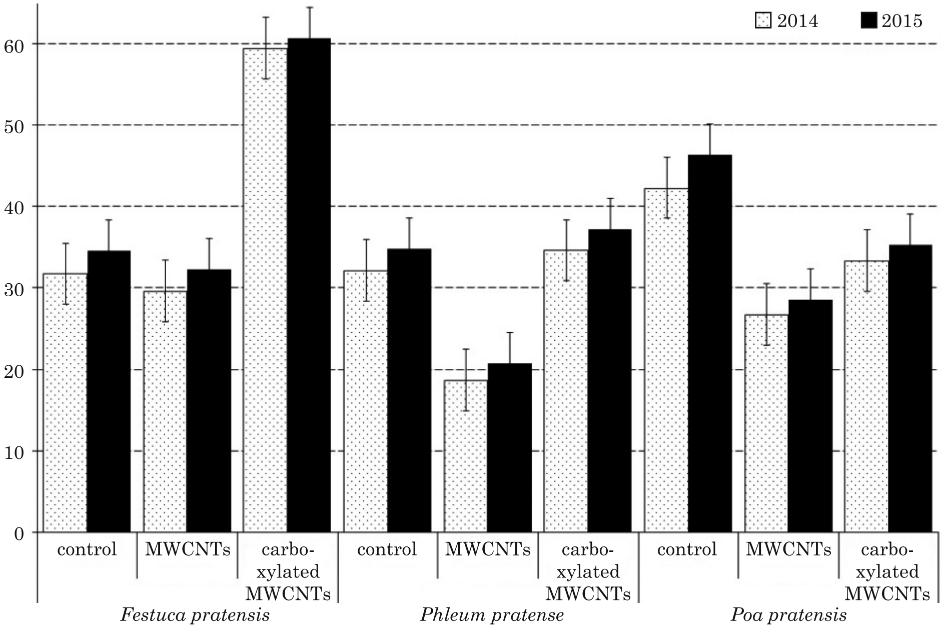

Fig. 2. Dry matter yield (g pot^{-1}) of the studied forage grass species depending on the type of carbon nanotubes. Coefficient of variation (%) in 2014: 31.7, in 2015: 29.7, $LSD_{0.05}$ year: 0.69, species: 0.84, CNT: 0.84, year×species: 1.19, year×CNT: 1.19, species×CNT: 1.46, year×species×CNT: 2.06

The root mass of the forage grasses ranged from 3.52 to 4.70 g pot^{-1} for timothy grass, from 2.38 to 5.72 g pot^{-1} for common meadow grass, and from 2.37 to 16.93 g pot^{-1} for meadow fescue (Figure 3). Compared with the control, the greatest increase in the root mass of the plants treated with carboxylated CNTs was observed in meadow fescue, but it was also noticeable for timothy grass and common meadow grass. The addition of raw CNTs resulted in a significant decrease in root mass in common meadow grass and meadow fescue as compared with the control.

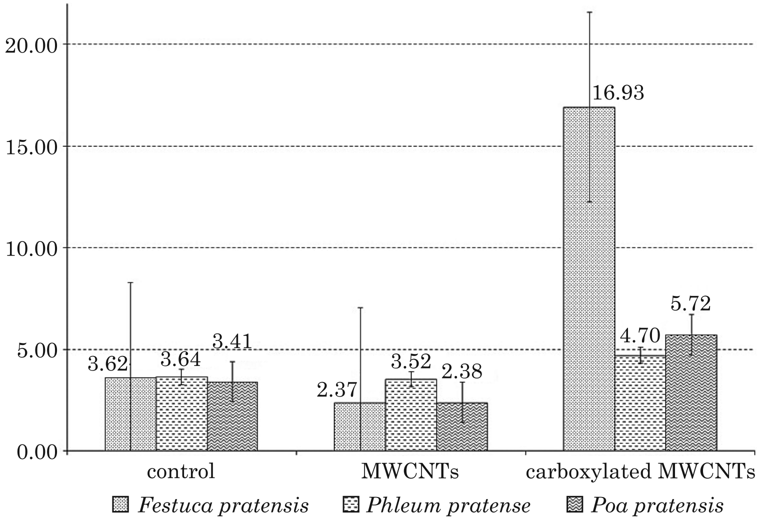

Fig. 3. Root mass (g pot^{-1}) of the studied forage grasses treated with carbon nanotubes. $LSD_{0.05}$ species: 0.45, CNT: 0.44, species×CNT: 0.79

The weighted mean macroelement content for the years 2014-2015 depended on a species and the type of CNTs, ranging as follows: 26.2-37.6 g N kg^{-1} DM, 3.9-5.8 g P kg^{-1} DM, 26.6-40.6 g K kg^{-1} DM, 4.0-6.8 g Ca kg^{-1} DM, 1.9-2.8 g Mg kg^{-1} DM, and 0.7-2.0 g Na kg^{-1} DM (Figure 4). These results indicate considerable differences in the content of the macroelements. The greatest variability was found for Na (CV=35.1%), and the smallest one – for K (CV = 12.3%). In most cases, soil supplementation with carboxylated MWCNTs increased the content of the macroelements studied, whereas the presence of raw MWCNTs caused a decrease in the content of the macroelements as compared with the control.

The weighted mean microelement content for the entire study period depended on a species and the type of CNTs (Figure 5), ranging as follows: 8.6-13.0 mg Cu kg^{-1} DM, 54.2-100.9 mg Mn kg^{-1} DM, 102.4-183.5 mg Fe kg^{-1} DM, and 72.6-120.6 mg Zn kg^{-1} DM. The greatest variability in the content of microelements in grasses was determined for Fe (CV = 23.1%), and the smallest one – for Cu (CV = 14.1%). In the control plants, the highest concentrations of Cu, Fe and Zn were detected in timothy grass, and Mn was the most abundant in meadow fescue. In the plants treated with carboxylated

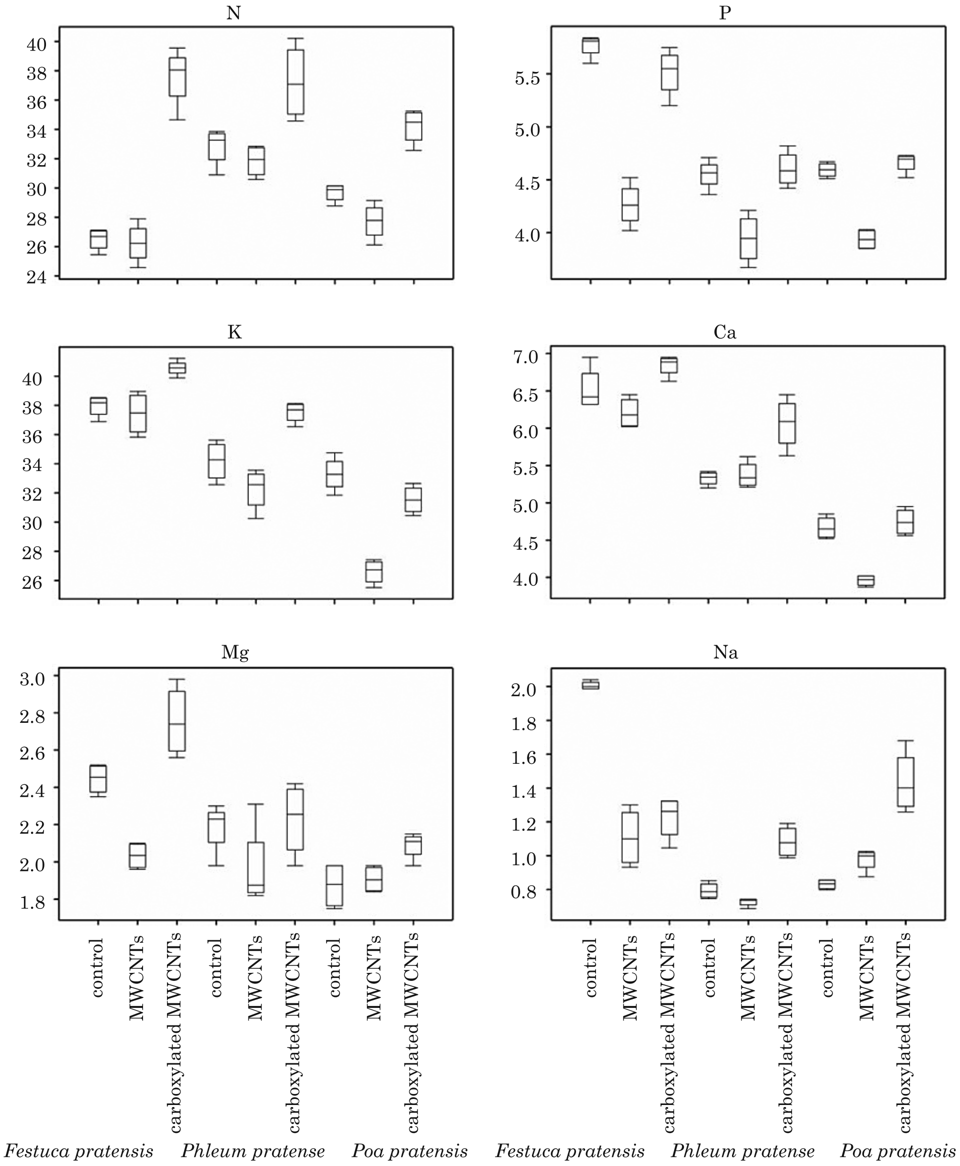

Fig. 4. Content of macroelements (g kg^{-1} DM) in the studied forage grasses depending on the type of carbon nanotubes (means from the 2-year study). LSD$_{0.05}$ for N species: 1.26, CNT: 1.26, species×CNT: 2.18, LSD$_{0.05}$ for P species: 0.14, CNT: 0.14, species×CNT: 0.24, LSD$_{0.05}$ for K species: 0.92, CNT: 0.92, species×CNT: 1.59, LSD$_{0.05}$ for Ca species: 0.17, CNT: 0.17, species×CNT: 0.30, LSD$_{0.05}$ for Mg species: 0.12, CNT: 0.12, species×CNT: 0.21, LSD$_{0.05}$ for Na species: 0.09, CNT: 0.09, species×CNT: 0.15

MWCNTs, the highest content of Cu, Fe and Zn was determined in common meadow grass, and meadow fescue accumulated the highest amounts of Mn. In plants supplemented with MWCNTs, the greatest increase in Cu, Mn and

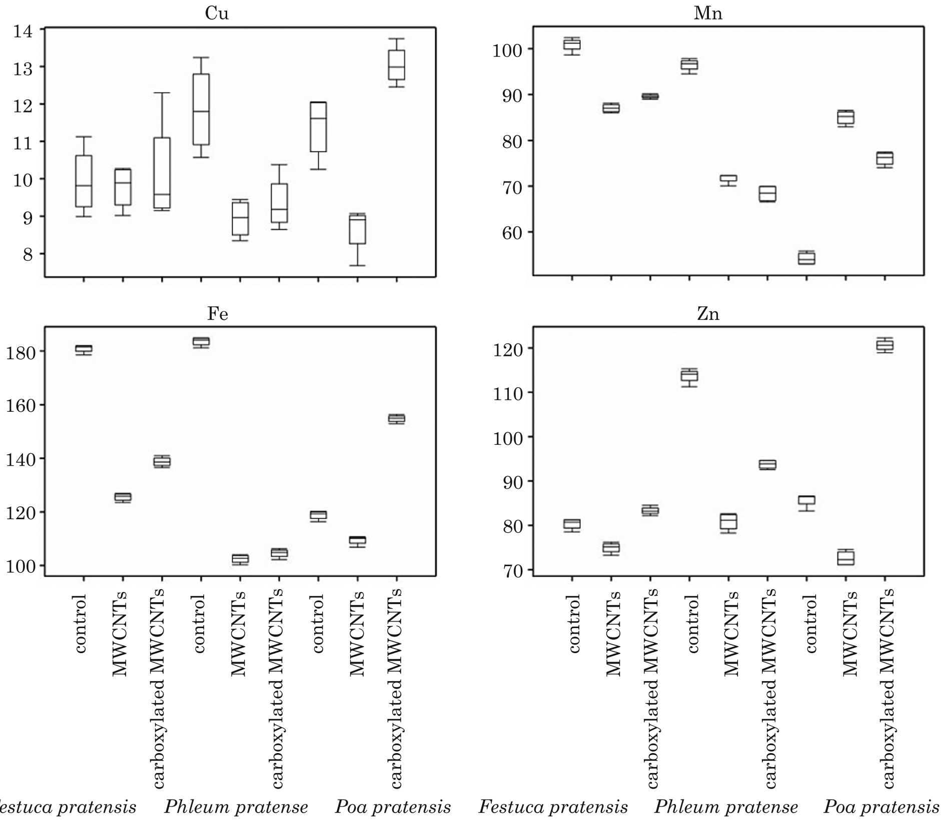

Fig. 5. Content of microelements (g kg^{-1} DM) in the studied forage grasses depending on the type of carbon nanotubes (means from the 2-year study). LSD$_{0.05}$ for Cu species: 0.74, CNT: 0.74, species×CNT: 1.28, LSD$_{0.05}$ for Mn species: 1.15, CNT: 1.15, species×CNT: 1.99, LSD$_{0.05}$ for Fe species: 1.39, CNT: 1.39, species×CNT: 2.40, LSD$_{0.05}$ for Zn species: 1.24, CNT: 1.24, species×CNT: 2.14

Fe was observed in meadow fescue, and in the case of Zn – in timothy grass. Both types of CNTs reduced the content of Cu, Mn, Fe and Zn in timothy grass as compared with the control. Contrary to that, concentrations of these elements in common meadow grass treated with carboxylated CNTs were higher than in the control. In meadow fescue, an increase in Zn and a decrease in Mn and Fe were noticed following carboxylated CNT supplementation.

DISCUSSION

Reports concerning the effects of CNTs on seed germination, plant growth and development are ambiguous. Plant toxicity studies have demonstrated positive (Cañas et al. 2008, Mondal et al. 2011, Khodakovskaya et al. 2013), negative (Cañas et al. 2008, Lin et al. 2009) and neutral (Lin, Xing 2007, Wild, Jones 2009) effects of various types of CNTs on germination and selected physiological parameters of different species.

This study does not provide a definite answer to the question of ecotoxicity of MWCNTs towards the studied grass species. Both stimulatory and inhibitory effects of CNTs on the studied species were observed. Inconclusive results concerning the effects of CNTs on plants were also published by Cañas et al. (2008), who studied root elongation in cucumber, lettuce, tomato, cabbage, carrot and onion treated with functionalized and nonfunctionalized single-walled CNTs. Nonfunctionalized SWCNTs inhibited root elongation in tomato and accelerated this process in onion and cucumber. Functionalized SWCNTs inhibited root elongation in lettuce, and no effects of any type of CNTs were found in cabbage and carrot. Positive response of plants to CNTs has been confirmed in numerous studies. Smirnova et al. (2012) reported a stimulatory effect of the engineered nanomaterial Taunit, containing MWCNTs, on the germination of *Onobrychis arenaria*. They investigated the effect of aqueous solution of Taunit (100 or 1000 μg ml^{-1}) on the germination energy and germination rate. Germination energy of *Onobrychis arenaria* in the presence of MWCNTs was 14% higher than in the control, irrespective of the MWCNT concentration. The germination rate was 2% higher for the seeds treated with 100 μg ml^{-1} MWCNT and 7% higher in those treated with MWCNTs at 1000 μg ml^{-1}. Moreover, Taunit stimulated the growth of roots and stems. The average stem length in the control plants was 14 mm, and in nanotube-treated variants it was nearly 26 mm, irrespective of the CNT concentration. The average length of the roots treated with Taunit was 29-32 mm, and of the control roots – 18 mm. Khodakovskaya et al. (2013) reported a much higher germination rate in tomato seeds placed on agar medium enriched with CNTs at 10, 20 or 40 μg ml^{-1}. The germination rate in the control seeds was 32% after 12 days, and 71% after 20 days, while for CNT-supplemented variant it was 74-82% and 90%, respectively. Total biomass of the seedlings grown in a CNT-containing medium increased 2.5-fold as compared with the control. Wang et al. (2012) investigated the effects of oxidized MWCNTs (at concentrations ranging from 10 to 160 μg ml^{-1}) on seed germination, root elongation, stem length and vegetative biomass of common wheat. The study showed a higher germination rate in the seeds treated with o-MWCNTs, but the reported changes were not significant. A significant increase in vegetative biomass was observed in wheat seedlings grown on substrates supplemented with o-MWCNTs. Total plant biomass (roots, stems, leaves) of wheat germinating and growing on plates with o-MWCNTs was 30-40% higher than in the control variant. Miralles et al. (2012) assessed the effect of industrial-grade MWCNTs on the growth and development of common wheat and alfalfa. CNT concentrations ranging from 40 to 2560 mg l^{-1} were not toxic to any of the species, and seed germination was not inhibited even at 2560 mg l^{-1} CNT. Furthermore, industrial-grade MWCNTs were found to accelerate root elongation in both common wheat and alfalfa. Larue et al. (2012) studied the effects of MWCNTs on the germination, root elongation, and plant biomass of hydroponically grown common wheat and rape. Concentrations of MWCNTs were 0, 10, 50, and 100 mg l^{-1}. The study showed that the examined type of nanotubes used at these concentrations did not affect seed germination, root elongation or dry matter of the studied plants.

CONCLUSIONS

1. Timothy grass was the least sensitive species to the presence of MWCNTs and carboxylated MWCNTs in the substrate during germination.

2. The highest concentration of carboxylated MWCNTs (16.7 mg ml^{-1}) resulted in germination inhibition in all the studied species of grasses.

3. The addition of MWCNTs caused a decrease in the mean SPAD value of timothy grass and common meadow grass as compared with the control.

4. In all the studied species, the presence of carboxylated MWCNTs in the substrate caused an increase in the SPAD value and root mass.

5. The grasses which were treated with MWCNTs had lower dry matter yield and lower root mass.

6. In most cases, the addition of MWCNTs caused a decrease in the content of macroelements and microelements, and the addition of carboxylated MWCNTs increased their content.

Acknowledgments

The authors would like to thank Intermag LLC from Olkusz for their assistance in conducting this experiment, particularly Mr. Piotr Lubaszka, Sales Manager.

REFERENCES

Baughman R.H., Zakhidov A.A., De Heer W.A. 2002. *Carbon nanotubes – the route toward applications*. Science, 297(5582): 787-792. DOI: 10.1126/science.1060928

Cañas J.E., Long M., Nations S., Vadan R., Dai L., Luo M., Ambikapathi R., Lee E. H., Olszyk D. 2008. *Effects of functionalized and nonfunctionalized single-walled carbon nanotubes on root elongation of select crop species*. Environ. Toxicol. Chem., 27(9): 1922-1931. https://www.ncbi.nlm.nih.gov/pubmed/19086209

De Volder M.F., Tawfick S.H., Baughman R.H., Hart A.J. 2013. *Carbon nanotubes: present and future commercial applications*. Science, 339(6119): 535-539. DOI: 10.1126/science.1222453

Eitan A., Jiang K., Dukes D., Andrews R., Schadler L. S. 2003. *Surface modification of multi-walled carbon nanotubes: toward the tailoring of the interface in polymer composites*. Chem. Mater., 15(16), 3198-3201. DOI: 10.1021/cm020975d

He H., Pham-Huy L.A., Dramou P., Xiao D., Zuo P., Pham-Huy C. 2013. *Carbon nanotubes: applications in pharmacy and medicine*. BioMed Res. Int. http://dx.doi.org/10.1155/2013/578290

International Rules for Seed Testing (ISTA). 2010. IHAR-PIB, Radzików. (in Polish) http://www.ihar.edu.pl/polska_wersja_miedzynarodowych_przepisow_oceny_nasion_ista.php

Jackson P., Jacobsen N.R., Baun A., Birkedal R., Kühnel D., Jensen K.A., Vogel U., Wallin H. 2013. *Bioaccumulation and ecotoxicity of carbon nanotubes*. Chem. Cent. J., 7: 154. DOI: 10.1186/1752-153X-7-154

Khodakovskaya M.V., Kim B.S., Kim J.N., Alimohammadi M., Dervishi E., Mustafa T., Cernigla C.E. 2013. *Carbon nanotubes as plant growth regulators: effects on tomato growth, reproductive system, and soil microbial community*. Small, 9(1): 115-123. DOI: 10.1002/smll.201201225

Lam C. W., James J.T., McCluskey R., Arepalli S., Hunter R.L. 2006. *A review of carbon nanotube toxicity and assessment of potential occupational and environmental health risks*. Crit. Rev. Toxicol., 36(3): 189-217. https://www.ncbi.nlm.nih.gov/pubmed/16686422

Larue C., Pinault M., Czarny B., Georgin D., Jaillard D., Bendiab N., Mayne-L'Hermite M., Taran F., Dive V., Carrière M. 2012. *Quantitative evaluation of multi-walled carbon nanotube uptake in wheat and rapeseed*. J. Hazard. Mat., 227: 155-163. DOI: 10.1016/j.jhazmat.2012.05.033

Lin S., Reppert J., Hu Q., Hudson J.S., Reid M.L., Ratnikova T.A., Rao A.M., Luo H., Ke, P.C. 2009. *Uptake, translocation, and transmission of carbon nanomaterials in rice plants*. Small, 5(10): 1128-1132. DOI: 10.1002/smll.200801556

Lin D., Xing B. 2007. *Phytotoxicity of nanoparticles: Inhibition of seed germination and root growth*. Environ. Pollut., 150: 243-250. DOI: 10.1016/j.envpol.2007.01.016

Miralles P., Johnson E., Church T.L., Harris A.T. 2012. *Multiwalled carbon nanotubes in alfalfa and wheat: toxicology and uptake*. J. R. Soc., Interface, 9(77): 3514-3527. DOI: 10.1098/rsif.2012.0535

Mondal A., Basu R., Das S., Nandy P. 2011. *Beneficial role of carbon nanotubes on mustard plant growth: an agricultural prospect*. J. Nanopart. Res., 13: 4519-4528. DOI: 10.1007/s11051-011-0406-z

Petersen E.J., Zhang L., Mattison N.T., O'Carroll D.M., Whelton A.J., Uddin N., Nguyen T., Huang Q., Henry T.B., Holbrook R.D., Chen K.L. 2011. *Potential release pathways, environmental fate, and ecological risks of carbon nanotubes*. Environ. Sci. Technol., 45(23): 9837-9856. DOI: 10.1021/es201579y

Roth S., Carroll D. 2015. *One-dimensional metals: conjugated polymers, organic crystals, carbon nanotubes and graphene*. Weinheim: Wiley-VCH. DOI: 10.1002/9783527690176

Russier J., Ménard-Moyon C., Venturelli E., Gravel E., Marcolongo G., Meneghetti M., Doris E., Bianco A. 2011. *Oxidative biodegradation of single-and multi-walled carbon nanotubes*. Nanoscale, 3(3): 893-896. DOI: 10.1039/C0NR00779J

Scown T.M., Van Aerle R., Tyler C. R. 2010. *Review: Do engineered nanoparticles pose a significant threat to the aquatic environment?* Crit. Rev. Toxicol., 40(7): 653-670. DOI: 10.3109/10408444.2010.494174

Smirnova E., Gusev A., Zaytseva O., Sheina O., Tkachev A., Kuznetsova E., Lazareva E., Onishchenko G., Feofanov A., Kirpichnikov A. 2012. *Uptake and accumulation of multiwalled carbon nanotubes change the morphometric and biochemical characteristics of Onobrychis arenaria seedlings*. Front. Chem. Sci. Eng., 6(2): 132-138. DOI: 10.1007/s11705-012-1290-5

Sweeney S., Grandolfo D., Ruenraroengsak P., Tetley T.D. 2015. *Functional consequences for primary human alveolar macrophages following treatment with long, but not short, multiwalled carbon nanotubes*. Int. J. Nanomed., 10: 3115-3129. https://doi.org/10.2147/IJN.S77867

Wang X., Han H., Liu X., Gu X., Chen K., Lu D. 2012. *Multi-walled carbon nanotubes can enhance root elongation of wheat (Triticum aestivum) plants*. J. Nanopart. Res., 14(6): 1-10. DOI: 10.1007/s11051-012-0841-5

Wild E., Jones K. C. 2009. *Novel method for the direct visualization of in vivo nanomaterials and chemical interactions in plants*. Environ. Sci. Technol., 43: 5290-5294. DOI: 10.1021/es900065h