Journal of Elementology ISSN 1644-2296

Radkowski A., Radkowska I., Bocianowski J., Galus-Barchan A. 2019.
Effect of multi-walled carbon nanotubes (MWCNTs) on counts of microorganisms in soil as exemplified by the cultivation of selected fodder grasses.
J. Elem., 24(1): 47-59. DOI: 10.5601/jelem.2018.23.2.1588

RECEIVED: 2 January 2018
ACCEPTED: 17 June 2018

ORIGINAL PAPER

EFFECT OF MULTI-WALLED CARBON NANOTUBES (MWCNTS) ON COUNTS OF MICROORGANISMS IN SOIL AS EXEMPLIFIED BY THE CULTIVATION OF SELECTED FODDER GRASSES*

Adam Radkowski[1], Iwona Radkowska[2], Jan Bocianowski[3], Agnieszka Galus-Barchan[4]

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

Abstract

The aim of this study was to evaluate the effect of two types of multi-walled CNTs: raw MWCNTs and carboxylated MWCNTs, on the microbiology of soil on which three species of fodder grasses were cultivated: timothy grass, common meadow grass and meadow fescue. A two-year pot experiment evaluated the effect of MWCNTs (at a dose of 15 g pot^{-1}, i.e. 4.77 t ha^{-1}) and carboxylated MWCNTs (at a dose of 80 g pot^{-1}, i.e. 25.5 t ha^{-1}) on the number of microorganisms in soil and in root matter. The total number of bacteria, number of dormant bacteria, actinomycetes and fungi were investigated. Considerable diversity in the counts of microorganisms was observed, depending on a grass species, type of fraction and type of nanotubes added. When analyzing the total results from the three fractions (the rhizosphere, outside the rhizosphere and roots), the highest number of most of the microorganisms studied was determined in samples from control treatments. The lowest values were obtained in samples from treatments with the addition of carboxylated MWCNTs, and intermediate values were achieved from treatments with raw MWCNTs. The total number of bacteria was positively correlated with the number of dormant bacteria, actinomycetes and fungi. The number of dormant bacteria correlated with the growth of actinomycetes and fungi. Soil contamination even with relatively high amounts of MWCNTs or carboxylated MWCNTs does not cause massive mortality of soil microorganisms. That is why it is important to assess their potential effect on the environment.

Keywords: *Phleum pratense*, *Poa pratensis*, *Festuca pratensis*, MWCNTs, soil microorganisms.

dr inż. Adam Radkowski, Department of Grassland Management, Institute of Plant Production, University of Agriculture in Krakow, al. Mickiewicza 21, 31-120 Krakow, Poland, e-mail: adam.radkowski@urk.edu.pl

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

INTRODUCTION

Carbon nanotubes (CNTs) are single atoms of hexagonal carbon rolled up into hollow cylinders, and they are classified as single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs). They are used as innovative raw materials in the medical, pharmaceutical and technical industries, as well as for production of energy storage devices (Biercuk et al. 2002, Chung et al. 2011). Global production of carbon nanotubes is increasing by about 25% every year. However, there are no strict rules regulating production, use and disposal of CNTs. Therefore, there is a great risk that their considerable amounts could be released to the environment, which may have an adverse effect on its functioning and, in consequence, on human health. Earlier studies showed that SWCNTs are more toxic to macroorganism cells, whereas MWCNTs have lower toxicity. Although CNTs exhibited low toxicity at the physiological, cellular and genetic level in artificial plant cultures, there is still very limited knowledge about the release of CNTs into the environment and the direct risk they pose to microorganisms that live in that environment (Mukherrjee et al. 2016). Soil microbiological communities play an important role in soil ecosystem activities. Microorganisms that live in soil ecosystems are responsible for the cycling of nutrients and elements. Their biodiversity largely depends on numerous chemical factors. Information about the interactions between CNTs and soil microorganisms is very limited but it is assumed that they can have a direct toxic effect on the soil community (Kerfahi et al. 2015). Nevertheless, literature suggests that they are neutral or slightly toxic to soil environment and microorganisms living in it. It is justified to investigate the effect of CNTs on soil microorganisms. This article presents their impact on selected groups of microorganisms in the cultivation of meadow grasses in a two-year seasonal study.

MATERIAL AND METHODS

Characteristics of nanotubes

MWCNTs were bought from CNT Co., Ltd., 806 Mecarium Officetel, 593 Yeonsu 2-Dong Yeonsu-Gu, Incheon, Korea. The MWCNTs provided by the manufacturer had the following commercial characteristics: 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}. Inorganic residue after MWCNT sample combustion, 3.3%. An analysis of the inorganic residue after MWCNT sample combustion showed the presence of Fe 360.3 g kg^{-1}, Cr 98.0 g kg^{-1}, Mn 25.0 mg kg^{-1}, Mg 53.2 mg kg^{-1} and Ca 57.0 mg kg^{-1}. Commercial raw MWCNTs were carboxylated. Raw MWCNTs (80 g) were boiled in a triple-neck, round-bottom flask equipped with a reflux condenser and thermometer

in 1.7 L 68% HNO_3 for 60 h. The mixture was cooled and filtered through a glass filter and then abundantly rinsed with distilled water up to pH >5, as measured in the filtrate. The entire sample was then filter dried for 2 h, the black cake-like mass was disintegrated and dried for one day at 80°C and then one day at 120°C to obtain solid mass. The mass of the product was 31.8 g, and the process efficiency was 39.7%. The carboxylated MWCNTs obtained in this way did not exhibit the presence of inorganic content after combustion.

A more detailed description can be found in the paper by Radkowski et al. 2018.

Plant material

The following species were used in the conducted experiment: timothy grass (*Phleum pratense* L.) cv. Owacja – entered in the National Court Register on 28 Feb 2014, meadow fescue (*Festuca pratensis* Huds.) cv. Fantazja – entered in the National Court Register on 01 Mar 2006, common meadow grass (*Poa pratensis* L.) cv. Struga – entered in the National Court Register on 07 Mar 2011. All the species were grown by the Małopolska Plant Breeding Company in Kraków, Poland. The germinability before sowing into pots was 98% for timothy grass, 92% for meadow fescue and 82% for common meadow grass.

Pot experiment

A two-year pot experiment was conducted in the years 2014-2015, 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 (growing season: from April to September), the temperature at night and during the day was 8-15°C and 22-32°C, respectively. The length of a day ranged from 12 h (April) to 16 h (July-August). Relative air humidity in the greenhouse was between 55 and 60%.

5.02 dm^3 polyethylene pots were filled with soil material, topsoil collected from the depth of 30 cm of loess-based degraded chernozem. All pots were fertilized each year with NPK at 0.3 g N; 0.08 g P; and 0.2 g K 1 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 plants, and their solutions were thoroughly mixed with the substrate. In the second year, the fertilizers were applied in early spring before the growing season started.

For each species, the following treatments were used: control without CNTs (4.7 kg soil), soil enriched with carboxylated MWCNTs (80 g pot^{-1}, which corresponded to a dose of 25.5 t ha^{-1}), soil with raw MWCNTs (15 g pot^{-1}, which corresponded to a dose of 4.77 t ha^{-1}). Concentrations of nanotubes were chosen arbitrarily so that they were the largest addition to the soil, not the other way round. The difference in concentrations

between raw MWCNTs and carboxylated MWCNTs was due to a large difference in their bulk density. Raw MWCNTs have a very low bulk density and even a small addition significantly increases the volume of the substrate. In all combinations, nanotubes were thoroughly mixed with topsoil to the depth of about 10 cm, directly before seed sowing. The investigated grass species were sown (30 seeds per pot) on 27 March 2014. While growing, the plants were watered with redistilled water, maintaining the soil moisture at 60% of the maximum water capacity.

The analyses

After completion of the experiment, in order to determine the quantity of microorganisms present in the studied samples (soil from rhizosphere, soil outside the rhizosphere and roots), a microbiological analysis of the soil was carried out using the Koch's plate dilution method (LIBUDZISZ et al. 2007). The microorganisms were cultivated according to the protocol shown in Table 1.

Table 1

Conditions of the incubation of the microorganisms analysed

Group of microorganisms	Temperature (°C)	Incubation time (h)
Bacteria – total number	37	72
Bacteria – dormant forms	37	72
Actinomycetes	28	120
Fungi	28	120

Dormant forms of bacteria were obtained by pasteurizing appropriate dilutions at 85°C for 10 minutes.

After incubation, the number of grown colonies of microorganisms was counted and converted into the number of colony-forming units (cfu) in 1 g DM of soil.

Moreover, the following were determined in the soil material: pH with the potentiometric method in H_2O and 1 mol KCl dm^{-3}; organic C with the Tiurin method; total N with the Kjeldahl method; available phosphorus and potassium with the Egner-Riehm method; content of magnesium, zinc, manganese and copper using atomic absorption spectrometry (AAS). Prior preparation of the soil samples consisted in mineralization in pressure systems.

Statistical analysis

A three-way analysis of variance (ANOVA) was carried out to determine the effects of species, CNTs, rhizosphere and the following interactions: species×CNTs, species×rhizosphere, CNTs×rhizosphere, species×CNTs×rhizosphere on the variability of total bacteria, number of dormant bacteria, actinomycetes and fungi. The *post-hoc* least significant differences (LSDs)

test was used to distinguish significant treatments for analyses with significant exposure. The relationships between total bacteria, dormant bacteria, actinomycetes and fungi were estimated using Pearson correlation coefficients. Data analysis was carried out using the statistical package GenStat 17.

RESULTS

Chemical properties of the soil where the experiments were conducted are presented in Figure 1. The value of pH of the soil after the experiment decreased by about 3% compared to that before the experiment. As for the total N and organic C content, they tended to increase; their content increased (in relative numbers) by 7% compared to the amount before the experiment.

The content of available forms of phosphorus, potassium and magnesium changed after the experiment (increasing by 12%, 5% and 14%, respectively). The microelement content in the soil after the experiment also showed an increasing tendency. The amounts of zinc, manganese and copper in the soil after the experiment increased by 3%, 3% and 4%, respectively.

Results of ANOVA show statistically significant effects of all factors (species, rhizosphere and CNTs) and of all interactions, on the variability of the all traits observed (Table 2).

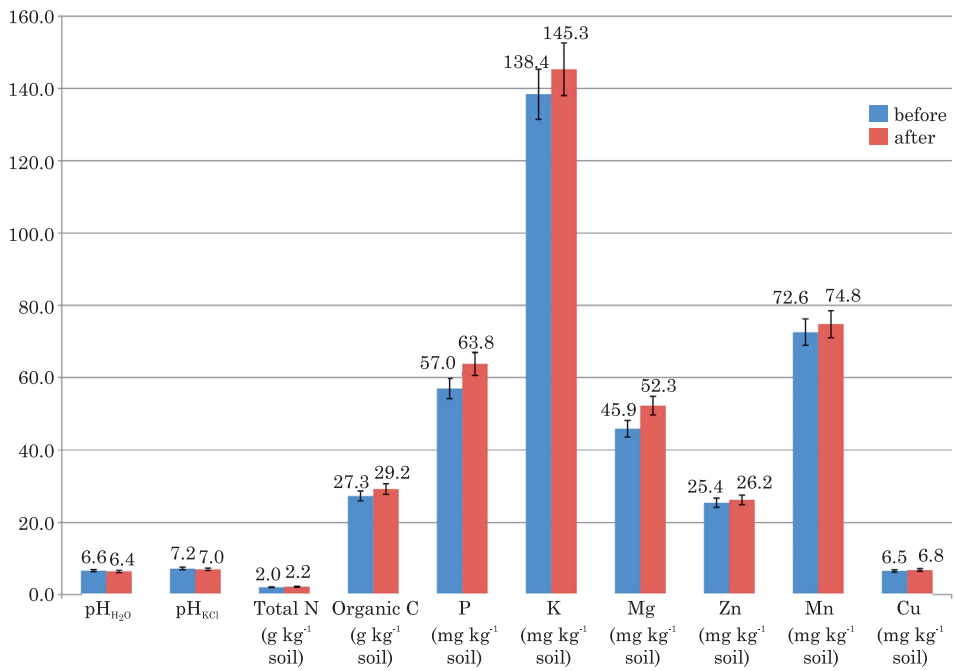

Fig. 1. Chemical properties of the soil (average values)

Table 2

Values of *F*-statistics from three-way analysis of variance for total bacteria, number of dormant bacteria, actinomycetes and fungi

Source of variation	d.f.	Total no. of bacteria	Number of dormant bacteria	Actinomycetes	Fungi
Species	2	429.6***	193.11***	229.77***	856.74***
CNTs	2	186.34***	89.11***	304.65***	65.19***
Rhizosphere	2	5911.18***	1033.88***	2050.82***	1055.56***
Species × CNTs	4	20.5***	102.52***	54.84***	367.29***
Species × rhizosphere	4	180.32***	103.93***	202.41***	165.43***
CNTs × rhizosphere	4	74.89***	23.65***	191.82***	121.55***
Species × CNTs × rhizosphere	8	90.6***	39.26***	45.23***	148.02***

d.f. – degrees of freedom, *** statistically significant at 0.001 level, CNTs – carbon nanotubes

Tables 3, 4, 5 and 6 show mean values (as well as standard deviations) from the microbiological analysis of the soil and roots from the second year of the study. Considerable diversity in the counts of microorganisms was observed, depending on a grass species and type of nanotubes added. When analyzing the total results from the three fractions (the rhizosphere, outside the rhizosphere and roots), the highest number of most of the studied microorganisms was determined in samples from control treatments. The lowest values were obtained in samples from treatments with the addition of carboxylated MWCNTs and intermediate values in treatments with raw MWCNTs.

A similar relationship was determined for total bacteria. A different pattern was observed for dormant forms of bacteria: the lowest values were determined in treatments with raw MWCNTs and intermediate in treatments with the addition of carboxylated MWCNTs. On the other hand, the highest number of actinomycetes was recorded in treatments where raw MWCNTs were added to the soil, and the lowest in treatments with the addition of carboxylated MWCNTs. Moreover, the highest number of fungi was recorded in treatments with the addition of carboxylated MWCNTs and the lowest – in treatments with raw MWCNTs. The total number of bacteria was very significantly positively correlated with the number of dormant bacteria ($r = 0.6767$), actinomycetes ($r = 0.7549$) and fungi ($r = 0.3153$). In addition, an increase in the number of dormant bacteria determined the growth of actinomycetes ($r = 0.5027$) and fungi ($r = 0.3148$) – Table 7.

Table 3

Total number of bacteria ($\cdot 10^6$) in soil and in root matter (number of colony-forming units (cfu) in 1 g DM of soil)

Species	CNT	Roots		Outside rhizosphere		Rhizosphere		Total
		mean	SD	mean	SD	mean	SD	
Fescue	0	1.235	0.170	2.670	0.188	14.470	0.391	18.375
	MWCNTs	3.540	0.295	2.320	0.322	11.300	0.332	17.160
	(COOH) MWCNTs	3.970	0.309	2.151	0.151	8.950	0.295	15.071
Timothy	0	3.456	0.338	1.561	0.295	8.760	0.322	13.777
	MWCNTs	1.885	0.125	1.551	0.261	6.540	0.345	9.976
	(COOH) MWCNTs	1.430	0.157	1.241	0.217	4.550	0.325	7.221
Meadow-grass	0	3.150	0.160	2.250	0.229	9.650	0.795	15.050
	MWCNTs	3.431	0.274	2.680	0.293	9.520	0.389	15.631
	(COOH) MWCNTs	0.341	0.039	1.780	0.157	9.973	0.520	12.094
Mean		2.493	1.246	2.023	0.536	9.301	2.688	
$LSD_{0.05}$	for species, CNT, rhizosphere: 0.149; for species · CNT, species · rhizosphere, CNT · rhizosphere: 0.258, for species · CNT · rhizosphere: 0.447							

CNT – carbon nanotube, MWCNTs – multi-walled carbon nanotubes, SD – standard deviation

Table 4

Number of dormant bacteria (· 10^5) in soil and in root matter (number of colony-forming units (cfu) in 1 g DM of soil)

Species	CNT	Roots		Outside rhizosphere		Rhizosphere		Total
		mean	SD	mean	SD	mean	SD	
Fescue	0	11.607	1.660	4.432	0.159	12.304	1.054	28.343
	MWCNTs	0.573	0.008	1.340	0.074	11.205	0.885	13.118
	(COOH) MWCNTs	9.303	0.227	3.773	0.081	15.560	1.213	28.636
Timothy	0	2.456	0.101	3.545	0.235	6.750	1.368	12.751
	MWCNTs	2.065	0.037	3.765	0.142	8.876	0.808	14.706
	(COOH) MWCNTs	1.275	0.143	1.285	0.176	11.005	1.550	13.565
Meadow-grass	0	2.575	0.185	7.783	0.203	12.771	1.365	23.129
	MWCNTs	2.672	0.103	5.517	0.218	8.250	0.146	16.439
	(COOH) MWCNTs	1.245	0.037	4.203	0.201	6.850	0.286	12.298
Mean		3.752	3.77	3.960	1.906	10.397	2.975	28.343
$LSD_{0.05}$	for species, CNT, rhizosphere: 0.33; for species · CNT, species · rhizosphere, CNT · rhizosphere: 0.57, for species · CNT · rhizosphere: 0.99							

CNT – carbon nanotube, MWCNTs – multi-walled carbon nanotubes, SD – standard deviation

DISCUSSION

The growing use of carbon nanotubes in industrial production is accompanied by their increasing presence in the natural environment, hence the need for research allowing us to determine potential economic benefits and risks posed by these new materials, which have never before been present in the environment of living organisms (Jackson et al. 2013). Carbon nanotubes can be released into the environment directly during the use of materials containing CNTs or as waste from sewage treatment plants, waste incineration plants and landfills (Petersen et al. 2011). In respect of soil contamination with carboxylated MWCNTs, in the initial phase it is possible to lower soil pH, which over time is neutralized through chemical exchange reactions with calcium carbonate or calcium hydrogencarbonate ($CaCO_3$ or $Ca(HCO_3)_2$) present in soil, or through reactions with other alkaline constituents of soil. These reactions will be accompanied by the formation of extremely stable (under conditions close to neutral pH) complex compounds of carboxylated MWCNTs salt character with ions of metals, Ca^{2+}, Mg^{2+}, and with ions

Table 5

Number of actinomycetes ($\cdot 10^5$) in soil and in root matter (number of colony-forming units (cfu) in 1 g DM of soil)

Species	CNT	Roots		Outside rhizosphere		Rhizosphere		Total
		mean	SD	mean	SD	mean	SD	
Fescue	0	1.064	0.082	0.445	0.037	1.774	0.125	3.283
	MWCNTs	0.966	0.068	0.333	0.023	2.770	0.214	4.069
	(COOH) MWCNTs	0.445	0.033	0.313	0.022	1.250	0.096	2.008
Timothy	0	0.885	0.015	0.342	0.006	1.231	0.033	2.458
	MWCNTs	0.764	0.013	0.356	0.007	1.105	0.017	2.225
	(COOH) MWCNTs	0.634	0.013	0.213	0.012	0.774	0.014	1.621
Meadow-grass	0	1.234	0.087	0.227	0.016	1.041	0.012	2.502
	MWCNTs	0.854	0.074	0.399	0.028	1.672	0.124	2.925
	(COOH) MWCNTs	0.664	0.054	0.665	0.048	0.832	0.019	2.161
Mean		0.834	0.234	0.366	0.130	1.383	0.599	
$LSD_{0.05}$	for species, CNT, rhizosphere: 0.03; for species · CNT, species · rhizosphere, CNT · rhizosphere: 0.05, for species · CNT · rhizosphere: 0.09							

CNT – carbon nanotube, MWCNTs – multi-walled carbon nanotubes, SD – standard deviation

of d-block metals which are present in soil, i.e. Fe^{3+}, Cu^{2+}, Mn^{2+}, Zn^{2+}. In this respect, carboxylated MWCNTs should behave in soil similarly to carboxylic ion-exchange resins (Rafati et al. 2010, Rengaraj et al. 2001).

Kuźniar et al. (2011) observed that multi-walled carbon nanotubes do not cause mortality of larvae of insecticidal nematodes. However, they partially reduce their activity against test insects. Multi-walled carbon nanotubes (MWNCTs and MWCNT(COOH)s) are non-toxic to infective juveniles of entomopathogenic nematodes of *Steinernema feltiae* (Owinema, Namasys, Nemaplus) and *Heterorhabditis bacteriphora* (Namatop) species, and they are a factor in limiting the activity of infective juvenile larvae of entomopathogenic nematodes of *Steinernema feltiae* (Owinema, Namasys, Nemaplus) and *Heterorhabditis bacteriphora* (Namatop) species.

Gorczyca et al. (2009) showed that raw carbon nanotubes greatly stimulated the superficial growth of *P. fumosoroseus* mycelium and reduced its sporulation as compared with the control. On the other hand, carboxylation changed the effect of carbon nanotubes on spores of the studied fungus. The effect of MWCNT(COOH) was clearly weaker and the linear growth and sporulation obtained did not differ significantly from the control. There

Table 6

Number of fungi ($\cdot 10^5$) in soil and in root matter (number of colony-forming units (cfu) in 1 g DM of soil)

Species	CNT	Roots		Outside rhizosphere		Rhizosphere		Total
		mean	SD	mean	SD	mean	SD	
Fescue	0	1.232	0.093	1.021	0.079	1.887	0.133	4.140
	MWCNTs	0.821	0.063	0.945	0.049	1.432	0.094	3.198
	(COOH) MWCNTs	1.754	0.103	1.356	0.098	2.274	0.197	5.384
Timothy	0	2.210	0.090	1.072	0.076	2.405	0.169	5.687
	MWCNTs	3.321	0.201	2.894	0.158	4.356	0.238	10.571
	(COOH) MWCNTs	2.786	0.114	2.231	0.157	3.997	0.163	9.014
Meadow-grass	0	2.282	0.145	1.342	0.094	7.750	0.482	11.374
	MWCNTs	1.754	0.084	1.453	0.109	2.134	0.117	5.341
	(COOH) MWCNTs	2.564	0.164	2.223	0.146	3.950	0.260	8.737
Mean		2.080	0.752	1.615	0.653	3.354	1.878	
$LSD_{0.05}$	for species, CNT, rhizosphere: 0.078; for species · CNT, species · rhizosphere, CNT · rhizosphere: 0.135, for species · CNT · rhizosphere: 0.234							

CNT – carbon nanotube, MWCNTs – multi-walled carbon nanotubes, SD – standard deviation

Table 7

Correlations coefficients between the traits observed

Traits	Total no. of bacteria	Number of dormant bacteria	Actinomycetes	Fungi
Total no. of bacteria	1			
Dormant bacteria	0.6767***	1		
Actinomycetes	0.7549***	0.5027***	1	
Fungi	0.3153***	0.3148***	0.0887	1

*** statistically significant at 0.001 level

was no significant effect of MWCNTs on the increase in the biomass of *P. fumosoroseus* fungus. In another study, Gorczyca et al. (2014) showed that carbon nanotubes can damage cell membranes of fungal spores and can cause considerable deterioration of fungal functions.

There are numerous reports in the literature about the negative impact of nanomaterials on living organisms, especially invertebrates (Handy et al.

2008). That is why it important to assess their potential environmental impact. In order to estimate changes in counts of soil microorganisms under the effect of carbon nanotubes, three physiological groups were selected: bacteria, fungi and actinomycetes. During the two-year pot experiment, little effect on the microbiological community was observed. Tong et al. (2007) obtained similar results when investigating the effect of fullerene C_{60} on soil microorganisms. When assessing the growth of selected strains of the *Bacillus* genus bacteria, Płaza et al. (2009) showed that carbon nanotubes did not cause growth inhibition of the bacteria or inhibition of morphological changes, but they stimulated the production of bacterial spores.

Soil environment can be one of the final recipients of contamination with nanomaterials. Hence, it is justified to investigate the effect of CNTs on soil living organism. Research carried out by Chung et al. (2011) shows that short-term use of CNTs may decrease biomass of microorganisms in soil. Numerous scientific reports inform about short-term use of treated CNTs and raw MWCNTs (Jin et al. 2012). Taking into account the fact that soil microorganisms are believed to be a slow-growing group and one with a large number of inactive cells, it was required to conduct a study where nanotubes would be used for a longer period of time. In the research conducted by Shrestha et al. (2013), after 90-day exposition to MWCNTs, there was no change in the number of soil microorganisms compared with the control.

Kerfahi et al. (2015) showed that samples with treated CNTs had a larger effect on selected groups of microorganisms, whereas lower deviations from the control occurred in soils with raw MWCNTs. Very similar results were obtained in a two-year pot study on grassland soils with plant species such as timothy grass, meadow fescue and common meadow grass, where a smaller number of soil microorganisms was determined after an application of carboxylated MWCNTs than raw MWCNTs. However, the differences were not significant in relation to the control without the addition of CNTs. Similar relations were observed for actinomycetes. A considerable reduction in the abundance of *Actinobacteria* was observed after an application of carboxylated MWCNTs, which in consequence may reduce the cycling of carbon in soil, since it is a group of microorganisms responsible for the decomposition of cellulolytic and pectinolytic compounds.

Literature reports indicate that effects of exposition of soil bacteria to treated CNTs are quite persistent because microflora is restored and recovers its metabolic properties after about 8 weeks (Johansen et al. 2008, Nannipieri et al. 2003). Application of raw MWCNTs does not have a noticeable effect on the majority of microbial populations living in soil, which was also confirmed in the two-year pot study presented in this article.

CONCLUSIONS

The presence of MWCNTs or carboxylated MWCNTs in soil causes developmental fluctuations of microorganisms such as bacteria and fungi in cultivation of fodder grasses (timothy grass, meadow fescue and common meadow grass) which are in excess of a measurement error. However, soil contamination with even relatively high amounts of MWCNTs or carboxylated MWCNTs does not cause massive mortality of soil microorganisms.

REFERENCES

Biercuk M.J., Llaguno M.C., Radosavljevic M., Hyun J.K., Johnson A.T., Fischer J.E. 2002. *Carbon nanotube composites for thermal management*. Appl. Phys. Lett., 80: 2767-2769. https://doi.org/10.1063/1.1469696

Chung H., Son Y., Yoon T.K., Kim S., Kim W. 2011. *Effect of multi-walled carbon nanotubes on soil microbial activity*. Ecotoxicol Environ. Saf., 74: 569-575. https://doi.org/10.1016/j.ecoenv.2011.01.004

Gorczyca A., Kasprowicz M.J., Lemek T. 2009. *Physiological effect of multi-walled carbon nanotubes (MWCNTs) on conidia of the entomopathogenic fungus, Paecilomyces fumosoroseus (Deuteromycotina: Hyphomycetes)*. J. Environ. Sci. Health A Tox Hazard Subst. Environ. Eng., 44(14): 1592-1597. DOI: 10.1080/10934520903263603

Gorczyca A., Kasprowicz M.J., Lemek T. 2014. *Physiological aspects of the interaction of multi-walled carbon nanotubes with Beauveria bassiana (Balsamo) Vuillem in entomopathogenic spores*. J. Environ. Sci. Health. A Tox. Hazard. Subst. Environ. Eng., 49(7): 863-968. DOI: 10.1080/10934529.2014.882684

Handy R.D., von der Kammer F., Lead J.R., Hassellöv M., Owen R., Crane M. 2008. *The ecotoxicology and chemistry of manufactured nanoparticles*. Ecotoxicology, 17: 287-314. https://doi.org/10.1007/s10646-008-0199-8

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(1): 154. DOI: 10.1186/1752-153X-7-154

Jin L., Son Y., Yoon T.K., Kang Y.J., Kim W., Chung H. 2012. *High concentrations of single-walled carbon nanotubes lower enzyme activity and soil microbial biomass*. Ecotoxycol. Environ. Safety, 88: 9-15. https://doi.org/10.1016/j.ecoenv.2012.10.031

Johansen A., Pedersen A.L., Jensen K.A., Karlson U., Hansen B.M., Scott-Fordsmand J.J., Winding A. 2008. *Effects of C_{60} Fullerene nanoparticles on soil bacteria and protozoans*. Environ. Toxicol. Chem. / SETAC, 27: 1895-1903. DOI: 10.1897/07-375.1

Kerfahi D., Tripathi B.M., Singh D., Kim H., Lee S., Lee J., Adams J.M. 2015. *Effects of functionalized and raw multi-walled carbon nanotubes on soil bacterial community Composition*. PLOS One, 10(3): 1-18. https://doi.org/10.1371/journal.pone.0123042

Kuźniar T., Ropek D., Lemek T. 2011. *Impact of multi-walled carbon nanotubes on viability and pathogenicity of enthomopathogenic nematodes*. ECEA, 18(5-6): 757-762.

Libudzisz Z., Kowal K., Żakowska Z. 2007. *Technical microbiology*. Part I. *Microorganisms and environments of their occurrence*. PWN, Warszawa, pp. 354. (in Polish)

Mukherrjee A., Majumdar S., Servin A.D., Pagano L., Dhankher O.P., White J.C. 2016. *Carbon nanomaterials in agriculture: a critical review*. Front. Plant Sci., 7: 172: 1-16. DOI: 10.3389/fpls.2016.00172

Nannipieri P., Ascher J., Ceccherini M., Landi L., Pietramellara G., Renella G. 2003. *Microbial diversity and soil functions*. Europ. J. Soil Sci., 54: 655-670. DOI: 10.1046/j.1351-0754.2003.0556.x

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

Płaza G., Kowalska E., Radomska J., Czerwosz E., Jangid K., Gawior K., Ulfig K., Janda-Ulfig K. 2009. *Effect of multiwalled carbon nanotubes on the growth of bacteria of the genus Bacillus and on the production of biosurfactants*. Ochr. Środ., 1(1): 21-24. (in Polish)

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

Rafati L., Mahvi A.H., Asgari A.R., Hosseini S.S. 2010. *Removal of chromium(VI) from aqueous solutions using Lewatit FO36 nano ion exchange resin*. Int. J. Environ. Sci. Tech., 7(1): 147-156. https://doi.org/10.1007/BF03326126

Rengaraj S., Yeon K.-H., Moon S.-H. 2001. *Removal of chromium from water and wastewater by ion exchange resins*. J. Hazardous Mater. 87: 273-287. https://doi.org/10.1016/S0304--3894(01)00291-6

Shrestha B., Acosta-Martinez V., Cox S.B., Green M.J., Li S., Canas-Carrell J.E. 2013. *An evaluation of the impact of multiwalled carbon nanotubes on soil microbial community structure and functioning*. J. Hazard. Mater. 261: 188-197. DOI: 10.1016/j.jhazmat.2013.07.031

Tong Z., Bischoff M., Loring Nies L., Applegate B., Turco R.F. 2007. *Impact of fullerene (C_{60}) on a soil microbial community*. Environ. Sci. Technol., 41(8): 2985-2991. DOI: 10.1021/es061953l