Journal of Hazardous Materials 186 (2011) 436–442

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

The toxicity to plants of the sewage sludges containing multiwalled carbon nanotubes

Patryk Oleszczuk[a,*], Izabela Jośko[a], Baoshan Xing[b]

[a] Institute of Soil Science and Environmental Management, University of Life Sciences in Lublin, ul. Leszczyńskiego 7, 20-069 Lublin, Poland
[b] Department of Plant, Soil and Insect Sciences, University of Massachusetts, Amherst, MA 01003, USA

ARTICLE INFO

Article history:
Received 4 September 2010
Received in revised form 4 November 2010
Accepted 5 November 2010
Available online 12 November 2010

Keywords:
Sewage sludge
Carbon nanotubes
Phytotoxicity
Nanotechnology
Nanoparticles

ABSTRACT

The aim of this study was to evaluate the toxicity of sewage sludges containing multiwalled carbon nanotubes (CNTs) with an outer diameter <10 nm (CNT10) or 40–60 nm (CNT60) to *Lepidium sativum* (cress), *Sorghum saccharatum* (sorgo), *Solanum lycopersicon* (tomato), *Raphanus sativus* (radish) and *Cucumis sativus* (cucumber). CNTs were also incubated in sewage sludge for 7 or 31 days to determine the effect of CNT aging on sewage sludge phytotoxicity. The influence of CNTs on 4 different sewage sludges was tested. The CNTs' influence on sludge toxicity varied with respect to the CNTs' outer diameter, type of sewage sludges and the plants tested. No significant influence of CNT concentration on phytotoxicity was noted. In the case of two sludges, a positive influence of CNTs on seed germination and root growth was observed. Depending on the CNTs' outer diameter, CNT aging decreased (CNT10) or increased (CNT60) sewage sludge phytotoxicity.

1. Introduction

Nanotechnology is currently the fastest developing branch of science [1,2]. An increasing use of nanomaterials can lead to random/conscious inclusions of nanoparticles to the natural environment. The impact that wastewater treatment has on nanomaterials, or conversely, the impact that nanomaterials have on wastewater treatment, is largely unknown [3]. Sewage sludges constitute a very important element in sewage management. A noted increase in sewage sludge production requires measures to ensure its utilisation. Frequently, agricultural application of sewage sludges is limited because of the organic and inorganic pollutants present. The influence of pollutants contained in sewage sludges on their toxicity has not, so far, been demonstrated unequivocally. It is assumed, however, that the presence of such compounds as polycyclic aromatic hydrocarbons, polychlorinated biphenyls and compounds affecting the human endocrinal system can exert a significant influence (both direct and indirect) on sewage sludge toxicity [4]. Potentially, nanomaterials constitute a new group of pollutants which can become a sewage sludge component as a result of sewage sludge treatment. Carbon-based nanomaterials represent one type of manufactured nanomaterial. Fullerenes and carbon nanotubes, including multiwalled carbon nanotubes (CNTs) are among the most widely used carbon-based nanomaterials. CNTs and their derivatives are used in plastics, catalysts, battery and fuel cell electrodes, supercapacitors, water purification systems, orthopedic implants, conductive coating, adhesives and composites, sensors and components in the electronics [2]. Their widespread use means that they can get into municipal sewage and then, due to their hydrophobic properties, sewage sludges.

CNTs present in sewage sludges can influence sewage sludge properties in many ways. The CNTs' presence in sewage sludges can (1) increase sewage sludge toxicity; (2) influence the toxicity, accumulation and migration of other pollutants; and (3) limit the mobility/bioavailability of nutrients present in sewage sludges. A number of authors have published data on toxicological information of nanomaterials [5,6]. However the research concerning phytotoxicity is mainly related with metal nanoparticles [7–10] rather than multiwalled carbon nanotubes [9,11]. Supplementing the studies conducted so far with information on to what degree CNTs influence the toxicity of environmental matrices is an important element in evaluating the trust of such materials in the environment. This issue is relatively new and as yet poorly recognized in the literature.

The aim of the present study was therefore to evaluate the phytotoxicity of sewage sludges containing CNTs with respect to their concentration and outer diameter, type of sewage sludge and test plant, as well as contact period between CNTs and sewage sludges (aging).

---

* Corresponding author. Tel.: +48 81 5343548, fax: +48 81 5322632.
E-mail addresses: patryk.oleszczuk@ar.lublin.pl, patryk@psis.umass.edu (P. Oleszczuk).

0304-3894/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2010.11.028

Table 1
Selected properties of the multiwalled carbon nanotubes (CNTs) used in the experiment.

Carbon nanotubes	Purity[a]	OD[b]	A_{surf}[c]	V_{meso}[c]	V_{micro}[c]	Ash[d]	EC[e]		
							C	H	O
MWCNT10	>95%	9.4	357	0.951	0.142	2.97	96.41	0.42	0.2
MWCNT60	>95%	42.7	73	0.162	0.029	2.09	97.68	0.14	0.09

[a] Provided by the supplier.
[b] Outer diameter (OD) measured by TEM (nm), $n = 100$.
[c] Surface area (A_{surf}) ($m^2\,g^{-1}$), mesopore volume (V_{meso}) ($cm^3\,g^{-1}$), and micropore volume (V_{micro}) ($m^3\,g^{-1}$) were calculated from the adsorption–desorption isotherm of N_2 at 77 K by the multipoint BET method.
[d] Ash content (%) were measured by heating the CNTs at 900 °C for 10 h.
[e] Bulk dry weight-based elemental contents (EC) (%) of the CNTs were determined using an elemental analyzer; O contents were calculated by mass difference.

2. Materials and methods

2.1. CNT properties

CNTs were purchased from Shenzhen Nanotech Co., China. They are multiwalled CNTs with outer diameters of <10 nm (MWCNT10) and 40–60 nm (MWCNT60). Selected properties of CNTs are presented in Table 1. The CNTs were synthesized by chemical vapor deposition from the CH_4/H_2 mixture at 700 °C using Ni as a catalyst. The synthesized CNTs were purified by mixed HNO_3 and H_2SO_4 solution for reducing the content of metal catalyst and amorphous carbon.

2.2. Sewage sludge sampling and properties

Sewage sludge samples were collected from four municipal-industrial sewage treatment plants (mechanical–biological treatment system). The selected treatment plants were characterised by their differentiation with catchment area as well as the industrial character of the areas (quantity and variety of industrial plants). The sludge samples (about 3 kg) were collected at endpoints of the technological line of sewage sludge digestion (non stabilized). The collected samples were stored in glass bottles and immediately transported to the laboratory after collecting. All sewage sludge samples were air-dried and milled to obtain representative samples. The physico-chemical properties of the sewage sludge samples as well as heavy metal and polycyclic aromatic hydrocarbons contents are presented in Table 2.

Table 2
The properties, heavy metals ($mg\,kg^{-1}$) and PAH ($mg\,kg^{-1}$) content in sewage sludges used in the experiment.

Properties	SL1	SL2	SL3	SL4
pH	7.2	7	5.8	6.6
TOC	253	208	229	177
N_t	36.4	40.6	40.6	39.2
CEC	725	547	1196	491
TEB	746	563	1222	513
K	2.47	3.84	3.16	3.24
P	24.1	31.5	35.4	18.2
EC	0.005	5.07	2.13	5.19
Pb	10.6	18.5	20.1	28.2
Cd	0.7	2.3	2.4	3.66
Cr	44.1	40	56.9	28.8
Cu	98.4	94.1	161	149
Ni	27	20.2	15.7	26.1
PAHs 16	9.8	4.8	14.7	27.9
Non-carcinogenic	4.30	2.17	5.08	11.9
Carcinogenic	5.49	2.59	9.62	16.0

pH – reactivity in KCl, TOC – total organic carbon content ($g\,kg^{-1}$), N_t – total nitrogen content ($g\,kg^{-1}$), CEC – cation exchange capacity ($mmol\,kg^{-1}$), TEB – the total of the exchangeable bases ($mmol\,kg^{-1}$), P and K – available forms of phosphorous and potassium ($mg\,kg^{-1}$), EC – electrical conductivity (mS/cm). All reported values are expressed on a dry weight basis of sewage sludges.

The chemical properties of sewage sludges studied were determined by standard methods. The pH was measured potentiometrically in 1 M KCl after 24 h in the liquid/soil ratio of 10 [12], the total of the exchangeable bases (TEB) and cation exchange capacity (CEC) were determined in the 0.1 M HCl extraction [12]. The total nitrogen (N_t) was determined by the Kjeldahl's method [13] without the application of Dewarda's alloy (Cu–Al–Zn alloy-reducer of nitrites and nitrates). Available potassium and phosphorus were determined according to Egner et al. [14].

2.3. Phytotoxicity testing

The Phytotoxkit microbiotest [15] measures the decrease (or the absence) of seed germination and of the growth of the young roots after three days of exposure of seeds of selected higher plants to a matrix in comparison to the controls in a reference soil. The Phytotoxkit makes use of flat and shallow transparent test plates composed of two compartments, the lower one of which contains soil saturated to the water holding capacity. Ten plant seeds (*Lepidium sativum*, *Sorghum saccharatum*, *Cucumis sativus*, *Raphanus sativus*, *Solanum lycopersicon*) were positioned at equal distance near the middle ridge of the test plate on a filter paper placed on top of the hydrated soil. After closing the test plates with their transparent cover, the test plates were placed vertically in a holder and incubated at 25 °C for 3 days. At the end of the incubation period, a digital picture was taken of the test plates with the germinated plants. The analyses and length measurements were performed using the Image Tool 3.0 for Windows (UTHSCSA, San Antonio, USA).

The percent inhibition of seed germination (GI) and root growth inhibition (RI) for plant were calculated with the formula:

$$\mathrm{GI/RI} = 100 - \left(\frac{B}{A}100\right) \qquad (1)$$

where A is the mean seed germination or root length in the OECD soil and B is the mean seed germination or root length in soil with sewage sludge or with soil with sewage sludge and CNTs.

2.4. Preparation of sewage sludge-CNT mixtures

Sewage sludges (SL1 and SL2) were spiked with CNTs (CNT10 and CNT60) at the dose 0.01%, 0.1% and 0.5% (w/w). The sewage sludge (5 g) and CNTs (depending on the dose used) were thoroughly mixed with a Teflon-coated spatula. Due to the characteristics of sewage sludges, their adaptation for phytotoxicity assay requires mixing of sewage sludge with inert material. Standard OECD soil (1984) was used as inert material. OECD artificial soil is a widely used substrate in soil toxicity tests. It has been recommended as a medium for ecotoxicological tests and it is a "reference soil" in the testing of complex solid samples (e.g. wastes or contaminated soils). OECD artificial soil is defined as a mixture of 70% fine quartz sand (50% particles 0.05–0.2 mm), 20% kaolin clay (kaolinite content preferably above 30%), and finely ground Sphag-

Table 3
Inhibition (%) of seed germination (GI) of *L. sativum* depending on the type of sewage sludge, and CNTs' outer diameter and concentration calculated on the basis of Eq. (1).

Sewage sludge	No CNTs	CNT10			CNT60		
		0.50%	0.10%	0.01%	0.50%	0.10%	0.01%
SL1	10 ± 0.9	40 ± 0.9	60 ± 5.9	10 ± 0.6	60 ± 8.0	70 ± 5.2	50 ± 1.8
SL2	0	22 ± 1.6	0	22 ± 3.4	22 ± 1.4	11 ± 1.6	0
SL3	40.0 ± 4.5	–	0.0	–	–	0.0	–
SL4	30.0 ± 3.1	–	20.0 ± 1.8	–	–	10.0 ± 0.9	–

± – relative standard deviation (%), RSD ($N = 3$).

num peat [16]. OECD soil was mixed with sewage sludge or sewage sludge-CNT mixture at a ratio of 19:1 (w/w), which represented the contribution of sewage sludge in soil at a level of 5%. Such prepared material was then used in the phytotoxicity experiment.

In the experiment with different sewage sludges, four sewage sludges (two from the first experiment named SL1 and SL2 as well as two different sewage sludges named SL3 and SL4) were used. Sewage sludges were mixed with CNTs with outer diameter (CNT10 or CNT60) at the dose of 0.1%. In experiment with different plants sewage sludges (SL2 and SL3) were mixed with CNT60 at the dose of 0.1%. In the aging experiment the sewage sludge (SL3) was mixed with CNT10 or CNT60 at the dose of 0.1% and stored in the dark for 7 or 31 days. The mixing procedures were the same as described at the beginning of this section.

3. Results

3.1. Sewage sludge characteristics

Table 2 shows the basic physical–chemical properties of the sewage sludges, the heavy metal content and the content of polycyclic aromatic hydrocarbons (PAHs). The pH of sewage sludges ranged from slightly acidic to neutral (pH 5.8–7.2). SL3 was only one sewage sludge which could negatively affect plant growth and development with regard to pH level. In most sewage sludges, the content of organic carbon did not exceed 200 g/kg. The highest level was noted for sludge SL1, while sludge SL4 had a TOC content level of 177 g/kg. The total nitrogen content (N_t) did not differ significantly among individual sludges. However, a relatively low level of potassium content and an increased level of available phosphorus were noted. The above data is typical for municipal sewage sludges. Electrical conductivity in sewage sludges did not exceed the values recommended by VLACO (3 mS/cm) [17]. All the physico-chemical parameters determined for tested sewage sludges closely reflect those values noted in the literature [18].

Threshold values of heavy metals established in the European Union [19] were not exceeded in any of the studied sewage sludges. The highest content levels of lead and cadmium were noted in the case of sludge SL4, whereas the SL3 sewage sludge had the highest content of chromium and copper (Table 2). The content of PAHs in the sewage sludges was characterised by a clear differentiation. The sum of 16 PAHs ranged from 10 to 27 mg/kg. The highest content of PAHs was noted for sewage sludge SL4. Half of this value was determined in sewage sludge SL3. In these sewage sludges a significantly higher contribution of potentially carcinogenic PAHs was also observed compared to non-carcinogenic compounds (Table 2). Taking into consideration the standards of the European Union [20] concerning the maximum content of a total of 11 PAHs in sewage sludge (6 mg/kg) for two sewage sludges (SL3 and SL4) the PAH content exceeded the allowable value.

3.2. Effect of the concentration and outer diameter of CNTs on sewage sludge phytotoxicity

Table 3 presents the influence of CNTs on seed germination (GI) of *L. sativum* depending on outer diameter and concentration of CNTs added to sewage sludges. No significant relation was noted between CNT concentration in sewage sludge and the toxic effect observed. However, in the case of both sludges SL1 and SL2 a clear negative effect of CNTs on the seed germination of *L. sativum* was observed. CNTs presence in sludge SL1 resulted in considerable inhibition of seed germination. Inhibition of seed germination

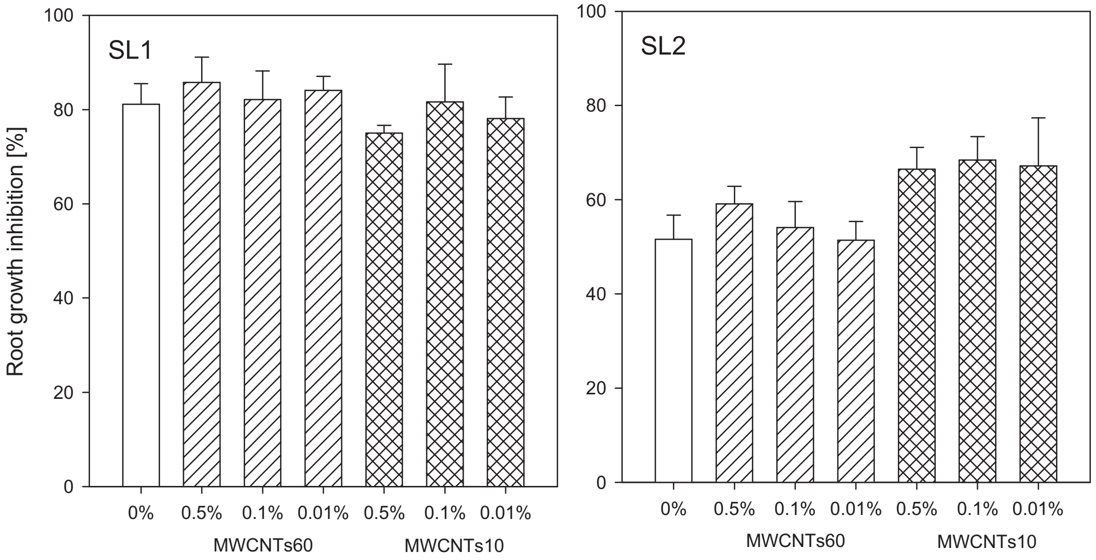

Fig. 1. *Lepidium sativum* root growth inhibition (RI) in soil amended by sewage sludge containing multiwalled carbon nanotubes. Error bars represent standard deviation error (SD, $n = 3$ determinations).

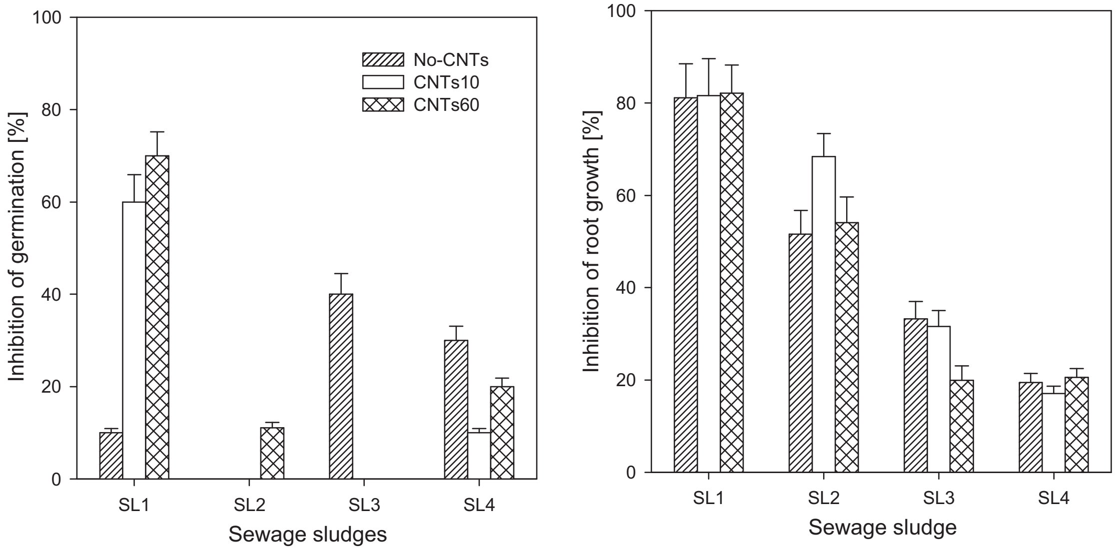

Fig. 2. Inhibition of germination (GI) and root growth (RI) of *Lepidium sativum* in different sewage sludges containing multiwalled carbon nanotubes. Error bars represent standard deviation error (SD, $n = 3$ determinations).

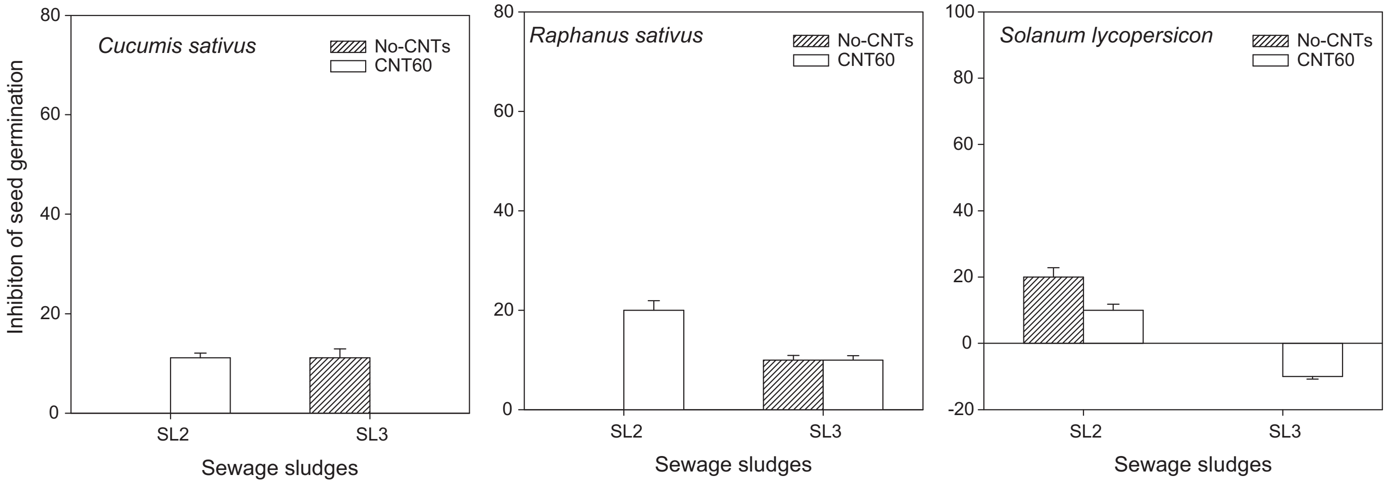

Fig. 3. Influence of CNTs on inhibition of seed germination (GI) depending on plants tested. Error bars represent standard deviation error (SD, $n = 3$ determinations).

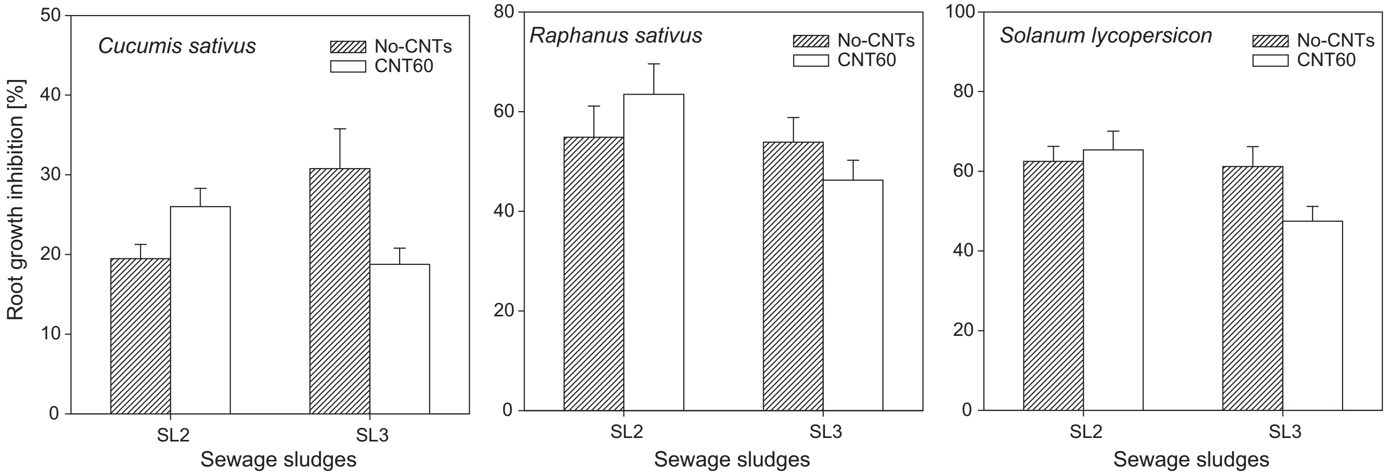

Fig. 4. Root growth inhibition (RI) in CNT-amended sewage sludge depending on plants tested. Error bars represent standard deviation error (SD, $n = 3$ determinations).

ranged from 50 to 70% in all CNT60 doses tested. Considerably lower values, at 40, 60 and 10%, were observed for CNT10 for a dose of 0.5, 0.1 and 0.01% CNTs, respectively. A significant inhibition of germination of *L. sativum* was noted in sludge SL2 which contained CNTs. The above mentioned influence was noted at a concentration of 0.5 and 0.1% in CNT60, and 0.5 and 0.01% in CNT10.

The presence of CNTs in sludge SL1 did not influence the root length (RI) of *L. sativum* (Fig. 1). Irrespective of the CNT concentration and outer diameter, no significant differences were observed with respect to root length inhibition in the OECD soil fertilised with sewage sludge and sewage sludge with the addition of CNTs. In contrast to sludge SL1, a significant influence of CNTs on root length was observed in sludge SL2 containing CNT10 (Fig. 1). As with sludge SL1, the effect observed did not depend on the CNT concentration in sewage sludge. The values observed were similar for various concentration levels, ranging from 66 to 68%. These values were about 30% higher in comparison to the soils fertilised with sewage sludge without CNTs. As in the case of sludge SL1, the addition of CNT60 to sludge SL2 did not exert any significant influence on root length inhibition (Fig. 1).

3.3. Influence of CNTs on the phytotoxicity of different sewage sludges

In order to evaluate to what degree composition and properties can determine the toxicity of sewage sludges containing CNTs, four different sludges with differentiated properties and pollutant contents were selected (Table 2). In contrast to sludges SL1 and SL2 described above, the other two sludges used for these studies (SL3 and SL4) showed a clear positive influence on the germination capacities of *L. sativum*. Moreover, a positive influence of CNT10 on root growth was observed in sludge SL3 (Fig. 2). For most of the sewage sludges, a clear influence of CNTs' outer diameter on the inhibition of seed germination and root growth was observed. Statistical analysis did not show any significant relationship between sludge properties (Table 2) and phytotoxic parameters tested (Fig. 2).

3.4. Influence of CNTs on the phytotoxicity of sewage sludge to different plants

In the present study, the influence of sewage sludges containing CNTs was tested not only on *L. sativum* but also on 4 other plant species such as: *S. saccharatum*, *S. lycopersicon*, *R. sativus* and *C. sativus*. Only in the case of *S. saccharatum*, was no significant influence of CNTs on the parameters tested (seed germination and growth inhibition) noted. Therefore, the following discussion will be focused on the results obtained for three other plants. The addition of CNTs to sewage sludges (SL2 and SL3) clearly influenced both seed germination and root growth inhibition of *S. lycopersicon*, *R. sativus* and *C. sativus* (Figs. 3 and 4). Seed germination of individual plants depended on the sewage sludge tested. CNTs presence in sludge SL2 resulted in the inhibition of germination in *C. sativus* and *R. sativus*. On the other hand, CNTs presence increased germination capacity in *S. lycopersicon* (Fig. 3). In sludge SL3, the presence of CNTs did not influence the germination capacity of *R. sativus* when compared to sewage sludge without CNTs. An increase in germination capacity in the presence of CNTs was observed for *C. sativus* and *S. lycopersicon*.

The trend observed while evaluating seed germination was then repeated in relation to almost all plants when evaluating their root growth. CNT60 presence in sludge SL2 increased its toxicity while CNTs presence in sludge SL3, as a rule, resulted in a decrease in root growth inhibition (Fig. 4). The only exception was observed in sludge SL2 for *S. lycopersicon*, in which no significant differences were observed between sewage sludge without the addition of CNTs and sewage sludge with CNTs.

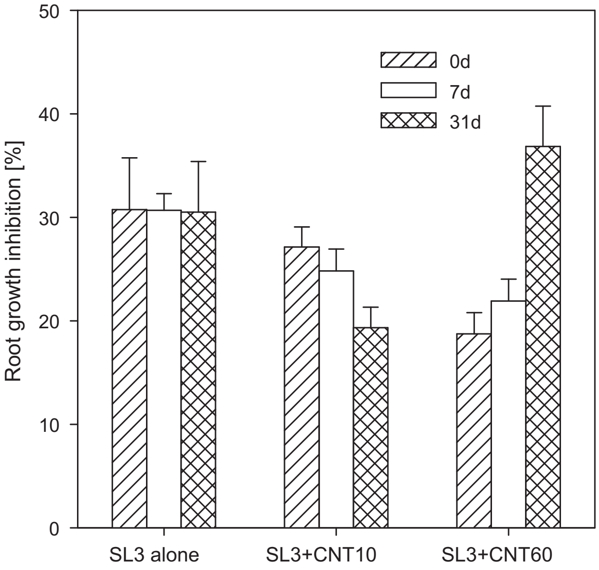

Fig. 5. Effect of CNT aging in sewage sludges on root growth (RI) of *C. sativus*. Error bars represent standard deviation error (SD, $n = 3$ determinations).

3.5. Effect of CNT aging on sewage sludge toxicity

No significant influence of CNT aging on seed germination of *C. sativus* was found (data not presented). On the other hand, CNT aging had a clear influence on the increase in root length. The observed influence depended on the outer diameter of CNTs (Fig. 5). Immediately after the addition of CNTs to sewage sludge (SL3) a significant lowering of root growth inhibition (0 d) was noted both in CNT10 and CNT60. 7-d aging resulted in further lowering of root growth inhibition in the sludge containing CNT10. However, in the case of sludge containing CNT60, a slight increase in root growth inhibition (not statistically significant) was noted in relation to CNT60 aged for 0-d. A marked aging effect was observed when CNTs were aged for 31 days (Fig. 5). CNT10 aged for 31 days resulted in a decrease in root growth inhibition of *C. sativus* of 29% in relation to CNT10 aged for 0-d. In the case of CNT60 aged for 31 d, there was a significant increase in root growth inhibition, exceeding 97% compared with the level noted for CNT60 aged for 0 d (Fig. 5).

4. Discussion

Studies on the phytotoxicity of carbon nanotubes remain scarce [9–11,21–23]. The effect of functionalized and non-functionalized single-walled carbon nanotubes on plants (cabbage, carrot, cucumber, lettuce, onion and tomato) was investigated by Canas et al. [11]. Nonfunctionalized nanotubes inhibited root elongation in tomato and enhanced root elongation in onion and cucumber. In the research carried out by Stampoulis et al. [22] multiwalled carbon nanotubes did not affect seed germination of *Cucurbita pepo*. However the biomass of plants exposed to CNTs was significantly reduced. In the above mentioned studies it is difficult to compare CNT toxicity on the plant tested due to the fact that sewage sludges can also influence phytotoxicity significantly. However, the very fact of varying toxicity of sewage sludges containing CNTs is worth noting. With respect to sludges SL1 and SL2, a visible negative influence of CNTs was observed. This influence was manifested both in the inhibition of seed germination and root growth of *L. sativum*. In the case of root growth inhibition, a statistically significant negative influence of CNTs was only noticeable for nanotubes with a diameter less than 10 nm. In none of the cases studied did the CNTs' influence on the observed plants depend on the CNT dose. Toxicity of CNTs to plants may be explained by the work of Canas et al. [11] who observed root surfaces partially covered by nanotubes, often in the format of nanotube sheets. These CNT sheets may alter the surface chemistry of the root, e.g. microbial–root interaction and nutrient uptake as well as microbial toxicity and different biochemical processes necessary for plant growth and survival [11]. Moreover Lin et al. [23] have shown that CNTs have the ability to inhibit cell growth of *Arabidopsis*. Another factor increasing sewage sludge toxicity in the presence of CNTs can be nutrient sorption by CNTs, which can lead to limitation of their bioavailability for plants. Nanomaterials are likely in an unsteady thermodynamic state, so they could change under certain conditions. Allen et al. [24] provided the evidence of the biodegradation of single walled carbon nanotubes by incubating the nanotubes with a natural horseradish peroxidase and low concentrations of H_2O_2 over 12 weeks under static conditions. In our research, CNTs were mixed with sewage sludges. CNTs could be degraded under these conditions. However extended studies are needed to examine the degradation of CNTs in the environment because biotic and abiotic alteration of CNTs will affect their environmental and toxicological behaviour.

The positive effect of CNTs observed on seed germination and root growth inhibition of sewage sludges SL3 and SL4 could be explained by a strong binding of pollutants which are potentially toxic to plants by the CNTs present in sewage sludges. Numerous studies have shown that CNTs are characterised by a strong affinity for organic pollutants [25–28] and heavy metals [29,30]. The above mentioned pollutants often occur in sewage sludges [31] and can be a factor determining sludge toxicity [4]. Hence, pollutant binding by CNTs can significantly limit their bioavailability, and consequently, also their negative influence on the living organisms including plants. Organic matter can play a role in limiting pollutant bioavailability [32]. The influence of organic carbon on pollutants sorption can already be seen when its concentration in the soil is at a level of 0.1%. It is the CNT level applied in this study. Sludges SL3 and SL4, in which there was a lowering of phytotoxicity after the addition of CNTs, were characterised by a clearly increased content of PAHs and some heavy metals (Table 2) when compared to sludges SL1 and SL2. Our earlier studies [4] showed that PAHs present in sewage sludges can negatively influence organisms. The bioavailability of these compounds plays a significant role in this process. The results presented here confirm our earlier assumptions. PAH binding by CNTs could significantly limit their bioavailability, at the same time reducing their toxic influence on plants. A similar effect on CNTs could also be observed with heavy metals. It is possible that the reduction of the toxic effect of organic pollutants observed in sewage sludges SL3 and SL4 is related to a simultaneous blocking of the toxic effect of CNTs. Naturally, further explanation of this phenomenon requires additional detailed study.

A prolonged contact of the sewage sludge and CNTs had some bearing on changes in the sewage sludge toxicity. A decrease in toxicity probably results from the sequestration of organic contaminants present in sewage sludges. Sequestration of contaminants is common in the natural environment [33,34], and organic matter plays an important role in this process [35]. In time, the contaminant becomes less available for organisms and hence, less toxic [36]. However, it is surprising that CNT60 aged for 31 days caused an increase in sludge phytotoxicity. This may suggest that a prolonged contact time between sewage sludge and some of the CNTs (in this case CNT60) influence CNT properties to a certain degree, which is then indirectly expressed by a negative influence on plants. Differences observed between CNT10 and CNT60 in their influence on phytotoxicity depending on the contact time between sludges and CNTs result from varied outer CNT diameters. The outer diameter of CNTs has a significant influence both on sorption and CNT behaviour in the environment [28]. However, explanation of the above differences calls for further study.

5. Conclusion

The study results require a complex evaluation of CNT toxicity taking into account not only their properties but also the type of matrix in which they occur. A real danger exists that as a result of industrial processes and everyday human activities, CNTs, similar to other pollutants, will become an additional component of sewage sludges. The studies presented above showed that CNT influence on sewage sludge toxicity can vary, and this deserves special attention. Depending on the sewage sludge type, CNTs can increase or reduce their toxicity. Such a differentiated influence of CNTs on sewage sludge toxicity is especially important in the conditions of sewage sludge application in the environment. Explanation of the above mentioned phenomenon, however, requires further study.

Acknowledgments

The work was funded in the frame of Grant no. N N523 616639 financed in 2010–2012 from the budget of Ministry of Science and Information Society Technologies (Poland), and by USDA NIFA MAES 00978.

References

[1] G. Bystrzejewska-Piotrowska, J. Golimowski, P.L. Urban, Nanoparticles: their potential toxicity, waste and environmental management, Waste Manage. 29 (2009) 2587–2595.
[2] M.S. Mauter, M. Elimelech, Environmental applications of carbon-based nanomaterials, Environ. Sci. Technol. 42 (2008) 5843–5859.
[3] S.K. Brar, M. Verma, R.D. Tyagi, R.Y. Surampalli, Engineered nanoparticles in wastewater and wastewater sludge – evidence and impacts, Waste Manage. 30 (2010) 504–520.
[4] P. Oleszczuk, The Tenax fraction of PAHs relates to effects in sewage sludges, Ecotoxicol. Environ. Saf. 72 (2009) 1320–1325.
[5] T.M. Scown, R. Van Aerle, C.R. Tyler, Review: do engineered nanoparticles pose a significant threat to the aquatic environment, Crit. Rev. Toxicol. 40 (2010) 653–670.
[6] S.J. Klaine, P.J.J. Alvarez, G.E. Batley, T.F. Fernandes, R.D. Handy, D.Y. Lyon, S. Mahendra, M.J. McLaughlin, J.R. Lead, Nanomaterials in the environment: behavior, fate, bioavailability, and effects, Environ. Toxicol. Chem. 27 (2008) 1825–1851.
[7] F.S. Hong, J. Zhou, C. Liu, F. Yang, C. Wu, L. Zheng, P. Yang, Effect of nano-TiO_2 on photochemical reaction of chloroplasts of spinach, Biol. Trace Elem. Res. 105 (2005) 269–279.
[8] Z. Lei, H. Fashui, L. Shipeng, L. Chao, Effect of nano-TiO_2 on strength of naturally aged seeds and growth of spinach, Biol. Trace Elem. Res. 104 (2005) 83–92.
[9] D. Lin, B. Xing, Phytotoxicity of nanoparticles: inhibition of seed germination and root growth, Environ. Pollut. 150 (2007) 243–250.
[10] D. Lin, B. Xing, Root uptake and phytotoxicity of ZnO nanoparticles, Environ. Sci. Technol. 42 (2008) 5580–5585.
[11] J.E. Canas, M. Long, S. Nations, R. Vadan, L. Dai, M. Luo, R. Ambikapathi, E.H. Lee, D. Olszyk, Effects of functionalized and nonfunctionalized single-walled carbon nanotubes on root elongation of select crop species, Environ. Toxicol. Chem. 27 (2008) 1922–1931.
[12] M. Misztal, H. Smal, A. Wójcikowska-Kapusta, Lithosphere and Its Protection, Wydawnictwo AR, Lublin, 1997.
[13] L.P. van Reeuwijk, Procedures for Soil Analysis, ISRIC, Wageningen, 1995.
[14] J. Drozd, M. Licznar, S.E. Licznar, J. Weber, Soil Science with Mineralogy and Petrography Elements, Wydawnictwo AR, Wroclaw, 1998.
[15] Phytotoxkit, Seed germination and early growth microbiotest with higher plants, in: Standard Operation Procedure, MicroBioTests Inc., Nazareth, Belgium, 2004, pp. 1–24.
[16] J. Hofman, I. Hovorková, J. Machát, Comparison and Characterization of OECD Artificial Soils, Springer, 2009.
[17] VLACO, Vlamse Organisatie ter bevordering van de Afzet van Groenten Fruit Tuin en Groencompost, Aktiviteitenverslag, Mechelen, Belgium, 1995.
[18] E. Epstein, Land Application of Sewage Sludge and Biosolids, Lewis, Boca Raton, 2003.
[19] C.C.o.t.E. Communities, Directive of 12 June 1986 on the protection of the environment and in particular of the soil, when sewage sludge is used in soil, Brussels, 1986.
[20] UE, European Union Draft Directive on Sewage Sludge, UE, Brussels, 2000, pp. 1–20.

[21] C.W. Lee, S. Mahendra, K. Zodrow, D. Li, Y.C. Tsai, J. Braam, P.J.J. Alvarez, Developmental phytotoxicity of metal oxide nanoparticles to *Arabidopsis thaliana*, Environ. Toxicol. Chem. 29 (2010) 669–675.
[22] D. Stampoulis, S.K. Sinha, J.C. White, Assay-dependent phytotoxicity of nanoparticles to plants, Environ. Sci. Technol. 43 (2009) 9473–9479.
[23] C. Lin, B. Fugetsu, Y. Su, F. Watari, Studies on toxicity of multi-walled carbon nanotubes on *Arabidopsis* T87 suspension cells, J. Hazard. Mater. 170 (2009) 578–583.
[24] B.L. Allen, P.D. Kichambare, P. Gou, I.I. Vlasova, A.A. Kapralov, N. Konduru, V.E. Kagan, A. Star, Biodegradation of single-walled carbon nanotubes through enzymatic catalysis, Nano Lett. 8 (2008) 3899–3903.
[25] P. Oleszczuk, B. Pan, B. Xing, Adsorption and desorption of oxytetracycline and carbamazepine by multiwalled carbon nanotubes, Environ. Sci. Technol. 43 (2009) 9167–9173.
[26] K. Yang, L. Zhu, B. Xing, Adsorption of polycyclic aromatic hydrocarbons by carbon nanomaterials, Environ. Sci. Technol. 40 (2006) 1855–1861.
[27] D.H. Lin, B. Xing, Adsorption of phenolic compounds by carbon nanotubes: role of aromaticity and substitution of hydroxyl groups, Environ. Sci. Technol. 42 (2008) 7254–7259.
[28] B. Pan, B. Xing, Adsorption mechanisms of organic chemicals on carbon nanotubes, Environ. Sci. Technol. 42 (2008) 9005–9013.
[29] K. Pyrzyńska, M. Bystrzejewski, Comparative study of heavy metal ions sorption onto activated carbon, carbon nanotubes, and carbon-encapsulated magnetic nanoparticles, Colloid Surf. A 362 (2010) 102–109.
[30] Y.C. Sharma, V. Srivastava, V.K. Singh, S.N. Kaul, C.H. Weng, Nano-adsorbents for the removal of metallic pollutants from water and wastewater, Environ. Technol. 30 (2009) 583–609.
[31] S.R. Smith, Organic contaminants in sewage sludge (biosolids) and their significance for agricultural recycling, Philos. Transact. R. Soc. A 367 (2009) 4005–4041.
[32] L. Beesley, E. Moreno-Jiménez, J.L. Gomez-Eyles, Effects of biochar and greenwaste compost amendments on mobility, bioavailability and toxicity of inorganic and organic contaminants in a multi-element polluted soil, Environ. Pollut. 158 (2010) 2282–2287.
[33] M. Alexander, Aging, bioavailability, and overestimation of risk from environmental pollutants, Environ. Sci. Technol. 34 (2000) 4259–4265.
[34] B. Xing, J.J. Pignatello, Mechanisms of slow sorption of organic chemicals to natural particles, Environ. Sci. Technol. 30 (1996) 1–11.
[35] R.G. Luthy, G.R. Aiken, M.L. Brusseau, S.D. Cunningham, P.M. Gschwend, J.J. Pignatello, M. Reinhard, S.J. Traina, W.J. Weber Jr., J.C. Westall, Sequestration of hydrophobic organic contaminants by geosorbents, Environ. Sci. Technol. 31 (1997) 3341–3347.
[36] R.H. Anderson, N.T. Basta, R.P. Lanno, Partitioning species variability from soil property effects on phytotoxicity: ECx normalization using a plant contaminant sensitivity index, J. Environ. Qual. 37 (2008) 1701–1709.