



PHYTOTOXICOLOGICAL TESTS - APPLICATIONS OF FOILS BASED ON GRAPHENE (GRAPHENE OXIDE)

Petra ROUPCOVÁ¹, Hana KUBÁTOVÁ², Karel KLOUDA³, Petr LEPÍK⁴

Research article

Abstract: This paper discusses the problematics of phytotoxicity of chemicals. It mainly focuses on the phytotoxicity of nanomaterials made of graphene. It describes phytotoxicological tests performed with foils from materials belonging to the graphene family. It also describes testing the influence of plants on these films. Furthermore, the paper discusses the issues of mutual influence between plants and tested nanomaterials.

Keywords: Phytotoxicity, substances made from graphene, graphene oxide, white mustard, elongation of the root.

Introduction

We use ecotoxicity tests to determine or estimate the effects of chemicals on individual constituents of the environment. Where the tests relate to the toxic effects of chemicals on plants, we call them phytotoxicity tests. Phytotoxicity is expressed as harmful deviations from the normal appearance and growth of plants on the basis of exposure to the chemical. These deviations are determined both by measurement and visual assessment. (OECD, 2006) Due to the fact that germination and early growth stages are a critical stage of plant development and in this period the plants are sensitive to exposure to chemicals, the phytotoxicity tests with terrestrial plants are focused only on the stage of germination, root elongation and growth of seedlings. (OECD, 2006; Fletcher et al., 1991; Ratsch, 1983) The advantages of phytotoxicity tests are their simplicity, versatility, material and inexpensiveness. Variability refers to the species of tested plants and the method of application of the tested substance. Variability, however, might also bring some problems e.g. the results obtained for certain plant species could not always be fully extrapolated to other species. Therefore it is always necessary to carefully choose the criteria for selecting species of tested plants. Generally speaking, the proposed test format

(not just the plant species, but also the method of application of the tested substance, pH or salinity) can significantly affect the interpretation of results. (OECD, 2006)

Phytotoxicity tests are used to evaluate the effects of chemicals on the environment by many organizations, e.g. the Organization for Economic Cooperation and Development (Organization for Economic Cooperation and Development, OECD), the European and Mediterranean Plant Protection Organisation (European and Mediterranean Plant Protection Organization, EPPO), the United States Environmental Protection Agency (United States Environmental Protection Agency, EPA) or the United States Food and Drug Administration (Food and Drug Administration, FDA). These organizations have created their own lists of testing plants (both monocots and dicots), and agree on e.g. the use of the following species: maize (*Zea mays*), oats (*Avena sativa*), ryegrass (*Lolium perenne*), lettuce (*Lactuca sativa*) and carrot (*Daucus carota*). The other importance of phytotoxicity tests is the fact that they are required at registration or re-registration of commercially produced chemicals.

Just as industrially produced substances can negatively affect the growth and development of plants, the plants may also affect industrially produced substances and materials which interact

¹ VŠB - Technical University of Ostrava, Faculty of Safety Engineering, Ostrava, Czech Republic, petra.roupcova@vsb.cz

² State Office for Nuclear Safety, Senovazne nam. 9, Praha, hana.kubatova@sujb.cz

³ VŠB - Technical University of Ostrava, Faculty of Safety Engineering, Ostrava, Czech Republic, karel.klouda@vsb.cz

⁴ VŠB - Technical University of Ostrava, Faculty of Safety Engineering, Ostrava, Czech Republic, petr.lepik@vsb.cz

with them. Degradation processes caused or conditionally effected by living organisms are known as biodegradation or biocorrosion. This article not only deals with the possible impact of substances from the graphene group on plants but also the action of the plants on these compounds.

Exotoxicity of substances made of graphene

The graphene group (*graphene family*) are compounds derived from graphene - the foil consisting of only one layer of carbon atoms arranged in a hexagon (2D) isolated from graphite (3D). Many studies have investigated the toxic effects of nanomaterials from graphene. Detailed research studies (Seabra et al., 2014; Guo et al., 2014; Jastrzebska et al, 2015) summarize recent findings regarding toxic effects and possible mechanisms of toxicity and conclude that graphene, graphene oxide (GO) and reduced graphene oxide (rGO) cause toxic effects in tests both *in vitro* and *in vivo*. Interpreted results of toxicity vary and depend on many factors such as the physical appearance of the tested substance (solid phase, suspension, surface, dimensions, concentrations in the suspension, etc.), the method of preparation (pollution by chemical preparation or reduction), the environment in which the toxicity test was made (water, soil, aerosol), or the biological substance on which the tests were performed (bacteria, green algae, nematodes, crustaceans, mammalian cells, etc.). For example, for GO in an aqueous environment the following data about its toxicity have been published:

- toxic to crustaceans *Amphibalanus Amphitrite* (Mesarič et al, 2013);
- toxic to microbial communities in wastewater (Ahmed et al., 2013);
- non-toxic to bacteria *Shewanella oneidensis* (Wang, G. et al., 2011);
- slightly toxic to zebrafish embryos (*Danio rerio*) (Liqiang et al., 2012);
- non-toxic to zebrafish embryos (*Danio rerio*) (Zhou et al., 2012);
- both toxic and non-toxic to broad bean seeds (*Vicia faba*) (Anjum et al., 2014).

From this brief summary of published results we can see some disunity of outputs. Interaction with living systems is affected by the concentration of the tested substance, the functional groups on its surface, particle size, exposure time, type of exposed cells (Seabra et al., 2014) and in our opinion also the purity of the tested substance. All of these variables can affect the results of toxicity tests.

Phytotoxicity of graphene and GO was analysed on the basis of tests carried out on seeds and seedlings of tomato, cabbage, spinach and red lettuce. (Begum et al., 2011) Concentrations of 0, 500 mg/L, 1000 mg/L and 2000 mg/L were used for the tests; exposure time was 20 days. The test results show that at the highest concentration a significant reduction in the growth, number and size of sheets was observed in all plants, except salad, while increasing the production of reactive oxygen species (*Reactive oxygen species*, ROS) and necrotic symptoms.

Similar tests were performed by Anjum et al. on broad bean seeds (*Vicia faba*). The seeds were exposed to different concentrations of GO suspension (the suspensions contained particles of 0.5-5 μ formed of a single bilayer of GO). GO suspension was tested at concentrations ranging from 0 to 1600 mg/L. The study revealed both positive and negative influences on the growth parameters. (Anjum et al., 2014) A positive effect was detected at concentrations of GO at 400 and 800 mg/L. For other concentrations, both lower and higher, the effect on growth parameters of the beans was negative. The negative impact was indicated by a decline in growth parameters, increased activity of enzymes decomposing hydrogen peroxide and an increased level of leakage (a violation of the stability of cell membranes), elevated levels of hydrogen peroxide and oxidation of lipids and proteins. These results demonstrate the difficulty in interpreting phytotoxicity results. The question remains, why did the indicated concentrations of GO optimize the physiological process of germination and growth?

Plant effect on substances made of graphene

As mentioned above, the functional groups on the surface of graphene do affect the interaction of living systems with substances made of graphene. In the case of GO there are active oxygen functional groups (e.g. carbonyl, carboxyl, epoxy or hydroxyl) that can react with a variety of biological materials. The reaction of these functional groups of GO is the principle of ecological preparation of graphene using a so-called *green reduction* of GO, thus reducing GO to graphene by using substances that are not harmful to the environment. (Agharkar et al., 2014; Wang, Y. et al., 2011a) The reduction is usually carried out in order to restore electrical conductivity and at the same time there is a change of other properties of GO.

The reason for the use of *green reduction* of GO is the fact that classical reducing agents are often toxic or explosive. In classical chemical reduction,

hydrides, salts of hydrazine, oxygen, hydrohalide acid, sodium bisulphite and the like are used as reducing agents, (Agharkar et al., 2014) whereas during *green reduction* of GO phytoextracts are used, from leaves, stems, fruit or bark of plants including agricultural crops. (Wang, Y. et al., 2011; Kuila et al., 2012; Haighighi et al., 2013).

The *green reduction* of GO is used in cases where the intention is to create a reduced GO with varying ratios of C/O used in medicine (Guo et al., 2014; Li at al., 2016) or in tissue engineering (Ku et al., 2013), e.g. as a carrier for drugs, cytostatics, biosensors, etc. (An et al., 2013; Pan et al., 2012; Liu et al., 2010; Wang, Y. et al., 2011 b). The green reduction utilizes the ability to form a physical (π - π , electrostatic interaction), or chemical conjugate between carbon nanomaterial and the biomolecule (peptide, protein, DNA, but also bacteria, mammalian cells, etc.). This procedure is chosen to unclog other chemicals (inorganic) in the molecule of GO or to create a partially reduced GO.

Chemicals produced by plants ("plant chemicals") may be used for the reduction of GO, such as ascorbic acid (vitamin C) or tannic acid. (Luo et al., 2016; Bo et al., 2014) A large number of plant chemicals (Agharkar et al., 2014; Thakur et al., 2012) (e.g. ascorbic acid, gallic acid, caffeic acid, vitamin E, luteolin, apigenin, tannins, flavonoids and others) have a polyphenolic structure that allows transition to the stable quinones associated with the reduction of the oxygen functional groups of GO. We assume an S_N2 nucleophilic attack by an oxygen anion of polyphenols on the carbon of the oxygenated group. (Xu et al., 2015)

In the extract from fenugreek seeds (*Trigonella foenum-graecum*) the ability to jointly reduce both the GO and ions of palladium to Pd nanoparticles was described, to form a common composite Pd-rGO. (Singh et al., 2016) Similarly, the reduction of GO and silver ions was reported on a common composite Ag-rGO using an extract of *Pulicaria glutinosa* (Al-marri et al., 2015) (a plant of the genus husk). GO reduction was also caused by the pollen extract of the exotic ornamental tree *Peltophorum pterocarpum*. (Rahman et al., 2014) By reduction of *Ginkgo biloba* (Gurunathan et al., 2014) rGO which had no toxic properties was prepared, while it was biocompatible with human tumour cells.

Phytotoxicity tests of foils made of graphene

Phytotoxicity of substances made of graphene was tested by a contact germination seed test and seedlings with GO foils "The film/foil is a thin layer

of small, partially overlapping slices (flocks) of the individual materials used. These slices are mutually connected by physical and chemical bonds." and its hybrid compounds: GO-fluorine graphite (GO-CF), GO- fullerene C_{60} (GO- C_{60}) and GO-Biochar. Preparation and identification of the films of GO and its hybrid compounds was described in our previous publications. (Gembalová et al., 2016; Roupcová et al., 2016) These films can be differently stratified from each other and combined among each other. It is also possible to prepare the joint foil with nanofibers. For testing of phytotoxicity we have not solely used films made of graphene but we have also tested joint foils with nanofibers of polycaprolactone (PCL). Inside the films prepared from GO and its hybrids we identified by means of IR spectra the consistent presence of oxygenated functional groups (the foil GO-CF has an extra bond C-F) which with help of hydrogen bridges and π - π interactions can form layered materials, see Figure 1. Simultaneously we characterized the foils during their process of thermal stability and during their decomposition, which is similar for the tested foils, see Figure 2. Characterization of the films is discussed in meticulous details in different papers. (Friedrichová et al., 2016) The layered structure of the films is evident from the cuts in Figure 3.

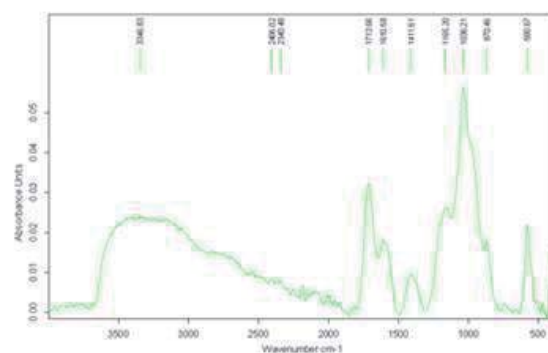


Fig. 1 FT-IR spectrum of the foil GO- Biochar

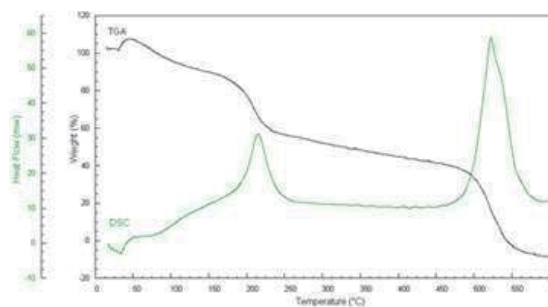


Fig. 2 Thermal Analysis of the foil GO-Biochar (TGA and DSC curves)

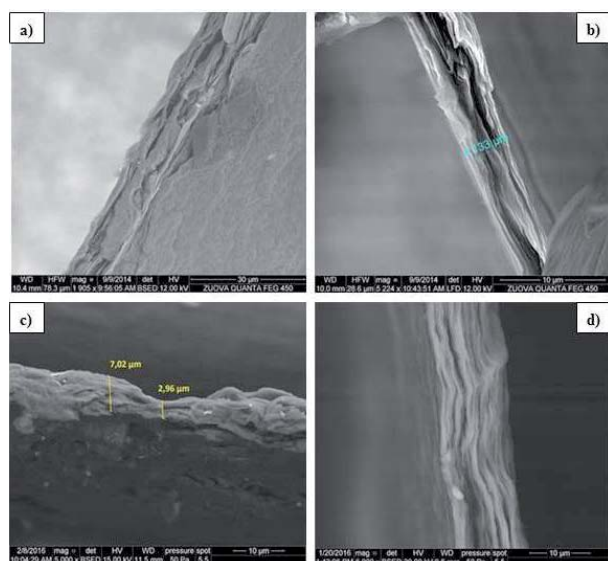


Fig. 3 SEM cuts of foils: GO (a), GO-C₆₀ (b), GO-CF (c), GO-Biochar (d)

Materials and methods

For the pilot test to determine phytotoxicity of the film made of graphene we chose a contact germination seed test and a test of inhibition of root growth. White mustard (*Sinapis alba* L.) was chosen as a test organism. The tests were made according to the modified procedure given in Appendix no. 1 of the Waste Department Guideline to determine the ecotoxicity of waste (ČR, 2007 a). For a control sample 20 seeds were spaced out on Petri dishes lined with moistened filter paper. For the test samples, due to the limited size of the prepared films, 20 x 20 mm sheets were inserted in each dish. 10 seeds were placed straight onto the foils and in the area around the film 10 seeds were evenly spaced similarly to the control sample, see Fig. 4. The seeds were incubated in the dark at 21°C for 72 hours.

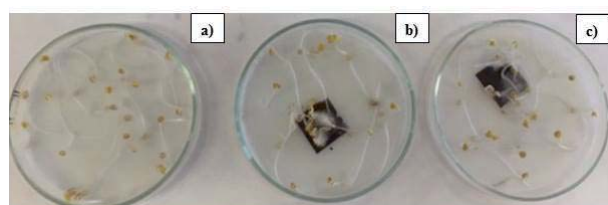


Fig. 4 Germination seed test on *Sinapis alba* L.; blank determination (a), parallel determination for the foil GO-CF (b, c)

Results and discussion

A double determination has always been done for each type of film. The value of the root growth inhibition for seeds placed on the film and beyond the foil was calculated based on measuring the length of root (root elongation) of seedlings by computing the relation (1).

$$IC = \frac{(L_c - L_v)}{L_c} \cdot 100 \quad (1)$$

where: IC - is the root growth inhibition [%], L_c - is the arithmetical average length of the control roots [mm], L_v - is the arithmetical average length of roots in the test solution [mm]. (ČR, 2007 a; ČR, 2007 b)

Tab. 1 The average values of germination seeds test and root growth inhibitions (IC)

Material	Average weight of foils [g]	Average seed germination on/outside foil [%]	Average IC of seeds on foils* [%]	Average IC of seeds outside foils* [%]
foil GO-C ₆₀	0.00979	100/100	75.00	-12.50
foil GO-CF	0.00220	100/100	69.12	7.76
foil GO-Biochar	0.01045	95/95	70.02	33.34
foil C ₆₀ -GO-PCL	0.01705	100/95	65.77	20.85
foil GO-C ₆₀ -PCL	0.02305	90/95	71.16	10.46
foil GO	0.00709	95/95	22.33	-32.52

As can be seen from Tab. 1 and from the graphs in Figure 5, the films which least affected root growth were the foils prepared from GO. Other films slowed elongation of the root to a much greater extent and their inhibitory effect is substantially comparable. For seeds placed in tested samples outside the film no significant inhibitory effect was observed except for the film GO-Biochar. Around the foils GO-C₆₀ and GO there was even a positive effect on root growth, which was also the most significant for films prepared from GO. Positive and negative effects on the seeds placed in tested samples outside the foil are in accordance with the results of the experiments described by Anjumem et al. (Anjum et al., 2014). A positive effect can be explained by leach of material from the foil. In a pilot test, however, only a limited number of seeds have been used and the results are particularly informative.

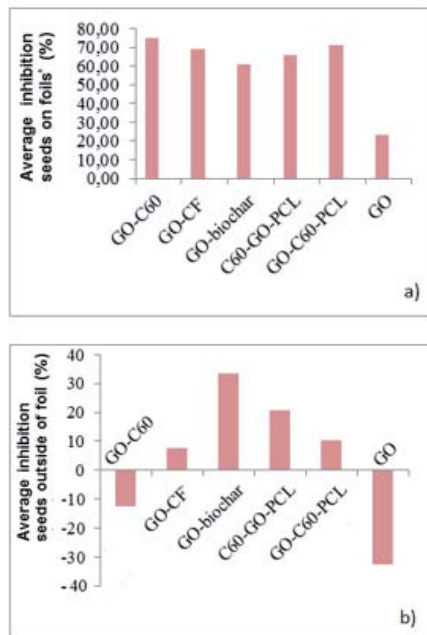


Fig. 5 Graphical expression of the average root growth inhibition for the seeds placed on the film (a) and outside the film (b)

We have observed morphological changes after the removal of seedlings from the foils that were used for the phytotoxicity tests. See Figures 6 and 7. The changes that have occurred are evident even on cuts of pieces of the foils. See Figure 8.

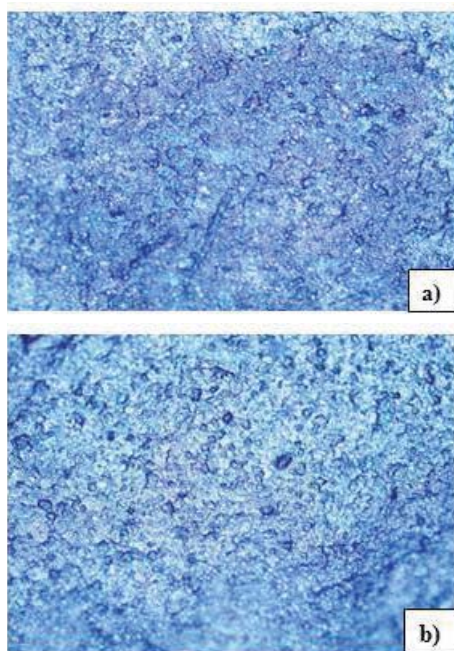


Fig. 6 Microscopic image of the foil GO-CF, 10x zoomed: in places where there was seed (a), no seed (b)

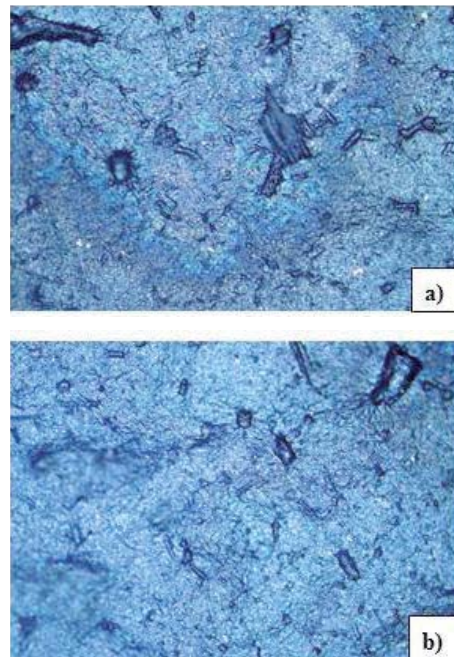


Fig. 7 Microscopic image of the foil GO-Biochar, 10x zoomed: in places where there was seed (a), no seed (b)

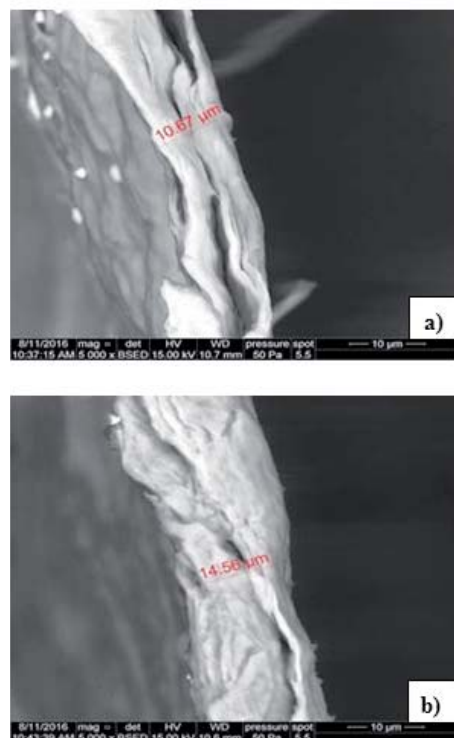


Fig. 8 SEM cuts of foils, in a places where the seed was located (a) and in a place located away from the seed (b)

The effect of extract from white mustard seeds on foils made of graphene

As indicated above, the extract from fenugreek seeds (*Trigonella foenum-graecum*) has the ability to reduce GO (Singh et al., 2016). Fenugreek seeds like mustard seeds are used in the food industry as spices and are referred to as natural antioxidants. If we compare the composition of the seeds and extracts prepared from seeds, it can be stated that they contain plenty of similar substances and display antioxidant activity (Dixit et al., 2014; Řezníčková et al., 2014). Based on this fact we assume that an extract of germinating seeds of white mustard would be able to reduce GO in a similar way to fenugreek seed extract.

The seeds of white mustard were suffused with distilled water and allowed 48 hours to germinate. Subsequently the germinated seeds were crushed in the mortar, suffused with distilled water and extracted for 24 hours at room temperature. The extract was used for our experiments as the pure filtered state. The foil of GO-Biochar (2:1) was chosen for testing; 10 x 30 mm sheets of these foils were used. The films were placed in Petri dishes and were topped with distilled water (blank determination), suspension of extract and the filtrate extract (using 10 mL of each). See Figure 9. The films were then incubated for 72 hours at 6°C. Subsequently the films were removed from the Petri dishes and dried at 35°C.

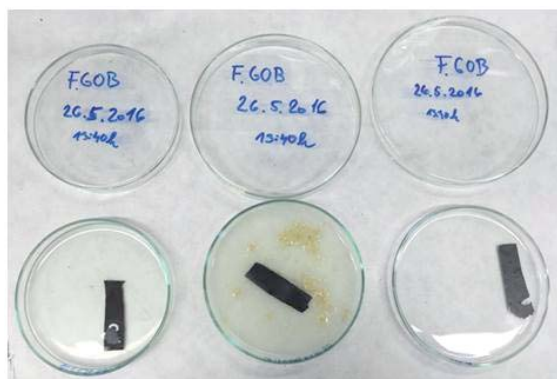


Fig. 9 Arrangement of the experiment focused on the influence of the extract from the seeds on foils of substances from a graphene; from the left, film was put in distilled water, in the pure extract from the seeds, and in the filtrate extract

In the extract from the incubated seeds, apparent morphological changes were visible even to the human eye. These changes were made manifest by enlargement under the microscope. The changes are clear from the microscopic images, see Figure 10. The effect of substances extracted from germinating

seeds was damage to the surface structure of the foils.

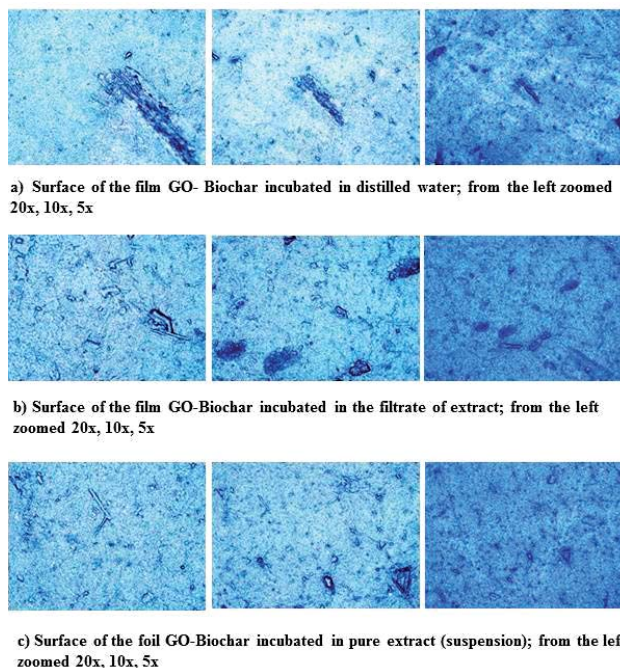


Fig. 10 Comparisons of the film surfaces of GO-Biochar incubated in distilled water (a), in the filtrate extract (b), and in the pure extract (suspension) (c) at various extensions

Conclusion

The results of our experiments confirmed the variability of the results in published studies, even if they are used for testing just one type of tested organism (plants) and examine the influence of one chemical. The results show that phytotoxicity is dependent on the particle size of the tested substance, its concentration and its way into the organism. When considering the impact of nanomaterials from the graphene group on terrestrial plants, we should also consider the fact that these substances interact with biomolecules such as proteins, carbohydrates, lipids and nucleic acids (Rodrigues-Gonzales et al., 2015). Derivatives of graphene, for example, adsorb proteins to form bioconjugates which destabilize lipid membranes (Ku et al., 2013). Similarly, there are on the surface of the graphene derivatives which strongly adsorbed single-stranded DNA and RNA (Rodrigues-Gonzales et al., 2015), which could be one of the causes of the genotoxicity of nanomaterials.

The toxic effect of substances from the graphene group can be explained by the increased production of ROS induced by its presence at both the surface

and interior of the cells of the tested organism. Besides, the increased production of ROS, whose appearance is created by hydrogen peroxide, may also cause a massive leakage of electrolytes. It indicates that oxidative stress occurs. At the same time, there is visible damage in the form of necrotic lesions (Begum et al., 2011; Aslani et al., 2014). The formation of ROS in contact with substances from the graphene group could be associated with a hypersensitive reaction as the response of plant cells to infection by pathogenic microorganisms. During this reaction, increased ROS production stands at the beginning of programmed cell death (Govrin et al., 2000; Heath, 2000).

On the basis of the results of experiments described in publications concerning *green reduction*, it can be presumed that a similar reduction reaction occurred even during the processes of our experiments. In places where the seeds and roots of seedlings were in contact with the foils, the morphological changes were evident at first sight. This was also confirmed by microscopic images of the foils. Extract from the germinating seeds had a similar effect on the film

as the seeds themselves. Therefore it is obvious that the chemicals produced by germinating plants react with substances which belong to the graphene family, and change their character. The question is whether its long-term effect may be the initial stage of the biodegradation process. All the tests carried out so far were short in duration and took place under conditions that rarely resemble the real potential fate of industrially manufactured nanomaterials in the environment.

Acknowledgements

This article has been created within the Student Grant Competition "Assessment of the environmental impact of selected nanomaterials and products of thermal degradation on materials after contact with water in eco-toxic terms". Project number SP2016 / 92.

The authors furthermore thank the team who have contributed to this article: A. Fojtikova, L. Gembalova, J. Pavlovsky and R. Jones.

References

- AGHARKAR, M.; KOCHREKAR, S.; HIDOURI, S.; AZEEZ, M.A. (2014).: *Trends in green reduction of graphene oxides, issues and challenges: a review*. Material Research Bulletin (59). p. 323-328.
- AHMED, F.; RODRIGUES, D.F. (2013).: *Investigation of acute effects of graphene oxide on wastewater microbial community: a case study*. J Hazard Mater. p. 256-257:3-39.
- AL-MARRI, A.H.; KHAN, M.; KHAN, M.; ADIL, S.F.; AL-WARTHAN, A.; ALKHATHLAN, H.Z.; TREMEL, W.; LABIS, J.P.; SIDIQI, M.R.H.; TAHIR, M.N. (2015).: *Pulicaria glutinosa Extract: A Toolbox to Synthesize Highly Reduced Graphene Oxide-Silver Nanocomposites*. Int. J. Mol. Sci. 16. p. 1131-1142.
- AN, J.; GOU, Y.; YANG, C. et al. (2013).: *Synthesis of biocompatible gelatin functionalized graphene nanosheets and its application for drug delivery*. Mater SciEng C Mater BiolAppl. (33), p. 2827-2837.
- ANJUM, N.A.; SINGHT, N.; SINGHT, M.K.; SAYEED, I.; DUARTE, A.C.; PEREIRA, E.; AHMAD, I. (2014).: *Single-bilayer graphene oxide sheet impacts and underlying potential mechanism assessment in germinating faba bean (Vicia faba L.)*. Science of the Total Environment, Vol. 472, p. 834-841.
- ASLANI, F.; BAGHERI, S.; MUHD, JULKAPLI, N.; JURAIMI, A.S.; HASHEMI, F.S.; BAGHDADI, A. (2014).: *Effects of engineered nanomaterials on plants growth: an overview*. ScientificWorldJournal.2014:641759. doi: 10.1155/2014/641759.
- BEGUM, P.; IKHTIARI, R.; FUGETSU, B. (2011).: *Graphene phytotoxicity in the seedling stage of cabbage tomato, red spinach and lettuce*. Carbon. Vol. 49, I: 12, p. 3907-3919.
- BO, Z.; SHUAI, X.; MAO, S.; YANG, H.; QIAN, J.; CHEN, J.; YAN, J.; CEN, K. (2014).: *Green preparation of reduced graphene oxide for sensing and energy storage applications*. Scientific reports 4, 4684.
- ČR (2007 a).: Metodický pokyn odboru odpadů ke stanovení ekotoxicity odpadů. [Methodical instruction of Waste Department to determine the ecotoxicity of waste.] *Věstník Ministerstva životního prostředí, ročník XVII, částka 4.* (in Czech)
- ČR (2007 b).: ČSN EN 14735 - Charakterizace odpadů - Příprava vzorků odpadu pro testy ekotoxicity [Characterization of waste - Preparation of waste samples for ecotoxicity tests]. (in Czech)
- DIXIT, P.; GHASKADBI, S.; MOHAN, H.; DEVASAGAYAM, T.P. (2005).: *Antioxidant properties of germinated fenugreek seeds*. Phytother Res. 19(11), p. 977-983.

- FLETCHER, J.S.; RATSCH, H.C. (1991).: *Plant tier testing: A workshop to evaluate non target plant testing in Subdivisions*. J.Pesticide Guidelines. EPA/600/9-91/041. Corvallis, OR.
- FRIEDRICHOVÁ, R.; KLOUDA, K.; WEISHEITELOVÁ, M.; ROUPCOVÁ, P. (2016).: Tepelná stabilita biocharu a jeho modifikovaných verzí [Thermal Stability of Biochar and its Modifications], In: *Fire protection 2016: Transactions of the contributions of the international conference*. Ostrava: SPBI, 2016, p-76-85. ISBN 987-80-7385-177-4. (in Czech)
- GEMBALOVÁ, L.; KLOUDA, K.; ROUPCOVÁ, P.; RUSÍN, J.; PRYSZCZ, A.; WEISHEITELOVÁ, M. (2016).: Biochar- ekologický product a jeho uplatnění v ochraně životního prostředí. [Biochar- ecological Product and its Application in Environmental Protection], In: *Civil protection- Medical Rescue Work in Protection of population 2016: Transactions of the contributions of the international conference*. Ostrava: SPBI, 2016, p. 24-31. ISBN 978-80-73-85-171-2. (in Czech)
- GOVRIN, E.M.; LEVINE, A. (2000).: *The hypersensitive response facilitates plant infection by the necrotrophic pathogen Botrytis cinerea*. Curr Biol. 10(13), p. 751-757.
- GUO, X.; MEI, N. (2014).: *Assessment of the toxic potential of graphene family nanomaterials*. JFDA: 22, p. 105-115.
- GURUNATHAN, S.; HAN, J.W.; PARK, J.H.; EPPAKAYALA, V.; KIM, J.H. (2014).: *Ginkgo biloba: a natural reducing agent for synthesis of cytocompatible graphene*. Int J Nanomedicine. 9. p. 363-377.
- HAIGHIGHI, B.; TABRIZI, M.A. (2013).: *Green synthesis of reduced graphene oxide nanosheets using rose water and a survey on their characteristics and applications*. RSC Adv. 3, p. 13365-13371.
- HEATH, M.C. (2000).: *Hypersensitive response-related death*. Plant Mol. Biol. 44(3), p. 321-333.
- JASTRZEBSKA, A.M.; OLSZYNA, A.R. (2015).: *The ecotoxicity of graphene family materials: current status, knowledge gaps and future needs*, J Nanopart Res. 17:40.
- KU, S.H.; LEE, M.; PARK, C.B. (2013).: *Carbon-based Nanomaterials for Tissue Engineering*. Adv. Healthcare Mater. 2, p. 244-260.
- KUILA, T.; BOSE, S.; KHANRA, P.; A. MISHRA, K.; KIM, N.H.; LEE, J.H. (2012).: *A green approach for the reduction of graphene oxide by wild carrot root*. Carbon 50, p. 914-921.
- LI, H.; FIERENS, K.; ZHANG, Z.; VANPARIJS, N.; SCHUIJS, M.J.; VANSTEENDAM, K.; GRACIA, N.F.; RYCKE, R.D.; BEER, T.D.; BEUCKELAER, A.D.; KOKER, S.D.; DEFORCE, D.; ALBERTAZZI, L.; GROTEN, J.; LAMBRECHT, B.N.; GEEST, B- G.D. (2016).: *Spontaneous Protein Adsorption on Graphene Oxide Nanosheets Allowing Efficient Intracellular Vaccine Protein Delivery*. Appl. Mater. Interfaces 8 (2) p. 1147-1155.
- LIQIANG, C.; PINGPING, H.U.; LI, Z.; SIZHOU, H.; LINGFEI, L.; CHENGZHI, H. (2012).: *Toxicity of graphene oxide and multi-walled carbon nanotubes against human cells and zebrafish*. Sci China Chem 55, p. 2209-2216.
- LIU, Y.; YU, D.; ZENG, C. et al. (2010).: *Biocompatible graphene oxide-based glucose biosensors*. Langmuir (26), p. 6158-6160.
- LUO, J.; LAI, J.; ZHANG, N.; LIU, Y.; LIU, R.; LIU, X. (2016).: *Tannic Acid Induced Self- Assembly of Three-Dimensional Graphene with Good Adsorption and Antibacterial Properties*. ACS Sustainable Chem. Eng. 4 (3), p. 1404-1413.
- MESARIČ, T.; SEPČIČ, K.; PIAZZA, V.; GAMBARDELLA, C.; GARAVENTA, F.; DROBNE, D.; FAIMALI, M. (2013).: *Effects of nano carbon black and single-layer graphene oxide on settlement, survival and swimming behavior of Amphibalanus Amphitrite larvae*. Chem Ecol. 29. p. 643-652.
- OECD (2006).: *Test No. 208: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test*. OECD Publishing, Paris.
- PAN, Y.; SAHOO, N.G.; LI, L. (2012).: *The application of graphene oxide in drug delivery*. Expert Opin Drug Deliv. (9) 1365-1376.
- RAHMAN, O.S.A.; CHELLASAMY, V.; PONPANDIAN, N.; ARMIRTHAPANDIAN, S.; PANIGRAHI, B.K.; THANGADURAI, P. (2014).: *A facile green synthesis of reduced graphene oxide by using pollen grains of Peltophorum pterocarpum and study of its electrochemical behavior*. RCS Adv. 4. 56910-56917.

- RATSCH, H.C. (1983).: *Interlaboratory root elongation testing of toxic substances on selected plant species*. EPA/600/3-83/051. U.S. Environmental Protection Agency, Corvallis, OR.
- ROUPCOVÁ, P.; KLOUDA, K.; PAVLOVSKÝ, J. (2016).: Příspěvek k monitorování ekotoxicity u uhlíkatých nanočástic na bázi grafenu. [Contribution to the Monitoring of Ecotoxicity of Carbon Nanoparticles Based on Graphene] In *Occupational health and safety 2016: Transactions of the contributions from the international conference*. Sepetná: SPBI, 2016,p. 74-77. ISBN 978-80-7385-175-0. (in Czech)
- ŘEZNÍČKOVÁ, P. (2014).: *Olejnata semena, bakalářská práce* [Oilseeds, bachelor thesis], LF, Masarykova univerzita Brno. (in Czech)
- RODRIGUES-GONZALES, C.; BRIZUELA-COLMENARES, N.; SALAS, P.; KHARISSOVA, O.V.; CASTANO, V.M. (2015): Chapter 25. Graphene Oxide: Grafting Biomolecules onto Graphene Oxide Sheets. In *CRC Concise Encyclopedia of Nanotechnology*. CRC Press 2015, p. 285-303 ISBN 978-1-4665-8034-3.
- SEABRA, A.B.; PAULA, A.J.; DE LIMA, R.; ALVES, O.L.; DURAN, N. (2014).: *Nanotoxicity of Graphene and Graphene Oxide*. Chem. Res. Toxicol., 27 (2), pp 159-168.
- SINGH, C.; ALI, Md. A.; SUMANA, G. (2016).: *Green synthesis of graphene based Biomaterial Using Fenugreek Seeds for lipid Detection*. ACS Sustainable Chem. Eng., 4 (3), p. 871-880.
- THAKUR, S.; KARAK, N. (2012).: *Green reduction of graphene oxide by aqueous phytoextracts*. Carbon (50), p. 5331-5339.
- WANG, G.; QUIAN, F.; SALTIKOW, C.W.; JIAO, Y.; LI, Y.: (2011).: *Microbial reduction of graphene oxide by Shewanella*. Nano Res 4, p. 563-570.
- WANG, Y.; SHI, Z.; YIN, J. (2011 a).: *Facile Synthesis of Soluble Graphene via a Green Reduction of Graphene Oxide in Tea Solution and Its Biocomposites*. ACS Appl. Mater. Interfaces 3 (4), p. 1127-1133.
- WANG, Y.; ZHAOUHI, L.; WANG, J.; JINGHONG, L.; YUEHE, L. (2011 b).: *Graphene and graphene oxide: biofunctionalization and applications in biotechnology*. Trends in Biotechnology Vol. 29, No. 5, p. 205-212.
- XU, C.; SHI, X.; JI, A.; SHI, L.; ZHOU, C.; CUI, Y. (2015).: *Fabrication and Characteristics of Reduced Graphene Oxide Produced with Different Green Reductants*. PLoS One. 10(12):e0144842. doi: 10.1371/journal.pone.0144842. eCollection 2015.
- ZHOU, X.; LAROCHE, F.; LAMERS, G.E.M.; TORRACA, V.; VOSKAMP, P.; LU, T.; CHU, F.; SPAINK, H.P.; ABRAHAMS J.P.; LIU, Z. (2012).: *Ultra - small graphene oxide functionalized with polyethylenimine (PEI) for very efficient gene delivery in cell and Zebrafish embryos*. Nano Res 5: 2012.