EFFECTIVITY APLICATION OF NANOMATERIAL CEO_2 INTO SOIL CONTAMINATED BY CHLORINATED COMPOUNDS

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Abstract
In recent years, more and more studies focused on research into various applications of nanomaterials in contaminated soil to remove pollutants. In this study, we conducted research on the impact of the application of nanomaterials CeO_2 in soil contaminated with chlorinated pesticides. The influence of the presence of nanoparticles on the toxicity and the concentration of pollutants into the soil were studied. The results indicate reduction of chlorinated compounds in the soil.

Key words:
Nanomaterials, CeO_2, ecotoxicity, chlorinated compounds

Introduction
Increasing amounts of xenobiotic and potentially harmful substances released into the environment is a major environmental problem. Many of these pollutants are chlorinated substances that are very persistent, bioaccumulative and toxic, and it is not easy to remove them from the environment. In recent years, many studies have been published on the use nanoiron just to remediation purposes (e.g. Yang and Chang, 2011; Hosseini and Tosco, 2015). However, many new studies describes that nanoiron may be harmful for the different organisms and cause them e.g. oxidative stress (Sacco et al., 2014; Rocha et al., 2015). Another of nanomaterials, which falls due to its properties for the removal of chlorinated substances from the environment is CeO_2.

Cerium oxide exhibits high thermal stability and is practically insoluble in water, but it can be dissolved in strong mineral acids. It is paramagnetic (Vohlídal et al., 1999). Some studies have shown that $nCeO_2$ acts as a great antioxidant and it may, for certain systems, a low toxic effect of this substance (Tourino, et al., 2015). CeO_2 is used in UV filter, as an additive in paints and coatings, in fuel cells, as an additive for fuels, etc. Regarding ecotoxicity, $Ce0_2$ has been previously tested on plants and microorganisms. The results indicated that CeO_2 had no effect on plant growth of corn, soybeans, rice and tomatoes. It was found, however, that this nanomaterial accumulate in the roots and other plant parts and thus getting into the edible parts (Rui et al., 2015). On the other hand, $nCeO_2$ remained in the roots of monocots (Zhao et al., 2015). Also proved negative effect on some strains of aerobic and anaerobic bacteria, other strains but not. It was also observed sorption on the surface of bacteria of the genus *Escherichia coli* (Thill et al., 2006).

Methods
In this work was used the so-called method for the preparation of nanostructured material of CeO_2 in the aqueous phase when cerium sulphate is reacted with an aqueous ammonia (25%), which is added dropwise while stirring, to produce cerium oxide. The particles then have a size range of 1-2 nm. To gain larger nanoparticles was used dimethylformamide (DMF) and dimethyl sulfoxide (DMSO). This has provided a size 4 and 7 nm CeO_2. The particle size was determined by XRD analysis (Poštulka, 2013).

The natural soil purchased in building materials was used as a test soil, which have been artificially contaminated with chlorinated compounds mixture at a concentration of 1 g/kg. PH and a maximum water capacity of the soil (WHC) was analysed. WHC was then wetted to its 70% by distilled water (control) or with a solution of cerium oxide. Into the soil with a height of 2-3 cm were then deployed pre-germinated seeds of lettuce varieties Sapphire - ten pieces per each test vessel. From each concentration and control were prepared in three replicates. Test wells were capped and placed in an incubator under continuous light (600-1000 lux) and temperature (22 ^{0}C). The test lasted for five days. After exposure, the plants were pulled out with tweezers lettuce from the soil and for each plant was measured by ruler root length in mm. From the obtained data was calculated root growth inhibition compared to control. The plants were then transferred to test tubes with methanol to extract chlorophyll that took place in the dark in the refrigerator for several hours. The amount of chlorophyll was then determined spectrophotometrically at wavelengths 666 nm and 653 nm. Obtained values were substituted into the following formulas (Laboratorní návod č. 4, 2015).

$$CChl\ a = 15.65 \times A666 - 7.34 \times A653 \quad (1)$$
$$CChl\ b = 27.05 \times A653 - 11.21 \times A666 \quad (2)$$
$$CChl\ a{+}b = (CChl\ a + CChl\ b) \times 10 \quad (3)$$

where *CChl a = concentration of chlorophyll a, CChl b = concentration of chlorophyll a*

After removing the plants from the each soil sample, part of the soil sample was collected from each test vessel for the analytical determination of α-δ HCB, HCH and PCBs (28, 52, 101). Before the determination, it was necessary sample (2.5 g) extract in n-hexane (10 mL). Extraction took place under normal conditions in an ultrasonic bath for 20 min to allow the successful extraction of soil pesticides. The separation of any solids was n-hexane then centrifuged in a centrifuge (4500 rpm, 10 min). From this sample, 10 L was collected in pre-sealed vials with 1 ml of n-hexane and analyzed by GC-ECD detection (provide other research group).
All statistical data processing was done in this work using GraphPad InStat®. Tukey-Kramer test (difference is significant when the P value less than 0.05) was used. For the removal of outliers was used Dean Dixon test (Q-test).

Results

Analytical determination of HCH, HCB and PCB (Tab. 1) after exposure to $nCeO_2$ show that there was a significant loss of these pesticides (efficacy in average always above 50%).

Tab. 1: Removal efficiency (%) of HCH, HCB and PCB.

	HCB	sum HCH	sum PCB
The addition of CeO_2 (4 nm) in a mixture of soil contaminated with chlorinated substances			
10 mg/kg	90.7 ± 10.7	61 - 89	83 - 90
100 mg/kg	89.3 ± 9.7	63 - 89	85 - 91
The addition of CeO_2 (7 nm) in a mixture of soil contaminated with chlorinated substances			
10 mg/kg	89.6 ± 4.1	62 - 88	84 - 91
100 mg/kg	90.4 ± 5.3	57 - 90	84 - 91

Other results indicate that there was no difference in the amount of chlorophyll in plants from the soil, where the nanomaterial CeO_2 was present in comparison with the soil contaminated with pesticides alone. Inhibition compared with the control in all the tested variants were around 50%. Lettuce root growth was reduced compared to control variation and statistically significant difference in this case was shown, although not very noticeable due to the relatively large variance in the values of the sample where the soil was added CeO_2 size of 7 nm (Tab. 2). In samples with nanomaterial he was also found branched root.

Tab. 2: Inhibition (%) concentration of chlorophyll ($\mu g.cm^{-3}$) and the root growth of lettuce (mm) compared with the control.

sample	chlorophyll	SD	root	SD
The addition of CeO_2 (4 nm) in a mixture of soil contaminated with chlorinated substances				
10 mg/kg	54.2	1.9	38.7	7.5
100 mg/kg	52.0	4.0	45.3	11.1
The addition of CeO_2 (7 nm) in a mixture of soil contaminated with chlorinated substances				
10 mg/kg	48.2	11.7	24.4	15.0
100 mg/kg	46.6	1.9	44.4	3.7
Soil contaminated with a mixture of chlorinated compounds without the addition of CeO_2 (control)				
Contaminated soil	44.2	0	-40.5	0

Soil spiked by cerium oxide only was tested before performing this experiment. The minimal inhibition of root growth of lettuce (max. 20%) after the addition of one or other the size of prepared CeO_2 (Poštulka, 2015) was found. However, results from the previous (Poštulka, 2015) and the current study cannot be unambiguously compared because in these experiments were used different soils.

Soil tested in a present experiment was mainly clayey nature with a very fine grain. Thanks to this, after moistening soil became very plastic to solid. This had an impact on the results of the test, especially on the shape and length of the root. Root salad had tended to grow over the surface of the soil, due to the high resistance that appeared to be its plasticity. The underground portion of the root in this case at first sight more robust and tended in the presence CeO_2 divide. It could be a defense mechanism in which the plant is trying to enlarge the area from which it can extract water and nutrients.

Conclusion

This work was aimed at studying the behavior of organochlorine compounds in the presence of cerium oxide nanoparticles through ecotoxicological tests. To evaluate the ecotoxicological effects pesticide mixture was used test root growth of lettuce (*Lactuca sativa*) and determining the amount of chlorophyll in plants. It showed a significant decrease in chlorinated pesticides in soil containing nanoparticles of cerium oxide. The results also suggest that the presence of nanoparticles CeO_2 in soil may affect plant growth, despite a previous experiment (Poštulka, 2015) demonstrated a rather neutral or stimulatory effects of the application itself $nCeO_2$ to clean soil. Influence on the amount of chlorophyll was not demonstrated. The similar studies dealing with antagonistic effects of nanoparticles in soil spiked with chlorinated pesticides have not been published and this work is to present a new field of research. In any case, it is advisable to continue testing ecotoxicity of soils with different physico-chemical properties after the addition of CeO_2 nanoparticles in connection with the (bio) remediation potential.

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