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学 位 論 文 審 査 の 要 旨

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学 位 論 文 題 名

Studies on phytotoxicities of carbon nanomaterials in seedling stage
（植物の実生段階における炭素ナノ材料の毒性評価に関する研究）

The candidate, in this study, had evaluated phytotoxicities of carbon nanomaterials (CNMs) in seedling stage of several selected plants. Graphene oxide (GO) and multiwall carbon nanotubes (MWNTs) typify a class of CNMs; those materials, in fact, have been widely involved in the so-called nanotechnology based products. The uncontrolled utilization of CNMs is concerned to rise contributions for negative consequences related to their environmental exposure to human, animal and plant. Therefore the biological effects of CNMs have been evaluated intensively and experimental data suggested that the studies of their environmental fate are very important in the field of nano-toxicology.

The candidate had firstly studied the detrimental effects of GO on root and shoot growth, biomass, shape, cell death, and reactive oxygen species (ROS) of cabbage, tomato, red spinach, and lettuce with a concentration range from 500 to 2000 mg/L. Morphological and physiological data indicated that after 20 days of GO exposure plant growth and biomass in comparison with the control groups were inhibited. The number and size of leaves of the GO-treated plants were reduced in a dose-dependent manner. Furthermore, a concentration dependent manner was observed for ROS (reactive oxygen species); identical trends were also observed for cell death as well as the visible symptoms of necrotic lesions. The GO-induced adverse effects on cabbage, tomato, and red spinach was attributed to the oxidative stress necrosis induced by ROS. However, little or no toxic effect was observed for lettuce seedlings under the identical experimental conditions. The toxic effect of GO was found to be largely dependent on the dose, exposure time, and the plant species. The candidate had then evaluated phytotoxicities with MWNTs as the typical CNMs. It was reported in previous studies that MWCNTs are toxic to plants, but the potential impacts of exposure remain unclear. In this study, the candidate selected red spinach, lettuce, rice, cucumber, chili, lady's finger, and soybean as the targeted plants with MWNTs at 0, 20, 200, 1000, and 2000 mg/L as the exposure doses. The toxicity was evaluated based on root and shoot growth, cell death, and electrolyte leakage at the seedling stage. After 15 days of the hydroponic culture, the root and shoot lengths of red spinach, lettuce, and cucumber were significantly reduced with the exposure to

1000 mg/L and 2000 mg/L MWNTs. Identical trends in cell death and electrolyte leakage were also observed. Red spinach and lettuce were most sensitive to MWNTs, followed by rice and cucumber. However, very little or no toxic effects were observed for chili, lady's finger, and soybean. For further evaluation of the toxic mechanism, the candidate had cultured the selected plants using Hoagland's Media treated with MWNTs hydroponically. After two weeks, toxic symptoms for plants exposed with MWNTs were observed and the toxicity was also found in a dose-dependent manner. Microscopic observation found that MWNTs attached firmly on the root surface area; epidermical injury, root tip damage and uptake of MWNTs into plant cells were also observed. Through the *in situ* detection of hydrogen peroxide, the candidate suggested a ROS-induced model to explain the possible toxic mechanism of CNMs involved in the plant seedling stage: CNMs induce the over production of ROS through oxidative stress mechanism which visibly detected as necrotic lesions.

In conclusion, the candidate showed familiarity with, and critical understanding of the relevant literatures; the methods adopted were appropriate to the subject matter and properly applied; the research findings were suitable set out, accompanied by adequate exposition and discussion, and the quality of English and the general presentation were satisfactory. The recommendation of the examination committee was that "the degree be awarded".