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博士（環境科学）Ikhtiari Refi

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)

学 位 論 文 内 容 の 要 旨

Carbon nanomaterials (CNMs) which represented by graphene oxide (GO) and multiwall carbon nanotubes (MWNTs) have been widely applied in nano science and industry. The uncontrolled utilization of CNMs was supposed to give contribution for negative consequences related to their environmental exposure to human, animal and plant. Therefore the biological effects of CNMs have been evaluated intensively and urgently suggested that the studies of their environmental fate would be very important in the field of nano-toxicology.

The effects of GO on root and shoot growth, biomass, shape, cell death, and reactive oxygen species (ROS) of cabbage, tomato, red spinach, and lettuce, were investigated using a concentration range from 500 to 2000 mg/L. The results of the combined morphological and physiological analyses indicate that after 20 days of exposure under our experimental conditions, GO significantly inhibited plant growth and biomass compared to a control. The number and size of leaves of the GO-treated plants were reduced in a dose-dependent manner. Significant effects also were detected showing a concentration dependent increase in ROS and cell death as well as visible symptoms of necrotic lesions, indicating GO-induced adverse effects on cabbage, tomato, and red spinach mediated by oxidative stress necrosis. Little or no significant toxic effect was observed with lettuce seedlings under the same conditions. The potential effect of GO largely depends on dose, exposure time, and plant species.

Abundant experimental data have shown that MWNTs are toxic to plants, but the potential impacts of exposure remain unclear. The objective of the present study was to evaluate

the possible phytotoxicity of MWNTs at 0, 20, 200, 1000, and 2000 mg/L with red spinach, lettuce, rice, cucumber, chili, lady's finger, and soybean based on root and shoot growth, cell death, and electrolyte leakage at the seedling stage. After 15 days of hydroponic culture, the root and shoot lengths of red spinach, lettuce, and cucumber were significantly reduced following exposure to 1000 mg/L and 2000 mg/L MWNTs. Similar toxic effects occurred regarding cell death and electrolyte leakage. Red spinach and lettuce were most sensitive to MWNTs, followed by rice and cucumber. Very little or no toxic effects were observed for chili, lady's finger, and soybean.

Further, we investigated the phytotoxicity of MWNTs on lettuce regarding inhibition effect on plant growth development as well as cell damage on root and leaves. We have cultured the plant seedling of lettuce using Hoagland's Media treated with MWNTs hydroponically. After two weeks, we observed the toxic symptoms in the presence of MWNTs in a dose-dependent manner as compared to carbon black (CB). Statistically, MWNTs could inhibit the seed germination, plant growth and plant biomass. Microscopic analysis showed the attachment of MWNTs on the root surface area, epidermical injury, root tip damage and an image of MWNTs uptake into plant cell. We also showed that MWNTs caused cell death on root and leaves. *In situ* detection of hydrogen peroxide has proposed the reactive oxygen species (ROS) for mechanism of toxic effect of MWNTs.

In general conclusion, at low concentration, CNMs may not negatively affect all crop species. In the other hand, at high concentration, CNMs could induce the over production of ROS through oxidative stress mechanism which visibly detected as necrotic lesions.