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Carboxylated multi-walled carbon nanotubes exacerbated oxidative damage in roots of *Vicia faba* L. seedlings under combined stress of lead and cadmium

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ABSTRACT

Multi-walled carbon nanotubes (MWCNTs) and heavy metals could be absorbed and bioaccumulated by agricultural crops, implicating ecological risks. Herein, the present study investigated the ecotoxicological effects and mechanisms of individual carboxylated MWCNTs (MWCNTs-COOH) (2.5, 5.0 and 10 mg/L) and their combination with 20 μM Pb and 5 μM Cd (shortened as Pb + Cd) on roots of *Vicia faba* L. seedlings after 20 days of exposure. The results showed that the tested MWCNTs-COOH induced imbalance of nutrient elements, enhanced isozymes and activities of superoxide dismutase (SOD), guaiacol peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX), resulting in accumulation of carbonylated proteins, elevation of endoproteases (EPs) isozymes, and reduction of HSP70 synthesis in the roots. However, the tested MWCNTs-COOH facilitated the enrichment of Cd, Pb and Na elements, contributing to the decrease of SOD, CAT and APX activities, and the reduction of HSP70 synthesis, whereas the elevation of carbonylated proteins, EP activities and cell necrosis in the roots when Pb + Cd was combined in comparison to the treatments of MWCNTs-COOH, or Pb + Cd alone. Thus, the tested MWCNTs-COOH not only caused oxidative stress, but also aggravated the oxidative damage in the roots exposed to Pb + Cd in the culture solution.

1. Introduction

Carbon nanotubes (CNTs) are a type of manufactured nanomaterials and are generally grouped into single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) (Vaisman et al., 2006). MWCNTs have many potential industrial applications due to their unique physicochemical properties (Stark and Stoessel, 2015; Vlaanderen et al., 2017), and they are more widely applied than SWCNTs by virtue of their facile synthesis. Moreover, surface functionalization of MWCNTs can change their chemical and physical properties, and hence possess much better solubility and dispersion in medium than non-functionalized ones (Wang et al., 2014). Additionally, functionalization of MWCNTs has better biocompatibility and stronger effect on organisms (Zhang et al., 2015). Thus, carboxylated MWCNTs (MWCNTs-COOH), as representative of functionalized-MWCNTs, have gained increasing attention of researchers (Chen and Hsiue, 2013; Entezari et al., 2014; Yan et al., 2016).

Rapid and broad application of MWCNTs in industrial fields has led to their continuous input into the environment, and thus increasing attention has been paid to the ecological safety of MWCNTs (Khodakovskaya et al., 2012; Long et al., 2012; Simon-deckers et al., 2009; Zhao and Liu, 2012). Moreover, the residue of MWCNTs can sorb and interact with heavy metals owing to their high specific surface area (Boncel et al., 2015; Pyrzyñska and Bystrzejewski, 2010; Sharma et al., 2009). MWCNTs and heavy metals uptake together by plants could be bioaccumulated and biomagnified along the food chain (Zhao et al., 2017), eventually leading to potential risks for the environmental, ecological systems and public health. However, the relevant studies are still limited.

Exposure to MWCNTs or heavy metals has been confirmed to cause reactive oxygen species (ROS) burst in organisms (Patlolla et al., 2011). To alleviate the occurrence of oxidative damage, organisms have developed antioxidant enzymes, including superoxide dismutase (SOD), guaiacol peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APX) (Mittler, 2002), which work together with other enzymes to promote the scavenging of ROS and keep them at a low level. In addition, each of these antioxidant enzymes has distinct isozymes, cooperating to protect organelles and minimize tissue injury in plants

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Table 1
Contents of Cd, Pb and some mineral nutrient elements in the roots*.

Treatments	K content (μg/g)	Mg content (μg/g)	Mn content (μg/g)	Na content (μg/g)
Control	39429.8 ± 6872.7[a]	899.1 ± 128.3[abc]	13.61 ± 1.96[ab]	2109.9 ± 303.9[bc]
2.5 mg/L MWCNTs-COOH	37432.6 ± 5777.6[ab]	972.4 ± 120.0[ab]	12.84 ± 2.13[ab]	2052.1 ± 320.0[bc]
5.0 mg/L MWCNTs-COOH	32641.2 ± 5480.2[abc]	1037.4 ± 167.9[a]	14.45 ± 2.53[a]	2499.4 ± 400.3[ab]
10 mg/L MWCNTs-COOH	30423.9 ± 5616.0[abcd]	882.2 ± 119.5[abc]	11.12 ± 1.98[b]	2722.3 ± 437.7[a]
Cd + Pb*	28014.2 ± 5601.0[bcde]	610.0 ± 104.2[d]	5.10 ± 0.96[c]	1578.8 ± 299.3[c]
Cd + Pb + 2.5 mg/L MWCNTs-COOH	22027.9 ± 3521.4[de]	668.4 ± 107.0[cd]	5.79 ± 1.00[c]	1672.1 ± 277.5[c]
Cd + Pb + 5.0 mg/L MWCNTs-COOH	20250.5 ± 3570.0[e]	782.6 ± 125.7[bcd]	6.91 ± 1.23[c]	1719.2 ± 264.5[c]
Cd + Pb + 10 mg/L MWCNTs-COOH	25637.7 ± 4863.4[cde]	681.1 ± 127.5[cd]	7.26 ± 1.23[c]	1788.8 ± 306.1[c]

Treatments	Cu content (μg/g)	Fe content (μg/g)	P content (μg/g)	Zn content (μg/g)
Control	60.88 ± 10.00[ab]	725.4 ± 105.0[ab]	4514.0 ± 745.3[a]	226.4 ± 39.4[a]
2.5 mg/L MWCNTs-COOH	57.58 ± 10.28[ab]	679.9 ± 117.0[ab]	4453.7 ± 534.5[a]	265.7 ± 40.4[a]
5.0 mg/L MWCNTs-COOH	65.00 ± 10.21[a]	751.8 ± 123.7[ab]	4707.4 ± 688.1[a]	249.2 ± 46.4[a]
10 mg/L MWCNTs-COOH	43.92 ± 8.01[b]	565.0 ± 102.9[b]	3997.3 ± 522.2[a]	203.4 ± 33.5[ab]
Cd + Pb*	47.91 ± 9.80[ab]	789.4 ± 127.1[a]	2600.2 ± 454.8[b]	152.9 ± 25.5[bc]
Cd + Pb + 2.5 mg/L MWCNTs-COOH	48.07 ± 7.61[ab]	711.8 ± 115.0[ab]	2334.0 ± 340.7[b]	162.9 ± 25.3[bc]
Cd + Pb + 5.0 mg/L MWCNTs-COOH	53.04 ± 10.56[ab]	617.2 ± 105.4[ab]	2581.7 ± 434.4[b]	144.7 ± 25.8[bc]
Cd + Pb + 10 mg/L MWCNTs-COOH	55.33 ± 13.22[ab]	542.8 ± 115.5[b]	2721.8 ± 471.8[b]	132.6 ± 25.4[c]

Treatments	Ca content (μg/g)	Cd content (μg/g)	Pb content (μg/g)
Control	4185.5 ± 587.6[a]	Not Detected	Not Detected
2.5 mg/L MWCNTs-COOH	3904.8 ± 457.2[a]	Not Detected	Not Detected
5.0 mg/L MWCNTs-COOH	4286.5 ± 416.5[a]	Not Detected	Not Detected
10 mg/L MWCNTs-COOH	3745.9 ± 557.1[a]	Not Detected	Not Detected
Cd + Pb*	3441.7 ± 562.3[a]	92.55 ± 12.72[b]	1256.7 ± 220.0[a]
Cd + Pb + 2.5 mg/L WCNTs-COOH	3563.2 ± 477.7[a]	98.30 ± 14.60[b]	1126.4 ± 146.5[a]
Cd + Pb + 5.0 mg/L WCNTs-COOH	3507.1 ± 519.7[a]	133.48 ± 22.91[a]	1299.3 ± 174.9[a]
Cd + Pb + 10 mg/L MWCNTs-COOH	3628.6 ± 560.4[a]	150.27 ± 23.63[a]	1332.6 ± 184.1[a]

*Cd + Pb denotes 20 μM Pb + 5 μM Cd, the same letter are not significantly different, $p < 0.05$.

(Mittler, 2002). Among antioxidant enzymes, SOD converts super oxide radicals ($O_2^{\cdot-}$) to hydrogen peroxide (H_2O_2) and oxygen (O_2), and then H_2O_2 is reduced to H_2O and O_2 by CAT, POD and APX in cytoplasm and other cellular compartments (Nahakpam and Shah, 2011; Xu et al., 2015). However, ROS overproduction may exceed the capacity of antioxidant defense systems and then damage cellular components, such as proteins, resulting in oxidatively damaged proteins by directly oxidation of amino acid side chains (Habib et al., 2014; Romero-Puertas et al., 2002; Stadtman and Levine, 2003). When the damaged proteins accumulated in cells, they could impair cell division and might lead to cell death (Davies and Shringarpure, 2006).

In a previous study, we confirmed that MWCNTs-COOH aggravated biochemical and subcellular damages in leaves of *Vicia faba* L. seedlings under combined stress of lead (Pb) and cadmium (Cd) (Wang et al., 2014). Plant roots, directly adsorbing contaminants in soil or water, have differential physiological properties and defense mechanisms compared with leaves (Wang et al., 2011). Thus, roots of *Vicia faba* L. seedlings were preferentially employed as the tested materials in present experiments. The objectives were to (1) investigate the impact of MWCNTs-COOH on uptake of Cd, Pb and mineral nutrient elements, (2) determine the synergistic toxicity and related mechanisms of MWCNTs-COOH on alterations in antioxidant and defense responses, oxidatively damaged proteins, and detoxification, as well as in cell death and growth in roots of *Vicia faba* L. seedlings under combined stress of Cd and Pb. To the best of our knowledge, data concerning the combined effects of MWCNTs-COOH and heavy metals on roots of crop seedlings are still very limited.

2. Materials and methods

2.1. MWCNTs-COOH properties, pretreatment and culture solution preparation

MWCNTs-COOH was purchased from Chengdu Organic Chemicals Co. Ltd., Chinese Academy of Sciences. The properties, pretreatment, and culture solution preparation of the MWCNTs-COOH were the same as previously described (Wang et al., 2014). The final treatments includes control (Hoagland solution, 0.5 ×), 2.5 mg/L MWCNTs-COOH, 5 mg/L MWCNTs-COOH, 10 mg/L MWCNTs-COOH, 20 μmol L^{-1} Pb + 5 μmol L^{-1} Cd (abbreviated as Pb + Cd below), Pb + Cd + 2.5 mg/L MWCNTs-COOH, Pb + Cd + 5 mg/L MWCNTs-COOH, and Pb + Cd + 10 mg/L MWCNTs-COOH. The pH values were maintained between 5.5 and 5.8, and zeta potentials were ranged at − 10.92 ± 1.09 mV.

2.2. Plant material and exposure

The sterilization, pregermination and culture of *V. faba* L. seeds were the same as previously reported by Wang et al. (2014). The seedlings were cultured under controlled conditions (15-hr photoperiod with active radiation of 240 μmol m^{-2} s^{-1}, 75% relative humidity, and 22 °C/20 °C day/night regime), and aerated consecutively. Each treatment was conducted in triplicate, and 24 containers were employed in each of two independent experiments. The solutions were renewed every three days. Roots were harvested after exposure of 20 days for tests as below.

2.3. Determination of root lengths and cell death in root tips in situ

Root lengths were measured from stem base to primary root tips. Root necrosis was detected according to method described by Turner and Novacky (1974). The stained root tips were photographed with microscope (Leica, BX51, Japan).

2.4. Measurement of metal contents by ICP-OES

Contents of Cd, Pb and mineral elements were determined by ICP–OES (Perkin-Elmer, Optima 5300 DV) according to method of Wang et al., (2008, 2010), and expressed as μg g^{-1} dry weight. Certified standard samples (GBW07429) and triplicates of all samples were used to ensure accuracy and precision. Detection limit of 0.02 μg L^{-1}