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# Accumulation of phenanthrene and its metabolites in lettuce (*Lactuca sativa* L.) as affected by magnetic carbon nanotubes and dissolved humic acids

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**Synthesis of magnetic CNTs**

The CNTs were immersed in 1M acidic solution ($H_2SO_4$: $HNO_3$; *v/v* =3:1) for 24 h and then sonicated for 20 min.[1] The homogeneous mixture was heated at 100 °C in water bath for 40 min. After cooling, the CNTs were washed and brought to pH 7.0 and then dried for 12 h at 100 °C in oven.

Two hundred milligrams of acidified CNTs, 200 mg of $FeCl_2$ and 400 of mg $FeCl_3$ were mixed in a 200 mL flask containing 80 mL deionized water.[1] The mixture was sonicated for 30 min and heated with stirring at 70 °C, while 6% $NH_3 \cdot H_2O$ (30 mL) was adding to the mixture drop-by-drop. The magnetic ferric oxides were slowly formed and precipitated onto the surface of CNTs under the alkaline heating environment. The magnetic CNTs were then washed with deionized water until the pH was stable at 7.0 and dried for 24 h at 50 °C in oven.

**Sample cleanup**

The silica gel column consisted of the following: from the bottom to top was filled with 2 g silica, 0.5 g graphitized carbon black and 1 g anhydrous sodium sulfate. Combined extracts were filtered through the silica gel column followed by 10 mL hexane to elute impurities. Then using 15 mL hexane-dichloromethane (v:v=7:3) to elute and collect the solvent fractions. The eluent was concentrated under nitrogen waiting for Phe and metabolites determination.

**Photosynthesis analysis**

Lettuce were dark-adapted for 20 min before measurement with IMAGING- PAM.[2, 3] First, a leaf fixed in the clip was used to record the chlorophyll fluorescence kinetic parameter

$F_v/F_m$ (the maximum quantum efficiency of PSII in the dark-adapted state). Then the leaf was measured with 120s illumination periods gradually increasing in a sequence from 0 to 2430 μmol photons $m^{-2}$ $s^{-1}$. The parameters of the rapid light curve were measured: $\Phi_{PSII}$- practical quantum efficiency of PSII; $F_v/F_m$- maximum quantum efficiency of PSII; qP- intensity of photochemical quenching coefficient (Swamp model); qL- intensity of photochemical quenching coefficient (Lake model); Y(NO)- Non-regulatory energy dissipation coefficient.

**References**

[1] Jia W, Zhai S, Ma C, Cao H, Wang C, Sun H, et al. The role of different fractions of humic acid in the physiological response of amaranth treated with magnetic carbon nanotubes. Ecotoxicology and Environmental Safety 2019;169:848-55.
[2] Maxwell K, Johnson GN. Chlorophyll fluorescence—a practical guide. Journal of Experimental Botany 2000;51:659-68.
[3] Müller P, Li XP, Niyogi KK. Non-photochemical quenching. A response to excess light energy. Plant Physiol 2001;125:1558-66.

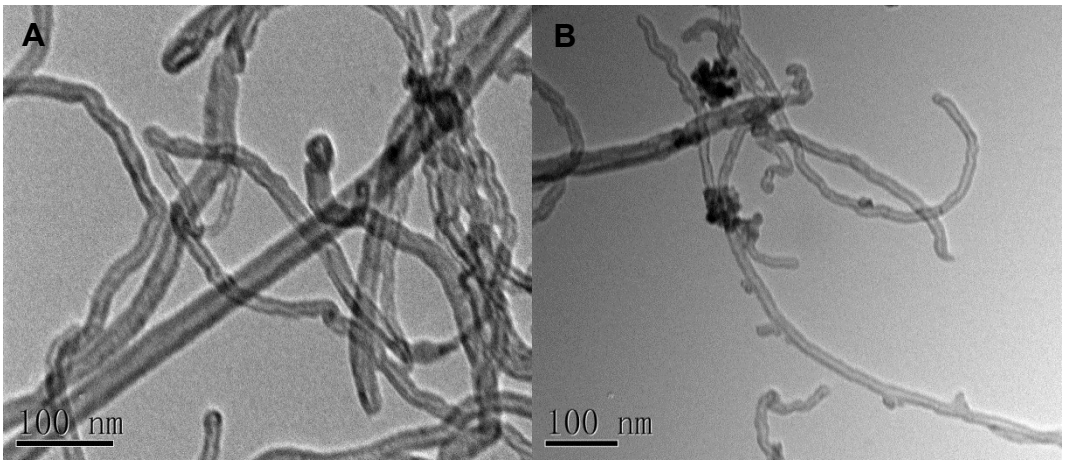


**Figure S1.** TEM images of carbon nanotubes (A) and magnetic carbon nanotubes (B).

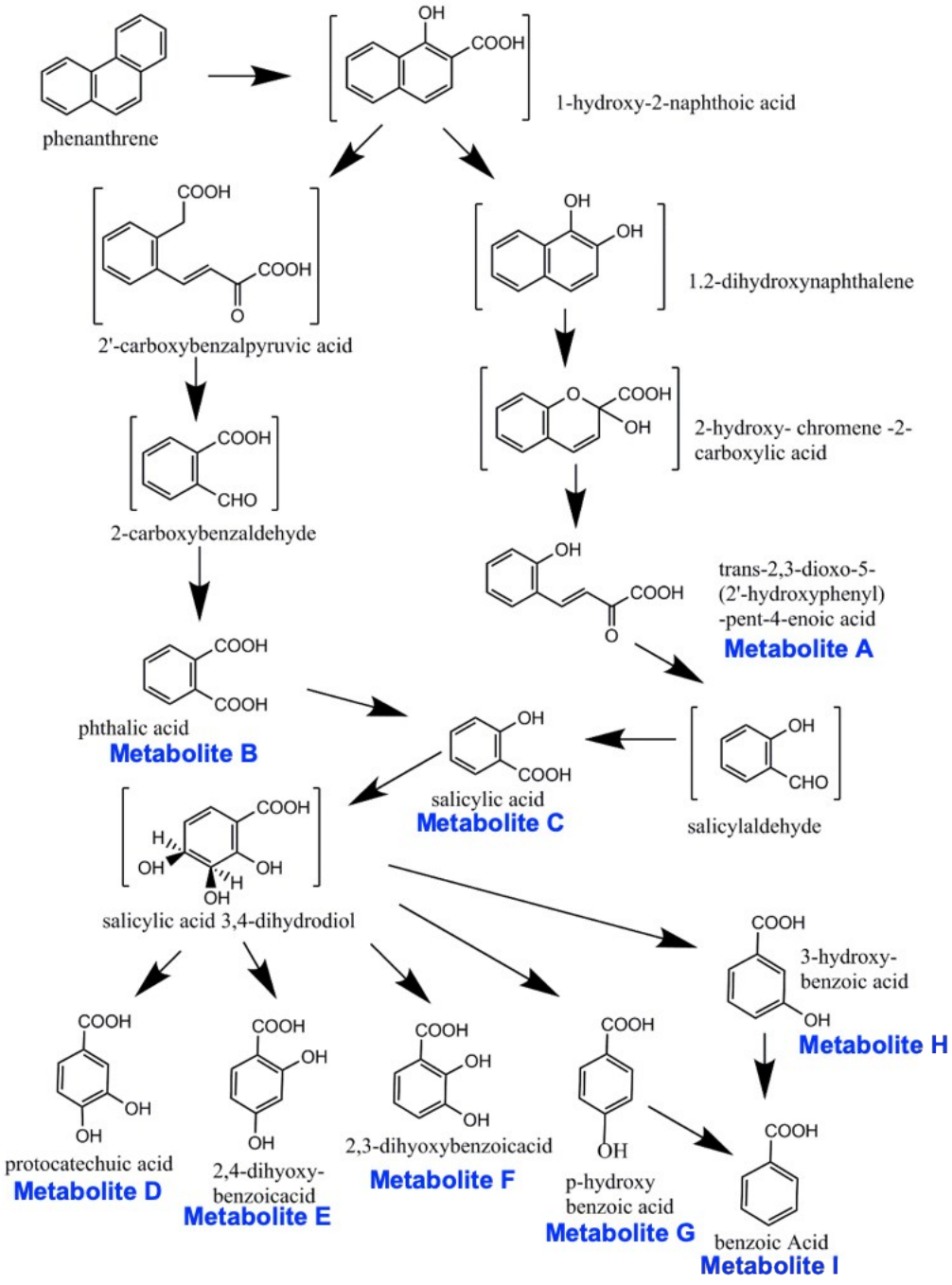


**Figure S2**. Proposed metabolic pathways of phenanthrene

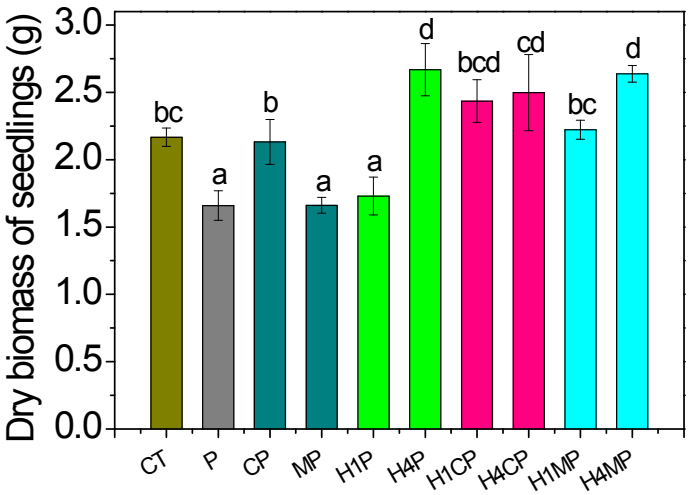


**Figure S3.** Dry biomass of lettuce seedlings as affected by Phe (P), carbon nanotubes (C), magnetic carbon nanotubes (M), and different fractions of DHA (H1 and H4). Different letters in each panel represent that the data points are significantly different at $p < 0.05$ (Duncan's test).

**Table S1.** Experimental design

| Treatment | Detailed information | Exposure concentration |
|---|---|---|
| CT | Control | 0 |
| P | phenanthrene | 1 mg/L |
| CP | CNT+ phenanthrene | 25 mg/L+1 mg/L |
| MP | MCNT+ phenanthrene | 25 mg/L +1 mg/L |
| H1P | DHA1+ phenanthrene | 10 mg/L+1 mg/L |
| H1CP | DHA1+ CNT+phenanthrene | 10 mg/L+25 mg/L +1 mg/L |
| H1MP | DHA1+ MCNT+phenanthrene | 10 mg/L+25 mg/L +1 mg/L |
| H4P | DHA4+ phenanthrene | 10 mg/L+1 mg/L |
| H4CP | DHA4+ CNT+phenanthrene | 10 mg/L+25 mg/L +1 mg/L |
| H4MP | DHA4+ MCNT+phenanthrene | 10 mg/L+25 mg/L +1 mg/L |

**Table S2.** Phenanthrene metabolites in lettuce.

| Phenanthrene metabolites number | Phenanthrene metabolites name |
|---|---|
| A | *trans*-2,3-dioxo-5-(2'-hydroxyphenyl)-pent-4-enoic acid |
| B | phthalic acid |
| C | salicylic acid |
| D | protocatechuic acid |
| E | 2,4-dihyoxybenzoicacid |
| F | 2,3-dihyoxybenzoicacid |
| G | p-hydroxybenzoic acid |
| H | 3-hydroxybenzoic acid |
| I | benzoic Acid |

**Table S3**. A list of gene primers

| Gene name | Sequence |
|---|---|
| LsCCD1 F | GAA ACC ACA AAC CGT CAC AAC |
| LsCCD1 R | GGA TCA ACC GAA ACC CTA CTC |
| LsNCED1 F | TCT TCC AAA CCC TAC AAT CCG |
| LsNCED1 R | CTT TGT AAG AGG TTC CAT TGC AG |
| Ls petB F | GTT GGT TAA TCC GAT CAG TTC ATC |
| Ls petB R | AAT TTG GTC CCG AGG TAA GG |
| Ls psbA F | TGG TTG ACA CGG GCA TAT AAG |
| Ls psbA R | GTT ACA GAA GCG ACC CCA TAG |
| Ls psbC F | GGC TTT GGC GGT ATT TAT CAT G |
| Ls psbC R | CGG AGC CCA AGT ATC ATA TAC G |
| LsACTIN7 F | TGC GTG ACA TGA AGG AGA AG |
| LsACTIN7 R | GAA GGC TGG AAT AGA ACC TCA G |