



Biochar Extracts Can Modulate the Toxicity of Persistent Free Radicals in the Nematode *Caenorhabditis elegans*

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Text S1. Media and buffer. In order to maintain pH balance, the exposure medium was prepared by adding M9 buffer to K-medium, then mixed well with OP50 just before the exposure experiments, with a volume ratio of K medium: M9 Buffer: OP50 = 3:3:1. K-medium: 2.36 g potassium chloride (KCl), 3 g sodium chloride (NaCl), in 1 L double-distilled H₂O (ddH₂O). M9 buffer: 3 g potassium dihydrogen phosphate (KH₂PO₄), 7.5 g disodium phosphate (Na₂HPO₄), 5 g NaCl, in 1 L ddH₂O.

Test S2. 96 mm-chemotaxis-assay plates. To test the attraction of NaCl, chemotaxis agars were prepared without adding NaCl. Add 10 g agar and 2.3 mL of 1 M potassium phosphate buffer in the bottle, fill up to 500 mL with the ddH₂O. After sterilization, mix 0.5 mL of 1 M calcium chloride (CaCl₂) and 0.5 mL of 1 M magnesium sulfate (MgSO₄), then distribute in 96 mm Petri dishes.

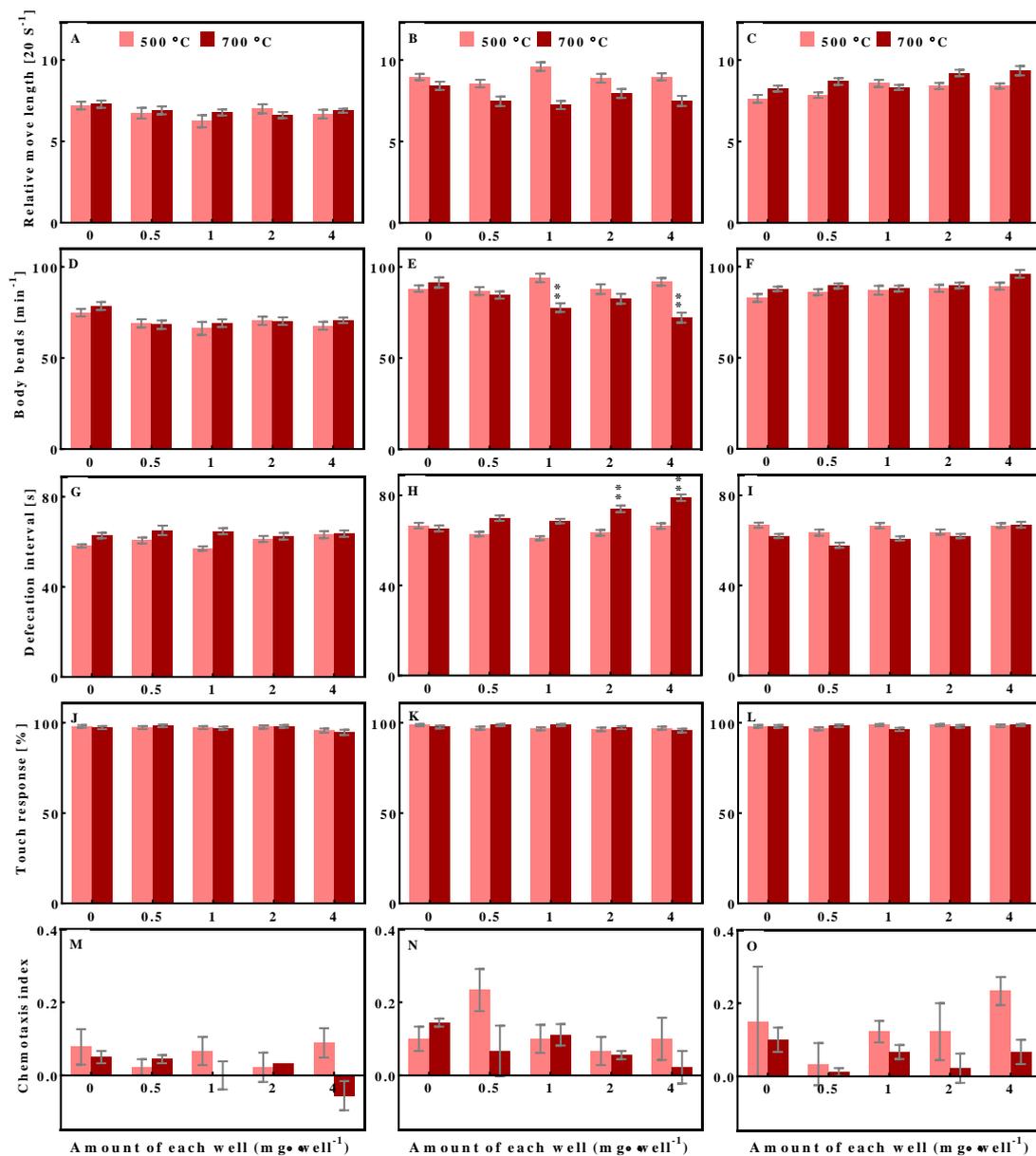


Figure S1. Neurobehavior of *C. elegans* after 24h exposure to unwashed biochars (A, D, G, J, and M), washed biochar particles (B, E, H, K, and N, these data were copied from Figure 2A–2E for a better comparison), and supernatants (C, F, I, L, and O) at 20 °C. Relative move length (A, B, and C), body bend (D, E, and F), defecation interval (G, H, and I), touch response (J, K, and L), and chemotaxis index (M, N, and O) were determined. * p < 0.05, ** p < 0.01.

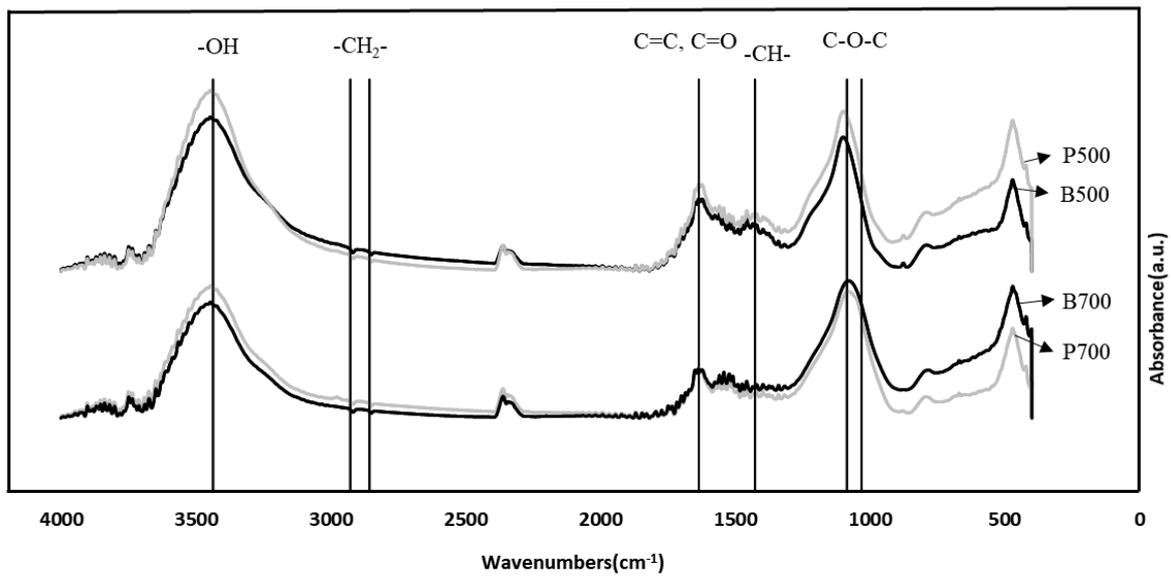


Figure S2. Fourier transform infrared (FTIR) spectra of unwashed biochar and washed biochar. Samples were prepared with potassium bromide (mass ratio =1:1500) and detected by ThermoFisher spectrometer (NICOLET iS50 FT-IR, USA). The dark line means unwashed biochar, the grey line means washed biochar particles.

Table S1. Total of carbon concentration in the supernatant.

TOC	S500	S700
mg·L ⁻¹	143.76	66.80

Note: 40 mg of biochar was added in 15 mL sterile water and shaken for 24 h at 20°C. The total carbon in the liquid phases was detected by TOC Analyzer (vario TOC cube, Elementar Analysensystem, Germany).

Table S2. The elemental composition in unwashed biochar and washed biochar.

%	C	H	O	N	S	H/C	O/C	(N + O) /C
biomass	37.42	5.58	34.82	0.65	0.00	1.79	0.70	0.71
B500	33.53	1.89	16.37	0.83	0.75	0.68	0.37	0.39
B700	24.33	1.20	13.95	0.50	0.88	0.59	0.43	0.45
P500	36.66	2.13	20.61	0.92	0.00	0.70	0.42	0.44
P700	27.37	1.39	16.62	0.57	0.09	0.61	0.46	0.47

Note: The elemental composition was determined by CHNOS Elemental Analyzer (vario MICRO, Elementar Analysensystem, Germany).