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ARTICLE

Effect of Dissolved Organic Matters on Napropamide Availability and Ecotoxicity in Rapeseed (*Brassica napus*)

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Napropamide is a herbicide widely used for controlling annual weeds. Substantial use of napropamide in recent years has led to its bioaccumulation in ecosystems and thus contamination to crops. Meanwhile, application of dissolved organic matters (DOMs) to soils in the form of compost, sludge, or plant residues has become a popular practice in agriculture management owning to its low cost and recycling of nutrients. However, whether DOMs affect environmental behaviors of herbicides in soil−plant systems is poorly understood. This study investigated napropamide accumulation and biological responses as affected by DOMs in *Brassica napus*. Plants exposed to 0−16 mg/kg napropamide show inhibited growth and oxidative damage. Treatment with 50 mg of DOC/kg DOMs derived from either sludge or straw improved plant growth and reduced napropamide accumulation in plants. Both DOMs reduced the production of reactive oxygen species (ROS) and the activities of antioxidative enzymes in napropamide-exposed plants. Analysis of FT-IR spectra confirmed the difference between structures of the two DOMs. Additional evidence was provided by three-dimensional excitation−emission matrix (EEM) fluorescence spectra to demonstrate the DOM−napropamide complex formed during the process of the interaction.

KEYWORDS: DOM; napropamide; ecotoxicology; bioavailability; *Brassica napus*

INTRODUCTION

Herbicides have become indispensable elements in modern agriculture. Although they have brought great benefits to crop production by controlling weeds, they also have detrimental effects on environmental quality. Because intensively used herbicides have become one of the most frequently occurring organic pollutants in farmland, great concern has arisen from the possible effect of herbicides on crop production safety and human health (*1*−*5*). Because the majority of herbicides are applied to soils, they constitute the major source of contamination to crops and groundwater (*6*−*8*). Napropamide [*N*,*N*-diethyl-2-(1-naphthalenyloxy)propanamide] is a pre-emergence herbicide killing many annual monocotyledon grasses and broad-leaf weeds (*9*). It is a polar herbicide, and its water solubility is 73 mg/L; the median lethal dose (LD_{50}) for mice is >5 g/kg when administered orally and >1 g/kg when injected intraperitoneally (*10*). Because commercial napropamide can easily pass into tissues of living organisms, it readily accumulates in crops and may cause biological toxicity (*11*). Hence, there is a need to estimate its behavior and fate in plant−soil systems.

Dissolved organic matters (DOMs) derived from soil organic matter can interact with organic pollutants (*7*, *12*−*14*). DOMs consist of a mixture of organic compounds including small molecular organic acids and macromolecule compositions (e.g., enzymes, humic acids, polyphenol, and amino sugar) (*15*). Due to their dynamic property, DOMs have multiple capacities of modifying mobility, transformation, and binding to organic compounds in soils (*12*, *14*, *16*). It is most likely that DOMs target pesticides to influence their behaviors. Recent studies have demonstrated that soil DOMs affected sorption or desorption of organic compounds (*17*−*19*). DOMs may reduce organic pollutant sorption onto soils and promote desorption of pesticides from soils (*20*,*21*). In contrast, DOMs can enhance pesticide sorption if sorbed DOMs on soil particles provide additional sites for pesticide sorption (*22*) and, thereby, availability of hydrophobic organic contaminants is reduced (*3*, *12*, *23*). In this case, DOMs may mitigate the effect of organic contaminants by reducing their concentrations as free species in solution and uptake by organisms. Moreover, DOMs may interact with organic chemicals and reduce directly their bioavailability to crops.

Application of compost, sludge, and crop residues in soils is a popular practice for improving soil organic carbon or fertilizers (*8*, *24*). Such a management is particularly important in China and other developing countries for cultivation of cereal and other crops. Rapeseed (*Brassica napus*) is one of the most economically important crops in China, Europe, and other areas of the world. In Asia, the seeds of rapeseed crops are used as a primary source of edible oil. Therefore, it is necessary to estimate the bioavailability/bioaccumulation of napropamide in soils and possible role of DOMs. Pioneering studies have shown that DOMs derived from soils affected significantly the soil sorption/desorption of napropamide (*25*,*26*). Due to the strong interaction between DOMs and napropamide, the transport of the pesticide

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through soils was facilitated (*27*, *28*). As a consequence of the mobility, it is necessary to evaluate the bioavailability of napropamide to organisms in the presence DOM. To date, most studies have focused on the effect of DOMs on ecotoxicology of hydrophobic organic chemicals in aquatic organisms (*29*, *30*). Very few have been reported on the biological accumulation of pesticides in crops as affected by DOMs in the soil. Also, the process of DOM interaction with crops is largely unknown. In this study, rapeseed plants were employed to investigate the napropamide-responsive stress and interaction between DOMs and napropamide in plants. We examined the regulatory capacity of DOMs in the process. The purpose of the study is to understand how DOMs affect bioavailability of the herbicide to plant−soil systems.

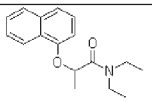
MATERIALS AND METHODS

Materials and Treatment Set. Napropamide was obtained from Rudong Pesticide Institute, Jiangsu, China. The major properties of napropamide are summarized in **Table 1**. Some chemical properties of the soil are listed as follows: pH 7.55; organic carbon, 1.28%; total N, 1.23 g/kg; available P, 35.6 mg/kg; available K, 90.1 mg/kg; sand, 32.15%; silt, 38.36%; clay, 29.52%; and CEC, 17.1 cmol/kg. Soil was collected from the surface layer (0−20 cm) of uncontaminated soils at the Experimental Station of the Academy of Agricultural Science in Jiangsu, Nanjing, China. The collected soil was air-dried at room temperature, gently crumbled, and sieved through a 3 mm mesh before use. Concentrations of napropamide applied to soil were set at 0, 1, 2, 4, 8, 12, and 16 mg/kg of soil (for biomass assay) or 0, 2, 4, 8, 12, and 16 mg/kg of soil (for metabolite determination) on the basis of the previous data applied to weeds, with the optimal concentrations at 4 or 8 mg/kg (*31*). DOM concentration was set at 50 mg of DOC/kg. Each treatment was repeated three times.

Seeds of *B. napus* were placed on Petri dishes with wet filter paper and germinated in darkness at 25 °C for 24 h. The germinating seedlings were transferred to a plastic pot with soil with 60% relative water content and grown at 22/18 °C (day/night), with a light intensity of 200 μmol m^{-2} s^{-1}. After 10 days of growth (the third leaf emergence), roots and shoots (or leaves) were separately harvested and immediately frozen in liquid nitrogen or stored in an −80 °C freezer for analysis.

DOM Extraction. Two types of dissolved organic matter were employed, one of which was extracted from sludge (SL) and the other from straw (ST). The DOMs were extracted from sludge and straw with double-distilled water using a solid-to-water ratio of 1:10 (w/v, dry weight) on a reciprocal shaker at 200 rpm and 4 °C for 16 h. After that, the suspensions were centrifuged at 10000*g* and 4 °C for 15 min and filtered through a 0.45 μm sterilized membrane (GN-6 Mctrice, Gelman Sciences, Ann Arbor, MI) (*21*). The major properties of DOMs are shown in **Table 2**. The freshly prepared DOM extracts were used immediately. DOMs extracted from the two sources contained a small amount of salts or other metals that may influence biological effect of DOMs. However, as the concentration used in this study was very low, the significance was thought to be of least importance.

Table 1. Some Properties of Napropamide

Name	Formula	Chemical struture	H_2O solubility (mg L^{-1})	Melting point (20 °C)	Vapor pressure (20 °C)	Molecular weight (g mol^{-1})
Napropamide	$C_{17}H_{21}NO_2$		73	75	0.53Pa	271.35

Determination of Growth and Metabolites. Accumulation of lipid peroxides in tissues was determined in terms of thiobarbituric acid reactive substances (TBARS) according to the method of Song et al. (*7*).

Histochemical Detection of $O_2^{\bullet-}$ and H_2O_2. Superoxide was visually detected in leaves by using nitroblue tetrazolium (NBT) as a substrate according to the method of Frahry and Schopfer (*32*). Leaves were excised at the base of stems with a razor blade and supplied through the cuts with 10 mM sodium citrate buffer (pH 6.0) containing 6 mM NBT under light at 25 °C for 8 h. After treatment, the leaves were immersed in boiling ethanol (95%) for 10 min to remove the green background.

Histochemical detection of cellular H_2O_2 was performed in leaves using 3,3-diaminobenzidine (DAB) as substrate (*33*). The treated plants were loaded through the stem cut with a 1 mg/mL solution of DAB, pH 3.8, under light at 25 °C for 8 h. The leaves were then immersed in the boiling ethanol (95%) for 10 min to remove the green background. This allowed the deep brown polymerization product (reaction of DAB with H_2O_2) to be visualized.

Antioxidant Enzyme Assay. Plant tissues (0.3 g) were homogenized in 3 mL of 50 mM potassium phosphate buffer (pH 7.8) containing 1 mM EDTA and 1% w/w polyvinyl polypyrrolidone (PVP), with the addition of 5 mM ascorbate in the case of APX assay. The homogenate was centrifuged at 10000*g* at 4 °C for 20 min. The resulting supernatant was used as the crude extract for enzyme assay. Activities of superoxide

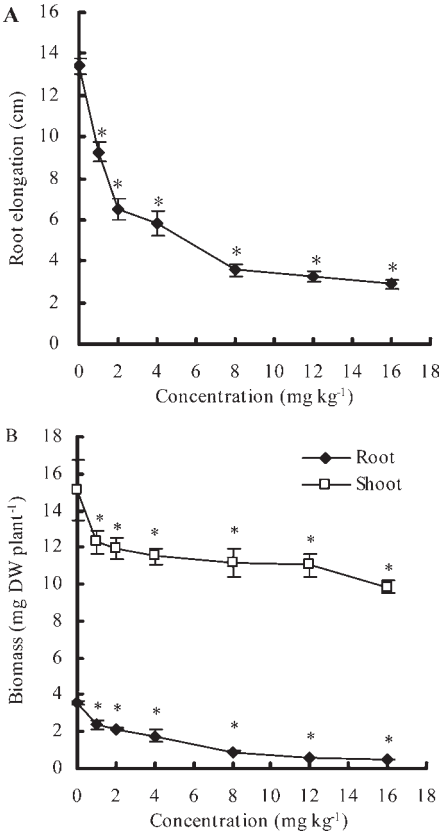


Figure 1. Effect of napropamide on growth of *B. napus*. Seedlings were cultured in soil containing napropamide at 0, 1, 2, 4, 8, 12, and 16 mg/kg for 10 days. Then, elongation (**A**) of roots and biomass (**B**) of plants were measured. Values are the means ± SD (n = 3). Asterisks indicate significant differences between the treatments and the control ($P < 0.05$).

Table 2. Properties of Two Dissolved Organic Matters

	elemental composition (%)			atomic ratio				
sample	C	H	N	H/C	N/C	ash content (%)	total organic carbon (mg of DOC/L)	pH
DOM-SL	6.144	3.730	7.242	0.607	1.179	41.40	934	7.14
DOM-ST	23.81	4.972	4.277	0.209	0.178	30.13	3270	6.81

dismutase (SOD, EC 1.15.1.1) were assayed by determining its capacity of inhibiting photochemical reduction of NBT (*7*). One unit of SOD was defined as the enzyme activity that inhibited the photoreduction of NBT to blue formazan by 50%. Catalase (CAT, EC 1.11.1.6) activity was determined by the consumption of H_2O_2 (extinction coefficient of 39.4 mM/cm) at 240 nm for 30 s (*34*), and guaiacol peroxidase (POD, EC 1.11.1.7) activity was assayed for 1 min (*35*).

Ascorbate peroxidase (APX, EC 1.11.1.11) activity was assayed in the reaction mixture containing 50 mM sodium phosphate buffer (pH 7.0), 0.5 mM ascorbate, 0.1 mM H_2O_2, and 100 μL of enzyme extract in a volume of 3 mL (*35*). The reaction mixture for glutathione *S*-transferase (GST, EC 2.5.1.18) assay contained 5 μL of GST enzyme solution extract, 5 μL of 30 mM CDNB in ethanol, 45 μL of 3.3 mM GSH, and 95 μL of 0.1 M potassium phosphate buffer (pH 7.4) (*36*).

Analysis of Bioaccumulation. Plant tissues were homogenized with 10 mL of extraction solvent (acetone/water = 3:1). The homogenate was ultrasonically crushed for 15 min and filtered. Acetone in filtered solution was evaporated on a rotary vacuum evaporator at 30 °C. Then, 2 g of NaCl was added and the mixture was extracted by 10, 5, and 5 mL of hexane, respectively. The organic phase was combined and concentrated.

The LC-C_{18} cartridge (Supelco Park, Bellefonte, PA; 300 mg of sorbent) was reactivated with 5 mL of methanol and 5 mL of water, respectively. The residues were dissolved in the 50 mL mixture solvent (distilled water/acetonitrile (v/v) = 50:1) and loaded onto an LC-C_{18} cartridge. Elutes were discarded. The column of the LC-C_{18} cartridge was washed with 4 mLof methanol, and washing methanol was collected for HPLC analysis. The precleaning of tissue was repeated three times. To confirm the precise analysis of napropamide residues in soil and plant tissues, a recovery experiment was carried out. Three concentrations of napropamide, 0.5, 1, and 2 mg/kg, were selected. The spiked recoveries of the root sample ranged from 86.4 to 101.2% and those of the leaf sample from 90.4 to 96.6%. The relative standard deviations (RSD) of roots and leaves were 4.11%−11.17 and 2.41−6.43%, respectively.

Dry soil (10 g) was ground and extracted with 40 mL of acetone/water (v/v = 3:1) solution for 1.5−2 h using a shaker. The extract was centrifuged. Acetone in the extract was evaporated under vacuum at 30 °C. The residue water phase was extracted with hexane (3 × 10 mL), and the organic phase was transferred to a rockered flask and vaporized to dryness. The glass column tube was filled with 5 g of silica gel, and the upper layer was anhydrous sodium sulfate (2 g) for further purification. The column was reactivated with 10 mL of petroleum ether. The residue was dissolved in 3 mL of acetone and transferred into the column. Afterward, the column was eluted with 30 mL of mixed acetone/hexane (v/v = 1:4) solution. The collected elutes were evaporated at 40 °C to dryness. Finally, the residue was redissolved in 5 mL of methanol for quantitative analysis by HPLC. The precleaning of soil was repeated three times. At 0.2, 1, and 5 mg/kg of napropamide, the spiked recoveries of soil samples ranged from 88.5 to 94.9% and the RSD from 2.11 to 2.87%, respectively.

Napropamide in soil and plant was determined using external standard calibration by HPLC (Waters 515, 2487 dual k ultraviolet (UV) absorbance detector, Waters Technologies Co. Ltd.) under the following conditions: wavelength, 230 nm; Hypersil ODS column (250 × 4.6 mm i.d., 5 μm); mobile phase, methanol/water (80:20, v/v) at a flow rate of 0.8 mL/min; injection volume, 20 μL; and temperature, 25 °C. Under these conditions, the retention time of napropamide was 9.1 min and the range of calibration curves was 0.1−20 mg/L. Bioconcentration factors (BCF) for napropamide were calculated on the basis of tissue concentrations/soil concentrations. Translocation factors (TF) were calculated as the ratio of chemical concentrations in shoots to those in roots.

Structural Analysis of DOM. Solutions containing 1000 mg of C/L DOMs were freeze-dried for FT-IR spectral analysis (Bruker Vector-22 infrared spectrophotometer, Optics Inc., Germany). FT-IR spectra of the samples, which were obtained from a 200 mg KBr disk containing 1 mg of sample, were recorded within the range of 4000−400 cm^{-1} for scanning three times. The producing disk and FT-IR determination of freeze-dried samples were repeated three times.

Spectroscopy of three-dimensional excitation−emission matrix (EEM) fluorescence was performed. All fractions of DOMs were determined by a model F-4600 fluorescence spectrophotometer (Hitachi, Japan) with a

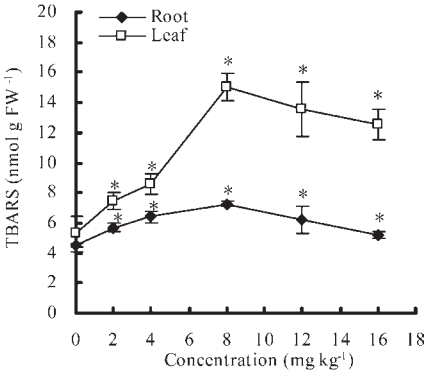


Figure 2. Effect of napropamide on TBARS accumulation in *B. napus*. Seedlings were cultured in soil containing napropamide at 0, 2, 4, 8, 12, and 16 mg/kg for 10 days. Then, the contents of TBARS were measured. Values are the means ± SD (n = 3). Asterisks indicate significant differences between the treatments and the control (P < 0.05).

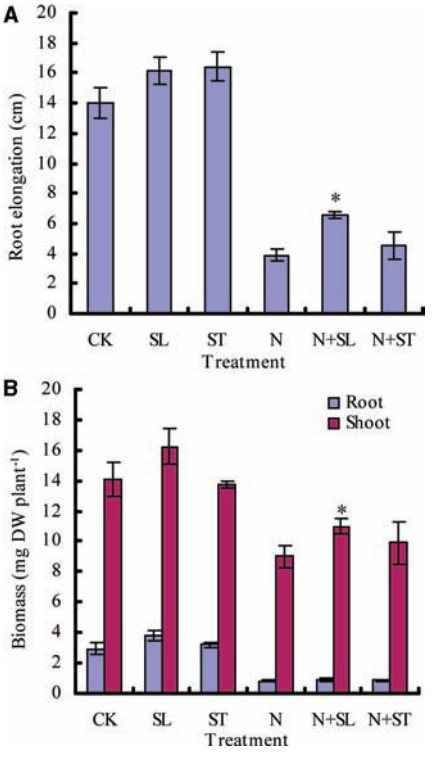


Figure 3. DOMs regulate the growth of *B. napus* exposed to napropamide. CK, soil only; SL, DOM from sludge; ST, DOM from straw; N, napropamide; N+SL, napropamide + DOM from sludge; N+ST, napropamide + DOM from straw. Plants were cultured in soil containing napropamide at 8 mg/kg and DOM-SL and DOM-ST at 50 mg of DOC/kg for 10 days. Then, the elongation (**A**) of roots and biomass (**B**) of both roots and leaves were measured, respectively. Values are the means ± SD (n = 3). Asterisks indicate significant differences between the N+SL/N+ST treatments and N treatment alone (P < 0.05).

150 W Xe arc lamp. The filtrate was analyzed for total organic carbon by TOC-5000A and used for fluorescence analysis. The DOMs and DOM−napropamide complex were diluted with 0.1 mol/L phosphate buffer (pH 7.0). The final concentration of DOC was made up to 2.5 mg of C/L. Prior to analysis, concentrations of all samples were adjusted to make them comparable to each other. In this study, the emission (Em) wavelength range was fixed from 200 to 500 nm in a 5 nm step, whereas the excitation (Ex) wavelength increased from 200 to 500 nm in a 5 nm step. The excitation and emission slits were maintained at 10 nm, and the

scanning speed was set at 1200 nm/min. Analysis of EEM fluorescence for all samples was repeated three times.

Statistical Analysis. Each result shown in this paper was the mean of at least three replicated treatments. Significant differences between the treatments were statistically evaluated by standard deviation and Student *t*-test methods.

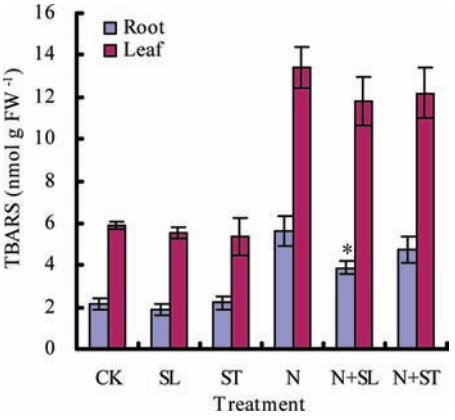


Figure 4. Effect of DOMs on accumulation of TBARS in *B. napus* exposed to napropamide. CK, soil only; SL, DOM from sludge; ST, DOM from straw; N, napropamide; N+SL, napropamide + DOM from sludge; N+ST, napropamide + DOM from straw. Plants were cultured in soil containing napropamide at 8 mg/kg and DOM-SL and DOM-ST at 50 mg of DOC/kg for 10 days. Then, the content of TBARS was measured. Values are the means ± SD ($n = 3$). Asterisks indicate significant differences between the N+SL/N+ST treatments and N treatment alone ($P < 0.05$).

Table 3. Effects of DOM on Napropamide Accumulation in Soil and *B. napus* Tissues[a]

treatment	soil (mg/kg of dry soil)	root (mg/kg of fresh plant)	shoot (mg/kg of fresh plant)
N	6.775 ± 0.256b	2.995 ± 0.262a	0.768 ± 0.072a
N+DOM-SL	7.458 ± 0.394a	2.203 ± 0.079c	0.653 ± 0.090a
N+DOM-ST	7.135 ± 0.046a	2.535 ± 0.126b	0.663 ± 0.095a

[a] N, napropamide; N+DOM-SL, napropamide + DOM from sludge; N+DOM-ST, napropamide + DOM from straw. Plants were cultured in soil containing napropamide at 8 mg/kg with DOM-SL and DOM-ST at 50 mg of DOC/kg for 10 days. Values are the means ± SD, and those followed by the same letter are not significantly different at the 0.05 level.

RESULTS AND DISCUSSION

Effect of Napropamide on Growth and Metabolite Accumulation. After exposure of *B. napus* to napropamide at 0−16 mg/kg for 10 days, the growth of plants was significantly inhibited. Treatment with napropamide resulted in a progressive decrease in root elongation with napropamide concentrations (**Figure 1A**). A similar response was observed for biomass of *B. napus* with napropamide. Inhibition of root biomass was more pronounced than that of shoots. At 1 mg/kg of napropamide, the root and shoot biomasses were reduced to 66.8 and 81.3% of the control, respectively (**Figure 1B**), indicating that roots were more affected by napropamide than shoots.

To estimate napropamide-induced toxicity in *B. napus*, the oxidative damage to plasma membrane lipids was examined by quantifying TBARS, an indicator of lipid peroxidation. TBARS level was elevated with napropamide concentrations from 0 to 16 mg/kg (**Figure 2**). The maximal accumulation in roots and leaves was observed at 8 mg/kg of napropamide, where the TBARS contents increased by 1.61-fold in roots and by 2.85-fold in leaves, respectively. However, increased TBARS accumulation was not observed with higher napropamide concentrations.

Effect of DOM on Growth and Metabolite Accumulation under Napropamide Stress. DOMs may influence behaviors of many organic compounds including contaminants in soil (*12*, *16*, *21*, *37*). We initially analyzed the effect of DOMs on biomass and root elongation in the presence of napropamide. Analyses revealed that application of DOMs at 50 mg of C/L enhanced the root elongation and biomass accumulation in the presence of napropamide (**Figure 3**). However, only DOM-SL induced significant alteration, suggesting that DOMs can differentially affect biological responses of plants to napropamide.

To understand whether DOMs altered physiological parameters, TBARS and reactive oxygen species (ROS) were measured. It is shown that DOMs depressed the production of TBARS with napropamide (**Figure 4**). One of the reasons was the lower uptake of napropamide in shoot and root than that of the control (**Table 3**). However, DOMs from SL appeared to be more effective than those from ST because the uptake content of napropamide with DOM-SL was lower than that with DOM-ST.

Residues of chemical contaminants in soils caused a variety of physiological changes in plants (*38*). Of these, production of cellular ROS is a major response. Superoxide radical (O_2^-) is one

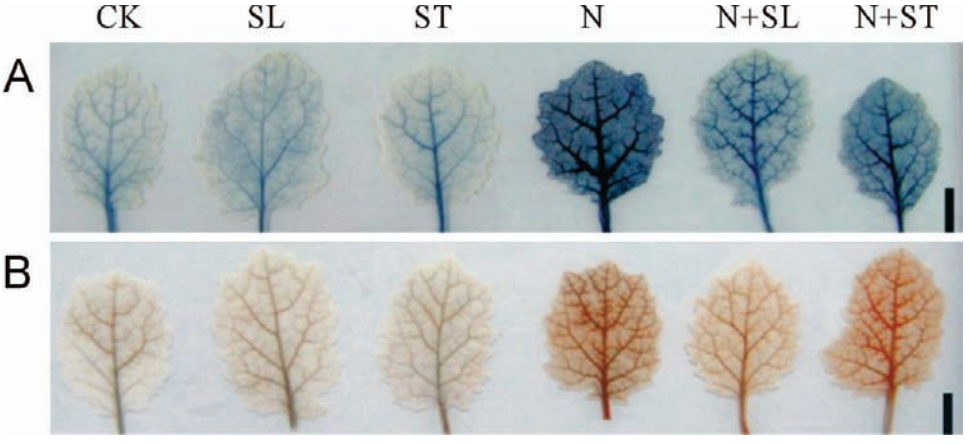


Figure 5. Effect of DOMs on napropamide-induced superoxide radical (**A**) and hydrogen peroxidase (**B**) accumulation in *B. napus* leaves. CK, soil only; SL, DOM from sludge; ST, DOM from straw; N, napropamide; N+SL, napropamide + DOM from sludge; N+ST, napropamide + DOM from straw. Plants were cultured in soil containing napropamide at 8 mg/kg and DOM-SL and DOM-ST at 50 mg of DOC/kg for 10 days. The harvested plants were excised at the base of stems and supplied through the cut stems with NBT or DAB solutions for 8 and 6 h, respectively. Afterward, the leaves were photographed. The scale bar in the graph indicates 5 mm.

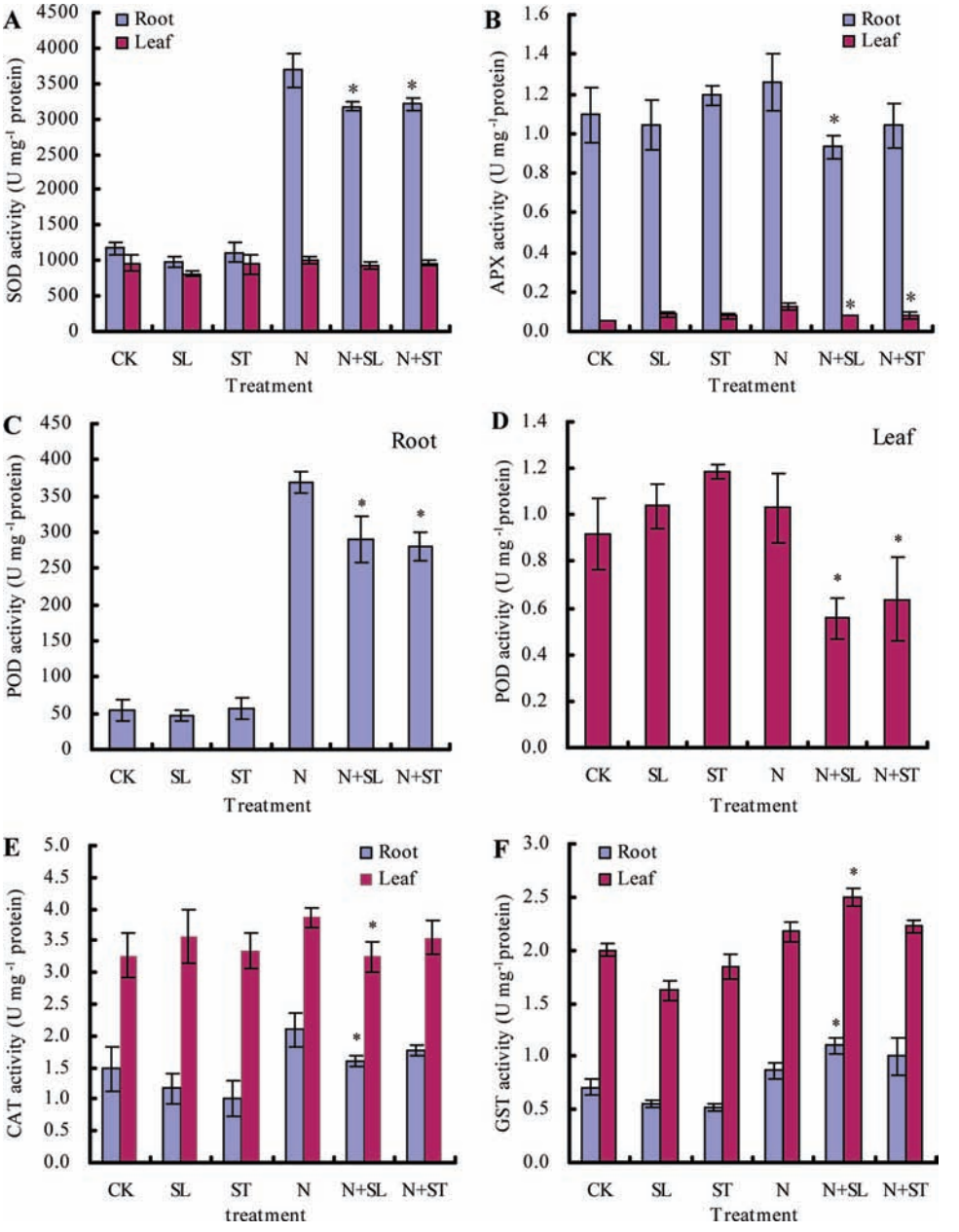


Figure 6. DOMs regulate the activities of SOD (**A**), APX (**B**), POD (**C**, root), POD (**D**, leaf), CAT (**E**), and GST (**F**) in *B. napus* exposed to napropamide. CK, soil only; SL, DOM from sludge; ST, DOM from straw; N, napropamide; N+SL, napropamide + DOM from sludge; N+ST, napropamide + DOM from straw. Plants were cultured in soil containing napropamide at 8 mg/kg and DOM-SL and DOM-ST at 50 mg of DOC/kg for 10 days. Then, the enzyme activities were assayed. Values are the means ± SD ($n = 3$). Asterisks indicate significant differences between N+SL/N+ST treatments and N treatment alone ($P < 0.05$).

Table 4. Bioconcentration Factors (BCF) and Translocation Factors (TF) for Napropamide with Two Types of DOMs[a]

treatment	shoot BCF	root BCF	TF
N	0.113 ± 0.006a	0.442 ± 0.042a	0.256 ± 0.032a
N+DOM-SL	0.097 ± 0.017a	0.325 ± 0.018c	0.296 ± 0.038a
N+DOM-ST	0.098 ± 0.017a	0.374 ± 0.019b	0.262 ± 0.043a

[a] Concentrations of napropamide were determined in soil and plant tissues after soil was exposed to napropamide (8 mg/kg napropamide) and DOMs for 10 days. Values are the means ± SD; those followed by the same letter are not significantly different at the 0.05 level. BCF, fresh weight ratio of napropamide concentration in plant to the soil; TF, ratio of shoot BCF to root BCF.

of the important ROS, which is frequently used to indicate the intracellular degree of oxidative stress (*35*, *38*). To understand whether napropamide triggered the oxidative stress through enhancement of ROS abundance in rapeseed, generation of O_2^- in *B. napus* leaves was measured. Histochemical staining of O_2^- with NBT shows that treatment with napropamide induced intense staining compared to the control (**Figure 5A**). However, simultaneous treatment with DOMs suppressed the O_2^- accumulation, showing DOMs exerted an apparent antioxidative effect against napropamide. Excessive hydrogen peroxide (H_2O_2) also damages many biomolecules and can be visually detected in tissue using 3,3-diaminobenzidine (DAB) as a substrate (*35*). Leaves of rapeseed treated with napropamide alone were stained extensively, and those pretreated with DOMs (SL) had light staining (**Figure 5B**), suggesting that DOMs conferred the tolerance of plants to napropamide toxicity. Also, DOM-SL appeared to be more effective than DOM-ST.

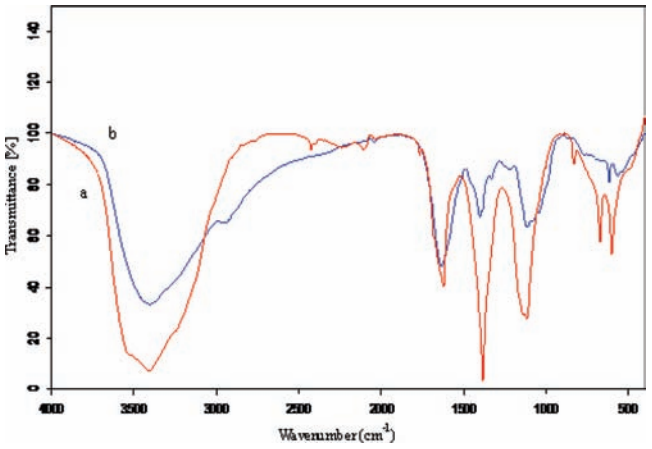


Figure 7. FT-IR spectra of DOMs derived from sludge (a) and straw (b).

Table 5. Main Infrared Absorption Peaks in the FT-IR Spectra of DOMs

main infrared absorption frequencies (cm^{-1})	
DOM-SL	DOM-ST
3408	3405
2426	
1764	
1622	1634
	1401
1384	
	1322
1116	1110
832	
	773
673	
602	618
	567

Effect of DOM on Enzyme Activities in Napropamide-Treated Plants. Napropamide-induced oxidative stress in *B. napus* was linked to the enhanced production of ROS (**Figures 4** and **5**), and regulation of ROS levels can be achieved by a group of enzymes such as SOD, CAT, and APX (*36*, *38*−*40*). SOD is an essential component of antioxidative defense system in plants responsible for catalyzing the dismutation of superoxide radicals (O_2^-) to O_2 and H_2O_2 (*41*). It is shown that total activities of SOD drastically increased upon exposure to 8 mg/kg napropamide (**Figure 6A**). However, SOD activities in napropamide-treated roots were significantly reduced by DOMs, suggesting that DOMs suppressed the activity of SOD under napropamide stress. The activity of SOD is frequently used to indicate the extent of oxidative stress in plants (*33*, *35*, *38*), because the enhanced activity of SOD is stimulated by the production of ROS. In this study, the correlation between the SOD activity and generation of ROS was well established. Compared to SOD activities in roots, the leaf activity was not significantly affected by DOMs.

Within the enzymes for the removal of ROS, POD can be considered one of the key enzymes in higher plants, because both of its extra- and intracellular forms participate in the breakdown of H_2O_2 and lignin biosynthesis in the presence of H_2O_2 (*33*, *42*). APX is an enzyme that uses ascorbate as electron donor in the first step of the ascorbate−glutathione cycle and is considered as the most important plant peroxidase in H_2O_2 detoxification (*41*). In this study, both POD and APX activities displayed a pattern similar to SOD in response to napropamide (**Figure 6B**−**D**).

CAT is one of the key enzymes involved in the removal of toxic hydrogen peroxides. Treatments with napropamide slightly, but not significantly, increased its activities compared to the control (CK). Under napropamide exposure, its activity was decreased by DOMs. However, only DOM-SL treatment decreased the CAT activity significantly (**Figure 6E**). GST is believed to be a typical detoxifying enzyme. It plays an important role in plant protection against oxidative damage (*43*). The intracellular level of glutathione *S*-transferase can be induced by a variety of contaminants (*36*, *44*). The enzyme catalyzes the conjugation of glutathione to several electrophilic substrates and transfers the complexes into vacuoles for detoxification and is considered as a good biomarker of xenobiotic-induced stress (*45*). Napropamide exposure slightly, but not significantly, stimulated the activity of GST in *B. napus* (**Figure 6F**). However, the marked enhancement of its activities was observed in plants when DOM-SL was added, suggesting that the GST activity was improved by DOM treatment. Increased GST activities were reported under herbicide stress in wheat (*36*) and rice (*46*), but to our best knowledge, there has been no report indicating that DOMs activate GST-based detoxification system in plants under herbicide stress.

Bioaccumulation of Napropamide. To get insights into the napropamide toxicity to *B. napus* and regulating role of DOMs, concentrations of napropamide in plant tissues were measured. Rapeseed plants were cultured in soil containing napropamide at 8 mg/kg with DOMs (50 mg of DOC/kg) for 10 days. The soil contained higher levels of napropamide than plants (**Table 3**). The roots accumulated more napropamide than shoots, with 3.9-fold higher accumulation than shoots. This was attributed to the direct exposure of roots to napropamide. Compared to the napropamide treatment alone, concomitant treatment with DOMs reduced the napropamide accumulation in roots and shoots. However, significant difference was found in only roots. Therefore, more napropamide was possibly retained in DOM-treated soil. DOMs in soils could form complexes with napropamide (*25*, *27*) and consequently might reduce the accumulation of napropamide in corps. Also, it was most likely that the herbicide−DOM complex was polar or too large in size, which prevents its transfer into the root cells (*21*). Overall, the reduced amount of napropamide in the presence of DOMs could confer the resistance of rapeseed plants to napropamide-induced oxidative damage to plasma membrane.

Bioconcentration factors (BCF) and translocation factors (TF) were calculated on the basis of napropamide accumulation in plant and soil. BCF is defined as the quotient between the organism and medium substance concentrations (*47*). It was used to compare the relative abilities of cultivars under different treatments to accumulate napropamide in shoots and roots, for example, the ratio of napropamide concentrations in harvested plant materials to napropamide concentrations in soil. All BCF values were lower than 1.0, and BCF values with exposure to napropamide with DOMs were even lower than those with exposure to napropamide without DOMs (**Table 4**). This result suggests that addition of DOMs reduced the capacity of napropamide translocation from soil to tissues, which is consistent with our previous data with chlorotoluron in wheat plants (*21*).

TF can be used to evaluate the plant capability of accumulating napropamide. TF for napropamide was expressed by the ratio of napropamide concentration in shoots to roots and shows napropamide translocation properties from roots to shoots. TF values were in the order N+DOM-SL > N+DOM-ST > N (**Table 4**), indicating that addition of DOMs enhanced napropamide accumulation in shoots. The result might be explained by enhanced napropamide desorption with DOM in plants.

FT-IR Spectroscopy Analysis of DOM. FT-IR spectroscopy is frequently used to characterize DOMs and can provide valuable information on the structural and functional properties of DOM molecules (*21*). In this study, information on the molecular basis

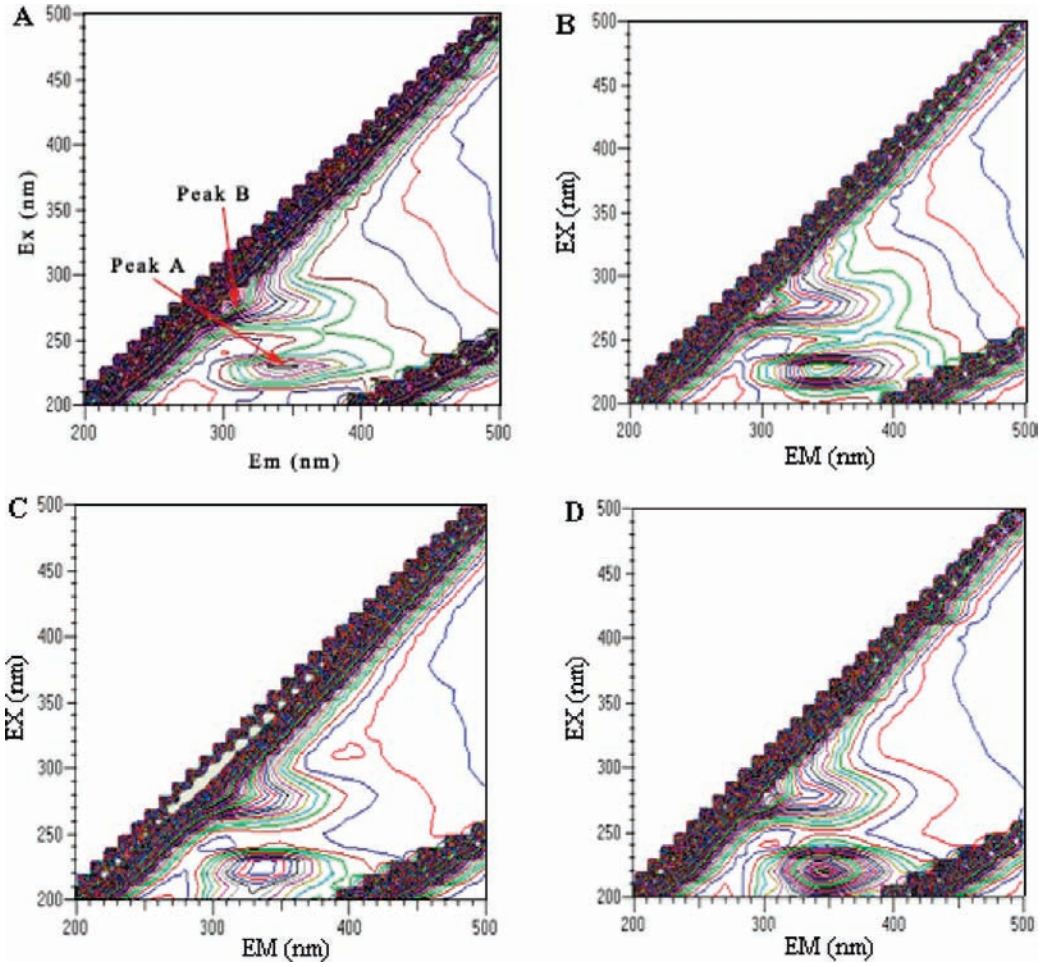


Figure 8. EEM fluorescence spectra of DOMs or DOM−napropamide: (**A**) DOM-SL; (**B**) DOM(SL)−napropamide; (**C**) DOM-ST; (**D**) DOM-(ST)−napropamide. Concentrations of DOMs were set at a 2.5 mg of C/L.

Table 6. Fluorescence Spectral Parameters of DOMs and DOM−Napropamide Complexes

	peak A (on a per carbon basis)		peak B (on a per carbon basis)		
sample	Ex/Em	intensity	Ex/Em	intensity	peak intensity ratio A/B
DOM-SL	230/340	109.0	280/310	221.6	0.49
DOM-ST	230/340	137.6	280/305	307.0	0.45
DOM(SL)−napropamide complex	230/340	179.8	280/310	203.6	0.88
DOM(ST)−napropamide complex	220/340	253.6	280/300	207.6	1.22

of DOMs was obtained from the comparative analysis of FT-IR spectra (**Figure 7** and **Table 5**). The FT-IR spectra show signals at 3408 and 3405 cm^{-1}, which were attributed to the stretching of hydroxyl group absorption and N—H stretching of various functional group peaks. DOM-SL with absorption at 2426 cm^{-1} could be interpreted by the C═N stretching vibration absorption peak. Similarly, DOM-SL with absorption at 1764 cm^{-1} was noted by the C═O stretching vibration absorption peak.

The bands at 1622 cm^{-1} for DOM-SL and 1634 cm^{-1} for DOM-ST resulted from the stretching C═C bonds in aromatic ring modes and alkenes. Signals at 1384 and 1401 cm^{-1} for SL and ST, respectively, were preferentially ascribed to N−H or C−H deformation. Moreover, signals at 1322 cm^{-1} for DOM-ST were preferentially ascribed to C−N or −OH deformation. The bands at 1116 and 1110 cm^{-1} for SL and ST, respectively, were attributed to the stretching C−O of alcohols, ethers, and carbohydrates. Absorption peaks near 870−600 cm^{-1} were the peaks for benzene ring.

The greatest change was the decrease in the relative intensity of absorption bands at 3408 and 3405 cm^{-1}, 1384 and 1401 cm^{-1}, and 1167 and 1110 cm^{-1} for DOM-SL and DOM-ST, respectively (**Figure 7**). The structural information indicated the direct involvement of these groups in the interaction with DOM by H-bond formation with suitable functional groups (e.g., carboxylic and/or phenolic hydroxyls) of humic substance molecules. The effect was more evident for DOM-SL than for DOM-ST. More detailed studies will be required to elucidate the change in DOM structures in the presence of the herbicide.

EEM Fluorescence Spectroscopy Analysis of DOM and DOM−Napropamide Complex. Due to its high sensitivity and nondestruction of samples, three-dimensional EEM fluorescence spectroscopy is frequently employed to analyze the material structures, particularly the structure of DOM and definition of its fluorescence properties (*48*−*50*). Our results with DOM-SL, DOM-ST, DOM(SL)−napropamide, and DOM(ST)−napropamide indicated that the main peak was located at the Ex/Em of 220−230/340 nm (peak A), and the second main peak was observed at

the Ex/Em of 280/300−310 nm (peak B) (**Figure 8** and **Table 6**). The first peak A fell in the region defined as shorter excitation wavelength (< 250 nm) and shorter emission wavelength (< 350 nm) related to the aromatic protein-like substances (*51*). Peak B was related to the tryptophan protein-like substances (*52*). Therefore, both DOM-SL and DOM-ST contained aromatic protein-like substances and tryptophan protein-like substances. Compared to the DOM-ST, DOM-SL shows a relatively lower intensity, suggesting that DOM-SL contained fewer unsaturated components (such as aromatic amino acids).

In peak A, DOM−napropamide complexes (both SL and ST) had a higher intensity than DOM alone, suggesting the effective incorporation and adsorption of napropamide into DOMs. However, the opposite intensity between the two categories was observed in peak B. It was possible that after DOMs chemically interacted with napropamide, some new molecular bonds would be formed, which resulted in the decrease of intensity. It is noted that there are several peaks (e.g., 320/360 nm) with relatively low fluorescence intensity for DOM-SL, suggesting that DOM-SL contained soluble microbial byproduct such as protein-like or phenol-like organics (*50*). These substances, whose molecule mass is small, may readily combine with napropamide.

Conclusion. The current study provides initial evidence that exogenous DOMs could alleviate toxicity of napropamide to *B. napus* grown in soil. Although the precise mechanism by which the two DOMs differentially affect napropamide-induced toxicity in rapeseed is unknown, application of DOMs to soil decreased the accumulation of napropamide in tissues and thereby reduced the production of ROS (H_2O_2 and O_2^-) and oxidative damage to plant cells. Also, DOMs improved plant growth under napropamide stress by reducing the activities of enzymes SOD, POD, CAT, and APX (indicators of oxidative stress) and accumulation of napropamide in plants.

To get insights into the interaction between DOMs and napropamide, experiments were performed focusing on the identification of DOMs or DOM−napropamide compound structures using FT-IR and three-dimensional EEM fluorescence spectroscopic approaches. Our results provide direct evidence that DOMs could interact with napropamide. These results support the importance that assessment of herbicide availability in soil−plant systems is necessary due to the application of DOMs to soils and their potential effects on yield and safety of crops under natural conditions.

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