Supplementary Information

Fungal degradation of selected medium to highly polar pesticides by *Trametes versicolor*: kinetics, biodegradation pathways, and ecotoxicity of treated waters

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Text S1. UPLC-ESI-HRMS analyses

An ultra-high performance liquid chromatography (UPLC) system (Acquity, Waters, Milford, MA, USA) coupled to a high-resolution mass spectrometer (HRMS) (a hybrid quadrupole-Orbitrap analyser Q Exactive, Thermo Fisher Scientific, San Jose, CA, USA) was used to identify the biodegradation products formed during the fungal degradation process and malathion abatement.

Chromatographic separation of 10 μ L of the sample extract was performed with a Purospher® STAR RP-18 endcapped Hibar® HR (150 $\times$ 2.1 mm, 2 μ m) column (Merck, Darmstadt, Germany) and a linear organic gradient. Two chromatographic runs were performed to analyse independently the samples under the electrospray positive ionization (ESI+) and negative ionization (ESI-) modes. The mobile phase used for ESI+ analyses consisted of water with and methanol, both with 0.1% of formic acid at a flow rate of 0.2 mL/min. In the ESI- analyses, a mobile phase consisting of water and acetonitrile at a flow rate of 0.3 mL/min was used. The linear organic gradient used was as follows: 5% for 1 min, increased to 20% in 2 min, to 80% in 4 min, and to 100% in 1 more min. Pure organic conditions were maintained for 2 min and then decreased to the initial conditions (5%) in 0.5 min, held for 4.5 min for column re-equilibration.

The ESI interface was operated using the following specific conditions: spray voltage, 3.0 kV; sheath gas and auxiliary gas flow rates, 40 and 10 arbitrary units, respectively; capillary and vaporizer temperatures, 350 °C and 400°C, respectively. HRMS acquisition was conducted in data-dependent acquisition (DDA) mode. For this, a full scan over the m/z range 70 to 1,000 with a full width at half maximum (FWHM) resolution of 70,000 (at m/z 200) was done, and data-dependent MS/MS scan events (FWHM resolution of 17,500 at m/z 200) using a 40% normalized collision energy were recorded for the most intense ions detected in each scan (with an Automatic Gain Control (AGC) target of 10e5 or a maximum IT of 50 ms). Data acquisition was controlled by Xcalibur 2.2 software (Thermo Fisher Scientific).

Malathion was analysed in the positive ionization mode and quantified using the full scan response of its molecular ion $[M+H]^+$. The quantification of malathion residual concentrations in the samples was performed by the internal standard method using its deuterated analogue (malthion-d10), and a 6-point solvent-based calibration curve.

Table S1. Structure and physical-chemical properties of the investigated pesticides.

Analyte	MW (g/mol)	Molecular formula	CAS number	IUPAC name	Solubility ^a	K _{oc} ^a	K _{ow} ^a	GUS ^a	Henry's law constant ^a	DT50 ^a
Acetamiprid	222.67	C ₁₀ H ₁₁ ClN ₄	135410-20-7	<i>N</i> -[(6-chloro-pyridin-3-yl)methyl]- <i>N'</i> -cyano- <i>N</i> -methyl-ethanimidamide	2950	200	0.80	0.40	5.30 X 10 ⁻⁰⁸	1.6
Imidacloprid	255.66	C ₉ H ₁₀ ClN ₅ O ₂	138261-41-3	(<i>NE</i>)- <i>N</i> -[1-[(6-chloropyridin-3-yl)methyl]imidazolidin-2-ylidene]nitramide	610	262 ^β	0.57	3.69	1.7 X 10 ⁻¹⁰	191
Malathion	330.36	C ₁₀ H ₁₉ O ₆ PS ₂	121-75-5	diethyl 2-dimethoxyphosphinothioylsulfanylb utanedioate	148	1800	2.75	0.00	1.00 X 10 ⁻⁰³	0.17

^a The PPDB, Pesticide Properties Database, <http://sitem.herts.ac.uk/aeru/footprint/index2.htm>. - Lewis, K.A., Tzilivakis, J., Warner, D. and Green, A. (2016). An international database for pesticide risk assessments and management. Human and Ecological Risk Assessment: An International Journal, 22(4), 1050-1064.

^β Kegley, S.E., Hill, B.R., Orme S., Choi A.H., PAN Pesticide Database, Pesticide Action Network, North America (Oakland, CA, 2016), <http://www.pesticideinfo.org>.

MW: molecular weight; Solubility: solubility in water at 20 °C; K_{oc}: organic carbon partition coefficient; K_{ow}: octanol-water partition coefficient; GUS: leaching potential index; Henry's law constant: Henry's law constant at 25 °C (Pa m³ mol⁻¹); DT50: soil degradation potential, expressed as half-life in days.

Table S2. Toxicity classification according to the Globally Harmonized System of Classification and Labelling of Chemicals.

Toxicity range (mg/L)	Interpretation
LC50/EC50^a ≤ 1	Very toxic
1 < LC50/EC50 ≤ 10	Toxic
10 < LC50/EC50 ≤ 100	Harmful
LC50/EC50 > 100	Not harmful

^a LC50, 50% lethal concentration; EC50, 50% effective concentration.

Figure S1. Evolution of the TPs identified during *T.versicolor*-mediated degradation of malathion.

Figure S2. Evolution of the TPs identified during *T.versicolor*-mediated degradation of a) acetamiprid and b) imidacloprid.