Accessory publication

1-D and 2-D NMR metabolomics of earthworm responses to sub-lethal trifluralin and endosulfan exposure

Jimmy Yuk,[A] *Myrna J. Simpson*[A] *and André J. Simpson*[A,B]

[A]Department of Chemistry, University of Toronto, Scarborough College, 1265 Military Trail, Toronto, ON, M1C 1A4, Canada.

[B]Corresponding author. Email: andre.simpson@utoronto.ca

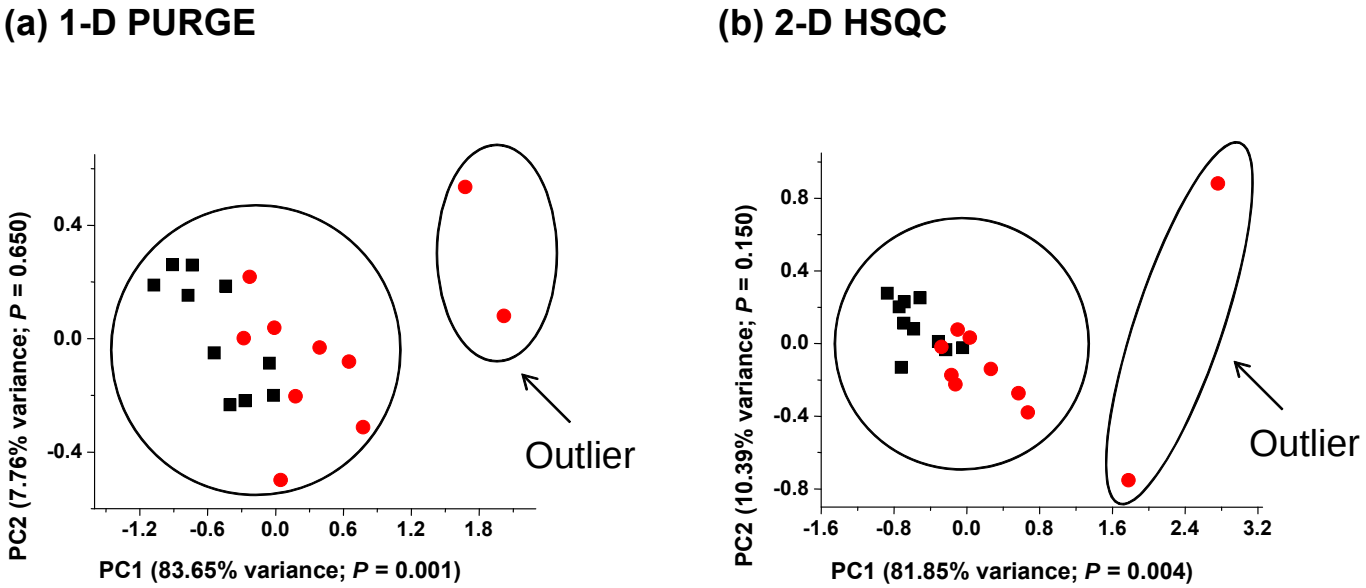

Fig. A1. PCA scores plot of PC1 *v.* PC2 of endosulfan-exposed *Eisenia fetida* (●) at 2.0 μg cm^{-2} and control *E. fetida* (■) (n = 10) using: (a) 1-D PURGE and (b) 2-D ^{1}H–^{13}C HSQC NMR spectra. The P-value for control- and endosulfan-exposed earthworms for the PCA components are reported using a two-sample t-test. Two earthworms at the highest endosulfan concentration (2.0 μg cm^{-2}) were identified to be outside the Hotelling's T2 ellipse at the 95% confidence interval and thus were removed from the dataset prior to subsequent analysis.

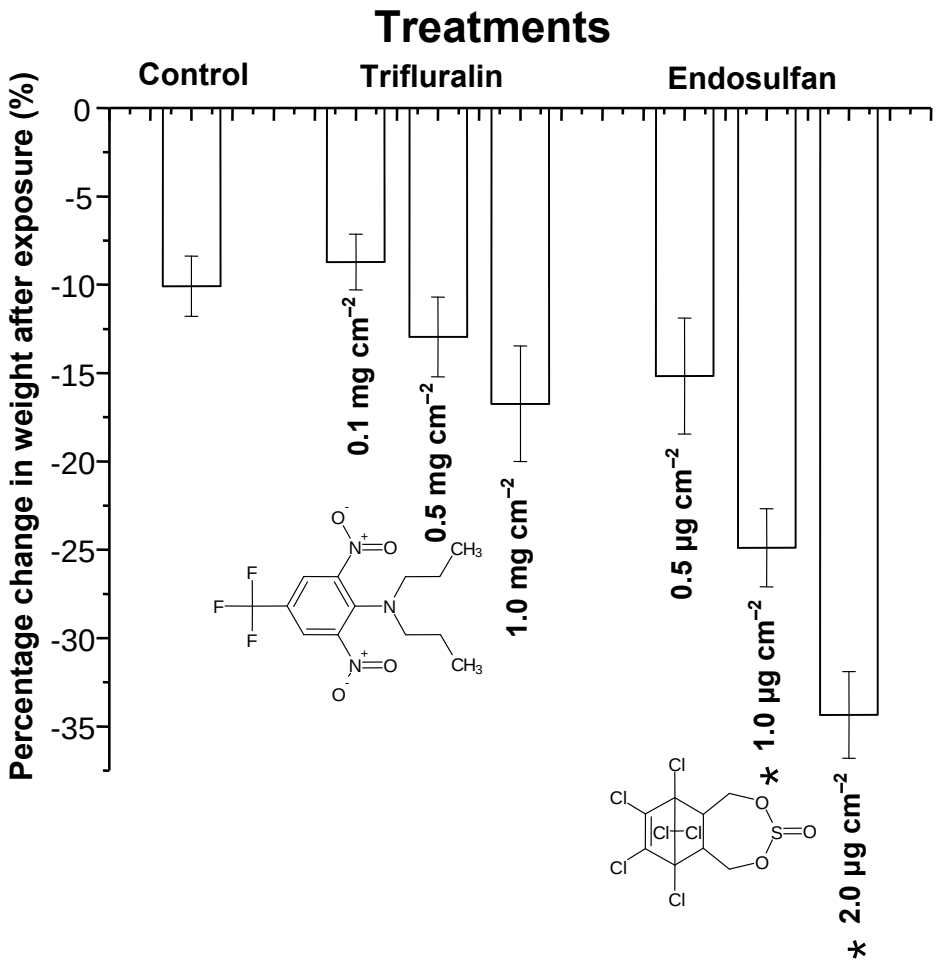

Fig. A2. Average percentage weight change of *Eisenia fetida* after exposure to trifluralin and endosulfan. The exposure concentrations are given below each bar and each asterisk represents significant weight change compared to control. Significance was determined by a two-sample *t*-test with a confidence interval of 95% ($P < 0.05$). The chemical structures of trifluralin and endosulfan are shown below their respective bar graphs.

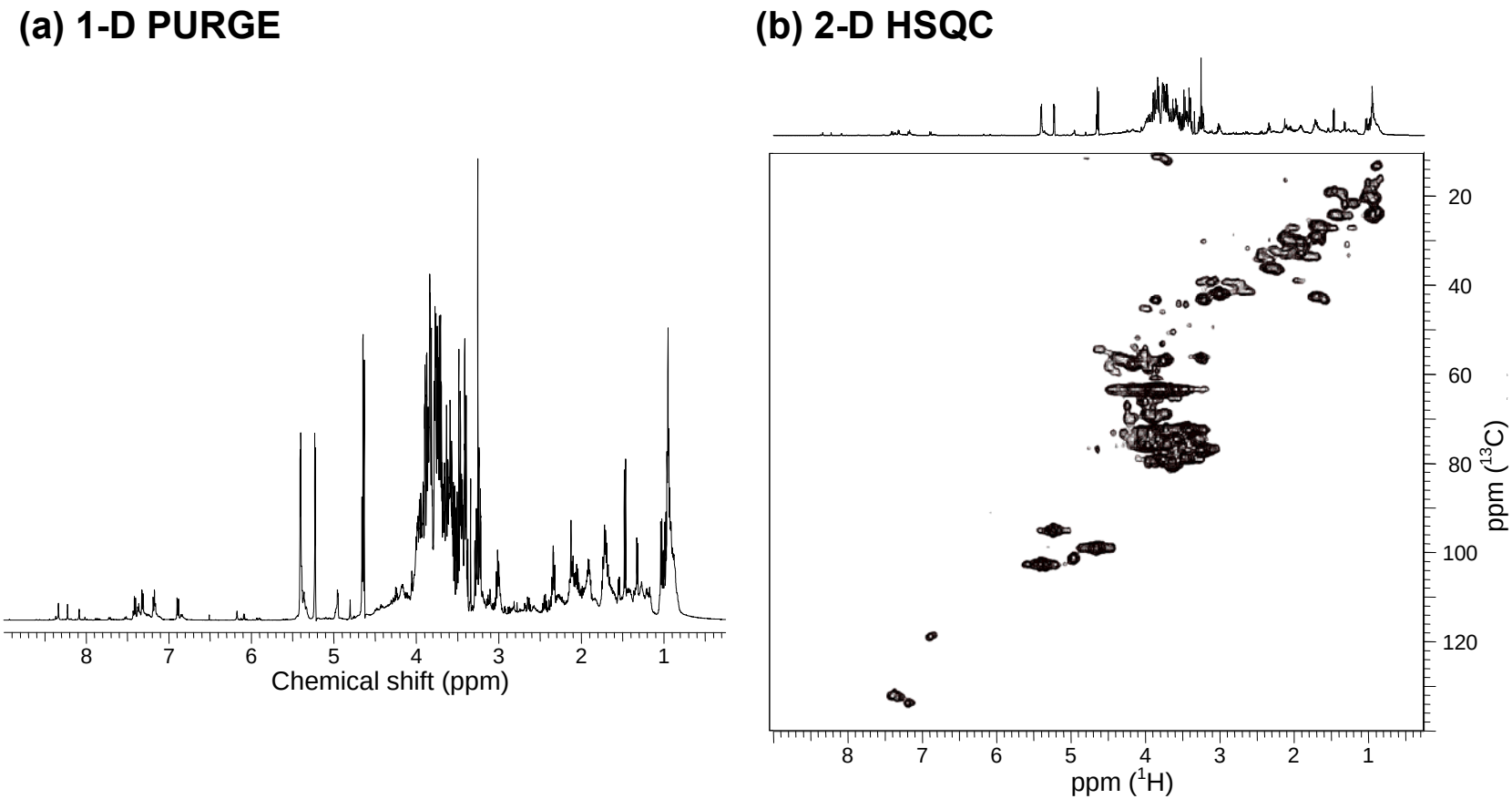

Fig. A3. 1-D and 2-D NMR spectra of control worm tissue extracts acquired using (a) 1-D PURGE and (b) 2-D ^{1}H–^{13}C HSQC NMR spectroscopy.

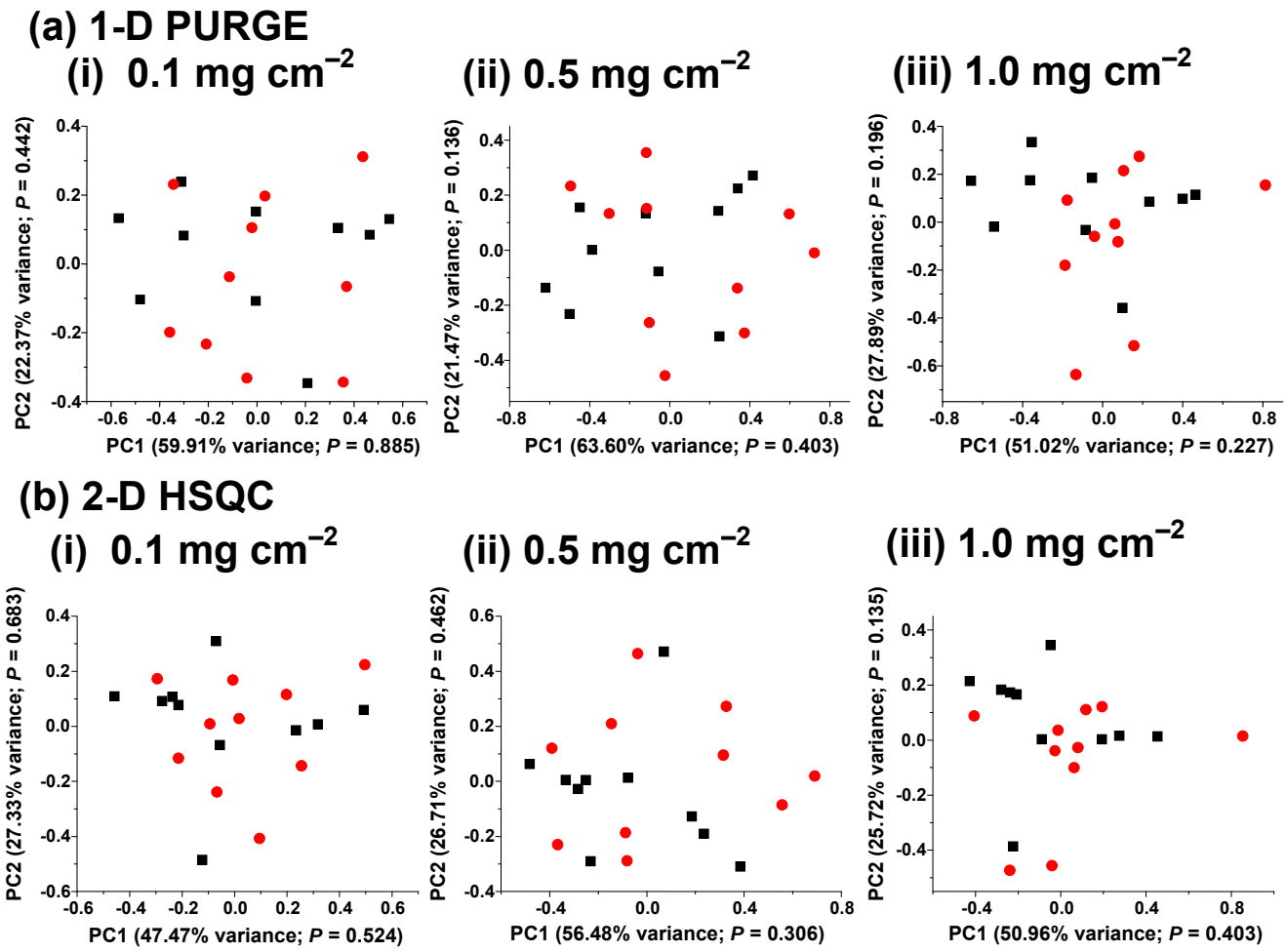

Fig. A4. PCA scores plot of PC1 *v.* PC2 of trifluralin-exposed *Eisenia fetida* (●) and control *E. fetida* (■) (n = 10) using (a) 1-D PURGE and (b) 2-D $^{1}H–^{13}C$ HSQC NMR spectra at: (i) 0.1 mg cm^{-2}; (ii) 0.5 mg cm^{-2}; and (iii) 1.0 mg cm^{-2}. The P-value for control- and endosulfan-exposed earthworms for the PCA components are reported using a two-sample t-test.

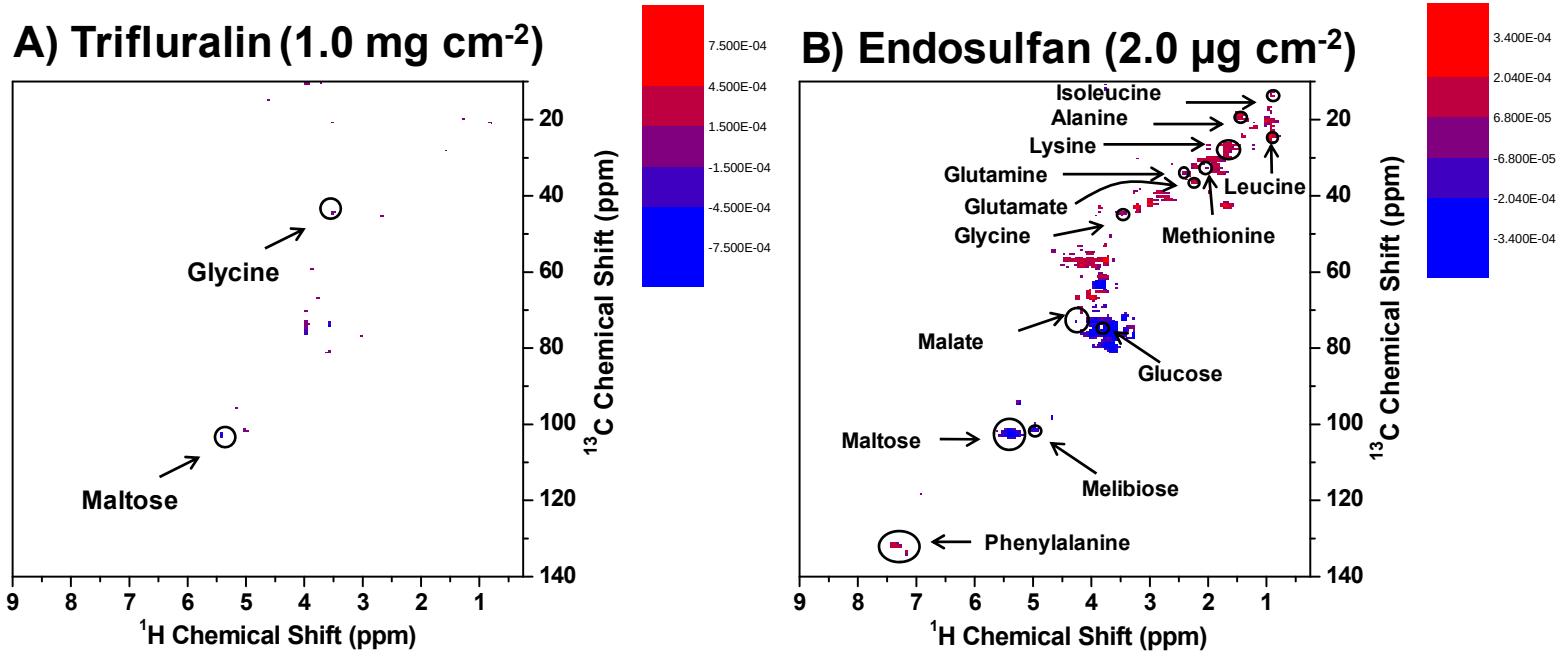

Fig. A5. *t*-test filtered ^{1}H-^{13}C HSQC difference spectra of *Eisenia fetida* tissue extracts were obtained by subtracting the mean buckets of: (a) trifluralin-exposed earthworms at 1.0 mg cm^{-2} concentration and (b) endosulfan-exposed earthworms at 2.0 µg cm^{-2} concentration, with the mean buckets of the control earthworms. Signals that were significantly different from the control ($P < 0.05$) were retained while everything else were excluded

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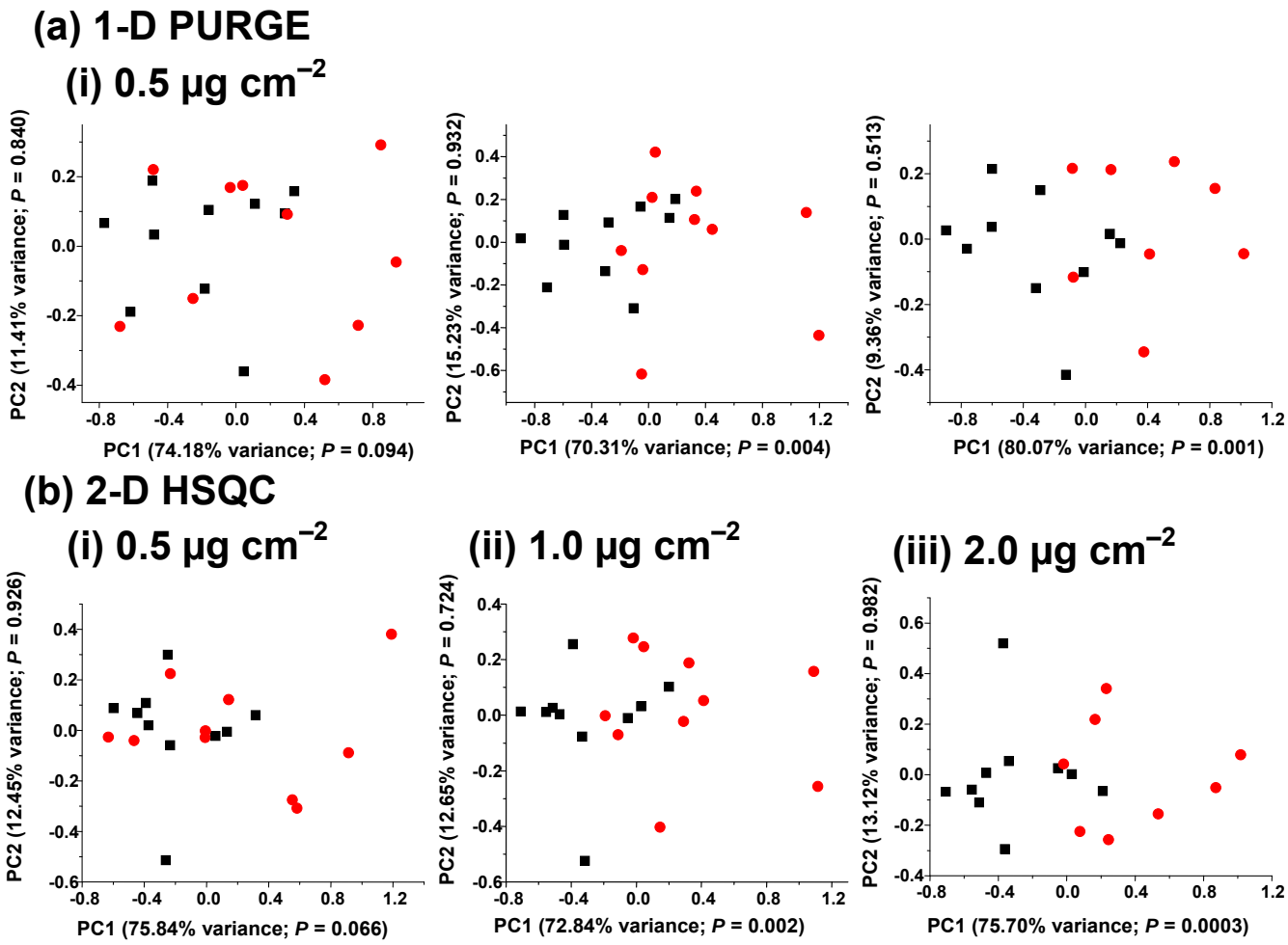

Fig. A6. PCA scores plot of PC1 *v.* PC2 of endosulfan-exposed *Eisenia fetida* (●) and control *E. fetida* (■) (n = 10) using (a) 1-D PURGE and (b) 2-D ^{1}H–^{13}C HSQC NMR spectra at: (i) 0.5 µg cm^{-2}; (ii) 1.0 µg cm^{-2}; and (iii) 2.0 µg cm^{-2}. The P-value for control- and endosulfan-exposed earthworms for the PCA components are reported using a two-sample t-test.