

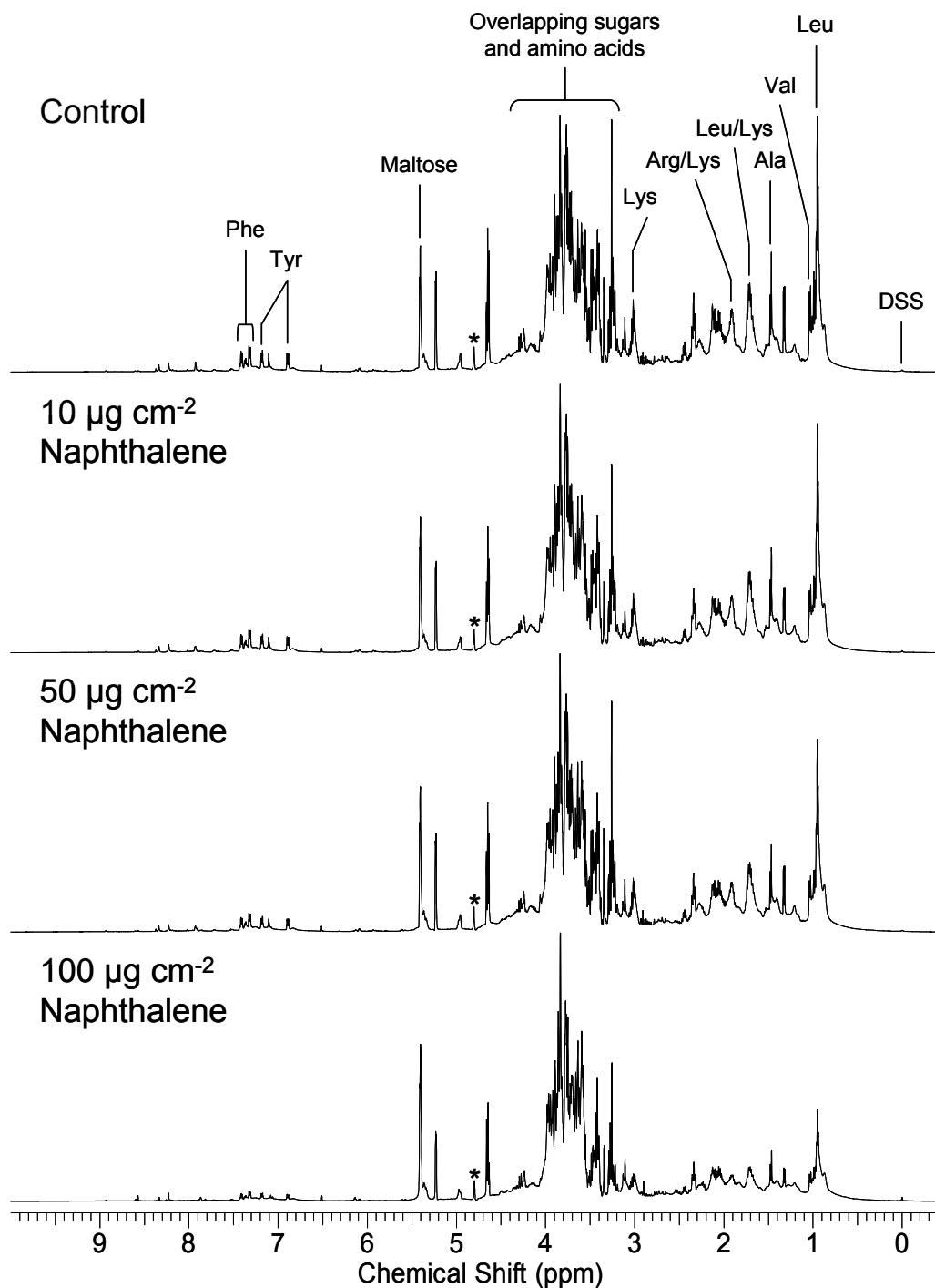
**Accessory publication**

**<sup>1</sup>H NMR metabolomics of earthworm responses to sub-lethal PAH exposure**

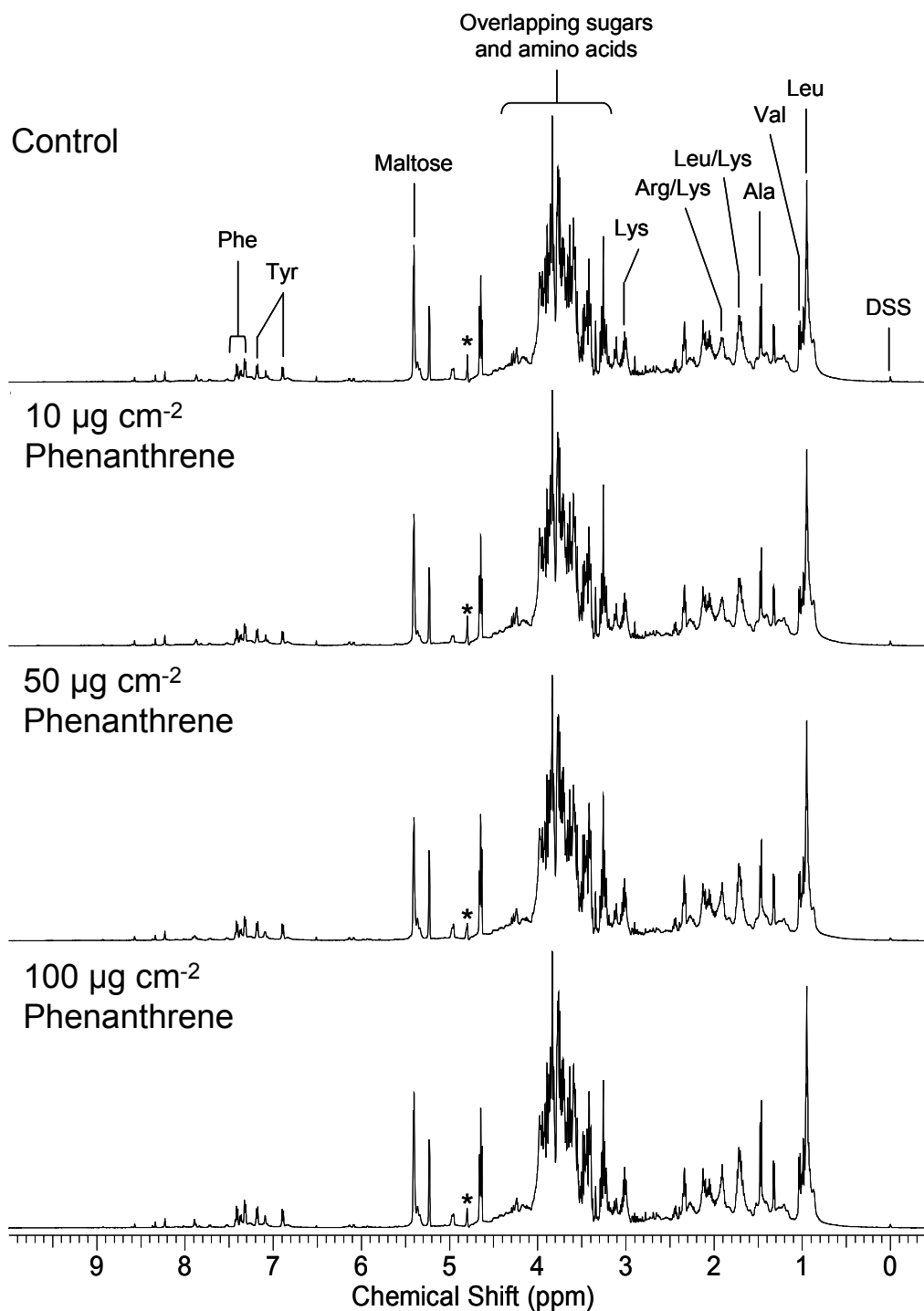
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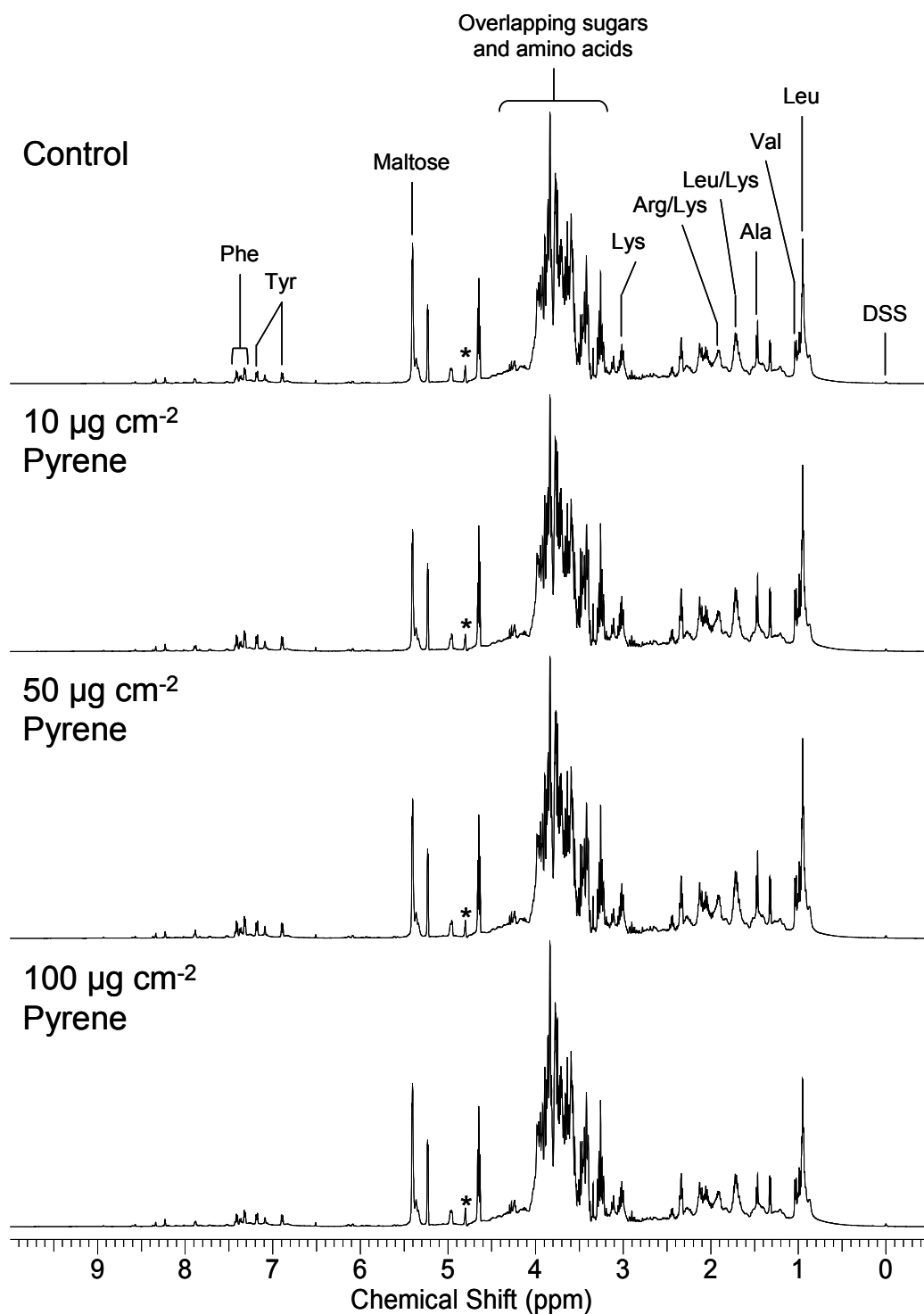
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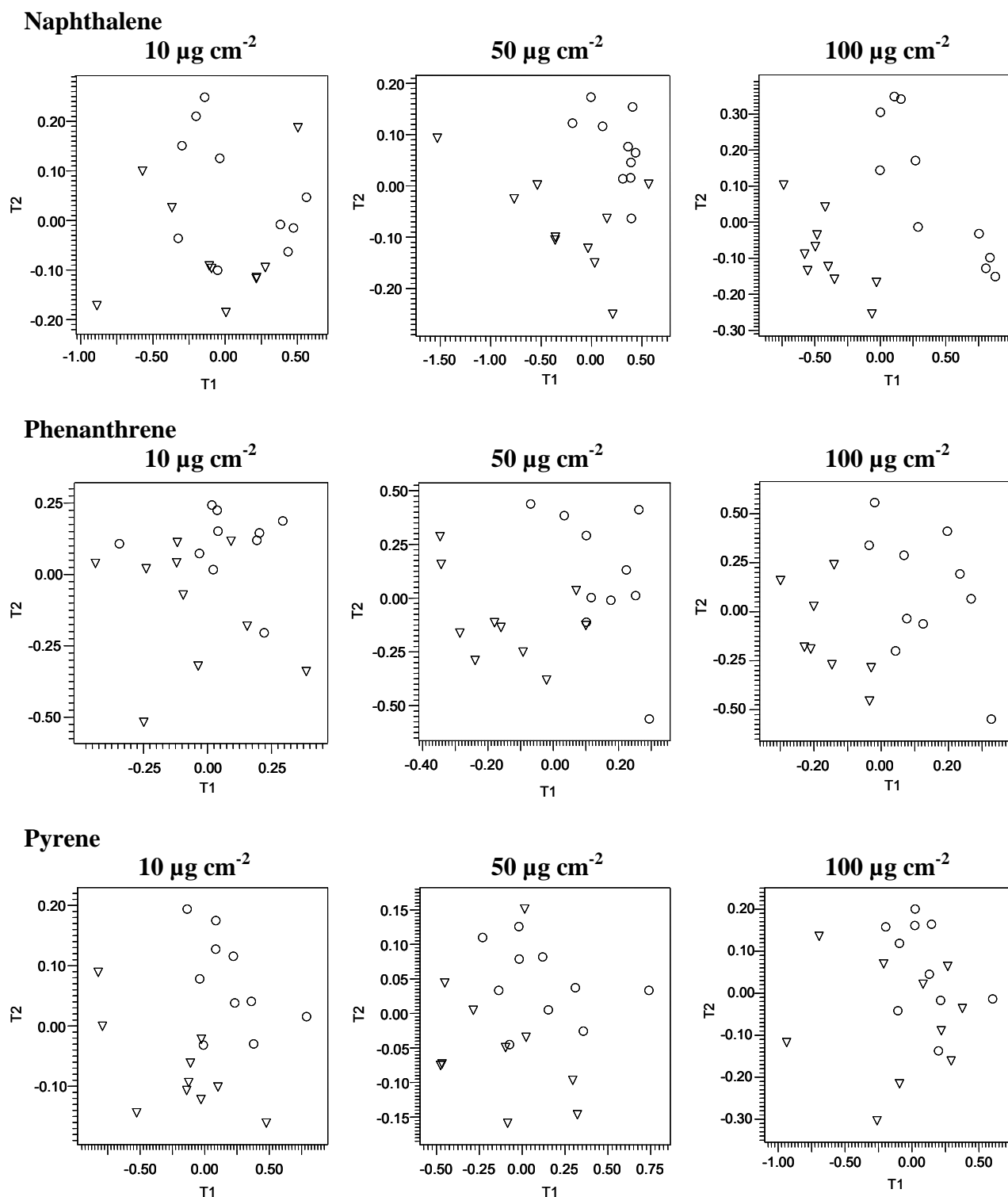
**Fig. A1.** The mean ( $n = 10$ ) <sup>1</sup>H NMR spectra of *Eisenia fetida* control (unexposed) earthworm D<sub>2</sub>O buffer extracts compared to earthworms exposed to 10, 50 and 100 μg cm<sup>-2</sup> naphthalene. HOD/H<sub>2</sub>O is labelled with an asterisk. Selected signals from several major metabolites and the internal standard (DSS) are labelled. Leu, leucine; Val, valine; Ala, alanine; Arg, arginine; Lys, lysine; Tyr, tyrosine; and Phe, phenylalanine.



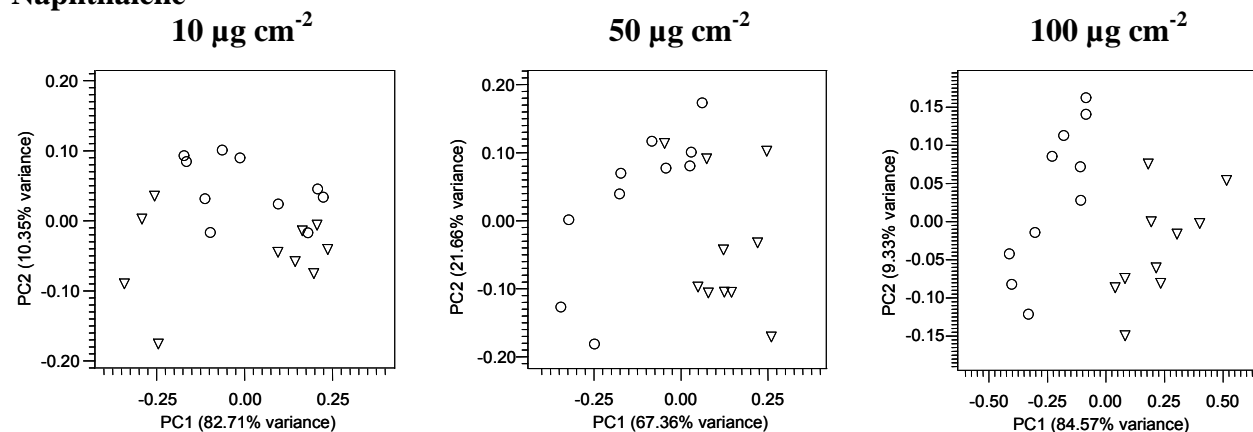
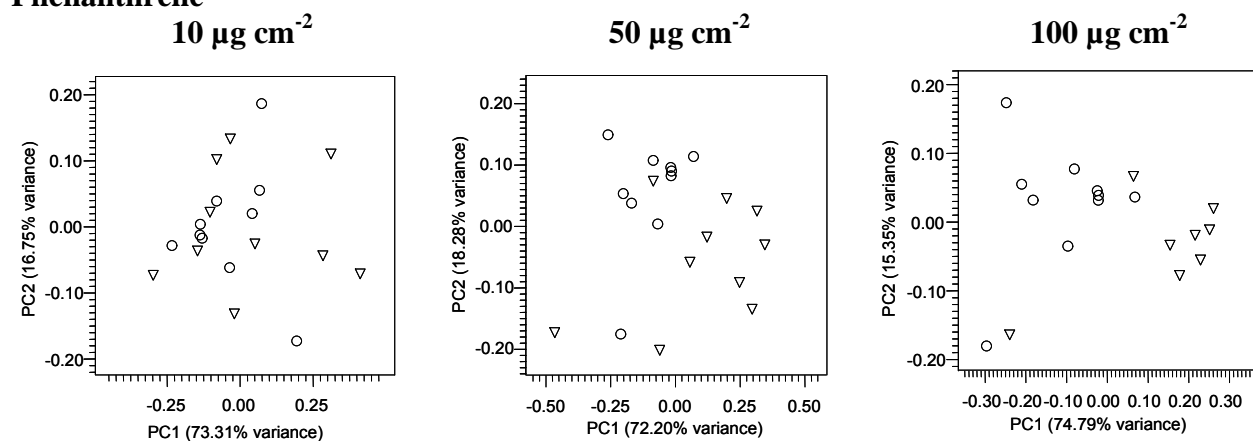
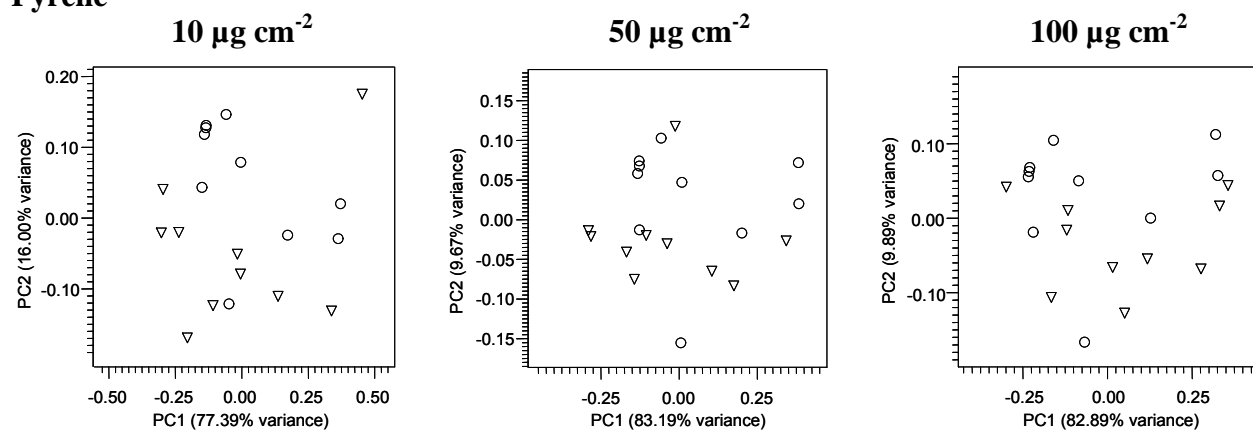
**Fig. A2.** The mean ( $n = 10$ )  $^1\text{H}$  NMR spectra of *E. fetida* control (unexposed) earthworm  $\text{D}_2\text{O}$  buffer extracts compared to earthworms exposed to 10, 50 and 100  $\mu\text{g cm}^{-2}$  phenanthrene.  $\text{HOD}/\text{H}_2\text{O}$  is labelled with an asterisk. Selected signals from several major metabolites and the internal standard (DSS) are labelled. Two of the ten earthworms exposed to 100  $\mu\text{g cm}^{-2}$  phenanthrene did not survive exposure and their data were not used in calculating the average  $^1\text{H}$  NMR spectrum. Leu, leucine; Val, valine; Ala, alanine; Arg, arginine; Lys, lysine; Tyr, tyrosine; and Phe, phenylalanine.



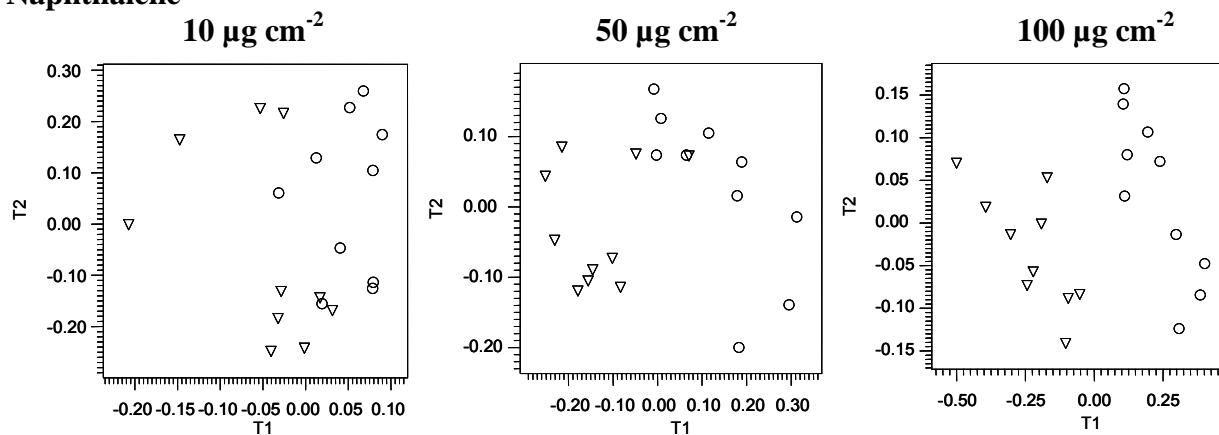
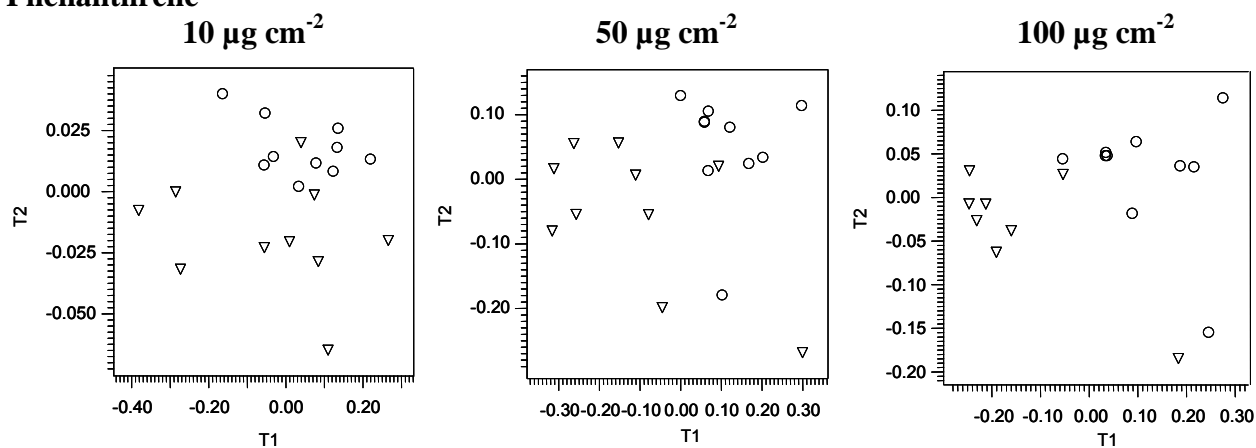
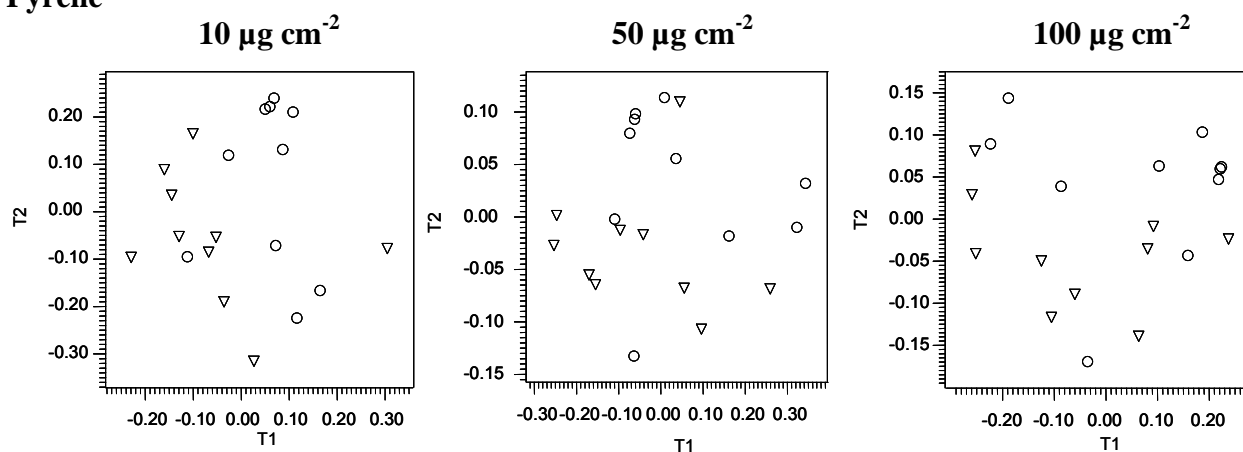
**Fig. A3.** The mean ( $n = 10$ )  $^1\text{H}$  NMR spectra of *E. fetida* control (unexposed) earthworm  $\text{D}_2\text{O}$  buffer extracts compared to earthworms exposed to 10, 50 and 100  $\mu\text{g cm}^{-2}$  pyrene. HOD/ $\text{H}_2\text{O}$  is labelled with an asterisk. Selected signals from several major metabolites and the internal standard (DSS) are labelled. Leu, leucine; Val, valine; Ala, alanine; Arg, arginine; Lys, lysine; Tyr, tyrosine; and Phe, phenylalanine.



**Fig. A4.** PLS-DA T-scores of the integrated  $^1\text{H}$  NMR spectra (0.50–10.00 ppm) for *E. fetida* control earthworms (○) and earthworms exposed to naphthalene, phenanthrene and pyrene (▽) at concentrations of 10, 50 and 100  $\mu\text{g cm}^{-2}$ . Two of the ten earthworms exposed to 100  $\mu\text{g cm}^{-2}$  phenanthrene did not survive exposure and their data were not used in calculations.

**Naphthalene****Phenanthrene****Pyrene**

**Figure A5:** PCA scores plots of the low-field region (0.50–3.10 ppm) of the integrated  $^1\text{H}$  NMR for *E. fetida* control earthworms (○) and earthworms exposed to naphthalene, phenanthrene and pyrene (▽) at concentrations of 10, 50 and 100  $\mu\text{g cm}^{-2}$ . Two of the ten earthworms exposed to 100  $\mu\text{g cm}^{-2}$  phenanthrene did not survive exposure and their data were not used in calculations

**Naphthalene****Phenanthrene****Pyrene**

**Fig. A6.** PLS-DA T-scores of the low-field region (0.50–3.10 ppm) of integrated  $^1\text{H}$  NMR spectra for *E. fetida* control earthworms (○) and earthworms exposed to naphthalene, phenanthrene and pyrene (▽) at concentrations of 10, 50 and 100  $\mu\text{g cm}^{-2}$ . Two of the ten earthworms exposed to 100  $\mu\text{g cm}^{-2}$  phenanthrene did not survive exposure and their data were not used in calculations.