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CHANGES IN THE METABOLIC PROFILE OF THE EARTHWORM EISENIA FETIDA AFTER EXPOSURE TO POLYCYCLIC AROMATIC HYDROCARBONS

Sarah A.E. BROWN, Andre J. SIMPSON and Myrna J. SIMPSON

Environmental NMR Centre and Department of Physical and Environmental Sciences, University of Toronto, 1265 Military Trail, Toronto, Ontario, Canada M1C 1A4

Earthworms are useful indicators of soil health and are frequently used in ecotoxicological studies (Lenz et al., 2005). In the past many of these studies focused on the lethal dose of contaminants, however there is increasing interest in determining the consequences of sublethal exposure. An innovative new technique useful for this type of study is metabonomics. Metabonomics is defined as "the quantitative measurement of the time-related multi-parametric metabolic response of living systems to pathophysiological stimuli or genetic modification" (Robertson, 2005). In metabonomics, the major metabolites are identified and quantified and provide information on how contaminant exposure affects the organisms' biochemical function (Robertson, 2005).

One of the most common types of contaminant found in soil is polycyclic aromatic hydrocarbons (PAHs). These contaminants are known to cause health problems in humans and soil dwelling organisms (Wilcke, 2000). In this research, ^{1}H NMR spectroscopy was used to determine the metabolic profile of *Eisenia fetida* (*E. fetida*) exposed to three common PAHs; naphthalene, phenanthrene and pyrene. The earthworm *E. fetida* was used because it is considered as a representative species for ecotoxicological studies even in environments where they are not typically found (OECD 1984). Filter paper tests were performed to determine the changes in the metabolic profile of the earthworms after exposure to sub-lethal doses of each contaminant. Sets of 10 worms were exposed to each dose of the PAHs; the doses administered were 0.1 mg/cm^2, 0.5 mg/cm^2 and 1 mg/cm^2. The earthworms were exposed to the PAHs for 48 hours, lyophilized and extracted into D_2O-based phosphate buffer. The ^{1}H NMR spectra of the extracts were acquired with a Bruker Avance 500 MHz spectrometer. Principal component analysis (PCA) was performed on the data to determine whether exposure to PAHs was causing a change in the metabolic profile of the earthworms.

It was determined that exposure to sub-lethal doses of naphthalene, phenanthrene and pyrene does cause a change in the metabolic profile of *E. fetida*. A dose-dependent response can be seen with phenanthrene and naphthalene. However, in the phenanthrene-dosed worm extracts there are greater differences in the metabolic profile with the higher dose of the PAH, but the opposite is seen with exposure to naphthalene. A dose-dependent response was not

observed in the earthworms' metabolic profiles after exposure to pyrene. In addition, this study demonstrated that the changes in the metabolic profile caused by exposure are different for each PAH. This is illustrated by the PCA plot of the datasets for exposure to 1mg/cm^2 of each PAH, shown in Figure 1. Analysis of ^{1}H-NMR spectra determined that exposure to naphthalene caused an overall decrease in the amount of glucose, maltose, phenylalanine, tyrosine, leucine, valine, lysine, arginine and alanine in the D_2O extract of the earthworms. Exposure to phenanthrene caused a decrease in the amount of glucose and maltose in the D_2O extract of the earthworm, but an increase in the amount of leucine, lysine, arginine, alanine, phenylalanine and tyrosine. Similarly, exposure to pyrene caused an increase in alanine, arginine, leucine, lysine and valine. The amount of glucose and maltose was also reduced, but the reduction was less than that observed after exposure to phenanthrene.

Additional experiments are planned that will examine the changes in the metabolic profile of *E. fetida* when the worms are exposed to mixtures of PAHs, PAH-contaminated humic acid, PAH-contaminated soil and remediated soils.

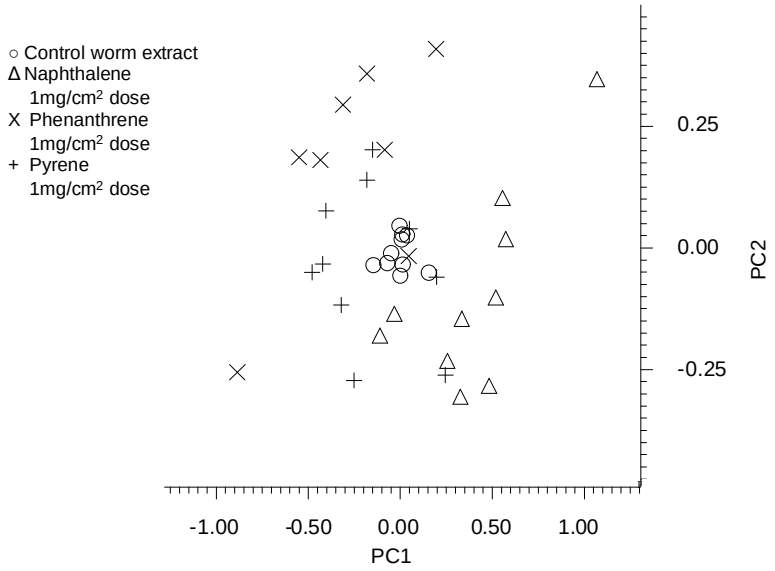

Figure 1. PCA plot of *E. fetida* control worm D_2O extracts and D_2O extracts of *E. fetida* dosed with 1 mg/cm^2 naphthalene, phenanthrene and pyrene.

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