Determining the Potential of Soil Communities to Metabolize Emerging Environmental Contaminants using Metagenomics

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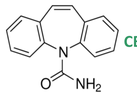
Background

Many contaminants of emerging concern (CECs) are not degraded during wastewater treatment and may enter the environment through **irrigation with wastewater effluents and biosolids** application.

From the many CECs being released into the environment, four are particularly common: the anti-inflammatory drug diclofenac (**DCF**), the anti-seizure drug carbamazepine (**CBZ**), and the antimicrobials triclocarban (**TCC**) and triclosan (**TCS**).



CBZ Carbamazepine (5*H*-dibenzo [*b*,*f*]azepine-5-carboxamide)

Diclofenac (2-(2,6-dichloranilino) phenylacetic acid) **DCF**

Triclocarban (3-(4-chlorophenyl)-1-(3,4-dichlorophenyl)urea **TCC**

Triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol) **TCS**

DCF, **CBZ**, **TCC** and **TCS** in soils represent a risk for surface water and groundwater contamination as well as a risk for uptake into plants.

To date, limited information exists on the potential for these chemicals to biodegrade in soils. The microorganisms and pathways responsible for **DCF**, **CBZ**, **TCC** and **TCS** biodegradation in soils are largely unknown.

Objectives

To determine the susceptibility of **DCF**, **CBZ**, **TCC** and **TCS** to biodegrade in different agricultural soils under **aerobic and anaerobic conditions** at environmentally relevant concentrations (**50ng CECs/g of soil**).

To identify which microbial phylotypes are enriched during **DCF**, **CBZ**, **TCC** and **TCS** biodegradation and therefore may be **associated with the biodegradation of these chemicals.**

To determine which **metabolic pathways** are associated with **DCF**, **CBZ**, **TCC** and **TCS** biodegradation in agricultural soils.

Methods

- Batch soil microcosms spiked with **DCF** or **CBZ** or **TCC** were established with four soils collected from agricultural site close to MSU (hereafter soils **A, B, C, D**) under **aerobic or anaerobic conditions**.
- Another soil batch microcosms spiked with **CBZ** or **TCC** or **TCS** were established with four soils collected from the KBS LTER Main Cropping System Experiment site for Treatments 1, 2, 3 and 4 (**T1-T4**) under **aerobic conditions**.
- **T1-T4** consist of corn/soybean/wheat rotations subject to different levels of chemical inputs and plot managements.
- The experimental design included triplicates of live sample microcosms, abiotic controls and live controls (no **DCF** or **CBZ** or **TCC** or **TCS**).
- At select time points, microcosms were sacrificed for nucleic acid extraction and **DCF** or **CBZ** or **TCC** or **TCS** extraction.
- High throughput sequencing (Illumina MiSeq platform) was performed on the extracted DNA and the data were analyzed using **Mothur**[1], **PICRUSt**[2] (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States), and **STAMP**[3] (Statistical Analysis of Taxonomic and Functional Profiles) (**Figure 1A**).
- **DCF**, **CBZ** ,**TCC**, **TCS** were extracted using QuEChERS (quick, easy, cheap, effective, rugged & safe), followed by purification using solid phase extraction (SPE) and analysis by liquid chromatography mass spectrometry (LC-MS/MS) (**Figure 1B**).

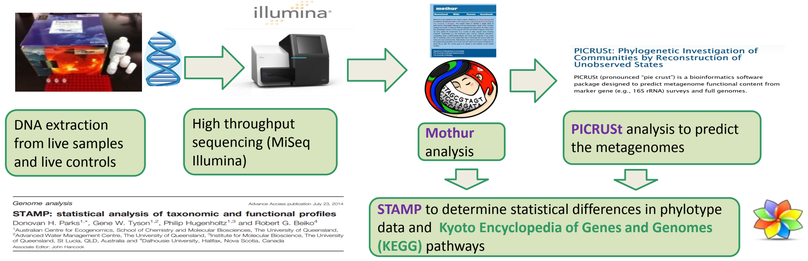


Figure 1A. Summary of molecular analysis

Methods

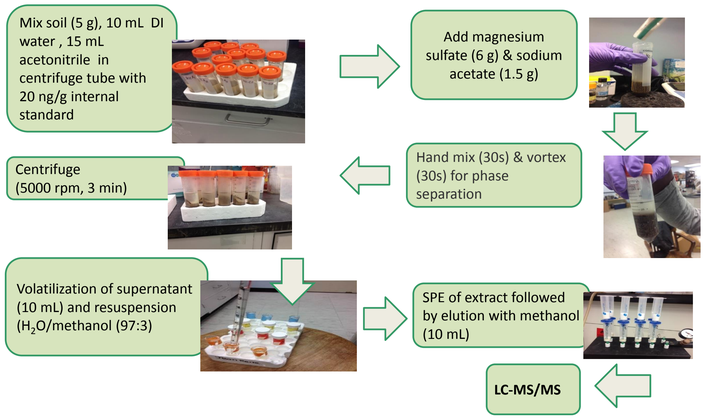


Figure 1B. Summary of **DC F**, **CBZ** , **TCC** and **TCS** extraction and analysis

Results: Biodegradation

***Diclofenac* Biodegradation**

DCF was rapidly biodegraded in all four soils tested **under aerobic conditions** (**Figure 2 A**).

Under anaerobic conditions, **insignificant** **DCF** biodegradation occurred in the majority of conditions and soils tested (**Figure 2B-D**).

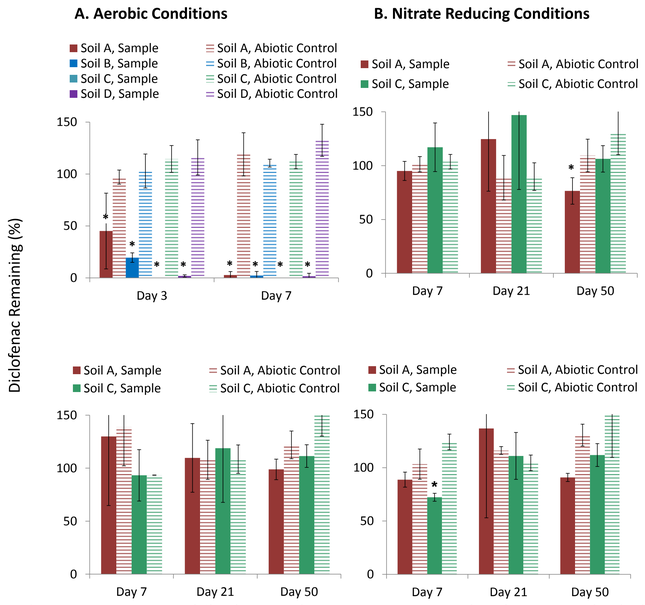


Figure 2. **DCF** remaining (%) in live sample microcosms compared to abiotic controls. The bars represent standard deviations from two or three microcosms. Asterisks indicate a significant difference between samples and controls ($p < 0.05$). The initial **DFC** concentration was 50 ng/g soil.

***Carbamazepine* & *Triclocarban* Biodegradation**

- ✓ **CBZ** & **TCC** were only **slowly** biodegraded in all four soils under aerobic conditions (**Figure 3**).
- ✓ However, significant difference was found between samples and controls for **CBZ** biodegradation on all days (except day 4, soil B).
- ✓ **TCC** biodegradation was significantly different compared to the controls on day 50 for all the soils.

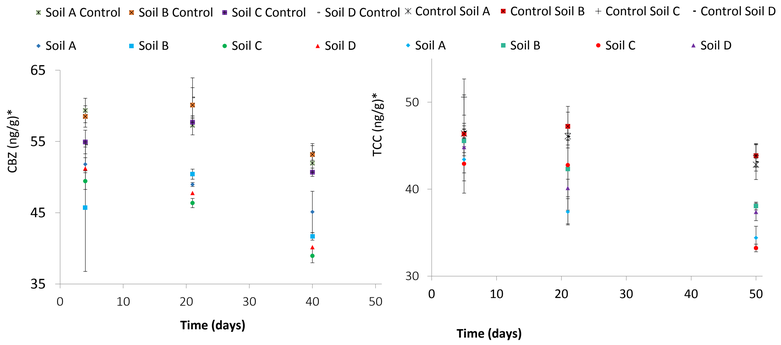


Figure 3. **CBZ** and **TCC** remaining (ng/g) under aerobic conditions in four soils live sample microcosms compared to abiotic controls. The bars represent standard deviations from three microcosms. The initial **CBZ** or **TCC** concentration was 50 ng/g soil. *non-zero axis.

Results: KEGG Pathways

***Enriched Pathways:* *Diclofenac* Biodegradation**

The predicted metagenomics (**PICRUSt**) were analyzed to determine which KEGG pathways were enriched during **DFC** biodegradation in the live samples compared to the controls (e.g. **Figure 5**).

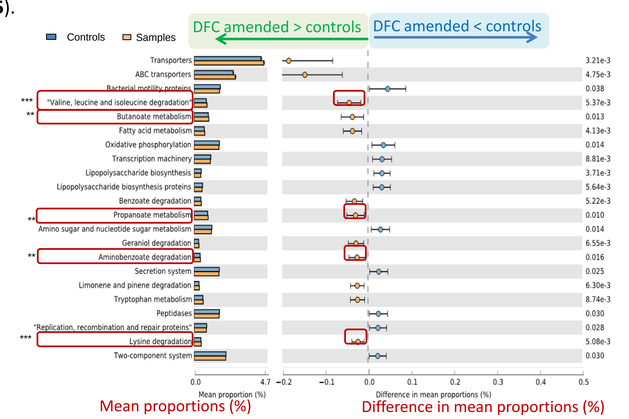


Figure 4. KEGG pathways more abundant in the DFC samples (n=6) versus the controls (n=6) for soil A. Two and three asterisks indicate the pathways were enriched in three and two soils, respectively.

When all four soils were compared, **two KEGG pathways** were enriched in three soils, including those involved with "*valine, leucine and isoleucine degradation*" and "*lysine degradation*" (**Figure 5**).

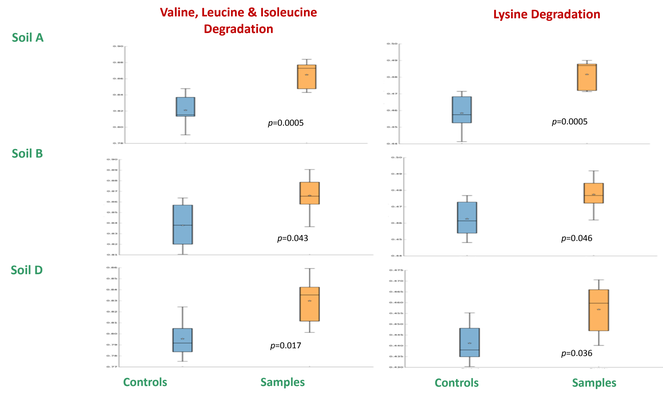


Figure 5. Comparison of the proportion of sequences (%) between the samples and controls (no DCF) for the KEGG pathways valine, leucine, isoleucine degradation (left) and lysine degradation (right) for soils A, B and D.

Biodegradation experiments for **CBZ** , **TCC** and **TCS** using the soils collected from the KBS LTER Main Cropping System Experiment site for Treatments 1, 2, 3 and 4 are still ongoing.
The average percent recovery (%) for these chemicals is presented in Table 1.

Table 1. Carbamazepine, triclocarban and triclosan average percent recovery (%) (n=3) in the KBS LTER soils (50ng/g **CBZ** or **TCC** or **TCS**) for the treatments 1, 2, 3 & 4. * To be determined.

Treatments	Carbamazepine	Triclocarban	Triclosan
1	83.5 ±6.5	93.4±3.5	TBD*
2	79.7±2.4	89.6±7.0	TBD
3	84.7±2.7	99.6±1.0	TBD
4	96.2±7.0	93.5±8.0	TBD

Conclusions & Ongoing Research

- ✓ **DCF** biodegradation was **limited under anaerobic conditions**.
- ✓ Aerobic **DCF** biodegradation was rapid (< 7 days) and **associated with KEGG pathways commonly present in soils** (those encoding for valine, leucine, isoleucine and lysine biodegradation).
- ✓ Aerobic **CBZ** and **TCC** biodegradation was slow (20-30% in 40-50 days).
- ✓ A **large number of phylotypes** were associated with **DCF** biodegradation.
- ✓ Aerobic **Ongoing research** is exploring the microorganisms associated with **CBZ** and **TCC** biodegradation as well as the pathways involved for soils **A**,**B**, **C**, and **D**.

✓Additional experiments are underway to examine the biodegradation of **CBZ**, **TCC** and **TCS** using soils from the KBS LTER Main Cropping System Experiment site for **T1-T4**.

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[2]Langille MGI, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, et al. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nature Biotechnology 2013; 31: 814-+.
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[4]Thelusmond, J.R., Strathmann, T. J. and A. M. Cupples. 2016. The identification of carbamazepine biodegrading phylotypes and phylotypes sensitive to carbamazepine exposure in two soil microbial communities. Science of the Total Environment. 571: 1241-1252.