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# Degradation of roxarsone in a silt loam soil and its toxicity assessment

Tengfang Liang [a,b], Zhengchen Ke [a,b], Qing Chen [b], Li Liu [a,b], Guowei Chen [a,b,*]

[a] Department of Civil Engineering, Hefei University of Technology, Hefei 230009, China
[b] School of Civil and Hydraulic Engineering, Hefei University of Technology, Hefei 230009, China

## HIGHLIGHTS

- Higher moisture and temperature promoted roxarsone degradation.
- Pentavalent arsenic was detected out.
- Inorganic arsenic other than roxarsone, exerted moderate toxicity to soil microbes.

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## ABSTRACT

The land application of poultry or swine litter, containing large amounts of roxarsone, causes serious arsenic pollution in soil. Understanding biotransformation process of roxarsone and its potential risks favors proper disposal of roxarsone-contaminated animal litter, yet remains not achieved. We report an experimental study of biotransformation process of roxarsone in a silt loam soil under various soil moisture and temperature conditions, and the toxicity of roxarsone and its products from degradation. Results showed that soil moisture and higher temperature promoted roxarsone degradation, associating with emergent pentavalent arsenic. Analysis of fluorescein diacetate (FDA) hydrolysis activity revealed that roxarsone does not exert acute toxic on soil microbes. With the release of inorganic arsenic, FDA hydrolysis activity was inhibited gradually, as evidenced by ecotoxicological assessment using *Photobacterium leiognathi*. The results shade new lights on the dynamic roxarsone biotransformation processes in soil, which is important for guiding appropriate disposal of poultry or swine litter in the environment.


## 1. Introduction

Roxarsone (3-nitro-4-hydroxyphenylarsonic acid) has been extensively used as animal feed additives to control coccidial intestinal parasites, and to increase weight and feed efficiency (Bednar et al., 2003; Andra et al., 2010; Mestrot et al., 2013). The majority of roxarsone is usually excreted unchanged in fresh manure, and enters into environment with fertilizer or litter (Garbarino et al., 2003; Cortinas et al., 2006; Stolz et al., 2007). More than 11.4 million tons of poultry litter was produced in the United States each year and of which approximately 90% was disposed through land application (Jackson et al., 2003), resulting in potential arsenic (As) pollution. The total As concentration in the poultry litter varied from 16 to 50 mg $kg^{-1}$ (Arai et al., 2003; Jackson et al., 2003).

Although roxarsone is less toxic than inorganic arsenic, its mineralization may result in more toxic species such as As (III) and As (V) (Jackson et al., 2003, 2006). Various arsenic species, such as arsenate, arsenite, monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), 3-amino-4-hydroxyphenylarsonic acid (HAPA) and other arsenic species have been detected in animal manure and roxarsone amended soil (Rosal et al., 2005; Makris et al., 2008; Huang et al., 2013). Arsenite could be cleaved photolytically from roxarsone moiety at pH 4–8, and the reaction rate increased with nitrate and natural organic matter concentration in poultry litter (Bednar et al., 2003).

Notwithstanding the ambiguity of detailed biochemical degradation pathways of roxarsone, it is undoubtedly that the biotransformation of arsenic species occurs after roxarsone enters the environment matrix. It depends greatly on the geophysics, geochemistry and microbial activity (Huang et al., 2011). Soil microbes play important roles in environmental arsenic cycling processes such as oxidation, reduction, methylation, and demethylation (Oremland and Stolz, 2003); and on the other hand with inhibitory arsenical compounds influencing microbial activity and their

* Corresponding author at: Department of Civil Engineering, Hefei University of Technology, Tunxi Road 193, Hefei 230009, China. Tel.: +86 551 62904144; fax: +86 551 62902066.
*E-mail address:* gwchen@hfut.edu.cn (G. Chen).

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ecological performance (Sierra-Alvarez et al., 2004). For example, *Clostridium* species, common in the chicken cecum and litter, has been identified to be involved in the biotransformation of roxarsone (Stolz et al., 2007; Guo et al., 2013). Jiang et al. (2013) reported that the presence of roxarsone greatly affected the diversity of indigenous microbial community and their metabolic activity in soil. Studies (Garbarino et al., 2003; Jackson et al., 2003) have revealed that roxarsone could be partly degraded to As (V), and other unidentified As species upon composting of the poultry litter. At the catalysis of particulate copper species, organoarsenicals could be degraded directly or indirectly under aerobic conditions (Andra et al., 2010).

Nevertheless, due to the extremely complex and dynamic soil environmental conditions, e.g., spatial heterogeneity and unsaturated and dynamic water condition (Stotzky, 1997; Or et al., 2007; Wang and Or, 2010, 2013), most previous studies focused either on saturated systems (Sierra-Alvarez et al., 2004; Stolz et al., 2007; Andra et al., 2010; Guo et al., 2013) or on direct sampling-analyzing of roxarsone contaminated soils (Garbarino et al., 2003; Jiang et al., 2013). Knowledge about the biotransformation processes and dynamics of roxarsone in soils, as well as the toxicity impacts of its degradation compounds on microbial activities remained unclear. We investigated the biotransformation of roxarsone in a silt loam soil under various environmental conditions, in terms of soil temperature and moisture, and assessed its toxicity on soil microbes.

## 2. Materials and methods

### 2.1. Soil sample

Soil samples were obtained from top layer in lakeshore of Hubintang in Hefei, China, and then transported to the laboratory for air-dried immediately. After manual sorting to remove gravels and plant residues, the soil was screened through sieve with 1 mm mesh. The physicochemical properties of the tested soil were summarized in Table 1, which could be classified as silt loam according to USDA soil texture classification (1951). The soil pH is 7.2, and the contents of organic carbon, total ferrum, and moisture were 0.78%, 3.29%, and 3.15%, respectively. The background value of arsenic and roxarsone in the tested soil was 0.73 and 0 mg $kg^{-1}$, respectively. Soil was enriched by a nutrient broth medium at pH 7.0, which contains basal solution per liter: pepton 5.0 g, beef extract 30.0 g, NaCl 5.0 g, $MnSO_4{\cdot}H_2O$ 5.0 mg. Then, the enriched soil was air-dried and used for further experiments. Sterilized soil was prepared by autoclaving soil at 121 °C for 60 min each time for a total of 3 times. .

### 2.2. Experimental procedures

To investigate roxarsone biotransformation in sterilized and non-sterilized soil, 4.0 mL of 1.0 g $L^{-1}$ roxarsone solution was added to 80.0 g (dry weight) sterilized or non-sterilized soil samples and then mixed thoroughly, respectively, keeping a spiking concentration of 50.0 mg $kg^{-1}$ dry weight. Each portion of soil was weighed accurately into 50.0 mL polypropylene centrifugal tubes at 4.2 g per tube. Sterilized water was added to adjust soil moisture to 50%. Tubes were then covered with plastic caps and placed in an incubator at 35 ± 0.5 °C in dark.

All the other experimental conditions were kept the same as above unless specially stated.

For the experiment scenarios of moisture effect, soil moistures were maintained at 10%, 20%, and 50%, respectively; and for scenarios of temperature dependence, soil incubation temperature was set to 15 ± 0.5, 25 ± 0.5, and 35 ± 0.5 °C, respectively.

For fluorescein diacetate (FDA) hydrolytic activity tests, bioluminescence inhibition and bacterial enumeration assays, roxarsone solution was added to 50.0 mL polypropylene tubes containing soil (4.0 g equivalent dry weight) to give roxarsone concentration ranging from 0 to 200.0 mg $kg^{-1}$ dry weight. The moisture content was adjusted to 35% and then the soils were incubated at 35 ± 0.5 °C in the dark.

**Table 1**
Physicochemical properties of the test soil used in this study.

| Parameter | Mean[a] | Parameter | Mean[a] |
|---|---|---|---|
| Soil pH | 7.2 | Roxarsone | ND[b] |
| Moisture | 3.29% | Sand | 20.0% |
| Organic carbon | 7.80 g $kg^{-1}$ | Silt | 65.9% |
| Total ferrum | 31.50 g $kg^{-1}$ | Clay | 14.1% |
| Total arsenic | 0.73 mg $kg^{-1}$ | Texture class[c] | Silt loam |

[a] Values are means of triplicate samples.
[b] ND, not detected.
[c] Classified as silt loam according to USDA soil texture classification (1951).

### 2.3. Sample extraction of roxarsone and other arsenic species from soil

Roxarsone, HAPA and inorganic arsenics in soils were extracted according to Huang et al. (2013) with slight modification. One drop of mercuric chloride solution and 10.0 mL of the mixture of 0.1 M $H_3PO_4$ and 0.1 M $NaH_2PO_4{\cdot}2H_2O$ (1:9, v/v) solution were added to each tube. Tubes were kept in water bath at 55 ± 0.5 °C for 10 h, and then sonicated for 10 min. After centrifugation at 10,000 rpm for 5 min, the supernatant was collected, filtered through 0.22 μm mixed cellulose membrane and the filtrate was stored at 4 °C for further analysis.

### 2.4. Chemical analysis

The concentrations of roxarsone and HAPA were determined using high performance liquid chromatography (HPLC 1260, Agilent Technology). We used a 4.6 mm × 15 cm Hypersil ODS-2 5 μm analytical column, with column temperature set to 30 °C. The mobile phase was composed of 90% $KH_2PO_4$ solution (0.05 M), 9% methanol and 1% formic acid, and the flow rate was 1.0 mL $min^{-1}$. Sample injection volume was 10.0 μL, and the detector wavelength was set to 264 nm.

Other As speices were detected by high-performance liquid chromatography with hydride generation atomic fluorescence spectrometry (HPLC-HG-AFS). Experimental conditions are summarized in Table 2.

### 2.5. Assay of FDA hydrolytic activity

The activity of FDA hydrolase was determined based on Green et al. (2006). Fifty mL polypropylene tubes containing 4.0 g soil

**Table 2**
Conditions of HPLC-HG-AFS for As (III) and As (V) detection analysis.

| | |
|---|---|
| *HPLC procedure* | |
| Anion exchange column | Hamilton PRP-X100 (250 mm × 4.1 mm id, 10 μm) |
| Mobile phase | 15 mM $(NH_4)_2HPO_4$; pH 6.0 |
| Flow rate | 1.0 mL $min^{-1}$ |
| Injection volume | 100 μL |
| *HG-AFS procedure* | |
| Acid solution | 7.0% HCl (v/v) |
| Reducing agent | 2.0% (w/v) $KBH_4$ in 0.5% (w/v) KOH |
| Main argon flow rate | 400 mL $min^{-1}$ |
| Auxiliary argon flow rate | 600 mL $min^{-1}$ |
| Primary current | 80 mA |
| Boost current | 40 mA |