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Integration of Chemical Methods and Biomarkers for Assessment of Chlorimuron-Ethyl Bioavailability in Soil

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ABSTRACT

Bioavailability is a critical factor for assessing the environmental risk of organic pollutants in soil. In this study, extractions with 3 different solvents, including 2 aqueous solutions, calcium chloride ($CaCl_2$) and a phosphate buffer solution (PBS), and a mixture of aqueous solution and organic solvent, a PBS-methanol (8:2, volume/volume) mixture (PBS-M), were performed to assess the bioavailability of chlorimuron-ethyl in soil in comparison to a battery of toxicity tests in wheat seedlings. The results indicated that the peroxidase (POD) activity in wheat leaves after 7 d of exposure was one of the sensitive biomarkers of chlorimuron-ethyl in soil. The extractability of chlorimuron-ethyl by all the 3 solvents decreased with exposure time, and the rate of decrease of the PBS-M extraction between 1 and 7 d of exposure was substantially higher than those of the aqueous solution extractions. Chlorimuron-ethyl gradually changed from a water-soluble form into a soil organic matter (SOM)-bound form in the soil. The PBS extraction correlated best with the POD activity in the leaves after 7 d of exposure.

Key Words: chemical extraction, environmental risk, organic pollutants, organic solvent, peroxidase activity, phosphate buffer solution, toxicity test

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INTRODUCTION

Agricultural soil contamination has become increasingly ubiquitous with the widespread use of pesticides and fertilizers, resulting in major environmental concerns. The current risk assessments of soil contaminants are predominantly based on their total concentrations. However, a growing number of reports have demonstrated that the chemical residues in soil are not completely bioavailable to organisms due to the interactions between contaminants and soil, such as sorption, sequestration, and the formation of bound residues (Kelsey *et al.*, 1997; Gevao *et al.*, 2001; Latawiec *et al.*, 2011). Therefore, current risk assessments may lead to overly conservative assessments and, subsequently, unnecessary remediation, or they may hinder the redevelopment of brownfield sites (Gomez-Eyles *et al.*, 2012). The bioavailable fraction has been gradually accepted as a key factor in actual risk assessments of soil contamination.

In recent decades, two empirical approaches have been developed to estimate the bioavailability of contaminants: a biological method and a chemical method. The biological method, as a basic approach for estimating bioavailability, must be performed to justify the feasibility of other methods (Katayama *et al.*, 2010); however, it is often laborious and time consuming. Therefore, a number of chemical methods have been proposed. Mild solvent extraction is one of the most widely applicable chemical methods because of its convenience and ease of use. It has been demonstrated as an effective method for estimating the bioavailability of many organic pollutants, including butachlor and myclobutanil (Yu *et al.*, 2005), polycyclic aromatic hydrocarbons (PAHs) (Gomez-Eyles *et al.*, 2011), and chlorobenzenes (Song *et al.*, 2011).

A chemical method can be considered feasible only when the concentration of pollutants extracted by the

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chemicals is correlated with biological endpoints (Lanno *et al.*, 2004). Accumulation in organisms and concentrations in the tissues have been suggested as biological endpoints in many studies on the bioavailability of organic pollutants such as PAHs, polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethanes (DDTs), and atrazine (Morrison *et al.*, 2000; Tao *et al.*, 2008; Song *et al.*, 2011). These pollutants are persistent organic pollutants (POPs), which could easily accumulate in plants or animals. Besides, they usually have relatively high concentrations in the environment and their detection limits are very low. In these circumstances, the concentration of the pollutants accumulated by organisms could be easily measured and compared with the quantity determined by chemical extraction. However, for those chemicals without the properties stated above, their accumulation or uptake may be difficult to determine and may not be applicable for use as biological endpoints. For example, sulfonylurea herbicides, as a type of highly effective herbicide, can be effective at a very low application rate; therefore, these herbicides exist at very low concentrations in the environment. Moreover, they are prone to be metabolized in plants or invertebrates. Consequently, they are difficult to detect in plants or invertebrates, which suggests that accumulation may not be a suitable biological endpoint for these pollutants. In this case, it has occurred to scientists that some of the biomarkers identified through toxicity testing may be good biological endpoints because they can provide the most direct measure of a hazard (Smith *et al.*, 2010). For example, Frassinetti *et al.* (2012) adopted phytochelatins in the marine diatom *Thalassiosira weissflogii* as a biomarker of metal bioavailability and found that a significantly positive relationship existed between the phytochelatin concentration in the cells of *T. weissflogii* and the total dissolved concentration of Cd in the elutriates of marine sediments. Maderova *et al.* (2011) determined that the expression of bioluminescence by the Cu specific biosensor enabled the quantification of Cu bioavailability in the soil pore waters and was thus a definitive measure of exposure. Smith *et al.* (2012) considered the progeny production to be a biomarker and observed that the $CaCl_2$ and simulated earthworm gut extractions were well correlated with progeny production.

Toxicity testing directly responds to the bioactive fraction of pollutants in soils and proves to be the most commonly applicable tool for measuring bioavailability (Kammenga *et al.*, 2000). Recently, most studies that have used biomarkers to determine the predictive availability of surrogate measures of bioavailability have examined heavy metals (Morrison *et al.*, 2000; Berthelot *et al.*, 2008). As a result, there is a clear need to investigate whether this method is applicable for organic pollutants, particularly for those chemicals noted above.

Chlorimuron-ethyl, a sulfonylurea herbicide, has been widely applied to control annual broadleaf weeds on soybean fields. Because of the low application rates, the concentration of chlorimuron-ethyl in soil is quite low. Nevertheless, it has been reported that chlorimuron-ethyl is detrimental to rotational crops even at very low concentrations because of its long persistence and high activity (Papiernik *et al.*, 2005). To our knowledge, no research about chlorimuron-ethyl bioavailability in soil has been reported. The aim of this study was to evaluate the applicability of toxicological and chemical measures for the assessment of chlorimuron-ethyl bioavailability in soil.

MATERIALS AND METHODS

Chemicals used and soil preparation

Chlorimuron-ethyl formulated as a 25% dispersible granule was bought from the Shanghai Branch, DuPont de Nemours & Co. (Shanghai, China). A stock solution was prepared by dissolving the formulated granule in methanol and was used to prepare the contaminated soil for wheat cultivation. Chlorimuronethyl (98.4% purity) purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany) was used as the standard for high performance liquid chromatography (HPLC). Methanol and glacial acetic acid were HPLC grade, and the other chemicals (acetone, methylene chloride, sodium dihydrogenphosphate and disodium hydrogen phosphate) were analytic grade.

Samples of a brown soil were collected from the Shenyang Experimental Station of Ecology in Liaoning Province, northeastern China. The soil samples were air-dried and sieved through 2-mm mesh prior to physicochemical characterization. The basic physicochemical properties of the soil are summarized in Table I. Air-dried (1 kg) soil was spiked with the above-mentioned stock solution to a final concentration of 0.052–1.970 mg kg^{-1} and was placed in a plastic pot (15 cm top diameter, 10 cm bottom diameter, 15 cm depth) for wheat cultivation.

Wheat cultivation

Wheat (*Triticum aestivum* L.) seeds obtained from Shenyang Agricultural University, China were first surface sterilized in 3% (volume/volume) H_2O_2, soaked in