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 (CaCl2) 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 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
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. Chlorimuron-ethyl (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 Liao’ning Province, northeastern China. The soil samples were air-dried and sieved through 2-mm mesh prior to physiochemical 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\(_2\)O\(_2\), soaked in
TABLE I

| pH<sup>a)</sup> | Organic C | Organic N | C/N ratio | CEC<sup>b)</sup> | Texture | Fe<sub>0</sub><sup>c)</sup> |
|----------------|------------|------------|-----------|-------------------|---------|-----------------|
| 5.70           | 11.4       | 1.08       | 10.56     | 17.99             | Sand    | 31.4            |
|                |            |            |           |                   | Silt    | 60              |
|                |            |            |           |                   | Clay    | 8.6             |
|                |            |            |           |                   |         | 15.12           |

<sup>a)</sup>Soil/water = 1:2.5.
<sup>b)</sup>Cation exchange capacity.
<sup>c)</sup>Dithionite-citrate-extractable iron.

distilled water for 4 h, and then germinated at 25 °C in a cultivation dish with moist filter paper. Subsequently, 15 uniformly germinated seeds in three replicates were transferred to each of the plastic pots containing soil spiked with different concentrations of chlorimuron-ethyl. The seedlings were grown under greenhouse conditions for 12-h light/12-h dark cycles at a constant temperature of 25 °C. The plants were irrigated with distilled water twice a day to maintain the soil moisture at 40%.

Soil samples were collected for chemical extractions after 1, 4 and 7 d, and wheat seedlings were harvested after 7 and 14 d of growth. After the wheat seedlings harvested were washed thoroughly with tap water and then with distilled water, they were wiped with clean filter paper. Finally, the leaves and roots were separated and ready for use in the analysis of toxicity endpoints.

**Determination of enzyme activity, soluble protein and malondialdehyde in wheat seedlings**

Approximately 0.1 (± 0.02) g of the leaf and root tissues in five pseudo-replicates were homogenized in 1 mL of 50 mmol L<sup>-1</sup> Na-phosphate buffer solution (pH = 7.8) containing 0.1 mmol L<sup>-1</sup> ethylenediamine tetraacetic acid (EDTA) and 1.0% (weight/volume) polyvinylpyrrolidone (PVP). The whole process was performed in an ice bath. Subsequently, the homogenate was centrifuged at 10000 r min<sup>-1</sup> at 4 °C for 15 min. The supernatant was used for enzyme assays and the determination of malondialdehyde (MDA) and soluble protein contents.

Enzyme activity was measured according to Wu and von Tiedemann (2002). The peroxidase (POD) activity was estimated by the increase in absorbance of oxiguaiacol at 470 nm. The catalase (CAT) activity was determined as a decrease in absorbance at 240 nm following the consumption of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). All enzyme activity data were related to the plant fresh weight (FW), and the activity of the enzymes was expressed as units (U) g<sup>-1</sup> FW.

The soluble protein content was determined by a spectrophotometric method at 595 nm according to the procedure of Bradford (1976) using a calibration curve with bovine serum albumin for quantification. The MDA content was determined as described by Hegedüs et al. (2001) and expressed as nmol g<sup>-1</sup> FW.

**Soil extraction of chlorimuron-ethyl with different solvents**

Extraction of chlorimuron-ethyl from the soil samples taken after different days of wheat seedling growth was carried out in triplicate using 4 different types of solvents including two aqueous solutions, 0.01 mol L<sup>-1</sup> CaCl<sub>2</sub> and a phosphate buffer solution (PBS) (pH 7.8), a mixture of aqueous solution and organic solvent, a PBS-methanol (8:2, volume/volume) mixture (PBS-M), and an organic solvent, a methylene dichloride-acetone (1:1, volume/volume) mixture (D-A). A proportion of 10 g (dry weight, DW) of soil was transferred to a 50-mL glass centrifuge tube with 20 mL of the 4 different solvents added. The centrifuge tubes were then shaken on a rotary shaker at 50 r min<sup>-1</sup> for 2 h at room temperature in the dark. The samples were centrifuged at 3000 r min<sup>-1</sup> for 15 min. The extraction process was repeated 3 times, and the supernatant was collected in a 100-mL beaker for the cleanup procedure. However, for extraction with D-A, the supernatant was transferred to a 100-mL round-bottom flask and concentrated using evaporation in a vacuum near dryness. The concentrate was dissolved in 5 mL of methanol and 40 mL of PBS for the cleanup procedure.

**Pre-concentration and cleanup of chlorimuron-ethyl from extraction solution**

Pre-concentration and cleanup of chlorimuron-ethyl from the extraction solution was accomplished using solid phase extraction (SPE). The solution pH was adjusted to 3.5 before loading an SPE cartridge.
The SPE cartridge (Oasis HLB, Waters Milford, USA) was preconditioned with 5 mL of methanol, followed by 5 mL of PBS that had been acidified to a pH of 3.5. The aqueous sample acidified to a pH of 3.5 was loaded onto the cartridge at a flow rate of 1 mL min$^{-1}$ in a vacuum. After washing with 5 mL of an aqueous methanol solution (30%, volume/volume), the cartridge was dried in a vacuum for approximately 30 min. The analytes retained on the cartridge were eluted with 10 mL of methanol, and the extract was evaporated to 2 mL under a stream of dry nitrogen gas. The residue was then filtered through a 0.45-µm filter membrane before analysis with HPLC.

**Conditions for HPLC analysis of chlorimuron-ethyl**

Chlorimuron-ethyl determination using HPLC was carried out according to a modified method from Choudhury and Dureja (1998). The HPLC system, assembled from modular components (Waters 2695-2696, Milford, USA), consisted of an in-line degasser, a 600E pump, a diode array detector (DAD), and an autosampler with an electric sample valve. A Zorbax SB-C18 column (15 cm length × 4.6 mm inner diameter, 5.0 µm particle size, Agilent, Foster City, USA) was used for separation. The applied mobile phase was a methanol-water (70:30, volume/volume) mixture acidified with the addition of 0.3% (volume/volume) glacial acetic acid at a flow rate of 1.0 mL min$^{-1}$. The injection volume was 10 µL, the oven temperature was 30 °C and the detector wavelength was 254 nm.

**Statistical analysis**

Descriptive statistics (i.e., means and standard deviations) were calculated for all data. Analysis of variance (ANOVA) was performed on the toxicity data. If ANOVA tests indicated a significant difference among the toxicity endpoints at different initial concentrations, two-sided least significant difference tests were applied to discriminate the differences between means. The assumptions of normality, homogeneity of variance and independence were examined; if the data failed the assumptions, non-parametric procedures (Dunnett’s C test) were applied. The relationship between each bioavailability measure and the observed toxicity endpoints was examined using univariate linear regression. The results of the univariate regression were preferred to assess the strength of each measure of bioavailability. Significance levels for each relationship were determined from the $P$ value associated with the slopes. SPSS (version 16.0) was used for all of the statistical analyses.

**RESULTS**

**Toxicity assessment of chlorimuron-ethyl to wheat seedlings**

The effects of different initial chlorimuron-ethyl concentrations on the soluble protein content and various antioxidant enzymes of wheat plants were as described in Fig. 1. The soluble protein content in the leaves did not exhibit a significant change after 7 d of exposure to contaminated soil by different concentrations of chlorimuron-ethyl ($P > 0.05$), even with the presence of chlorimuron-ethyl at the highest concentration, but it significantly decreased after 14 d of exposure. Compared with that in the leaves, the soluble protein content in the roots was much more susceptible to treatment with chlorimuron-ethyl. With increases in chlorimuron-ethyl concentration, the soluble protein content in the roots after 7 d of exposure decreased noticeably ($P < 0.05$), and this effect was transient because no significant difference was observed after 14 d of exposure.

The wheat seedlings grown in soil treated by chlorimuron-ethyl exhibited significantly ($P < 0.05$) higher levels of POD activity compared to the control. When the initial chlorimuron-ethyl concentration in soil was 1.970 mg kg$^{-1}$, the POD activity in the leaves increased by 80% over the control. With increases in chlorimuron-ethyl concentration, the POD activity in the leaves exhibited a consistent increasing trend throughout the growth period. In general, the POD activity was much higher in the roots than in the leaves, but the sensitivity of POD activity in the roots was much lower than that in the leaves. Moreover, after 14 d of exposure, the POD activity in the roots fluctuated a little.

The CAT activity was also markedly ($P < 0.05$) enhanced by the chlorimuron-ethyl treatments. The CAT activity in the leaves after 7 d of exposure to 1.970 mg kg$^{-1}$ chlorimuron-ethyl was twice that of the control. Similar to the change in the soluble protein content in the roots, the induction of CAT activity in the leaves by chlorimuron-ethyl diminished with the time of exposure. Both the CAT activity and the effect of chlorimuron-ethyl on it were much lower in the roots than in the leaves.

The treatment of chlorimuron-ethyl significantly ($P < 0.05$) increased the MDA contents in the leaves and roots after 7 d of exposure. When the concentrations of chlorimuron-ethyl were low, no apparent difference was observed in the MDA contents. As the concentrations increased to a high level, the MDA con-
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Fig. 1 Toxicity data in the leaves and roots of wheat seedlings after 7 and 14 d of exposure to contaminated soil by different concentrations of chlorimuron-ethyl in the range from 0 (control, CK) to 1.970 mg kg\(^{-1}\) dry weight (DW). The error bars indicate one standard deviation of the mean (n = 3). Bars with the same letter(s) indicate no significant difference at \(P > 0.05\). FW = fresh weight; U = units; POD = peroxidase; CAT = catalase; MDA = malondialdehyde.

Contents increased significantly. Especially for the highest concentration (1.970 mg kg\(^{-1}\)), the MDA contents increased by 37% and 43% in the leaves and roots, respectively, as compared with the control. However, different change trends were observed in the roots and the leaves exhibited little fluctuation between different concentrations after 14 d of exposure.

Chemical extractions

The recovery of the D-A extraction used to measure the total concentration of chlorimuron-ethyl in soil was 84%–94% in the preliminary experiments (relative standard deviation (RSD) = 2.1%–7.5%) (data not shown). As shown in Fig. 2, the total concentration of chlorimuron-ethyl decreased substantially to only 49%–62% of the initial spiking concentration after 1 d of exposure. The total concentration decreased dramatically from the first day to the fourth day, whereas it changed insignificantly from the fourth day to the seventh day. The other 3 solvents (CaCl\(_2\), PBS and PBS-M) were applied to assess the bioavailability concentration of chlorimuron-ethyl in soil. Most of the extractions by the 3 solvents had significant decreases from the first day to the fourth day. However, from the fourth day to the seventh day, they showed different decrease rates: The PBS-M extraction showed no obvious difference, but the extractions with the 2 aqueous solutions decreased significantly (Fig. 2).

In order to quantitatively show the change, the extraction percentage of the other 3 solvents were calculated as the ratio of the extraction amount by the solvent to the total amount determined by the D-A extraction. The chlorimuron-ethyl extractability of the other 3 solvents decreased in the following order: PBS-M > PBS > CaCl\(_2\). After exposure for 1 d, the extraction rates were 39%–66%, 48%–68%, and 68%–98%, respectively. The extractability of chlorimuron-ethyl decreased with the exposure time. The extractable percentages after 7 d were significantly (\(P < 0.05\)) reduced to 19%–51%, 24%–48%, and 47%–93% of the total amount for the PBS-M, PBS, and CaCl\(_2\) extractions, respectively. For the higher initial concentrations of chlorimuron-ethyl, an obvious (\(P < 0.05\)) decline of almost 10% was observed for the CaCl\(_2\) and PBS extractions between 1 and 7 d of exposure; however, the extractability of PBS-M did not decline significantly (Fig. 3).
Fig. 2 Concentrations of chlorimuron-ethyl extracted from the soil under wheat seedlings after 1, 4 and 7 d of exposure to contaminated soil by different concentrations of chlorimuron-ethyl in the range from 0.052 to 1.970 mg kg\(^{-1}\) dry weight (DW), using CaCl\(_2\) (a), a methylene dichloride-acetone mixture (D-A) (b), a phosphate buffer solution (PBS) (c) and a PBS-methanol mixture (PBS-M) (d). Error bars indicate one standard deviation of the mean (\(n = 3\)). Bars with the same letter(s) indicate no significant difference at \(P > 0.05\).

Fig. 3 Percentages of chlorimuron-ethyl extracted from the soil under wheat seedlings after 1, 4 and 7 d of exposure to contaminated soil by chlorimuron-ethyl at 0.58 mg kg\(^{-1}\) dry weight, using CaCl\(_2\), a phosphate buffer solution (PBS) and a PBS-methanol mixture (PBS-M).

Comparison of chemical assessment with wheat-based toxicity endpoints

Table II lists the adjusted regression coefficients between the chemical extractions and biological endpoints after 7 d of exposure. Univariate regressions were only performed using toxicity data, for which there was a significant difference among at least three initial chlorimuron-ethyl concentrations.

After 7 d of exposure, the amount of chlorimuron-ethyl extracted using all three chemicals were significantly \((P < 0.05)\) correlated with the POD activity in the leaves (Table II). Among all of the extractions, the extractable amount using PBS had the largest adjusted regression coefficient \((R^2)\) with the POD activity in the leaves, particularly after 4 d of exposure (adjusted \(R^2 = 0.908, P < 0.001\)). No extraction was adequately correlated with the CAT activity in the leaves, except the CaCl\(_2\) extraction, for which the adjusted \(R^2\) was also low (0.578). None of these extractions after 3 d of exposure were correlated well with the MDA content in the leaves. However, it seems much better for the soluble protein content in the roots, as those of all the extractions were well correlated with the soluble protein content in the roots (adjusted \(R^2 > 0.65, P < 0.05\)) after 4 d of exposure. A regression of all of the extractions with the MDA content in the roots fit very well, with adjusted \(R^2\) being greater than 0.88 \((P < 0.01)\).

DISCUSSION

When plants are exposed to different stress states, active oxygen species (AOS) can be generated in their
### TABLE II

Adjusted regression coefficient $R^2$ values of univariate regressions between chlorimuron-ethyl in soil extracted with different solvents and toxicity data$^a$ of wheat seedlings after 7 d of exposure to contaminated soil by different initial chlorimuron-ethyl concentrations.

| Extraction solvent | Exposure time | Leaves | Roots |
|--------------------|--------------|--------|-------|
|                    |              | Soluble protein content | POD activity | CAT activity | MDA content | Soluble protein content | POD activity | CAT activity | MDA content |
| CaCl$_2$           | 1d           | ND$^c$ | 0.866*** | 0.489 | 0.513 | 0.562 | ND | ND | 0.926*** |
|                    | 4d           | ND | 0.870*** | 0.519 | 0.559 | 0.616* | ND | ND | 0.946*** |
|                    | 7d           | ND | 0.691* | 0.578* | 0.705* | 0.516 | ND | ND | 0.882*** |
| PBS                | 1d           | ND | 0.880** | 0.486 | 0.595* | 0.648* | ND | ND | 0.968*** |
|                    | 4d           | ND | 0.908** | 0.478 | 0.445 | 0.601* | ND | ND | 0.926*** |
|                    | 7d           | ND | 0.834* | 0.488 | 0.499 | 0.568 | ND | ND | 0.949* |
| PBS-M              | 1d           | ND | 0.864* | 0.533 | 0.466 | 0.593 | ND | ND | 0.941** |
|                    | 4d           | ND | 0.884** | 0.515 | 0.537 | 0.688* | ND | ND | 0.938*** |
|                    | 7d           | ND | 0.835* | 0.498 | 0.504 | 0.573 | ND | ND | 0.949** |

$^a$POD = peroxidase; CAT = catalase; MDA = malondialdehyde.

$^b$PBS = a phosphate buffer solution; PBS-M = a PBS-methanol mixture; D-A = a methylene dichloride-acetone mixture.

$^c$Not determined because there were no significant differences among at least three initial chlorimuron-ethyl concentrations.

* *, **, ***Significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

AOS bodies. Accordingly, plants act with various self-protection mechanisms to eliminate or reduce the AOS caused by damages and stresses (Wu and von Tiedemann, 2002). One of the most important protection mechanisms is the adjustment of the enzymatic antioxidant system, including POD, CAT, etc (Hegedüs et al., 2001). To complement a general toxicity assessment, a multi-marker approach was applied using indicators from different physiological systems. Several endpoints, including soluble protein content, POD activity, CAT activity, and MDA content, were investigated in this study. Except for the soluble protein content, all of the toxicity endpoints in the leaves and the MDA content in roots after 7 d of exposure were sensitive to the presence of chlorimuron-ethyl, which was similar to a study by Wang and Zhou (2006). Furthermore, compared with their results, the change in our study was much more significant because the concentration range was larger. Moreover, the increasing trend in these endpoints was relatively consistent with the increasing concentration of chlorimuron-ethyl. However, after 14 d of exposure, even if there was also a significant difference in these endpoints between different initial concentrations, abnormal points against the general trend always existed, except with the POD activity in the leaves. This phenomenon might be due to the recovery of the defensive effect of antioxidant enzymes with increasing exposure time (Yu et al., 2012). In this way, the results of these endpoints after 7 d of exposure proved to be reliable biomarkers for the detection of wheat exposure to chlorimuron-ethyl in soil, especially the POD activity, which was very consistent in each exposure time measured. Considering the shorter duration of the toxicity experiment, the POD activity in the leaves after 7 d of exposure was considered the best biomarker for the assessment of chemical extractions.

The total chlorimuron-ethyl concentration of D-A extraction decreased dramatically to only 49%–62% of the initial concentration after 1 d of exposure, which may be due to the biodegradation and hydrolysis of chlorimuron-ethyl. In particular, hydrolysis was predominant for the breakdown of sulfonyleurea herbicides in soil and the hydrolysis rate was found to increase with increasing soil temperature and moisture content in soil (Fuesler and Hanafey, 1990; Sarmah and Sabadie, 2002). However, as the exposure time increased from 4 to 7 d, the total concentration of chlorimuron-ethyl did not obviously change and was likely to stabilize.

The chemical extractions applied in this study could be classified into two groups: extraction with an aqueous solution (CaCl$_2$ and PBS) and extraction...
with a mixture of aqueous solution and organic solvent (PBS-M). Even if CaCl₂ and PBS were both aqueous solutions, PBS had a much higher extraction rate than CaCl₂ for chlorimuron-ethyl (Fig. 2), which was consistent with extractions for other sulfonylurea herbicides (Wu et al., 2010). This is because chlorimuron-ethyl is an acidic compound and is prone to having a higher solubility in a basic solution, such as PBS (pH = 7.8). As expected, the extraction with PBS-M was significantly more exhaustive than those with the aqueous solutions (Fig. 2). A CaCl₂ solution is usually considered to be a “soft” extraction method for the labile fraction of chlorimuron-ethyl in soil (Barriuso et al., 2004): for example, it provided an indication of the amount of chlorimuron-ethyl that could be desorbed or that was water soluble and exchangeable. A mixture of aqueous solution and organic solvent, such as PBS-M, was expected to measure the fraction of chlorimuron-ethyl bound to soil organic matter (SOM) (Wu et al., 2011). As shown in Fig. 3, chlorimuron-ethyl was more readily available in a water-soluble form, but its availability in this form decreased rapidly with increasing soil-chlorimuron-ethyl contact time. Meanwhile, the SOM-bounded form increased, which suggested that the chlorimuron-ethyl in the soil changed from a water-soluble form into an SOM-bounded form.

The time-dependent reduction in the extractability of chlorimuron-ethyl was observed for all of the solvents, but the rate at which the extraction with organic solvents decreased with time was prominently higher than that with aqueous solutions between 1 and 7 d of exposure. This phenomenon might result from the change in the contamination states of chlorimuron-ethyl molecules in microsites within the soil matrix (Khan et al., 2011). For instance, chlorimuron-ethyl was redistributed from weaker to stronger sorption sites, which sequestrated or formed bound residues in soil and thus made the extraction very difficult.

A high regression coefficient between the POD activity and chemical extractions revealed that these extraction techniques would be efficient for predicting the bioavailability of chlorimuron-ethyl to wheat. Although these 3 extractions were expected to release different fractions of chlorimuron-ethyl from the soil, the relative contributions of chlorimuron-ethyl in the soluble fraction and adsorbed by organic matter to toxicity may have been similar. This was consistent with the findings of Smith et al. (2012), who observed that CaCl₂ extraction and simulated earthworm gut test extractions best predicted the bioavailability of heavy metals to earthworms, although CaCl₂ and earthworm gut simulations could extract different fractions of metals from soil. Compared with other extractions, the PBS extraction was most predictive of these endpoints after 7 d of exposure with the highest regression coefficients.

Though toxicity testing does not explicitly quantify the bioavailable fraction of chlorimuron-ethyl, measuring the toxicity of chlorimuron-ethyl in the soil automatically accounts for soil characteristics such as pH, cation exchangeable capacity, and organic matter content (Contardo-Jara and Wiegand, 2008). Moreover, the manifestation of a toxic effect is directly influenced by chlorimuron-ethyl bioavailability, which makes it a useful tool for comparing other measures of bioavailability. Evaluating bioavailability by integrating biomarkers and chemical extractions has been investigated by several researchers and is considered to be a valuable tool for identifying the potential hazards of pollutants (Berthelot et al., 2008; Smith et al., 2010; Frassinetti et al., 2012).

CONCLUSIONS

The POD activity in the leaves of wheat seedlings after 7 d of exposure was one of the most sensitive biomarkers of chlorimuron-ethyl in wheat. The chlorimuron-ethyl extractability of all 3 extraction solvents tested decreased with its residence time in soil. The amount of chlorimuron-ethyl recovered from soil with the 3 extractions decreased in the order of PBS-M > PBS > CaCl₂. None of the chemical extraction techniques used in this study reliably predicted the effects of chlorimuron-ethyl on all of the toxicity endpoints. Based on the results from the POD activity in the leaves of wheat seedlings after 7 d of exposure, PBS extraction could predict the bioavailability of chlorimuron-ethyl in the soil very well.

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