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Effects of adsorption on degradation and bioavailability of metolachlor in soil

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Abstract

The ability of soil to adsorb metolachlor strongly influences its environmental fate, but little information is available on the correlation of its soil adsorption with degradation and bioavailability. The present study was conducted to characterize adsorption, degradation and bioavailability of metolachlor in five soils with different properties, and to investigate the effect of soil adsorption on degradation and bioavailability. Metolachlor was weakly adsorbed to the tested soils with adsorption coefficients ranging from 0.36 to1.18 $\mu g^{1-n}mL^{n}g^{-1}$, suggesting its potential to move downward with percolating water. Adsorption followed a Freundlich isotherm and was positively correlated with soil organic matter (OM) content ($p < 0.01$). Degradation of metolachlor in soils obeyed the first-order kinetics, yielding the half-life varying from 37.9 to 49.5 days, which was significantly influenced by soil OM content ($p < 0.01$). The prolonged half-life by sterilization indicated that biodegradation was the dominant pathway for metolachlor degradation in soils. Uptake and bioaccumulation of metolachlor in soils by *Eisenia foetida* was also mainly controlled by soil properties, especially OM. Adsorption coefficients were negatively related to half-lives ($p < 0.01$) and bioaccumulation factors ($p < 0.05$), indicating that adsorption coefficients might be useful for predicting degradation and bioavailability of metolachlor in soils.

Keywords: Metolachlor, soil, adsorption, degradation, bioavailability

1. Introduction

Herbicides are generally considered the most economical and effective method for controlling noxious weeds in both agricultural and non-crop environments. However, increasing use of herbicides has resulted in water pollution and other ecological problems (Kalkhoff *et al.*, 1998). Adsorption and degradation are key processes determining whether herbicide use will have any effect on environmental quality as well as efficacy for weed control (Wang *et al.*, 1999; Si *et al.*, 2009). Furthermore, adsorption is often considered a process that governs and regulates herbicide degradation in soil. However, a growing body of evidence indicates that the effect of adsorption on degradation is much more complicated and depends on many factors, such as microbes, soil properties, characteristics of a chemical itself (Ogram *et al.*, 1985; Si *et al.*, 2009; Xu *et al.*, 2009). Completely opposite impacts were observed for herbicides with different degradation routes and mechanisms. For example, Armstrong and Chesters (1968) demonstrated that degradation of atrazine was accelerated by adsorption, while Ogram *et al.* (1985) showed that degradation of 2, 4-D was inhibited by adsorption. Thus, it is important to characterize adsorption and degradation of a certain herbicide in soil and their correlations, to increase the precision with which safer herbicide uses and potential issues of concern can be identified.

The bioavailability of a chemical is a measure of its accessibility to biota in the environment. It is a key factor controlling the uptake of the soil-associated contaminants in the body of soil-dwelling organisms and food crops, and the transfer of these chemicals in the food chain. It is thus an important consideration in the risk assessment of the soil contaminants and in the selection of appropriate remediation technologies for polluted sites. Bioavailability of organic chemicals such as herbicides is determined by the complex interactions of numerous factors, both abiotic and biotic, such as chemical characteristics, soil properties, partitioning of chemicals, and biological characteristics of the organisms (Lanno *et al.*, 2004). Specifically, the bioavailability of an organic contaminant is dependent on physicochemical processes such as sorption, transport, as well as biological processes (Lawrence *et al.*, 2000; Yu *et al.*, 2006). Of these processes, adsorption is generally recognized as a key to controlling the extent or rate of uptake of the chemical in soil by a receptor (Lawrence *et al.*, 2000; Yu *et al.*, 2006). Consequently adsorption and bioavailability of a chemical in soil may be linked. Nevertheless, limited information is available about the effect of adsorption on bioavailability of chemicals in soil. To fully understand the potential of a specific herbicide for causing environmental pollution and ecotoxicity, it is necessary to evaluate the relationship between adsorption and bioavailability of the herbicide in soil ecosystems.

Metolachlor (2-chloro-N-(2-ethyl 6-methylphenyl) -N-(2-methoxy-1-methylethyl) acetamide), a selective chloroacetamide herbicide, is heavily used in China and other countries for control of broadleaf and annual grassy weeds in a wide range of crops such as corn, soybean, peanut, potato, and tobacco. The fate of metolachlor has caused concern due to the relatively long persistence in soil, the relatively high water solubility and the significant toxicological properties (USEPA, 1988). However, the study of environmental behavior and fate of metolachlor was mainly focused on its respective adsorption and degradation in soil environment (e.g., Wang *et al.*, 1999; Rice *et al.*, 2002; Si *et al.*, 2009). There is very little information in the open literature on the correlation of metolachlor soil adsorption with degradation and bioavailability,

especially the latter. Therefore, the present study was undertaken to i) characterize adsorption and degradation of metolachlor in five soils with the different properties, and its bioavailability to earthworm *Eisenia foetida* that is widely used as bioindictors of soil health and in toxicity testing for chemicals, ii) identify by correlation analysis the main soil parameters which affect the adsorption, degradation and bioavailability of metolachlor, and iii) investigate the effect of adsorption on degradation and bioavailability of the herbicide involved in soil.

2. Materials and methods

2.1. Herbicide, soil and earthworm

Analytical standard of metolachlor with the purity of 98.8% was obtained from Dima Technology Inc., USA. This herbicide has a relative molecular mass of 283.8 g mol^{-1} and a solubility of 500 μg mL^{-1} in water at 20ºC. Its octanol/water partition coefficient is 2.26 (log *Kow*). The five surface soils (0~15 cm) were collected from the soils on the agricultural fields in southwest China. The soils were air-dried and sieved to 2-mm. All samples were stored at 20ºC in the dark before use. Soil pH was measured in deionised water using a 1:2.5 soil: solution ratio with a glass pH electrode. Particle size distribution was evaluated using the sieve-pipette method (Day, 1965) and organic matter content (OM) was measured by a colorimetric method using chromic acid (NSISAS, 1978). The cation exchange capacity (CEC) was determined by following the procedure reported by Hendershot and Duquette (1986). Selected soil physical and chemical properties of the five soils are given in Table 1. Mature earthworm (*Eisenia foetida*) was obtained from the Wandong flower-bird market, Guiyang City, Guizhou province, China. An average earthworm had a mass of 0.35 g (wet weight). All earthworms were allowed to acclimatize to the laboratory conditions for 14 days before the test.

Table 1. Selected characteristics of the five soils used in this study.

Soil	OM (%)	Clay 0-2 μm	Sand 2-50 μm	Silt 50-2000 μm	CEC (cmol kg^{-1})	pH
		(%)				
A	3.94±0.23a	26.23±0.73a	53.26±0.89a	20.51±0.78d	22.31±0.72a	6.32±0.04b
B	2.72±0.20b	18.41±0.70c	25.75±0.78d	55.84±0.92a	18.25±0.65b	5.15±0.03d
C	2.08±0.22c	19.32±0.81c	47.49±0.91b	33.19±0.84c	12.16±0.60c	6.62±0.04a
D	2.01±0.17c	21.24±0.79b	41.59±0.93c	37.17±0.87b	18.45±0.69b	6.24±0.03b
E	1.22±0.16d	17.46±0.69c	50.35±0.94ab	32.19±0.79c	16.02±0.68b	5.96±0.04c

All data are the means ± SD. Means in rows followed by the same letter are not statistically different ($p < 0.05$).

2.2. Adsorption experiment

Adsorption kinetics and adsorption isotherms of metolachlor were determined using the batch equilibration technique. Metolachlor solutions were prepared directly in a background solution of 0.01 Mol L^{-1} $CaCl_2$ and 10^{-4} Mol L^{-1} NaN_3. The initial concentration of metolachlor was 0, 5, 10, 15, 20, and 25 μg mL^{-1}. A 10 mL aliquot of metolachlor solution was added into a 20 mL polyethylene centrifuge tube with 2 g soil

sample. The tube was closed with a plug, shaken automatically for 24 h and centrifuged at 5000 rpm for 15 min at 20ºC. Triplicate samples were prepared for each concentration level. Preliminary studies showed that all adsorption equilibrations could be reached within 24 h. The supernatant of 2 mL was taken, filtered with a 0.45 μm membrane filter and analyzed using HPLC (described below). Amounts of metolachlor sorbed to soil were calculated from the differences between the initial and the equilibrium concentrations in the aqueous phase. Adsorption isotherms of the herbicide in the five soils were described with the Freundlich equation: $\log S = \log K_f + 1/n \log C_e$. Where S is the amount of the herbicide sorbed by soil (μg g^{-1}), C_e is the concentration of the herbicide in the solution at equilibrium (μg mL^{-1}), and K_f ($\mu g^{1-n} mL^n g^{-1}$) and $1/n$ represents the intercept and the slope of the isotherm, respectively.

2.3. Degradation experiment

Laboratory incubation experiments were conducted to investigate the degradation of metolachlor in soils. Soil sample of 10 g was placed in a 50 mL flask and were spiked aseptically with 1 mL of metolachlor stock standard solution in acetone to attain the initial concentration of 6 μg g^{-1}, which corresponds to the agricultural dose. The metolachlor-spiked soils were agitated on a reciprocating shaker for 48 h at room temperature in the dark to ensure thorough mixing and evaporate acetone. Sterile distilled water was added to keep about 60% of the water holding capacity (WHC). The soil samples were incubated at 20ºC. Soil moisture contents were measured and maintained to constant weight by adding an appropriate amount of sterile distilled water, determined by weighing once each week. The remaining levels of metolachlor in soils were determined by extracting samples on days 0, 7, 14, 30, and 60. Triplicate soil flasks were removed from each treatment at each sampling time point. The soil samples were mixed with 30 mL acetone-water (25:5, v/v), shaken for 2 h on a reciprocating shaker and ultrasonically extracted for 20 min at 20ºC, respectively. After filtration, acetone within the filtrate was allowed to evaporate on a vacuum rotary evaporator. Solution of 2 mL was taken and passed through a 0.45 μm membrane filter before HPLC analysis.

To investigate the effect of microorganisms on degradation of metolachlor in soils, the degradation was carried out under sterilized conditions. These tests were undertaken only in soil A and E with the highest and lowest OM content, respectively. Sterilization was achieved by autoclaving twice at 121ºC for 60 min. Prior to and after the incubation period, samples of the sterilized soils were incubated on nutrient agar for 7 days, no microbial growth was observed. The degradation data in the sterilized and unsterilized soils were fitted to the first-order reaction kinetics model: $C_t = C_0 \times \exp^{(-kt)}$. Where C_0 is the herbicide concentration in the soil at the application time (μg g^{-1}), t is the time (days), C_t is the herbicide concentration detected in the soil at time t (μg g^{-1}), and k is the first-order rate coefficient ($days^{-1}$). The degradation data were summarized by calculating the degradation half-life time ($T_{1/2,}$ days) from k with the equation: $T_{1/2} = ln2/k$.

2.4. Bioassay

Bioavailability of metolachlor in soil to *E.foetida* was evaluated in a microcosm. For this purpose, 100 g soil sample was placed in a 250-mL flask and sterilized twice using autoclave at 121ºC for 60 min. Addition of metolachlor (6 μg g^{-1}) and adjustment of the soil moisture level (60% of WHC) for each soil was achieved using the same procedures as described above for the degradation experiment. After ten earthworms were added to the soil surface, the flasks were covered with

aluminum foil (ten small holes were cut in the foil for aeration) and incubated for 7 days in the dark at 20ºC. Four replicates were prepared for each soil. At the end of incubation period, earthworms were removed from the soil and kept for 24 h on the moistened filter paper to purge the gut contents. Earthworms were weighed and sealed in petri dishes and frozen at -10ºC for 24 h. Following being ground with anhydrous sodium sulphate, the earthworm tissues were placed in the Soxhlet apparatus and extracted with 80 mL methanol for 12 h. The extracts were concentrated to about 2 mL and purified by a column containing 5 g of 5% deactivated florisil. After the columns were eluted with 5 mL acetone and 10 mL petroleum ether, respectively, the extracts were added into the columns, and eluted with 30 mL acetone-petroleum ether (7:3, v/v). The resulting elutes were concentrated to dryness on a vacuum rotary evaporator. The residues of metolachlor were recovered by rinsing the flask with 5 mL acetonitrile. A 2 mL aliquot of metolachlor solution was taken and filtered with a 0.45 μm membrane filter before analysis by HPLC.

To determine the bioaccumulation factor (*BAF*) of earthworms for metolachlor in soil, the quantities of metolachlor in soil were also detected. After the earthworms were removed from the soil, triplicate soil samples of 10 g (wet weight) was mixed with 30 mL acetone-water (25:5, v/v) and the subsequent extraction and analysis processes were carried out as described above for the degradation experiment. Triplicate additional 10 g of each soil was weighed into the individual aluminum tins and placed in a 105ºC oven for 24 h for determination of moisture content. Quantities of metolachlor detected in soil and earthworm samples were expressed as μg g^{-1} on the basis of dry and wet weight, respectively. The bioaccumulation factor was calculated as the ratio of the metolachlor concentration in the earthworm tissues (C_{et}) and the metolachlor concentration in the soil.

2.5. HPLC analysis

Metolachlor was quantified on Wasters 600E PHLC equipped with Waters 2487 ultraviolet absorbance detector and a reversed phase C_{18} column (150×4.6 mm i.d., 5 μm).The eluting solvent was acetonitrile-water (80-20, v/v) at a flow rate of 1.2 mL min^{-1}. The wavelength was set at 230 nm and the column temperature was kept at 30ºC for detection purpose. The injection volume was 5 μL. Each sample was analyzed in duplicate. The retention time for metolachlor under these conditions was 7.2 min.

Recovery was evaluated by spiking herbicide-free soil and earthworm samples at thee concentration levels of 0.05, 0.5, and 5 μg g^{-1}. In all fortification levels, recovery was higher than 86% for both soil and earthworm samples. The minimum detection limit of metolachlor was 0.015 μg g^{-1}. Specificity was demonstrated by the absence of interferences at the retention time of the analyte of interest.

2.6. Statistical analysis

The yield data were analyzed using SPSS 12.0 statistical software package. Origin 8.0 graphing software package was used to plot figures from adsorption, degradation, and bioassay experiments. The differences between treatments were evaluated using one-way analysis of variance (ANOVA) followed by Least significant difference test at $p < 0.05$. The stepwise regression analysis was employed to determine the correlations between adsorption, degradation, bioavailability of metolachlor and soil physical-chemical property parameters. The effect of adsorption on degradation and bioavailability of metolachlor were assessed using the linear regression procedure and Pearson correlation coefficient test. A two-tailed p value < 0.05 was considered statistically significant.

3. Results and discussion

3.1. Adsorption

The adsorption isotherms of metolachlor in the five tested soils are shown in Figure 1. According to classification of adsorption isotherms, the adsorption isotherm of metolachlor for each soil was L-type, suggesting a minor competition between solute and solvent molecules for the adsorbing sites of the surface, which is in accordance with the previous results reported by Spongberg and Lou (2000) and Wang *et al.*(1999). Sorption of metolachlor to soils was well described by the Freundlich equation over the range of equilibrium concentrations from 0 to 25 μg mL^{-1} with r^2 value > 0.94 (Table 2). Adsorption isotherms for all soils had slopes of (1/*n*) less than 1, indicating that the percentage of this herbicide adsorbed by soil decreased with increasing solution concentration, and that there was a potential for the herbicide leaching particularly at higher application rates.

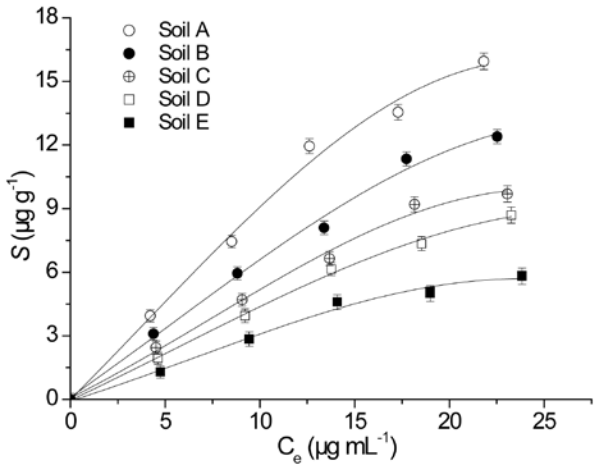

Figure 1. Adsorption isotherms of metolachlor in five soils.

Table 2. Parameters of adsorption, degradation, and bioavailability of metolachlor in soils.

Soil	Adsorption			Degradation			Bioavailability	
	Kf	1/*n*	r^2	$T_{1/2}$	*k*	r^2	*Cet*	*BAF*
A	1.18±0.03a	0.865	0.984	37.9±0.52d	0.0183	0.938	0.86±0.023c	0.23±0.005b
B	0.89±0.02b	0.861	0.995	40.5±0.77c	0.0171	0.980	0.97±0.020bc	0.24±0.007b
C	0.63±0.02c	0.897	0.991	43.9±0.89b	0.0158	0.988	0.96±0.025bc	0.24±0.006b
D	0.55±0.02c	0.876	0.982	44.4±0.86b	0.0156	0.985	1.09±0.024b	0.25±0.007ab
E	0.36±0.02d	0.856	0.947	49.5±.091a	0.0140	0.994	1.29±0.027a	0.27±0.008a

All data are the means ± SD. Means in rows followed by the same letter are not statistically different ($p < 0.05$).

The Freundlich adsorption coefficient K_f ranged from 0.36 to 1.18 $\mu g^{1-n}mL^n g^{-1}$, implying that metolachlor was weakly adsorbed to soils for the concentration range tested in this study (Table 2 and Figure 1). From this K_f value, organic carbon (OC) adsorption coefficient (K_{oc}, mL g^{-1}) can be calculated according to the equation $K_{oc} = K_f/(W_{oc})\times 100$, where K_f is Freundlich adsorption coefficient and W_{oc} is the percentage of organic carbon of the soil. Using the measured K_f and W_{oc} values, the calculated K_{oc} values were 51.63 mL g^{-1} for soil A, 56.41 mL g^{-1} for soil B, 52.22 mL g^{-1} for soil C, 47.17 mL g^{-1} for soil D, and 50.87 mL g^{-1} for soil E. The calculated K_{oc} values for metolachlor in this study were lower than the range of published results of 67.82 to 269.77 mL g^{-1} of the six soils with OC content between 1.10% and 2.10% (Zheng and Cooper, 1996), 173.70 to 195.90 mL g^{-1} of the two soils with OC content of 2.50% to 4.20% (Krutz *et al.*, 2002), and 1078.00 to 1389.00 mL g^{-1} of the three soils with OC content ranging from 0.27% to 0.51% (Obrigawitch, 1981). Obviously, K_{oc} values for metolachlor in soil were depended not only on soil OC content, but also on other soil prosperity parameters such as pH, CEC, as well as clay, sand and silt content.

Furthermore, statistical analysis of the influence of soil properties on the adsorption parameters (K_f) was performed. The results show that adsorption coefficient was significantly correlated with the soil OM content ($r = 0.990$, $p < 0.01$) (Table 3), showing that the organic matter was the main factor governing the extent to which the sorption processes occurred. In previous studies to investigate soil adsorption of metolachlor, Spongberg and Lou (2000), Wang *et al.* (1999) and Si *et al.* (2009) also found that adsorption of metolachlor was mainly dependent on OM and was generally weak in soils. The weak adsorption reveals that metolachlor may have a high potential to move downward with percolating water, especially in light textured soils. Studies by Frank *et al.* (1990) indicated that this sorption did not prevent movement of metolachlor and its metabolites to aquatic systems, as evidenced by the presence of parent compound and/or its metabolites in streams, ponds, and wells. They had been frequently found in surface waters throughout the United States (Frank *et al.*, 1990). For example, the detected concentration in 12 stream sites located in eastern Iowa was 0.15 μg L^{-1} for metolachlor, 3.0 μg L^{-1} for metolachlor ethanesulfonic acid and 0.7 μg L^{-1} for metolachlor oxanilic acid (Kalkhoff *et al.*, 1998). The presence of metolachlor metabolites in these systems have aroused enhancing concern.

Table 3. Correlation coefficients between K_f, $T_{1/2}$, C_{et} and *BAF* of metolachlor and soil properties.

Parameter	OM%	Clay%	Sand%	Silt%	CEC	pH
Kf	0.990**	0.741	-0.069	-0.143	0.673	-0.112
$T_{1/2}$	-0.963**	-0.718	0.208	0.018	-0.604	0.108
Cet	-0.880*	-0.660	0.110	0.086	-0.333	-0.119
BAF	-0.885*	-0.673	0.126	0.076	-0.360	-0.110

* and ** represent $p < 0.05$ and $p < 0.01$, respectively.

3.2. Degradation

The degradation of metolachlor in all unsterilized soils was fitted to the first-order reaction kinetics model and showed good performance for all treatments (Figure 2), with r^2 values ranging from 0.938 to 0.994 (Table 2). The observed half-life (from 37.9 to 49.5 days) for metolachlor in unsterilization soils were similar to those previously reported varying from 9.6 to 81 days (Rice *et al.*, 2002). In all cases, metolachlor was more persistent in soils with lower OM content, compared to soils with higher OM content (Table 3). A significantly negative correlation was observed between the half-life and the soil OM content (r = -0.993, $p < 0.01$), suggesting that soil OM is a predominant factor determining the persistence of metolachlor in soils although the degradation of metolachlor in soils also is dependent on clay content and other property parameters of soils (Table 3). The decreasing persistence of metolachlor in soils with increasing OM content was in agreement with the previous results reported by Rice *et al.* (2002) who attributed the increase in degradation rate of metolachlor in soils with the relative high OM content to the relative large microbial population degrading metolachlor.

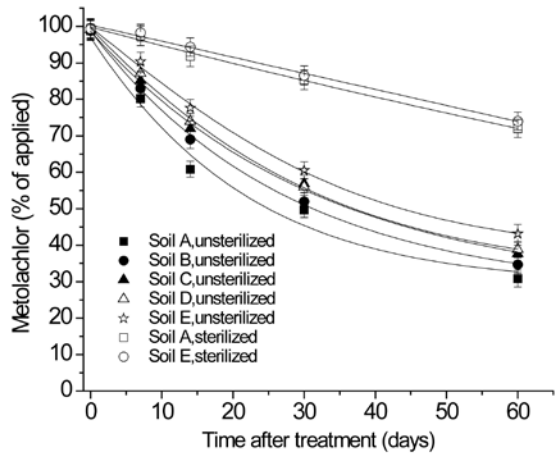

Figure 2. Degradation kinetics of metolachlor in unsterilized and sterilized soils.

The degradation of metolachlor in sterilized soil A and E also obeyed well the first-order kinetics with r^2 values of 0.994 and 0.991, respectively. As expected, sterilization treatment resulted in a significant decrease in degradation rate of metolachlor in the two soils investigated ($p < 0.05$). In soil A, sterilization increased the half-life from 37.9 to 126.0 days, or by 69.92%. In soil E, the persistence increased from 49.5 to 135.9 days or by 63.57%. The degradation rate coefficient k for metolachlor in sterilized soils A and E was 3.3 and 2.7 times smaller (slower) than in corresponding unsterilized soil, indicating that microbial degradation may be the dominant pathway for metolachlor degradation in soils. The results obtained here comparing the sterilized and unsterilized soils confirm the findings of Rice *et al.* (2002) who have demonstrated that the degrada-

tion rate of metolachlor was significantly decreased in autoclaved soils, as compared with unsterilized soils. Microbial degradation has been shown to be the primary mechanism of metolachlor dissipation or disappearance in soil (Rice *et al.*, 2002).

Adsorption and persistence are usually the predominant factors influencing the leaching potential of a pesticide in soil. Leaching potential of metolachlor in soils was calculated using the following groundwater ubiquity score (GUS) (Gustafson, 1989):

$$GUS = \log (T_{1/2}) \times (4 - \log K_{oc})$$

Where K_{oc} is organic carbon partition coefficient (mL g^{-1}); $T_{1/2}$ is half-life in the soil (days). A chemical with GUS > 2.8 is considered of high leaching potential, while a chemical with GUS < 1.8 is defined as a low leaching candidate. When GUS of a chemical is between 1.8 and 2.8, it belongs to a "transition zone". Using the measured K_{oc} and $T_{1/2}$ values, the estimated GUS index was 3.61 for soil A, 3.61 soil B, 3.75 for soil C, 3.83 for soil D, and 3.89 for soil E. For the herbicide metolachlor, GUS (3.61 to 3.89) was substantially higher than 2.8, which corresponds to a high-leacher compound. Therefore, it may be concluded that metolachlor may leach easily through soils under conducive conditions, especially in soils with relatively low OM. These results confirm the observations of the adsorption studies. However, many other factors may alter the actual dissipation rate of a pesticide under field conditions, and such factors include volatilization and photolysis, among others. The exact leaching risk of metolachlor must therefore be investigated under field conditions.

3.3. Bioavailability

Uptake of metolachlor from soil by *E.foetida* is presented Table 2. For the five soils tested, metolachlor uptaken by earthworms appeared to decrease with the increase in the OM content. According to the regression analysis result, there was a significant correlation ($p < 0.05$) between concentrations in earthworm tissues and the soil OM contents (Table 3). This suggests that OM also was a dominant parameter in earthworm availability of metolachlor in soils with widely varying OM, clay, sand, silt, CEC, and pH. Investigating the bioavailability of another chloroacetamide herbicide butachlor in the five soils with different properties to earthworms, Yu *et al.* (2006) also showed that uptake of butachlor by *Allolobophora caliginosa* decreased as soil OM content increased. In an experiment to examine the availability of anthracene, chrysene, pyrene, and benzo (a) pyrene in the five soils to earthworms, Tang *et al.* (2002) also found that concentrations of these compounds detected in *E.foetida* tissues were the greatest in the soil with the lowest OM. In other studies, earthworm uptake of pesticides varying with soil properties were also observed with DDT, DDE, DDD, and dieldrin (Morrison *et al.*, 2000). These results indicate that accessibility of a certain contaminant to earthworm was dependent on both characteristics of a contaminant (e.g., solubility, K_{ow}, molecular structure) and soil properties such as OM, clay content, CEC and pH.

The bioaccumulation factor of earthworms for metolachlor is listed in Table 2. Like concentrations detected in earthworm tissues, the soil organic matter also had significant influence on bioaccumulation of metolachlor by earthworms ($p < 0.05$) (Table 3).There was a similar variation trend for bioaccumulation and uptake of metolachlor by earthworms with soil OM content. Moreover, the bioaccumulation factor was significantly correlated with the earthworm tissue concentration ($p < 0.01$). The bioaccumulation of a contaminant in soil by earthworms was found to be influenced by a complex interaction of physicochemical and biological factors (Morrison *et al.*,

2000; Tang *et al.*, 2002; Lanno *et al.*, 2004; Yu *et al.*, 2006). In general, a crucial component in determining the rate of entry of an organic molecule into an organism is its octanol/water partition coefficient since this determines its ability to traverse the cell membrane (Simkiss, 1996). The octanol/water partition coefficient is perhaps the single most important factor that is used in predicting the bioavailability of environmental contaminants (Lawrence *et al.*, 2000). For the herbicide metolachlor, the partitioning of metolachlor in soils appeared to be governed by its relatively low log K_{ow}(2.26) and hence resulted in weak sorption to soils, as evidenced by L-type isotherms and low adsorption coefficients (Figure 1 and Table 2). Presumably, metolachlor available to *E.foetida* was possibly the fraction in pore-water and/or weakly associated with the surface of soils (Yu *et al.*, 2006). As a result, the increase in adsorption of metolachlor with the soil OM content makes it less 'bioavailable' to earthworms. It is, therefore, not surprising that there was a significantly correlation between bioaccumulation and uptake of metolachlor in soil by earthworms.

3.4. Effect of adsorption on degradation

The adsorption coefficient K_f and the degradation half-life $T_{1/2}$ of metolachlor increased and decreased with the soil OM content, respectively. This sgguests that they may be inversely related. As expected, there was a negative correlation between K_f and $T_{1/2}$ (r = -0.970) based on the result provided by a linear regression analysis. The regression equation was: $T_{1/2}$ = 52.875 – 13.345 K_f (Figure 3). The Pearson correlation coefficient test show that the degree of the linearly relation between adsorption extent and degradation rate of metolachlor was statistically significant at $p < 0.01$ level. It seems that the use of the adsorption coefficient would be useful in predicting the corresponding degradation or persistence for metolachlor in soil.

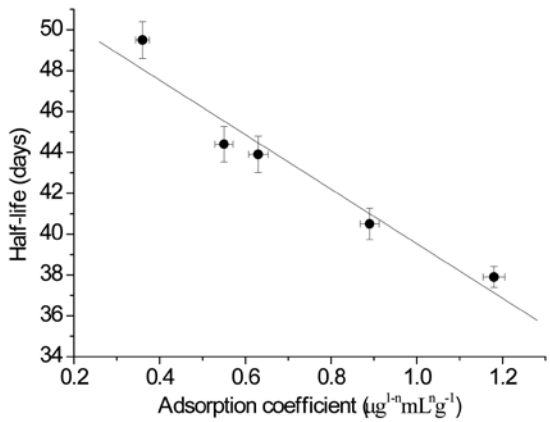

Figure 3. Relationship between the degradation half-life and the adsorption coefficient of metolachlor. Error bars indicate ± SD.

Adsorption is a governing process determining the persistence of organic chemicals in soil, and their parameters derived from standard laboratory tests can be used for the parametrization of mathematical models to assess chemical leaching potential (Pantelelis *et al.*, 2006). Thus, numerous laboratory studies have been carried out to investigate the effect of adsorption on degradation of herbicides or other chemicals. In general, adsorption is often considered to decrease degradation by limiting the availability of organic chemicals to microbial or chemical transformations (e.g.,Ogram *et al.*, 1985; Xu *et al.*, 2009). However, based on the data presented herein, the degradation rate of metolachlor in soils increased with the enhancement of sorption due to an increase in soil OM content (Tables 1 and 2). For instance, although the largest adsorption capacity for metolachlor was observed in soil A, the half life of metolachlor in soil A was the shortest. Soil E, on the other hand, had the weakest sorption capacity for metolachlor, the half life of metolachlor in soil E was the longest. Similar phenomena that the increase in degradation rate of herbicides with adsorption strength were also observed on napropamide (Hurle and Lang, 1981). These findings suggest that degradation of herbicides also may be influenced by the microbial population, and thereby by the soil OM content because the soil OM was known to support microbial growth (Gaultier *et al.*, 2008). It is possible that the lowest OM content in the soil E resulted in the relatively low microbial population degrading metolachlor, and hence the longest half-life of metolachlor in this soil although there was the lowest adsorption capacity for the herbicide. This should be the result of the balance between extents of both adsorption and biodegradation.

It has been reported that metolachlor can be utilized by soil indigenous fungi (*Aspergillus flavus* and *A. terricola*) as a carbon and nitrogen source and converted to 6-methyl 2-ethyl acetanilide and 6-methyl 2-ethyl aniline (Sanyal and Kulshrestha, 2002). Saxena *et al.* (1987) also found strains of *Bacillus circulans*, *B. megaterium*, *Fusarium* sp., *Mucor racemosus*, and an actinomycete can transform metolachlor. Thus, it is possible that microbial metabolism of metolachlor in the solution phase was creating a concentration gradient thereby stimulating metolachlor desorption. This additional solubilized metolachlor was then metabolised rapidly by microorganisms. A more likely explanation is that microorganisms are attached to the surface of the soil matrix. It was evident from microscope observation that soil microorganisms and OM flocculated and were in intimate association with each other (McGhee *et al.*, 1999). Under these circumstances metolachlor desorption would be facilitated (and almost instant degradation by surface associated microbes). This phenomenon was described for degradation of phenanthrene (Aronstein, 1991) and 2, 4-D (McGhee *et al.*, 1999). Another possible explanation for the increase of metolachlor degradation with adsorption intensity is that soil microorganisms were accessing and metabolizing sorbed metolachlor. It is possible to conclude that a metolachlor was exposed to surface associated microorganisms and that this was being degraded. However, in our study, we simply measured the dissipation of metolachlor over time, and thus did not provide mechanistic information about the degradation pathway. There are many direct and indirect processes that could account for the increase in degradation rate and further experiments are underway that may allow an understanding of the mechanism of the effect of adsorption on degradation of metolachlor in soil.

3.5. Effect of adsorption on bioavailability

Adsorption may reduce bioavailability of metolachlor by decreasing uptake rate of metolachlor in soil by earthworms, as indicated in Table 2. According to the result of the regression analysis, the bioaccumula-

tion factor of earthworms for metolachlor was closely correlated with the Freundlich adsorption coefficient, with *r* value of -0.897. The corresponding linear regression equation was: $BAF = 0.276 - 0.042\ K_f$ (Figure 4). The strength of association between *BAF* and K_f was also statistically significant ($p < 0.05$) on the basis of the Pearson correlation coefficient test result, suggesting that the Freundlich adsorption coefficient might be used as a predictor of bioavailability of metolachlor in soils to earthworms.

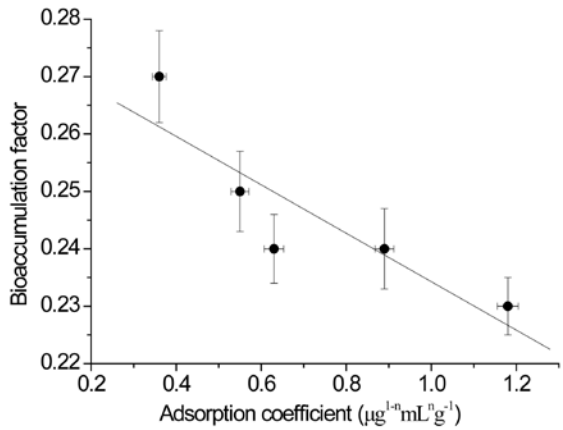

Figure 4. Relationship between the bioaccumulation factor and the adsorption coefficient of metolachlor. Error bars indicate ± SD.

The bioavailability of contaminants to earthworms is of interest because these organisms contact soil directly and can act as a conduit through which pollutants enter food webs. Moreover, they are the model organisms used in certain standardized tests designed to evaluate the risk of contaminated soil (Lanno *et al.*, 2004). However, there have been few detailed studies on the correlation of contaminant adsorption with bioavailability to earthworms in soil. In attempting to describe an existing relationship between soil sorption and bioavailability of pesticides, Yu *et al.* (2006) investigated the effect of soil adsorption on bioavailability of pesticides such as butachlor, myclobutanil and chlorpyrifos to *A. caliginosa*. In their experiment, the bioavailability of both butachlor and myclobutanil with relative low K_{ow} to *A. caliginosa* could be predicted by the adsorption coefficient, whilst the adsorption coefficient could not be used as a predictor of the bioconcentration of *A. caliginosa* for chlorpyrifos with relative high K_{ow}. It is conceivable that octanol/water-partitioning coefficient should be a crucial factor in determining sorption, advective-dispersive transport and biological processes, and hence the bioavailability of pesticides (as mentioned above).

The bioavailability is a critical factor in the success of biologically based remediation technologies for polluted sites. Adsorption is generally considered a key to controlling the bioavailability of contaminants in soil to receptors. Thus, knowledge of contaminant adsorption mechanism is necessary for predicting the

bioavailability and fate of contaminants in soil ecosystems. Using independent datum sets and understanding how adsorption influences bioavailability of a contaminant may result in more accurate prediction of its bioavailability in biota. Based on data presented in the current study, a Freundlich adsorption coefficient K_f may be used as a model for assessing the bioavailability of metolachlor in soil to earthworms. However, the present investigation merely represents a step in the direction of attempts to use parameters from standard adsorption tests to predict the bioavailability of metolachlor to receptors because there were only five soils and one test organism involved. Additional studies are warranted to investigate more test organisms and soils with different characteristics to obtain a more reliable predictability of metolachlor bioavailability based on information of adsorption.

4. Conclusions

An understanding of adsorption mechanism is fundamental for predicting the environmental fate of many organic contaminants in soil ecosystems. Experiment data presented here indicate that the adsorption isotherms of metolachlor were L-type, and described well by the Freundlich equation. Adsorption of metolachlor in the tested soils was weak but appeared to increase with the soil organic matter content. The degradation of metolachlor in soils, following the first-order kinetics, was strongly controlled by soil organic matter. Biodegradation was the dominant pathway for metolachlor degradation in soils. Uptake and bioaccumulation of metolachlor in soils by earthworm *E. foetida* was also mainly depended on soil properties, especially organic matter. The results of linear regression procedure and Pearson correlation coefficient test show that the Freundlich adsorption coefficient might be useful for predicting degradation and earthworm bioavailability of metolachlor in soils.

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