Science of the Total Environment 707 (2020) 135476

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Behavior of imidazolinone herbicide enantiomers in earthworm-soil microcosms: Degradation and bioaccumulation

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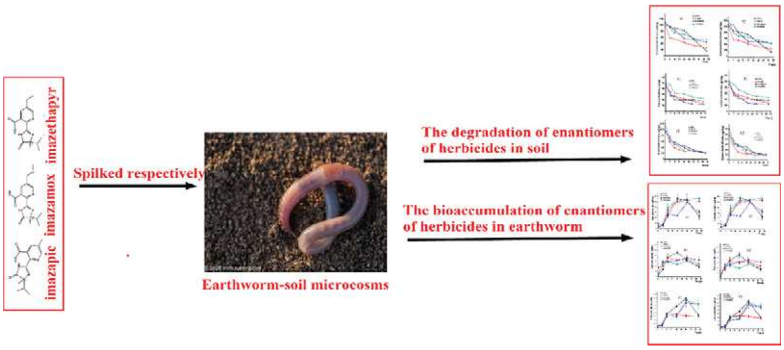
HIGHLIGHTS

- The bioaccumulation of three imidazolinone herbicides in earthworm tissues was not enantioselective.
- The degradation of imazapic in soils was enantioselective, and no significant stereoselectivity occurred for imazamox and imazethapyr.
- The BSAFs values were negatively correlated with the log K_{OW} values of herbicides and were positively correlated with organic matter content of soil in earthworm-soil microcosms.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 5 September 2019
Received in revised form 30 October 2019
Accepted 9 November 2019
Available online 21 November 2019

Editor: Yolanda Picó

Keywords:
Imidazolinone herbicides
Bioaccumulation
Degradation
Earthworm-soil microcosms

ABSTRACT

Imidazolinone herbicides are a group of chiral herbicides that are widely used to control weeds in crops. Despite their wide use, few studies on the behavior of enantiomers in terrestrial systems have been reported. In this study, the bioaccumulation of imazamox, imazapic, and imazethapyr enantiomers in earthworm and their degradation in soils were assessed using earthworm-soil microcosms. The bioaccumulation of the three herbicides in earthworm was not significantly enantioselective. Imazamox and imazethapyr did not significant stereoselective degradation in soil ($p > 0.05$), while the enantioselectivity of the degradation of imazapic was significant ($p < 0.05$). Furthermore, biota to soil accumulation factor (BSAF) values were also calculated for three herbicides. Relationships between BSAF values and organic matter content of soil and log K_{OW} of herbicides were investigated. The BSAFs values were negatively correlated with the log K_{OW} of herbicides, and were positively correlated with organic matter content of soil in earthworm-soil microcosms. These relationships indicated that chemical hydrophobicity (K_{ow}) and organic matter content of soil were good predictors to estimate the bioavailability of imidazolinone herbicides to earthworm.

1. Introduction

Pesticides are widely used in modern agriculture to achieve high levels of agricultural production. In addition, some studies have

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https://doi.org/10.1016/j.scitotenv.2019.135476
0048-9697/

reported pesticide efficiencies were between approximately 20% and 30% in spring orchards and fields where seedlings grow, while the pesticide efficiencies ranged from 50% to 60% in summer orchards and fields when the crops were at the full-leaf stage for conventional spraying (Yuan et al., 2011); thus, the most of the pesticides were dispersed into the environment. Soil is considered to be one of the most important sinks for pesticides, because these pesticides can reach the soil via various ways, including field application, air deposition or water leaching (Navarro et al., 2007). Soil organisms may accumulate amounts of pesticides from the soil, which are toxic or transferred to food chains, and subsequently pose potential risks to human and animal health. Earthworms are one of most important organisms in soil ecosystems. Earthworms live in contact with soil particles, which make up about 80% of the total soil biomass (Liu et al., 2015), so earthworms are widely used as a biomarker to assess the impacts of organic contaminants (e.g., pesticides) on the soil and environment. Studies on the bioaccumulation of various contaminants (including pesticides, pharmaceutical and organic pollutants) in earthworms is successively reported (Carter et al., 2016; Navarro et al., 2016; Liu et al., 2015). Furthermore, many studies have also indicated that some contaminants could be bioaccumulated by earthworms, and then threaten the health of humans and other animals via the food chain (Frouke et al., 2010; Zhu and Kannan, 2019). Thus, information on the behaviors and effects (including degradation, bioaccumulation and toxicity to earthworms) of the pesticides on earthworms is vitally important for human health and environmental safety.

Approximately 30% of pesticides in use worldwide are chiral and chiral pesticides may account for over 40% of the currently used pesticide in China (Li et al., 2017; Albuquerque et al., 2018). The racemic mixtures of chiral herbicides comprise two or more enantiomers. Although the enantiomers of chiral compounds have the same physicochemical properties, they usually have different biological activities. Various biological activities including the environmental fate, carcinogenicity, teratogenicity, and toxicity of chiral compounds are generally enantioselective (Lehmler et al., 2009; Sanganyado et al., 2017; Liu et al., 2018). A credible environmental risk assessment is only possible when the differences between the chiral enantiomers are considered. Therefore, it is essential to obtain stereospecific information on the chiral pesticides to ensure a more accurate benefit-risk evaluation.

A significant class of chiral pesticides is the imidazolinone herbicides, all of which comprise two enantiomers. The imidazolinone herbicides are widely used to control weeds in peanuts, soybeans, cereal crops, and potatoes fields worldwide because they have low mammalian toxicity, selectivity, high efficiency, and long-term broad spectrum activity advantages (Kemmerich et al., 2015). However, a main characteristic of these herbicides is their high soil persistence, and they degrade slowly in the soil, with half-life values in the range of 0–360 days for imazethapyr (Aichele and Penner, 2005; Goetz et al., 1990), 21–39 days for imazapic (Bajrai et al., 2017), 30–45 days for imazapyr (Wang et al., 2005; Gianelli et al., 2014), 22–31 days for imazamox (Milan et al., 2017), and 1337 days for imazaquin (Aichele and Penner, 2005). Imidazolinone residues in soil not only affect rotational sensitive crops, but also pose potential threats to non-target organisms (Kemmerich et al., 2015; Martini et al., 2013). Unfortunately, to date, there have been very few reports on the degradation and bioaccumulation of the imidazolinone herbicides in earthworm-soil systems, and specifically, data on the enantioselective degradation and bioaccumulation of the enantiomers in soil and earthworm are lacking. There have been only a few reports on analysis and enantiomeric separation methods ((Lao and Gan, 2006; Li et al., 2019), toxicities (Qian et al., 2009; Xie et al., 2018), and enantiomeric degradation in soil (Ramezani et al., 2010; Buerge et al., 2019b) have been reported. For example, Buerge et al. demonstrated that biodegradation (in the dark) and photodegradation (under sunlight) were the predominant degradation processes for imazamox in soils (Buerge et al., 2019a). Furthermore, Buerge et al. also reported that degradation was enantioselective in neutral soils, finding faster degradation of (+)-imazamox; differences between enantiomers were not pronounced in slightly acidic soils, and degradation was again enantioselective in strongly acidic soils (Buerge et al., 2019b). Xie et al. showed that *R*-imidazolinones were more active than *S*-imidazolinones in the inhibition of target organisms (Xie et al., 2018). Hence, studying the degradation and bioaccumulation of enantiomers of three imidazolinone herbicides in earthworm-soil microcosms is important and of interest to environmental and public health.

In this paper, the bioaccumulation and degradation behaviors of enantiomers of three imidazolinone herbicides in earthworm-soil microcosms were assessed. The relationships between BSAF values and soil and herbicide properties were investigated.

The results from these analyses will be of potential benefit to regulatory agencies, manufacturers, and natural resource managers.

2. Materials and methods

2.1. Chemicals and reagents

Analytical standards of rac-imazapic (>99.0% purity), rac-imazethapyr (>99.0% purity), and rac-imazamox (>99.0% purity) were purchased from the Dr. Ehrenstorfer Co. (Augsburg, Germany). The physicochemical properties and chemical structures of the herbicides are shown in Table 1 and the Supplementary information (Fig. S1), respectively. Water was purified with a Milli-Q system. Analytical grade acetonitrile, sodium chloride (NaCl), acetic acid, and anhydrous magnesium sulfate ($MgSO_4$) were purchased from the Tianjin Kermel Chemical Reagent Co. Ltd. (Tianjin, China). Acetonitrile, methanol, and aqueous ammonia (all HPLC-grade) were obtained from Merck KGaA (Darmstadt, Germany).

2.2. Earthworm and soil

Adult earthworms (*E. fetida*) with a well-developed clitellum and a body weight of 400–600 mg were purchased from the College of Animal Science and Technology, Guangxi University. The earthworms were kept in a glass breeding box with clean soil in the dark at 20 °C and fed 50% cow manure for at least 2 weeks to allow them to acclimate to the laboratory. Before testing, the worms were removed from the breeding box and stored on damp filter paper for 24 h to purge their gut, after which they were washed with tap water.

Four different agricultural soils with different physicochemical properties were collected from the 0–15 cm surface layer in fields from four districts in China: Heilongjiang (HLJ), Hunan (HN), Chongqing (CQ), and Guangxi (GX) provinces. No pesticides had been applied to these soils in the previous 5 years. The soils were air-dried at room temperature and then sieved (mesh size, 2 mm). All soil samples were kept in black plastic bags at 25 ± 2 °C in the dark to stabilize the soil chemistry and structure. Before experiment, soil preincubated under the experimental conditions for two weeks in large polyvinyl chloride (PVC) tanks. The characteristics of the soils are listed in Table 2.

2.3. Establishment of earthworm-soil microcosms

Earthworm-soil microcosms were established to study the bioaccumulation and degradation of each of the three pesticides. The soils were

Table 1
Test herbicides physico-chemical properties.

Name	CAS no.	Molar mass (g/mol)	$\log K_{ow}$	pKa	Sorption coefficient (Koc)(L/kg)
Imazapic	104098-48-8	275.30	2.47	2.0	137
Imazamox	114311-32-9	305.34	5.36	2.3	11.6
Imazethapyr	81335-77-5	289.33	1.49	2.1	52

Note: these data obtained from Pesticide Properties Data Base.

Table 2
Physicochemical property of four tested soils.

Number	Site	Particle size				pH	Organic matter (g/kg)	Cation exchange capacity (cmol/kg)
		Sand (%)	Silt (%)	Clay (%)	Texture			
1	GX	72.8	11.9	15.3	Sand loam	7.44	8.8	5.89
2	HN	40.0	18.2	41.8	Silty clay	6.24	5.37	10.7
3	CQ	56.1	28.4	15.5	Loam	6.35	7.52	26.1
4	HLJ	57.8	12.3	29.9	Loam	6.21	14.6	20.7

spiked with each of the three herbicides at 2 mg/kg. There were two steps to this procedure: first, 5 mL racemic stock solution (400 mg/mL dissolved in acetone) was added to dry soil (50 g) and mixed for 30 min. The spiked soil samples were left in a fume cupboard for at least 2 h to allow the acetone to evaporate. Second, 950 g soil was mixed thoroughly with the contaminated 50 g dry soil to uniformly distribute the herbicide, and the 1000 g of contaminated soil was fully stirred with a glass rod, and then transferred into a glass bottle. To assess the uniform distribution of pesticide throughout each bottle, three soil samples were randomly collected from the bottle and then analyzed using UPLC-MS/MS, Average concentrations (in all soils) ranged from 0.85 mg/kg to 1.1 mg/kg, with coefficients of variance were <10% for the two enantiomers of three herbicides. Deionized water was added every day to the bottle to adjust the water content to 36%, and water was added to compensate for evaporated water. Fifty grams of earthworm was placed into each bottle. For each herbicide, three independent replicates were prepared. A negative control (no solvent or herbicide) and a solvent control (no herbicide) with three replicates were also prepared. All bottles were placed in a climate-controlled chamber (day:night = 16:8) and maintained at 20 ± 2 °C and 50% humidity, and the water lost by evaporation was replaced with tap water every day. At each sampling time (0 (2 h), 7, 14, 28, 42, and 60 d), about 20 g soil and 5 g earthworm samples were randomly collected from each bottle. The earthworm samples were rinsed with tap water to remove soil and placed on damp filter paper for about 2 h to purge their gut contents. Then, the earthworm samples were dried on filter paper. Finally, the earthworm and soil samples were frozen and stored at −20 °C until analysis.

2.4. Chemical analysis

2.4.1. Instrumental analysis

A triple quadrupole mass spectrometer (6460B, Agilent Technologies Inc., Santa Clara, CA, USA) coupled to an Agilent 1260 UPLC system was then used for the chromatographic analysis. The UPLC system was equipped with an Agilent 1200 Series Autosampler, a binary pump (Agilent Bin Pump SL), and a column oven (Agilent G7116B). For separation, we used a CHIRALCEL® OJ-3R column (0.46 cm I.D. ×15 cm L × 3 μm; Daicel, Japan). The mobile phase was 0.1% *v*/v formic acid in water / chromatography grade acetonitrile (75:25, *v*/v) with a flow rate of 0.4 mL/min. The injected sample was maintained at 3 μL. The column temperature was kept at 30 °C·As showed in the Supplementary information (Fig. S2), the three target analytes were well separated. The Agilent 6460B QqQ LC/MS/MS system equipped with an electrospray ionization (ESI) source was used to analyze the samples for all compounds in the positive ion and multiple reaction monitoring (MRM) mode. All of the mass spectrometry parameters were separately optimized to achieve a satisfactory sensitivity and listed in the Supplementary information (Table S1).

The absolute configuration of the enantiomers of each imidazolinone herbicide was determined using a CHIRALCEL® OJ-3R column (150 mm long, 4.6 mm i.d., 3 μm particle size) according to previously described methods (Lao and Gan, 2006; Lao and Gan, 2007; Ramezani et al., 2010). For all three imidazoline herbicides, the *S*-enantiomer was eluted first, followed by the *R*- enantiomer.

2.4.2. Soil and earthworm extraction and cleanup

For soil samples, the sample preparation procedures followed a previously developed method with minor modifications (Inês et al., 2019). First, 10 g soil was accurately weighed into a 100 mL centrifuge tube, and then 40 mL $Ca(OH)_2$ (0.00005 mol/L; pH = 10) was added. The mixture was swirled for 5 min, and then 20 mL acetonitrile was added, after which the mixture was swirled for another 5 min. The mixture was then acidified to pH 2.0–3.0 with HCl before adding 8 g NaCl and 10 g anhydrous $MgSO_4$. The tubes were capped and immediately vortexed for 5 min and then centrifuged at RCF 2077 ×g for 5 min. The 2 mL upper layer was evaporated to dryness under a gentle stream of nitrogen gas, and the analyte was dissolved in 2 mL acetonitrile. The residue was filtered through a 0.22-μm nylon syringe filter, and then analyzed by UPLC-MS/MS.

For the earthworm samples, a 2 g sample was blended with 10 mL acetonitrile and homogenized with Ultra-Turrax T18 homogenizer for 1 min. The sample tube was vortexed for 3 min, and then, 2 g NaCl and 2 g anhydrous Na_2SO_4 were added. The tubes were capped and immediately vortexed for 5 min and then centrifuged at RCF 2077 ×g for 5 min. Next, 1.5 mL of the upper layer was transferred into a single-use centrifuge tube containing 100 mg C18 and 200 mg of anhydrous $MgSO_4$ sorbent. The tube was capped, vortexed vigorously again for 2 min, and then centrifuged at RCF 2077 ×g for 5 min. The residue was filtered through a 0.22-μm nylon syringe filter and then analyzed by UPLC-MS/MS.

2.4.3. Quality assurance and quality control

The limit of detection (LOD) and limit of quantification (LOQ) values of the compounds were calculated for both earthworms and the soil matrix. The LOQs values for the three herbicides were estimated to be 1.01–1.88 μg/kg in soil, and 0.74–1.22 μg/kg in earthworms. The LODs values were estimated to be 0.34–0.61 μg/kg in soil, and 0.26–0.37 μg/kg in earthworms. The correlation coefficient (R^2), intercept, and slope ratio of the calibration curves for each stereoisomer of each herbicide are listed in Table S2. Linear regression analysis was performed with a concentration range 2.5–1000 μg/L for each enantiomer. Good linearity was observed for all herbicide' enantiomers in solvent, earthworms, and soil matrices, with R^2 values >0.9972 in all matrices (Table S2). Three chiral herbicides enantiomers gave excellent mean recoveries in the range of 86.5%–101.2% at three fortification levels in both soil and earthworm matrixes. And a good repeatability was obtained with the RSDs <8.9% (Table S3). The results of the recovery studies indicated that the developed chiral LC–MS/MS method achieved a satisfactory precision and accuracy for the enantiomeric analysis of the three herbicides in soil and earthworm (Table S3).

2.5. Statistical analysis

The enantiomer fraction (EF) was used to assess the enantioselectivity behavior of herbicides in soil and earthworm. The defined EF values ranged from 0 to 1, with EF = 0.5 representing the racemate. The EF values were calculated using the following equations:

$$\text{EF} = \text{peak area of } (+)/\text{peak area of } [(-)+(+)] \quad (1)$$

where (+) is the first eluted chromatograph peak of the S-compound and (−) is the second eluted peak of the R-compound.

The degradation of chemicals in soil was calculated using the following first-order equation:

$$C = C_0 e^{-kt} \tag{2}$$

where C_0 and C are the concentrations of the compound at times zero and t, respectively, and k is the rate constant. The half-life ($T_{1/2}$, in days) was estimated as follows:

$$T_{1/2} = \ln(2)/k \tag{3}$$

The significance of the correlations of the enantioselectivity (SE) was used to reflect the overall trend of enantioselective dissipation, as reported by Diao et al. (Diao et al., 2009), The ES of dissipation was defined as follows:

$$ES = (k_{(S)} - k_{(R)})/(k_{(S)} + k_{(R)}) \tag{4}$$

where $k_{(S)}$ and $k_{(R)}$ are the first-order rate constants of the dissipation of the *S*-enantiomer and *R*- enantiomer in soil, respectively. An ES value that ranged from 0 to 1 indicated a more rapid dissipation of the S-enantiomer in the soil, and a value of $-1 < ES < 0$ indicated a more rapid dissipation of the R-enantiomer. ES = 0 indicated that dissipation was not enantioselective, and ES = 1 or ES = −1 indicated that dissipation was fully enantioselective.

BSAF (bio-soil accumulation factors) was used to assess the bioaccumulation of herbicides in earthworm (Chang et al., 2016). BSAFs(kg_{oc}/kg_{lip}) was normalized to the lipid content in earthworm and organic carbon (OC) in soil (Liu et al., 2015). The equations used were as follows:

$$BSAF = C_{EW}/C_S \tag{5}$$

$$BSAFs\left(kg_{oc}/kg_{lip}\right) = [C_{EW}\,F_{OC}(soil)]/[C_S\,F_{lip}(earthworm)] \tag{6}$$

C_S and C_{EW} are the concentrations of herbicide in soil and earthworm, respectively. F_{lip} (earthworm) is the fraction of lipid in earthworm and it was assumed to be 1%.F_{OC} (soil) is the fraction of organic carbon in soil (Diao et al., 2011; Liu et al., 2015).

3. Results and discussion

3.1. Degradation of three herbicides in soil

The concentrations of the three analyzed herbicides in soil in earthworm-soil microcosms decreased over time. The initial concentrations were 1 mg/kg in soil for the two enantiomers. An acceptable fit to first-order reaction kinetics was obtained for the degradation of the two enantiomers in soil in 60 d. Table 3 shows the half-lives of the two enantiomers of each herbicide in soil. As shown in Table 3, the half-lives of the two enantiomers of imazapic ranged from 22 to 49.5 d (R^2, 0.8292–0.9299), those of imazamox ranged from 31.5 to 53.3 d (R^2, 0.6504–0.9086), and those of imazethapyr ranged from 25.7 to 33 d (R^2, 0.6722–0.9127). These half-life values of the three herbicides in soils were very similar to those previously reported in the literature. For example, previously reported values for the half-life of imazethapyr in soils range from 27.3 d to 60 d under laboratory conditions. For imazamox, its soil half-life was reported to range from 23.5 d to 43.3 d in clay loam, from 19.6 d to 39.8 d in sandy loam soil (Rani et al., 2019). The previously reported half-life range of imazapic was 21–64.4 d (Inês et al.,2019; Yavari et al., 2019).

As shown in Table 3 and Fig. 1, imazapic showed enantioselective degradation in soils, with the *S*-enantiomer degrading faster in the HN and GX earthworm–soil systems (ES values of 0.06 and 0.03, respectively). However, the *R*-enantiomer degraded more rapidly than the *S*-enantiomer in the HLJ and CQ earthworm-soil microcosms. The respective half-lives of the *S*-enantiomer and *R*-enantiomer were 49.5 d and 46.2 d in the HLJ soil-earthworm microcosm, and 63 d and 49.5 d in the CQ soil–earthworm microcosm, (ES values of 0.03 and 0.12, respectively). Imazamox and imazethapyr did not show enantioselective degradation in soils in the HN, HLJ, and CQ earthworm-soil microcosms, with ES values of 0. Furthermore, the EF was also used to assess the enantioselectivity behavior of herbicides in soil, and the EF values of imazamox and imazethapyr in soil in all treatments fluctuated around 0.5 (n = 3) during the incubation period. The results also indicated that there was no significant enantioselectivity ($p > 0.05$) for the degradation of imazamox and imazethapyr in soil in earthworm-soil microcosms.

The persistence of imazamox and imazethapyr in soil was similar in the HN and CQ earthworm-soil microcosms possibly because the HN and CQ soils had similar pH values and organic matter contents. Although the HLJ soil also had a very similar pH to that of HN, the organic matter contents the HLJ soil differed greatly from those of CQ and HN. Some studies have indicated that organic matter in soil has a strong effect on the persistence of contaminants in soil (Webb and Aylmore, 2002). Furthermore, some studies also have demonstrated that the soil pH can substantially affect the degradation rate of pesticides. Specifically for ionizable herbicides, such as imidazolinones, soil pH was found to be one of the most the important factors affecting their degradation in soil (Ramezani et al., 2010). Wu et al. showed that soil pH influenced the selective degradation of imidazolinone herbicides, because the soil pH strongly affected the structure of the soil microbial communities, including the bacterial ratio, fungal and bacterial taxonomic groups, and the distribution of functional groups (Wu et al., 2017). Some studies have also reported that soil pH affected the degradation of imidazolinone herbicides (Wang et al., 2006; Buerge et al., 2019a, 2019b). However, the soils used in this study varied little in their soil properties. Further research should be conducted to analyze in detail the relationship between soil properties and the degradation rates of these herbicides.

Table 3

First-order rate constants (k),ES, half-lives ($t_{1/2}$) a and correlation coefficient (R^2) values as derived from the regression models for the degradation of imidazolinone enantiomers in different soils.

Soils	Enantiomers	Herbicides											
		Imazapic				Imazamox				Imazethapyr			
		K(day^{-1})	T1/2(day)	R^2	ES	K(day^{-1})	T1/2(day)	R^2	ES	K(day^{-1})	T1/2(day)	R^2	ES
HN	*S*	0.033(±0.0007)	21	0.8787	0.06	0.013(±0.001)	53.3	0.6504	0	0.027(±0.0006)	25.7	0.9016	0
	R	0.029(±0.0005)	23.9	0.8689		0.013(±0.0009)	53.3	0.6818		0.027(±0.0008)	25.7	0.9003	
GX	*S*	0.02(±0.0006)	34.7	0.9299	0.03	0.021(±0.0008)	33	0.9544	−0.02	0.022(±0.0009)	31.5	0.7935	0.02
	R	0.019(±0.0012)	36.5	0.9834		0.022(±0.0007)	31.5	0.9657		0.021(±0.0011)	33	0.6722	
HLJ	*S*	0.014(±0.001)	49.5	0.8962	−0.03	0.022(±0.0005)	31.5	0.8517	0	0.025(±0.0009)	27.7	0.9544	0
	R	0.015(±0.0009)	46.2	0.8861		0.022(±0.0006)	31.5	0.8253		0.027(±0.0007)	25.7	0.9542	
CQ	*S*	0.011(±0.0007)	63	0.8292	−0.12	0.013(±0.0009)	53.3	0.8669	0	0.027(±0.0006)	25.7	0.9126	0
	R	0.014(±0.0008)	49.5	0.8665		0.013(±0.0007)	53.3	0.9086		0.027(±0.0004)	25.7	0.9127	

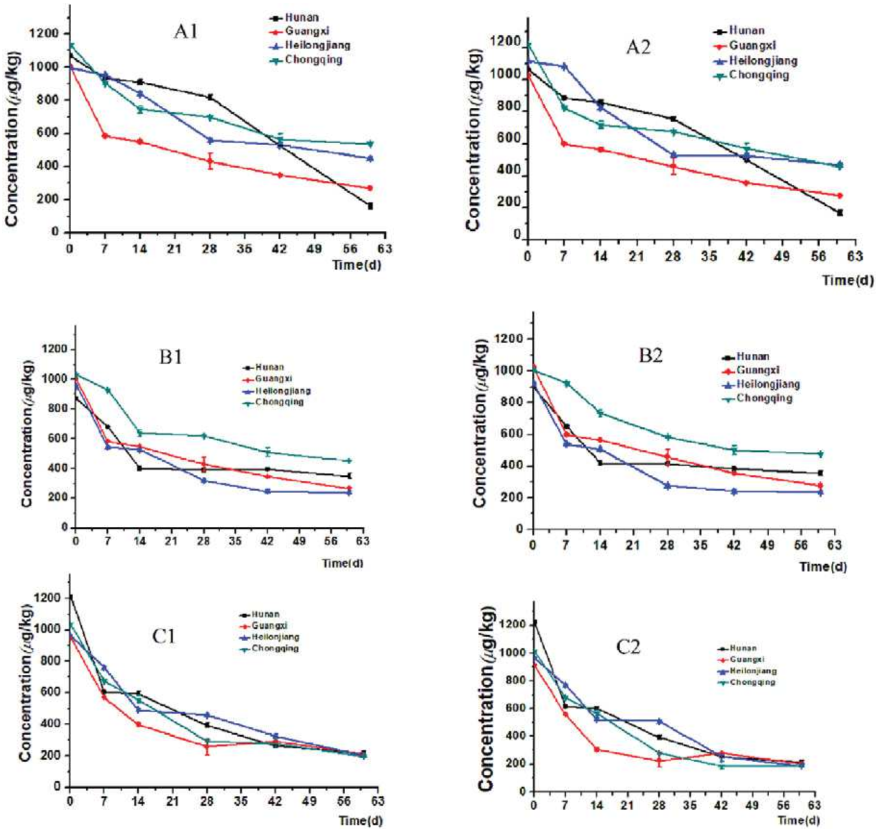

Fig. 1. Concentrations of two enantiomers of three imidazolinone herbicides in soil samples in four earthworm-soil microcosms (A1; *S*-imazapic; A2; *R*-imazapic; B1: *S*-imazamox; B2: *R*-imazamox; C1: *S* -imazethapyr; C2: *R* -imazethapyr).

3.2. Bioaccumulation of the enantiomers of three imidazolinone herbicides in earthworm

Fig. 2 shows the accumulation of the two enantiomers of herbicides in earthworms during exposure, and these results indicate that the uptake of herbicides by earthworms differed among the compounds and soil types.

For imazapic, at the first sampling point, *S*-imazapic and *R*-imazapic were rapidly taken up by earthworms, with concentrations of 8.31 and 10.58 μg/kg, 26.58 and 26.55 μg/kg, 6.81 and 8.33 μg/kg, and 18.05 and 24.16 μg/kg in the HN, GX, HLJ, and CQ earthworm-soil microcosms, respectively. After 28 days of exposure, the concentrations of *S*-imazapic and *R*-imazapic in earthworms were 167.77 and 155.79 μg/kg, 148.12 and 145.21 μg/kg, and 159.01 and 159.33 μg/kg in the HN, GX, and CQ earthworm-soil microcosms, respectively, and they had reached equilibrium. However, in the HLJ earthworm-soil microcosm, the concentrations of *S*-imazapic and *R*-imazapic in earthworms were 146.01 and 147.62 μg/kg, respectively, and these concentrations had stabilized by 42 days of exposure. The herbicides took longer to reach steady state levels in the HLJ earthworm–soil microcosm than in the other earthworm-soil microcosms. These data indicated that imazapic was taken up faster and more easily by earthworms in the HN, GX, and CQ earthworm-soil microcosms than in the HLJ earthworm-soil microcosm. The amount of organic matter was much higher in the HLJ earthworm-soil system than in the other soil systems. This suggested that organic matter may negatively affect the bioaccumulation of imazapic in earthworms. If a higher soil organic matter in soil led to a lower bioavailability of the pesticides, then it would take longer to achieve an equilibrium concentration. Previous studies showed that soils with a low organic matter content bind some herbicides less strongly than do soils with a high organic matter content (Thomas and Bjarne, 2008). Differences in herbicide uptake rates among different soils have been observed in other studies (Qu et al., 2014).

After the initial 1 d of exposure, the amounts of *S*-imazamox and *R*-imazamox in earthworms were 9.28 and 10.15 μg/kg, 49.72 and 46.12 μg/kg, 9.10 and 8.20 μg/kg, and 19.64 and 20.02 μg/kg in the HN, GX, HLJ, and CQ earthworm-soil microcosms, respectively. After 7 d of exposure, the concentrations of both the *S*-enantiomer and *R*-enantiomer in earthworms reached steady state levels in the HLJ and CQ earthworm-soil microcosms, corresponding to concentrations of 86.07 and 80.59 μg/kg and 76.46 and 69.17 μg/kg, respectively. However, the concentrations of these two enantiomers took 14 d to stabilize in earthworms in the HN and GX earthworm-soil microcosms, at concentrations of 109.15 and 105.37 μg/kg and 101.07 and 102.05 μg/kg, respectively.

For imazethapyr, at the first sampling time, the concentrations of the *S*-enantiomer and *R*-enantiomer in earthworms were 9.23 and 7.88 μg/kg, 10.07 and 8.54 μg/kg, 15.64 and 15.21 μg/kg, and 15.41 and 13.66 μg/kg in the HN, HLJ, CQ, and GX earthworm-soil microcosms, respectively. In the GX earthworm-soil microcosm, the concentrations of *S*-imazethapyr and *R*-imazethapyr in earthworms reached steady state levels of 106.89 μg/kg and 102.27 μg/kg, respectively, at day 14. However, in the CQ, HN, and HLJ earthworm-soil microcosms, equilibrium was not reached until 42 days, when the concentrations of the *S*- and *R*-enantiomers in earthworms stabilized at 217.11 and 211.23 μg/kg, 237.14 and 224.43 μg/kg, and 206.89 and 199.11 μg/kg, respectively.

To investigate whether the bioaccumulation of the three chiral herbicides was enantioselective, we calculated the EF values in earthworms (Fig. S3). The values were 0.5 at the start of the experiment. A one sample *t*-test was used to compare the means of the EF values in earthworm samples with EF = 0.5. As shown in Fig. 4, the EF values of the three herbicides in earthworms remained around 0.5, and did not change significantly

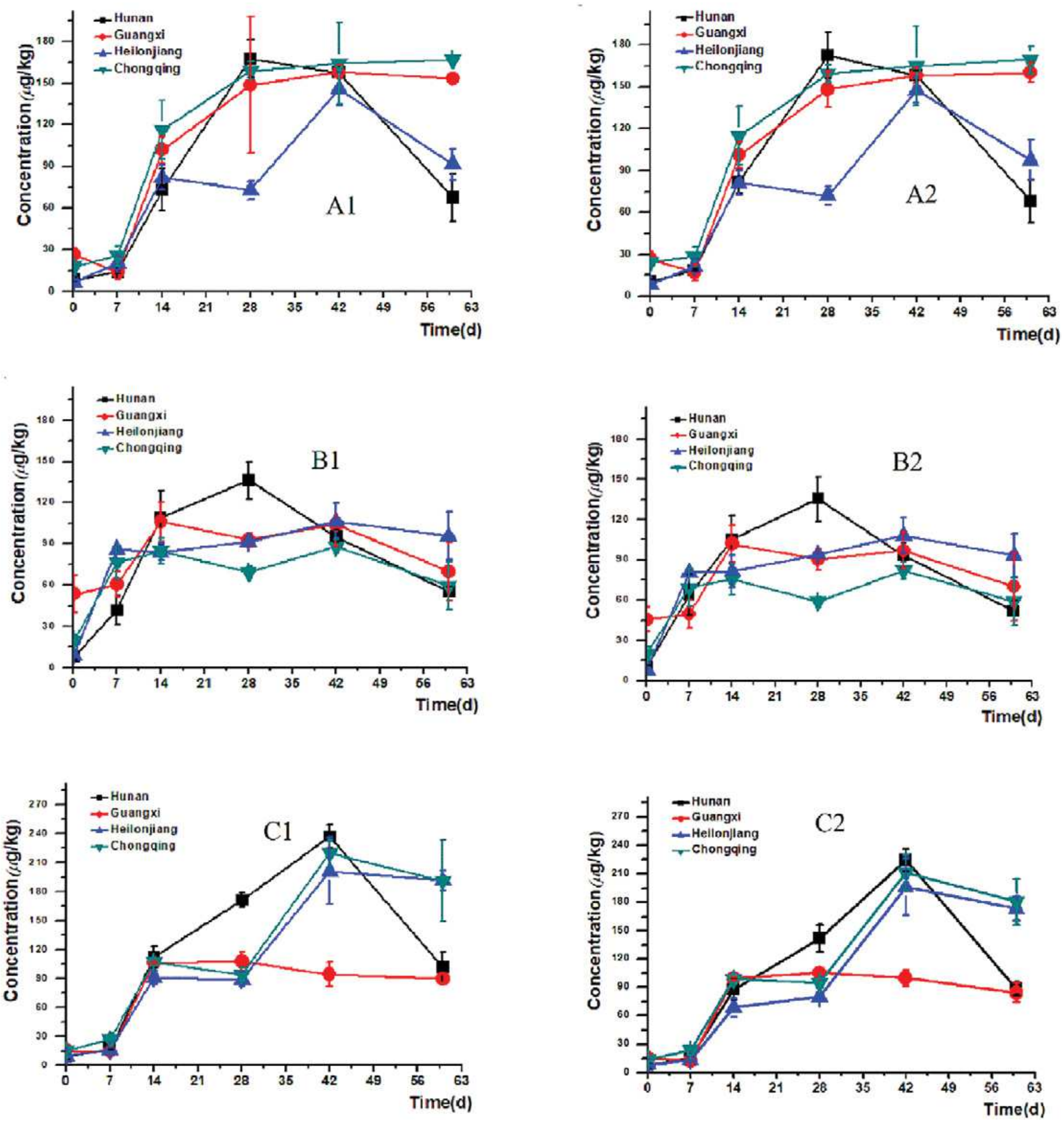

Fig. 2. Concentrations of two enantiomers of three imidazolinone herbicides in earthworm samples in four earthworm-soil microcosms (A1; *S*-imazapic; A2; *R*-imazapic; B1: *S*-imazamox; B2: *R*-imazamox; C1: *S* -imazethapyr; C2: *R*-imazethapyr).

($p > 0.01$). According to previously published results, the bioaccumulation of chiral pesticides in earthworms is not enantioselective if the EF values are 0.5 or is weakly enantioselective if the EF values are between 0.37 and 0.61 (Chang et al., 2016; Xu et al., 2017; Xu et al., 2009). Therefore, we concluded that three chiral herbicides did not show enantioselective bioaccumulation in earthworms in these earthworm-soil microcosms.

3.3. Bioconcentration factor

In accumulation experiments, BSAFs values are calculated to express the bioaccumulation of contaminants. As shown in Fig. 3, the BSAFs values increased with exposure time in all soil microcosms, with relationships that could be described by simple linear regressions. As shown in Table 4, for imazapic, satisfactory linearities were obtained with mean coefficients of determination (R^2) >0.8 in the HN, CQ, and GX earthworm-soil microcosms, and lower R^2 values of 0.7764 and 0.7768 were obtained in the HLJ earthworm-soil microcosm for the *S*- and *R*-enantiomers, respectively. For imazamox, an $R^2 > 0.9$ was obtained in the CQ and GX earthworm-soil microcosms, but an $R^2 < 0.8$ was obtained in the HLJ and HN earthworm-soil microcosm for the two enantiomers. For imazethapyr, good linearities were obtained for the two enantiomers in all earthworm-soil microcosms with $R^2 > 0.9$, except in the HN earthworm-soil microcosm, with the $R^2 < 0.8$.

As illustrated in Table 4, the result of $R^2 < 0.8$ indicated that the BSAFs values reached their maximum during the exposure period and then decreased over the following days, with an "increase-decrease" trend. There are several possible explanations for this trend. First, at the start of exposure, the high concentration of herbicide in the soil and the low concentration of herbicide in earthworms resulted in very low BSAFs values. Second, with increasing exposure time, the herbicide concentration in earthworms increased and the BSAFs values increased, and then, the BSAFs values decreased slowly after reaching a maximum. The reason for the decrease after the BSAFs values reached a maximum may be that the herbicides were degraded in soil, reducing their availability to earthworms. Alternatively, if the herbicides were adsorbed by the soil particles, they would be unavailable to earthworms, and the BSAFs values would decrease.

The result of $R^2 > 0.8$ for herbicides in the earthworm-soil systems indicated that the BSAFs values in the earthworm-soil microcosms increased during the whole incubation period for these herbicides. One explanation for this trend is that although herbicides were degraded in soil and the concentrations of herbicides in soil decreased, there was sufficient availability of herbicides for continuous uptake by

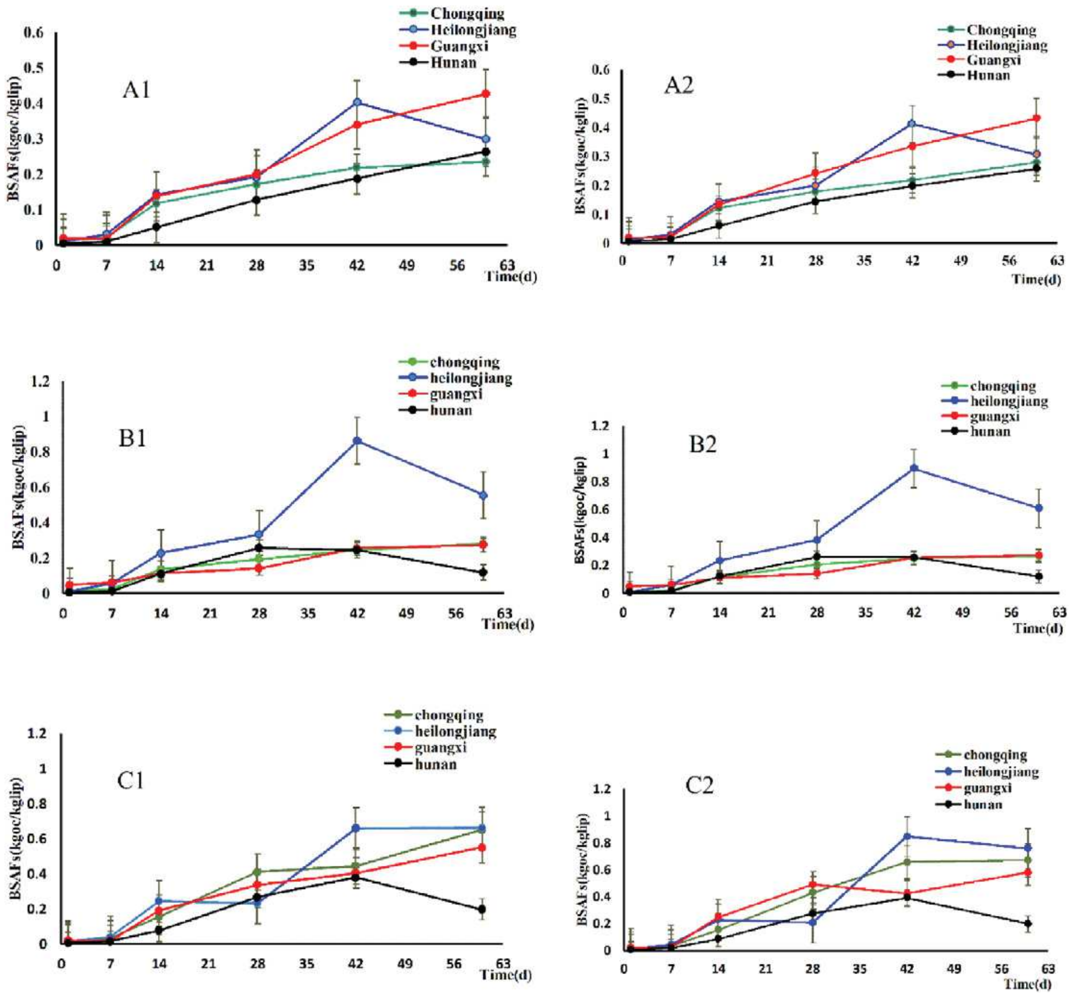

Fig. 3. Calculated BSAFs of two enantiomers for three imidazolinone herbicides in four earthworm-soil microcosms (A1; *S*-imazapic; A2; *R*-imazapic; B1: *S*-imazamox; B2: *R*-imazamox; C1: *S*-imazethapyr; C2: R -imazethapyr).

Table 4
the relationship between BSAF values and exposed time.

Compounds	Soil	Regression equation	R^2
R-imazapic	CQ	Y = 0.004x + 0.0275	0.8846
	HLJ	Y = 0.006x + 0.0285	0.7764
	HN	Y = 0.0046x−0.0097	0.993
	GX	Y = 0.0073x + 0.0049	0.9738
S-imazapic	CQ	Y = 0.0045x + 0.024	0.9421
	HLJ	Y = 0.0061x + 0.0283	0.7768
	HN	Y = 0.0045x−0.0017	0.983
	GX	Y = 0.0074x + 0.0096	0.9769
R-imazamox	CQ	Y = 0.0046x + 0.0307	0.9034
	HLJ	Y = 0.0012x + 0.348	0.7098
	HN	Y = 0.0028x + 0.0532	0.3461
	GX	Y = 0.0041x + 0.0429	0.9486
S-imazamox	CQ	Y = 0.0046x + 0.0294	0.8927
	HLJ	Y = 0.013x + 0.036	0.7454
	HN	Y = 0.0029x + 0.0585	0.34
	GX	Y = 0.041x + 0.0429	0.9486
R-imazethapyr	CQ	Y = 0.0111x + 0.003	0.961
	HLJ	Y = 0.0121x + 0.0007	0.8906
	HN	Y = 0.005x + 0.0309	0.5548
	GX	Y = 0.0093x + 0.0169	0.9566
S-imazethapyr	CQ	Y = 0.0128x−0.0019	0.9274
	HLJ	Y = 0.0147x−0.0229	0.8319
	HN	Y = 0.005x + 0.0362	0.5372
	GX	Y = 0.0097x + 0.0514	0.5866

earthworms. Finally, based on the BASFs values and the pattern of herbicide degradation in soil, we could predict the concentration of herbicides in earthworms at given times during exposure.

3.4. Relationship between herbicide properties and earthworm uptake

According to previous studies, chemicals with a log K_{OW} between 2 and 5 show bioaccumulation potential. For chemicals with log $K_{OW} < 5$, the major exposure route is the skin, while hydrophobic chemicals (log $K_{OW} > 6$) are absorbed mainly through the gut, and the gut begins to become an important route for chemicals with a log Kow above approximately 5 and then is the dominate route above 6 (Jager et al., 2003; Xu et al., 2017). As shown in Table 1, the log K_{OW} values of imazapic, imazamox, and imazethapyr were 2.47, 5.36, and 1.49, respectively, indicating that all three herbicides were absorbed mainly via the skin. To further characterize the relationship between the uptake and pharmaceutical properties of herbicides in the earthworm–soil microcosms, the BSAFs values were plotted against the log K_{OW} values for the three herbicides. As shown in Fig. 4, the BSAFs values in earthworms were negatively correlated with the log K_{OW} values for all herbicides in all earthworm-soil microcosms, except for the HLJ earthworm-soil microcosm. Compounds with high K_{OW} values may strongly adsorb to organic matter and become less bioavailable to earthworms, resulting in relatively lower BSAFs values (Shang et al., 2013). Similar trends were reported for bifenthrin, lambda-cyhalothrin, and cypermethrin (Chang et al., 2016). However, this correlation was not evident for earthworms

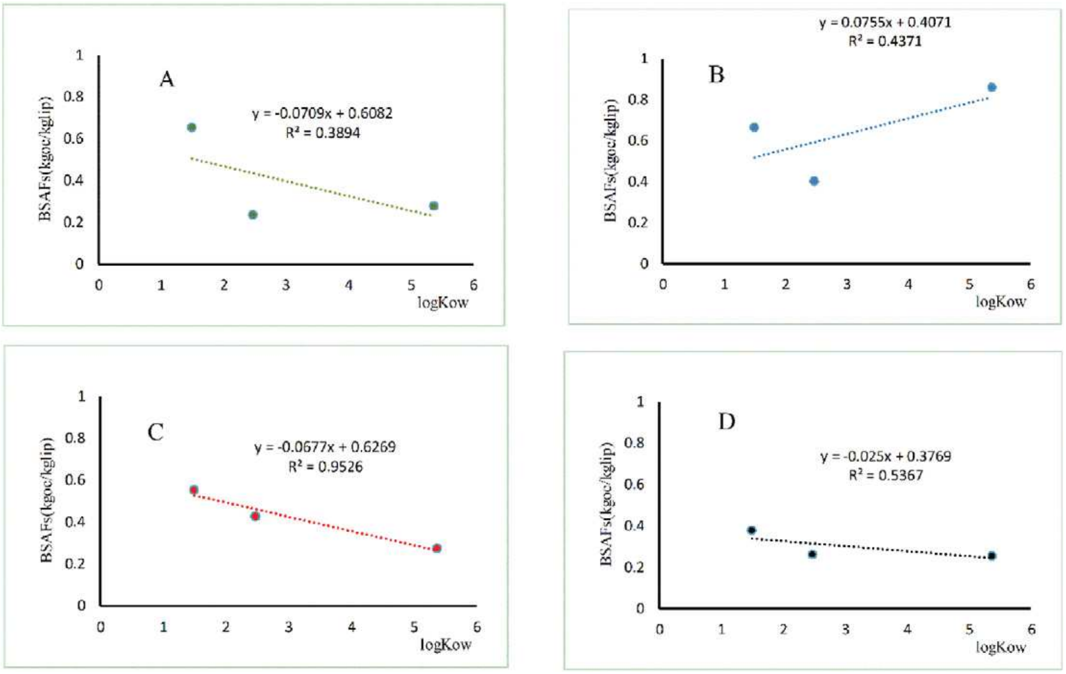

Fig. 4. Linear relationship between BSAFs and log K_{OW} for three imidazolinone herbicides under four earthworm-soil system (A: CQ-earthworm-soil microcosms; B: HLJ-earthworm-soil microcosms; C: GX-earthworm-soil microcosms; D: HN-earthworm-soil microcosms).

in the HLJ earthworm-soil microcosm. The reason maybe that the three studied herbicides were ionizable herbicides, soil pH may have affected their adsorption behavior (Stougaard et al., 1990).

3.5. Relationship between soil properties and earthworm uptake

The bioaccumulation of organic pollutants from soils in earthworms is strongly dependent on their bioavailability, which is a function of soil properties (such as organic matter, pH, cation exchange capacity, and texture), the compound's properties, and the organism's physiology and ecology. Organic matter content of soil has been shown to greatly influence the bioavailability of organic pollutants in soils (Carter et al., 2016). The relationship between BSAFs values and organic matter content of soil was assessed using simple linear regression analyses. As shown in Fig. 5, organic matter content of soil positively affected the bioaccumulation of these herbicides in earthworms. Other studies have shown that the higher the organic matter content, the more readily the accumulation of hydrophobic organic pollutants in earthworms reaches equilibrium (Miao et al., 2018). This may be because earthworms are more active in soils with a higher organic carbon content, resulting in greater herbicide uptake. Moreover, log $K_{OW} < 6$ for the three herbicides, and these herbicides were absorbed mainly via the skin. In this study, the R^2 values ranged from 0.4511 to 0.8877, indicating a poor relationship between BSAFs values and organic matter for these herbicides. The reason for the poor correlation may be that the bioaccumulation of compounds in earthworms is a complex interaction affected by a variety of factors and processes, and does not exclusively rely on a single soil parameter. For example, cation exchange capacity, moisture content, clay content and character, temperature, pore size and quantity, pH, and particle size distribution are also important soil characteristics affecting bioavailability (Bielská et al., 2012; Nam et al., 1998). Although only four soils were studied and statistical analyses would therefore be inappropriate, this study performed for the first time a comparison of earthworm uptake of imidazolinone herbicides in four earthworm-soil microcosms with characterized soil properties.

4. Conclusions

In this study, the bioaccumulation and degradation behaviors of enantiomers of three imidazolinone herbicides in earthworm-soil microcosms were examined. Significant stereoselective degradation in soil occurred in earthworm-soil microcosms for imazapic, However, imazamox and imazethapyr showed no significant stereoselectivity in their degradation. The three imidazolinone herbicides did not show enantioselective bioaccumulation in earthworms in the earthworm-soil microcosms. The results also indicated that the BSAFs values were negatively correlated with the log K_{OW} values of herbicides, but were positively correlated with organic matter content of soil. Chemical hydrophobicity (Kow) and organic matter content of soil may serve as good predictors of bioavailability for imidazolinone herbicides in earthworm. Finally, these data provide information on the bioaccumulation and degradation behaviors of three chiral imidazolinone herbicides in earthworm–soil microcosms, and will be useful for accurate risk assessments.

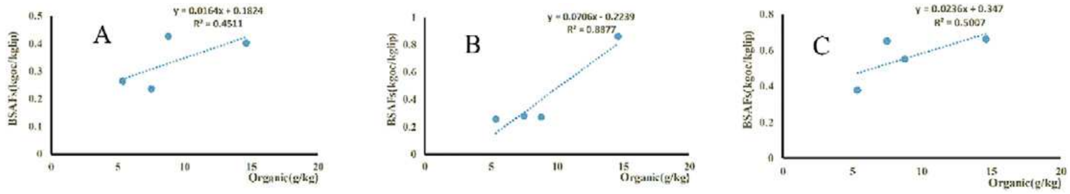

Fig. 5. Linear relationship between BSAFs and organic matter content of soil for three imidazolinone herbicides under four earthworm-soil microcosms (A: imazapic; B: imazamox; C: imazethapyr).

Acknowledgements

This work was supported by Natural Science Foundation of China (31101464, 31872000).

Declaration of competing interest

The authors have no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.135476.

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