

## Reveal the metal handling and resistance of earthworm *Metaphire californica* with different exposure history through toxicokinetic modeling

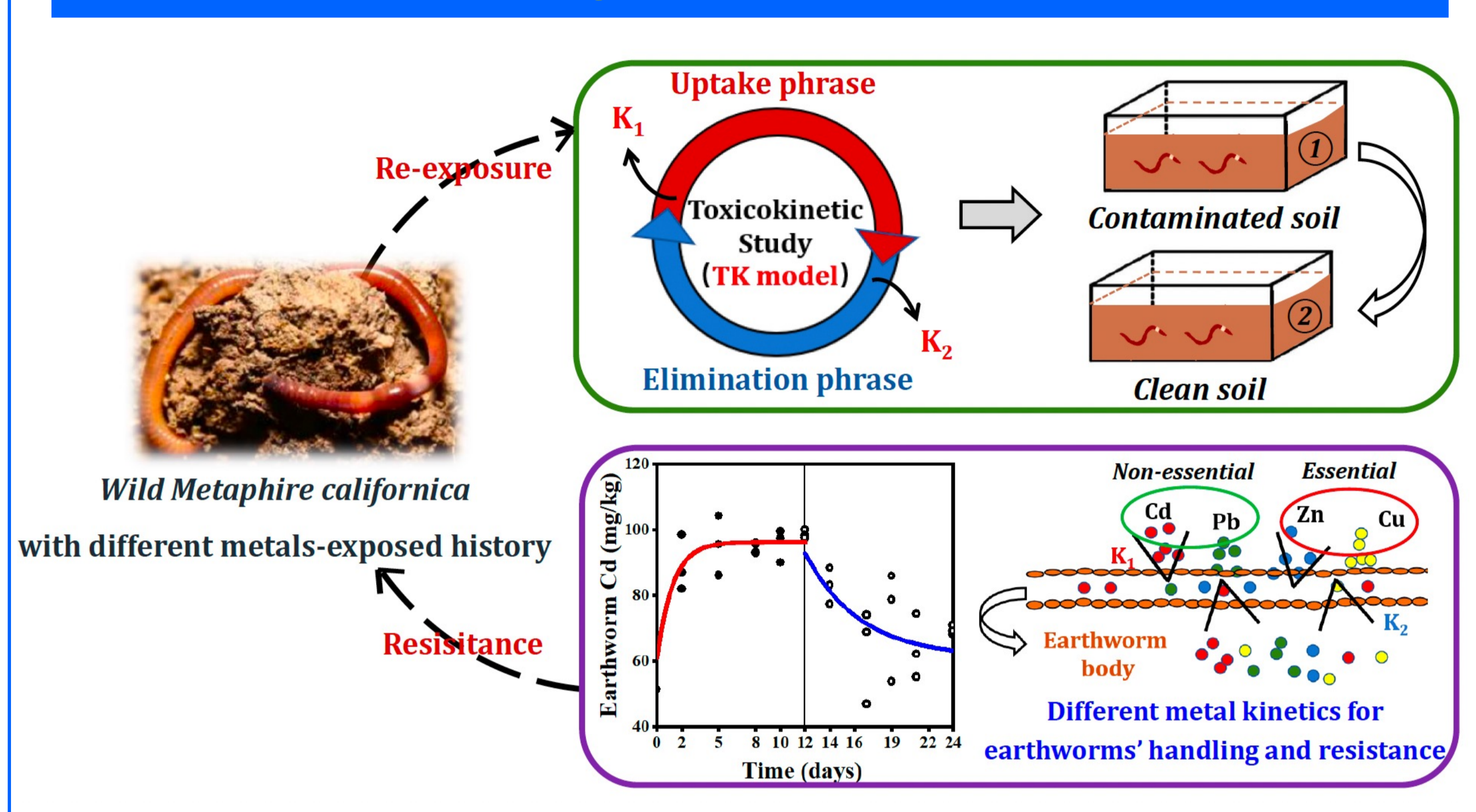
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### Graphical abstract



### Abstract

Toxicokinetic (TK) model provides a new approach to mechanistically elucidate the natural variation of metal handling strategy by adaptive and sensitive earthworm populations. Here, TK model was applied to explore the metal handling and resistance strategy of wild *Metaphire californica* with different historical exposure history through a 12-day re-exposure and another 12-day elimination incubation. *M. californica* populations showed different kinetic strategies for non-essential metals (Cd and Pb) and essential metals (Zn and Cu), which were closely related to their exposure history. *M. californica* from the most serious Cd-contaminated soil showed the fastest kinetic rates of both Cd uptake ( $K_1 = 0.78 \text{ gsoil/gworm/day}$ ) and elimination ( $K_2 = 0.23 \text{ day}^{-1}$ ), and also had the lowest Cd half-life ( $t_{1/2} = 3.01 \text{ day}$ ), which demonstrated the potential Cd-resistance of wild *M. californica* from Cd-contaminated soils. Besides, the comparative experiment showed totally different metal kinetics of laboratory *Eisenia fetida* from field *M. californica*, suggesting the impacts of distinct exposure history and species-specific sensitivities. These findings provide a novel approach to identify and quantify resistance using TK model and also imply the risk of overlooking existing exposure background and interspecies extrapolation in ecotoxicological studies and risk assessments.

### Methods

#### 1. Sampling sites

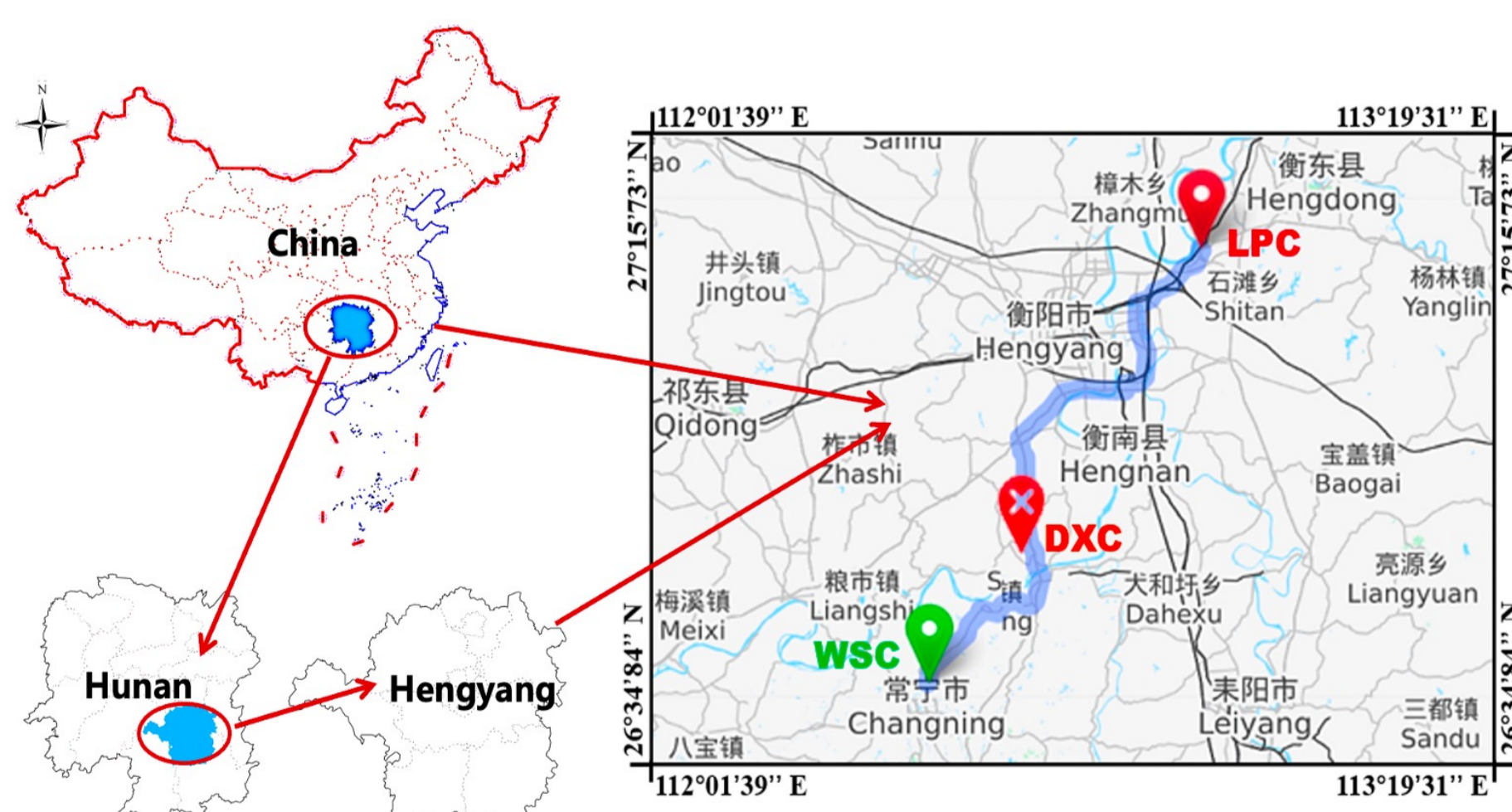


Fig. 1. Location of the sampling sites in Hengyang, Hunan Province, China.

Wild earthworm *M. californica* sampled from WSC, DXC, and LPC sites in Hunan Province

#### 2. Soil contamination

Table 1. Soil properties and metal concentrations of three different sampling plots

Sites	pH	Cd (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	Pb (mg/kg)
WSC	6.49±0.04a	1.50±0.07c	45.4±6.63b	148±4.28b	107±2.08a
DXC	6.69±0.24a	5.08±0.10b	83.0±0.60a	178±42.14b	70.4±0.83c
LPC	6.89±0.01a	12.6±0.13a	37.3±0.54c	290±33.8a	95.1±0.60b

Total soil metal contamination: LPC > DXC > WSC

### Results and discussion

#### 1. Non-essential - Cd

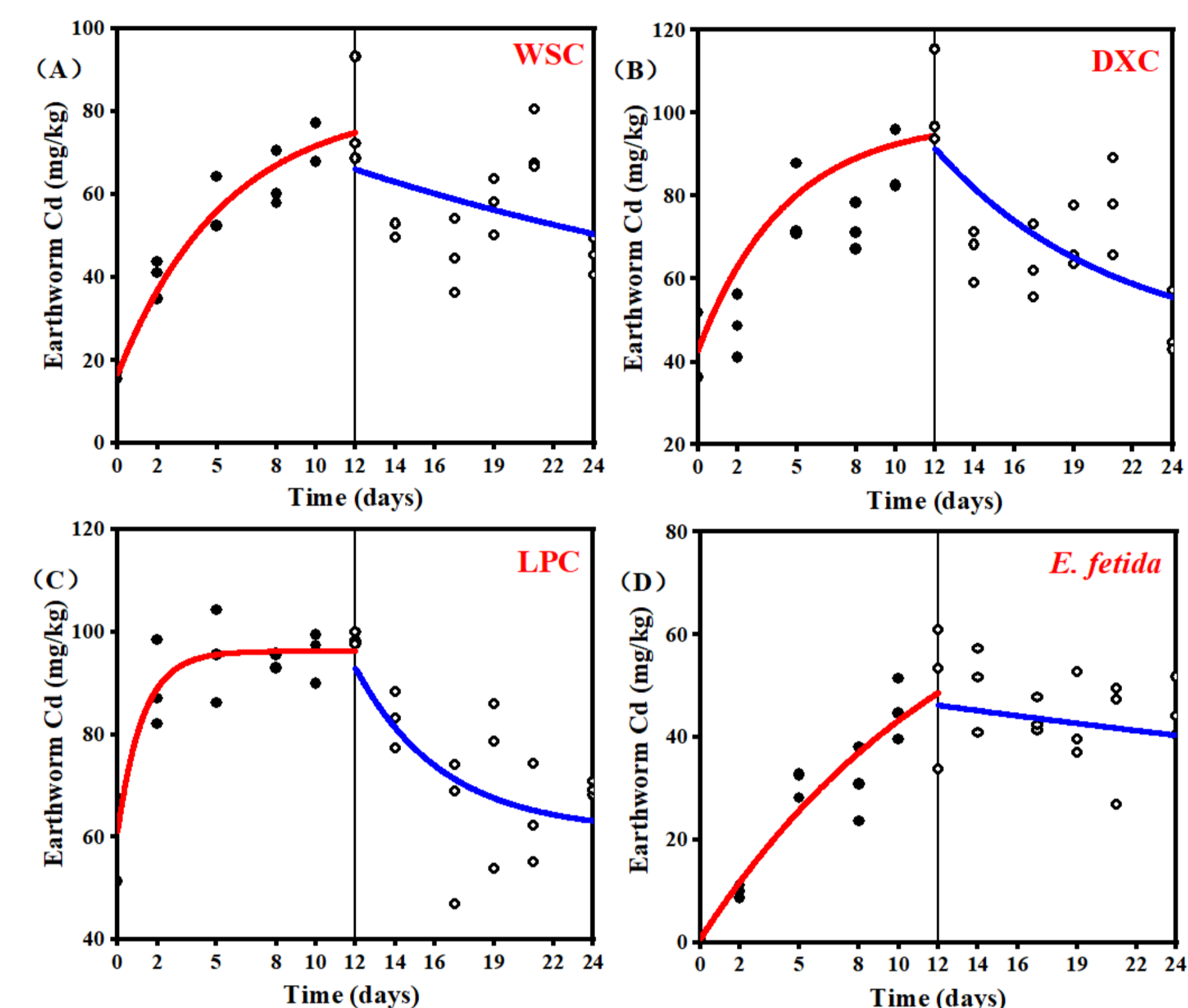


Fig. 2. Kinetic pattern of Cd in earthworms after 12-day exposure and 12-day elimination

*M. californica* from LPC showed the maximum  $K_1$  and  $K_2$  values and the shortest Cd half-life, suggesting efficiently Cd handling ability

#### 2. Essential - Zn

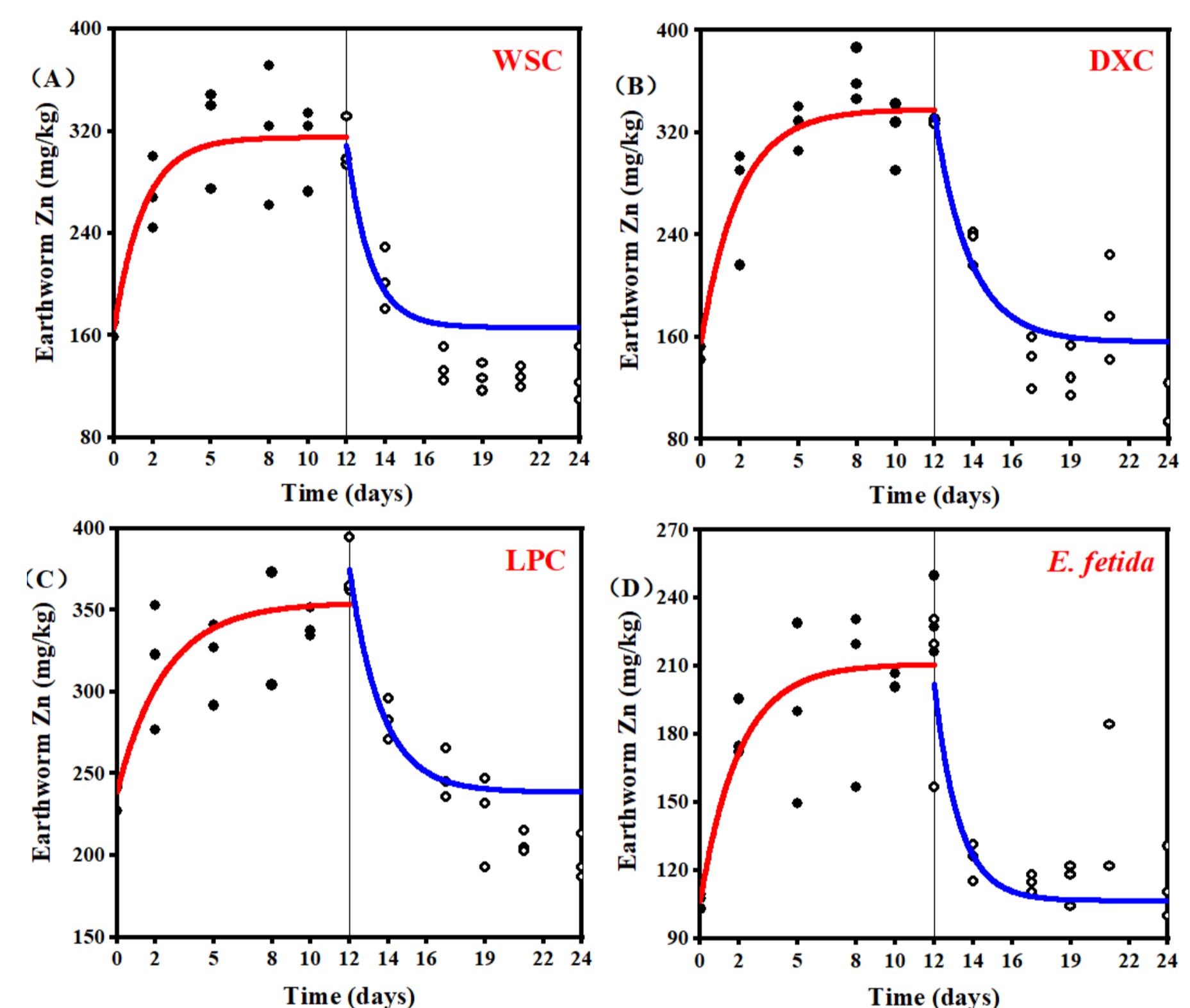


Fig. 3. Kinetic pattern of Zn in earthworms after 12-day exposure and 12-day elimination

*M. californica* showed rapid Zn kinetics, quickly reached the internal equilibrium either in the uptake or elimination phases

#### 3. TK parameters

Table 1. Toxicokinetic model parameters for the metal uptake and elimination phases

Treatment	$C_0$ (mg/kg)	$K_1$ (gsoil/gworm/day)	$K_2$ (day <sup>-1</sup> )	Half-life (d)	BAF	
Cd	WSC	16.8±1.12c	0.33 (0.28-0.39) b	0.03 (0.01-0.05) c	23.1	11.0
	DXC	42.8±8.08b	0.35 (0.19-0.51) b	0.11 (0.08-0.15) b	6.30	3.18
	LPC	61.1±8.56a	0.78 (0.54-1.01) a	0.23 (0.14-0.31) a	3.01	3.39
Zn	WSC	166±6.48b	0.20 (0.13-0.26) a	0.82 (0.44-1.20) a	0.85	0.24
	DXC	156±16.7b	0.19 (0.15-0.23) a	0.55 (0.38-0.72) a	1.26	0.35
	LPC	239±10.8a	0.09 (0.06-0.13) b	0.61 (0.41-0.80) a	1.14	0.15

TK parameter showed sites-specific features, suggesting the impacts of distinct exposure history and metal handling strategies

### Conclusions

- Toxicokinetic study was performed using wild earthworms to reveal their different metal handlings.
- Faster Cd uptake and elimination in *M. californica* indicated its Cd resistance and related mechanisms need more exploration.

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