

# Selenium Speciation in the Sediments and the Oligochaete *Lumbriculus Variegatus*

**Jichen Yang**

South China Normal University

**Xin Yan**

Guangxi Academy of Fishery Sciences

**Hongsong Liu**

South China Normal University

**Hongxing Chen**

South China Normal University

**Yongzhuang Wang**

Nanning Normal University

**Wei Zhang**

Guangzhou University

**Wu Dong**

Inner Mongolia University for Nationalities

**Dan Li**

South China Normal University

**Lingtian Xie** (✉ [lingtian.xie@m.scnu.edu.cn](mailto:lingtian.xie@m.scnu.edu.cn))

South China Normal University <https://orcid.org/0000-0002-6962-3957>

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## Research Article

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# Abstract

Sediments are the major sink for selenium (Se) in aquatic environments. Se speciation in sediments is crucial for its bioavailability and the toxicity in benthos but this is relatively understudied. In this study, selenite (Se(IV)) and seleno-L-methionine (Se-Met) were respectively added to sediments for 90 and 7 days, and the accumulation and depuration of Se in the oligochaete *Lumbriculus variegatus* was investigated. The background levels of Se in the river sediments, fish flakes, and *L. variegatus* were also detected. Without the presence of worms, the levels of Se(IV) in the sediments were relatively stable within 7 d but showed a decreasing trend during the 90 d of aging. In contrast, Se-Met in the sediments showed a sharp decrease within 3 d of aging. The LC<sub>50</sub>-96h values of Se(IV) and Se-Met in *L. variegatus* were 372.6 and 9.4 µg/g. Interestingly, the dominant Se species in Se(IV)- or Se-Met-treated *L. variegatus* was Se-Met, whose levels were increased with time in 7 days of exposure. Se was barely depurated from *L. variegatus* during the 8 d of depuration period. This study has provided indispensable data on the levels of total Se in the abiotic and biotic matrices and the biodynamics of Se in a representative benthos, which could better understand the ecological risk of Se to the freshwater benthic communities.

## 1. Introduction

Selenium (Se) is an essential trace element required for various functions in animals including maintaining immunity, stimulation of growth and reproduction (Mansour et al., 2017), and has been widely used in the livestock industry (Tinggi, 2003). However, due to its relatively narrow safety range for animals in the organisms (Debruyne and Chapman, 2007), it can be highly toxic to aquatic organisms. Conclusive evidence for its toxicity at elevated concentrations to aquatic organisms has been obtained from the two heavily Se-contaminated aquatic ecosystems, i.e., the Belews Lake and Kesterson Reservoir, which have witnessed the extinction of a number of apex predatory species (fish and birds) in the aquatic food chain (Hamilton, 2004; Lemly, 2004). Meanwhile, laboratory experiments have documented a variety of adverse effects of Se in different aquatic organisms, including teratogenicity (Wang et al., 2020), neurotoxicity (Li et al., 2021), oxidative stress (Janz et al., 2010), the composition of microbial community (Liu et al., 2022), etc.

Elemental selenium is insoluble in water and is generally difficult to be oxidized and reduced, Se from natural and anthropogenic sources is present in the aquatic ecosystem mainly as highly soluble inorganic (i.e., selenite (Se(IV)) and selenate (Se(VI))) and organic Se (seleno-methionine (Se-Met), selenocystine (Se-Cys), and other Se-substituted analogs of organosulfur compounds) (Hyne et al., 2002; Maier et al., 1988). Inorganic Se derived from anthropogenic activities such as the coal fly ash and agricultural drainage (Schwartz et al., 2016) can adsorb onto surfaces of sediment minerals and organic matter once it is discharged into aquatic environments (Wang and Chen, 2003). Organic Se, such as Se-Met and Se-Cys, mainly biogenically derives from the death of organisms (Hyne et al., 2002) and is preserved in the sediments under moderately reduced conditions (Hamilton, 2004). It is well accepted that sediment is a major sink or source of Se (Canton and Van Derveer, 1997). Se in the sediments has been ubiquitously detected at ~ 1.0 µg/g ranging from < 0.08 to 39 µg/g (Lemly, 1997; May et al., 2008; Sun et al., 2021),

with maximum values of 100 µg/g (Lemly, 1997) and 210 µg/g (Presser and Ohlendorf, 1987) in the sediments from the Belews Lake and Kesterson Reservoir, respectively. Meanwhile, Se in the sediments is readily bioaccumulated by benthic organisms via the dietary exposure route, and could be subsequently transferred to higher trophic level species (Orr et al., 2006). Se levels in aquatic organisms range from 0.2 to 70 µg/g (Hamilton, 2004; Jasonsmith et al., 2008), and could be up to 250 µg/g in Se-contaminated environments (Jasonsmith et al., 2008).

Se speciation in the sediments is a complicated process (Fujita et al., 2005), which is crucial for its bioavailability and the ensuing toxicity in animals. The toxicity of organic Se has been documented to be higher than inorganic Se in aquatic organisms (Davis, 1988; Maier et al., 1993; Xie et al., 2016), most probably because it is more readily bioavailable than the latter. For example, the level of total Se in the *Lumbriculus variegatus* exposed to 15 µg/L Se-Met for 2 weeks is ~ two orders of magnitude higher than that exposed to 15 µg/L Se(IV) and Se(VI) (Xie et al., 2016). However, the speciation and toxicity of different Se species in the sediments are largely unknown. The main objectives of the present study were to explore 1) the speciation of different Se species in the sediments for short and long duration; 2) the biodynamics of Se and acute lethal toxicity of Se(IV) and Se-Met in the oligochaete *L. variegatus*.

## 2 Materials And Methods

### 2.1 Chemicals, sediments, and organisms

Sodium selenite (Se(IV), purity > 99%, Shenyang Ruifeng Fine Chemicals Ltd., Shenyang, Liaoning, China) and seleno-L-methionine (Se-Met, purity > 98%, Ryon Biological Technology Co., Ltd., Shanghai, China) were utilized in this study as representative inorganic and organic selenium species. Protease and lipase were purchased from Biosharp, Labgic Bio-Technology Co., Ltd (Beijing, China) and Shanghai Yuanye Bio-Technology Co., Ltd (Shanghai, China). All other reagents of analytical grade were purchased from Guangzhou Chemical Reagent Factory (Guangzhou, China). All glassware was acid-washed with 50% nitric acid, rinsed three times with deionized water, and oven-dried at 70°C prior to use.

The sediments were collected from the upper stream of the Songhua River in Heilongjiang Province (45.8°N, 126.5°E), and stored at -20°C for over weeks to eliminate all invisible benthic organisms. Before spiking, the overlying water was removed, and the sediments were wetly sieved (500 µm) after thawing at the room temperature (25°C) (Xie et al., 2016). The sediment had an organic content of 6.2 ± 0.1%, a silt/clay content of 76.8 ± 1.3%, a sand content of 15.4 ± 0.7% and a moisture content of 51.7 ± 0.2% (mean ± SE, n = 3).

The oligochaete *Lumbriculus variegatus* were maintained in the Aquatic Toxicology Laboratory of South China Normal University for many generations following the Guide for the Care and Use of Laboratory Animals and approved by the Research Ethics Committee of South China Normal University. They were cultivated in a glass container (18 L) with sediments (~ 2 cm depth)/water (12 L) carbon filtered dechlorinated tap water) at 26 ± 1°C under a 14 h light/10 h dark cycle, and fed ~ 100 mg of fish flakes

(Shenyang, China) once per day. Prior to the test, approximately 300 adult worms with similar size (wet weight of  $4.0 \pm 0.5$  mg, body length of  $22.0 \pm 2.0$  mm) were randomly selected and transferred into a glass container holding ~ 2 cm depth of sediments and 12 L dechlorinated tap water. The acclimation lasted for 7 days without food and aeration.

## 2.2 Experimental design

Before the experiment, the background levels of total Se in the sediments from Songhua River, food for the worms (fish flakes in this study) and *L. variegates* in the lab were quantified by inductively coupled plasma mass spectrometry (ICP-MS). The background levels of different Se species in the three matrices were identified by high performance liquid chromatography (HPLC) in combination with ICP-MS (details in below). The target concentrations of Se in the sediments were prepared with the stock solutions of Se(IV) or Se-Met (10 mg/L in Milli-Q water) in 60 g (~ 2 cm depth) sediments and stirred evenly with limited volume of dechlorinated tap water in a glass beaker (250 mL). All experimental glass beakers with sediments were equilibrated for 24 hours after gentle addition of 150 mL of the dechlorinated tap water. No food and aeration were provided during the acclimation and exposures. In addition, unless otherwise stated, the Se levels in the sediments were expressed on a dry weight basis, and those in the oligochaetes were expressed on a wet weight basis.

### 2.2.1 Se speciation in the sediments without worms

For the determination of Se speciation after addition of Se(IV) and Se-Met in sediments without worms for 90 days, 0 (Cont), 3, 30, and 100  $\mu\text{g Se/g}$  sediments were assigned. These concentrations were chosen based on the environmental relevance of Se(IV) and Se-Met in clean and Se contaminated ecosystems (Lemly, 2004). Each treatment had 3 replicates. The sediments/overlying water were sampled on the first eight consecutive days and on day 14, 21, 30, 45, 60, and 90 by taking 2 mL overlying water (1 mL for the quantification of total Se and 1 mL for Se speciation) and ~ 40 mg sediments (half for the quantification of total Se and another half for Se speciation). For the Se-Met treatments, water and sediments were sampled for the first 7 days but ceased due to the relatively stable of total Se at the day 3 and loss of > 99% Se-Met in the sediments at the day 7. All samples were stored at  $-80^\circ\text{C}$  prior to the quantification of total Se and Se species (details in below).

### 2.2.3 Acute toxicity and accumulation of Se in the worms

For the 96 h acute lethal toxicity, a preliminary experiment was performed according to the modified guidelines of OECD no. 225 (OECD, 2007) for screening the ranges of lethal nominal concentrations of Se(IV) or Se-Met in *L. variegatus*. Based on the preliminary results, nominal concentrations of Se(IV) at 0, 250, 287.5, 330.6, 380.2, 437.3, 502.8  $\mu\text{g Se(IV)/g}$  and Se-Met (0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0  $\mu\text{g Se-Met/g}$ ) were chosen for an accurate estimation of the lethal concentrations in 96 h. Each treatment had 3 replicates with 10 worms. The worms were determined to be dead if the body turned white and no reaction after a gentle mechanical stimulus within 10 s, recorded per 12 hours, and removed to prevent a toxic bystander effect in the small volume of the beaker. The exposure lasted for 96 h.

For the biodynamics of Se in the worms and the Se speciation in the sediments/overlying water in the Se(IV) or Se-Met treatments with the presence of *L. variegatus*, 0 (Cont), 0.1 (low, abbreviated as L treatment), 0.3 (median, abbreviated as M treatment), and 1.0 (high, abbreviated as H treatment)  $\mu\text{g Se/g}$  sediments were set. Each treatment had three replicates each with 30 acclimated worms. On day 0, 1, 3, 5, and 7, similar regime to the previous experiment without the presence of worms was adopted for the collections of the water and sediment samples. For the worm samples, 2 worms (1 for the quantification of total Se and the other for Se speciation) were taken from each beaker, blotted dry, and their wet weight and length were recorded. All samples were stored at  $-80^{\circ}\text{C}$  for later quantification of total Se and for Se speciation (details in below).

### ***2.2.4 Se depuration in the oligochaetes L. variegatus***

For the depuration of Se in *L. variegatus* after 7 days of uptake, the remained worms in each beaker were transferred into clean dechlorinated tap water without sediments. During the depuration period, 2 worms (1 for the quantification of total Se and 1 for Se speciation) were taken on day 2, 4, 6, and 8. After the measurements of weight and body length of the blotted dry worms, all samples were stored at  $-80^{\circ}\text{C}$  for the quantification of total Se and Se species (details in below).

## **2.3 Analysis of the total Se in the samples**

The overlying water samples were filtered with  $0.22\ \mu\text{m}$  PVDF membranes (ANPEL, Shanghai) and digested in  $\text{HNO}_3$  at  $165^{\circ}\text{C}$  for 30 min using a Microwave Digestion System (Model MARS6 240/50, CEM Corporation, Mathews, NC). The sediment samples were oven-dried to obtain a constant weight for  $\sim 24$  hours, followed by digestion in 10 mL mixed acid (5 mL HF, 3 mL  $\text{HNO}_3$  and 2 mL HCl). The thawed worms (without the drying process) were digested in 8 mL  $\text{HNO}_3$ . The sediments and worm samples were digested at  $190^{\circ}\text{C}$  for 40 min in the microwave. The digestate was further diluted with Milli-Q water to a final volume of 50 mL, and then was filtered with  $0.22\ \mu\text{m}$  PVDF membranes. Total Se concentrations in the samples were determined by ICP-MS (Agilent, 7900, USA) following a previous method (Jagtap et al., 2016). The ICP-MS operating parameters for were as follows: RF power = 1200 W, plasma gas flow = 15 L/min, carrier gas flow = 1 L/min, helium gas flow = 5 mL/min, sampling depth = 6 mm, octopole bias =  $-18\ \text{V}$ , energy discrimination = 5 V, integration time per mass = 0.3 s, and the monitored isotopes comprised  $^{78}\text{Se}$  and  $^{82}\text{Se}$ . The standard reference materials (SRM) (GBW10024, GBW07379, National Research Center for Certified Reference Materials, Beijing, China) were used for the recovery of total Se. The recovery for Se in the SRM was 101% and 99%. Other QA/QC included spiked samples and acid blanks. Blanks were analyzed at a rate of 1 per 5 samples.

## **2.4 Analysis of Se species**

The filtered overlying water samples were directly subject to the HPLC-ICP-MS analysis following a previous method with modifications (Y et al., 2001). Briefly,  $\sim 20.0\ \text{mg}$  sediment samples in all treatments were transferred into 50 mL centrifuge tubes with 20 mL EDTA (4 mM, pH 7.5), and filtered with  $0.22\ \mu\text{m}$  membranes after shaken on a shaker at 100 rpm at  $40^{\circ}\text{C}$  for 10 hours (Zhang, 2013). The worm samples were homogenized in 1 mL 30 mM Tris-HCl buffer (pH 7.5) with 0.2 ml of 7 M urea solution and 2 mg

lipase, and shaken for 1 h. After added 4  $\mu\text{l}$  of 0.5 M dithiothreitol and 16.5  $\mu\text{l}$  of 0.5 M iodoacetamide, solutions incubated at 25°C in the dark. After 1 h, added 22.5  $\mu\text{l}$  of the dithiothreitol solution and the mixture was shaken for 1 h. 2 mg protease type XIV were added and the samples were incubated on a shaker at 60 rpm at 37°C in the dark for 20 h. After enzymatic hydrolysis, extracts were centrifuged for 10 min at 3000 rpm (Jagtap et al., 2016). The supernatants were filtered with 0.22  $\mu\text{m}$  membranes. After extraction, the Se species in the sediments and worms were separated on a Hamilton PRP-X100 column (4.1  $\times$  250 mm, 10  $\mu\text{m}$ , Hamilton, USA) using an Agilent 1260 Infinity  $\infty$  Ultra Performance LC system (Agilent, USA). The mobile phase was 6 mM citrate buffer (pH 5.0) with a flow rate at 1.5 mL/min at 25°C. The conditions of ICP-MS were the same with those for the determination of total levels of Se. The injection volume was 50  $\mu\text{L}$ . The limit of detection (LOD) and the limit of quantification (LOQ) in the present study were 0.04 and 0.05  $\mu\text{g/L}$ , respectively. The recoveries were 102% for water samples, 96.2% for sediments, and 92.8% for worms.

## 2.5 Statistical analysis

Data were presented as mean  $\pm$  standard deviation (SD), and checked for normality by Kolmogorov-Smirnov test and homogeneity of variances by Levene's test. No significant deviations from normality or homoscedasticity were detected. One-way analysis of variance (ANOVA), followed by the Tukey's test, was conducted to detect the differences among treatments. Statistical significance was accepted at  $p < 0.05$ . The statistical analyses were performed using SPSS 22.0.

The 95% confidence interval of the 96-h LD50 was obtained by linear regression method. Taking the concentration of selenium as the independent variable (X) and the mortality rate at the corresponding concentration as the dependent variable (Y), the analysis software SPSS 20.0 was used for data processing. The linear equation of "concentration-mortality" was established, and the regression equation and related parameters were obtained.

## 3 Results

### 3.1 Determination of the background levels of total Se and Se species in different matrices

The levels of total Se in the sediments from the Songhua River were  $0.21 \pm 0.01$   $\mu\text{g/g}$ , dominating by Se(IV) with  $0.17 \pm 0.01$   $\mu\text{g/g}$  (Table 1). The body burden of total Se in *L. variegatus* was  $4.4 \pm 0.2$   $\mu\text{g/g}$ , dominant by Se-Met with  $3.2 \pm 0.2$   $\mu\text{g/g}$ . In the fish flakes, the levels of total Se, Se(IV), Se(VI), Se-Met, and Se-Cys were  $3.4 \pm 0.9$ ,  $1.71 \pm 0.32$ ,  $0.05 \pm 0.01$ ,  $1.33 \pm 0.24$ , and  $0.85 \pm 0.32$   $\mu\text{g/g}$ , respectively.

Table 1

The background levels of different selenium species (n = 3) in sediments, fish flakes, and the oligochaete *Lumbriculus variegatus*, and the measured levels in the sediments of the selenite (Se(IV)) and seleno-L-methionine (Se-Met) treatments (n = 3) determined by a high-performance liquid chromatography inductively coupled plasma mass spectrometry (mean  $\pm$  SE, n = 3). The Se levels in the sediments and food were expressed on a dry weight basis, while those in the oligochaetes were expressed on a wet weight basis.

Environmental matrix	Se(IV) ( $\mu\text{g/g}$ )	Se(VI) ( $\mu\text{g/g}$ )	Se-Met ( $\mu\text{g/g}$ )	Se-Cys ( $\mu\text{g/g}$ )	Total Se ( $\mu\text{g/g}$ )
Water	ND	ND	ND	ND	ND
Sediments (Control)	0.17 $\pm$ 0.01	0.02 $\pm$ 0.01	0.07 $\pm$ 0.03	ND	0.21 $\pm$ 0.01
Fish flakes	1.71 $\pm$ 0.32	0.05 $\pm$ 0.01	1.33 $\pm$ 0.24	ND	3.43 $\pm$ 0.87
<i>Lumbriculus variegatus</i>	0.10 $\pm$ 0.02	ND	3.21 $\pm$ 0.17	0.85 $\pm$ 0.12	4.36 $\pm$ 0.24
<b>Treatments with nominal conc.s</b>					
Se(IV) - L (0.1 $\mu\text{g/g}$ )	0.23 $\pm$ 0.01	0.01 $\pm$ 0.00	ND	ND	0.35 $\pm$ 0.00
Se(IV) - M (0.3 $\mu\text{g/g}$ )	0.41 $\pm$ 0.01	0.01 $\pm$ 0.00	ND	ND	0.55 $\pm$ 0.00
Se(IV) - H (1.0 $\mu\text{g/g}$ )	1.14 $\pm$ 0.05	ND	ND	ND	1.25 $\pm$ 0.00
Se-Met - L (0.1 $\mu\text{g/g}$ )	0.16 $\pm$ 0.00	0.01 $\pm$ 0.00	0.10 $\pm$ 0.00	ND	0.34 $\pm$ 0.01
Se-Met - M (0.3 $\mu\text{g/g}$ )	0.14 $\pm$ 0.00	0.01 $\pm$ 0.00	0.30 $\pm$ 0.00	ND	0.55 $\pm$ 0.00
Se-Met - H (1.0 $\mu\text{g/g}$ )	0.14 $\pm$ 0.01	ND	1.01 $\pm$ 0.01	ND	1.25 $\pm$ 0.00

The measured concentrations of Se(IV) and Se-Met in the sediments on the day of the exposure initiation (day 0) is shown in the Table 1. In general, the measured concentrations of Se-Met were very close to its nominal concentrations. After the subtraction of the background levels of Se(IV), the measured levels of Se(IV) approximated to its nominal concentrations as well (Table 1).

### 3.2 The levels of Se species in the sediment and overlying water

In the absence of worms, the total Se levels in the Se(IV)-treated sediments in general exhibited a slow but gradual decrease over the 90 d of aging (Figs 1A-1C), more pronounced in the 100  $\mu\text{g/g}$  treatment, in which the levels of Se(IV) in were retained  $\sim$  75.7% after 60 d and  $\sim$  38.4% after 90 d (Fig. 1C). The levels

of Se(IV) in the Se(IV) treatments were sharply decreased (41.4% – 68.9%) in the 14 days with the detectable Se(IV) ranging from 0.03 to 1.32 µg/g (Fig.s 1A-1C). Organic Se species were generally not or barely detected (Fig. 1).

In the absence of worms, for the Se-Met treatments, the total Se levels were decreased rapidly within the first 3 days, approaching ~ 60.2%, 20.4%, and 31.3% of their nominal concentrations for the 3, 30, and 100 µg/g treatments, respectively (Fig.s 2A-2C), but maintained relatively stable for the remaining 5 d. However, the Se-Met levels in the sediments showed a more rapid decrease than Se(IV) levels, with an almost complete reduction of ~ 99.8%, 99.5%, and 99.5% for the 3, 30, and 100 µg/g treatments on day 7 (Fig. 2). The levels of Se(IV) were comparable to its background levels, while Se(VI) and Se-Cys were hardly detected in the sediments (Fig. 2).

In the sediments with the presence of worms, the total Se levels in the Se(IV)-treated sediments exhibited a slow decrease during the 7 d of exposure, with a reduction of  $25.0 \pm 1.9\%$  on day 7 (Fig.s 3A-3C). Meanwhile, the levels of Se-Met showed a marked increase on day 3, while Se(VI) and Se-Cys were at the background levels. On the other hand, the total Se levels in the Se-Met-treated sediments showed a similar decreasing pattern but seemingly more rapid than those in the Se(IV) treated sediments (Fig.s 3D-3F). Interestingly, the levels of Se-Met were decreased during the 7 d of exposure but maintained a minimum of 0.04 µg/g after 7 d of exposure, which was contrast to those levels in the sediments with the absence of worms. Se(IV), Se(VI), and Se-Cys sustained at their background levels (Fig.s 3D-3F).

The levels of Se in the overlying waters of the Se(IV) and Se-Met treatments with and without the presence of the oligochaetes were fairly low and negligible (Fig.s. S1-S3).

### **3.3 Acute toxicity and Se biodynamics in *L. variegatus***

The mortality of *L. variegatus* exposed to sediment borne Se(IV) and Se-Met was dose dependent. The LC<sub>50</sub>-96h values of Se(IV) and Se-Met were estimated to be 372.6 (334.5–410.7) µg/g and 9.4 (7.9–10.9) µg/g (Tab.s 1), respectively.

When *L. variegatus* were exposed to sediment borne Se(IV), the body burden of total Se and Se-Met showed a time-dependent increase during the 7 d of exposure for all three concentrations (Fig.s 4A-4C), while the differences in the body burden of total Se (4.15–4.61 µg/g) and Se-Met (5.3–5.8 µg/g) were not significantly different among the three concentrations. The body burden of Se-Cys was similar in the oligochaetes among all treatments (1.1–1.2 µg/g) (Fig.s 4A-4C). Se(IV) and Se(VI) were hardly detected in the oligochaetes. During the 8-d depuration period, the body burden of all Se species remained relatively unchanged.

When *L. variegatus* were exposed to sediment borne Se-Met, the body burden of total Se and Se-Met was increased with exposure duration during the 7 d of exposure for all three concentrations (Fig.s 4D-4F). The body burden of Se-Cys was similar among all 6 treatments, and Se(IV) and Se(VI) were hardly detected in the oligochaetes (Fig. 4). However, total Se and Se-Met levels were increased more rapidly during the sediment borne Se-Met experiment than the sediment borne Se(IV), averaging  $10.9 \pm 0.8$  µg/g

and  $8.5 \pm 0.3 \mu\text{g/g}$  for the  $1 \mu\text{g/g}$  Se-Met (Fig. 4F) compared to an average of  $4.62 \pm 0.05$  and  $5.75 \pm 0.22 \mu\text{g/g}$  for  $1 \mu\text{g/g}$  Se(IV) (Fig. 4C). During the 8-d depuration period, the body burden of all Se species showed no obvious changes (Fig.s 4D-4F).

The BAFs of the total Se in the worms exposed to either Se(IV) or Se-Met were increased with exposure duration (Fig. 5), with the largest BAF being  $12.7 \pm 1.5$  at Se-Met-H treatment. Interestingly, it seemed that the BAFs of total Se in the  $1 \mu\text{g/g}$  Se(IV) treatments were lower than those of the other two Se(IV) treatments (Fig. 5A).

## 4. Discussion

### 4.1 The background levels of Se in the environmental matrices.

The sediments for this study were collected from the Songhua River (Heilongjiang Province, China), where low levels of Se are expected since this region is Se deficient (Dinh et al., 2018). The measured levels of total Se was  $0.2 \mu\text{g/g}$ , which is close to  $0.3 \mu\text{g/g}$  ( $0.1\text{--}0.5 \mu\text{g/g}$ ) in the sediments of the Daihai lake in Inner Mongolia, a Se-deficient area in northern China (Sun et al., 2021), and to  $0.3 \mu\text{g/g}$  in the sediments of a stream in Finland (Wang et al., 1994). On the contrast, up to  $23.5 \mu\text{g/g}$  of Se has been reported in the sediments of the Xiangjiang River (Hunan Province, China), a relatively Se-sufficient area in China (Wang et al., 1994). Meanwhile, the level of total Se in the fish flakes used in this study was  $3.4 \mu\text{g/g}$ . This value is close to the recommended value of Se in aquaculture (BCMOE, 2018) where Se is often added as a mineral nutrient supplement to enhance fish growth and immunity. Similarly,  $2.6 \mu\text{g/g}$  (Li et al., 2021) and  $1.1 \mu\text{g/g}$  (Chen et al., 2020) of total Se have been documented in different brand of fish flakes. These results suggest that Se is often added in the fish flakes or the raw materials (i.e., fish meal) to prepare fish flakes contain Se. Meanwhile, in this study, the levels of total Se in the oligochaetes *L. variegatus* was  $\sim 4.4 \mu\text{g/g}$ . The levels of total Se are  $\sim 0.2 \mu\text{g/g}$  in *L. variegatus* fed the Se-free diet (Xie et al., 2016) and  $2.6 \mu\text{g/g}$  in *L. variegatus* exposed to  $1.4 \mu\text{g/g}$  sedimentborne Se for 28 days (Winger et al., 1999). Part of the reason for the relatively high level of total Se is probably due to the elevated level of Se in the flakes used in this study. The above results on the Se content in the abiotic (sediments and fish flakes) and biotic matrices suggest its ubiquitousness of Se in environmental matrices.

### 4.2 Se speciation in the sediments with or without worms

In this study, Se in the sediments was  $\sim 98\%$  in the sediment/overlying system, which is close to  $90\%$  of Se in the sediments settled from the water column in a clean river (Canton and Van Derveer, 1997). It is also documented that the ratio of Se in the aquatic environments is  $\sim 97$  (sediment) :  $3$  (water) :  $0.1$  (biota) (Bowie et al., 1996). These data have consolidated that the sediment is the major sink for Se (Canton and Van Derveer, 1997). Meanwhile, in this study, the total Se levels in the Se(IV)-treated sediments remained relatively constant for the 60 d during the 90 d of aging. Generally, Se(IV) has a high adsorption affinity to organic matter, clay minerals and iron oxides in the sediments (Schroeder et al.,

2002), and is a major inorganic Se species in the sediments with a moderate redox potential (Séby et al., 2001). However, total Se levels in the Se(IV)-treated sediments showed a decrease trend after 60 d of aging, probably due to that Se(IV) is reduced into particulate element Se or gaseous Se (though they were not measured in this study) (Bowie et al., 1996) after elongated exposure duration. These results imply that measuring Se(IV) is not a necessity when conducting a sediment-borne Se(IV) experiment with relatively short exposure duration (i.e., shorter than 2 months). In contrast, in this study, the levels of Se-Met in the Se-Met treatments showed a sharp decrease after 3 d and almost completely vanished after 7 d of exposure. It is known that Se-Met is readily to be transformed into volatile Se (dimethyl selenide) by microorganisms (Fujita et al., 2005; Maier et al., 1988). Therefore, it is likely that the sharp decrease and complete vanishment of Se-Met could be due to the rapid biotransformation. However, biotransformation of Se in the sediments was not evident in this study since organic Se was barely detected during the 90 d of aging. Apparently, more research is warranted to better understand the speciation processes of Se in the sediments.

With the presence of worms in the sediments, the levels of Se-Met decreased much slower than those in the sediments without worms. It is worth noting that the exposure concentrations of Se-Met (0.1–1 µg/g) in this experiment were much smaller than those (30–100 µg/g) in the aging experiment, which is closed to those in a previous study (Zhang and Moore, 1997). It is likely that the bioturbation from *L. variegatus* may increase the oxygen levels in the sediments, therefore slowing down the reduction of Se-Met absorbed on the sediments (Fujita et al., 2005). It is well known that Se-Met is the predominant organic Se species in the Se-contaminated ecosystems (Hamilton, 2004). Our results imply Se-Met supplied in the artificial or live diet might be more appropriate than in sediments in term of maintaining a relatively constant exposure level, while Se-Met should be measured frequently during dissolved and dietary Se-Met exposure experiments to ensure the exposure concentrations of Se-Met.

### 4.3 Se-accumulation and speciation in *L. variegatus*

In this study, the values of LC<sub>50</sub>-96h of Se(IV) and Se-Met in the oligochaete *L. variegatus* were 372.6 and 9.4 µg/g, respectively. As discussed early, the LC<sub>50</sub>-96h could be lower than 9.4 µg/g due to the loss of Se-Met during the 96 h exposure time. Data on the lethality of Se on benthic organisms from the dietary exposure route are very scarce. However, the acute toxicity data reveal that Se-Met is generally much more toxic than Se(IV), as also shown in the water flea *Daphnia magna* (Davis, 1988), in *L. variegatus* (Xie et al., 2016), which is accounted for by the much faster accumulation of Se-Met than Se(IV).

Indeed, in this study, the accumulation of Se-Met was more rapid than Se(IV) from the sediment exposure. Since benthic organisms rely on sediment processing for their nutrition (Orr et al., 2006), sediment processing (i.e., dietary exposure) might be the predominant exposure route for pollutants including Se and other particle bound pollutants in the sediments for benthos. Previous studies have shown that the Se body burden in the oligochaetes *Tubifex tubifex*, *L. variegatus* and *Limnodrilus hoffmeisteri* is 7.1, 11.0 and 27.8 µg/g after the exposure to 1.2 (Duboi and Landis, 2008), 20 (Xie et al., 2016) and 40 µg/g (Chen et al., 2019) of Se(IV) in the sediments for 14 days, respectively. These results demonstrate that the

oligochaetes might be capable of accumulating Se and coping with the stress resulted from overload of Se. In the meantime, in this study, the body burden of Se in the oligochaete *L. variegatus* exposed to Se(IV) and Se-Met were increased with time, and the accumulation of Se in the worms was faster for Se-Met than Se(IV), which is consistent with the results from previous studies using freshwater oligochaetes (Chen et al., 2019; Xie et al., 2016). In addition, the depuration of Se was barely noticeable in *L. variegatus* exposed to either Se(IV) or Se-Met. This strongly suggests that upon entry into the cells, the accumulated Se is rapidly utilized by the organisms via biotransformation into different intermediate metabolites (Suzuki, 2005), thereby manifesting the essentiality of Se in animals (Burk and Hill, 2015). Nonetheless, depuration of Se in aquatic organisms has seldom been reported. Finally, the BAFs of both Se(IV) and Se-Met were greater than 1 and increased with exposure duration. This implies that at one hand, *L. variegatus* might be able to handle the stress from overload of Se; on the other hand, this species is a good vector of Se and other pollutants for the trophic transfer research, as demonstrated by early studies (Xie et al., 2016).

Se speciation after entry into the cells could provide valuable information on the biochemical functions of Se in animals (Burk and Hill, 2015). In this study, a slow but steady increase in the levels of Se-Met was found in *L. variegatus* exposed to sediment borne Se(IV). Again, this implies that Se(IV) could be rapidly biotransformed into other Se species inside the cells. However, the exact mechanisms for the increased levels of Se-Met in Se(IV) treated *L. variegatus* remain unexplored and need further investigation.

## 5. Conclusion

Taken together, this study has provided important data on the levels of total Se and Se species in different environmental matrices. The levels of Se(IV) are relatively stable during aging, while those of Se-Met are decreased sharply within a few days. The presence of the oligochaetes could help slow down the reduction of Se-Met in the sediments. Se-Met is the predominant Se species in *L. variegatus* and Se is barely depurated once taken up by the oligochaetes. Our results could help better evaluate the risk of Se in the benthic communities.

## Declarations

### Author contribution

**Jichen Yang:** Methodology, Formal Analysis, Investigation, Writing - Original draft; **Xin Yan:** Methodology, Data Curation, Conceptualization; **Hongsong Liu:** Methodology, Data Curation; **Hongxing Chen:** Project Administration, Conceptualization, Funding Acquisition; **Yongzhuang Wang:** Methodology, Visualization; **Wei Zhang:** Conceptualization, Supervision; **Wu Dong:** Conceptualization, Supervision; **Dan Li:** Conceptualization, Methodology, Writing Review & Editing, Supervision, Funding Acquisition; **Lingtian Xie:** Writing Review & Editing, Supervision, Funding Acquisition.

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### **Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the first author on reasonable request.

### **Compliance with ethical standards**

#### **Ethics approval**

Not applicable.

#### **Consent to participate**

Not applicable.

#### **Consent for publication**

All of the authors have approved the final version to publish this paper.

#### **Conflict of interest**

The authors declare no competing interests.

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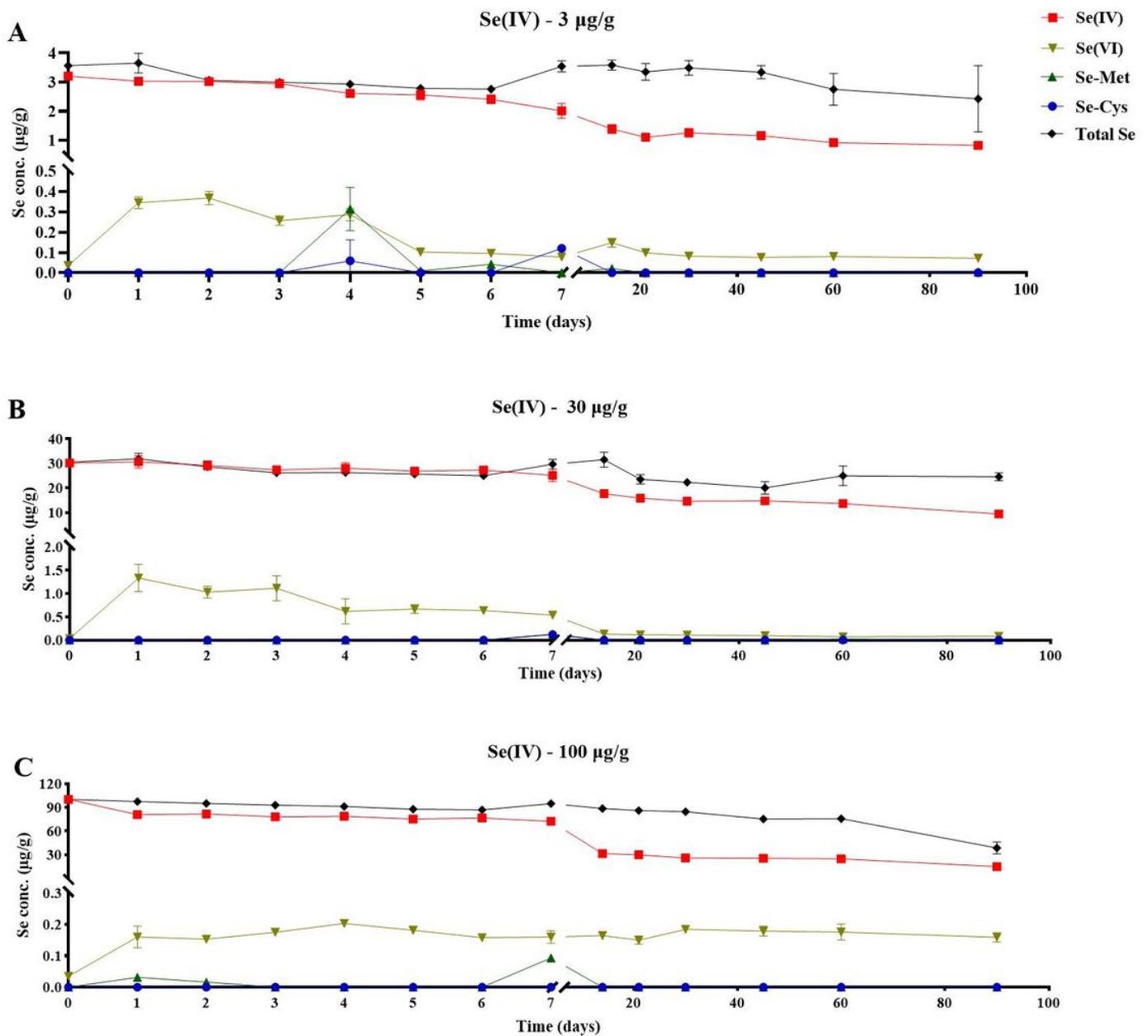
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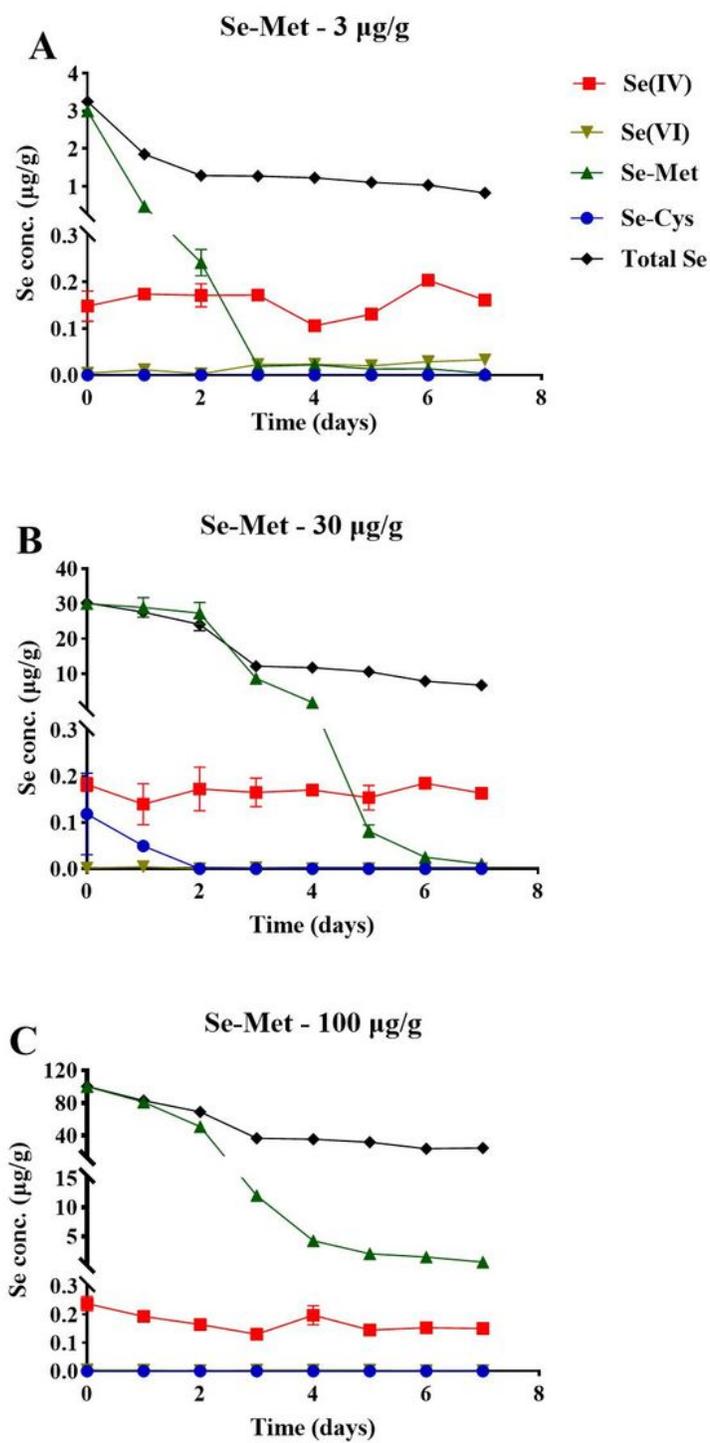
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## Figures



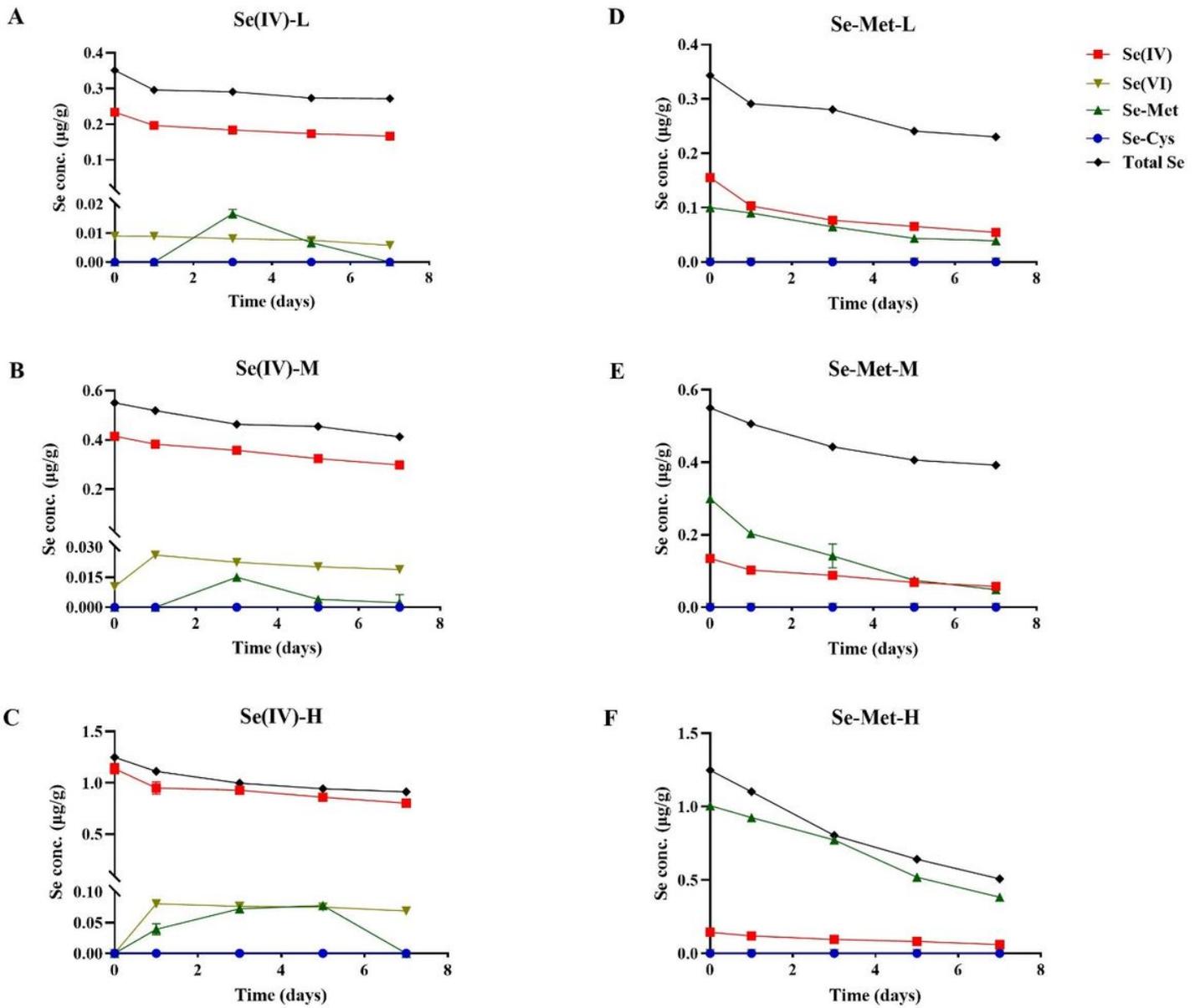
**Figure 1**

The levels of total Se and Se species in the sediments (panels A - C) in the beakers (n = 3) with different levels of Se(IV) at 3.0, 30.0, and 100.0 µg/g for 90 days of aging without the presence of *Lumbriculus variegatus* (mean ± SE, n = 3). Samples were taken for 7 consecutive days and at the day 14, 21, 30, 45, 60, and 90.



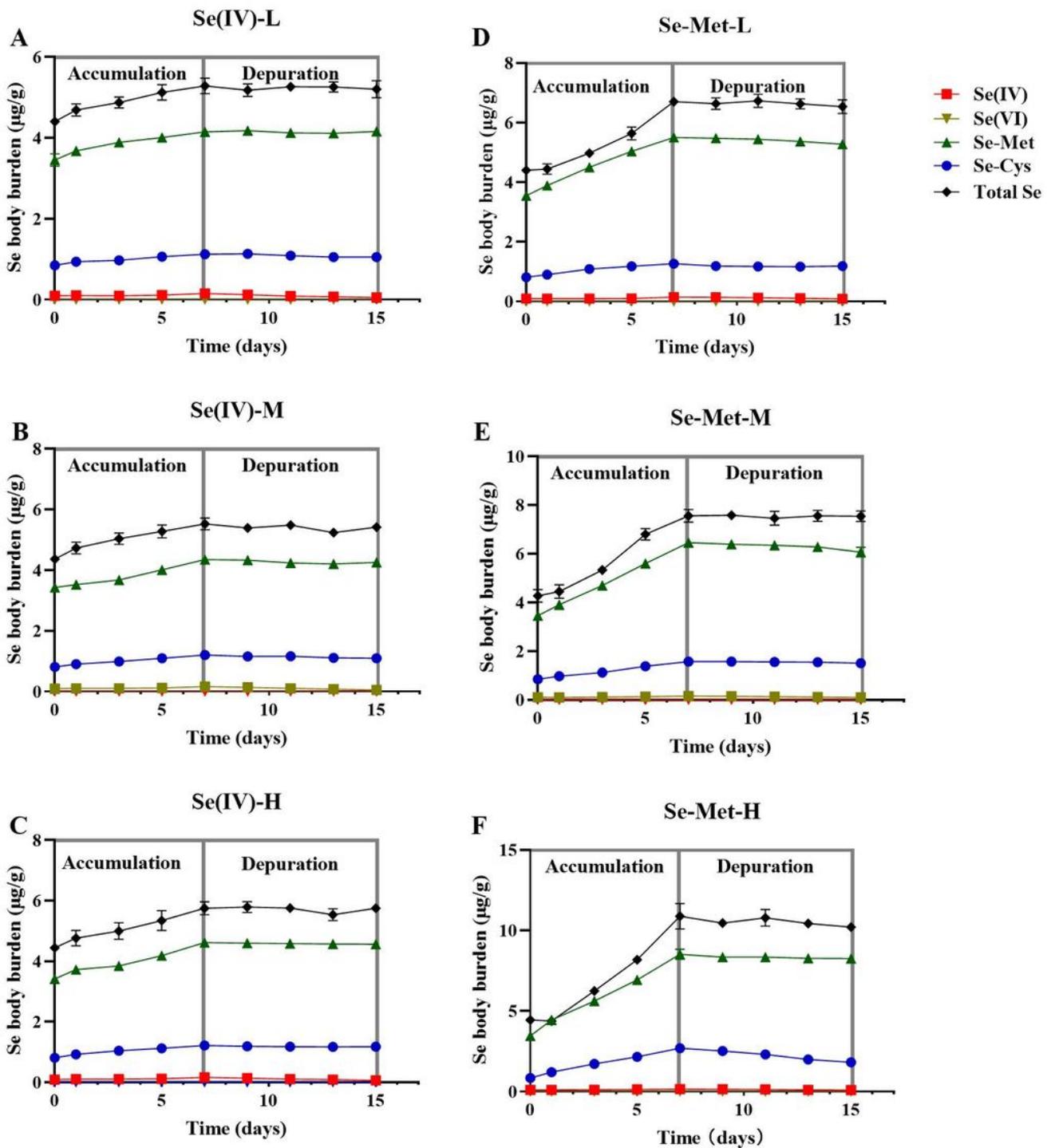
**Figure 2**

The levels of total Se and Se species in the sediments in the beakers (n = 3) with different levels of Se-Met at 3.0, 30.0, and 100.0 µg/g for 7 days without the presence of *L. variegatus* (mean ± SE, n = 3).



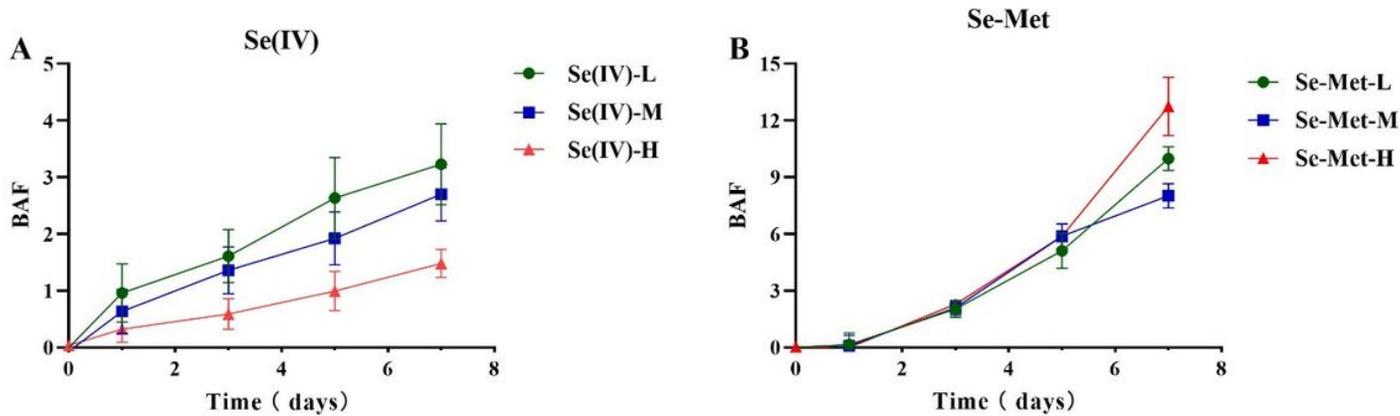
**Figure 3**

The levels of total Se and Se species in the sediments in the beakers ( $n = 3$ ) with different levels of Se(IV) or Se-Met at 0.1 (L, panel A), 0.3 (M, panel B), and 1.0 (H, panel C)  $\mu\text{g/g}$  for 7 days with the presence of *L. variegatus* (mean  $\pm$  SE,  $n = 3$ ).



**Figure 4**

The accumulation (for 7 days) and depuration (for 8 days) of total Se and different Se species in the oligochaete *L. variegatus* in the exposure beakers (n = 3) with either Se(IV) (panel A) or Se-Met (panel B) at 0.1 (L), 0.3 (M), and 1.0 (H) µg/g (mean ± SE, n = 3).



**Figure 5**

The bioaccumulation factors (BAFs) of the total Se in the oligochaete *L. variegatus* exposed to Se(IV) (panel A) and Se-Met (panel B) for 7 days (mean  $\pm$  SE, n = 3).

## Supplementary Files

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