Ecotoxicity stress and bioaccumulation in *Eisenia fetida* earthworms exposed to vanadium pentoxide in soil

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Abstract

As an important commercial form of vanadium, vanadium pentoxide (V_2O_5) is widely used in various modern industries, and its environmental impacts and ecotoxicity have been extensively studied. In this research, the ecotoxicity of V_2O_5 to earthworms (*Eisenia fetida*) in soil was tested by exposure to V_2O_5 at a series of doses, and biochemical response indices, such as the superoxide dismutase (SOD) and catalase (CAT) enzyme activity and malondialdehyde (MDA) content, were analysed to determine the mechanism by which antioxidant enzymes respond to V_2O_5 exposure. The bioaccumulation factor (BAF) of vanadium pentoxide in the earthworms and soil was also measured to explore the bioaccumulation process of V_2O_5 in the test period. The results showed that the acute and chronic lethal toxicity values of V_2O_5 towards *E. fetida* were 21.96 mg/kg (LC_{50} , 14 days) and 6.28 mg/kg (LC_{10} , 28 days), respectively. For the antioxidant enzymes, SOD and CAT were synchronously induced or inhibited within the time period, and the enzyme activity had a dose-effect relationship with the V_2O_5 concentration. MDA analysis indicated that lipid peroxidation in earthworms mainly occurred at the early stage and was eliminated slowly in the later stage during the test time. In addition, the BAFs were much less than 1, which indicated that V_2O_5 did not easily accumulate in earthworms, and the BAF was positively correlated with the exposure time and negatively linearly correlated with the V_2O_5 concentration in the soil. These results indicated that the bioconcentration and metabolic mechanism of V_2O_5 in earthworms differed with the different exposure concentrations, and bioaccumulation became balanced after 14-28 days in earthworms exposed to a relatively lower dose of V_2O_5 . The analysis of the integrated biomarker response (IBR) index indicated that the trends of IBR values were positively related to the changing V_2O_5 concentration, and the IBR index could reflect the organism's sensitivity to the external stimulus of V_2O_5 . The toxicity of V_2O_5 is mainly caused by V^{5+} , which is also an important factor in formulating guidelines regarding vanadium levels in soil, and the test earthworm species (*Eisenia fetida*) is a sensitive biological indicator for risk assessments of vanadium oxidation in the soil.

Highlights

- The ecotoxicity of V_2O_5 to earthworms (*Eisenia fetida*) in soil was tested by exposure to V_2O_5 at a series of doses, and biochemical response indices such as the superoxide dismutase (SOD) and catalase (CAT) enzyme activity and malondialdehyde (MDA) content were analysed to discuss the toxicity stress response mechanism of antioxidant enzymes under V_2O_5 exposure.
- The BAFs of vanadium pentoxide in earthworms and soil were also measured to probe the bioaccumulation process of V_2O_5 in soil organisms.
- SOD and CAT were synchronously induced or inhibited within the test period, and enzyme activity had a dose-effect relationship with V_2O_5 . MDA analysis indicated that lipid peroxidation of earthworms mainly occurred in the early stage and was reduced in the later stage.

- The BAF values were far less than 1, which indicated that V_2O_5 did not easily accumulate in the organism and that the BAF in earthworms may be a sensitive predictor for describing the pollution risk posed by V_2O_5 in the soil.
- The IBR values were positively related to the V_2O_5 concentrations and reflected the organism's sensitivity to external stimuli from V_2O_5 in the soil.

1. Introduction

With the development of modern industry, vanadium and vanadium pentoxide (V_2O_5) have been used in various products. Vanadium is primarily employed in steel to improve its strength and corrosion resistance; it is also used in many other products, such as pigments, medicines, and catalysts (Huang et al., 2015; IARC, 2006; Schlesinger et al., 2017), and vanadium-based selective catalyst reduction (V-SCR) has been widely used in recent years with tougher requirements for oxynitride control for industries and vehicles (Hoej et al., 2014). Therefore, a considerable amount of vanadium compounds could enter the soil, water or air and may cause environmental pollution with the extensive use of vanadium products (Hindy et al., 1990; Imtiaz et al., 2015; Xiao et al., 2017). V_2O_5 , as the main form of high valance vanadium oxide, is suspected to be carcinogenic to humans according to the China National Occupational Health Standard GBZ 2.1-2019 (NHC, 2019), and the International Association for Research on Cancer (IARC) classifies the compound into Group B2, members of which may cause cancer to humans (Assem and Levy, 2009; IARC, 2006). The American Conference of Governmental Industrial Hygienists (ACGIH) classified V_2O_5 as an A3 compound, members of which are confirmed animal carcinogens with unknown relevance to humans (ACGIH, 2009). Acute exposure of humans, primates and rodents to V_2O_5 has been confirmed to have adverse respiratory effects (USEPA, 2011), human exposure to dust containing high levels of V_2O_5 could induce wheezing, chest pain, bronchitis, and impaired lung function symptoms (Hauser et al., 2001), and the toxicity of V_2O_5 to plants has also been studied (Fortoul et al., 2014; Smith et al., 2013). However, studies on the toxicity and risk to terrestrial soil animals have rarely been reported.

Soil organisms are generally exposed to various environmental pollutants, and earthworms are important soil mollusc species that play key roles in improving soil fertility (Lee et al., 1985; Kula, 1997). Earthworms have been exposed to chemicals for soil risk assessment due to their natural role in recycling nutrients for the food chain (Van Gestel et al., 1988), and earthworms are recognized as test organisms for soil risk evaluation under the guidelines from the Organization for Economic Co-operation and Development (OECD, 2004). As a group of biomarkers of external chemical toxicity, the antioxidant defence system, which is made up of antioxidant enzymes and nonenzymatic substances, is often used in early warning and ecotoxicity stress evaluations of low-concentration pollutants (Van der Oost, 2003; Li et al., 2019). Antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) play a main role in eliminating or detoxifying free radicals and reactive oxygen species (ROS), which result in environmental contamination (Tripathi et al., 2006; Asagba et al., 2008). Moreover, as an important index of lipid peroxidation, the level of malondialdehyde (MDA) is also a sensitive indicator of cellular oxidative

damage (Avci et al., 2005; Chen et al., 2012; Aihemaiti et al., 2019). Some evidence has shown that antioxidant enzymes are effective biomarkers of toxicological stress responses (Song et al., 2009; Xue et al., 2009; Zhang et al., 2009). Considering the potential ecotoxicity of vanadium in soil, the toxic stress effects and bioaccumulation of vanadium in earthworms should be tested to predict the possible effects of soil contamination on the food chain. In this study, the mechanism of ecotoxicity stress caused by V_2O_5 in the earthworm, including the analysis of the integrated biomarker response (IBR) index, the activity of catalase (CAT) and superoxide dismutase (SOD), the content of malondialdehyde (MDA), and the bioaccumulation factor (BAF), was investigated. The toxic effects of V_2O_5 on the terrestrial earthworm (*Eisenia fetida*) were measured to reveal the acute and chronic toxicity, including oxidative stress, of V_2O_5 to the species and to understand the bioaccumulation process of V_2O_5 in soil organisms.

2. Materials And Methods

2.1. Chemicals and materials

Vanadium pentoxide (V_2O_5), carbon ($CaCO_3$, analytical reagent), quartz sand (SiO_2 , analytical reagent), kaolin ($Al_2O_3 \cdot 2SiO_2 \cdot 2H_2O$, chemically pure reagent), Turfy soil, a superoxide dismutase (SOD) test kit, catalase (H_2O_2) test kit, malondialdehyde (MDA) test kit, and all other enzyme activity test kits were purchased from Beijing Solarbio Science and Technology Co., Ltd. (Beijing, China). All reagents in the tests were of analytical grade.

2.2. Test organism

Earthworms (*Eisenia fetida*) were purchased from a commercial breeder in Beijing, China. Adult worms with well-developed clitella, above 2 months of age, and body weight of 300–500 mg were used for toxicity tests. Before the experiment, all test earthworms were bred on dry cow dung for 7 days in test artificial soil. The characteristics of the artificial soil were as follows: temperature of $20 \pm 1^\circ C$ and moisture content of $35 \pm 2\%$.

2.3. Test soil

The preparation of an artificial soil was conducted according to the OECD guidelines for testing chemicals No. 207 and No. 222 (OECD, 2004). The artificial soil consisted of a mixture of 70% quartz sand, 20% kaolin and 10% Turfy soil (measured by dry mass), adjusted with carbonate to a pH of 6.0~6.5 and adjusted with ultrapure water to a moisture content of approximately 35% of the maximum water holding capacity.

2.4. Eco-toxicity test

According to the guidelines for earthworm acute toxicity tests (OECD, 2004), V_2O_5 exposure was performed in artificial soil based on pre-experiment results, and certain amounts of V_2O_5 were added to 500 g (dry weight) artificial soil to obtain the required V_2O_5 concentrations in a series as follows: 0, 2.94, 5, 8, 14.45, 24.57, 41.79, 71.05 mg/kg soil. The soil was mixed for at least 30 min to obtain a

homogeneous test soil, and the moisture content was adjusted to 35% before the soil was put into the test boxes. Each treatment group included 3-4 repeats. Then, 10 earthworms were added to 500 g of soil and cultivated in an artificial climate incubator under controlled temperature ($20 \pm 1^\circ\text{C}$), humidity (80%), light intensity (500-600 lux), etc. with deionized water blank groups. The toxicity test period was 28 d, and the soil moisture content was kept at the initial level by spraying ultrapure water regularly. During the exposure period from 14 to 28 days, the test earthworms were counted to derive the 50% and 10% lethal concentration ($\text{LC}_{50}/\text{LC}_{10}$) values for exposure to V_2O_5 .

2.5. Biochemical enzyme assays

The enzyme activity measurements were taken at the same time as in the above earthworm toxicity test. During the exposure periods of 3, 7, 14, and 28 d, the test earthworms were collected from each treatment group for biochemical and metal determination. The gut contents of selected earthworms were emptied by placing the animals in separate beakers with filter paper for 24 h. Earthworm tissues were extracted and homogenized, and the SOD and CAT activities and MDA content were measured. The extracts were obtained from the corresponding enzyme activity test kits with extraction solution, and the test earthworms frozen in liquid nitrogen were removed and homogenized in an ice-cold extraction solution at a ratio of earthworm weight to extraction solution of 1:10 (w/v). The homogenates were centrifuged at 8000 g for 10 min at 4°C . After centrifugation, the supernatant was removed for determination of the SOD and CAT activity and MDA content. CAT and SOD activities were measured as described previously (Lyttle and DeSombre 1977; McCord and Fridovich 1969), and lipid peroxidation was determined by measuring the MDA content with the thiobarbituric acid test (Ohkawa et al., 1979).

2.6. Analysis of V_2O_5 in soil and earthworms

The collected earthworms were cleaned and put on damp filter paper for 24-48 h to empty their intestines (Cao et al., 2017). The worms and soils were freeze-dried and stored at -80°C . The concentration of vanadium in the soil and worms was determined according to the following steps: the soils were soaked in 5 ml HNO_3 and 1 ml HCl overnight and digested at 180°C for 8 hours. During this period, the H_2O_2 was decreased, the samples were cooled, and the digested solution was stabilized to a volume of 50 ml with HNO_3 . The earthworms were soaked in 5 ml HNO_3 and 2 ml HClO_4 overnight, digested at 180°C for 8 hours, cooled, and the digested solution was stabilized to a volume of 50 ml with HNO_3 (Ji et al., 2011). Finally, both digested solutions were analysed by inductively coupled plasma–mass spectrometry (ICP–MS 7900, Agilent). In addition, the amount of vanadium measured was converted to the concentration of vanadium pentoxide according to the test substance.

2.7. Statistical analysis

Data were first tested for homogeneity of variances and expressed in terms of the mean $\pm$ standard deviation (SD). One-way analysis of variance (ANOVA) was used for statistical analysis. The least significant differences (LSD) test was used to determine significant differences between different treatments, and $P < 0.05$ was considered statistically significant using SPSS 20.0 for data analysis. The

calculation of the integrated biomarker response (IBR) index was based on a previously published method (Pytharopoulou et al., 2008; Oliveira et al., 2010). Test data were standardized, and star charts were used to visualize the integrated results of the biomarkers, and the IBR values were calculated and analysed; the larger was the IBR value was, the more the biological influence. The IBR index was calculated with the following steps: (1) calculation of the total mean value (m), the standard deviation (s) of the test time points, and the mean value (X) of the enzyme biomarker at each time point; (2) the mean value X of the time point was normalized by the formula $Y = (X-m)/s$; (3) if the test biomarker was activated, then $Z = Y$, and if it was not activated, then $Z = -Y$, and $|X_{min}|$ represented the absolute minimum value of the biomarker uniformity data for the time point. The biomarker score S_i for the time point was calculated as $S_i = Z + |X_{min}|$. The S_i value of the biomarker for a specific period is represented by the length of the radiation in the star chart (Li et al., 2020). The IBR value for a specific period was obtained by calculating the area of the star chart, which shows the total area A_i of the triangle enclosed by the radiation of the adjacent biomarker, and analytical indices were described as follows:

$$\beta = \arctan [s_i \sin \alpha / (S_i - S_{i+1} \cos \alpha)] \quad (1)$$

$$A_i = S_i / [2 \sin \beta (S_i \cos \beta + S_{i+1} \sin \beta)] \quad (2)$$

$$IBR = \sum_{i=1}^n A_i \quad (3)$$

where n is the quantity of selected biomarkers, α is the angle between two nearby radiation lines, and $\alpha = 2\pi/n$, $S_{i+1} = S_i$.

3. Results And Discussion

3.1 Acute and chronic toxicity of V_2O_5 to earthworms

It is important to perform toxicity tests in the laboratory to correctly assess the chemical risks associated with contaminated sites. In this work, all test earthworms had a mortality of less than 5%, which occurred in the blank group after 14 days of exposure, and no new deaths occurred after 14-28 days. The earthworms exposed to V_2O_5 showed a correlation between concentration and the lethal effects, as shown in Fig. 1. The acute 50% lethal concentration (LC_{50}) of the V_2O_5 to test earthworms was 21.96 mg/kg in 14 days, and the earthworm mortality increased from 56.67–100% with V_2O_5 concentrations from 24.57 mg/kg to 41.76 mg/kg. Additionally, the chronic LC_{50} was 18.71 mg/kg in 28 days. The acute and chronic toxicity values of earthworms were calculated with lethal effects as an end point (Fig. 1). The acute toxicity value of LC_{10} at 14 days was 7.32 mg/kg, and the chronic toxicity value of LC_{10} at 28 days was 6.28 mg/kg. However, the soil quality guideline value for vanadium released by the Canadian Council of Ministers of the Environment for environmental health (CCME, 2007) was 130 mg/kg, which is less than the interim soil quality criterion. Therefore, this test result showed that the toxicity stress from vanadium oxide V_2O_5 was greater than that of vanadium to the soil organism. It is known that vanadium

(V) exists in two oxidation states in soil: V^{4+} and V^{5+} (Khan et al., 2011). The results also showed that the toxicity stress of vanadium oxide was mainly caused by V^{5+} from V_2O_5 . Furthermore, the vanadium oxide state of V^{5+} is the main toxic form, and this is an important factor when formulating the guideline values for vanadium in soil. Moreover, the test earthworm species (*Eisenia fetida*) may be a sensitive biological indicator for toxicity assessments of vanadium oxide forms in the soil.

3.2 Accumulation of V_2O_5 in earthworms and soil

Table 1 shows that the measured concentrations differed from the spiked concentrations by less than 20%, and the V_2O_5 concentration ratio from earthworms to soil was used to calculate the bioaccumulation factor (BAF). The V_2O_5 concentrations in earthworms ranged from 0.115 to 0.732 mg/kg, becoming higher with increasing exposure time and spiked concentration after 3, 7, 14, and 28 d of exposure. However, the concentration in earthworms decreased first and then increased with longer exposure times in some lower concentration groups, such as 2.94, 5.00, and 8.50 mg/kg. Additionally, the bioaccumulation increased with longer times in the higher concentration spiked groups, such as 14.45 and 24.57 mg/kg. In addition, the content of V_2O_5 in earthworms was positively correlated with the concentration in soil, and the BAF values decreased with increasing concentrations in the spiked groups. This result was similar to that of a study (Cao et al., 2017) on copper, zinc, cadmium and lead, and the BAF values of metals were greater with lower metal contents in the test organism and soil. The BAF of vanadium pentoxide was positively correlated with exposure time and negatively linearly correlated with the total V_2O_5 concentration in soil. In addition, the BAFs of V_2O_5 were far less than 1 (from 0.026 to 0.090), as shown in Table 1, which indicated that V_2O_5 could not easily accumulate in earthworms in the test. It is important to measure BAFs to describe the environmental behaviour and toxic effects of V_2O_5 , and research has shown that the BAFs of vanadium in aquatic organisms range from 61 to 242 (Jardine et al., 2019). Because the oxidation state and toxic activity of vanadium in the soil environment could be affected by some main factors, such as the organic matter content, cation exchange capacity, organism characteristics and bioavailability for biochemical reaction processes, the BAF values decreased with increasing exposure concentrations of V_2O_5 in the soil (Table 1). At relatively lower-dose exposure levels, such as 2.94-8.50 mg/kg, the V_2O_5 concentration in earthworms and BAF values first increased for 3 days, then changed from decreasing to increasing and tended to balance at approximately 14-28 d. For the relatively higher-dose exposure, such as 14.45-24.57 mg/kg, the V_2O_5 concentration in earthworms and BAF values increased, becoming balanced from 14-28 days. These results indicated that the bioconcentrations and metabolic mechanisms of V_2O_5 in earthworms differed with the different exposure concentrations in soil. Relatively lower-dose V_2O_5 exposure can more easily allow for balanced bioaccumulation in 14-28 days in earthworms, which plays a role in the test soil system. In addition, it was shown that the BAF values of V_2O_5 showed a positive relationship with the exposure time from 7-14 days and a negative correlation with the relative lower-dose level of V_2O_5 in soil. It has also been indicated that the bioaccumulation characteristics in earthworms may be a sensitive predictor for describing the pollution risk of V_2O_5 in soil.

Table 1
Bioaccumulation factors (BAFs) and concentrations of V₂O₅ in earthworms and soil.

soil concentration		earthworm concentration				BAF			
spikes measurement (mg/kg)		(mg/kg), days				(V ₂ O ₅ to earthworms in soil), days			
		3	7	14	28	3	7	14	28
control	nd	–	–	–	–	–	–	–	–
2.940	2.924	0.146	0.139	0.151	0.147	0.089	0.085	0.092	0.090
5.000	4.941	0.148	0.115	0.164	0.157	0.054	0.042	0.059	0.057
8.500	8.338	0.195	0.147	0.230	0.213	0.042	0.031	0.049	0.046
14.450	14.117	0.231	0.296	0.331	0.312	0.029	0.037	0.042	0.039
24.570	23.637	0.339	0.398	0.689	0.732	0.026	0.030	0.052	0.055
The test data were significantly different compared with the control p < 0.05.									

3.3 Biochemical enzyme effects

Early biochemical reactions are important information for evaluating the potential adverse impact of chemicals on the environment (Gao et al., 2007). The biochemical effects of V₂O₅ exposure on the activities of two antioxidant enzymes (SOD and CAT) and the MDA content in earthworms are shown in Fig. 2. Exogenous pollutants can induce the production of oxygen free radicals (OFRs) in organisms. ORFs can cause oxidative stress, which leads to protein denaturation, enzyme inactivation, biofilm damage, DNA replication errors and even cell death (Silva et al., 1999; Halliwell et al., 2004; Asagba et al., 2008), as the OFR level exceeds the ability of the antioxidant enzyme defence system to resist external pressure. Fig. 2 (1) shows that the SOD activity of earthworms exposed to V₂O₅ decreased on the 3rd day. Then, the activity increased in the test groups on the 7th and 14th days, except that the higher concentration group of 24.57 mg/kg had a slight decrease on the 14th day, and all concentration groups showed inhibition of SOD activity on the 28th day. Superoxide anion free radicals are catalysed by SOD enzymes to convert hydrogen peroxide (H₂O₂) to oxygen in cells for removal, so this enzyme plays an important role in protecting the dynamic balance of oxygen free radical generation in organisms (Shao et al., 2018). In this study, SOD activity was inhibited by V₂O₅ exposure for the first 3 days. Then, SOD activity was increased at almost all doses at 7 to 14 days and inhibited after 14-28 days of exposure. It can be speculated that this reactive mechanism may be related to the effect of vanadium on the metal prosthetic group of SOD, which could affect SOD activity by changing the molecular structure of the enzyme protein. As the test time increased, the oxidative stress of vanadium increased on the 7th and 14th days, which might have caused increases in SOD products. However, the regulation of enzyme activity in the test earthworm was limited during V₂O₅ exposure for 14-28 days, it did not increase indefinitely, and finally, the enzyme activity was inhibited. The enzyme activity was generally attributed to

the special physiological effect of vanadium on the test organisms, and the enzyme activity tendency indicated that SOD activity had a certain relationship with the dose at the test time.

As shown in Fig. 2 (2), CAT activity was increased by V_2O_5 at relatively lower doses of V_2O_5 , such as 5.0-14.45 mg/kg, and inhibited at a higher dose of 24.57 mg/kg at the test time of 28 days. The response trends of the CAT activity in different test groups were similar at 5.0-14.45 mg/kg from 3-28 days. CAT was inhibited in the first 3 days, induced from 7 to 14 days with a peak concentration on the 14th day, and then inhibited on the 28th day. The main product in the catalysis process of SOD is hydrogen peroxide, which has an adverse effect on cells (Saint-Denis et al., 2001). H_2O_2 is eliminated or converted into H_2O and O_2 by the antioxidant defence effects of CAT. The CAT activity of the test earthworm had a response mechanism similar to that of SOD under V_2O_5 exposure in the soil. Moreover, the CAT enzyme activity was promoted at relative lower-dose exposure over the 28 days process compared with the inhibitory effect in the higher-dose V_2O_5 (24.57 mg/kg) group from 7-28 days. In addition, the CAT activity showed a decreasing trend within the same exposure time. The lower-dose (2.94 mg/kg) group had a larger peak than the higher-dose (24.57 mg/kg) group with lower CAT activity. This result indicated that the V_2O_5 dose had a significant influence on the activity of CAT, and CAT activity was inhibited in a relatively short time at a higher dose.

The level of malondialdehyde (MDA) is a main indicator of lipid peroxidation, and the oxidative stress induced by V_2O_5 is presented in Fig. 2 (3). The MDA content from the higher-dose groups such as 14.45 mg/kg and 24.57 mg/kg increased noticeably on the 3rd day by 2.69-fold and 2.81-fold compared to the control groups. MDA showed a positive trend with increasing V_2O_5 concentration in the same time period. In the subsequent exposure time (3-28 days), the MDA content from the same V_2O_5 concentration group showed a downwards trend, while it still increased compared to the control group. The MDA content trend was related to the delayed increase in SOD and CAT activity, as shown in Fig. 2 (1) and (2). It could be speculated that SOD or CAT decreased with oxygen free radicals or MDA increased under the V_2O_5 exposure early on (first 3 days), then the antioxidant defence system cleared the oxygen free radicals and the MDA decreased with the subsequent V_2O_5 exposure time. In addition, the MDA content trends indicated that the lipid peroxidation of earthworms under V_2O_5 exposure mainly occurred during the early stage and gradually weakened in the later stage. This may be an effective process in which antioxidants or detoxification system enzymes scavenge free radicals and induce self-repair in earthworms. During the same exposure time, the MDA content showed an increasing trend, which indicated that the V_2O_5 dose could obviously influence the MDA content, and MDA was increased in a relatively short time at a higher dose.

3.4 Analysis of the IBR

Because various enzymes from different stress conditions showed inhibitory or inducing effects and the responses were not synchronized, the sensitivity of enzyme activity was different in the organism system. It was difficult to quantitatively evaluate the comprehensive effect of lethal toxicity by considering only a single enzyme activity. The integrated biomarker response (IBR) index can be quantified to describe the

incorporated biological effects of various biological indicators by integrating relative enzymes or proteomic biomarkers. The IBR index method has been extensively used to analyse the impact of environmental pollutants on a variety of biomarkers to determine the impact of environmental contaminants on organisms (Liu et al., 2015; Cao et al., 2019). In this study, Fig. 3 shows that the IBR index assessment method was appropriate to integrate the active effects of the three selected biomarkers (SOD, CAT and MDA) in different time periods and to evaluate the toxicity of V_2O_5 to earthworms. In the same time periods, the IBR values were positively related to the changing V_2O_5 concentrations, and the IBR value was the largest at the highest test concentration of 24.57 mg/kg. Over a range of time periods, the trend of IBR value had no distinct linear relationship with the test time extension, and it was relatively higher on the 3rd day and the 28th day. As the IBR values were significantly higher than those of the control group, the test earthworms had a significant stress response to V_2O_5 exposure, and the higher the V_2O_5 concentration was, the greater the toxicity to the organism. To a certain extent, this IBR index could also reflect the organism's sensitivity to defensive stress from external stimuli of V_2O_5 . At the same concentration, the IBR values on the 7th and 14th days were lower than those on the 3rd day. This may have been due to the enzyme activity and MDA response to V_2O_5 stress at the test time. The main reaction process predicted that the enzyme activity gradually recovered after 3 days of V_2O_5 exposure, while the IBR value decreased to a certain extent. The IBR value increased after 14 days, which indicated that the organism's enzymes had an obvious stress response under prolonged V_2O_5 exposure. As shown in Fig. 3, the IBR value was the largest on the 14th day under the highest concentration of 24.57 mg/kg. The reason for this phenomenon may be that the relatively higher stress and longer exposure time induced a difference in sensitivity of the enzymes to the V_2O_5 stress response. In terms of the relationship between the time effect and dose effect, the higher the concentration of V_2O_5 was, the larger the biological toxicity impact. However, at the same exposure concentration, the largest IBR impact was not observed at the longest test time (the 28th day), which indicated that the V_2O_5 concentration had a more visible influence on the oxidative stress of the earthworms than time.

4. Conclusion

This investigation can contribute to the evaluation of the adverse effects of V_2O_5 on the soil environment. The results showed that the acute and chronic toxicity values of V_2O_5 to earthworms were 21.96 mg/kg (LC_{50} , 14 days) and 6.28 mg/kg (LC_{10} , 28 days), respectively, the toxicity of V_2O_5 is mainly caused by V^{5+} , which is an important factor when formulating guideline values for vanadium oxide in soil, and the test earthworm species (*Eisenia fetida*) may be a sensitive biological indicator for risk assessments of vanadium oxide in the soil. For the biochemical responses, the SOD and CAT antioxidant enzyme activities from earthworms showed some different trends from certain V_2O_5 concentration groups, but there was synchronous variation in the induction or inhibition of these enzymes within the test time period. SOD activity was inhibited by V_2O_5 exposure for the first 3 days, and the activity increased from 7 to 14 days and then was inhibited after 14-28 days. The CAT activity of the test earthworms had a response mechanism similar to that of SOD enzymes in the soil, and the CAT activity was inhibited in a

relatively short time at a higher dose. The enzyme activity was generally attributed to the special physiological effects on the test organisms, and the SOD and CAT activities had a certain relationship with the dose. MDA analysis indicated that lipid peroxidation of V_2O_5 in earthworms mainly occurred at the early stage and was eliminated slowly in the later stage. The MDA content showed an increasing trend, which indicated that the V_2O_5 dose could obviously influence MDA, and the MDA would be increased in a relatively short time with a dose induced by V_2O_5 .

In addition, the results also showed that the bioconcentrations and metabolic mechanisms of V_2O_5 in earthworms have some differences with the different exposure concentrations in the soil, and bioaccumulation in earthworms is more readily balanced in 14-28 days under relatively lower-dose V_2O_5 exposure. The BAF values of V_2O_5 tended to have a positive relationship with the exposure time from 7-14 days and a negative correlation with the relative lower-dose level of V_2O_5 . Moreover, the BAF values were far less than 1, which indicated that V_2O_5 did not easily accumulate in earthworms. The BAF of vanadium pentoxide was positively correlated with exposure time and negatively linearly correlated with the V_2O_5 concentration in the soil, and bioaccumulation characteristics in earthworms may be a sensitive predictor for describing the pollution risks posed by V_2O_5 . Finally, the integrated biomarker response (IBR) index showed that the biological stress response was greater at higher V_2O_5 concentrations at different times, and the index particularly reflected the organism's sensitivity to external stimuli from V_2O_5 . The IBR method was appropriate to assess the active effects of the selected biomarkers (SOD, CAT and MDA) in different time periods, and it could evaluate the toxicity of V_2O_5 to earthworms. For the same test periods, the trends of IBR values were positively related to the changing V_2O_5 concentrations, and the IBR value had no distinct linear relationship over diverse time periods. As the IBR values were significantly higher than those of the control group, the earthworms had a significant stress response to V_2O_5 exposure, and the higher the V_2O_5 concentration was, the greater the toxicity to the organism.

Declarations

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Author contributions

Zhao Shiqing: Conceptualization, Writing - Original draft, investigation

Ni Hong: Writing - Original draft, investigation

Li Ji: Investigation

Ai Shunhao: Investigation

Wang Xiaonan: Investigation

Gao Xiangyun: Investigation

Xu Qianyun: Investigation

Liu Zhengtao: Project administration, funding acquisition

Data availability

The data that support the findings of this study are openly available at [DOI].

Conflict of interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical approval. The manuscript has not been submitted to more than one journal for simultaneous consideration. The submitted work was original and has not been published elsewhere in any form or language. Authors adhered to discipline-specific rules for acquiring, selecting and processing data.

Consent to participate. The authors have been personally and actively involved in substantive work leading to the manuscript and will hold themselves jointly and individually responsible for its content.

Consent for publication. The authors consent to publish this research.

References

1. Aihemaiti A, Gao Y, Meng Y, Chen X, Liu J, Xiang H, Xu Y, Jiang J (2019) Review of plant-vanadium physiological interactions, bioaccumulation, and bioremediation of vanadium-contaminated sites[J]. *Sci Total Environ* 712:135637
2. American Conference of Governmental Industrial Hygienists (ACGIH) (2009) Vanadium Pentoxide: TLV Chemical Substances 7th Edition 393 Documentation, 9 pp
3. Asagba SO, Eriyamremu GE, Igberaese ME (2008) Bioaccumulation of cadmium and its biochemical effect on selected tissues of the catfish (*Clarias gariepinus*). *Fish Physiol Biochem* 34:61–69
4. Assem FL, Levy LS (2009) A Review of Current Toxicological Concerns on Vanadium Pentoxide and Other Vanadium Compounds: Gaps in Knowledge and Directions for Future Research. *Journal of Toxicology and Environmental* 12(4):289–306
5. Avci A, Kaçmaz M, Durak I (2005) Peroxidation in muscle and liver tissues from fish in a contaminated river due to a petroleum refinery industry. *Ecotoxicol Environ Saf* 60:101–105
6. Canadian Council of Ministers of the Environment (CCME) (2007) Canadian soil Quality Guidelines for the Protection of Environmental and Human Health. Summary Tables
7. Cao R, Zhang T, Li X, Zhao Y, Wang Q, Yang D, Qu Y, Liu H, Dong ZJ, Zhao JM (2019) Seawater acidification increases copper toxicity: a multi-biomarker approach with a key marine invertebrate,

- the Pacific Oyster *Crassostrea gigas*. *Aquat Toxicol* 210:167–178
8. Cao X, Bi R, Song Y (2017) Toxic responses of cytochrome P450 sub-enzyme activities to heavy metals exposure in soil and correlation with their bioaccumulation in *Eisenia fetida*, vol 144. *Ecotoxicology and Environmental Safety*, pp 158–165
 9. Chen F, Gao J, Zhou QX (2012) Toxicity assessment of simulated urban runoff containing polycyclic musks and cadmium in *Carassius auratus* using oxidative stress biomarkers. *Environ Pollut* 162:91–97
 10. Fortoul TI, Rodriguez-Lara V, González-Villalva A, Rojas-Lemus M, Cano-Gutiérrez G, Ustarroz-Cano M, Colín-Barenque L, Bizarro-Nevarés P, García-Pealea I, Montaña LF, Jimenez-Martinez RS, Lopez-Valdez N, Ruiz-Guerrero ML, Meléndez-García NA, García-Ibarra FA, Martínez-Baez V, Zapata Alfaro D, Muñoz-Rivera-Cambas A, López-Zepeda LS, Quezada-Maldonado EM, Cervantes-Yépez S (2014) Inhalation of vanadium pentoxide and its toxic effects in a mouse model. *Inorg Chim Acta* 420:8–15
 11. Gao YH, Sun ZJ, Sun XS, Bao YZ (2007) Toxic effect of olaquinox antibiotic on *Eisenia fetida*. *Eur J Soil Biol* 43:252–255
 12. Halliwell B, Whiteman M (2004) Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean? *Br J Pharmacol* 142:231–255
 13. Hindy KT, Shafy HIA, Farag SA (1990) The role of the cement industry in the contamination of air, water, soil and plant with vanadium in Cairo. *Environ Pollut* 66(3):195–205
 14. Hoej J, Beier MJ, Vanadium-Based SCR, Systems (2014) : Release of Vanadium and Tungsten during Operation and Possibilities for Reducing Vanadium Emissions. *SAE International Journal of Engines*, 7(3),1397–1400
 15. Huang JH, Huang F, Evans L, Glasaue S (2015) Vanadium: global (bio) geochemistry. *Chem Geol* 417:68–89
 16. Imtiaz M, Rizwan MS, Xiong S, Li H, Ashraf M, Shahzad SM, Shahzad M, Rizwan M, Tu S (2015) Vanadium, recent advancements and research prospects: a review. *Environ Int* 80:79–88
 17. International Agency for Research on Cancer (IARC) (2006) Cobalt in hard metals and cobalt sulfate, gallium arsenide, indium phosphide and vanadium pentoxide. Lyon, France, vol 41186. in: IARC Monographs, pp 1–294
 18. Jardine TD, Doig LE, Jones PD, Bharadwaj L, Carr M, Tendlaer B, Lindenschmidt KE (2019) Vanadium and thallium exhibit biodilution in a northern river food web. *Chemosphere* 233(OCT):381–386
 19. Ji P, Sun T, Song Y, Ackland ML, Liu Y (2011) Strategies for enhancing the phytoremediation of cadmium-contaminated agricultural soils by *Solanum nigrum* L. *Environ Pollut* 159:762–768
 20. Khan S, Kazi TG, Kolachi NF, Baig JA, Afridi HI, Shah AQ, Kumar S, Shah F (2011) Hazardous impact and translocation of vanadium (V) species from soil to different vegetables and grasses grown in the vicinity of thermal power plant. *J Hazard Mater* 190(1–3):738–743
 21. Kula H, Larink O (1997) Development and standardization of test methods for the prediction of sublethal effects of chemicals on earthworms. *Soil Biol Biochem* 29:635e639

22. Lee KE (1985) Earthworms: Their Ecology and Relationships with Soils and Land Use. Academic Press, London, p 411
23. Li M, Xu G, Yu R, Wang Y, Yu Y (2019) Bioaccumulation and toxicity of pentachloronitrobenzene to earthworm (*Eisenia fetida*), vol 174. Ecotoxicology and Environmental Safety, pp 429–434. JUN.
24. Li W, Wang S, Li J, Wang X, Cui L, Chen J, Liu Z (2020) Antioxidative enzyme activities in the *Rhodeinae sinensis Gunther* and *Macrobrachium nipponense* and multi-endpoint assessment under tonalide exposure. Ecotoxicology Environmental Safety 199:110751
25. Liu J, Qu R, Yan L, Wang LS, Wang ZY (2015) Evaluation of single and joint toxicity of perfluorooctane sulfonate and zinc to *Limnodrilus hoffmeisteri*: acute toxicity, bioaccumulation and oxidative stress. Journal of Hazard Mater 301:342–349
26. Lyttle CR, DeSombre ER (1977) Uterine peroxidase as a marker for estrogen action. Proceedings of the National Academy of Sciences, 74, 3162–3166
27. McCord JM, Fridovich I (1969) Superoxide dismutase an enzymic function for erythrocyte (hemocuprein). J Biol Chem 244:6049–6055
28. National Health Commission (NHC) of P.R.C., GBZ2.1-2019, 2019, Occupational exposure limits for hazardous agents in the workplace-Part 1: Chemical hazardous agents. Standards Press of China
29. National Toxicology Program (NTP) (2002) NTP Technical Report on the Toxicology and Carcinogenicity Studies of 413 Vanadium Pentoxide (CAS NO. 1314-62-1) in F344/N Rats and B6C3F1 Mice 414 (Inhalation Studies). National Toxicology Program, Research Triangle Park NC. 415 NTP TR 507, NIH Publication No. 03-4441, dated December 2002
30. Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95:351–358
31. Oliveira M, Ahmad I, Maria VL, Pacheco M, Santos MA (2010) Monitoring pollution of coastal lagoon using *Liza aurata* kidney oxidative stress and genetic endpoints: an integrated biomarker approach. Ecotoxicology 19:643–653
32. Organization for Economic Co-operation and Development OECD (2004) Test 207: earthworm, acute toxicity tests; Test 222: Earthworm Reproduction Test. OECD Guidelines for Testing of Chemicals, Paris, France
33. Pytharopoulou S, Sazakli E, Grintzalis K, Georgiou CD, Leotsinidis M, Kalpaxis DL (2008) Translational responses of *Mytilus galloprovincialis* to environmental pollution: integrating the responses to oxidative stress and other biomarker responses into a general stress index. Aquat Toxicol 89:18–27
34. Hauser R, Eisen EA, Pothier L, Christiani DC A prospective study of lung function among boilermaker construction workers exposed to combustion particulates. American Journal of Industrial Medicine, 39,454–462
35. Saint-Denis M, Narbonne JF, Arnaud C, Ribera D (2001) Biochemical responses of the earthworm *Eisenia fetida andrei* exposed to contaminated artificial soil: effects of lead acetate. Soil Biology Biochemistry 33:395–404

36. Schlesinger WH, Klein EM, Vengosh A (2017) Global biogeochemical cycle of vanadium. *Proc. Natl. Acad. Sci. USA* 114, E11092–E11100
37. Shao Y, Wang J, Du Z, Li B, Zhu L, Wang J, Zhang S (2018) Toxic effect of Omim BF₄ and Omim Br on antioxidant stress and oxidative damage in earthworms (*Eisenia fetida*). *Environ Toxicol Pharmacol* 60:37–44
38. Silva AMM, Novelli ELB, Fascineli ML, Almeida JA (1999) Impact of an environmentally realistic intake of water contaminants and superoxide formation on tissues of rats. *Environ Pollut* 105:243–249
39. Smith PG, Céline B, Loren K (2013) Vanadium pentoxide phytotoxicity: effects of species selection and nutrient concentration. *Archives of Environmental Contamination Toxicology* 64(1):87–96
40. Song Y, Zhu LS, Wang J, Wang JH, Liu W, Xie H (2009) DNA damage and effects on antioxidative enzymes in earthworm (*Eisenia foetida*) induced by atrazine. *Soil Biology Biochemistry* 41:905–909
41. Tripathi B, Mehta S, Amar A, Gaur J (2006) Oxidative stress in *Scenedesmus* sp. during short-and long-term exposure to Cu²⁺ and Zn²⁺. *Chemosphere* 62:538–544
42. US EPA (2011) Draft Toxicological Review of Vanadium Pentoxide: In Support of 436 Summary Information on the Integrated Risk Information System (IRIS) FRL-4379474-1; Docket ID No. EPA-HQ-ORD-2011-0738
43. van der Oost R, Beyer J, Vermeulen NPE (2003) Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environmental Toxicology Pharmacology* 13:57–149
44. Van Gestel CAM, Van Dis WA (1988) The influence of soil characteristics on the toxicity of four chemicals to the earthworm *Eisenia fetida* andrei (Oligochaeta), vol 6. *Biology and Fertility of Soils*, pp 262–265
45. Xiao XY, Wang MW, Zhu HW, Guo ZH, Han XQ, Zeng P (2017) Response of soil microbial activities and microbial community structure to vanadium stress. *Ecotoxicity Environment Safety* 142:200–206
46. Xue Y, Wang X, Gu X, Sun C (2009) Acute toxicity of tetrabromobisphenol A to earthworm *Eisenia fetida* and its effects on antioxidant defense system enzymes. *Asian Journal Ecotoxicology* 4:93–100
47. Zhang Y, Shen GQ, Yu YS, Zhu HL (2009) The hormetic effect of cadmium on the activity of antioxidant enzymes in the earthworm *Eisenia fetida*. *Environ Pollut* 157:3064–3068

Figures

Figure 1

The correlation between concentration and lethal effects to earthworms of V_2O_5

Figure 2

Effects of V_2O_5 on the activity of SOD and CAT and content of MDA in earthworms shown separately in (1), (2), and (3). Data are presented as the means $\pm$ standard deviations (SDs). Capital A represents significant differences compared with the blank control group at $p < 0.01$. Lowercase letters represents significant differences compared with the a (0.0 mg/kg), b (5.0 mg/kg), c (14.45 mg/kg), and d (8.5 mg/kg) control groups at $p < 0.05$.

Figure 3

Star chart representing the IBR under V_2O_5 exposure to earthworms