https://doi.org/10.31871/IJNTR.8.4.5 International Journal of New Technology and Research (IJNTR)
ISSN: 2454-4116, Volume-8, Issue-4, April 2022 Pages 22-25

Short-Term Toxicity of Nano-Vanadium Dioxide To Pea Seedlings

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***Abstract*—Vanadium dioxide (VO_2) is an inorganic thermochromic compound. At 68 ℃, VO_2 exhibits a crystalline phase transition accompanied by a thermochromic effect, so it has broad application prospects in thermochromic smart windows and so on. VO_2 will inevitably enter the environment in the process of extensive research, application and production. In order to ensure its safe production and use, a systematic environmental toxicity assessment of VO_2 should be carried out. Plants are important producers in the ecosystem. In this study, pea was selected as a plant model exposed to commercially produced nano-vanadium dioxide (S-VO_2) for experimental research. The pea seedlings were exposed to S-VO_2 for 4 days, and then transferred to a stress-free culture system . After incubation in the stress-free culture system for 15 days, the effects of short-term S-VO_2 exposure on growth of pea seedlings were evaluated by observing the growth of the plants, measuring and analyzing the root length, the length of the upper part of the root, the number of lateral roots, the number of leaves, the leaf area and the biomass content. Theeffects of S-VO_2 on plant physiological characteristics were assessed by measuring leaf photosynthesis and chlorophyll content. The accumulation of V in rhizomes and leaves, and oxidative damage in leaves and roots were measured to evaluate the toxic effects of VO_2 on pea plants.**

***Index Terms*— VO_2; Peas; Nanotoxicity.**

INTRODUCTION

Vanadium dioxide (VO_2) is an inorganic thermochromic compound. At 68 ℃, VO_2 exhibits a crystalline phase transition accompanied by a thermochromic effect, which can regulate heat flux by automatically responding to temperature [1]. Therefore, it has great potential in smart windows and other fields, and there are many patents on the application of VO_2 to make smart window coatings [2-4]. However, the application of VO_2 in smart windows has been hindered by its high critical temperature, low light transmittance, and poor solar modulation ability.. It thus has been proposed to introduce suitable dopants into the VO_2 system to reduce the temperature transition and improve the reflection of solar heat flux [5]. For example, Wu et al. improved the thermochromic properties of VO_2 and provided a better protective layer for the VO_2 film by encapsulating the VO_2 film in a hafnium dioxide (HfO_2) layer. [6,7]. Liang et al. studied the hybrid film by combining electrochromic LC with thermochromic VO_2 to make the film with 2.5 wt% W-VO_2 reduce the transmittance in the near-infrared spectral when temperature increased from 25 °C to 55 °C [8]. These studies indicated that VO_2 easily entered the environment with its wide applicatioin, and interacted with microbe, plants and animals. It has been reported that VO_2 NPs are toxic to bacteria, cells and animals [9-12]. However, there is currently no article investigating the phytotoxicity of VO_2.

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In this study, pea plant was used as a plant model, and pea seedlings were exposed to the commercially produced nano-vanadium dioxide (S-VO_2) with a concentration of 0-1000 mL. After 4 days, they were taken out and transferred to a non-stressed cultivation system for further cultivation. The pea plants were collected after 15 days to measure the number of roots, number of leaves, root, upper part of the root length, leaf area, fresh weight and dry weight. Chlorophyll content and photosynthesis parameters were detected. The environmental toxicity of S-VO_2 was assessed by quantifying vanadium bioaccumulation in roots, rhizomes, and leaves using inductively coupled plasma mass spectrometry (ICP-MS).

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EXPERIMENTAL

Materials preparation

VO_2 was purchased from Jikang New Materials Co., Ltd., Hangzhou, China. Pea (Pisum sativum L.) seeds were purchased from Beijing Lily Technology Co., Ltd., China. The modified Hoagland nutrient solution is used for seed germination and seedling. The improved Hoagland nutrient solution formula is: $Ca(NO_3)_24H_2O$ (945 mg/L)、KNO_3 • $4H_2O$ (506 mg/L)、$MgSO_4$ (493 mg/L)、KH_2PO_4 (136 mg/L))、NH_4NO_3 (80 mg/L)、$MnSO_4$ (22.3 mg/L)、EDTA • 2Na (18.65 mg/L)、$FeSO_4$ • $7H_2O$ (13.9 • mg/L)、$ZnSO_4$ • $7H_2O$ (8.6 mg/L)、H_3BO_3 (6.2 mg/L)、KI (0.83 mg/L)、Na_2MoO_4 • $2H_2O$ (0.25 mg/L)、$CuSO_4$ • $5H_2O$ (0.025 mg/L) and $CoCl_2$ • $6H_2O$ (0.025 mg/L). Other chemicals are of analytical grade and can be used without purification.

Seedlings cultivation

Pea seeds were soaked in 15% NaCl for half an hour, washed with distilled water, and germinated at 25 °C and 80% relative humidity. After 5 days, the germinated pea seedlings were transferred to a 100 mL beaker for cultivation (each concentration set 3 parallel samples, 5 bean sprouts in each beaker). Supplement with modified Hoagland's nutrient solution daily to maintain a volume of 100 mL. The culture conditions were a day-night cycle of 12 h/12 h, a sunshine intensity of 2400 lx, a humidity of 80%, and a day-night temperature of 25 °C. After 15 days, the growth of the seedlings was observed, and the pea plants were collected for toxicity assessment.

Toxicity of VO_2 to pea seedlings

The pea seedlings were exposed to S-VO_2 (0-1000 mg/L), the seedlings were taken out after 4 days to take pictures of the seedlings, and then they were transferred to a stress-free

culture system for further cultivation. After 15 days, the pea seedlings were collected to take a photo of the plants. They were then placed on a multi-function analyzer (LA-S, Hangzhou Wanshen Testing Technology Co, Hangzhou, China) to analyze leaf number, root number, root length, length of the upper part of the root, and leaf area. , The root weight and the fresh weight of the upper part of the roots were weighed after removing the surface moisture, and the plants were dried in an oven at 60 °C for 24 hours and then taken out and weighed for dry weight. To measure oxidative stress, five kits including total protein, glutathione (GSH), malondialdehyde (MDA), catalase (CAT), and H_2O_2 were purchased from Jiancheng Bioengineering Institute, Nanjing, China. The measurement was performed strictly according to the recommended procedure, which can be obtained from the official website http://www.njjcbio.com/.

Effects of VO_2 on photosynthesis of pea plant leaves

The chlorophyll contents of pea leaves were measured by a chlorophyll meter (LA-S, Hangzhou Wanshen Testing Technology Co., Hangzhou, China). The net photosynthetic rate, stomatal conductance, transpiration rate and intercellular CO_2 concentration of leaves were measured using a portable photosynthetic system (Yaxin-1102, YaXin Liyi Technology Co., Ltd., Beijing, China).

Bioaccumulation of vanadium in pea shoots

The roots, stems and leaves of the pea plants were separated to determine the bioaccumulation of vanadium in pea shoots. They are then frozen, lyophilized, ground separately. 5.0 mg of each sample was weighed into a mixture of HNO_3 and $HClO_4$ (5:1 v/v ratio) and placed on an electric furnace for digestion. After heating to boiling for 10 minutes, the heating was stopped, and after cooling to room temperature, the digestion solution was diluted to volume with 2% HNO_3. The concentration of vanadium in the digestion solution was measured by ICP-MS, and the data was converted into the content of vanadium in fresh tissue.

RESULTS AND DISCUSSION

Toxicity of VO_2 to pea seedlings

We exposed the pea seedlings to S-VO_2 at a wide concentration of 0-1000 mg/L, and after 4 days, the pea seedlings were taken out to take pictures of the seedlings. As shown in Fig. 1a, the growth of lateral roots of seedlings was inhibited when the pea seedlings exposed to S-VO_2 at 10 mg/L ,and the roots were blackened at S-VO_2 of 100 mg/L. At 1000 mg/L, the roots obviously became black and almost no lateral roots grew. After being transferred to the culture system without stress for 15 days, the early root blackening of pea seedlings disappeared and lateral roots grew, and there was almost no difference of pea growth compared with the control group (Fig.1b).

Figure 1.The inhibitory effect of S-VO_2 on pea plants, the value is expressed as the concentration of S-VO_2 in mg/L.(a) Photograph of pea seedlings exposed to VO_2 on day 4;(b) Photographs of pea seedlings exposed to S-VO_2 for 4 days and transferred to a centipede stress culture system for 15 days.

To better understand the effects of S-VO_2 on pea seedlings, we measured the root number, leaf number, root length, root length, root fresh weight, root fresh weight, fresh weight, and dry weight of pea plants after 15 days. As shown in Fig. 2a, the lateral root growth of pea seedlings was promoted by S-VO_2 at low concentrations (0.1, 10 mg/L), while the promoting effects disappeared at 1 mg/L and 100 mg/L, and the root growth was inhibited when the concentration increased to 1000 mg/L, S-VO_2 significantly promoted the root length of plants at low concentrations (0.1-100 mg/L), but inhibited the root length at 1000 mg/L. Compared with the control group, S-VO_2 had almost no effect on the leaf number of pea plants at 0.1-100 mg/L, and significantly inhibited the leaf number of plants at 1000 mg/L. S-VO_2 had severe effects on pea plants shown as it promoted root growth at 1 mg/L, but inhibited root growth at concentrations of 10-1000 mg/L. This may be because the roots of pea seedlings directly contacted the material when exposed to S-VO_2 for 4 days, which seriously affected the growth of seedling roots.

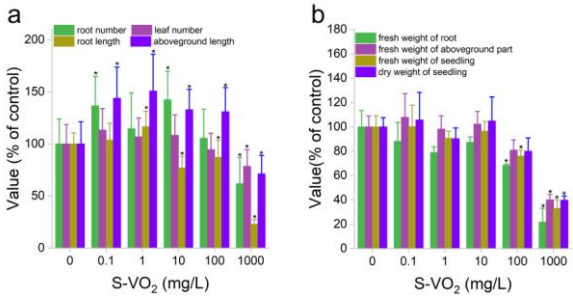

Figure 2 Effects of S-VO_2 on pea seedling growth and seedling weight. (a) The effect of S-VO_2 on the number of young roots, leaves, root length and upper part of the root of pea; (b) Effects of S-VO_2 on the fresh weight of root, fresh weight of root, fresh weight of seedling and dry weight of seedling of pea seedlings. (* p <0.05 compared with the control group. n=15.)

Photosynthesis is the process that plants convert CO_2 into their own organic matter, and particularly important for plant carbon fixation and growth. As shown in Figure 3a, a concentration of 1000 mg/L promoted net photosynthesis in leaves. At 10-1000 mg/L, S-VO_2 significantly increased leaf transpiration rate and intercellular CO_2 concentration. The

stomatal conductance of leaves decreased significantly at 1000 mg/L.

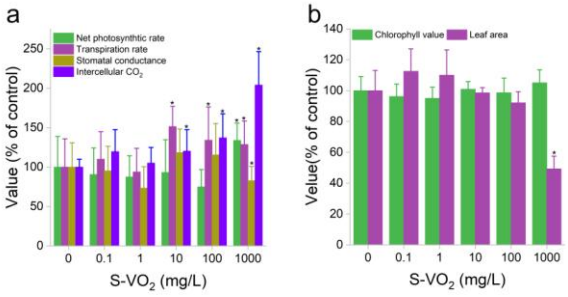

Figure 3 (a) The effect of S-VO_2 on photosynthesis of pea plants ;(b) Effects of S-VO_2 on leaf chlorophyll and leaf area. (* p <0.05 compared with the control group. n=15).

As can be seen in Figure 3b, pea plants had chlorophyll content similar to that of the control group after they exposed to S-VO_2 for 4 days and transferred to a non-stressed culture system, and there was almost no effects of the highest concentration (1000 mg/L) on pea plants. While the leaf area of the plants decreased significantly at S-VO_2 of 1000 mg/L, .

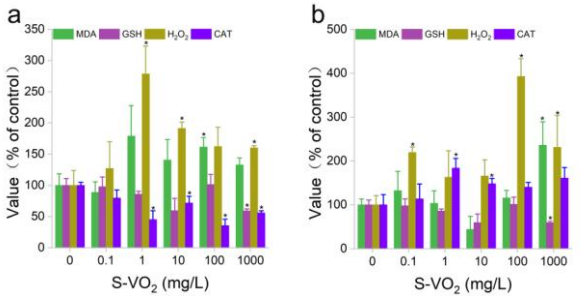

Figure 4 Toxicological mechanism of S-VO_2 on pea seedlings. (a) Oxidative stress in roots; (b) Oxidative stress in leaves. (* p <0.05 compared with the control group. n=3).

Oxidative stress is a well-recognized mechanism for the negative effects of exogenous organisms. Therefore, we measured oxidative stress parameters to explore the toxicological mechanism of VO_2 particles on pea seedlings . The pea seedlings were exposed to S-VO_2 and transferred to a non-stressed culture system after 4 days. After 15 days, the MDA in the roots of the seedlings increased significantly at 100 mg/L, but this phenomenon disappeared at 1000 mg/L. While GSH had no significant change at 0.1-100 mg/L, and decreased significantly at 1000 mg/L. H_2O_2 significantly increased at 1-1000 mg/L, correspondingly, CATsignificantly decreased at 1-1000 mg/L, indicating that there was slight oxidative stress in the roots at 1-1000 mg/L. As shown in Figure 4b, at 1000 mg/L, MDA significantly increased, while GSH significantly decreased. H_2O_2 significantly increased at 0.1 mg/L, and this phenomenon disappeared at 1 mg/L. When the concentration was 100-1000 mg/L, S-VO_2 again caused a significant increase in H_2O_2. CAT increased significantly at 1 and 10 mg/L and no significant change at other concentrations. These phenomena indicated that mild oxidative stress also appeared in leaves and leaves at high concentrations (1000 mg/L), but the roots and leaves of pea plants had not been damaged.

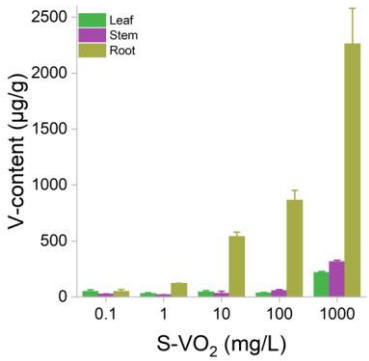

Figure 5 Bioconcentration of V ions in peas. (* p <0.05 compared with the control group. n=3).

The bioaccumulation data showed that the accumulation of V ions in plants increased with increasing concentrations even pea seedlings were transferred to a non-stressed culture system after 4 days of S-VO_2-exposed. At low concentrations (0.1 and 100 mg/L), the intake of V ions was lower and had no significant effect on plant growth, physiology and toxicity compared with the control group. Leaf mobility was also lower (<45.8 μg/g) and had no significant effect on plant growth photosynthesis. At high concentration (1000 mg/L), root uptake and leaf migration further increased (Fig.5), and the accumulation in roots was 2262.25 μg/g, the accumulation in stems was 315.0 μg/g, and the accumulation in leaves was 218.7 μg/g. At this concentration, both roots and leaves were damaged, inhibiting the growth of pea plants.

CONCLUSION

In this study, pea seedlings were exposed to S-VO_2 for 4 days, and the roots of the plants were washed and transferred to a non-stress culture system for further cultivation. Plant growth was observed after 15 days, chlorophyll content, photosynthesis, oxidative stress in roots and leaves, and bioaccumulation of V ions in pea plants were measured. It was found that when pea seedlings were exposed to S-VO_2 on the 4th day, S-VO_2 had a significant inhibitory effect on the development of seedling roots. However, it is worth noting that the inhibition of roots was alleviated after transfer to a non-stressed culture system. Indeed, at high concentration (1000 mg/L), the growth of pea seedlings was still inhibited, the leaf area was reduced, and the bioaccumulation of V ions in the plant was significantly increased. Therefore, we should pay more attention to the environmental safety of S-VO_2 in commercial use and production in the future.

ACKNOWLEDGMENT

We acknowledge financial support from the Fundamental Research Funds for the Central Universities, Southwest Minzu University (No. 2021PTJS36).

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