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Accumulation and Biotransformation of Vanadium in *Opuntia microdasys*

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Abstract The accumulation and biotransformation of vanadium (V) in *Opuntia microdasys* were investigated under hydroponic conditions to determine the toxicity of pentavalent V [i.e., V(V)] to the plant and the mechanism of tolerance by the plant to V. Results showed that the concentration of V(V) in nutrient solution was negatively correlated to plant biomass. Moreover, the water content of cladodes decreased under V(V) stress. In V(V)-treated plants, most of the adsorbed V remained in the roots and in the cell wall compartment. In the cladodes, the ratios of V(V) to V_{total} were lower in V(V)-treated plants than those in the control plants. These results indicate that a high concentration of V(V) is toxic to *O. microdasys* but that the plants may limit this toxicity through the compartmentalization of V in the cell wall and the biotransformation of V from V(V) to tetravalent V [i.e., V(IV)].

Keywords Heavy metal · Plant · Uptake · Toxicity · Detoxification

Vanadium (V) is a natural element found in air, soil, water, plants, and animals (Minelli et al. 2000). A main natural source of V is titaniferous magnetite, which contains 1.5 %–2.5 % of V pentoxide. This source is largely mined in South Africa, Russia, and China (The Health and Safety Executive 2002). The concentrations of anthropogenic sourced V in the environment have increased significantly, mostly as a result of increased demand for V in high-temperature industrial activities, including steel-iron refining, electronics and dyeing (Ringelband 2001; Yang et al. 2011). The increased concentrations of V in the environment may affect local ecosystems; in fact, plants have been damaged directly because of contact with V-rich ashes (Vaccarino et al. 1983).

The biological image of V is highly contradictory in terms of toxicity and essentiality (Mukherjee et al. 2004). Arnon and Wessel (1953) suggest that V is essential for some plants; for example, V is necessary in the growth of the green algae *Scenedesmus obliquus*. However, other studies reveal that V is generally toxic to terrestrial plants at levels greater than picomolar (Singh 1971; Olness et al. 2000, 2005). Although V uptake processes have been investigated in some plant species, little is known about V distribution, speciation and detoxification in plants. The mechanisms involved are also highly speculative.

A study was conducted on *Opuntia microdasys* (Cactaceae), a species commonly found in the mine tailing disposal area of Panzhihua, China. This area is among the largest reserves of V-titano-magnetite minerals in the world as a result of over five decades of mining and smelting (Teng et al. 2006). The aims of the study were to determine the uptake and accumulation of V in this plant species, and to elucidate possible mechanisms for its tolerance to V.

Materials and Methods

Healthy *O. microdasys* plants were obtained from Panzhihua Plant Seed Company Limited, Panzhihua, China. The plants contained two cladodes and were similar in terms of weight and height. At the beginning of the experiment, the cladodes were green and plump, and the roots were white. For the first 5 days, the plants were transplanted into 1 L black polyethylene pots that contained deionized water. For

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the next 7 days, they were placed in an untreated, half-strength modified Hoagland solution (Hoagland and Arnon 1950) to recover from transplant shock. The Hoagland solution was mixed with sodium metavanadate ($NaVO_3$) solution to produce five treatment levels of 0, 2.5, 5, 25, and 50 mg L^{-1} V(V) in the nutrient solution. Each treatment was replicated three times. The pots were arranged in a completely randomized design, and their positions were changed occasionally. The plants were grown from July to December under the respective room conditions: average day/night temperatures of 32/22°C and 24/10°C. Growth was sustained until the newly generated biomass of the plants was sufficient for chemical analysis. The solution in the pots was brought up to 1 L volume with deionized water, aerated daily, and adjusted to pH 6.5 ± 0.1 twice every week. The nutrient solution was changed every 7 days.

At the end of the exposure period, the plants were segmented into the lower cladode (C1), middle cladode (C2), upper cladode (C3), and root. These parts were obtained from each of the three plants in the same treatment pots, and they were composited and homogenized. All of the plant samples were washed separately with both tap and deionized water. The fresh weights of the plants were recorded immediately using an analytical balance (accuracy of 0.0001 g) after the residual deionized water on the plant surface was wiped off. Plant height was measured from the bottom of the plant to the top of the cladode. The length of the longest root was considered to be the root length. Moreover, the threshold of V(V) in the nutrient solution which induced 50 % biomass decrease (IC_{50}) was calculated. The IC_{50} was determined by constructing a dose–response curve and examining the effect of different concentrations of V(V) on decreasing plant biomass. The water content of the plants was determined through the gravimetric method following 30 min of fixation at 90°C and subsequent drying to a constant weight at 70°C. The oven-dried plant samples were milled using an agate mortar and pestle and then stored in airtight polyethylene sachets until analysis.

The concentrations of V_{total} in the plant samples were measured after wet digestion in a mixture of nitric acid and hydrogen peroxide (Lu 1999). Approximately 0.1000 g (depending on the biomass of the samples) of the plant samples was immersed in 7 mL HNO_3 for 2–4 h. Subsequently, 2 mL H_2O_2 were added, and the sample was digested on an electric hot plate until the volume of the clear aliquots was roughly 1 mL. The solution was then boiled with 5 mL of 1.5 mol L^{-1} HNO_3 for 2 min. The clear solution was cooled and diluted to 25 mL with deionized water. Meanwhile, three blanks were also prepared. Concentrations of V in the digest were measured by a graphite furnace atomic absorption spectrophotometer (model AAnalyst 800, Perkin- Elmer Corp., Norwalk, CT, USA) using the hollow cathode lamp for V (Perkin-Elmer). The entire system was controlled using AAWinlab control software. A standard stock solution (GSB04-1759-2004) that contained 1000 mg L^{-1} of V was purchased from Chinese Standard Material Center, Beijing, China, to prepare the working standards (0–200 μg L^{-1}). Green tea [GBW10052 (GSB-30)] purchased from the National Research Center for the Geoanalysis of China (Beijing, China) was used as the standard reference material. All of the analyses were performed in triplicate. The blanks were run simultaneously. For plant samples, the recovery of reference material was 91.8 ± 7.2 %. We also determined the biological transfer coefficient (BTC), which is the ratio of heavy metal concentration in the plant shoot to that in the plant root (Zu et al. 2005). It evaluated the effectiveness of the transfer of V from the plant roots to the above-ground parts.

To further explore the transformation of the plant-absorbed V, concentrations of V species and the cell wall-binding V concentration in the cladodes were examined. The V in plant tissue can be categorized into V(V) and V(IV) according to the $(NH_4)_2HPO_4$ solution extraction method. Once the samples are treated with $(NH_4)_2HPO_4$, all of the V(V) species goes into the solution (Mandiwana and Panichev 2006). The distribution of V between the cell walls and symplast of cladode was further estimated after isolating the cell walls using the method developed by Hart et al. (1992). Washed cladodes were soaked in a mixture of methanol:chloroform (2:1, v/v) solution for 3 days, and then washed extensively with deionized water. The materials were dried at 90°C and subsequently dried to a constant weight at 70°C, and then acid-digested with a mixture of HNO_3 and H_2O_2 for V analysis. All concentrations were based on oven-dried mass.

Results and Discussion

V(V) reduced the number of newly generated cladodes in the plants. Three new cladodes (C3, C4, and C5) developed in the control plants; two cladodes (C3 and C4) at the 2.5 and 5 mg L^{-1} V(V) treatments; and either only one cladode (C3) or none at the 25 and 50 mg L^{-1} V(V) treatments. The biomass produced by the C4 and the C5 was low; hence, C1, C2, and C3 alone were considered in chemical analysis. Both root length and plant height were reduced with increased concentrations of V(V) (Fig. 1). At the 2.5–50 mg L^{-1} V(V) treatment, root length and plant height decreased from 41 % and 76 % to 9.8 % and 40 % of the control, respectively (Fig. 1a). The IC_{50} values for root length and plant height in the present study were 4.5 and 18.0 mg L^{-1} V, respectively. Meanwhile, under V(V) stress, the cladodes were flaccid and slightly yellowed, and the roots darkened.

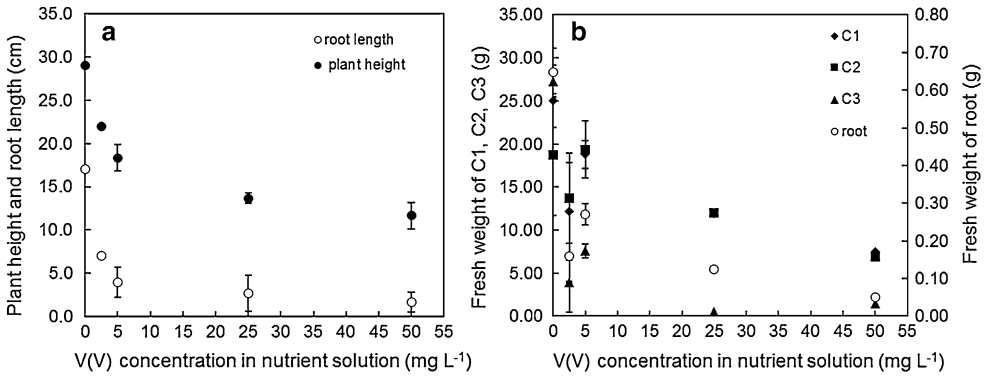

Fig. 1 Effect of V(V) on the biomass of *O. microdasys*. The mean values and standard deviations of the three replicates are shown; *C1* represents the lower cladode; *C2* denotes the middle cladode; and *C3* indicates the upper cladode

The fresh weights of the cladodes and the roots were negatively correlated with the V(V) concentrations in the solution (Fig. 1b). Root weight decreased by 92 % relative to that of the control when the plants were grown in the 50 mg L^{-1} V(V)-treated nutrient solution. Furthermore, the total weight of the cladodes decreased by 78 %. These results agreed with those of the previous studies conducted by Olness et al. (2005), who concluded that root length decreased by approximately 50 % of that of the control when the cuphea was grown in 0.153 mM V hydroponic culture and dry weight decreased by ≥75 %. The IC_{50} values that refer to fresh root and cladode weights were 9.0 and 14.5 mg L^{-1} V, and 21.5 and 39.0 mg L^{-1} V for C1 and C2, respectively. These findings indicate that roots are affected to a greater extent by hydroponic V(V) stress than cladodes. In addition, old cladodes are more sensitive to V(V) stress than young ones.

When the concentration of V(V) in the nutrient solution increased, the water content in the cladodes of the plants decreased (Fig. 2). At the end of the experiment, the water percentages were 94.8 % and 95.2 % in the C1 and C2 of the control plant. When V(V) concentration in the nutrient solution increased to 50 mg L^{-1}, the water content decreased to 90.6 % (C1) and 92.5 % (C2). As V(V) concentration increased from 0 to 50 mg L^{-1}, C2 consistently maintained a higher water content than C1. Under the stress of high V(V) concentration, the biomass of the plants decreased. This decrease may be related to the disturbed water metabolism balance in the plant. This hypothesis is expected to be verified through further physiological tests. Plant water content also decreases during natural senescence, but V stress enhances this process.

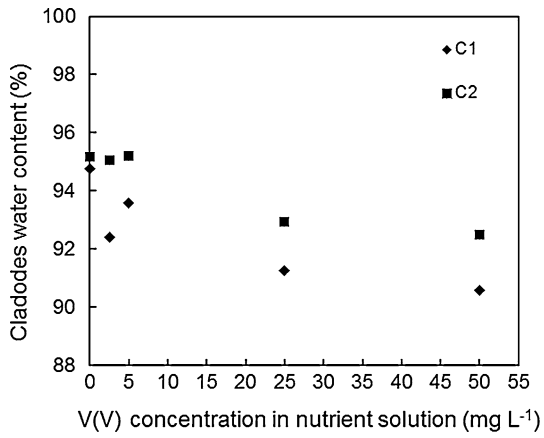

Fig. 2 Effect of V(V) on the water contents in the cladodes of *O. microdasys*. *C1* represents the lower cladode and *C2* denotes the middle cladode

The V_{total} concentrations in the root, C1, and C2 were positively correlated with V(V) concentration in the nutrient solution. When the V(V) concentration was 2.5 mg L^{-1}, the V_{total} concentrations in the root, C1, C2, and C3 were 100, 29, 2, and 2 times the respective tissue concentrations in control plants. When V(V) concentration in the nutrient solution increased to 50 mg L^{-1}, the corresponding values were 797, 554, 22, and 23 times those in the control (Table 1). This result agrees with the finding of Wallace et al. (1977). This study reported decreased dry matter production in plants grown with 0.1 mM vanadate, and the V levels of the tissue increased in relation to supply rate. This result also agrees with the results of a study by Tian et al. (2014), who demonstrated that V concentrations in the roots of Chinese cabbages were positively related to the V concentrations in the soils. The relative high V concentration in the root and in C1 during high V treatment may be related to the solution V that adheres to the plant surface. Nonetheless, most of the V remained in the roots, and only a small portion was transferred to the new generation of cladodes in the V(V)-treated plants. In the control plants, the BTCs of C1, C2, and C3 were 63 %, 54 %, and 32 %, respectively. The BTCs of the plants treated with V(V) were significantly lower than those of the control, especially those of C2 and C3. This supports the finding of Hara et al. (1976), who noted that V was retained in cabbage roots at levels of up to 2500 mg kg^{-1}, representing 95 %–98 % of the total V absorbed.

The V(V) concentrations in the C1 and C2 of the control plants were 3.05 and 3.34 mg kg^{-1}, respectively. When V(V) concentration in the nutrient solution increased from

Table 1 Total V concentrations and transfer coefficients of V in either the root or the cladodes of *O. microdasys*

Solution V (mg L^{-1})	V_{total} concentration in plants (mg kg^{-1})				Biological transfer coefficient (%)		
	C1	C2	C3	Root	C1	C2	C3
0	4.67 ± 0.12[a]	3.97 ± 0.37	2.36[b]	7.41	63.0	53.61	31.9
2.5	136.49 ± 14.52	9.32 ± 0.89	5.76	746.35	18.3	1.25	0.77
5	186.11 ± 9.18	25.29 ± 1.54	8.73	2329.49	7.99	1.09	0.37
25	1214.04 ± 3.60	29.45 ± 0.91	7.08	2652.95	45.8	1.11	0.27
50	2586.33 ± 4.80	87.90 ± 16.27	56.14	5908.51	43.8	1.49	0.95

C1 represents the lower cladode; C2 denotes the middle cladode; and C3 indicates the upper cladode

[a] Average ± standard deviation

[b] The biomasses of C3 and the root were insufficient for replicate experiments

2.5 to 50 mg L^{-1}, the V(V) concentrations in the C1 and C2 increased from 4.29 to 1363.32 mg kg^{-1} and 3.83 to 29.99 mg kg^{-1}, respectively (Fig. 3). The ratios of V(V) to V_{total} were 65 % and 84 % in the C1 and C2 of the control plants, respectively, and decreased to 3 %–53 % and 34 %–69 % in the C1 and C2 of the V-treated plants. A previous study conducted with electron spin resonance showed that barley roots grown in non-paramagnetic NH_4VO_3 also generated the spectrum characteristic of V(IV) (Deiana et al. 1983). This process may be initiated by the oxidation of the acid groups of cell wall polysaccharides (Deiana et al. 1983; Morrell et al. 1986). This mechanism may also help explain the low BTC of the *O. microdasys* under V(V) stress given that V(V) is generally mobile and is considered an active component in the biogeochemical cycle of V in the surface environment (Baken et al. 2012). The importance of this transformation of V is heightened when the effect of the vanadate ion on Na–K ATPases is considered. V(V) is a potent inhibitor of Na–K ATPases, which are often unaffected by the reduced form of V(IV) (Macara 1980). Thus, V(V) effectively limits potential disruption in membrane systems (Morrell et al. 1986). Tian et al. (2014) also demonstrated the predominance of V(IV) in leaves (60 %–80 % of the V_{total}). Garcia et al. (2013) suggested that when the biomass made contact with the V(V) species, the surface level of the V(IV) coordinated by the oxygen donors of the biomass may experience sorption, reduction, and retention.

Aside from biotransformation, other strategies of metal tolerance and accumulation may also be involved, such as cell wall binding or localization in the apoplast (Krämer et al. 2000). In the current study, roughly 90 % and 86 % of the V was detected in the cell walls of the C1 and C2 of the control plants. The concentration of cell wall-binding V increased with V(V) concentration in the nutrient solution. Moreover, a higher percent of V was obtained in the apoplasm fraction in C1 than that in C2 (Fig. 4). When V(V) concentration in the nutrient solution increased from 5.0 to 50 mg L^{-1}, more than 76 % and 68 %–76 % of V were determined in the apoplasm fractions in C1 and C2, respectively. Symplasm exclusion may also influence the V tolerance of *O. microdasys*. However, V was toxic to *O. microdasys* even though most of the V remained in association with the cell walls.

The results from the present study showed that V(V) in the nutrient solution was toxic to *O. microdasys* at concentrations of 2.5 to 50 mg L^{-1}. V(V) toxicity inhibited biomass production and reduced cladodes water content.

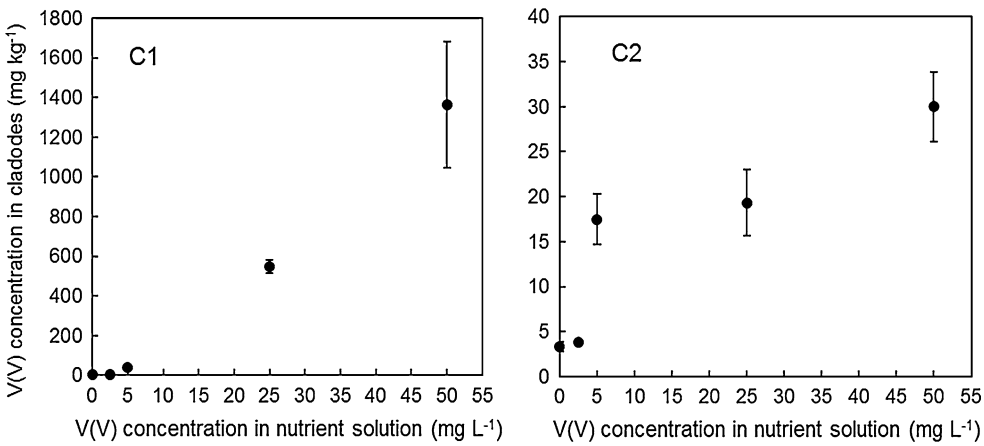

Fig. 3 V(V) concentrations in the cladodes of *O. microdasys*. The mean values and standard deviations of the three replicates are shown; *C1* represents the lower cladode and *C2* denotes the middle cladode

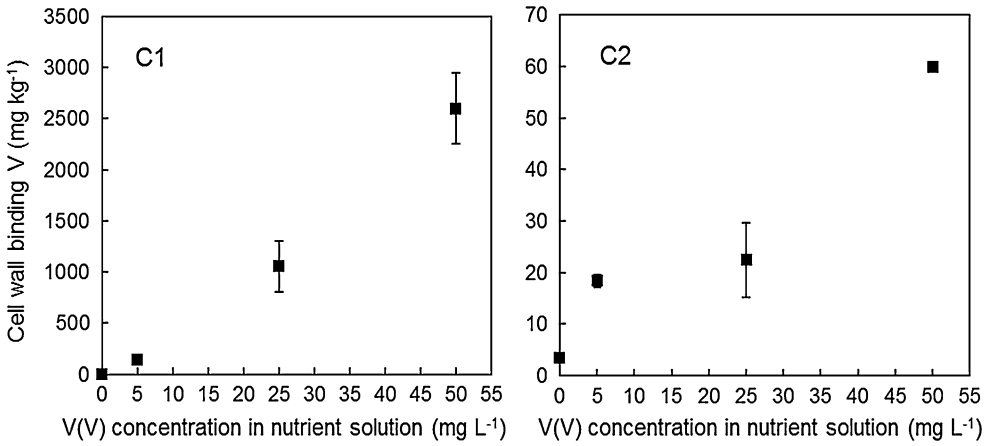

Fig. 4 Cell wall-binding V concentrations in the cladodes of *O. microdasys*. The mean values and standard deviations of two replicates are shown; *C1* represents the lower cladode and *C2* denotes the middle cladode. The biomass of the *C2* under 50 mg L^{-1} V(V) treatment was insufficient for replicate experiments

However, plants survived even the highest V(V) treatment. This survival may primarily be ascribed to the immobilization of the V that was mostly adsorbed in the root, the fixation of V to the cell wall, and the biotransformation of V(V) into the less toxic V(IV).

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