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Toxicity of vanadium in soil on soybean at different growth stages☆

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ABSTRACT

Vanadium(V) is present in trace amounts in most plants and widely distributed in soils. However, the environmental toxicity of V compound in soils is controversial. A greenhouse study with soybean from germination to bean production under exposure to pentavalent V [V(V)] was conducted to elucidate the interaction of plants and V fractions in soils and to evaluate the toxicity of V at different plant growth stages. Soybean growth has no effect on non-specific-bond and specific-bond fractions of V in soils, but V fractionation occurred in more extraction-resistant phases at high V concentrations. High concentrations of V(V) postponed the germination and growth of the soybeans. Bean production was less than half of that of the control at 500 mg kg^{-1} spiked V(V). For the 0 mg kg^{-1} spiked V(V) treated plants, the root was not the main location where V was retained. Vanadium in the soils at $\leq$ 250 mg kg^{-1} did not significantly affect the V concentration in the shoot and leaf of soybeans. With the increase in V concentration in soil, V concentrations in roots increased, whereas those in beans and pods decreased. From vegetative growth to the reproductive growth, the soybeans adsorbed more V and accumulated more V in the roots, with <20% transported to the aboveground parts. Hence, the analysis of V concentration in vegetative tissues or beans may not be a useful indicator for V pollution in soil. Meanwhile, the ratio of V concentration in cell wall to the total V concentration in the root increased with the increase in V(V) concentration in soils. Our results revealed that high concentrations of V inhibited soybean germination and biomass production. However, plants may produce self-defense systems to endure V toxicity.

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1. Introduction

Vanadium(V) presents in trace amounts in most plants and widely distributed in soils (Panichev et al., 2006). One of the main sources of V in soil is the parent rock from which it is formed (Nriagu, 1998), such as titaniferrous magnetites, certain deposits of phosphate rock, shales, some uranium ores, and asphaltic deposits (Adriano, 2001). Except for the natural sources, the compounds of V are also released in the atmosphere by burning of fossil fuels and from various industrial processes (Pyrzynska and Wierzbicki, 2004; Khan et al., 2011). Vanadium is considered as one of the most important element for the 21st century given its extensive use in industries, addition in fertilizers, and part of different medicines (Imtiaz et al., 2015). However, based on previous studies, V has been recognized as a potentially dangerous pollutant in the same class as mercury, lead, and arsenic (Lazaridis et al., 2003). The U.S. Environmental Protection Agency put V on the top list of candidate contaminants (U.S. Environmental Protection Agency, 2006).

Vanadium is a trace element of highly critical role in biochemical processes (Crans et al., 2004). Vanadium acts as a growth-promoting factor and participates in the fixation and accumulation of nitrogen in plants (Taylor and Staden, 1994). However, the essentiality of V for higher plants and crops had yet to be unequivocally accepted by biologists and biomedical scientists (Mukherjee et al., 2004). Several reports reveal the direct effects of plant growth by V and ultimately damage food quality (Vaccarino et al., 1983), and these events may possibly repeated in future (Imtiaz et al., 2015). A similar concern was raised by Panichev et al. (2006) because of the structural analogy between vanadate ($H_2VO_4^-$) and phosphate ($H_2PO_4^-$) ions (Rehder, 1999), and the consumption of V enriched contaminated grass by mammals would ultimately lead to the replacement of PO_4^{3-} in their bones (Panichev

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et al., 2006).

The increase in environmental levels of V in soil has raised various concerns (Hope, 1997). China, the largest vanadium producing and consumption country in the world, contributed 57% to the world's vanadium production (GCVIR, 2014). Due to the increased mining and smelting activities, 26.49% of soils were contaminated by V scattered in southwest of China (Yang et al., 2017). Although the total content of V remains useful in many areas, several reports indicated that V mobility, transport, toxicity, bioavailability, and bioaccumulation are highly dependent upon its oxidation state (Imtiaz et al., 2015). In general, V exists in two oxidation states: the tetravalent [V(IV)] and pentavalent [V(V)] in the environmental samples; V(V) is more mobile and more toxic to both plants and animals than V(IV) compounds (Panichev et al., 2006; Xiao et al., 2015). Therefore, understanding the biological roles of V(V) is important for evaluating the potential risk of V to the environmental and biological systems.

A number of studies have indicated that V should be listed as toxic heavy metal (Imtiaz et al., 2015). However, information on the environmental biogeochemistry of V is generally insufficient. To date, the knowledge related to V in soils and plants, as well as the tolerance mechanism of plants to V, is still limited. Consequently, accurate risk assessment of V on the environment in V-contaminated regions is extremely difficult. Determination of the relationship between soil V fractions and their bioavailability in the soil–plant system is highly desirable. Thus, we started with V(V), which is considered most toxic, to elucidate the toxicity or role of V in soil to leguminous plants. This study aims to: (1) elucidate the potential toxicity of V to plant, (2) evaluate bioavailability of V in the soil and subsequently understand the translocation and accumulation of V from soils to plants at different stages of plant growth, and (3) recognize the possible mechanisms of plant tolerance to V.

2. Materials and methods

2.1. Germination and root length test

For the germination test, seeds of soybean (*Glycine* max (L.) Merr.) were surface sterilized for 10 min with NaClO (approximately 2% of active chlorine), and thereafter rinsed in distilled water for the first day and in the presence of $NaVO_3$ solutions (0, 0.05, 0.10, 0.50, 1.00, 5.00, and 10.0 mmol L^{-1}, separately; equivalent to 0, 2.55, 5.09, 25.5, 50.9, 255, and 509 mg L^{-1}) for the next 8 days in a growth chamber in randomized order. The conditions of the growth chamber were 16 h light: 8 h dark regime, with a day/night temperature of 28/20 °C, and a relative humidity of 80%. Three replicate plates per treatment were set and each plate was filled with ten soybean seeds. The positions of the plates were occasionally changed. The germination rate (percentage of germinated seeds out of all the seeds planted over a given period), survival rate (percentage of survived seedlings out of all the seeds planted over a given period), and the longest root of each seedling were measured and analyzed after 5 and 9 days of growth, respectively.

2.2. Soil ageing experiment

The top soils (0–20 cm depth) from an agricultural site were taken for soybean pot experiments. After sampling, the soils were air-dried and sieved through a 2 mm sieve for use. The soil contained 16% loam, 26% silt, and 58% sand. Soil $pH_{(H_2O)}$ (V:V = 1:1) was 6.54. The C and N content was 1.37% and 0.10%, respectively. The cation exchange capacity was 13.6 cmol kg^{-1}. The concentration of total V in soil was 61.2 mg kg^{-1} as determined by X-ray fluorescence spectrometry (SPECTRO X-LAB 2000, SPECTRO Analytical Instruments, Germany). The total concentration of Fe, Si, and Al in the soil was 1.98%, 24.9%, and 4.28%, respectively.

Air-dried soil samples were rewetted with deionized water to 60% of field water capacity, and incubated in growth chamber for 28 days before V treatment. The growth chamber was conditioned as mentioned above. Based on result of the germination test and our previous field study (data not shown) that less than 20% of the soil total V was bioavailable, 5 different levels (0, 50, 100, 250, and 500 mg V kg^{-1} soil) of V(V) as $NaVO_3$ solution with exactly the same volume were spiked into the soils and homogenized carefully. Metavanadate reacts quickly with water to form orthovanadate (Crans, 1995). This salt was preferred to sodium orthovanadate (Na_3VO_4) because Na_3VO_4 would cause a greater change in both salinity and pH (Baken et al., 2012).

Soil ageing was conducted in pots (19 cm diameter × 17 cm height) containing 1.5 kg of soil in the green chamber mentioned above for another 28 days. The pots were arranged in a completely randomized design with each treatment replicated 6 times. Moisture loss was compensated by calculating the difference of soil weight. During ageing, 1, 7, 14, 21, and 28 days after V(V) addition, soil was thoroughly mixed and 20 g sub-samples were collected. Pentavalent V extracted with Na_2CO_3 (solid: liquid ratio = 1 g: 25 mL, boiled 15 min) were determined according to Mandiwana and Panichev (2004). The water soluble fraction of V (solid: liquid ratio 1 g: 5 mL, 24 h end-over-end shaking) was analyzed at the 21 and 28 days after V(V) addition (Mandiwana and Panichev, 2004). Vanadium concentrations in the solution were measured by ICP-OES (inductively coupled plasma–optical emission spectroscopy) using an Optima 3300 DV (Perkin Elmer, Waltham, MA, USA).

2.3. Soybean growth experiment

After 28 days of rewetting and 28 days of V ageing, surface-sterilized soybean seeds were planted in the soils in the same growth chamber. Ten healthy seeds per pot were sown into the treated soils and rooted approximately 2–3 cm deep under the ground. For the plant growth assays, no fertilizer was applied to avoid the interaction of these chemicals with V. After 5 days of seeding, germination rate was recorded every 5 days until 25 days after seeding. All the pots were arranged in a completely randomized block design and the positions were changed occasionally. Collection trays were placed under each pot to retain any leachate. Each pot was watered with 100 mL of deionized water in the morning per day. Two pots of each treatment were harvested till the end of vegetative stage (V4 stage) of the soybean, the other two pots were harvested till one normal pod on the main stem that has reached its mature pod color (R7 stage), and the left two pots did not plant soybean throughout.

Plants were carefully removed from the soils and were separated into leaves, stems, and roots in the first harvest; additionally, fruits were collected in the second harvest. Similar plant parts from each treatment were composited and homogenized. The above-ground parts and roots were washed carefully with distilled water. The fresh weights of the plants were recorded immediately using an analytical balance after the residual deionized water on the plant surface was wiped off. Part of the plant samples was weighed and oven dried at 60 °C for 4 days; and part of the samples was analyzed immediately. For chlorophyll analysis, fresh leaf samples at the same place from each treated sample, avoiding major veins, were cut into small pieces immediately following collection, and then treated with mixture of ethanol, acetone, and deionized water (4.5:4.5:1, V/V/V) at 45 °C in the dark till the leaves turned gray. The chlorophyll *a*, chlorophyll *b*, and chlorophyll *a*+b concentrations

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