Biogeochemical characterization of Vanadium in soils and its interaction with Fungi

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Summary

Vanadium widely disperses in soils, water and organisms. Natural vanadium in soils is primarily from parent rock, with average concentration of 90 mg/kg. Human activities such as fossil fuels, mining and industrial emissions often increase vanadium content in soils, and also its environmental risk and biological risk. Although vanadium has been recognised as the same class of pollutant as arsenic, mercury and lead, the researches of vanadium in soils are limited.

To better understand the biological risk of vanadium in soils and support pollutant control, we investigated the biogeochemical properties of vanadium in soils, and its interaction with fungi. The research is based on a vanadiferous titanomagnetite ore site in Panzhihua, China. The thesis comprises of three main chapters.

An optimized eight-step sequential extraction for vanadium fractionation in soils was proposed according to the urgent requirement of a sequential extraction specified for vanadium (Chapter 2). The suitability of the new scheme was confirmed by its higher selectivity against the target phases and higher extraction efficiencies (55%-77% of total vanadium) with model minerals and 6 soils of different properties than previous scheme.

We then analyzed binding phases, oxidation species, and bioavailable species and related control factors of vanadium in soil samples through the complementary methods including wet chemical experiments and X-ray techniques (Chapter 3). Proportion of bioavailable V was less than 10%, and negatively related with hydroscopic moisture content of soils. Vanadium(IV) was the dominant oxidation states in soils, while the ratio of H2O/EDTA extractable vanadium(IV) to vanadium(V) was negatively related with soil pH value. Furthermore, anthropogenic sources potentially increase both bioavailability and mobility of vanadium along soil verticals.

Finally, fungi (Amanita muscaria, Armillaria cepistipes, Xerocomus badius and Bjerkandera adusta) were cultured with a series of vanadium oxides (III, IV, V), vanadate, vanadyl and vanadium containing slag, separately (Chapter 4). Fungal accumulation ability was diverse according to the oxidation states and compound structures of vanadium. Conversely, biochemical composition, hyphae morphology and related biomass of fungi were influenced in different extents. A. cepistipes showed generally higher removal efficiency of vanadium than the other three species.
Zusammenfassung


Eine achtstufige sequenzielle Extraktionsmetode für die Vanadiumfraktionierung aus Böden wurde optimiert um dem Bedarf für eine vanadiumspezifische sequenzielle Extraktionsmetode decken (Kapitel 2). Die Tauglichkeit der neuen Methode wurde durch die höhere Selektivität für die extrahierbaren Phasen und einer höherer Extraktionseffizienz (55-70 % des gesamten Vanadiums) gegenüber älteren Methoden aus künstlichen Standartmineralien und sechs Böden mit unterschiedlichen Eigenschaften bestätigt.

Wir haben dann die unterschiedlichen Bindungsformen, Oxidationsstufen und bioverfügbare Komponenten und verwandte Kontrollfaktoren von Vanadium in den Bodenproben mit komplementären Methoden wie nasschemischen Verfahren und Röntgenverfahren untersucht (Kapitel 3). Der Anteil von bioverfügbarem V war kleiner als 10 % und negativ korreliert mit dem hygroskopischen Feuchtigkeitsgehalt der Böden. Vanadium(IV) war die dominante Oxidationsstufe in Böden und das Verhältnis von mit H₂O/EDTA extrahierbarem Vanadium(IV) und Vanadium(V) war negativ korreliert mit dem pH-Wert der Böden. Zudem erhöhen anthropogene Quellen potentiell die Bioverfügbarkeit und Mobilität von Vanadium in Bodenprofilen.

Im letzten Kapitel wurden die Pilze Amanita muscaria, Armillaria cepistipes, Xerocomus badius und Bjerkandera adusta separat mit einer Serie von Vanadiumoxiden (III, IV, V), Vanadaten, Vanadyl und vanadiumhaltiger Schlacke kultiviert (Kapitel 4). Die Akkumulierung in Pilzen war unterschiedlich zwischen den verschiedenen Oxidationsstufen und Bindungsformen von Vanadium. Im Gegensatz dazu wurde die biochemische
Zusammensetzung, die Morphologie der Hyphen und die entsprechende Biomasse der Pilze in unterschiedlichem Ausmass beeinflusst. *A. cepistipes* zeigte generell eine höher Mobilisierungseffizienz von Vanadium als die drei andern Arten.
Chapter 1

General introduction
Soil biogeochemistry

Biogeochemistry was founded by Ukrainian scientist Vladimir Vernadsky in 1926. American limnologist and geochemist G. Evelyn Hutchinson outlined its broad scope and principles. More recently, British scientist and writer James Lovelock restated the basic elements of the discipline of biogeochemistry. As defined by Journal of Nature, biogeochemistry is the study of how chemical elements flow through living systems and their physical environments. Physical, chemical, physical, biological and geological processes and reactions which influence the composition of natural environment (including the biosphere, the cryosphere, the hydrosphere, the pedosphere, the atmosphere, and the lithosphere) are involved. Generally, biogeochemistry concerns chemical cycles which are either have an impact on or driven by biological activity.

Soils are heterogeneous system mixed by air, water, inorganic and organic solids, and microorganisms (Sparks, 2003). These phases are necessary for the chemical reactions in soils, and also affected in the way of microorganisms function, plant growth, water quality and even air quality. The research of soils traditionally focused on the chemical reactions that affect plant growth and plant nutrition. Beginning in the 1970s and certainly in the 1990s, the concerns increased about inorganic and organic contaminants in soils and their impact on plant, animal and human health. The emphasis of soil chemistry is now on soil biogeochemistry like phytoremediation, micro-bioremediation, carbon sequestration and biogeochemical prospecting for ore deposits, et al (Alloway, 1995; Gadd, 2010; Lombi et al., 2001; Pulford and Watson, 2003; Sparks, 2003).

Metals in soils

Heavy metals are those elements having density greater than 5.0 g/cm³, e.g., arsenic, chromium, cadmium, lead, zinc, cooper, mercury and vanadium. A heavy metal is kind of a trace element which presents at a level <0.1% in natural materials such as the lithosphere (Sparks, 2003), and can be toxic to living organisms if the concentration is high enough (Adriano, 1986). Natural source of heavy metals in soils is primarily parent rock; other anthropogenic sources include liming materials, commercial fertilizers, irrigation waters, biosolids, auto emissions, coal combustion residues, smelting industries, and others (Sparks, 2003). Excessive input of anthropogenic metals results in soil pollution.

Metal speciation is an important concept in soil biogeochemistry since the toxicity, mobility and bioavailability of a metal largely depend on its chemical speciation. The
speciation of a metal includes: (1) the identity of a metal under investigation; (2) the oxidation states; (3) associations and possible complexes to solids and dissolved species (surface complexed, metal-ligand bonds, surface precipitates); (4) the molecular geometry and coordination environment of the metal (Sparks, 2003).

![Figure 1](image.png)

**Figure 1.** The mechanisms of ion sorption at the mineral-solution interface. Picture is modified after Charlet and Manceau (Charlet and Manceau, 1993).

As shown in Figure 1, metals in soils could exit as free ions in soil solution or complex with soil complexants including organic ligands and inorganic surface functional groups. Organic ligand could be an atom, functional group, or molecule attached to the central atom of coordination compound, such as carboxyl and phenolic groups of soils organic matter. Inorganic surface functional groups in soils mainly include the siloxane surface groups associated with the plane of oxygen atoms bound to the silica tetrahedral layer of a phyllosilicate, and hydroxyl groups associated with the edges of inorganic materials e.g. kaolinite, amorphous materials, metal oxides, oxyhydroxides, and hydroxides (Sparks, 2003). All the processes forming metal complex in soils could be called sorption phenomena. Sorption is a process describing a substance or material captured at an interface between the solid surface and bathing solution. It includes three phenomena which are adsorption, surface precipitation and polymerization. The movement of nutrients and contaminants in soils could
be affected by sorption condition. Hereby, the quantity of plant nutrients, pesticides, metals, and other organic chemicals in soils will be influenced.

Various mechanisms including physical and chemical forces are involved in sorption phenomena in soils (Figure 1). Free ion in soil solutions could be adsorbed and form outer-sphere complex via Waals forces (e.g., partitioning) and electrostatic interaction (e.g., ion exchange). After loss of hydration water, an outer-sphere complex will transform to inner-sphere complex. And then the inner-sphere complex could diffuse and form isomorphic substitute within mineral lattice. On another hand, rapid lateral diffusion could help the inner-sphere complex to form a surface polymer or adsorb on a ledge which maximizes the number of bonds to the atom. And then, surface polymers will end up embedded in lattice structure as a result of the particle growth. Finally, the adsorbed ion can also diffuse back in solution as a result of dynamic equilibrium or as a product of surface redox reactions (Sparks, 2003). The stability of a complex could be affected by the electron availability (Bruemmer et al., 1988; Fendorf et al., 1997; Gehring et al., 1993; Kaur et al., 2009; Manning et al., 1998). It explains why the oxidation states of a metal affect its sorption condition in soils. Oxidation states of the metals are in close contact with their solubility and mobility in soil and aqueous environments. Another variable affecting the solubility and availability of a metal in soils is soil pH. Soil pH affects many soil chemical reactions and processes (Sauve et al., 2000; Sparks, 2003).

**X-ray absorption spectroscopy**

X-ray absorption spectroscopy was developed in the early 1970s (Sayers et al., 1971). X-ray is electromagnetic radiation (“light”) with specific particle termed photon and wave properties, and therefore is characterized by a range of wavelengths and photon energies. X-ray produced by synchrotron, a particular type of cyclic particle accelerator, will penetrate matter and interact with the core electrons of an atom. When the core electron is excited into higher energy electron orbitals that are unoccupied or into the continuum where the electron is no longer associated with the atom, X-ray absorption happens (Figure 2). As the electron leaves, a hole is left in the inner atomic shell, and subsequently filled by an electron from an outer shell. The energy is therefore released as a fluorescence photon. The energy of the fluorescent X-ray for a given electronic transition is unique for each element as the electron orbital energies of each element are unique (Kelly et al., 2008). Quantitatively absorption is given by the so-called absorption coefficient $\mu$. A specific energy is required for an electron to escape to an unoccupied orbital. When the incident X-ray energy achieves this requirement, the probability
for absorption sharply increases. These steps appeared as absorption edge in the absorption coefficient.

**Figure 2.** Bohr model of an atom. Bohr model was built by Niels Bohr in 1913. Picture is from Kelly et al., 2008.

As shown in an example of an X-ray absorption edge of NiO (Figure 3), very close to the edge \( (E_0) \) the absorption coefficient exhibits a very intense peak so-called “white line”. Absorption spectrum near the absorption edge, ranging from approximately -50 to +200 eV relative to the edge energy, named X-ray absorption near edge structure (XANES). The energy of the edge position for a given element increases with increasing valence of the absorbing atom, thus XANES is useful for extracting the oxidation state and the densities of unoccupied states, as well as the binding geometry (e.g. bonding angles and coordination) of the element in soil samples (Kelly et al., 2008). At X-ray energies above the absorption edge, the photoelectron is excited into the continuum and it will interact with the surrounding atoms. Extended X-ray absorption fine structure (EXAFS) is the normalized oscillatory part of the absorption coefficient above the absorption edge to about 1000 eV or higher. The border between the XANES and the EXAFS region is smooth rather than being clearly defined. The information of the types and numbers of atoms in coordination with absorber atoms, their interatomic distances, and the degree of local molecular bonding disorder could be explained through EXAFS spectrum (Kelly et al., 2008). As a result, the molecular bonding environments of elements in soils could be determined.
**Microorganisms and metals**

Interaction between microorganisms and metals/minerals is the basis of geomicrobiology (Banfield and Nealson, 1997; Ehrlich et al., 2015; Konhauser, 2009) and bioremediation (Gadd, 2010; Romantschuk et al., 2000; Watanabe, 2001). The interaction happens both inside and outside cell (Figure 4). As a response to metal stress, microorganisms produce metabolisms and hereby change the physic-chemical microenvironmental conditions around the biomass. Metabolic reactions can solubilize metals, e.g., chemolithotrophic (autotrophic) and chemooorganotrophic (heterotrophic) leaching, siderophores and other complexing agents, methylation and demethylation, and biodegradation of organo-radionuclide complexes. These processes are the keys of bioweathering. On another hand, metabolic products such as sulfide and oxalate, and redox reactions can cause metal precipitation and form biominerals. Metals can also be adsorbed to cell walls, exopolymers which are secreted by organisms, and other structural components and derived/excreted products; and retained as colloids and particulates. Most metals are essential nutrients for microorganisms; inessential metal species can also be taken up (Gadd, 2010). Intracellular accumulation including deposition, localization and sequestration is another kind of metal immobilization. Besides the mobility and bioavailability
Introduction

of metals can be reduced through the transformation by microorganisms, detoxification of metals is also possible through redox reaction or precipitation. This is the rationale for the application of microorganisms to bioremediation. Furthermore, a number of mechanisms could be operated based on organism and cellular environment (Gadd, 2010). As a feedback of metal detoxification and transformation, microbial growth, activity and survival will be affected by metals and minerals (Fomina et al., 2005; Meharg, 2003; Op De Beeck et al., 2015; Rathnayake et al., 2013). Metal tolerance therefore is an important parameter which should be considered during the application of microorganism for soil pollution treatment and metal recycling.

Fungi are eukaryotes with complex cell structures. Fungi can form tissues and organs, and comprise what is arguably the most pivotal kingdom of organisms with about 1.5 million species on the earth (Hawksworth, 2001; Moore et al., 2011). Fungi widely spread in every terrestrial ecosystem as mutualist partners, pathogens, parasites or saprotrophs (Moore et al., 2011). They plan an important role in element cycling and mineral formation. Such properties provide the rationality of the application of fungi to metal cycling in industry and soil bioremediation.

Figure 4. Mechanisms involved in the transformation of metals by microorganisms.
Vanadium

Vanadium, with symbol V and atomic number 23, is a transition element in group 5 of periodic table of elements. Its density is 6.1 g/cm³ at 20 °C. The melting point of vanadium is 1910 °C; the boiling point is 3407 °C. To a similar level of zinc and nickel, average crustal abundance of V is approximately 150 μg/g (Byerrum et al., 1974). The amount is also about twice amount of copper, 10 times amount of lead, and 100 times amount of molybdenum (Reimann and De Caritat, 2012). However, vanadium is more dispersed in crust rather than concentrated in mineral deposits (Huang et al., 2015). Compounds of V were discovered by Andrés Manuel del Río in Mexico in 1801. Swedish chemist Nils Gabriel Sefström proved the new element and named “vanadium” after the Scandinavian goddess of beauty and fertility in 1830. However, the first report of V in organism was published by German chemist Martin Henze until 1911. The high levels of V in blood cells was firstly reported thanks to the ascidian (tunicate) collected in the Gulf of Naples.

Vanadium is an essential element for organisms, but it will also be toxic at a specific concentration (Lazaridis et al., 2003). Anthropogenic sources such as mining and smelting activities, industrial emissions, batteries discarding, and fossil fuel combustion have increased the content of vanadium in environments as well as the biological risk for organisms. Vanadium is widely used in high strength-low alloy steels, non-ferrous metals, alloys, batteries, and chemical industry. These used productions have regarded as “waste materials” because of the low rates of recycling rather than “surface mines” (Graedel, 2011; Reck and Graedel, 2012). Recycling metals is also an important way to reduce the emissions of pollutants (Reck and Graedel, 2012). Recently, the end-of-life recycling rate (the percentage of a metal in discards that is actually recycled) of vanadium is less than 1% (Graedel, 2011).

Panzhihua is one of the most important vanadiferous titanomagnetite ore in the world. It locates in south-western China of 26°05’~27°21’ N and 102°15’~108°08’ E (Figure 5). Teng et al. (Teng et al., 2011a; Teng et al., 2011b) has found a high amount of vanadium in soils in Panzhihua. It is meaningful to study vanadium based on this region.
Introduction

Figure 5. Location of Panzhihua in China (National Administration of Surveying, Mapping and Geoinformation, GS (2008) 1153).

Research hypothesis and procedure

Original research questions include two parts: (i) what is the vanadium mobility and bioavailability in soils? (ii) are there any possibilities to apply fungi to remediate polluted soils and recycle vanadium in wastes? What is the role of fungi in transport and transform of vanadium in environments? We performed field investigation and sample collection in Panzhihua in 2011. The experiments were carried out in the lab based on analyses and reviews of literature. The content of each main chapter is following, Chapter 2: development the analysis method of vanadium phases in soils; Chapter 3: biogeochemical characterization of vanadium in soils based on a set of complex techniques; Chapter 4: investigation of fungal interaction with vanadium. Findings from chapter 2 and chapter 3 are the basis of chapter 4.
References

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Chapter 2

An optimised sequential extraction scheme for the evaluation of vanadium mobility in soils

(Journal of Environmental Sciences, in press)
Chapter 3

Mobility and bioavailability of vanadium in soils from vertical and horizontal geochemical characterization
Abstract

A series of soils from vanadiferous titanomagnetite ore mining area and smelting area, wetland, forest, and farmland were collected for the biological risk analysis of vanadium in soils. Comprehensive experimental methods including sequential extraction, high-performance liquid chromatography and X-ray absorption spectroscopy were applied for the analysis. Results of sequential extraction indicated that dominant phases of vanadium in soils were crystalline (32±6.5%) and residual (35±11%) fractions, while the least phases were water-soluble vanadium (0.41±0.34%) and adsorbed vanadium (0.79±0.94%). Average proportion of organic matter fraction was 7.5±8.4%. Multiple linear regression suggested that the proportion of bioavailable vanadium was negatively related with hydroscopic moisture content of soils. Vanadium(IV) was the dominant oxidation states in soils, but vanadium(V) was the main part of H$_2$O/EDTA extractable vanadium for most soil samples. Further analysis of multiple linear regression suggested that the higher ratio of H$_2$O/EDTA extractable vanadium(IV) to vanadium(V) was significantly related with lower soil pH value. Furthermore, anthropogenic sources such as deposition of smelting fly ash, raising dust of mineral and slags were observed containing more bioavailable vanadium and toxic vanadium(V) than mining soils, and potentially increasing both bioavailability and mobility of vanadium along soil verticals.

Introduction

Vanadium (V) occurs commonly but not uniformly in the earth’s crust, with average concentration of 150 μg/g (Byerrum et al., 1974). Global average V concentration in soils is 90 mg/kg (Huang et al., 2015). Enrichment of V in soils is resulted from geogenic enrichment and anthropogenic activities such as fossil fuels, mining and industrial activities (Reimann and De Caritat, 2012; Teng et al., 2011). Vanadium has been listed on the United States Environment Protection Agency candidate contaminant list 2. Although soil V pollution has not a global scale and an urgent environmental threat, the presence of V in soils raises concerns because of its toxicological effects to ecosystems (Yang et al., 2014).

Biological risks of V in soils are not only related with V content in soils, but also on V phases and oxidation valence (Alloway, 2013; Panichev et al., 2006; Shaheen and Rinklebe, 2014; Stalikas et al., 1999; Tian et al., 2015; Zhang et al., 2014). Metals in soils could be adsorbed to soil particles by cation/anion exchange, complexed with soil organic matter or precipitated with iron, aluminum and manganese oxides (Alloway, 2013; Bacon and Davidson, 2008; Xu et al.). However, uptake and availability of metals by plants is greatly affected by the
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metal solubilized, exchanged and bound with soil organic matter (Sparks, 2003). At the same time, mobile metal pool could also potentially be leached from the contaminated soil to groundwater and surface water (Sauve et al., 2000). Vanadium formed compounds are dominated by valence states IV and V rather than valence states II and III which are rare and oxidized by air easily (Wehrli and Stumm, 1989; WHO, 2000). Vanadium(V) are considered particularly more toxic than V(IV). It has been proved that vanadate ion is a strong inhibitor than V(IV) for enzyme Na and K-ATPase (Patel et al., 1990). Mutagenic effects, respiratory tract toxicity and possible carcinogenic activity have also been found for V(V) (Shafer et al., 2011).

Major methods for determining oxidation states of V are high-performance liquid chromatography (HPLC) and X-ray absorption spectroscopy (XAS). Spectrophotometry is widely used due to its high sensitivity and selectivity (Taylor and Van Staden, 1994), but it has a limitation of potential redox reaction caused by high temperature and aqueous condition during the sample preparation (Huang and Kretzschmar, 2010). Synchrotron analysis does not need to change the material status but it is not easy to access. The extended X-ray absorption fine structure (XAFS) detection of V in soils will be interfered by the associated titanium (Ti) since Ti K-edge (4966 eV) is very close to V K-L3 (4953 eV), but X-ray absorption near edge structure (XANES) has been applied successfully for the detection of V oxidation states in natural soil and dust samples (Burke et al., 2012; Shafer et al., 2011). In this research, we planned to integrate both methods for the analysis of V species in soils.

Binding phases and oxidation species of V in soils could be affected by soil pH, soil organic matter as well as soil particles (Huang et al., 2015; Larsson et al., 2013; Reijonen et al., 2016; Sauve et al., 2000). Sauvé et al. (Sauve et al., 2000) compiled over 70 researches of various origins of cadmium, copper, lead, nickel and zinc, and found that the mobility and bioavailability of metals in soils were best predicted with pH and either the log of soil organic matter or the log of the total metal. Wang et al. (Wang and Mulligan, 2006) found that arsenic sorption in soils was influenced by arsenic speciation and site geochemical conditions such as pH, redox potential, co-occurring ions and even microbial activity. Lin et al. (Lin and Puls, 2000) also found a lower adsorption of clay minerals to arsenic(III) than arsenic(V). The adsorption was affected by pH.

Few studies performed in the greenhouse have analyzed the relationship between V species and plants (Larsson et al., 2013; Panichev et al., 2006; Reijonen et al., 2016; Tian et al., 2015; Tian et al., 2014), but the complex findings of V binding phases, bioavailable species and control factor based on field investigation are still limited. Based on a group of soils with
various V content, soil properties and anthropogenic influences, we investigate V binding phases and oxidation species in soils through spectrophotometry and synchrotron. Oxidation species of V in bioavailable and mobile fractions are further analyzed. Finally, factors affecting mobility and bioavailability of V in soil profiles or different sites are discussed, separately.

Materials and methods

Sample collection

Fifteen soil samples of diverse geochemical characteristics were used in this study (Table 1). A permanently anoxic wetland soil (WL, 50-60 cm, Fibric Histosol,) and two well-drained forest soil (FA, 0-12 cm; FB, 12-30 cm; Haplic Podzol) were sampled in the Lehstenbach catchment in northeast Bavaria, Germany. A seasonal paddy soil (PA, 30-35 cm, Hydragric Anthrosol) was sampled after rice harvest in the Munshiganj district where is about 30 km south of Dhaka, Bangladesh. A purple soil (PP, 0-20 cm) was collected from a wild land in Maotai, China. The sample SB (0-20 cm) was a greenhouse soil after harvesting soybean (provided by Prof. Jinyan Yang from Sichuan University, China). The other nine soil samples (Xigeda soil) were all from Panzhihua, China, which is a representative vanadiferous titanomagnetite (V-Ti) ore mining and smelting area. Sample group of M1, M2 and M3 was a soil profile (0-20 cm, 20-40cm, 40-60 cm, respectively) from a wild land covered by shrubs and grass besides the opencast pit. Sample group of S1, S2 and S3 was also a soil profile with same depth but from the smelting processing area. Sample S0 (0 cm) was the overlaying black fine material on top of S1. Another wild land soil (MY, 0-20 cm) and farmland soil (FY, 0-20 cm) were also collected in Panzhihua. As the most famous base of V-Ti production in the world, Panzhihua V-Ti magnetite \([\text{Fe(V,Ti)}_3\text{O}_4]\) deposit provides 20% Fe, 64% V, and 53% Ti supply for China. All the soil samples were homogeneously mixed with three random samples from each sample site. Soil samples were frozen dried and then sieved to 2 mm. All the samples were stored at 4°C before analysis.

Soil V content and fraction analysis

All reagents used in this study were of at least analytical grade and diluted to the required concentration with de-ionized water. Total V in soils was detected by X-ray fluorescence spectrometry (XRF; Spectro-X-Lab 2000, Kleve, Germany). Soil hydroscopic moisture content and organic carbon content were measured according to ASTM D 2974-87 standard (ASMTD2974-87, 1993). Soil pH value was measured by the 1 M KCl method (NATESC, 2006). Combination species of V in soils were analysed via an optimized sequential extraction
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(53) (Xu et al.) which sequentially extracted soil V into water-soluble, strongly adsorbed, organic matter binding, Mn oxides, very poorly crystalline, poorly crystalline, crystalline Fe and Al (hydr)oxides and residual fractions. The SE experiment involved lab-shaker (LS-W, Kühner AG, Basel, Switzerland), centrifuge (Sigma 4K10, VWR International, Germany) and microwave pressure digestion system (MWS-1, Berghof, Florida, USA). Analyses of V in every extractant was performed with an ICP-MS (Agilent 7500ce, USA) by monitoring $^{51}\text{V}$ and using He collision cell to eliminate the ClO$^+$ interference (D’Illo et al., 2011; Rousis et al., 2014).

Analysis of Vanadium Oxidation Species

Pentavalent vanadium (V(V)) in soils was extracted by boiled 0.1 M Na$_2$CO$_3$ (Mandiwana and Panichev, 2004) and detected by ICP-MS. EDTA-extractable and H$_2$O-extractable tetravalent vanadium V(IV) and V(V) were also detected, separately. We firstly added 15 ml of 0.05 M EDTA to 0.5 g dry soil in a 50 ml centrifuge tube and vortex mixed the mixture. After 10 min sonication, we took 0.5 ml suspension to combine with 0.5 ml mobile phase solution (3% acetonitrile, 2 mM EDTA and 80 mM NH$_4$HCO$_3$ at pH 6) (Li and Le, 2007) in a micro-tube. The mixture was then heated at 60°C for 1 hour and centrifuged for 4 min at 100% (Hettich Zentrifugen D-7200, Tuttlingen, Germany). The supernatant was collected for HPLC-ICP-MS (Agilent 7500ce, Cetac ASX-510, USA) detection of V(IV) and V(V) immediately, following the method modified from Li et al (Li and Le, 2007). H$_2$O extraction was produced by 0.5 g dry soil with 7.5 ml deionized water. The other procedures were as same as the EDTA extraction.

Vanadium species analysis with X-ray absorption spectroscopy

We collected XANES data at V K-edge (5465 eV) for selected soil samples and reference materials at station BL17C1 in National Synchrotron Radiation Research Center (NSRRC), Taiwan. Soil samples M1, S0, S1 and S3 were measured. Related soils after the sequential extraction step of 0.2 M oxalate buffer (crystalline and residual fractions) and 4 M HCl (residues), separately, were also applied for the detection. Standard spectra were collected from a range of laboratory chemicals as V$_2$O$_5$, VO$_2$, V$_2$O$_5$, VOSO$_4$ and NaVO$_3$. Additional standards including goethite-V(IV)/V(V), boehmite-V(IV)/V(V) and EDTA-V(IV)/V(V) were prepared via fully mixing each adsorbent with vanadyl/vanadate and de-ionized water (1:1:10), respectively, and then collecting the precipitate after centrifuging. Finely powered soil samples and reference materials were thin layered to $\Phi$ 1 cm round on Kapton polyamide film before measurement. The normalization and analysis of XANES spectra were processed via software
Athena 0.9.20. Linear combination fitting (LCF) of Athena was applied to figure out the quantities of V oxidation states and potential molecular structures in soil samples. Function termed “fitted combinations” of LCF was performed for each sample; and then the fit with higher R-factor and spectra coincidence were chosen.

**Statistical Analysis**

Multiple linear regression was applied to figure out the factor affecting the bioavailability of V and the distribution of oxidation species of V in soils, separately. The data were natural log transformed before analysis. Regression method was “enter”. The statistical analyses were performed through software SPSS 22.

**Results and discussion**

**Soil characteristics**

As shown in Table 1, vanadium content in soils from mining area (M1-M3, MY) were generally higher than other samples. Vanadium content from M1 to M3 lightly increased along the soil layers. The overlaying material S0 from smelting area was rich in V and even higher than the V content in mining soils. However, the V content in its following soil layer S1 suddenly decreased and then gently decreased in the deeper depth from S2 to S3. Content of Fe, Mn in soils, soil pH and moisture content presented significant positive correlations (P < 0.01) with V content, respectively, while the content of Al and Si in soils was negatively correlated with soil V content at 0.01 levels. Soils from V-Ti mining area (M1-M3, MY, FY), smelting area (S1-S3) and purple soil (PP) presented much higher pH (>7) and higher content of V, Fe and Mn than other samples.

**Sequential distribution of vanadium in soils**

The least phases of V among all the soils samples were water-soluble V (0.41±0.34%, mean±SD) and adsorbed V (0.79±0.94%). The dominant phases were crystalline (32±6.5%) and residual (35±11%) fractions. The proportion of V corresponding with Mn oxides (1.8±2.2%) was much less than the proportion of V related with very poorly crystalline (7.6±5.2%), poorly crystalline (15±12%) and crystalline (32±6.5%) Fe and Al (hydr)oxides. The average proportion of organic fraction was 7.5±8.4%. Sample SB, WL and FA presented much more organic V than other soils, while sample FB appeared higher proportion of strongly adsorbed and Mn oxides fractions (Figure 1).
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Table 1. Characteristics of Soil Samples. M1-3: 0-20 cm, 20-40cm and 40-60 cm samples from V mining area, S0-3: 0 cm, 0-20 cm, 20-40cm and 40-60 cm samples from V smelting area, PP: 0-20 cm purple soil, MY: 0-20 cm wild land soil, FY: 0-20 cm farmland soil, PA: 30-35 cm paddy soil, SB: 0-20 cm soybean soil, WL: 50-60 cm wetland soil, FA: 0-12 cm forest soil, FB: 12-30 cm forest soil.

<table>
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<tr>
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<th>V (mg/kg)</th>
<th>Hydroscopic moisture content (%)</th>
<th>Organic C content (%)</th>
<th>pH</th>
<th>Fe (%)</th>
<th>Mn (%)</th>
<th>Al (%)</th>
<th>Si (%)</th>
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As shown in Figure 1, vanadium in mining soils was mainly related with Fe and Al (hydr)oxides. The sequential distribution was generally independent with soil depth except the organic fraction which presented a lightly increasing trend following the soil depth. Compared to mining soils, the proportion of water-soluble, strongly adsorbed, organic, Mn oxides and very poorly crystalline fractions were much higher in the overlaying material S0, while the proportion of crystalline and residual fractions were less. In the following soil layers S1, the proportion of former six fractions gently decreased and the proportion of crystalline and residual fraction increased. This trend continued in the deeper layers S2 and S3 except the proportion of organic fraction which lightly increased along soil layers.

Water soluble, strongly adsorbed and organic matter fractions are together termed bioavailable fraction. Sample S0 contained the highest amount of bioavailable V, and the smelting soils contained more bioavailable V than the mining soils (Figure 2). Although the V
content in SB was much less than most samples, the bioavailable V concentration in SB was more than other soils.

![Figure 1](https://via.placeholder.com/150)

**Figure 1.** Relative amount of V sequential extracted from soils. Sample definitions are given in Table 1.

![Figure 2](https://via.placeholder.com/150)

**Figure 2.** Amount of bioavailable V in soils. Sample definitions are given in Table 1.

**Vanadium(IV) and Vanadium(V) Species in Soils**

Besides sample S0, SB, FA and FB, other soil samples only contained less than 10% V(V) (Figure 3) which is less absorbed than V(IV) (Huang et al., 2015). Like the trend of V content,
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The proportion of V(V) varied little along soil profiles in mining area (M1-M3), and the content of V(V) sharply decreased under the surface material S0 in smelting area.

Figure 3. Relative amount of V(V) in soils. Sample definitions are given in Table 1.

Figure 4. Relative amount of H2O extractable and EDTA extractable V(IV) and V(V) in soils. Sample definitions are given in Table 1.

Among H2O extractable V, sample SB, WL, FA and FB were dominated by V(IV), while other soils mainly contained H2O-V(V) (Figure 4). Among EDTA extractable V, vanadium(V) was the domination although sample SB, WL, FA, FB and S0 showed relatively more V(IV) than others. Along the vertical soil layers, the relative amount of V(IV) and V(V)
did not change a lot in both tow sites (M and S). Only the surface material S0 contained much more EDTA-V(IV).

Synchrotron based speciation

Vanadium XANES spectra can not only discriminate vanadium valence states of V(III), V(IV) and V(V), but also distinguish molecular structure between different compounds with the same oxidization state, i.e., VO$_2$ and VSO$_5$, V$_2$O$_5$ and NaVO$_3$ (Figure S1). Besides the diversity of the absorption edge position, peak density and spectra shape, the intensity of pre-edge peak increased with higher V oxidation states and its coordination (Burke et al., 2012; Shafer et al., 2011; Wong et al., 1984).

As shown in Figure 5, soil from mining area (M1) presented a sharp resonance peak but very weak pre-edge peak, while the spectrum of S0 was opposite. The crystalline and residual fractions of soils also showed differences at the peak intensity compared to the original soils. Such differences were more significant among smelting soils, of which the intensity of pre-edge peak was gradually lower while the absorption peak became sharper in crystalline and residual fractions.

Although we analyzed through the method of fit all combination, there were still some mismatches between the best fit and XAS data (Figure 5). It indicated that the chemical compositions of samples were more complex than the references presented (Shafer et al., 2011). As a result, we simplified the fit and focused on the oxidation states firstly. The proportion of oxidation states in soils showed that the dominant states was V(IV) and then V(III) (Figure 6). The proportion of V(III) was more in mining soils than smelting soils. Vanadium(V) was only appeared in sample S0 and S1, and the proportion decreased along the layer depth. These findings were in accordance with the analysis of oxidation species from Na$_2$CO$_3$-ICP-MS and HPLC. Furthermore, the proportion of V(III) gradually increased from original sample to extracted samples (CR and R) especially for smelting soils. This is reasonable as V(III) is mainly captured in mineral lattice which is difficult to be extracted (Gehring et al., 1993; Klein et al., 1993; Schwertmann and Pfab, 1994).

The combination fit of all the references could also give the information about the differences in V binding phases and prove the diverse of V fractions according to sample site and extraction steps (Figure 5 and Table S1). Only fit of S0 included NaVO$_3$, reflecting the high content of soluble V(V). This finding was consistent with higher amount of water soluble, strongly adsorbed and organic matter fractions in S0 from sequential extraction analysis, as well as the higher amount of H$_2$O/EDTA extractable V in S0. The XANES species further identified that S0 was input material which could be attributed by anthropogenic sources such
as deposition of smelting fly ash, raising dust of mineral and slags from the smelting area. The spectra comparison between S0 and S1, S3 also certificated the influence of the surface material to the under layers. And the influence mainly caused by the high mobile fractions, e.g. soluble and organic matter fractions. It was suggested that the synchrotron techniques was effective to identify the more stable and major component of metal species in soils, while the bulk extraction method was better effective for the more labile phases (Terzano et al., 2007).

On the other hand, there were some divergences of the proportion of oxides references between the simple and complex fits although they presented the same distribution of the oxidation species. The weight of each reference was not the absolute but relative amount depending on the real amount and structure of V chemical compounds in samples.
Figure 5. V K-edge XANES spectra of selected soils and their precipitate after specific extraction. Red dot lines are the best fits among all the references analyzed through fit all combination of LCF; Sample definitions are given in Table 1. Based on sequential extraction, CR represented crystalline and residual fraction of soil after the extraction of 0.2 M NH$_4^+$-oxalate buffer; R represented residual fraction of soil after the extraction of 4M HCl.
Figure 6. Proportion of V oxidation states in soil samples analyzed by LCF with oxides. The weights sum was forced to 1. Sample definitions are given in Figure 5.

Vanadium mobility in vertical soil profiles

Vanadium could leach from soils, shown as the increasing of V content and bioavailable V along soil depth from M1 to M3 (Table 1 and Figure 2). The leaching ability could be promoted by drought and rewetting (Yang et al., 2014). The clear division of dry season and wet season (June to September) in Panzhihua region contributes to the leaching ability of V along soil layers. The overlaying material S0 contained much more V in both movable and less stable fractions than soil M (Figure 1 and Figure 5). Material S0 improved the bioavailable V content in soil S1, but the amount of each fraction decreased following the soil depth (from S1 to S3). It indicated that the interception as adsorption and precipitation of V caused by soil organic matter, Mn oxides and Fe-Al oxides (Martin and Kaplan, 1998) surpassed the V leaching from S0. However, the bioavailable amount of V in soil S was still higher than soil M even the V concentration in soil S was less (Figure 2).

Compared to V(V), the vertical migration of V(IV) was weak (Figure 4). It is known that V(IV) oxycation VO$_2^+$ forms more stable complexes with organic compounds, as compared to vanadate oxyanions (Wehrli, 1987). Furthermore, vanadium(IV) could be oxidized to V(V) in the condition of pH value greater than 8 (Huang et al., 2015; Wehrli and Stumm, 1989), or reduced to form hydroxide of V(III), V(OH)$_3$(s), at relatively low concentrations at 25 °C (Huang et al., 2015). Another reason could be the stronger sorption for
V(IV) by the crystalline lattice as the structural stability of goethite-V(IV) is higher than goethite-V(V) (Kaur et al., 2009).

Compared to V(IV), vanadium(V) is more toxic (Patel et al., 1990) and more active to be involved in the soil-plant interaction (Tian et al., 2015). Generally, the anthropogenic input could increase not only the bioavailable amount of V but also the proportion of V(V) in soils. As a result, the biological risk of V in soils could be increased, especially in the shallow layers.

Vanadium bioavailability affected by soil characterization

Soil properties are important factors modifying metal bioavailability to ecological receptors (Bradham et al., 2006). Multiple linear regression analysis for soil properties omitted sample S0 which was mainly minerals rather than soils. From the result of the regression, the ratio of H₂O/EDTA extractable V(IV) to V(V) was significantly affected by pH value rather than organic C and hydroscopic moisture of soils (Table 2). Most soil samples were dominant with bioavailable V(V), but the proportion of bioavailable V(IV) increased as the soil pH value declining (Figure 7 a). Only soil WL, FA and FB presented the ratio of more than 1 (Figure 4). Soil WL, the wetland soils, even contended 3 times of H₂O/EDTA-V(IV) compared to H₂O/EDTA-V(V) (Figure 4). In reducing environments, V exists as vanadyl (VO²⁺) and strongly complexed by organic matter (Amrhein et al., 1993). Anaerobic condition combining low pH promoted the reduction of V(V) to V(IV) in wetland soils. On another hand, the paddy land soils (PA) was of neutral pH, but H₂O/EDTA-V(V) was in a more dominant position in PA than other soils. The growth of rape (Brassica juncea L.) substantially reduced the concentrations of V(V) but not V(IV) in the rhizosphere soil (Tian et al., 2015). Our results indicated that the growth of paddy promoted the oxidation of bioavailable V(IV) to V(V) and therefore increased the biological risk of V in soils. The V concentration and distribution in paddy plant could be worth further investigation.

<table>
<thead>
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<th>Table 2. Results of Multiple Linear Regression</th>
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<td>Sum of Squares</td>
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<td>----------------</td>
</tr>
<tr>
<td>H₂O/EDTA-V(IV)/V(V) Regression</td>
</tr>
<tr>
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</tr>
<tr>
<td>Bioavailable V Regression</td>
</tr>
<tr>
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*: the significant level is at 0.05  
**: the significant level is at 0.01
The relative amount of bioavailable V was negatively related with hydrosopic moisture content of soils rather than soil pH and organic C content (Table 2). Higher hydrosopic moisture content reflects stronger adsorption ability of soil particles which is caused by the molecular attraction and naturally occurring electrical forces (Veihmeyer, 1956). Stronger sorption ability of soils decreased the relative amount of bioavailable V (Figure 7 b). Larsson et al. (2013) also found that the V toxicity to plants decreased with a stronger soil sorption strength. Among all the soil samples, the soil grown with soybean showed the highest proportion of bioavailable V ignoring the hydrosopic moisture content. The structural and electronic properties of vanadate are in analogy to nutrient element phosphorus (Bowman, 1983). A bacteria strain (*Pantoea agglomerans*) which solubilized various insoluble inorganic phosphates was isolated from soybean rhizosphere (Son et al., 2006). Our result indicated that the growth of soybean potentially increased the mobility and bioavailability of V in soils. The vertical movement of V could be increased, but the V accumulated in soybean should be further studied in order to better understand the feedback to soybean growth.

![Figure 7. Scatter plots with fit lines and confidence bonds. a: linear regression between soil pH and ratio of H$_2$O/EDTA extractable V(IV) to V(V); b: linear regression between soil hydrosopic moisture and relative amount of bioavailable V.](image)

**Acknowledgements**

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Bayreuth, Germany) for the support with HPLC and ICP-MS measurement. This work was supported by the China Scholarship Council (No. 2011624130).
References


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Supporting information

Figure S1. V K-edge XANES spectra of standards.
Table S1. Weights of standards from V K-edge XANES linear combination fitting.

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<tr>
<th></th>
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<th>Boehmite-V(V)</th>
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<th>Goethite-V(V)</th>
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<th>VSO_5</th>
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*: the weight was forced between 0 and 1
Chapter 4

Interaction of Basidiomycota with vanadium

(Submitted)
Abstract

Basidiomycota present an important function in metal cycle in terrestrial environments. A group of Basidiomycota including *Amanita muscaria*, *Armillaria cepistipes*, *Xerocomus badius* and *Bjerkandera adusta* was exposed to a series of vanadium oxides (III, IV, V), vanadate, vanadyl and vanadium containing slag which represented various oxidation states and molecular structures of vanadium. All species were able to accumulate vanadium although the ability varied according to the oxidation states and compound structures of vanadium. The competitive biosorption of vanadate compared to phosphorus should be involved as an important process. Oxidation reaction was also observed for vanadium of lower oxidation states. Organic acids excreted by fungi were able to solubilize the vanadium oxides. However, vanadium in slag did not be significantly solubilized and mobilized by fungi, which could be caused by the stable crystalline structure and the interference of other metals in the slag. The pigment produced by *B. adusta* under the stress of vanadyl was supposed to be a complexing agent for the extracellular crystal particles. Biochemical compositions, hyphae morphology and related biomass were influenced to different extents based on fungal species and vanadium compounds. Combining accumulation capacity and tolerant biomass, *A. cepistipes* presented generally higher removal efficiencies. Our research demonstrates for the role of Basidiomycota in vanadium cycling, such as mobilization, biosorption, transformation and biomineral formation. In perspective, the findings might indicate the potential of Basidiomycota in the bioremediation of contaminanted sites and in the microbe-mediated industrial recycling of vanadium.

Introduction

Fungi play an important role in environmental processes like biogeochemical cycling of element, metal and mineral transformations, bioweathering and mycogenic biomineral formations (Ceci et al., 2015; Gadd, 2010, 2013; Wei et al., 2013). Organic acids and other metabolisms excreted by fungi can change the pH of the substrate, induce redox reaction of metal, exchange protons, and form nanoparticles (Gadd, 2000, 2010, 2013). Solid mineral consituents could be solubilized altering metal mobility and speciation (Ehrlich et al., 2015; Gadd, 2010, 2013). Such metabolic also suggest potential contributions of fungi to bioremediation and biotechnological techniques for metal recycle from solid wastes (Brandl, 2008). However, fungal metabolism production, hyphal size, mycelia morphology, and the interaction with metals might be affected by metal species and molecular structures related to
vanadium (Ceci et al., 2012; Fomina, 2002; Gadd, 2010; Jarosz-Wilkołazka and Grą, 2006; Nies, 1999).

Vanadium (V) is a bright white ductile metal belonging to group 5 of the periodic system of elements (WHO, 2000a). The average concentration of V is 150 μg/g in the earth’s crust (Byerrum et al., 1974), but varies 3-310 μg/g in soils (Waters, 1977). Natural sources of V in soils are mainly crustal weathering and volcanic emissions. Human activities in recent years (e.g., combustion of fossil fuels, mining, steel-iron refining, dyeing) heterogeneously added V to soils and often result in soil contamination (Reimann and De Caritat, 2012; Taner, 2002; Teng et al., 2011a; WHO, 2000b). The toxicity of V is in the same order of mercury, lead and arsenic (Lazaridis et al., 2003). Besides the excess amount, element speciation and binding states are determining mobility and biological risk of V in soils (Bacon and Davidson, 2008; Pueyo et al., 2008; Stalikas et al., 1999). Normally, metals in soluble phase or bound with organic matter show a higher biological risk because of the increased bioavailability and mobility (Sparks, 2003). Most V in soils are relatively stable as captured in crystalline structures (Teng et al., 2011a; Teng et al., 2011b; Yang et al., 2014), but this part of V might also be released via the pH change of soils, ion exchange and redox process (Sparks, 2003). Vanadium in soils mainly in the valence states of IV and V (Yang et al., 2010), and V(III) can also be found in the lattice of soils minerals (Klein et al., 1993; Schwertmann and Pfab, 1994).

Some fungi can accumulate V or leach V from solid wastes. A group of saprotrophic fungi have developed a certain tolerance towards vanadate concentration of up to 6 mmol/L (Ceci et al., 2012). Coprinus comatus is able to grow well with vanadate concentration of 0.4% and accumulate the metal with a yield of approximately 3.5 mg/g of biomass (Han et al., 2009; Han et al., 2008). Aspergillus niger has the capability of vanadinite transformation (Ceci et al., 2015). In other studies, cell-free liquid medium containing organic acids excreted after growth of A. niger was used to leach V from thermal power plant ash, catalyst, ore residue or converter slag (Aung and Ting, 2005; Mirazimi et al., 2015; Rasoulnia and Mousavi, 2016; Safdari et al., 2013). However, fungal reactions to various oxidation states and molecular structures of V are investigated only marginally.

Basidiomycetes are widely spread and often in relation to the element transportation between soils and plants. Amanita muscaria, Armillaria cepistipes, Xerocomus badius and Bjerkandera adusta can grow on the ground or wood and are often associated with various deciduous trees and coniferous trees or mixed wood. A. muscaria is well known for V complexation through amavadine in its fruit bodies. A. cepistipes, X. badius and B. adusta are able to accumulate heavy metals (Falandysz et al., 2012; Falandysz et al., 2013; Kojta et al., 2013;
2012; Rigling et al., 2006) or excrete organic acids (Galkin et al., 1998; Jarosz-Wilkołazka and Graż, 2006). *B. adusta* can also grow well in silver nanoparticle solutions although silver is toxic (Bigall et al., 2008).

In this research, *A. muscaria*, *A. cepistipes*, *X. badius* and *B. adusta* were cultured in the presence of V oxides (III, IV and V) and a series of vanadate and vanadyl at different concentration. Additionally, vanadium containing slag was also included. Goals of the research were (i) to investigate tolerance, accumulation capacities, fungal morphological reactions and changes in chemical composition in the presence of V; (ii) to better understand the fungal role in V related biogeochemical process; and (iii) to set a base for the future implementation of fungi to bioremediation and V recycling.

**Experimental procedures**

**Fungal strains, medium and growth conditions**

Four fungal strains belonging to Basidiomycota were used. Strains of *A. muscaria*, *A. cepistipes* and *X. badius* were from Forest Soils and Biogeochemistry Group of Swiss Federal Institute for Forest, Snow and Landscape Research (WSL; Birmensdorf, Switzerland). *B. adusta* strain was isolated from soils and identified by Leibniz-Institute DSMZ-German Collection of Microorganisms and Cell Cultures (DSMZ; Braunschweig, Germany). Growth medium MMN - 0.03% aspartate - agar medium was modified from former used (Guo et al., 2008; Kottke et al., 1987). Cultures were inoculated by an agar plug (5 mmol/L in diameter) with actively growing mycelium and grown in 90 mmol/L Petri dishes. To determine bioaccumulation of V, strains were grown in 35 mmol/L petri dishes. Prior to inoculation, a dialysis membrane (MEMBRA-CEL®) was placed on the agar surface to facilitate the removal of the biomass at the end of the incubation period. Before use, the dialysis membranes were soaked in 10% (w/v) EDTA solution overnight to remove metal contaminants and then washed three times with diluted water. Grown mycelia were peeled off the membrane for further analysis. All the experiments were carried out in triplicate at 23 °C in dark.

The medium were amended by specific V compounds as well as V containing slag according to the desired final concentration. Sodium metavanadate (NaVO₃; Sigma-Aldrich, Buchs, Switzerland) and vanadyl sulfate (VSO₅; Sigma-Aldrich, Buchs, Switzerland) were added to the medium at final V concentration of 1, 3, 6 and 10 mmol/L, respectively. The pH of VSO₅ medium was adjusted to 5.50 by 5 mol/L NaOH before sterilization in order to solidify. Vanadium(III, IV) oxides (V₂O₃, VO₂; Sigma-Aldrich, Buchs, Switzerland), vanadium(V) oxide (V₂O₅; ChemPUR, Karlsruhe, Germany) oxide and V slag were added to
the medium after sterilization at V concentration of 10 mmol/L, respectively. The V slag (containing 0.28% V, 6.22% Ti, 47.5% Fe and 0.23% Mn) was collected from a vanadium-titanium magnetite mining area in Panzhihua, China.

**Fungi growth**

Diametric expansion and dried biomass of colonies were determined when the cultures entered the stationary phase. Mycelia were dried to constant weight at 80 °C for at least 48 hours. Tolerance index (TI, %), calculated by dry weight of treated mycelium / dry weight of control mycelium × 100, was used to assess fungal metal tolerance (Ceci et al., 2012; Wei et al., 2013). Pearson Correlation analysis between fungal diameter (ln transformed) and biomass (ln transformed) were carried out through SPSS 22. Final surface pH of the agar medium after biomass removal was measured by a glass electrode with fixed ground-joint (Metrohm, Herisau, Switzerland).

**Vanadium bioaccumulation**

The colonies were harvested in their stationary phase and then air-dried. Samples were digested in mix of 0.8 ml HNO₃, 0.2 ml H₂O₂ and 0.2 ml HSO₄ at 200 °C until solution was clear (Ceci et al., 2012; Tüzen, 2003) followed by the addition of dilution buffer containing 0.1% (v/v) LaCl/CsCl and 1% (v/v) HNO₃ to a final volume of 25 ml. Vanadium content was analyzed using a high-resolution continuum source atomic absorption spectrometer (contrAA® 300, Analytik Jena, Germany). Removal efficiency (R, %), calculated as (V amount of fungi grown with V addition - V amount in control fungi) / amended V amount × 100, was taken as indicator of the fungal ability to accumulate V from medium. Multiple linear regression in SPSS 22 was applied to analyze the relationship and contribution of original medium pH, final medium pH, amended V concentration to fungal V content. The data except pH values were ln transformed before analysis.

**Metabolic fingerprints**

Metabolic fingerprints of freeze-dried mycelia were measured using fourier transform infrared spectroscopy (FTIR; JASCO 4200, Brechbühler AG, Schlieren, Switzerland) in attenuated total reflection (ATR) mode using an ATR accessory equipped with a zinc selenide (ZnSe) prism (average of fifty scans with a resolution of 4 cm⁻¹ over a wave number range from 650 to 4000 cm⁻¹). Raw spectral data were processed with JASCO Spectra Manager 2.02.02. Each spectrum was treated with baseline correction (linear), smoothing (Savitzky-Golay, width = 15 (Ammann and Brandl, 2011)), truncation of 2400 - 2250 cm⁻¹ and normalization (highest value
Second derivative of each spectrum was then calculated using a Savitzky-Golay algorithm with nine smoothing points. Comparison of second derivative transformed spectra was performed through the function termed Qcheck in OMINIC software (version 8.2, Thermo Electron Corporation). The degree of spectral similarity between fungi grown with V addition and control fungi was expressed as a correlation value from 0.0 (no similarity) to 1.0 (the spectra are identical).

**Mycelial morphology**

Mycelia harvested from medium with V concentration of 10 mmol/L were applied for morphological analysis. The analysis was carried out in Center for Microscopy and Image Analysis in University of Zurich (ZMB UZH; Zurich, Switzerland), using a field emission scanning electron microscope (SEM; Zeiss SUPRA 50 VP, Carl Zeiss AG, Germany) at acceleration voltage of 10 kV. Specimens were prepared via the procedure of fixation, critical point drying, mounting on aluminum stubs using conductive carbon cement, and coating with 66 nm Au/Pd by a high-vacuum sputter coater (SCD500, Bal-tec AG, Balzers, Switzerland).

**Results**

**Diametric growth and biomass formation**

Inhibition effects on diametric growth varied according to V compounds and concentration applied. In addition, fungal species also reacted differently upon exposure to V (Fig. 1 - Fig. 5). Insoluble V(III), soluble V(IV) and V(V) generally reduced the diametric growth, while V slag did not significantly restrain but even promoted fungal growth. At a concentration of 10 mmol/L, soluble V compounds were more critical for fungal propagation than insoluble V compounds. Furthermore, the inhibitory effect of soluble V on *B. adusta* was much weaker than that on other three fungi suggest a higher tolerance.

Pearson Correlation analysis indicated that only *A. muscaria* and *X. badius* showed a significant relationship between diameter and biomass (P < 0.01). The lowest TI was found about 20% when *A. muscaria* treated with 10 mmol/L V₂O₃, VSO₅ and NaVO₃ (Table 1). Biomass of *A. muscaria*, *A. cepistipes* and *B. adusta* grown with 10 mmol/L V slag were all higher than that in the controls. *B. adusta* grown on 10 mmol/L soluble V(IV) produced extremely more biomass than control and showed the highest TI.
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Figure 1. Natural logarithm and error bar of maximum diametric growth size of fungal mycelium on the petri dish, under different vanadium emendation. O: control, no additional V; ●: 10 mmol/L V from V₂O₃, VO₂, V₂O₅ and V-Ti magnetite, respectively; △: 1, 3, 6, 10 mmol/L V(IV) from VSOS₅, respectively; ▼: 1, 3, 6, 10 mmol/L V(V) from NaVO₃, respectively.
Table 1. Tolerance index (TI) of fungi grown on metal-amended medium.

<table>
<thead>
<tr>
<th></th>
<th>V₂O₃</th>
<th>VO₂</th>
<th>V₂O₅</th>
<th>Slag</th>
<th>VSO₅</th>
<th>NaVO₃</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
<td>1</td>
<td>3</td>
<td>6</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>3</td>
<td>6</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>A. muscaria</td>
<td>%</td>
<td>25</td>
<td>68</td>
<td>48</td>
<td>119</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.3</td>
<td>0.1</td>
<td>0.1</td>
<td>0.8</td>
<td>0.1</td>
</tr>
<tr>
<td>A. cepistipes</td>
<td>%</td>
<td>37</td>
<td>56</td>
<td>35</td>
<td>102</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>X. badius</td>
<td>%</td>
<td>65</td>
<td>72</td>
<td>47</td>
<td>90</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.0</td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>B. adusta</td>
<td>%</td>
<td>57</td>
<td>57</td>
<td>33</td>
<td>119</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.3</td>
<td>0.4</td>
<td>0.3</td>
<td>0.5</td>
<td>0.9</td>
</tr>
</tbody>
</table>

a: V concentration in medium, the unit is mmol/L.

Morphological reaction of fungi

Cultivation conditions caused alterations in both colony appearances and mycelia morphology (Fig. 2 – Fig. 5). The colony of *A. muscaria* which grew in the presence of 10 mmol/L VSO₅, NaVO₃ or V₂O₃ was not able to spread over the agar (Fig. 2). Mycelia forming on the culture of 10 mmol/L VSO₅ or NaVO₃ were more complicated rather than simple tube-shape of control mycelia. Mycelia from V₂O₅ medium showed distinct morphological changes (shrinkage). Cubic crystalloids were found in the colonies cultivated on 10 mmol/L NaVO₃ amended medium (see red arrows in Fig. 2).

Colonies *A. cepistipes* cultivated on slag showed similar branched structure and rhizomorphs like the V-free control (Fig. 3). Fine hairs were observed on the mycelia in the samples cultivated on 10 mmol/L NaVO₃, V₂O₃, VO₂, V₂O₅, or slag amended medium. Length and density of the fine hair were further found diverse according to the V compounds. Bulbous structures were found in the samples growing with V₂O₃, VO₂ or V₂O₅.

Both VSO₅ and V₂O₃ showed significant degeneration effects on *X. badius* as the mycelia were shortened and formed bulbous structures, and formed a light brown pigment (Fig. 4). Mycelia of *B. adusta* (Fig. 5) was packed much denser and tighter on V₂O₃, VO₂ or V₂O₅ as compared to the control. Shortened mycelia and star-shaped crystalloids (indicated by red arrows in Fig. 5) were found in the samples cultured with VSO₅. A green diffusible pigment was formed in the presence of 10 mmol/L VSO₅ and NaVO₃ which was not observed at 1 and 3 mmol/L V (not shown).
Figure 2. Colony images and SEM visualization of mycelia of *A. muscaria* cultured on V-amended medium. The concentration of V emendation was 10 mmol/L for each.
Figure 3. Colony images and SEM visualization of mycelia of *A. cepistipes* cultured on V-amended medium. The concentration of V emendation was 10 mmol/L for each.
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Figure 4. Colony images and SEM visualization of mycelia of *X. badius* cultured on V-amended medium. The concentration of V emendation was 10 mmol/L for each.
Figure 5. Colony images and SEM visualization of mycelia of *B. adusta* cultured on V-amended medium. The concentration of V emendation was 10 mmol/L for each.
**Fungal vanadium accumulation**

Table 2 shows the pH values of each kind of medium before and after fungal growth as well fungal V concentrations and removal efficiencies. When growth media were amended by V oxides, VSO$_5$ or NaVO$_3$, all the fungi species were able to accumulate V as compared to the controls. When the medium was amended by a series of either soluble V(IV) or V(V), the V concentration in all fungi gradually increased with increasing V concentration. Vanadium accumulated in the presence of 10 mmol/L soluble V(IV) were higher than on 10 mmol/L insoluble VO$_2$, while the V concentration in 10 mmol/L soluble V(V) condition was similar or even less than 10 mmol/L insoluble V$_2$O$_5$. When V containing slags was added, significant increase in mycelial V concentration was not observed. X-ray powder diffraction (XRD) patterns of the slags after cultivation were not significantly different from the original slag sample confirming the fungal inability to mobilize V from the solid matrix (data not shown). Vanadium removal efficiencies of *A. cepistipes* and *X. badius* were significantly higher than these of the other two fungi strains.

According to the results of multiple linear regression (Table 3), vanadium concentration accumulated by *A. muscaria* and *B. adusta* was negatively related to final pH of medium; while fungal V concentrations of *A. cepistipes* and *X. badius* were positively related to V content of emendation. Removal efficiencies of fungi species were primarily decided by amended V in medium. Fungi cultured with lower V concentration presented higher removal efficiencies.

**Table 2.** Medium pH after cultivation and V content in fungi grown on metal-amended medium.

<table>
<thead>
<tr>
<th>Amended V (mmol/L)</th>
<th>Initial pH of medium</th>
<th><em>A. muscaria</em></th>
<th><em>A. cepistipes</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH $^a$</td>
<td>V (mg/g) $^b$</td>
<td>R (%) $^c$</td>
</tr>
<tr>
<td>Control 0.9 $\times 10^{-3}$</td>
<td>5.4</td>
<td>7.0</td>
<td>0.09 ± 0.0 $^e$</td>
</tr>
<tr>
<td>V$_2$O$_3$ 10</td>
<td>3.2</td>
<td>3.8</td>
<td>40.4 ± 13.5</td>
</tr>
<tr>
<td>VO$_2$</td>
<td>3.4</td>
<td>4.6</td>
<td>9.2 ± 3.8</td>
</tr>
<tr>
<td>V$_2$O$_5$</td>
<td>3.9</td>
<td>4.4</td>
<td>12.2 ± 5.2</td>
</tr>
<tr>
<td>Slag</td>
<td>5.7</td>
<td>6.5</td>
<td>NA</td>
</tr>
<tr>
<td>VSO$_5$</td>
<td>5.5</td>
<td>4.7</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td>3</td>
<td>5.5</td>
<td>4.1</td>
<td>4.0 ± 2.2</td>
</tr>
<tr>
<td>6</td>
<td>5.5</td>
<td>3.4</td>
<td>23.1 ± 19.6</td>
</tr>
<tr>
<td>10</td>
<td>5.5</td>
<td>3.1</td>
<td>51.3 ± 19.8</td>
</tr>
<tr>
<td>NaVO$_3$</td>
<td>1</td>
<td>5.5</td>
<td>5.3</td>
</tr>
<tr>
<td>Amended V (mmol/L)</td>
<td>Initial pH of medium</td>
<td>A. muscaria</td>
<td>A. cepistipes</td>
</tr>
<tr>
<td>--------------------</td>
<td>----------------------</td>
<td>------------</td>
<td>--------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pH a</td>
<td>V (mg/g) b</td>
</tr>
<tr>
<td>3</td>
<td>5.7</td>
<td>4.5</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>6</td>
<td>5.8</td>
<td>4.3</td>
<td>9.4 ± 3.5</td>
</tr>
<tr>
<td>10</td>
<td>5.9</td>
<td>3.8</td>
<td>14.4 ± 3.2</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Amended V (mmol/L)</th>
<th>Initial pH of medium</th>
<th>X. badius</th>
<th>B. adusta</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>pH</td>
<td>V (mg/g)</td>
</tr>
<tr>
<td>Control 0.9 ×10⁻³</td>
<td>5.4</td>
<td>8.1</td>
<td>0.02 ± 0.0</td>
</tr>
<tr>
<td>V₂O₃</td>
<td>3.2</td>
<td>3.4</td>
<td>5.4 ± 1.7</td>
</tr>
<tr>
<td>VO₂</td>
<td>3.4</td>
<td>6.3</td>
<td>3.6 ± 1.2</td>
</tr>
<tr>
<td>V₂O₅</td>
<td>3.9</td>
<td>6.1</td>
<td>9.5 ± 5.8</td>
</tr>
<tr>
<td>Slag</td>
<td>5.7</td>
<td>6.2</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>5.5</td>
<td>7.4</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>VSO₅</td>
<td>3.5</td>
<td>6.9</td>
<td>3.8 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>6.5</td>
<td>6.8</td>
<td>10.9 ± 6.5</td>
</tr>
<tr>
<td></td>
<td>10.5</td>
<td>3.2</td>
<td>17.2 ± 6.9</td>
</tr>
<tr>
<td></td>
<td>5.5</td>
<td>7.3</td>
<td>2.0 ± 0.3</td>
</tr>
<tr>
<td>NaVO₃</td>
<td>3.5</td>
<td>7.4</td>
<td>5.2 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>6.5</td>
<td>7.4</td>
<td>4.3 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>10.5</td>
<td>7.2</td>
<td>5.3 ± 0.2</td>
</tr>
</tbody>
</table>

a: medium pH after cultivation.
b: Fungal V concentration; For metal amended condition, the fungal V concentration in the table is the measured V concentration in biomass subtracted by the V concentration in control biomass.
c: removal efficiency.
d: V content of the medium without V emendation.
e: mean ± SD, n=3.
NA: not applicable.
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Table 3. Results of multiple linear regression between fungal V concentration and removal efficiency and original medium pH, final medium pH, and amended V, separately.

<table>
<thead>
<tr>
<th></th>
<th>initial medium pH</th>
<th>final medium pH</th>
<th>amended V</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sig. R²</td>
<td>Sig. R²</td>
<td>Sig. R²</td>
</tr>
</tbody>
</table>
| **A. muscaria**         | fungal V<sup>a</sup> ** 0.282 | ** 0.624 | / / /
|                         | R<sup>b</sup> / / / / | * 0.173 |
| **A. cepistipes**       | fungal V / / / / | ** 0.489 |
|                         | R / / ** 0.244 | ** 0.368 |
| **X. badius**           | fungal V / / / / | ** 0.427 |
|                         | R / / ** 0.283 |
| **B. adusta**           | fungal V / / ** 0.429 | / / |
|                         | R / / ** 0.673 | ** 0.653 |

a: V concentration in fungi.
b: Removal efficiency of fungi to V.
*: significant as P < 0.05.
**: significant as P < 0.01.
/: Not significant correlation.

**FTIR spectra of fungi**

FTIR spectra of *A. muscaria, A. cepistipes, X. badius* and *B. adusta* are shown in Fig. 6. Compared to other fungal species (Jilkine et al., 2008; Kaminskyj et al., 2008; Santos et al., 2010; Sompong et al., 2013; Wang et al., 2012), strong absorptions appearing in specific spectral regions characterized the major cellular components. In the region 3000 - 2800 cm<sup>-1</sup>, a peak around 2920 cm<sup>-1</sup> was assigned to C-H stretching of lipids. In the region 1720 - 1480 cm<sup>-1</sup>, the peak around 1640 cm<sup>-1</sup> was assigned to Amide I, and peaks at 1541, 1521 and 1555 cm<sup>-1</sup> were assigned to Amide II. The peaks in the region 1480 - 1320 cm<sup>-1</sup> were attributed to deformation C-H and O-H bending (Movasaghi et al., 2008; Nie et al., 2007). Region 1320 - 1180 cm<sup>-1</sup> represented Amide III because of the peaks at 1254 and 1315 cm<sup>-1</sup>. The peaks shown in region 1180 - 820 cm<sup>-1</sup> were mainly due to carbohydrates from glucan and chitin (Nie et al., 2007; Szeghalmi et al., 2007). After fungal cultivation in the presence of different V compounds, the alternations of peak positons and intensities were observed (Table 4 and Figure S1-S4). Compared to *A. muscaria, A. cepistipes* and *B. adusta*, spectra of *X. badius* after V amendment were distinctly more different to the controls in the absence of V (Table 4). When VSO<sub>5</sub> was added to the medium, the lowest V concentration (1 mmol/L) showed the strongest influence to the spectra of *X. badius*. 
Figure 6. FTIR spectra of fungal hyphae grown on medium without vanadium addition. Red lines are the spectra of fungi grown on medium containing 10 mmol/L VSO$_5$.

Discussion

Fungal biosorption of soluble V

Tested Basidiomycota (*A. muscaria*, *A. cepistipes*, *X. badius* and *B. adusta*) can grow and accumulate V from medium supplemented with different amounts of vanadate and vanadyl. Vanadium concentrations in fungal biomass correspondingly increased with the increasing V content added in medium. Fungal V concentrations from vanadyl amended medium were higher than that from vanadate medium, particularly at the higher concentration.
# Interaction of Basidiomycota with vanadium

## Table 4. Correlation values of second derivative transformed FTIR spectra comparison between treated fungi cultures and control fungi cultures.

<table>
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<th>Designation</th>
<th>Wavenumber Region (cm⁻¹)</th>
<th>V₂O₅</th>
<th>VO₂</th>
<th>V₂O₅</th>
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<th>VSO₃</th>
<th>10ᵃ</th>
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ᵃ: vanadium concentration in medium, the unit is mmol/L.
ᵇ: The correlation value is from 0.00 (no similarity) to 1.00 (identical spectra); the cell color gradually changes from white (low similarity) to black (high similarity).
Vanadium can act as counterion for protein, DNA, RNA as well as in various biological organelles (Crans et al., 2004). Vanadium ions function also as an enzyme cofactor. V-containing chloroperoxidases has been found in fungi, *Curvularia inaequalis* (Messerschmidt et al., 1997; Messerschmidt and Wever, 1996). The structure of chloroperoxidases of *C. inaequalis* showed that vanadate covalently linked to the protein and embedded in the protein through an extensive hydrogen-bonding network (Messerschmidt et al., 1997; Messerschmidt and Wever, 1996). Amavadine is a natural V(IV) complex present in *A. muscaria*. We have detected V content in all the fungal biomass cultivated from control medium. This could be an evidence of the V participation in the biological activities of the four species. Furthermore, the structural and electronic character of V is analogy to phosphorus. Bowman (1983) has found that vanadate is taken up by *Neurospora crassa* through the phosphate transport system II when cells are starving for phosphate. As a result, we suppose that the competitive biosorption of vanadate compared to phosphorus should be an important process for fungi in the presence of amended V. On another hand, our experiment has shown that the fungal V concentrations from vanadyl medium were higher than that from vanadate medium, especially at the higher concentrations of 6 to 10 mmol/L V. The vanadyl ion has a strong tendency to coordinate with oxygen donor atoms and is thus capable of both forming strong complexes with soluble organic chelates (Wehrli and Stumm, 1989). The oxidation from V(IV) to V(V) could happen as indicated by color of vanadyl medium from blue to yellow.

At the same time, Vanadium-compounds including V oxidation states IV and V are also a potent inhibitor (and sometimes stimulator (Stankiewicz et al., 1995)) for phosphorylases like phosphatase, ribonuclease and ATPases (Bowman, 1983; Chasteen, 1990; Crans et al., 2004; Rehder, 1999). Vanadate, vanadyl cation, and their respective complexes potently inhibit hydrolysis of the phosphodiester in RNA and DNA (Crans et al., 2004). Vanadate was presumed to inhibit the ATPase enzymes, which include many membrane enzymes, as a transition state analogue for the phosphoryl group transfer (Aureliano, 2000; Beaugé, 1988; Sauna et al., 2001). Vanadyl cation is the inhibitor of alkaline phosphatase for *Escherichia coli* although the interaction has not been elucidated (Lopez et al., 1976). The inhibition could result in impeding growth of fungi which showed different tolerances related to coordination number and emended V concentration.

Extrahyphal crystals have been observed in *A. muscaria* in the presence of 10 mmol/L vanadate. Like most ectomycorrhizal fungi, *A. muscaria* is able to release oxalate and protons (Riedlinger et al., 2006). Gadd et al. (2014) have revealed that oxalate precipitation is an ability widely associated with fungi that are capable of oxalic acid formation. Our result
showed the substrate acidification by *A. muscaria* in the presence of 10 mmol/L vanadate. We would hypothesize that the crystals produced by *A. muscaria* with vanadate medium could be the precipitated V-oxalate.

**Formation and function of fungal pigments**

*X. badius* produced yellow-brown pigments at higher concentration of vanadyl or V$_2$O$_3$, and *B. adusta* produced green pigments at higher concentration of vanadate or vanadyl. Such production of pigments have also been observed in *Penicillium terverticillate* and *Fusarium solani* with lead, copper and cadmium (Kowshik and Nazareth, 2000; Nazareth and Marbaniang, 2008), and *A. niger* with V compounds (Ceci et al., 2015). The pigments reflect the extent of environmental stress (Hanson, 2008). Vanadium can cause an oxidative stress because of changes in glutathione as well as antioxidant enzyme activities and the formation of reactive oxygen species (Cortizo et al., 2000; Saxena and Flora, 2004). However, the pigments could also play a role in helping fungi accumulate V from medium as some of the fungal pigments are less highly colored quinol or phenol which is able to form a metal complex (Gadd, 2000; Hanson, 2008).

Extrahyphal crystals have been observed of *B. adusta* in the presence of 10 mmol/L vanadyl. The diametric size of *B. adusta* colonies on vanadyl containing medium was similar to the vanadate amended medium and less than the controls, but its biomass was more than twice as high as that under vanadate and control condition. Furthermore, the fungal V concentration on vanadyl amended medium was more than four times higher compared to vanadate treatment. *Fusarium oxysporum* has shown the synthesis of extracellular silver nanoparticles when the biomass exposed to Ag$^+$ ions (Ahmad et al., 2003). The color change of the colonies has also been observed. Both *B. adusta* and *F. oxysporum* are plant pathogens and able to degrade lignin and azo dye (Rodriguez et al., 1996; Heinfling et al., 1997; Solís et al., 2012; Łebkowska and Załęśka-Radziwiłł, 2014). According to the analogy of metabolic application and colony morphology after growth in the presence of metals, extracellular particles observed are likely V particles which are synthesized by *B. adusta*.

**Fungal mobilization and accumulation of insoluble V**

All the tested species have shown the abilities of solubilization and accumulation of insoluble V oxides (V$_2$O$_3$, VO$_2$ and V$_2$O$_5$). Organic acids like oxalic and citric acid produced by fungi were able to dissolve metal containing minerals (Gadd, 1999; Safdari et al., 2013). *B. adusta* has been found to produce oxalic acid in ZnO amended medium, but in very low concentrations (Jarosz-Wilkołazka and Grąż, 2006). Sayer et al. (1999) and Fomina et al.
(2004) have observed that acidolysis was the major mechanism involved in the fungal solubilization of both pyromorphite and zinc phosphate. Excreted oxalic acid by *A. niger* was supposed to result in the solubilization of combinations of V$_2$O$_5$ and lead compounds (Ceci et al., 2015). However, all four species showed a very weak mobilization and biosorption of V from V-Ti slag amended medium. Adding V slags lightly increased the initial pH of the medium compared to control. The slag also contains many other elements such as Ti, Fe and Mn. Vanadium is mainly captured in a very stable V-Ti crystalline structure. The pH and buffering capacity of the substrate and the presence of certain metals can affect the type and amount of organic acids excreted by fungi (Fomina et al., 2004; Gadd, 1999; Gadd and Raven, 2010; Safdari et al., 2013). As a result, the pH of the slag medium increased after cultivation of the tested species. The mobilization of V from slag has been impeded. This was also confirmed by XRD patterns which have not shown the significant difference between the original slag and slags treated by fungi. Consequently, the four fungal species hardly cause bioweathering of a comprehensive V-Ti mineral, at least in a short time.

**Morphological reactions and metabolic fingerprints**

Fungal morphology could be corresponding to the biosorption ability of fungi. For *A. cepistipes*, fine filamentous structures emanating from the hyphae appeared in cultures grown in the presence of 10 mmol/L V$_2$O$_3$, VO$_2$, V$_2$O$_5$, V slag and vanadate. The corresponding V concentrations in these cultures were relatively higher than that in vanadyl amended culture. The projection structure should be the positive variation of *A. cepistipes* to promote the biosorption of V or other elements (in the presence of V slag) from the medium. Morphological changes of fungi could be the results of metabolic reactions to V. As shown by FTIR, lipid content of cell membranes, cell proteins and cell wall polysaccharides of each species in amended medium appeared significant different compared to the control cultures. Similar results can also be found in other fungi to heavy metal stress (Fomina, 2002; Nazareth and Marbaniang, 2008). However, comparing morphological malformation and variations of each species with their related FTIR fingerprints, it was difficult to generate a rule to describe the relationship between the morphological changes and the changes in molecular composition.

**Environmental and industrial implications**

Our findings have demonstrated that *A. muscaria, A. cepistipes, X. badius* and *B. adusta* can tolerate V oxidation states from III to V, and accumulate V from both soluble and insoluble V compounds. However, V-Ti slag was only poorly transformed. These capabilities suggested environmental and industrial implications. The tested species are widely spread basidiomycetes
which can grow on ground or wood and are associated with various deciduous trees, coniferous trees, or mixed wood. Vanadium in soils mainly occurs as oxidation states of IV and V. Most V in soils is in a crystalline structure which cannot easily be transformed and uptaken by organisms (Xu et al.). However, there is still a certain amount of soluble V, bioavailable V and V in a weak crystalline structure, which could be absorbed and transformed relatively easier (Xu et al.). In relation to the capabilities of these fungi, they may play a role in the V cycling in soils. Fungi may increase the biological risk of V in soils by transforming immobile V to mobile V. However, they may also immobilize V via biosorption into mycelium or form the extracellular V containing crystals under certain conditions. In perspective, fungi might be applied in the recycling of V containing wastes. According to the diverse removal efficiencies for the different V compounds and concentration, we would suggest some pretreatments for the implications. For example, improve the fungal removal efficiency by adjusting the V concentration in wastes; stimulate the production of organic acids by just the pH of the substrate. Roasting the solids could also improve the removal of V (Mirazimi et al., 2015). At last, the control the phosphorus supply for fungi should be considered.

**Acknowledgements**

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Interaction of Basidiomycota with vanadium


Supporting information

Figure S1. FTIR spectra of *A. muscaria* grown on medium amended with vanadium.
**Figure S2.** FTIR spectra of *A. cepistipes* grown on medium amended with vanadium.
Figure S3. FTIR spectra of *X. badius* grown on medium amended with vanadium.
Figure S4. FTIR spectra of *B. adusta* grown on medium amended with vanadium.
Chapter 5

General discussion
Optimised sequential extraction scheme

According to the review of current sequential extraction schemes used to fractionate V in soils, the most commonly used scheme was exchangeable (sorbed)/carbonate/reducible (or Fe and Mn (hydr)oxides)/oxidisable (or organic matter and sulphides) fractions. Almost all studies showed that less than 1% of total V in soils was exchangeable, suggesting the general low mobility of V in soils. When excluding residual fraction, most schemes in literature presented low extraction efficiencies as 10%-30% of total V extracted in natural soils. These suggest the current sequential extraction methods being still unsuitable for fractionation of V in soils, especially when V is associated with recalcitrant phases.

When we proposed our scheme, we avoided using very strong oxidants, acids and toxic reagents for safety reason. First, we extracted the soil with de-ionised water to quantify the pool of readily mobilisable V. Then, we applied 5 mmol/L phosphate and shortened the extraction time to 8 hr, which successfully extracted more than 80% of exchangeable arsenic in soils (Huang and Kretzschmar, 2010). Pyrophosphate was then used to destabilise the soil organic matter in complex with polyvalent cations and released V associated. Since pyrophosphate may also exchange adsorbed vanadate from soils, phosphate extraction was done prior to pyrophosphate extraction. We utilised NH2OH·HCl to quantify Mn oxide instead of Fe and Al (hydr)oxide bound V as NH2OH·HCl has been found inefficient to dissolve amorphous ferrihydrite in the given time, whereas complete dissolution of Mn oxides in 30 min with 0.4 mol/L NH2OH·HCl have been proved in preliminary experiments. Given that V(III) and V(IV) may incorporate into the lattice of soil minerals of different crystallinity, we refined the fraction associated with Fe and Al (hydr)oxides by separating it into very poorly crystalline, poorly crystalline and crystalline. The corresponding extraction reagents were 1 mol/L HCl, 0.25 mol/L oxalate buffer and 4 mol/L hot HCl, respectively. Higher HCl concentrations or hot HCl should be avoided to minimise ligand-promoted dissolution of crystalline Fe (hydr)oxides (Huang and Kretzschmar, 2010). Increasing temperature to 95°C, complete dissolution of goethite and boehmite was achieved with 4 mol/L HCl in one hour and therefore was used to extract crystalline Fe and Al (hydr)oxide associated V.

To verify the suitability of the eight-step sequential scheme, we proposed a second scheme which was a combination of the most commonly used schemes, and compared the two schemes through two soil samples of very different characteristics. The eight-step sequential scheme has been quantified better extraction efficiencies for both samples. Furthermore, another four soils of different characteristics at 23.5 to 784 mg/kg V were analysed to check
the adaptability and reproducibility of the eight-step sequential extraction scheme. When exclude residue, the newly proposed scheme is highly superior to the previous schemes by increasing the total extraction recovery from averagely 27% of total V (8.4%-48%) in 192 soils in literature to 65% (55%-77%) in 6 soil samples investigated here. Satisfactory V mass balance was shown with averagely 95% of V in soils was quantified with the new SE scheme (including residue) as compared to XRF values. The variation coefficients of each extraction step averaged 3.8%, indicating our extraction method was highly precise. We have demonstrated that our newly-proposed eight-step SE was able to target the distinct and environmentally important forms of V in soils. The new scheme efficiently refined the fraction of V bound to Mn, Fe and Al (hydr)oxides, which can be useful for the evaluation of V mobilisation under reducing conditions. The largely improved extractability against crystalline Fe and Al (hydr)oxide enabled for the first time to identify the presence of noticeable amounts of geogenic V incorporated in the lattice of soil minerals.

Biogeochemical characterization of vanadium in soils

Fifteen soil samples with diverse geochemical characteristics were used for the biogeochemical characterization analysis of V. The results of sequential extraction showed that the least phases of V among all the soils samples were water-soluble V (0.41±0.34%, mean±SD) and adsorbed V (0.79±0.94%). The dominant phases were crystalline (32±6.5%) and residual (35±11%) fractions. The proportion of V corresponding with Mn oxides (1.8±2.2%) was much less than the proportion of V related with very poorly crystalline (7.6±5.2%), poorly crystalline (15±12%) and crystalline (32±6.5%) Fe and Al (hydr)oxides. The average proportion of organic fraction was 7.5±8.4%. Most soil samples only contained less than 10% V(V). The domination of V(IV) in soils was also verified by the results of XAS.

Vanadium could leach from soils, shown as the increasing of V content and bioavailable V content along soil depth in mining area. The clear division of dry season and wet season (June to September) in Panzhihua region could contributes to the leachability of V along soil layers through drought and rewetting (Yang et al., 2014). In the smelting area, the overlaying material which was related to the anthropogenic input such as mining and smelting dust contained much more V as well as mobile and less stable fractions than that of mining soils. Vanadium content and bioavailable V content in the underlaying horizon suddenly decreased and then gently decreased in the deeper horizons. Overall, the bioavailable amount of V in smelting soil was still higher than mining soil even the V concentration in smelting soil was less. The vertical migration of V(IV) was weaker than V(V). It is related to the stronger
sorption ability of V(IV) than V(V) (Kaur et al., 2009; Wehrli, 1987). Furthermore, V(IV)
could be oxidized to V(V) in the condition of pH value greater than 8 (Huang et al., 2015;
Wehrli and Stumm, 1989), or reduced to form hydroxide of V(III), V(OH)$_3^{(s)}$, at relatively low
concentrations at 25 °C (Huang et al., 2015). Compared to V(IV), V(V) is more toxic (Patel et
al., 1990) and more active to be involved in the soil-plant interaction (Tian et al., 2015).
Generally, the anthropogenic input could increase not only the bioavailable amount of V but
also the proportion of V(V) in soils, especially in the shallow layers.

The ratio of H$_2$O/EDTA extractable V(IV) to V(V) was significantly affected by pH
value rather than organic C and hydroscopic moisture of soils. Most soil samples were dominant
with bioavailable V(V), but the proportion of bioavailable V(IV) increased as the soil pH value
declining. In reducing environments, V exists as vanadyl (VO$^{2+}$) and strongly complexed by
organic matter (Amrhein et al., 1993). Anaerobic condition combined with low pH promoted
the reduction of V(V) to V(IV) in wetland soils. On the other hand, the paddy land soils were
of neutral pH, but the H$_2$O/EDTA-V(V) in paddy soil was more dominant than others. It
indicated that the growth of paddy promoted the oxidation of bioavailable V(IV) to V(V). The
V concentration and distribution in paddy plant could be worth further investigation.

The relative amount of bioavailable V was negatively related with hydroscopic
moisture content of soils rather than soil pH and organic C content. Higher hydroscopic
moisture content reflects stronger adsorption ability of soil particles which is caused by the
molecular attraction and naturally occurring electrical forces (Veihmeyer, 1956). Stronger
sorption ability of soils decreased relative amount of bioavailable V. Soil grown with soybean
showed the highest proportion of bioavailable V ignoring the hydroscopic moisture content.
The vertical movement of V could be increased by the soybean growth, but the amount of V
accumulated in soybean should be further studied in order to better understand the feedback to
soybean growth.

**Fungal interaction with vanadium**

*A. muscaria, A. cepistipes, X. badius and B. adusta* can grow and accumulate V from medium
supplemented with different amounts of vanadate and vanadyl. Vanadium concentrations in
fungal biomass correspondingly increased with the increasing V content added in medium.
Vanadium ions can act as counterions for protein, DNA, RNA and are present in different
biological organelles (Crans et al., 2004). Vanadium ions function also an enzyme cofactor.
The structural and electronic properties of V are in analogy to phosphorus. We suppose that the
competitive biosorption of vanadate compared to phosphorus might be an important process
Discussion

for fungi in the presence of V. On the other hand, fungal V concentrations from vanadyl medium were higher than that from vanadate medium, especially at the higher concentrations of 6 to 10 mmol/L V. The vanadyl ion has a strong tendency to coordinate with oxygen donor atoms and is thus capable of both forming strong complexes with soluble organic chelates (Wehrli and Stumm, 1989). The oxidation from V(IV) to V(V) could happen as indicated by color of vanadyl medium from blue to yellow. At the same time, vanadium-compounds including V oxidation states IV and V are also a potent inhibitor for phosphorylases like phosphatase, ribonuclease, and ATPases (Bowman, 1983; Chasteen, 1990; Crans et al., 2004; Rehder, 1999). The inhibition could result in impeding growth of fungi which showed different tolerances related to coordination number and emended V concentration.

Oxalate precipitation is an ability widely associated with fungi that are capable of oxalic acid formation (Gadd et al., 2014). The substrate acidification was observed in *A. muscaria* in the presence of 10 mmol/L vanadate. We hypothesize that the extrahyphal crystals produced by *A. muscaria* with vanadate medium could be precipitated V-oxalate. *X. badius* produced yellow-brown pigments at higher concentration of vanadyl or V$_2$O$_3$, and *B. adusta* produced green pigments at higher concentration of vanadate or vanadyl. The pigments reflect the extent of environmental stress (Hanson, 2008). However, the pigments also play a role in helping fungi accumulate V from medium as some of the fungal pigments are less highly colored quinol or phenol which is able to form a metal complex (Gadd, 2000; Hanson, 2008). Extrahyphal crystals have been observed of *B. adusta* in the presence of 10 mmol/L vanadyl.

All the tested species have shown the abilities of solubilization and accumulation of insoluble V oxides (V$_2$O$_3$, VO$_2$ and V$_2$O$_5$). Organic acids like oxalic and citric acid produced by fungi were able to dissolve metal containing minerals (Gadd, 1999; Safdari et al., 2013). However, all four species hardly cause bioweathering of a comprehensive V-Ti mineral, at least in a short time. This was also confirmed by XRD patterns which have not shown the significant difference between the original slag and slags treated by fungi.

Vanadium tolerance, solubilization and accumulation by *A. muscaria*, *A. cepistipes*, *X. badius* and *B. adusta* in the presence of V oxidation states of III to V, and both vanadate and vanadyl might suggest future environmental and industrial implications. The tested species are widely spread basidiomycetes which can grow on ground or wood and are associated with various deciduous trees, coniferous trees, or mixed wood. In relation to the capabilities of these fungi, they may play a role in the V cycling in soils. Fungi may transform immobile V to mobile V, but they may also immobilize V via biosorption into mycelium or form the
extracellular V containing crystals under certain conditions. In perspective, fungi might be applied in the recycling of V containing wastes.
Discussion

References


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