Ageing of vanadium in soils and consequences for bioavailability

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Summary

Total vanadium (V) concentrations in soils commonly range from 20 to 120 mg kg^{-1}. Vanadium directly added to soils is more soluble than geogenic V, and can be phytotoxic at doses within this range of background concentrations. However, it is unknown how slow sorption reactions change the fate and effect of added V in soils. This study addresses the changes in V solubility, toxicity, and bioavailability in soils over time. Four soils were amended with pentavalent V in the form of a soluble vanadate salt, and extractable V concentrations were monitored over 100 days. The toxicity to barley and tomato plants was evaluated in freshly spiked soils and in the corresponding aged soils that were equilibrated for up to 330 days after spiking. The V concentrations in 0.01 M $CaCl_2$ soil extracts decreased approximately twofold between 14 and 100 days after soil spiking, and the reaction kinetics were similar for all soils. The phytotoxicity of added V decreased on average twofold between freshly spiked and aged soils. The reduced toxicity was associated with a corresponding decrease of V concentrations in the isolated soil solutions and in the shoots. The V speciation in the soil solution of the aged soils was dominated by V(V); less than 8 % was present as V(IV). Oxalate extractions suggest that the V(V) added to soils is predominantly sorbed onto poorly crystalline oxyhydroxides. It is concluded that the toxicity of V measured in freshly spiked soils may not be representative of soils subject to a long-term V contamination in the field.

Introduction

The transition metal vanadium (V) is among the 20 most abundant elements in the earth's crust (Nriagu, 1998a), and therefore naturally occurs in soils. The total V concentrations in European soils, measured in hydrogen fluoride digests, are on

average 68 mg kg^{-1} (with a 10^{th} and 90^{th} percentile of 18 and 123 mg kg^{-1}), and the aqua regia extractable V concentrations are about twofold lower (Salminen, 2005). Vanadium in the environment may also be of anthropogenic origin. Anthropogenic sources of V include mining activities, fossil fuel combustion, and the metal industry where V is an important component of alloys. These sources may directly or indirectly cause emissions of V into the environment (Gustafsson & Johnsson, 2004; Panichev *et al.*, 2006).

Vanadium in soils generally occurs in two redox forms which have contrasting geochemical properties: V(IV) and V(V). Under oxic conditions, V(V) is the most stable redox form, but it may be reduced to V(IV) by humic substances (Lu *et al.*, 1998). Vanadium(IV) mainly occurs as the vanadyl oxocation VO^{2+}, which is strongly bound by different organic ligands including humic substances (Lu *et al.*, 1998; Gustafsson *et al.*, 2007). Vanadium(V) commonly occurs as vanadate anions (HVO_4^{2-} or $H_2VO_4^-$) and is strongly bound by iron oxides and hydroxides (Blackmore *et al.*, 1996; Peacock & Sherman, 2004). The sorption of added V(V) across different soils increases with increasing clay, organic matter, and poorly crystalline Fe and Al oxyhydroxide contents, but appears unrelated to soil pH in the range between pH 4 and 7 (Gäbler *et al.*, 2009). This is in line with the fairly constant affinity of V(V) for goethite across this pH range (Peacock & Sherman, 2004).

Elevated V concentrations in the environment may adversely affect biota, including humans, plants, aquatic organisms, and micro-organisms (Nriagu, 1998b; Gustafsson & Johnsson, 2004). At elevated concentrations, V causes reddening of the aerial parts, stunted growth, and death (Cannon, 1963). The phytotoxic effects of V(V) may in part be explained by its capacity to inhibit phosphate-metabolising systems (Seargeant & Stinson, 1979; Perlin & Spanswick, 1981). The reduction of

V(V) to V(IV) in plant roots has been observed and interpreted as a detoxification mechanism since V(IV) is presumably less toxic to plants than V(V) (Morrell *et al.*, 1986). In culture media, phytotoxicity has been observed at dissolved V concentrations of 3 and 6 mg litre^{-1} (Kaplan *et al.*, 1990a; Kaplan *et al.*, 1990b). In soils, phytotoxic concentrations of added V may be within the range of natural background V concentrations due to the different solubility of both pools, but data are scarce. Toxic effects may occur at added V concentrations as low as 30 mg added V kg^{-1} (Wang & Liu, 1999), whereas in other cases no effects were observed at levels of up to 100 mg added V kg^{-1} (Kaplan *et al.*, 1990b).

Ageing reactions in soils, *i.e.* the long-term changes in solubility that occur after prolonged reaction times, have been observed for many trace metals (e.g. Barrow, 1998). Such ageing reactions may reduce the mobility and bioavailability of chemicals. If ageing reactions are pronounced, toxicity data based on freshly spiked soils have little environmental relevance and may yield limit concentrations below natural background concentrations (Smolders *et al.*, 2009). Therefore, quantitative knowledge of such ageing processes is crucial for setting adequate limit concentrations. Gradual immobilisation reactions of phosphate, an anion structurally similar to vanadate, are well known and have been attributed to diffusion into soil particles (van der Zee & van Riemsdijk, 1988; Barrow, 1991), but ageing of V in soils has rarely been explored. Martin & Kaplan (1998) showed that V concentrations in acid soil extracts of a field plot decreased fivefold over 18 months after spiking with V(IV). No further decrease occurred after 12 additional months. Vangheluwe *et al.* (2007) noted that 24 weeks after soil spiking with V(V), the V concentrations in the pore waters of incubated soils had decreased by factors between 1.5 and 3.4 compared

to the V concentrations two weeks after spiking. The limited available data on V ageing in soils, and on the toxicity of V in soils, warrant further studies.

The goal of this study was to extend the knowledge on ageing of V in soils, and to evaluate the consequences of such ageing reactions on V solubility, bioavailability and toxicity. Such knowledge is currently lacking, but is crucial for regulators in order to set adequate limit concentrations. The objectives were to determine V sorption kinetics in different soils, to compare V phytotoxicity and plant uptake between freshly spiked and aged soils, and to relate the observed trends to differences in solubility.

Materials and methods

Soils were sampled from the top 20 cm layer at four European locations. The soil samples were air-dried, sieved (4 mm), and stored in plastic drums. Selected soil properties are summarised in Table 1. The effective cation exchange capacity (eCEC) was determined in a 0.01 M silver thiourea (AgTU) extract (Pleysier, 1980), and oxalate extractable metals were determined in a 0.2 M ammonium oxalate extract at pH 3 (solid:liquid ratio 1 g:50 ml, 2 hours equilibration in darkness) (Schwertmann, 1964). The soil pH was measured in a 0.01 M $CaCl_2$ soil extract (2 h end-over-end shaking, solid:liquid ratio 1 g:5 ml). Approximately 200 mg of soil material was digested in aqua regia at 140°C in a hot block for 3 hours, the digests were then diluted to 10 ml, and element concentrations were measured by ICP-OES (Inductively Coupled Plasma – Optical Emission Spectroscopy) using a Perkin Elmer Optima 3300 DV. Vanadium was measured at a wavelength of 290.880 nm. The standard reference material NRC Canada LKSD-4 (certified aqua regia-extractable V concentration of 32 mg V kg^{-1}, standard deviation 10 mg V kg^{-1}, n = 31, Lynch, 1990) and the soil sample WEPAL 921 from the WEPAL international soil-analytical exchange program

(consensus value of acid extractable V concentration of 51.2 mg V kg^{-1}, standard deviation 6.6 mg V kg^{-1}, n = 136) were included on a regular basis in the aqua regia digestions. The recovery of V was on average 108 % for LKSD-4 (standard deviation 1.4 mg V kg^{-1}, n = 4) and 96 % for WEPAL 921 (standard deviation 2.5 mg V kg^{-1}, n = 3).

Experiment 1: Vanadium reaction kinetics

Air-dry samples of all four studied soils (about 500 g) were wetted with deionised water, incubated at 20°C in darkness for one week, and then amended with dissolved analytical-grade sodium metavanadate ($NaVO_3$) to nominal concentrations of 32 and 100 mg added V kg^{-1}. Metavanadate reacts quickly with water to form orthovanadate (VO_4^{3-}) (Crans *et al.*, 1995). This salt was preferred to sodium orthovanadate (Na_3VO_4) because the latter would cause a greater change in both salinity and pH. Soil spiking was performed on the bulk soil sample by spraying a spiking solution (deionised water containing the adequate amount of dissolved $NaVO_3$) over the soil using a pipette. The volume of liquid added to each treatment of a soil was exactly the same. After spiking, the soil samples were thoroughly mixed. The soil V concentrations were measured as described earlier (ICP-OES after aqua regia digestion) and were within 20 % of the nominal values. In preliminary experiments, it was ascertained that this spiking method yielded homogenously spiked soils: the variability in soil V concentrations in different subsamples of about 1 g was not greater than the variability inherent to the digestion and ICP-OES analyses. After spiking, the soil moisture content was increased with deionised water to approximately 75 % of that at pF 2.0, and the soil samples were incubated at 20°C in darkness in plastic pots.

142 The soil samples were extracted between 3 and 100 days after soil spiking with
143 0.01 M $CaCl_2$ (solid:liquid ratio 1 g:1 ml, 4 hours end-over-end shaking). The
144 conditions in such extracts are assumed to mimick those in the soil solution (Degryse
145 *et al.*, 2003) and such extracts have previously also been used for the quantification of
146 short-term V mobility (Cappuyns & Slabbinck, 2012). The extractions undertaken 100
147 days after soil spiking were performed in duplicate; at other times only one replicate
148 was extracted. The low replication of the experiment somewhat compromises the
149 reliability of the results. However, the repeatability between the replicate extractions
150 after 100 days was excellent: the coefficients of variation were below 0.03 for all
151 treatments except one. The unspiked and spiked Pustnäs, Säby, and Ter Munck soils
152 were also extracted with 0.2 M ammonium oxalate at pH 3 (solid:liquid ratio
153 1 g:50 ml, 2 hours equilibration in darkness) (Schwertmann, 1964) in an attempt to
154 quantify the V bound to poorly crystalline oxyhydroxides. The V concentrations in the
155 $CaCl_2$ and oxalate extracts were measured by ICP-OES after centrifugation (3000 g,
156 15 min) and filtration of the supernatant (0.45 µm, disposable regenerated cellulose
157 filter). The V concentrations in both extractants and in blank extractions were below
158 the limit of quantification (approximately 3 µg $litre^{-1}$) and therefore no blank
159 corrections were applied.

160 *Soil spiking and pretreatment for toxicity testing*

161 The toxicity assays were performed in freshly spiked and aged Pustnäs, Säby, and Ter
162 Munck soils. An unspiked control and seven treatment levels were established with
163 nominal added V concentrations of 3.2, 10, 32, 100, 320, 1000, and
164 3200 mg added V kg^{-1} dry soil. For the freshly spiked soils, air dry soils were
165 rewetted two weeks before toxicity testing to a moisture content of about 50 % of that
166 at pF 2.0 using deionised water. These soils were then incubated for one week at 20°C

in darkness. The soil samples were subsequently spiked in the same manner as described above, except that for the 3200 mg V kg^{-1} treatment, the spiking was performed using a suspension. The moisture content of the soil samples was increased to approximately 75 % of that at pF 2.0 using deionised water. For the plant growth assays, the soils were fertilised with 50 mg P kg^{-1} as dissolved KH_2PO_4 and 100 mg N kg^{-1} as dissolved KNO_3. The freshly spiked soils were then equilibrated for one more week at 20°C in darkness prior to toxicity testing.

For the aged soils, the spiking was carried out in the same manner and at the same seven doses of $NaVO_3$ as described above. The control and spiked soils were placed in pots (5 kg soil per pot) with free drainage in outdoor conditions. The Ter Munck soil was spiked in April 2010 and aged in Belgium for approximately 150 days. The Pustnäs and Säby soils were spiked in October 2009 and aged in Sweden for approximately 330 days. After that, the aged soils were air-dried, sieved, further air-dried, and stored. Two weeks prior to the toxicity tests, the air-dried aged soils were wetted to a moisture content of about 50 % of that at pF 2.0, and thenceforth treated in the same manner as the freshly spiked soils. The V concentrations in the freshly spiked and aged soils were measured with ICP-OES after aqua regia digestion as described above.

Experiment 2: Root elongation assay

The root elongation assay (ISO 11269-1) evaluates treatment effects on root formation and was performed on summer barley (*Hordeum vulgare* L.). Three replicate pots per treatment were filled with approximately 500 g of soil. Barley seeds were pregerminated in a wet cloth at 20°C in the dark for 24 hours, and five pregerminated seeds were sown in each pot. The soil surface was covered with a 1 cm layer of inert polyethylene beads to reduce evaporation. The pots were placed in randomised order

192 in a growth cabinet under the following conditions: 16—8 hour light-dark regime
193 (light intensity approximately 650 mol photons m^{-2} s^{-1}), 20—16°C temperature
194 regime, and a constant humidity of 70 %. Moisture loss was replaced daily. After 5
195 days of growth, the longest root of each seedling was measured. For each pot, the
196 average length of the longest root of 5 seedlings was calculated.

197 *Experiment 3: Plant growth assay and soil solution analysis*

198 The plant growth assay (ISO 11269-2) assesses the toxic effect of V on the early
199 stages of growth of higher plants and was performed on summer barley and tomato
200 (*Lycopersicon esculentum* Miller). Four replicate pots per treatment were filled with
201 approximately 500 g of soil. Ten pregerminated barley seeds or 20 tomato seeds were
202 uniformly sown in each pot. The soil surface was covered with a 1 cm layer of inert
203 beads. The pots were placed in randomised order in a growth cabinet under the same
204 conditions as described above, and moisture loss was replaced daily. As soon as 70 %
205 of the seeds had emerged in each control pot (*i.e.* after 3 and 8—11 days for barley
206 and tomato, respectively), seedlings were thinned to yield five evenly spaced
207 representative specimens per pot. After an additional 13—15 days of growth, shoots
208 were cut and dry shoot mass in each pot was recorded after oven drying at 65°C for at
209 least one day. The dried barley plant material was crushed and approximately 200 mg
210 were digested with 3 or 4 ml of 67 % nitric acid at 180°C in a hot block. Digests were
211 diluted to 5 ml and element concentrations were measured by ICP-OES. The tomato
212 leaf sample NIST 1573a (certified total V concentration of 0.835 mg V kg^{-1}, 95 %
213 confidence limits ± 0.010 mg V kg^{-1}) was included in each batch and its recovery was
214 on average 91 % (standard deviation 0.08 mg V kg^{-1}, n = 6).

215 After the plant growth assay, the soils of the control treatment and of at least two
216 treatment levels around the EC_{50} (added V concentration at which 50 % reduction in

response variable is observed, see below) of both freshly spiked and aged soils were sampled in duplicate, *i.e.* from two different replicate pots. Their moisture content was increased to between 80 and 90 % of that at pF 2.0 in order to extract a sufficient amount of soil solution, and the soils were incubated for 3 days. Thereafter, the soil solution was extracted using a direct centrifugation method (Merckx *et al.*, 2001): approximately 50 g of soil sample was centrifuged at approximately 3000 g for 15 minutes during which the soil solution drained through a glasswool plug into a collecting vial below. The soil solutions of the freshly spiked soils were extracted between 26 and 33 days after spiking, and those of the aged soils about 190 (Ter Munck) or 370 (Pustnäs, Säby) days after spiking. The soil solution pH was measured and V concentrations were determined with ICP-OES.

The V speciation was measured in one treatment level close to the EC_{50} of each aged soil. The centrifugation method did not yield enough soil solution volume for the V speciation analysis. Therefore, the V speciation was measured in a 0.01 M $CaCl_2$ soil extract (4 h end-over-end shaking, solid:liquid ratio 1 g:1 ml, one replicate), and it was assumed that the speciation in such extracts was similar to that in the soil solution. The V(V) and V(IV) concentrations were measured within a week according to the method of Aureli *et al.* (2008). The V(V) and V(IV) species were stabilised by converting them into V–EDTA complexes and determined by anion exchange liquid chromatography-inductively coupled plasma mass spectrometry (HPLC-ICP-MS), using a Perkin Elmer Series 200 chromatographic system and an Elan DRC II ICP-MS. Post-column recovery was evaluated by comparing the sum of the V species determined by HPLC-ICP-MS with total V determined by ICP-MS and was 102 % on average.

Statistical analysis

The sorption kinetics, *i.e.* the V concentrations in $CaCl_2$ extracts, were fitted using a reversible first order kinetic model: $[V] = A \cdot \exp(-k \cdot t) + [V_{eq}]$, where k is a rate constant (the sum of the forward and backward first order rate constants), and $[V_{eq}]$ is the V concentration at equilibrium. The concentration profiles over time (Figure 1; see below) suggest that the V concentration is close to equilibrium in all soils after 100 days, and therefore it was assumed that the $[V_{eq}]$ was equal to the V concentration in the extract prepared 100 days after spiking (averaged over two replicate extracts). The linearised form of the above model, $\log([V] - [V_{eq}]) = \log(A) - k \cdot t$, was then used to fit the V concentrations in the extracts prepared between 3 and 30 days after spiking with a least-squares algorithm. The assumption of near-equilibrium after 100 days is not backed by longer term data, but is made here only for the purpose of fitting the first order kinetic model using two instead of three parameters ($[V_{eq}]$ is not fitted but fixed). When all three parameters were fitted, unrealistic fits were obtained that did not follow the trend suggested by the data. Since there is no further data available, the results should not be extrapolated beyond 100 days after spiking.

A 3-parameter log-logistic dose-response model was fitted to the dose-response plots of toxicity assays (Doelman & Haanstra, 1989): $Y = C \cdot [1 + \exp(b \cdot (\ln X - \ln EC_{50}))]^{-1}$, where Y is the response variable, C the upper limit of the response variable, b the slope parameter, X the dose variable, and EC_{50} the dose at which a 50 % reduction in the response variable was obtained. The soil added V concentration was used as the dose variable since native V in soils is much less soluble than added V (see below). It was calculated as the measured V concentration in aqua regia digests minus the background V concentration. However, for treatments with nominal added V concentrations of 3.2 and 10 mg kg^{-1}, *i.e.* lower than the background V concentration, the precision of this difference was low, and therefore nominal added

V concentrations were used. An arbitrary small value of 1 mg added V kg^{-1} was assigned to the control treatment because the dose is expressed in log units in the empirical model. Model parameters and their standard errors were estimated with the Marquardt method (Marquardt, 1963) using the NLIN procedure of the statistical software SAS. The difference between pairs of EC_{50} estimates was tested for significance by estimating its variance as the sum of the variance of each separate EC_{50} value, and by then performing a single sided t-test at $P = 0.05$.

Sorption curves were drawn by plotting the soil added V concentrations (as measured in aqua regia digests) against the V concentrations measured in the isolated soil solutions. These data were fitted with a Freundlich-type sorption model, $V_S = K \cdot [V]^n$, where $[V]$ is the V concentration in the soil solution, and V_S the sorbed V concentration. The measured soil added V concentration was used here as a surrogate for the sorbed V concentration V_S. The NLIN procedure (SAS) was used to calculate parameter estimates and their standard errors with a least squares algorithm.

Results and discussion

Vanadium reaction kinetics (experiment 1)

The V concentrations in dilute $CaCl_2$ extracts decreased over time (Figure 1). The fitted rate constants for the sorption of V in soils varied surprisingly little across the four studied soils and were between 0.03 and 0.08 day^{-1} (Table 2). The fitted curve was used to calculate the soluble V concentration 14 days after spiking, $[V_{14}]$, and this value was compared to the $[V_{100}]$ measured after 100 days. The quotient $[V_{14}]$:$[V_{100}]$ was calculated, and these ageing factors ranged between 1.6 and 2.5 (average 1.9, standard error 0.1) across all treatments. The replication in this assay was low and therefore reliability is somewhat compromised. However, agreement with other assays

291 is excellent (see below), and the ageing factor of about 2 is also in good agreement
292 with earlier work (Vangheluwe *et al.*, 2007). Martin & Kaplan (1998) reported a
293 fivefold solubility difference between freshly spiked and aged soils, but they spiked
294 with a V(IV) salt and at much lower concentrations which may explain the difference.
295 The pH of the soil extracts after 100 days was between 0.1 and 0.5 units lower
296 compared to the corresponding values obtained 7 days after spiking, likely due to
297 microbial activity. This acidification may have affected V sorption, but pH effects on
298 V sorption in soils are generally small between pH 4 and 7 (Gäbler *et al.*, 2009).
299 Therefore, this effect is assumed to be of limited importance.

300 In the oxalate extracts prepared 3 days after soil spiking, the mean V recovery
301 was 98 % of the nominal added V with a standard error of 4 %. Oxalate extractions
302 are routinely used for the quantification of poorly crystalline Fe, Al and Mn
303 oxyhydroxides because oxalate dissolves such oxyhydroxides (Schwertmann, 1964).
304 Therefore, the near complete recovery indicates that added V(V) in these soils was
305 predominantly sorbed onto poorly crystalline oxyhydroxides, either in reversible or in
306 irreversible form. This finding is in line with previous studies on phosphate which is
307 structurally similar to vanadate (van der Zee & van Riemsdijk, 1988). It is also in
308 agreement with the well documented high affinity of V(V) for oxyhydroxides
309 (Blackmore *et al.*, 1996; Peacock & Sherman, 2004), and with Gäbler *et al.* (2009)
310 who found a strong correlation between V sorption in soils and poorly crystalline
311 oxyhydroxide content.

312 In the unspiked soils, mean recoveries of V in oxalate extracts varied between 13
313 and 35 % of the aqua regia soluble V. The much lower recovery of the background V
314 shows that it reacts in a different way from added V. This agrees with earlier studies
315 (Gustafsson & Johnsson, 2004; Gäbler *et al.*, 2009). We speculate that in the

environment a large fraction of the naturally present V is essentially unreactive in soils at timescales shorter than the chemical weathering processes of minerals. This view is supported by the fact that average aqua regia extractable V concentrations in soils are twofold lower compared to total (HF extractable) V concentrations (Salminen, 2005).

Root elongation and plant growth assays (experiments 2 and 3)

The aged soils were assessed for changes in V concentration, V speciation, and pH. Such changes should ideally be minor in order to allow a reliable comparison between freshly spiked and aged treatments. The V concentrations in the aqua regia digests indicate that, during the ageing process outdoors, a large fraction of the added V in the high treatment levels was removed, likely due to leaching. This effect was the most pronounced in the Pustnäs soil: approximately 160 mg added V kg^{-1} was left in the three highest treatment levels which were initially amended with 320, 1000, and 3200 mg V kg^{-1}. However, this does not pose a problem for the comparison of toxicity in freshly spiked and aged treatments: leaching effects are accounted for by using the measured soil added V concentration after ageing as the dose variable. The speciation measurements show that only a small amount of the soluble V in aged soils (< 8 %) was present as V(IV), the remainder being present as V(V) (Table 3). The reduction of V(V) to V(IV) may render it less toxic (Morrell *et al.*, 1986), but our results show that even after prolonged ageing periods, this reaction was not important in the studied soils. The pH of the aged soils generally did not differ more than 0.3 units from that of the freshly spiked soils. Overall, no important changes in soil chemical properties were detected that would compromise a reliable comparison between freshly spiked and aged treatments.

The plant response data and their fitted dose-response curves for freshly spiked and aged soils are shown in Figure 2. The corresponding fitted EC_{50} estimates and their standard errors are shown in Table 4. The EC_{50} estimates are in line with earlier data on V toxicity in soils (Kaplan *et al.*, 1990b; Wang & Liu, 1999). Considerable differences are observed depending on the endpoint and on the soil. Barley root elongation was generally the least sensitive endpoint, followed by barley growth and tomato growth. Vanadium toxicity was generally the least pronounced in the Säby soil, followed by the Ter Munck and the Pustnäs soils. The clay and poorly crystalline Fe contents increased in the order Pustnäs < Ter Munck < Säby, and therefore the toxicity differences between the studied soils are in agreement with the strong correlation between clay content and V sorption, and between poorly crystalline Fe content and V sorption (Gäbler *et al.*, 2009). A comparison of the toxicity data for freshly spiked and aged treatments shows that the EC_{50} estimates of aged soils exceeded those of freshly spiked soils by factors between 1.3 and 2.9 (average 1.9, standard error 0.2, Table 4). All these pairs of EC_{50} estimates differed significantly ($P < 0.05$). In other words, ageing reduced V toxicity approximately twofold. The three studied soils showed no difference in ageing factors, but more rigorous studies are needed before this finding can be extended to other soil types. The above results are in good agreement with the twofold decrease in $CaCl_2$-extractable V concentrations between 14 and 100 days after soil spiking (experiment 1). The extractions at day 14 and day 100 may be considered to represent the situation in freshly spiked soils and aged soils, respectively. It is concluded that measurements of V toxicity in freshly spiked soils may not be representative of long-term contaminated soils in the field.

The average measured V concentrations in barley shoots are plotted against the soil added V concentrations (Figure 3). Variability between replicate experiments was low: the coefficient of variation between seven treatments performed in duplicate or triplicate was between 0.01 and 0.12. The shoot V concentrations in the control treatments varied little across soils and ranged from 0.2 to 0.3 mg V kg^{-1} dry plant tissue. This agrees well with the range of 0.18—0.42 mg V kg^{-1} dry plant tissue reported for common dry weight based V concentrations in grass shoots grown on unpolluted soils (Kabata-Pendias & Pendias, 2001). At low soil added V concentrations, shoot V concentrations were not or marginally increased compared to the control treatment. As soil added V concentrations increase (to about half the EC_{50} value and above), shoot V concentrations increased to values of 1 mg V kg^{-1} and above. At these elevated added V concentrations, shoot V concentrations in aged treatments were significantly ($P < 0.05$) lower than those in the corresponding freshly spiked treatments. This confirms that V toxicity is associated with an increased V translocation to the shoot, and that ageing reactions result in a reduced bioavailability and translocation of V. It is concluded that, over time, ageing reactions cause V added to soils to become less bioavailable and toxic.

Soil solution analysis (experiment 3)

Analysis of the soil solutions of unamended soils isolated after the barley growth assay revealed V concentrations between 0.005 and 0.020 mg $litre^{-1}$. The partition coefficients of geogenic V in the unamended soils ($K_d = V_S / [V]$) were between 10 and 60 times greater than those of freshly added V in the soils spiked with 32 mg V kg^{-1} (a concentration within the range of the geogenic V concentrations of the studied soils). The low solubility of V in soils is in agreement with earlier studies

(Cappuyns & Slabbinck, 2012). This again highlights the difference between the background V and the V added to soils as also discussed earlier.

The added V concentrations are plotted against the V concentrations in isolated soil solutions, and fitted Freundlich-type isotherms are shown (Figure 4). The EC_{50} estimates for barley growth in each freshly spiked soil are indicated with a horizontal line. Freundlich parameters for freshly spiked and aged treatments differed ($P < 0.05$), showing greater V solubility in the freshly spiked treatments. The difference in solubility between treatments was quantified by evaluating fitted isotherms at V_S concentrations equal to the EC_{50} estimates for barley growth in freshly spiked soils (horizontal line in Figure 4). These V_S concentrations were selected because they represent the toxic range of V in soils. The V concentrations in the soil solutions of aged treatments calculated in this manner were 1.7, 2.6, and 2.3 times lower than those in the corresponding freshly spiked treatments of the Pustnäs, Säby, and Ter Munck soils, respectively. These factors are in excellent agreement with and confirm the results discussed earlier. Phytotoxicity in aged soils is approximately twofold lower compared to freshly spiked soils, and this is associated with a twofold lower V solubility.

Conclusions

Taken together, it has been shown that soluble V concentrations in four different soils decreased approximately twofold between 14 and 100 days after soil spiking with V(V). These results were modelled using a simple reversible first-order model with a kinetic rate constant between 0.03 and 0.08 day^{-1}. After ageing reaction times from 150 to 330 days, V phytotoxicity was reduced approximately twofold compared to the corresponding freshly spiked soils. Dissolved V concentrations in the isolated soil solutions of such aged soils were also about twofold lower than those in freshly

spiked soils. The decreased phytotoxicity in aged soils was accompanied by a decreased V translocation to the shoot. Overall, the effects of V ageing reactions across the four studied soils were surprisingly similar, but more studies are warranted in order to check if this finding can be extrapolated to other soil types. Extractions with oxalate suggest that V(V) added to soils is predominantly bound to poorly crystalline oxyhydroxides, whereas this is only true for a small fraction of the naturally present V in soils. The naturally present V in the investigated soils is much less soluble than the freshly added V. If EC_{50} values are expressed as added V, they often are within the common range of background V concentrations in soils. Toxicity data measured in freshly spiked soils may not be representative for long-term and well equilibrated soil contaminations in the field.

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References

F. Aureli, S. Ciardullo, M. Pagano, A. Raggi & F. Cubadda. 2008. Speciation of vanadium(IV) and (V) in mineral water by anion exchange liquid chromatography-

436 inductively coupled plasma mass spectrometry after EDTA complexation. *Journal*
437 *of Analytical Atomic Spectrometry*, **23**, 1009-1016.

438 N.J. Barrow. 1991. Testing a mechanistic model. XI. The effects of time and of level
439 of application on isotopically exchangeable phosphate. *Journal of Soil Science*, **42**,
440 277-288.

441 N.J. Barrow. 1998. Effects of time and temperature on the sorption of cadmium, zinc,
442 cobalt, and nickel by a soil. *Australian Journal of Soil Research*, **36**, 941-950.

443 D.P.T. Blackmore, J. Ellis & P.J. Riley. 1996. Treatment of a vanadium-containing
444 effluent by adsorption-coprecipitation with iron oxyhydroxide. *Water Research*, **30**,
445 2512-2516.

446 H.L. Cannon. 1963. The biogeochemistry of vanadium. *Soil Science*, **96**, 196-204.

447 V. Cappuyns & E. Slabbinck. 2012. Occurrence of vanadium in Belgian and
448 European alluval soils. *Applied and Environmental Soil Science*, **2012**.

449 D.C. Crans, M. Mahroof-Tahir & A.D. Keramidas. 1995. Vanadium chemistry and
450 biochemistry of relevance for use of vanadium compounds as antidiabetic agents.
451 *Molecular and Cellular Biochemistry*, **153**, 17-24.

452 F. Degryse, K. Broos, E. Smolders & R. Merckx. 2003. Soil solution concentration of
453 Cd and Zn can be predicted with a CaCl2 soil extract. *European Journal of Soil*
454 *Science*, **54**, 149-157.

455 P. Doelman & L. Haanstra. 1989. Short- and long-term effects of heavy metals on
456 phosphatase activity in soils: an ecological dose response model approach. *Biology*
457 *and Fertility of Soils*, **8**, 235-241.

458 H.E. Gäbler, K. Gluh, A. Bahr & J. Utermann. 2009. Quantification of vanadium
459 adsorption by German soils. *Journal of Geochemical Exploration*, **103**, 37-44.

J.P. Gustafsson & L. Johnsson. 2004. Vanadin i Svensk miljö - förekomst och toxicitet (Vanadium in the Swedish environment - occurrence and toxicity). KTH, Stockholm, Available online at http://www2.lwr.kth.se/Publikationer/PDF_Files/LWR_REPORT_3009.pdf.

J.P. Gustafsson, I. Persson, D.B. Kleja & J.W.J. Van Schaik. 2007. Binding of iron(III) to organic soils: EXAFS spectroscopy and chemical equilibrium modeling. *Environmental Science & Technology*, **41**, 1232-1237.

A. Kabata-Pendias & H. Pendias. 2001. *Trace elements in soils and plants, 3rd edition*. CRC Press.

D.I. Kaplan, D.C. Adriano, C.L. Carlson & K.S. Sajwan. 1990a. Vanadium - Toxicity and accumulation by beans. *Water Air and Soil Pollution*, **49**, 81-91.

D.I. Kaplan, K.S. Sajwan, D.C. Adriano & S. Gettier. 1990b. Phytoavailability and toxicity of beryllium and vanadium. *Water Air and Soil Pollution*, **53**, 203-212.

X.Q. Lu, W.D. Johnson & J. Hook. 1998. Reaction of vanadate with aquatic humic substances: An ESR and V-51 NMR study. *Environmental Science & Technology*, **32**, 2257-2263.

J. Lynch. 1990. Provisional elemental values for eight new geochemical lake sediment and stream sediment reference materials LKSD-1, LKSD-2, LKSD-3, LKSD-4, STSD-1, STSD-2, STSD-3 and STSD-4. *Geostandards Newsletter*, **14**, 153-167.

D.W. Marquardt. 1963. An algorithm for least-squares estimation of nonlinear parameters. *Journal of the Society for Industrial and Applied Mathematics*, **11**, 431-441.

H.W. Martin & D.I. Kaplan. 1998. Temporal changes in cadmium, thallium, and vanadium mobility in soil and phytoavailability under field conditions. *Water Air and Soil Pollution*, **101**, 399-410.

R. Merckx, K. Brans & E. Smolders. 2001. Decomposition of dissolved organic carbon after soil drying and rewetting as an indicator of metal toxicity in soils. *Soil Biology and Biochemistry*, **33**, 235-240.

B.G. Morrell, N.W. Lepp & D.A. Phipps. 1986. Vanadium uptake by higher plants - some recent developments. *Environmental Geochemistry and Health*, **8**, 14-18.

J.O. Nriagu ed. 1998a. *Vanadium in the environment. Part 1: Chemistry and biochemistry*. Wiley.

J.O. Nriagu ed. 1998b. *Vanadium in the environment. Part 2: Health effects*. Wiley.

N. Panichev, K. Mandiwana, D. Moema, R. Molatlhegi & P. Ngobeni. 2006. Distribution of vanadium(V) species between soil and plants in the vicinity of vanadium mine. *Journal of Hazardous Materials*, **137**, 649-653.

C.L. Peacock & D.M. Sherman. 2004. Vanadium(V) adsorption onto goethite (alpha-FeOOH) at pH 1.5 to 12: A surface complexation model based on ab initio molecular geometries and EXAFS spectroscopy. *Geochimica et Cosmochimica Acta*, **68**, 1723-1733.

D.S. Perlin & R.M. Spanswick. 1981. Characterization of ATPase activity associated with corn leaf plasma-membranes. *Plant Physiology*, **68**, 521-526.

J.L. Pleysier. 1980. A single-extraction method using silver-thiourea for measuring exchangeable cations and effective CEC in soils with variable charges. *Soil Science*, **129**, 205-211.

R. Salminen ed. 2005. *Geochemical atlas of Europe, part one*. Geological Survey of Finland, Espoo, Finland.

U. Schwertmann. 1964. Differenzierung der Eisenoxide des Bodens durch Extraktion mit Ammoniumoxalat Lösung. *Zeitschrift für Pflanzenernährung, Düngung, Bodenkunde*, **105**, 194-202.

L.E. Seargeant & R.A. Stinson. 1979. Inhibition of human alkaline-phosphatases by vanadate. *Biochemical Journal*, **181**, 247-250.

E. Smolders, K. Oorts, P. Van Sprang, I. Schoeters, C.R. Janssen, S.P. McGrath, *et al.* 2009. Toxicity of trace metals in soil as affected by soil type and aging after contamination: using calibrated bioavailability models to set ecological soil standards. *Environmental Toxicology and Chemistry*, **28**, 1633-1642.

S.E.A.T.M. van der Zee & W.H. van Riemsdijk. 1988. Model for long-term phosphate reaction kinetics in soil. *Journal of Environmental Quality*, **17**, 35-41.

M. Vangheluwe, M. Vandenbroele, P. Van Sprang, E. Smolders, F. Degryse, A. Ruttens, *et al.* 2007. Evaluatie normstelling bodem en secundaire grondstoffen voor bijkomende metalen (Evaluation of standards for additional metals in soil and secondary raw materials). Report. Arcadis, Katholieke Universiteit Leuven & Limburgs Universitair Centrum.

J.F. Wang & Z. Liu. 1999. Effect of vanadium on the growth of soybean seedlings. *Plant and Soil*, **216**, 47-51.

Figure 1. Vanadium concentrations in 0.01 M $CaCl_2$ soil extracts prepared during soil incubation at 20°C in soils spiked with 32 (diamonds) and 100 (crosses) mg V kg^{-1}. Dashed lines are first-order model fits. Extractions after 100 days were performed in duplicate but these data points overlap.

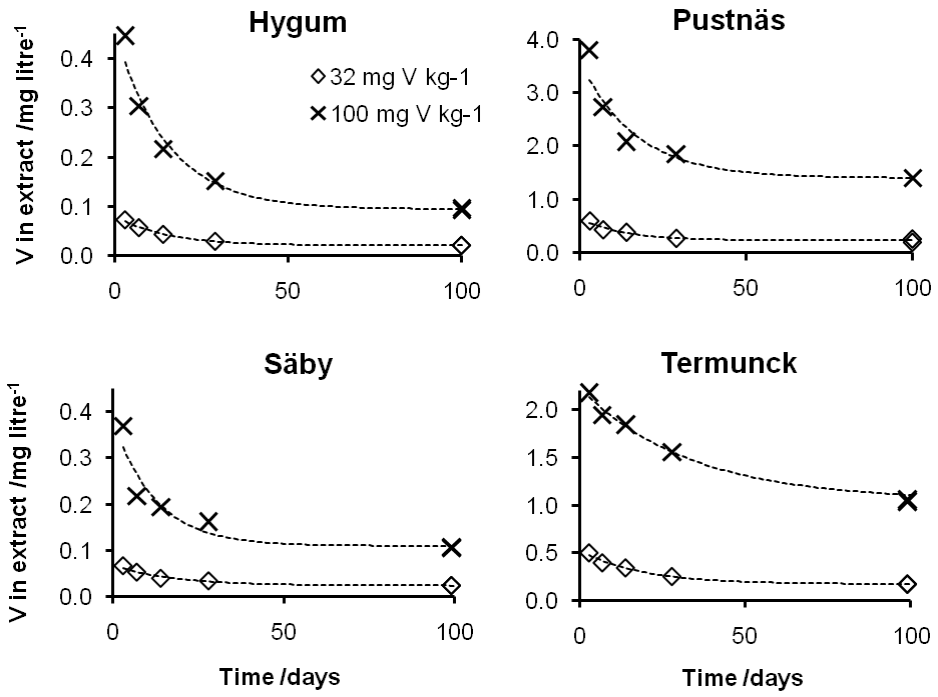
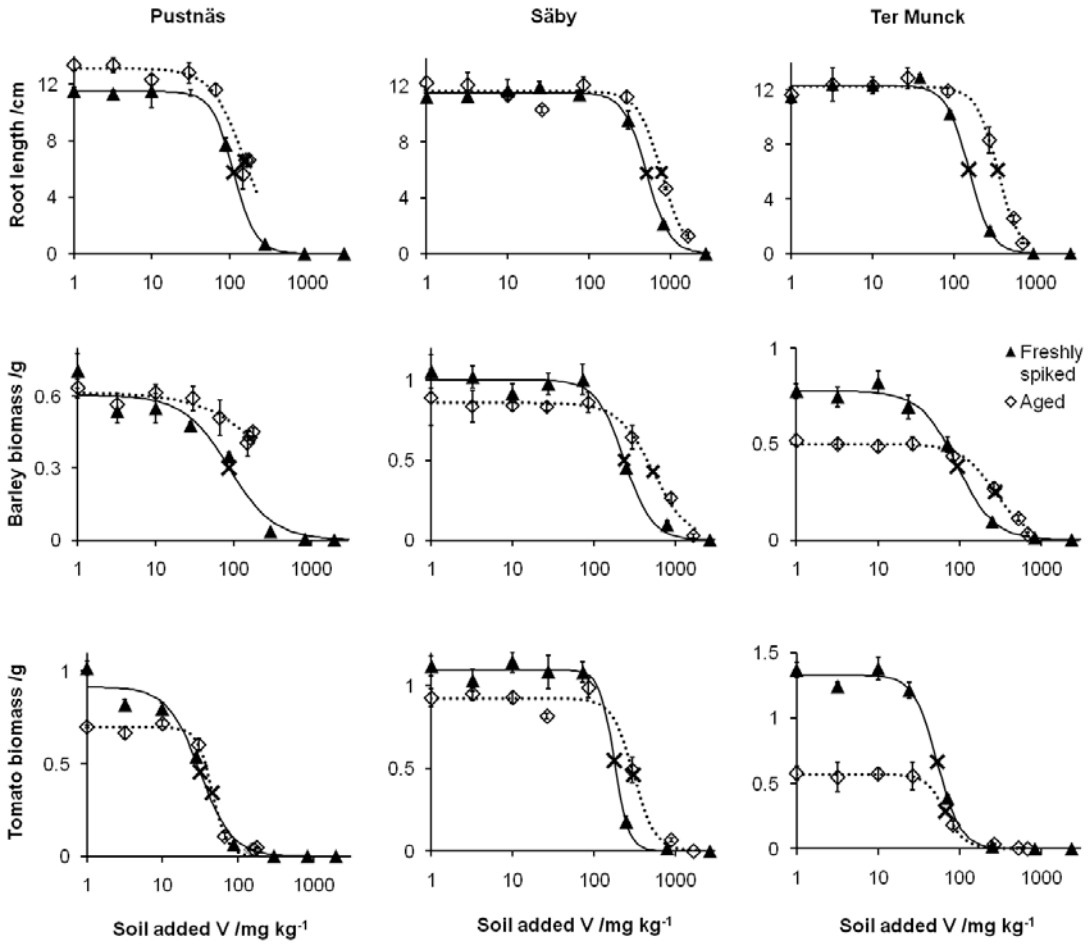


Figure 2. Dose-response relationships for the root elongation (top), barley growth (middle), and tomato growth (bottom) endpoints in the freshly spiked and aged Pustnäs (left), Säby (middle), and Ter Munck (right) soils. The x-axis values are soil added V concentrations (background corrected) measured in aqua regia digests. Freshly spiked soils: closed triangles (data points) and full line (model fit); aged soils: open diamonds and dotted line. The error bars represent standard deviations. The EC_{50} estimates are marked with a cross (X).



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549 **Figure 3** Average barley shoot V concentrations plotted against soil added V
550 concentrations. Freshly spiked soils: closed triangles connected with full lines; aged
551 soils: open diamonds connected with dashed lines. Coefficients of variation between
552 replicate measurements were between 0.01 and 0.12.

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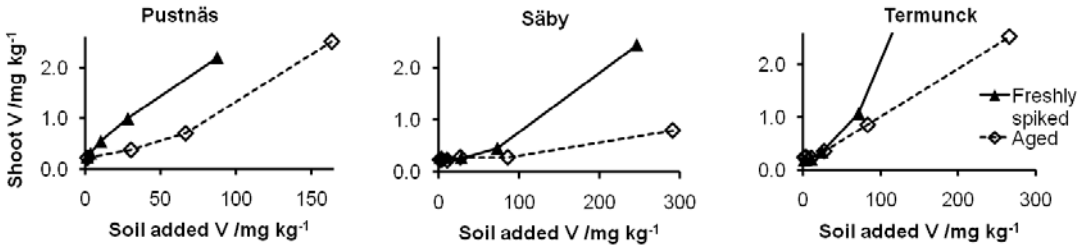

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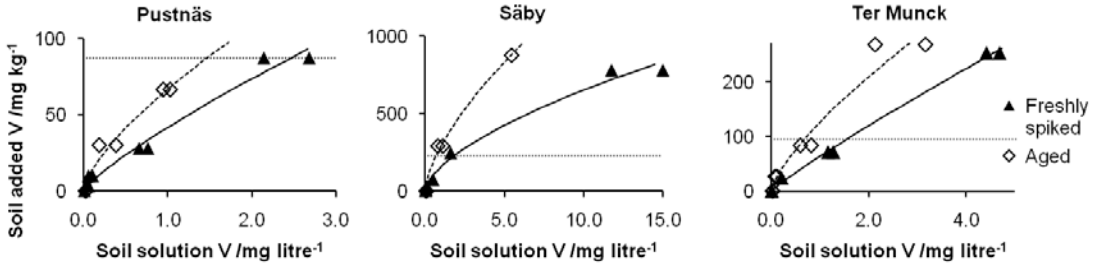
Figure 4 Sorption isotherms with the soil added V (background corrected) plotted against the V concentration in isolated soil solutions. Freshly spiked soils: closed triangles (data points) + full line (fitted Freundlich isotherm); aged soils: open diamonds + dashed line. The horizontal line indicates the EC_{50} for barley growth in the freshly spiked soils.



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569 **Table 1** Characteristics of unspiked soils

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		Hygum	Pustnäs	Säby	Ter Munck
Location		Denmark	Sweden	Sweden	Belgium
Soil type		n.d.	Eutric regosol	Eutric cambisol	Haplic luvisol
pH		5.2	5.9	5.5	6.6
eCEC /cmol$_c$ kg^{-1}		7.6	4.3	10.2	7.3
Texture	**sand /%**	56	86	34	19
	silt /%	31	3	37	64
	clay /%	13	11	29	17
Oxalate extractable	**Al /g kg^{-1}**	1.8	0.8	1.3	0.6
	Fe /g kg^{-1}	3.4	1.4	4.4	2.2
	Mn /g kg^{-1}	0.7	0.1	< 0.1	0.4
	V /mg kg^{-1}	7	4	11	12
Aqua regia	**V /mg kg^{-1}**	31	27	58	38

n.d.: not determined

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575 **Table 1** Fitted first order rate constants (*k*) and their standard errors (SE) describing
576 the kinetics of V solubility in 0.01 M $CaCl_2$ soil extracts between 3 and 100 days after
577 soil spiking. The $[V_{14}]$:$[V_{100}]$ is the ratio of soluble V 14 days after spiking to that 100
578 days after spiking.

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Soil	**Nominal added V /mg kg^{-1}**	***k* ± SE /day^{-1}**	**$[V_{14}]$:$[V_{100}]$**
Hygum	32	0.070 ± 0.002	2.1
Hygum	100	0.067 ± 0.009	2.5
Pustnäs	32	0.078 ± 0.008	1.6
Pustnäs	100	0.060 ± 0.016	1.7
Säby	32	0.056 ± 0.011	1.8
Säby	100	0.053 ± 0.019	1.9
Ter Munck	32	0.054 ± 0.005	1.9
Ter Munck	100	0.030 ± 0.003	1.6

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584 **Table 2** Vanadium speciation in 0.01 M $CaCl_2$ extracts of soils spiked with V(V) and

585 subsequently aged for 5—11 months.

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	added V /mg kg^{-1}	**V(IV) extracted** /mg $litre^{-1}$	**V(V) extracted** /mg $litre^{-1}$
Pustnäs	150	0.11	2.92
Säby	290	0.055	0.59
Ter Munck	270	0.14	3.02

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591 **Table 3** EC_{50} estimates and their standard errors fitted using the log-logistic dose-
592 response model in freshly spiked soils and in aged soils, in mg added V kg^{-1}. All pairs
593 of EC_{50} estimates for freshly spiked and aged soils differ significantly ($P < 0.05$).

594

		Root elongation	Barley growth	Tomato growth
Pustnäs	**freshly spiked**	110 ± 4	87 ± 12	31 ± 2
	aged	160 ± 7	> 180 [a]	46 ± 1
	ratio	1.4	> 2.1 [a]	1.5
Säby	**freshly spiked**	510 ± 18	230 ± 14	180 ± 24
	aged	780 ± 44	530 ± 50	310 ± 14
	ratio	1.5	2.3	1.7
Ter Munck	**freshly spiked**	150 ± 9	94 ± 6	53 ± 2
	aged	340 ± 11	270 ± 14	68 ± 7
	ratio	2.3	2.9	1.3

595 [a] The EC_{50} for barley growth in the aged Pustnäs soil is unbounded, *i.e.* no 50 % reduction in biomass
596 yield was observed at the highest treatment level of 180 mg V kg^{-1}.

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