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Ageing of vanadium in soils and consequences for bioavailability

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24 **Summary**

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26 Total vanadium (V) concentrations in soils commonly range from 20 to 120 mg kg^{-1}.

27 Vanadium added directly to soils is more soluble than geogenic V, and can be

28 phytotoxic at doses within this range of background concentrations. However, it is

29 unknown how slow sorption reactions change the fate and effect of added V in soils.

30 This study addresses the changes in V solubility, toxicity and bioavailability in soils

31 over time. Four soils were amended with pentavalent V in the form of a soluble

32 vanadate salt, and extractable V concentrations were monitored over 100 days. The

33 toxicity to barley and tomato plants was evaluated in freshly spiked soils and in the

34 corresponding aged soils that were equilibrated for up to 330 days after spiking. The

35 V concentrations in 0.01 M CaCl$_2$ soil extracts decreased by approximately twofold

36 between 14 and 100 days after soil spiking, and the reaction kinetics were similar for

37 all soils. The phytotoxicity of added V decreased on average by twofold between

38 freshly spiked and aged soils. The reduced toxicity was associated with a

39 corresponding decrease in V concentrations in the isolated soil solutions and in the

40 shoots. The V speciation in the soil solution of the aged soils was dominated by V(V);

41 less than 8% was present as V(IV). Oxalate extractions suggest that the V(V) added to

42 soils is predominantly sorbed onto poorly crystalline oxyhydroxides. It is concluded

43 that the toxicity of V measured in freshly spiked soils may not be representative of

44 soils subject to a long-term V contamination in the field.

45

46

47 **Introduction**

48

49 The transition metal vanadium (V) is among the 20 most abundant elements in the

50 earth's crust (Nriagu, 1998a), and therefore occurs naturally in soils. The total V

51 concentrations in European soils, measured in hydrogen fluoride digests are, on

52 average, 68 mg kg^{-1} (with a 10th and 90th percentile of 18 and 123 mg kg^{-1}), and the

53 *aqua regia* extractable V concentrations are about twofold smaller (Salminen, 2005).

54 Vanadium in the environment may also be of anthropogenic origin which include

55 mining activities, fossil fuel combustion and the metal industry where V is an

56 important component of alloys. These sources may directly or indirectly cause

57 emissions of V into the environment (Gustafsson & Johnsson, 2004; Panichev *et al.*,

58 2006).

59   Vanadium in soils generally occurs in two redox forms, V(IV) and V(V), which

60 have contrasting geochemical properties. Under oxic conditions, V(V) is the most

61 stable redox form, but it may be reduced to V(IV) by humic substances (Lu *et al.*,

62 1998). Vanadium(IV) mainly occurs as the vanadyl oxo-cation VO^{2+}, which is

63 strongly bound by different organic ligands including humic substances (Lu *et al.*,

64 1998; Gustafsson *et al.*, 2007). Vanadium(V) commonly occurs as vanadate anions

65 (HVO$_4^{2-}$ or H$_2$VO$_4^-$) and is strongly bound by iron oxides and hydroxides (Blackmore

et al., 1996; Peacock & Sherman, 2004). The sorption of added V(V) in 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 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 affect biota, including humans, plants, aquatic organisms, and micro-organisms adversely (Nriagu, 1998b; Gustafsson & Johnsson, 2004). At elevated concentrations, V causes reddening of the aerial parts, stunted growth and eventual death of plants (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 because V(IV) is presumed to be 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; 1990b). In soils, phytotoxic concentrations of added V may be within the range of natural background V concentrations because of the different solubility of both pools, but data for this are scarce. Toxic effects may occur at added V concentrations as small 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, the long-term changes in solubility that occur after prolonged periods, have been observed for many trace metals (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 immobilization 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 by 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 with 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 test the theory that such ageing reactions influenced 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 summarized 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, two hours equilibration in darkness) (Schwertmann, 1964). The soil pH was measured in a 0.01 M $CaCl_2$ soil extract (two hours 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 three hours, the digests were then diluted to 10 ml, and elemental concentrations were measured by ICP-OES (Inductively Coupled Plasma – Optical Emission Spectroscopy) using a Optima 3300 DV (Perkin Elmer, Waltham, MA, United States of America). 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 (consensus value of acid extractable V concentration of 51.2 mg V kg^{-1}, standard deviation 6.6 mg V kg^{-1}, $n = 136$) (WEPAL, 2007) 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}, $n = 3$). All mentioned concentrations are on oven-dry mass basis.

Experiment 1: Vanadium reaction kinetics

Air-dry samples of all four studied soils (about 500 g) were wetted with deionized 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 solution (deionized 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 method yielded homogenously spiked soils: the variability in soil V concentrations in different 1-g sub-samples was not greater than the variability inherent to the digestion and ICP-OES analyses. After spiking, the soil moisture content was increased with deionized water to approximately 75% of that at pF 2.0, and the soil samples were incubated at 20° C in darkness in plastic pots.

154   The soil samples were extracted between three and 100 days after soil spiking
155   with 0.01 M $CaCl_2$ (solid:liquid ratio 1 g:1 ml, four hours end-over-end shaking). The
156   conditions in such extracts are assumed to mimic those in the soil solution (Degryse *et*
157   *al.*, 2003) and such extracts have previously also been used for the quantification of
158   short-term V mobility (Cappuyns & Slabbinck, 2012). The extractions undertaken 100
159   days after soil spiking were performed in duplicate; at other times only one replicate
160   was extracted. The poor replication of the experiment somewhat compromises the
161   reliability of the results. However, the repeatability between the replicate extractions
162   after 100 days was excellent: the coefficients of variation were below 0.03 for all
163   treatments except one. The unspiked and spiked Pustnäs, Säby, and Ter Munck soils
164   were also extracted with 0.2 M ammonium oxalate at pH 3 (solid:liquid ratio
165   1 g:50 ml, two hours equilibration in darkness) (Schwertmann, 1964) in an attempt to
166   quantify the V bound to poorly crystalline oxyhydroxides. The V concentrations in the
167      $CaCl_2$ and oxalate extracts were measured by ICP-OES after centrifugation (3000 g,
168   15 minutes) and filtration of the supernatant (0.45 µm, disposable regenerated
169   cellulose filter). The V concentrations in both extractants and in blank extractions
170   were below the limit of quantification (approximately 3 µg $litre^{-1}$) and therefore no
171   blank corrections were applied.
172
173   *Soil spiking and pre-treatment for toxicity testing*
174   The toxicity assays were performed in freshly spiked and aged Pustnäs, Säby, and Ter
175   Munck soils. An unspiked control and seven treatment levels were established with
176   nominal added V concentrations of 3.2, 10, 32, 100, 320, 1000 and
177   3200 mg added V kg^{-1} dry soil. For the freshly spiked soils, air dry soils were
178   rewetted two weeks before toxicity testing to a moisture content of about 50% of that
179   at pF 2.0 using deionized water. These soils were then incubated for one week at
180   20° C in darkness. The soil samples were subsequently spiked in the same manner as
181   described above, except that for the 3200 mg V kg^{-1} treatment, the spiking was with a
182   suspension. The moisture content of the soil samples was increased to approximately
183   75% of that at pF 2.0 using deionized water. For the plant growth assays, the soils
184   were fertilized with 50 mg P kg^{-1} as dissolved KH_2PO_4 and 100 mg N kg^{-1} as
185   dissolved KNO_3. The freshly spiked soils were then equilibrated for one more week at
186   20° C in darkness prior to toxicity testing.
187   For the aged soils, the spiking was carried out in the same manner and at the same
188   seven doses of $NaVO_3$ as described above. The control and spiked soils were placed
189   in pots (5 kg soil per pot) with free drainage in outdoor conditions. The Ter Munck
190   soil was spiked in April 2010 and aged in Belgium for approximately 150 days. The
191   Pustnäs and Säby soils were spiked in October 2009 and aged in Sweden for
192   approximately 330 days. After that, the aged soils were air-dried, sieved, further air-
193   dried, and stored. Two weeks prior to the toxicity tests, the air-dried aged soils were
194   wetted to a moisture content of about 50% of that at pF 2.0, and thenceforth treated in
195   the same manner as the freshly spiked soils. The V concentrations in the freshly

196 spiked and aged soils were measured with ICP-OES after *aqua regia* digestion as
197 described above.

198

199 *Experiment 2: Root elongation assay*
200 The root elongation assay ISO 11269-1 (International Organisation for
201 Standardisation ISO, 1993) evaluates treatment effects on root formation and was
202 conducted with summer barley (*Hordeum vulgare* L.). Three replicate pots per
203 treatment were filled with approximately 500 g of soil. Barley seeds were pre-
204 germinated in a wet cloth at 20° C in the dark for 24 hours, and five pre-germinated
205 seeds were sown in each pot. The soil surface was covered with a 1-cm layer of inert
206 polyethylene beads to reduce evaporation. The pots were placed in randomized order
207 in a growth cabinet under the following conditions: 16–8 hour light-dark regime (light
208 intensity approximately 650 mol photons $m^{-2} s^{-1}$), 20–16° C temperature regime, and a
209 constant humidity of 70%. Moisture loss was replaced daily. After five days of
210 growth, the longest root of each seedling was measured. For each pot, the average
211 length of the longest root of five seedlings was calculated.

212

213 *Experiment 3: Plant growth assay and soil solution analysis*
214 The plant growth assay ISO 11269-2, (International Organisation for Standardisation
215 ISO, 2005) assesses the toxic effect of V on the early stages of growth of higher plants
216 and was performed on summer barley and tomato (*Lycopersicon esculentum* Miller).
217 Four replicate pots per treatment were filled with approximately 500 g soil. Ten pre-
218 germinated barley seeds or 20 tomato seeds were uniformly sown in each pot. The soil
219 surface was covered with a 1-cm layer of inert beads. The pots were placed in
220 randomized order in a growth cabinet under the same conditions as described above,
221 and moisture loss was replaced daily. As soon as 70% of the seeds had emerged in
222 each control pot (after 3 and 8–11 days for barley and tomato, respectively), seedlings
223 were thinned to yield five evenly spaced representative specimens per pot. After an
224 additional 13–15 days of growth, shoots were cut and dry shoot mass in each pot was
225 recorded after oven drying at 65° C for at least one day. The dried barley plant
226 material was crushed and approximately 200 mg were digested with 3 or 4 ml 67%
227 nitric acid at 180° C in a hot block. Digests were diluted to 5 ml and element
228 concentrations were measured by ICP-OES. The tomato leaf sample 1573a (National
229 Institute of Standards & Technology NIST, 1995) with a certified total V
230 concentration of 0.835 mg V kg^{-1}, 95% confidence limits $\pm$ 0.010 mg V kg^{-1} was
231 included in each batch. Its recovery was, on average, 91% (standard deviation
232 0.08 mg V kg^{-1}, $n = 6$).
233 After the plant growth assay, the soils of the control treatment and of at least two
234 treatment levels around the EC_{50} (added V concentration at which 50% reduction in
235 response variable is observed, see below) of both freshly spiked and aged soils were
236 sampled in duplicate from two different replicate pots. Their moisture content was
237 increased to between 80 and 90% of that at pF 2.0 in order to extract sufficient soil
238 solution, and the soils were incubated for three days. Thereafter, the soil solution was
239 extracted using a direct centrifugation method (Merckx *et al.*, 2001): approximately

240   50 g of soil sample was centrifuged at approximately 3000 g for 15 minutes during

241   which the soil solution drained through a glass-wool plug into a collecting vial below.

242   The soil solutions of the freshly spiked soils were extracted between 26 and 33 days

243   after spiking, and those of the aged soils about 190 (Ter Munck) or 370 (Pustnäs,

244   Säby) days after spiking. The soil solution pH was measured and V concentrations

245   were determined with ICP-OES.

246   The V speciation was measured in one treatment level close to the EC_{50} of each

247   aged soil. The centrifugation method did not yield enough soil solution volume for the

248   V speciation analysis. Therefore, the V speciation was measured in a 0.01 M CaCl$_2$

249   soil extract (four hours end-over-end shaking, solid:liquid ratio 1 g:1 ml, one

250   replicate), and it was assumed that the speciation in such extracts was similar to that

251   in the soil solution. The V(V) and V(IV) concentrations were measured within a week

252   according to the method of Aureli *et al.* (2008). The V(V) and V(IV) species were

253   stabilized by converting them into V–EDTA complexes and determined by anion

254   exchange liquid chromatography-inductively coupled plasma mass spectrometry

255   (HPLC-ICP-MS), using a Perkin Elmer Series 200 chromatographic system and an

256   Elan DRC II ICP-MS. Post-column recovery was evaluated by comparing the sum of

257   the V species determined by HPLC-ICP-MS with total V determined by ICP-MS and

258   was 102% on average.

259

260   *Statistical analysis*

261   The sorption kinetics, *i.e.* the V concentrations in CaCl$_2$ extracts, were fitted with a

262   reversible first order kinetic model:

263        $[V] = A \cdot \exp(-k \cdot t) + [V_{eq}]$,   (1)

264   where k is a rate constant (the sum of the forward and backward first order rate

265   constants), and $[V_{eq}]$ is the V concentration at equilibrium. The concentration profiles

266   over time (Figure 4; see later) suggested that the V concentration was close to

267   equilibrium in all soils after 100 days, and therefore it was assumed that the $[V_{eq}]$ was

268   equal to the V concentration in the extract prepared 100 days after spiking (averaged

269   over two replicate extracts). The linearised form of the above model,

270        $\log([V] - [V_{eq}]) = \log(A) - k \cdot t$,   (2)

271   was then used to fit the V concentrations in the extracts prepared between 3 and 30

272   days after spiking with a least-squares algorithm. The assumption of near-equilibrium

273   after 100 days is not backed by longer-term data, but is made here only for the

274   purpose of fitting the first order kinetic model using two instead of three parameters

275   ($[V_{eq}]$ is not fitted but fixed). When all three parameters were fitted, unrealistic fits

276   were obtained that did not follow the trend suggested by the data. Since there is no

277   further data available, the results should not be extrapolated beyond 100 days after

278   spiking.

279   A three-parameter log-logistic dose-response model was fitted to the dose-

280   response plots of toxicity assays (Doelman & Haanstra, 1989):

281        $Y = C \cdot [1 + \exp(b \cdot (\ln X - \ln EC_{50}))]^{-1}$,   (3)

282   where Y is the response variable, C the upper limit of the response variable, b the

283   slope parameter, X the dose variable, and EC_{50} the dose at which a 50% reduction in

the response variable was obtained. The concentration of V added to soil was used as the dose variable since native forms are much less soluble than added V (see later). This variable was calculated as the measured concentration in *aqua regia* digests minus the background concentration. However, for treatments with nominal added V concentrations of 3.2 and 10 mg kg^{-1} (less than the background V concentration) the precision of this difference was poor, 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,

$$V_S = K \cdot [V]^n, \tag{4}$$

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 with 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. There was no replication in this assay except for the measurement after 100 days, and therefore reliability is somewhat compromised. However, agreement with other assays was excellent (see later), and the ageing factor of about 2 is also in good agreement with earlier work (Vangheluwe *et al.*, 2007). Martin & Kaplan (1998) reported a fivefold solubility difference between freshly spiked and aged soils, but they spiked with a V(IV) salt and at much smaller concentrations which may explain the difference. The pH of the soil extracts after 100 days was between 0.1 and 0.5 units smaller compared with the corresponding values obtained seven days after spiking, probably because of microbial activity. This acidification may have affected V sorption, but pH effects on V sorption in soils are generally small between pH 4 and 7 (Gäbler *et al.*, 2009). Therefore, this effect is assumed to be of limited importance.

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

In the un-spiked soils, mean recoveries of V in oxalate extracts varied between 13 and 35% of the *aqua regia* soluble V. The much poorer recovery of the background V shows that it reacts in a different way from added V agreeing with earlier studies (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 time-scales 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 smaller than 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 treatments with large added V concentrations was removed, probably by leaching. This effect was the most pronounced in the Pustnäs soil: approximately 160 mg added V kg^{-1} was left in the treatments 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 our 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 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 by 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 were plotted against the soil added V concentrations (Figure 3). Variability between replicate experiments was small: 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 dry-weight based V concentrations in grass shoots grown on unpolluted soils (Kabata-Pendias & Pendias, 2001). At small added V concentrations, shoot V concentrations were not, or only marginally, increased compared with the control treatment. As added V concentrations increased (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 ($P < 0.05$) less 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)

Soil solutions of un-amended soils isolated after the barley growth assay had V concentrations of between 0.005 and 0.020 mg litre^{-1}. The partition coefficients of geogenic V in the un-amended 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 our soils). The

poor 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.

The added V concentrations were plotted against the V concentrations in isolated soil solutions, and Freundlich-type isotherms fitted (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 less 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 less than in freshly spiked soils, and this is associated with a twofold smaller V solubility.

Conclusions

Our results show that, over time, ageing reactions cause V added to soils to become less soluble, bioavailable and toxic. The soluble V concentrations in four different soils decreased by approximately twofold between 14 and 100 days after soil spiking with V(V). These results were modelled by 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 decreased by approximately twofold compared to the corresponding freshly spiked soils, and this decrease was accompanied by a decreased V translocation to the shoot. Toxicity data measured in freshly spiked soils may therefore not be representative for long-term and well equilibrated soil contaminations in the field. Overall, the effects of V ageing reactions across the four studied soils were surprisingly similar, but more studies are warranted in order to investigate if this finding can be extrapolated to other soils. Oxalate extractions suggested that the V(V) added to soils was predominantly bound to poorly crystalline oxyhydroxides. The naturally present V in soils was much less soluble than the added V(V), and only a small fraction of it was associated with poorly crystalline oxyhydroxides.

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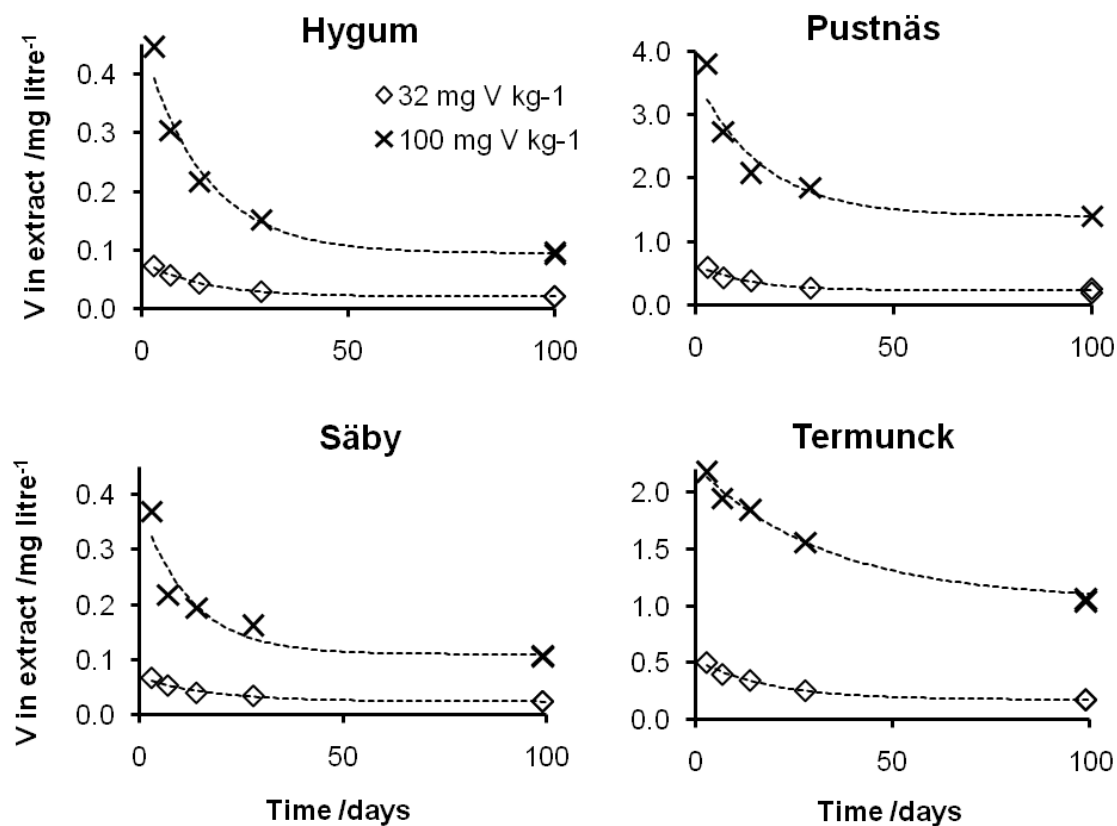603 *Plant & Soil*, **216**, 47-51.

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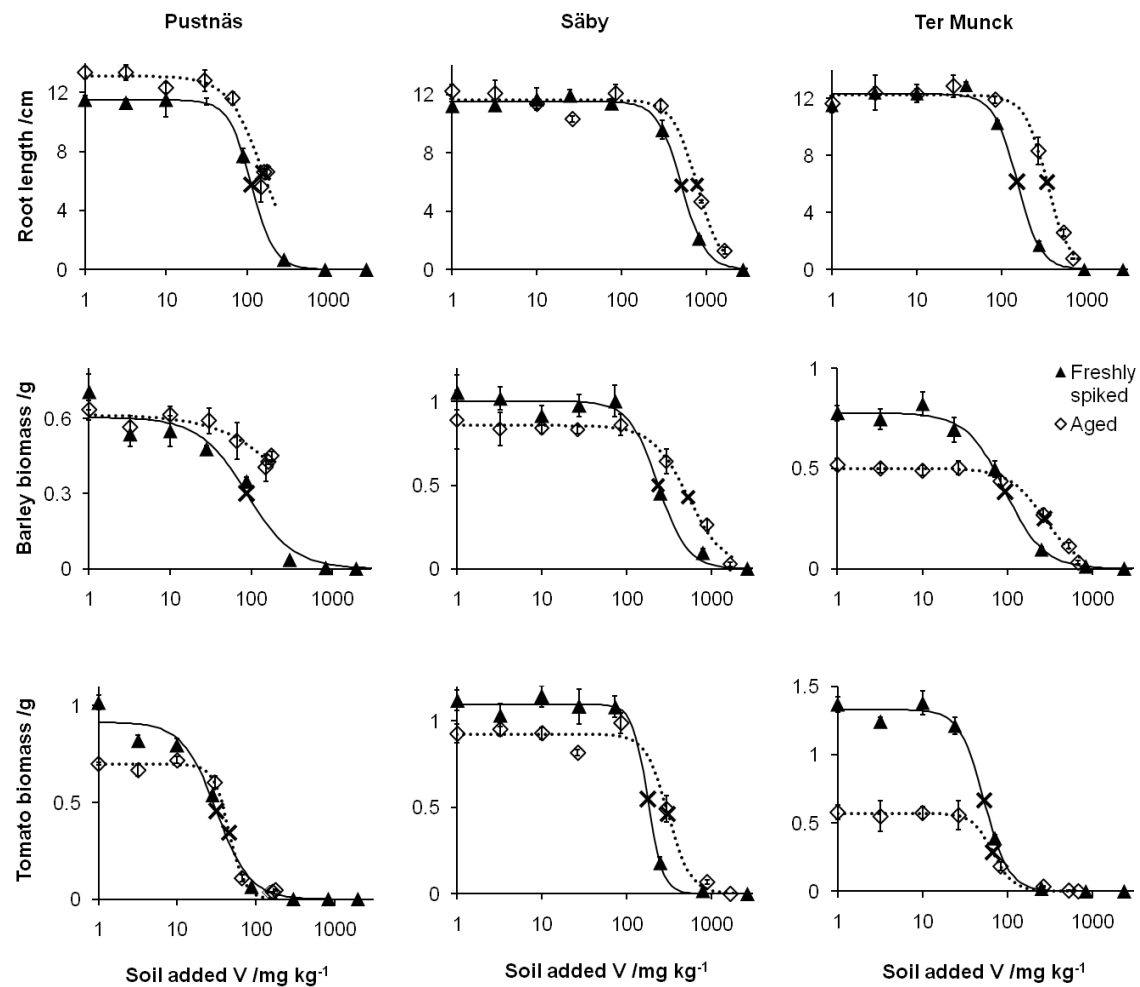
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FIGURE CAPTIONS

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 added V concentrations in the soil (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 by X

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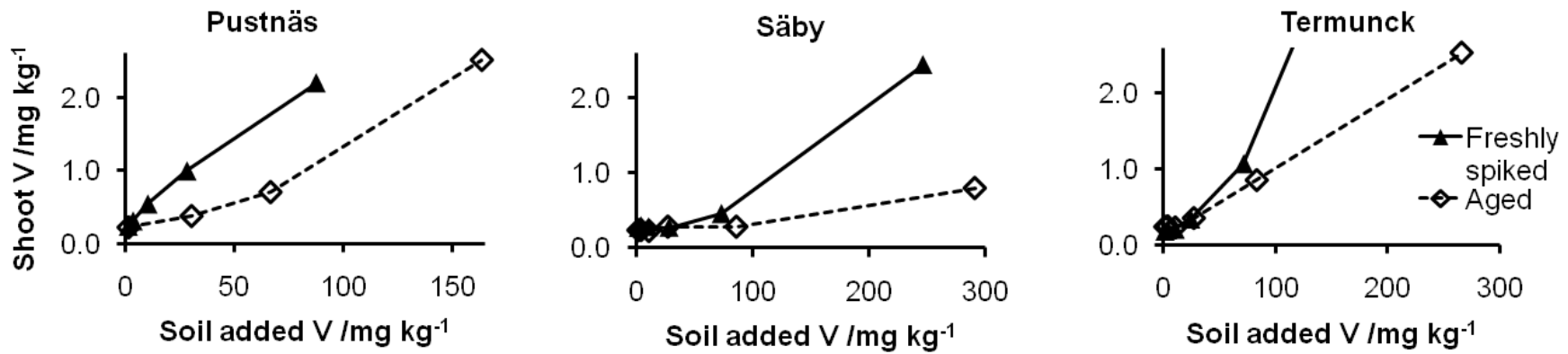
Figure 3 Average barley shoot V concentrations plotted against soil added V concentrations. Freshly spiked soils: closed triangles connected with full lines; aged soils: open diamonds connected with dashed lines. Coefficients of variation between replicate measurements were between 0.01 and 0.12
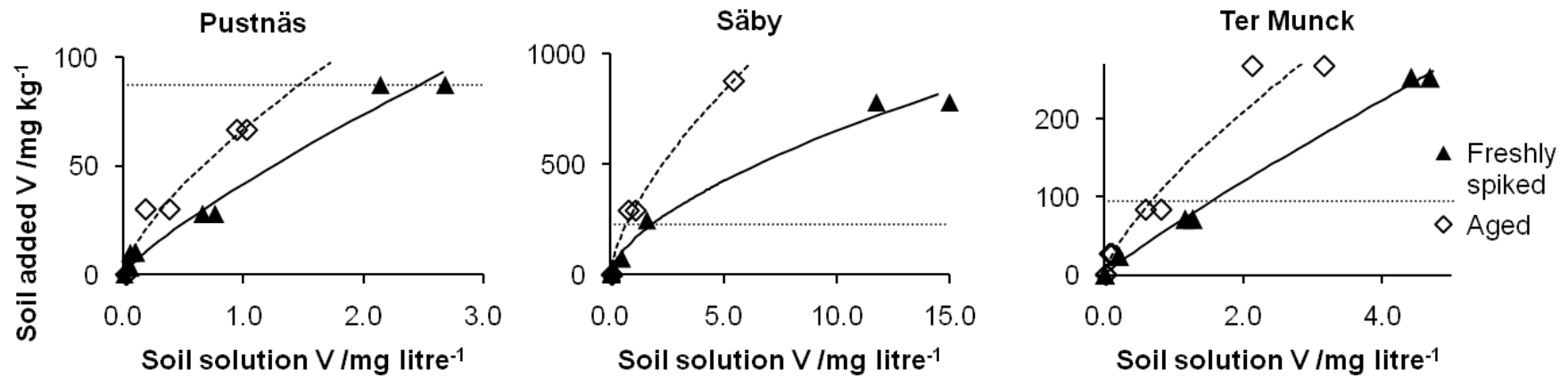
Figure 4 Sorption isotherms with the soil added V (background corrected) plotted against the V concentration in isolated soil solutions. Freshly spiked soils: closed triangles (data points) + full line (fitted Freundlich isotherm); aged soils: open diamonds + dashed line. The horizontal line indicates the EC_{50} for barley growth in the freshly spiked soils.

632 **TABLES**

633

634 **Table 1** Characteristics of unspiked soils used for toxicity and sorption experiments

		Hygum	Pustnäs	Säby	Ter Munck
Country		Denmark	Sweden	Sweden	Belgium
Region		Velje County	Uppland	Uppland	Vlaams-Brabant
Coordinates		55'46'25" N, 9 '25'50" E	59°48'17" N, 17°40'27" E	59°49'59" N, 17°42'11" E	50°52'42'' N, 4°39'24'' E
Soil type		n.d.	Eutric regosol	Eutric cambisol	Haplic luvisol
Land use		grassland	grassland	arable land	arable land
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

635

636 **Table 2** Fitted first order rate constants (k) and their standard errors (SE) describing
637 the kinetics of V solubility in 0.01 M CaCl$_2$ soil extracts between 3 and 100 days after
638 soil spiking. The [V_{14}]:[V_{100}] is the ratio of soluble V 14 days after spiking to that 100
639 days after spiking

Soil	Nominal added V /mg kg^{-1}	$k \pm$ 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

640

641

Table 3 Vanadium speciation in 0.01 M CaCl$_2$ extracts of soils spiked with V(V) and subsequently aged for 5–11 months.

644

	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

645

646

Table 4 EC_{50} estimates and their standard errors fitted using the log-logistic dose-response model in freshly spiked soils and in aged soils, in mg added V kg^{-1}. All pairs of EC_{50} estimates for freshly spiked and aged soils differ significantly ($P < 0.05$).

650

		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

651 [a] The EC_{50} for barley growth in the aged Pustnäs soil is unbounded, because no 50% reduction in
652 biomass yield was observed at the largest treatment concentration of 180 mg V kg^{-1}.