APPLIED ORGANOMETALLIC CHEMISTRY, VOL. 8, 141–147 (1994)

The Association between Selenium and Humic Substances in Forested Ecosystems—Laboratory Evidence

Jon Petter Gustafsson* and Lars Johnsson†
* Division of Land and Water Resources, Royal Institute of Technology, S-100 44 Stockholm, Sweden, and † Department of Soil Sciences, Box 7014, S-750 07 Uppsala, Sweden

In the soils and aquatic systems of coniferous forests, selenium is usually associated with humic substances. To clarify further some of the mechanisms involved, labelled and unlabelled selenite were added to two forest floors and to a brown-water lake. Sequential extraction procedures and chromatographic methods were used to evaluate the resulting association between selenium and humic substances. It was observed that the forest floors fixed most of the added selenite by means of microbial reductive incorporation and that selenium was preferentially incorporated into low-molecular-weight fractions of the humic substances. By contrast, selenium reduction was much slower in the brown-water lake and instead, inorganic complexation of selenite to metal–humic complexes was important during the experiment, provided that the concentrations of competing ligands were low.

Keywords: Selenium, humic substances, soils, aquatic systems, coniferous forests

INTRODUCTION

Selenium is an essential micronutrient for animals, but it is deficient in large areas of northern Europe.[1] Selenium has also been proved to counteract mercury poisoning in Swedish lakes.[2] For these reasons, research concerning the biogeochemical cycling of naturally occurring selenium in northern coniferous forest ecosystems has been conducted in Sweden over the past few years.[3,4]

The occurrence of humic substances (HS) seems to be of fundamental importance in the selenium cycle of slightly acidic forest ecosystems. Previous authors have reported a high degree of association between selenium and HS in aquatic and terrestrial environments.[5–8] This association decreases selenium availability for plants.[9] We have earlier shown that podzolic HS, especially those of the B horizons, are highly enriched with selenium as compared with plant biomass.[4]

The mechanism of the interaction between selenium and HS is not understood. One possibility is that selenite [Se(IV)] can be adsorbed by iron–HS complexes,[10,11] a mechanism previously documented for phosphate.[12] The failure to extract significant amounts of selenite from HS using pyrophosphate solutions indicates that this mechanism is not important for explaining the bulk of the native humic-bound selenium in soils, however.[4] Recently, Cohen *et al.*[13] have suggested that selenite ions could be specifically adsorbed to amine groups of organic constituents such as amino acids.

Other hypotheses centre around biochemical mechanisms for explaining the selenium–HS association. Selenium oxyanions are more easily reduced than their sulphur analogues.[14] Microbial reductive incorporation of selenite into biogenic material is an important feature of the marine biogeochemical selenium cycle.[15,16] Much evidence for the microbial reduction of selenite in soils exists; selenite reduction and the volatilization of reduced selenium forms is strongly enhanced after C amendments.[17,18] Van Dorst and Peterson[19] identified selenoglutathione after adding selenite to soil extracts. To what degree reduced selenium species (elemental selenium and selenide) may be incorported into stable HS molecules is, however, far from clear.

Our intention was to clarify further the interaction between selenium and HS. Thus selenite was added in a series of experiments to (i) a brown-water lake with a high HS content and (ii) two coniferous forest floors. The resulting selenium–HS association was studied using sequential extractions, column chromatography and gel filtration.

CCC 0268-2605/94/020141-07
© 1994 by John Wiley & Sons, Ltd.

Received 3 November 1993
Accepted 17 December 1993

EXPERIMENTAL

Samples used

The lake water used in the experiments originated from Lake Snuggan, a small brown-water lake 20 km north of Stockholm. The catchment surrounding the lake consisted of lithic mor soils with frequent rock outcrops. The water had low alkalinity, a pH of 5.6, and a dissolved organic carbon (DOC) content of 20–35 mg dm^{-3}, depending on the sampling time. The hydrophobic acid percentage was high, about 60–65%. The total dissolved selenium content was 0.21 µg dm^{-3}. The DOC probably originated mainly from seeps draining the Norway spruce (*Picea abies*) mor soils, but also to some extent from a small Sphagnum peat area, located in the discharge zone along the shore. Freshly sampled water, which had been passed through a 0.45 µm membrane filter, was always used in the experiments.

The forest floors originated from two Norway Spruce forests. One was an acid mor layer from Stråsan, which is described elsewhere.[4] The other was from a young Norway spruce plantation at Ängsjö, 20 km northwest of Stockholm. This plantation was growing on a calcareous till soil, which is why the mor layer was only slightly acid (pH 5.2 in water). Fresh, field-moist samples from these sites were used in the experiments.

Addition of selenite to forest floors

A number of experiments were conducted in which Se(IV) (as dilute Na_2SeO_3) was added to soil suspensions (1:10 soil/solution ratio), either labelled (^{75}Se; Amersham Inc.) or unlabelled, as follows:

(1) *Effect of contact time on Se(IV) incorporation*: 1000 µg dm^{-3} Se(IV) was added to Stråsan soil suspended in deionized water (DW). The suspensions were equilibrated for 1, 2, 4, 8, 16, 24, 40, and 64 h on a reciprocating shaker. After centrifugation and filtration, the Se(IV) content in the extract was analysed by hydride AA, as described previously.[4]

(2) *Effect of phosphate and of an antimicrobial agent (sodium azide)*: ^{75}Se(IV) (0.23 µg dm^{-3}) was added to Ängsjö soil suspensions containing (i) DW only, (ii) 0.05 M KH_2PO_4 (pH 4.4), or (iii) 0.05 M KH_2PO_4/0.05 M NaN_3 (pH 5.6). After equilibration for 24 h, ^{75}Se was analysed using a well-type scintillation counter in the centrifuged supernatants. KH_2PO_4 (0.2 M) was then added to the soil residue from treatment (i) and equilibrated for an additional 30 min. After withdrawal of the supernatant (which was later analysed for ^{75}Se), 0.1 M NaOH was added to all soil residues and equilibrated for 18 h. After this equilibration, ^{75}Se was analysed both in the resulting supernatants and in the soil residue. Results were corrected for interstitial solutions remaining in the sample between each extraction. The total recovery of ^{75}Se was always 100 ± 1%, indicating that selenium volatilization was not important during the short duration of the experiments. The procedures described were repeated on another set of samples which were equilibrated for only 1 h.

(3) *Effect of selenite concentration*: Experiment 2 was duplicated, except that unlabelled Se(IV) was premixed with the labelled ^{75}Se, giving a final concentration of 10 µg dm^{-3} in the spiked sample.

(4) *Effect of pH*: ^{75}Se(IV) (0.23 µg dm^{-3}) was equilibrated with a DW soil suspension that was (i) pH-adjusted to 4.3, using 1.6 mM HCl, (ii) unadjusted at pH 5.2, or (iii) pH-adjusted to 6.3, using 1.6 mM NaOH.

The NaOH extract from experiment 2(i) was fractionated into humate-^{75}Se, hydrophobic fulvate-^{75}Se, and hydrophilic fulvate-^{75}Se, according to Gustafsson and Johnsson.[4] Selenium-75 in hydrophobic acids was also analysed in extracts from experiments 4(ii) and 4(iii). Moreover, the water and KH_2PO_4 extracts from experiment 3(i) were analysed for Se(IV) using hydride AA, to estimate the contribution of inorganic selenite to the selenium detected by scintillation counting.

Addition of selenite to lake water

Two experiments were conducted. One involved the addition of unlabelled selenite and subsequent XAD-8 column chromatography, and the other involved addition of ^{75}Se and subsequent gel filtration, as follows.

(1) Se(IV) (5 µg dm^{-3}) was added to differently treated lake water and left to stand in the dark at the ambient laboratory temperature (21 °C) for seven days. The treatments consisted of (i) no special treatment (control), (ii) 0.02 M NaH_2PO_4 that was premixed with the lake water 1 h before selenite addition, and (iii) 1 mg dm^{-3} Fe^{3+}. After the equilibration time, sample aliquots were

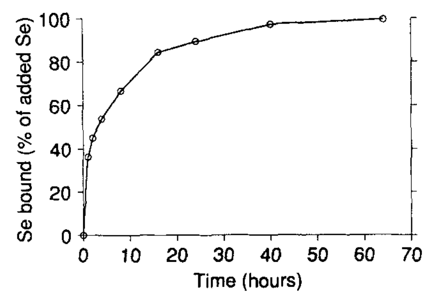

Figure 1 Retention of Se(IV) in the Stråsan mor layer as a function of time.

eluted through an XAD-8 column ($k'=9$). Hydrophobic acids were eluted from the column using 0.1 M NaOH. The hydrophobic acids were digested and their total selenium content was analysed using hydride generation.[3]

(2) ^{75}Se(IV) (1.1 μg dm^{-3}) was added to lake water and equilibrated for 7 d. The sample was then processed through a TSK SW guard column 7.5 cm long and a TSK G3000SW column 30 cm long connected in sequence (TosoHaas Corporation, Germany) using an HPLC system. The gel filtration was conducted according to the methodology of Berdén and Berggren.[20] UV absorbance at 260 nm was monitored and 0.50 cm^3 fractions of the eluate were analysed for ^{75}Se. The void volume, $v(0)$, was 6.5 cm^3 while the total permeation volume, $v(t)$, was 14.8 cm^3. As in experiment 1, separate samples were treated with iron or phosphate.

Organic carbon was analysed using plasma emission spectrometry (Jobin-Yvon JY24) after acidification of the sample to pH 1–2. All experiments were performed in duplicate, and the values reported represent means. Standard errors were always below 10%.

RESULTS

The incorporation of selenite into soil humic substances

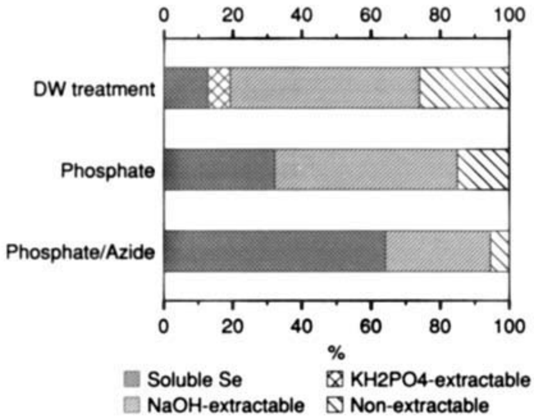

Figure 2 Selenium-75 in different fractions after the addition of 0.23 μg dm^{-3} ^{75}Se(IV) and an equilibration time of 24 h. See text for definitions.

Selenium added to the mor was retained rather slowly; however, after a few days the retention was nearly complete (Fig. 1). After 64 h, 99.4% of the 1000 μg dm^{-3} Se(IV) added to the Stråsan soil had been withdrawn from solution, even though this addition represented selenium concentrations at least six to seven orders of magnitude higher than those expected under natural conditions. In the B horizon of the same soil, added selenium was retained considerably faster—the same amount (>99.4%) was retained in less than 1 h (data not shown). The reason for this kinetic difference is probably that much of the retention in the mor proceeded through reductive incorporation into biogenic matter, while anion sorption was more important in the B horizon.

The addition of ^{75}Se(IV) to the Ängsjö soil confirmed that most of the added selenium had been converted to organic forms. When 0.23 μg dm^{-3} ^{75}Se(IV) was added, only 13% remained in a water-soluble form after equilibration (Fig. 2); the corresponding value for the 10 μg dm^{-3} treatment was 16% (Fig. 3). The small difference shows that selenite incorporation was essentially concentration-dependent. Although

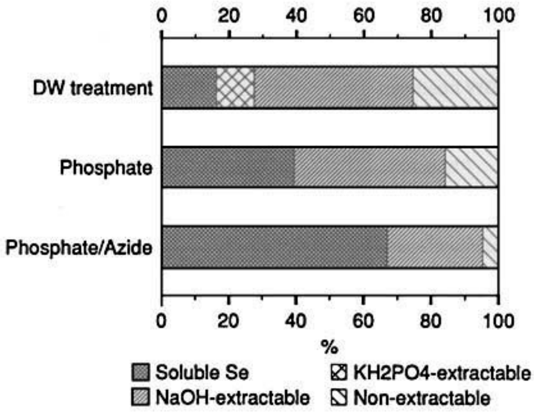

Figure 3 Selenium-75 in different fractions after the addition of 10 μg dm^{-3} Se(IV) and an equilibration time of 24 h.

Table 1 Organic carbon and ^{75}Se incorporated in different humic fractions after 24 h equilibration [initial addition 0.23 μg dm^{-3} ^{75}Se(IV)]

Fraction	Carbon (g kg^{-1})	^{75}Se (μg kg^{-1})	C: ^{75}Se ($\times 10^6$)[a]
Humates	63	1.41	45
Hydrophobic fulvates	24	0.84	29
Hydrophilic fulvaters	17	1.17	15

[a] As mass (not molar) ratios.

16% of the selenium was water-soluble, only 3.7% was detected when the water extract was analysed specifically for Se(IV) using AA. The low $NaBH_4$-reducibility showed that most of the Se(IV) had been reduced to lower valence states, e.g. to elemental selenium or organic selenide. As could be expected, selenium had remained unreduced and water-soluble to a much greater degree after only 1 h equilibration (Fig. 4): 24% had remained as inorganic Se(IV) when 10 μg dm^{-3} has been added.

Even though only 30% of the total organic carbon could be extracted by NaOH, most of the insoluble ^{75}Se had been incorporated into the NaOH-extractable fraction. Further fractionation of the NaOH extract revealed that most selenium had been incorporated into the fulvate fractions, even though the humates comprised most of the NaOH-extractable organic carbon (Table 1).

Abiotic sorption of Se(IV) proved to be of minor importance in the retention process, except as a first, transitory, retention step. After 1 h, roughly 20% was KH_2PO_4-extractable, most of which was inorganic Se(IV). After 24 h, however, only a small amount (5 or 10%, depending on the initial selenite concentration) was extracted by KH_2PO_4 (Figs 2, 3); moreover, most of this selenium was in a reduced organic form. Equilibration in a phosphate medium should have precluded any abiotic sorption of Se(IV); in fact, less Se(IV) was incorporated into non-KH_2PO_4-extractable forms than in the DW soil suspensions. The last observation may be due to any of the following three reasons: (i) the incorporation of selenium into organic matter was catalysed by selenite adsorption to humic matter as a first step; (ii) the KH_2PO_4 extraction of the soil residue from the DW equilibrations failed to recover all adsorbed selenite; (iii) the phosphate solution might have reduced microbial activity.

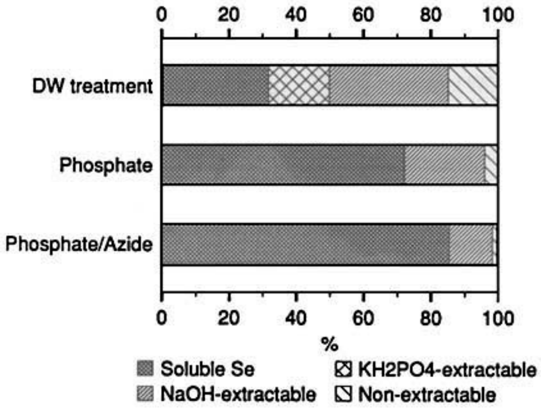

Figure 4 Selenium-75 in different fractions after the addition of 0.23 μg dm^{-3} ^{75}Se(IV) and an equilibration time of 1 h.

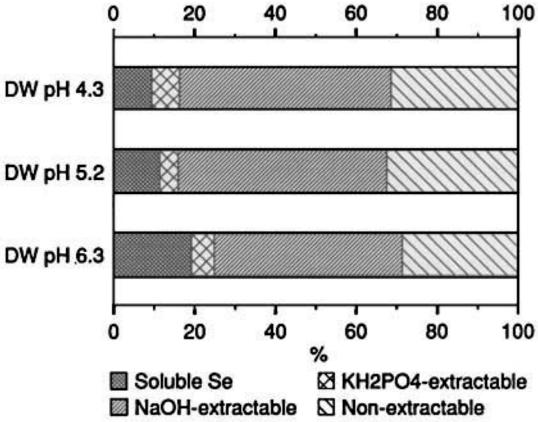

Figure 5 Selenium-75 in different fractions after the addition of 0.23 μg dm^{-3} ^{75}Se(IV) and an equilibration time of 24 h.

The addition of sodium azide to the soil suspension efficiently reduced selenium incorporation (Figs 2, 3), indicating that microbes were an

Table 2 Selenium concentration in the hydrophobic acids of Lake Snuggan after different treatments

Treatment	Se (μg dm^{-3} lake water)
No Se added	0.05
5 μg dm^{-3} Se(IV)	1.75 (34% retention efficiency)
5 μg dm^{-3} Se(IV) + NaH_2PO_4	0.32 (5.5%)
5 μg dm^{-3} Se(IV) + Fe^{3+}	3.0 (60%)

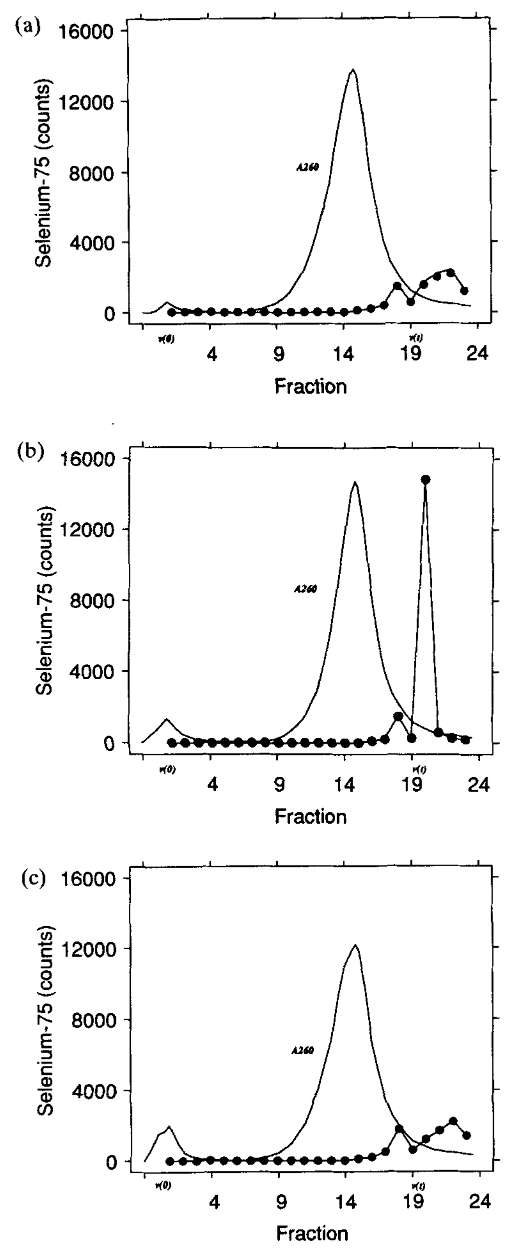

Figure 6 Gel filtration patterns of selenium-75 added to lake water (dotted line) and UV absorbance (solid line) after 7 d. (a) Control treatment; (b) NaH_2PO_4 treatment; (c) Fe^{3+} treatment.

important driving force. This was confirmed when another antimicrobial agent, chloroacetic acid, was used in parallel experiments (data not shown). The fact that the addition of sodium azide resulted in a slightly higher pH probably did not affect the selenium partition to any considerable degree. Only small differences due to pH were observed when the pH was varied from 4.2 to 6.3 (Fig. 5). At pH 6.3, more selenium was soluble than at low pH. This effect was mainly brought about by the increased dissolution of selenium containing organic matter at high pH. This was evidenced by the increased colouring of the extracts with higher pH and by the observation that 54% of the soluble selenium at pH 6.3 was bound to hydrophobic acids. The corresponding value for pH 5.2 was 41%.

Retention of selenite by lake water humic substances

Analysis of the NaOH eluate from the XAD-8 resin suggested that a mechanism of inorganic complexation to the retained hydrophobic acids accounted for a considerable part of the Se(IV) withdrawal. Selenium retention by the isolated HS was drastically reduced when the experiment was conducted in a phosphate medium (Table 2). However, some selenium was still retained which was probably either specifically adsorbed to amine groups,[13] or fixed to the hydrophobic acids by reductive incorporation. Conversely, selenium was enhanced after the iron addition.

In the gel filtration experiment, about 10% of the added selenium was incorporated into relatively low-molecular-weight fractions, as compared with the bulk of the HS (Fig. 6).. The inorganic selenite was eluted after *v*(*t*), albeit slowly when no phosphate had been added (compare Figs 6a and 6c with Fig. 6b). The retardation was most likely due to adsorption/desorption interactions with solid surfaces in the HPLC system. Because the organic selenium was eluted shortly before *v*(*t*), it was impossible to determine molecular weights accurately with polystyrene sulphonates. An acetate/nitrate solution at pH 7 was used as a mobile phase.[20] Most of the inorganically complexed selenite was displaced from the HS during the mixing with the mobile phase and a result eluted as dissolved inorganic selenite. Therefore, the addition of iron had no visible effect on selenium retention in this experiment (Fig. 6c).

DISCUSSION

The results demonstrate clearly that the prevailing mechanism for selenium retention in the surface horizons of forest soils involves the microbially mediated reduction to lower selenium valence states [e.g. lower than Se(IV)] and subsequent incorporation into HS. In these soil horizons, surface complexation of inorganic selenite is unimportant except as an initial (and very transitory) step whereby dissolved selenite is retained by the soil matrix.

It is certainly premature to speculate too much about the pathway for selenium incorporation by HS. However, we suggest that selenium may be reduced by microbes to H_2Se or elemental selenium which is either (i) incorporated into selenoamino acids or other selenium-organic compounds which are then added to the HS pool or (ii) incorporated directly into HS, possibly by a reaction between selenides and peripheral hydroxyl groups of the HS, in a manner parallel to that observed for sulphur in brackish peats.[21,22]

Given that selenium reduction is a key process in podzolic forest floors, it was not surprising to find that the addition of an antimicrobial agent such as sodium azide drastically suppressed Se retention. The pH as such did not influence selenium retention markedly. It is possible, however, that pH changes arising from (for example) soil acidification may, in the long term, change the composition of the microbial community and hence affect selenium retention.

Selenium was preferentially incorporated into relatively low-molecular-weight fractions of the organic carbon pool (e.g. humates and fulvates), while rather small amounts were recovered in the solid-phase humin. Probably, the surface-to-volume ratio of the HS molecules is crucial in determining selenium incorporation patterns. Hydrophilic fulvates would therefore be the most selenium enriched humic fraction in short-term experiments. In contrast, earlier research has shown that hydrophobic fulvates are the most enriched fraction with respect to native selenium.[4] Possibly, this discrepancy is due to different turnover times of the various humic fractions—part of the operationally defined hydophilic fulvate pool probably consisted of simple organic acids and short-chain aliphatics which are susceptible to rapid degradation.[23]

In brown-water lake systems, selenium reduction probably proceeds, but the rates are much slower than in soils. Only about 10% of a 1.1 $\mu g\,dm^{-3}$ Se(IV) addition was recovered after seven days in an organic form. Moreover, as we did not determine the actual valence state of the organically bound selenium, it cannot be excluded that part of these 10% was Se(IV) adsorbed specifically according to the mechanism proposed by Cohen *et al.*[13] The organically bound selenium was of a lower molecular weight than the bulk of the HS, suggesting either (i) that most of this selenium was not bound to HS but rather present as selenotrisulphide intermediates or other low-molecular-weight compounds (e.g. amino acids), or (ii) that the selenium had been bound to low-molecular-weight HS because of the higher surface-to-volume ratios of these fractions. The observation that some incorporated selenium eluted as hydrophobic acids seems to support hypothesis (ii), since most low-molecular-weight non-humic selenium compounds, such as amino acids, would elute as hydrophilic constituents. This is, however, by no means conclusive, and more detailed studies involving gel filtration and thin-layer chromatogaphy would be needed to clarify it.

A large proportion of the Se(IV) added to humic lake water was complexed by HS, as evidenced by XAD-8 chromatography. As the addition of trivalent iron increased the retention, and as the addition of phosphate decreased it very considerably, it is suggested that most of the complexation occurred by means of iron or aluminium bridging to carboxylic and phenolic functional groups. There are several weaknesses with the XAD-8 method that made it impossible to determine properly the extent of selenium–HS complexation in the water. Firstly, only the selenium binding by the hydrophobic acid fraction could be analysed. Secondly, pH adjustment to pH 2 probably destroyed most iron–aluminium–organic complexes. Thirdly, hydrogen bonding by protonated organic surfaces would instead add new complexation sites.[24] Nevertheless, the results indicate strongly that selenite may bind to metal–organic complexes in a similar way to phosphate.

CONCLUSIONS

This study demonstrates that the rapid and efficient selenium retention by surface horizons of forest soils is primarily due to microbially mediated reductive incorporation, whereby selenium is reduced to low valence states and then incorported into low-molecular-weight HS fractions. Abiotic sorption may be an important retention mechanism for 'trapping' dissolved selenite, but is quantitatively unimportant as a storage mechanism. In brown-water lakes, the reductive incorporation of selenium to HS probably proceeds also but the rates are very much slower than in podzolic forest floors. Instead, inorganic complexation of Se(IV) to iron–HS complexes is important, provided that the concentrations of competing inorganic ligands (especially phosphate) are low. The exact mechanism for the incorporation of selenium into HS in soils still remains to be elucidated.

Acknowledgements We thank Kjell Svärdström, Gunnar Henriksson and Maria Berdén for constructive suggestions and for providing some of the analytical facilities. We are also indebted to the National Swedish Environmental Protection Board for financial support.

REFERENCES

1. G. Gissel-Nielsen, C. G. Umesh, M. Lamand and T. Westermarck, *Adv. Agron.* **37**, 397 (1984).
2. K. Paulsson and K. Lundbergh, *Water Air Soil Pollut.* **56**, 833 (1989).
3. L. Johnsson, *Swed. J. Agric. Res.* **19**, 21 (1989).
4. J. P. Gustafsson and L. Johnsson, *J. Soil Sci.* **43**, 461 (1992).
5. A. A. Hamdy and G. Gissel-Nielsen, *Z. Pflanzenaehr. Bodenkd.* **6**, 697 (1976).
6. M. A. Turner and J. W. M. Rudd, *Can. J. Fish. Aquat. Sci.* **40**, 2228 (1983).
7. B. T. Christensen, F. Bertelsen and G. Gissel-Nielsen, *J. Soil. Sci.* **40**, 641 (1989).
8. D. Tanzer and K. G. Heumann, *Anal. Chem.* **63**, 1984 (1991).
9. L. Johnsson, *Plant Soil* **133**, 57 (1991).
10. M. Levesque, *Can. J. Soil Sci.* **54**, 205 (1974).
11. M. Remberger, Förekomst och omsättning av selen i inlandsvatten, IVL Rapport B564. IVL, Stockholm, Sweden (1980).
12. R. I. Jones, K. Salonen and H. de Haan, *Freshwater Biol.* **19**, 357 (1989).
13. R. Cohen, D. Schuhmann, F. Sinan and P. Vanel, *Mar. Chem.* **40**, 249 (1992).
14. H. Vokal-Borek, *Selenium*, USIP Report 79-16. Institute of Physics, University of Stockholm, Sweden (1979).
15. G. A. Cutter, *Nature (London)* **217**, 829 (1982).
16. G. A. Cutter and K. W. Bruland, *Limnol. Oceanogr.* **29**, 1179 (1984).
17. U. Karlson and W. T. Frankenberger Jr, *Soil Sci. Soc. Am. J.* **53**, 749 (1989).
18. R. H. Neal and G. Sposito, *J. Environ. Qual.* **20**, 808 (1991).
19. S. H. Van Dorst and P. J. Peterson, *J. Sci. Food Agric.* **35**, 601 (1984).
20. M. Berdén and D. Berggren, *J. Soil Sci.* **41**, 61 (1990).
21. K. A. Brown, *Soil Biol. Biochem.* **18**, 131 (1986).
22. L. E. Lowe, *Sci. Total Environ.* **113**, 133 (1992).
23. E. M. Thurman, *Organic Geochemistry of Natural Waters*. Martinus Nijhoff/Dr W. Junk, Dordrecht, Netherlands (1985).
24. J. L. Fio and R. Fujii, *Soil Sci. Soc. Am. J.* **54**, 363 (1990).