INFLUENCE OF SALINITY AND EUTROPHICATION ON BIOACCUMULATION OF 99Tc TECHNETIUM IN DUCKWEED

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Abstract—This study concerns the bioaccumulation of the long-lived nuclear waste product 99Tc in duckweed (Lemna minor L.). 99Tc was present as the oxyanion TcO4−, being the main chemical form of technetium in aerobic water systems. In contrast with terrestrial plants, bioaccumulation in duckweed proved to be independent of the nitrate concentration in the medium. However, uptake is controlled by electrostatic effects in the cell wall, which affects the bioaccumulation of 99Tc in duckweed in natural environments. These waters are characterized by a range of salinity and hardness, and this study suggests that this may result in up to a threefold difference in 99Tc accumulation. Because of screening of negative charges in the cell wall, the highest accumulation may be expected in hard, brackish water. This behavior can be described by a general model, which includes electrostatic effects and binding of cations at the cell wall. The model also explains why cationic radionuclides are preferably taken up in soft, fresh water while anionic species are concomitantly taken up in hard, chlorine-rich waters.

Keywords—Cell wall Duckweed Electrostatic effects Technetium Water hardness

INTRODUCTION

One of the elements that contribute to global contamination is the long-lived fission product technetium (99Tc, β−-emitter, E_{max} = 294 keV, half-life = 213,000 years). The 99Tc is considered as rather mobile and abundant among the long-lived radionuclides generated in nuclear fission [1]. Releases of 99Tc into the environment include discharges from the nuclear fuel cycle, atomic weapons tests, accidents (Chernobyl), and medical diagnostic use of its metastable form 99mTc (half-life, 6 h) [1].

In aerobic environments, Tc is present as pertechnetate (TcO4−) [1], the only Tc form known to be taken up by plants [2,3]. Accumulation of Tc by terrestrial vegetation is characterized by plant–soil concentration ratios of 1 to 36 on a dry weight basis [4,5]. Most of the observed variations in plant–soil concentration ratios can be explained by the soil solution concentration of TcO4− and NO3− [2,6]. NO3− inhibits TcO4− accumulation by competitive behavior for the same uptake route [6]. On the other hand, marine algae, having accumulation ratios up to 10^5 L/kg dry weight [1], show enhanced uptake with increasing salinity [7]. It was suggested that electrostatic factors in the cell wall may be responsible for these effects. Earlier work indicates that anionic competition was negligible or absent in the aquatic macrophyte duckweed (Lemna minor) [8].

Nutrient uptake in aquatic plants may occur by root uptake and foliar absorption (rooted macrophytes) or by foliar absorption alone (floating macrophytes). In the case of Tc uptake, aquatic plants most likely absorb Tc through their leaves since Tc is reduced to nonbioavailable forms in anoxic sediments [9]. Nitrate inhibition and enhanced uptake by cationic interactions may play a role in the uptake of Tc in aquatic plants, especially in estuaries and eutrophic areas.

This work focuses on the effects of eutrophication and salinity on the accumulation of Tc in duckweed. Duckweed was chosen because of its world-wide distribution, covering shallow water from mesohypertrophic to highly eutrophic, which may range in chlorinity from 0.1 to 47% and range in total hardness from 0.22 to 50 meq/L [10]. Duckweed exhibits only foliar absorption [10]. The hypothesis tested is that the accumulation by the fronds (leaf-like structures) is regulated by the cell wall (electrostatic effects) while a competitive behavior of nitrate is absent or plays only a minor role. This hypothesis is based on some initial experiments [8]. Cationic effects were quantified using a generalized theoretical approach for the uptake of electrolytes by higher plants [11].

MATERIAL AND METHODS

General

Three experiments were performed to study the effect of the anions NO3− and Cl− and the cations Na+, K+, Mg2+, and Ca2+. In the first experiment, Tc uptake was studied with the bathing solutions at equal ionic strength but differing in potassium and calcium concentrations and nitrate and chloride concentrations. In the second experiment, Tc uptake was studied in a concentration range of nitrate or chloride salts of calcium, magnesium, potassium, and sodium, respectively. Potassium showed a behavior that was different than expected from the uptake model (see Results and Discussion). Therefore, the third experiment included the sorption of pertechnetate in dead duckweed material as a function of potassium concentration in order to study electrostatic effects only.

Materials

All reagents applied were of analytical grade (Sigma-Aldrich Chemicals, Bornem, Belgium; Merck, Darmstadt, Germany; J.T. Baker BV, Deventer, The Netherlands). Demineralized or Milli-Q® water (Millipore, Bedford, MA, USA) was used throughout the experiments. 99Tc (specific activity 6.2 × 10^6 Bq/L) was obtained from a reactor of the Technical University of Delft.
Bioaccumulation of $^{99}$Tc in duckweed

$10^{10}$ Bq/mol was obtained from Amersham (Amersham, Buckinghamshire, UK) as K$\text{TcO}_4$ in 1 M NH$_4$OH.

**Duckweed culture**

A strain of *L. minor* L. (common duckweed) was provided by Jenner (KEMA, Arnhem, The Netherlands) and cultured in 150 ml of nutrient solution [12]. Duckweed was placed on fresh nutrient solution every two weeks. Cultures were kept in a climate room at 25°C and a light intensity of 120 μmol photons/m²/s (Phillips PLL 83; Phillips Semiconductor, Amsterdam, The Netherlands) during a 16:8 h light:dark period. Typical doubling time under these conditions was 2.6 d. Cultures up to four weeks old were used as experimental material.

**Radioactivity measurement**

The $^{99}$Tc was measured on a Packard liquid scintillation counter (Packard Instrument BV, Groningen, The Netherlands) using the appropriate correction for quenching. Energy windows were set at 5 to 290 keV, with a counting efficiency ≥95%. Before measuring $^{99}$Tc in duckweed, samples were digested with boiling HNO$_3$/H$_2$O$_2$, evaporated to 1 ml and cooled, after which 10 ml Ultima Gold$^\circledR$ scintillation cocktail (Sigma-Alrich Chemicals) was added. Standards were prepared similarly to the samples.

**Experiments: General procedures**

Before accumulation, about 0.15 g of fresh duckweed was placed on 150 ml of the desired solution for 3 d under continuous light (120 μmol photons/m²/s). The amount of fronds was determined by simply counting them. The $^{99}$Tc was added using an autoanalyzer (Cobas Mira, Roche Diagnostics, Issy Les Moulineaux, France). No changes in pH, $^{99}$Tc, NO$_3^-$, or Cl$^-$ concentrations were observed in the nutrient solution at the end of the experiments.

**Effect of nitrate versus chloride on the Tc accumulation**

To study the effect of nitrate on Tc accumulation, nitrate was applied as either KNO$_3$ or Ca(NO$_3$)$_2$. To correct for anions, KCl and Ca(Cl)$_2$, respectively, were used to balance the sum of nitrate and chloride in the nutrient solution at $9 \times 10^{-3}$ mol/L. Consequently, the sum of calcium and potassium ranged from $5.5 \times 10^{-3}$ to $9.5 \times 10^{-3}$ mol/L, and mannitol was used to maintain equal osmotic pressure. Samples were treated as described above.

**Effect of (earth) alkali metals on the Tc accumulation**

A range of nitrate and chloride salt concentrations of the desired cation was used to study the effect of (earth) alkali metals (calcium, magnesium, potassium, and sodium) on Tc uptake. No attempt was made to correct for osmotic pressure. Standard nutrient solutions were made without the cation of interest and nitrate. In all experiments, $10^{-3}$ mol/L KH$_2$PO$_4$ and at least $0.5 \times 10^{-3}$ mol/L NO$_3^-$ and $0.5 \times 10^{-3}$ mol/L Ca$^{2+}$ were present. In the magnesium treatment, MgSO$_4$ was replaced by Na$_2$SO$_4$. Samples were treated as described above.

**Effect of potassium on the Tc accumulation**

Since it was hypothesized that potassium might influence the Tc uptake by interaction with the cell membrane potential (see Results and Discussion), additional experiments were carried out with dead, fixed duckweed. Duckweed plants were placed in the dark the day before experiments were performed to decrease the reducing capacity. Then the duckweed was placed on a fresh $0.5 \times 10^{-3}$ mol/L CaCl$_2$ solution for 1 h, also in the dark. This washing step was repeated three times to remove the nutrient solution. Subsequently, duckweed was fixed by bathing in 2.5% glutaraldehyde solution for 2 h. Glutaraldehyde forms methyl bridges between free protein amino groups, resulting in conservation of the shape of duckweed, but all biological processes are terminated. The glutaraldehyde solution contained also $0.5 \times 10^{-3}$ mol/L CaCl$_2$ and $10^{-3}$ mol/L NaOH to avoid enhanced polyelectrolyte effects, and the desired potassium concentration was applied as nitrate or chloride salt. The pH in this experiment was 4.65 because of the presence of aldehyde groups. After a 2-h fixation time, $^{99}$Tc was added to a final concentration of 0.6 μmol/L. After an additional 3-h accumulation period, the duckweed was centrifuged, weighed, and analyzed for $^{99}$Tc as described above.

**Data analysis**

Accumulation was related to the biomass, expressed as kilograms fresh weight, at the start of the experiment. This was done in order to correct for differences in biomass dilution by growth between treatments. Relative growth rates under continuous light were between 0.25/d and 0.40/d (corresponding to doubling times between 1.73 and 2.77 d), with a mean of 0.30/d (doubling time 2.31 d). The relative growth rate was calculated, assuming exponential growth, according to

$$\text{relative growth rate} = \frac{\ln(n^{0.05}_{\text{final}}) - \ln(n^{0.05}_{\text{initial}})}{\Delta t}$$

where $n^{0.05}_{\text{final}}$ is the number of fronds at the start ($t = 0$ d), $n^{0.3}_{\text{final}}$ is the number of fronds at the end ($t = 3$ d) of the accumulation period, and $\Delta t$ is the length of the experiments (3 d). Within an experiment, relative growth rates were accurate to 7% and independent of the medium composition ($F$ test, $\alpha = 0.05$). Differences between experiments may be explained by differences between the batches used, i.e., the time needed to adapt to the new situation. Biomass at the start of the experiment was determined by multiplication of the initial frond numbers and fresh weight per frond. Weight per frond was determined at the end of the accumulation period. Random measurements of the weight per frond after the 3-d preincubation and the weight per frond after the 3-d accumulation period showed no significant differences (Student’s $t$ test, $\alpha = 0.05$). Mean weight per frond during the experiments was $1.65 \pm 0.05$ mg.

**Bioaccumulation model**

Figure 1 shows a schematic diagram of the model. The mathematical formula describing the accumulation of Tc in duckweed follows from earlier work on accumulation dynamics of Tc in duckweed [8] and from work of Sentenac and Grignon on the behavior of electrolytes in the cell wall [11]. Therefore, only the most important equations are given. In general, uptake of an electrolyte is regulated by diffusion in the unstirred layer, diffusion and sorption in the cell wall, and transport across the cell membrane. The diffusive flux through
Due to negative carboxyl groups in the cell wall, anions like pertechnetate are excluded while cations are attracted (Donnan theory) [11]. The presence of cations results in a screening of the negative charges or, if binding occurs at the carboxyl groups, reduction of the cell wall potential. This implies that, in duckweed, the concentration of carboxyl groups in the cell wall rises, resulting in higher uptake rates.

The general Donnan condition for the concentration of the electrolyte inside and outside the cell wall is [11]

$$ f = \frac{[\bar{e}c_1]}{[ec_1]} = \frac{[\bar{e}c_2]}{[ec_2]} = \cdots = \frac{[\bar{e}c_j]}{[ec_j]} \quad (2) $$

where $f$ represents the ratio of the concentration of the solute $j$ in the cell wall and nutrient solution, respectively. Equation 2 is only valid if one assumes that chemical activities can be replaced by concentrations and that the swelling pressure is negligible or at least does not result in swelling of the cell wall within the experimental conditions [13].

In the cell wall, cations may form complexes with negatively charged groups. These complexes are dominated by complexes of (earth) alkali metals with pectic carboxyl groups and may be characterized by formation constants ($K_f$). Alkali metals (group 1: H, Na, K) are associated with one carboxyl group per molecule. Binding of alkaline earth metals (group 2, including Ca and Mg) can be described by the egg-box model, which states that one divalent cation binds with two single carboxyl groups from different pectin molecules [14]. Here, following Sentenac and Grignon [11], we consider this behavior as a first-order binding process with respect to two carboxyl groups. This is in contrast with multidissociation kinetics, often applied to the binding of divalent cations with carboxyl groups [15]. The first-order formation constant can be expressed as [11]

$$ K_f = \frac{[MR^+]}{[M^{n+}][R^-]} \quad (3) $$

where $n$ is 1 (alkaline metals) or 2 (alkaline earth metals), $R^-$ is the concentration of unassociated carboxyl groups in the cell wall (in mol/L cell wall water), and $M^{n+}$ is the concentration of the (earth) alkali metal (in mol/L). $K_f$ is the formation constant in L/mol. The concentration of unassociated carboxyl groups, $R^-$, can be calculated from the mass action law. Combining Equation 2 with the electroneutrality rule for the cell wall, the Donnan factor, $f$, can be found by iteratively solving

$$ \frac{[R_{tot}]}{1 + \sum_{j=1}^{\infty} f_j K_{fj} c_j} - \sum_{j=1}^{\infty} z_j f_j c_j = 0 \quad (4) $$

where $R_{tot}$ is the total amount of carboxyl groups in the cell wall (in mol/L), $m$ is the number of complexes, $n$ is the total number of anions present, $z_j$ denotes the charge of the (earth) alkali metal $M$ (principally $z_j$ is 1 or 2), and $j$ is the number of cations. The cell wall pertechnetate concentration now can be calculated from Equation 2. Actually, the pertechnetate concentration must be corrected for the fractions of aqueous pertechnetate complexes. Especially KTcO$_4$ complexes might result in a lower bioavailability; the log($K_f$) is 0.91, which is, compared with other pertechnetate complexes, relatively high [16]. Here, formation of the potassium pertechnetate complex was only considered when technetium uptake was studied at increasing potassium concentrations.

From the cell wall, Tc$^{VII}$O$_4^-$ is transported over the cell membrane by a (pseudo) first-order process and is subsequently reduced and immobilized. If we assume that the amount of pertechnetate present in the cell wall and in the plant are negligible compared with the total accumulation and if we correct the results for growth dilution, the accumulation of technetium can be described by

$$ [\text{Tc}]_{\text{duckweed}}(t) = k \alpha \beta [\text{TcO}_4^-]_{\text{bulk solution}} \quad (5) $$

After combining Equation 5 with Equation 2 and rewriting,

$$ CF_{\text{Tc}} = \frac{[\text{Tc}]_{\text{duckweed}}}{[\text{Tc}]_{\text{bulk solution}}} = \frac{k \alpha \beta}{f} \quad (6) $$

where $CF_{\text{Tc}}$ is the concentration factor, i.e., the technetium concentration in duckweed normalized against the technetium concentration in the bulk solution, $k$ is the accumulation constant (h$^{-1}$), $t$ is the accumulation period (h), $[\text{TcO}_4^-]$ is the free pertechnetate concentration in the cell wall (mol/L), $\alpha$ is the fraction of water in the cell wall (expressed as volume of cell wall water/mass cell wall), $\beta$ is the weight fraction of the cell wall (kg cell wall/kg duckweed). The accumulation constant, $k$, comprises all processes regulating the accumulation; thus, the constant includes the transport across the cell membrane and the reduction of Tc. Simulations were carried out using Mathcad 4.0 (Mathsoft, Cambridge, UK). To simulate uptake, the $K_f$ values for binding of (earth) alkali metals and the accumulation constant $k$ should be known. These were derived in two ways, specifically, $K_f$ values were derived from literature and the accumulation constant was taken from earlier work [8] or the whole data set was used to fit the $K_f$ values and the accumulation constant. The second approach was applied to summarize the experimental data in model parameters, which can be compared with parameters derived from literature.

Literature data on binding constants for in situ binding of (earth) alkali metals are scarce. Most papers report exchange curves and selectivity constants or use binding constants of (earth) alkali metals for mono/dicarboxylic, gluconic, and uronic acids. The $K_f$ values measured for soy (Glycine max)
hull cell wall material [17] and the $K_f$ value for Ca binding in a pectic gel [18] were used as boundary values. Exchange curves for Ca-Mg, Ca-K, Na-K, and Na-K for horse bean (Vicia faba), lupine (Lupinus luteus), and duckweed (L. minor) were used to derive $K_f$ values for Na, K, and Mg [19] (see Table 1). The $K_f$ values were chosen in such a way that the exchange behavior observed in literature could be simulated. It can be proven that these values are proportional to the binding constant of calcium. The accumulation constant was derived from earlier determined flux constants, as described previously [8]. The measured pertechnetate concentration factor, i.e., the ratio of $TcO_4^-$ in duckweed and in the bulk solution, for duckweed grown in standard conditions (characterized by high cation concentrations; sum of cations is $\sim 6 \times 10^{-3}$ mol/L) was determined to be 0.21 $\pm$ 0.03 L/kg [8]. Since high cation concentrations are present, we may assume that the pertechnetate concentration in the nutrient solution equals the pertechnetate concentration in the cell wall solution ($f \approx 1$). The reduction rate constant, $k_{acc}$, was determined to be 0.65 $\pm$ 0.06 h$^{-1}$. From these data, the accumulation constant $k$ can be calculated as

$$k = \frac{[TcO_4^-]_{\text{duckweed}}}{[TcO_4^-]_{\text{bulk solution}}/ka}$$

i.e., 8.1 $\pm$ 1.4/h, where $\alpha$ and $\beta$ were taken from the literature (see Table 1).

**RESULTS AND DISCUSSION**

**Effect of nitrate**

Figure 2 plots Tc accumulation factors against the anion concentration. Tc uptake seems to be independent of the ratio of nitrate and chloride. However, the literature reports a competitive behavior of nitrate with the uptake of technetium in tomato and spinach plants (55% inhibition at $9 \times 10^{-3}$ mol nitrate/L), while chloride did not affect the Tc accumulation [2,6]. It was suggested that pertechnetate is transported across the cell membrane via a nitrate transport system [6]. The uptake of nitrate is regarded as biphasic, involving high-affinity and low-affinity transport systems [20]. Krijger et al. suggested that the uptake of pertechnetate is regulated by the low-affinity nitrate transport system [6]. However, this transporter might not be present or induced in Lemna, naturally growing in eutrophic areas and, in this study, cultivated at relatively high nitrate concentrations.

The transport of pertechnetate across the cell membrane may also be regulated by other (semi) specific transporters responsible for the transport of $TcO_4^-$, Evidence for more than one transporter can be found in the study of Krijger et al. [6] and in an earlier study [8]. Krijger et al. couldn’t inhibit the uptake of pertechnetate completely by nitrate only; a constant influx of pertechnetate was established at nitrate concentrations above $20 \times 10^{-3}$ mol/L. The earlier study showed higher Tc uptake rates from solutions without micronutrients than from solutions with micronutrients, which may be explained by the competitive behavior of $TcO_4^-$ with $MoO_4^{2-}$, as was shown for Tc uptake in soybean (Glycine max) [21].

**Effect of (earth) alkali metals on the Tc accumulation**

Figure 3 replots the data from Figure 2 against the cations present. Tc accumulation increases with increasing calcium and decreasing potassium concentrations. This is also predicted by the model, although the magnitude of the phenomena is underestimated.

To focus on cationic effects only, the uptake of Tc was...
studied in a concentration range of Na, K, Mg, or Ca salts of nitrate and chloride, as shown in Figure 4. Both calcium and potassium were applied as well as nitrate and chloride salts (Fig. 4A and B), while magnesium and sodium were only applied as nitrate salts (Fig. 4C and D). In experiments with magnesium, concentrations above the $8 \times 10^{-3}$ mol/L showed significant lower growth rates compared with cultures or were even lethal. Therefore, these magnesium concentrations were not considered further. The general trend in Figure 4 is comparable with that in Figure 3. An excess of cations results in higher accumulation (Fig. 4A, C, and D). However, application of potassium leads to a small decrease in accumulation (Fig. 4B). Although the variance is relatively high at low potassium concentrations, the decrease is significant (linear model, slope significantly less then zero, $t$ test, $\alpha = 0.05$).

Chloride and nitrate salts show the same effect on the uptake of Tc (Fig. 4A and B). If both competitive and electrostatic effects play a significant role, competitive effects should become visible at high cation concentrations. At that point, the cell wall is saturated with cations and the anion concentration in the cell wall equals the concentration in the bulk solution (Eqn. 2). Then competitive effects will not be overshadowed by electrostatic effects and a decrease in technetium accumulation should result. However, this pattern is not present in Figure 4. Instead, a plateau is observed, indicating that the uptake is not influenced by the anions present. This behavior supports the observations in Figure 2 where the osmotic pressure was constant in all nitrate concentrations. Thus, inhibition of Tc uptake by nitrate is absent in duckweed.

The lines in Figures 3 and 4 present the model as discussed. When literature values for complexation constants are used, the model tends to predict the curvature but does not match the data points. To overcome this difference, the data were fitted against the whole data set, excluding the potassium data. A maximum concentration factor, $CF_{\text{m}}$, of $10.1 \pm 1.0 \text{ L/kg}$ was obtained. From this concentration factor, the accumulation constant, $k$, can be calculated as $7.9 \pm 0.8/h$. This value is in good agreement with the constant of $8.1 \pm 1.4/h$ determined from the experimental data obtained in earlier work [8]. A $K_f$ for calcium of $10^{4.7}$ and associated $K_v$ values for the other binding constants were fitted to the data.

The fitted $K_f$ value for calcium and carboxyl groups is about one order of magnitude higher than the reported $K_v$ values for cell wall material and pectin [18]. Reported $K_v$ values for binding of calcium with single carboxylic acid groups range from $10^{6.6}$ to $10^{3.6}$ [22]. However, most of these values are determined in solutions. Tibbits and coworkers showed that calcium binding for pectin was higher in gel phases than in solutions [18]. Thus, a comparison with these values might be questionable.

An explanation of the relatively high $K_v$ values may be found in the assumption of a homogeneous distribution of pectic groups in the model, which is an ideal situation. In reality, pectic groups are heterogeneously distributed. Together with the cell wall cellulose, the cell wall consists of pores (the so-called water-free space, WFS) and charge-influenced patches (the Donnan free space, DFS) [23]. In the WFS, the pertechnetate concentration equals that in the nutrient solution. In the DFS, pertechnetate concentration is described by Equation 2. At increasing cation concentrations, the concentration of cation-pectic complexes also rises. This results not only in a decrease in cell wall potential but also in an increase in WFS; i.e., if all the negative groups are complexed, the whole cell wall becomes WFS.
netate concentrations at the cell membrane than expected from the model simulations. To correct for this effect, 10-fold higher binding constants are fitted to the data. The difference between simulation and fit provides information about the WFS. The difference appeared to be constant up to 8 mmol/L cations. The WFS fractions were calculated as 18 ± 7%, 14 ± 2%, and 9 ± 5% for Ca, Mg, and Na, respectively, for the simulations based on values reported by Tibbits et al. [18]. Simulations based on Laszlo [17] resulted in 28 ± 6%, 24 ± 3%, and 17 ± 5%, respectively. The effect of potassium on the Tc accumulation in duckweed deviates from the model and is therefore excluded from the WFS calculations.

Summarizing, between 14 and 23% of the concentration present at the cell membrane may be a result of Tc transport in the WFS. Above the 8 mmol/L cation, these fractions decrease. This is probably not a result of a physiological process but of the small fraction of free carboxyl groups. This results in a pertechnetate concentration in the DFS that approaches the bulk solution concentration. In models describing the cationic accumulation in the cell wall, the DFS is much larger since cations are accumulated in the DFS. Therefore, WFS effects may be less abundant and may be neglected.

Above 8 mmol/L, hydrophobic interactions may also begin to play a role in regulating the TCO₄⁻ cell wall concentration. Hydrophobic interactions of anions with organic phases may be explained by the water arrangement around the anion and the organic phase. This effect is often used in chromatographic separations of anions. Harms et al. [24] showed that the distribution coefficient of TCO₄⁻ over a chromatographic column increased with increasing chloride concentrations in the eluents.

Potassium shows a completely different behavior from that of Tc uptake. From the model, it can be expected that the behavior is similar to sodium (compare Fig. 4B and D). This is presented in Figure 4B by the solid line. As is clear, instead of an increasing uptake, the data show a decreasing trend. Since potassium tends to complex with pertechnetate [16], this behavior was included in the model (Fig. 4B). As can be seen, the formation of pertechnetate complexes leads to a decreased uptake, but not to the extent that the data suggest. It might be possible that potassium, having a high permeability for the cell membrane compared with sodium, magnesium, and calcium [25], will depolarize the cell membrane and influence the pertechnetate transport across the membrane.

Potassium behaves differently: Effect on the cell membrane

In order to eliminate the effect of the cell membrane, duckweed was fixated with glutaraldehyde. Figure 5 shows the accumulation in this fixated material. The data are presented as pooled data since the effect of nitrate and chloride salts on the adsorption showed no significant differences. Clearly, adsorption increases at higher potassium concentrations. This adsorption could be attributed mainly to absorption of pertechnetate from screening of negative charges by potassium. The fraction of total absorbed pertechnetate in the form of a neutral KTCO₄ complex was estimated as 20% at the most. However, the curvature of Figure 5 did not change significantly if the data were corrected for this fraction (data not shown). Figure 5 supports our hypothesis that the transport of pertechnetate across the cell membrane is coupled to the cell membrane potential that is regulated by the potassium concentrations inside and outside the cell. However, model outcomes, using a binding constant for potassium of 10⁻¹⁸ mol/L, deviate 11 to 38% from the data points (dotted line, Fig. 5). This may be a result of the presence of glutaraldehyde, which was not included as a separate species in simulations.

Physiological effects of applied salts

In this study, we assumed no additional physiological effects from the applied salts. However, all of the ions used are involved in physiological functions, ranging from amino acid and protein biosynthesis to osmoregulation to solute transport across the biomembrane [26]. Growth, as a parameter, incorporates all these effects but was not affected by the application of the nitrate and chloride salts over the 6-d experimental period. These observations will not exclude the possibilities of altered biochemical reactions that may possibly affect the Tc accumulation. However, the data strongly suggest that cell wall effects dominate the uptake behavior.

Environmental implications

Radionuclides released may impact the local environment. The degree of impact, or radiobiological hazard, can be related to the bioavailability and mobility of the radionuclides [26]. Among the long-lived radionuclides released into the environment, ⁹⁹Tc is considered to be one of the important nuclides in safety analysis due to its high environmental mobility [1]. Previously, information about the relationship of Tc in aquatic environments to aquatic plants was very scarce. However, aquatic plants can accumulate large amounts of radionuclides and influence the biogeochemical fate of these [27], but the magnitude of accumulation depends on the composition of the water [28]. This study shows that hardness and salinity are positively correlated with the accumulation of technetium in duckweed when supplied as TCO₄⁻. This contrasts with the general behavior of many (metallic) radionuclides, which often appear in a cationic form [26]. Such radionuclides are adsorbed less when hardness and salinity increase [28] as a result of a competition with other cations, mainly (earth) alkaline metals, for binding places located in the cell wall [11,29]. This is also demonstrated by our model. The intracellular uptake of TCO₄⁻ in duckweed was independent of the nitrate and chloride con-
centrations. In contrast, TeO\textsuperscript{4–} uptake was inhibited by nitrate in terrestrial plants [2,6].

The electrostatic effects observed in this study are relevant to the natural environments where duckweed can be found [10]. Differences in bioaccumulation factors for \textsuperscript{99}Tc in duckweed between (polluted) areas may be explained by these effects. Bioaccumulation factors may vary by a factor up to three as a result of these electrostatic effects, and thus related to the hardness of the water. However, this difference is too small to induce significant differences in radioecological effects.

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