

Modeling of cesium-137 and strontium-90 accumulation in the freshwater algae cells

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Abstract. Using the model object, freshwater Charophytae alga cells *Nitella flexilis*, the mechanisms of ^{137}Cs and ^{90}Sr uptake have been studied. The curves of radionuclide accumulation and washout in time have been measured, and the effects of uni- and divalent cations, pH of a solution, illumination, fulvic and humic acids on the curve parameters have been investigated. The results obtained have been interpreted on the basis of the currently known mechanisms of ion transport in plasmalemma: H^+ -ATPase pump, potassium channels, nonselective ion leakage, surface charges and the apoplast - cation exchanging cell wall. It has been shown, that nuclides enter into a cell through potassium channels and nonselective ionic leakage. While a share of each pathway in a total ^{137}Cs influx is identical, the influx of ^{90}Sr is primarily through leakage. The basic ion transport mechanisms were assumed to be located separately on plasmalemma because of discrete nature of the molecule structures their forming, and can interact by superposition of local electric fields, generated by each of ion-transport mechanisms. So the intro-membrane electrical field may be vary for different plasmalemma sites. Quantitative model based on above mentioned notions was developed and validated: it describes steady state distribution of ^{137}Cs and ^{90}Sr between cell and medium.

1. INTRODUCTION

It is conventional guess that the share of water reservoir radionuclides (RN), containing in algae, is not big: it does not exceed 10-15%. But the data show the much more important role of algae in process of RN exchange between water body and others compartments of water reservoir, because of large the general RN flux through algae including phytoplankton [1]. On the other side, algae are the first rings in human food chain through fish. It is evidently, that to predict RN level in water body and in a fish, understanding the process of RN uptake and accumulation in algae is necessary. So, the aim of this work is by use earlier received information and obtained experimental data of RN uptake by alga cells to clear the adequate picture of the process and to construct the quantitative model of ^{137}Cs and ^{90}Sr accumulation into the cell.

Radionuclides ^{137}Cs and ^{90}Sr , as mineral substances, being present in water body as the ions, enter into the alga cells through an ion transport mechanisms, localized on cell membranes. The main of them are H^+ -pump, potassium channels both outward- and inward-rectified (ORPC, IRPC), unselective ionic leakage [4, 5]. Being structurally independent, they are linked by a membrane electric field (or potential difference, PD), and in its turn each mechanism contributes to PD: so, they are functioning as a system.

Unidirectional flux (e.g. into a cell) of some ion is proportional to its concentration in a near membrane layer of the outer solution, permeability and to the exponential of PD. The resultant flux is a sum of those through ionic channels and leakage. A near membrane layer of the solution is located in the pores of a cell wall (apoplast, AP). Being a weakly-acidic cation exchanger (pK 5.1), AP is capable to increase the cation concentrations in the pores as compared to the bulk of solution [2, 3]. Divalent ions are preferably absorbed in the AP, then univalent ones [3]. Its selectivity for each from the ion pairs (Cs^+ and K^+ , Sr^{2+} and Ca^{2+}) is equal, due to closeness of their ionic diameters. It is significant that the cation-exchange capacity of AP is saturated when the solution contains more than 10^{-3} M of Ca^{2+} or 10^{-2} M K^+ ions.

Passing over a cell wall, ions come to the plasmalemma, through which they pass by several ways. The PD value is a result of electrically-parallel connection of those ways: H^+ -pump, channels and leakage with their electromotive forces (EMF) and conductance. The EMF of H^+ -pump is the most negative one, those of the channels and leakage depend on the ionic concentration in a medium. For selective to K^+ IRPC and ORPC (the channel's cation selectivity is the following series, identical for both types: $\text{K}^+ >$

$Rb^+ > Na^+ = Li^+ > Cs^+ > Mg^{2+} > Sr^{2+} = Ca^{2+} = Cd^{2+} = Ba^{2+}$ [4, 5]) the EMF is determined by the ratio between the K^+ concentration in outer solution and the cytoplasmic one in accordance with Nernst's equation. The same dependence for leakage is more complex, but its EMF is lower than for the channels [4, 6].

Conductance (permeability) of the channels is controlled by the PD: for ORPC it is increased at less negative PD, and for IRPC it is, on the contrary, decreasing [4, 5]. Leakage conductance is PD- independent [6]. Surface fixed anions are located only near the ORPC at the outer plasmalemma side; that is why their modification by uni- and polyvalent cations leads to change the conductance only those channels.

Thus, the concrete tasks of this work were to determine (1) which of the above mechanisms are responsible for the RN transport through the plasmalemma; (2) what is the share each of them in the total RN influx; (3) what are the effects of the main environmental factors: the content of uni- and divalent cation, pH values, the presence of water-soluble humus components (fulvic and humic acids, FA and HA), light exposure of the cell and finally, on the basis of information received, to construct the quantitative model, describing steady-state distribution of radionuclides between cell interior and medium.

2. OBJECTS AND METHODS

As an object the cells of freshwater Charophytae alga *Nitella flexilis*, cultivated in laboratory conditions, have been used [4]. They are typical freshwater algae, poses all structural elements – cell wall, plasma membrane, cytoplasm and vacuole. Due to big size (up to 0,5 mm in diameter and 3-5 cm length) they are convenient for the electrophysiological experiments (by which the above-mentioned mechanisms of ion transport have been studied), and direct studies of ion fluxes using a radioisotope as well.

In the course of experiments the curves of radionuclides accumulation and washing-out in an isolated cell in time have been obtained (Fig. 1). The cells, placed in solution, containing either ^{137}Cs or ^{90}Sr , in a

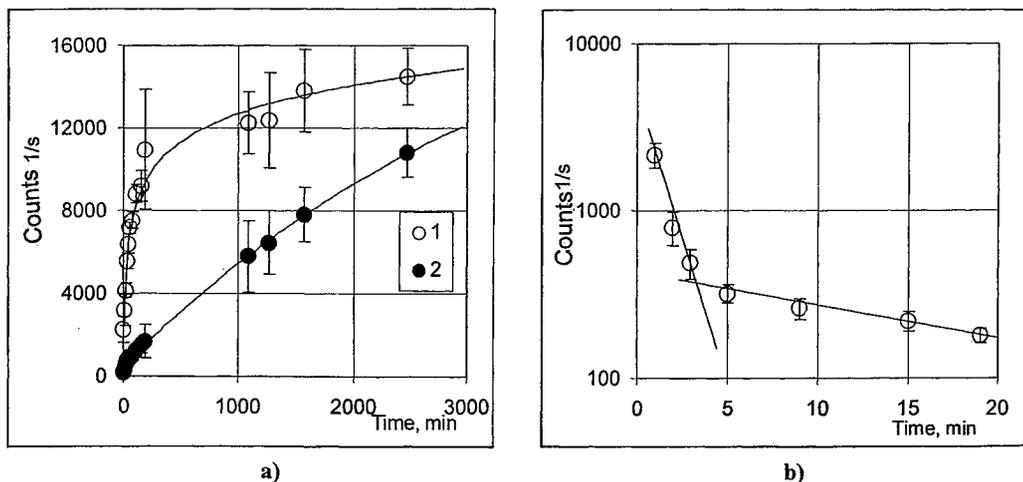


Figure 1: Time course of radionuclides accumulation and washout in the alga *Nitella* cells.

a): Strontium-90 (1) and Yttrium-90 (2) accumulation in the cells;

b): Caesium-137 washout from the cell. Average data of at least seven cells are shown.

defined interval of time, were periodically withdrawn from solutions for measuring their activity by appropriate counters. The value of the RN volumic activity in solutions was adjusted to 10-20 kBq/ml for ^{90}Sr and 50-100 - for ^{137}Cs . The nuclides have been used as $CsCl$ and $SrCl_2$, their concentrations being less than 10^{-7} M. This value is higher then in the water body of polluted water reservoir [7], but lower, than the independence thresholds of ion transport through the membrane [5], and the membrane effects of these cation (adsorption on fixed anions of Sr^{2+} and blocking of potassium channels by Cs^+ [3-5]).

The time course of nuclides accumulation and washout (Fig. 1) were analyzed graphically and revealed at least three exponential phases. The first two are shown in Fig. 1, b. According to axial symmetric structure of the cell – cell wall, cytoplasm, and central vacuole – the first two phase correspondingly show the process in AP and cytoplasm. The slowest phase (Fig. 1, a – close to saturation) reflects the nu-

clides exchange in vacuole. As we can see from Fig. 1, b, AP exchange for ^{137}Cs takes no more than 5 min. So, experimental procedures for ^{137}Cs , were the following: the cells, withdrawn from solution for the activity measuring, after rinsing in distillate, were successively held for 2,5 min in two solutions of artificial pond water (APW) for the ^{137}Cs removal from the AP. For the estimation of its cation-exchanging capacity no soaking has been used before the first measuring, and then above procedure applied.

It is well known, that as a result of decay ^{90}Sr arise ^{90}Y ; the both exist simultaneously and penetrate into a cell differently due to their different ion charges. The measurements were conducted using an Al filter to chop 0.99 of ^{90}Sr radiation and 0.50 - ^{90}Y , and without the filter - to obtain estimation of ^{90}Sr content. The cell activity has been determined immediately upon rinsing, the apoplast capacity has been estimated by the counting rate after the first 5 min of exposure in a labeled solution. The APW solution contained (in M): 10^{-4} KCl, 10^{-3} NaCl, 10^{-4} CaCl₂; pH 8.2 and 5.5 were adjusted by TRIS and MES buffers.

3. RESULTS AND DISCUSSION

An alga cell comprises a number of successively positioned axially symmetrical compartments: cell wall, cytoplasm, vacuole. The analysis has shown that the accumulation curves as well as the washout ones represent a sum of the two exponential phases provided a cell wall phase is excluded [8]. To reveal its, the logarithmic dependence of the difference between steady-state (for a long time) and current activity values has been constructed. It is clear, that during the first hours of the experiments a slope (time constant, T) of the dependences reflects the radionuclides influx or outflux to (from) cytoplasm across the plasmalemma. A subsequent, slowest phase is more difficult to interpret and not discusses here.

Experimental results for ^{137}Cs are summarized in Table 1. Analysis has demonstrated that in case of

Table 1. Parameters of the ^{137}Cs accumulation by single cell (alga *Nitella*) under varying environmental conditions. T - time constant of the first stage of the processes of ^{137}Cs accumulation (in), or washing (out) by the cell, when the cell wall (apoplast) stage was rejected. N - cell activity, divided on the cell surface area. K_D - distribution coefficient as the ratio of specific cell activity (without apoplast, which is done as $K_{D,ap}$) and one of the solutions. Each of the data is an average of at least 5 cells. pH was 8.2. Notation D, H and N correspond to dark, high and normal illumination.. Ionic concentrations are indicated in mM.

Solutions	APW	APW	APW	APW+5K ⁺	APW+5Ca ²⁺	APW+5K ⁺ +5Ca ²⁺	APW+5Ba ²⁺	APW+5Cs ⁺	APW+10 ⁻² % FA	APW+10 ⁻² % HA
Light conditions	H	N	D	N	N	N	N	N	N	N
Parameters										
T _{in} , min	550	950	795	1350	940	1540	2000	2050	1700	2490
T _{out} , min.	580	1100	920	850	-	1510	1900	1560	-	-
N _{st} , s ⁻¹	880±190	510±200	520±160	360±80	400±110	120±40	250±60	340±80	1120±210	480±130
N _{ap} , s ⁻¹	-	1390±240	-	1100±250	-	960±210	-	-	2150±300	1240±150
K _{D,ap}	-	231±40	-	183±41	-	160±35	-	-	358±50	207±35
K _D	14.4±3.1	8.2±1.4	8.5±2.6	6.0±2.1	6.7±1.3	2.1±0.7	4.1±1.1	5.6±1.5	18.3±3.0	7.8±1.7

^{137}Cs transport decelerations of the influx only through the channels would be three-fold with an addition of $5 \cdot 10^{-3}$ M K⁺ in solution and never equal to 40 %, as in experiment (Table 1). Considering all the above-said, a two-fold deceleration in case of potassium channel block by $5 \cdot 10^{-3}$ M Ba²⁺ and Cs⁺ means that only a half of incoming nuclide is transported through the channels, and the other half seems to be entered through leakage. Actually, a rise of the Ca²⁺ content in APW makes no change in the influx rate, but decelerate the influx still further in case of the increased K⁺ content when ORPC are partially opened. This effect is non existent in APW, when only IRPC are opened, in the vicinity of which there are not fixed surface anions. The nonselective leakage participation in ^{137}Cs transport is indicated by more considerable decrease in K_D in presence of K⁺ and Ca²⁺ as compared to the presence of channel blockers (Ba²⁺ and Cs⁺), in the sense that Ca²⁺ in the first case is responsible for the inactivation of ORPC and K⁺ decreases the PD thus reducing the ^{137}Cs influx not only through channels, but through leakage as well. The blockers cause decrease of the influx only through the channels.

The results show that high light exposure accelerates the influx by twice, while transition from darkness to weak lighting causes no change. The direction and magnitude of the effects correspond to the electrophysiological reaction of H^+ -pump on illumination, when the plasmalemma PD decreased [4]. However, outflux acceleration, observed at the same time, could be a result of both change in cytoplasm absorption-exchange properties upon photosynthesis and involving of the H^+ -pump in the ^{137}Cs transport.

Special experiments have shown the FA and HA in the used concentrations cause no electrophysiological effects. The decelerated ^{137}Cs influx seems to be a result of radionuclides binding at their ion-exchange sites. Obviously, the molecules of FA, penetrating the pores of a cell wall, become bound; that lead to the increasing both exchangeable and no exchangeable capacity of apoplast to Cs^+ . As a result of this effect K_D is increased. The larger molecules of HA are unable to penetrate the pores resulting in the unchanged K_D and apoplast capacity. Some others membrane effects of FA and HA are possible, which are not revealed electrophysiologically, and are probably associated with their surface activity.

As it is seen from Table 2, an addition of K^+ to APW (pH 8.2) brings about a purely electrophysiological effect: the ^{90}Sr influx is decelerated more than twice, while its outflux is accelerated and the K_D is lowered. A small effect of the channel blocker – Ba^{2+} (deceleration of the influx and outflux by 30% and 50%) is an evidence of the insignificant role of the channels in the total ^{90}Sr influx. A simple quantitative

Table 2. Parameters of the ^{90}Sr accumulation by single alga *Nitella* cells under varying environmental conditions. (The notations used are the same, as in Table 1).

Solutions	APW	APW	APW	APW	APW+ 5K ⁺	APW+ 5K ⁺	APW+ 5Ca ²⁺	APW+ 5Ba ²⁺	APW+ 5Ba ²⁺	APW+ 5K ⁺ + 5Ca ²⁺	APW+ 5K ⁺ + 5Ba ²⁺	APW+ 10 ⁻² % FA	APW+ 10 ⁻² % HA
pH	8.2	8.2	8.2	5.5	8.2	5.5	8.2	8.2	5.5	8.2	8.2	8.2	8.2
Light conditions	D	N	H	N	N	N	N	N	N	N	N	N	N
Parameters													
T _{in} , min	925	1140	-	1210	2120	1400	2160	1450	1500	2040	2770	860	2130
T _{out} , min.	550	1300	1610	1600	830	2580	380	1900	1300	-	-	710	940
N _{st} , s ⁻¹	9000 1690±	10100 ±2100	14360 ±3520	5050 ±1500	8300 ±1650	9160 ±1680	7300 ±1430	3620 ±1150	6700 ±940	7690 ±1250	3200 ±840	4830 ±1130	4390 ±1530
N _{ap} , s ⁻¹	1700 ±320	1770 ±290	1520 ±180	1390 ±270	1710 ±320	1580 ±410	880 ±170	550 ±110	910 ±250	800 ±190	390 ±150	960 ±120	1670 ±330
K _D ap	425±80	442±72	380±45	347±63	427±80	395±102	220±42	137±24	227±62	200±45	97±37	240±30	417±83
K _D	180±35	210±42	320±75	88±25	160±32	182±32	156±30	74±23	140±25	166±30	68±18	94±23	80±27

analysis of these data determines a relative share of the channels, which is no greater than 0.3. The relative channel permeability to Cs^+ and Sr^{2+} is about 1:0.5 [4], that is in accordance with the found share of the channels as 0.5 for $^{137}Cs^{2+}$ and 0.3 for $^{90}Sr^{2+}$. Further analysis of the results, given in Table 2, provides support for this estimates. Actually, in presence of the K^+ the accelerating effect of Ca^{2+} upon the influx is lacking, K_D also remains unchanged. Being a blocker, Ba^{2+} decelerates in APW (pH 8.2) the RN influx and outflux. Under pH 5.5, when the ORPC share in the total membrane permeability is less [5], the effect of Ba^{2+} particularly on the K_D value is less too, while with K^+ present a share of the channels is greater and deceleration is more significant (Table 2).

Positive correlation of the apoplast capacity with the influx rate and the K_D value for ^{90}Sr is obvious. Selectivity of AP to divalent cations has been noted above. Evidently, AP more strongly controls the divalent cation concentrations near the plasmalemma than the univalent ones. In such way, the decrease of AP capacity for ^{90}Sr causes the lowering of K_D due to the addition of FA to APW. At the same time HA, leaving apoplast unchanged (as with ^{137}Cs), leads to the decreased K_D and decelerates the influx.

As in case of ^{137}Cs , transition to severe light exposure activates H^+ -pump, thus decelerating the ^{90}Sr outflux and increasing K_D . However, sharp acceleration of the influx in darkness is never accompanied

with the influx decelerations (as with K^+ addition). This effect seems to be induced not by the lowering PD of the plasmalemma upon the H^+ -pump inactivation in darkness; besides, K_D is practically unchanged. Just probably this is caused by the change in the cytoplasmic adsorption-exchange properties. Similar effects are found when Ca^{2+} is added into the solution, i.e. the rate ^{90}Sr outflux also grows. It is well known that divalent cations, Ca^{2+} in particular, occur in cytoplasm in a bound state - at concentrations of the order of 10^{-2} M; the free Ca^{2+} activity is not more than 10^{-6} M [9]. This seems to be the cause for the large K_D value for ^{90}Sr (of the order of 200) as compared to K_D for ^{137}Cs (of the order 10).

4. MODEL

Using idea of lateral segregation of ion-transport mechanisms on plasmalemma [6], we attempt quantitatively describe distribution of cations-radionuclides, observed in experiment, not using assumption of active pumping them from a cell.

Cations pass plasmalemma through potassium channels and leakage, which have their own local potential difference. So, for ion content in cytoplasm ($C_{i,Cyt}$):

$$dC_{i,Cyt}/dt = \Phi_{lk\ in} + \Phi_{ch\ in} - \Phi_{lk\ out} - \Phi_{ch\ out}, \text{ and in steady state } \Phi_{lk\ in} + \Phi_{ch\ in} - \Phi_{lk\ out} - \Phi_{ch\ out} = 0 \quad (1)$$

for each ion. $\Phi_{lk\ in}$, $\Phi_{lk\ out}$ and $\Phi_{ch\ in}$, $\Phi_{ch\ out}$ - in- and outflow through unselective ionic leakage, and accordingly through potassium channels.

According to the constant field (Goldman's) theory, for leakage:

$$\begin{aligned} \Phi_{lk\ in} &= P_{lk\ i} C_0 (zV_{lk} F/RT) \exp(-zV_{lk} F/RT) (\exp(-zV_{lk} F/RT) - 1)^{-1}, \\ \Phi_{lk\ out} &= -P_{lk\ i} C_i (zV_{lk} F/RT) (\exp(-zV_{lk} F/RT) - 1)^{-1} \end{aligned} \quad (2)$$

and for potassium channels:

$$\begin{aligned} \Phi_{ch\ in} &= P_{ch} C_0 (zV_{ch} F/RT) \exp(-zV_{ch} F/RT) (\exp(-zV_{ch} F/RT) - 1)^{-1}, \\ \Phi_{ch\ out} &= -P_{ch} C_i (zV_{ch} F/RT) (\exp(-zV_{ch} F/RT) - 1)^{-1} \end{aligned} \quad (3)$$

Finally, substituting (2) and (3) to (1), we receive:

$$K_D = C_i/C_0 = [(P_{lk}/P_{ch})(V_{lk}/V_{ch}) \cdot \exp(zV_{lk} F/RT) \cdot (\exp(-zV_{lk} F/RT) - 1)^{-1} + \exp(-zV_{ch} F/RT) \times (\exp(-zV_{ch} F/RT) - 1)^{-1}] / [(P_{lk}/P_{ch})(V_{lk}/V_{ch}) \times (\exp(-zV_{lk} F/RT) - 1)^{-1} + \exp(-zV_{ch} F/RT) - 1]^{-1}$$

When the main parameters for channels and leakage are the same, (spatial non-uniformity of a plasmalemma electric field is lacking) the received expression transforms in Nernst's equation; when the permeability of any ion-transport mechanism to the given cation dominates, the equation shows, that the cation is distributed according to its local potential difference. The results of calculations on model are shown by curves in a fig. 2.. It is seen, that the cell accumulates much more divalent ^{90}Sr , than univalent

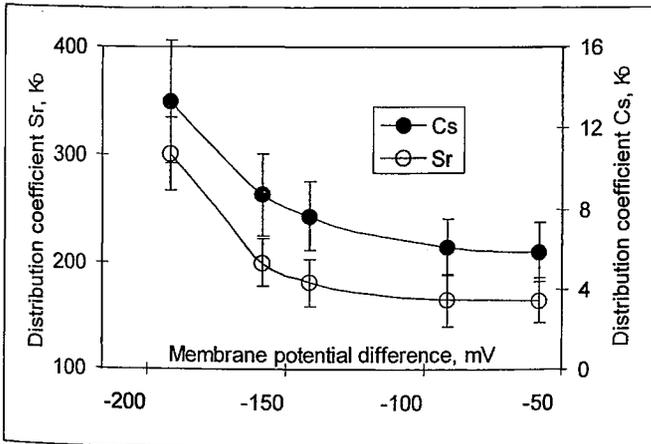


Figure 2: Potential dependence of distribution coefficients (K_D) of radionuclides between an internal part of a cell and medium. The lines are drawn according to the model developed.

^{137}Cs ; K_D for caesium as a whole is notably smaller, than for strontium (6-15 and about 300 accordingly).

The results demonstrate, that steady state distribution of ^{90}Sr and especially ^{137}Cs between a cell and medium is far from equilibrium, predicted according Nernst's equation: Nernst's K_D rise from 23 to 2742 for ^{137}Cs and $150 - 7,5 \cdot 10^5$ for ^{90}Sr when

the potential difference decrease from -50 to -200 mV. So, discrepancy dramatically rises when, for instance, potassium level in water decrease and illumination increase.

5. CONCLUSIONS

The presented data show, that when potassium level in medium is lowered, any "high-affinity" system of cations transport (like higher plants) is not activated, and radionuclides pass plasmalemma through potassium channels and unselective ionic leakage. The large apoplast K_D for the both nuclides have to be pointed out. It is important, in particular for ^{137}Cs , that relative amount of nuclides in apoplast will increase and can become dominant when the cell size decrease; the effect for ^{90}Sr is not so significant.

The presented picture reflects relatively short-time processes (within tens of hours) of RN accumulation and involved mechanisms. In reality the alga cells grow in the water for a long time with RN permanently presented and are included in a cell structures. Nevertheless, above picture is valid for ^{137}Cs , because in cell compartments ^{137}Cs is mostly in a free ionic form. Divalent ^{90}Sr , as was pointed out above, like Ca^{2+} , can be bound in sorption-exchange complex of cytoplasm. However, the very low Sr^{2+} level, used in above experiment, and particularly in contaminated water reservoirs [7], let us to consider, that we are dealing with distribution free ionic ^{90}Sr between cell and medium, when sorption-exchange processes in cytoplasm do not noticeably contribute.

Hence, we can conclude the following. In dilute solutions (APW) a half of the ^{137}Cs influx pass the plasmalemma through potassium channels and another half - through ionic leakage. In the alike conditions ^{90}Sr is transported mainly through ionic leakage - about 0.7 of the total influx. The accumulation parameters of ^{90}Sr are controlled by the apoplast capacity and also by the sorption-exchange properties of cytoplasm.

Fulvic acids at the concentration of 0,01 % bring about the increase in K_D for ^{137}Cs and decrease of that for ^{90}Sr . Hindering the influx of ^{90}Sr , humic acids at the same concentration lead to the decreased K_D .

Severe light-exposure (up to 200 lx) stimulates H^+ -pump thus accelerating the influx of ^{137}Cs and leading to a considerable increase in K_D . The effect of lighting on the accumulation parameters of ^{90}Sr is weaker.

Quantitative model based on presented data was developed and validated: it describes steady state distribution of ^{137}Cs and ^{90}Sr between cell and medium under various conditions. The model could be easily extended for heavy metals and other polyvalent cations.

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