

Root Absorption and Transport Behavior of Technetium in Soybean¹

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ABSTRACT

The absorption characteristics and mechanisms of pertechnetate (TcO_4^-) uptake by hydroponically grown soybean seedlings (*Glycine max* cv Williams) were determined. Absorption from 10 micromolar solutions was linear for at least 6 hours, with 30% of the absorbed TcO_4^- being transferred to the shoot. Evaluation of concentration-dependent absorption rates from solutions containing 0.02 to 10 micromolar TcO_4^- shows the presence of multiphasic absorption isotherms with calculated K_s values of 0.09, 8.9, and 54 micromolar for intact seedlings. The uptake of TcO_4^- was inhibited by a 4-fold concentration excess of sulfate, phosphate, selenate, molybdate, and permanganate; no reduction was noted with borate, nitrate, tungstate, perchlorate, iodate, or vanadate. Analyses of the kinetics of interaction between TcO_4^- and inhibiting anions show permanganate to be a noncompetitive inhibitor, while sulfate, phosphate, and selenate, and molybdate exhibit characteristics of competitive inhibitors of TcO_4^- transport suggesting involvement of a common transport process.

Technetium-99 is currently projected to be one of the most important isotopes associated with the nuclear fuel cycle based on potential long-term dose to man. This is based on a number of criteria including fission yield (6%), long half-life (2.13×10^5 year), mobility and persistence in soils as the pertechnetate ion (TcO_4^-), and its comparatively high availability for plant absorption (11, 19). Wildung *et al.* (18) have shown that TcO_4^- added to soil was readily accumulated by soybean and wheat seedlings over a 30-d period with reported CR² values (CR = activity/g tissue dry weight ÷ activity/g soil dry weight) of 380 to 170 for soil concentrations of 0.001 to 0.1 μg Tc/g soil. At concentrations above 0.1 $\mu\text{g}/\text{g}$, severe toxicity and seedling death resulted. At soil concentrations of 1 pg Tc/g soil, CR values have been reported to range from 50 to 400 for cheatgrass and tumbleweed grown on arid soils (16). Landa *et al.* (12) showed a similar range in CR values for six plant species grown hydroponically and for wheat grown on 11 different soils.

While CR values for TcO_4^- are high compared with values for fission products and non-nutrient pollutant elements (<1), they are of a magnitude observed for trace nutrient ions such as Mo, Mn, Cu, Fe, and Zn (4). The solubility of TcO_4^- in soils, although an important aspect of its soil-plant behavior, does not totally explain the high affinity of plants for TcO_4^- .

The observed phytotoxicity of ⁹⁹Tc was investigated by Berlyn *et al.* (2). Their results failed to demonstrate cytological evidence

for radiation-induced damage and suggested an interaction or substitution of Tc for an essential nutrient. Pertechnetate has been shown to be toxic to a number of algae and bacteria, and interfere with both respiration and photosynthesis (9) at concentrations in excess of 1 mM. The potential interaction of Tc with nutrient ion metabolism is supported by uptake inhibition studies which showed ⁹⁹ TcO_4^- uptake to be reduced by the presence of sulfate, selenate, phosphate, and molybdate (6).

The purpose of the present study is to evaluate the parameters associated with TcO_4^- absorption by plant roots, and the kinetics of its interaction with essential nutrient elements and non-nutrient elements with chemical similarities.

MATERIALS AND METHODS

Plant Culture. Seeds of *Glycine max* cv Williams were germinated on moist filter paper and individual seedlings transferred to 600-ml beakers containing 500 ml of aerated nutrient solution 3 d following germination. The nutrient solution contained 150 mg of KCl, 120 mg of MgSO_4 , 946 mg of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 68 mg of KH_2PO_4 , 0.06 mg of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.69 mg of H_3BO_3 , 0.017 mg of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, 0.024 mg of $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, 0.022 mg of $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, and 0.60 mg of Fe^{+3} (as Fe EDDHA) per liter. The pH was adjusted to 6.8 with KOH and solutions changed three times a week. Plants were maintained in controlled-environment chambers with a 16-h light cycle ($\sim 500 \mu\text{E m}^{-2} \text{s}^{-1}$, PAR, at leaf surface), a day/night temperature cycle of 26/22°C, and 50% RH.

Technetium Uptake. Evaluation of the absorption behavior of Tc was performed using 10- to 12-d-old plants. Prior to use, plants were transferred from nutrient solutions to 0.5 mM CaCl_2 solutions (pH 6.8) for 12 h to allow for desorption of possible interfering ions from root surfaces. Individual plants were transferred to aerated solutions containing 500 ml 0.5 mM CaCl_2 (MCB, suprapure) and various concentrations of $\text{NH}_4^{99}\text{TcO}_4$. Following the absorption period, which was 2 h except as noted, plants were transferred to either complete nutrient solution or 0.5 mM CaCl_2 solutions containing 10 mM KH_2PO_4 or K_2SO_4 . Ions were desorbed from roots using three changes of solution for a total of 20 min.

The influence of H^+ concentration on Tc absorption and transfer from root to shoot was determined using 0.5 mM CaCl_2 solutions containing 0.2 μM TcO_4^- . The solutions were adjusted to pH 3 to 8.5 using either HCl or KOH. After the 2-h absorption period, solution pH varied by no more than ± 0.2 pH units for any given pH.

Ion competition studies were performed using the above protocols. Preliminary competition studies employed 0.25 μM solution of ⁹⁹ TcO_4^- in the absence or presence of 1.0 μM competing anions. Detailed kinetic analyses of anion interactions were performed using 0.25, 0.33, 0.4, 0.67, and 1.33 μM ⁹⁹ TcO_4^- solutions in the absence and presence of 10 μM K_2SO_4 , KH_2PO_4 , Na_2MoO_4 , Na_2SeO_4 , and KMnO_4 . Kinetic constants (K_s , V_{max})

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² Abbreviation: CR, concentration ratio.

were determined by linear regression of Lineweaver-Burk plots.

Radioanalysis. Tissues containing ^{99}Tc were oven dried, weighed, wetted with 10 ml of concentrated NH_4OH , covered and digested for 12 to 20 h on low heat ($\sim 60^\circ\text{C}$). These were brought to dryness, placed in a muffle furnace at 200°C for 1 to 2 h, then 450°C for 24 h. Samples were cooled, wetted with 1 ml concentrated NH_4OH , evaporated to dryness, and re-muffled at 450°C for 20 h. Samples were then cooled and suspended in 2 to 5 ml 0.1 N NH_4OH for analysis. This procedure resulted in no loss of Tc as determined by spike recovery on control plant tissues. Activity of ^{99}Tc was determined on a 250- μl aliquot by liquid scintillation spectrometry, with appropriate quench correction. Uptake rates for Tc were expressed on the basis of root dry weight, which included only lateral roots; fibrous roots were assayed separately, and included in total for uptake rates.

RESULTS AND DISCUSSION

Preliminary studies of pH effects on absorption, desorption from root surfaces, and time-dependent uptake were undertaken to define the absorption parameters for TcO_4^- . The absorption of TcO_4^- from solutions of pH 3.0 to 8.5, while decreasing with increasing pH (Fig. 1), exhibited no distinctive pH optimum. Since TcO_4^- is a stable species in aqueous solution over this pH range (1, 15), especially for the short time periods employed, the observed pH effect on root absorption most likely represents a generalized effect of H^+ concentration on the absorption process. Hydrogen ion concentration may also aid in the chemical reduction of TcO_4^- since, as the pH decreases, reduction can occur at progressively higher oxidation potentials. The apparent effect of pH on the transfer of Tc between root and shoot tissues, although qualitatively similar to that of root absorption, results from a relatively constant proportion of Tc transfer from root to shoot at each pH. This transfer ranged from 25% at pH 8.5 to 31% at pH 3.0. Subsequent studies were routinely conducted at pH 6.8, which is more suitable for plant growth, and to assure chemical stability of TcO_4^- over long time periods.

The absorption of TcO_4^- by seedlings was linear for at least 6 h (Fig. 2). Transfer of TcO_4^- from root to shoot was rapid (detected in <15 min), with accumulation being linear with time and accounting for 29% of the total accumulated in 2 h. Evident in the time course data is an irreversibly bound, nonmetabolic component of ~ 70 nmol Tc/g root dry weight. Desorption of

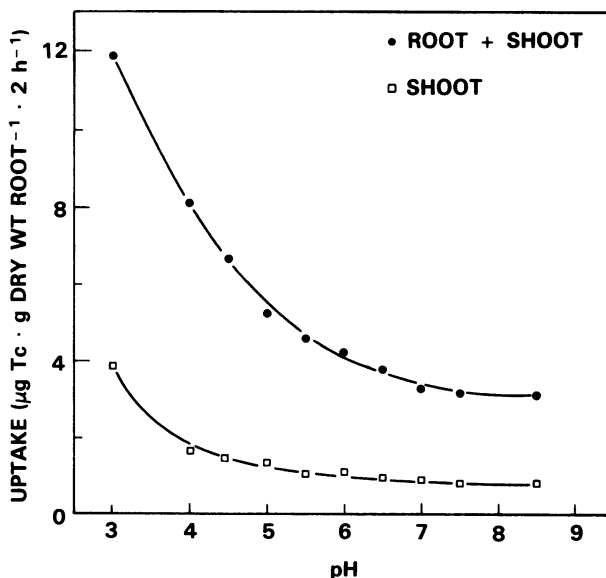


FIG. 1. Effect of pH on absorption of $^{99}\text{TcO}_4^-$ from 0.2 μM solutions. Desorption in 10 mM K_2SO_4 ; values represent average of three replicates.

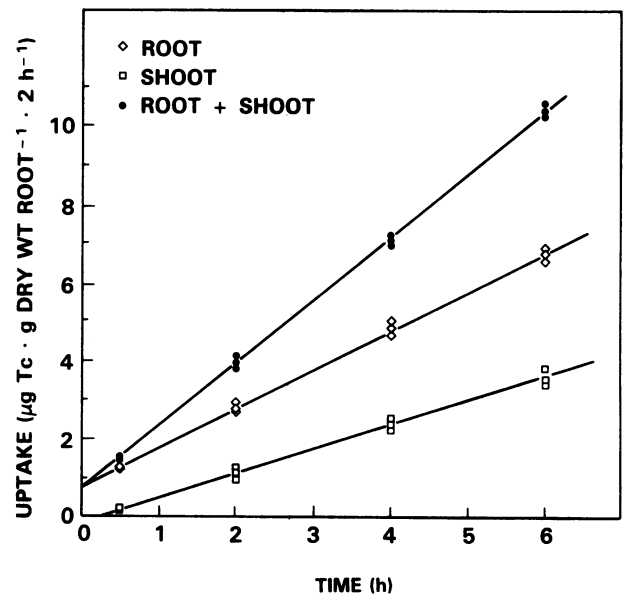


FIG. 2. Time course of $^{99}\text{TcO}_4^-$ absorption from 0.25 μM solutions for seedlings and transfer to shoots. Desorption in 10 mM K_2SO_4 .

Table I. Effect of Metabolic Inhibitors on Absorption of $^{99}\text{TcO}_4^-$ by 11-Day-Old Soybean Plants

Treatment	Concn.	Absorption ^a	
		Whole plant	Shoot
	<i>M</i>	% of control ^b	
Control		100 ± 17	100 ± 32
Na azide	10 ⁻⁴	21 ± 2	43 ± 14
Dinitrophenol	10 ⁻⁴	12 ± 4	27 ± 6
Succinate	5 × 10 ⁻²	21 ± 10	38 ± 4
FCCP ^c	10 ⁻⁵	7 ± 1	15 ± 2

^a Absorption was for 1 h from 0.1 μM $\text{NH}_4^{99}\text{TcO}_4$ in 0.5 mM CaCl_2 , pH 6.8.

^b Control absorption rates were 0.574 ± 0.097 and 0.081 ± 0.026 $\mu\text{g g}^{-1}$ root dry wt h⁻¹.

^c FCCP, carbonylcyanide-*p*-trifluoromethoxy-phenylhydrazine.

exchangeable TcO_4^- from root surfaces using 0.5 mM CaCl_2 was rapid and resulted in the removal of 6% of the total Tc accumulated by the root after 2 h of uptake. Desorption in either complete nutrient solution, or 10 mM KH_2PO_4 or K_2SO_4 increased the fraction removed to only 7 to 9%. The fraction not readily desorbed is assumed to represent metabolic absorption for purposes of quantitating uptake characteristics in subsequent studies.

To determine whether the absorption of TcO_4^- is metabolically mediated, plants were allowed to accumulate Tc in the presence of several metabolic inhibitors (Table I). In each case, absorption was depressed, and ranged from 21% of control rates for azide and succinate to 7% for carbonylcyanide-*p*-trifluoromethoxy-phenylhydrazine. The transfer of Tc from root to shoot tissues was similarly inhibited and ranged from 43 to 15% of control rates. This would indicate that energy requirements exist for Tc absorption as with nutrient elements.

The absorption of TcO_4^- by intact seedlings, from solutions containing 0.02 to 10 μM Tc, yielded multiphasic absorption isotherms (Fig. 3) characteristically seen for nutrient ions in higher plants. The absorption isotherms for both the intact plant and for transfer from roots to shoot showed discontinuous transitions, with similarities in saturation kinetics. The fraction of Tc transported to shoot tissues over the 2-h treatment period ranged from 24 to 28% of the total absorbed over the concentra-

tions employed. This is a substantially higher rate of transfer from root to shoot than the 3 to 9% reported for Ni (5) and ~1% for Cd (7), under similar experimental conditions. It should be noted that, in these studies, a reduction in growth and toxicity symptoms appeared following 24 h of root absorption at concentrations in excess of 10 μM. This toxicity response is similar to that reported for plants grown on soils amended with TcO₄⁻ (18).

Kinetic constants were calculated for each of the isotherms observed for intact plants and for transfer from root to shoot (Table II). For the intact plant, values of *K_s* for the three isotherms were 0.09, 8.9, and 54 μM, with *V_{max}* values increasing from 2.9 to 830 μg Tc g root dry weight 2h⁻¹. These kinetic constants are within the range reported by Vange *et al.* (17) for sulfate and phosphate using excised roots, and Bowen (3) for borate absorption by excised sugarcane leaves. Kinetic constants for transfer from root to shoot were based on TcO₄⁻ concentrations in root bathing solutions, and not its concentration in cytoplasm of the stelar parenchyma. However, even with this limitation, isotherms 2 and 3 conform to Michaelis-Menten kinetics and suggest an

active metabolic transfer between root and shoot which is concentration dependent. Data for isotherm 1 did not exhibit saturation behavior, possibly due to variability in ⁹⁹Tc quantitation at these levels.

Since the absorption behavior of TcO₄⁻ is consistent with that observed for nutrient and non-nutrient ions, its absorption by plants may, in fact, result from its behavior as a nutrient analog. To determine whether TcO₄⁻ shares absorption processes with other anions, absorption was measured in the absence and presence of a 4-fold excess of sulfate, phosphate, molybdate, selenate, borate, tungstate, orthovanadate, permanganate, perchlorate, and iodate. Under the conditions of these short term studies, and pH 6.8, the species present should be SO₄²⁻, H₂PO₄⁻/HPO₄²⁻, MoO₄²⁻, HSeO₃⁻/SeO₃²⁻/SeO₄²⁻, H₃BO₃, NO₃⁻, WO₄²⁻, H₂VO₄⁻, MnO₄⁻, ReO₄⁻, and IO₃⁻. Similarly, technetium should be as TcO₄⁻ (pK HTcO₄ is ~0). Borate, nitrate, tungstate, iodate, perchlorate, and vanadate had no significant effect on TcO₄⁻ uptake from 0.25 μM solutions. It should be noted that perchlorate (ReO₄⁻), is often used to predict the long term geological behavior of TcO₄⁻ since they have chemical and physical similarities (10, 13). In the present studies, perchlorate supplied in 4- and 30-fold excess failed to reduce TcO₄⁻ absorption. This provides a good example of differences between chemical and biological specificity in that chemically similar species such as TcO₄⁻, permanganate, and perchlorate are not necessarily biological analogs. Absorption of TcO₄⁻ was significantly (P < 0.05) reduced by sulfate, selenate, molybdate, phosphate, and permanganate (Table III). Absorption of TcO₄⁻ was inhibited by 27 to 43% in the presence of the nutrient ions sulfate, molybdate, and phosphate. Selenate, a non-nutrient analog of sulfate (17), reduced TcO₄⁻ uptake by 20%. Permanganate, although not normally found in this form in the environment and not considered as the biologically available form of Mn, has chemical similarities to TcO₄⁻ (13) and dramatically reduced Tc absorption by 68%.

The interaction of these anions with TcO₄⁻ absorption was further resolved by evaluation of kinetic constants associated with TcO₄⁻ absorption using double reciprocal plots over a concentration range of 0.25 to 1.3 μM (isotherm 2) in the absence and presence of 10 μM of individual competing anions. Interpretations of anion interactions with respect to absorption were based on parameters describing their respective saturation kinetics, namely slope and intercepts (8, 14). Based on graphic representations and computed constants for absorption in the absence and presence of competing anions, sulfate, selenate, molybdate, and phosphate appear to be competitive analogs (common *V_{max}*, increased *K_s*) in the absorption of TcO₄⁻ by plant roots; calculated *K_i* values were (7, 160, 17, and 14 μM, respectively). Permanganate exhibits characteristics of a non-competitive inhibitor (reduced *V_{max}*, common *K_s*). The latter interaction may

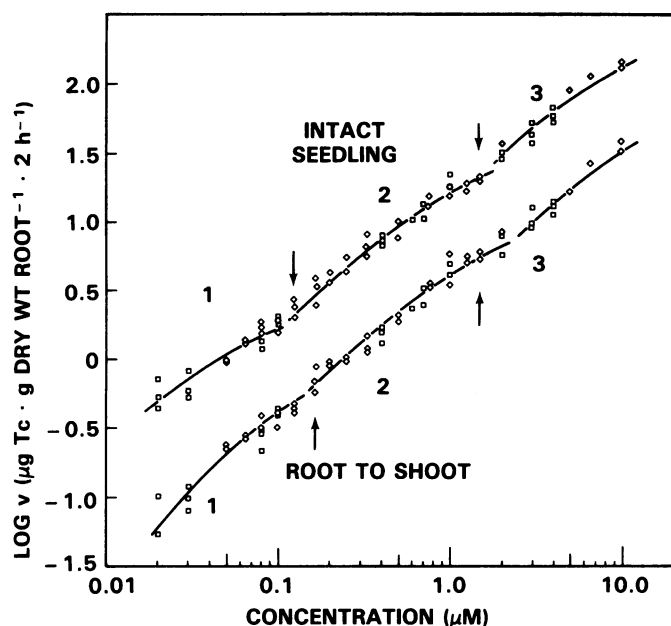


FIG. 3. Concentration-dependent absorption of ⁹⁹TcO₄⁻ by seedlings and transfer from root to shoot. Results are for two separate batches of plants. Arrows indicate concentration limits for isotherm 2 used in subsequent experiments.

Table II. Calculated Kinetic Constants for Absorption of TcO₄⁻ by Intact Soybean Seedlings and for Transfer from Root to Shoot Tissues

Isotherm	Kinetic Parameters ^a			Coefficient of Determination
	Concn. μM	<i>K_s</i> μg Tc g ⁻¹ root dry wt 2 h ⁻¹	<i>V_{max}</i>	
Intact plant				
1	0.02–0.10	0.094	2.9	0.78
2	0.125–1.5	8.9	160	0.93
3	1.5–10	54	830	0.89
Root to shoot				
1	0.02–0.125	ND ^b	ND	ND
2	0.167–1.5	7.1	31	0.92
3	1.5–10	77	270	0.91

^a *K_s*, *V_{max}*, and *r*² determined from double reciprocal plots.

^b Not determined.

Table III. Influence of Nutrient and Non-Nutrient Anions on TcO₄⁻ Absorption by Soybean Seedlings

Treatment ^a	Whole Plant Accumulation of Technetium	
	% of control ^b	
TcO ₄ ⁻ (control)	100 ± 6	(15)
TcO ₄ ⁻ + sulfate	73 ± 1	(3)
TcO ₄ ⁻ + selenate	80 ± 5	(3)
TcO ₄ ⁻ + molybdate	57 ± 20	(3)
TcO ₄ ⁻ + phosphate	61 ± 5	(3)
TcO ₄ ⁻ + permanganate	32 ± 7	(3)

^a Solutions contained 0.25 μM TcO₄⁻ in the absence and presence of 1.0 μM co-anion.

^b Control absorption rate was 2.68 μg Tc g⁻¹ root dry wt 2 h⁻¹; values are mean ± SD.

result from the behavior of permanganate as an oxidant and its effect on the absorption process.

CONCLUSIONS

The present investigation shows TcO_4^- absorption by intact, hydroponically grown plants to exhibit characteristics of carrier-mediated transport commonly associated with the absorption of nutrient ions in higher plants. These data suggest that the high CR values reported for transfer of TcO_4^- from soil to plant result from the efficient absorption of TcO_4^- by mechanisms already in place for essential nutrient elements. Kinetic analyses of TcO_4^- absorption in the absence and presence of individual nutrient anions show TcO_4^- absorption to be competitively inhibited by sulfate, phosphate, selenate, and molybdate indicating use of common transport mechanisms. The effective use of carrier mechanisms in place for nutrient anions provides a basis for understanding the environmental behavior of Tc and suggests the use of competing anions to ameliorate the effects of localized Tc contamination situations.

LITERATURE CITED

- ANDERS E 1960 Radiochemistry of Technetium. United States Atomic Energy Commission Report, NAS-NS-3021
- BERLYN GP, SS DHILLON, EE KOSLOW 1980 Technetium: a toxic waste product of the nuclear fuel cycle. Effects on soybean growth and development. *Environ Manag* 4: 149-156
- BOWEN JE 1968 Borate absorption in excised sugarcane leaves. *Plant Cell Physiol* 9: 467-478
- CATALDO DA, RE WILDUNG 1978 Soil and plant factors influencing the accumulation of heavy metals by plants. *Environ Health Pers* 27: 149-159
- CATALDO DA, TR GARLAND, RE WILDUNG 1978 Nickel in plants. I. Uptake kinetics using intact soybean seedlings. *Plant Physiol* 62: 563-565
- CATALDO DA, RE WILDUNG, TR GARLAND 1978 Technetium accumulation, fate, and behavior in plants. In DC Adriano, TL Brisbin, Jr, ed, *Environmental Chemistry and Cycling Processes*. Department of Energy Symposium Series No. 45, CONF-760429. National Technical Information Service, Springfield, VA
- CATALDO DA, TR GARLAND, RE WILDUNG 1983 Cadmium uptake kinetics in intact soybean plants. *Plant Physiol* 73: 844-848
- EPSTEIN E, CE HAGEN 1952 A kinetic study of the absorption of alkali cations by barley roots. *Plant Physiol* 27: 457-74
- GEARING P, C VAN BAALEN, PL PARKER 1975 Biochemical effects of technetium-99-perchnetate on microorganisms. *Plant Physiol* 55:240-246
- KORKISCH J 1969 *Modern Methods for Separation of Rarer Metal Ions*. Pergamon Press, New York, pp 504-523
- LANDA ER, LJ THORVIG, RG GAST 1977 Effect of selective dissolution, electrolytes, aeration and sterilization on technetium-99 sorption by soils. *J Environ Qual* 6: 181-187
- LANDA ER, LJ HART, RG GAST 1977 The uptake and distribution of Tc-99 in higher plants. In H Drucker, RE Wildung, eds, *Biological Implications of Metals in the Environment*. USERDA Symposium Series, CONF-750959, National Technical Information Service, Springfield VA, pp 390-401
- MCFADDEN KM 1980 *The Chemistry of Technetium in the Environment*. NTIS, PNL-2579. Pacific Northwest Laboratory, Richland, WA
- PLOWMAN KM 1972 *Enzyme Kinetics*. McGraw-Hill, New York, pp 56-73
- POURBAIX M 1966 *Atlas of Electrochemical Equilibria in Aqueous Solution*. Pergamon Press, New York
- ROUTSON RC, DA CATALDO 1978 Accumulation of ^{99}Tc by tumbleweed and cheatgrass grown on arid soils. *Health Physics* 34:685-690
- VANGE MS, K HOLMERN, P NISSEN 1974 Multiphasic uptake of sulfate by barley roots. I. Effects of analogues, phosphate, and pH. *Physiol Plant* 31: 292-301
- WILDUNG RE, TR GARLAND, DA CATALDO 1977 Accumulation of technetium by plants. *Health Physics* 32: 314-317
- WILDUNG RE, KM MCFADDEN, TR GARLAND 1979 Technetium sources and behavior in the environment. *J Environ Qual* 8: 156-161