Radioprotection, vol. **44**, n° 5 (2009) 65–69

DOI: 10.1051/radiopro/20095018

Accumulation and distribution of ^{241}Am in the biomass of freshwater plant *Elodea canadensis*

T.A. Zotina, G.S. Kalachova and A.Ya. Bolsunovsky

Institute of Biophysics SB RAS, Akademgorodok, 660036 Krasnoyarsk, Russia
e-mail: t_zotina@ibp.ru

Abstract. Accumulation of transuranic element ^{241}Am by photoassimilating organs and its distribution in fractions of the biomass of aquatic plant *Elodea canadensis* have been investigated in laboratory batch experiments. Americium was taken up by apical shoots of *Elodea* from water. Of the activity accumulated by the shoots, up to 80% of ^{241}Am was detected in the leaves and 20% in the stems. Americium concentration in apical leaves was several times higher than in distal leaves. Of the ^{241}Am activity concentrated in the shoots, 95% was bound to cell walls, membranes and organelles and 5% of the radionuclide was dissolved in cytoplasm. Less than 1% of americium accumulated in the biomass was found in the lipid extract; up to 10% of americium was bound to proteins and carbohydrates; the major part of the radionuclide (about 90%) was registered in the cell wall fraction that mainly contained cellulose-like polysaccharides. Biomass treatment with hydrochloric acid resulted in desorption of about 70% of americium, mostly from the polysaccharides.

1. INTRODUCTION

Plants are an important link in migration of artificial radionuclides in the aquatic environment. Artificial radionuclides, similarly to stable metals, are distributed in plant biomass non-uniformly [1–4]. Radionuclide localization in the biomass determines the dose to a specific cellular structure and migration of isotopes in an aquatic ecosystem in general and along the food chain in particular [5].

The transuranium element ^{241}Am is one of the most radiotoxic long-lived artificial radionuclides. This radionuclide is detected in bottom sediments and biomass of aquatic plants in the radioactively contaminated part of the Yenisei River [6]. Our laboratory experiments showed that *Elodea canadensis*, one of the most frequently occurring submerged macrophytes in the Yenisei River [7], can accumulate high ^{241}Am activity in its biomass [8] from water.

The purpose of this study was to estimate the distribution of ^{241}Am among photoassimilating organs of *E.canadensis*, among cell compartments, and fractions of lipids, proteins and polysaccharides.

2. MATERIALS AND METHODS

The apical shoots: long (15–20-cm-long) and short (3-cm-long) of *Elodea* were used for laboratory experiments. The shoots were maintained in filtered (0.2 μm RC-membranes, Schleicher & Schuell) Yenisei River water in the presence of ^{241}Am (added as $^{241}Am(NO_3)_3$) at pH = 7. The initial activity of ^{241}Am was 1300–1400 Bq/L. The cylinders were illuminated for 14 h per day (2 klx on the surface). Water temperature was 19 °C.

To estimate the distribution of americium between different parts of a shoot, each long shoot was divided into several parts based on its morphology and function: the stem, the apical leaves (2 cm from the apex top), and the distal leaves. The apical leaves were of gradually varying sizes, while distal leaves were of equal sizes.

The experiments with short apical shoots were performed to investigate the distribution of americium in the biomass of elodea. The shoots were sampled from water after being maintained for 4 and 5 days in the presence of americium. The dry biomass of one shoot was 0.017 ± 0.001 mg.

Before the extraction the short shoots were washed with distilled water at pH 7 for about 30 sec, and dried at 80 °C. Lipids were extracted from finely ground samples with a mixture of isopropyl alcohol and trichloromethane (1:1 v/v) [9]. The remaining biomass was treated with 1M NaOH for 1 h in boiling water bath to extract proteins. The extracts were separated by filtration through glass fibre filters (GF6, Schleicher&Schuell).

To determine the dissolved intracellular portion of ^{241}Am, the washed fresh short shoots were finely homogenized in a glass cylinder homogenizer with a glass pestle in the presence of a tiny amount of distilled water, pH = 7. The homogenized biomass was separated by filtering through membranes (0.2 μm, RC, S&S).

The desorption of ^{241}Am from the washed fresh biomass was done with a 1 M HCl solution (by soaking for 15 min). After the treatment the shoots were rinsed with distilled water and dried. The extractions were performed as described above.

The ashing of biomass was done at 500 °C for 2 h.

To measure americium activity in samples of biomass, the samples were dissolved in a mixture of H_2O_2 (30%) and HNO_3 while heating.

^{241}Am activity was measured in samples using a hyper-pure-Ge-gamma-spectrometer (Canberra, USA). The concentration of protein in the samples was calculated as total Kjeldahl nitrogen × 6.25. The protein content in liquid samples was also determined according to Lowry et al. [10]. The carbohydrate content in samples was determined with the anthron assay [11].

To determine the surface of plant leaves and steams, the size parameters of 17 long apical shoots were measured. The size of each third leaf was measured on every shoot.

3. RESULTS AND DISCUSSION

3.1 Distribution of ^{241}Am between the leaves and the stem

The accumulation of ^{241}Am by the biomass of *Elodea* from water in batch experiments was represented by a typical saturation curve [8]. The uptake of ^{241}Am by *Elodea* from water stopped after 2 days of exposure.

The activity of ^{241}Am in an entire long apical shoot of *Elodea* in equilibrium was about 3 kBq/g of dry biomass. Up to 80% of ^{241}Am was detected in leaves and 20% in stems (Table 1). The biomass of leaves comprised 67% of the biomass of a shoot. Activity concentration of americium per gram of biomass of leaves was twice higher than that determined for the stem, while there was no difference in activity per unit area of the leaves and the stem.

Activity concentration of ^{241}Am depended on the maturity of leaves. Activity of americium in distal (mature) leaves was 3.5 and 3.3 times higher than its activity in young apical leaves per unit of biomass and unit of surface, respectively. The distribution of americium among parts of the shoot did not change with time (Fig. 1) within 8 days of experiment. New apices, which were formed on the long shoots during the experiment, did not accumulate any essential amounts of americium (Fig. 1).

Table 1. Distribution of ^{241}Am among different parts of *E. canadensis* (mean ± SD, n = 4) after one day of exposure.

Part of the shoot	^{241}Am content		Am the biomass	^{241}Am per unit area
	Bq	% of total	kBq/g	Bq/cm^2
Apical leaves	8.7 ± 0.7	10.3 ± 1.2	1.5 ± 0.4	0.8 ± 0.1
Distal leaves	58.7 ± 3.6	69.5 ± 2.9	5.2 ± 1.1	2.6 ± 0.6
Stem	17.1 ± 2.5	20.2 ± 2.9	2.0 ± 0.4	2.3 ± 0.4
All leaves on the stem	67.4 ± 3.3	79.8 ± 2.9	4.0 ± 0.9	2.0 ± 0.3
Entire shoot	84.5 ± 2.7	100	3.3 ± 0.7	2.0 ± 0.3

Microscopic examination demonstrated that distal leaves contained much more epiphytic organisms (mainly diatoms) and mineral incrustations (mainly calcium carbonate) than apical leaves. Our previous experiments showed that freshwater diatoms can absorb ^{241}Am from the water of the Yenisei River. ^{241}Am activity concentration in biomass of diatoms (7–20 kBq/g of dry mass) is up to an order of magnitude higher than its concentration in plant biomass [12]. Epiphytes can produce extracellular organic compounds [13], and, thus, may enhance biosorption of the radionuclide on the surface of *Elodea* as well.

Hence, organic-mineral incrustations precipitated on the surface of the leaves considerably enhance the ^{241}Am biosorption capacity of *Elodea* biomass.

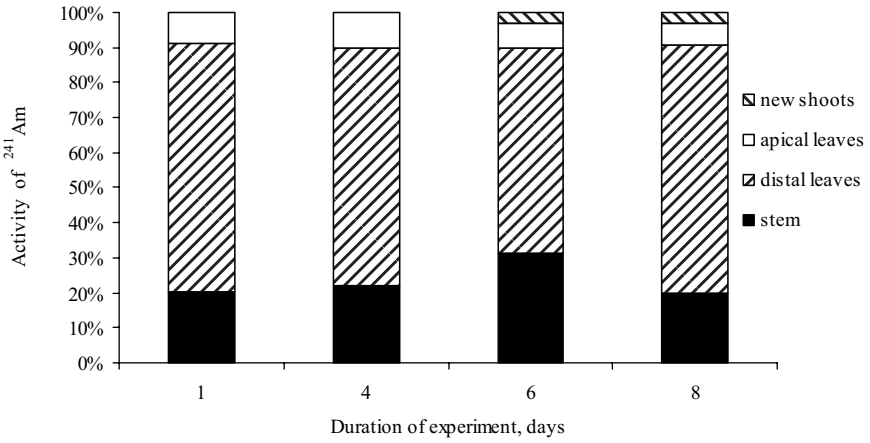

Figure 1. Distribution of ^{241}Am between parts of *Elodea* shoots (% of total activity in the entire shoot) after 1, 4, 6 and 8 days of exposure. Mean value of two replicates.

3.2 Distribution of ^{241}Am among cell compartments

Microscopy showed that homogenization of *Elodea* biomass destroyed both the cells and the majority of chloroplasts. The major part ($94.8 \pm 1.2\%$) of americium was bound in particles of homogenated *Elodea* biomass larger than 0.2 μm, i.e. cell walls, membranes and organelles. About 5% of ^{241}Am was registered in the filtrate, which contained particles smaller than 0.2 μm, i.e. cytozol and dissolved macromolecules.

Similar results were reported for marine microalgae [1, 5]. Up to 7% of ^{241}Am was registered in cytoplasm, and the major part of the radionuclide was bound to cell walls and plasmalemma.

^{241}Am, as well as plutonium, has a higher affinity to oxygen than to nitrogen or sulfur, therefore, penetration of ^{241}Am into cytoplasm is less effective than that of, e.g., Cd and Zn [5].

Studies addressing assimilation of a number of radionuclides by crustaceans from microalgae showed that the larger amounts of the element are dissolved in cytoplasm, the more effectively it is transferred along the trophic chain. Since just small amounts of ^{241}Am penetrated into the cytoplasm of microalgae, the efficiency of ^{241}Am assimilation by copepods was low (0.9%) [5]. Some marine bivalves, however, assimilated up to 40% of ^{241}Am from microalgal biomass [14].

3.3 Distribution of ^{241}Am among biomass fractions

The extracellular unbound (distilled water removable) fraction of ^{241}Am in the biomass of short apical shoots of *Elodea* made up $6.3 \pm 1.5\%$. Hence, 94% of ^{241}Am was in some way bound in the biomass. The activity concentration of bound americium was 0.97 ± 0.19 kBq/g of dry biomass. The bound

americium was distributed among biomass extracts as follows. The lipid fraction contained less than 1% americium, the alkali extract of protein – 9%, and the biomass residue – 90% (Fig. 2). The alkali extract contained not only protein but also the major part of carbohydrates. The remaining fraction (biomass residue) was considered to be mainly cellulose-like polysaccharides. That fraction also contained 5% cellular protein and 11% carbohydrates (Table 2).

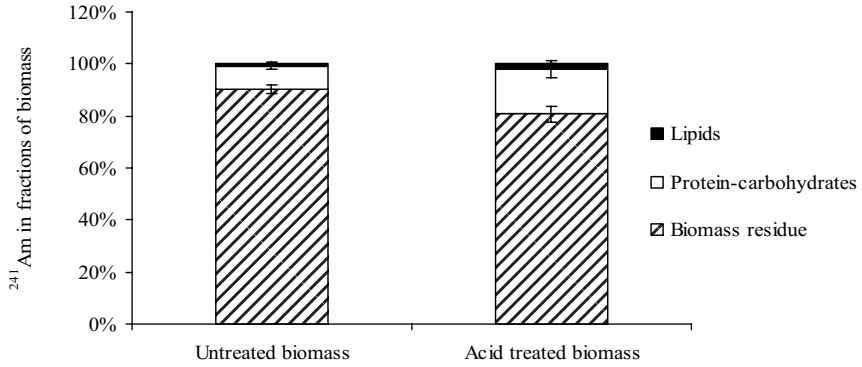

Figure 2. Distribution of ^{241}Am among fractions of *Elodea* biomass (% of total activity): untreated and acid treated before extraction. Mean value of three replicates, sd.

Hence, of the molecules constituting *Elodea* biomass, cellulose-like polysaccharides are the principal concentrators of ^{241}Am. Other authors reported that accumulation of such artificial isotopes as ^{137}Cs, ^{65}Zn, ^{60}Co and ^{51}Cr in biomass of *Elodea* and some other aquatic plants occurs mainly in sugars and lipids [3, 4].

The biosorption of americium by the cell wall is independent of metabolism, as it does not require energy expenditures. It has been shown recently that biosorption of ^{241}Am by *Saccharomyces* can occur due to nonspecific adsorption on cell wall, ion exchange and complexation [15] simultaneously.

Table 2. Composition of total biomass and biomass fractions (%).

Component	Total biomass	Fractions of biomass		
		Extract of lipids	Extract of protein	Biomass residue
Lipids	3.6 ± 0.5	100	-	-
Protein (N_{total} ×6.25)	30.0 ± 3.5	-	95	5 ± 2
Carbohydrates	6.1	-	89 ± 13	11
Cellulose-like polysaccharides	19.2 ± 4.0	-	-	100
Ash	15.6 ± 0.1	-	-	-

It has been experimentally shown before that the major part of americium registered in the biomass of *Elodea* was present in the acid removable form [8].

In this experiment the acid removable fraction of americium made up 72 ± 4% of the activity bound in biomass. The treatment with acid removed 20% of plant mass as well. About 0.2% of lipids, 24% of protein, 17% of carbohydrates were removed from plant biomass by the acid.

The remaining activity concentration of americium in the acid treated biomass was 0.293 ± 0.033 kBq/g of dry mass. Americium was distributed in the fractions of the biomass similarly to its distribution in untreated biomass (Fig. 2). That is, 2% of americium was registered in the extract of lipids and 17% – in the extract of protein. The major portion of americium was registered in the biomass residue – cellulose-like polysaccharides of cell walls – 81%.

The calculations showed that after desorption with acid, americium activity in the lipid fraction decreased by 19%, in the protein-carbohydrates fraction – 42% and in the biomass residue – 73%. Hence, americium was mainly desorbed from polysaccharides of cell walls.

The acid treatment might be considered as a model of biomass processing in the digestive system of plant grazing fauna.

4. CONCLUSIONS

Americium activity taken up from the water by *Elodea* apical shoots was non-uniformly distributed between the leaves and the steam. The major activity of americium (80%) was concentrated in leaves.

Organic-mineral incrustations and epiphytic organisms accumulated on the surface of *Elodea* leaves considerably increased americium adsorption.

^{241}Am taken up by *Elodea*'s photoassimilating organs from the water was mainly adsorbed by cell walls, plasmalemma and organelles. A small portion of americium (about 5%) could be dissolved in cytoplasm.

Of the molecules that constitute *Elodea* biomass, americium had the highest affinity to cellulose-like polysaccharides.

The treatment of biomass with acid led to desorption of 70% americium accumulated in biomass, mostly from the polysaccharides of cell walls.

The data obtained can provide the basis for predicting further migration of ^{241}Am in the ecosystem, including its transfer along the trophic chain.

Acknowledgments

The research was supported by the grant of the President of Russian Federation No. MK-5961.2006.4; RFBR-KSF Grant No. 07-04-96813, and the Russian Science Support Foundation.

The authors are grateful to L.G. Bondareva (IBP SB RAS, Krasnoyarsk) for her help in experiments.

References

[1] Fisher N.S., Burns K.A., Cherry R.D., Heyraud M., *Mar. Ecol. Prog. Ser*. **11** (1983) 233-237.
[2] Vazquez M.D., Lopez J., Carballeira A., *Ecotox. Environ. Safe*. **44** (1999) 12-24.
[3] Cecal A., Popa K., Caraus I., Potoroaca V., *Isotopes Environ. Health Stud*. **38** (2002) 33-37.
[4] Cecal A., Popa K., Potoroaca V., Melniciuc-Puica N., *J. Radioanal. Nucl. Ch.* **251** (2002) 257-261.
[5] Reinfelder J.R. and Fisher N.S., *Science* **251** (1991) 794-796.
[6] Bolsunovsky A. and Bondareva L., *J. Alloy. Compd*. **444-445** (2007) 495-499.
[7] Zotina T.A., *J of Siberian federal university. Biology* **1** (2008) 102-108.
[8] Bolsunovsky A., Zotina T., Bondareva L., *J. Environ. Radioactivity* **81** (2005) 33-46.
[9] Novitskaya G.V. and Rutskaya L.A., *Phisiologia Rastenii (Plant physiology)* **23** (1976) 889-905.
[10] Lowry O.M., Rosenbrough W.J., Farr A.L., Randall R.J., *J. Biol. Chem*. **193** (1951) 265-275.
[11] Dreywood R., *Ind. Eng. Chem*. **18** (1946) 499-504.
[12] Zotina T.A., Bolsunovsky A.Ya., Bondareva L.G., *Radiobiologia. Radioekologia. (Radiobiology. Radioecology)* **48** (2008) 131-136.
[13] Chappel K.R. and Goulder R., *Arch. Hydrobiol.* **132** (1994) 237-253.
[14] Reinfelder J.R., Wang W.X., Luoma S.N., Fisher N.S., *Mar. Biol*. **129** (1997) 443-452.
[15] Liu N., Liao J., Yang Y., Luo S., Luo Q., An Z., Duan Y., Liu M., Zhao P., *J. Radioanal. Nucl. Ch.* **275** (2008) 173-180.