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Environmental Aspects of Radiopharmaceuticals: Extraction and Translocation of Ra-223 in Plants

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Abstract*— Radiopharmaceuticals represent an attractive and efficient treatment of oncological diseases. Medical radionuclide use might bring a particular safety issue with penetration of a radioactive material into environment via urinal and colonal excretion. Therefore, the waste water cleaning and decontamination of food chain ought to be studied. Radium-223 is FDA and EMA approved therapeutic radionuclide for the treatment of bone metastases originating from castration resistant prostate cancer. Its introduction to clinical praxis opened the possibility of Radium retention and translocation into roots and shoot plant parts in the ecosystem. Though ^{223}Ra uptake was investigated *in vitro* on cultivated plants *Avena sativa* and *Zea mays* using electronic autoradiography. Stimulators (Atonik®, Racine®, Rexan®, Sunagreen®, Stimulator Z®) increasing the water transport, the plant stress management additives (Vermaktiv Stimul® and Vermaktiv RP®), together with the chelating agent ethylenediaminetetraacetic acid (EDTA) were added to the cultivars. Results of plants growth without any regulators indicate over 90% uptake of ^{223}Ra in root system with minimal translocation into other parts. An addition of growth regulators decreased the overall uptake, however significantly increased Radium translocation into shoot parts. Surprisingly, an addition of EDTA decreases the overall retention in oat under the lowest detectable limit, nevertheless an increased translocation to shoot parts was observed. Experiments reveal potential of phytoextraction technologies for waste water cleaning in hospitals, on the other hand, indicates possibility of food chain contamination particularly when growth regulators were used.

***Keywords*—uptake, radium-223, radiopharmaceuticals. environment contamination**

I. Introduction

Radiopharmaceuticals represent modern and attractive tool in diagnostics and therapy. Mostly, they are used in oncology, however their use in cardiology, neurology and others is also widespread. In a lot of cases radiopharmaceuticals allow to prolong patient survival or at least keep the quality of life on an acceptable level. In the past few years targeted alpha therapy became very efficient tool together with stereotactic irradiation of tumor tissues with targeted short-range charged particles with higher linear energy transfer (LET). Several results indicate the effectivity of this synergism and significant therapeutical potential [1]. Besides ^{212}Bi, ^{213}Bi, ^{211}At also ^{223}Ra (half-life of 11.4 days) became promising therapeutical radionuclide, desired primary and also US Food and Drug Administration and European Medicines Agency approved to palliative treatment of bone metastases originating from castration resistant prostate cancer [2, 3] and several bio-nanocariers for targeted alpha therapy is being studied [4, 5]. Notwithstanding effective precautions which ought to prevent undesired or accidental release of the radioactivity to the ecosystem from residual and contaminated materials, this situation may occur. Therefore, the systematic study of new medical isotopes translocation together with its relation and impact to environment and possibility of food chain contamination is needed. One of the possibilities of waste water cleaning is a technology based on a phytoremediation which offer also an opportunity to evaluate a contribution of eventual food chain contamination. Phytoremediation of metals and heavy metals or radioactive elements has been theoretically studied and its efficiency and applicability was already proved [6-8].

The aim of this work was to prove the retention of cationic radium-223 by roots as a phytoextraction technology model and to perform a translocation study of $^{223}Ra^{2+}$ into shoot plant parts as a marker of eventual food chain contamination. Regarding very often application of growth regulators, stimulators and stress management agents, an influence of these modulators was also investigated in relation to the retention and translocation of $^{223}Ra^{2+}$. The application of Atonik®, Racine®, Sunagreen®, Rexan®, Stimulator Z® and Vermaktiv® might increase the translocation and subsequently increase the contamination of plant upper parts. The influence of ethylenediaminetetraacetic acid increasing retention of heavy metal elements was also studied.

II. Experimental

A. *Materials and Methods*

In vitro cultures of plants *Zea mays* cv. DKC 4014 (Monsanto CZ) and *Avena sativa* cv. Cat Grass (Johnson seeds UK) were initiated from sterilized seeds and cultivated in Murashige-Skoog basal salt mixture [9] without hormones and sugar under sterile conditions. Cultivations were carried out at 24 °C

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and lighting with fluorescence tubes (40 W/m^2, 40 cm distance, Philips Cool White), 16 hours per day.

Solution of ^{223}Ra nitrate (approx. 50 kBq in 1 mL) was eluted from ^{227}Ac/^{227}Th/^{223}Ra generator [10, 11]. Actinium-227 in a secular equilibrium with its decay products was loaded on the column filled with 0.5 g of Dowex-18 anion exchanger utilized as a generator of ^{223}Ra. Aqueous methanol-nitric acid solution (0.7 M HNO_3 in 80 % methanol) was used as an eluent for the separation of ^{223}Ra from the ^{227}Ac/^{227}Th parents. Eluate was evaporated under vacuum and $^{223}Ra(NO_3)_2$ was reconstituted in water. Radioactivity was measured by Berthold LB 122 and well-type NaI(Tl) coulter (Capintec, USA). Translocation experiments were evaluated using Instant Imager electronic autoradiography (Packard) with the mixture of argon-methane-isobutane counting gas (Linde Gas). Samples were measured for 1 hour.

Plant growth regulators Atonic®, Racine® (Asahi chemical MFG, Co. Ltd, Japan) and Stimulator Z®, Sunagreen®, Rexane® (Biosphor s.r.o., Czech Republic), were prepared directly before use from commercially available chemicals (Sigma Aldrich) according to published compositions [12]. Vermaktiv Stimul® and Vermaktiv RP® were obtained from Enzymix s.r.o., Czech Republic. Etylenediaminetetraacetic acid (EDTA) was purchased from Lach-Ner, Czech Republic.

Plants were cultivated under above mentioned conditions during three weeks and afterwards cultivation media were replaced with sterilized water, 50 mL per cultivation. Solution of reconstituted $^{223}Ra(NO_3)_2$ (50 kBq) and a corresponding stimulator (together with blank without stimulator) was added to each plant in overall concentration 100 μL/L. Plants were cultivated for one week then were taken-off from the cultivation containers, washed with distilled water and dried for three days at laboratory temperature. The translocation in plants was evaluated by electronic autoradiography. Residual activity in media was measured on a well-type detector with time correction on decay. Each experiment was performed with 4-5 plants to reach conformable weight of cultivated material.

B. ***Results and Discussion***

Study of the environmental risk of residual radionuclides excreted by patients after hospitalization represent peremptory issue due to utilized radionuclides and its daughter products released in the environment. Retention in plants and especially translocation into shoot parts represents the risk how contaminants might enter to the food chain. On the other hand, suitable plant phytoextraction might be utilized as a cheap and technologically unassuming process for effective radionuclide extraction from the water after wastewater treatment with conventional technologies. Although strict rules existing in the risk assessment in hospital manipulating with radioactive waste, random contamination of waste water on outflow cannot be excluded and additive phytoextraction unit might be useful tool against random and unwanted ecosystem contamination.

Therefore, the cultivation experiments were evaluated regarding radioactivity retention degree as well as ratios between root and shoot parts. These data, provide an important information about the contamination degree of food chains in nature as well as in the human environment. An example of electronic autoradiography result is shown on the Figure 1.

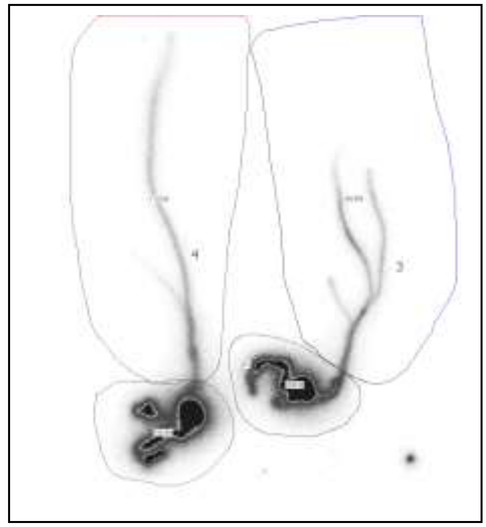

Figure 1 Electronic autoradiogram of *Avena sativa* cultivated in water containing ^{223}Ra (50 kBq) and Stimulator Z® (100 μL/L) for one week.

Experiments were carried-out with cationic ^{223}Ra and an addition of standard growth stimulators to increase the translocation of water and root system activation as well as the biomass growth was tested. Also stress reducing compounds were applied to the cultivated plants. Regarding the phytoextraction efficiency, there is a direct relation with translocated and transpirated water amount and thus increased phytoextraction effectivity might be expected. Ethylenediaminetetraacetic acid, a chelating agent, was also studied as an additive utilized to increase the phytoextraction of heavy elements. The results of *Avena sativa* phytoextraction experiments are listed in Table I.

TABLE I. PHYTOEXTRACTION OF $^{223}Ra^{2+}$ WITH *AVENA SATIVA IN VITRO* CULTIVATED PLANTS.

Additive	**Uptake**		
	% in roots[a]	*% in shoots*[b]	*% Total* [c]
Blank	94	6	84
Atonic®	86	14	20
EDTA	75	25	<1
Racine®	88	12	51
Rexan®	82	18	29
Stimulator Z®	82	18	31
Sunagreen®	96	4	83
Vermaktiv®	77	23	59
Vermaktiv RP®	89	11	41

a,b - per cent of activities in root and shoot parts according autoradiography counting;

c - per cent uptake from initial activity

Plants revealed relatively high value of $^{223}Ra^{2+}$ uptake, while over 90 % of radioactivity remained absorbed/adsorbed in roots. Stimulators addition usually increasing plant water transport, surprisingly decreased the overall retention, nevertheless significant translocation increase was observed in shoot parts. Vermaktiv® antistress stimulator decreases an overall retention of radioactive ions, however increased translocation was observed.

Maize phytoextraction experiments results are listed in Table II. In primary experiment without any additives, the overall uptake of 75 % radioactivity was observed, with predominant uptake in roots. Further trends are in general comparable to *Avena sativa* results, while the uptake decrease with EDTA addition was not so extensive.

TABLE II. PHYTOEXTRACTION OF 223-RA IONS WITH *ZEA MAYS IN VITRO* CULTIVATED PLANTS.

Additive	Uptake		
	% in roots[a]	*% in shoots[b]*	*% Total [c]*
Blank	93	7	75
Atonic®	86	14	41
EDTA	79	21	24
Racine®	74	26	69
Rexan®	81	19	41
Stimulator Z®	74	26	57
Sunagreen®	86	14	48
Vermaktiv®	83	17	65
Vermaktiv RP®	85	15	71

a,b - per cent of activities in root and shoot parts according autoradiography counting

c - per cent uptake from starting activity

It is evident from our results that the ^{223}Ra ions are effectively trapped by the root systems of the studied plants. Some differences in the uptake are given by the variance of plant species, e.g. their morphology and different metabolic pathways. From the metabolic pathways point of view, the *Avena sativa* belongs to the *C3* plants, while the *Zea mays* belongs to *C4* plants.

The influence of the additives is based on the increased water transport, causing higher contaminants translocation. Furthermore, Radium as an analogue of Calcium has similar chemical properties, and may follow the Calcium metabolism and its natural uptake by the plants.

The decrease in the overall uptake is probably linked to possible formation of Radium-additive complexes, thus lowering its bioavailability for root uptake and transport. Such decrease was observed with also Vermaktiv® additive that is based on natural extracts containing humic and fulvic acids, certainly offering a variety of complexing functional groups.

III. Conclusion

The results of our experiments showed the possibility of low concentration ^{223}Ra retention by the plant root systems. Selected plants thus may be used for the decontamination of hospital waste waters. Since the Radium uptake efficiency is practically independent on the plant metabolic type (*C3* vs. *C4*), it would be possible to employ also other plant species with more distinctly developed root system, that are better cultivable in the environment of hydroponic root waste water treatment plants or constructed wetlands. Further, the results indicate the possibility of Radium translocation to the shoot parts of the plants, particularly when auxiliary agrochemical products are employed. Thus the potential risk of food chain contamination should be carefully evaluated. Particular regimes for the patients medicated with Radium radiopharmaceuticals should be proposed and proper regulations for handling, treatment and a disposal of radioactive waste waters should be required.

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