

STUDY OF Cs UPTAKE BY PLANTS UNDER CONDITIONS OF SEVERE POTASSIUM DEFICIENCY AND RADIOACTIVE STRESS

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Abstract

A possibility of a significant increase in the uptake of cesium by plants up to levels sufficient for effective phytoremediation was examined. We employed a novel method to enhance vital functions of plants under conditions of strong potassium deficiency. Moreover, methods of processing newly generated waste from the phytoremediation of contaminated soils were also studied. The efficiency of radiocesium uptake was assessed using microorganisms and bio-energy-activators added to soils. A significantly increased uptake of Cs by plants was observed when the available potassium content in the soils was as low as $5 \pm 1 \text{ mg kg}^{-1}$. This increased uptake practically vanished at levels of $25 \pm 5 \text{ mg kg}^{-1}$ depending on the type of plant. This study has proved that the radioactivity of the "phytoremediation waste" byproducts was lower than is given in acting regulations on permitted levels in many countries. Less than 10% of the harvested plant mass used for phytoremediation is expected to undergo special management as radioactive byproducts. Pretreatment of seeds before planting using the same bio-energy-activators and inoculates of microorganisms brought to significant increase of plants mass and, hence, to increase of uptake of cesium for about 100% compared to adding of preparations to soil. Adding bio-energy-activators and microorganisms-based fertilizers to soils and pretreatment of seeds before planting can become a highly efficient mean for rapid phytoremediation of ^{137}Cs contaminated soils.

Keywords: ^{137}Cs , phytoremediation, potassium deficiency, waste, bio-energy-activators

1. Introduction

Since the early 1950s, the problem of the transfer of ^{137}Cs and other hazardous radionuclides into the food chain has been extensively studied. Potassium fertilizers have been successfully used to prevent the uptake of Cs by plants following radioactive pollution of food [1, 2]. After that time, the possibility of remediating radioactive Cs-polluted soils using the uptake of radionuclides by plants ("phytoremediation") has been investigated and discussed by many researchers. Most of them [e.g., 3] made conclusions about the low efficiency or practical inefficiency of phytoremediation due to low Transfer Coefficients characterizing the transfer of ^{137}Cs from soil to plants. On the other hand, the phytoremediation of ^{137}Cs from LLNW (low-level nuclear waste) using *Catharanthus roseus* was demonstrated [4]. However the phytoremediation of Cs-contaminated soils has not been widely spread, mainly because of relatively low decontamination factors (an exorbitant amount of time required for decontamination) and the problem and costs of "secondary" radioactive waste treatment. We recently employed a new approach to the phytoremediation which is based on the effect of potassium deficiency on the uptake of cesium [e.g., 5]. It is to enhance vital functions, and then, accelerate plant metabolism under conditions of abiotic stresses, including a strong potassium deficiency ("potassium starvation") for soils contaminated with radiocesium. The potassium deficiency may cause a considerable decline in the yield of plants, which, in turn, prevents an increase in the total volume of cesium taken up from soils, despite the increase of the coefficients characterizing the transfer from soils to plants. Enhancing the vital functions (metabolism) of plants was examined in this study by means of the addition of plant hormones, bio-energy-activators and bacterial preparations. Our present method is a direct way of retaining the convenient yield of crops and achieving highly efficient phytoremediation under conditions of potassium deficiency.

2. Methods

2.1 Vegetation and soils

After screening plants for the selection of the objects of study and considering the results of previous studies [1-5], and a preliminary greenhouse study involving six months of growth in low contaminated soil ($\leq 100 \text{ Bq kg}^{-1}$), the three following plants showed a comparative simplicity in growth, a higher yield, and a higher average ^{137}Cs content in

roots and shoots and were chosen for the detailed study: sugar beet, sweet clover (white) and creeping red fescue. Yellow-brown forest soils from Western Georgia heavily contaminated by Cs after the Chernobyl incident ($900 - 9000 \text{ Bq kg}^{-1}$) with a very low potassium content ($\leq 5 \pm 1 \text{ mg kg}^{-1}$) were used for preparing model soils with three different levels of contamination (500 ± 50 , 1500 ± 100 and $4000 \pm 200 \text{ Bq kg}^{-1}$), and potassium chloride was added to obtain six different levels of available potassium content (5 ± 1 , 10 ± 1 , 15 ± 2 , 20 ± 2 , 25 ± 2 and $50 \pm 5 \text{ mg kg}^{-1}$). A soil with $50 \pm 5 \text{ mg kg}^{-1}$ of available potassium was also utilized in control experiments.

2.2 Vital function enhancer

To enhance the vital functions of plants, a combination of two organic preparations – Bacterial-organic meliorating fertilizer (BOMF) and Bio-energy-activator (“Biorag”) developed in Georgia (in proportion by weight – 100 parts of bacterial preparation to 1 part of bio-energy activator) – was added to the modeled soils in amounts of 1 ± 0.2 , 2 ± 0.4 and $5 \pm 1 \text{ g}$ in a combined preparation to 1 kg of soil. Bacterial-organic meliorating fertilizer (BOMF) was developed in Georgia and successfully tested through field-scale testing at the number of scientific institutions in Georgia and abroad. It has been registered by Georgian Ministry for Agriculture and Food and recommended for use in farming and the agriculture industry. The organic Bio-energy-activator “Biorag” is commercially produced and widely used in Georgia to accelerate vegetation, increase yield and enhance responses to abiotic stresses. Soils for the control experiment did not contain the combined preparation.

2.3 Cultivation and sample collection

The plants in the soils were cultivated using convenient agricultural measures and NP fertilizers. The plants and soils were sampled three times during the growth period and after the harvest and prepared for the analysis by a standard methodology [1]. The radionuclide content (in Bq kg^{-1}) was determined using a Canberra HpGe detector with an electrical chilling system (Electrically Refrigerated Cryostat – Cryo-Pulse5).

3. Pretreatment of seeds with BOMF and bio-energy-activator “Biorag”.

Pretreatment of seeds before planting is widely ubiquitous way to increase productivity and strengthen the resistivity of plants to biotic and abiotic stresses. Therefore, investigation of Cs uptake by plants utilizing pretreatment of seeds before planting (using BOMF inoculate and “Biorag” solution) was carried out both with and without adding the preparations to the soil. The results of the experimental research are represented in Tab 2.

4. Results and Discussions

4.1 Evaluation of remediation efficiency

To characterize the dependence of the remediation capacity of plants on the amount of the combined preparation in soils, a dimensionless factor $K = F/F_0$ was calculated, where $F = Y (\text{kg m}^{-2}) \times C (\text{Bq kg}^{-1})$ (Y – the total yield of plants, C – the averaged radioactivity concentration of ^{137}Cs in plant leaves and roots), and F_0 is the value of F for the control soils. The harvested sugar beet crops were processed by means of vacuum distillation using a 2 l vacuum distillation unit. The alcohol waste slurry (distillery dreg) and plant mass were chemically analyzed and composted in the modified lab-scale biogas production plant similar to that described and tested in [6]. Biogas production using thermophilic strains was performed at 35°C . The liquid residue, which can be used as a high-efficient fertilizer and a raw material for B_{12} vitamin-enriched feed additives for livestock and poultry, was dried. The radioactivity concentration (Bq l^{-1} and Bq kg^{-1}) of the produced alcohol, biogas and residue was determined according to standard procedures. A microwave installation with regulated power (1.5 to 20 kW) and a conventional resistive oven were used as heating sources during all distillery and biogas production processes, and the processing times were compared.

4.2 Remediation efficiency under enhanced vital function

The dependence of the dimensionless factor K , characterizing the total remediation capacity of plants, on the potassium content of soils with different Cs and combined preparation (BOMF and “Biorag”) contents are provided in Figures 1, 2, and 3, for sugar beet, sweet clover (white) and creeping red fescue, respectively. The remediation capacity increased with the content of combine preparation in the soil. The significantly increased uptake of Cs by plants, characterized by the non-dimensional factor F , was observed when the available potassium content in the soils was

approximately 5 mg kg^{-1} , and it practically vanished at levels between 20-25 mg/kg , depending on the type of plants and the radioactivity of the soils.

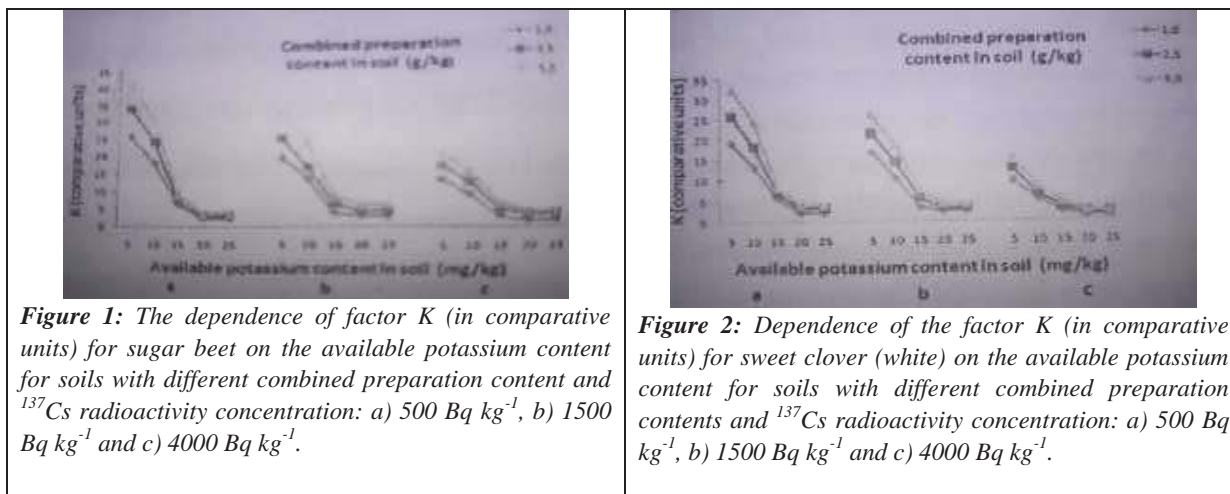


Figure 1: The dependence of factor K (in comparative units) for sugar beet on the available potassium content for soils with different combined preparation content and ^{137}Cs radioactivity concentration: a) 500 Bq kg^{-1} , b) 1500 Bq kg^{-1} and c) 4000 Bq kg^{-1} .

Figure 2: Dependence of the factor K (in comparative units) for sweet clover (white) on the available potassium content for soils with different combined preparation contents and ^{137}Cs radioactivity concentration: a) 500 Bq kg^{-1} , b) 1500 Bq kg^{-1} and c) 4000 Bq kg^{-1} .

Sweet clover (white) is characterized by lower values of factor F, but it can also be regarded as a prospective plant for the remediation of ^{137}Cs -polluted soils. It is also important that it can be composted together with distillery bards to produce biogas.

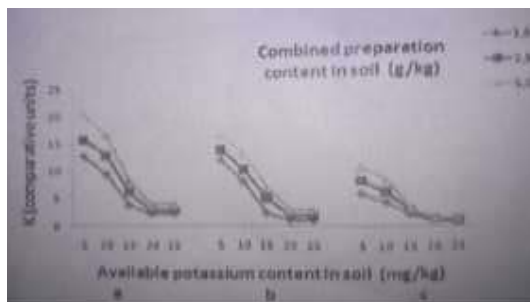


Figure 3: Dependence of the factor K (in comparative units) for creeping red fescue on the available potassium content for soils with different combined preparation contents and ^{137}Cs radioactivity concentration: a) 500 Bq kg^{-1} , b) 1500 Bq kg^{-1} and c) 4000 Bq kg^{-1} .

Creeping red fescue showed the lowest remediation capacity compared with sugar beet and sweet clover (white); however, it was of the same order of magnitude. Considering the simplicity of growing the grass and the capacity of the Rubra variety to spread easily and widely across cool seasons and shadowed areas, its tolerance to full-sun conditions and low fertilizer content in soils, it can be regarded as a prospective plant for phytoremediation. Similar to the case of sugar beet and clover, a tendency toward the saturation of remediation capabilities is observed and additional research is required. Similar to other plants, it can be composted together with vacuum distillery dregs to produce biogas and other byproducts.

4.3. Remediation efficiency in case of pretreatment of seeds

The comparative dimensionless factor K_R was used to characterize the change of remediation capacity of plants caused by pretreatment of seeds in BOMF inoculate and “Biorag” solution. The experiments showed that the optimal pretreatment conditions are the same as recommended by producers and suppliers. Pretreatment both in BOMF inoculate and “Biorag” solution and adding BOMF and “Biorag” preparations to the soil (case A) provides the maximum increase of K_R for all three kinds of plants (about 80-100%). Combination of pretreatment of seeds in BOMF inoculate and “Biorag” solution with adding “Biorag” preparation the soil (case B) leads to increase of K_R for 60-80%, while the combination of pretreatment of seeds in BOMF inoculate and “Biorag” solution with adding BOMF inoculate preparation to the soil (case C) leads to increase of K_R for only 30-50% (in comparison with method without pretreatment of seeds). The obtained results for sugar beet, sweet clover (white) and creeping red fescue are given in Table 1.

Table 1. Increase of the comparative factor K_R for different combinations of treatment means (cases A, B, S)

Treatment \ Plants	Sugar beet	Sweet clover (white)	Creeping red fescue
Case A	1.96±0.07	1.89±0.06	1.81±0.08
Case B	1.76±0.06	1.71±0.05	1.65±0.06
Case C	1.45±0.05	1.39±0.06	1.34±0.07

It can be considered that the increase of ^{137}Cs uptake capacity is almost equally due to the increase of yields and the extracting of plants.

The experience of all nuclear incidents shows that developing relevant methods of utilizing plant mass to generate low or very low volumes of VLLW or/and exempted waste is an urgent problem. Even in the case of the development of high-efficiency methods of phytoremediation, instead of vast contaminated areas, we are left with an enormous amount of phytoremediation waste, sometimes with even higher concentrations of contaminants (heavy metals, hydrocarbons, radionuclides, among others). The disposal of these wastes requires large financial and labor costs.

Therefore, effective processing, which allows such costs to be reduced or even completely compensated, is an urgent current need. The major decontamination costs are mainly associated not with the remediation of contaminated territories, but with the management of generated radioactive waste. Hence, much attention has to be paid to generating little or no radioactive waste (i.e., generating a low volume of VLLW or/and exempted waste).

The microwave-enhanced vacuum distillation of crops (accidentally polluted or utilized for phytoremediation) into high-purity alcohol and the follow-up production of biogas from distillery bards and the green mass of plants can become a good solution for this problem, lowering both the volume of radioactive waste and the costs of waste management due to the possibility of producing marketable products. To evaluate the prospective of methods proposed and preliminarily tested in the present paper, the data on the comparison of different national permissible levels of radioactive Cs in food and feed products, provided in Table 1, can be used.

On the other hand, a significant problem of modern energetic and environmental science is the low-cost production of renewable fuels without the use of edible plants. A solution to this problem is becoming increasingly urgent because of the rapid decrease in the stock of non-renewable energy sources and because of the escalating food crisis. Notably, the product obtained from the crops used in our remediation experiments contains less acetone, volatile acids and aromatic alcohol than the product distilled using a convenient technology.

The radioactivity concentration caused by ^{137}Cs in the product is approximately 1-2 Bq kg⁻¹, indicating that it can be freely used for technical purposes, which must yield a lower demand for ethanol produced from edible crops. The implementation of vacuum distillation and microwave heating also provides the following advantages. The viability and activity of yeast remains at the initial level throughout and even after the fermentation cycle. When working in the fermentation vat with increased hydronic modules, foreign micro-flora is virtually nonexistent, and no impurities usual for contaminating microorganisms, such as isopropyl alcohol, are in the mixture. The semi-liquid distillery dreg containing up to 30-40% proteins is essentially ready for further compost production of biogas, natural fertilizers and livestock feed additives. It can also be used as an additive while co-fermenting different substrates.

The used fermenting yeast cultures remain active in the bards after fermentation and can promote the intensification of biogas production. Clearly, the rational application of co-fermentation and the correct selection of different types of substrates can significantly improve the process parameters. The concentration of radioactivity in the liquid dregs was 10-12 times lower than in the harvested crops, whereas in the residues taken from the biogas reactor it was lower than 150-200 Bq kg⁻¹.

The radioactivity of the produced biogas obtained from the composted mass containing alcohol bard and the green mass of the "phytoremediation plants" was below the detection limit. According to our research results, less than 10% of the harvested plant mass used for phytoremediation is expected to undergo special management as radioactive waste after the application of the above methods. Considering the international regulations on the permitted levels of radioactivity, we hope to reduce this value using the optimized treatment of soils with combined preparation and microwave heating, significantly accelerating the chemical reactions and increasing the efficiency of processing.

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მცენარეების მიერ ცეზიუმის რადიონუკლიდების შთანთქმის კვლევა კალიუმის მნიშვნელოვანი დეფიციტის და რადიაციული სტრესის პირობებში

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მეტალურგიის ფაკულტეტი

რეზიუმე

განხილულია მცენარეების მიერ ცეზიუმის შთანთქმის დონის მნიშვნელოვანი ზრდის შესაძლებლობა მაღალეფექტური ფიტორემედიაციის განსახორციელებლად. გამოყენებულ იქნა კალიუმის მწვავე დეფიციტის პირობებში მცენარეთა სასიცოცხლო ფუნქციების გაძლიერების ახალი მეთოდი. ამასთანავე, კვლევის საგანი იყო რადიოაქტიურად დაბინძურებული ნიადაგების ფიტორემედიაციის პროცესში ახლად წარმოქმნილი ნარჩენების გადამუშავების მეთოდები. ნიადაგებიდან ცეზიუმის ამოღების დაჩქარება ხდებოდა მიკროორგანიზმების და ბიოაქტივატორების გამოყენებით, ნიადაგში მათი შეტანის ან სათესლე მასალის გადამუშავების გზით. მცენარეთა მიერ კალიუმის ამოღების სიჩქარის მნიშვნელოვანი ზრდა აღინიშნებოდა, როდესაც ათვისებადი (ე.წ. მოძრავი) კალიუმის შემცველობა ნიადაგში იყო არაუმეტეს 5 ± 1 მგ/კგ. ეს ეფექტი პრაქტიკულად ქრებოდა კალიუმის შემცველობის 25 ± 5 მგ/კგ დონეზე და დამოკიდებული იყო მცენარის გვარობაზე. კვლევამ აგრეთვე გვჩვენა, რომ გამოყენებული მეთოდებით ფიტორემედიაციის პროცესში გენერირებული ნარჩენების გადამუშავების შედეგად მიღებული პროდუქტების რადიოაქტიურობა უფრო დაბალია, ვიდრე მსოფლიოს მრავალი ქვეყნის მოქმედი ნორმატიული აქტით დადგენილი ზღვრული მნიშვნელობები. ამ მეთოდების გამოყენების შემთხვევაში, ფიტორემედიაციისთვის გამოყენებული მცენარეული მასის მხოლოდ 10%-მდე საჭიროებს განსაკუთრებულ მართვას, როგორც რადიოაქტიური ნარჩენი. ბიოაქტივატორებით სათესლე მასალის დამუშავება ბევრად უკეთეს შედეგს იძლევა, ვიდრე იგივე პრეპარატების ნიადაგში შეტანა. იზრდება მცენარეების საერთო მასა და, შესაბამისად, ცეზიუმის ამოღების ჯამური მანუალებელი (დაახლოებით 100%-ით), ნიადაგში შეტანასთან შედარებით. ბიოენერგოაქტივატორების და მიკროორგანიზმების შეტანა ნიადაგში და მათი გამოყენებით სათესლე მასალის დამუშავება შეიძლება გახდეს რადიოაქტიური ცეზიუმით (^{137}Cs) დაბინძურებული ნიადაგების რემედიაციის ოპტიმალური საშუალება.

საკვანძო სიტყვები: ^{137}Cs , ფიტორემედიაცია, კალიუმის დეფიციტი, რადიოაქტიური ნარჩენები, ბიოენერგოაქტივატორები

ИЗУЧЕНИЕ ПОГЛОЩЕНИЯ РАДИОНУКЛИДОВ CS РАСТЕНИЯМИ В УСЛОВИЯХ СЕРЕЗНОГО ДЕФИЦИТА КАЛИЯ И РАДИОАКТИВНОГО СТРЕССА

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Резюме

Была рассмотрена возможность значительного увеличения поглощения цезия растениями до уровней, достаточных для эффективной фиторемедиации. Мы использовали новый метод повышения жизнеспособности растений в условиях сильного дефицита калия. Кроме того, изучались методы переработки вновь образующихся отходов фиторемедиации загрязненных почв. Эффективность извлечения радиоцезия оценивали с использованием микроорганизмов и биоэнергетических активаторов, добавленных к почвам. Наблюдалось значительное увлечение извлечения Cs растениями, когда доступное содержание калия в почвах было всего лишь 5 ± 1 мг кг⁻¹. Это увлечение поглощения практически исчезало на уровне 25 ± 5 мг кг⁻¹ в зависимости от типа растееия. Это исследование показало, что радиоактивность побочных продуктов отходов фиторемедиации ниже, чем указано в действующих нормах допустимых уровней во многих странах мира. Ожидается, что менее 10% собранной растительной массы, используемой для фиторемедиации, будет подвергаться специальному управлению в качестве радиоактивных пвбчных продуктов. Предварительная обработка семян перед посадкой с использованием тех же биоэнергетических активаторов и инокулятов микроорганизмов привела к значительному увеличению массы растений и следовательно, к увеличению поглощения цезия примерно на 100% по сравнению с добавлением препаратов в почву. Добавление биоэнергетических активаторов и удобрений на основе микроорганизмов в почву и предварительная обработка семян перед посадкой может стать высокоэффективным средством для быстрой фиторемедиации почв, загрязненных ¹³⁷Cs.

Ключевые слова: ¹³⁷Cs, фиторемедиация, дефицит калия, радиоактивные отходы, биоэнергетические активаторы.