PLANT UPTAKE OF SELECTED NATURAL RADIONUCLIDES FROM CONTAMINATED ENVIRONMENT

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Doctoral Dissertation
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PRIVZEM IZBRANIH NARAVNIH RADIONUKLIDOV IZ KONTAMINIRANEGA OKOLJA V RASTLINE

Doktorska disertacija

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I love those who can smile in trouble, who can gather strength from distress, and grow brave by reflection. 'Tis the business of little minds to shrink, but they whose heart is firm, and whose conscience approves their conduct, will pursue their principles unto death.
Leonardo da Vinci

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Abstract

Naturally occurring radioactive materials (NORM) are wide spread in the environment and may pose a threat to humans and other beings as a consequence of discharging radionuclides into the environment due to human activities and natural processes. The knowledge about mobility and bioavailability rates and ratios of NORM is crucial. Thus it is important to acquire new knowledge and upgrade the current one on the transfer of natural radionuclides in the environment. On the other hand, radiotoxicity as well as chemical toxicity of deposited radionuclides are important characteristics regarding health concern. Therefore it is necessary to have appropriate radiochemical analytical methods to obtain reliable results of measurements. Common problems related to determination of radionuclides in biological materials are small amounts, complex matrices, inhomogeneity of the sample, great individual variability, low activity concentrations etc.

In the first part of the doctoral dissertation the method optimisation was accomplished while in the second part the soil-to-plant transfer of natural radionuclides was studied.

A comparison of different dissolution techniques (conventional wet dissolution, microwave dissolution and alkaline fusion) for determination of uranium in soil samples was performed. For testing the effectiveness of the dissolution procedures, reference materials and soil samples from the surroundings of a former uranium mine were investigated. It was observed that the content of uranium in the residues determined by Instrumental neutron activation analysis (INAA) represents a significant contribution to its total concentration in the sample, especially in the case of microwave dissolution. Next, three wet digestion procedures (wet ashing over a gas flame in a long-necked Kjeldahl flask, acids digestion in an Erlenmeyer flask and microwave digestion in a Teflon vessel) for dissolution of biological samples in the determination of ^{210}Po were compared. In addition, special attention was given to potential polonium losses after dissolution due to its volatilisation. The results obtained showed that the activity concentrations of ^{210}Po found in the samples analysed were comparable for all the procedures used. In the third part, optimisation of procedure for consecutive determination of ^{210}Po, ^{226}Ra, ^{234,235,238}U and ^{230,232}Th by alpha-particle spectrometry from a single sample test portion was carried out. The method was optimised by using certified reference materials. The obtained results showed fairly good agreement between the assigned and measured values. Therefore the method was used for determination of selected radionuclides in various plant samples obtained during the pot experiment.

In the second part of the doctoral dissertation, the purpose was to provide new knowledge about transfer of selected natural radionuclides (^{238}U, ^{230}Th, ^{226}Ra and ^{210}Pb) from uranium mill tailing (UMT) contaminated soil to eatable plants (radish, savoy and rocket) as well as to critically evaluate impact of soil parameters on bioavailability of NORM. The results obtained indicated significant linear correlation between the content of the radionuclides in soil and plants as well as between the content of the radionuclides in plants and soil parameters, while radiological contamination had no effect on

photochemical efficiency and content of photosynthetic pigments in the selected plants. In addition, the results obtained confirmed analogous behaviour of radium and calcium as well as indicated that UMT does not have a negative influence on plant biomass production. The obtained concentration ratios (CR) were lower than 1 for all investigated plants, which leads to the conclusion that radish, savoy and rocket cannot be used for phytoremediation purposes under the investigated growth conditions. The assessed total annual effective ingestion dose due to ingestion of studied vegetables did not exceed the world ingestion average radiation dose of 0.29 mSv y^{-1}. However, the total annual effective ingestion dose was linearly correlated with the content of UMT.

Povzetek

Radioaktivne snovi naravnega izvora se nahajajo povsod v okolju in so lahko potencialno nevarne ljudem kot tudi ostalim živim bitjem, saj se naravni radionuklidi lahko izločajo v okolje zaradi različnih človekovih dejavnosti kot tudi naravnih procesov. Poznavanje mobilnosti in biološke dostopnosti v okolju je zato bistvenega pomena, za kar pa je potrebno zagotoviti nova znanja ter nadgrajevati obstoječa. Radiotoksičnost kot tudi kemijska toksičnost radionuklidov sta glavni lastnosti, ki definirata njihovo potencialno škodljivost zdravju, zato je pomembno, da so na voljo primerne radioanalitske metode, ki lahko zagotovijo verodostojne rezultate. Pogoste težave pri določitvi radionuklidov v bioloških vzorcih so majhne mase, kompleksne matrice, nehomogenost vzorca, velika variabilnost med vzorci, majhne aktivnostne koncentracije idr.

Doktorsko delo je razdeljeno v dva dela: v prvem delu sem naredila optimizacijo metod, medtem ko sem v drugem preučevala prenos naravnih radionuklidov iz onesnaženih tal v rastline.

Optimizacija metod je obsegala primerjavo treh različnih razkrojev (klasičen kislinski razkroj, razkroj z mikrovalovnim sistemom in razkroj z alkalno talino) za določitev urana v vzorcih tal. Za testiranje učinkovitosti posameznega razkroja smo uporabili referenčne materiale kot tudi vzorce tal iz okolice nekdanjega rudnika urana. Ugotovili smo, da vsebnost urana v ostanku (določitev z INAA metodo) po razkroju ni zanemarljiva in lahko predstavlja pomemben delež celokupnega urana v vzorcu, kar se je še posebej izkazalo pri razkroju z mikrovalovnim sistemom. Namen drugega dela optimizacije je bil primerjati rezultate koncentracij aktivnostnih ^{210}Po, dobljene v bioloških vzorcih ob uporabi različnih razkrojev (kislinski razkroj nad odprtim ognjem v Kjeldahl epruvetah, kislinski razkroj v Erlenmeyer steklenicah in razkroj z mikrovalovnim sistemom). Pozornost ni bila namenjena le učinkovitosti razkroja, pač pa tudi potencialnim izgubam polonija tekom razkroja zaradi njegove hlapnosti. Dobljeni rezultati so pokazali, da so vse tri uporabljene tehnike ustrezne za razkroj bioloških vzorcev za določitev polonija. V tretjem delu sem naredila optimizacijo za sočasno določitev ^{210}Po, ^{226}Ra, ^{234,235,238}U in ^{230,232}Th v istem vzorcu s spektrometrijo alfa. Optimizacija je potekala z uporabo certificiranih referenčnih materialov. Rezultati so pokazali zadovoljivo ujemanje med certificiranimi in izmerjenimi vrednostmi. Metodo sem kasneje uporabila za določitev izbranih radionuklidov v rastlinskih vzorcih, pridobljenih z lončnim poskusom.

Namen drugega dela doktorskega dela je bil pridobiti nova znanja o prenosu izbranih naravnih radionuklidov (^{238}U, ^{230}Th, ^{226}Ra in ^{210}Pb) iz rastnega substrata, ki mu je bila dodana hidrometalurška jalovina (HMJ), v različne rastline, kot so redkev, ohrovt in rukola. Prav tako je bil namen kritično ovrednotiti vpliv pedoloških parametrov na biološko dostopnost naravnih radionuklidov. Rezultati so pokazali statistično signifikantno linearno povezavo med vsebnostjo radionuklidov v tleh in rastlinah. Prav tako sem pokazala linearno povezavo med vsebnostjo radionuklidov v rastlinah in pedološkimi lastnostmi rastnega substrata. Nasprotno pa radiološka onesnaženost ni imela nobenega vpliva na fotokemično učinkovitost kot tudi ne na vsebnost fotosinteznih pigmentov v preučevanih rastlinah. Dobljeni rezultati so potrdili podobnost obnašanja

radija in kalcija v rastlinah kot tudi da vsebnost HMJ nima vpliva na produkcijo biomase rastlin. Ocenjeni prenosni faktorji (CR) so bili pri vseh preučevanih rastlinah pri določenih eksperimentalnih razmerah manjši od 1, kar pomeni, da redkev, ohrovt in rukola niso primerni za fitoremediacijske namene. Zaradi uživanja preučevane zelenjave ocenjena kombinirana letna efektivna ingestijska doza ne presega povprečne svetovne doze 0,29 mSv leto^{-1}. Medtem ko je bila ocenjena letna efektivna ingestijska doza linearno sorazmerna z vsebnostjo HMJ v rastnem substratu.

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Abbreviations

ANOVA	... Analysis of variance
ATSDR	... Agency for Toxic Substances and Disease Registry
BAC	... Biological absorption coefficient
BCF	... Bioconcentration factor
CEC	... Cation exchange capacity
CR	... Concentration ratio
D_{10}	... The dose which is necessary to reduce the living population by factor of ten
DA	... Discriminant analysis
EFSA	... European Food Safety Authority
ENSREG	... European Nuclear Safety Regulators Group
EPA	... Environmental Protection Agency
HMJ	... Hidrometalurška jalovina
HPGe	... High purity gamma detector
IAEA	... International Atomic Energy Agency
ICP-MS	... Inductively coupled plasma mass spectrometry
IDC	... Individual dose criterion
INAA	... Instrumental neutron activation analysis
IPS	... International Postgraduate School
IUR	... International union of radioecology
JSI	... Jožef Stefan Institute
MDA	... Minimum detectable activity
NORM	... Naturally occurring radioactive material
RM	... Reference material
SI units	... International System of Units
SOM	... Soil organic matter
SRM	... Standard reference material
TF	... Transfer factor
UMT	... Uranium mill tailing
WHO	... World health organisation

Symbols

E_h	... Oxidoreduction potential
K_2O	... Available potassium in soil
P_2O_5	... Available phosphorus in soil
MeV	... Megaelectron-volt
$b.m.$	... Body mass
$d.m.$	... Dry mass
$f.m.$	... Fresh mass
mSv	... Milisievert
$p\ value$	... the level of marginal significance within a statistical hypothesis test, representing the probability of the occurrence of a given event
r	... Pearson correlation coefficient
α	... Alpha radiation
β	... Beta radiation
γ	... Gamma radiation

Chapter 1

Introduction

1.1 Natural Radionuclides

Naturally occurring radionuclides (NORM) are present in many natural resources. High concentrations of these radionuclides are often found in certain geological materials, i.e. ingenious rocks and ores. Human activities that exploit these resources may lead to enhanced concentrations of radionuclides or enhanced potential exposure [1]. The main causes of discharging of natural radionuclides into the environment are the mining and the milling of raw materials containing uranium and thorium and their decay chain products. The other sources of enhanced natural radionuclides are coal combustion, cement production, phosphate fertilizers production and its use in agriculture management and in the nuclear fuel cycle production [1], [2]. Dispersed radionuclides in the environment may pose a threat to the humans, due to inhalation of contaminated aerosols, ingestion of contaminated food or water and external radiation [3].

As mentioned above, elevated levels of NORM can be found in the uranium mining and milling wastes, like wastes from the former uranium mine at Žirovski vrh, Slovenia. Industrial excavation of the uranium ore at the area of Žirovski vrh (Slovenia) lasted for six years in 1980s. During that period about 600 000 tons of uranium-ore with content of approximately 840 g t^{-1} of U_3O_8 were processed. The by-products of the yellow cake production were different kinds of wastes deposited in the mine environment such as U-mill tailings deposited on the so-called Boršt site and red mud on the Jazbec site [4]. Activity concentrations of key radionuclides from the uranium decay products in the U-mill tailing at the Boršt site are 995 Bq kg^{-1} d.m. of ^{238}U, 3930 Bq kg^{-1} d.m. of ^{230}Th, 8630 Bq kg^{-1} d.m. of ^{226}Ra and 7400 Bq kg^{-1} d.m. of ^{210}Pb, on average [4], [5]. It has to be mentioned that remediation actions were finished in 2008 and 2010 for Jazbec and Boršt repositories, respectively [6]. However, emission of radionuclides from the waste disposal sites may lead to elevated activities in the nearby soil due to dry or wet deposition of re-suspended radioactive material or discharge of radionuclides by tailings seepage waters, which leads to adsorption of radionuclides on soil colloids and consequently their weaker or stronger retention in the soil. Released radionuclides can be further taken up by different organisms, such as plants and consequently also transferred into foodstuffs [7], [8].

1.2 Radionuclides Plant Uptake

The transfer of radionuclides along food chains has been studied extensively over the past 50 years [9]. In the literature review it can be noticed that there are different approaches

for quantifying the behaviour of contaminants, i.e. natural radionuclides, in the soil–plant relation or within the plant.

The usual method for expressing the overall degree of incorporation of radionuclides into plant parts from soils or, in other words, the availability of soil-containing radionuclides for plant uptake is the soil to plant transfer factor (TF) or concentration ratio (CR). Both terms are used and are defined as the ratio between plant activity concentration (Bq kg^{-1}, d.m.) and total soil activity concentration (Bq kg^{-1}, d.m.) [3], [10].

The most concerned efforts in this field have been in Europe and the USA. Accordingly, the two principal databases of TF have been established by the US Nuclear Regulatory Commission and by the International Union of Radioecology (IUR) in Europe [3]. They especially pointed out the depth of soil over which the average radionuclide concentration is measured since it has an important influence on the result of the calculation. Thus, instead of the real rooting depth, IUR proposes standardized soil layer depth. For grass, this soil depth value is 10 cm and for all other crops (including fruit trees) is 20 cm [3], [9].

TF values are statistically uncertain and uncertainty should be as low as possible. Reliable average TF values can be achieved with replicated TF values for individual combinations of radionuclide, soil type and crop. These values are further applied in radiological assessment models for calculations of human exposure via the food chain [3]. For example, radionuclides plant uptake can be studied by a hydroponic or a soil system. An obvious difference between those two systems was shown. Plant tissue concentrations of radionuclides have rarely shown a linear relationship to radionuclide concentrations in soil [10], [11], [12], whereas in water grown plants linear correlation between the concentration of the element in water and accumulation of the element in plant occurs more often [13], [14]. To minimize uncertainty these differences should be considered while the experiment is planned.

However, various plant families were used for the study of plant uptake of natural radionuclides. One of them is the family of Brassicaceae crops which are considered as plants with higher capability to accumulate contaminants such as radionuclides in the edible parts [15]–[19]. As mentioned before, the capability of accumulation of contaminates is an important parameter in case of ingestion [20]. Furthermore, such characteristics of plants can represent their potential use for phytoremediation. In this case, the metal biological absorption coefficient (BAC), i.e. the plant-(harvestable)-to-soil metal concentration ratio needs to be determined [21]. In addition, bioconcentration factor (BCF, root-to-soil metal concentration ratio) as well as the translocation factor (TF, shoot-to-root metal concentration ratio) are important factors; plants with BAC greater than 1 are suitable for phytoextraction while those with BCF greater than 1 and TF lower than 1 can be potentially used for phytostabilisation [22]. Here we need to point out that in the literature abbreviation TF can be defined as transfer or translocation factor.

So, there are many factors and processes that influence the transfer of natural radionuclides to vegetation and can be listed as follows:

- radionuclide properties,
- soil type and characteristics,
- agricultural practice (fertiliser application, ploughing),
- plant properties [3], [23].

The ^{238}U decay radionuclides characteristics are presented in Chapter 1.3, while soil properties and agricultural practice are presented below (Chapter 1.2.1 and 1.2.1.5).

1.2.1 Soil type and characteristics

1.2.1.1 Physical soil properties

Physical soil properties such as soil texture, structure/porosity and derived properties such as surface area, permeability, diffusivity and tortuosity primarily control metal transport in soils via their effect on water transport in the soil profile. Soil texture and aggregation are also a source of microheterogeneity of metal distribution in soils. It should be noted that soil aggregation and porosity are not only determined by abiotic properties derived from the parent material but can also be in a significant manner a result of the activity of the plant roots and soil biota [24]. However, the inorganic fraction of the soils can be divided into various textural classes, depending on the percentage of principal constituents; i.e. sand, silt and clay. Sand consists largely of primary minerals such as quartz and has a particle size ranging from 60 μm to about 2 mm. Silt consists of particles in the range of 2 to 60 μm and clay particles are smaller than 2 μm in diameter [25].

One of the factors that significantly influence the radionuclides mobility and bioavailability is soil texture. It was reported that activity concentration of ^{238}U, ^{226}Ra and ^{230}Th was decreased as the particle size increased. The activity concentration of the finer fractions presented a strong correlation with the activity concentration of the bulk soil. Moreover, uranium was found to be more available in the coarser fractions (sand and fine sand) while for radium it was in the finest fractions [26].

1.2.1.2 Physico-chemical and mineralogical soil parameters

1.2.1.2.1 Cation exchange capacity

The cation exchange capacity (CEC) is defined as a degree to which a soil can adsorb and exchange ions and it is highly dependent on the soil type [27]. It is primarily dependent on the surface areas which are determined by soil texture, and the amount and kind of organic matter, clay minerals and oxides [24]. CEC has an important role in soil fertility since a soil with higher CEC generally has a larger reserve of mineral nutrients [27]. Furthermore, soils with higher fertility generally retain radionuclides more strongly [23] and, as a consequence, have a negative impact on radionuclides plant uptake, which was shown by several studies [28]–[30].

1.2.1.2.2 Organic matter

Humus and soil organic matter (SOM) can be thought of as synonyms, and include the total organic compounds in soils (excluding undecayed plant and animal tissues, their partial decomposition products and the soil biomass). Organic matter includes humic substances and resynthesis products of microorganisms which are stable and a part of the soil. Humic substances are a series of relatively high-molecular-mass, brown to black coloured substances formed by secondary synthesis reactions, such as humin, humic acid and fulvic acid. Soil organic matter contents range from 0.5 to 5 % (mass basis) in the surface horizon of mineral soil to 100 % in organic soils.

Soil organic matter has a high specific surface (800–900 $m^2\ g^{-1}$) and CEC (150–300 cmol kg^{-1}). So, the most (up to 80 %) of a surface soil's CEC originate from SOM. Due to all these facts, organic matter is an important sorbent of plant macro- and micro-nutrients, organic materials, heavy metals and consequently radionuclides. However, the complexation of metals ions as well as radionuclides by SOM is important in affecting the retention, mobility and bioavailability of contaminants in soil [31].

1.2.1.2.3 Clay minerals and metal oxides

Clay is an inorganic material that is < 2 μm in size, whereas clay mineral refers to a specific mineral which mainly occurs in the clay-sized fraction of the soil. The main characteristic of clay particles is the abundance of negative surface charges and consequently the ability to attract ions, especially positive ions, to their surface [25], [31]. Further, the capacity of clay minerals to adsorb metals arises from isomorphic substitution. For example, clay minerals with 2:1 structure such as vermiculite and montmorillonite have larger specific surfaces and more abundant isomorphic substitution than 1:1 clay minerals such as kaolinite. However, clays are typically not present in soils as pure minerals, but are partially coated by hydroxide precipitates and form colloidal organo-mineral complexes with humic substances that modify their interaction with metals [24].

The metal oxides (aluminium, iron, manganese), as one of the constituents of soil minerals, especially need to be mentioned since they play important roles in the chemistry of the soils. The general term oxides refers to metal hydroxides, oxyhydroxides and hydrous oxides. In spite of their minor quantities in soil they have significant effects on many soil chemical processes because of their high specific surface areas and reactivity [31]. They are present in a variety of forms that range from amorphous to crystalline minerals. The chemisorption of metals onto hydrous oxides depends on the degree of crystallinity and surface morphology. Processes that are involved in metal fixation by hydrous oxides are immobilizing metalloids, co-precipitation, surface precipitation, particle diffusion and mediation of various metals and metalloids, resulting either in decreased or increased mobility [24].

As already mentioned in the previous chapter (1.2.1.2.2), a major component of a soil's CEC is attributable to the organic matter fraction while the other major component are soil minerals. The CEC of soil minerals depends on their type. For example, the montmorillonite has a high CEC (80–150 cmol kg^{-1}) that is due to substantial isomorphic substitution and to the presence of fully expanded interlayers that promote exchange of ions. On the other hand, the kaolinite has low CEC (2–15 cmol kg^{-1}) but can be variable, depending on the degree of impurities in the clay and the pH at which the CEC is measured [31]. Therefore, higher content of clay minerals and metal oxides in soil provides more sites for adsorption of metals and in this way reduces the amount of directly bioavailable metals as well as radionuclides [25].

1.2.1.2.4 pH value

Soil pH has often been considered as the major variable of the soils which significantly affects numerous soil chemical reactions and processes. The most important factors of soil acidity in mineral-organic soils are Al and H, with Al being more important in soils except for those with very low pH values (< 4) [31]. Generally, the metal uptake decreases with increasing pH in soil but this is not always the case. For example, molybdate is taken up as an anion and consequently the uptake is enhanced by increased pH [32]. In the case of radionuclides, i.e. uranium, Vandenhove et al. [33] reported that at low pH the uranium solubility was higher but due to the other factors influencing solubility this was not always observed. In addition, they inferred that alkaline pH (> 6.9), high total inorganic carbon content, low amorphous Fe, low organic matter and clay content, low CEC and low phosphate levels appear to be conducive to increased U concentrations in the soil solution. So, simple correlation between uranium (and also other radionuclides) in the soil and single soil characteristics cannot be done since the behaviour of radionuclides is complex.

1.2.1.2.5 Redox potential

Soil chemical reactions involve some combination of proton and electron transfer. Oxidation occurs if there is a loss of electrons in the transfer process while reduction occurs if there is a gain of electrons. The oxidised component or oxidant is the electron acceptor and the reduced component or reductant is the electron donor. So, redox potential is a measure of the tendency of a chemical species to acquire or donate electrons and thereby be reduced or oxidised. Redox potential can provide information on conditions that are favourable for increased bioavailability of metals. Moreover, changes in the oxidation state of the metals associated with the oxides (Chapter 1.2.1.2.3) can greatly affect their solubility and mobility in soil. The reductants can be either inorganic or organic [31]. For example, the oxidation state of uranium has a significant effect on its mobility in the natural environment. The U(VI) is soluble (oxidising to mildly reducing conditions) while U(IV) is insoluble (reducing conditions) and is considered as relatively immobile since it forms slightly soluble minerals such as uraninite (UO_2) [34].

1.2.1.3 Interaction of radionuclides with soil

Soils are heterogeneous systems combining three incompatible phases (solid, liquid and gaseous) in different and changing proportions depending on the humidity level. Each phase is highly complex and variable in composition and also in physico-chemical characteristics. Soil properties and thickness are also highly variable in space. Soils are often stratified in layers named horizons which lie on parent bedrock. The top layer, named topsoil, is rich in organic matter, while underlying layers are essentially inorganic [2].

Moreover, soils are dynamic systems. They depend on the joint actions of natural factors (variations in temperature and humidity, erosion) and application practices. Radionuclides deposited on the ground or dispersed within soil are first dissolved in soil water. Once in solution, radionuclides can:

- sorb on the sorption complex by exchange processes,
- (co-)precipitate as hydroxides, sulphides, carbonates or insoluble oxides,
- form complexes with organic molecules,
- or remain in the water phase in ionic form [2].

Therefore, by using chemical extractions, it was found that radionuclides are associated with soil solids in four major ways. Firstly, a very small proportion is retained in adsorbed or exchangeable forms which are available for plant uptake. Secondly, the elements are bound by the soil organic matter and by the organic materials. Organically bound elements are not readily available to plants, but can be released over a period of time. Thirdly, the association of radionuclides in soil is with carbonates and with oxides of iron and manganese. These forms are less available to plants than either the exchangeable or organically bound forms. The last association is commonly known as the residual form, which consists of sulphides and very insoluble compounds that are less available to plants than any of the other forms [35], [36].

However, the dynamics of radionuclides in soils not only depend on the abiotic components such as organic and inorganic constituents and physico-chemical characteristics, but also on their interactions with the biological components, namely the presence of soil microflora, mycorrhizal fungi associated with plants and the physiological status of plants [7]. Figure 1.1 presents a scheme for radionuclides movement in soil which was proposed by Igwe et al. [35].

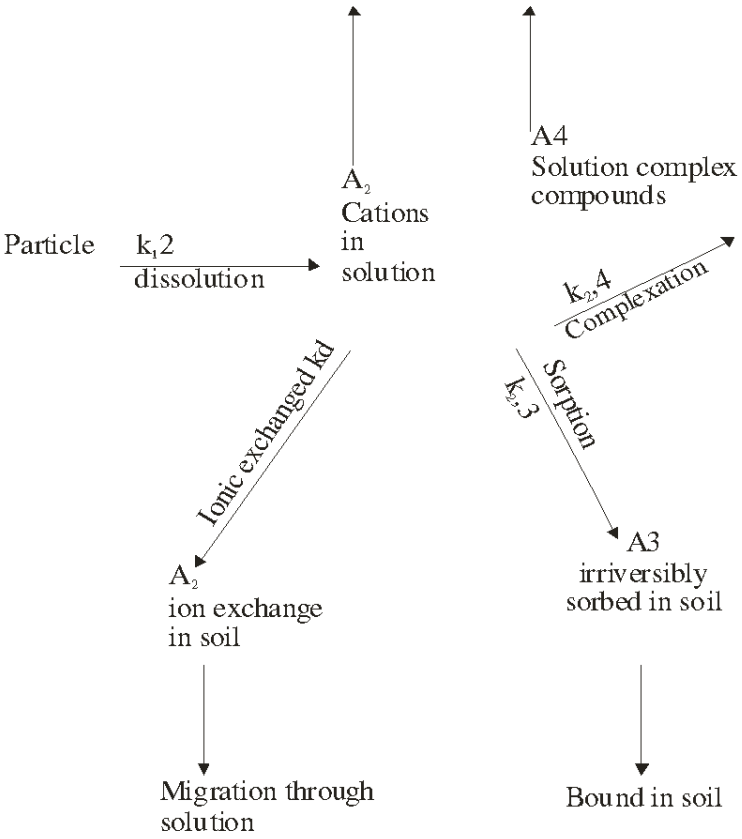


Figure 1.1: Radionuclide transformation processes in soil (k_i – *reaction rate*) [35].

The most important components of the soil are the colloids which may be either inorganic matter, that is clay minerals, or organic matter, that is humus. The colloids make up the active fraction because of their high surface area and chemical activity [37]. So that soil colloids can adsorb ions and reduce their overall mobility in the soil. They contain a high specific density of negative charges acting as cation exchange sites. All positively charged ions in the solution compete to fill these sites, yet the tendency to bind to a solid surface is ion-specific. Hence, averaged over the entire solid surface, the composition of the adsorbed ions at equilibrium is depended on the amount of each ion in solution and the selectivity of the sites for each ion. The total number of sites for adsorption is often described by the cation exchange capacity which records the amount of cations a solid can sorb expressed in equivalents [2], [3].

For most ions, adsorption of ions is reversible and equilibrium tends to be achieved between the concentration in the soil solution and on the adsorption complex. This equilibrium is generally expressed as the solid-liquid distribution coefficient concept (Kd values), which is the ratio of the concentration of radionuclide sorbed on a specified solid to the radionuclide concentration in a specified liquid phase at equilibrium. This model does not assume any knowledge of sorption mechanisms, nor does it contain a term to quantify the capacity and selectivity of the sorption sites or the competition with other ions to fill the sorption sites [38]. But it is useful for studying heterogeneous solids, such as soils and sediments [3]. This approach is the simplest sorption model available and relates the concentration of solute in water to the amount of solute sorbed [2], [3] (Eq.(1.1)):

$$M_{aq} \leftrightarrow M_{ads} \tag{1.1}$$

where M_{aq} and M_{ads} indicate the sorbed and aqueous species, respectively. K_d (L kg^{-1}) is the ratio of the concentration of exchangeable ion sorbed on solid at equilibrium and the concentration of free ion in solution at equilibrium [3] (Eq.(1.2):

$$K_d = \frac{[M]_{ads}}{[M]_{aq}} \tag{1.2}$$

Immobilizing ions by fixation onto the soil phase or precipitation delays or prevents their leaching with percolation water down to below roots. However, immobilization might be a transient process. In other words, if the solid phase can be a sink for radioactivity, it may become a source depending on changes in concentration gradients between the solid and liquid phases or variations in the soil chemical properties, for example variation in pH or oxidoreduction potential (E_h) [2].

1.2.1.4 Microbial soil activity

Microorganisms are highly resistant to radiation. The range of D_{10} values (the dose which is necessary to reduce the living population by a factor of ten) is between 0.34 and 1.68 kGy. Therefore, microorganisms are able to control the behaviour of radionuclides in the environment in a different kind of ways. The level, direction and nature of the radionuclide fluxes are partly depended on the presence of microorganisms which act through four principal ways:

- *Biodegradation* of radionuclide-loaded organic matter: Living, resting and dead microbial cells immobilise free and bound radionuclides which can further enter the higher levels of the trophic chain through micro-predation. Radionuclides can moreover be transformed by the indirect consequences of microbial activity, such as pH and E_h can induce the precipitation of dissolved radionuclides.
- *Bio-accumulation:* Just as essential, nonessential and toxic elements, radioelements can also be taken up and stored by microorganisms. These uptakes are controlled by cell metabolism and consequently by microbial growth.
- *Bio-adsorption:* The cell surface acts as an adsorbent on which radionuclides fix by physico-chemical bonds and are exchanged with the dissolved free forms. This is appeared on the cell surface of living as well as dead microorganisms.
- *Precipitation* of dissolved elements: The precipitation of dissolved radionuclides can occur due to changes in the chemical speciation or chemical form of the ion after modification of the pH or E_h. These modifications can be induced by microbial metabolism and favour precipitation as carbonate, sulphide or phosphate [3].

1.2.1.5 Ion competition in the soil

The competition between ions is also an important factor that influences the uptake of trace elements such as radionuclides and is usually not taken into account in assessments of transfer factors. The radionuclides that need caution are those which chemical behaviour is similar to some of macro-nutrients and a number of other substances naturally present in soils [39]. Therefore, the most often studied analogue element-pairs are K – Cs, Ca – Sr, Ba – Ra, Re – Tc [40]. For example, the correlation coefficient between Ca and Sr in suspended particulate matter was found to be 0.999 according to the study conducted by Varnavas et al. [41]. Gerzabek et al. [42] showed the effect of soil Ca on plant uptake of radium where significant negative correlation between transfer

factor and Ca content in soil was observed. On the other hand, the similarity of the two elements could be used as a tracer in studies of the environmental behaviour of the chosen radionuclides, such as ^{133}Ba tracer for studies of ^{226}Ra behaviour in the environment [43].

1.2.2 Agricultural practice

Cultivation of agricultural crops involves various technologies of soil management, different rates and combinations of fertilizers, irrigation in dry areas, drainage in marshy environment, etc. Agricultural practises may affect soil properties or cause redistribution of radionuclides in the root zone, and consequently change accumulation of radionuclides in plants [23]. For example, in arable land the radioactive contamination in the upper 20 cm layer is nearly the same, because of the regular ploughing of the soil, and the activity of the isotopes causing radioactive contamination gradually decreases with depth. On the other hand, in uncultivated land the largest part of radioactive pollution is accumulated in the upper 5 cm layer of soil. Furthermore, the flow of radioactive isotopes from the soil to plants is also influenced by the applied artificial fertilizers. The nitrogen fertilizers, i.e. ammonium ions, promote the flow of ^{137}Cs from the soil to plant while phosphate fertilizers inhibit the accumulation of ^{90}Sr. The usage of artificial fertilizers may also cause elevated soil radioactivity since they contain a significant amount of radioactive isotopes of natural origin such as ^{40}K, uranium, thorium, ^{226}Ra, ^{210}Pb and ^{210}Po [44].

1.2.3 Plant properties

Among all the key factors influencing the uptake of radionuclides in soil-plant relation, the biological variability inherent in plants and diversity between different varieties and species is also the source of variability in transfer/concentration factors. The sources of this variability are different metabolic and biochemical mechanisms of radionuclides uptake by plants, like requirements for certain nutrients [23]. Due to this fact there can be an analogue behaviour of radionuclides and nutrients, for example K for Cs, Ca and Mg for Sr or Ra [23], [45], [46]. Further mechanisms that influence the variability are detoxification and exclusion mechanisms, distribution of roots in the soil and rhizosphere properties. Duration of vegetative period and yield may also influence radionuclide transfer factors [23], [47]. Radionuclides usually transfer in maximum concentration to roots, in greater concentration to leaves and stems and in much lower concentration to generative parts [10], [11], [14], [47]–[52]. In addition, strontium and radium are more effectively accumulated by legumes than by cereals [23], [53], [54].

1.3 ^{238}U Decay Radionuclides: Basic Information and Plant Uptake Characteristics with Case Studies

1.3.1 Uranium

Uranium is one of the main elements contributing to natural terrestrial radioactivity. Among all uranium isotopes, only three of them are naturally occurring, namely ^{238}U ($t_{½}$ = 4.47 × 109 y), ^{235}U ($t_{½}$ = 7.04 × 108 y) and ^{234}U ($t_{½}$ = 2.45 × 105 y) [55], [56].

The most common oxidation states of uranium in nature are 4+ and 6+. Compounds containing U^{4+} are insoluble in mildly acidic to alkaline conditions. On the other hand,

those containing U^{6+}, such as UO_2^{2+} (uranyl), are highly soluble and mobile. In solution, uranyl forms soluble complexes with carbonate, oxalate and hydroxide. Uranyl is complexed by organic matter, iron oxyhydroxides or precipitated by various anions, such as hydroxide, silicate, vanadate, arsenate, and phosphate. Uranium (IV) and uranium (VI) form very strong fluoride complexes, while those formed with Cl^-, Br^- and I^- are week. All these reactions are strongly pH dependent. [57]

Uranium shows a strongly lithophile characteristic, because of its position in the periodic table and electronegativity [58] it appears in a large number of mineral species (at least 200 are known) [59]. The most important uranium compounds are the oxides such as U_3O_8 and UO_2, both are solids with low solubility in water [34], [59]. There are also hydrides, phosphates, carbonates, sulphates, arsenates, vanadates, molybdenates and silicates, and U tends to have affinities with hydrocarbon complexes [58].

The earth's crust contains approximately 3–4 mg kg^{-1} U, which is comparable with arsenic or boron. The specific radioactivity of natural uranium makes it a week radiological hazard. On the other hand, it is chemically toxic; it can affect reproduction and fetal development [60].

1.3.1.1 Uranium mobility in soil

1.3.1.1.1 Phosphorus

Phosphorus occurs in the soil solution as negatively charged phosphate ion $H_2PO_4^-$ in acid soil and or HPO_4^{2-} in alkaline soils. These anions react rapidly with positively charged soil compounds and heavy metals, such as uranium. Phosphate forms with uranium low soluble uranyl phosphates or minerals, such as autunite. For example, results in a study by Rufyikiri et al. [61] showed that adding P resulted in reduced U solubility and extractability in soil, thus decreasing risks of U dispersion. Uranyl phosphate complexes are most stable at neutral pH conditions. On the other hand, Jerden et al. [62] reported that even if uranyl phosphates are not stable in low pH soils (e. g. pH 4–6) uranium may be retained through incorporation into aluminium phosphates and the formation of uranium phosphate complexes with iron oxides (nano-precipitates). Furthermore, results showed [61] that the plant biomass production can be greatly enhanced with P fertilization, while in opposite, the uranium plant uptake was regularly decreased by increasing P content in soil. Thus, P fertilizations can be used in phyto-stabilization strategies for U contaminated areas.

1.3.1.1.2 Organic matter

In general, organic matter increases the sorption properties in soils. Bednar et al. [63] showed that this assumption is based on stationary organic matter in soils (e. g. attached to soil particles), which can supply additional sorption sites. Soil particles can interact with dissolved organic matter, such that the soluble organic matter competes with other dissolved components for sorption sites on soil particles. The humic acid (dissolved organic matter) does not promote sorption, but could hinder it. Probably, the dissolved humic acid competes with uranium for sorption sites on oxide and clay particles. Additionally, humic acid promotes subsequent desorption of uranium [33]. Thus, dissolved organic matter may cause higher uranium mobility, but in general organic matter decreases uranium mobility [33], [48], [63].

1.3.1.2 Uranium plant uptake

Uranium present in soil enters the plants through roots and is distributed in different parts of the plants. Several studies [11], [47], [48], [64] were carried out and here some results are presented.

It has been reported in numerous publications that uranium concentration in roots is much higher than in leaves and seeds [11], [47]–[50]. For example, Sight et al. [47] studied uranium plant uptake in some plants. All the studied plants were provided with similar watering and other environmental conditions and were grown in the laboratory with six different uranium concentrations mixed in the soil. It was confirmed that the root system is entry path for the uranium and its distribution in plants is heterogeneous with maximum in the roots and generally with minimum in the leaves. Furthermore, it was found that the uranium content in the plant depends upon the amount of the uranium in the soil over which the plant was grown, while a complete uptake was not observed. With the growth period, the uranium content in various plant parts increases and becomes nearly constant for all plant parts after growth period of about 3 months. In addition, Shtangeeva [11] studied temporal variations of U in plants and showed that short-term dynamics (Figure 1.2) of radionuclide plant concentrations are rather significant, regular and species-specific. Thus the uranium uptake in plants depends upon the nature and age of plant and complete uptake of uranium is not possible in plants [47].

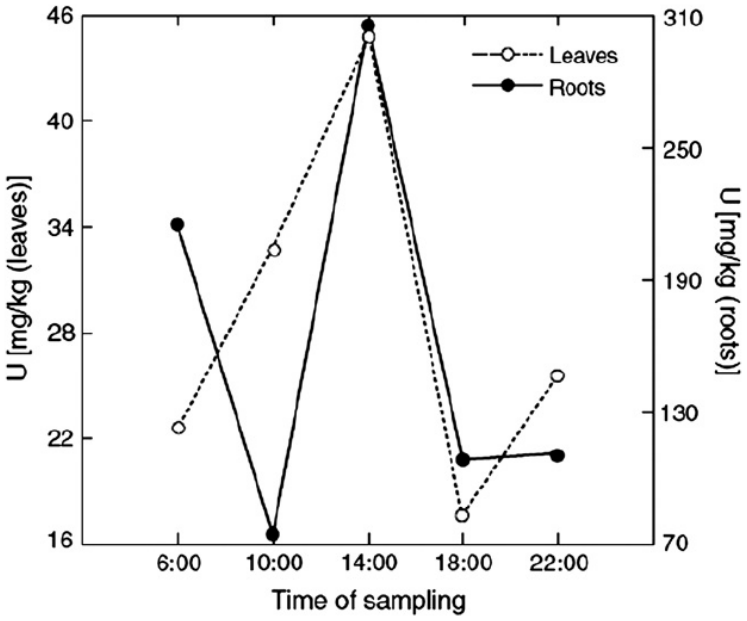


Figure 1.2: Dynamics of U concentrations in leaves and roots of couch-grass (*Elytrigia repens* (L.) Desv. ex Nevski) [11].

Transfer characteristics of radionuclides, such as linear or non-linear, are important for radiological assessments. Numerous publications have reported linear relationship between total radionuclide concentration in the hydroponic solution and total amount of the radionuclide in the plant roots, independently of growth medium pH [14], [50]. However, these experiments give only approximate estimates of the processes occurring in the complex soil solution and do not reflect the real field situations. So, radionuclide

uptake prediction is not related to simple bioavailability parameters but needs to be considered along with several soil characteristics [65].

1.3.2 Thorium

Natural thorium consists only of ^{232}Th ($t_{½}$ = 14.02 × 10^9 y [56]) which is the parent nuclei of thorium decay series [60]. Uranium-238 decay chain also contains ^{234}Th ($t_{½}$ = 24.1 d [56]) and ^{230}Th ($t_{½}$ 0= 7.5 × 10^4 y [66]) [55]. Thorium occurs in only one oxidation state as Th^{4+} and is generally very insoluble in water. It shows strong colloidal characteristics, and may form hydroxide and silicate colloids. Because of its large size (ionic radium of 1.08 Å [67]) and ionic charge, Th^{4+} has a strong tendency to form complex ions with chloride, fluoride, nitrate, sulphate, carbonate and hydroxide [58].

1.3.2.1 Thorium mobility in soil

The stable elements present in the soils may be associated with the mobility and distribution of the radionuclides through the formation of more or less insoluble compounds. Vera et al. [68] showed a correlation of thorium in soils with iron, aluminium and manganese which were co-precipitated or adsorbed onto particles of insoluble oxides. Thorium can form insoluble compounds with magnesium and calcium (as calcium carbonate) [68], [69]. Organic matter also reduces Th mobility due to forming strong complexes with humic and fulvic acids [70]. On the other hand, the presence of carbonates (i.e. Na_2Co_3) in solution greatly increases the solubility of thorium such as thorium dioxide (ThO_2) [70].

The soil pH is also an important factor controlling Th mobility in soils. In the presence of acid radical ions in soil the solubility of thorium oxide is enhanced by forming thorium-sulphate, fluoride and phosphate complexes below pH 7 [71]. On the other hand, the presence of calcium carbonates decreases Th solubility [69].

1.3.2.2 Thorium plant uptake

Shtangeeva and Ayrault [14] extensively studied thorium wheat seedling uptake from soil and water media and here we present their main findings. Firstly, Th plant accumulation has not affected the biomass of the plants. This indicates that a short-term Th exposure was not very toxic for the plants. On the other hand, Shtangeeva et al. [51] reported that young wheat seedlings on the germination in Th enriched medium were decreased in calcium content in all parts of the seedlings. Calcium has an important role as a cell wall structure component and it is also an effective regulator of various processes in plant cells. The decrease of calcium may have a harmful effect on the plant.

Secondly, Th uptake depends on the rhizosphere-specific micro-environment caused by roots excretes. The organic roots exudates are able to enhance acquisition of nutrients by plant and many fungi dwelling on plant roots excrete oxalic acid which can dissolve a large part of Th bounded to a solid phase; consequently concentration of Th in roots of the experimental plants was increased approximately to the level on Th content in the soil with Th contamination. Therefore, Th plant uptake can be enhanced by forming Th–organic complexes and pH decrease by oxalic acid [14].

Thirdly, it was found that Th concentration in non-filtered water was higher than that in filtered water, as a result of seedling growth in the media. The simplest explanation is that a large part of Th in the water may be potentially bound to dissolved organic matter. If the solution contains sulphates, which may form insoluble complexes with Th, they may precipitate on the glass wall surface of the jars. This phenomenon results in decreased Th content in solution [14].

Many studies conducted by Shtangeeva et al. [11], [14], [51] concluded that Th main accumulation target organs are roots, while Th concentrations in upper plant parts are rather low.

1.3.3 Radium

Naturally occurring radium radioisotopes are ^{223}Ra, ^{224}Ra, ^{226}Ra and ^{228}Ra with half-lives 11.4 d, 3.6 d, 1600 y and 5.7 y, respectively [56], [72].

It is highly electropositive, it reacts readily with many agents; most of these products are insoluble. It can be co-precipitated with barium and strontium to form insoluble sulphate and with calcium carbonates and iron hydroxides to form insoluble complexes. It can be absorbed by clay minerals, colloidal silicic acid, manganese oxides and organic matter. Consequently, these reactions decrease Ra availability to plants. On the other hand, the conditions where radium migration in water (water is also part of soil) may occur, are in case of low pH, high total dissolved solids (in particular due to group II) or a low redox potential [23], [64], [73], [74].

1.3.3.1 Radium plant uptake

When radium is in solution it can be taken up by plants. Like mentioned above, high total dissolved solids such as Be, Mg, Ca, Sr and Ba may cause radium migration in water [74]. So that, Lauria et al. [54] studied radium levels in vegetables and found the highest radium levels in vegetables with the highest calcium content, i. e. beans. Correlation between radium content in plants and alkaline earth metals was also confirmed by Chao and Chuang [53].

Just like other radionuclides radium is also taken up mainly by roots into the plants and concentrations are higher in roots than in aerial parts [10]. Radium is more mobile in plats compared to U and Th as shown by Rodríguez et al. [50]. The lowest concentration of ^{226}Ra compared to ^{238}U and ^{210}Pb in leaves and stems was also shown by Černe et al. [52].

1.3.4 Polonium and lead

Seven polonium radioisotopes can be found in nature but most are very short lived and decay rapidly. The most important is ^{210}Po with half-life of 138.4 d. It is relatively ubiquitous in the environment but tends to be elevated in uraninite/pitchblende (approx. 10–100 ng kg^{-1}) and in phosphate related materials [56], [75].

Lead occurs in five oxidation states –4, –2, 0, +2 and +4 [55]. It has four stable isotopes and among several radioactive isotopes only seven have a half-life longer than 10 h. It is a chalcophile element that is slightly volatile at high temperature. The solubility of lead in water solutions is very low because it is easily oxidized to Pb(II). Pb(II) is a predominant inorganic form in the environment and the most significant from the toxicological point of view [76]. However, in the presence of complexing agents like chlorides, iron oxides, sulphides, carbonates or organic ligands, its solubility is enhanced [55], [75], [76].

^{210}Po and ^{210}Pb in the terrestrial environment arise from ^{226}Ra ($t_{½}$ = 1600 y [56]) which further decays in a noble gas ^{222}Rn ($t_{½}$ = 3.82 d [56]) [77]. However, the natural distribution of ^{210}Po in the environment is integrally dependant on its progeny, ^{210}Pb. Ingrowth of ^{210}Pb ($t_{½}$ = 22.23 y) is slow and its environmental transport largely controls the distribution of ^{210}Po [56], [78].

1.3.4.1 Polonium and lead mobility in soil

The polonium content in soil varies with soil type. Karanukara et al. [79] have reported that organic soils contain three times as much ^{210}Po as mineral soils and it was found that there are differences between the ^{210}Po contents of black and brown soils. This is due to high organic matter cation exchange capacity. Polonium is very immobile in soil and likely to be irreversibly sorbed on clay and organic colloids. It was observed that silt content and soil pH are very important to absorb polonium and lead in soil and thus reduce mobility of these radionuclides [80]. The lead solubility is low and therefore bioavailability of soil Pb to plants is limited, but may be enhanced by reduced soil pH, organic matter and inorganic colloids, iron oxides and phosphorus content [75].

1.3.4.2 Polonium and lead plant uptake

Polonium and lead transfer to plant is not only via roots of the plant which is limited [75], but also via atmospheric deposition due to decay of gaseous ^{222}Rn. Consequently, it results in higher TF values, especially where there is a high content of ^{226}Ra and ^{238}U in soil, and therefore more radon gas is produced [81]. Higher ^{210}Po activity in leaves was also observed by Karunakara et al. [79] proving contribution of atmospheric precipitation to the overall polonium activity. Pietrzak-Flis et al. [82] reported that the contribution of the above-ground interception to the total amount of radionuclides in and on the plants is up to 95 % and enters into the plants mostly when they are dissolved in water. Furthermore, Ekdal et al. [77] reported lower activity concentrations of ^{210}Po and ^{210}Pb measured in root vegetables compared with leafy vegetables, which results from the fact that they can mainly take up the radionuclides directly from associated soil without any considerable contribution from the atmosphere. Thus, wet atmospheric deposition on plants is the main contributor to the concentrations of ^{210}Po and ^{210}Pb in the plants and this should be considered when plant uptake in natural environment is calculated.

1.4 Effects of Ionising Radiation on Plants

Radiation effects in natural and agricultural ecosystems are of particular interest in situations where the ionising radiation results from radionuclides present in the natural environment. This might be the case for releases of man-made radionuclides as well as for natural radionuclides in regions where their concentrations are high. The worst case with consequences for the ecosystem is the radiation accident with uncontrolled release of radioactive materials into the environment. In such a situation the ionising radiation may cause radiation injury and even extinction of the whole ecosystem; depending on the type of radioactive material and dimension of the release [83]. On the other hand, the long-term (chronic) exposure of plants to low dose levels, especially internal alpha-particle exposure, is not studied that well [84].

Plant response to radiation exposure can be considered at different levels of organisation: from DNA and chromosome to whole organism, population and community. In the Geras'kin et al. [84] study it was found that plant populations growing in areas with relatively low levels of radioactive pollution are characterized by an increased level of both cytogenetic alterations and genetic diversity which may be related to higher-order effects like mortality or reproduction. It has to be noted that all the mentioned effects cannot be explained only by radiation exposure due to the fact that the naturally occurring radionuclides can also act as toxic chemicals [85]. In addition, climate conditions, soil types, chemical pollution as well as adaptation processes can significantly influence the effects of low dose rate exposure in natural plant population [86]. Moreover,

natural populations can respond to radioactive contamination not only by an enhanced level of genetic alterations and reduction in reproduction ability but also by radio-adoption, which means physiological acclimation or changes in sexual, age or genetic structure of population [84].

1.5 Theoretical Background for Optimization of Radio-Analytical Procedures

1.5.1 Specific characteristics of uranium in soil matrix

One of the most important requirements in radiochemical analysis is that before isolation of the radionuclides of interest the sample must be completely dissolved. It is well known that soil samples are often considered as one of the most difficult matrices to dissolve. Uranium is incorporated into the structure of difficultly soluble minerals – such as zircon, apatite, titanite, allanite etc. These minerals are part of the soil and it is very important that they are completely decomposed during chemical treatment. The literature review of the accomplished study is presented in Jurečič et al. [87] (Appendix A) in more detail.

1.5.2 Volatilisation loses of ^{210}Po from biological matrixes

^{210}Po is a pure alpha emitter and due to its short half-life of 138.4 days [56] and physiological role in organisms, it is known as one of the radionuclides with the highest radiotoxicity and one of the most important sources of the internal dose received by humans from ingestion of food [88]. It is widely distributed in the environment with the result that ^{210}Po enters food chains through direct uptake, ingestion or inhalation. Accuracy in the determination of the activity concentrations of ^{210}Po in various samples depends heavily on the sample dissolution techniques used. The analyst has to be aware of a possible loss occurring during the dissolution procedures and achieve the best performance to minimize or avoid this effect since polonium is known as volatile. The literature review of the accomplished study is presented in Planinšek et al. [89] (Appendix A) in more detail.

Chapter 2

Aims and Hypothesis

In general, the doctoral dissertation is divided in two parts: method optimisation and plant pot experiment. However, the results of the study of uranium concentration in the residue after sample dissolution and study of potential polonium losses after dissolution due to its volatilisation are just briefly presented since they were already published. The accomplished studies are presented in the attached publications (Appendix A) in more detail.

2.1 Aims of the Accomplished Studies

Generally, radiotoxicity as well as chemical toxicity of deposited radionuclides determine their potential detrimental health effects [20]. Common problems related to determination of radionuclides in botanical materials are often small available sample masses, complex matrices, inhomogeneity of the samples, great individual biological variability, low activity concentrations, etc. Therefore, reliable radiochemical analytical methods are required to obtain accurate measurement results. Additionally, a literature survey shows that researchers usually chemically separate only radionuclides of one element or maximum three elements from each sample portion for alpha particle spectrometry analyses [5], [64], [90]–[93].

After the method optimization, three different eatable plants from the Brassicaceae family were selected as tested plants for the laboratory pot experiment. Radish (*Raphanus sativus* L.) was chosen as a root vegetable, while savoy (*Brassica oleracea* L. ssp. *oleracea var. sabauda* L.) has an edible plant part above ground. Rocket (*Diplotaxis tenuifolia* (L.) DC.) was chosen according to the increasing importance as a vegetable and spice [94]. The experiment was designed in the way to demonstrate different contamination scenarios with uranium mill tailing. The specific goals of the thesis are as follows:

- to compare various dissolution techniques for determination of uranium in soil and sediment samples with emphasis on the content of uranium in the residues obtained after dissolution of the samples;
- to compare three wet ashing techniques for determination of ^{210}Po in biological samples, particularly regarding volatilisation losses;
- to optimize a method for consecutive determination of several members of uranium series decay chain that are enriched in sites contaminated with NORM;
- to evaluate the transfer of natural radionuclides to eatable plants, i.e. radish, savoy and rocket, from commercially available plant substrate contaminated with uranium mill tailing;

- to investigate transfer of natural radionuclides to the plants in different contamination scenarios (control, 20 %, 40 %, 60 % and 80 % content of UMT);
- to study the influence of siil parameters on uptake of NORM radionuclides;
- to estimate the potential use of the studied plants under the investigated growing conditions;
- to assess annual effective doses received from the selected radionuclides by the studied vegetables consumption.

2.2 Hypothesis and Goals of the Accomplished Studies

The following hypotheses were tested:

- the uranium concentration in the residue after sample dissolution will represent a significant portion of the total uranium in the sample;
- the polonium found in biological samples will depend on a dissolution technique;
- uptake of ^{226}Ra will be the highest comparing to ^{238}U, ^{210}Pb and ^{230}Th;
- activity concentrations of all studied radionuclides (^{238}U, ^{230}Th, ^{226}Ra and ^{210}Pb) in plants will be correlated with activity concentrations in soils and their behaviour will follow a linear function;
- the soil parameters (pH, content of organic matter, CEC, available P_2O_5 and K_2O, total N, organic C, C/N ratio) will have impact on availability of NORM radionuclides to plants;
- activity concentrations of ^{238}U, ^{230}Th, ^{226}Ra and ^{210}Pb will be the highest in the root system comparing to the shoots system.

Chapter 3

Materials and Methods

3.1 Optimization of Radio-Analytical Procedures

3.1.1 Uranium in soil matrix

Conventional open-vessel wet dissolution with mixtures of the acids HNO_3, $HClO_4$ and HF, closed-vessel microwave dissolution using HNO_3 and HF followed by open-vessel dissolution with $HClO_4$, and alkaline fusion with Na_2CO_3 and Na_2O_2 in a glassy carbon crucible followed by mineralization with HNO_3, HCl, HF and H_2SO_4 were used. For determination of uranium in the insoluble residues, instrumental neutron activation analysis was applied. The studied samples included one soil and one sediment RMs and six real soil samples from the vicinity of a former uranium mine and mill. The soil samples were collected at six locations in the surroundings of the former Uranium mine at Žirovski vrh, Slovenia. The materials and methods of the accomplished study are described in Jurečič et al. [87] (Appendix A) in more detail.

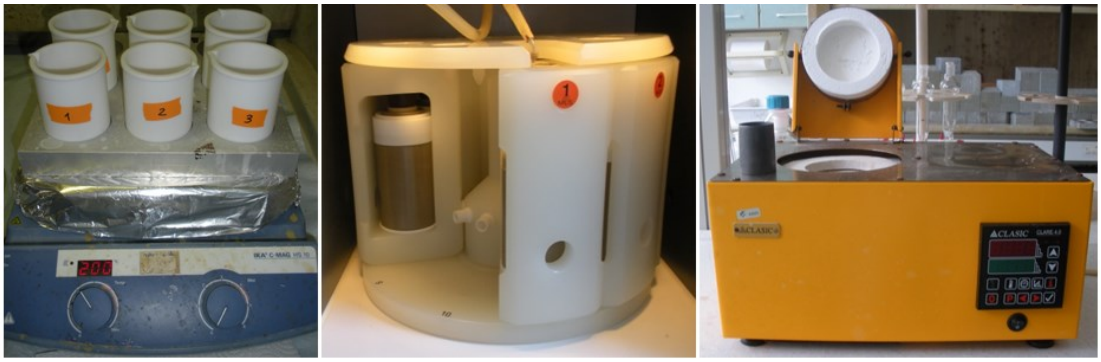


Figure 3.1: Dissolution of the sample by conventional wet dissolution, in the microwave system and by alkaline fusion.

3.1.2 ^{210}Po in biological samples

Three dissolution techniques used in determination of ^{210}Po in biological samples are described and compared. The dissolution techniques involved classical wet ashing with acids in a 100 mL long-necked Kjeldahl flask by heating vigorously over a gas flame, digestion with acids in an Erlenmeyer flask at temperatures up to 200 °C and wet digestion by a microwave system in Teflon vessels up to 200 °C. For the study of the best applicable dissolution procedure for determination of ^{210}Po in biological samples,

seven plant and six real mussel (*Mytilus galloprovincialis* Lamarck, 1819) samples were used. The plant samples included four reference and three real lichen samples. Lichen samples were collected in Slovenia, while three mussel samples were bought on the domestic market, and three mussel samples were from the Croatian Adriatic coast. The materials and methods of the accomplished study are described in Planinšek et. al. [89] (Appendix A) in more detail.



Figure 3.2: Dissolution of the sample in a quartz Kjeldahl flask, in an Erlenmeyer flask and in a microwave system.

3.1.3 Radiochemical procedure for consecutive determination of ^{210}Po, ^{226}Ra, ^{234,235,238}U and ^{230,232}Th in plants

All reagents used in the radiochemical procedure were of analytical grade. The tracer solutions of ^{232}U, ^{229}Th, ^{133}Ba and ^{209}Po used as working solutions in the study were prepared from calibrated solutions purchased from Analytics, Inc. (Atlanta, GA, USA). The producer maintains traceability to NIST standards.

3.1.3.1 Dissolution of plant samples

Up to 2 g of plant sample was transferred to a Teflon beaker and an appropriate volume of ^{133}Ba, ^{209}Po, ^{232}U and ^{229}Th tracers was added. Then 20 mL of concentrated HNO_3 acid was added and the sample was digested over night at room temperature. The next day, sample was heated at 150 °C near dryness and then 10 mL of concentrated HNO_3, 10 mL of concentrated $HClO_4$ and 10 mL of concentrated HF were added and the solution was heated again at 200 °C until dryness. The step was repeated once more to assure sufficient dissolution. The residue was dissolved with 2 mL of concentrated HCl and again evaporated until dryness at 200 °C. This step was repeated once more to convert the solution into a chloride form and finally prepare the sample for spontaneous deposition of polonium radionuclides.

3.1.3.2 Consecutive separation of selected radionuclides

After the sample dissolution spontaneous deposition of polonium radioisotopes onto silver discs was performed. The residue as described in the previous section (3.1.3.1) was dissolved with 2 mL of concentrated HCl and diluted with deionised water up to 100 mL. Then 0.5 g of ascorbic acid and 0.5 g of hydroxyl ammonium chloride were added to the solution to reduce Fe(III) and prevent co-plating of iron and interfering

elements. Finally, the spontaneous deposition of polonium was carried out at 100 °C for 4 h [95].

Then the solution was prepared for consecutive separation of Ra, U and Th radionuclides. The solution remained after polonium separation was transferred into a glass beaker; then 20 mL of concentrated HNO_3 was added to digest organic compounds and evaporated until dryness at 200 °C. Then 5 mL of H_2O_2 was added and evaporated until dryness at 150 °C. This step was repeated once more to assure complete decomposition of organic reagents. In the last step residue was dissolved with 2 mL of concentrated HCl and then the radionuclides were co-precipitated with iron hydroxide after the addition of a sufficient amount of ammonia solution.

Extraction chromatographic separation techniques based on UTEVA and TEVA resins (Eichrom Technologies) were utilized to separate uranium, thorium and radium isotopes in the plant samples. An extraction chromatographic resin UTEVA was used for the separation of uranium. A TEVA resin was chosen to retain thorium isotopes, while radium isotopes passed both columns [96]. In more detail, the iron precipitate was dissolved with 6 mL 3 M HNO_3 – 1 M $Al(NO_3)_3$ and loaded onto TEVA and UTEVA resins in tandem (Figure 3.3). The columns were first washed with 30 mL of 3 M HNO_3 to elute radium. Then the columns were separated and the UTEVA column was washed with 6 mL 3 M HNO_3 to remove residual radium. Radium isotopes and a barium tracer were co-precipitated from the radium-barium containing eluate with $PbSO_4$ as described by Lozano et al. [97]. In the next step a clean beaker for collecting thorium radioisotopes was placed under the TEVA column. The columns were washed with 20 mL of 9 M HCl and 5 mL of 6 M HCl. The collected solution was evaporated until dryness, the residue dissolved with 15 mL 1 M HCl and then micro-precipitation of thorium radioisotopes was performed. The UTEVA column was further washed with 5 mL of 9 M HCl and 25 mL 5 M HCl – 0.05 M oxalic acid in order to wash out residues of thorium and other interfering radioisotopes. Then uranium radioisotopes were washed out with 15 mL 1 M HCl. The scheme of consecutive radiochemical separation of polonium, radium, thorium and uranium is presented in Figure 3.3. After the separation processes, alpha emitting sources were prepared by micro-co-precipitation [98].

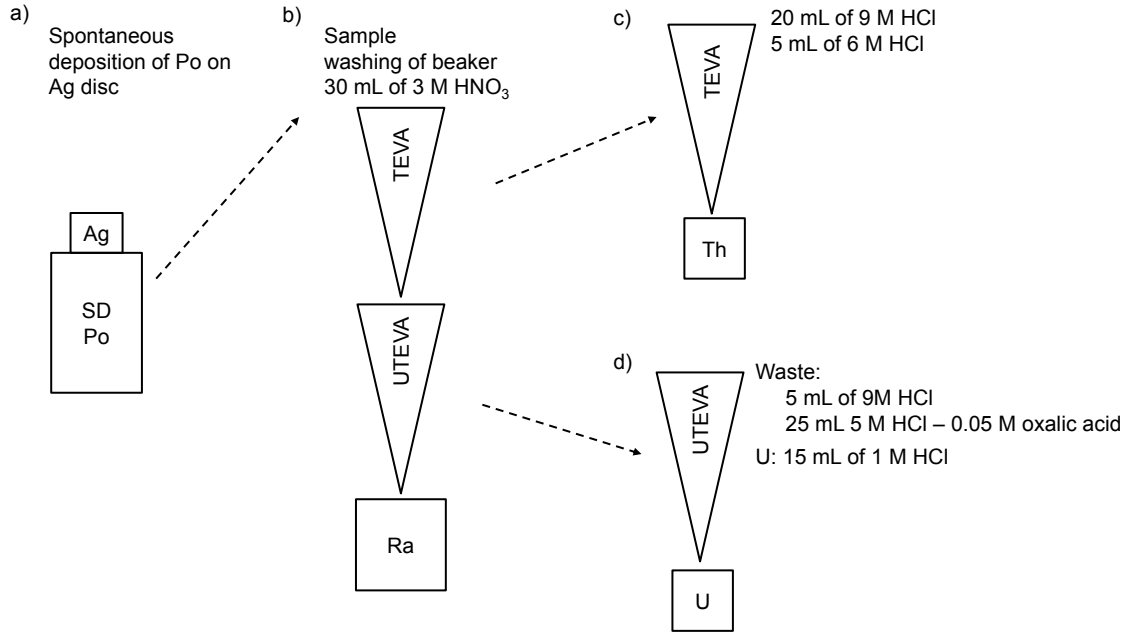


Figure 3.3: Scheme of radiochemical separation procedure for polonium, radium, thorium and uranium determination in plants.

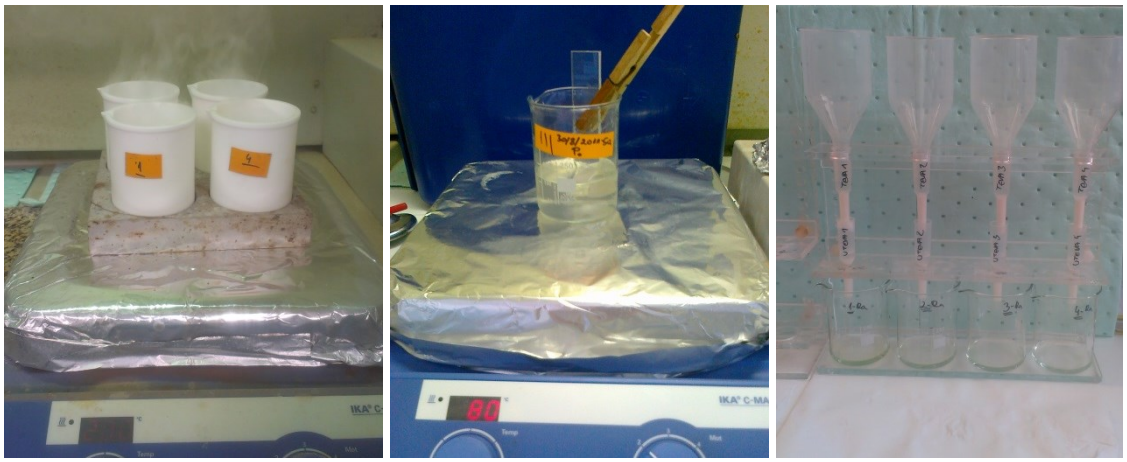


Figure 3.4: Dissolution of the sample in a Teflon beaker (left), spontaneous deposition of polonium (middle) and consecutive separation of radium, uranium and throium (right).

3.1.3.3 Alpha-particle spectrometry

An alpha spectrometer system (Alpha Analyst, Canberra, USA), with passivated implanted planar silicon (PIPS) semiconductor detectors was used for alpha particle spectrometric measurements of the deposited polonium, radium, uranium and thorium radioisotopes. The selected radioisotopes were measured at energies of 4.15 MeV, 4.65 MeV, 4.69 MeV and 5.31 MeV for ^{238}U, ^{230}Th, ^{226}Ra and ^{210}Po, respectively (Figure 3.6). The tracers were measured at energies of 5.28 MeV, 4.89 MeV and 4.88 MeV for ^{232}U, ^{229}Th and ^{209}Po, respectively. The counting efficiency was 29 %, on average. The calibration of the detector was made with a standard radionuclide source containing ^{238}U,

^{234}U, ^{239}Pu and ^{241}Am (code: 67978–121), obtained from Analytics, Inc. The calibration was made for a range of energies from 3900 to 4290 keV for ^{238}U, from 4580 to 4860 keV for ^{234}U, from 4950 to 5240 keV for ^{239}Pu and from 5275 to 6690 keV for ^{241}Am. Data acquisition analysis was performed using Genie 2000 spectroscopy system software. The disc sources with deposited radioisotopes were measured until a statistically sufficient number of counts was accumulated, that is to reach counting uncertainty less than 10 % or maximum counting time of one week.

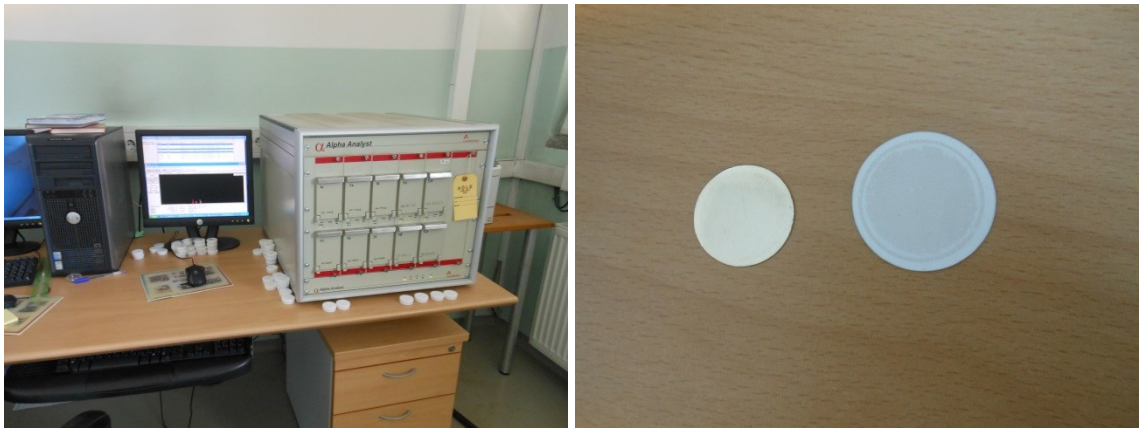


Figure 3.5: An alpha spectrometer system (left). Silver disc with deposited polonium radioisotopes and polypropilen filter with micro co-precipitated uranium, thorium and radium radionuclides, respectively (right).

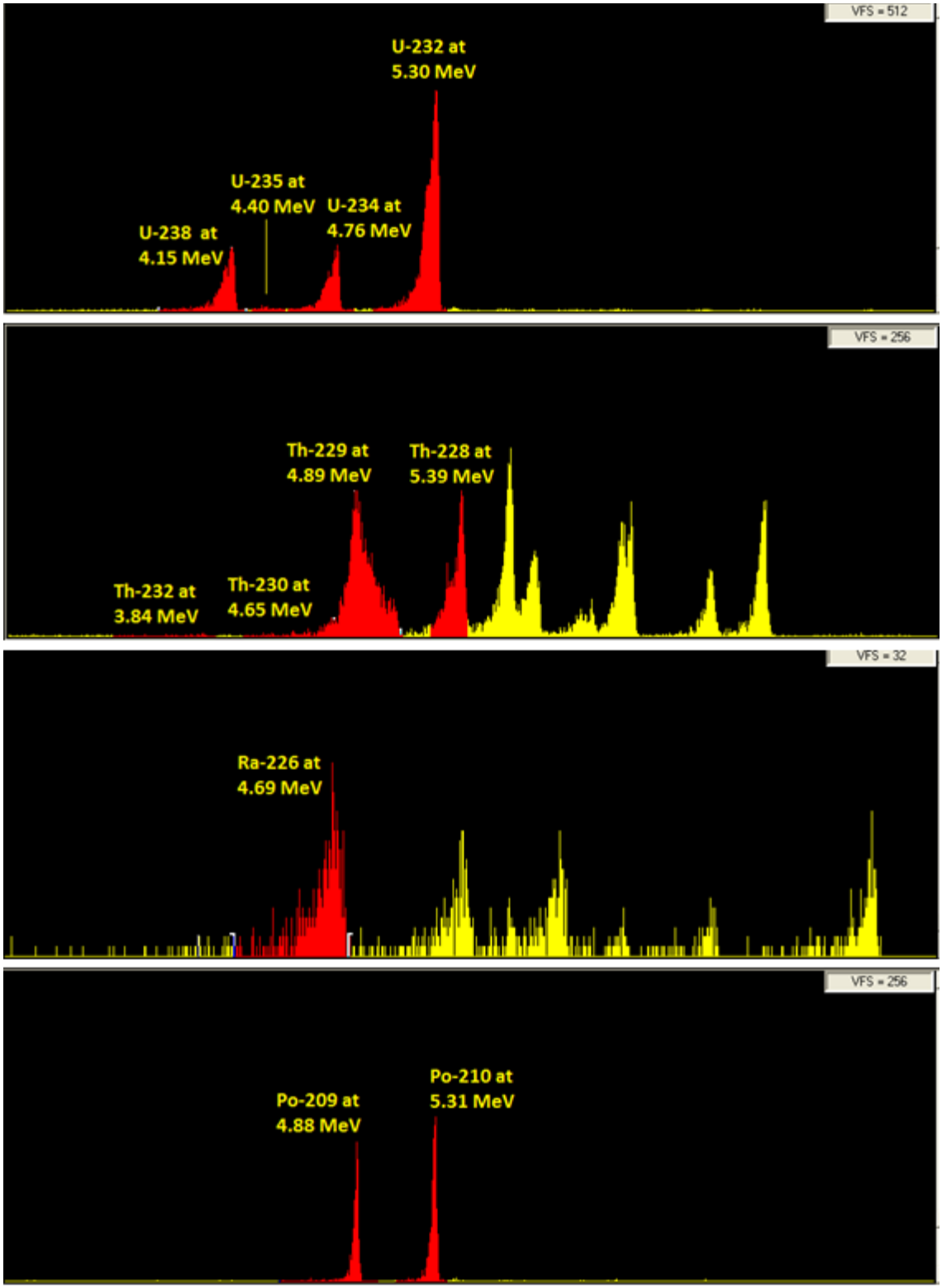


Figure 3.6: Alpha spectrum of uranium, thorium, radium and polonium radioisotopes.

3.1.3.4 Method validation

Two certified reference materials were used for quality control of the alpha-particle spectrometric measurements; IAEA 447 (Moss soil) and NIST 4359 (Seaweed). The uranium measurements results for IAEA 447 were slightly lower than the certified values,

probably due to incomplete digestion of the soil sample using the proposed dissolution method, as uranium is expected to be incorporated into the structure of difficultly soluble minerals. Moreover, the results and discussion of the study of uranium in the residues after decomposition of soil samples are presented in more detail in Appendix A.1 [87]. However, for the plant matrix of NIST 4359 the obtained results were within uncertainties (Table 3.1). Fairly good agreement was found for the other radionuclides in both reference materials and the results mostly comply with the certified values despite some relatively high measurement uncertainties. The solely exception was ^{210}Po in IAEA 447, which was probably due to the applied dissolution procedure, as already discussed in the previously published research (Appendix A.2) [89].

Table 3.1: Comparison of assigned/certified and measured activity concentrations (Bq kg^{-1} dry mass) obtained by alpha-spectrometric determinations in reference materials.

Reference material	Radionuclide	Certified value[a]	Information value	Measured value[b]
IAEA 447	^{238}U	22.2 ± 1.6		19.5 ± 2.0 (4)
(moss soil)	^{234}U	21.8 ± 1.6		17.4 ± 1.8 (4)
	^{226}Ra	25.1 ± 4.0		33.1 ± 2.6 (4)
	^{232}Th		37.3 ± 4.0	32.6 ± 3.8 (4)
	^{210}Pb(^{210}Po)	424 ± 40		349 ± 29 (4)
NIST 4359	^{238}U	8.67 ± 0.54		8.44 ± 0.89 (6)
(seaweed)	^{235}U	0.400 ± 0.047		0.348 ± 0.135 (6)
	^{234}U	9.5 ± 1.1		8.91 ± 0.86 (6)
	^{226}Ra		5.7	5.86 ± 0.77 (6)
	^{230}Th		3.3	5.19 ± 0.53 (6)
	^{210}Pb(^{210}Po)	21.0 ± 3.0		22.4 ± 2.9 (6)

[a] From the reference sheet (IAEA) or certificate (NIST); uncertainty values shown as combined expended (k = 2) uncertainties.

[b] The values are presented as averages with standard deviations (number of replicates in parentheses).

3.2 Plant Pot Experiment

3.2.1 Design of pot experiment

Three different types of crops from the Brassicaceae family were sown on contaminated and non-contaminated soil with high organic content (45 %). Soil mixtures were placed in 2 L plastic pots. Radish (*Raphanus sativus* 'Poletna bela', Semenarna Ljubljana d.d.) was chosen as a root vegetable, while savoy (*Brassica oleracea* L. ssp. *oleracea* var. *sabauda* 'Eisenkopf', Semenarna Ljubljana d.d.) has an above ground edible plant part. Rocket (*Diplotaxis tenuifolia* (L.) DC., Semenarna Ljubljana d.d.) was chosen according to its increasing importance as a vegetable and spice [94]. The plant pots were randomly placed on a shelf, arranged in five blocks (one block for one replicate) to minimize influence of random experimental factors (potential inhomogeneity of soil and UMT mixtures or non-steady lighting, heat or humidity). Design of the pot experiment without random placing is shown in Figure 3.7. Plants were irradiated by fluorescent lamps (Fluora, Osram, 58 W) with a daily light phase of 12 h. Previous studies showed that

the 12 h ventilation per day is sufficient for plant growth. Additionally, it is enough for prevention of contamination of leaves with gaseous ^{222}Rn decay products [99]. The experiment was performed in the Hot Cells Facility of the Jožef Stefan Institute (Ljubljana, Slovenia) as the facility is licensed for work with radioactive material, such as UMT.

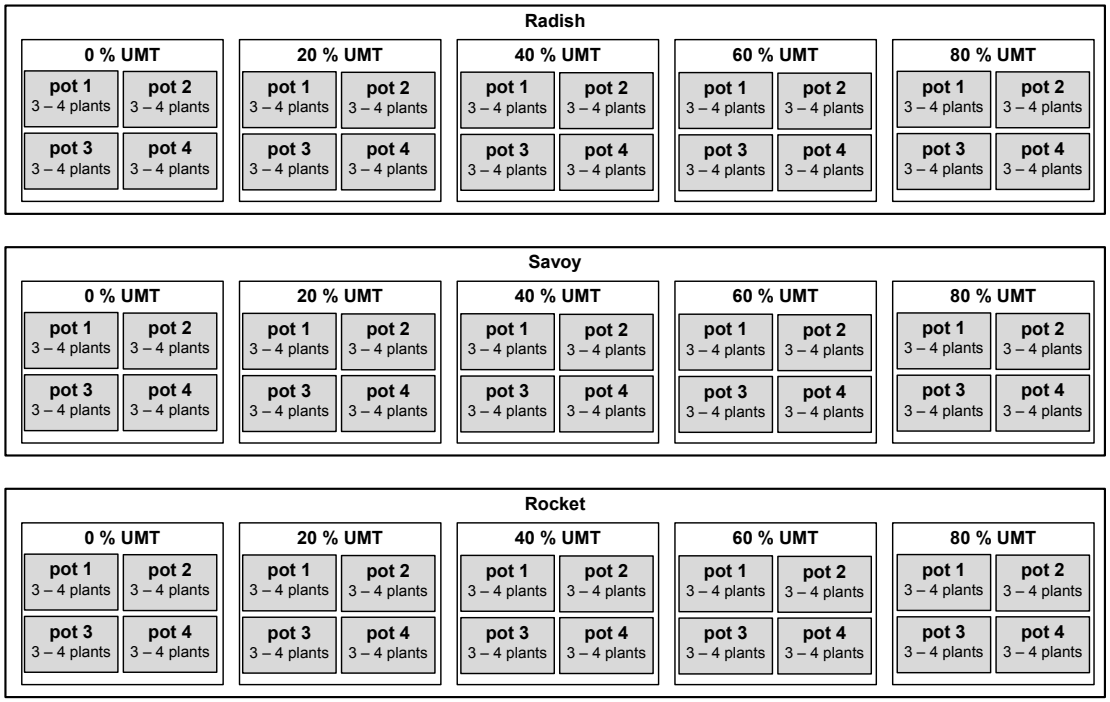


Figure 3.7: Scheme of pot experiment with indicated amounts of UMT.

3.2.2 Preparation of various contamination scenarios

Contaminated soils were prepared by mixing commercially available substrate (Humko, Kompomix) with UMT (Figure 3.8) at four different mass ratios, representing different contamination scenarios. The non-contaminated soil was used as a control. The tailings samples were taken from the Boršt tailings pile [4] of the former Žirovski vrh uranium mill and mine (latitude: 46°05′46.10″N; longitude: 14°10′38.19″E; altitude 617 m) [28]. Soil and tailings samples were air-dried for three weeks, sieved to pass through 5 mm sieve and mixed in mass ratios, representing 20, 40, 60 and 80 % of UMT content in the contaminated soil, respectively.



Figure 3.8: Comercialy availabe substrate (left) and U-mill tailings after sieving (right).

3.2.3 Controlled experimental conditions

Seeding of the selected plants was performed in February 2012, while the harvest of fully-grown plants was in July, i.e. after 5 months of growth (Figure 3.9 and Figure 3.10). The plants were grown in the temperature range from 22 to 25 °C, relative humidity ranged from 40 to 60 % and light irradiation time was from 8 a.m. to 8 p.m. with photosynthetic photon flux density ranging from 53 to 94 µmol s^{-1} m^{-2} (LI–COR Quantum sensor, USA) at the plant surfaces (30 cm below the lamps). In every pot there were up to 4 plants grown to gain sufficient sample mass. The plants in each pot were checked daily and irrigated with an appropriate amount of deionised water, if necessary. To prevent contamination of plants by the soil dust from the pots, all possible pathways of external contamination were considered. At the beginning of seeding, garden mats were put onto the soil already placed in pots to ensure the maximum protection of plants against the soil dust resuspension. In addition, the ventilation of the Hot Cell Facility was enabled during daytime phase (from 8 a.m. to 8 p.m.) to prevent potential contamination of plants by ^{222}Rn decay products due to the presence of relatively high quantities of UMT in the pots and consequently presence of ^{222}Rn in the air [28]. However, one week of measurements for situation without and one week of measurements with ventilation (24 h per day) were performed in February 2013. The pots with UMT were present at time of ^{222}Rn measurements. The mean activity concentrations of ^{222}Rn in the air were 21 and 156 Bq m^{-3} for situations with and without ventilation, respectively. ^{222}Rn was measured as described by Vaupotič et al. [100].



Figure 3.9: Savoy (in the front of left picture) at 1 month, radish at 1 month (middle) and radish at 2 months (right) of growth.



Figure 3.10: Fully grown radish (left) and rocket (right).

3.2.4 Sample preparation for radionuclides analyses

3.2.4.1 Soils

Samples of soil mixtures as well as control soil were taken before the plants seeding. Three soil samples were randomly taken for each experimental treatment (control, 20, 40, 60 and 80 % of UMT content) from the amount of 3 kg of contaminated and non-contaminated soil prepared for the pots. Collected soil samples were dried in an oven (ST–05, Instrumentaria, Zagreb) for 3 days at 60 °C, sieved to pass through 2 mm sieve (Fritsch) and left at room temperature to achieve an ambient humidity, i.e. constant mass [28].

3.2.4.2 Plants

After the growing period, plants were harvested and prepared for the determination of selected radionuclides. Plant parts were separated on shoot (leaves, stems) and root system (roots or tubers) [101]. This separation seemed most optimal in order to have sufficient sample mass. The chosen separation presents underground and aboveground plant parts. Further, plants were carefully washed with tap water, dried in an oven (SP–

260, Kambič) for 3 days at 40 °C and dry mass was recorded after reaching equilibrium with ambient conditions. Dried plant leaves and stems were milled by knife mill (Retsch, Grandomix GM 200, Germany) while tubers and roots were milled by rotor mill (Pulverisette 7, Fritsch, Idar-Oberstein, Germany) and homogenized as solid.



Figure 3.11: Oven SP–260, Kambič (left), knife mill Retsch, Grandomix GM 200 (middle) and rotor mill, Pulverisette 7, Fritsch (right).

3.2.4.3 Biochemical and physiological conditions

3.2.4.3.1 Photochemical efficiency

Fluorescence measurement is a non-destructive, simple and sensitive method for detecting the photoinhibitory effects of environmental stressors on higher plants [102], [103]. Chlorophyll fluorescence was measured in situ on vital plants, at least on 5 plants from each replicate with fluorometer (PAM 2100 Chlorophyll Fluorometer, Heinz Walz GmbH, Germany). Measurements of chlorophyll fluorescence were made after 10 min of darkness, provided by dark-adaptation clips. Fluorescence was excited with a saturating beam of "white light" (PPFD = 8000 μmol m^{-2} s^{-1}, 0.8 s) [104].

3.2.4.3.2 Photosynthetic pigments

To assess the content of chlorophylls a and b and carotenoids, leaves of five plants from every replicate were measured according to the Lichtenthaler and Buschmann procedure [105], [106]. For each replicate to be tested at least 0.1 g (f.m.) of chosen leaf was placed in a mortar, 10 mL of acetone was added and ground with a pestle. The turbid pigment extract comprising chlorophylls and total carotenoids was transferred to graduated glass centrifuge tubes and centrifuged at 4000 rpm at 4 °C for 4 min. After the centrifugation step, the exact volume of solution was taken down. For the spectrophotometric analyses, an aliquot of the clear extract (supernatant) was transferred to a 1 cm path length cuvette and absorbance readings against a solvent blank in a UV–VIS spectrometer at three wavelengths were taken: 662 nm, 645 nm and 470 nm for chlorophyll a, chlorophyll b and carotenoids, respectively. The water content of the chosen leaf was determined prior to spectrophotometric analyses. The pigment content was calculated using Eq. (3.1), Eq. (3.2) and Eq. (3.3) for chlorophyll a, chlorophyll b and carotenoids, respectively:

$$c_a = \frac{((11.24 \times A_{662}) - (2.04 \times A_{644})) \times V}{m_{d.m.}} \tag{3.1}$$

$$c_b = \frac{((20.13 \times A_{645}) - (4.19 \times A_{662})) \times V}{m_{d.m.}} \tag{3.2}$$

$$c_{car.} = \frac{((A_{470} \times 1000) - (1.9 \times c_a) - (63.14 \times c_b)) \times V \times 214}{m_{d.m.}} \tag{3.3}$$

where c is the concentration of the pigment; A is the absorbance at a specific wavelength; V is the volume of the pigment extract; $m_{d.m.}$ is the mass of the sample on a dry mass basis.



Figure 3.12: Homogenised plant sample for further analysis (left) and plant pigment extracts for determination of photosynthetic pigments (right).

3.2.4.3.3 Anthocyanins

The total anthocyanins content was determined as described by Drumm and Mohr [107]. The appropriate mass (at least 0.1 g of f.m.) of selected leaf was placed in a mortar, 10 mL of extraction media (methanol:HCl = 99:1) was added and ground with a pestle. The turbid pigment extract comprising anthocyanins was transferred to graduated glass centrifuge tubes and centrifuged at 4000 rpm at 4 °C for 4 min. After the centrifugation step, the exact volume of solution was written down. The solution was stored in refrigerator for 24 h. Next day, absorbance against a solvent blank in a UV–VIS spectrometer at wavelength of 530 nm was measured. The water content of the chosen leaf was determined prior to spectrophotometric analyses. The anthocyanins content was calculated using Eq. (3.4):

$$c_{ant} = A_{530} \times V \times m_{d.m.} \tag{3.4}$$

where c_{ant} is the concentration of anthocyanins; A is the absorbance at a specific wavelength; V is the volume of the pigment extract; $m_{d.m.}$ is the mass of the sample on a dry mass basis.

3.2.4.4 Determination of soil parameters

Organic matter content, pH value, the content of available P_5O_5 and K_2O, exchangeable Ca and Mg, cation exchange capacity (CEC), organic C, total N and C/N ratio were analysed by standardised methods at Jurana Company, Maribor. Four randomly taken samples from each bulk soil mixture and control soil were analysed. Organic matter

content was analysed according to ISO 10694:1995 (Walkely–-Black modification) [108], while total N was determined as described in ISO 13878:1999 [109]. pH was analysed as described in ISO 10390:2006 [110]. The content of available P_5O_5 and K_2O was determined by the calcium-acetate-lactate (CAL) method with inter-laboratory modification [111]. The exchangeable Ca, Mg, K and Na were extracted with ammonium acetate extraction method and finally, cation exchange capacity (CEC) was calculated [112]. The exchangeable acidity was determined according to Peech modification of the Mehlich buffer method (H^+) [112].

3.2.4.5 Gamma-ray spectrometric measurements of soils and ^{133}Ba tracer

Despite of optimisation of method for dissolution of soil samples for alpha particle spectrometry (Chapter 3.1.1), gammy-ray spectrometry was used for determination of selected radionuclides in soil samples since this method is less time and chemicals consuming as well as less labour intensive. On the other hand, alpha spectrometry is more appropriate for low activity concentrations and small sample mases. However, direct gamma-ray spectrometry was applied for measurements of ^{238}U, ^{226}Ra, ^{230}Th and ^{210}Pb in contaminated and control soils. ^{238}U was determined by measuring in equilibrium via its daughter product ^{234}Th (E_γ = 63.28 keV, P_γ = 3.75 % [56]), ^{226}Ra via its decay products ^{214}Pb (E_γ = 295.22 keV, P_γ = 18.41 % and E_γ = 351.93 keV, P_γ = 35.60 % [56]), ^{210}Pb at energy of 46.54 keV (P_γ = 4.25 % [56]) and ^{230}Th at 67.67 keV (P_γ = 0.38 % [113]). Three soil samples from each bulk soil mixtures and control were taken for the gamma-ray spectrometry. Properly prepared samples were measured by a coaxial HPGe detector (Canberra, USA) connected to a multichannel analyser (Canberra, USA). The procedure of preparation of soil samples for gamma-ray spectrometry is described in detail by Smodiš et al. [28]. However, cylindrical polystyrene containers of 5.9 cm inner diameter and 3.8 cm height (sample height) were filed with dry soil material and sealed hermetically using a glue gun, and stored for four weeks to allow radioactive equilibrium of the ^{226}Ra series to be established. The samples were measured until a statistically sufficient number of counts was accumulated, that is to reach counting uncertainty less than 5 % or maximum counting time of 28 h (100800 s).

Two IAEA reference materials (IAEA-447 Moss-soil and IAEA-434 Phosphogypsum) were used for quality control of the gamma-ray spectrometric measurements (Table 3.2). The obtained results showed fairly good agreement between the assigned and measured values.

Table 3.2: Comparison of assigned/certified and measured activity concentrations (Bq kg^{-1} dry mass) obtained by gamma-spectrometric determinations in reference materials.

Reference material	Radionuclide	Certified value[a]	Measured value[b]
IAEA 447	^{238}U	22.2 ± 1.6	17.4 ± 5.4 (4)
(moss soil)	^{226}Ra	25.1 ± 4.0	27.0 ± 1.8 (4)
	^{210}Pb	424 ± 40	364 ± 34 (4)
IAEA 434	^{238}U	120 ± 22	82 ± 19 (4)
(phosphogypsum)	^{226}Ra	780 ± 124	730 ± 30 (4)
	^{210}Pb	680 ± 116	591 ± 52 (4)
	^{230}Th	211 ± 18	<MDA[c] (4)

[a] From the reference sheet; uncertainty values shown as combined expended (k = 2) uncertainties

[b] The values are presented as averages with standard deviations, (number of replicates in parentheses).

[c] Minimum detectable activity (MDA = 292 Bq kg^{-1} for m = 99.8 g, $t_{measurement}$ = 100.000 s).

Radiochemical recovery for ^{226}Ra in plants determined by alpha particle spectrometry was also determined via gamma-ray measurements of the added ^{133}Ba tracer. ^{133}Ba was measured at energy of 356.02 keV on a gamma-ray spectrometer consisting of HPGe detector connected to a multichannel analyser (Spectrum Master 919, Ortec, UK). ^{133}Ba activity was determined relative to the ^{133}Ba certified standard solution with activity traceable to SI units, which was evaporated on aluminium disc to achieve the same geometry as the sample [92].

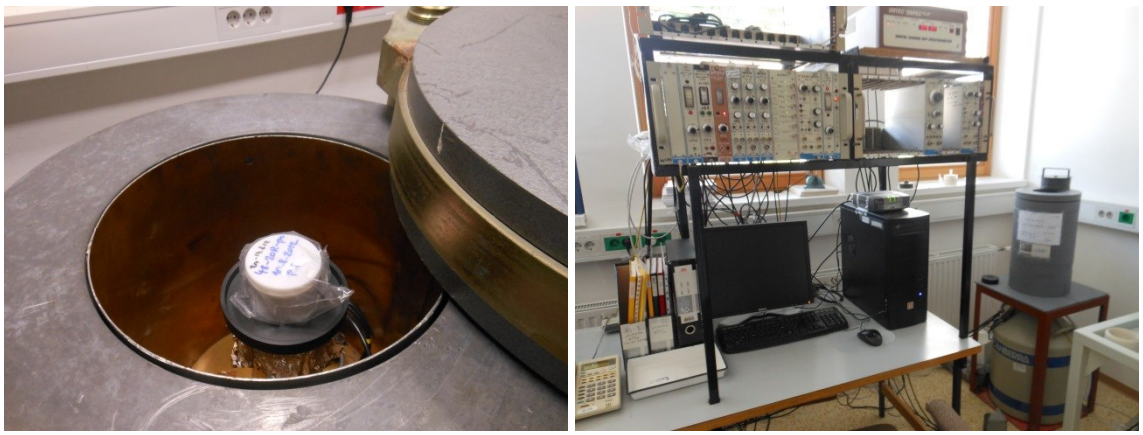


Figure 3.13: HPGe detector and cylindrical polystyrene container filled with soil sample (left) and HPGe gamma detector connected to a multichannel analyser for ^{133}Ba activity measuremnt (right).

3.2.4.6 Alpha-particle spectrometry

Optimised radiochemical procedure for consecutive determination of ^{210}Po, ^{226}Ra, ^{234,235,238}U and ^{230,232}Th in plants was used for determination of selected radionuclides in plant samples (Chapter 3.1.3.3). ^{210}Po measurement was used to determine content of its parent ^{210}Pb in the samples since they were in secular equilibrium [114], i.e. approximately two years from harvesting. Additionally, only the results corresponding to the ^{238}U isotope were considered since the behaviour of the uranium isotopes ^{238}U and ^{234}U is similar (they are in secular equilibrium).

3.3 Determination of CR Factors

The radionuclide concentration ratios (CR) were determined as the ratios of the measured activity concentrations (Bq kg^{-1} d.m.) in the plants (leaves or tubers) to the measured activity concentrations in the soils [10].

3.4 Assessment of Annual Effective Dose

For the calculation of the annual effective ingestion dose due to ^{238}U, ^{230}Th, ^{226}Ra and ^{210}Pb in the studied vegetables Eq. (3.5) and Eq. (3.6) were used.

$$E_{ing} = e(g)_{radioisotope} \times a_{radioisotope} \times m_{avg} \tag{3.5}$$

$$E_{ing,max} = e(g)_{radioisotope} \times a_{radioisotope} \times m_{max} \tag{3.6}$$

where E_{ing} and $E_{ing,max}$ are the annual effective ingestion doses due to ^{238}U, ^{230}Th, ^{226}Ra or ^{210}Pb in Sv y^{-1}; $e(g)$ is the committed effective dose per unit of intake for adults and is 4.5×10^{-8}, 2.1×10^{-7}, 2.8×10^{-7}, 6.9×10^{-7} Sv Bq^{-1} [115] for ^{238}U, ^{230}Th, ^{226}Ra and ^{210}Pb, respectively; $a_{radioisotope}$ is the activity concentration of ^{238}U, ^{230}Th, ^{226}Ra or ^{210}Pb in the sample in Bq kg^{-1} fresh mass; m_{avg} and m_{max} are the average or maximum annual intake of radish, savoy or rocket in kg y^{-1}.

The total annual effective ingestion doses (ΣE_{ing}) considered average intake of studied vegetables and total annual effective ingestion doses ($\Sigma E_{ing,max}$) considered maximum intake of studied vegetables due to ^{238}U, ^{230}Th, ^{226}Ra and ^{210}Pb were calculated using Eq. (3.7) and Eq. (3.8), respectively:

$$\sum E_{ing} = E_{^{238}U} + E_{^{230}Th} + E_{^{226}Ra} + E_{^{210}Pb} \tag{3.7}$$

$$\sum E_{ing,max} = E_{^{238}U,max} + E_{^{230}Th,max} + E_{^{226}Ra,max} + E_{^{210}Pb,max} \tag{3.8}$$

The data for average and maximum annual intake of the selected vegetables were obtained from EFSA (European Food Safety Authority) Comprehensive European Food Consumption Database [116]. For the conservative dose assessment, the average annual intake of radish, savoy and rocket was determined as average of 23, 14 and 6 individual dietary surveys from different European countries, respectively. The maximum annual intake of radish, savoy and rocket represents the maximum consumption among considered studies. Data for adults as well as for lactating mothers and pregnant women were taken into consideration since there is a relatively small number of consumers of radishes, savoy and rocket. So, lactating mothers and pregnant women were considered as adults for establishing a dataset for average/maximum consumption calculation, as in the investigated databases a relatively small number of studies that included data about savoy and rocket consumption occurred. The annual intake was calculated for adult persons with 60 kg of body mass since in EFSA Comprehensive European Food Consumption Database the consumption is presented in grams per kg of body mass per

day. For the calculation of annual intake of savoy, data for kale (*Brassica oleracea* L. ssp. *oleracea* convar. *acephalea* (DC.) Alef.) food category was used, assuming that the consumption of savoy and kale is in the same range. The calculated average and maximum annual intakes as well as calculated average and maximum annual intakes used for dose assessments are presented in Table 3.3.

Table 3.3: The calculated average and maximum annual intakes obtained from EFSA database, and calculated average and maximum annual intakes used for dose assessments.

	Data from EFSA database		Data used in dose assessment	
Plant	Average annual intake [g kg^{-1} b.m. per day]	Maximum annual intake [g kg^{-1} b.m. per day]	Average annual intake [kg y^{-1}]	Maximum annual intake [kg y^{-1}]
Radish	0.007 ± 0.012	0.054	0.154	1.183
Savoy	0.010 ± 0.019	0.066	0.215	1.445
Rocket	0.005 ± 0.003	0.011	0.113	0.241

3.5 Statistical Analysis of Data

Statistical analysis of data was performed using Microsoft Excel 2010, version 14.0.7153.5000 (64 bit). A Pearson's product-moment correlation (r) with corresponding significance level (p) was used for evaluation of linear relationship between two sets of variables. The value of r is always between +1 and –1. If the r value was > 0.5 the linear correlation was considered to be strong, between 0.3–0.5 moderate and between 0.1–0.3 small. A t-test was applied to determine whether the correlation coefficient was "highly significant" ($p < 0.001$), "significant" ($0.001 < p < 0.05$) or "not significant" ($p > 0.05$). The level of statistical significance of correlation coefficient was described by the p value (two-tailed). If the r value was greater than 0.1 (small correlation) and p value was greater than 0.05 (correlation not statistically significant), the correlation was reported as not linear. For the data analysis purposes the results below the respective minimum detectable activities (MDAs) were substituted by half the values of the MDAs. Throughout the text correlations among all concerned variables are presented and discussed.

The Box-Cocx transformation of data was applied in order to ensure conditions for ANVA usage, such as normal distribution and homogeneity. After the data transformation, simple statistics including analysis of variance by ANOVA with Duncan's test for comparisons of means were carried out in order to find out statistically significant parameters for possible discrimination of various radionuclides uptake in leaves and roots among plants under investigations. Statistical calculations were carried out using the XLSTAT software package (Addinsoft, New York, USA). According to the ANOVA test all radionuclide parameters were found statistically significant ($p < 0.05$) for possible discrimination of radionuclides activity concentrations in root and aboveground plant's parts. Further, for discrimination of each observation within the same studied vegetable type, the multivariate analysis by discriminant analysis (DA) was used.

Chapter 4

Results

4.1 Optimization of Radio-Analytical Procedures

The results of the accomplished studies are presented in Jurečič et al. [87] and Planinšek et. al. [89] (Appendix A) in more detail.

4.2 Soil

4.2.1 Radionuclides in soils

The activity concentrations of particular radionuclides in the soils mixed with UMT were obtained by gamma-ray spectrometry. The results are presented in Table 4.1 and are shown as average values with standard deviations of 3 independently measured samples randomly taken from each soil/UMT mixture before plant seeding. Standard combined measurement uncertainties for the measured radionuclides in soils were 5–11 % and 31–58 % relative for contaminated and control soil samples, respectively, for ^{238}U; 13–26 % relative for contaminated soil samples for ^{230}Th; 2 % and 5–6 % relative for contaminated and control soil samples, respectively, for ^{226}Ra; 4–5 % and 9–10 % relative for contaminated and control soil samples, respectively, for ^{210}Pb, all with k = 1. These activity concentrations were also used for the calculation of concentration ratios of ^{238}U, ^{226}Ra, ^{230}Th and ^{210}Pb in the cultivated plants.

Table 4.1: Activity concentrations (Bq kg^{-1}, d.m.) of selected radionuclides in UMT–contaminated and control soils.

UMT content	^{238}U	^{226}Ra	^{230}Th	^{210}Pb
Control	19 ± 6	27 ± 4	< MDA[a]	90 ± 5
20 %	283 ± 26	2000 ± 100	1100 ± 140	2100 ± 150
40 %	454 ± 47	3500 ± 70	2000 ± 230	3600 ± 190
60 %	731 ± 37	5300 ± 150	2200 ± 200	5000 ± 100
80 %	895 ± 88	6700 ± 170	3400 ± 800	6300 ± 90

[a] For statistical analysis of ^{230}Th in the control, ½ of MDA was used. The MDA was 210 Bq kg^{-1}.

Selected plants were cultivated on UMT-contaminated soil containing ^{238}U, ^{230}Th, ^{226}Ra and ^{210}Pb. ^{238}U, ^{230}Th, ^{226}Ra and ^{210}Pb activity concentrations in contaminated soils were 15–47, 11–32, 75–250 and 23–71 times higher compared to controls (non-contaminated soil), respectively.

4.2.2 Properties of prepared soil mixtures

Properties (pH, content of available P_2O_5 and K_2O, organic matter, organic C, total N, C/N, cation exchange capacity and exchangeable cations) of the prepared soil mixtures for different contamination scenarios and control are shown in Table 4.2. The values are presented as average values with standard deviations of four replicates taken from each contamination treatment in the beginning of the experiment, i.e. before seeding. Large variances in properties among soil mixtures can be observed as a consequence of specific properties of UMT having a large inorganic fraction; UMT is not really soil but the by-product of intensive acid treated uranium ore during the uranium yellow cake production process.

Table 4.2: Properties of UMT-contaminated and control soils.

Parameter[a]	Control soil	20 % of UMT in the soil	40 % of UMT in the soil	60 % of UMT in the soil	80 % of UMT in the soil
pH in $CaCl_2$	6.7 ± 0.1	6.6 ± 0.0	6.5 ± 0.0	6.6 ± 0.0	6.7 ± 0.1
Available P_2O_5 [mg kg^{-1}]	800 ± 37	632 ± 42	485 ± 19	303 ± 24	148 ± 6
Available K_2O [mg kg^{-1}]	1205 ± 57	919 ± 19	728 ± 64	437 ± 29	273 ± 29
Organic matter [%]	46 ± 6	35 ± 2	28 ± 6	18 ± 3	8.0 ± 2.9
CEC [cmol kg^{-1}]	86 ± 2	88 ± 3	91 ± 2	96 ± 1	108 ± 0.4
C [%]	26 ± 2	20 ± 1	16 ± 1	10 ± 1	4.6 ± 0.4
N [%]	1.1 ± 0.01	0.93 ± 0.03	0.72 ± 0.04	0.43 ± 0.02	0.24 ± 0.02
C/N	24 ± 3	22 ± 2	22 ± 1	24 ± 3	20 ± 3
Exchangeable Ca [%]	66 ± 1	73 ± 2	78 ± 1	87 ± 3	93 ± 2
Exchangeable Mg [%]	16 ± 0.1	12 ± 0.2	9.2 ± 0.6	6.0 ± 0.3	2.9 ± 0.3
Exchangeable K [%]	3.0 ± 0.1	2.4 ± 0.1	1.7 ± 0.1	1.1 ± 0.1	0.45 ± 0.10
Exchangeable Na [%]	0.60 ± 0.00	0.50 ± 0.00	0.40 ± 0.00	0.20 ± 0.00	0.10 ± 0.00

[a] The measured values were reported by chosen institution.

A Pearson's product-moment correlation assessment showed strong negative linear relationship between UMT content and most of soil parameters (available P_2O_5 and K_2O, organic matter, organic C, total N). A strong positive linear correlation was observed between UMT content and cation exchange capacity. However, no linear correlation was found between UMT content and pH as well as between UMT content and C/N ratio. The r and p values are presented in Table 4.3 in more detail.

Table 4.3: Pearson correlation coefficients with p values between UMT content in soil and several soil parameters (number of observations in parenthesis).

Parameter	UMT content
pH in $CaCl_2$	No correlation
Available P_2O_5 [mg kg^{-1}]	r (20) = –0.994, p = 1.12×10^{-18}
Available K_2O [mg kg^{-1}]	r (20) = –0.992, p = 1.68×10^{-17}
Organic matter [%]	r (20) = –0.991, p = 4.67×10^{-17}
CEC [cmol kg^{-1}]	r (20) = 0.893, p = 1.19×10^{-7}
C [%]	r (20) = –0.991, p = 4.09×10^{-17}
N [%]	r (20) = –0.995, p = 1.04×10^{-19}
C/N	No correlation

4.3 Radionuclides in Plants

The activity concentrations of selected radionuclides in the studied plants were measured by alpha particle spectrometry. The average activity concentrations with standard deviations for ^{238}U, ^{226}Ra, ^{230}Th and ^{210}Pb in radish, savoy and rocket grown in commercially available substrate at different UMT contamination levels are presented in Table 4.4. There were some large changes of activity concentrations observed in plants grown in mixtures containing 60 % UMT and 80 % UMT. These changes were particularly extreme for ^{226}Ra and ^{210}Pb. Therefore, this phenomenon requires further investigations to determine, along with the combination of various soil properties, the crucial parameters responsible for the observed changes in the uptake pattern of the radionuclides.

In further subchapters statistical analysis of data is presented. A Pearson's product-moment correlation (r) with corresponding significance level (p) was used for evaluation of linear relationship between two sets of variables. The value of r is always between +1 and –1. If the r value was > 0.5 the linear correlation was considered to be strong, between 0.3–0.5 moderate and between 0.1–0.3 small. A t-test was applied to determine whether the correlation coefficient was “highly significant” ($p < 0.001$), “significant” ($0.001 < p < 0.05$) or “not significant” ($p > 0.05$). The level of statistical significance of correlation coefficient was described by the p value (two-tailed). If the r value was greater than 0.1 (small correlation) and p value was greater than 0.05 (correlation not statistically significant), the correlation was reported as not linear.

Table 4.4: Activity concentrations (Bq kg^{-1} d.m.) of radionuclides in plants (number of measured samples in parenthesis).

Plant	UMT content	^{238}U	^{230}Th	^{226}Ra	^{210}Pb
Radish – leaves	Control	0.44 ± 0.11 (4)	2.1 ± 0.8 (4)	4.0 ± 1.9 (4)	3.1 ± 0.7 (4)
	20 %	0.58 ± 0.19 (4)	1.9 ± 0.4 (4)	63 ± 14 (4)	5.8 ± 1.8 (4)
	40 %	0.83 ± 0.63 (4)	1.7 ± 0.7 (4)	56 ± 24 (4)	8.0 ± 4.2 (4)
	60 %	1.2 ± 1.0 (4)	1.9 ± 0.7 (4)	43 ± 11 (4)	11 ± 7 (4)
	80 %	0.53 ± 0.16 (4)	2.0 ± 0.7 (3)	121 ± 11 (4)	32 ± 9 (4)
Radish – tubers	Control	0.19 ± 0.07 (4)	2.4 ± 0.7 (4)	1.2 ± 0.2 (4)	0.60 ± 0.38 (4)
	20 %	0.24 ± 0.05 (4)	2.4 ± 0.8 (4)	27 ± 8 (4)	4.4 ± 0.8 (4)
	40 %	0.37 ± 0.22 (3)	3.2 ± 1.1 (4)	34 ± 8 (4)	14 ± 5 (4)
	60 %	0.46 ± 0.19 (4)	3.9 ± 1.0 (4)	24 ± 5 (4)	18 ± 3 (4)
	80 %	0.98 ± 0.16 (4)	4.7 ± 1.6 (4)	74 ± 11 (4)	70 ± 6 (3)
Savoy – leaves	Control	0.32 ± 0.18 (4)	1.6 ± 0.5 (4)	2.4 ± 0.3 (4)	1.6 ± 0.3 (4)
	20 %	0.41 ± 0.11 (4)	1.6 ± 0.4 (4)	27 ± 9 (4)	3.0 ± 0.5 (4)
	40 %	0.50 ± 0.13 (4)	1.5 ± 0.3 (4)	21 ± 3 (4)	3.6 ± 0.3 (4)
	60 %	0.34 ± 0.18 (4)	2.1 ± 0.7 (4)	34 ± 4 (4)	7.0 ± 0.9 (4)
	80 %	0.43 ± 0.07 (4)	2.3 ± 0.5 (4)	67 ± 3 (4)	24 ± 4 (4)
Savoy – roots	Control	3.4 ± 1.0 (3)	14 ± 5 (4)	6.0 ± 1.1 (1)[a]	2.4 ± 0.7 (4)
	20 %	6.2 ± 1.7 (3)	22 ± 4 (4)	34 ± 5 (4)	26 ± 1 (4)
	40 %	9.5 ± 0.1 (3)	40 ± 13 (4)	63 ± 5 (4)	61 ± 10 (4)
	60 %	12 ± 5 (3)	55 ± 12 (4)	95 ± 27 (4)	101 ± 14 (4)
	80 %	29 ± 2 (3)	106 ± 11 (4)	232 ± 30 (4)	276 ± 36 (4)
Rocket – leaves	Control	0.39 ± 0.06 (4)	2.4 ± 0.7 (4)	1.9 ± 0.3 (4)	3.1 ± 0.6 (4)
	20 %	0.55 ± 0.08 (3)	3.7 ± 1.7 (4)	62 ± 14 (4)	6.1 ± 0.5 (3)
	40 %	0.52 ± 0.18 (4)	3.2 ± 0.6 (4)	61 ± 17 (4)	7.2 ± 2.2 (4)
	60 %	0.85 ± 0.28 (3)	4.5 ± 1.8 (4)	89 ± 14 (4)	12 ± 2 (4)
	80 %	1.8 ± 1.0 (3)	8.3 ± 1.4 (3)	87 ± 11 (3)	22 ± 4 (4)
Rocket – roots	Control	3.7 ± 1.8 (3)	21 ± 10 (4)	6.0 ± 3.2 (3)	2.2 ± 0.9 (4)
	20 %	13 ± 7 (3)	56 ± 30 (4)	53 ± 22 (4)	26 ± 6 (4)
	40 %	12 ± 22 (3)	51 ± 15 (3)	94 ± 26 (4)	79 ± 28 (4)
	60 %	3.5 ± 1.1 (4)	34 ± 10 (4)	94 ± 14 (4)	72 ± 8 (4)
	80 %	39 ± 65 (4)	250 ± 148 (4)	322 ± 102 (4)	247 ± 48 (4)

[a] The uncertainty value as standard combined uncertainty of one measure.

Statistical analyses of the differences between the radionuclides activity concentration in root system and shoot system of radish, savoy and rocket with ANOVA Duncan's Multiple Range test are presented in Table 4.5, Table 4.6 and Table 4.7.

Table 4.5: Statistical analysis of the differences between the radionuclides activity concentration in root system and shoot system of radish (Duncan's Multiple Range test, confidence interval of 95 %).

Contrast	UMT content	Difference	Standardized difference	Critical value	Pr > Diff	alpha (Modified)	Significant
^{238}U in	Control	1.0117	2.8440	2.2644	0.0575	0.1855	Yes
roots vs	20 %	0.9770	2.7464	2.3006	0.0962	0.2262	Yes
^{238}U in	40 %	0.7364	1.9165	2.3006	0.4128	0.2262	No
leaves	60 %	0.8998	2.5293	2.3288	0.1861	0.2649	Yes
	80 %	0.6692	1.8813	2.2165	0.2580	0.1426	No
^{230}Th in	Control	0.1583	0.8537				No
roots vs	20 %	0.1414	0.7627				No
^{230}Th in	40 %	0.5098	2.7495	2.3515	0.1481	0.3017	Yes
leaves	60 %	0.5429	2.9283	2.3515	0.1033	0.3017	Yes
	80 %	0.6413	3.2022	2.3515	0.0572	0.3017	Yes
^{226}Ra in	Control	1.6062	1.6710	2.0423	0.1051	0.0500	No
roots vs	20 %	4.5742	4.7586	2.2617	0.0004	0.1855	Yes
^{226}Ra in	40 %	2.5962	2.7009	2.1463	0.0295	0.0975	Yes
leaves	60 %	2.7249	2.8347	2.2136	0.0385	0.1426	Yes
	80 %	3.8480	4.0031	2.0423	0.0004	0.0500	Yes
^{210}Pb in	Control	1.8181	5.0478	2.0452	< 0,0001	0.0500	Yes
roots vs	20 %	0.2985	0.8287				No
^{210}Pb in	40 %	0.8610	2.3905	2.1492	0.0593	0.0975	Yes
leaves	60 %	0.9928	2.7564	2.1492	0.0263	0.0975	Yes
	80 %	1.4139	3.6343	2.0452	0.0011	0.0500	Yes

Table 4.6: Statistical analysis of the differences between the radionuclides activity concentration in root system and shoot system of savoy (Duncan's Multiple Range test, confidence interval of 95 %).

Contrast	UMT content	Difference	Standardized difference	Critical value	Pr > Diff	alpha (Modified)	Significant
^{238}U in	Control	2.995	3.310	2.045	0.003	0.050	Yes
roots vs	20 %	5.839	6.453	2.329	< 0,0001	0.265	Yes
^{238}U in	40 %	9.055	10.008	2.329	< 0,0001	0.265	Yes
leaves	60 %	11.969	13.228	2.329	< 0,0001	0.265	Yes
	80 %	28.865	31.902	2.329	< 0,0001	0.265	Yes
^{230}Th in	Control	1.7177	11.0201	2.2617	< 0,0001	0.1855	Yes
roots vs	20 %	2.0450	13.1200	2.2617	< 0,0001	0.1855	Yes
^{230}Th in	40 %	2.4540	15.7441	2.3493	< 0,0001	0.3017	Yes
leaves	60 %	2.3982	15.3861	2.2981	< 0,0001	0.2262	Yes
	80 %	2.6220	16.8215	2.2981	< 0,0001	0.2262	Yes
^{226}Ra in	Control	4.050	0.433	2.035	0.668	0.050	No
roots vs	20 %	0.000	0.000	2.206	1.000	0.143	No
^{226}Ra in	40 %	33.688	3.602	2.206	0.005	0.143	Yes
leaves	60 %	66.188	7.078	2.255	< 0,0001	0.185	Yes
	80 %	165.750	17.725	2.139	< 0,0001	0.098	Yes
^{210}Pb in	Control	0.3697	4.1582	2.0423	0.0003	0.0500	Yes
roots vs	20 %	1.6661	18.7376	2.2617	< 0,0001	0.1855	Yes
^{210}Pb in	40 %	2.0333	22.8663	2.2617	< 0,0001	0.1855	Yes
leaves	60 %	1.7783	19.9993	2.2617	< 0,0001	0.1855	Yes
	80 %	1.4256	16.0321	2.2617	< 0,0001	0.1855	Yes

Table 4.7: Statistical analysis of the differences between the radionuclides activity concentration in root system and shoot system of rocket (Duncan's Multiple Range test, confidence interval of 95 %).

Contrast	UMT content	Difference	Standardized difference	Critical value	Pr > Diff	alpha (Modified)	Significant
^{238}U in	Control	2.6954	8.8172	2.3437	< 0,0001	0.2649	Yes
roots vs	20 %	3.2132	9.8319	2.3437	< 0,0001	0.2649	Yes
^{238}U in	40 %	3.0790	10.0718	2.3437	< 0,0001	0.2649	Yes
leaves	60 %	1.8735	6.1286	2.1678	< 0,0001	0.0975	Yes
	80 %	2.4609	8.0499	2.3166	< 0,0001	0.2262	Yes
^{230}Th in	Control	1.4955	7.9700	2.3034	< 0,0001	0.2262	Yes
roots vs	20 %	1.7136	9.1327	2.3034	< 0,0001	0.2262	Yes
^{230}Th in	40 %	1.8003	8.8826	2.3538	< 0,0001	0.3017	Yes
leaves	60 %	1.3322	7.0998	2.2195	< 0,0001	0.1426	Yes
	80 %	1.6998	8.3868	2.3034	< 0,0001	0.2262	Yes
^{226}Ra in	Control	1.5939	1.6908	2.0484	0.1020	0.0500	No
roots vs	20 %	0.7818	0.8958	2.1524	0.6474	0.0975	No
^{226}Ra in	40 %	1.9171	2.1966	2.2674	0.2104	0.1855	No
leaves	60 %	0.2330	0.2670				No
	80 %	7.4393	7.8914	2.2674	< 0,0001	0.1855	Yes
^{210}Pb in	Control	0.3738	2.3878	2.0452	0.0237	0.0500	Yes
roots vs	20 %	1.1903	7.0400	2.2644	< 0,0001	0.1855	Yes
^{210}Pb in	40 %	1.9117	12.2120	2.3006	< 0,0001	0.2262	Yes
leaves	60 %	1.4173	9.0538	2.2165	< 0,0001	0.1426	Yes
	80 %	1.7986	11.4896	2.2644	< 0,0001	0.1855	Yes

In Figure 4.1 results of DA modeling of the data set of selected radionuclides in shoot system and root system of studied vegetables (radish, savoy, and rocket) are presented. The “L” in the graph legend represents shoot system at different contamination level, where “c” is for control whilst “R” is mark for root system. Results obtained showed that the groups of root systems comparing to shoot systems for all three plants are well separated. For radish, 58.09 % of total variance was explained by first discriminant function (F1) and 37.62 % by second discriminant function (F2). Total prediction ability for radish was 95.71 %. The distinction among savoy root and shoot samples is demonstrated along the F1 (77.13 %) and F2 (19.52 %) with total prediction ability of 96.65 %. It can be observed that root and shoot groups for rocket were also successfully separated with first discriminant function (F1) of 65.71 % and second discriminant function (F2) of 31.20 %. Total prediction ability was 96.91 %. The difference was significant for the most pairs of root system – shoot system as it is shown in Table 4.5, Table 4.6 and Table 4.7.

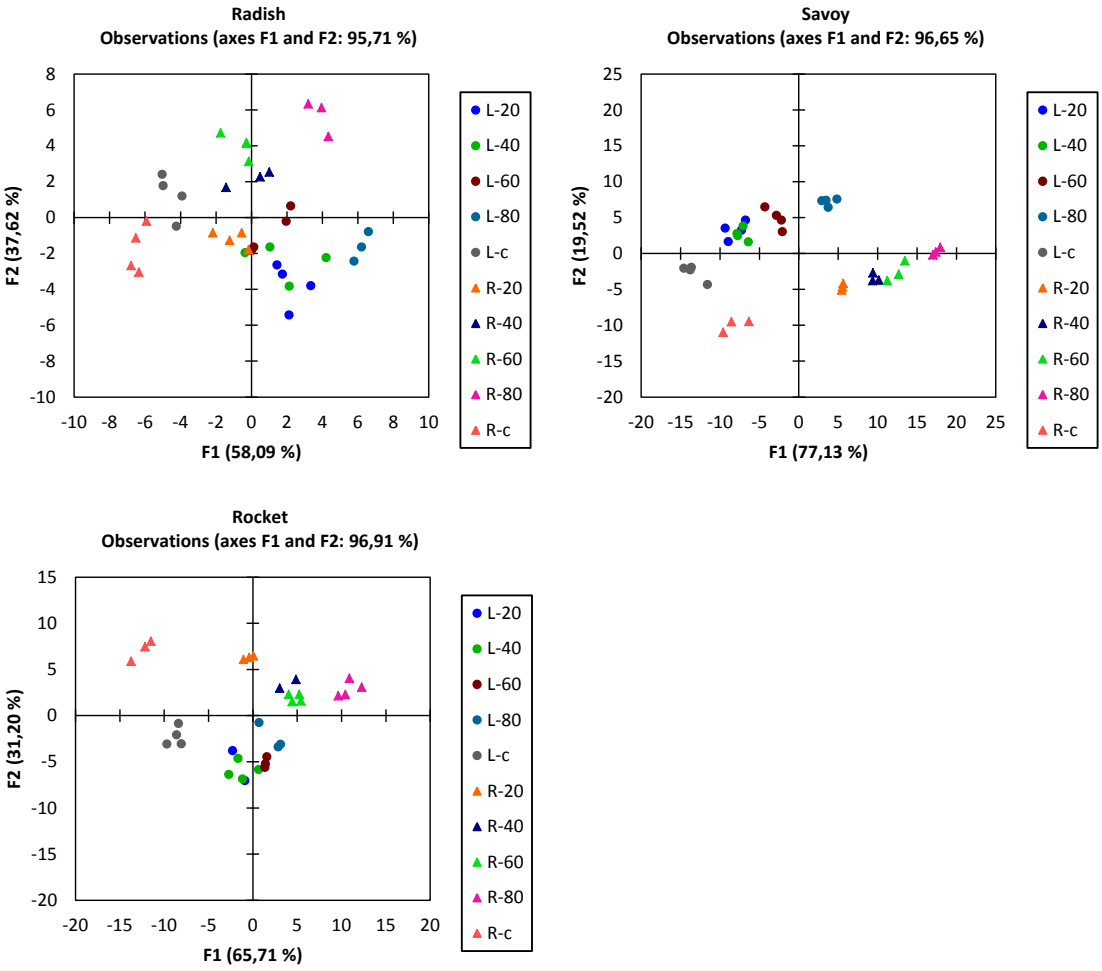


Figure 4.1: Results of DA modeling of the data set of selected radionuclides in shoot system (L) and root system (R) of studied vegetables (radish, savoy, and rocket).

4.3.1 ^{238}U in radish, savoy and rocket

^{238}U activity concentrations in shoot and root systems of radish, savoy and rocket are presented in Figure 4.2. Average activity concentrations of ^{238}U varied from 0.24–39 Bq kg^{-1} for different contamination scenarios while for the controls they were lower than 3.7 Bq kg^{-1} (Table 4.4). Standard combined measurement uncertainties for ^{238}U in plants were 4–29 % (12 %) and 9–26 % (14 %) relative for plants grown in contaminated and control soil, respectively (average in parenthesis, k = 1).

A Pearson's product-moment correlation with corresponding significance level was used for evaluation of linear relationship between ^{38}U activity concentrations in plants and ^{238}U content in soil as well as ^{238}U activity concentrations in different plant's parts and soil parameters in order to confirm impact of soil parameter on uranium plant uptake. The results of statistical analysis are shown in Table 4.8 and Table 4.9. A positive linear correlation between ^{238}U activity concentration in rocket shoot system and ^{238}U activity concentration in the contaminated soil was observed. No significant correlation was found for radish and savoy shoot systems (Table 4.8). Further, statistical analysis showed strong correlation between ^{238}U activity concentration in tubers of radish and ^{238}U activity concentration in growing substrate as well as between ^{238}U activity concentration in savoy root system and ^{238}U activity concentration in growing substrate.

No correlation was observed between ^{238}U activity concentration in rocket root system and ^{238}U activity concentration in soil (Figure 4.2).

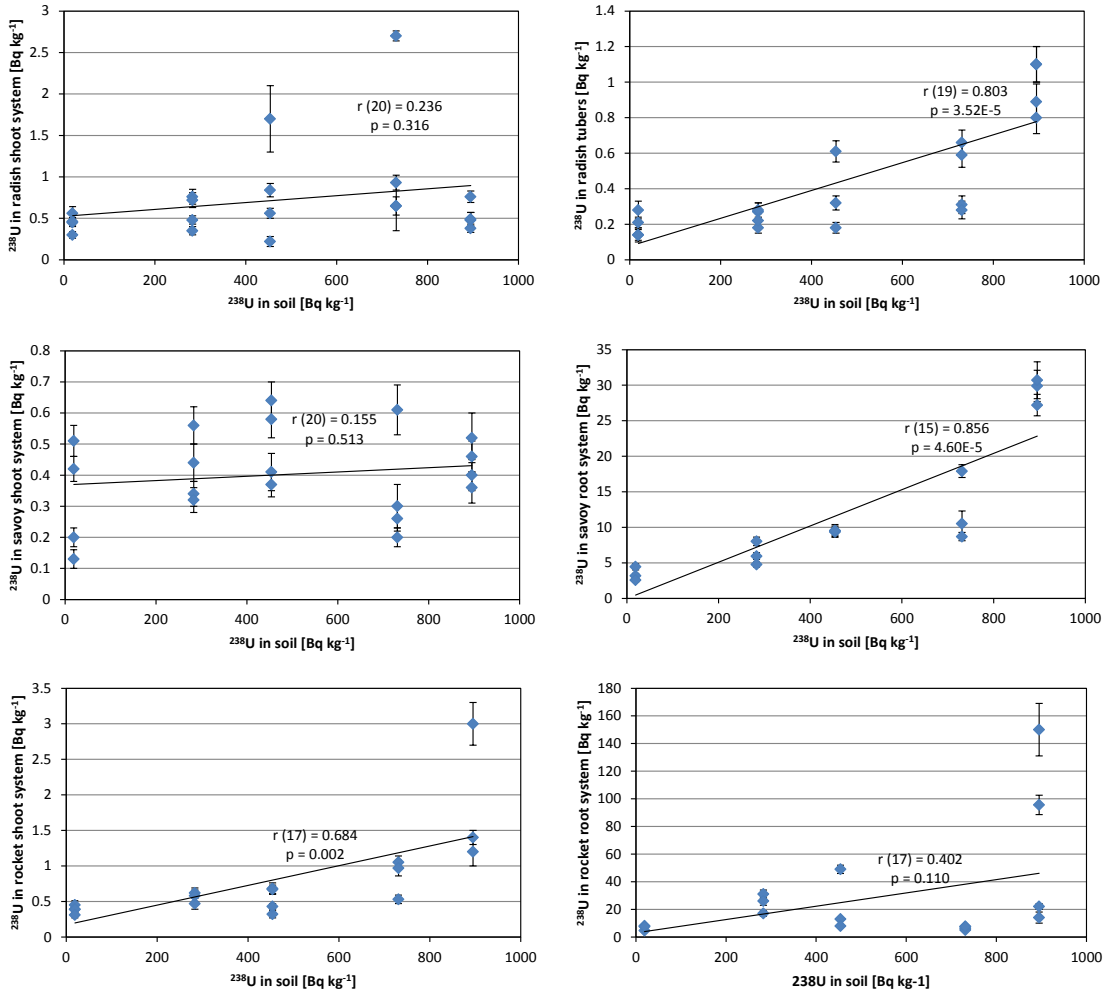


Figure 4.2: ^{238}U activity concentrations (d.m.) in shoot and root systems of radish, savoy and rocket (with Pearson correlation coefficient and *p* value, number of observations in parenthesis).

The ^{238}U activity concentrations in rocket shoot system and most of the soil parameters (available P_2O_5 and K_2O, organic matter content, total N, organic C and C/N ratio in the growing substrate) were strongly negatively correlated, except for pH where there were no correlations. ^{238}U activity concentrations in radish shoot system and soil parameters as well as ^{238}U activity concentrations in savoy shoot and soil parameters were not correlated. Moreover, the negative correlation was observed between ^{238}U activity concentrations in tubers of radish and most of the soil parameters (available P_2O_5 and K_2O, organic matter content, total N, organic C and C/N ratio in the growing substrate) as well as between ^{238}U activity concentrations in savoy root system and most of the soil parameters (available P_2O_5 and K_2O, organic matter content, total N, organic C and C/N ratio in the growing substrate), except for pH where there were no correlations. ^{238}U activity concentrations in rocket root system and soil parameters were not correlated, except for C/N where significant strong correlation was observed. However, ^{238}U activity concentrations in most of the studied plants (rocket shoot system,

tubers of radish, savoy and rocket root system) were positively correlated with CEC, otherwise no correlation was obtained.

Table 4.8: Pearson correlation coefficients with p values for ^{238}U activity concentrations in plants leaves and several tested parameters (number of observations in parenthesis).

Tested parameter	A ($^{238}U_{plant}$)		
	Radish – leaves	Savoy – leaves	Rocket – leaves
A ($^{238}U_{soil}$)	No correlation	No correlation	r (17) = 0.684, p = 0.002
pH	No correlation	No correlation	No correlation
P_2O_5	No correlation	No correlation	r (17) = –0.700, p = 0.002
K_2O	No correlation	No correlation	r (17) = –0.677, p = 0.003
Organic matter	No correlation	No correlation	r (17) = –0.706, p = 0.002
CEC	No correlation	No correlation	r (17) = 0.806, p = 9.47×10^{-5}
C	No correlation	No correlation	r (17) = –0.707, p = 1.50×10^{-3}
N	No correlation	No correlation	r (17) = –0.700, p = 0.002
C/N	No correlation	No correlation	r (17) = –0.659, p = 0.004

Table 4.9: Pearson correlation coefficients with p values for ^{238}U activity concentrations in underground plant parts and several tested parameters (number of observations in parenthesis).

Tested parameter	A ($^{238}U_{plant}$)		
	Radish – tubers	Savoy – roots	Rocket – roots
A ($^{238}U_{soil}$)	r (19) = 0.803, p = 3.52×10^{-5}	r (15) = 0.856, p = 4.60×10^{-5}	No correlation
pH	No correlation	No correlation	No correlation
P_2O_5	r (19) = –0.825, p = 1.37×10^{-5}	r (15) = –0.875, p = 1.65×10^{-5}	No correlation
K_2O	r (19) = –0.797, p = 4.42×10^{-5}	r (15) = –0.850, p = 6.06×10^{-5}	No correlation
Organic matter	r (19) = –0.829, p = 1.14×10^{-5}	r (15) = –0.884, p = 1.20×10^{-5}	No correlation
CEC	r (19) = 0.909, p = 7.14×10^{-8}	r (15) = 0.966, p = 5.30×10^{-9}	r (17) = 0.539, p = 0.026
C	r (19) = –0.830, p = 1.11×10^{-5}	r (15) = –0.885, p = 1.16×10^{-5}	No correlation
N	r (19) = –0.821, p = 1.66×10^{-5}	r (15) = –0.873, p = 2.20×10^{-5}	No correlation
C/N	r (19) = –0.733, p = 3.59×10^{-4}	r (15) = –0.801, p = 3.35×10^{-4}	No correlation

4.3.2 ^{230}Th in radish, savoy and rocket

^{230}Th activity concentrations in shoot and root system of radish, savoy and rocket are presented in Figure 4.3. Average activity concentrations of ^{230}Th varied from 1.5–250 Bq kg^{-1} and were lower than 21 Bq kg^{-1} in the controls (Table 4.4). Standard combined measurement uncertainties for ^{230}Th in plants were 3–12 % (8 %) and 5–12 % (8 %) relative for plants grown in contaminated and control soil, respectively (average in parenthesis, k = 1).

Pearson correlation coefficients with corresponding p values among activity concentrations of ^{230}Th in plants and ^{230}Th activity concentrations in soil, as well as the soil parameters, are presented in Table 4.10 and Table 4.11. The aim of statistical analysis was to evaluate the influence of soil parameters on thorium plant uptake form radioactively contaminated soil. However, a positive linear correlation between ^{230}Th activity concentration in rocket shoot system and ^{230}Th activity concentration in the contaminated soil was observed. No significant correlation was found for radish and savoy shoot systems (Figure 4.3). The activity concentrations of ^{230}Th in plants and ^{230}Th in soil were positively correlated and statistically significant for all root systems (tubers of radish, root system of savoy and rocket) as shown in Figure 4.3.

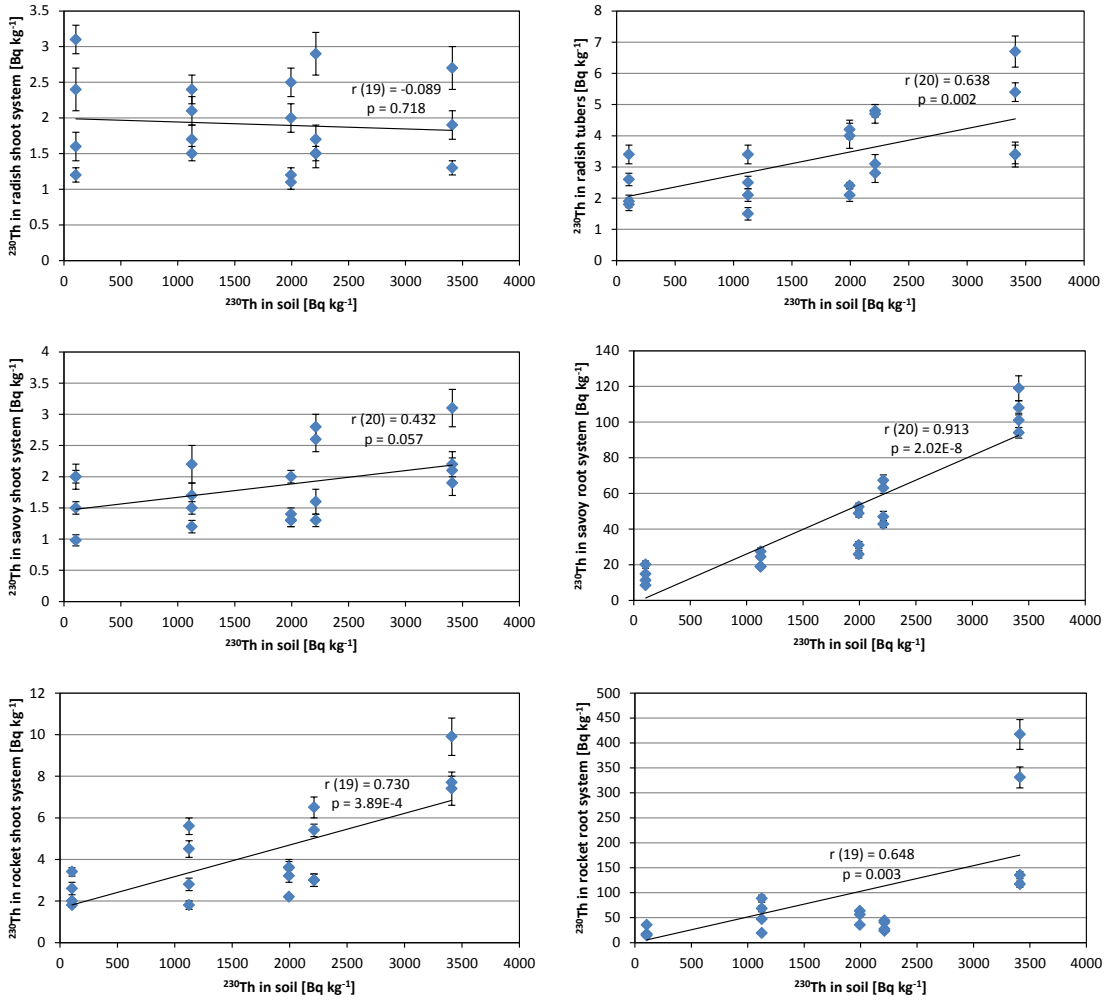


Figure 4.3: ^{230}Th activity concentrations (d.m.) in shoot and root systems of radish, savoy and rocket (with Pearson correlation coefficient and p value, number of observations in parenthesis).

Negative correlations were found between ^{230}Th activity concentrations in savoy shoot system and most of soil parameters (available P_2O_5 and K_2O, organic matter content, total N and organic C) (except: pH and C/N ratio – no correlation) as well as between ^{230}Th activity concentrations in rocket shoot system and most of soil parameters (available P_2O_5 and K_2O, organic matter content, total N, organic C and C/N ratio) (except: pH – no correlation). However, no correlations were found between soil parameters and ^{230}Th in the shoot system of radish. In case of underground plant's parts, negative linear correlations were found between ^{230}Th activity concentrations in all investigated root systems (radish, savoy and rocket) and most of the soil parameters (available P_2O_5 and K_2O, organic matter content, total N, organic C and C/N ratio) (except: pH – no correlation for all three plants, C/N ratio – no correlation for radish) of the growing substrates. ^{230}Th content in all investigated plants was positively correlated with CEC in the soil, except for the radish shoot system where there were no linear correlations at all.

Table 4.10: Pearson correlation coefficients with p values for ^{230}Th activity concentrations in plant leaves and several tested parameters (number of observations in parenthesis).

Tested parameter	A ($^{230}Th_{plant}$)		
	Radish – leaves	Savoy – leaves	Rocket – leaves
A ($^{230}Th_{soil}$)	No correlation	No correlation	r (19) = 0.730, p = 3.89×10^{-4}
pH	No correlation	No correlation	No correlation
P_2O_5	No correlation	r (20) = –0.479, p = 0.032	r (19) = –0.728, p = 4.13×10^{-4}
K_2O	No correlation	r (20) = –0.472, p = 0.036	r (19) = –0.706, p = 7.38×10^{-4}
Organic matter	No correlation	r (20) = –0.480, p = 0.032	r (19) = –0.737, p = 3.19×10^{-4}
CEC	No correlation	r (20) = 0.524, p = 0.018	r (19) = 0.830, p = 1.11×10^{-5}
C	No correlation	r (20) = –0.480, p = 0.032	r (19) = –0.738, p = 3.10×10^{-4}
N	No correlation	r (20) = –0.487, p = 0.029	r (19) = –0.722, p = 4.82×10^{-4}
C/N	No correlation	No correlation	r (19) = –0.686, p = 1.20×10^{-3}

Table 4.11: Pearson correlation coefficients with p values for ^{230}Th activity concentrations in underground plant parts and several tested parameters (number of observations in parenthesis).

Tested parameter	A ($^{230}Th_{plant}$)		
	Radish – tubers	Savoy – roots	Rocket – roots
A ($^{230}Th_{soil}$)	r (20) = 0.638, p = 0.002	r (20) = 0.913, p = 2.02×10^{-8}	r (19) = 0.648, p = 0.003
pH	No correlation	No correlation	No correlation
P_2O_5	r (20) = –0.657, p = 0.002	r (20) = –0.912, p = 2.27×10^{-8}	r (19) = –0.597, p = 0.007
K_2O	r (20) = –0.645, p = 0.002	r (20) = –0.888, p = 1.75×10^{-7}	r (19) = –0.563, p = 0.012
Organic matter	r (20) = –0.655, p = 0.002	r (20) = –0.915, p = 1.68×10^{-8}	r (19) = –0.608, p = 0.006
CEC	r (20) = 0.662, p = 1.46×10^{-3}	r (20) = 0.964, p = 8.44×10^{-12}	r (19) = 0.736, p = 3.28×10^{-4}
C	r (20) = –0.655, p = 0.002	r (20) = –0.915, p = 1.60×10^{-8}	r (19) = –0.609, p = 0.006
N	r (20) = –0.661, p = 1.50×10^{-3}	r (20) = –0.909, p = 2.94×10^{-8}	r (19) = –0.584, p = 0.009
C/N	No correlation	r (20) = –0.741, p = 1.86×10^{-4}	r (19) = –0.754, p = 1.93×10^{-4}

4.3.3 ^{226}Ra in radish, savoy and rocket

^{226}Ra activity concentrations in the shoot and root system of radish, savoy and rocket are presented in Figure 4.4. Average activity concentrations of ^{226}Ra varied from 21–322 Bq kg^{-1} and were lower than 6 Bq kg^{-1} in the controls (Table 4.4). Standard combined measurement uncertainties for ^{226}Ra in plants were 4–10 % (6 %) and 5–18 % (11 %) relative for plants grown in contaminated and control soil, respectively (average in parenthesis, k = 1). In addition, there were some large changes of ^{226}Ra activity concentrations observed in plants grown in mixtures containing 60 % UMT and 80 % UMT. Therefore, this phenomenon requires further investigations to determine, along with the combination of various soil properties, the crucial parameters responsible for the observed changes in the uptake pattern of the radium.

Statistical analysis of linear dependency between ^{226}Ra activity concentrations in plants and its content in soil, as well as between activity concentrations of ^{226}Ra in plants and the soil parameters of a growing substrate are shown in Table 4.12 and Table 4.13. The tested hypothesis was that soil parameters will have impact on availability of radium to plants. Thus, positive linear correlations were found between ^{226}Ra activity concentrations in plants and in soil for all investigated plants and plant parts (Figure 4.4).

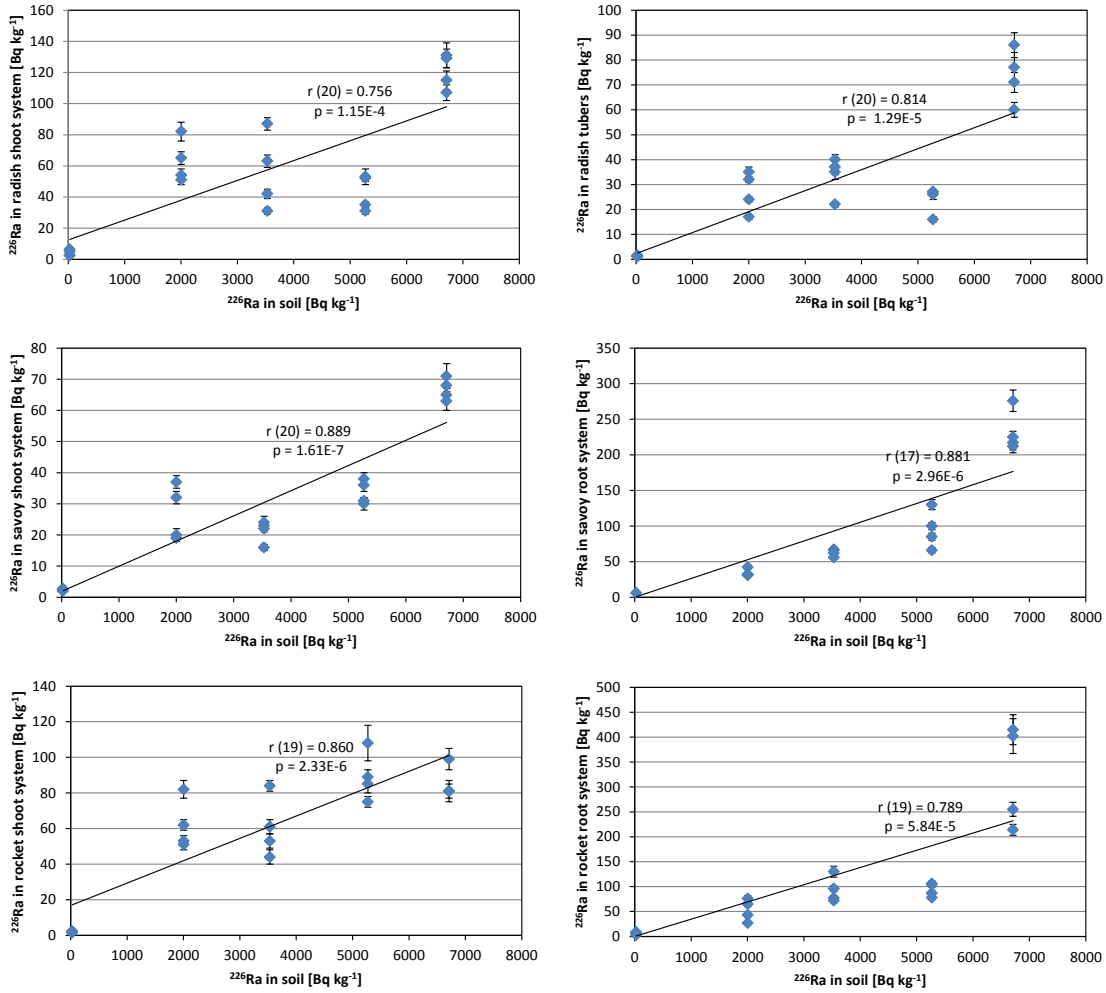


Figure 4.4: ^{226}Ra activity concentrations (d.m.) in shoot and root systems of radish, savoy and rocket (with Pearson correlation coefficient and p value, number of observations in parenthesis).

Strong negative linear correlations were found between ^{226}Ra activity concentrations in all tested plants and most determined soil parameters (available P_2O_5 and K_2O, organic matter content, total N, organic C and C/N ratio) of the growing contaminated substrate. However, no correlations were observed between ^{226}Ra activity concentrations in most plants and pH of the growing substrate. The linear correlation between ^{226}Ra activity concentration in plant and pH of growing substrate was only found for the savoy root system. ^{226}Ra activity concentrations in all the studied plants were positively correlated with CEC of the contaminated soils. Additionally, strong positive linear correlation was observed between ^{226}Ra activity concentrations in all tested plants and exchangeable Ca while correlation among ^{226}Ra activity concentrations in all tested plants and exchangeable Mg was negative.

Table 4.12: Pearson correlation coefficients with p values for ^{226}Ra activity concentrations in plant leaves and several tested parameters (number of observations in parenthesis).

Tested parameter	A ($^{226}Ra_{plant}$)		
	Radish – leaves	Savoy – leaves	Rocket – leaves
A ($^{226}Ra_{soil}$)	r (20) = 0.756, p = 1.15×10^{-4}	r (20) = 0.889, p = 1.61×10^{-7}	r (19) = 0.860, p = 2.33×10^{-6}
pH	No correlation	No correlation	No correlation
P_2O_5	r (20) = –0.754, p = 1.21×10^{-4}	r (20) = –0.895, p = 9.81×10^{-8}	r (19) = –0.843, p = 5.86×10^{-6}
K_2O	r (20) = –0.739, p = 1.97×10^{-4}	r (20) = –0.883, p = 2.62×10^{-7}	r (19) = –0.868, p = 1.49×10^{-6}
Organic matter	r (20) = –0.772, p = 6.79×10^{-5}	r (20) = –0.906, p = 3.70×10^{-8}	r (19) = –0.849, p = 4.39×10^{-6}
CEC	r (20) = 0.789, p = 3.59×10^{-5}	r (20) = 0.928, p = 3.99×10^{-9}	r (19) = 0.657, p = 0.002
C	r (20) = –0.773, p = 6.55×10^{-5}	r (20) = –0.907, p = 3.40×10^{-8}	r (19) = –0.849, p = 4.40×10^{-6}
N	r (20) = –0.729, p = 2.65×10^{-4}	r (20) = –0.884, p = 2.40×10^{-7}	r (19) = –0.832, p = 1.00×10^{-5}
C/N	r (20) = –0.920, p = 9.69×10^{-9}	r (20) = –0.837, p = 4.21×10^{-6}	r (19) = –0.514, p = 0.024
Exchangeable Ca [%]	r (20) = 0.746, p = 1.59×10^{-4}	r (20) = 0.898, p = 7.59×10^{-8}	r (19) = 0.844, p = 5.69×10^{-6}
Exchangeable Mg [%]	r (20) = –0.771, p = 6.95×10^{-5}	r (20) = –0.898, p = 7.51×10^{-8}	r (19) = –0.861, p = 2.22×10^{-6}

Table 4.13: Pearson correlation coefficients with p values for ^{226}Ra activity concentrations in underground plant parts and several tested parameters (number of observations in parenthesis).

Tested parameter	A ($^{226}Ra_{plant}$)		
	Radish – tubers	Savoy – roots	Savoy – roots
A ($^{226}Ra_{soil}$)	r (20) = 0.814, p = 1.29×10^{-5}	r (17) = 0.881, p = 2.96×10^{-6}	r (19) = 0.789, p = 5.84×10^{-5}
pH	No correlation	r (17) = 0.598, p = 0.011	No correlation
P_2O_5	r (20) = –0.816, p = 1.15×10^{-5}	r (17) = –0.894, p = 1.33×10^{-6}	r (19) = –0.803, p = 3.54×10^{-5}
K_2O	r (20) = –0.794, p = 2.96×10^{-5}	r (17) = –0.794, p = 2.96×10^{-5}	r (19) = –0.771, p = 1.10×10^{-4}
Organic matter	r (20) = –0.829, p = 6.24×10^{-6}	r (17) = –0.904, p = 7.30×10^{-6}	r (19) = –0.812, p = 2.46×10^{-5}
CEC	r (20) = 0.862, p = 1.05×10^{-6}	r (17) = 0.970, p = 1.31×10^{-10}	r (19) = 0.897, p = 1.91×10^{-7}
C	r (20) = –0.830, p = 6.00×10^{-6}	r (17) = –0.904, p = 6.22×10^{-7}	r (19) = –0.813, p = 2.37×10^{-5}
N	r (20) = –0.795, p = 2.83×10^{-5}	r (17) = –0.882, p = 2.76×10^{-6}	r (19) = –0.791, p = 5.55×10^{-5}
C/N	r (20) = –0.916, p = 1.46×10^{-8}	r (17) = –0.739, p = 7.00×10^{-4}	r (19) = –0.807, p = 3.00×10^{-5}
Exchangeable Ca [%]	r (20) = 0.805, p = 1.87×10^{-5}	r (17) = 0.891, p = 1.64×10^{-6}	r (19) = 0.797, p = 4.40×10^{-5}
Exchangeable Mg [%]	r (20) = –0.826, p = 7.16×10^{-6}	r (17) = –0.892, p = 1.49×10^{-6}	r (19) = –0.800, p = 3.92×10^{-5}

Biomass and mass conversion factors of studied plants as well as Pearson correlation coefficients with p values among ^{226}Ra activity concentrations in plants and biomass are presented in Table 4.14. The statistical analysis showed moderate correlation for radish and savoy shoot system while for other plant/plant parts no linear correlation was observed.

Table 4.14: Biomass and mass conversion factors of studied plants (results are presented as averages with standard deviations) as well as Pearson correlation coefficients with p values for ^{226}Ra activity concentrations in plants and biomass (number of observations in parenthesis).

Plant	UMT content	Biomass [g, d.m.]	Conversion factor (d.m. ↔ f.m.)	Ra_{plant} – Biomass correlation
Radish – leaves	Control	5.72 ± 3.36	12 ± 1	r (20) = 0.587, p = 0.007
	20 %	13 ± 1		
	40 %	13 ± 2		
	60 %	13 ± 2		
	80 %	13 ± 1		
Radish – tubers	Control	4.82 ± 2.17	19 ± 2	No correlation
	20 %	6.62 ± 1.62		
	40 %	4.69 ± 1.41		
	60 %	5.82 ± 1.54		
	80 %	4.68 ± 1.35		
Savoy – leaves	Control	9.78 ± 2.66	9.85 ± 0.95	r (20) = 0.472, p = 0.036
	20 %	15 ± 2		
	40 %	17 ± 2		
	60 %	16 ± 2		
	80 %	15 ± 2		
Savoy – roots	Control	0.472 ± 0.110	4.42 ± 0.39	No correlation
	20 %	0.477 ± 0.049		
	40 %	0.440 ± 0.108		
	60 %	0.464 ± 0.021		
	80 %	0.407 ± 0.126		
Rocket – leaves	Control	2.02 ± 0.28	9.22 ± 1.40	No correlation
	20 %	1.88 ± 0.85		
	40 %	2.19 ± 0.53		
	60 %	3.54 ± 0.15		
	80 %	1.55 ± 0.47		
Rocket – roots	Control	0.295 ± 0.083	4.37 ± 0.50	No correlation
	20 %	0.208 ± 0.195		
	40 %	0.249 ± 0.111		
	60 %	0.590 ± 0.063		
	80 %	0.156 ± 0.099		

4.3.4 ^{210}Pb in radish, savoy and rocket

^{210}Pb activity concentrations in shoot and root system of radish, savoy and rocket are presented in Figure 4.5. Average activity concentrations of ^{210}Pb varied from 3.0–276 Bq kg^{-1} and were lower than 3.1 Bq kg^{-1} in the controls (Table 4.4). Standard combined measurement uncertainties for ^{210}Pb in plants were 2–14 % (8 %) and 7–56 % (17 %) relative for plants grown in contaminated and control soil, respectively (average in parenthesis, k = 1). In addition, there were some evident changes of ^{210}Pb activity concentrations observed in plants grown in mixtures containing 60 % UMT and 80 % UMT. Further investigations are needed to explain this unexpected anomaly.

Statistical analysis of linear correlation between ^{210}Pb activity concentrations in plants and its content in soil, as well as between activity concentrations of ^{210}Pb in plants is presented in Table 4.15 and Table 4.16. The purpose of statistical analysis was to investigate the transfer of lead to the plants among different contamination scenarios as well as to confirm influence of soil parameter on plant uptake of lead. Positive linear correlations were found between ^{210}Pb activity concentrations in plants and in soil for all investigated plants and plant parts (Figure 4.5).

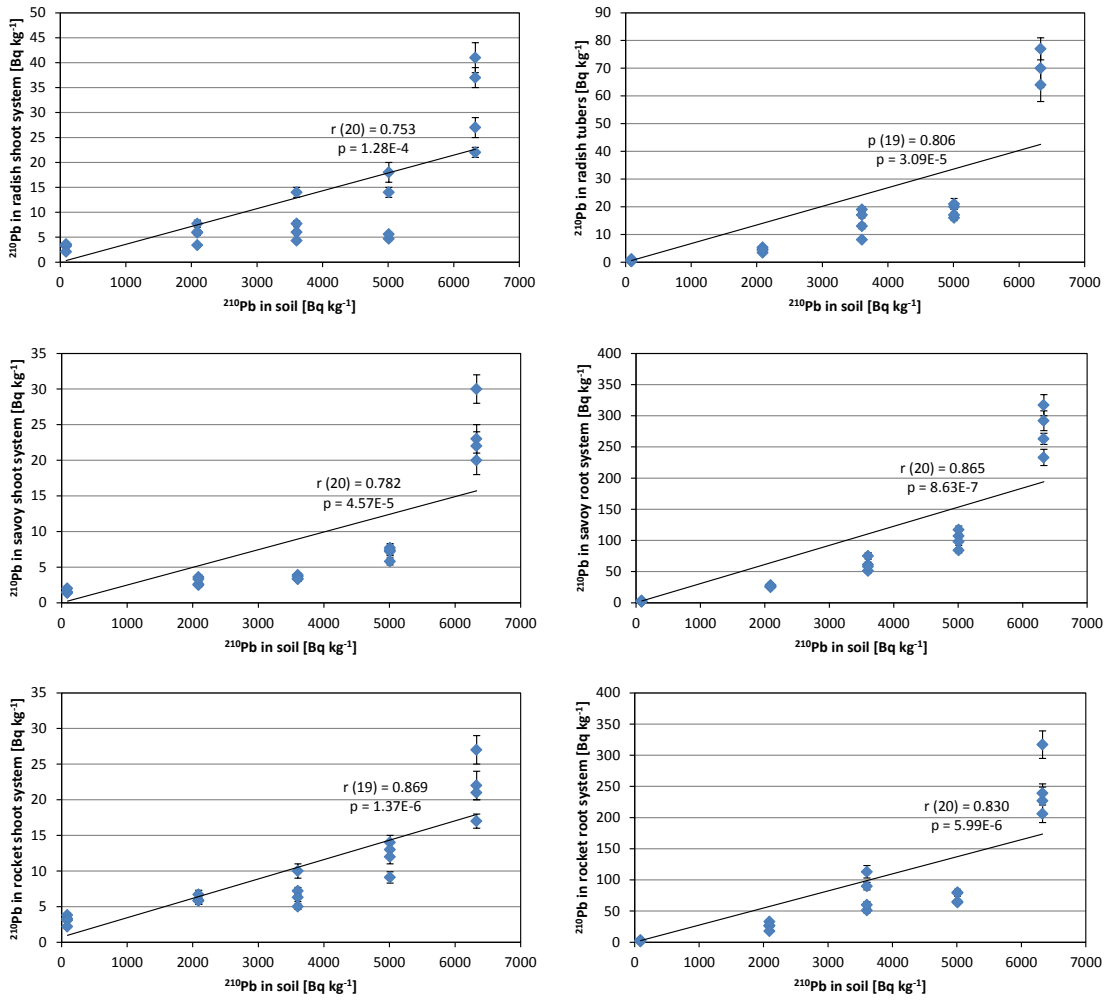


Figure 4.5: ^{210}Pb activity concentrations (d.m.) in shoot and root systems of radish, savoy and rocket (with Pearson correlation coefficient and p value, number of observations in parenthesis).

Strong negative linear correlations were found between ^{210}Pb activity concentrations in all tested plants and most determined soil parameters (available P_2O5 and K_2O, organic matter content, total N, organic C and C/N ratio) of the growing contaminated substrate. However, no correlations were observed between ^{210}Pb activity concentrations in all plants and pH of the growing substrate. ^{210}Pb activity concentrations in all the studied plants were positively correlated with CEC of the contaminated soils.

Table 4.15: Pearson correlation coefficients with p values for ^{210}Pb activity concentrations in plant leaves and several tested parameters (number of observations in parenthesis).

Tested parameter	A ($^{210}Pb_{plant}$)		
	Radish – leaves	Savoy – leaves	Rocket – leaves
A ($^{210}Pb_{soil}$)	r (20) = 0.753, p = 1.28×10^{-4}	r (20) = 0.782, p = 4.57×10^{-5}	r (19) = 0.869, p = 1.37×10^{-6}
pH	No correlation	No correlation	No correlation
P_2O_5	r (20) = –0.780, p = 4.91×10^{-5}	r (20) = –0.818, p = 1.06×10^{-5}	r (19) = –0.895, p = 2.33×10^{-7}
K_2O	r (20) = –0.750, p = 1.41×10^{-4}	r (20) = –0.784, p = 4.28×10^{-5}	r (19) = –0.875, p = 9.74×10^{-7}
Organic matter	r (20) = –0.788, p = 3.74×10^{-5}	r (20) = –0.898, p = 7.73×10^{-8}	r (19) = –0.900, p = 1.60×10^{-7}
CEC	r (20) = 0.892, p = 1.28×10^{-7}	r (20) = 0.981, p = 2.31×10^{-14}	r (19) = 0.952, p = 3.60×10^{-10}
C	r (20) = –0.789, p = 3.62×10^{-5}	r (20) = –0.899, p = 7.28×10^{-8}	r (19) = –0.900, p = 1.51×10^{-7}
N	r (20) = –0.774, p = 6.18×10^{-5}	r (20) = –0.889, p = 1.63×10^{-7}	r (19) = –0.893, p = 2.71×10^{-7}
C/N	r (20) = –0.767, p = 8.08×10^{-5}	r (20) = –0.789, p = 3.56×10^{-5}	r (19) = –0.748, p = 2.29×10^{-4}

Table 4.16: Pearson correlation coefficients with p values for ^{210}Pb activity concentrations in underground plant parts and several tested parameters (number of observations in parenthesis).

Tested parameter	A ($^{210}Pb_{plant}$)		
	Radish – tubers	Savoy – roots	Rocket – roots
A ($^{210}Pb_{soil}$)	r (19) = 0.806, $p = 3.09 \times 10^{-5}$	r (20) = 0.865, $p = 8.63 \times 10^{-7}$	r (20) = 0.830, $p = 5.99 \times 10^{-6}$
pH	No correlation	No correlation	No correlation
P_2O_5	r (19) = –0.837, $p = 8.07 \times 10^{-6}$	r (20) = –0.893, $p = 1.19 \times 10^{-7}$	r (20) = –0.850, $p = 2.12 \times 10^{-6}$
K_2O	r (19) = –0.801, $p = 8.83 \times 10^{-5}$	r (20) = –0.864, $p = 9.02 \times 10^{-7}$	r (20) = –0.818, $p = 1.05 \times 10^{-5}$
Organic matter	r (19) = –0.843, $p = 5.79 \times 10^{-6}$	r (20) = –0.898, $p = 7.73 \times 10^{-8}$	r (20) = –0.856, $p = 1.46 \times 10^{-6}$
CEC	r (19) = 0.965, $p = 2.42 \times 10^{-11}$	r (20) = 0.981, $p = 2.31 \times 10^{-14}$	r (20) = 0.937, $p = 1.13 \times 10^{-9}$
C	r (19) = –0.844, $p = 5.54 \times 10^{-6}$	r (20) = –0.899, $p = 7.28 \times 10^{-8}$	r (20) = –0.857, $p = 1.41 \times 10^{-6}$
N	r (19) = –0.830, $p = 1.11 \times 10^{-5}$	r (20) = –0.899, $p = 1.63 \times 10^{-7}$	r (20) = –0.840, $p = 3.54 \times 10^{-6}$
C/N	r (19) = –0.789, $p = 5.89 \times 10^{-5}$	r (20) = –0.789, $p = 3.56 \times 10^{-5}$	r (20) = –0.825, $p = 7.54 \times 10^{-6}$

4.4 Concentration Ratios of Selected Radionuclides

The average concentration ratios of ^{238}U, ^{230}Th, ^{226}Ra and ^{210}Pb for radish, savoy and rocket grown in commercially available substrate at different UMT contamination levels are presented in Table 4.17. Concentration ratios for ^{238}U varied from 4.7×10^{-4}–8.7×10^{-2} and 1.0×10^{-2}–3.7×10^{-1} for plants grown in contaminated and control soils, respectively. Concentration ratios for ^{230}Th varied from 5.8×10^{-4}–7.3×10^{-2} and 1.5×10^{-2}–2.0×10^{-2} for plants grown in contaminated and control soils, respectively. Concentration ratios for ^{226}Ra varied from 4.6×10^{-3}–4.8×10^{-2} and 4.7×10^{-2}–2.2×10^{-1} for plants grown in contaminated and control soils, respectively. Concentration ratios for ^{210}Pb varied from 9.9×10^{-4}–4.4×10^{-2} and 6.6×10^{-3}–3.4×10^{-2} for plants grown in contaminated and control soils, respectively.

Table 4.17: Average concentration ratios of radionuclides in plant parts.

Plant	UMT content	CR ^{238}U	CR ^{230}Th	CR ^{226}Ra	CR ^{210}Pb
Radish – leaves	Control	2.3×10^{-2}	2.0×10^{-2}	1.5×10^{-1}	3.4×10^{-2}
	20 %	2.0×10^{-3}	1.7×10^{-3}	3.2×10^{-2}	2.8×10^{-3}
	40 %	1.8×10^{-3}	8.5×10^{-4}	1.6×10^{-2}	2.2×10^{-3}
	60 %	1.7×10^{-3}	8.6×10^{-4}	8.1×10^{-3}	2.1×10^{-3}
	80 %	5.9×10^{-4}	5.8×10^{-4}	1.8×10^{-2}	5.0×10^{-3}
Radish – tubers	Control	1.0×10^{-2}	2.3×10^{-2}	4.6×10^{-2}	6.6×10^{-3}
	20 %	8.4×10^{-4}	2.1×10^{-3}	1.4×10^{-2}	2.1×10^{-3}
	40 %	8.2×10^{-4}	1.6×10^{-3}	9.5×10^{-3}	4.0×10^{-3}
	60 %	6.3×10^{-4}	1.7×10^{-3}	4.6×10^{-3}	3.7×10^{-3}
	80 %	1.1×10^{-3}	1.4×10^{-3}	1.1×10^{-2}	1.1×10^{-2}
Savoy – leaves	Control	1.7×10^{-2}	1.5×10^{-2}	9.1×10^{-2}	1.8×10^{-2}
	20 %	1.5×10^{-3}	1.5×10^{-3}	1.4×10^{-2}	1.4×10^{-3}
	40 %	1.1×10^{-3}	7.5×10^{-4}	6.0×10^{-3}	9.9×10^{-4}
	60 %	4.7×10^{-4}	9.4×10^{-4}	6.4×10^{-3}	1.4×10^{-3}
	80 %	4.9×10^{-4}	6.8×10^{-4}	1.0×10^{-2}	3.8×10^{-3}
Savoy – roots	Control	1.8×10^{-1}	1.3×10^{-1}	2.2×10^{-1}	2.7×10^{-2}
	20 %	2.2×10^{-2}	2.0×10^{-2}	1.7×10^{-2}	1.3×10^{-2}
	40 %	2.1×10^{-2}	2.0×10^{-2}	1.8×10^{-2}	1.7×10^{-2}
	60 %	1.7×10^{-2}	2.5×10^{-2}	1.8×10^{-2}	2.0×10^{-2}
	80 %	3.3×10^{-2}	3.1×10^{-2}	3.5×10^{-2}	4.4×10^{-2}
Rocket – leaves	Control	2.0×10^{-2}	2.3×10^{-2}	7.1×10^{-2}	3.4×10^{-2}
	20 %	2.0×10^{-3}	3.3×10^{-3}	3.1×10^{-2}	2.0×10^{-3}
	40 %	1.2×10^{-3}	1.6×10^{-3}	1.7×10^{-2}	2.0×10^{-3}
	60 %	1.2×10^{-3}	2.0×10^{-3}	1.7×10^{-2}	2.4×10^{-3}
	80 %	2.1×10^{-3}	2.4×10^{-3}	1.3×10^{-2}	3.4×10^{-3}
Rocket – roots	Control	3.6×10^{-1}	2.0×10^{-1}	2.2×10^{-1}	2.4×10^{-2}
	20 %	8.7×10^{-2}	4.9×10^{-2}	2.6×10^{-2}	1.4×10^{-2}
	40 %	5.1×10^{-2}	2.6×10^{-2}	2.7×10^{-2}	2.2×10^{-2}
	60 %	9.0×10^{-3}	1.5×10^{-2}	1.8×10^{-2}	1.4×10^{-2}
	80 %	7.9×10^{-2}	7.3×10^{-2}	4.8×10^{-2}	3.9×10^{-2}

4.5 Photochemical Efficiency and Content of Photosynthetic Pigments

Results of photochemical efficiency and content of photosynthetic pigments in selected plants are presented in Table 4.18. Statistical analysis of linear correlations among activity concentrations of radionuclides and photochemical efficiency, content of photosynthetic pigments (chlorophyll a, b) and anthocyanins showed no correlation for all tested plants. There was no clear effect of activity concentrations of the selected radionuclides in soils on photochemical efficiency, content of photosynthetic pigments and anthocyanins values, as all values were comparable with the controls taking into account the uncertainties (Table 4.18). Statistical analysis of linear correlations among activity concentrations of the studied radionuclides and photochemical efficiency, content

of photosynthetic pigments (chlorophyll a, b) and anthocyanins showed no correlation for all tested plants.

Table 4.18: Photochemical efficiency and content of photosynthetic pigments in plant leaves.

Parameter[a]	UMT content	Radish	Savoy	Rocket
Photochemical	Control	0.64 ± 0.16	0.74 ± 0.04	0.62 ± 0.07
efficiency	20 %	0.75 ± 0.03	0.77 ± 0.03	0.66 ± 0.12
	40 %	0.74 ± 0.04	0.74 ± 0.03	0.68 ± 0.08
	60 %	0.75 ± 0.05	0.77 ± 0.03	0.67 ± 0.05
	80 %	0.72 ± 0.05	0.77 ± 0.03	0.63 ± 0.05
Chlorophyll a	Control	1.12 ± 0.26	1.21 ± 0.36	1.39 ± 0.27
(mg g^{-1} f.m.)	20 %	1.30 ± 0.27	1.18 ± 0.18	1.70 ± 0.50
	40 %	1.57 ± 0.53	1.32 ± 0.04	1.40 ± 0.17
	60 %	1.38 ± 0.13	1.32 ± 0.20	1.69 ± 0.18
	80 %	1.45 ± 0.42	1.38 ± 0.12	1.44 ± 0.21
Chlorophyll b	Control	0.44 ± 0.10	0.45 ± 0.20	0.56 ± 0.09
(mg g^{-1} f.m.)	20 %	0.51 ± 0.12	0.42 ± 0.09	0.68 ± 0.25
	40 %	0.60 ± 0.17	0.51 ± 0.04	0.49 ± 0.06
	60 %	0.56 ± 0.06	0.56 ± 0.14	0.64 ± 0.10
	80 %	0.60 ± 0.16	0.56 ± 0.07	0.56 ± 0.12
Carotenoids	Control	0.39 ± 0.09	0.43 ± 0.15	0.54 ± 0.10
(mg g^{-1} f.m.)	20 %	0.48 ± 0.09	0.41 ± 0.08	0.67 ± 0.23
	40 %	0.57 ± 0.18	0.48 ± 0.02	0.54 ± 0.07
	60 %	0.50 ± 0.06	0.50 ± 0.09	0.66 ± 0.08
	80 %	0.54 ± 0.14	0.51 ± 0.04	0.57 ± 0.09
Anthocyanins	Control	226 ± 19	137 ± 6	215 ± 63
(rel. unit g^{-1} f.m.)	20 %	264 ± 21	120 ± 19	182 ± 30
	40 %	230 ± 35	160 ± 18	203 ± 27
	60 %	214 ± 37	191 ± 28	226 ± 24
	80 %	202 ± 71	170 ± 29	198 ± 43

[a] The measured values were reported by chosen institution.

4.6 Assessment of Annual Effective Dose

The annual effective ingestion doses for average and maximum annual intake of radish, savoy and rocket for each contamination scenario, including controls, are presented in Table 4.19. The results are displayed as averages with standard deviations where uncertainties arise from determination of activity concentration of selected radionuclides. The dose assessment is complex and the numerical estimation of uncertainty is difficult since there are many influencing factors such as estimation of intake (food type, amount of ingestion), time between harvesting of vegetables and consumption, determination of radionuclides, etc. It has to be pointed out that calculated uncertainties are rather small while the expected uncertainties are below 50 % with main contribution form supposed annual intakes of studied vegetables. The annual intakes were calculated as average of individual dietary surveys from different European countries. The data were obtained from EFSA Comprehensive European Food Consumption Database [116] since for the Slovenia such a study is not accomplished yet.

In the calculation of annual effective ingestion dose ^{210}Po was not taken into account as it was not specifically determined. The analyses of plant samples were performed after approximately two years from harvest and ^{210}Po measurement was used to determine content of its parent ^{210}Pb in the samples as they were in secular equilibrium [114]. It has to be aware that ^{210}Po activity concentration is rather higher at time of harvesting when vegetables are usually consumed. In spite of that, if we suppose that ^{210}Po and ^{210}Pb in the investigated samples are in equilibrium, the annual effective dose would be increased for approximately up to 50 % but would still be lower than 4 % of the world ingestion average radiation dose of 0.29 mSv y^{-1} [117].

The calculated total annual effective ingestion dose for average annual intake, considering all four radionuclides, varied from 0.01–0.54, 0.04–0.74, 0.04–0.49 μSv y^{-1} for radish, savoy and rocket, respectively (Figure 4.6). The calculated total annual effective ingestion dose for maximum annual intake considering all four radioisotopes varied from 0.07–4.16, 0.30–4.98, 0.08–1.04 μSv y^{-1} for radish, savoy and rocket, respectively (Figure 4.7).

Table 4.19: Annual effective ingestion dose (nSv y^{-1}) due to ^{238}U, ^{230}Th, ^{226}Ra and ^{210}Pb for adults with average and maximum annual consumption of selected vegetables (results are presented as averages with standard deviations).

Sample	UMT content	$E_{ing,U-238}$	$E_{ing,max,U-238}$	$E_{ing,Th-230}$	$E_{ing,max,Th-230}$	$E_{ing,Ra-226}$	$E_{ing,max,Ra-226}$	$E_{ing,Pb-210}$	$E_{ing,max,Pb-210}$
Radish - tubers	Control	0.07 ± 0.02	0.54 ± 0.19	3.6 ± 0.6	28 ± 5	2.8 ± 0.5	22 ± 4	3.1 ± 2.0	24 ± 15
	20 %	0.09 ± 0.02	0.67 ± 0.13	4.0 ± 1.4	31 ± 11	62 ± 18	480 ± 140	23 ± 4	180 ± 30
	40 %	0.13 ± 0.07	1.0 ± 0.5	5.2 ± 1.6	40 ± 13	85 ± 5	660 ± 40	73 ± 25	560 ± 190
	60 %	0.17 ± 0.07	1.3 ± 0.5	6.6 ± 1.8	51 ± 14	55 ± 12	420 ± 90	100 ± 10	730 ± 110
	80 %	0.36 ± 0.06	2.8 ± 0.5	8.1 ± 2.8	62 ± 22	170 ± 30	1300 ± 200	360 ± 30	2790 ± 210
Savoy - leaves	Control	0.31 ± 0.18	2.1 ± 1.2	7.4 ± 2.2	50 ± 15	15 ± 2	100 ± 10	22 ± 4	150 ± 30
	20 %	0.40 ± 0.11	2.7 ± 0.7	7.5 ± 1.9	51 ± 13	170 ± 60	1110 ± 380	41 ± 8	280 ± 50
	40 %	0.49 ± 0.13	3.3 ± 0.9	6.7 ± 1.5	46 ± 10	130 ± 20	880 ± 140	49 ± 4	330 ± 30
	60 %	0.34 ± 0.18	2.3 ± 1.2	9.4 ± 3.2	63 ± 22	200 ± 20	1380 ± 150	100 ± 10	650 ± 80
	80 %	0.42 ± 0.07	2.9 ± 0.5	11 ± 2	72 ± 16	410 ± 20	2740 ± 140	320 ± 60	2190 ± 400
Rocket - leaves	Control	0.21 ± 0.03	0.46 ± 0.06	6.3 ± 1.9	13 ± 4	6.5 ± 1.1	14 ± 2	24 ± 5	51 ± 11
	20 %	0.31 ± 0.04	0.65 ± 0.08	9.5 ± 4.4	20 ± 9	190 ± 20	410 ± 40	48 ± 3	100 ± 10
	40 %	0.29 ± 0.10	0.62 ± 0.21	8.2 ± 1.7	17 ± 4	180 ± 20	390 ± 50	56 ± 17	120 ± 40
	60 %	0.47 ± 0.13	1.0 ± 0.3	12 ± 5	25 ± 10	290 ± 20	610 ± 40	93 ± 16	200 ± 30
	80 %	1.0 ± 0.5	2.2 ± 1.0	21 ± 3	46 ± 6	300 ± 30	640 ± 60	170 ± 30	360 ± 70

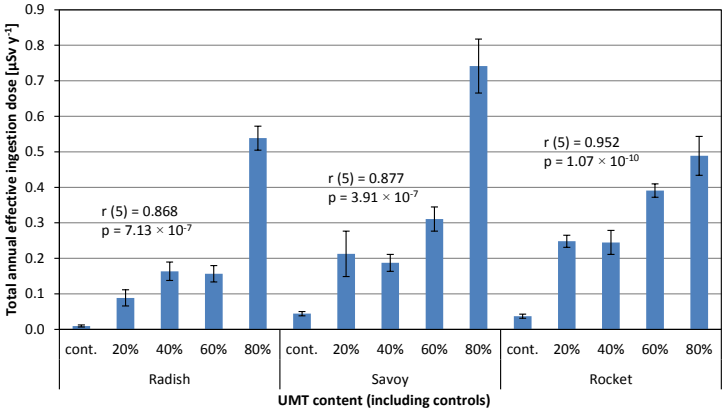


Figure 4.6: Total annual effective ingestion doses due to ^{238}U, ^{230}Th, ^{226}Ra and ^{210}Po recived by studied vegetables ingestion (average intake) for adult population (results are presented as averages with standard deviations), with Pearson correlation coefficient and p value (number of observations in parenthesis).

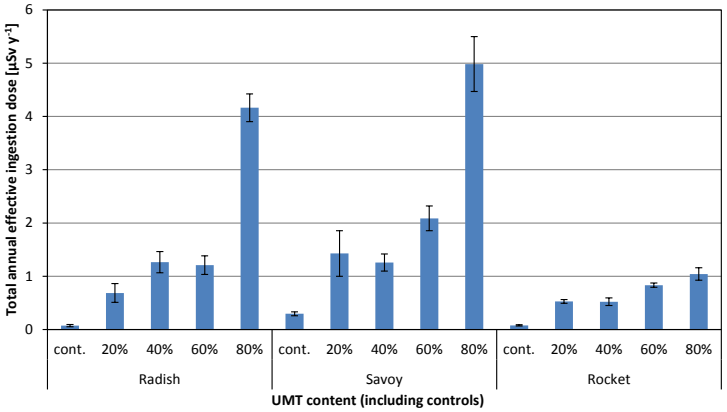


Figure 4.7: Total annual effective ingestion doses due to ^{238}U, ^{230}Th, ^{226}Ra and ^{210}Po received by studied vegetables ingestion (maximum intake) for adult population (results are presented as averages with standard deviations), with Pearson correlation coefficient and p value (number of observations in parenthesis).

Chapter 5

Discussion

5.1 Optimization of Radio-Analytical Procedures

The results of the accomplished studies are briefly discussed below, and in Jurečič et al. [87] and Planinšek et. al. [89] (Appendix A) in more detail.

A comparison of different dissolution techniques for determination of uranium in soil samples was carried out. Complete dissolution of soil samples without residues was obtained only by alkaline fusion with Na_2CO_3 and Na_2O_2. The same result was also reported by Jia et al. [118]. In the case of conventional wet ashing and microwave digestion, significant residues were observed. As has already been reported in some papers [119]–[121], decomposition of a soil sample depends on its mineral composition. It is known that uranium is incorporated in the structure of the various minerals such as illite and montmorillonite which contain more than 20 % of silicon. However, the results obtained show that the samples were not completely dissolved by acid treatment as well as by microwave dissolution and that a significant amount of uranium was found in the residues. In addition, due to the very poor results obtained by microwave digestion, the efficiency of dissolution by this technique should be improved by the use of larger volumes of acids, a longer time at the final temperature and then leaving the sample overnight in acid at room temperature.

In the next study, the aim was to compare three wet digestion procedures for dissolution of biological samples in the determination of ^{210}Po. The results obtained for reference materials NIST-4359 and IAEA-307 all lay very close to the certificate or information value, respectively. Results for ^{210}Po in most plant materials (seaweed, sea plant, Virginia tobacco leaves, lichen samples), where dissolution in Kjeldahl flask was carried out, showed slightly higher values as those obtained by dissolution in an Erlenmeyer flask and dissolution in the microwave system. ^{210}Po was also determined in six real mussel samples (*M. galloprovincialis*). The activity concentrations of ^{210}Po in the mussel samples are comparable between all measured samples and dissolution techniques used. For one mussel sample higher variations in results were found which is probably due to relatively low activity concentration of ^{210}Po (between 2.2–5.1 Bq kg^{-1}) in this sample. However, the ANOVA test showed that there is no statistical difference between the three dissolution techniques. Therefore for determination of ^{210}Po in biological samples all of the compared dissolution procedures can be used. The choice of the dissolution technique depends on sample and method characteristics, as presented in Planinšek et. al. [89] (Appendix A.1) in more detail.

The optimisation of method for consecutive determination of selected radionuclides in plant samples with small masses (up to 2 g of dried sample) was carried out. For quality control of the alpha-particle spectrometric measurements IAEA 447 and NIST 4359

(Seaweed) certified reference materials were used. The uranium measurements results for IAEA 447 were slightly lower than the certified values, probably due to incomplete digestion of the soil sample using the proposed dissolution method, as uranium is expected to be incorporated into the structure of difficultly soluble minerals. Moreover, the results and discussion of study of uranium in the residues after decomposition of soil samples are presented in more detail in Appendix A.1 [87]. The obtained results for the NIST 4359 (plant matrix) were within uncertainties (Table 3.1). Fairly good agreement was found for the other radionuclides in both reference materials and the results mostly comply with the certified values despite some relatively high measurement uncertainties. The sole exception was ^{210}Po in IAEA 447, which was probably due to the applied dissolution procedure, as already discussed in the previously published publication by Planinšek et. al [89] (Appendix A.2).

5.2 Soil-to-Plant Transfer of Selected Radionuclides and Impact of Soil Parameters

Comprehensive description of work and procedures used as well as discussion of main achievements are described elsewhere (Appendix A.3) [122] while in more detail discussion is in following chapters.

5.2.1 ^{238}U uptake by radish, savoy and rocket

The results obtained showed that a transfer of ^{238}U to the studied plants from the contaminated growing substrate has occurred. ^{238}U uptake in plants (rocket shoot system, tubers of radish, savoy root system) was directly related with ^{238}U in soil (Figure 4.2). The correlations between uranium content in plants and in soil were also observed by Sigh et al. [47]. Similarly to our finding, the same authors also observed relatively low uptake of ^{238}U compared to the other radionuclides (Table 4.4). Our finding of nonlinearity between ^{238}U activity concentrations in UMT containing soils and ^{238}U activity concentrations in several plant parts (shoot system of radish and savoy, rocket root system) was also observed in other studies [11], [12]. Those findings indicate the possibility of each plant and soil combination having a unique curvilinear relationship. Some literature data suggests that it is hardly possible to use laboratory experimental results for reliable prediction of uptake and translocation of radionuclides in the environment as studies of radionuclides uptake under controlled conditions may give only approximate information about their behaviour in the natural environment [11].

In general, roots serve as a natural barrier preventing the transport of many trace metals, including radionuclides to upper plant parts. Moreover, the rate of radionuclides translocations from roots to shoot is probably species dependent. It may be different for different species and even cultivars [11]. For example, it has been reported that U concentration in roots of different plants collected from the same site was 30–50 times higher than U concentration in shoots [123]. However, uranium activity concentration in different parts of the plant is different, being in general minimum in the shoot system and maximum in the root system as it was reported in several other publications [11], [47], [48]. The statistical analyses of obtained results showed that the difference among activity concentration in root system and activity concentration in shoot system was statistically significant for radish (Table 4.5), savoy (Table 4.6) and rocket (Table 4.7) at most contamination levels. The difference was not statistically significant only for radish at 40 % contamination. In addition, in Shtangeeva's [11] publication a warning is given that even careful washing of the plant roots would hardly allow removing all soil particles

from the root surface. On the other hand, a stronger treatment of plants may result in dissolving of a certain part of the plant tissue and it is not recommended [124]. Nevertheless, it has to be taken in consideration that higher concentration of many elements in roots compared to leaves is typical not only for soil-grown plants, but also for plants grown in nutrient solutions [11].

The found linear correlations between ^{238}U activity concentrations in plants (rocket shoot system, tubers of radish, savoy root system) and most of studied soil parameters (available P_2O_5 and K_2O, organic matter content, total N, organic C and C/N ratio) were negative (Table 4.8 and Table 4.9). It seems that, in general, all these soil factors decrease uranium mobility in soil due to its rapid reaction with those compounds, resulting in reduced uranium bioavailability [33], [48], [61], [63].

Literature survey shows that an important soil parameter in radionuclides uptake is CEC [29]. ^{238}U content in most plant and plant parts (rocket shoot system, tubers of radish, savoy and rocket root system) was positively correlated with CEC in the soil (Table 4.8 and Table 4.9), as already shown by Smodiš et al. [28]. This observation was explained as a consequence of higher exchange of uranium from soil colloids to the soil solution and consequently higher root uptake [28], [29].

The observed scattered CR values might be attributed to a different UMT content in the substrates that impacted their chemical composition and soil properties, which could affect the plants' capabilities for taking up uranium. Further, the obtained concentration ratios for uranium in radish, savoy and rocket are generally for one or two orders of magnitude lower compared to the reported average CRs, being 2.0×10^{-2} for leafy vegetables and 5.0×10^{-3} for tubers [9]. The reported CR values are only for eatable plant parts. However, the low uptake of uranium may be attributed to the fact that the majority of uranium had been extracted from uranium ore and consequently the added UMT was technologically altered and eventually biologically less available, as reported by Smodiš et al. [28].

5.2.2 ^{230}Th uptake by radish, savoy and rocket

The results obtained indicated that the transfer of ^{230}Th from UMT containing growing substrate to tested plants can be observed. The activity concentrations of ^{230}Th in plants and ^{230}Th in soil (with different % of UMT content) were positively correlated and statistically significant for several plants (tubers of radish, shoot and root system of savoy, root system of rocket). These findings as well as the observation that behaviour of thorium in different plant parts may be different are in agreement with the reported literature data [40].

Several studies [11], [14], [51] concluded that Th main accumulation target organs are roots, while Th concentrations in upper plant parts are rather low which was also the case in our study, whenever the difference among thorium in roots and aboveground plants parts was significant. In more detail, the statistical analyses of differences among ^{230}Th activity concentration in root system and ^{230}Th activity concentration in shoots system revealed that the difference was statistically significant for radish at 40, 60 and 80 % of UMT content (Table 4.5), for savoy and rocket the difference was significant at all contamination levels (Table 4.6 and Table 4.7). However, the difference was statistically significant at the highest contamination level for all tested plants.

The obtained relatively low activity concentrations of ^{238}U and ^{230}Th in comparison with ^{226}Ra and ^{210}Pb content in the studied plants are in agreement with the literature data, since thorium and uranium are considered as elements with similar behaviour in soil, i.e., both are assumed as relatively immobile in soil and with low bioavailability [58], [70].

Accordingly, negative linear correlations were found between ^{230}Th activity concentrations in all investigated plants (and most plants' parts), and most of the parameters of the growing substrates (available P_2O_5 and K_2O, organic matter content, total N, organic C and C/N ratio). The importance of soil properties on thorium uptake was also shown by Guo et al. [71]. As mentioned before, CEC is an important soil parameter in the case of radionuclides uptake from soil [29]; ^{230}Th content in all investigated plants was positively correlated with CEC in the soil.

The observed scattered CR values might be attributed to a different UMT content in the substrates that impacted their chemical composition and soil properties, which could affect the plants' capabilities for taking up thorium. Additionally, for the broad leafy vegetables category that includes savoy and rocket, the IAEA reported CR average value for Th is 1.2×10^{-3} and for tubers 2.0×10^{-4} [9]. It has to be pointed out that the reported CR values are only for the eatable plant part. The obtained CR values were generally lower for one or two orders of magnitude for leaves and tubers of radish and leaves of savoy. The CR values for leaves of rocket were in agreement with the values reported by IAEA [9].

5.2.3 ^{226}Ra uptake by radish, savoy and rocket

The obtained results indicated that transfer of ^{226}Ra from UMT containing growing substrate to tested plants was occurred. The activity concentrations of ^{226}Ra in plants and ^{226}Ra in soil (with different % of UMT content) were positively correlated and statistically significant for all plants (radish, savoy and rocket) as shown in Figure 4.4. Contrary to our findings, Madruga et al. [10] found that the plant uptake response to the total radium activity concentration in the tailings follows a non-linear function while Smodiš et al. [28] and Blanco Rodríguez et al. [125] also found linear correlations. Despite of statistically confirmed linear dependency, there were some large changes of ^{226}Ra activity concentrations observed in plants grown in mixtures containing 60 % UMT and 80 % UMT (Table 4.4 and Figure 4.4). The similar observation was found by Markose et al.[126]. In order to explain this apparent anomaly, they suggested determination of available radium in soil. Therefore, this phenomenon requires further investigations to determine, along with the combination of various soil properties, the crucial parameters responsible for the observed changes in the uptake pattern of the radium.

Like other radionuclides radium is also accumulated mainly by roots; and consequently, concentrations are higher in roots than in aerial parts [10], [91]. However, the statistical analyses of results obtained showed that the ^{226}Ra activity concentration was higher in roots compared to the aboveground plant parts for radish and savoy at most contamination levels (20, 40, 60, 80 % of UMT and 40, 60, 80 % of UMT, respectively) (Table 4.5 and Table 4.6) while for the rocket the difference was significant only at 80 % UMT content (Table 4.7).

Strong negative linear correlations were found between ^{226}Ra activity concentrations in all investigated plants and several parameters of the growing substrates (available P_2O_5 and K_2O, organic matter content, total N, organic C and C/N ratio) (Table 4.12 and Table 4.13). The correlations indicate that the reduced ^{226}Ra uptake may be a consequence of increased values of the investigated soil parameters in the UMT contaminated soils [28]. ^{226}Ra activity concentrations in the studied plants were positively correlated with CEC in the contaminated soils, which may indicate that the elevated CEC values might be one of the reasons for increased ^{226}Ra transfer to the plants despite its non-essential role in them [28].

It is suggested that plants take up radium as a substitute ion of essential macro elements such as Ca and Mg [127], [128], due to their analogue chemical behaviour [42].

To confirm that assumption, correlation between ^{226}Ra in plants and Ca/Mg content in soil was tested. The positive linear correlation was found between ^{226}Ra in plants and exchangeable Ca content in soil; oppositely, negative linear correlation was observed between ^{226}Ra in plant and exchangeable Mg content in soil. Similar relationship was found in previously reported works [10], [54], [129], [130], indicating that plants growing in high Ca concentration in soils had the lowest uptake of ^{226}Ra, and vice versa. The authors reported similar correlations for Mg in soil, which is opposite to our finding, i.e. negative linear correlation.

The correlations between biomass of various parts of plants and radium activity concentrations in plants were reported by Smodiš et al. [28]. In our study, the correlation between ^{226}Ra activity concentration in plants and biomass was observed only for the radish and savoy shoot system. In case of our experiment it can be concluded that radioactivity or radium content has no impact on biomass, as found by other authors [131].

It can be observed that CR values are scattered. The interpretation would be analogous to that for uranium and thorium, i.e. it might be attributed to a different UMT content in the substrates that impacted their chemical composition and soil properties, which could affect the plants' capabilities for taking up radium. For the broad leafy vegetables category, the IAEA reports CR average value for Ra 9.1×10^{-2} and for tubers 1.1×10^{-2} [9]. The observed CR values for savoy were one order of magnitude lower for most contamination scenarios while for tubers of radish and rocket they were at the same order of magnitude as the IAEA values [9].

5.2.4 ^{210}Pb uptake by radish, savoy and rocket

Transfer of ^{210}Pb to studied plants was enhanced at all plants from the contaminated substrate compared to control as shown by statistical analyses. However, positive linear correlations were found between ^{210}Pb activity concentrations in plants and in soil for all investigated plants and plant parts (Figure 4.5) which indicate that in our study the main path of ^{210}Pb uptake was via the root system. It has been documented that ^{210}Pb can be transferred from the environment to plants either via the root system or as above-ground interception [82]. Therefore, an experiment was carried out in a controlled closed system with as much as minimized atmospheric ^{222}Rn contribution onto external plant surface contamination by ^{210}Pb-bounded aerosols. The measured average ^{222}Rn activity concentrations were relatively low, 156 and 21 Bq m^{-3} at the situation without and with ventilation, respectively. Therefore, atmospheric impact on ^{210}Pb activity concentrations in plants was assumed negligible. ^{222}Rn was measured as described by Vaupotič et al. [100].

In spite of statistically significant linear correlation between ^{210}Pb activity concentrations in plants and its content in soil, there were some evident changes of ^{210}Pb activity concentrations observed in plants grown in mixtures containing 60 % UMT and 80 % UMT (Table 4.4 and Figure 4.5). Similarly to the radium, further investigations are needed to explain this unexpected anomaly since rational explanation was not found.

The statistical analyses showed that the higher ^{210}Pb activity concentration in roots was significantly higher compared with ^{210}Pb activity concentration in shoot system for radish, savoy and rocket for most contamination levels, except for radish at 20 % of contamination (Table 4.5, Table 4.6 and Table 4.7). The observation confirmed the assumption about negligible atmospheric impact on ^{210}Pb activity concentrations in plants. However, it can be concluded that like other radionuclides lead is also taken up mainly by roots into the plants and concentrations are higher in roots than in aerial parts.

Similarly to the other three investigated radionuclides, strong negative linear correlations were found between ^{210}Pb activity concentrations in all tested plants and most determined parameters of the growing contaminated substrate (available P_2O5 and K_2O, organic matter content, total N, organic C and C/N ratio). ^{210}Pb activity concentrations in all the studied plants were positively correlated with CEC of the contaminated soils. The correlations indicate that reduced ^{210}Pb uptake occurs at increased values of the analysed soil parameters in the UMT contaminated soils. In the literature there is evidence for several elements that CEC may be one of the important controlling factors for their transfer from soil to plants [28], [29], [58]. Therefore, the observed correlation between CEC and ^{210}Pb activity concentration in plants (Table 10) is in agreement with the previously published data [9].

It can be observed that CR values are scattered which might be attributed to a different UMT content in the substrates that impacted their chemical composition and soil properties, which could affect the plants' capabilities for taking up ^{210}Pb. The obtained CR values for savoy and rocket were from one to two orders of magnitude lower than the IAEA [9] reported average ^{210}Pb values for leafy vegetables (8.0×10^{-1}) and for tubers (1.5×10^{-3}), and in the same order of magnitude for tubers of radish, generally for all contamination scenarios. It has to be stressed that the reported CR values are only for the eatable plant part.

5.3 Photochemical Efficiency and Content of Photosynthetic Pigments

Statistical analysis of linear correlations among activity concentrations of the studied radionuclides and photochemical efficiency, content of photosynthetic pigments (chlorophyll a, b) and anthocyanins showed no correlation for all tested plants. There was no clear effect of activity concentrations of the selected radionuclides in soils on photochemical efficiency, content of photosynthetic pigments and anthocyanins values, as all values were comparable with the controls taking into account the uncertainties; the results obtained are summarised in Table 4.18. Since the chemical toxicity of metals has a much bigger effect on physiological parameters of plants than the radiological one [132]–[135], the observation that radiological toxicity had no effect on physiological parameters of the plants was within expectations. Measurements of photosynthetic pigments as well as anthocyanins are an important tool to determine whenever the plant is under stress or not. The response to the stress is expressed in a lower content of photosynthetic pigments and in a higher content of anthocyanins compared to control [136], [137].

5.4 Assessment of Annual Effective Dose Received from Selected Radionuclides by Studied Vegetables Consumption

The calculated total annual effective ingestion dose for average annual intake, considering all four radionuclides, varied from 0.01–0.54, 0.04–0.74, 0.04–0.49 μSv y^{-1} for radish, savoy and rocket, respectively (Figure 4.6). The calculated total annual effective ingestion dose for maximum annual intake considering all four radioisotopes varied from 0.07–4.16, 0.30–4.98, 0.08–1.04 μSv y^{-1} for radish, savoy and rocket, respectively (Figure 4.7). The main contributors to the total annual effective ingestion dose are ^{226}Ra and

^{210}Pb; these two radionuclides have the highest annual effective ingestion doses for all contamination scenarios. Statistical analyses of linear correlation among total annual effective ingestion dose and UMT showed highly significant correlation (Figure 4.6). Therefore, the caution due to potential radiological risk should be recommended whenever vegetables would be grown in a highly radioactively contaminated soil. Many international institutions such as ENSREG (European Nuclear Safety Regulators Group), IAEA (International Atomic Energy Agency) and ATSDR (Agency for Toxic Substances and Disease Registry) conducted several recommendations and regulations due to radiological health risk [138]–[140]. For example, ATSDR published recommendations on how can public lower their exposure to uranium. They recommend avoiding eating root vegetables grown in soils with high levels of uranium as well as washing fruits and vegetables grown in that soil and discard the outside portion of root vegetables [140].

Nevertheless, all the assessed annual effective ingestion doses do not exceed the world ingestion (food and drinking water) average radiation dose of 0.29 mSv y^{-1} and represent less than 2 % of it, as well as it does not exceed IDC (individual dose criterion) value of 0.1 mSv y^{-1}, which represents a very low level of risk that is not expected to give rise to any detectable adverse health effect [117]. In this calculation ^{210}Po was not taken into account as it was not specifically determined. If we suppose that ^{210}Po and ^{210}Pb in the investigated samples are in equilibrium, the annual effective dose would be increased for approximately up to 50 % but would still be lower than 4 % of the world ingestion average radiation dose of 0.29 mSv y^{-1}, and would also not exceeded the IDC value [117]. Therefore, the consumption of the studied vegetables grown at various contamination scenarios does not represent any health hazard concerning radioactivity. However, caution due to potential radiological risk would be needed at higher activity concentrations and at different soil characteristics of growing substrate, as well as with other types of radioactive contamination as it was shown by several authors. Al-Kharouf et al. [141] studied the natural radioactivity in the area of superficial uranium deposits which is regularly used to produce vegetables and fruits consumed by the public. They determined the uranium uptake by crops and hence estimated the effective dose equivalent to human consumption. The assessed annual effective dose per kilogram of watermelon pulp intake was estimated 3.1 nSv y^{-1} for ^{238}U which is in the same range as annual effective ingestion dose for maximum annual intake of savoy (Table 4.19). The annual effective ingestion dose for maximum annual intake of other two vegetables (radish and rocket) for ^{238}U was one order of magnitude lower. Moreover, Shanthi et al. [142] evaluated the radioactivity concentration in the food crops grown in high-level natural radioactive area in south west India. The total annual internal dose due to ^{226}Ra, ^{228}Ra, ^{228}Th, ^{40}K from ingestion of food samples in the study was 1.8 mSv y^{-1} which is much higher than our estimated values where other radionuclides and vegetables were considered. Different approach for dose assessment was used by Carvalho et al. [143]. To assess the ingestion of radionuclides from uranium mining and milling tailings and other sources related to the legacy of uranium mining, homemade soups prepared with vegetables and legumes grown in the area of Urgeiriça mining and milling (Portugal) were analysed. The ingestion radiation doses were 0.036 and 0.04 mSv y^{-1} for soup and main dish, respectively. From the our accomplished study and presented literature survey can be observed that the dose assessment is complex and the numerical estimation of uncertainty is difficult since there are many influencing factors such as estimation of intake (food type, amount of ingestion), time between harvesting of vegetables and consumption, determination of radionuclides, etc.

Chapter 6

Conclusions

In the first part of the doctoral dissertation the method optimisation was accomplished while in the second part the soil-to-plant transfer of natural radionuclides was studied. Instrumental neutron activation analysis was used for the determination of uranium in SRMs, real soil samples and their residues obtained after three dissolution techniques (open-vessel wet dissolution with mixtures of the acids HNO_3, $HClO_4$ and HF, closed-vessel microwave dissolution using HNO_3 and HF followed by open-vessel dissolution with $HClO_4$, and alkaline fusion with Na_2CO_3 and Na_2O_2 followed by mineralization with HNO_3, HCl, HF and H_2SO_4). Complete decomposition of the soil sample was achieved only with alkaline fusion by Na_2CO_3 and Na_2O_2. After conventional wet and microwave dissolution soil samples are not decomposed completely and the residue can represent more than 10 % of the mass of the sample. Given the different mineral composition of samples, the residue can thus represent a highly significant proportion of the ultimate analytical outcome – an up to 60 % contribution to the total content of uranium. Better results were achieved with conventional wet dissolution, where residues represented up to 35 % less by mass and up to 50 % lower by mass content compared to microwave dissolution. The amount of uranium dissolved is influenced by the presence of hydrofluoric acid, which decomposes silicates and releases uranium linked to their structure. Preliminary results showed that leaving the residue after microwave dissolution overnight in hydrofluoric acid at room temperature reduced the deficit of uranium compared to immediate evaporation of acid by up to 6 %, but the losses are still unacceptable.

Next, three dissolution techniques used in determination of ^{210}Po in biological samples were compared. The dissolution techniques involved classical wet ashing with acids in a 100 mL long-necked Kjeldahl flask by heating vigorously over a gas flame, digestion with acids in an Erlenmeyer flask at temperatures up to 200 °C and wet digestion by a microwave system in Teflon vessels up to 200 °C were investigated. The results obtained showed that the activity concentrations of ^{210}Po found in biological samples were not statistically different between the three digestion methods. The choice of dissolution techniques hence mainly depends on the required sample mass and its matrix.

Often problems related to determination of radionuclides in botanical materials are small available sample masses, which was also the case in our plant pot experiment. Therefore, the optimisation of radiochemical analytical method was needed to obtain as much information as possible from a certain sample. During the optimisation process it was shown that the method for consecutive determination of selected radionuclides is appropriate for plant matrixes and small sample masses (up to 2 g of dried sample) and can be further used for determination of selected radionuclides in cultivated plants.

To summarise, the optimised dissolution techniques as well as consecutive method for determination of selected natural radionuclides by alpha-particle spectrometry are appropriate for low activity concentration, small sample masses and natural matrixes.

The selected tested Brassica plants, namely radish, savoy and rocket, proved to be appropriate for the study of natural radionuclides transfer from soil to plants. The obtained results showed correlations among soil and plant activity concentrations of selected radionuclides. The ANOVA based experiment design turned out to be applicable for further statistical analyses of obtained data.

Radish, savoy and rocket can accumulate natural radionuclides, such as ^{238}U, ^{230}Th, ^{226}Ra and ^{210}Pb, from substrate contaminated with UMT to eatable parts of plants with the highest activity concentrations in root systems as it was confirmed with statistical analyses of linear correlation and Duncan's Multiple Range test, respectively. The highest soil-to-plant transfer of ^{226}Ra, compared with the other three investigated radionuclides, can partially be explained with the reported fact that Ra has a similar chemical behaviour as Ca, which is known as an essential macro element for plants. However, the results obtained suggested the analogue chemical behaviour of radium and calcium while for magnesium this could not be confirmed. Further, it was shown that in general UMT has no negative effect on biomass production of cultivated plants under established experimental conditions.

The impact of soil parameters on the availability of the studied natural radionuclides was found as an important controlling factor, as also confirmed by other authors. The activity concentrations of radionuclides in plants were positively correlated with CEC in the contaminated soils, which may indicate that CEC is an important controlling factor in case of radionuclides plant uptake. On the other hand, the found correlation among other soil parameters (available P_2O_5 and K_2O, organic matter content, total N, organic C and C/N ratio) was negative.

The obtained CR ratios were lower than 1 for all investigated plants, which leads to the conclusion that radish, savoy and rocket cannot be used for phytoremediation purposes under the investigated growth conditions. However, the understanding of soil-to-plant transfer behaviour of natural radionuclides in contaminated areas or in areas with naturally elevated values of NORM radionuclides is crucial for the correctness of dose assessments and in developing appropriate radiological models, as well as it is very important for upgrading the global database for the human and biota dose-assessment modelling.

The study also revealed that radiological toxicity has no clear effect on physiological parameters of plants (photochemical efficiency, content of photosynthetic pigments and anthocyanins content) at the given activity concentration levels of the selected radionuclides. In addition, the values of physiological parameters of plants showed that plants were not under stress since these parameters are an important tool to determine plant stress.

The assessed total annual effective ingestion dose received by humans from the studied radionuclides of uranium, thorium, radium and lead did not exceed any of the threshold limit values and represented less than 2 % world ingestion average dose. In addition, assuming that ^{210}Po and ^{210}Pb in the investigated samples are in equilibrium, the annual effective dose would still not exceed any of the threshold limit values. Therefore, the consumption of radish, savoy and rocket grown under the described experimental conditions does not pose a health hazard. However, the total annual effective ingestion dose was linearly correlated with the content of UMT. Therefore, the caution due to potential radiological risk should be recommended whenever vegetables would be grown in a highly radioactively contaminated soil.

Appendix A

Publications Describing Details of Decomposition Techniques

A.1 **Analysis of Uranium in the Insoluble Residues After Decomposition of Soil Samples by Various Techniques.** ***Appl. Radiat. Isot.: 87, 61–65 (2014).***

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Analysis of uranium in the insoluble residues after decomposition of soil samples by various techniques

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HIGHLIGHTS

- Various most often used dissolution techniques for soil samples were investigated.
- Conventional wet ashing, microwave dissolution and alkaline fusion were tested.
- The residues obtained after dissolution were tested for uranium content.
- Instrumental neutron activation analysis was used for uranium determination.
- The results showed that the tested dissolution techniques must be improved.

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ABSTRACT

A comparison of different dissolution techniques for determination of uranium in soil samples was made. Conventional wet dissolution with mixtures of HNO_3, $HClO_4$ and HF acids, microwave dissolution using HNO_3 and HF, and alkaline fusion with Na_2CO_3 and Na_2O_2 were evaluated. For testing the effectiveness of the dissolution procedures, two reference materials and six soil samples from the surroundings of a former uranium mine were investigated. It was observed that the content of uranium in the residues determined by INAA represents a significant contribution to its total concentration in the sample, especially in the case of microwave dissolution.

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1. Introduction

One of the most important requirements in radiochemical analysis is that before isolation of the radionuclides of interest the sample must be completely dissolved. It is well known that soil samples are often considered one of the most difficult matrices to dissolve. Uranium is incorporated into the structure of difficultly soluble minerals—such as zircon, apatite, titanite, allanite etc. These minerals are part of the soil and it is very important that they are completely decomposed during chemical treatment.

Conventional wet dissolution and microwave dissolution are two of the most often techniques used for dissolution of soil and sediment. But these techniques, in spite of using combinations of acids such as HF, HNO_3 and $HClO_4$, do not achieve complete dissolution for the majority of actinide-containing oxides. It was proven, that zircon ($ZrSiO_4$), can contain up to 15% of the uranium content. Therefore for total dissolution authors suggested alkaline fusion based on the use of a $LiBO_2$/LiBr melt in combination with extraction chromatography and ICP-MS analysis (Milliard et al., 2011). $LiBO_2$/$Li_2B_4O_7$, NaOH/Na_2O_2 and $LiBO_2$/$Li_2B_4O_7$/LiBr are also widely used melting reagents (Croudace et al., 1998; Galindo et al., 2007; Saidou et al., 2008). The leachability of some natural and man-made radionuclides from soil and sediments on acid attack was also previously investigated in our laboratory (Benedik et al., 1999).

The concentration of uranium in soil depends on its mineral composition. Due to the fact that many minerals containing uranium are hard to decompose, the measured concentration of uranium in soil samples may be lower than the actual. A comparison of the use of H_2SO_4 and the combination of HF and HNO_3 (Selvig et al., 2005) showed a smaller recovery of uranium using H_2SO_4, which dissolves smaller proportions of the minerals apatite, zircon and titanite compared to the combination of HF and HNO_3. The same problem also occurs in reference samples such as Rocky Flats Soil I (SRM 4353), Rocky Flats Soil II (4353a), Columbia River Srediment (SRM 4350B) and Ocean Sediment (SRM 4357) (Motabar et al., 2009). It may be noted that some NIST standard reference materials (SRMs) contain only 0.08% of such minerals, which are very difficult to degrade, but because they contain a significant amount of uranium, they have a significant

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impact on the concentration determined in the sample. It was determined that in some cases ~0.03% insoluble residue contains 15% U (Inn, 2012).

In determining Mn, Ni, Zn, Pb, Cr, Cd, Cu, In, Mg, Ca, Fe and Al in soil samples by ICP-AES the authors compared microwave dissolution of the sample with a mixture of HNO_3 and HF, and mixture of HNO_3, HF and HCl. The combination of all three acids brought better results for most metals (Sandroni and Smith, 2002).

Various authors (Sastre et al., 2002; Marin et al., 2002; Sandroni and Smith, 2002) reported when determining elements in soil samples using microwave dissolution with a combination of the acids HCl, HNO_3 and HF, that after decomposition the sample was filtered, and the residues discarded and the analysis performed only with the filtered solution. No data on the concentrations of the investigated elements in the residue was given.

The aim of our work was to compare various dissolution techniques for determination of uranium in soil and sediment samples with emphasis on the content of uranium in the residues obtained after dissolution of the samples. Conventional open-vessel wet dissolution with mixtures of the acids HNO_3, $HClO_4$ and HF, closed-vessel microwave dissolution using HNO_3 and HF followed by open-vessel dissolution with $HClO_4$, and alkaline fusion with Na_2CO_3 and Na_2O_2 in a glassy carbon crucible followed by mineralization with HNO_3, HCl, HF and H_2SO_4 were used. For determination of uranium in the insoluble residues, instrumental neutron activation analysis was applied. Samples studied included one soil and one sediment RMs and six real soil samples from the vicinity of a former uranium mine.

2. Experimental work

2.1. Reagents

All reagents used in the radiochemical procedures were of analytical grade. A working standard solution of 5.073 g g^{-1} uranium was prepared by diluting a uranium standard solution obtained from NIST (SRM 3164 Uranium (U) Standard Solution).

2.2. Samples and sample preparation

For this study two reference materials (NIST-4353a Rocky Flats Soil and NBS-4354 Freshwater Lake Sediment) with certified uranium values, and six real soil samples were used. The soil samples were collected at six locations in the surroundings of the former Uranium mine at Žirovski vrh, Slovenia. The samples of soils were prepared in our laboratory (Štrok and Smodiš, 2010) and their elemental composition was given in Table 2. Pre-treatment of the samples was performed according to ISO 11464 (ISO, 1994). After sampling the collected soils were dried at 80 °C. Samples were hand ground and sieved through a 2 mm sieve. A part of the soil samples was additionally ground with an agate ball grinder (Fritsch, Planetary Micro Mill pulverisette-7) and sifted with an orbital laboratory seed drill (Fritsch, Pulversiette-0), which had a pore size of 90 μm.

2.3. Sample dissolution procedures

Prior to dissolution, the samples were first ashed at 650 °C in a muffle furnace to remove organic matter. Ashed samples were then decomposed by three different techniques as follows: conventional wet dissolution with mixtures of the acids HNO_3, $HClO_4$ and HF, microwave dissolution using HNO_3 and HF, and alkaline fusion with Na_2CO_3 and Na_2O_2. The study of the decomposition of soil samples included the two different grain sizes of 2 mm and 0.09 mm.

2.3.1. Conventional wet dissolution with mixtures of HNO_3, $HClO_4$ and HF acids

Up to 1 g of sample was weighed in a Teflon beaker, and then additions of the acids HNO_3 (2 × 5 mL) and two times a mixture of HNO_3 (5 mL)+$HClO_4$ (10 mL)+HF (10 mL) were successively added. Samples were heated to 250 °C to near dryness. The dry sample residues were then redissolved in 10 mL of 3 M HNO_3. The residue was then separated from the solution by centrifugation. The residues were dried and prepared for neutron irradiation.

2.3.2. Microwave dissolution using HNO_3 and HF

An ETHOS 1 (MILESTONE SN 130471) microwave system with a maximum power of 1300 W and a Teflon vessel (HPR-1000/10S high pressure segment rotor) was used for digestion. Up to 1 g of sample was weighed into each vessel and then 5 mL of concentrated HNO_3 and 10 mL of HF were added. The microwave heating conditions are given in Table 1.

After microwave dissolution the contents were transferred to another Teflon beaker and treatment on a hot plate with $HClO_4$ in the open-vessel followed. The content was evaporated to dryness and redissolved in 3 M HNO_3. The residue from this solution was then separated by centrifugation. The residues were dried and prepared for neutron irradiation.

2.3.3. Dissolution by alkaline fusion with Na_2CO_3 and Na_2O_2

Up to 1 g of the sample was added to graphite crucible together with 2 g of Na_2CO_3 and 2 g of Na_2O_2 and fused for 5 min at 900 °C in a Clasic Clare 4.0 oven. The fused sample was removed from the oven, cooled to room temperature and the melt dissolved in 2 mL of HNO_3 and 2 mL of H_2O. The solution was transferred to a Teflon beaker and treated with a mixture of HNO_3, HCl and HF by heating in the open-vessel. At the end, a portion of H_2SO_4 was added, and the solution evaporated to dryness and dissolved in 3 M HNO_3. Using this technique soil samples were completely dissolved without any residue.

2.4. Instrumental neutron activation analysis

Instrumental neutron activation was applied for the determination of ^{238}U by ^{239}Np ($t1/2=2.356$ d) induced via the reaction $^{238}U(n,\gamma)^{239}U$ ($t1/2=23.46$ min)→^{239}Np (LNHB, 2013; recommended data). The samples and residues were weighed into polythene ampoules, doubly encapsulated to avoid superficial contamination and irradiated with uranium standards in the Institute's TRIGA Mark II reactor. The time of irradiation was up to 12 h in the rotary specimen rack at a neutron fluence of 1.5×10^{12} n cm^{-2} s^{-1}. The irradiated samples were cooled for up to 3 days and then transferred to a measuring vial and counted on a HP Ge well-type detector connected to a Canberra MCA by Genie 2000 software. The activity of the induced nuclide ^{239}Np was measured at 277.599 keV ($P\gamma=14.4\%$) (LNHB, 2013; recommended data). For evaluation of gamma spectra, the HyperLab (2005) program was used.

Table 1
Microwave heating conditions for digestion by the microwave system.

Step	Power (W)	Hold time (min)	Temperature in vessel (°C)	Temperature in system (°C)
1	1300	20	130	75
2	1300	20	130–200	75–130
3	1300	40	200	130

2.5. Determination of elemental composition by X-ray fluorescence spectrometry

Elemental composition of reference materials and soil samples was determined by nondestructive energy dispersive X-ray fluorescence spectrometry (EDXRF). Samples were excited by radiation sources ^{55}Fe, ^{109}Cd and ^{241}Am1 (Nečemer et al., 2008).

3. Results and discussion

Complete dissolution of soil samples without residues was obtained only by alkaline fusion with Na_2CO_3 and Na_2O_2. The same result was also reported by Jia et al. (2004). In the case of conventional wet ashing and microwave digestion, significant residues were observed. As has already been reported in some papers (Selvig et al., 2005; Motabar et al., 2009; Milliard et al., 2011), decomposition of a soil sample depends on its mineral composition. Soil from the surroundings of the former uranium mine at Žirovski vrh consists of illite, calcite, montmorillonite, brightly coloured siltstones, sandstones, tuffites and limestone (Omaljev, 1982). It is known that uranium is incorporated in the structure of the minerals of illite and montmorillonite which contain more than 20% of silicon. The concentration of elements determined by X-ray fluorescence spectrometry in the reference materials investigated and the chosen soil samples are presented in Table 2.

The content of silicon in the investigated samples is shown in Fig. 1 and represents more than 25% of the composition of soil.

The mass concentration of uranium in the investigated samples and in the residues after conventional wet dissolution and microwave dissolution with mineral acids was determined by INAA via its induced nuclide ^{239}Np

$$^{238}U(n,\gamma)^{239}U \xrightarrow{\beta^-} {}^{239}Np \rightarrow$$

The mass concentration was converted to an activity concentration using the factor 1 mg ^{238}U ≡ 12.4 Bq. The uranium content in the samples and in the residues obtained are presented in Table 3, after conversion to activity units. For the real soil samples the values presented for uranium were obtained in samples and their residues for material grain sizes of 2 mm and 0.09 mm. Reference materials were analysed as received; the results for the residues represent INAA analyses after the two different dissolution techniques.

The results obtained show that the samples were not completely dissolved by acid treatment and that a significant amount of uranium was found in the residues. When conventional wet ashing was used, the reference material NBS 4354 Fresh Lake Sediment was completely dissolved, while for the reference material NIST 4353a Rocky Flat Soils a residue was observed. Its mass represented less than 1%, with approximately 1.6% of the content of uranium. From Table 3 and Fig. 2 is evident that the grain size affects the level of uranium in the residues of decomposed samples obtained by classical wet digestion. In five cases out of six the values of uranium in residue were approximately 5% lower for smaller grains (bellow <0.09 mm) than those where grain size was <2 mm when classical wet digestion was applied. The mass of the residues obtained after classical wet ashing of real soil samples in three cases represented less than 10% of the total mass of the samples analysed but in three cases more than 10%.

The microwave technique gave worse results for dissolution of the samples. A small mass of 0.2% of residue was observed after dissolution of NBS 4354 Fresh Lake Sediment with a content of uranium less than 2% of the total activity. The reference material NIST 4353a Rocky Flat Soils was not completely dissolved, as also described Inn (2012). The mass of residue obtained was 4.4% of the total sample analysed, but the uranium content in the residue represented approximately 35% of the uranium analysed in the sample. The mass of residue obtained after microwave dissolution

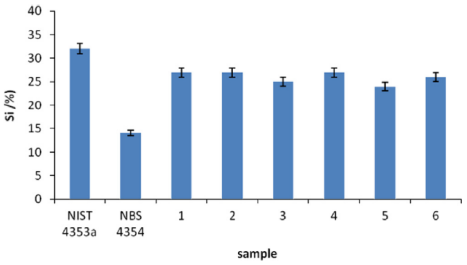


Fig. 1. Silicon content (in %) in the investigated samples obtained by X-ray fluorescence spectrometry (uncertainty values shown are reported as combined uncertainty expanded by a coverage factor $k=2$).

Table 2
Elemental composition of reference materials and soil samples in g g $^{-1}$ determined by X-ray fluorescence spectrometry.

	NIST 4353a	NBS 4354	Soil 1	Soil 2	Soil 3	Soil 4	Soil 5	Soil 6
Al	4.4 E−02	1.4 E−02	1.1 E−01	1.0 E−01	9.0 E−02	1.2 E−01	1.1 E−01	1.1 E−01
Si	3.2 E−01	1.4 E−01	2.5 E−01	2.7 E−01	2.7 E−01	2.7 E−01	2.6 E−01	2.4 E−01
P	9.7 E−03	2.9 E−03	6.0 E−03	4.0 E−03	6.8 E−03	5.0 E−03	5.7 E−03	/
K	1.6 E−02	1.7 E−03	1.5 E−02	1.5 E−02	1.8 E−02	1.5 E−02	1.7 E−02	1.6 E−02
Ca	3.9 E−03	9.2 E−03	9.8 E−04	2.6 E−03	2.2 E−03	3.9 E−03	4.7 E−03	25 E−02
Ti	2.9 E−03	7.3 E−04	5.9 E−03	5.5 E−03	5.1 E−03	4.6 E−03	5.7 E−03	4.0 E−03
Mn	1.9 E−05	5.6 E−04	2.0 E−02	2.4 E−04	3.6 E−04	3.1 E−04	4.2 E−04	1.3 E−04
Fe	2.8 E−02	2.7 E−02	4.5 E−04	3.0 E−02	2.8 E−02	3.4 E−02	2.7 E−02	2.6 E−02
Cu	1.6 E−05	6.6 E−06	1.9 E−05	1.6 E−05	2.6 E−05	3.1 E−05	1.9 E−05	1.7 E−05
Zn	8.7 E−05	2.9 E−05	1.0 E−04	5.1 E−05	1.0 E−04	1.4 E−04	1.4 E−04	3.9 E−05
Ga	1.9 E−05	1.0 E−05	5.0 E−06	2.0 E−05	1.5 E−05	2.6 E−05	1.6 E−05	2.3 E−05
Pb	8.1 E−05	5.1 E−05	2.3 E−05	5.6 E−05	7.6 E−05	7.1 E−05	9.8 E−05	3.8 E−05
Br	1.1 E−06	4.2 E−06	3.9 E−05	1.4 E−06	5.1 E−06	5.0 E−06	4.1 E−06	1.1 E−06
Rb	7.6 E−05	7.4 E−05	8.1 E−06	8.0 E−05	1.2E−04	9.0 E−05	9.1 E−05	8.4 E−05
Sr	4.1 E−05	1.6 E−05	7.5 E−05	4.8 E−05	5.8 E−05	5.5 E−05	5.4 E−05	4.9 E−05
Th	1.5 E−05	1.6 E−05	2.0 E−06	3.7 E−05	1.1 E−05	2.4 E−05	1.7 E−05	2.1 E−05
Y	4.2 E−05	2.2 E−05	2.8 E−05	2.0 E−05	2.6 E−05	3.4 E−05	1.4 E−05	29 E−05
U	3.0 E−05	3.9 E−06	2.0 E−06	6.9 E−06	4.9 E−06	1.1 E−04	4.7 E−05	3.8 E−06
Zr	3.6 E−04	3.5 E−04	3.1 E−05	4.0 E−04	2.8 E−04	4.2 E−04	2.9 E−04	4.1 E−04
Nb	9.5 E−05	1.2 E−04	4.4 E−06	2.0 E−05	2.1 E−05	3.9 E−05	2.7 E−05	1.9 E−05

Table 3
Activity concentration of uranium (in Bq kg $^{-1}$) [a] in investigated samples and the residues obtained by INAA after conventional wet ashing and microwave digestion.

Sample	Uranium in residue after dissolution[c]							
	Total uranium	Conventional wet ashing				Microwave digestion			
	Bq kg $^{-1}$	Grain size <2 mm		Grain size <0.09 mm		Grain size <2 mm		Grain size <0.09 mm	
		Bq kg $^{-1}$	%	Bq kg $^{-1}$	%	Bq kg $^{-1}$	%	Bq kg $^{-1}$	%
1	67 ± 5	10.5 ± 1.0	15.7 ± 3.0	16.3 ± 1.0	24.3 ± 3.0	28.7 ± 1.9	42.8 ± 5.7	36.4 ± 2.7	54.3 ± 8.1
2	74 ± 5	25.1 ± 1.5	33.9 ± 4.1	20.3 ± 1.5	27.4 ± 4.1	18.6 ± 1.2	25.1 ± 3.2	43.0 ± 3.1	58.1 ± 8.4
3	126 ± 9	47.0 ± 3.0	37.3 ± 4.8	26.4 ± 1.8	21.0 ± 2.9	49.8 ± 3.4	63.3 ± 5.4	57.8 ± 4.1	49.9 ± 6.5
4	497 ± 32	51.1 ± 3.3	10.3 ± 1.3	31.2 ± 2.3	6.3 ± 0.9	269 ± 19	54.1 ± 7.6	273 ± 19	54.9 ± 7.6
5	670 ± 42	39.3 ± 2.7	5.9 ± 0.8	18.1 ± 1.5	2.7 ± 0.5	364 ± 24	54.3 ± 7.2	364 ± 39	54.3 ± 11.6
6	1900 ± 120	82.5 ± 5.4	4.4 ± 0.6	58.7 ± 4.4	3.1 ± 0.5	624 ± 40	33.2 ± 4.3	543 ± 26	28.9 ± 2.8
NIST 4353a Rocky Flats Soil	45.8 ± 3.0 (39.6 ± 3.0)[b]	0.73 ± 0.04	1.6 ± 0.2			16.0 ± 1.0	34.9 ± 2.2		
NBS 4354 Freshwater Lake Sediment	16.3 ± 1.2 (17.4 ± 1.4)[b]					0.27 ± 0.02	1.6 ± 0.1		

[a] Average $\pm$ standard deviation of minimum three measurements.
[b] Certified reference value.
[c] Value calculated on activity in original sample.

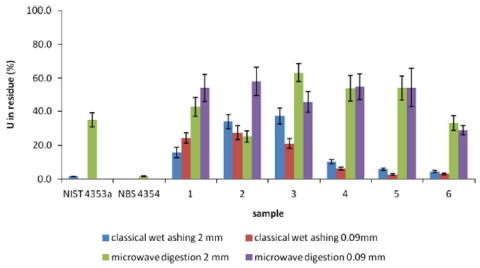


Fig. 2. Percentage of uranium in residues obtained after classical wet ashing and microwave digestion compared to the total content in the samples (uncertainty values reported as combined uncertainty expanded by a coverage factor $k=2$).

of real soil samples was in all cases more than 10%, and the uranium content in these residues represented from 25 to 63% of the total amount of uranium in the samples. In four cases out of six the values of uranium in residue were lower for smaller grains (bellow <0.09 mm) than those where soil with grain size <2 mm was analysed.

Due to the very poor results obtained by microwave digestion, the efficiency of dissolution by this technique should be improved by the use of larger volumes of acids, a longer time at the final temperature and then leaving the sample overnight in acid at room temperature. The another option is addition of H3BO3 to dissolve insoluble fluorides which obviously appear in case of microwave digestion as it is described by García and Kahn (2001). For conventional wet dissolution, grinding in most cases proved to be a good way to increase the efficiency of the extraction. From Table 3 and Fig. 2 it is clearly seen that the amount of uranium in the residues obtained in conventional wet dissolution are much lower than the activities obtained by microwave dissolution. While dissolution by the microwave procedure is less time consuming (about 4 h in microwave system and 12 h for evaporation) than classical wet ashing (about 2 days), the loss of uranium is inacceptable, and the procedure should be improved.

4. Conclusions

Instrumental neutron activation analysis was used for determination of uranium in SRMs, real soil samples and their residues obtained after three dissolution techniques. Complete decomposition of the soil sample was achieved only with alkaline fusion by Na_2CO_3 and Na_2O_2. After conventional wet and microwave dissolution soil samples are not decomposed completely and the residue can represent more than 10% of the weight of the sample. Given the different mineral composition of samples, the residue can thus represent a highly significant proportion of the ultimate analytical outcome- an up to 60% contribution to the total content of uranium. Better results were achieved with conventional wet dissolution, where residues represented up to 35% less by weight and up to 50% lower by mass content compared to microwave dissolution.

The amount of uranium dissolved is influenced by the presence of hydrofluoric acid, which decomposes silicates and releases uranium linked to their structure. Preliminary results showed that leaving the residue after microwave dissolution overnight in hydrofluoric acid at room temperature reduced the deficit of uranium compared to immediate evaporation of acid by up to 6%, but the losses are still unacceptable.

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References

Benedik, L., Pintar, H., Byrne, A.R., 1999. The leachability of some natural and man-made radionuclides from soil and sediments on the acid attack. J. Radioanal. Nucl. Chem. 240, 859–865.
Croudace, I., Warwick, P., Taylor, R., Dee, S., 1998. Rapid procedure for plutonium and uranium determination in soils using a borate fusion followed by ion-exchange and extraction chromatography. Anal. Chim. Acta 371, 217–225.
Galindo, C., Mougin, L., Nourreddine, A., 2007. An improved radiochemical separation of uranium and thorium in environmental samples involving peroxide fusion. Appl. Radiat. Isot. 65 9–6.
García, R., Kahn, B., 2001. Total dissolution of environmental and biological samples by closed-vessel microwave digestion for radiometric analysis. J. Radioanal. Nucl. Chem. 250, 85–91.
HyperLab, 2005. System, Installation and Quick Start Guide, HyperLabs Software, Budapest, Hungary.
Inn, K. G.W., 2012. Analysis of uranium and thorium in soil and sediments—how come we sometimes get the wrong results? In: Sixth International Conference on Radionuclide Metrology Low-level Radioactivity Measurement Techniques, 17–21 September 2012, Jeju, Korea, Abstracts of ICRM-LLRMT12, O-062.
ISO, 1994. ISO 11464: 1994(E) Soil Quality—Pretreatment of Samples for Physico-chemical Analyses. International Organization for Standardization, Geneva, Switzerland.

A.1. Analysis of Uranium in the Insoluble Residues After Decomposition of Soil Samples by Various Techniques. Appl. Radiat. Isot.: 87, 61–65 (2014). 75

Jia, G., Torri, G., Innocenzi, P., 2004. An improved method for the determination of uranium isotopes in environmental samples by alpha-spectrometry. J. Radioanal. Nucl. Chem. 262, 433–441.
LNHB, 2013. Recommended data: (⟨http://www.nucleide.org/DDEP_WG/DDEPdata.htm⟩).
Marin, B., Chopin, E.I.B., Jupinet, B., Gauthier, D., 2002. Comparison of microwave-assisted digestion procedures for total trace element content determination in calcareous soils. Talanta 77, 282–288.
Milliard, A., Durand-Jézéquel, M., Lariviere, D., 2011. Sequential automated fusion/extraction chromatography methodology for the dissolution of uranium in environmental samples for mass spectrometric determination. Anal. Chim. Acta 684, 40–46.
Motabar, P., Inn, K.G.W., Davis, J., LaRosa, J., 2009. Identification and characterization of resistate minerals containing uranium and thorium. J. Radioanal. Nucl. Chem. 282, 335–341.
Nečemer, M., Kump, P., Ščančar, J., Jačimović, R., Simčič, J., Pelicon, P., Budnar, M., Jeran, Z., Ponger, P., Regvar, M., Vojel – Mikuš, K., 2008. Application of X-ray fluorescence analytical techniques in phytoremediation and plant biology studies. Spectrochim. Acta, Part B 63. (1260-1247).
Omaljev, V., 1982. Metallogenetic characteristics of the uranium ore deposit of Žirovski vrh. Monographs vol. 7. (Geoinstitut, Beograd).
Saidou, F.B., Laedermann, J.P, Kwato Njock, M.G., Froidevaux, P., 2008. A comparison of alpha and gamma spectrometry for environmental natural radioactivity surveys. Appl. Radiat. Isot. 66, 215–222.
Sandroni, V., Smith, C.M.M., 2002. Microwave digestion of sludge, soil and sediment samples for metal analysis by inductively coupled plasma-atomic emission spectrometry. Anal. Chim. Acta 468, 335–344.
Sastre, J., Sahuquillo, A., Vidal, M., Rauret, G, 2002. Determination of Cd, Cu, Pb and Zn in environmental samples: microwave-assisted total digestion versus aqua regia and nitric acid extraction. Anal. Chim. Acta 462, 59–72.
Selvig, L.K., Inn, K.G.W., Outola, I.M.J., Kurosaki, H.K., Lee, A., 2005. Dissolution of resistate minerals containing uranium and thorium: environmental implications. J. Radioanal. Nucl. Chem. 263, 341–348.
Štrok, M., Smodiš, B., 2010. Fractionation of natural radionuclides in soils from the vicinity of a former uranium mine Žirovski vrh, Slovenia. J. Environm. Radioact 101, 22–28.

A.2 Comparison of Various Dissolution Techniques for Determination of Po-210 in Biological Samples. *Appl. Radiat. Isot.: 81, 53 – 56 (2013).*

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Comparison of various dissolution techniques for determination of Po-210 in biological samples

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HIGHLIGHTS

- We compared three dissolution techniques for determination of Po-210 in biological samples.
- Classical wet ashing, digestion in an Erlenmeyer flask and by microwave digestion were used.
- The maximum digestion temperature was 200 °C.
- The results obtained showed that all the used procedures are comparable.
- The choice of dissolution techniques depends on required sample mass and matrix of the sample.

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ABSTRACT

The aim of the present study was to compare three wet digestion procedures for dissolution of biological samples in the determination of Po-210. Classical wet ashing over a gas flame with acids in a long-necked Kjeldahl flask, digestion with acids in an Erlenmeyer flask and microwave digestion in a Teflon vessel at temperatures at up to 200 °C were investigated. The results obtained showed that the activity concentrations of Po-210 found in the samples analysed were comparable for all the procedures used.

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1. Introduction

Polonium-210 is a pure alpha emitter and due to its short half life of 138.4 days and physiological role in organisms, it is known as one of the radionuclides with the highest radiotoxicity and one of the most important sources of the internal dose received by humans from ingestion of food (IAEA, 2003). Because of its widespread distribution in the environment, Po-210 enters food chains through direct uptake, ingestion or inhalation. Accuracy in the determination of the activity concentrations of Po-210 in various samples depends heavily on the sample dissolution techniques used. Due to the volatility of polonium, the analyst has to be aware of possible loss occurring during the dissolution procedures and achieve the best performance to minimise or avoid this effect. Data on the volatilization of polonium during wet ashing with regard to the temperature used are scarce. Henricsson et al. (2011) investigated the loss of activity from wet digested samples by using a double-tracer method. Wet digestions were carried out in a glass beaker or a Kjeldahl flask on hot plates at 70–80 °C or in a microwave system at up to 200 °C. As reported by Louw et al. (2009), foodstuff samples were dissolved using an open vessel microwave digestion system at 100 °C. For dissolution of tobacco samples, Khater (2004) employed wet acid digestion using a mixture of HNO_3 and HF acids on a sand bath at a temperature of about 80 °C, while Savidou et al. (2006) used only leaching of up to 10 g of dried sample for 1 h with nitric acid. Recovery of polonium using a microwave bomb digestion procedure was evaluated by Towler and Smith (1994). The digestion of plant and animal tissues in concentrated nitric acid in a closed vessel at up to 1380 kPa took up to 2 h. Before transformation to hydrochloric acid medium, the nitric acid was removed by evaporation in an open beaker at a temperature below 150 °C. In our previous work (Vrečak et al., 2004) we investigated hot acid dissolution and cold leaching for determination of Pb-210 and Po-210 in sediments, soils and biological materials. During dissolution, the temperature was controlled and did not exceed 80 °C. Meli et al. (2009) reported that for determination of Po-210 in urine samples preconcentration was performed by evaporation of the sample up to 150 °C, and the residue was treated at a controlled temperature of 85–90 °C. Germain et al. (1995) reported dissolution of powdered biological samples in a Teflon bomb using concentrated nitric acid over a period of 48 h at 80 °C.

Due to the fact that the literature data on the recovery of different dissolution procedures is scarce and conflicting, the aim of the work presented was to compare three wet ashing techniques for determination of Po-210 in biological samples, particularly regarding volatilisation losses.

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2. Experimental

2.1. Reagents

All reagents used in the radiochemical procedures were of analytical grade. The tracer solution of Po-209 used as a working solution in the study (0.3 Bq g^{-1}) was prepared from calibrated solutions purchased from Analytics, Inc. (Atlanta, GA, USA). The producer maintains traceability to NIST standards.

2.2. Samples

For this study seven plant and six real mussel (*Mytilus galloprovincialis*) samples were used. The plant samples included four reference materials (NIST-4359 Seaweed Radionuclide Standard, IAEA-307 Sea Plant, CTA-OTL-1 Oriental Tobacco Leaves, CTA-VTL-2 Virginia Tobacco Leaves) and three real lichen samples. The samples of lichen and mussels 1–3 were prepared in our laboratory. Lichen samples were collected in Slovenia while, mussel samples 1–3 were bought on the domestic market. Lichen and mussel samples 1–3 were then lyophilised to constant weight and homogenised by grinding in a mill with agate balls. Mussel samples 4–6 were prepared as described by Rožmarić et al. (2012).

2.3. Radiochemical procedure

After addition of a Po-209 tracer, the samples were decomposed by three different wet ashing techniques as follows: classical wet ashing with acids in a 100 mL long-necked quartz Kjeldahl flask by heating vigorously over a gas flame; digestion with acids in an Erlenmeyer flask at a temperature on a hot plate up to 200 °C, and wet digestion by a microwave system in a Teflon vessel up to 200 °C. After dissolution, the dry sample residues were redissolved in 2 mL of concentrated HCl, diluted with deionised water to 100 mL and filtered if necessary. 0.5 g of ascorbic acid and 0.5 g of hydroxylammonium chloride were added to the solution to reduce Fe(III) and prevent co-plating of iron and interfering elements. The spontaneous deposition of polonium on a 19 mm diameter silver disk was carried out at 90 °C for 4 h (Benedik and Vreček, 2001).

2.3.1. Dissolution in a Kjeldahl flask

For dissolution of biological materials a procedure similar to that for determination of uranium by radiochemical neutron activation analysis was used (Repinc and Benedik 2005). Up to 1 g of the sample was transferred to a 100 mL long-neck quartz Kjeldahl flask already containing 10 mL of concentrated nitric acid and Po-209 tracer. The sample was wet-ashed by heating over a gas flame, as shown in Fig. 1. The distance between the flame and the Kjeldahl flask was controlled so that during dissolution the temperature of the fumes at the end of the long-neck did not exceed 120 °C. A few millilitre of concentrated nitric acid and 30% H_2O_2 were added several times and evaporated to dryness. The dissolution procedure was finished when the residue obtained did not darken on heating. The flask with dry residue was cooled by plunging it into water and the sample was transferred with 2 mL of concentrated HCl and deionised water to a glass beaker for spontaneous deposition of polonium radioisotopes.

2.3.2. Dissolution in an Erlenmeyer flask

This procedure for dissolution is based on that developed by Flynn (1968) and modified in our lab (Štrok and Smodiš, 2012). Up to 1 g of sample was digested overnight at room temperature after addition of 6 mL of conc. HNO_3, 2 mL of conc. HCl and Po-209 tracer. The dissolution is shown in Fig. 2. The next day, the sample was digested for 30 min at 200 °C on a hot plate. After cooling, 3 mL of 30% H_2O_2 were added and heated for 10 min at 100 °C. When the sample had cooled to room temperature, it was filtered

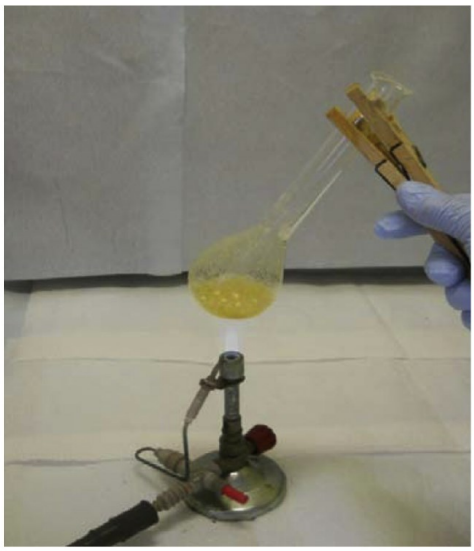


Fig. 1. Dissolution of the sample in quartz Kjeldahl flask.



Fig. 2. Dissolution of the sample in an Erlenmeyer flask.

through a black ribbon filter paper into a glass beaker. The filter with the residue was again introduced into the same Erlenmeyer flask and the whole digestion procedure was repeated. The sample solution was again filtered and the filtrate was combined with previous one. The sample solution obtained was evaporated to dryness at 100 °C. Then 2 mL of concentrated HCl was added and the sample was again evaporated to dryness. This step was repeated twice. After that, spontaneous deposition was performed as described above.



Fig. 3. Dissolution of the sample in the microwave system.

Table 1
Microwave heating conditions for digestion by the microwave system.

Step	Power (W)	Hold time (min)	Temperature in vessel (°C)	Temperature in system (°C)
1	1300	10	130	75
2	1300	10	130–200	75–130
3	1300	40	200	130

2.3.3. Digestion by microwave system

An ETHOS 1 (MILESTONE SN 130471) microwave system (Fig. 3), with a maximum power of 1300 W and Teflon vessel (HPR-1000/10S high pressure segment rotor) was used for digestion. Up to 1 g of sample was weighed into each vessel with an appropriate amount of Po-209 tracer and then 5 mL of concentrated HNO_3 and 2 mL of H_2O_2 were added. The microwave heating conditions are given in Table 1.

After cooling the vessels, the acid solution was transferred into a glass beaker and evaporated to dryness at 100 °C. 2 mL of concentrated HCl was added and the sample was again evaporated. This step was repeated twice. Finally, the spontaneous deposition of polonium radioisotopes was performed as before.

2.4. Alpha-particle spectrometry

An alpha spectrometer (Alpha Analyst, Canberra, USA), with passivated implanted planar silicon (PIPS) semiconductor detectors (active area of 450 mm^2 and approximately 28% efficiency for a 25 mm diameter disk) were used for alpha particle spectrometric measurements of polonium radioisotopes. The calibration of the detector was made with a standard radionuclide source containing U-238, U-234, Pu-239 and Am-241 (code: 67978-121), obtained from Analytics, Inc. The calibration was made for a range of energies from 3900 to 4290 keV for U-238, from 4580 to 4860 keV for U-234, from 4950 to 5240 keV for Pu-239 and from 5275 to 6690 keV for Am-241. Data acquisition analysis was performed using Genie 2000 spectroscopy system software.

3. Results and discussion

The results with their corresponding standard deviations for the determination of the activity concentration of Po-210 in the samples analysed, obtained using the three dissolution procedures are presented in Table 2. Most of the samples were analysed in two or more replicates; however two mussel samples were analysed once because of the insufficient sample mass. Due to the fact that all of the analysed reference materials were prepared many years

Table 2
Activity concentrations of Po-210 in Bq kg^{-1} in samples obtained using various dissolution procedures.

Plant sample		Certified or information value	Results[a]		
			Kejdahl flask	Erlenmeyer flask	Microwave system
1	NIST-4359 Seaweed Radionuclide Standard	21.0 ± 0.8	21.9 ± 0.8, n=2	19.9 ± 0.1, n=2	18.8 ± 1.2, n=2
2	IAEA-307 Sea plant	58.5[b]	56.0 ± 0.1, n=2	48.6 ± 0.3, n=3	49.1 ± 4.8, n=3
3	CTA OTL-1 Oriental tobacco leaves		15.7 ± 1.5, n=2	16.2 ± 1.7, n=2	17.5 ± 2.8, n=2
4	CTA VTL-2 Virginia tobacco leaves		15.2 ± 0.1, n=2	11.4 ± 1.3, n=3	11.3 ± 1.1, n=3
5	Lichen 1		251 ± 7, n=2	225 ± 5, n=2	230 ± 6, n=2
6	Lichen 2		147 ± 2, n=2	148 ± 1, n=2	152 ± 2, n=2
7	Lichen 3		742 ± 5, n=2	689 ± 31, n=2	705 ± 26, n=2
Mussel sample					
Mussel 1			2.2 ± 0.1, n=2	5.1 ± 0.2, n=2	3.8 ± 0.4, n=2
Mussel 2			298 ± 13, n=2	307 ± 4, n=2	293 ± 11, n=2
Mussel 3			353 ± 29, n=2	354 ± 23, n=2	367 ± 2, n=2
Mussel 4			137 ± 5, n=1[c]	143 ± 6, n=1[c]	143 ± 9, n=1[c]
Mussel 5			83 ± 3, n=1[c]	91 ± 3, n=1[c]	85 ± 4, n=1[c]
Mussel 6			34.5 ± 0.5, n=2	38.8 ± 1.4, n=2	36.4 ± 1.5, n=2

[a] Average ± standard deviation of independent measurements (if n=2, $sd = |x_1 - x_2|/2$), k=1.
[b] Information value, confidence interval 40–91 Bq kg^{-1}.
[c] Result ± uncertainty, k=1 (only one measurement due to an insufficient amount of sample).

Table 3
Comparison of various parameters dependent on dissolution procedure.

Parameter	Kjeldahl flask	Erlenmeyer flask	Microwave system
Mass of the sample (g)	<1	>1	<1
Total time of dissolution	~1 h	~2 days	~2 h
Radiochemical recovery (%)	70–100	80–100	80–100
Advantage	Better digestion of fats	Large mass of sample	Better digestion of fats; can dissolve more than one sample at same time
Disadvantage	Poor control of the temperature	Losses during filtration	Mass limitation
Results	Comparable (one way ANOVA, $p=0.998$)		

ago, it is assumed that the activity concentrations of Po-210 in the samples are in equilibrium with Pb-210.

The results obtained for certified reference material NIST-4359 all lie very close to the certificate value. For reference material IAEA-307 Sea Plant only an information value of 58.5 Bq kg^{-1} for Pb-210 is reported and the activity concentrations of Po-210 found were in good agreement with this value. Results for Po-210 in most plant materials (seaweed, sea plant, Virgina tobacco leaves, lichen 1, lichen 3) where dissolution in Kjeldahl flask was carried out showed slightly higher values as those obtained by dissolution in an Erlenmeyer flask and dissolution in the microwave system. For the CTA-VTL-2 Virginia Tobacco Leaves aggregation of particles, occurring non-homogeneously as reported by Dybczyński et al. (1998) could have affected the results. Certified or information values for Pb-210 for the reference material CTA-OTL-1 and CTA-VTL-2 Virginia Tobacco Leaves do not exist. Po-210 was also determined in six real mussel samples (*M. galloprovincialis*). The activity concentration of Po-210 in the mussel sample 1 is relatively low, between 2.2–5.1 Bq kg^{-1}, which probably contributes to the high variation in results for the three dissolution techniques.

For statistical analyses of results One-Way Analysis of Variance was used. The ANOVA test showed that there is no statistical difference between the three dissolution techniques ($p=0.998$). Therefore for determination of Po-210 in biological samples all of the dissolution procedures compared can be used. The choice of dissolution technique depends on sample and method characteristics, as presented in Table 3.

The recoveries of the radiochemical procedures, determined with Po-209 tracer, were between 70–100% for dissolution in a Kejdahl flask, between 80–100% for dissolution in an Erlenmeyer flask on a hot plate and between 80–100% for the microwave system.

4. Conclusion

Three dissolution techniques used in determination of Po-210 in biological samples are described and compared. The dissolution techniques involved classical wet ashing with acids in a 100 mL long-necked Kjeldahl flask by heating vigorously over a gas flame, digestion with acids in an Erlenmeyer flask at temperatures up to 200 °C and wet digestion by a microwave system in Teflon vessels up to 200 °C were investigated. The results obtained showed that the activity concentrations of Po-210 found in biological samples were not statistically different between the three digestion methods. The choice of dissolution techniques hence mainly depends on the required sample mass and its matrix.

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References

Benedik, L., Vreček, P., 2001. Determination of Pb-210 and Po-210 in environmental samples. Acta Chim. Slov. 48, 199–213.
Dybczyński, R., Polkowska-Motrenko, H., Samczyński, Z., Szopa, Z., 1998. Virginia Tobacco Leaves (CTA-VTL-2)—new polish CRM for inorganic trace analysis including microanalysis. Fresenius J. Anal. Chem. 360, 384–387.
Flynn, W.W., 1968. The determination of low levels of polonium-210 in environmental materials. Anal. Chim. Acta 43, 221–227.
Germain, P., Leclerc, G., Simon, S., 1995. Transfer of polonium-210 into *Mytilus edulis* (L.) and *Fucus vesiculosus* (L.) from the Baie de Seine (channel coast of France). Sci. Total Environ. 164, 109–123.
Henricsson, F., Ranebo, Y., Holm, E., Ross, P., 2011. Aspects on the analysis of Po-210. J. Environ. Radioact. 102, 415–419.
IAEA, 2003. International Basic Safety Standards for Protection Against Ionizing Radiations and for the Safety of Radiation Sources, Safety Series No. 115. IAEA, Vienna.
Khater, Ashraf E.M., 2004. Polonium-210 budget in cigarettes. J. Environ. Radioact. 71, 33–41.
Louw, L, Faanhof, A., Kotze, D., 2009. Determination of polonium-210 in various foodstuffs after microwave digestion. Radioprotection 44 (5), 89–95.
Meli, M.A., Desideri, D., Roselli, C., Feduzi, L., 2009. Po-210 determination in urines of people living in central Italy. J. Environ. Radioact. 100, 84–88.
Repinc, U., Benedik, L., 2005. Simultaneous determination of trace uranium and vanadium in biological samples by radiochemical neutron activation analysis. J. Radioanal. Nucl. Chem. 264, 77–81.
Rožmarić, M., Rogić, M., Benedik, L., Štrok, M., Barišić, D., Gojmerac-Ivšić, A., 2012. Po-210 and Pb-210 activity concentrations in *Mytilus galloprovincialis* from the Croatian Adriatic coast with the related dose assessment to the coastal population. Chemosphere 87, 1295–1300.
Savidou, A., Kehagia, K., Eleftheriadis, K., 2006. Concentration levels of Pb-210 and Po-210 in dry tobacco leaves in Greece. J. Environ. Radioact. 85, 94–102.
Štrok, M., Smodiš, B., 2012. Transfer of natural radionuclides from hay and silage to cow's milk in the vicinity of a former uranium mine. J. Environ. Radioact. 110, 64–68.
Towler, P.H., Smith, J.D., 1994. Recovery of polonium from microwave bomb digestions. Anal. Chim. Acta 292, 209–212.
Vreček, P., Benedik, L., Pihlar, B., 2004. Determination of Pb-210 and Po-210 in sediment and soil leachates and in biological materials using a Sr–resin column and evaluation of column reuse. Appl. Radiat. Isot. 60, 717–723.

A.3 Simultaneous Determination and Uptake Assessment of Selected Radionuclides in Plants Grown in Substrate Contaminated with U-mill Tailings. *J. Radioanal. Nucl. Chem.: 309, 351-365 (2016).*

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Simultaneous determination and uptake assessment of selected radionuclides in plants grown in substrate contaminated with U-mill tailings

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Abstract The aim of this study was to investigate transfer of ^{238}U, ^{230}Th, ^{226}Ra and ^{210}Pb from substrate contaminated with different amounts of uranium-mill tailings to eatable plants (radish, savoy and rocket). The applied radiochemical procedure allows for simultaneous determination of all investigated nuclides from a single sample test portion. The results obtained indicate significant linear correlation between content of the radionuclides in soil and plants as well as between content of the radionuclides in plants and pedological parameters, while contamination has no effect on photochemical efficiency and content of photosynthetic pigments in the selected plants.

Keywords Radish · Savoy · Rocket · NORM radionuclides · Uptake

Introduction

Industrial excavation of the uranium ore in the area of Žirovski vrh (Slovenia) lasted for 6 years in 1980s. During that period about 600,000 tons of U—ore with low content of U_3O_8 were processed. The by—products of the yellow cake production are different kinds of wastes deposited in the mine environment such as U-mill tailings deposited on the Boršt site and red mud on the Jazbec site [1]. Activity concentrations of key radionuclides in the U-mill tailing at the Boršt site are 995 Bq kg^{-1} d.w. of ^{238}U, 3930 Bq kg^{-1} d.w. of ^{230}Th, 8630 Bq kg^{-1} d.w. of ^{226}Ra and 7400 Bq kg^{-1} d.w. of ^{210}Pb, on average [1, 2]. Activity concentrations of the studied NORM (naturally occurring radioactive materials) radionuclides are highly elevated compared to natural levels and therefore potential contaminants of the nearby environment. Emission of radionuclides from the waste disposal sites may lead to their elevated levels in the nearby soil either by dry or wet deposition of re-suspended radioactive material, or by discharge by tailings seepage waters. Those radionuclides can then be adsorbed on soil colloids and subsequently retained in the soil. The radionuclides could finally be taken up by different organisms (e.g., plants) and eventually transferred into foodstuffs [3, 4].

The family of *Brassicaceae* crops are considered as plants with higher capability to accumulate contaminants such as radionuclides in the edible parts [5–9], which is an important factor in case of ingestion [10]. Furthermore, such characteristics of plants can represent their potential use for phytoremediation. In this case, the metal biological absorption coefficient (BAC), i.e. the plant-(harvestable)-to-soil metal concentration ratio needs to be determined [11]. Bioconcentration factor (BCF, root-to-soil metal concentration ratio) and translocation factor (TF, shoot-to-root metal concentration ratio) are also important factors; plants with BAC greater than 1 are suitable for phytoextraction while those with BCF greater than 1 and TF lower than 1 can be potentially used for phytostabilisation [12]. Frequently used parameter is concentration ratio (CR), defined as the ratio between plant activity concentration (Bq kg^{-1}) and total soil activity concentration (Bq kg^{-1}). It determines the availability of soil—containing radionuclides for plant uptake [13].

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Generally, radiotoxicity as well as chemical toxicity of deposited radionuclides determine their potential detrimental health effects [10]. Common problems related to determination of radionuclides in botanical materials are often small available sample masses, complex matrices, inhomogeneity of the samples, great individual biological variability, low activity concentrations, etc. Therefore, reliable radiochemical analytical methods are required to obtain accurate measurements results. Literature survey shows that researchers usually chemically separate only radionuclides of one element or maximum three elements from each sample portion for alpha particle spectrometry analyses [2, 14–18]. If the available sample mass is for whatever reason small, the set of radionuclides to be radiochemically determined, is consequently rather limited.

The aim of this study was therefore to optimize a method for simultaneous determination of several members of uranium series decay chain that are enriched in sites contaminated with NORM: polonium (^{210}Po for assessment of ^{210}Pb content), radium (^{226}Ra), uranium (^{234,235,238}U) and thorium (^{230,232}Th). The second aim was to quantify the transfer of ^{238}U, ^{230}Th, ^{226}Ra and ^{210}Pb to radish (*Raphanus sativus L.*), savoy (*Brassica oleracea var. sabauda*) and rocket (*Diplotaxis tenuifolia*) from commercially available plant substrate contaminated with different amounts of UMT.

Experimental

Plants and growth conditions

Three different types of crops from *Brassicaceae* family were sown on contaminated and non-contaminated soil with high organic content (45 %). Soil mixtures were placed in 2 L plastic pots. Radish (*Raphanus sativus L.*, Semenarna Ljubljana d.d.) was chosen as a root vegetable, while savoy (*Brassica oleracea var. sabauda*, Semenarna Ljubljana d.d.) has an above ground edible plant part. Rocket (*Diplotaxis tenuifolia*, Semenarna Ljubljana d.d.) was chosen according to its increasing importance as a vegetable and spice [19]. The plant pots were randomly placed on a shelf, arranged in five blocks to minimize influence of random experimental factors (potential inhomogeneity of soil and UMT mixtures or non-steady lighting, heat or humidity). Design of the pot experiment without random placing is shown in Fig. 1. Plants were irradiated by fluorescent lamps (Fluora, Osram, 58W) with a daily light phase of 12 h.

Contaminated soils were prepared by mixing commercially available substrate (Humko, Kompomix) with UMT at four different mass ratios, representing different contamination scenarios. The non-contaminated soil was used as a control. The tailings samples were taken from the Boršt tailings pile [1] of the former Žirovski vrh uranium mill and mine (latitude: 46°05′46.10″N; longitude: 14°10′38.19″E; altitude 617 m) [20]. Soil and tailings samples were air dried for three weeks, sieved to pass through 5 mm sieve and mixed in mass ratios, representing 20, 40, 60 and 80 % of UMT content in the contaminated soil, respectively. The experiment was performed in the Hot Cells Facility of the Jožef Stefan Institute (Ljubljana, Slovenia) as the facility is licensed for work with radioactive material, such as UMT.

Seeding of selected plants was performed in February 2012, while the harvest of fully-grown plants was in July, i.e. after 5 months of growth. The plants were grown in the temperature range from 22 to 25 °C, relative humidity ranged from 40 to 60 % and light irradiation ranged from 53 to 94 μmol s^{-1} m^{-2} (LI-COR Quantum sensor, USA) at the plant surfaces (30 cm below the lamps). In every pot there were up to 4 plants grown to gain sufficient sample mass. Each pot was daily checked and irrigated with appropriate amount of deionised water, if necessary. To prevent contamination of plants by the soil dust from the pots, all possible pathways of external contamination were considered. At the beginning of seeding, garden mats were put onto the soil already placed in pots to ensure the maximum protection of plants against the soil dust resuspension. The ventilation of the Hot Cell Facility was enabled during the daytime phase (from 8 a.m. to 8 p.m.) to prevent potential contamination of plants by ^{222}Rn decay products due to the presence of relatively high quantities of UMT and consequently also ^{226}Ra in the pots [20].

After the growing period, plants were harvested and prepared for the determination of radionuclides. Plant parts were separated on underground parts (tubers, roots) and leaves. Each part was carefully washed with tap water, dried in an oven (SP-260, Kambič) for 3 days at 40 °C and dry mass was recorded after reaching equilibrium with ambient conditions. Dried plant leaves were milled by knife mill (Retsch, Grandomix GM 200, Germany) while tubers and roots were milled by rotor mill (Pulverisette 7, Fritsch, Idar-Oberstein, Germany) and homogenized as solid.

Samples of soil mixtures as well as control soil were taken before the plants seeding. Three soil samples were randomly taken for each experimental treatment (control, 20, 40, 60 and 80 % of UMT content) from the amount of 3 kg of total mass of contaminated and non-contaminated soil prepared for the pots. Collected soil samples were dried in an oven (ST-05, Instrumentaria, Zagreb) for 3 days at 60 °C, sieved to pass through 2 mm sieve (Fritsch) and left at room temperature to achieve an ambient humidity [20].

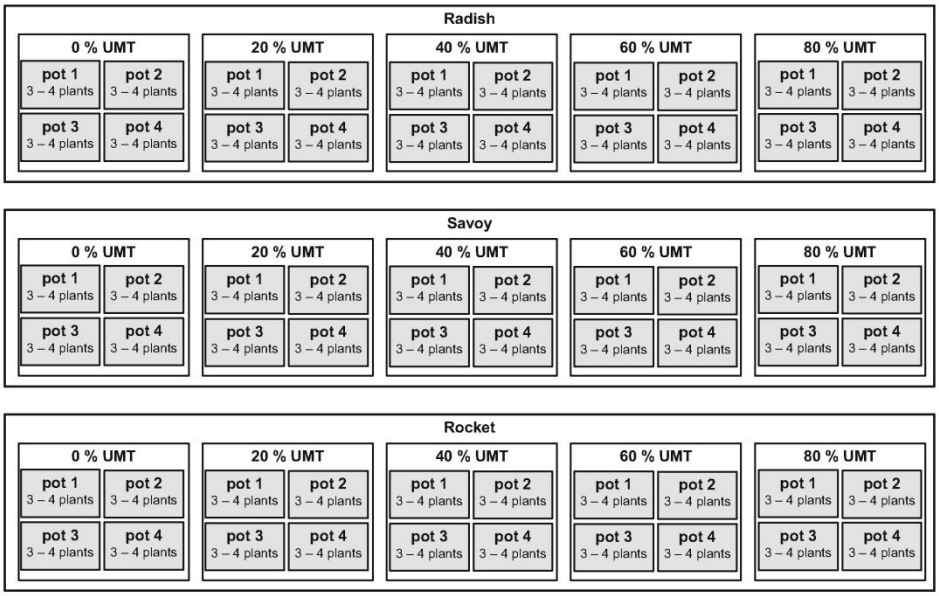


Fig. 1 Scheme of pot experiment with indicated amounts of UMT

Pedological parameters of soils

Organic matter content, pH value, the content of available P_5O_5 and K_2O, cation exchange capacity (CEC), organic C, total N and C/N ratio were analysed by standardised methods at the Jurana Company, Maribor [21–25]. Four randomly taken samples from each bulk soil mixture and control soil were analysed.

Biochemical and physiological parameters of plants

Photochemical efficiency

Fluorescence measurement is a non-destructive, simple and sensitive method for detecting the photoinhibitory effects of environmental stressors on higher plants [26, 27]. Chlorophyll fluorescence was measured in situ on vital plants on 5 plants from each replicate with fluorometer as described in more detail by Mechora et al. [28].

Photosynthetic pigments and anthocyanins

Measurements of photosynthetic pigments as well as anthocyanins are important tool to determine whether the plant is under stress or not. The response to stress is expressed in lower content of photosynthetic pigments and in higher content of anthocyanins comparing to control [29, 30]. To assess the content of chlorophylls a and b and carotenoids, leaves of five plants from every replicate were measured. The amount of pigments was determined as described by Lichtenthaler and Buschmann [31, 32]. The total anthocyanin content was measured as described by Drumm and Mohr [33].

Gamma-ray spectrometric measurements of soils and ^{133}Ba tracer

Direct gamma-ray spectrometry was applied for measurements of ^{238}U, ^{226}Ra, ^{230}Th and ^{210}Pb in contaminated and control soils. ^{238}U was determined by measuring its daughter product ^{234}Th at 63.28 keV, ^{226}Ra via its decay product ^{214}Pb at 295.2 and 351.9 keV, ^{210}Pb at 46.52 keV and ^{230}Th at 67.67 keV. Three soil samples from each bulk soil mixture and control were taken for the gamma-ray spectrometry. The samples were measured by a coaxial HPGe detector (Canberra, USA) connected to a multi-channel analyser (Canberra, USA). The procedure is described in detail by Smodiš et al. [20].

Chemical recovery for ^{226}Ra in plants determined by alpha-particle spectrometry was also determined via

gamma-ray measurements of the added ^{133}Ba tracer. ^{133}Ba was measured at the energy of 356.02 keV on a gamma-ray spectrometer consisting of HPGe detector connected to a multichannel analyser (Spectrum Master 919, Ortec, UK). ^{133}Ba activity was determined relative to the ^{133}Ba certified standard solution with activity traceable to SI units, which was evaporated on aluminium disc to get the same geometry as the sample [34].

Reagents

All reagents used in the radiochemical procedures were of analytical grade. The tracer solutions of ^{232}U, ^{229}Th, ^{133}Ba and ^{209}Po used as working solutions in the study were prepared from calibrated solutions purchased from Analytics, Inc. (Atlanta, GA, USA). The producer maintains traceability to NIST standards.

Dissolution of plant samples

Up to 2 g of plant sample was transferred to a Teflon beaker and appropriate volume of ^{133}Ba, ^{209}Po, ^{232}U and ^{229}Th tracers were added. Then 20 mL of concentrated HNO_3 acid was added and the sample was digested over night at room temperature. The next day, sample was heated at 150 °C near dryness and then 10 mL of concentrated HNO_3, 10 mL of concentrated $HClO_4$ and 10 mL of concentrated HF were added, and the solution was heated again at 200 °C until dryness. The step was repeated once more to assure sufficient dissolution. The residue was dissolved with 2 mL of concentrated HCl and again evaporated until dryness at 200 °C. This step was repeated once more to convert the solution into a chloride form and finally prepare the sample for spontaneous deposition of polonium radionuclides.

Radiochemical procedure for simultaneous determination of ^{210}Po, ^{226}Ra, ^{234,235,238}U and ^{230,232}Th in plants

After the sample dissolution spontaneous deposition of polonium radioisotopes onto silver discs was performed (^{210}Po measurement was used to determine content of its parent ^{210}Pb in the samples). The residue as described in the previous section was dissolved with 2 mL of concentrated HCl and diluted with deionised water up 100 mL. Then 0.5 g of ascorbic acid and 0.5 g of hydroxyl ammonium chloride were added to the solution to reduce potentially present Fe(III) and prevent co-plating of iron and interfering elements. Finally, the spontaneous deposition of polonium was carried out at 100 °C for 4 h [35].

Then the solution was prepared for simultaneous separation of Ra, U and Th radionuclides. The solution remained after Po separation was transferred into a glass beaker; then 20 mL of concentrated HNO_3 was added to digest organic compounds and evaporated until dryness at 200 °C. Then 5 mL of H_2O_2 was added and evaporated until dryness at 150 °C. This step was repeated once more to assure complete decomposition of organic reagents. In the last step residue was dissolved with 2 mL of concentrated HCl and then the radionuclides were co-precipitated with iron hydroxide after addition of sufficient amount of ammonia solution.

Extraction chromatographic separation techniques based on UTEVA and TEVA resins (Eichrom Technologies) were utilized to separate uranium, thorium and radium isotopes in the plant samples. An extraction chromatographic resin UTEVA was used for the separation of uranium. A TEVA resin was chosen to retain thorium isotopes, while radium isotopes passed both columns [36]. In more detail, the iron hydroxide precipitate was dissolved with 6 mL 3 M HNO_3–1 M $Al(NO_3)_3$ and loaded onto TEVA and UTEVA resins in tandem (Fig. 2). The columns were first washed with 30 mL of 3 M HNO_3 to elute Ra. Then the columns were separated and the UTEVA column was washed with 6 mL 3 M HNO_3 to remove residual radium. Radium isotopes and barium tracer were co-precipitated from the radium–barium containing eluate with $PbSO_4$ as described by Štrok and Smodiš [14]. In the next step a clean beaker for collecting thorium radioisotopes was placed under the TEVA column. The columns were washed with 20 mL of 9 M HCl and 5 mL of 6 M HCl. The collected solution was evaporated until dryness, the residue dissolved with 15 mL 1 M HCl and then microprecipitation of thorium radioisotopes was performed. The UTEVA column was further washed with 5 mL of 9 M HCl and 25 mL 5 M HCl-0.05 M oxalic acid in order to wash out residues of thorium and other interfering radioisotopes. Then uranium radioisotopes were washed out with 15 mL of 1 M HCl. The scheme of simultaneous radiochemical separation of polonium, radium, thorium and uranium is presented in Fig. 2. After separation processes, alpha emitting sources were prepared by microcoprecipitation [14].

Alpha-particle spectrometry

An alpha spectrometer system (Alpha Analyst, Canberra, USA), with passivated implanted planar silicon (PIPS) semiconductor detectors was used for alpha-particle spectrometric measurements of the deposited Po, Ra, U and Th radioisotopes. The selected radioisotopes were measured at energies of 4.15, 4.65, 4.69 and 5.31 MeV for ^{238}U, ^{230}Th, ^{226}Ra and ^{210}Po, respectively. The tracers were measured at energies of 5.28, 4.89 and 4.88 MeV for ^{232}U, ^{229}Th and ^{209}Po, respectively. The counting efficiency was 29 %, on

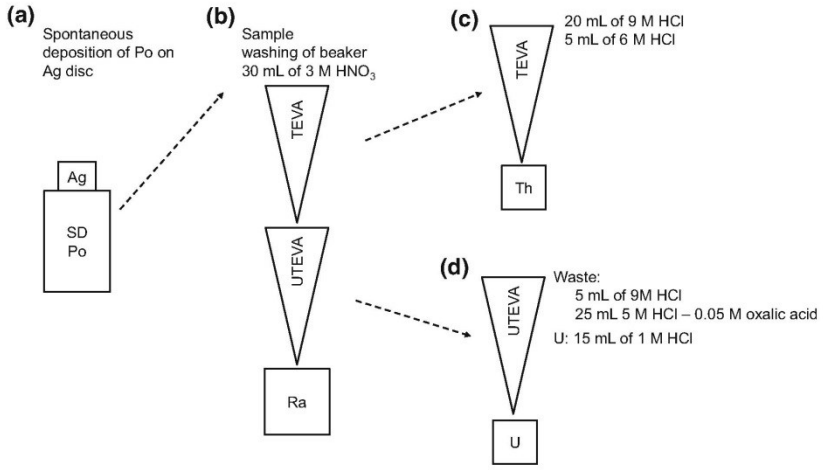


Fig. 2 Scheme of radiochemical separation procedure for polonium, radium, thorium and uranium determination in plant samples

average. Data acquisition analysis was performed using Genie 2000 spectroscopy system software. The disc sources with deposited radioisotopes were measured until a statistically sufficient number of counts (counting uncertainty <10 %) were achieved. The actual count time was up to one week.

Determination of CR factors

The concentration ratio (CR) of particular radionuclide was calculated as the ratio of the measured activity concentration (Bq kg^{-1} dry weight) in the plant (leaves or tubers) to the measured activity concentrations in the soil [13].

Data analysis

Statistical analysis of data was performed using Microsoft Excel 2010, version 14.0.7153.5000 (64 bit). A Pearson's product—moment correlation (r) with corresponding significance level (p) was used for evaluation of linear relationship between two sets of variables. The value of r is always between $+1$ and -1. If the r value was >0.5 the linear correlation was considered to be strong, between 0.3 and 0.5 moderate and between 0.1 and 0.3 small. ANOVA test was applied to determine whether the correlation coefficient was "highly significant" ($p < 0.001$), "significant" ($0.001 < p < 0.05$) or "not significant" ($p > 0.05$). The level of statistical significance of correlation coefficient was described by the p value (two-tailed). If the r value was greater than 0.1 (small correlation) and p value was greater than 0.05 (correlation not statistically significant), the correlation was reported as not linear. For the data analysis purposes the results below the respective minimum detectable activities (MDAs) were substituted by half the values of the MDAs. Throughout the text correlations among all concerned variables are presented and discussed.

Results and discussion

Methods validation

Two IAEA reference materials (IAEA-447 Moss-soil and IAEA-434 Phosphogypsum) were used for quality control of the gamma-ray spectrometric measurements (Table 1). The obtained results showed fairly good agreement between the assigned and measured values.

For quality control of the alpha-particle spectrometric measurements IAEA 447 and NIST 4359 (Seaweed) certified reference materials were used. The uranium measurements results for IAEA 447 were slightly lower than the certified values, probably due to incomplete digestion of the soil sample using the proposed dissolution method, as uranium may be incorporated into the structure of difficultly soluble minerals [37], while for plant matrix of NIST 4359 the obtained results were within the uncertainties (Table 1). Fairly good agreement was found for the other radionuclides in both reference materials and the results mostly comply with the certified values despite of

Table 1 Comparison of assigned/certified and measured activity concentrations (Bq kg^{-1} dry mass) obtained by gamma-spectrometric and alpha-particle spectrometric determinations in reference materials

Reference material	Method	Radionuclide	Certified value[a]	Information value[a]	Measured value[b]
IAEA 447 (moss soil)	Gamma-ray spectrometry	^{238}U	22.2 ± 1.6		17.4 ± 5.4 (4)
		^{226}Ra	25.1 ± 4.0		27.0 ± 1.8 (4)
		^{210}Pb	424 ± 40		364 ± 34 (4)
IAEA 434 (phosphogypsum)		^{238}U	120 ± 22		82 ± 19 (4)
		^{226}Ra	780 ± 124		730 ± 30 (4)
		^{210}Pb	680 ± 116		591 ± 52 (4)
		^{230}Th	211 ± 18		<MDA[c] (4)
IAEA 447 (moss soil)	Alpha-particle spectrometry	^{238}U	22.2 ± 1.6		19.5 ± 2.0 (4)
		^{234}U	21.8 ± 1.6		17.4 ± 1.8 (4)
		^{226}Ra	25.1 ± 4.0		33.1 ± 2.6 (4)
		^{232}Th		37.3 ± 4.0	32.6 ± 3.8 (4)
		^{210}Pb (^{210}Po)	424 ± 40		349 ± 29 (6)
NIST 4359 (seaweed)		^{238}U	8.67 ± 0.54		8.44 ± 0.89 (6)
		^{235}U	0.400 ± 0.047		0.348 ± 0.135 (6)
		^{234}U	9.5 ± 1.1		8.91 ± 0.86 (6)
		^{226}Ra		5.7	5.86 ± 0.77 (6)
		^{230}Th		3.3	5.19 ± 0.53 (6)
		^{210}Pb (^{210}Po)	21.0 ± 3.0		22.4 ± 2.9 (6)

[a] From the reference sheet (IAEA) or certificate (NIST); uncertainty values shown as combined expanded ($k = 2$) uncertainties

[b] The values are presented as averages with standard deviations, (number of replicates in parentheses)

[c] Minimum detectable activity (MDA = 292 Bq kg^{-1})

some relatively high measurement uncertainties. The solely exception is ^{210}Pb in IAEA 447, which is probably due to the applied dissolution procedure, as already discussed in the previously published research [37].

For the study of behaviour of uranium in soil-plant systems only activity concentrations of ^{238}U were considered. Determination of ^{228}Th was not included in the proposed procedure as ^{228}Th is the daughter product of ^{232}U tracer and appropriate correction of activity concentration for ^{228}Th should be made. Similarly, ^{232}Th was not considered in the study, as it is a member of another decay chain, which is not of radiological importance in areas contaminated with UMT. Therefore, in the present study only ^{230}Th (a member of ^{238}U decay chain) was taken into consideration.

Soils

The selected radionuclides were determined by gamma-ray spectrometry. Activity concentrations of particular radionuclides in the soils mixed with UMT are presented in Table 2. The results are shown as average values with standard deviations of three measured samples randomly taken from each soil/UMT mixture before plants seeding. The activity concentrations taken from Tables 2 and 4 were used for calculation of concentration ratios for ^{238}U, ^{226}Ra, ^{230}Th and ^{210}Pb in the cultivated plants.

Pedological properties of the prepared mixtures for different contamination scenarios are shown in Table 3. The values are presented as average values with standard deviations of four replicates taken from each contamination treatment in the beginning of the experiment, i.e. before seeding. Large variances in pedological properties among soil mixtures can be observed as a consequence of specific properties of UMT having a large inorganic fraction; UMT is not really soil but the by-product of intensive acid treated uranium ore during the uranium yellow cake production process.

A Pearson's product-moment correlation assessment showed strong linear relationship between UMT content and most of pedological parameters ($r > 0.5$, $p < 0.001$, $n = 20$), except for pH and C/N ratios where no correlations ($0.1 < r < 0.3$, $p > 0.05$, $n = 20$) were found.

Plants

The average activity concentrations with standard deviations for ^{238}U, ^{226}Ra, ^{230}Th and ^{210}Pb in radish, savoy and rocket grown in commercially available substrate at different UMT contamination levels are presented in Table 4. There were some large changes of activity concentrations observed in plants grown in mixtures containing 60 % UMT and 80 % UMT. These

Table 2 Activity concentrations (Bq kg^{-1} dry weight) of selected radionuclides in UMT–contaminated and control soils (arithmetic mean values with standard deviations of three replicates)

UMT content [mass %]	^{238}U	^{226}Ra	^{230}Th	^{210}Pb
Control	19 ± 6	27 ± 4	<MDA[a]	90 ± 5
20 %	283 ± 26	2000 ± 100	1100 ± 140	2100 ± 150
40 %	454 ± 47	3500 ± 70	2000 ± 230	3600 ± 190
60 %	731 ± 37	5300 ± 150	2200 ± 200	5000 ± 100
80 %	895 ± 88	6700 ± 170	3400 ± 800	6300 ± 90

[a] For statistical analysis of ^{230}Th in the control, ½ of MDA was used. The MDA was 210 Bq kg^{-1} for the given sample mass and measurement time

Table 3 Pedological properties of UMT–contaminated and control soils (arithmetic mean values with standard deviations of four replicates)

Pedological parameter	Control soil	20 wt% of UMT in the soil	40 wt% of UMT in the soil	60 wt% of UMT in the soil	80 wt% of UMT in the soil
pH in $CaCl_2$	6.7 ± 0.1	6.6 ± 0.0	6.5 ± 0.0	6.6 ± 0.0	6.7 ± 0.1
Available P_2O_5 [mg kg^{-1}]	800 ± 37	632 ± 42	485 ± 19	303 ± 24	148 ± 6
Available K_2O [mg kg^{-1}]	1205 ± 57	919 ± 19	728 ± 64	437 ± 29	273 ± 29
Organic matter [%]	45.6 ± 5.7	35.1 ± 1.9	27.6 ± 6.4	17.6 ± 2.9	8.0 ± 2.9
C [%]	26.4 ± 1.8	20.3 ± 1.2	16.0 ± 0.9	10.2 ± 0.8	4.6 ± 0.4
N [%]	1.13 ± 0.01	0.93 ± 0.03	0.72 ± 0.04	0.43 ± 0.02	0.24 ± 0.02
C/N	24.1 ± 2.5	21.9 ± 1.9	22.2 ± 1.1	23.6 ± 2.8	19.7 ± 3.2
CEC [cmol kg^{-1}]	86.3 ± 1.5	87.7 ± 2.7	90.5 ± 1.9	96.0 ± 0.8	107.9 ± 0.4

Table 4 Activity concentrations (Bq kg^{-1} dry mass) of radionuclides in plants (arithmetic mean values with standard deviations of four replicates)

Plant	UMT content [mass %]	^{238}U	^{226}Ra	^{230}Th	^{210}Pb
Radish-leaves	Control	0.44 ± 0.11	4.0 ± 1.9	2.1 ± 0.8	2.8 ± 0.6
	20 %	0.58 ± 0.19	63 ± 14	1.9 ± 0.4	5.3 ± 1.7
	40 %	0.83 ± 0.63	56 ± 24	1.7 ± 0.7	5.5 ± 1.5
	60 %	1.2 ± 1.0	43 ± 11	1.9 ± 0.7	9.6 ± 6.0
	80 %	0.53 ± 0.16	121 ± 11	2.0 ± 0.7	29 ± 8
Radish-tubers	Control	0.19 ± 0.07	1.2 ± 0.2	2.1 ± 0.5	0.55 ± 0.35
	20 %	0.24 ± 0.05	27 ± 8	2.4 ± 0.8	4.1 ± 0.7
	40 %	0.37 ± 0.22	37 ± 3	3.2 ± 1.1	13 ± 4
	60 %	0.46 ± 0.19	24 ± 5	3.9 ± 1.0	17 ± 2
	80 %	0.98 ± 0.16	74 ± 11	4.7 ± 1.6	64 ± 6
Savoy-leaves	Control	0.32 ± 0.18	2.4 ± 0.3	1.7 ± 0.4	1.4 ± 0.3
	20 %	0.41 ± 0.11	27 ± 9	1.6 ± 0.4	2.7 ± 0.5
	40 %	0.50 ± 0.13	21 ± 3	1.5 ± 0.3	3.3 ± 0.3
	60 %	0.34 ± 0.18	34 ± 4	2.1 ± 0.7	6.5 ± 0.8
	80 %	0.43 ± 0.07	67 ± 3	2.3 ± 0.5	22 ± 4
Rocket-leaves	Control	0.39 ± 0.06	1.9 ± 0.3	2.4 ± 0.7	2.8 ± 0.6
	20 %	0.55 ± 0.08	55 ± 6	3.7 ± 1.7	5.6 ± 0.5
	40 %	0.52 ± 0.18	53 ± 9	3.2 ± 0.6	6.6 ± 2.0
	60 %	0.85 ± 0.28	83 ± 7	4.5 ± 1.8	11 ± 2
	80 %	1.8 ± 1.0	87 ± 11	8.3 ± 1.4	20 ± 4

changes where particularly extreme for ^{226}Ra and ^{210}Pb. Therefore, this phenomenon requires further investigations to determine, along with combination of various pedological properties, the crucial parameters responsible for the observed changes in the uptake pattern of the radionuclides.

Results of photochemical efficiency and content of photosynthetic pigments in the studied plants are presented in Table 5.

The average concentration ratios of ^{238}U, ^{226}Ra, ^{230}Th and ^{210}Pb for radish, savoy and rocket grown in commercially available substrate at different UMT contamination levels are presented in Table 6. It can be observed that the calculated CR values are quite scattered, but nevertheless mostly in agreement with the existing literature data [38]. The scattered CR values might be attributed to different UMT content in the substrates, possibly impacting on their chemical composition, biological variability and pedological properties, which could affected plants' capabilities for taking up the radionuclides. The differences among uptake patterns for the studied radionuclides could further be consequence of their different uptake patterns that is characteristic for each element.

^{238}U uptake by radish, savoy and rocket

Activity concentrations of ^{238}U varied from 0.24 to 1.8 Bq kg^{-1} for different contamination scenarios while for the control, i.e. soil containing no UMT, they were lower than 0.44 Bq kg^{-1} (Table 4). Linear correlations between activity concentrations of ^{238}U in plants and U-mill tailing content in soil, as well as the pedological parameters, were tested as shown in Table 7. The results showed that ^{238}U activity concentrations in tubers of radish and UMT content in soil as well as ^{238}U activity concentrations in leaves of rocket and UMT content in soil were strongly correlated. There were no correlations found between UMT content in soil and ^{238}U activity concentration in leaves of radish and savoy. The correlations between uranium content in plants and in soil were also observed by Sigh et al. [39]. Similarly to our finding, the same authors also observed relatively low uptake of ^{238}U compared to the other radionuclides (Table 4). Our finding of nonlinearity between ^{238}U activity concentrations in UMT containing soils and ^{238}U activity concentrations in leaves of radish and savoy was also observed in other studies [40, 41]. Those findings indicate the possibility of each plant and soil combination having a unique curvilinear relationship. Some literature data suggests that it is hardly possible to use laboratory experimental results for

Table 5 Photochemical efficiency and content of photosynthetic pigments in plants (arithmetic mean values with standard deviations of five replicates)

Parameter	UMT content [mass %]	Radish	Savoy	Rocket
Photochemical efficiency	Control	0.64 ± 0.16	0.74 ± 0.04	0.62 ± 0.07
	20 %	0.75 ± 0.03	0.77 ± 0.03	0.66 ± 0.12
	40 %	0.74 ± 0.04	0.74 ± 0.03	0.68 ± 0.08
	60 %	0.75 ± 0.05	0.77 ± 0.03	0.67 ± 0.05
	80 %	0.72 ± 0.05	0.77 ± 0.03	0.63 ± 0.05
Chlorophyll a (mg g^{-1} f.w.[a])	Control	1.12 ± 0.26	1.21 ± 0.36	1.39 ± 0.27
	20 %	1.30 ± 0.27	1.18 ± 0.18	1.70 ± 0.50
	40 %	1.57 ± 0.53	1.32 ± 0.04	1.40 ± 0.17
	60 %	1.38 ± 0.13	1.32 ± 0.20	1.69 ± 0.18
	80 %	1.45 ± 0.42	1.38 ± 0.12	1.44 ± 0.21
Chlorophyll b (mg g^{-1} f.w.[a])	Control	0.44 ± 0.10	0.45 ± 0.20	0.56 ± 0.09
	20 %	0.51 ± 0.12	0.42 ± 0.09	0.68 ± 0.25
	40 %	0.60 ± 0.17	0.51 ± 0.04	0.49 ± 0.06
	60 %	0.56 ± 0.06	0.56 ± 0.14	0.64 ± 0.10
	80 %	0.60 ± 0.16	0.56 ± 0.07	0.56 ± 0.12
Carotenoids (mg g^{-1} f.w.[a])	Control	0.39 ± 0.09	0.43 ± 0.15	0.54 ± 0.10
	20 %	0.48 ± 0.09	0.41 ± 0.08	0.67 ± 0.23
	40 %	0.57 ± 0.18	0.48 ± 0.02	0.54 ± 0.07
	60 %	0.50 ± 0.06	0.50 ± 0.09	0.66 ± 0.08
	80 %	0.54 ± 0.14	0.51 ± 0.04	0.57 ± 0.09
Anthocyanins (rel. unit g^{-1} f.w.[a])	Control	226 ± 19	137 ± 6	215 ± 63
	20 %	264 ± 21	120 ± 19	182 ± 30
	40 %	230 ± 35	160 ± 18	203 ± 27
	60 %	214 ± 37	191 ± 28	226 ± 24
	80 %	202 ± 71	170 ± 29	198 ± 43

[a] Fresh mass

Table 6 Average concentration ratios of radionuclides in plant parts

Plant	UMT content [mass %]	CR ^{238}U	CR ^{226}Ra	CR ^{230}Th	CR ^{210}Pb
Radish-leaves	Control	2.33×10^{-2}	1.47×10^{-1}	1.98×10^{-2}	3.14×10^{-2}
	20 %	2.04×10^{-3}	3.15×10^{-2}	1.71×10^{-3}	2.53×10^{-3}
	40 %	1.83×10^{-3}	1.58×10^{-2}	8.53×10^{-4}	1.53×10^{-3}
	60 %	1.69×10^{-3}	8.11×10^{-3}	8.59×10^{-4}	1.92×10^{-3}
	80 %	5.89×10^{-4}	1.80×10^{-2}	5.79×10^{-4}	4.62×10^{-3}
Radish-tubers	Control	1.01×10^{-2}	4.59×10^{-2}	1.98×10^{-2}	6.06×10^{-3}
	20 %	8.39×10^{-4}	1.35×10^{-2}	2.11×10^{-3}	1.96×10^{-3}
	40 %	8.15×10^{-4}	1.05×10^{-2}	1.59×10^{-3}	3.63×10^{-3}
	60 %	6.29×10^{-4}	4.55×10^{-3}	1.74×10^{-3}	3.34×10^{-3}
	80 %	1.09×10^{-3}	1.10×10^{-2}	1.39×10^{-3}	1.02×10^{-2}
Savoy-leaves	Control	1.66×10^{-2}	9.04×10^{-2}	1.60×10^{-2}	1.58×10^{-2}
	20 %	1.46×10^{-3}	1.35×10^{-2}	1.46×10^{-3}	1.27×10^{-3}
	40 %	1.10×10^{-3}	6.02×10^{-3}	7.52×10^{-4}	9.16×10^{-4}
	60 %	4.82×10^{-4}	6.40×10^{-3}	9.38×10^{-4}	1.29×10^{-3}
	80 %	4.86×10^{-4}	9.95×10^{-3}	6.82×10^{-4}	3.40×10^{-3}
Rocket-leaves	Control	2.03×10^{-2}	7.13×10^{-2}	2.33×10^{-2}	3.17×10^{-2}
	20 %	1.96×10^{-3}	2.76×10^{-2}	3.27×10^{-3}	2.70×10^{-3}
	40 %	1.16×10^{-3}	1.49×10^{-2}	1.58×10^{-3}	1.82×10^{-3}
	60 %	1.18×10^{-3}	1.57×10^{-2}	2.02×10^{-3}	2.22×10^{-3}
	80 %	2.07×10^{-3}	1.30×10^{-2}	2.44×10^{-3}	3.13×10^{-3}

Table 7 Pearson correlation coefficients with p values for ^{238}U activity concentrations in plants and several tested parameters (number of observations in parenthesis)

Tested parameter	^{238}U			
	Radish-leaves	Radish-tubers	Savoy-leaves	Rocket-leaves
UMT content	No correlation	$r\ (20) = 0.826$, $p = 7.09 \times 10^{-6}$	No correlation	$r\ (20) = 0.730$, $p = 2.62 \times 10^{-4}$
pH	No correlation	No correlation	No correlation	No correlation
P_2O_5	No correlation	$r\ (20) = -0.824$, $p = 7.84 \times 10^{-6}$	No correlation	$r\ (20) = -0.731$, $p = 2.51 \times 10^{-4}$
K_2O	No correlation	$r\ (20) = -0.800$, $p = 2.68 \times 10^{-5}$	No correlation	$r\ (20) = -0.706$, $p = 5.03 \times 10^{-4}$
Organic matter	No correlation	$r\ (20) = -0.829$, $p = 6.37 \times 10^{-6}$	No correlation	$r\ (20) = -0.737$, $p = 2.07 \times 10^{-4}$
CEC	No correlation	$r\ (20) = 0.909$, $p = 2.92 \times 10^{-8}$	No correlation	$r\ (20) = 0.832$, $p = 5.54 \times 10^{-6}$
C	No correlation	$r\ (20) = -0829$, $p = 6.19 \times 10^{-6}$	No correlation	$r\ (20) = -0.738$, $p = 2.02 \times 10^{-4}$
N	No correlation	$r\ (20) = -0.821$, $p = 9.35 \times 10^{-6}$	No correlation	$r\ (20) = -0.728$, $p = 2.73 \times 10^{-4}$
C/N	No correlation	$r\ (20) = -0.731$, $p = 2.54 \times 10^{-4}$	No correlation	$r\ (20) = -0.677$, $p = 0.001$

reliable prediction of uptake and translocation of radionuclides in the environment as studies of radionuclides uptake under controlled conditions may give only approximate information about their behaviour in the natural environment [40].

The ^{238}U activity concentrations in tubers of radish and most of the pedological parameters (available P_2O_5 and K_2O, organic matter content, total N, organic C and C/N ratio in the growing substrate) as well as ^{238}U activity concentrations in leaves of rocket and most of the

pedological parameters (available P_2O_5 and K_2O, organic matter content, total N, organic C and C/N ratio in the growing substrate) were strongly negatively correlated, except for pH where the p values were not significant ($p > 0.05$) in both cases. ^{238}U activity concentrations in leaves of radish and pedological parameters as well as ^{238}U activity concentrations in leaves of savoy and pedological parameters were not correlated.

It seems that, in general, all these pedological factors decrease uranium mobility in soil due to its rapid reaction with those compounds, resulting in reduced uranium bioavailability [42–45]. Considering this fact, as well as higher uranium activity concentrations in the growing substrates due to UMT content, negative linear correlations were observed, as expected. Literature survey shows that an important pedological parameter in radionuclides uptake is CEC [46]. ^{238}U content in tubers of radish and leaves of rocket was positively correlated with CEC in the soil (Table 7), as already shown by Smodiš et al. [20]. This observation was explained as a consequence of higher exchange of uranium from soil colloids to the soil solution and consequently higher root uptake [20, 46].

Concentration ratios for ^{238}U varied from 5.89×10^{-4}–2.33×10^{-2}, 6.29×10^{-4}–1.01×10^{-2}, 4.82×10^{-4}–1.66×10^{-2} and 1.16×10^{-3}–2.03×10^{-2} for leaves of radish, tubers of radish, leaves of savoy and leaves of rocket grown in contaminated substrate, respectively; the average CRs are presented in Table 6. The observed scattered CR values might be attributed to different UMT content in the substrates that impacted on their chemical composition and pedological properties, which could affected plants' capabilities for taking up uranium.

The obtained concentration ratios for uranium in radish, savoy and rocket are generally for one or two orders of magnitude lower comparing with the reported average CRs, being 2.0×10^{-2} for leafy vegetables and 5.0×10^{-3} for tubers [38]. However, the low uptake of uranium may be attributed to the fact that majority of uranium had been extracted from uranium ore and consequently the added UMT was technologically altered and eventually biologically less available, as reported by Smodiš et al. [20].

^{230}Th uptake by radish, savoy and rocket

Activity concentrations of ^{230}Th varied from 1.5 to 8.3 Bq kg^{-1} and were lower than 2.4 Bq kg^{-1} in the controls (Table 4). Pearson correlation coefficients with corresponding p values among activity concentrations of ^{230}Th in plants and U-mill tailing content in soil, as well as the pedological parameters, are presented in Table 8. The activity concentrations of ^{230}Th in plants and ^{230}Th in soil (with different % of UMT content) were positively correlated and statistically significant for several plants (tubers of radish, leaves of savoy and leaves rocket). There was no correlation observed between ^{230}Th in soil and ^{230}Th activity concentrations in leaves of radish. These findings as well as the observation that behaviour of thorium in different plant parts may be different, are in agreement with the reported literature data [40]. Strong correlations were found between ^{230}Th activity concentrations in tubers of radish and all pedological parameters (except pH— no correlation, C/N ratio—moderate correlation) as well as between ^{230}Th activity concentrations in leaves of rocket and all pedological parameters (except pH—no correlation). Moderate correlations were observed between ^{230}Th in leaves of savoy and all pedological parameters (except pH and C/N ratio—no correlation). However, no correlations were found between pedological parameters and ^{230}Th in leaves of radish.

The obtained relatively low activity concentrations of ^{238}U and ^{230}Th in comparison with ^{226}Ra and ^{210}Pb content in the studied plants are in agreement with the literature data, since thorium and uranium are considered as elements with similar behaviour in soil, i.e., both are assumed as relatively immobile in soil and with low bioavailability [47, 48]. Accordingly, negative linear correlations were found between ^{230}Th activity concentrations in all investigated plants (and plants' parts), and most of the pedological parameters (available P_2O_5 and K_2O, organic matter content, total N, organic C and C/N ratio) of the growing substrates. The importance of soil properties on thorium uptake was also shown by Guo et al. [49]. As mentioned before, CEC is an important pedological parameter in the case of radionuclides uptake from soil [46]; ^{230}Th content in all investigated plants was positively correlated with CEC in the soil.

Concentration ratios for ^{230}Th varied from 5.79×10^{-4}–1.98×10^{-2}, 1.39×10^{-3}–1.98×10^{-2}, 6.82×10^{-4}–1.60×10^{-2} and 1.58×10^{-3}–2.33×10^{-2} for leaves of radish, tubers of radish, leaves of savoy and leaves of rocket grown in contaminated substrate, respectively; the average CRs are presented in Table 6. The observed scattered CR values might be attributed to different UMT content in the substrates that impacted on their chemical composition and pedological properties, which could affected plants' capabilities for taking up thorium.

For the broad leafy vegetables category that includes savoy and rocket, the IAEA reported CR average value for Th is 1.2×10^{-3} and for tubers 2.0×10^{-4} [38]. The obtained CR values were generally lower for one or two orders of magnitude for leaves and tubers of radish and leaves of savoy. The CR values for leaves of rocket were in agreement with the values reported by IAEA [38].

Table 8 Pearson correlation coefficients with p values for ^{230}Th activity concentrations in plants and several tested parameters (number of observations in parenthesis)

Tested parameter	^{230}Th			
	Radish-leaves	Radish-tubers	Savoy-leaves	Rocket-leaves
UMT content	No correlation	r (20) = 0.717, $p = 3.71 \times 10^{-4}$	r (20) = 0.451, p = 0.035	r (20) = 0.758, $p = 1.09 \times 10^{-4}$
pH	No correlation	No correlation	No correlation	No correlation
P_2O_5	No correlation	r (20) = −0.717, $p = 3.80 \times 10^{-4}$	r (20) = −0.457, p = 0.033	r (20) = −0.760, $p = 1.01 \times 10^{-4}$
K_2O	No correlation	r (20) = –0.708, p = 4.82×10^{-4}	r (20) = –0.446, p = 0.037	r (20) = –0.737, p = 2.09×10^{-4}
Organic matter	No correlation	r (20) = −0.715, $p = 4.00 \times 10^{-4}$	r (20) = −0.456, p = 0.033	r (20) = −0.769, $p = 7.51 \times 10^{-5}$
CEC	No correlation	r (20) = 0.700, $p = 6.45 \times 10^{-4}$	r (20) = 0.514, p = 0.014	r (20) = 0.856, $p = 1.48 \times 10^{-6}$
C	No correlation	r (20) = −0.715, $p = 3.96 \times 10^{-4}$	r (20) = −0.456, p = 0.033	r (20) = −0.770, $p = 7.25 \times 10^{-5}$
N	No correlation	r (20) = −0.718, $p = 3.68 \times 10^{-4}$	r (20) = −0.466, p = 0.029	r (20) = −0.754, $p = 1.22 \times 10^{-4}$
C/N	No correlation	r (20) = −0.468, p = 0.038	No correlation	r (20) = −0.732, $p = 2.45 \times 10^{-4}$

^{226}Ra uptake by radish, savoy and rocket

Activity concentrations of ^{226}Ra were below 4 Bq kg^{-1} and from 21 to 121 Bq kg^{-1} for the controls and various contamination scenarios, respectively (Table 4). Positive linear correlations were found between ^{226}Ra activity concentrations in plants and in soil (UMT content) for all investigated plants (Table 9). Contrary to our findings, Madruga et al. [13] found that the plant uptake response to the total radium activity concentration in the tailings follows a non–linear function while Smodiš et al. and Blanco Rodríguez et al. [20, 50] also found linear correlations.

Pearson correlation coefficients between ^{226}Ra activity concentrations in plants and pedological parameters are shown in Table 9. Strong linear correlations were found between ^{226}Ra activity concentrations in all tested plants and most determined pedological parameters (available P_2O_5 and K_2O, organic matter content, total N, organic C and C/N ratio) of the growing contaminated substrate. The correlations indicate that the reduced ^{226}Ra uptake may be a consequence of increased values of the investigated pedological parameters in the UMT contaminated soils [20]. ^{226}Ra activity concentrations in the studied plants were positively correlated with CEC in the contaminated soils, which may indicate that the elevated CEC values might be one of the reasons for increased ^{226}Ra transfer to the plants despite of its non-essential role in them [20]. However, no correlations were observed between ^{226}Ra activity concentrations in all plants and pH of the growing substrate.

Concentration ratios for ^{226}Ra varied from 8.11×10^{-3}–1.47×10^{-1}, 4.55×10^{-3}–4.59×10^{-2}, 6.02×10^{-3}–9.04×10^{-2} and 1.30×10^{-2}–7.13×10^{-2} for leaves of radish, tubers of radish, leaves of savoy and leaves of rocket grown in contaminated substrate, respectively; the average CRs for ^{226}Ra are presented in Table 6. The observed scattered CR values might be attributed to different UMT content in the substrates that impacted on their chemical composition and pedological properties, which could affected plants' capabilities for taking up radium.

For the broad leafy vegetables category, the IAEA reports CR average value for Ra 9.1×10^{-2} and for tubers 1.1×10^{-2} [38]. The observed CR values for savoy were one order of magnitude lower for most contamination scenarios while for tubers of radish and rocket they were at the same order of magnitude as the IAEA value [38].

^{210}Pb uptake by radish, savoy and rocket

Activity concentrations of ^{210}Pb varied from 2.7 to 64 Bq kg^{-1} for different contamination scenarios while for the controls they were lower than 2.8 Bq kg^{-1} (Table 4). Positive linear correlations were found between ^{210}Pb activity concentrations in plants and ^{210}Pb in soils for all investigated plants (Table 10), which indicate that in our study the main path of ^{210}Pb uptake was via the root system. It has been documented that ^{210}Pb can be transferred from the environment to plants either via the root system or as above-ground interception [51]. Therefore, an experiment was carried out in a controlled closed system with as

Table 9 Pearson correlation coefficients with p values for ^{226}Ra activity concentrations in plants and several tested parameters (number of observations in parenthesis)

Tested parameter	^{226}Ra			
	Radish-leaves	Radish-tubers	Savoy-leaves	Rocket-leaves
UMT content	$r\ (20) = 0.758$, $p = 1.09 \times 10^{-4}$	$r\ (20) = 0.824$, $p = 7.81 \times 10^{-6}$	$r\ (20) = 0.896$, $p = 1.80 \times 10^{-8}$	$r\ (20) = 0.907$, $p = 3.45 \times 10^{-8}$
pH	No correlation	No correlation	No correlation	No correlation
P_2O_5	$r\ (20) = -0.754$, $p = 1.21 \times 10^{-4}$	$r\ (20) = -0.817$, $p = 1.09 \times 10^{-5}$	$r\ (20) = -0.900$, $p = 1.41 \times 10^{-8}$	$r\ (20) = -0.912$, $p = 2.15 \times 10^{-8}$
K_2O	$r\ (20) = -0.740$, $p = 1.97 \times 10^{-4}$	$r\ (20) = -0.795$, $p = 2.81 \times 10^{-5}$	$r\ (20) = -0.888$, $p = 3.50 \times 10^{-8}$	$r\ (20) = -0.933$, $p = 2.16 \times 10^{-9}$
Organic matter	$r\ (20) = -0.772$, $p = 6.79 \times 10^{-5}$	$r\ (20) = -0.830$, $p = 5.96 \times 10^{-6}$	$r\ (20) = -0.909$, $p = 4.57 \times 10^{-9}$	$r\ (20) = -0.917$, $p = 1.28 \times 10^{-8}$
CEC	$r\ (20) = 0.789$, $p = 3.59 \times 10^{-5}$	$r\ (20) = 0.852$, $p = 1.84 \times 10^{-6}$	$r\ (20) = 0.921$, $p = 1.24 \times 10^{-9}$	$r\ (20) = 0.758$, $p = 1.09 \times 10^{-4}$
C	$r\ (20) = -0.773$, $p = 6.55 \times 10^{-5}$	$r\ (20) = -0.831$, $p = 5.76 \times 10^{-6}$	$r\ (20) = -0.910$, $p = 4.16 \times 10^{-9}$	$r\ (20) = -0.917$, $p = 1.28 \times 10^{-8}$
N	$r\ (20) = -0.729$, $p = 2.65 \times 10^{-4}$	$r\ (20) = -0.800$, $p = 2.85 \times 10^{-5}$	$r\ (20) = -0.886$, $p = 4.04 \times 10^{-8}$	$r\ (20) = -0.904$, $p = 4.82 \times 10^{-8}$
C/N	$r\ (20) = -0.920$, $p = 9.69 \times 10^{-9}$	$r\ (20) = -0.921$, $p = 8.43 \times 10^{-9}$	$r\ (20) = -0.844$, $p = 7.78 \times 10^{-7}$	$r\ (20) = -0.599$, $p = 5.30 \times 10^{-3}$

much as minimized atmospheric ^{222}Rn contribution onto external plant surface contamination by ^{210}Pb—bounded aerosols. The measured average ^{222}Rn activity concentrations were relatively low, 156 and 21 Bq m^{-3} at situation without and with ventilation, respectively. Therefore, atmospheric impact on ^{210}Pb activity concentrations in plants was assumed negligible. ^{222}Rn was measured as described in Vaupotič et al. [52].

Pearson correlation coefficients between ^{210}Pb activity concentrations in plants and the determined pedological parameters are presented in more detail in Table 10. Strong correlations ($r > 0.5$) with high significances ($p < 0.001$) between ^{210}Pb activity concentrations in all investigated plants and UMT content were found. Similarly as for the other three investigated radionuclides, negative linear correlations were found between ^{210}Pb content in all tested

Table 10 Pearson correlation coefficients with p values for ^{210}Pb activity concentrations in plants and several tested parameters (number of observations in parenthesis)

Tested parameter	^{210}Pb			
	Radish-leaves	Radish-tubers	Savoy-leaves	Rocket-leaves
UMT content	$r\ (20) = 0.774$, $p = 6.14 \times 10^{-5}$	$r\ (20) = 0.855$, $p = 1.56 \times 10^{-6}$	$r\ (20) = 0.825$, $p = 2.34 \times 10^{-6}$	$r\ (20) = 0.896$, $p = 9.30 \times 10^{-8}$
pH	No correlation	No correlation	No correlation	No correlation
P_2O_5	$r\ (20) = -0.774$, $p = 6.12 \times 10^{-5}$	$r\ (20) = -0.852$, $p = 1.86 \times 10^{-6}$	$r\ (20) = -0.825$, $p = 2.36 \times 10^{-6}$	$r\ (20) = -0.898$, $p = 8.09 \times 10^{-8}$
K_2O	$r\ (20) = -0.744$, $p = 1.71 \times 10^{-4}$	$r\ (20) = -0.817$, $p = 1.09 \times 10^{-5}$	$r\ (20) = -0.792$, $p = 1.12 \times 10^{-5}$	$r\ (20) = -0.878$, $p = 3.58 \times 10^{-7}$
Organic matter	$r\ (20) = -0.782$, $p = 4.61 \times 10^{-5}$	$r\ (20) = -0.859$, $p = 1.26 \times 10^{-6}$	$r\ (20) = -0.831$, $p = 1.68 \times 10^{-6}$	$r\ (20) = -0.902$, $p = 5.43 \times 10^{-8}$
CEC	$r\ (20) = 0.899$, $p = 7.18 \times 10^{-8}$	$r\ (20) = 0.971$, $p = 1.36 \times 10^{-12}$	$r\ (20) = 0.957$, $p = 3.45 \times 10^{-12}$	$r\ (20) = 0.950$, $p = 1.44 \times 10^{-10}$
C	$r\ (20) = -0.783$, $p = 4.44 \times 10^{-5}$	$r\ (20) = -0.860$, $p = 1.20 \times 10^{-6}$	$r\ (20) = -0.832$, $p = 1.60 \times 10^{-6}$	$r\ (20) = -0.903$, $p = 5.13 \times 10^{-8}$
N	$r\ (20) = -0.770$, $p = 7.28 \times 10^{-5}$	$r\ (20) = -0.846$, $p = 2.67 \times 10^{-6}$	$r\ (20) = -0.822$, $p = 2.76 \times 10^{-6}$	$r\ (20) = -0.896$, $p = 9.58 \times 10^{-8}$
C/N	$r\ (20) = -0.761$, $p = 9.81 \times 10^{-5}$	$r\ (20) = -0.825$, $p = 7.44 \times 10^{-6}$	$r\ (20) = -0.786$, $p = 1.48 \times 10^{-5}$	$r\ (20) = -0.729$, $p = 2.68 \times 10^{-4}$

plants and specific pedological parameters (available P_2O_5 and K_2O, organic matter content, total N, organic C and C/N ratio) of the growing substrates. The correlations indicate that reduced ^{210}Pb uptake occurs at increased values of the analysed soil parameters in the UMT contaminated soils. In the literature there is evidence for several elements, that CEC may be one of the important controlling factors for their transfer from soil to plants [20, 46, 53]. Therefore, the observed correlation between CEC and ^{210}Pb activity concentration in plants (Table 10) is in agreement with the previously published data [38].

Concentration ratios for ^{210}Pb varied from 1.53×10^{-3}–3.14×10^{-2}, 1.96×10^{-3}–1.02×10^{-2}, 9.16×10^{-4}–1.58×10^{-2} and 1.82×10^{-3}–3.17×10^{-2} for leaves of radish, tubers of radish, leaves of savoy and leaves of rocket grown in contaminated substrate, respectively; the average CRs for ^{210}Pb are presented in Table 6. The observed scattered CR values might be attributed to different UMT content in the substrates that impacted on their chemical composition and pedological properties, which could affected plants' capabilities for taking up ^{210}Pb.

The obtained CR values for savoy and rocket were from one to two orders of magnitude lower than the IAEA [38] reported average ^{210}Pb values for leafy vegetables (8.0×10^{-1}) and for tubers (1.5×10^{-3}), and in the same order of magnitude for tubers of radish, generally for all contamination scenarios.

Photochemical efficiency and content of photosynthetic pigments

Statistical analysis of linear correlations among activity concentrations of the studied radionuclides and photochemical efficiency, content of photosynthetic pigments (chlorophyll a, b) and anthocyanins showed no correlation for all tested plants. There was no clear effect of activity concentrations of the selected radionuclides in soils on photochemical efficiency, content of photosynthetic pigments and anthocyanins values, as all values were comparable with the controls taking into account the uncertainties; the results obtained are summarised in Table 5.

Since the chemical toxicity of metals has much bigger effect on physiological parameters of plants than the radiological one [54–57], the observation that radiological toxicity had no effect on physiological parameters of the plants, was within expectations.

Conclusions

The optimised method for simultaneous determination of selected radionuclides is appropriate for plant matrixes and small sample masses (up to 2 g of dried sample).

Radish, savoy and rocket can accumulate natural radionuclides from substrate contaminated with UMT to eatable parts of plants as confirmed with statistical analyses.

The obtained knowledge on soil-to-plant transfer of natural radionuclides is very important for upgrading the global database for the human and biota dose-assessment modelling.

The highest soil-to-plant transfer of ^{226}Ra, compared with the other three investigated radionuclides, can partially be explained with the fact that Ra has similar chemical behaviour as Ca, which is known as an essential macro element for plants.

The impact of pedological parameters on availability of the studied natural radionuclides was found as an important controlling factor, as also confirmed by other authors. The study also revealed that radiological toxicity has no effect on physiological parameters of plants at the given activity concentration levels of the selected radionuclides.

The obtained CR ratios were lower than 1 for all investigated plants, which leads to the conclusion that radish, savoy and rocket cannot be used for phytoremediation purposes under the investigated growth conditions. However, the understanding of soil-to-plant transfer behaviour of natural radionuclides in contaminated areas or in areas with naturally elevated values of NORM radionuclides is crucial for correctness of dose assessments as well as in developing appropriate radiological models.

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References

1. Križman M, Byrne AR, Benedik L (1995) Distribution of ^{230}Th in milling wastes from the Zirovski vrh uranium mine (Slovenia), and its radioecological implications. J Environ Radioact 26:223–235
2. Vreček P, Benedik L (2002) Determination of ^{210}Pb and ^{210}Po in sediments, water, and plants in an area contaminated with mine waste. Mine Water Environ 21:156–159
3. Tamponnet C, Martin-garin A, Gonze M, Parekh N (2008) An overview of BORIS : bioavailability of radionuclides in soils. J Environ Radioact 99:820–830
4. Štrok M, Smodiš B (2012) Transfer of natural radionuclides from hay and silage to cow's milk in the vicinity of a former uranium mine. J Environ Radioact 110C:64–68
5. Duquène L, Vandenhove H, Tack F, Meers E, Baeten J, Wannijn J (2009) Enhanced phytoextraction of uranium and selected heavy metals by Indian mustard and ryegrass using biodegradable soil amendments. Sci Total Environ 407:1496–1505
6. Ebbs SD, Brady DJ, Kochian LV (1998) Role of uranium speciation in the uptake and translocation of uranium by plants. Nutrition 49:1183–1190

7. Huang JW, Blaylock MJ, Kapulnik Y, Ensley BD (1998) Phytoremediation of uranium-contaminated soils: role of organic acids in triggering uranium hyperaccumulation in plants. Environ Sci Technol 32:2004–2008
8. Soudek P, Petrová S, Benesová D, Kotyza J, Vágner M, Vanková R, Vanek T (2010) Study of soil-plant transfer of ^{226}Ra under greenhouse conditions. J Environ Radioact 101:446–450
9. Liu D, Jiang W, Liu C, Xin C, Hou W (2000) Uptake and accumulation of lead by roots, hypocotyls and shoots of Indian mustard [Brassica juncea (L.)]. Bioresour Technol 71:273–277
10. ATSDR (Agency for Toxic Substances and Disease Registry), toxicological profiles. http://www.atsdr.cdc.gov/. Accessed: 24 Jun 2015
11. Blaylock MJ, Salt DE, Dushenkob S, Zakharova O, Gussman C, Kapulnik Y, Ensley BD, Raskin I (1997) Enhanced accumulation of Pb in Indian mustard by soil- applied chelating agents. Environ Sci Technol 31:860–865
12. Yoon J, Cao X, Zhou Q, Ma LQ (2006) Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. Sci Total Environ 368:456–464
13. Madruga MJ, Brogueira A, Alberto G, Cardoso F (2001) Ra bioavailability to plants at the Urgeiriça uranium mill tailings site. J Environ Radioact 54:175–188
14. Štrok M, Smodiš B (2010) Fractionation of natural radionuclides in soils from the vicinity of a former uranium mine Žirovski vrh, Slovenia. J Environ Radioact 101:22–28
15. Tomé FV, Vargas MJ, Sánchez AM (1994) Yields and losses at each step in preparing uranium and thorium samples for alpha spectrometry. Appl Radiat Isot 45:449–452
16. Matveyeva I, Jaćimović R, Planinšek P, Smodiš B, Burkitbayev M (2016) Uptake of uranium, thorium and radium isotopes by plants growing in dam impoundment Tasotkel and the Lower Shu region (Kazakhstan). Radiochim Acta 104:51–57
17. Černe M, Smodis B, Štrok M, Jaćimović R (2010) Accumulation of ^{226}Ra, ^{238}U and ^{230}Th by wetland plants in a vicinity of U-mill tailings at Žirovski vrh (Slovenia). J Radioanal Nucl Chem 286:323–327
18. Rodríguez PB, Tomé FV, Lozano JC (2001) Concerning the low uranium and thorium yields in the electrodeposition process of soil and sediment analyses. Appl Radiat Isot 54:29–33
19. D'Antuono LF, Elementi S, Neri R (2009) Exploring new potential health-promoting vegetables: glucosinolates and sensory attributes of rocket salads and related Diplotaxis and Eruca species. J Sci Food Agric 89:713–722
20. Smodiš B, Černe M, Jaćimović R, Benedik L (2015) Transfer of uranium and radium to Chinese cabbage from soil containing elevated levels of natural radionuclides. J Radioanal Nucl Chem 306:685–694
21. ONORM L 1087 (2006) Chemical analysis of soils-determination of "plant-available" Phosphorus And Potassium By The Calcium-acetate-lactate (cal)-method. Osterreichisches Normungsinstitut
22. Soil Survey Laboratory Staff (1992) Soil survey laboratory methods manual, U.S. Department of Agriculture
23. SIST ISO 13878 (1999) Soil quality—determination of total nitrogen content by dry combustion ('elemental analysis'). http://www.sist.si/ecommerce/catalog/project.aspx?id=557ec62e-84bd-4932-8bfb-a38cad8acf0e. Accessed 14 Aug 2015
24. SIST ISO 10390 (2006) Soil quality—determination of pH. http://www.sist.si/ecommerce/catalog/project.aspx?id=89661280-0784-40a2-8412-81e4b13cefb4. Accessed 14 Aug 2015
25. SIST ISO 10694 (1995) Soil quality—determination of organic and total carbon after dry combustion (elementary analysis). http://www.iso.org/iso/catalogue_detail.htm?csnumber=18782. Accessed 14 Aug 2015
26. Lichtenthaler HK (1988) In: Lichtenthaler HK (ed) Applications of chlorophyll fluorescene in photosynthesis research, stress physiology, hydrobiology and remote sensing. Springer, Dordrecht
27. Schreiber U, Bilger W, Neubauer C (1995) In: Schulze ED, Caldwell MM (eds) Ecophysiology of photosynthesis. Springer, Berlin
28. Mechora S, Stibilj V, Germ M (2013) The uptake and distribution of selenium in three aquatic plants grown in Se(IV) solution. Aquat Toxicol 128–129:53–59
29. Hawrylak-Nowak B (2008) Changes in anthocyanin content as indicator of maize sensitivity to selenium. J Plant Nutr 31:1232–1242
30. Aggarwal A, Sharma I, Tripathi BN, Munjal A, Baunthiyal M, Sharma V (2012) Metal toxicity and photosynthesis. In: Itoh S, Mohanty P, Guruprasad KN (eds) Photosynthesis: overviews on recent progress & future perspective. IK International Publishing House, New Delhi
31. Lichtenthaler HK, Buschmann C (2001) Chlorophylls and carotenoids: measurement and characterization by UV-Vis spectroscopy. In: Wrolstad RE, Acree TE, Decker EA, Penner MH, Reid DS, Schwartz SJ, Shoemaker CF, Smith D, Sporns P (eds) Current protocols in food analytical chemistry. John Wiley & Sons Inc, New York
32. Lichtenthaler HK, Buschmann C (2001) Extraction of phtosynthetic tissues: chlorophylls and carotenoids. In: Wrolstad RE, Acree TE, Decker EA, Penner MH, Reid DS, Schwartz SJ, Shoemaker CF, Smith D, Sporns P (eds) Current protocols in food analytical chemistry. Wiley, New York
33. Drumm H, Mohr H (1978) Mode of interaction between blue (UV) light pho- toreceptor and phytochrome in anthocyanin formation of Sorghum seedling. Photochem Photobiol 27:241–248
34. Štrok M, Smodiš B, Petrinec B (2010) Natural radionuclides in sediments and rocks from Adriatic Sea. J Radioanal Nucl Chem 286:303–308
35. Benedik L, Tavčar P (2001) Determination of ^{210}Pb and ^{210}Po in environmental samples. Acta Chim Slov 48:199–213
36. Horwitz EP (1995) Separation and preconcentration of actinides by extraction chromatography using a supported liquid anion exchanger: application to the characterization of high-level nuclear waste solutions. Anal Chim Acta 310:63–78
37. Jurečič S, Benedik L, Planinšek P, Nečemer M, Kump P, Pihlar B (2014) Analysis of uranium in the insoluble residues after decomposition of soil samples by various techniques. Appl Radiat Isot 87:61–65
38. IAEA (2010) Handbook of parameter values for the prediction of radionuclide transfer in terrestrial and freshwater, no. 472, IAEA (International Atomic Energy Agency), Vienna
39. Singh S, Malhotra R, Bajwa BS (2005) Uranium uptake studies in some plants. Radiat Meas 40:666–669
40. Shtangeeva I (2010) Uptake of uranium and thorium by native and cultivated plants. J Environ Radioact 101:458–463
41. Sheppard SC, Evenden WG, Pollock RJ (1989) Uptake of natural radionuclides by field and garden crops. Can J Soil Sci 69:751–767
42. Vandenhove H, Van Hees M, Wouters K, Wannijn J (2007) Can we predict uranium bioavailability based on soil parameters? Part 1: effect of soil parameters on soil solution uranium concentration. Environ Pollut 145:587–595
43. Bednar AJ, Medina VF, Ulmer-Scholle DS, Frey BA, Johnson BL, Brostoff WN, Larson SL (2007) Effects of organic matter on the distribution of uranium in soil and plant matrices. Chemosphere 70:237–247
44. Chang P, Kim KW, Yoshida S, Kim SY (2005) Uranium accumulation of crop plants enhanced by citric acid. Environ Geochem Health 27:529–538
45. Rufyikiri G, Wannijn J, Wang L, Thiry Y (2006) Effects of phosphorus fertilization on the availability and uptake of uranium

and nutrients by plants grown on soil derived from uranium mining debris. Environ Pollut 141:420–427
46. Vandenhove H, Van Hees M (2007) Predicting radium availability and uptake from soil properties. Chemosphere 69:664–674
47. Durrance EM (1986) Radioactivity in geology: principles and applications. John Wiley, New York
48. Wickleder MS, Fourest B, Dorhout PK (2008) In: Morss LR, Edelstein NM, Fuger J, Katz JJ (eds) The chemistry of the actinide and transactinide elements. Springer, Dordrecht
49. Guo P, Jia X, Duan T, Xu J, Chen H (2010) Influence of plant activity and phosphates on thorium bioavailability in soils from Baotou area, Inner Mongolia. Environ Radioact 101:767–772
50. Rodríguez PB, Tomé FV, Lozano JC (2002) About the assumption of linearity in soil-to-plant transfer factors for uranium and thorium isotopes and ^{226}Ra. Sci Total Environ 284:167–175
51. Pietrzak-Flis Z, Skowrohska-Smolak M (1995) Transfer of ^{210}Pb and ^{210}Po to plants via root system and above-ground interception. Sci Total Environ 162:139–147
52. Vaupotič J, Bezek M, Kávási N, Ishikawa T, Yonehara H, Tokonami S (2012) Radon and thoron doses in kindergartens and elementary schools. Radiat Prot Dosim 152:247–252
53. Veresoglou DS, Barbayiannis N, Matsi T, Anagnostopoulos C, Zalidis GC (1996) Shoot Sr concentrations in relation to shoot Ca concentrations and to soil properties. Plant Soil 178:95–100
54. Juneau P, Popovic R (1999) Evidence for the rapid phytotoxicity and environmental stress evaluation using the PAM fluorometric method: importance and future application. Ecotoxicology 8:449–455
55. Vanhoudt N, Horemans N, Biermans G, Saenen E, Wannijn J, Nauts R, Van Hees M, Vandenhove H (2014) Uranium affects photosynthetic parameters in Arabidopsis thaliana. Environ Exp Bot 97:22–29
56. Kaur G, Singh HP, Batish DR, Kohli RK (2012) Growth, photosynthetic activity and oxidative stress in wheat (Triticum aestivum) after exposure of lead to soil. J Environ Biol 33:265–269
57. Dias MC, Monteiro C, Moutinho-Pereira J, Correia C, Gonçalves B, Santos C (2012) Cadmium toxicity affects photosynthesis and plant growth at different levels. Acta Physiol Plant 35:1281–1289

References

[1] IAEA, *Extent of Environmental Contamination by Naturally Occurring Radioactive Material (NORM) and Technological Options for Mitigation. Technical reports No. 419*. Vienna, 2003.

[2] M. Pöschl and L. M. L. Nollet, *Radionuclide Concentrations in Food and the Environment*. Boca Raton: CRC Press, Taylor and Francis Group, 2007.

[3] R. Van der Strich and R. Kirchmann, Eds., *Radioecology: Radioactivity & Ecosystems*. Liege: International Union of Radioecology, 2001.

[4] M. Križman, A. R. Byrne, and L. Benedik, "Distribution of 230Th in milling wastes from the Zirovski vrh uranium mine (Slovenia), and its radioecological implications," *J. Environ. Radioact.*, vol. 26, no. 3, pp. 223–235, Jan. 1995.

[5] P. Vreček and L. Benedik, "Determination of 210Pb and 210Po in sediments, water, and plants in an area contaminated with mine waste," *Mine Water Environ.*, vol. 21, pp. 156–159, 2002.

[6] "Žirovski vrh uranium mine." [Online]. Available: http://www.rudnik-zv.si/izvajanje-sanacijskih-del. [Accessed: 01-Aug-2016].

[7] C. Tamponnet, A. Martin-garin, M. Gonze, and N. Parekh, "An overview of BORIS : Bioavailability of Radionuclides in Soils," *J. Environ. Radioact.*, vol. 99, pp. 820–830, 2008.

[8] M. Štrok and B. Smodiš, "Transfer of natural radionuclides from hay and silage to cow's milk in the vicinity of a former uranium mine.," *J. Environ. Radioact.*, vol. 110C, pp. 64–68, Feb. 2012.

[9] IAEA, *Handbook of Parameter Values for the Prediction of Radionuclide Transfer in Terrestrial and Freshwater*, no. 472. Vienna: IAEA (International Atomic Energy Agency), 2010.

[10] M. J. Madruga, A. Brogueira, G. Alberto, and F. Cardoso, "Ra bioavailability to plants at the Urgeiriça uranium mill tailings site," *J. Environ. Radioact.*, vol. 54, pp. 175–188, 2001.

[11] I. Shtangeeva, "Uptake of uranium and thorium by native and cultivated plants," *J. Environ. Radioact.*, vol. 101, no. 6, pp. 458–463, Jun. 2010.

[12] S. C. Sheppard, W. G. Evenden, and R. J. Pollock, "Uptake of natural

radionuclides by field and garden crops," *Can. J. Soil Sci.*, vol. 69, no. 4, pp. 751–767, Mar. 1989.

[13] V. N. Jha, R. M. Tripathi, N. K. Sethy, and S. K. Sahoo, "Uptake of uranium by aquatic plants growing in fresh water ecosystem around uranium mill tailings pond at Jaduguda, India.," *Sci. Total Environ.*, vol. 539, pp. 175–84, Jan. 2016.

[14] I. Shtangeeva and S. Ayrault, "Phytoextraction of Thorium from Soil and Water Media," *Water, Air, Soil Pollut.*, vol. 154, no. 1–4, pp. 19–35, May 2004.

[15] S. D. Ebbs, D. J. Brady, and L. V Kochian, "Role of uranium speciation in the uptake and translocation of uranium by plants," *Nutrition*, vol. 49, no. 324, pp. 1183–1190, 1998.

[16] L. Duquène, H. Vandenhove, F. Tack, E. Meers, J. Baeten, and J. Wannijn, "Enhanced phytoextraction of uranium and selected heavy metals by Indian mustard and ryegrass using biodegradable soil amendments," *Sci. Total Environ.*, vol. 407, no. 5, pp. 1496–1505, 2009.

[17] J. W. Huang, M. J. Blaylock, Y. Kapulnik, and B. D. Ensley, "Phytoremediation of Uranium-Contaminated Soils: Role of Organic Acids in Triggering Uranium Hyperaccumulation in Plants," *Environ. Sci. Technol.*, vol. 32, no. 13, pp. 2004–2008, Jul. 1998.

[18] P. Soudek, S. Petrová, D. Benesová, J. Kotyza, M. Vágner, R. Vanková, and T. Vanek, "Study of soil-plant transfer of 226Ra under greenhouse conditions.," *J. Environ. Radioact.*, vol. 101, no. 6, pp. 446–50, Jun. 2010.

[19] D. Liu, W. Jiang, C. Liu, C. Xin, and W. Hou, "Uptake and accumulation of lead by roots, hypocotyls and shoots of Indian mustard [Brassica juncea (L.)]," *Bioresour. Technol.*, vol. 71, no. 3, pp. 273–277, 2000.

[20] ATSDR (Agency for Toxic Substances and Disease Registry), "Toxicological Profiles." [Online]. Available: http://www.atsdr.cdc.gov/. [Accessed: 24-Jun-2015].

[21] M. J. Blaylock, D. E. Salt, S. Dushenkob, O. Zakharova, C. Gussman, Y. Kapulnik, B. D. Ensley, and I. Raskin, "Enhanced Accumulation of Pb in Indian Mustard by Soil- Applied Chelating Agents," *Environ. Sci. Technol.*, vol. 31, no. 3, pp. 860–865, 1997.

[22] J. Yoon, X. Cao, Q. Zhou, and L. Q. Ma, "Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site," *Sci. Total Environ.*, vol. 368, no. 2–3, pp. 456–464, 2006.

[23] N. Sanzharova, S. Fesenko, and E. Reed, "Processes governing radionuclide transfer to plants," in *Quantification of Radionuclide Transfer in Terrestrial and Freshwater Environments for Radiological Assessments*, Vienna: IAEA (International Atomic Energy Agency), 2009, pp. 123–138.

[24] W. W. Wenzel, E. Lombi, and D. C. Adriano, "Biogeochemical Processes in the

Rhizosphere: Role in Phytoremediation of Metal-Polluted Soils," in *Heavy Metal Stress in Plants - From Molecules to Ecosystems*, Prasad, M.N.V. and J. Hagemeyer, Eds. Berlin: Springer, 1999, pp. 273–303.

[25] M. Eisenbud and T. Gesell, Eds., "Terrestrial and Aquatic Pathways," in *Cover image Environmental Radioactivity: From Natural, Industrial, and Military Sources*, 4th ed., San Diego, 1997, pp. 88–133.

[26] P. Blanco Rodríguez, F. Vera Tomé, J. C. Lozano, and M. A. Pérez-Fernández, "Influence of soil texture on the distribution and availability of 238U, 230Th, and 226Ra in soils," *J. Environ. Radioact.*, vol. 99, no. 8, pp. 1247–54, Aug. 2008.

[27] L. Taiz and E. Zeiger, Eds., *Plant Physiology*, 3th ed. Sunderland: Sinauer Associates, Inc., 2002.

[28] B. Smodiš, M. Černe, R. Jaćimović, and L. Benedik, "Transfer of uranium and radium to Chinese cabbage from soil containing elevated levels of natural radionuclides," *J. Radioanal. Nucl. Chem.*, 2015.

[29] H. Vandenhove and M. Van Hees, "Predicting radium availability and uptake from soil properties.," *Chemosphere*, vol. 69, no. 4, pp. 664–74, Sep. 2007.

[30] D. S. Veresoglou, N. Barbayiannis, T. Matsi, C. Anagnostopoulos, and G. C. Zalidis, "Shoot Sr concentrations in relation to shoot Ca concentrations and to soil properties," *Plant Soil*, vol. 178, no. 1, pp. 95–100, Jan. 1996.

[31] D. L. Sparks, *Environmental soil chemistry*. San Diego: Academic Press, Inc., 1995.

[32] M. Greger, "Metal Availability and Bioconcentration in Plants," in *Heavy Metal Stress in Plants - From Molecules to Ecosystems*, M. N. V. Prasad and J. Hagemeyer, Eds. Berlin: Springer, 1999, pp. 1–27.

[33] H. Vandenhove, M. Van Hees, K. Wouters, and J. Wannijn, "Can we predict uranium bioavailability based on soil parameters? Part 1: effect of soil parameters on soil solution uranium concentration.," *Environ. Pollut.*, vol. 145, no. 2, pp. 587–95, Jan. 2007.

[34] M. Gavrilescu, L. V. Pavel, and I. Cretescu, "Characterization and remediation of soils contaminated with uranium.," *J. Hazard. Mater.*, vol. 163, no. 2–3, pp. 475–510, Apr. 2009.

[35] J. C. Igwe, I. C. Nnorom, and B. C. Gbaruko, "Kinetics of radionuclides and heavy metals behaviour in soils: Implications for plant growth," *African J. Biotechnol.*, vol. 4, no. 13, pp. 1541–1547, 2005.

[36] M. Štrok and B. Smodiš, "Fractionation of natural radionuclides in soils from the vicinity of a former uranium mine Zirovski vrh, Slovenia.," *J. Environ. Radioact.*, vol. 101, no. 1, pp. 22–8, Jan. 2010.

[37] H. L. Bohn, B. L. McNeal, and G. A. O'Connor, *Soil Chemistry*. New York: John

Wiley, 1985.

[38] M. Vidal, A. Rigol, and C. J. Gil-García, "Soil-radionuclide interactions," in *Quantification of Radionuclide Transfer in Terrestrial and Freshwater Environments for Radiological Assessments*, Vienna: IAEA (International Atomic Energy Agency), 2009, pp. 71–102.

[39] S. Ehlken and G. Kirchner, "Environmental processes affecting plant root uptake of radioactive trace elements and variability of transfer factor data: a review," *J. Environ. Radioact.*, vol. 58, no. 2–3, pp. 97–112, Jan. 2002.

[40] B. Varga, E. Leclerc, and P. Zagyvai, "The role of analogues in radioecology.," *J. Environ. Radioact.*, vol. 100, no. 9, pp. 802–5, Sep. 2009.

[41] S. P. Varnavas, D. Panagiotaras, and G. A. Wolff, "Biogeochemical processes at the sediment–water interface in a Northeastern Atlantic abyssal locality (Porcupine Abyssal Plain)," *Prog. Oceanogr.*, vol. 50, no. 1–4, pp. 223–243, Jan. 2001.

[42] M. H. Gerzabek, F. Strebl, and B. Temmel, "Plant uptake of radionuclides in lysimeter experiments," *Environ. Pollut.*, vol. 99, no. 1, pp. 93–103, 1998.

[43] H. Barros, A. Laissaoui, and J. M. Abril, "Trends of radionuclide sorption by estuarine sediments. Experimental studies using 133Ba as a tracer.," *Sci. Total Environ.*, vol. 319, no. 1–3, pp. 253–67, Feb. 2004.

[44] A. S. Szabo, *Radioecology and Environmental Protection.* Budapest: Ellis Horwood Ltd, 1993.

[45] M. H. Gerzabek, F. Strebl, and B. Temmel, "Plant uptake of radionuclides in lysimeter experiments.," *Environ. Pollut.*, vol. 99, no. 1, pp. 93–103, Jan. 1998.

[46] M. Greger, "Trace elements and radionuclides in edible plants," in *Trace Elements as Contaminants and Nutrients: Consequences in Ecosystems and Human Health*, M. N. V. Prasad, Ed. New Jersey: John Wiley & Sons, Inc., 2008, pp. 121–136.

[47] S. Singh, R. Malhotra, and B. S. Bajwa, "Uranium uptake studies in some plants," *Radiat. Meas.*, vol. 40, no. 2–6, pp. 666–669, Nov. 2005.

[48] P. Chang, K.-W. Kim, S. Yoshida, and S.-Y. Kim, "Uranium accumulation of crop plants enhanced by citric acid," *Environ. Geochem. Health*, vol. 27, no. 5–6, pp. 529–38, Sep. 2005.

[49] S. B. Chen, Y. G. Zhu, and Q. H. Hu, "Soil to plant transfer of 238U, 226Ra and 232Th on a uranium mining-impacted soil from southeastern China," *J. Environ. Radioact.*, vol. 82, no. 2, pp. 223–36, Jan. 2005.

[50] P. Blanco Rodríguez, F. Vera Tomé, M. Pérez Fernández, and J. C. Lozano, "Linearity assumption in soil-to-plant transfer factors of natural uranium and radium in Helianthus annuus L.," *Sci. Total Environ.*, vol. 361, no. 1–3, pp. 1–7, May 2006.

[51] I. Shtangeeva, S. Ayrault, and J. Jain, “Thorium uptake by wheat at different stages of plant growth,” *J. Environ. Radioact.*, vol. 81, no. 2–3, pp. 283–93, Jan. 2005.

[52] M. Černe, B. Smodiš, and M. Štrok, “Uptake of radionuclides by a common reed (Phragmites australis (Cav.) Trin. ex Steud.) grown in the vicinity of the former uranium mine at Žirovski vrh,” *Nucl. Eng. Des.*, vol. 241, no. 4, pp. 1282–1286, Apr. 2011.

[53] J. H. Chao and C. Y. Chuang, “Accumulation of radium in relation to some chemical analogues in Dicranopteris linearis,” *Appl. Radiat. Isot.*, vol. 69, pp. 261–267, 2011.

[54] D. C. Lauria, F. C. a Ribeiro, C. C. Conti, and F. a Loureiro, “Radium and uranium levels in vegetables grown using different farming management systems.,” *J. Environ. Radioact.*, vol. 100, no. 2, pp. 176–83, Feb. 2009.

[55] B. Bourdon, M. G. Henderson, C. C. Lundstrom, and P. S. Turner, Eds., *Uranium-series Geochemistry*. Washington: Mineralogical Society of America, 2003.

[56] LNHB, “Recommended data,” 2015. [Online]. Available: http://www.nucleide.org/DDEP_WG/DDEPdata.htm. [Accessed: 24-Nov-2015].

[57] I. Grenthe, J. Drożdżyński, T. Fujino, E. C. Buck, T. E. Albrecht-Shmitt, and S. F. Wolf, “Uranium,” in *The chemistry of the actinide and transactinide elements*, 3th ed., L. R. Morss, N. M. Edelstein, J. Fuger, and J. J. Katz, Eds. Dordrecht: Springer, 2008, pp. 253–698.

[58] E. M. Durrance, *Radioactivity in geology: Principles and Applications*. New York: John Wiley, 1986.

[59] W. Loveland, D. J. Morrissey, and G. T. Seaborg, *Modern nuclear chemistry*. New York: John Wiley, 2006.

[60] G. Choppin, J. O. Liljenzin, and R. J., *Radiochemistry and nuclear chemistry*, 3th ed. Woburn: Butterworth-Heinemann, 2001.

[61] G. Rufyikiri, J. Wannijn, L. Wang, and Y. Thiry, “Effects of phosphorus fertilization on the availability and uptake of uranium and nutrients by plants grown on soil derived from uranium mining debris.,” *Environ. Pollut.*, vol. 141, no. 3, pp. 420–427, Jun. 2006.

[62] J. . Jerden, a. . Sinha, and L. Zelazny, “Natural immobilization of uranium by phosphate mineralization in an oxidizing saprolite–soil profile: chemical weathering of the Coles Hill uranium deposit, Virginia,” *Chem. Geol.*, vol. 199, no. 1–2, pp. 129–157, Aug. 2003.

[63] A. J. Bednar, V. . F. Medina, D. S. Ulmer-Scholle, B. A. Frey, B. L. Johnson, W. N. Brostoff, and S. L. Larson, “Effects of organic matter on the distribution of uranium in soil and plant matrices.,” *Chemosphere*, vol. 70, no. 2, pp. 237–247,

Dec. 2007.

[64] M. Černe, B. Smodis, M. Štrok, and R. Jaćimović, "Accumulation of 226Ra, 238U and 230Th by wetland plants in a vicinity of U-mill tailings at Žirovski vrh (Slovenia)," *J. Radioanal. Nucl. Chem.*, vol. 286, no. 2, pp. 323–327, 2010.

[65] H. Vandenhove, C. Gil-García, a Rigol, and M. Vidal, "New best estimates for radionuclide solid-liquid distribution coefficients in soils. Part 2: naturally occurring radionuclides.," *J. Environ. Radioact.*, vol. 100, no. 9, pp. 697–703, Sep. 2009.

[66] NNDC (National Nuclear Data Center), "Chart of Nuclides." [Online]. Available: http://www.nndc.bnl.gov/. [Accessed: 26-Nov-2015].

[67] F. Lazarini and J. Brenčič, *Splošna in anorganska kemija*. Ljubljana: DZS, 1984.

[68] F. Vera Tomé, P. B. Rodríguez, and J. C. Lozano, "Distribution and mobilization of U, Th and 226Ra in the plant-soil compartments of a mineralized uranium area in south-west Spain," *J. Environ. Radioact.*, vol. 59, pp. 41–60, Jan. 2002.

[69] P. Sar and S. F. D'Souza, "Biosorption of thorium (IV) by a Pseudomonas biomass," *Biotechnol. Lett.*, vol. 24, pp. 239–243, 2002.

[70] M. S. Wickleder, B. Fourest, and P. K. Dorhout, "Thorium," in *The chemistry of the actinide and transactinide elements*, L. R. Morss, N. M. Edelstein, J. Fuger, and J. J. Katz, Eds. Dordrecht: Springer, 2008, pp. 52–160.

[71] P. Guo, X. Jia, T. Duan, J. Xu, and H. Chen, "Influence of plant activity and phosphates on thorium bioavailability in soils from Baotou area , Inner Mongolia," *J. Environ. Radioact.*, vol. 101, no. 9, pp. 767–772, 2010.

[72] ATSDR, "Toxicological Profile for Radium," 1990.

[73] N. G. Rachkova, I. I. Shuktomova, and a. I. Taskaev, "The state of natural radionuclides of uranium, radium, and thorium in soils," *Eurasian Soil Sci.*, vol. 43, no. 6, pp. 651–658, Jun. 2010.

[74] A. C. Baker and C. Toque, "A review of the potential for radium from luminising activities to migrate in the environment.," *J. Radiol. Prot.*, vol. 25, no. 2, pp. 127–140, Jun. 2005.

[75] A. Kabata-Pendias and A. B. Mukherjee, *Trace elements from soil to human*. Verlag, Berlin, Heidelberg: Springer, 2007.

[76] J. S. C. Fernández and J. Sordo, Eds., *Lead: chemistry, analytical aspects, environmental impact and health effects*. Amsterdam: Elsevier, 2006.

[77] E. Ekdal, T. Karali, and M. M. Saç, "210Po and 210Pb in soils and vegetables in Kucuk Menderes basin of Turkey," *Radiat. Meas.*, vol. 41, pp. 72–77, 2006.

[78] K. M. Matthews, C. Kim, and P. Martin, "Determination of Po in environmental materials: A review of analytical methodology," *Appl. Radiat. Isot.*, vol. 65, pp.

267–279, 2007.

[79] N. Karunakara, D. N. Avadhani, H. M. Mahesh, H. M. Somashekarappa, Y. Narayana, and K. Siddappa, "Distribution and enrichment of Po in the environment of Kaiga in South India," *J. Environ. Radioact.*, vol. 51, no. 3, pp. 349–362, 2000.

[80] M. A. A. Aslani, S. Akyil, S. Aytas, G. Gurboga, and M. Eral, "Activity concentration of 210Pb (210Po) in soils taken from cultivated lands," *Act. Conc. 210 Pb (210 Po) soils Tak. from Cultiv. lands*, vol. 39, no. 2, pp. 129–135, 2005.

[81] M. S. Al-Masri, B. Al-Akel, a Nashawani, Y. Amin, K. H. Khalifa, and F. Al-Ain, "Transfer of (40)K, (238)U, (210)Pb, and (210)Po from soil to plant in various locations in south of Syria.," *J. Environ. Radioact.*, vol. 99, no. 2, pp. 322–31, Feb. 2008.

[82] Z. Pietrzak-Flis and M. Skowrohska-smolak, "Transfer of 210Pb and 210Po to plants via root system and above-ground interception," *Science (80-.).*, vol. 162, no. 2–3, pp. 139–147, 1995.

[83] R. M. Alexakhin, "Effects of ionising radiation on ecosystems," in *Radioecology: Radioactivity & Ecosystems*, E. Van der Stricht and R. Kirchmann, Eds. Liege: International Union of Radioecology, 2001, pp. 376–390.

[84] S. Geras'kin, T. Evseeva, and A. Oudalova, "Effects of long-term chronic exposure to radionuclides in plant populations.," *J. Environ. Radioact.*, vol. 121, pp. 22–32, Jul. 2013.

[85] S. C. Sheppard, M. I. Sheppard, M.-O. Gallerand, and B. Sanipelli, "Derivation of ecotoxicity thresholds for uranium.," *J. Environ. Radioact.*, vol. 79, no. 1, pp. 55–83, Jan. 2005.

[86] R. Mittler, "Abiotic stress, the field environment and stress combination.," *Trends Plant Sci.*, vol. 11, no. 1, pp. 15–9, Jan. 2006.

[87] S. Jurečič, L. Benedik, P. Planinšek, M. Nečemer, P. Kump, and B. Pihlar, "Analysis of uranium in the insoluble residues after decomposition of soil samples by various techniques," *Appl. Radiat. Isot.*, vol. 87, pp. 61–5, May 2014.

[88] IAEA, *International basic safety standards for protection against ionizing radiations and for the safety of radiation sources. Safety Series No. 115, IAEA*. Vienna: IAEA (International Atomic Energy Agency), 2003.

[89] P. Planinšek, L. Benedik, and B. Smodiš, "Comparison of various dissolution techniques for determination of Po-210 in biological samples.," *Appl. Radiat. Isot.*, vol. 81, pp. 53–6, Nov. 2013.

[90] P. Blanco Rodríguez, F. Vera Tomé, and J. C. Lozano, "Concerning the low uranium and thorium yields in the electrodeposition process of soil and sediment analyses," *Appl. Radiat. Isot.*, vol. 54, no. 1, pp. 29–33, 2001.

[91] I. Matveyeva, R. Jaćimović, P. Planinšek, B. Smodiš, and M. Burkitbayev, "Uptake of uranium, thorium and radium isotopes by plants growing in dam impoundment Tasotkel and the Lower Shu region (Kazakhstan)," *Radiochim. Acta*, vol. 104, pp. 51–57, 2015.

[92] M. Štrok, B. Smodiš, and B. Petrinec, "Natural radionuclides in sediments and rocks from Adriatic Sea," *J. Radioanal. Nucl. Chem.*, vol. 286, no. 2, pp. 303–308, Jul. 2010.

[93] F. Vera Tomé, M. Jurado Vargas, and A. Martín Sánchez, "Yields and losses at each step in preparing uranium and thorium samples for alpha spectrometry," *Appl. Radiat. Isot.*, vol. 45, no. 4, pp. 449–452, Apr. 1994.

[94] L. F. D'Antuono, S. Elementi, and R. Neri, "Exploring new potential health-promoting vegetables: Glucosinolates and sensory attributes of rocket salads and related Diplotaxis and Eruca species," *J. Sci. Food Agric.*, vol. 89, no. 4, pp. 713–722, 2009.

[95] L. Benedik and P. Tavčar, "Determination of 210Pb and 210Po in environmental samples," *Acta Chim. Slov.*, vol. 48, no. 2, pp. 199–213, 2001.

[96] E. P. Horwitz, "Separation and preconcentration of actinides by extraction chromatography using a supported liquid anion exchanger: Application to the characterization of high-level nuclear waste solutions," *Anal. Chim. Acta*, vol. 310, no. 1, pp. 63–78, 1995.

[97] J. C. Lozano, F. Fernandez, and J. M. G. Gomez, "Determination of radium isotopes by BaSO4 coprecipitation for the preparation of alpha-spectrometric sources," *J. Radioanal. Nucl. Chem.*, vol. 223, no. 1–2, pp. 133–137, Sep. 1997.

[98] M. Štrok and B. Smodiš, "Fractionation of natural radionuclides in soils from the vicinity of a former uranium mine Žirovski vrh, Slovenia.," *J. Environ. Radioact.*, vol. 101, pp. 22–28, Jan. 2010.

[99] M. Černe, "Transfer of natural radionuclides to selected organisms from soil contaminated with U-mill tailings - Doctoral Dissertation," Ljubljana, 2014.

[100] J. Vaupotič, M. Bezek, N. Kávási, T. Ishikawa, H. Yonehara, and S. Tokonami, "Radon and thoron doses in kindergartens and elementary schools," vol. 152, no. 1–3, pp. 247–252, 2012.

[101] C. B. Beck, *An Introduction to Plant Structure and Development: Plant Anatomy for the Twenty-First Century*. Cambridge University Press, 2010.

[102] H. K. Lichtenthaler, "In Vivo Chlorophyll Fluorescence as a Tool for Stress Detection in Plants," in *Applications of Chlorophyll Fluorescene in Photosynthesis Research, Stress Physiology, Hydrobiology and Remote Sensing*, H. K. Lichtenthaler, Ed. Dordrecht: Springer Netherlands, 1988, pp. 129–142.

[103] U. Schreiber, W. Bilger, and C. Neubauer, "Chlorophyll Fluorescence as a

Nonintrusive Indicator for Rapid Assessment of In Vivo Photosynthesis," in *Ecophysiology of Photosynthesis*, E.-D. Schulze and M. M. Caldwell, Eds. Berlin, Heidelberg: Springer Berlin Heidelberg, 1995, pp. 49–70.

[104] S. Mechora, V. Stibilj, and M. Germ, "The uptake and distribution of selenium in three aquatic plants grown in Se(IV) solution.," *Aquat. Toxicol.*, vol. 128–129, pp. 53–9, Mar. 2013.

[105] H. K. Lichtenthaler and C. Buschmann, "Extraction of Phtosynthetic Tissues: Chlorophylls and Carotenoids," in *Current Protocols in Food Analytical Chemistry*, R. E. Wrolstad, T. E. Acree, E. A. Decker, M. H. Penner, D. S. Reid, S. J. Schwartz, C. F. Shoemaker, D. Smith, and P. Sporns, Eds. Hoboken, NJ, USA: John Wiley & Sons, Inc., 2001, p. F.4.2.1-4.2.6.

[106] H. K. Lichtenthaler and C. Buschmann, "Chlorophylls and Carotenoids: Measurement and Characterization by UV-VIS Spectroscopy," in *Current Protocols in Food Analytical Chemistry*, R. E. Wrolstad, T. E. Acree, E. A. Decker, M. H. Penner, D. S. Reid, S. J. Schwartz, C. F. Shoemaker, D. Smith, and P. Sporns, Eds. Hoboken, NJ, USA: John Wiley & Sons, Inc., 2001, p. F.4.2.1-4.2.6.

[107] H. Drumm and H. Mohr, "Mode of interaction between blue (UV) light photoreceptor and phytochrome in anthocyanin formation of Sorghum seedling," *Photochem. Photobiol.*, vol. 27, no. 2, pp. 241–248, Feb. 1978.

[108] ISO 10694, "Soil quality -- Determination of organic and total carbon after dry combustion (elementary analysis)," 1995. [Online]. Available: http://www.iso.org/iso/catalogue_detail.htm?csnumber=18782. [Accessed: 14-Aug-2015].

[109] ISO 13878, "Soil quality - Determination of total nitrogen content by dry combustion ('elemental analysis')," 1999. [Online]. Available: http://www.sist.si/ecommerce/catalog/project.aspx?id=557ec62e-84bd-4932-8bfb-a38cad8acf0e. [Accessed: 14-Aug-2015].

[110] ISO 10390, "Soil quality -- Determination of pH," 2006. [Online]. Available: http://www.sist.si/ecommerce/catalog/project.aspx?id=89661280-0784-40a2-8412-81e4b13cefb4. [Accessed: 14-Aug-2015].

[111] "ONORM L 1087 - Determination of 'plant-available' phosphorus and potassium by the calcium-acetate-lactate (CAL)-method," 2006.

[112] Soil Survey Laboratory Staff, "Soil survey laboratory methods manual," vol. 2.0, no. 42, 1992.

[113] "National Nuclear Data Center, Brookhaven National Laboratory, based on ENSDF and the Nuclear Wallet Cards." [Online]. Available: http://www.nndc.bnl.gov/chart/chartNuc.jsp. [Accessed: 02-Aug-2016].

[114] J. Lehto and X. Hou, *Chemistry and analysis of radionuclides laboratory*

techniques and methodology. Wiley-VCH, 2010.

[115] IAEA, *Radiation protection and safety of radiation sources international basic safety standards, general safety requirements*. Viena: IAEA (International Atomic Energy Agency), 2014.

[116] EFSA, "The EFSA (European Food Safety Authority) Comprehensive European Food Consumption Database: Chronic food consumption statistics in grams per day per kilogram of body weight, All subjects," 2015. [Online]. Available: http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb. [Accessed: 14-Oct-2015].

[117] WHO, *Guidelines for drinking-water quality*, 4th ed. World Health Organization, 2011.

[118] G. Jia, G. Torri, and P. Innocenzi, "An improved method for the determination of uranium isotopes in environmental samples by alpha-spectrometry," *J. Radioanal. Nucl. Chem.*, vol. 262, no. 2, pp. 433–441, 2004.

[119] A. Milliard, M. Durand-Jézéquel, and D. Larivière, "Sequential automated fusion/extraction chromatography methodology for the dissolution of uranium in environmental samples for mass spectrometric determination.," *Anal. Chim. Acta*, vol. 684, no. 1–2, pp. 31–37, Jan. 2011.

[120] P. Motabar, K. G. W. Inn, J. Davis, and J. LaRosa, "Identification and characterization of resistate minerals containing uranium and thorium," *J. Radioanal. Nucl. Chem.*, vol. 282, no. 2, pp. 335–341, Sep. 2009.

[121] L. K. Selvig, K. G. W. Inn, I. M. J. Outola, H. Kurosaki, and K. a. Lee, "Dissolution of resistate minerals containing uranium and thorium: Environmental implications," *J. Radioanal. Nucl. Chem.*, vol. 263, no. 2, pp. 341–348, Jan. 2005.

[122] P. Planinšek, B. Smodiš, and L. Benedik, "Simultaneous determination and uptake assessment of selected radionuclides in plants grown in substrate contaminated with U-mill tailings," *J. Radioanal. Nucl. Chem.*, vol. 309, no. 1, pp. 351–365, 2016.

[123] H. Shahandeh and L. R. Hossner, "Role of Soil Properties in Phytoaccumulation of Uranium," *Water. Air. Soil Pollut.*, vol. 141, no. 1–4, pp. 165–180.

[124] P. Schleppi, L. Tobler, J. B. Bucher, and A. Wyttenbach, "Multivariate interpretation of the foliar chemical composition of Norway spruce (Picea abies)," *Plant Soil*, vol. 219, no. 1–2, pp. 251–262.

[125] P. Blanco Rodríguez, F. Vera Tomé, and J. C. Lozano, "About the assumption of linearity in soil-to-plant transfer factors for uranium and thorium isotopes and 226Ra.," *Sci. Total Environ.*, vol. 284, no. 1–3, pp. 167–75, Feb. 2002.

[126] P. M. Markose, I. S. Bhat, and K. C. Pillai, "Some characteristics of 226Ra transfer from soil and uranium mill tailings to plants," *J. Environ. Radioact.*, vol.

21, pp. 131–142, 1993.

[127] P. J. White and M. R. Broadley, "Calcium in plants.," *Ann. Bot.*, vol. 92, no. 4, pp. 487–511, Oct. 2003.

[128] C. Hermans, J. Chen, and N. Verbruggen, "Magnesium in Plants," in *Encyclopedia of Metalloproteins*, R. H. Kretsinger, V. N. Uversky, and E. A. Permyakov, Eds. New York, NY: Springer, 2013, pp. 1269–1276.

[129] V. A. Pulhani, S. Dafauti, A. G. Hegde, R. M. Sharma, and U. C. Mishra, "Uptake and distribution of natural radioactivity in wheat plants from soil," *Environment*, vol. 79, pp. 331–346, 2005.

[130] P. Medley, A. Bollhöfer, D. Parry, and P. Martin, "Radium concentration factors in passionfruit (Passiflora foetida) from the Alligator Rivers Region, Northern Territory, Australia.," *J. Environ. Radioact.*, vol. 126, pp. 137–46, 2013.

[131] M. M. Abreu, J. Lopes, E. S. Santos, and M. C. F. Magalhães, "Ecotoxicity evaluation of an amended soil contaminated with uranium and radium using sensitive plants," *J. Geochemical Explor.*, vol. 142, pp. 112–121, Jul. 2014.

[132] M. C. Dias, C. Monteiro, J. Moutinho-Pereira, C. Correia, B. Gonçalves, and C. Santos, "Cadmium toxicity affects photosynthesis and plant growth at different levels," *Acta Physiol. Plant.*, vol. 35, no. 4, pp. 1281–1289, Dec. 2012.

[133] P. Juneau and R. Popovic, "Evidence for the Rapid Phytotoxicity and Environmental Stress Evaluation Using the PAM Fluorometric Method: Importance and Future Application," *Ecotoxicology*, vol. 8, no. 6, pp. 449–455, 1999.

[134] G. Kaur, H. P. Singh, D. R. Batish, and R. K. Kohli, "Growth, photosynthetic activity and oxidative stress in wheat (Triticum aestivum) after exposure of lead to soil," *J. Environ. Biol.*, vol. 33, no. 2, pp. 265–269, 2012.

[135] N. Vanhoudt, N. Horemans, G. Biermans, E. Saenen, J. Wannijn, R. Nauts, M. Van Hees, and H. Vandenhove, "Uranium affects photosynthetic parameters in Arabidopsis thaliana," *Environ. Exp. Bot.*, vol. 97, pp. 22–29, Jan. 2014.

[136] B. Hawrylak-Nowak, "Changes in Anthocyanin Content as Indicator of Maize Sensitivity to Selenium," *J. Plant Nutr.*, vol. 31, no. 7, pp. 1232–1242, 2008.

[137] A. Aggarwal, I. Sharma, B. N. Tripathi, A. Munjal, M. Baunthiyal, and V. Sharma, "Metal toxicity and Photosynthesis," in *Photosynthesis: Overviews on Recent Progress & Future Perspective*, First., no. October, S. Itoh, P. Mohanty, and K. N. Guruprasad, Eds. IK International Publishing House. New Delhi, 2012, pp. 229–236.

[138] (ENSREG) European Nuclear Safety Regulators Group, "Basic safety standards directive." [Online]. Available: http://www.ensreg.eu/nuclear-safety-regulation/eu-instruments/Basic-Safety-Standards-Directive. [Accessed: 27-Oct-2016].

[139] IAEA (International Atomic Energy Agency), "Publications on Exposure to Radiation from Natural Sources." [Online]. Available: http://www-ns.iaea.org/publications/norm-publications.asp. [Accessed: 27-Oct-2016].

[140] ATSDR (Agency for Toxic Substances and Disease Registry), "Toxicological Profile for Uranium," *Toxicological profiles*, 2013. [Online]. Available: https://www.atsdr.cdc.gov/toxprofiles/TP.asp?id=440&tid=77. [Accessed: 27-Oct-2016].

[141] S. J. Al-Kharouf, I. F. Al-Hamarneh, and M. Dababneh, "Natural radioactivity, dose assessment and uranium uptake by agricultural crops at Khan Al-Zabeeb, Jordan.," *J. Environ. Radioact.*, vol. 99, no. 7, pp. 1192–9, Jul. 2008.

[142] G. Shanthi, J. T. T. Kumaran, G. A. G. Raj, and C. G. Maniyan, "Natural radionuclides in the South Indian foods and their annual dose," *Nucl. Instruments Methods Phys. Res. Sect. A Accel. Spectrometers, Detect. Assoc. Equip.*, vol. 619, no. 1–3, pp. 436–440, 2010.

[143] F. P. Carvalho, J. M. Oliveira, and M. Malta, "Intake of Radionuclides with the Diet in Uranium Mining Areas," *Procedia Earth Planet. Sci.*, vol. 8, pp. 43–47, 2014.

Bibliography

Publications Related to the Thesis

Journal Articles

P. Planinšek, B. Smodiš, L. Benedik, "Simultaneous determination and uptake assessment of selected radionuclides in plants grown in substrate contaminated with U–mill tailings", Journal of Radioanalytical and Nuclear Chemistry, vol 309, pp 351–365, 2016.

S. Jurečič, L. Benedik, P. Planinšek, M. Nečemer, P. Kump, B. Pihlar, "Analysis of uranium in the insoluble residues after decomposition of soil samples by various techniques", Applied Radiation and Isotopes, vol 87, pp 61–65, 2014.

P. Planinšek, L. Benedik, B. Smodiš, "Comparison of various dissolution techniques for determination of Po-210 in biological samples", Applied Radiation and Isotopes, vol 81, pp 53–56, 2013.

Other Publications (optional)

Journal Articles

I. Matveyeva, R. Jaćimović, P. Planinšek, B. Smodiš, M. Burkitbayev, "Uptake of uranium, thorium and radium isotopes by plants growing in dam impoundment Tasotkel and the Lower Shu region (Kazakhstan)",. Radiochimica Acta, vol 104, pp 51–57, 2016.

U. Kristan, P. Planinšek, L. Benedik, I. Falnoga, V. Stibilj, "Polonium-210 and selenium in tissues and tissue extracts of the mussel Mytilus galloprovincialis (Gulf of Trieste)", Chemosphere, vol 119, pp 231–341, 2015.

M. Rožmarić, M. Rogić, L. Benedik, D. Barišić, P. Planinšek, "Radiological characterization of tap waters in Croatia and the age dependent dose assessment", Chemosphere, vol 111, pp 272–277, 2014.

I. Matveyeva, R. Jaćimović, P. Planinšek, P. Stegnar, B. Smodiš, M. Burkitbayev, "Assessment of the main natural radionuclides, minor and trace elements in soils and sediments of the Shu valley (near the border of Kazakhstan and Kyrgyzstan", Journal of radioanalytical and nuclear chemistry, vol 299, pp 1399–1409, 2014.

B. Smodiš, M. Štrok, M. Černe, P. Planinšek, L. Benedik, "Radioanalytical techniques for the determination of [sup](238)U, [sup](226)Ra and [sup](210)Pb in the environment",.Radiochimica acta, vol 101, pp 519–524, 2013.

A. Miklavčič Višnjevec, P. Cuderman, D. Mazej, J. Snoj Tratnik, M. Krsnik, P. Planinšek, J. Osredkar, M. Horvat, "Biomarkers of low-level mercury exposure through fish consumption in pregnant and lactating Slovenian women", Environmental research, vol 111, pp 1201–1207, 2011.

Conference Paper

M. Rogić, M. Rožmarič Maćefat, D. Barišić, L. Benedik, P. Planinšek, "Koncentracije aktivnosti ^{210}Po I ^{210}Pb u dagnjama (Mytilus galloprovincialis) te procjena ukupne godišnje efektivne doze za priobalno stanovništvo Republike Hrvatske", In: Ž. Knežević (ed.), M. Majer (ed.), I. Krajcar Bronić (ed.). Zbornik radova 9. simpozija Hrvatskog društva za zaščitu od zračenja = Proceedings of the 9th Symposium of the Croatian Radiation Protection Association : HDZZ - CRPA : Zagreb 2013. Zagreb: Hrvatsko društvo za zaščitu od zračenja, 2013, str. 229-234.

A. Miklavčič Višnjevec, D. Mazej, J. Snoj Tratnik, A. B. Kobal, M. Krsnik, P. Planinšek, J. Osredkar, M. Horvat, "Mercury, selenium and arsenic exposure assessment trough fish consumption", In: Program and proceedings, 13th International Neutrotoxicology Association Meeting & 11th International Symposium on Neurobehavioral Methods and Effects in Occupational and Environmental Health, June 5-10, 2011, Xi'an. 2011, 168-169.

A. Miklavčič Višnjevec, D. Mazej, J. Snoj Tratnik, A. B. Kobal, M. Krsnik, P. Planinšek, J. OSredkar, M. Horvat, "Relation of fish consumption to mercury, selenium and arsenic levels in cord blood and breast milk from Slovenian pregnant women", In: TRACEL 2011, 3rd International Symposium on Trace Elements & Health, TRACEL 2011, Murcia, 24-27 May, 2011. Murcia, 2011, str. 75-76.

M. Štrok, P. Planinšek, B. Smodiš, "Content of natural radionuclides in sediments in the vicinicy of a former uranium mine", In: I. Krajcar Bronić (ed.). Zbornik radova VIII. simpozija Hrvatskog društva za zaščitu od zračenja = Proceedings of the 8th Symposium of the Croatian Radiation Protection Association : HDZZ - CRPA : Zagreb 2011. Zagreb: Hrvatsko društvo za zaščitu od zračenja, 2011, str. 431-436.

A. Miklavčič Višnjevec, P. Cuderman, D. Mazej, J. Snoj Tratnik, A. B. Kobal, M. Krsnik, P. Planinšek, J. Osredkar, M. Horvat, "Biomarkers of low-level mercury exposure in pregnant and lactating Slovenian women", In: Ž. Bargańska (ed.). Proceedings of the 15th International Conference on Heavy Metals in the Environmental, September 19-23, 2010, Gdańsk, Poland. Gdańsk: Chemical Faculty, Gdańsk University of Technology, 2010, str. 881-885.

Conference Paper – Abstract

P. Planinšek, B. Smodiš, L. Benedik, "Transfer of Th-230 from soil contaminated with U-mill tailing to radish, savoy and rocket", In: Book of abstracts, 24th International Conference Nuclear Energy for New Europe, NENE 2015, Portorož, September 14-17, 2015. Ljubljana: Nuclear Society of Slovenia, 2015, str. 59.

B. Smodiš, P. Planinšek, L. Benedik, "Determination of thorium radioisotopes in botanical materials", In: Program and abstracts, 14th International Conference on Modern Trends in Activation Analysis, Delft, August 23-28, 2015, 11th International Conference on Nuclear Analytical Methods in Life Sciences, Delft, August 23-28, 2015. Delft: Reactor Institute Delft, 2015, str. 195.ž

P. Planinšek, U. Kristan, B. Smodiš, L. Benedik, V. Stibilj, "Distribution of polonium-210 and selenium in marine mussel Mytilus Galloprovincialis", In: D. Lisjak (ed.), P. Dušak (ed.), S. Kralj (ed.). [Program and abstract book], 7th Young Researchers' Day, 19 February, 2013, Ljubljana, Slovenia. Ljubljana: Institut Jožef Stefan, 2013, str. 53.

P. Planinšek, B. Smodiš, L. Benedik, "^{210}Po and ^{210}Pb levels in mussels and fish from Slovenian market and the related dose assessment", In: N. Karunakara (ed.), M. Baskaran (ed.). Proceedings, 2nd International conference on Po and radioactive Pb

isotopes. February 10-13, 2013, Mangalore. Mangalore: Mangalore University, 2013, str. 130.

P. Planinšek, U. Kristan, B. Smodiš, L. Benedik, V. Stibilj, "^{210}Po and Se speciation in Mytilus galloprovincialis", In: N. Karunakara (ed.), M. Baskaran (ed.). Proceedings, f2nd International conference on Po and radioactive Pb isotopes. February 10-13, 2013, Mangalore. Mangalore: Mangalore University, 2013, str. 247.

P. Planinšek, L. Benedik, B. Smodiš, "Digestion procedures for determination of polonium-210 in biological samples", In: R. Žabar (ed.). [Book of abstracts], YISAC, Young Investigators' Seminar on Analytical Chemistry, June 27th - June 30st, 2012, Nova Gorica. Nova Gorica: University, 2012, str. 18.

P. Planinšek, L. Benedik, B. Smodiš, "Determination of natural radionuclides in soil samples from a contaminated site with emphasis on digestion procedures". In: T. Žagar (ed.), S. Fürst (ed.). Proceedings, 21st International Conference Nuclear Energy for New Europe, Ljubljana 2012, September 5-7. Ljubljana: Nuclear Society of Slovenia, 2012, str. 61.

P. Planinšek, B. Smodiš, L. Benedik, M. Štrok, "Procedures for determination of natural radionuclides in soil with emphasis on efficiency of dissolution", In: Proceedings, EU-NORM I International Symposium, 5-8 June 2012, Tallin. Tallin, 2012: Environmental Board, str. 40.

J. Snoj Tratnik, I. Mrak, I. Begić, A. Miklavčič Višnjevec, P. Planinšek, P. Cuderman, M. Krsnik, F. Barbone, M. Mariuz, K. Sofianou, Z. Špirić, M. Horvat, "Suitability of biomarkers of low-to-medium mercury exposure in pregnant women", In: 10th International Conference on Mercury as a Global Pollutant : Abstract Volume : July 24-29, 2011, Halifax, Nova Scotia, Canada. Halifax: ICMGP, 2011, str. 315.

P. Planišek, B. Smodiš, L. Benedik, M. Štrok, "Natural radionuclides in soil samples", In: V. Guzsvány (ed.), Z. Papp (ed.). Book of abstracts, 18th Young Investigators´ Seminar on Analytical Chemistry, YISAC 2011, Novi Sad, June 28th-July 1st, 2011. Novi Sad: University of Novi Sad, 2011, str. 65.

A. Miklavčič Višnjevec, P. Cuderman, D. Mazej, J. Snoj Tratnik, A. B. Kobal, M. Krsnik, P. Planinšek, J. Osredkar, M. Horvat, "Comparison of biomarkers of low-level mercury exposure", In: Proceedings : Resetting our priorities, Environmental Health 2011, Resetting our Priorities, 6-9 February 2011, Salvador. Salvador: Elsevier, 2011.

A. Miklavčič Višnjevec, P. Cuderman, D. Mazej, J. Snoj Tratnik, A. B. Kobal, M. Krsnik, P. Planinšek, J. Osredkar, M. Horvat, "Biološki kazalci izpostavljenosti nizkim koncentracijam živega srebra preko uživanja rib", In: I. Pribošič (ed.), K. Krnel (ed.). [Program in povzetki], 5. dan mladih raziskovalcev, Ljubljana, Februar 2011. Ljubljana: Institut "Jožef Stefan", 2011.

P. Planinšek, B. Smodiš, L. Benedik, M. Štrok, "Določanje ^{210}Po v okoljskih vzorcih", In: Z. Kravanja (ed.), D. Brodnjak–Vončina (ed.), M. Bogataj (ed.). Zbornik povzetkov referatov s posvetovanja, Slovenski kemijski dnevi 2011, Portorož, 14.-16. september 2011. Maribor: FKKT, 2011, str. 234.

A. Miklavčič Višnjevec, D. Mazej, J. Snoj Tratnik, A. B. Kobal,.M. Krsnik, P. Planinšek, J. Osredkar, M. Horvat, "Biomarkers of low-level mercury exposure", In: Book of abstracts, 8th International Symposium on Biological Monitoring in Occupational and Environmental Health, Helsinki, 6-8 September 2010. Helsinki: Finish Institute of Occupational Health, 2010.

Independent scientific component part or a chapter in a monograph

Il. Matveyeva, S. Nazarkulova, B. Satybaldiev, B. M. Uralbekov, P. Planinšek, R. Jaćimović, B. Smodiš, M. Burkitbayev, "Natural radionuclides in a peat core from

the Kamyshanovskoe uranium deposit in Kyrgyzstan", In: M. Burkitbayev (ed.), J. Lehto (ed.). Environmental radioactivity in central Asia. Almaty: Kazakh National University, 2012, str. 123-127.

Other educational material

L. Benedik, V. Stibilj, B. Smodiš, P. Planinšek, "Training in radiochemistry and radioactivity measurements for practitioners from countries eligible under the JRC enlargement&integration policy", Training manual, Reactor center, 11-22 junij, 2012, Ljubljana. Ljubljana: Reaktorski center, 2012.

L. Benedik, B. Smodiš, P. Planinšek, "Training in radiochemistry and radioactivity measurements for practitioners from countries eligible under the JRC enlargement&integration policy", Training manual, Reactor center, 15-26 October, 2012, Ljubljana. Ljubljana: Reaktorski center, 2012.

L. Benedik, B. Smodiš, P. Planinšek, V. Stibilj, "Training in radiochemistry and radioactivity measurements for practitioners from countries eligible under the JRC enlargement&integration policy", Training manual, Reactor Center, 16 - 27april, 2012. Ljubljana: Reaktorski center, 2012.

Treatise, preliminary study, study

P. Planinšek, Z. Jeran, B. Smodiš, "Rezultati nadzora radioaktivnosti okolja Rudnika urana Žirovski vrh v letu 2011", (IJS delovno poročilo, 10939). 2012.

M. Štrok, P. Planinšek, L. Benedik, Z. Jeran, B. Smodiš, G. Omahen, "Rezultati meritev radioaktivnosti po zaključku del končne ureditve odlagališča Boršt v letu 2010", (IJS delovno poročilo, 10681). 2011.

M. Štrok, P. Planinšek, L. Benedik, Z. Jeran, B. Smodiš, G. Omahen, "Rezultati nadzora radioaktivnosti okolja Rudnika urana Žirovski vrh v letu 2010", (IJS delovno poročilo, 10680). 2011.

M. Horvat, P. Planinšek, V. Fajon, J. Snoj Tratnik, A. Miklavčič Višnjevec, D. Mazej, "Vsebnost živega srebra v krvi, laseh, urinu in materinem mleku pri dojecih materah na območju Ljubljane", (IJS delovno poročilo, 10589). 2010.

Biography

Petra Planinšek is a senior adviser at the Slovenian Nuclear Safety Administration, Radiation Safety and Materials Division. She is a graduate of University of Nova Gorica (Slovenia), where she received a bachelor of science in ecology degree.

She finished her BSc study with diploma thesis titled "*Mercury content in blood, urine and breast milk samples of lactating mothers from Ljubljana*". During the last two years of her BSc study she was a Jožef Stefan Institute (JSI) scholarship holder as well as worked via a student employment service at the Department of Environmental Sciences at the JSI as a student expert in the analytical laboratory. After graduation, she launched her career at the Jožef Stefan Institute (Ljubljana, Slovenia), where she began her specialization in radioecology. She was a young researcher at the Department of Environmental Sciences for five years as well as a postgraduate doctoral student of Ecotechnology at the Jožef Stefan International Postgraduate School. During the young researcher position, she published nine original scientific articles as a first author or co-author and participated at twelve international conferences, workshops and courses all over the world (Estonia, Albania, India, Austria, Serbia and Slovenia). In the time of her specialisation in radioecology, she obtained eexpertise in radioanalytical separation techniques, alpha particle spectrometry, beta counting, gamma-ray spectrometry, neutron activation analyses. She will finish her PhD study with the defence of her doctoral dissertation with the proposed tile "*Plant uptake of selected natural radionuclides from contaminated environment*".