Journal of Environmental Radioactivity 231 (2021) 106554

Contents lists available at ScienceDirect

Journal of Environmental Radioactivity

journal homepage: http://www.elsevier.com/locate/jenvrad

Transfer and bioaccumulation of ^{210}Po from soil to water spinach (*Ipomoea aquatica Forrsk.*) in Vietnam

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ARTICLE INFO

Keywords:
Transfer factor
Activity concentration
Spinach
North vietnam

ABSTRACT

The water spinach (*Ipomoea aquatica Forrsk.*) is a common vegetable and a part of the staple diet in Vietnam. It has a well-known tendency for the high absorption of lead, including the radioactive isotope ^{210}Pb. ^{210} Pb day to ^{210}Po, which is one of the most toxic radioactive isotopes when ingested. Currently, there are few data available on the activity concentration or transfer parameters of water spinach grown in Vietnam. To provide this data, in this study, the ^{210}Po activity concentrations in the soil, water and the roots, stems, and leaves of two types of spinach (grown in soil flooded with water and grown in normal soil) in Hanoi, Vietnam were determined by alpha spectrometry. The order of activity concentrations for water spinach grown in unflooded was $C_{stem} < C_{leaf} < C_{root}$, while for flooded $C_{stem} \approx C_{root} < C_{leaf}$. The transfer factors followed a similar pattern.

1. Introduction

Polonium-210 (^{210}Po) primarily comes from the ^{238}U decay series and is one of the most toxic substances on earth. It has a comparatively short half-life of 138.4 days. Additionally, the ^{210}Po significantly contributes to the natural radiation dose to the population via food ingestion (Al-Masri et al., 2019; Musthafa et al., 2019; Guy et al., 2020; Van et al., 2020). Thus, the ^{210}Po activity concentration in various foodstuff, plants, and vegetables has attracted attention from researchers around the world and has been widely investigated (Kannan et al., 2001; Kovács et al., 2014; Kubalek et al., 2016; Iwaoka et al., 2019). In vegetables, the ^{210}Po activity concentration in different types of vegetables in Kalpakkam (India) was found to be less than or equal to 10–653 mBq/kg. In general, the activity concentration of ^{210}Po in leafy vegetables (28–653 mBq/kg) was higher than that in roots and other types of vegetables (≤10–180 mBq/kg) (Kannan et al., 2001). The research results of Ekdal et al. (2006) indicated that the activity concentration of ^{210}Po in different vegetables in Turkey varied from 150 to 9400 mBq/kg (Ekdal et al., 2006). Giri et al. (2010) also found that the ^{210}Po activity concentration in vegetables in Jharkhand, India ranged from <200 mBq/kg to 35600 mBq/kg (Giri et al., 2010). While leafy vegetables are known for absorbing ^{210}Pb and ^{210}Po from atmospheric fallout. Generally, seafood (19000–33000 mBq/kg fresh) is considered one of the main contributors of ^{210}Po in the human diet (more than 80%), however other foods, such as liver and cereal can reach considerable activity concentrations (Persson and Holm, 2011).

Ipomoea aquatica Forrsk. is known by many names, including water spinach, river spinach, water morning glory, water convolvulus, Chinese spinach, Chinese watercress, Chinese convolvulus and swamp cabbage in English and Kalmi shak, Thooti Koora, Dagoeblad or dagublad, Hayoyo, Kalmi saag, Kolmou xak, Trokuon, Gazun, Rau muong, Kongxincal, Vallal, Èng-chhài, Kangkong, Hokkien, Phak bung and Ong choy in various other languages. It is often referred to colloquially simply as spinach, which might cause some misunderstanding. It is a different species from *Spinacia oleracea*, which is commonly thought of if spinach is mentioned in English.

Water spinach can be cultivated in different ways, free-floating in lakes and ponds, or rooted in wet or moist soils. It can be grown in conditions similar to rice paddies, in fields, raised beds and pots as well. With optimal conditions this plant's growth could be several cm.day^{-1} and annual yields up to ~90 tons fresh weight (f.w.) ha^{-1} (Rubatzky and Yamaguchi, 1997). This vegetable is widely grown and consumed in Vietnam and several other tropical and subtropical countries in east, south, and Southeast Asia, especially in the rural areas. It is also cultivated in many countries, including the USA, Australia, and various countries in Africa. However, in some areas, it might be also considered

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https://doi.org/10.1016/j.jenvrad.2021.106554
Received 19 October 2020; Received in revised form 5 February 2021; Accepted 7 February 2021
Available online 22 February 2021

an invasive species and as a weed (Austin, 2007).

The water spinach is an important staple food, it has a high nutritional value, it is rich in vitamin A, and it is the second most commonly eaten food after rice in Vietnam (Rubatzky and Yamaguchi, 1997; Duc et al., 1999; Marcussen et al., 2008). The upper part of the stem with the foliage is bundled and sold for human consumption, while the rest of the plant is fed to livestock. A recent paper in Vietnamese by Vu and Dinh (2015) on the metal accumulation in water spinach reported that the Pb in water spinach can reach high concentrations, up to 16.2 mg/kg dry wt. Accordingly, the ^{210}Pb activity concentration could be high, and ^{210}Pb day to ^{210}Po, which is one of the most toxic radioactive isotopes when ingested (UNSCEAR 2000). Currently, there are few data available on the activity concentration or transfer parameters of water spinach grown in Vietnam. In Hanoi, Vietnam, the local people grow water spinach in some areas which can be polluted by household waste or industrial waste. Figuié (2003) carried out a survey and reported that the Vietnamese are aware and concerned about the potential risk due to residues of chemical contaminants in food and about ~89% of the interviewers perceived vegetables as the most unsafe food category and ~47% of the interviewers specified that water spinach is the most unsafe commodity.

In this study, the ^{210}Po activity concentration and its transfer factor (TF) from soil to two types of water spinach (grown in soil flooded with water (flooded spinach) and in normal soil (unflooded spinach) in different locations in Hanoi, Vietnam were determined.

2. Materials and methods

2.1. Study area

In this study, different locations of growing spinach in Hanoi (Capital of Vietnam), including Hoai Duc (HD), Hoang Mai (HM), Thanh Tri (TT), Dong Anh (DA), and Bac Tu Liem (BTL) were chosen for the investigation of ^{210}Po in water spinach. In each location, two types of water spinach, namely spinach growing in soil flooded with water (flooded spinach) (HD1, HM1, TT1, DA1, BLT1) and spinach growing in normal soil (common vegetables) (unflooded spinach) (HD2, HM2, TT2, DA2, BLT2) were collected for this investigation. The study locations are shown in Fig. 1.

2.2. Sampling

For the flooded fields, 5-L water samples were taken at three points of an equilateral triangle of 1 m in dimension (a few mL of 5 M HCl was added to the samples to avoid precipitation). The water spinach plants (later separated into the root, stem, and leaf) and soil were collected from the same points as the water samples. For the soil-grown variety, the same pattern was used. At the sampling points, the spinach and soil were harvested in 15 cm^2 square and from 0 to 10 cm depth from the surface, which corresponds to the space the spinach roots can reach. All of the studied samples were coordinated by GPS, the sampling locations are shown in Table 1. All samples were processed within one week from collection.

2.3. Water spinach samples

The water spinach plants were separated into roots, stems, and leaves. The root, stem, leaf spinach samples were washed to remove any traces of aerosol, soil, and surface contamination. Then, the samples were chopped into small pieces by a knife and dried in an oven at a temperature of 80 °C to a constant dry weight. The obtained samples were then milled into powder by a mortar and pestle under 0.02 mm mesh sieve in size and 0.5 g of each powdered sample form was used for analysis. The powdered samples were wetted by distilled water, then 50 mBq of ^{209}Po tracer solution (polonium nitrate in 1 M HNO_3, alpha emission at 4.88 MeV) was added. The samples were wet-digested using a mixed acid solution with a ratio of 1:3 of HNO_3: HCl, then H_2O_2 was added to complete the digestion. For the water and spinach samples, the Po solution was taken to co-precipitation with MnO_2, performed by adding a solution of $MnCl_2$ with $KMnO_4$ in a solution of NH_4OH at pH > 9 (Skwarzec, 1997). The obtained precipitate was washed with distilled water to reach pH ~7. Then it was dissolved by HCl 9 M, and the solution was passed through a column of Dowex 1 × 8 (100–200 mesh) anion-exchange resin for the separation of uranium contamination (Pacer, 1983). After separation the sample was evaporated to dryness at a temperature less than 80 °C, then re-dissolved in 0.5 M HCl acid solution before catalytic chemicals were added. ^{210}Po was spontaneously deposited on a polished silver disk from the solution for ~4 h at a temperature of ~80 °C. Quality control was performed using 0.5 g IAEA-446 (Baltic Sea Seaweed) reference material and the recovery rate of ^{209}Po tracer was up to 90%.

2.4. Soil samples

Soil samples were also dried at 80 °C in an oven to a constant weight. Then the samples were milled into powder under 0.02 mm mesh sieve in size. The preparation procedure for soil samples followed Le et al. (2019). To 0.5 g of the soil samples, 50 mBq of ^{209}Po tracer was added, then they were digested with 10 ml of HF acid (39%) in a microwave with two steps: (i) the temperature was raised to 200 °C for about 20min; (ii) then stabilized at this temperature for 120min. The obtained sample was left cooling at room temperature, then the remaining HF acid solution in the samples was dried at 50 °C. In the next step, 10 ml of HNO_3 acid (65%) was added to each sample and they were heated again with the same microwave cycle and evaporated to dryness at 50 °C. The

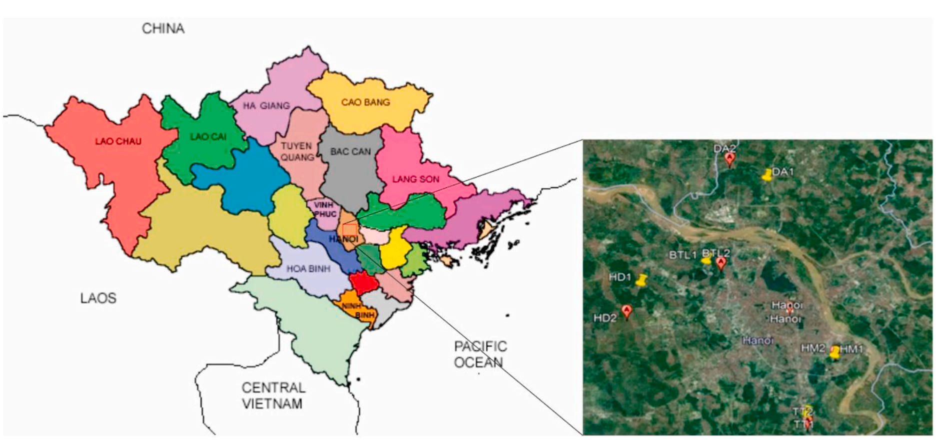

Fig. 1. The study locations.

Table 1
^{210}Po activity concentration in water spinach and associated environmental media under flooded and unflooded growing conditions.

	Leaf		Stem		Root		Soil		Water
	UnFl	Fl	UnFl	Fl	UnFl	Fl	UnFl	Fl	Fl
Mean water content [%]	90	88	89	91	65	58	–	–	–
AM±SD [Bq/kg_{dry}]	32.3 ±13.3	37.3 ±9.9	19.5 ±4.2	21.1 ±8.1	65.5 ±35.8	22.7 ±13.1	69.8 ±42.3	69.5 ±11.2	1.23 ±0.31
GM (GSD) [Bq/kg_{dry}]	29.6 (1.56)	36.0 (1.34)	19.0 (1.59)	19.3 (1.59)	58.1 (1.64)	18.7 (1.98)	60.4 (1.71)	68.7 (1.18)	1.19 (1.32)
Median [Bq/kg_{dry}]	28.7	39.1	19.6	26.3	54.8	20.0	52.4	73.4	1.37

AM: Arithmetic mean, SD: Standard deviation, GM: Geometric mean, GSD: Geometric standard deviation, UnFl: Unflooded, Fl: Flooded.

samples then were dissolved with HCl then the same procedure as the water and spinach samples was followed (as it is written above). Quality control was performed using 0.5 g of each sample of reference material IAEA-384 (Fangataufa Sediment) and the recovery rates of the ^{209}Po tracer were up to 85%.

All of the obtained silver disks (water, spinach, and soil samples) were left to dry at room temperature and measured using a high-resolution PIPS detectors ORTEC Alpha-Ensemble-4 spectrometer with AlphaVision software and a minimum detection limit of 0.5 mBq. The counting period was adjusted to obtain a relative standard error of approximately 5% (measurement time about 36–72 h). The ^{210}Po activity calculations were adjusted for the background (blank sample) and also for the collection date.

2.5. Methods

a. Soil to plant transfer factor

The transfer factor (TF) is commonly used to evaluate the transportation of radionuclides from soil to plant in general and in vegetable crops in particular (IAEA 2010; Azeez et al., 2019; Cengiz, 2019; Van et al., 2020). It is defined as the ratio of the activity concentration in the dry plant to that in the dry soil and is calculated according to the following equation:

$$TF = \frac{Activity\ concentration\ of\ nuclide\ of\ interest\ per\ kg\ dry\ plant\ mass}{Activity\ concentration\ of\ that\ nuclide\ in\ dry\ soil\ within\ the\ rooting\ area} \quad (1)$$

b. *Overall transfer factor (TF_{total})*

The overall transfer factor for radionuclides from soil to water spinach can be calculated as follows (Maiti and Jaiswal, 2008; Alloway, 2012). The activity concentration in the plant is calculated by dividing the total activity in Bq ($A_{root} + A_{stem} + A_{leaf}$) and the total mass in kg ($M_{root} + M_{stem} + M_{leaf}$).

$$TF_{total} = \frac{Activity\ concentration\ in\ the\ plant\ (Activity\ in\ the\ root + stem + leaf\ divided\ by\ the\ total\ mass)}{Activity\ concentration\ in\ soil} \quad (2)$$

c. Annual effective dose assessment

The annual effective dose E (μSv/year) of radionuclides to individuals due to the consumption of water spinach is calculated based on (UNSCEAR, 2000). Anh et al. (2004) carried out a household consumption survey in the urban and periurban areas of Hanoi and showed that the consumption of water spinach was comparatively high among different types of vegetables and the average consumption of water spinach in Hanoi was 77.3 g/person per day (~27 kg/person/year) (Anh et al., 2004).

3. Results and discussions

3.1. ^{210}Po activity concentration

The activity concentration of ^{210}Po in flooded and unflooded water spinach is shown in Table 1. The distribution of the various concentrations seems to be not significantly different from normal distribution according to the Kolmogorov-Smirnov Test of Normality. The differences between the variables were tested with ANOVA, and were confirmed by the non-parametric Kruskal-Wallis test as well. Regarding the edible parts for humans, the activity concentration of ^{210}Po in spinach leaves is higher than that in spinach stem, in both flooded (F 12.02, $p < .05$) and unflooded cases (F 12.69, $p < .05$). This tendency agrees well with the previous reports of high ^{210}Po activity concentration in leafy vegetables and the leaves having greater activity concentration than other parts of vegetable (Karunakara et al., 2000; Avadhani et al., 2001; Chang et al., 2009; Persson and Holm, 2011; Meli et al., 2014). Compared to other studies, the activity concentration of ^{210}Po in water spinach leaves in both water spinach types in this study is higher than spinach grown in Kalpakkam, India which varied from 0.10 to 0.65 Bq/kg (Kannan et al., 2001) or any other vegetable mentioned in that report. In addition, the measured ^{210}Po activity concentration in water spinach in this study is also higher than the ^{210}Po activity concentration in different vegetables in Turkey and Jharkhand, India, which range from 0.15 Bq/kg to 9.5 Bq/kg; <0.2 Bq/kg to 35.6 Bq/kg, respectively (Ekdal et al., 2006; Giri et al., 2010).

Figs. 2–4 show the variation of ^{210}Po activity concentration in water spinach tissues grown in different environmental conditions. The transfer of radionuclides from soil to plant tissues depends on the environmental ecological conditions (Marciulioniene et al., 2015). According to the Kolmogorov-Smirnov Test of Normality, the distribution of the activity concentration values does not differ significantly from normal distribution, which should allow for the use of ANOVA for the evaluation of the statistical significance of the differences between group means. Non-parametric tests, such as the Kruskal-Wallis test also indicate the same results. The ^{210}Po activity concentration in the root of the unflooded spinach is lower than those of flooded spinach (the difference is statistically significant, F 18.92, $p < .05$). For the leaves and the stem, the values observed in water spinach grown under flooded conditions tended to be higher, but the difference was not statistically significant (for leaves the F was 1.38, p .25, while for the stem F was 0.48 and p .50). Activity concentration in the soil of the flooded areas also

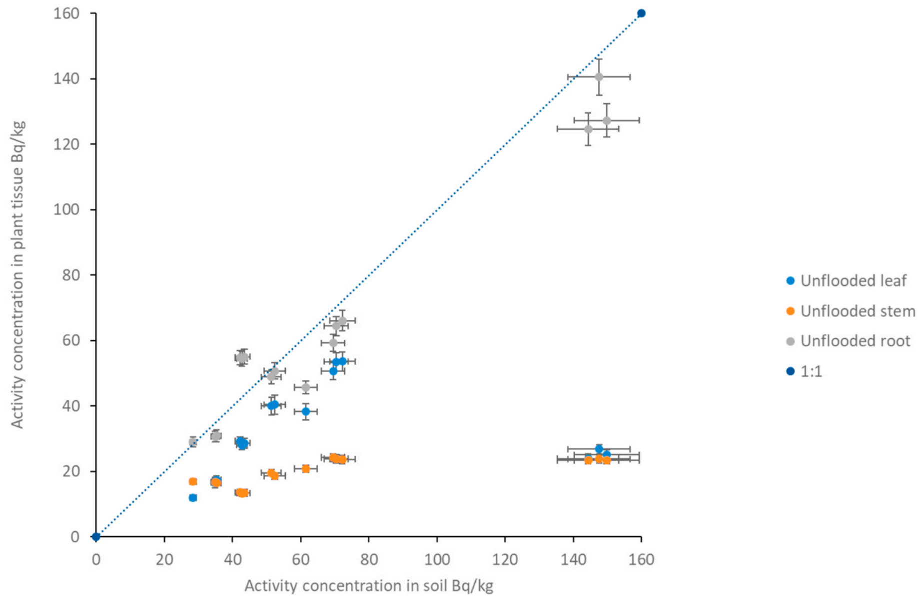

Fig. 2. Activity concentrations under unflooded growing conditions plotted against activity concentration in soil.

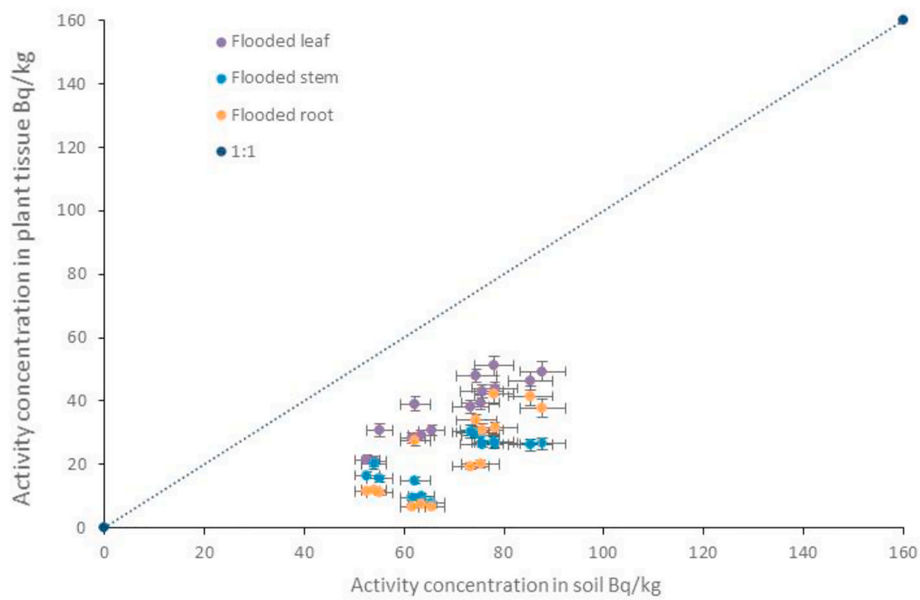

Fig. 3. Activity concentrations under flooded growing conditions plotted against activity concentration in soil.

tended to be higher, with the exception of one location, but the difference was not statistically significant ($F < 0.01$, p .98).

For the water spinach grown in unflooded soil, the order of activity concentrations is $C_{stem} < C_{leaf} < C_{root}$. The activity concentration of soil does not differ significantly from the activity concentration observed in the roots (F 0.09, p .77), but stem is significantly different from the roots (F 24.37, $p < .05$), and leaves are significantly different from the stem (F 12.69, $p < .05$). The Spearman's Rho test suggests there is a correlation between activity concentrations in the soil and activity concentrations in the root (r_s 0.89, $p < .05$) and in the stem (r_s 0.76, $p < .05$), which in turn are correlated with each other (r_s 0.57, $p < .05$). On the other hand the activity concentration in the leaves seems to be not significantly correlated to the soil (r_s 0.40, p .14), root (r_s 0.32, p .24) or stem (r_s 0.44, p .10) due to the comparatively low values observed at HD2. Without that sampling location the correlation is much improved, the correlation between leaf and stem was r_s 0.73, $p < .05$, between leaf and root r_s 0.80, $p < .05$ and between leaf and soil r_s 0.96, $p < .05$.

For the water spinach grown in unflooded soil, the order of activity concentrations is different, $C_{stem} \approx C_{root} < C_{leaf}$. Here there is no significant difference between activity concentrations in the stem and root (F 0.15, p .70), but there is a significant difference between leaf and stem (F 24.23, $p < .05$), leaf and root (F 12.02, $p < .05$), as well as soil and leaves (F 70.18, $p < .05$), stems (F 186.03, $p < .05$) and roots (111.81, $p < .05$). The Spearman's Rho test suggests there is a correlation between the activity concentrations of leaves and stems (r_s 0.63, $p < .05$), leaves and roots (r_s 0.89, $p < .05$), leaves and soil (r_s 0.88, $p < .05$), stems and roots (r_s 0.68, $p < .05$), stems and soil (r_s 0.54, $p < .05$)and root and soil (r_s 0.78, $p < .05$) as well.

For the correlations involving the activity concentration in water, the Spearman's Rho test indicated that there is a correlation between the activity concentration in water and the activity concentration in soil (r_s 0.59, $p < .05$), as well as water and leaf (r_s 0.57, $p < .05$), but there is

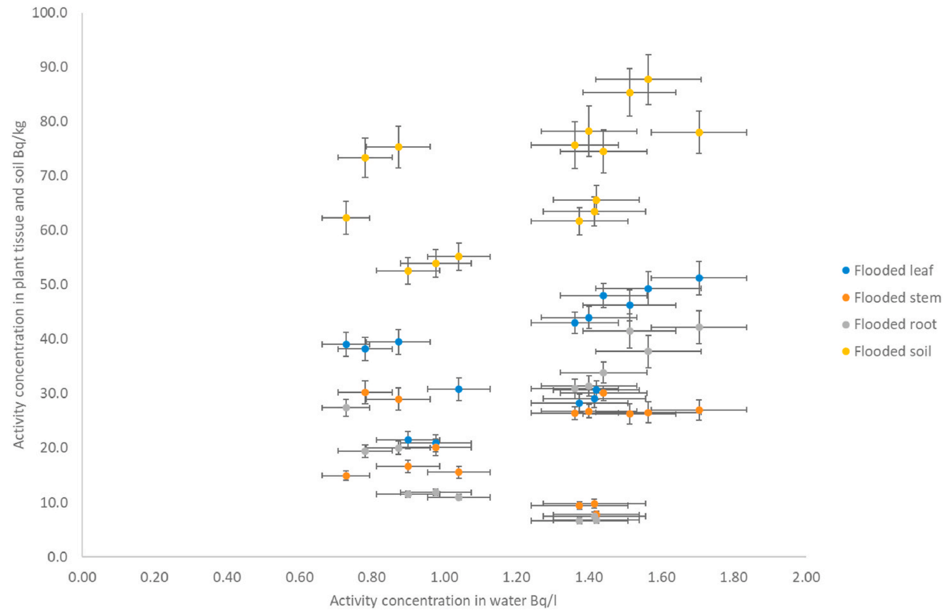

Fig. 4. Activity concentrations under flooded growing conditions plotted against activity concentration in water.

little correlation between water and root (r_s 0.44, p .10), and no correlation between water and stem (r_s 0.05, p .87).

It should be noted that the uptake pathways for polonium into plants include uptake through the root system and atmospheric fallout, which might be absorbed on the surface of the leaf and in some cases other above-ground parts as well, for example for parsley and mint it was reported that over 75% of the polonium uptake came via the atmosphere, and less than 25% through the roots (Al-Masri et al., 2010; Giri et al., 2010) The atmospheric route was deemed the major one for tobacco as well (Karunakara et al., 2000). However, there are some contrary findings (Tso and Fisenne 1968). For water spinach the specimens collected from the flooded fields had higher activity concentrations and transfer factors in the root than their counterparts grown in unflooded fields, indicating that the flooded condition might be beneficial for root uptake.

3.2. Transfer factor (TF) and overall transfer factor (TF_{whole})

Based on the activity concentration of ^{210}Po measured in different water spinach tissues and soil, the transfer factors (TFs) for ^{210}Po from soil to the leaf, stem, and root of water spinach were calculated and their variation in the different study locations are plotted in Fig. 5. In general, the calculated transfer factors presented in Fig. 5 are higher than those reported in the IAEA TRS472 (IAEA, 2010) for leafy vegetables in temperate environments (N = 12, average 7.4×10^{-3}, range 2.5×10^{-4} - 5.0×10^{-2}; there is no value presented for tropical or sub-tropical environments for ^{210}Po), but they were within the range reported by Vandenhove et al. (2009) for leafy vegetables (N = 31, geometric mean $2.11 \times 10^{\circ}$, range 3.21×10^{-3}- 2.46×10^{1}), which includes some articles regarding areas other than moderate as well.

The transfer factors show a somewhat different pattern from the

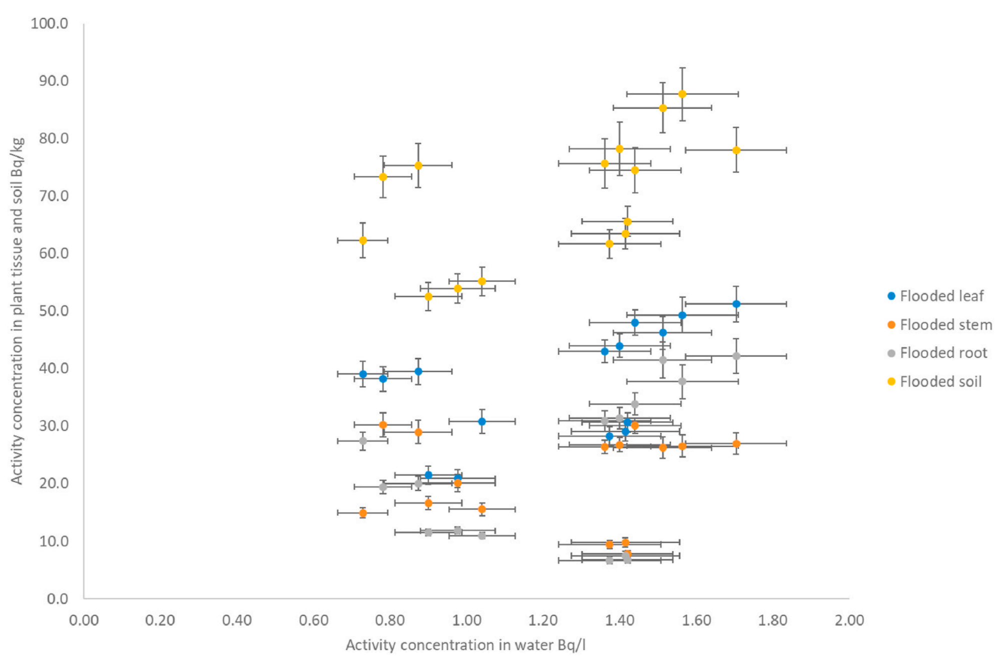

Fig. 5. Transfer factor for ^{210}Po from soil to spinach tissues.

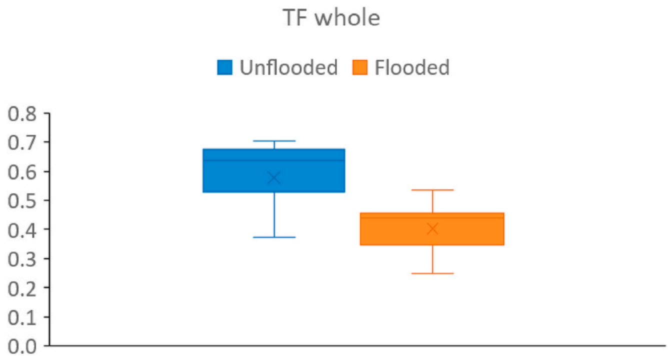

Fig. 6. TF_{whole} in flooded and unflooded spinach.

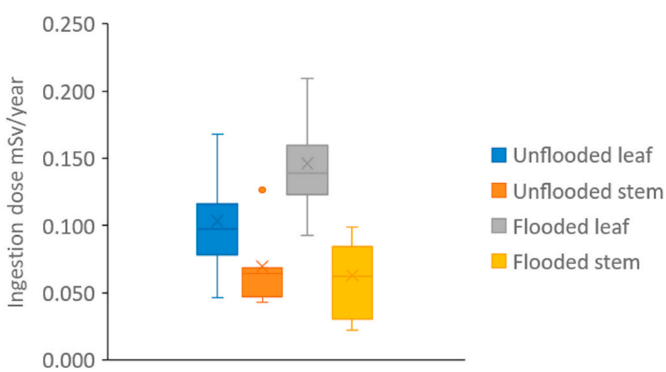

Fig. 7. Annual effective dose from ingesting ^{210}Po in flooded and unflooded water spinach.

concentrations. The Kruskal-Wallis Test indicates no significant difference between the transfer factors of the leaves (H 1.55, p .21) and stems (H 0.72, p .40) under flooded and unflooded growing conditions, but the unflooded roots have a higher transfer factor than those grown under flooded conditions (H 21.77, $p < .05$). Under unflooded conditions TF_{stem} was lower than TF_{leaf} (H 8.43, $p < .05$), which in turn was lower than TF_{root} (H 21.77, $p < .05$). Under flooded conditions the pattern was somewhat different, the TF_{stem} and TF_{root} are not significantly different from each other (H 0.11, p .74), and both are lower than TF_{leaf} (H 20.82, $p < .05$ for stem and H 14.40, $p < .05$ for root).

The overall transfer factor (TF_{whole}) can be used to evaluate the transfer of radionuclides from soil to plant biomass. In this study, TF_{whole} is calculated based on the activity of ^{210}Po in soil, root, stem, and leaf of water spinach and their respective mass. It is the ratio of ^{210}Po activity concentration in the whole water spinach (root + stem + leaf) to the activity concentration in soil. The calculated results of the average TF_{whole} in spinach vegetable are plotted in Fig. 6. Since the calculated TF_{whole} factors are less than unity, despite some specific observed TF values over one, the whole plant has a lower activity concentration than the soil, there is no bio-magnification effect. The Kruskal-Wallis test indicates there is a statistically significant difference (H 11.15, $p < .05$) between the two means, namely those grown in unflooded soil tend to have higher transfer factor values.

3.3. Annual effective dose

Fig. 7 shows the variation of annual effective dose due to the ingestion of flooded and unflooded water spinach. These levels are relatively high for a single food source compared to the total annual ingestion dose of about 300 μSv $year^{-1}$ reported by WHO (2011) and UNSCEAR (2000) resulting from the natural radionuclide content in food, but these levels of exposure are still arguably relatively safe in light of some recent publications on the health impact of low dose ionizing radiation (Vaiserman et al., 2018). There are two other comparison values that might be of interest, the WHO guideline levels for specific radionuclides having a generic criterion of 0.1 mSv per year for ingestion, which is exceeded in many of the leaf samples and some of the stem samples, and the IAEA 1 mSv per year reference level for public exposure in specific existing exposure situations including food, which is not exceeded in any of the samples (IAEA, 2018). Consuming the stem seems to result in lower doses than consuming the leaves, and the doses are similar between the two groups (H 0.00, p .96). Interestingly the differences in the water content resulted in the ingestion dose from the leaves of the water spinach grown in unflooded conditions being lower than that coming from the water spinach grown under flooded conditions (H 7.49, $p < .05$).(UNSCEAR, 1993) (Lee et al., 2009).

Additionally, it can be seen that the annual effective dose to people from ^{210}Po due to consumption of water spinach in the study area is higher than the annual effective dose from ^{210}Po due to the total consumption of leafy vegetables in Turkey, which was found to be 30.5 μSv.$year^{-1}$ (Ekdal et al., 2006).

As reported by Le et al. (2017), the total annual effective dose from the gross alpha of natural radionuclides due to the consumption of water spinach in Ho Chi Minh city with the assumed average consumption of water spinach of 10 kg/person/year ranged from 118 to 1365 μSv $year^{-1}$. At that level of consumption our results would yield lower exposure, a maximum of 77 ± 5 μSv $year^{-1}$.

Estimating the effects of feeding water spinach roots and and stems to animals requires further investigation on the scope, extent and effect of this practice due to the lack of available data.

4. Conclusions

The ^{210}Po activity concentration in leaves was found to be the highest in unflooded water spinach (*Ipomoea aquatica Forrsk.)*, while for flooded water spinach, the highest activity concentration of ^{210}Po was observed in the root. However, since the root part is not consumed, this does not seem to have a direct effect on the estimated radiological risk, but it might have some indirect effect due to it being used as feed.

The order of observed activity concentrations was $C_{stem} < C_{leaf} < C_{root}$ for the unflooded variety, while for the flooded one $C_{stem} \approx C_{root} < C_{leaf}$ was observed on a dry weight basis. The activity concentrations in the leaves and stems were somewhat similar between the two treatment groups, while the ^{210}Po activity concentration in the roots of the unflooded spinach is lower than those of flooded spinach.

Regarding the transfer factor, the difference in growing environment also leads to a difference in transfer factor for ^{210}Po. For flooded water spinach, the highest transfer factor is found for TF_{root}, whereas for unflooded water spinach, the highest one is observed for TF_{leaf}. The order of the observed transfer factors was $TF_{stem} < TF_{leaf} < TF_{root}$ for the unflooded variety, while for the flooded one $TF_{stem} \approx TF_{root} < TF_{leaf}$. Generally, the water spinach grown in unflooded soil tend to have higher overall transfer factor values.

The consumption of water spinach growing in Hanoi at levels reported for the area causes a comparatively high annual effective dose from ^{210}Po, which, often exceeds the WHO guideline levels generic criterion of 0.1 mSv per year for ingestion for specific radionuclides, but does not exceed in any case the IAEA 1 mSv per year reference level for public exposure in specific existing exposure situations.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The research work is supported by Grant of The National Foundation for Science and Technology Development (NAFOSTED), Vietnam, no 105.05-2019.10 and Hungarian National Research OTKA grant No. K128805 and K128818. The authors would like to thank the technicians in the laboratories of The Environmental Isotope Laboratory of Geophysics Department, (HUMG); The Institute for Nuclear Science and Technology (INST), Vietnam and in the Key Laboratory of Environmental and Climate Change Response, Vietnam University of Science (VNU) for supporting in conducting the experimental part of this work.

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