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Impact of fertilizers on the uptake of ^{226}Ra, ^{232}Th, and ^{40}K by pot-grown rice plants

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ABSTRACT: Fertilizers usually enhance potassium (K) content and other naturally occurring radioactive materials in agricultural fields that eventually enter the human food chain through plants. In this study, pot-grown rice plants planted in soil that is relatively high in natural radioactive content was used to estimate the individual influence of fertilizer applications on the uptake of ^{226}Ra, ^{232}Th, and ^{40}K using gamma-ray spectrometry. Three types of common fertilizers used in rice cultivation (with percentages) 17.5N:15.5P:10K, 17N:3P:25K+2MgO, and 46N (i.e., urea) were separately added to the potted-rice plants which were in three different growth stages: emergence stage (10 days), maximum tillering stage (40 days), and initiation stage (70 days). Fertilizers at various concentrations (0, 100, 200, 300, and 400 mg kg^{-1}) were applied in the first stage of plant growth, whereas only 200 mg kg^{-1} fertilizer was applied in the second and third stages. Results showed that the uptake of ^{226}Ra, ^{232}Th, and ^{40}K by rice grains was affected by different concentrations of fertilizer and its application time. However, these findings suggested insignificant health risk related to the ingestion dose of grains treated with selected fertilizers.

Keywords: fertilizer, pot, radioactivity, rice, uptake.

INTRODUCTION

Primordial radionuclides, namely, uranium (^{238}U), thorium (^{232}Th), and potassium (^{40}K), are the primary cause of natural radioactivity in soil. Gamma radiation of natural radioisotopes is the major source of irradiation for human body with a large contribution from terrestrial gamma radionuclides (UNSCEAR, 2000). These radionuclides may be absorbed by plants and transported to their edible parts, which become a source of internal exposure to the human body (Pulhani *et al.*, 2005). Therefore, transfer factor (TF) of radionuclides from soil to plants and finally, grains is studied around the world (IAEA, 1994). TF is an important parameter that encompasses influence of physicochemical properties of soil, environmental conditions, and types of radionuclides (Martinez-Aguirre and Perianez, 1998).

Fertilizers are vastly used to enhance yield of the crop by supporting nutrient reserves in soil, for example, NPK; however, these fertilizers interfere with physical, chemical, and biological properties of the soil. Consequently, inorganic fertilizers affect the production quality of soil (Acton and Gregorich, 1995). Furthermore, the utilization of chemical fertilizers alone is not very successful in maintaining high crop yield because of their adverse effect on pH of the soil, leaching of nutrient, and content of organic matter (Obi and Ebo, 1995). For example, Fageria *et al.* (2010) observed that compared to unfertilized soil, fertilization of

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soil using urea decreases pH of the soil. In another study (Asing *et al*., 2008), pH increased and decreased sequentially after addition of urea to the soil. Moreover, Takeda *et al*. (2006) found that the Uranium content in the soil of the fertilized experimental fields was higher than the soil of non-agricultural field. Shishkunova *et al*. (1989) stated that fertilizers with high phosphate (P) content changed constriction ratio of radionuclides that are available to plants to what is fixed in the soil. Belli *et al*. (1995), found that thorium isotope increased by a factor of 2 to 3 when 4,000 kg ha^{-1} ammophos fertilizer was added to the soil. The effect of NPK fertilizer on the uptake of cesium (^{137}Cs) by different species of plants and vegetation was studied by Belli *et al*. (1995). According to them, the absorption of ^{137}Cs in plants reduced when potassium (K) fertilizer was applied, while growth of the grass and absorption of radionuclide of cesium increased with the application of nitrogen (N) fertilizer. Natural radionuclides, namely, ^{226}Ra, ^{232}Th, and ^{40}K in fertilized soils, non-fertilized soils, and vegetables were measured by Bolca *et al*. (2007). They found that extensive use of phosphate (P) fertilizer may cause an increase in the activity of natural radionuclides. They also infer that fertilizers may cause chemical and biological change in the soil that influences transfer of radionuclides from soil to plants.

Rice is the primary food for more than half of world's population (Khush, 2005) influencing the economy of billions of people around the world. Several studies have investigated the uptake of radionuclide from soil by rice plants in various parts of the world (e.g., Alsaffar *et al*., 2015; Karunakara *et al*., 2013; Shanthi *et al*., 2012; Uchida *et al*., 2007). However, the effect of fertilizers on the uptake of radionuclides still needs further investigation. Therefore, in this study, we aimed to estimate the effect of common fertilizers on the uptake of ^{226}Ra, ^{232}Th, and ^{40}K radionuclides by rice grains.

MATERIALS AND METHODS

Soil and Pot Preparation

In this work, soil with relatively high levels of natural radioactivity was taken from a paddy field in Kampung Permatang Langsat, Malaysia (Alsaffar *et al*., 2015). The soil was collected from a depth of 0 to 20 cm of the selected site and was transferred to laboratory using plastic bags. The collected soil was air-dried at room temperature for 3 weeks; afterward, the soil was ground and screened to pass through a 2-mm mesh to remove pebbles and roots. Subsequently, the screened soil was mixed thoroughly to obtain a composite sample. Physicochemical characteristics of the screened soil were determined before applying fertilizers. Furthermore, pH, electrical conductivity (EC), organic matter (OM), and cation exchange capacity (CEC) of the composite soil were determined through relevant standard methods (Aprile and Lorandi, 2012; Jackson and Barak, 2005). In addition, soil type was classified based on the texture such as clayey, sandy, and silty, which was determined using the hydrometer method (Bouyoucos, 1962).

In this study, the experiment was performed during the 2014–2015 cropping season in Universiti Sains Malaysia under natural climatic conditions of the region (29–32°C temperature and 60.9–96.8% humidity) (Almayahi *et al*., 2012). Experiments performed using pot-grown plants are probably the most common method in plant research because this technique is fast, simple, and easily controlled when demonstrating effects of inputs on plant growth and yield. In our experiment, 19 plastic pots of equal size and cylindrical in shape, with a 20 cm inner diameter and 21.5 cm height were used. Each pot was filled with 4 kg of processed soil and was submerged for 2 weeks with limited water (about 1 in.) on soil surface to reach relative balance. Finally, all the pots were transferred and placed under a transparent polyethylene sheet to avoid influence of rainfall on the pots.

Rice Seedling and Application of Fertilizer

In this study, we used one of the widely planted rice seeds (Type MR 219) in Penang. We applied three common fertilizers, that is, 17.5N:15.5P:10K, 17N:3P:25K+2MgO, and 46N (*i.e.*, urea) in our experiments. After pot preparation, six pre-germinated seeds were sown in each pot. One week later, seedlings were thinned, and three uniform seedlings were placed in each pot. After placement of the rice seedlings, water elevation was raised to 3 in. and maintained at this level by daily addition of distilled water until the end of the growing period.

In our experiment, fertilizers were added in three different stages: after 10 days (rice emergence stage), after 40 days (maximum tillering stage), and after 70 days (panicle initiation stage). In the first stage, each type of fertilizer at a concentration of 100, 200, 300, and 400 mg kg^{-1} soil was separately added to three sets of pots (*i.e.*, four pots were used for each set). Subsequently, 200 mg kg^{-1} of each fertilizer was separately added to three pots in the second stage. This process was repeated in another three pots in the third stage.

One pot without any application of fertilizer was used as a control. Figures 1a and 1b show growth of rice plants during emergence and initiation stages, respectively. After 115 days, rice grains were harvested and transferred to the laboratory for processing before testing by gamma ray spectrometry. About 46 to 66 g un-hulled rice was produced from this experiment using pots.

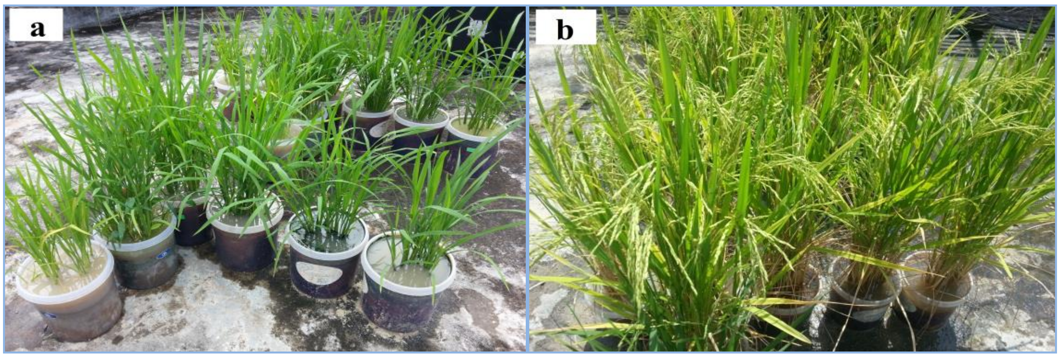

Fig. 1. Growth of rice plants during emergence and initiation stages

Sample Pretreatment and Analysis

A 500 g of composite dry soil was transferred into an air-tight container of uniform size (8.2 mm diameter × 8.2 mm height) and stored for 1 month to ensure equilibrium between ^{226}Ra as well as its short-lived decay products. Uniformly mixed fertilizer samples were prepared by mechanical crushing, sieving (through 2 mm mesh), and pulverizing. Afterward, each sample of fertilizer was pressed in a similar container and weighed with soil sample. In addition, all grain samples were oven-dried at 105°C to constant weight. Subsequently, a 40 g of each grain sample was ashed at 450°C for 5h. Approximately 36% of the original sample mass was obtained after ashing. Then, the ash sample was preserved in an air-tight container of uniform size (8.2 mm diameter × 1.2 mm height).

A high-resolution measurement system that consists of a high-purity germanium detector (GEM-F7040P4, Canberra Inc.) was used for radioactivity measurements. This system has the relative efficiency of 40% and 1.9% energy resolution at 1.33 MeV ^{60}Co. The detector was shielded with a lead cylinder of approximately 3.5 cm thickness, 21 cm inner diameter, and 25 cm length. The detector was calibrated for energy with standard gamma sources (^{241}Am, ^{137}Cs, ^{60}Co, ^{152}Eu, and ^{226}Ra) obtained from International Atomic Energy Agency (IAEA). Absolute efficiency was obtained using homogeneous ThO_2 powder mixture (at secular equilibrium) with $CaCO_3$ according to the method described by Lavi and Alfassi (2005). To quantify radionuclides with good precision and accuracy, the distribution and density for reference and samples were considered (Alsaffar *et al*., 2015). Each sample was counted for 86,400 s to reduce statistical uncertainty. Energy photopeaks 351.9 (^{214}Pb) and 609.3 keV (^{214}Bi) were used to determine ^{226}Ra activity; 238.6 (^{212}Pb) and 583.1 keV (^{208}Tl) were used for ^{232}Th activity; and 1461.8 keV photopeak was used for ^{40}K activity. Each radionuclide concentration was calculated using Equation (1) (IAEA, 1989):

$$A_S = \frac{N_t}{TP_\gamma \varepsilon M} \quad (1)$$

In this section, A_s, N_t, T, P_y, ε, and M are specific activities of radionuclide (Bq kg^{-1}), net counts under photopeak, counting time (s), gamma intensity of specific gamma-ray, absolute efficiency, and mass of sample (kg), respectively.

RESULTS AND DISCUSSION

Soil Properties

Physicochemical characteristics of the composite soil were as follows: pH was found to be 5.08, which tended toward an acidic behavior; soil CEC (soil's capacity to reserve nutrients for plants) was 18.35 meg/100 g, whereas its EC (standard measure of salinity) was 0.21 mS cm^{-1}; soil OM is found to be 14.7%, which shows that the sample can be classified as organic soil (Troeh and Thompson, 2005); clay, sand, and silt in soil were found to be 27.1, 63.4, and 9.5%, respectively. Based on the observed sand and clay content in soil, the sample can be classified as sandy clayey loamy soil (Shukla, 2013).

During planting time, pH was directly recorded for each pot using a portable pH meter after 4 days of fertilizer application. In case of control sample, pH was 6.71 (unfertilized pot); however, this parameter increased in three sets of pots with pH ranges of 7.67–7.89, 7.03–7.48, and 6.99–7.39 with different amounts of urea, NPK, and NPK+Mg, respectively. Subsequently, pH values for all fertilized pots gradually declined. The higher the percentage of ammonium (or urea) in the fertilizer, greater is the acidification potential of the soil. Addition of ammonium $\left(NH_4^+\right)$-based fertilizers and those that contain urea increased NH_4^+ *and* NO_3^- (nitrate) contents in the soil, which subsequently increased nitrogen content of the soil by the plant roots. The utilization of urea in flooded soil can increase and subsequently decrease pH of the soil. Initial increase in pH was caused by H^+ consumption during NH_4^+ formation (i.e., alkaline compound) during urea hydrolysis. Subsequent decrease in pH is caused by the uptake of NH_4^+ by the roots. In addition, neutral medium can create a good status for NH_4^+ conversion to NO_3^- during nitrification process and the release of $4H^+$ in soil; therefore, this conversion also increases soil's acidity. Furthermore, soil acidification can be caused by rapid conversion of NH_4^+ to NO_3^- as a result of bacterial activity. Hence, soil acidification can occur regardless of the uptake NH_4^+ by plant roots (Lambers and Colmer, 2005).

Radioactivity in Soil, Fertilizer, and Rice

Table 1 shows the activity concentrations of ^{226}Ra, ^{232}Th, and ^{40}K in composite soil and selected fertilizers. For fertilizer samples, the maximum radioactivity of ^{226}Ra and ^{232}Th were in NPK, whereas the maximum activity of ^{40}K was in NPK+Mg. These results indicate that each 100 mg NPK enhanced soil radioactivity by 2.4 × 10^{-3} and 1.6 × 10^{-3} Bq kg^{-1} for ^{226}Ra and ^{232}Th, respectively, whereas each 100 mg NPK+Mg enhanced soil radioactivity by 1.6 × 10^{-1} Bq kg^{-1} for ^{40}K. Consequently, the radioactivity produced by the addition of fertilizers to pots is apparently insignificant compared with that of soil alone.

Table 1. Activity of radionuclides in soil and selected fertilizers

Sample	Activity (Bq kg^{-1})		
	^{226}Ra	^{232}Th	^{40}K
Soil	191.75	187.56	592.05
Urea	9.32	7.14	81.7
NPK	24.94	16.51	1260.38
NPK+Mg	14.47	10.2	1611.52

Table 2 presents the activity concentrations of ^{226}Ra, ^{232}Th, and ^{40}K in rice grains that were harvested from fertilized and unfertilized pots. Results show that ^{226}Ra concentrations were increased with increasing urea (Fig. 2). Adsorption of cationic species, such as Ra, was driven by pH of the system. With each adsorbing cation, an H^+ ion is typically released that is enhanced in alkaline conditions while decreased in acidic conditions. Therefore, Ra shows great mobility in acidic media, and it appears to be more strong if there is a decrease in pH, which is caused by organic acids (Smith and Amonette, 2006). Sheppard and Sheppard (1988) found that by decreasing the pH of soil by 1 unit may increase the mobility of Ra by a factor of 2 or more, which consequently leads to increase in the uptake by plant. They also referred to the studies of Rusanova (1964) that a drop in pH from 5 to 4, rapid Ra desorption occurs and decreases with increasing in pH until pH 9 is reached.

Table 2. Activity of radionuclides in grains from fertilized and unfertilized pots

Stage of plant growth	Fertilizer (mg kg^{-1})	Activity (Bq kg^{-1})								
		UREA			NPK			NPK+Mg		
		^{226}Ra	^{232}Th	^{40}K	^{226}Ra	^{232}Th	^{40}K	^{226}Ra	^{232}Th	^{40}K
10 days	100	2.94	2.21	78.98	2.87	2.17	75.85	2.81	2.13	77.11
	200	3.05	2.28	87.31	2.97	2.25	78.3	2.92	2.2	80.85
	300	3.18	2.39	79.23	3.09	2.33	82.36	3.03	2.3	84.93
	400	3.23	2.43	74.21	3.15	2.4	70.76	3.11	2.35	75.31
40 days	200	2.97	2.24	83.04	2.9	2.19	75.31	2.84	2.13	76.06
70 days	200	2.86	2.15	80.13	2.81	2.11	73.36	2.78	2.06	74.79
Control	0	2.74[a]	2.08[a]	71.15[a]	---	---	---	---	---	---

[a] Activity in grains from unfertilized pot

Results show that ^{226}Ra concentrations also increased slightly when both NPK and NPK+Mg amounts were increased; however, ^{226}Ra concentration was found to be lower than that of urea because both fertilizers provided only 17.5 and 17% of N, respectively, that are significantly smaller than 46% of N contained by urea. Therefore, the effect of acidity due to NPK and NPK+Mg treatment to the soil has to be smaller than urea treatment. In addition, K is a major nutrient and is transported together with water from the root to the other parts of the plant, for example, grain. Lembrechts (1993) relates the radionuclide content in the overground phytomass with the supply of nutritive minerals and their composition in the soil. He states that, with

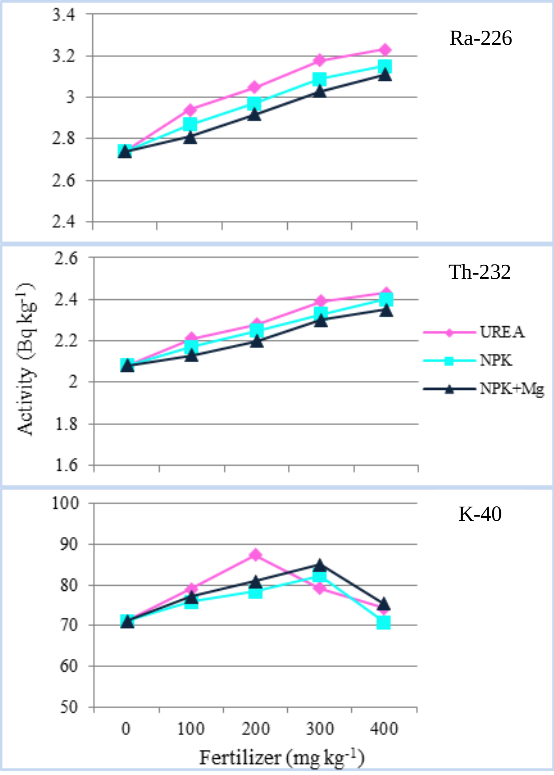

Fig. 2. Activity of radionuclides in grains with different fertilizer amounts

higher supply of nutritional mineral content in the soil with their composition closer to the optimum, the content of radionuclide in overground phytomass will be lower. Therefore, the smaller uptake of ^{226}Ra and ^{232}Th by rice plants grown in the soil treated with NPK and NPK+Mg fertilizers in comparison with urea (Fig. 2) might be due to the K content provided by these fertilizers. This is in accordance with other studies that observed the negative influence of K content on the absorption of other radionuclides, such as ^{238}U, ^{232}Th, ^{241}Am (Whicker *et al*., 1999), and ^{137}Cs (Kato *et al*., 2015; Nobori *et al*., 2014). In contrast, ^{226}Ra absorption caused by NPK fertilizer treatment was higher than NPK+Mg that can be attributed to the higher phosphorus concentration in former fertilizer (*i.e.*, 15.5% P) and its activity (Table 1). Erratic quantities of radionuclides can be logged in P fertilizers, which are derived from phosphate rock. Sedimentary phosphate rocks in marine environment are categorized on the basis of the concentration strength of ^{238}U, ^{232}Th, and its decay products along with the presence of phosphate minerals (Sahu *et al*., 2014). Several researchers reported that fertilizers that contain phosphate have high uranium concentrations (Hamamo *et al*., 1995; Khater, 2008; Yamazaki and Geraldo, 2003).

Similar behavior was observed for ^{232}Th absorption with urea, NPK, and NPK+Mg amounts (Fig. 2). This can be attributed to the decomposition of resistant minerals favored by strong acidic conditions and to the enhancement in the solubility of ThO_2 in conditions of pH below 7 by converting into thorium sulfate, fluoride, and phosphate complexes (Langmuir and Herman, 1980). Comparatively, Figure 2 shows that ^{226}Ra uptake by rice was higher than that of ^{232}Th. Results suggest that ^{226}Ra is more affected and soluble at high acidity (i.e., low pH) of the medium than ^{232}Th. Focazio *et al.* (2001) stated that ^{226}Ra, which is a ^{238}U progeny, has a relatively higher solubility than ^{228}Ra, which is a ^{232}Th progeny in groundwater.

^{40}K uptake at different amounts of fertilizers is relatively similar in behavior but different in magnitude (Fig. 2). Results show that ^{40}K uptake increases with increasing urea and falls off gradually from 300 mg kg^{-1} and higher urea quantities. In general, K is a major nutrient of fertilizers used in this study. Barak *et al*. (1997) infer that the elevation in exchangeable acidity related to N fertilization accompanies with a drop in CEC. The uptake of ^{40}K reflects its behavior in rice plant where this element has maximum value at intermediate pH and decreases toward acidic or alkaline soil. Our results on the uptake of ^{40}K agree with that of Chen and Barber (1990). The uptake of ^{40}K with NPK treatment was slightly lower than with NPK+Mg; this finding may be caused by higher K percentage in second fertilizer (25% K) compared with other. Results also show that ^{40}K absorption caused by urea treatments was more than other selected fertilizers. It indicates that the transfer of ^{40}K from soil to rice is affected by other factors (*i.e.*, soil properties) besides its concentration in the soil. The study of Karunakara *et al*. (2013) shows that ^{40}K concentration in rice plant does not have linear relationship with its concentration in soil. Furthermore, our results show that in rice grains there was a higher ^{40}K activity than other radionuclides, which can be attributed to the availability of ^{40}K in a natural abundance of 0.012% that is an essential mineral for growth of the plant (Atwood, 2013).

By adding the same amount of fertilizer (200 mg kg^{-1}) at different plant growth stages, uptake of ^{226}Ra, ^{232}Th, and ^{40}K in rice grains was found to be higher with the application of the fertilizer at first stage and gradually decreased through the second and to the third stage (Fig. 3). Soluble radionuclides in soil can be absorbed along with the nutrients via roots and transferred to different parts of the plant (Tsukada *et al.*, 2002). During application of the fertilizers in early season (rice emergence stage), soil can create a good medium for the plant growth with longer duration compared to other stages. Consequently, the potential of a particular fertilizer to acidify during the first treatment stage will make it available for a longer period of time and hence, soil may release and provide much more soluble radionuclides to the plant roots. Moreover, soil amelioration by using fertilizers positively influences the plant root absorption and likely become better structured. Therefore, early fertilizer application to soil allows the plant to absorb more nutrients, as well as soluble radionuclides.

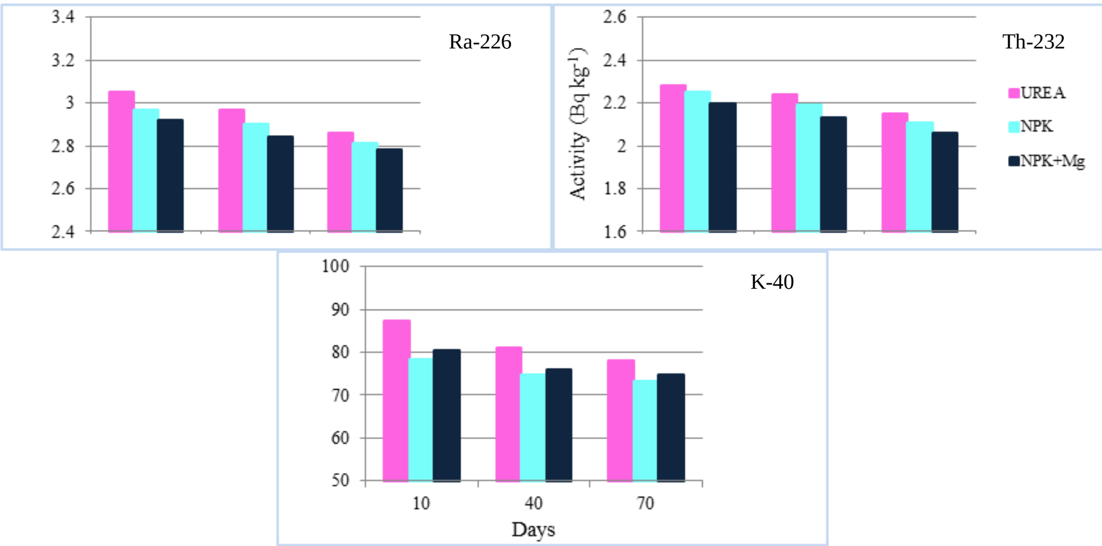

Fig. 3. Activity of radionuclides in grains at different time of application of fertilizer

Our results are consistent with the findings of Nain *et al*. (2013) that the transfer of alpha radioactivity from soil to potato plants increased with increase in the amount of fertilizer and plantation time. Compared to the previous study, urea can affect the uptake of natural radionuclides more than other selected fertilizers. This may be attributed to the rice planting method in which flooded soil is used that is more efficient for urea interaction through hydrolysis process.

Risk Assessment with Fertilizer Use

Overall results show that maximum ^{226}Ra, ^{232}Th, and ^{40}K concentrations in grains caused by enhanced fertilizers were 3.23, 2.43, and 87.31 Bq kg^{-1}, respectively. Compared to the control samples, maximum concentration of selected radionuclides was found to be 17, 16, and 22%, respectively. Risk from contaminated food consumption is calculated by estimating total amounts of ingested radioactivity (D'Mello, 2003). Thus, the total ingestion dose for adults can be estimated to be 0.063 mSv when maximum activity of selected radionuclide is multiplied by Malaysian rice consumption rate (95.9 kg y^{-1}) (USDA, 2013) and its dose conversion factor (2.8×10^{-7}, 2.2×10^{-7}, and 6.2×10^{-9} Sv Bq^{-1} for ^{226}Ra, ^{232}Th, and ^{40}K, respectively) (ICRP, 2012). Moreover, by comparing this value with the average annual ingestion dose worldwide (*i.e.*, 0.29 mSv) (UNSCEAR, 2000), results show that the ingestion of grains obtained from the plants grown in NPK, NPK+Mg, and urea-treated soils do not pose any serious health risk.

CONCLUSIONS

Planting using pots allows accurate estimation of fertilizer influence on uptake of natural radionuclides by rice plants. Increased fertilizers, namely, 17.5N:15.5P:10K, 17N:3P:25K+2MgO, and 46N (*i.e.*, urea) in rice plant pots leads to slightly increased ^{226}Ra and ^{232}Th uptake in rice grains. However, ^{40}K increased and decreased sequentially with enhanced selected fertilizers. Higher ^{226}Ra, ^{232}Th, and ^{40}K uptake by grains is noted during fertilizer application in the emergence stage; this level subsequently decreased from maximum tillering to the initiation stage. Urea can affect uptake of particular radionuclides more than other selected fertilizers. Thus, ^{226}Ra, ^{232}Th, and ^{40}K activities in rice grains were increased, as maximum, by 17, 16, and 22%, respectively.

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