

Original Research Article

Title: Study of Naturally Occurring Radioactive Materials and Potentially Toxic Elements from Intake of Some Nuts and Seeds in South-Western, Nigeria

Running Title: Radionuclides and heavy metals measurements in Nuts and Seeds in SW Nigeria

Abstract

Concentration of NORMs and some toxic metal in some nuts and seeds commonly consumed in the south-western state of Nigeria was investigated. Analysis was done with NaI(Tl) spectrometry and Inductively Coupled Plasma (ICP) respectively. The concentration of ^{226}Ra , ^{232}Th and ^{40}K in the samples are found to be below the world average. Their means are 6.6 ± 1.8 , 3.6 ± 1.0 and 98.2 ± 13.5 Bq/kg, respectively in nuts and 8.4 ± 2.6 , 2.6 ± 1.3 and 97.6 ± 15.0 Bq/kg in seeds. Effective dose in nuts is 13.99 and in seeds it is 12.0 μSv . The fatal and hereditary cancer risk estimated from the consumption of the nuts and seeds are lower than 1.0×10^{-6} which is the lowest limit. Concentration of metals in the samples descend as $\text{Fe} > \text{Zn} > \text{Cu} > \text{Cr}$. The Hazard Indices of heavy metals are lower than 1 except for in Cocoa which is higher than 1 due to high concentration of Cu and Fe. Therefore, the nuts and seeds analyzed in this study are contaminated with heavy metals and so must be taken with care so that consumers will not be exposed to excessive concentration of metals which may have undesirable effects.

Keywords: heavy metals, radionuclides, fatal cancer risk, nuts, seeds

Introduction

Human environment is radioactive and human beings are exposed anywhere in the environment either or both from natural radioactivity, artificial radioactivity and medical applications. Human beings are exposed from intake of water, air, soil and plants [1]. It is a well know fact that plants used as food commonly contain natural radionuclides and their progenies [2]. Also other contaminants, like heavy metals are added into plants through the applications of fertilizer, pesticides, herbicides in agriculture, deposition of dust from mining activities and other industrial activities such as wastes disposal and automobile exhaust [3].

Nuts and seeds are beneficial to human. It contains many nutrients beneficial to both adult and young one. It is consumed as delicacy and sometimes to treat some diseases and ailments. Medicinal application of nuts and seeds is limited due to presence of NORMs and some toxic metals in them [4,5]. Some important information on the level of radionuclides and heavy metals in medicinal plants, nuts and seeds have been reported through previous researches[6-8]. The process of transfer of radionuclides and heavy metals to human body is majorly through inhalation and ingestion of contaminated food stuffs. These contaminants especially radionuclides emit alpha particles which interact with water in the cells and result in some biological effects (mutation). Chronic effects are accompanied by lung cancer, tumour, genetic effects, kidney failure and vascular diseases [9].

In Nigeria, the side effects of orthodox medicine are changing people perception about their uses and there is increase urge in finding other natural means from consumption of natural plants, nuts and seeds to treat ailments. Therefore, it is imperative to assess the risk that may arise from the consumption of some nuts and seeds to checkmate food fraud[10] and to assess contaminants in them[11]. This study aimed at estimation of NORMS, some heavy metals and evaluation of hazard

in the consumption of some nuts and seeds in SW, Nigeria. The findings of this study would assist local and international agency in quality control of food products.

Materials and Method

Five samples each of ten nuts and seeds (cashew nut, groundnut, walnut, Kola nut, moringa seed, bitter kola nut, African Star Apple seed, avocado seed, watermelon seed and cocoa seed) commonly cultivated and consumed in south-western state of Nigeria was selected. The samples were collected from the farm sites and were packed with identification labels. Nuts and seeds were removed from shells and were thereafter left to dry at room temperature in the laboratory. The samples were crushed and sieved. 100g of the sample was weighed and transferred into a cylindrical plastic container and hermetically sealed for thirty days [12] for equilibrium between ^{238}U (^{226}Ra) and ^{232}Th (^{228}Ra) and their respective progenies to take place [13]. The samples were afterwards analysed using spectrometry method. For heavy metal, their concentration was measured using Inductively Coupled Plasma Spectrometry (ICPS)

Radionuclide Analysis

The analysis of radionuclide is achieved using NaI(Tl) detector. A Canberra multi-channel analyser coupled with a scintillating detector was used for the counting. The model no of the detector is 8020 and is manufactured in USA by Bicon Electronics Ltd. The detector is fixed to a multi-channel analyser by a coaxial cable. The detector is shielded with a lead shield of about 5cm thick to prevent radiation from other environmental sources present (background radiation). Gamma sources supplied by the IAEA were used for calibration and the energy-channel calibration was used to fit the linearity graph. A reference sources prepared by Rocketdyne Laboratories, Canoga Park, USA which is traceable to standard gamma source (No. 48722-356) was used for detection efficiency calibration. The resolution of the detector assembly is about 8% at 0.662 MeV of ^{137}Cs .

The net count was obtained by subtracting the background count from the gross count. The samples were counted for 36600s (10 h). Photo-peak energy 1.465 MeV from ^{40}K was used to determine ^{40}K while photopeak energy 1.765 MeV from ^{214}Bi was used to determine ^{226}Ra . ^{232}Th was determined from energy photopeak 2.615 MeV from ^{208}Tl and ^{228}Ra was determined from gamma-ray energies of its daughter ^{228}Ac (911.07 keV). The concentrations of NORMs in the samples was calculated using a spectrum acquisition and analysis software program (Genie 2K, manufactured by Canberra Industries Inc., USA).

Heavy Metal Analysis

Samples were digested using wet digestion method. One gram of each sample was weighed into 100mL tube and the tubes were labelled accordingly. Five millilitres of HNO₃ and H₂O₂ were added into the samples and H₂SO₄ was added in drops. The mixture was stirred until the solution become clear. The solution was poured out of the tube and mixed with deionized water up to 100mL and it was then filtered. The toxic metals concentration was obtained with inductively coupled plasma spectrometry (ICPS). Concentrations of metals in the digested samples were determined using plasma and a spectrometer. The solution is directed into spray chamber through nebulizer using peristaltic pump. Each element measured with spectrometer has its own characteristic emission spectrum. The light intensity on the wavelength is measured and with the ICPS calibrated, concentration of each element was evaluated.

Results

Activity Concentrations of Radionuclides and Calculation of Radiological Parameters

The concentration of the natural radionuclide in nuts and seeds is shown in Table 1. In nuts, concentration of ²²⁶Ra is lowest (3.2±1.0) in bitter kola nut and highest (10.9±3.2 Bq/kg) in kola nuts. In seeds, it ranged from 4.2±1.6 in African star apple seeds to 14.5±2.8 Bq/kg in Cocoa seeds. It was not detected in groundnut, walnut, moringa seeds and watermelon seeds. The concentration of ²²⁸Ra in nuts is minimum (2.4±0.5 Bq/kg) in Walnut and maximum (5.9±2.4 Bq/kg) in Groundnut. In seeds, the measured activity ranged from 1.8±1.1 in Cocoa seeds to 3.2±1.6 Bq/kg in Avocado seeds and was not detected in bitter kola nut, moringa and watermelon seeds. The concentration of ⁴⁰K in nuts ranged from 53.6± 4.2 in bitter kola nut to 126.2±22.1 Bq/kg in kola nut. In seeds it ranged from 50.6± 3.5 Bq/kg in Avocado to 123.1± 5.6 Bq/kg in African star apple seeds.

Effective dose

The annual effective dose from ingestion of nuts and seeds is estimated using the equation

$$E_{Dose} = C_R \cdot DCF_i \cdot A \quad 1$$

where DCF_i is dose conversion factor for ingestion of the three radionuclides analysed (i.e. 2.8 x 10⁻⁷ Sv/Bq, 2.3 x 10⁻⁷ Sv/Bq and 6.2 x 10⁻⁹ Sv/Bq for ²²⁸Ra, ²²⁶Ra and ⁴⁰K respectively for adult), C_R is the consumption rate of NORMs from ingestion of seeds and nuts, a rate from intake of 1.8 kg/year was assumed [14]. A is the concentration of NORMs in nuts and seeds. The E_{dose} estimated is presented in Table 3, column 1.

Fatal lung cancer risk and hereditary cancer risk

According to WHO, 2018 [15], Lung cancer causes about 30,000 deaths among non-smokers in United State alone in 2018. The International Commission on Radiological Protection had proposed several models for calculating lung cancer risk from exposure to radionuclides either through ingestion or inhalation. ICRP proposed 0.05 as value to calculate risk factor to the public from ingestion of radionuclides [16]. Fatal Cancer Risk (FCR) from the ingestion of the nuts and seeds was evaluated using equation 2 [17]

$$\text{Fatal Cancer Risk} = ED \times LE \times RF \quad 2$$

where ED is the mean effective dose ($\mu\text{Sv}/\text{y}$), LE is life expectancy (55years in Nigeria[15]) and RF is a factor for risk estimation (Sv^{-1}). The estimated average lifetime fatality cancer risk in nuts and seeds is presented in Table 3, column 2.

Hereditary cancer risk was estimated using Cancer risk methodology proposed by ICRP [17].

$$\text{Hereditary Cancer Risk} = ED \times LE \times RF \quad 3$$

where ED is the mean effective dose ($\mu\text{Sv}/\text{y}$), LE is expected life duration (55years) and RF is a factor for risk estimation (Sv^{-1}). The mean estimated lifetime hereditary cancer risk in the samples is presented in Table 3, column 3.

Heavy metal analysis

The concentration of some of the heavy metals in the samples is shown in Table 4. Pb and Cd were not detected in substantial amount or are below detection limit. The concentration of Cr is only found in kola nut and not detected in other nuts while the concentration of Iron (Fe) is the highest in all the samples. This might be due to that Fe is an essential element in human life and it bounds with haemoglobin. Cu and Zn concentrations are also higher than recommended in cashew nut.

In seeds, Fe concentration is very high especially in cocoa and watermelon than the WHO recommendation. High values of Cu, Zn and Cr were also established in cocoa seeds while Zn concentration is higher than WHO recommendation in African star Apple.

Calculation of risks in the samples

The health risk from the ingestion of heavy metals in the samples (HQ) was estimated as the fraction of quantity of metal taken in a day (ADD in mg/kg of the body weight/day) and quantity of metal recommended to be taken per day (RF Do; in mg/kg/day).

$$ADD = \frac{C_{\text{metal}} \times I}{D_{\text{average}}} \quad 4$$

where C_{metal} is the concentration of metal; I is daily intake of the metals; D_{average} , body weight of an adults. The value of ADD estimated for nuts and seeds samples is presented in Table 5. ADD for all metals in the samples is lower than 1

To estimate the health risks, equation 5 is used

$$HQ = \frac{ADD}{RfD} \quad 5$$

Where RfD is Oral RfDo. According to USEPA, oral RfD are 1.5 for Cr, 0.04 for Cu, 0.3for Zn, 0.7 for Fe [18]

If HQ is greater than 1, it means that risk is associated with the intake of the particular metal. The health risk (HQ) obtained from the ingestion of the samples is presented in Table 6. All the HQ obtained is lower than 1 except for Cu in Cocoa which is higher than 1 indicating a potential risk of exposure to Cu from consumption of Cocoa.

The Total Hazard Index in the samples is calculated using equation (9)[19]

$$HI = HQ_{\text{Fe}} + HQ_{\text{Cu}} + HQ_{\text{Zn}} + HQ_{\text{Cr}} \quad 6$$

If HI is <1, it means no hazard, if THI is 1.1 – 3, it means probable hazard from consumption of the samples. If HI is 3-10, it is likely to cause fatal risk [19].

Discussion

The concentration of NORMs in this study showed that ^{40}K has the highest contribution in all the samples. The concentration of ^{40}K obtained here is within the world average. The concentration of ^{226}Ra is highest in kola nut but below detection limit in groundnut and walnut. The concentration of ^{228}Ra is also below detection limit in bitter kola nut. The mean concentrations of the radionuclide analyzed in nuts are respectively, 98.3 ± 13.5 , 6.6 ± 1.8 and 3.6 ± 1.0 Bq/kg for ^{40}K , ^{226}Ra and ^{228}Ra . These values are within the recommended values of NORMs in food and food products [1]

In seeds, the concentration of ^{40}K is highest in African Star Apple and lowest in Avocado with mean of 95.7 ± 15.0 Bq/kg. ^{226}Ra concentration was below detection limit in Moringa and watermelon seeds. The maximum concentration of ^{226}Ra is from Cocoa seeds. The total mean concentration of ^{226}Ra in seeds is 8.4 ± 2.6 Bq/kg. The concentration of ^{228}Ra in the seeds is low in all the samples

with the highest from avocado. The concentration of ^{40}K , ^{226}Ra and ^{228}Ra obtained in this study are within the recommended average [1]. The concentration of NORMs in the samples follow the order $^{40}\text{K} > ^{226}\text{Ra} > ^{228}\text{Ra}$ as shown in Figure 1 and 2. The percentage concentrations of ^{226}Ra , ^{228}Ra and ^{40}K in the sample nuts and seeds is the same and so no significant difference in the concentration of radionuclides in nuts and seeds as shown in Figure 1 and 2. The mean activity of radionuclides measured in nuts and seeds were compared using 2-tail paired t-test at 95% level of significance (p -value = 1.645) and it was observed that there is no significant difference between their concentration (Calculated value = 0.29, -1.28 and 1.36, respectively).

Effective dose estimated from ingestion of the nuts and seeds confirmed that the largest effective dose was from kola nuts ($18.05 \mu\text{Svy}^{-1}$) while the lowest effective dose was from Avocado ($6.25 \mu\text{Svy}^{-1}$). The mean annual effective dose for nuts and seeds are 13.99 and $12.00 \mu\text{Svy}^{-1}$, respectively (Table 2). The average value obtained in this study is within the limit recommended by ICRP[17]

The Fatal Lung Cancer Risk and hereditary cancer risk estimated from ingestion of nuts and seeds in this study shows that average of 0.38 and 0.30 people respectively will contact cancer from consumption of nuts and 0.33 and 0.27 people respectively will contact cancer from the consumption of seeds. The highest risk is from consumption of kola nuts while the lowest risk is from consumption of bitter kola nuts. The values obtained in this study is below the world average of one person in a million per year [18,20].

The analyses of metals in the samples revealed that Cr is lowest in all samples and not detectable in Cashew nut, Bitter kola nuts, Groundnut and Walnut. The concentrations of Chromium obtained in these samples are lower than the regulation [21]. In Nuts, Fe is lowest in Groundnut (17.62 mg/kg) and highest in Walnut (28.86 mg/kg) and in seeds, Fe concentration is highest in Cocoa (542.49 mg/kg) and lowest in Moringa seed. It was observed that Fe concentration is higher in Cocoa and Mustard seeds than the World Health Organization recommendations [21]. Copper (Cu) concentration in the samples ranged from 3.07 mg/kg in Bitter kola nuts to 26.95 mg/kg in Cashew nut. The concentration is higher than 10mg/kg recommendation in Cashew and Walnuts [22]. Also in seeds, Cu is higher than the recommended concentration in Avocado, African star Apple and Cocoa seed[22]. The concentration of Zinc (Zn) ranged from 2.75 mg/kg in Bitter kola nuts to 50.4 mg/kg in Cashew nuts. Zn is higher than 40mg/kg which is the upper intake for healthy adult in Cashew, African star Apple and Cocoa seeds[20,23]. The concentrations of Cu, Cr and Zn were also observed to be higher than the WHO recommendations in Cocoa [21].

The Hazard Quotient (HQ) and Hazard Index (HI) estimated are lower than 1 except for Cocoa in which HI exceeded 1. This implies that there may be hazard in Cocoa seeds consumption due to

high concentration of Cu. Although, Copper is a vital dietary nutrient in which only small amounts is needed for human well-being. In high doses, Cu can be extremely toxic [24]. High dose of Zn may also lead to tachycardia, vomiting, diarrhea, pancreatitis and damage of hepatic parenchyma [25].

Conclusion

Radioactive analysis carried out showed that the concentrations of ^{226}Ra , ^{228}Ra and ^{40}K are within the recommended average. The estimated effective dose in nuts and seeds is also lower than the recommended values. The assessment of the cancer risks from the consumption of nuts and seeds show that the risk is lower than the safe limit of 1×10^{-6} to 1×10^{-4} [18,20]. Therefore, there is no radioactive health risk involved in the consumption of nuts and seeds if consumed within the 42g per day recommended.

The level of heavy metals obtained in the samples is in the order $\text{Fe} > \text{Zn} > \text{Cu} > \text{Cr}$. Zn is higher than recommended in Cashew, African star Apple and Cocoa seeds and also Cu in Cashew nuts and Walnuts. The Hazard Indices from consumption of heavy metals in the sample are lower than 1 except for in Cocoa which is higher than 1. Therefore, the nuts and seeds analyzed in this study are contaminated with heavy metals and so must be taken with care because excessive intake of iron may increase susceptibility to infection; excessive exposure to copper might result in liver cirrhosis and kidney damage [26].

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Table 1: Mean activity concentration (Bqkg^{-1}) of natural radionuclides in seeds and nuts

Samples	^{40}K	^{226}Ra	^{228}Ra
Kola nut (<i>Cola acominata</i>)	112	9.2	3.8
Cashew nut (<i>Anacardium occidentale</i>)	111	6.5	2.9
Bitter Kola nut (<i>Garcinia kola</i>)	74	4.0	ND
Groundnut (<i>Arachis hypogea</i>)	98	ND	5.0
Walnut (<i>Juglans Regia</i>)	96	ND	2.8
Average (Nut)	98.2	6.57	3.63

Avocado seed (<i>Persea americana</i>)	70	9.8	2.8
African Star Apple seed (<i>Chrysophyllum albidum</i>)	113	5.0	2.7
Cocoa seed (<i>Theobroma cacao</i>)	100	10.5	2.2
Moringa seed (<i>Moringa olifera</i>)	102	ND	ND
Watermelon seed (<i>Citrullus lanatus</i>)	93	ND	ND
Average (Seed)	95.6	8.43	2.57

Table 2: Mean annual effective dose (μSvy^{-1}), fatal cancer risk (MPY^{-1}) and hereditary cancer risk (MPY^{-1}) in nuts and seeds

Samples	E-dose	Fatal cancer risk	Hereditary cancer risk
Kola nut (<i>Cola acominata</i>)	18.05	0.50	0.40
Cashew nut (<i>Anacardium occidentale</i>)	16.36	0.45	0.36
Bitter Kola nut (<i>Garcinia kola</i>)	9.80	0.27	0.21
Groundnut (<i>Arachis hypogea</i>)	13.30	0.36	0.30
Walnut (<i>Juglans Regia</i>)	11.97	0.33	0.26

Average (Nut)	13.99	0.38	0.30
Avocado seed (<i>Persea americana</i>)	6.25	0.17	0.14
African Star Apple seed (<i>Chrysophyllum albidum</i>)	15.86	0.45	0.35
Cocoa seed (<i>Theobroma cacao</i>)	16.46	0.45	0.36
Moringa seed (<i>Moringa olifera</i>)	11.22	0.31	0.25
Watermelon seed (<i>Citrullus lanatus</i>)	10.23	0.28	0.23
Average (Seed)	12.00	0.33	0.27

Table 3: Mean concentration of heavy metals in nuts and seeds

Samples	Fe (mgkg ⁻¹)	Cu (mgkg ⁻¹)	Zn (mgkg ⁻¹)	Cr (mgkg ⁻¹)
Nuts				
Kola nut (<i>Cola acominata</i>)	9.28	9.28	27.67	0.08
Cashew nut (<i>Anacardium occidentale</i>)	26.95	26.95	50.40	ND
Bitter Kola nut (<i>Garcinia kola</i>)	18.68	3.07	2.75	ND
Groundnut (<i>Arachis hypogea</i>)	17.62	8.15	23.98	ND
Walnut	28.86	15.99	37.22	ND

<i>(Juglans Regia)</i>				
Seeds				
Avocado seed	93.45	10.58	14.22	0.52
<i>(Persea americana)</i>				
African Star Apple seed	63.46	13.82	46.37	0.36
<i>(Chrysophyllum albidum)</i>				
Cocoa seed	542.49	51.57	65.82	5.11
<i>(Theobroma cacao)</i>				
Moringa seed	51.96	3.96	32.29	0.08
<i>(Moringa olifera)</i>				
Watermelon seed	327.31	7.42	34.35	0.29
<i>(Citrullus lanatus)</i>				

Table 4: Average Daily Dose (ADD) of metals in the nuts and seeds samples

Samples	Fe (mgkg ⁻¹) x 10 ⁻³	Cu (mgkg ⁻¹) x 10 ⁻³	Zn (mgkg ⁻¹) x 10 ⁻³	Cr (mgkg ⁻¹) x 10 ⁻⁴
Nuts				
Kola nut	8.62	8.62	25.70	0.74
<i>(Cola acominata)</i>				
Cashew nut	25.1	25.1	47.80	ND
<i>(Anacardium occidentale)</i>				
Bitter Kola nut	17.40	2.85	2.55	ND

<i>(Garcinia kola)</i>				
Groundnut	16.36	7.57	22.27	ND
<i>(Arachis hypogea)</i>				
Walnut	26.79	14.85	34.56	ND
<i>(Juglans Regia)</i>				
Seeds				
<hr/>				
Avocado seed	86.77	9.82	13.20	4.83
<i>(Persea americana)</i>				
African Star Apple seed	58.92	12.83	43.06	3.34
<i>(Chrysophyllum albidum)</i>				
Cocoa seed	503.40	47.89	61.12	47.45
<i>(Theobroma cacao)</i>				
Moringa seed	14.80	0.37	0.30	0.74
<i>(Moringa olifera)</i>				
Watermelon seed	303.93	6.89	31.90	2.69
<i>(Citrullus lanatus)</i>				

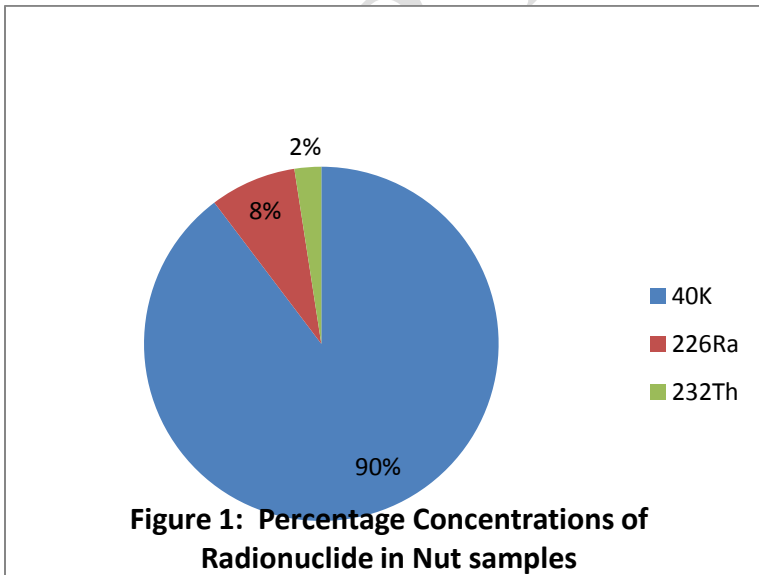
Table 5: Hazard Quotient (HQ, (mg/kg)) and Fractional Hazard Index (HI) of heavy metals in nuts and seeds samples

Samples	Fe (mgkg ⁻¹)	Cu (mgkg ⁻¹)	Zn (mgkg ⁻¹)	Cr (mgkg ⁻¹)x 10 ⁻¹	HI = \sum HQ
Nuts					
Kola nut	0.012	0.216	0.086	0.495	0.216
<i>(Cola acominata)</i>					
Cashew nut	0.036	0.626	0.156	ND	0.818
<i>(Anacardium occidentale)</i>					

Bitter Kola nut (<i>Garcinia kola</i>)	0.025	0.071	0.009	ND	0.105
Groundnut (<i>Arachis hypogea</i>)	0.023	0.189	0.074	ND	0.286
Walnut (<i>Juglans Regia</i>)	0.038	0.371	0.115	ND	0.524

Seeds

Avocado seed (<i>Persea americana</i>)	0.124	0.246	0.044	3.219	0.414
African Star Apple seed (<i>Chrysophyllum albidum</i>)	0.084	0.321	0.144	2.229	0.549
Cocoa seed (<i>Theobroma cacao</i>)	0.720	1.197	0.204	31.633	2.121
Moringa seed (<i>Moringa olifera</i>)	0.069	0.092	0.099	0.495	0.260
Watermelon seed (<i>Citrullus lanatus</i>)	0.434	0.172	0.106	1.795	0.712



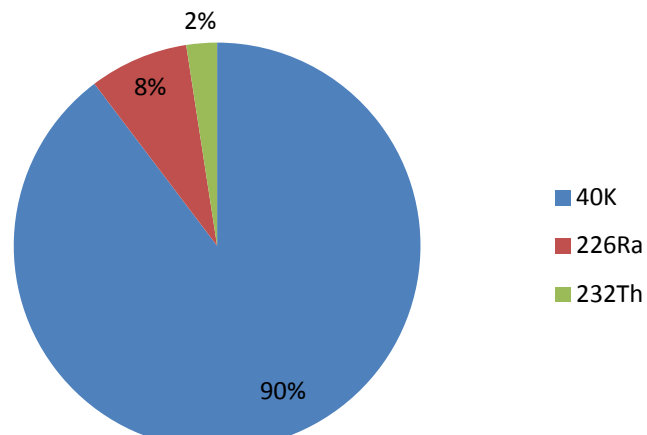


Figure 2: Percentage Concentrations of Radionuclide in Seed samples

UNDER PEER REVIEW