Title: Study of Naturally Occurring Radioactive Materials and some heavy metals 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

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

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

Introduction

The 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 the intake of water, air, soil, and plants [1]. It is a well-known 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 humans. It contains many nutrients beneficial to both adults and the young. It is consumed as a delicacy and sometimes to treat some diseases and ailments. Medicinal application of nuts and seeds is limited due to the 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 has been reported through previous researches[6-8]. The process of transfer of radionuclides and heavy metals to the human body is majorly through inhalation and ingestion of contaminated foodstuffs. These contaminants especially radionuclides emit alpha particles that interact with water in the cells and result in some biological effects (mutation). Chronic effects are accompanied by lung cancer, tumor, genetic effects, kidney failure, and vascular diseases [9].

In Nigeria, the side effects of orthodox medicine are changing people's 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 estimating NORMS, some heavy metals, and evaluation of hazard in the consumption of some nuts and seeds in South-West, Nigeria. The findings of this study would assist the local and international agencies 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 the south-western state of Nigeria were selected for this study. The samples were collected from the farm sites and were packed with identification labels. Nuts and seeds were removed from their shells and were thereafter left to dry at room temperature in the laboratory. The samples were crushed and sieved. 100g of each 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 afterward analyzed using spectrometry method. For heavy metal, their concentration was measured using Inductively Coupled Plasma Spectrometry (ICPS)

Radionuclide Analysis

The analysis of radionuclide in the samples was achieved using NaI(Tl) detector. A Canberra multi-channel analyzer coupled with a scintillating detector was used for the counting. The model no of the detector is 8020 and is manufactured in the USA by Bicron Electronics Ltd. The detector is fixed toa multi-channel analyzer 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 source prepared by Rocketdyne Laboratories, Canoga Park, in the 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 36000 (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 were calculated using a spectrum acquisition and analysis software program (Genie 2K, manufactured by Canberra Industries Inc. USA).

Heavy Metal Analysis

Samples were digested using the wet digestion method. One gram of each sample was weighed into a 100mL tube and the tubes were labeled accordingly. Five milliliters of HNO_3 and H_2O_2 was added into the samples and H_2SO_4 was added in drops. The mixture was stirred until the solution becomes 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 the spray chamber through a nebulizer using a peristaltic pump. Each element measured with spectrometer has its characteristic emission spectrum. The light intensity on the wavelength was measured and with the ICPS calibrated, the 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, the concentration of ^{226}Ra 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 ^{228}Ra 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 ^{40}K 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 was estimated using the equation

$$E_{Dose} = C_R \, . \, DCF_i \, . \, A \qquad 1$$

Where DCF_i is dose the conversion factor for ingestion of the three radionuclides analyzed (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 ^{228}Ra, ^{226}Ra and ^{40}K respectively for adults), C_R is the consumption rate of NORMs from ingestion of seeds and nuts, the 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 2, 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 the United States 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 factors to the public from the ingestion of radionuclides [16]. Fatal Cancer Risk (FCR) from the ingestion of the nuts and seeds was evaluated using equation 2 [17]

$$Fatal \quad Cancer \quad Risk = ED \times LE \times RF \qquad 2$$

where ED is the mean effective dose (μSv/y), LE is life expectancy (55years in Nigeria0[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 2, column 2.

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

$$Hereditary \quad Cancer \quad Risk = ED \times LE \times RF \qquad 3$$

where ED is the mean effective dose (μSv/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 2, column 3.

Heavy metal analysis

The concentration of some of the heavy metals in the samples is shown in Table 3. Pb and Cd were not detected in a substantial amount or are below the detection limit. The concentration of Cr is only found in the 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 bonds with haemoglobin. Cu and Zn concentrations are also higher than recommended in cashew nuts.

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).

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

where C_{metal} is the concentration of metal; I is daily intake of the metals; $D_{average}$, body weight of an adult. The value of ADD estimated for nuts and seeds samples are presented in Table 4. ADD for all metals in the samples is lower than 1

To estimate the health risks, equation 5 was used

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

Where RfD is Oral RfDo. According to USEPA, oral RfD is 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 5. All the HQs obtained in the samples were lower than 1 except for Cu in Cocoa in which HI is higher than 1 indicating a potential risk of exposure to Cu from consumption of Cocoa.

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

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

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 a fatal risk[19].

Discussion

The concentration of NORMs in this study showed that ^{40}K had the highest contribution in all the samples. The concentration of ^{40}K obtained in this study is within the world average. The concentration of ^{226}Ra was highest in kola nut but below the detection limit in groundnut and walnut. The concentration of ^{228}Ra was also below the detection limit in bitter kola nut. The mean concentrations of the radionuclide analyzed in nuts were 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 was highest in African Star Apple and lowest in Avocado with mean of 95.7±15.0 Bq/kg. ^{226}Ra concentration was below the detection limit in Moringa and watermelon seeds. The maximum concentration of ^{226}Ra was from Cocoa seeds. The mean concentration of ^{226}Ra in seeds was obtained as 8.4±2.6 Bq/kg. The concentration of ^{228}Ra in the seeds was found to be lower than the recommendation 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}K >^{226}Ra >^{228}Ra as shown in Figure 1 and 2. The percentage concentrations of ^{226}Ra, ^{228}Ra and ^{40}K in nuts and seeds is the same and so there is 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 was 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 the ingestion of nuts and seeds confirmed that the largest effective dose was from kola nuts (18.05 μSvy^{-1}) while the lowest effective dose was from Avocado (6.25 μSvy^{-1}). The mean annual effective dose for nuts and seeds are 13.99 and 12.00 μSvy^{-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 showed that average of 0.38 and 0.30 people will contract cancer from consumption of nuts and 0.33 and 0.27 people will contract cancer from the consumption of seeds. The highest risk estimated was from consumption of kola nuts while the lowest risk was 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 had the 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, The concentration of Fe was lowest in Groundnut (17.62 mg/kg) and highest in Walnut (28.86 mg/kg) and seeds, Fe concentration was highest in Cocoa (542.49 mg/kg) and lowest in Moringa seed. It was also observed that the concentration of Fe was 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 of Cu was found to be higher than 10mg/kg recommendation in Cashew and Walnuts [22]. Also in seeds, Cu was found to be 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 concentration was also found to be higher than 40mg/kg which is the upper intake for a 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 for all samples are lower than 1 except for Cocoa in which HI exceeded 1. This implies that there may be a 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, diarrhoea, pancreatitis and damage of hepatic parenchyma [25].

Conclusion

Measurement of natural radionuclides and some heavy metals in some nuts and seeds commonly consumed in south-western parts of Nigeria was carried out in this study. The Radioactive analysis was carried out using NaI(Tl) detector. The results of the analysis showed that the concentrations of ^{226}Ra, ^{228}Ra and ^{40}K were within the recommended average. The estimated effective doses were found to be lower than the recommended values. The assessment of the cancer risks from the consumption of nuts and seeds showed that the risks estimated are lower than the safe limit of 1 x

10^{-6} to 1 x 10^{-4}[18,20]. Therefore, no radioactive health risk was involved in the consumption of nuts and seeds if consumed within the 42g per day recommended.

The concentration of heavy metals was measured using Inductively Coupled Plasma (ICP). The concentration of heavy metals in the samples followed the order Fe > Zn > Cu > Cr. Concentration of Zn was found to be higher than the recommendation in Cashew, African star Apple and Cocoa seeds. The measured Cu concentration was found to be higher than the recommended value in Cashew nuts and Walnuts. The Hazard Indices estimated from consumption of heavy metals in the samples are lower than 1 except for in Cocoa where HI was higher than 1. Therefore, nuts and seeds analyzed in this study are contaminated with some 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] while high dose of Zn may cause tachycardia, vomiting, diarrhoea, pancreatitis and damage of hepatic parenchyma[25]

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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 (*Cola acominata*)	112	9.2	3.8
Cashew nut (*Anacardium occidentale*)	111	6.5	2.9
Bitter Kola nut (*Garcinia kola)*	74	4.0	ND
Groundnut (*Arachis hypogeal*)	98	ND	5.0
Walnut (*Juglans Regia*)	96	ND	2.8
Average (Nut)	98.2	6.57	3.63
Avocado seed (*Persea americana*)	70	9.8	2.8
African Star Apple seed (C*hrysophyllum albidum*	113	5.0	2.7
Cocoa seed (*Theobroma cacao*)	100	10.5	2.2
Moringa seed (*Moringa olifera*)	102	ND	ND
Watermelon seed (*Citrullus lanatus*)	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 (*Cola acominata*)	18.05	0.50	0.40
Cashew nut (*Anacardium occidentale*)	16.36	0.45	0.36
Bitter Kola nut (*Garcinia kola*)	9.80	0.27	0.21
Groundnut (*Arachis hypogeal*)	13.30	0.36	0.30
Walnut (*Juglans Regia*)	11.97	0.33	0.26
Average (Nut)	13.99	0.38	0.30
Avocado seed (*Persea americana*)	6.25	0.17	0.14
African Star Apple seed (*Chrysophyllum albidum)*	15.86	0.45	0.35
Cocoa seed (*Theobroma cacao*)	16.46	0.45	0.36
Moringa seed (*Moringa olifera*)	11.22	0.31	0.25
Watermelon seed (*Citrullus lanatus*)	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 (*Cola acominata*)	9.28	9.28	27.67	0.08
Cashew nut (Anacardiumoccidentale)	26.95	26.95	50.40	ND
Bitter Kola nut (*Garcinia kola*)	18.68	3.07	2.75	ND
Groundnut (*Arachis hypogeal*)	17.62	8.15	23.98	ND
Walnut (*JuglansRegia*)	28.86	15.99	37.22	ND
Seeds				
Avocado seed (*Persea americana*)	93.45	10.58	14.22	0.52
African Star Apple seed (*Chrysophyllum albidum*)	63.46	13.82	46.37	0.36
Cocoa seed (*Theobroma cacao*)	542.49	51.57	65.82	5.11
Moringa seed (*Moringa olifera*)	51.96	3.96	32.29	0.08
Watermelon seed (*Citrullus lanatus*)	327.31	7.42	34.35	0.29

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 (*Cola acominata*)	8.62	8.62	25.70	0.74
Cashew nut *(Anacardium occidentale)*	25.1	25.1	47.80	ND
Bitter Kola nut (*Garcinia kola*)	17.40	2.85	2.55	ND
Groundnut (*Arachis hypogeal*)	16.36	7.57	22.27	ND
Walnut (*Juglans Regia*)	26.79	14.85	34.56	ND
Seeds				
Avocado seed (*Persea americana*)	86.77	9.82	13.20	4.83
African Star Apple seed (*Chrysophyllum albidum*)	58.92	12.83	43.06	3.34
Cocoa seed (*Theobroma cacao*)	503.40	47.89	61.12	47.45
Moringa seed (*Moringa olifera*)	14.80	0.37	0.30	0.74
Watermelon seed (*Citrullus lanatus*)	303.93	6.89	31.90	2.69

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^{-4}	HI =∑HQ
Nuts					
Kola nut (*Cola acominata*)	0.012	0.216	0.086	0.495	0.216
Cashew nut (*Anacardium occidentale)*	0.036	0.626	0.156	ND	0.818
Bitter Kola nut (*Garcinia kola*)	0.025	0.071	0.009	ND	0.105
Groundnut (*Arachis hypogeal*)	0.023	0.189	0.074	ND	0.286
Walnut (*Juglans Regia*)	0.038	0.371	0.115	ND	0.524
Seeds					
Avocado seed (*Persea americana*)	0.124	0.246	0.044	3.219	0.414
African Star Apple seed (*Chrysophyllum albidum*)	0.084	0.321	0.144	2.229	0.549
Cocoa seed (*Theobroma cacao*)	0.720	1.197	0.204	31.633	2.121
Moringa seed (*Moringa olifera*)	0.069	0.092	0.099	0.495	0.260
Watermelon seed (*Citrullus lanatus*)	0.434	0.172	0.106	1.795	0.712

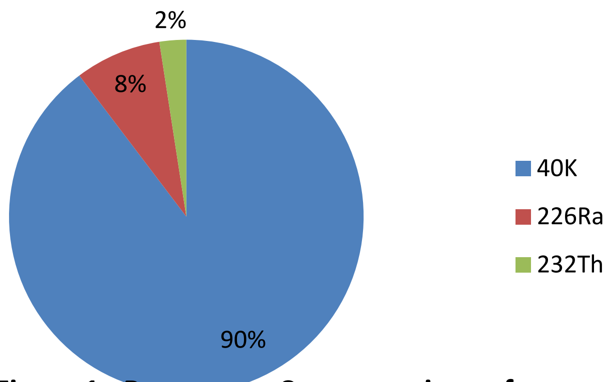

Figure 1: Percentage Concentrations of Radionuclide in Nut samples

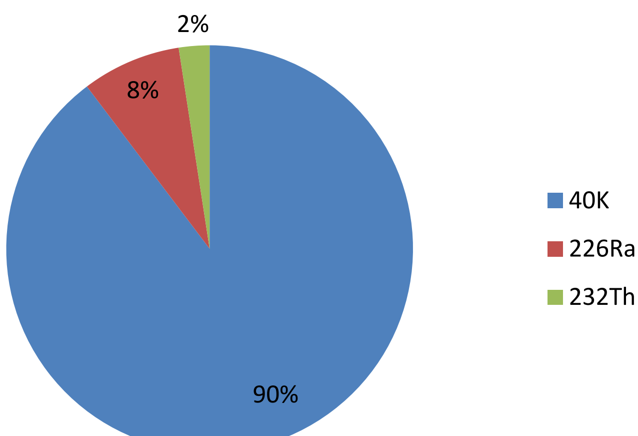

Figure 2: Percentage Concentrations of Radionuclide in Seed samples